Healthy elderly in clinical trials: how to define preclinical Alzheimer's Disease for clinical trial participation

Prins, S.

CHAPTER VII

Neurocognitive functions are not impaired in subjects with preclinical AD based on CSF Aβ and higher levels of CSF P-TAU217

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ABSTRACT

BACKGROUND Amyloid plaques in the brain and lowered levels of amyloid beta measured in cerebrospinal fluid (CSF) are used as biomarker evidence to diagnose patients with Alzheimer's disease (AD). Along with tauopathy and hyperphosphorylated tau, which can be measured as tau deposition in the brain and increased (hyperphosphorylated) tau levels in CSF, these are the hallmark for AD. Tau is expressed predominantly in the central and peripheral nervous systems, where it is abundant in nerve cell axons. Tau binds to microtubules, providing stability and facilitating axonal transport. Tau is encoded by the microtubule-associated protein tau (MAPT) gene and is naturally unfolded. Six tau isoforms are expressed in adult human brains. The current study investigated P-TAU181, P-TAU217 and P-TAU231 isoforms in CSF and P-TAU181 and P-TAU231 in plasma in subjects with preclinical AD and healthy elderly, to investigate whether phosphor-tau CSF can differentiate healthy elderly from preclinical AD subjects and study cognitive performance of subjects with preclinical AD based on CSF Aβ in combination with higher levels of P-TAU isoforms. Results could help identify the correct study populations for clinical trials investigating disease modifying treatments (DMTs) aimed at P-TAU.

METHODS Samples of 100 healthy male and female subjects of 65 years of age and older were selected from the main study in healthy elderly based on $A\beta_{1-42}$ status. All subjects were healthy volunteer with no cognitive complaints. Of the 100 subjects, 50 subjects were selected having CSF $A\beta_{1-42}$ profiles consistent with Alzheimer's disease and were classified as preclinical AD according to the NIA-AA standards from 2011. Blood and CSF samples were taken and analyzed on CSF P-TAU181, P-TAU217 and P-TAU231 and plasma P-TAU181 and P-TAU231. The following NeuroCart tests were performed: the Adaptive tracking test to measure attention and eye-hand coordination, the Face encoding and Recognition task (FACE) to measure visual memory, the Visual Verbal Learning Test (VVLT, 30 words) to measure the whole scope of learning behavior (i.e. acquisition, consolidation, storage and retrieval), the N-Back test was assessed to evaluate working memory, finger tapping for motor fluency, saccadic and smooth eye movement were also measured. Basic characteristics such as age, gender and ApoE ε4 status were reported per group. Visual checks on the ranges of biomarker scores for each group were done using scatter plots, as well as Tukey boxplots. To explore differences between groups the biomarker outcomes were tested with an ANCOVA

where age, sex and ApoE ε4 status were added in the model, or t-tests where applicable. Variables were Log transformed where applicable. Least square means were calculated for all P-TAU isoforms in both groups.

RESULTS The ApoE ε4 status was significantly different between Aβ+ and Aβ- subjects. CSF P-TAU181 and CSF P-TAU231 were significantly different on age, not on group difference between Aβ+ and Aβ-. Plasma P-TAU181 and P-TAU231 were not significantly different between Aβ+ and Aβ- subjects or any of the covariates. None of the cognitive assessments show significant difference per P-TAU concentration in CSF or plasma. Age was significantly higher in subjects with higher concentrations of CSF P-TAU181, P-TAU231 and P-TAU217. Age was also significantly higher in subjects with Aβ+ and CSF concentrations of P-TAU181 and P-TAU231. CSF P-TAU181 is strongly correlated with CSF P-TAU 217 and P-TAU231 (P=<0.0001) but also with plasma P-TAU181 (p=0.0184) and P-TAU231 (p=0.0189). CSF P-TAU217 and P-TAU231 are also strongly correlated (p=<0.0001). CSF P-TAU217 correlates with plasma P-TAU181 (P=0.0042) and P-TAU231 (p=0.0358). CSF P-TAU231 correlates with plasma P-TAU181 (P=0.0054) and P-TAU231 (p=0.0170). Plasma P-TAU181 correlates strongly with plasma P-TAU231 (p=<0.0001). None of the P-TAU biomarkers correlates with $A\beta_{1-42}$.

