Blood phosphorylated tau 181 as a biomarker for Alzheimer's disease: a diagnostic performance and prediction modelling study using data from four prospective cohorts

Thomas K Karikari, Tharick A Pascoal*, Nicholas J Ashton, Shorena Janelidze, Andréa Lessa Benedet, Juan Lantero Rodriguez, Mira Chamoun, Melissa Savard, Min Su Kang, Joseph Therriault, Michael Schöll, Gassan Massarweh, Jean-Paul Soucy, Kina Höglund, Gunnar Brinkmalm, Niklas Mattsson, Sebastian Palmqvist, Serge Gauthier, Erik Stomrud, Henrik Zetterberg, Oskar Hansson†, Pedro Rosa-Neto†, Kaj Blennow†*

Lancet Neurol 2020; 19: 422–33

See Comment page 373

*Co-first authors

†Contributed equally

Department of Psychiatry and Neurochemistry, Institute of Neuroscience and Physiology, Sahlgrenska Academy (T K Karikari PhD, N J Ashton PhD, J L Rodriguez MSc, M Schöll PhD, K Höglund PhD, G Brinkmalm PhD, Prof H Zetterberg MD, Prof K Blennow MD) **and Wallenberg Centre for Molecular and Translational Medicine** (N J Ashton, M Schöll), **University of Gothenburg, Gothenburg, Sweden; Translational Neuroimaging Laboratory, The McGill University Research Centre for Studies in Aging, Montreal, QC, Canada** (T A Pascoal MD, A L Benedet MSc, M Chamoun PhD, M Savard MSc, M S Kang BSc, J Therriault BSc, Prof S Gauthier MD, Prof P Rosa-Neto MD); **Montreal Neurological Institute, Montreal, QC, Canada** (T A Pascoal, M S Kang, G Masserweh PhD, J-P Soucy MD, Prof P Rosa-Neto); **Maurice Wohl Clinical Neuroscience Institute, Institute of Psychiatry, Psychology and Neuroscience, King's College London, London, UK** (N J Ashton); **National Institute for Health Research Biomedical Research Centre for Mental Health and Biomedical Research Unit for Dementia, South London and Maudsley NHS Foundation Trust, London, UK** (N J Ashton); **Clinical Memory Research Unit, Department of Clinical Sciences** (S Janelidze PhD, N Mattsson MD, S Palmqvist MD, E Stomrud PhD, Prof O Hansson PhD), **Wallenberg Center for Molecular Medicine**

Summary

Background CSF and PET biomarkers of amyloid β and tau accurately detect Alzheimer's disease pathology, but the invasiveness, high cost, and poor availability of these detection methods restrict their widespread use as clinical diagnostic tools. CSF tau phosphorylated at threonine 181 (p-tau181) is a highly specific biomarker for Alzheimer's disease pathology. We aimed to assess whether blood p-tau181 could be used as a biomarker for Alzheimer's disease and for prediction of 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 patients with Alzheimer's disease and age-matched controls. Two validation cohorts (TRIAD and BioFINDER-2) included cognitively unimpaired older adults (mean age 63–69 years), participants with mild cognitive impairment (MCI), Alzheimer's disease, and frontotemporal dementia. In addition, TRIAD included healthy young adults (mean age 23 years) and BioFINDER-2 included patients with other neurodegenerative disorders. The primary care cohort, which recruited participants in Montreal, Canada, comprised control participants from the community without a diagnosis of a neurological condition and patients referred from primary care physicians of the Canadian National Health Service 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 We studied 37 individuals in the discovery cohort, 226 in the first validation cohort (TRIAD), 763 in the second validation cohort (BioFINDER-2), and 105 in the primary care cohort (n=1131 individuals). In all cohorts, plasma p-tau181 showed gradual increases along the Alzheimer's disease continuum, from the lowest concentrations in amyloid β-negative young adults and cognitively unimpaired older adults, through higher concentrations in the amyloid β-positive cognitively unimpaired older adults and MCI groups, to the highest concentrations in the amyloid β-positive MCI and Alzheimer's disease groups (p<0·001, Alzheimer's disease *vs* all other groups). Plasma p-tau181 distinguished Alzheimer's disease dementia from amyloid β-negative young adults (AUC=99·40%) and cognitively unimpaired older adults (AUC=90·21–98·24% across cohorts), as well as other neurodegenerative disorders, including frontotemporal dementia (AUC=82·76–100% across cohorts), vascular dementia (AUC=92·13%), progressive supranuclear palsy or corticobasal syndrome (AUC=88·47%), and Parkinson's disease or multiple systems atrophy (AUC=81·90%). Plasma p-tau181 was associated with PET-measured cerebral tau (AUC=83·08–93·11% across cohorts) and amyloid β (AUC=76·14–88·09% across cohorts) pathologies, and 1-year cognitive decline (p=0·0015) and hippocampal atrophy (p=0·015). In the primary care cohort, plasma p-tau181 discriminated Alzheimer's disease from young adults (AUC=100%) and cognitively unimpaired older adults (AUC=84·44%), but not from MCI (AUC=55·00%).

Interpretation Blood p-tau181 can predict tau and amyloid β pathologies, differentiate Alzheimer's disease from other neurodegenerative disorders, and identify Alzheimer's disease across the clinical continuum. Blood p-tau181 could be used as a simple, accessible, and scalable test for screening and diagnosis of Alzheimer's disease.

Funding Alzheimer Drug Discovery Foundation, European Research Council, Swedish Research Council, Swedish Alzheimer Foundation, Swedish Dementia Foundation, Alzheimer Society Research Program.

Introduction

More than 50 million people worldwide have dementia, and the cost of dementia care reached US$1 trillion in 2018.[1] Amyloid β 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 CSF and PET biomarkers of amyloid β and tau are highly accurate for detecting Alzheimer's disease pathology, the costs and

Research in context

Evidence before this study

We searched PubMed for all articles published from database inception to Jan 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". Previous attempts to develop a reliable blood assay for p-tau181 have been challenging due to the very low concentrations in blood samples. Furthermore, initial unsuccessful efforts focused on applying established mid-region CSF p-tau181 immunoassays directly to blood. Recent evidence has shown that tau in blood and CSF might 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 few patient cohorts. However, some of these assays lack the analytical sensitivity for examining cognitively unimpaired individuals, some of whom might be in the preclinical phase of Alzheimer's disease. Moreover, it is unclear if previously described 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, characterised 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 good diagnostic performance for Alzheimer's disease in both plasma and serum. The blood p-tau181 assay identified Alzheimer's disease at the very early stages of disease and demonstrated high diagnostic accuracy, with stepwise increases across the Alzheimer's disease continuum. Similar to mid-region CSF p-tau181, our blood p-tau181 assay appeared to be specific to Alzheimer's disease, differentiating it from other neurodegenerative diseases with high accuracy. Additionally, blood p-tau181 predicted cognitive decline and hippocampal atrophy over a period of 1 year, making it suitable as an Alzheimer's disease progression marker. Furthermore, plasma p-tau181 performed better than the most well-known Alzheimer's disease risk factors (age, *APOE* ε4 genotype, or both) and other plasma biomarkers (total-tau, amyloid β_{1-42}, amyloid β_{1-42} to amyloid β_{1-40} ratio, and total-tau to amyloid β_{1-42} ratio) in predicting Alzheimer's disease diagnosis, increased tau PET, and increased amyloid β PET.

Implications of all the available evidence

The blood p-tau181 assay described in this study could represent the first simple, practical, and scalable test for the diagnosis of Alzheimer's disease. This technology has applications for diagnosis and recruitment for disease-modifying trials. The blood p-tau181 assay has the potential to be incorporated into clinical practice as a rapid screening test to identify or rule out Alzheimer's disease pathophysiology and to guide therapy and clinical management of patients with suspected neurodegenerative disorders. To facilitate widespread implementation of the blood p-tau181 assay, full clinical standardisation, including establishment of reference materials and methods to harmonise readouts across clinical laboratories, will be required.

(N Mattsson), **and Department of Neurology, Skåne University Hospital** (N Mattsson), **Lund University, Lund, Sweden; Clinical Neurochemistry Laboratory, Sahlgrenska University Hospital, Mölndal, Sweden** (K Höglund, Prof H Zetterberg, Prof K Blennow); **Department of Neurodegenerative Disease, Institute of Neurology and UK Dementia Research Institute, University College London, London, UK** (Prof H Zetterberg); **and Memory Clinic, Skåne University Hospital, Malmö, Sweden** (Prof O Hansson)

Correspondence to:
Prof Kaj Blennow, Department of Psychiatry and Neurochemistry, Institute of Neuroscience and Physiology, Sahlgrenska Academy, University of Gothenburg, Gothenburg SE-43180, Sweden
kaj.blennow@neuro.gu.se

low availability of the tools needed to detect these biomarkers 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 attractive for first-line clinical use and for facilitating 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, because abnormal increases are also reported in several other neurodegenerative disorders, such as multiple system atrophy, corticobasal syndrome, and progressive supranuclear palsy.[9]

Another advance in biomarkers for Alzheimer's disease is the use of mass spectrometry-based assays for plasma amyloid β (ratio of amyloid β_{1-42} to amyloid β_{1-40}), which reflects brain amyloidosis.[10,11] However, these assays have limitations, including substantial peripheral amyloid β expression,[12] which results in less pronounced decreases in the amyloid β_{1-42} to amyloid β_{1-40} ratio in plasma compared with CSF, and a larger overlap of amyloid β concentrations between individuals who are amyloid β PET-positive and PET-negative.[10] Furthermore, brain amyloidosis is present in 10–30% of individuals who are cognitively unimpaired.[13] By contrast, CSF tau phosphorylated at threonine 181 (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 had insufficient analytical sensitivity for examining preclinical and cognitively unimpaired individuals, and it is unclear whether Alzheimer-specific tau pathology was detected.

In this study, we report the performance of an ultrasensitive immunoassay for blood p-tau181 that can be implemented for a practical assessment of in-vivo Alzheimer's disease pathophysiology. We aimed to evaluate whether blood p-tau181 can: (1) differentiate Alzheimer's disease dementia from no cognitive impairment, mild cognitive impairment (MCI) due to Alzheimer's disease, and other neurodegenerative diseases; (2) reflect abnormalities in tau or amyloid PET scans; and (3) predict future cognitive decline and hippocampal atrophy.

	Discovery cohort (n=37)		Primary care clinical cohort (n=105)			
	Cognitively unimpaired older adults (n=18)	Alzheimer's disease (n=19)	Young adults (n=11)	Cognitively unimpaired older adults (n=72)	Mild cognitive impairment (n=12)	Alzheimer's disease (n=10)
Age, years	63·8 (11·4)	74·4 (5·4)*	23·5 (2·0)*	70·0 (9·1)†	71·7 (10·5)	62·7 (13·6)*
Sex						
Men	13 (72%)	9 (47%)	6 (55%)	23 (32%)	4 (33%)	6 (60%)
Women	5 (28%)	10 (53%)	5 (45%)	49 (68%)	8 (67%)	4 (40%)
APOE ε4 genotype	..	..	2/11 (18%)	23/69 (33%)	5/12 (42%)	4/10 (40%)
Education, years	..	..	17·8 (2·4)	15·1 (3·6)	14·1 (3·2)	13·0 (3·3)
CSF amyloid β_{1-42}, pg/mL	842·2 (175·9)	388·9 (72·1)*	..	..	..	..
CSF p-tau181, pg/mL	35·4 (10·1)	94·3 (28·6)*	..	..	..	..
CSF total-tau, pg/mL	223·3 (68·7)	669·5 (255·5)*	..	..	..	..

Data are mean (SD) or n (%). Cognitively unimpaired older adults in the discovery cohort additionally tested negative for the CSF core biomarkers (amyloid β, p-tau181, and total-tau). The individuals in the young adults group were cognitively unimpaired. Student's *t* test (in the discovery cohort) or analysis of variance followed by Tukey's post-hoc test (in the primary care cohort) was done to identify significant differences between groups for continuous variables. For sex and *APOE* ε4 genotype, contingency χ^2 tests were done. p-tau181=tau phosphorylated at threonine 181. *p<0·05 compared with cognitively unimpaired older adults. †p<0·05 compared with Alzheimer's disease.

Table 1: **Characteristics of the discovery and primary care cohorts**

Methods

Study design and population

We developed a new analytical method for blood p-tau181 and validated its performance for assessment of Alzheimer's pathophysiology using four independent, prospective cohorts recruiting consecutive cases. The discovery cohort included patients with Alzheimer's disease, with a typical Alzheimer's disease core CSF biomarker profile (CSF amyloid β_{1-42} <530 ng/L, p-tau181 >60 ng/L, and total-tau >350 ng/L[19]), and age-matched controls, who were patients examined at the memory or neurology clinics in the catchment area of the Sahlgrenska University Hospital (Gothenberg, Sweden) for minor neurological or psychiatric symptoms, and who had both basic and core CSF biomarker levels within normal ranges.

Two independent validation cohorts were evaluated, from the TRIAD (McGill University, Canada)[20] and BioFINDER-2 (Lund University, Sweden)[21] studies. Participants in both cohorts underwent detailed assessments, including of CSF (amyloid β_{1-42}, p-tau181, and total-tau) and PET (tau and amyloid β) biomarkers, and clinical and cognitive evaluations. Both cohorts included older people (>60 years) who were cognitively unimpaired, and participants with MCI, Alzheimer's disease dementia, and frontotemporal dementia. Additionally, TRIAD included young adults (20–30 years), and BioFINDER-2 included individuals with other neurodegenerative disorders. Amyloid β positivity in the TRIAD and BioFINDER-2 cohorts was independently determined using amyloid β PET uptake, on the basis of visual rating and a consensus of two neurologists blinded to the diagnosis for TRIAD,[22] and mixture modelling techniques for BioFINDER-2 (appendix pp 6, 7).[23]

See Online for appendix

Finally, we tested the feasibility of using the assay as a rapid screening tool in a primary care cohort in Montreal, Canada, that included controls from the community without a diagnosis of a neurological condition and patients referred from primary care physicians of the Canadan National Health System for specialist care at the McGill University Research Centre for Studies in Aging (Montreal, Canada). These patients had received a clinical diagnosis in the primary care setting, but had not yet undergone biomarker and clinical assessments in specialist centres.

All studies were approved by the relevant ethics committees, and written informed consent was obtained for all participants when necessary. Further details about the study participants are provided in the appendix (pp 5, 6).

Outcomes

In the discovery cohort, CSF p-tau181, total-tau, and amyloid β_{1-42} were measured between Feb 1 and March 30, 2019, using Innotest ELISA assays from Fujirebio (Tokyo, Japan), as described previously.[24] A biomarker-positive Alzheimer's disease diagnosis was determined using previously defined cutoffs.[19] The fully automated LUMIPULSE G1200 (Fujirebio) was used to measure CSF p-tau181, total-tau, and amyloid β_{1-42} for the TRIAD and primary care cohorts between Aug 1 and Dec 31, 2019. For the BioFINDER-2 cohort, Meso Scale Discovery assays (Meso Scale Diagnostics, Rockville, MD, USA) were used to measure CSF amyloid β_{1-42} and amyloid β_{1-40}.

In the TRIAD cohort, individuals were assessed using 3T MRI as well as amyloid β [^{18}F]AZD4694 PET and tau [^{18}F]MK-6240 PET between April 1, 2017, and June 30, 2019. In the BioFINDER-2 cohort, individuals had MRI, amyloid β [^{18}F]flutemetamol PET, and tau [^{18}F]RO948 PET between May 1, 2017, and Oct 30, 2019. We segregated individuals into Braak staging groups based on in-vivo tau PET deposition in regions corresponding to stages I–II, III–IV, and V–VI (postmortem Braak staging suggests that the accumulation of tau