CONCLUSION As P-TAU seems to emerge in the preclinical phase of AD as a response to upcoming Aβ misfolding in the brain, this could be the earliest possible intervention window for treatment before neurofibrillary tangles arise. Measuring P-TAU in plasma can be used for the measurement of target engagement of these specific anti-tau DMT and early phase removal or lowering of P-TAU might lead to less subjects progressing from preclinical AD to AD. As this study does not confirm the discriminating power of P-TAU in preclinical AD, more (longitudinal) research is needed to provide more insight into the usefulness of plasma P-TAU biomarkers for distinction between preclinical AD and healthy subjects.

BACKGROUND

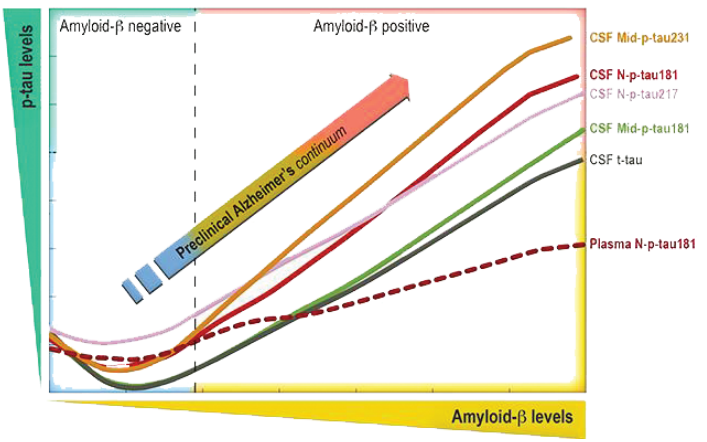
Amyloid plaques in the brain and lowered levels of amyloid beta measured in cerebrospinal fluid (CSF) are used as biomarker evidence to diagnose patients with Alzheimer's disease (AD). Along with tauopathy and hyperphosphorylated tau, which can be measured as tau deposition in the brain and increased (hyperphosphorylated) tau levels in CSF, these are the hallmark for AD.[1]

Tau is expressed predominantly in the central and peripheral nervous systems, where it is abundant in nerve cell axons.[2] Tau binds to microtubules, providing stability and facilitating axonal transport.[3]

Tau is encoded by the microtubule-associated protein tau (MAPT) gene and is naturally unfolded. Six tau isoforms are expressed in adult human brains. An imbalance in tau kinase and phosphatase activity is considered to be the reason for tau hyperphosphorylation in AD and other neurodegenerative diseases.[4] Previous research has focused on specific isoforms of phosphorylated tau to distinguish between healthy subjects and patients with AD with regard to increased CSF total and phosphorylated tau levels at threonine 181 (P-TAU181). However, P-TAU181 was shown not to be specific for AD and is increased in multiple neurodegenerative diseases.[5] Various other studies have found correlations between different phosphorylated tau isoforms and amyloid plaques in patients with Alzheimer's disease.[6–8] Especially the P-TAU isoforms P-TAU217, P-TAU231 and P-TAU 181 are found to be increased in CSF of subjects with amyloidosis related to AD. Barthelemy et al., (2020) describe that especially P-TAU217 measured in CSF is a highly specific biomarker for detecting preclinical and advanced forms of AD, more specific than P-TAU181. CSF P-TAU217 correlates strongly with presence of beta amyloid in the brain using PiB-PET imaging.[5] A publication by the same group describes significant differences in CSF and plasma P-TAU217 and P-TAU181 between amyloid beta (Aβ) positive and Aβ negative subjects, regardless of the cognitive status which indicates tauopathy in the preclinical stage of AD.[9] Preclinical AD refers to cognitively healthy subjects having lowered CSF $A\beta_{1-42}$ levels consistent with AD, so called Aβ positive subjects.[10] Palmqvist et al., (2020) tried to discriminate AD from other neurodegenerative disorders by using plasma P-TAU217 in populations ranging from healthy to AD. They found that plasma P-TAU217 performed better in discriminating AD than other plasma tau isoforms and MRI based biomarkers and was similarly effective as key CSF and PET based measures.[11] P-TAU has been suggested to correlate with cognitive impairment, better than Aβ related biomarkers.[12] Suarez-Calvet et al., (2020) published an illustration of the process of tau-phosphorylation compared to amyloidosis in CSF see, figure 1.[12]