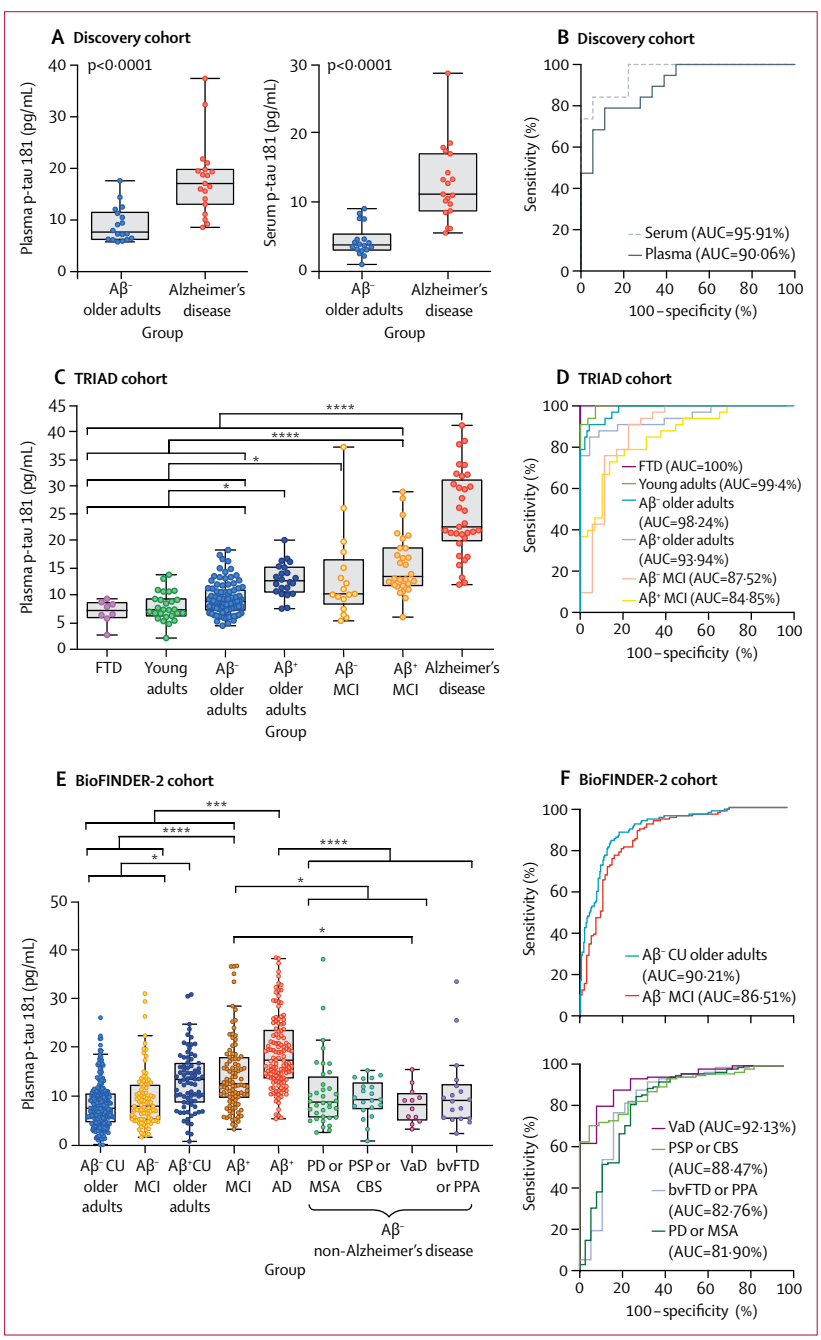
	TRIAD cohort (n=226)					BioFINDER-2 cohort (n=763)						
	Young adults (n=27)	CU older adults (n=113)	MCI (n=45)	Alzheimer's disease (n=33)	FTD (n=8)	CU older adults (n=337)	MCI (n=191)	Alzheimer's disease (n=126)	Behavioural variant FTD or PPA (n=18)	PD or MSA (n=36)	Vascular dementia (n=12)	PSP or CBS (n=21)
Age, years	22·7 (1·9)*†	69·2 (9·7)	72·6 (6·8)#	64·6 (9·2)	59·3 (8·5)*	63·1 (5·0)†	70·6 (8·1)*	74·0 (6·9)*	67·4 (7·4)	68·7 (11·0)	74·8 (6·5)*	69·0 (7·9)
Sex												
Men	10 (37%)	41 (36%)	22 (49%)	18 (55%)	1 (12%)	154 (46%)	106 (55%)	59 (47%)	5 (28%)	21 (58%)	8 (67%)	12 (57%)
Women	17 (63%)	72 (64%)	23 (51%)	15 (45%)	7 (88%)	183 (54%)	85 (45%)*	67 (53%)	13 (72%)	15 (42%)	4 (33%)	9 (43%)
APOE ε4	6/27 (22%)†	33/111 (30%)†	19/44 (43%)	17/32 (53%)*	0/8†	147/335 (44%)†	98/186 (53%)†	87/123* (71%)	3/17 (18%)*†	12/34 (35%)†	3/12 (25%)†	5/21 (24%)†
Education, years	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	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 amyloid $\beta_{1\text{-}42}$, pg/mL	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, pg/mL	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, pg/mL	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)
Amyloid β PET SUVR	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 region	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 region	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 region	1·1 (0·2)†	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, pg/mL	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)†

Data are mean (SD) or n (%). In the BioFINDER-2 cohort, amyloid β-positive non-Alzheimer's disease cases (n=22) are not shown; characteristics of the cohort including these individuals are shown in the appendix (p 20). In both cohorts, CU older adults were CSF biomarker-negative. Individuals in the young adults group were cognitively unimpaired and CSF biomarker-negative. All individuals in the FTD or PPA, PD or MSA, vascular dementia, and PSP or CBS groups were amyloid β-negative. Additional stratification by amyloid β is provided in the appendix (pp 19, 20). We used analysis of variance followed by Tukey's post hoc test to assess differences between groups for continuous variables. For sex and *APOE* ε4 genotype, we used contingency χ^2 tests. Data were unavailable for BioFINDER-2 participants for the following variables: *APOE* ε4 (n=13), education (n=6), MMSE (n=3), CSF amyloid β_{42} and total-tau (n=1), CSF p-tau181 (n=3), amyloid β PET (n=332), and tau PET (n=95). CU=cognitively unimpaired. MCI=mild cognitive impairment. FTD=frontotemporal dementia. PPA=primary progressive aphasia. PD=Parkinson's disease. MSA=multiple systems atrophy. PSP=progressive supranuclear palsy. CBS=corticobasal syndrome. MMSE=Mini-Mental State Examination. SUVR=standardised uptake value ratio. p-tau181=tau phosphorylated at threonine 181. *p<0·05 compared with cognitively unimpaired older adults. †p<0·05 compared with Alzheimer's disease.

Table 2: **Characteristics of the TRIAD and BioFINDER-2 cohorts**

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[25]). Tau PET standardised uptake value ratio was measured regionally in the transentorhinal (stage I–II), limbic (III–IV), and isocortical (V–VI) Braak regions, as previously described,[26] as well as globally in a composite area including the whole cortex (Braak stage I–VI regions), and tau positivity defined as an SD of 2·5 higher than the mean standardised uptake value ratio of older patients who are amyloid β-negative and cognitively unimpaired. Further details are provided in the appendix (pp 6, 7).

For individuals in TRIAD who had baseline plasma p-tau181 measures and baseline Mini Mental State Examination (MMSE) scores and structural MRI assessments (n=226), we assessed the associations between plasma p-tau181 and cognitive impairment and neurodegeneration at baseline. Furthermore, in individuals with baseline plasma p-tau181 as well as baseline and 1-year follow-up MMSE scores (n=85) and structural MRI assessments (n=88), we evaluated the associations between baseline plasma p-tau181 concentrations and 1-year longitudinal change in cognitive function and neurodegeneration. Brain atrophy was measured by grey matter density on T1-weighted MRI images using voxel-based morphometry.



(Figure 1 continues on next page)

Predictors

Plasma p-tau181 for the four cohorts was measured in the Clinical Neurochemistry Laboratory, University of Gothenberg (Mölndal, Sweden) during May to December, 2019, (one run for each cohort) in a blinded manner, on the Simoa HD-1 (Quanterix, Billerica, MA, USA). The AT270 mouse monoclonal antibody (MN1050; Invitrogen, Waltham, MA, USA) specific for the threonine-181 phosphorylation site[27] was coupled to paramagnetic beads (103207; Quanterix) and used for capture. This antibody recognises the tau sequence 176-PPAPKT(p)P-182 phosphorylated specifically at threonine-181.[28] As the detector, we used the anti-tau mouse monoclonal antibody Tau12 (806502; BioLegend, San Diego, CA, USA), which binds the N-terminal epitope 6-QEFEVMEDHAGT-18 on human tau protein.[29] Amino acid numbering follows that of the full-length tau 1-441 (Uniprot ID P10636-8). The detection antibody was conjugated to biotin (A3959; Thermo Fisher Scientific, Waltham, MA, USA) following the manufacturer's recommendations. Full-length recombinant tau1-441 phosphorylated in vitro by glycogen synthase kinase 3β (TO8–50FN; SignalChem, Vancouver, BC, Canada) was used as the calibrator. A detailed description of analytical procedures and assay validation is provided in 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, *APOE* ε4 genotype, or both) to correctly identify Alzheimer's disease diagnosis, increased amyloid β PET, and elevated tau PET uptake. *APOE* ε4 genotyping was done using the TaqMan real-time polymerase chain reaction assay externally at Applied Biosystems (Foster City, CA, USA). Furthermore, the performance of plasma p-tau181 to accurately identify Alzheimer's disease diagnosis and increased amyloid β and tau PET was compared with other plasma biomarkers (total-tau, amyloid β_{1-42}, amyloid β_{1-42} to amyloid β_{1-40} ratio, and total-tau to amyloid β_{1-42} ratio) using AUC analyses. Plasma total-tau, amyloid β_{1-42}, and amyloid β_{1-40} were

measured using the Neuro 3-Plex A kit (101995; Quanterix), following the manufacturer's instructions on the Simoa HD-1 instrument.

Statistical analysis

The prospective 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 version 3.1.2, MATLAB version 9.2 with VoxelStats package,[30] and SPSS version 26. Only individuals with complete data were included in each specific analysis. Unpaired t-tests and analysis of variance with Tukey's multiple comparisons test were used to compare continuous variables between groups. The χ^2 test was used to compare dichotomous variables between groups. Receiver operating curves (ROCs) comparing cohort subgroups provided the AUC for a diagnosis of Alzheimer's disease or biomarker positivity. AUC, sensitivity, specificity, and the representative best value for accuracy at an optimal cutoff 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 at baseline and 1-year change in cognition (MMSE score) and structural imaging (hippocampus grey matter density) data. The linear regressions were corrected for age, sex, *APOE* ε4 genotype, and years of formal education. p<0·05 was considered to indicate statistical significance. Further details of statistical analyses are provided 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. TKK, TAP, SJ, PR-N, and KB had access to all the data in the study and had final responsibility for the decision to submit for publication.

Results

We studied 37 individuals in the discovery cohort, 226 in the first validation cohort (TRIAD), 763 in the second validation cohort (BioFINDER-2), and 105 in the primary care cohort (total of 1131 individuals). Blood p-tau181 concentrations were not affected by sex. Demographic characteristics are presented in tables 1 and 2, and in the appendix (pp 19, 20).

The blood p-tau181 assay (appendix p 10) showed high analytical performance (appendix p 21), with high precision within and between runs, and between different batches of reagents (appendix pp 22–24). Mass spectrometric studies showed that the assay specifically measures N-terminal to mid-domain forms of p-tau181, and does not recognise non-phosphorylated forms of tau (appendix p 11).

In the discovery cohort, the mean p-tau181 concentrations in paired serum and plasma samples were approximately two-times and three-times higher, respectively, in CSF biomarker-positive patients with Alzheimer's disease compared with controls (p<0·0001; figure 1A). p-tau181 concentrations in paired serum and plasma were positively correlated (r=0·8202, p<0·0001; appendix p 12). p-tau181 in both plasma and serum showed high performance for the diagnosis of Alzheimer's disease (serum AUC=95·91%; plasma AUC=90·06%; figure 1B, appendix p 13), suggesting that plasma and serum are equally suitable for p-tau181 analysis.

In the TRIAD cohort, plasma p-tau181 was increased in the CSF amyloid β-positive Alzheimer's disease group compared with all other diagnostic groups (p<0·0001; figure 1C). Plasma p-tau181 concentrations in amyloid β-positive cognitively unimpaired older adults and amyloid β-negative and amyloid β-positive individuals with MCI were higher than in young adults, individuals with frontotemporal dementia, and amyloid β-negative cognitively unimpaired older adults (p<0·05; figure 1C, appendix p 19). Plasma p-tau181 distinguished Alzheimer's disease from frontotemporal dementia (AUC=100%), young adults and cognitively unimpaired older adults (AUC and accuracy >95%), and MCI (AUC >84% and accuracy >80%; figure 1D, appendix p 13). Plasma p-tau181 distinguished amyloid β-positive cognitively unimpaired older adults from amyloid β-negative cognitively unimpaired older adults (AUC=81·02%), and young adults (AUC=89·90%; appendix p 14).

In the BioFINDER-2 cohort, plasma p-tau181 concentrations gradually increased across the Alzheimer's disease clinical continuum, with the lowest concentration in the CSF amyloid β-negative cognitively unimpaired

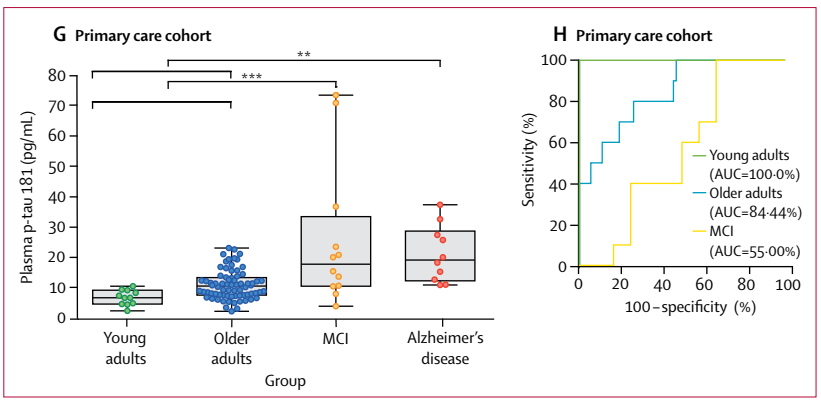


Figure 1: **Plasma p-tau181 concentrations in the four cohorts**

In box-and-whisker plots the central horizontal bar shows the median p-tau181 concentration, and the lower and upper boundaries show the 25th and 75th percentiles, respectively. Graphs show receiver operating curves. 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%. Data are presented separately for the discovery cohort (A, B), the TRIAD cohort (C, D), the BioFINDER-2 cohort (E, F), and the primary care cohort (G, H). For illustrative purposes only, four individuals with cognitive impairment with high plasma p-tau181 concentrations (50–90 pg/mL) were not shown in panel E, but they were fully included in the statistical analyses. In panels D, F, and H, receiver operating curves are calculated versus Alzheimer's disease. p values are indicated with asterisks: *p<0·05, **p<0·01, ***p<0·001, ****p<0·0001. Aβ=amyloid β. AUC=area under the curve. bvFTD=behavioural variant frontotemporal dementia. CBS=corticobasal syndrome. CU=cognitively unimpaired. FTD=frontotemporal dementia. MCI=mild cognitive impairment. MSA=multiple systems atrophy. p-tau181=tau phosphorylated at threonine 181. PD=Parkinson's disease. PPA=primary progressive aphasia. PSP=progressive supranuclear palsy. VaD=vascular dementia.