Measuring these isoforms in CSF and plasma in healthy elderly and in people with preclinical AD can help to identify pathological disease onset and can also be used to identify early AD pathology when selecting cognitively healthy elderly for participation in clinical trials with amyloid beta targeting drugs aimed at disease modifying effects and prevention of dementia. Additionally, very few studies have shown discrimination between healthy subjects and preclinical AD based on plasma P-TAU isoforms, so confirmation of the findings of the Barthelemy papers is needed. The current study investigated P-TAU181, P-TAU217 and P-TAU231 isoforms in CSF and P-TAU181 and P-TAU231 in plasma in subjects with preclinical AD and healthy elderly, to investigate whether phosphor-tau CSF can differentiate healthy elderly from preclinical AD subjects and study cognitive performance of subjects with preclinical AD based on CSF Aβ in combination with P-TAU isoforms. Results could help identify the correct study populations for clinical trials investigating disease modifying treatments (DMTs) aimed at P-TAU.

Figure 1 The CSF continuum of different P-TAU isotope levels compared to Aβ levels.[12]



METHODS

This was an exploratory sub-study of a previously performed study registered in the international trial register with ID number: ISRCTN79036545.[13] All study participants provided written consent for exploratory analyses of material obtained during study execution.

The main study was approved by the ethics committee of the Leiden University Medical Center (LUMC), the Netherlands. The study was conducted according to the Dutch act on Medical Research Involving Human Subjects (WMO) and in compliance with Good Clinical Practice (ICH-GCP) and the Declaration of Helsinki.

PARTICIPANTS Samples of 100 healthy male and female subjects of 65 years of age and older were selected from the main study in healthy elderly.[13] based on $A\beta_{1-42}$ status. All subjects were healthy volunteer with no cognitive complaints. Subjects responded voluntarily on recruitment advertisements asking for healthy elderly trial subjects. Of the 100 subjects, 50 subjects were selected having CSF $A\beta_{1-42}$ profiles consistent with Alzheimer's disease and were classified as preclinical AD according to the NIA-AA standards from 2011.[10] The remaining 50 subjects were selected on having high levels of CSF $A\beta_{1-42}$ as healthy control group. Lowered Aβ levels classified as amyloid abnormal and consistent with the presence of Alzheimer pathology were dichotomized by creating a group of 'Aβ positive subjects' (Aβ+ = < 1000 PG/mL) and 'Aβ negative subjects' (Aβ- = > 1700 PG/mL) using confirmed cut-offs.[14] $A\beta_{1-42}$ was measured in CSF using the fully automated Elecsys platform as this is widely used for diagnostics.[15] All the subjects visited Centre for Human Drug Research (CHDR) between October 2017 and November 2018. Main exclusion criteria were a diagnosis of a cognitive disorder (including but not limited to Mild Cognitive Impairment [MCI], AD, Lewy Body dementia, Frontotemporal dementia), history of psychiatric disease in the past 3 years, Mini Mental State Examination (MMSE) ≤ 24, Geriatric Depression Scale (GDS) ≥ 6, presence of drug or alcohol abuse (<2 standard drinks per day for female and <3 standard drinks per day for male), use of any medication that was expected to influence central nervous system function or is contraindicative of the performance of a lumbar puncture.

All subjects visited the clinical research unit once and underwent blood sampling at predefined time points (0, 2 and 4 hour[s]). A single lumbar puncture was performed for the collection of CSF (at 4 hours), for measurement of $A\beta_{1-42}$ as described below.

This is an exploratory study, therefore the sample size is not based on statistical considerations. Including 50 preclinical AD subjects and an equally sized healthy elderly control group (n=50) was considered appropriate for a comparative study. Previous comparable studies have been able to show differences between groups in smaller sample sizes.[11,16]

BLOOD SAMPLING Approximately 10mL blood was collected via an i.v. catheter placed in an antecubital vein in the arm in appropriate K2EDTA tubes at the predefined time points mentioned above. Following blood sample processing, the plasma fractions were stored at -80°C.

LUMBAR PUNCTURE A CSF sample of 4 mL was collected in a 10 mL polypropylene tube. CSF was centrifuged within one hour, at 2000g for 10 minutes at 4°C and stored at -80°C. Lumbar punctures were performed by a trained, physician with a 25G atraumatic lumbar puncture needle (Braun, 25G). The needle was placed at the L3-L4 or L4-L5 interspace with the subject in supine or sitting position.