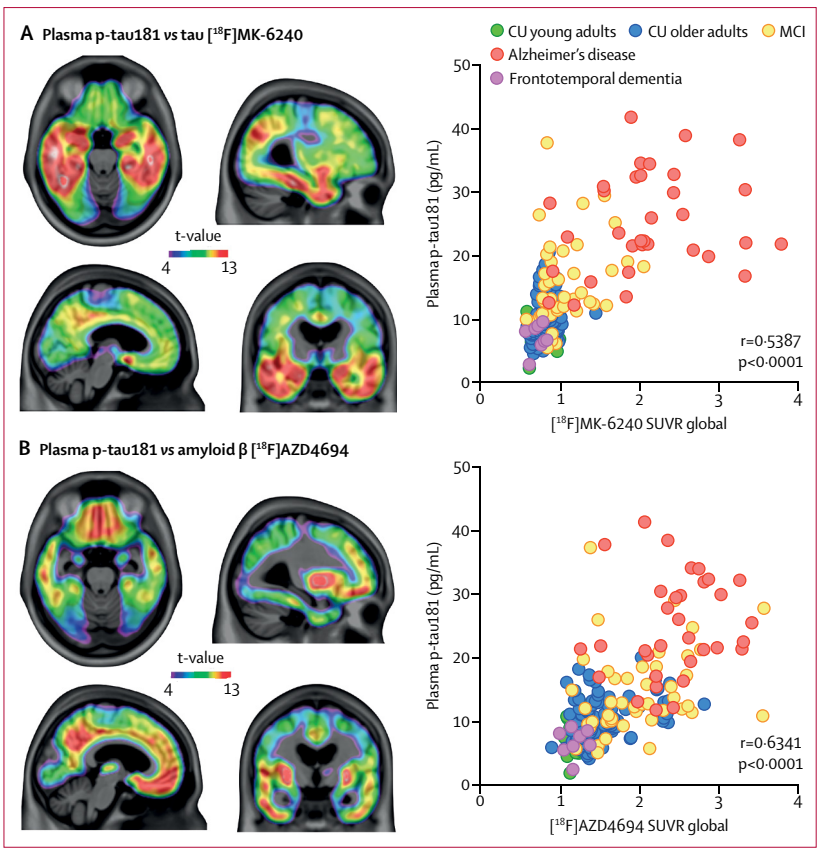


Figure 2: **Associations between plasma p-tau181 concentration and PET tau and amyloid β load**
(A) Association between plasma p-tau181 and PET-measured tau ([18F]MK-6240 tau PET). (B) Association between p-tau181 and PET-measured amyloid β load ([18F]AZD4694 amyloid β PET). Brain images show the results of voxel-wise regressions (false discovery rate corrected for multiple comparisons at p<0·05) overlaid on a structural MRI template. The strength of the association of plasma p-tau181 versus tau PET and amyloid β PET in different brain areas is shown in colour scales, with the areas with greatest association indicated in red. Graphs on the right show Spearman's rank correlations between plasma p-tau181 and tau PET (PET [18F]MK-6240) or amyloid β PET ligand ([18F]AZD4694) uptake (n=226). PET [18F]MK-6240 SUVR global values were estimated from Braak I–VI regions and [18F]AZD4694 SUVR global values were estimated from typical brain regions used to assess global PET amyloid β (appendix pp 6, 7). For tau PET, r=0·6280 and p<0·0001 for amyloid β-positive individuals, and r=0·1636 and p=0·0492 for amyloid β-negative individuals. For amyloid β PET, r=0·4454 and p<0·0001 for amyloid β-positive individuals, and r=0·2890 and p=0·0004 for amyloid β-negative individuals. p-tau181=tau phosphorylated at threonine 181. MCI=mild cognitive impairment. SUVR=standardised uptake value ratio.

older adults and amyloid β-negative MCI groups, an increased concentration in the amyloid β-positive cognitively unimpaired older adult and amyloid β-positive MCI groups, and the highest concentration in the CSF amyloid β-positive Alzheimer's disease group (p<0·0001 *vs* all other cognitively unimpaired and MCI groups; figure 1E). Plasma p-tau181 distinguished Alzheimer's disease from amyloid β-negative cognitively unimpaired older adults (AUC=90·21%) and amyloid β-negative MCI (AUC=86·51%; figure IF). Moreover, plasma p-tau181 was increased in Alzheimer's disease compared with several amyloid β-negative neurodegenerative disorders (p<0·0001; figure 1E). Plasma p-tau181 distinguished Alzheimer's disease from vascular dementia (AUC=92·13%), progressive supranuclear palsy or corticobasal syndrome (AUC=88·47%), behavioural variant frontotemporal dementia or primary progressive aphasia (AUC=82·76%), and Parkinson's disease or multiple systems atrophy (AUC=81·90%; figure 1F). Comparisons with amyloid β-positive neurodegenerative disorders (ie, with concomitant Alzheimer's disease-type pathology) are shown in the appendix (p 15).

In the primary care cohort, plasma p-tau181 concentration increased progressively from young adults to cognitively unimpaired older adults, MCI, and clinically diagnosed Alzheimer's disease patients with unknown CSF and PET biomarker status (figure 1G). Plasma p-tau181 distinguished Alzheimer's disease from young adults (AUC and accuracy=100%), cognitively unimpaired older adults (AUC=84·44% and accuracy >90%), but not from MCI (AUC=55·00%; figure 1H, appendix p 13).

In the TRIAD cohort, plasma p-tau181 was strongly correlated with PET-measured tau ([18F]MK-6240 PET) across the cortex, with the highest association in the temporal lobe (figure 2A), and also with PET-measured amyloid β ([18F]AZD4694 PET) across the cortex, with the highest associations in the precuneus, frontal cortex, and striatum (figure 2B). Plasma p-tau181 strongly predicted tau PET positivity (AUC and accuracy >90%, figure 3A) and amyloid β PET positivity (AUC=88·09% and accuracy >80%, figure 3B). Additionally, plasma p-tau181 distinguished individuals who were PET positive for both tau and amyloid β from individuals who were 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 (appendix p 16), and correlated better with both tau PET and amyloid β PET in amyloid β-positive cases than in amyloid β-negative individuals (figure 2). Plasma p-tau181 correlation with tau and amyloid β PET stratified by clinical diagnosis is shown in the 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 group, calculated as age at blood collection minus age of onset (r=0·3627, p=0·0252). Among tau PET-negative individuals (Braak 0), plasma p-tau181 distinguished amyloid β-positive from amyloid β-negative individuals (AUC=84·82% [data not shown]; figure 3E).

In the BioFINDER-2 cohort, plasma p-tau181 correlated with PET-measured tau ([18F]RO948 PET) in amyloid β-positive individuals (Braak I–II, r=0·445; Braak III–IV, r=0·488; Braak V–IV, r=0·446; all p<0·0001). Plasma p-tau181 differentiated tau PET-positive individuals from tau PET-negative individuals with high accuracy (Braak I–II, AUC=83·08%; Braak III–IV, AUC=85·08%; Braak V–VI, AUC=84·70%; appendix p 17). Additionally, plasma p-tau181 was higher for amyloid β PET-positive cases than amyloid β PET-negative participants (p<0·0001).

In the discovery cohort, plasma and serum p-tau181 (Simoa) were correlated with Innotest CSF p-tau181 (r=0·7055, p<0·0001 for plasma; r=0·7937, p<0·0001 for

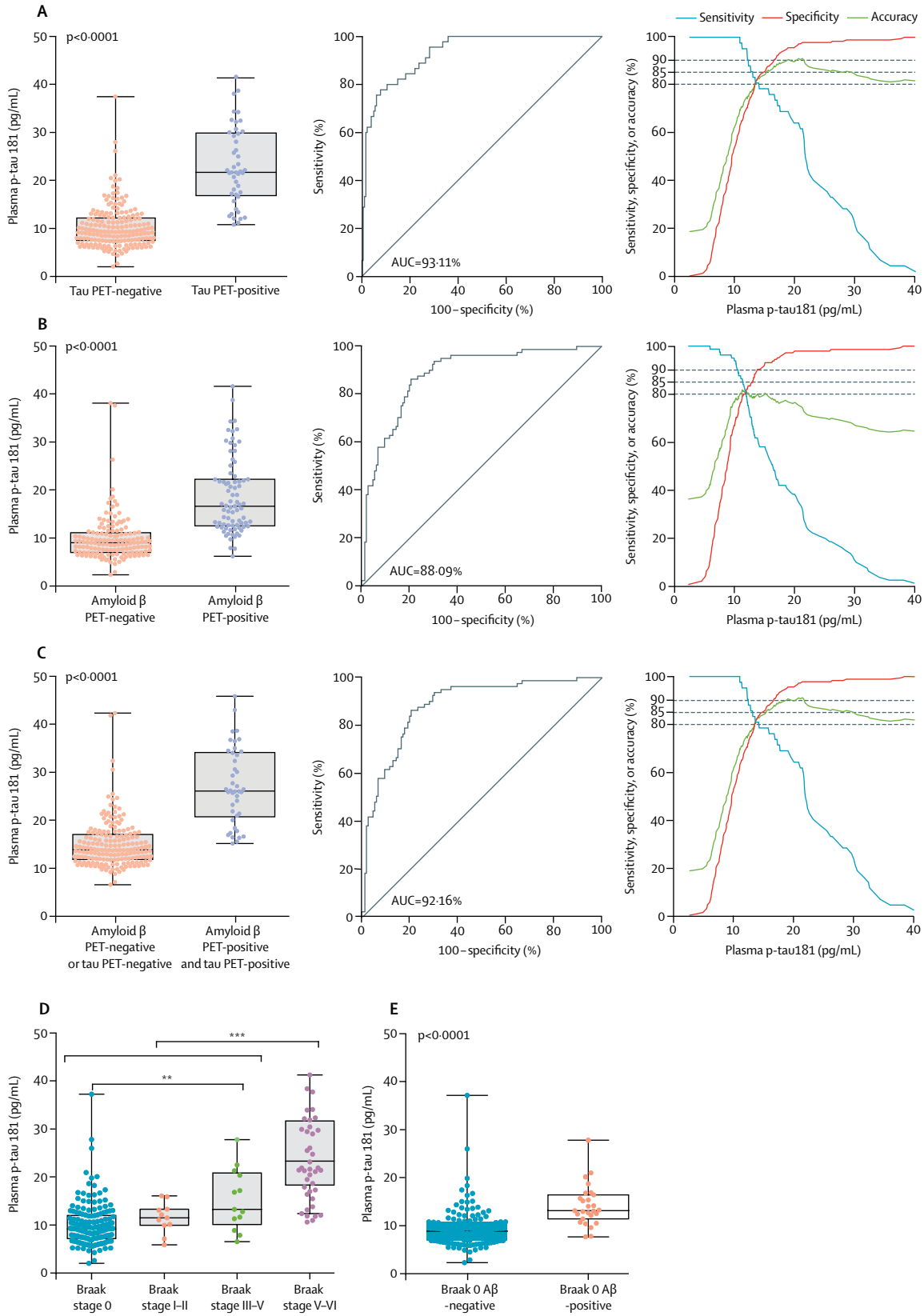


Figure 3: **Plasma p-tau181 concentration according to tau PET and amyloid β PET positivity**
(A) Plasma p-tau181 concentrations in tau PET-positive (n=45) and tau PET-negative (n=181) individuals. (B) Plasma p-tau181 concentrations in amyloid β PET-positive (n=81) and amyloid β PET-negative (n=145) individuals. (C) Plasma p-tau181 concentrations in individuals who were positive for both tau PET and amyloid β PET (n=42) and individuals who were negative for at least one of these biomarkers (n=184). (D) Plasma p-tau181 concentrations according to disease severity, as measured by tau PET Braak stages. (E) Plasma p-tau181 concentrations in tau PET-negative participants (Braak stage 0) separated into amyloid β-positive (n=139) and amyloid β-negative (n=29) individuals. p values are indicated with asterisks: **p<0·01, ***p<0·001. Aβ=amyloid β. AUC=area under the curve. p-tau181=tau phosphorylated at threonine 181.

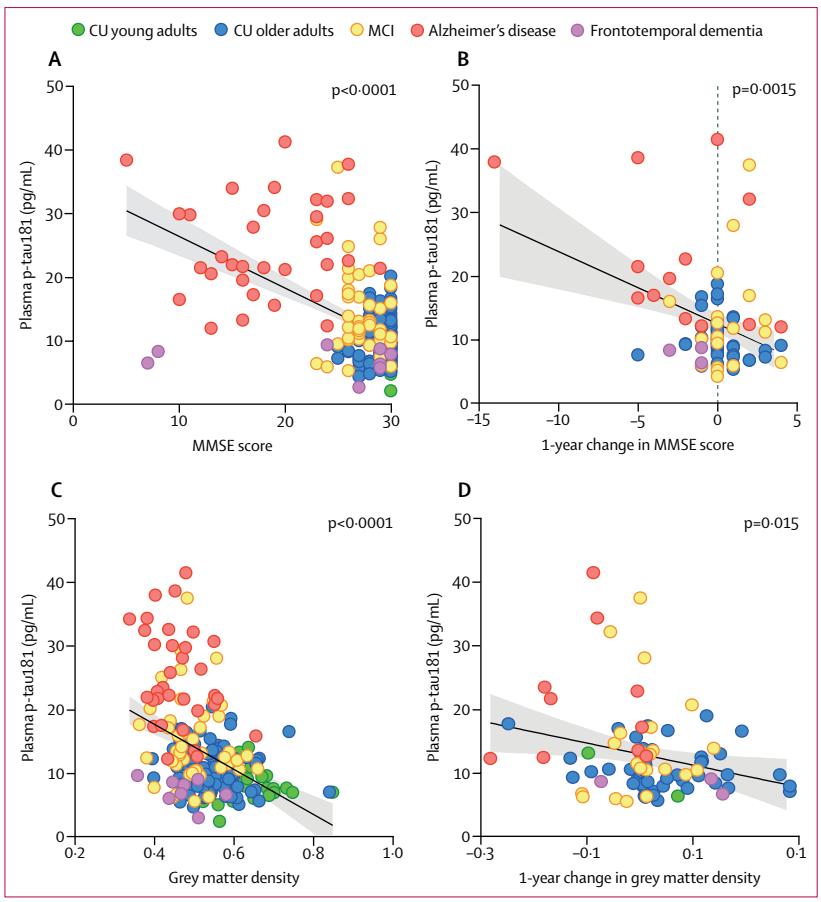


Figure 4: **Association between plasma p-tau181 concentration and 1-year longitudinal neurodegeneration and cognitive decline**

Left plots show data all individuals of the TRIAD cohort at baseline (n=226) and right plots show the subset who had 1-year follow-up assessments (n=85 for cognition, n=88 for structural MRI). Cognition was assessed with the MMSE score (A, B) and hippocampal volume was assessed as a measure of grey matter density (C, D). For longitudinal changes in MMSE score (B) and hippocampus atrophy (D), lower scores represent cognitive decline and a decrease in hippocampal volume (neurodegeneration), respectively. CU=cognitively unimpaired. MCI=mild cognitive impairment. MMSE=Mini-Mental State Examination. p-tau181=tau phosphorylated at threonine 181.

serum) and CSF amyloid β_{1-42} (r=–0·5936, p<0·0001 for plasma, r=–0·6830, p<0·0001 for serum; appendix p 18). In the TRIAD cohort, plasma p-tau181 correlated with CSF p-tau181, measured with either LUMIPULSE or Simoa (appendix p 18). Simoa and LUMIPULSE CSF p-tau181 were also correlated (r=0·8666, p<0·0001; appendix p 18). Simoa p-tau181 measured in paired plasma and CSF from the same individuals had a mean plasma p-tau181 to CSF p-tau181 ratio of around 5%.

Plasma p-tau181 was a better predictor of Alzheimer's disease diagnosis and increased amyloid β PET than age, *APOE* ε4 genotype, or age and *APOE* ε4 genotype combined. Adding age and *APOE* ε4 status made marginal or no improvement to the predictive accuracies of plasma p-tau181 (appendix p 25). Similarly, plasma p-tau181 predicted increased tau PET better than age, *APOE* ε4, or age and *APOE* ε4 status combined in all Braak regions when considering the entire cohort, as well as within the Alzheimer's disease group and the non-dementia groups (Braak III-IV and V-VI; appendix p 26). In *APOE* ε4-stratified analysis, plasma p-tau181 was a better predictor of Alzheimer's disease, increased amyloid β PET, and tau PET than age in both carriers and non-carriers of the *APOE* ε4 genotype (appendix pp 25, 26).

Plasma p-tau181 was a more accurate predictor of Alzheimer's disease, increased amyloid β PET, and increased tau PET (Braak I–VI) than plasma amyloid β_{1-42}, amyloid β_{1-42} /amyloid β_{1-40} ratio, total-tau, or total-tau/amyloid β_{1-42} ratio (appendix p 27).

A subset of individuals in the TRIAD cohort had 1-year follow-up structural MRI (n=88) and cognitive assessment (n=85). After correcting for age, sex, *APOE* ε4 genotype, and years of education, plasma p-tau181 correlated with both baseline (p<0·0001) and 1-year worsening (p=0·0015) in MMSE (figure 4A, B), and with both baseline (p<0·0001) and 1-year change (p=0·015) in hippocampal atrophy (figure 4C, D; analysis by diagnostic group is provided in the appendix, p 27).

Discussion

Our high-performance blood p-tau181 assay enabled the identification of brain tau pathology, showing increased concentrations of blood p-tau181 in individuals with amyloid β pathology who were tau PET-negative. Moreover, plasma p-tau181 provided high diagnostic accuracy for Alzheimer's disease in four independent cohorts, discriminated amyloid β-positive cognitively unimpaired older adults and amyloid β-positive individuals with MCI from amyloid β-negative cognitively unimpaired older adults and young adults, and showed high performance in identifying clinically diagnosed Alzheimer's disease patients with unknown brain amyloid status. Blood p-tau181 differentiated Alzheimer's disease from several other neurodegenerative diseases with high performance. Additionally, blood p-tau181 predicted cognitive decline and hippocampal atrophy over a period of 1 year.

The specificity of p-tau181 to Alzheimer's disease, as previously shown in CSF,[14] was corroborated in the blood in the present study, making it a 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-dementia controls.[15–18] However, these previously reported assays have not been applied to large, independent cohorts including non-Alzheimer's 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.[31] Two assays were not sensitive enough to measure p-tau181 concentrations in many participants, including control participants.[16,18] Furthermore, some assays were validated specifically for plasma,[16,17] limiting the choice of matrix. The ultrasensitive 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 concentrations of p-tau181 indicate that it specifically measures brain-derived p-tau181. Of note, blood p-tau181 distinguished amyloid β-negative cognitively unimpaired older adults from amyloid β-positive cognitively unimpaired older adults and amyloid β-positive individuals with MCI, suggesting that plasma p-tau181 can model the entire Alzheimer's disease continuum. Furthermore, similar to CSF p-tau181,[32] blood p-tau181 separated Alzheimer's disease from other neurodegenerative disorders with high accuracy, indicating that this assay might be a specific marker of tau pathology in Alzheimer's disease. The assay distinguished Alzheimer's disease from phenotypes of primary tauopathies, including progressive supranuclear palsy and corticobasal syndrome, both of which have tau pathology similar to that found in Alzheimer's disease. The aforementioned results indicate that blood p-tau181 could have the specificity and scalability required for effective population screening in Alzheimer's disease.

The blood p-tau181 test showed high accuracy for predicting in-vivo tau tangles and a predictive power to detect amyloid β plaque-positive individuals that was similar to high-performance mass spectrometry-based amyloid β plasma assays.[10,11] Of note, blood p-tau181 identifies individuals with brain tau and amyloid β pathology with an AUC of up to 90%. The strong correlation between plasma p-tau181 and amyloid β PET, together with the increased plasma p-tau181 in amyloid β PET-positive and 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 amyloid β plaques, in that plasma p-tau181 might detect a neuronal reaction to initial amyloid β aggregation,[30] supporting the amyloid cascade hypothesis. The high accuracy of the blood p-tau181 assay to identify brain tangle and plaque pathologies, both separately and combined, makes it an ideal biomarker in relation to the biological and clinical definitions of Alzheimer's disease.[4] The blood p-tau181 assay thus represents a rapid method of identifying in-vivo Alzheimer's disease pathophysiology, and could become a cost-saving and time-saving first-line test for the evaluation of patients with suspected Alzheimer's disease, irrespective of disease stage. The overlap between participants with MCI and Alzheimer's disease in the primary care cohort is likely to be driven by patients with MCI already having Alzheimer's disease dementia phenotypes, which cannot be excluded in this cohort without detailed PET or CSF biomarker data. The multicentre design, the larger and more diverse population (compared with the other cohorts), and the different PET ligands used in BioFINDER-2 could 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 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 1-year cognitive deterioration, as well as hippocampal atrophy, suggest that the p-tau181 blood assay could also serve as a predictor of disease progression, and thus could be used to select individuals most likely to progress during typically short clinical trial periods. The correlation between plasma p-tau181 and PET-measure tau ([^{18}F]MK-6240 tau) in the TRIAD cohort showed almost a bimodal distribution, with p-tau181 increasing steeply within the cognitively unimpaired and MCI groups and plateauing in the Alzheimer's disease group, 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 amyloid β PET-positive individuals who were still tau PET-negative (Braak stage 0). However, plasma p-tau181 does not appear to increase further in cases with moderate to severe tau pathology. Similarly, a previous study reported a poor correlation between p-tau181 and PET-measured tau ([^{18}F]AV1451 tau PET) in Alzheimer's disease dementia, but more robust correlations in amyloid β-positive cognitively unimpaired and MCI individuals.[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 plasma and CSF directly reflects brain tau phosphorylation state, which might not directly translate to tau aggregation status measured by PET.

A previous study[16] showed a modest correlation ($r=0\cdot45$) between plasma p-tau181 and CSF p-tau181 (Innotest) in a small cohort (n=11). However, to our knowledge, no previous study has shown that the plasma analyte measured by a p-tau181 assay can also be measured in serum and CSF. Moreover, one study showed that plasma p-tau181 predicts increased amyloid β PET with an AUC of 80% in participants with no cognitive impairment, with MCI, and with Alzheimer's disease combined,[15] but did not show whether plasma p-tau181 predicts tau PET positivity. Another study, using a discontinued commercial assay, reported poor performance for plasma p-tau181.[18] By contrast, we showed that our plasma p-tau181 assay can predict amyloid PET and tau PET, and we validated these findings in two large cohorts, each using a distinct set of PET ligands. Furthermore, contrary to the immunomagnetic reduction p-tau181 assay,[17,31] our blood p-tau181 assay appears to be specific to Alzheimer's disease-type tau pathology, showing no significant increases in several other tauopathies. This finding emphasises that not only is tau phosphorylation at threonine 181 important, but also that the species on which this phosphorylation site occurs is critical. Of note, blood p-tau181 has potential uses in three clinical settings:

primary care, clinical diagnosis, and in clinical trials. To our knowledge, we have shown for the first time that plasma and serum are similarly suitable for blood p-tau181 analysis.

The improved diagnostic performance of plasma p-tau181 compared with the most well known risk factors for amyloid deposition—age, *APOE* ε4, or both—suggest that use of this diagnostic test does not require prior knowledge of an individual's age and *APOE* genotype. The higher performance compared with other plasma biomarkers indicates that our new assay extends the clinical diagnostic potential of blood biomarkers for Alzheimer's disease.

Although our findings show that the blood p-tau181 assay can identify Alzheimer's disease in a primary care setting, the primary care cohort had no CSF or PET imaging assessments, precluding an aetiological diagnosis of individuals with MCI and identification of preclinical Alzheimer-type pathophysiology in cognitively normal older adults. These assessments would probably have reduced the overlap in p-tau181 concentrations between individuals with MCI and the Alzheimer's disease group. Furthermore, our findings suggest that baseline plasma p-tau181 has potential clinical applications for prognosis and longitudinal monitoring, but the relatively short duration of the longitudinal evaluations and the small number of individuals with longitudinal cognition and imaging biomarkers limits the interpretation of these results.

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

Contributors

TKK, TAP, NJA, HZ, OH, PR-N, and KB conceived the study. TKK developed and validated the blood assay for tau phosphorylated at threonine 181 with support from NJA, JLR, KH, GB, HZ, and KB. TKK, TAP, NJA, SJ, ALB, and OH performed the statistical analysis. TAP, SJ, ALB, MC, MSa, MSK, JT, NM, SP, ES, OH, and PR-N designed and implemented MRI and PET acquisition protocols, and 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, MSc, 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

HZ has served on scientific advisory boards for Wave, Samumed, CogRx, and Roche Diagnostics, and has given open lectures for Alzecure. HZ and KB are co-founders of Brain Biomarker Solutions in Gothenburg, 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, and has received consultancy or speaker fees (paid to the institution) from Biogen and Roche. KB has served as a consultant or on advisory boards for Axon, Biogen, CogRx, Lilly, MagQu, Novartis, and Roche Diagnostics. All other authors declare no competing interests.

Acknowledgments

The authors thank all participants of the study and staff at the University of Gothenburg, Sahlgrenska University Hospital, Lund University, Skåne University Hospital, McGill University Research Centre for Studies, and the Montreal Neurological Institute who supported this project. We particularly thank Victor Liman and Andreja Emeršič for assistance with assay development and validation. We thank Cerveau Technologies for MK-6240, GE Healthcare for providing the precursor of flutemetamol, and Roche for providing the precursor of RO948. TKK was supported by the Swedish Alzheimer Foundation, the Swedish Dementia Foundation (Demensfonden), Gamla Tjänarinnor, the Aina (Ann) Wallströms and Mary-Ann Sjöbloms Foundation, and the Anna Lisa and Brother Björnsson's Foundation. TAP was supported by the Alzheimer Society Research Program and the Canadian Institutes of Health Research (CIHR) Canadian Consortium on Neurodegeneration in Aging. NJA was supported by the Wallenberg Centre for Molecular and Translational Medicine. MS was supported by the Wallenberg Centre for Molecular and Translational Medicine, the Swedish Research Council, the Swedish Alzheimer's Foundation, and the Association for Frontotemporal Degeneration UK. HZ is a Wallenberg Academy Fellow supported by grants from the Swedish Research Council (2018-02532), the European Research Council (681712), the Swedish State Support for Clinical Research (ALFGBG-720931), and the UK Dementia Research Institute at University College London. OH is a Wallenberg Clinical Scholar supported by grants from by the Swedish Research Council, the Knut and Alice Wallenberg foundation, the Marianne and Marcus Wallenberg foundation, the Strategic Research Area MultiPark (Multidisciplinary Research in Parkinson's disease) at Lund University, the Swedish Alzheimer Foundation, the Swedish Brain Foundation, The Parkinson Foundation of Sweden, The Parkinson Research Foundation, the Skåne University Hospital Foundation, and the Swedish Federal Government under the *Avtal om läkarutbildning och forskning* (ALF) agreement. PR-N was supported by the Weston Brain Institute, CIHR, the CIHR Canadian Consortium on Neurodegeneration in Aging, the Fonds de Recherche du Québec–Santé (Chercheur boursier and 2020-VICO-279314 TRIAD/BIOVIE cohort), and the Canada Foundation for Innovation (project 34874). KB was supported by the Alzheimer Drug Discovery Foundation (RDAPB-201809–2016615), the Swedish Research Council (2017-00915), the Swedish Alzheimer Foundation (AF-742881), Hjärnfonden, Sweden (FO2017-0243), and a grant (ALFGBG-715986) from the Swedish Government and County Councils under the ALF agreement.

References

1 Patterson C. World Alzheimer report 2018. The state of the art of dementia research: new frontiers. September, 2018. https://www.alz.co.uk/research/WorldAlzheimerReport2018.pdf (accessed Jan 20, 2020).

2 Jack CR Jr, Knopman DS, Jagust WJ, et al. Tracking pathophysiological processes in Alzheimer's disease: an updated hypothetical model of dynamic biomarkers. *Lancet Neurol* 2013; **12:** 207–16.

3 Dubois B, Feldman HH, Jacova C, et al. Advancing research diagnostic criteria for Alzheimer's disease: the IWG-2 criteria. *Lancet Neurol* 2014; **13:** 614–29.

4 Jack CR Jr, Bennett DA, Blennow K, et al. NIA-AA Research Framework: toward a biological definition of Alzheimer's disease. *Alzheimers Dement* 2018; **14:** 535–62.

5 Lleó A, Cavedo E, Parnetti L, et al. Cerebrospinal fluid biomarkers in trials for Alzheimer and Parkinson diseases. *Nat Rev Neurol* 2015; **11:** 41–55.

6 Molinuevo JL, Ayton S, Batrla R, et al. Current state of Alzheimer's fluid biomarkers. *Acta Neuropathol* 2018; **136:** 821–53.

7 Hampel H, O'Bryant SE, Molinuevo JL, et al. Blood-based biomarkers for Alzheimer disease: mapping the road to the clinic. *Nat Rev Neurol* 2018; **14:** 639–52.

8 Mattsson N, Cullen NC, Andreasson U, Zetterberg H, Blennow K. Association between longitudinal plasma neurofilament light and neurodegeneration in patients with Alzheimer disease. *JAMA Neurol* 2019; **76:** 791–99.

9 Hansson O, Janelidze S, Hall S, et al. Blood-based NfL: a biomarker for differential diagnosis of parkinsonian disorder. *Neurology* 2017; **88:** 930–37.

10 Nakamura A, Kaneko N, Villemagne VL, et al. High performance plasma amyloid-β biomarkers for Alzheimer's disease. *Nature* 2018; **554:** 249–54.

11 Schindler SE, Bollinger JG, Ovod V, et al. High-precision plasma β-amyloid 42/40 predicts current and future brain amyloidosis. *Neurology* 2019; **93:** e1647–59.

12 Citron M, Vigo-Pelfrey C, Teplow DB, et al. Excessive production of amyloid beta-protein by peripheral cells of symptomatic and presymptomatic patients carrying the Swedish familial Alzheimer disease mutation. *Proc Natl Acad Sci USA* 1994; **91:** 11993–97.

13 Chételat G, La Joie R, Villain N, et al. Amyloid imaging in cognitively normal individuals, at-risk populations and preclinical Alzheimer's disease. *Neuroimage Clin* 2013; **2:** 356–65.

14 Skillbäck T, Farahmand BY, Rosén C, et al. Cerebrospinal fluid tau and amyloid-β1-42 in patients with dementia. *Brain* 2015; **138:** 2716–31.

15 Mielke MM, Hagen CE, Xu J, et al. Plasma phospho-tau181 increases with Alzheimer's disease clinical severity and is associated with tau- and amyloid-positron emission tomography. *Alzheimers Dement* 2018; **14:** 989–97.

16 Tatebe H, Kasai T, Ohmichi T, et al. Quantification of plasma phosphorylated tau to use as a biomarker for brain Alzheimer pathology: pilot case-control studies including patients with Alzheimer's disease and down syndrome. *Mol Neurodegener* 2017; **12:** 63.

17 Yang CC, Chiu MJ, Chen TF, Chang HL, Liu BH, Yang SY. Assay of plasma phosphorylated tau protein (threonine 181) and total tau protein in early-stage Alzheimer's disease. *J Alzheimers Dis* 2018; **61:** 1323–32.

18 Park J-C, Han S-H, Yi D, et al. Plasma tau/amyloid-β1-42 ratio predicts brain tau deposition and neurodegeneration in Alzheimer's disease. *Brain* 2019; **142:** 771–86.

19 Hansson O, Zetterberg H, Buchhave P, Londos E, Blennow K, Minthon L. Association between CSF biomarkers and incipient Alzheimer's disease in patients with mild cognitive impairment: a follow-up study. *Lancet Neurol* 2006; **5:** 228–34.

20 Therriault J, Benedet AL, Pascoal TA, et al. Association of apolipoprotein E ε4 with medial temporal tau independent of amyloid-β. *JAMA Neurol* 2019; published online Dec 20. DOI:10.1001/jamaneurol.2019.4421.

21 Smith R, Schöll M, Leuzy A, et al. Head-to-head comparison of tau positron emission tomography tracers [^{18}F]flortaucipir and [^{18}F] RO948. *Eur J Nucl Med Mol Imaging* 2020; **47:** 342–54.

22 Pascoal TA, Mathotaarachchi S, Kang MS, et al. Aβ-induced vulnerability propagates via the brain's default mode network. *Nat Commun* 2019; **10:** 2353.

23 Landau SM, Fero A, Baker SL, et al. Measurement of longitudinal β-amyloid change with 18F-florbetapir PET and standardized uptake value ratios. *J Nucl Med* 2015; **56:** 567–74.

24 Palmqvist S, Zetterberg H, Blennow K, et al. Accuracy of brain amyloid detection in clinical practice using cerebrospinal fluid β-amyloid 42: a cross-validation study against amyloid positron emission tomography. *JAMA Neurol* 2014; **71:** 1282–89.

25 Braak H, Braak E. Neuropathological stageing of Alzheimer-related changes. *Acta Neuropathol* 1991; **82:** 239–59.

26 Schöll M, Lockhart SN, Schonhaut DR, et al. PET imaging of tau deposition in the aging human brain. *Neuron* 2016; **89:** 971–82.

27 Goedert M, Jakes R, Crowther RA, et al. Epitope mapping of monoclonal antibodies to the paired helical filaments of Alzheimer's disease: identification of phosphorylation sites in tau protein. *Biochem J* 1994; **301:** 871–77.

28 Vanderstichele H, Vanmechelen E. Diagnosis of tauopathies determining tau/phospho-tau ratio. 2002. https://patents.google.com/patent/EP1250600A2/en (accessed Dec 3, 2019).

29 Horowitz PM, Patterson KR, Guillozet-Bongaarts AL, et al. Early N-terminal changes and caspase-6 cleavage of tau in Alzheimer's disease. *J Neurosci* 2004; **24:** 7895–902.

30 Sato C, Barthélemy NR, Mawuenyega KG, et al. Tau kinetics in neurons and the human central nervous system. *Neuron* 2018; **97:** 1284–98.e7.

31 Lin CH, Yang SY, Horng HE, et al. Plasma biomarkers differentiate Parkinson's disease from atypical parkinsonism syndromes. *Front Aging Neurosci* 2018; **10:** 123.

32 Rosso SM, van Herpen E, Pijnenburg YAL, et al. Total tau and phosphorylated tau 181 levels in the cerebrospinal fluid of patients with frontotemporal dementia due to P301L and G272V tau mutations. *Arch Neurol* 2003; **60:** 1209–13.