APOLIPOPROTEIN E GENOTYPING Apolipoprotein E (ApoE) genotyping was performed after isolating DNA from EDTA blood. DNA was isolated using QIAamp DNA Blood MINI kit after which a polymerase chain reaction (PCR) technique was applied on the clean DNA. A sequential analysis (according to the Sanger method) than determined the ApoE genotype. One or 2 ApoE ε4 alleles classified subjects as ApoE ε4 carriers, when no ApoE ε4 alleles were present a subject was classified as noncarrier.

MEASUREMENT OF CSF P-TAU181, P-TAU217 AND P-TAU231 AND PLASMA P-TAU181 AND P-TAU231 All blood samples for analyses of phosphorated tau were collected in a non-fasted state within one hour of collection of the CSF sample. After sample processing, the CSF and plasma fractions were stored at -80 °C until further analyses. $A\beta_{1-42}$ was measured in CSF using the fully automated Elecsys platform as this is widely used for diagnostics.[15] All P-TAU isoforms were analyses with Simoa HD-X using in-house assays at the Department of Psychiatry and Neurochemistry, University of Gothenburg, Mölndal, Sweden as described by Karikari et al., (2020).[17]

COGNITIVE ASSESSMENTS AND QUESTIONNAIRES The NeuroCart is a battery of CNS tests used to assess a wide range of CNS domains.[18] All measurements were performed in a quiet room with ambient illumination. Per session there was only one subject in the room. The following tests were performed using the NeuroCart: the Adaptive tracking test to measure attention and eye-hand coordination,[19] the Face encoding and Recognition task (FACE) to measure visual memory,[20] the Visual Verbal Learning Test (VVLT, 30 words) to measure the whole scope of learning behavior (i.e. acquisition, consolidation, storage and retrieval),[21] the N-Back test was assessed to evaluate working memory,[22] finger tapping for motor fluency,[23] saccadic and smooth eye movement were also measured.[24]

The clinical dementia rating scale (CDR).[25] was assessed via a semi-structured interview with the participating subject only, to rate impairment in six different cognitive categories (memory, orientation, judgement and problem solving, community affairs, home and hobbies and personal care).

STATISTICAL METHODOLOGY Subjects were grouped based on CSF amyloid beta status where Aβ+ equals preclinical AD and Aβ- equals healthy elderly as mentioned above. Basic characteristics such as age, gender and ApoE ε4 status were reported per group. Visual checks on the ranges of biomarker scores for each group were done using scatter plots, as well as Tukey boxplots. To explore differences between groups the biomarker outcomes were tested with an ANCOVA where age, sex and ApoE ε4 status were added in the model, or t-tests where applicable. Variables were Log transformed where applicable. Least square means were calculated for all P-TAU isoforms in both groups. All analyses were carried out using SAS for Windows V9.4 (SAS Institute, Inc., Cary, NC, USA).

RESULTS

DEMOGRAPHIC AND CLINICAL CHARACTERISTICS The mean age of the total group of study participants (n=100) was 72.6 (4.6) years old with 62 male and 38 female subjects. Mean overall MMSE score was 28.7 (0.49) and 32 subjects were ApoE ε4 carriers. All subjects had a CDR score of 0.

COMPARISON OF CSF P-TAU181, P-TAU217, P-TAU231 AND PLASMA P-TAU181 AND P-TAU231 BETWEEN Aβ+ AND Aβ- SUBJECTS Table 1 presents the cross-sectional demographics and clinical characteristics of the studied population based on Aβ+/ Aβ- groups. The ApoE ε4 status was significantly different between Aβ+ and Aβ- subjects. All other clinical characteristics do not differ significantly between the Aβ+ and Aβ- group. CSF P-TAU217 was significantly different between Aβ+ and Aβ- subjects, see Table 1 and Figure 1. CSF P-TAU181 and CSF P-TAU231 were significantly different on age, not on group difference between Aβ+ and Aβ- as the data shows more spreading, see Figure 2. Plasma P-TAU181 and P-TAU231 were not significantly different between Aβ+ and Aβ- subjects or any of the covariates.

COGNITIVE PERFORMANCE OF SUBJECTS WITH DIFFERENT P-TAU CONCENTRATIONS None of the cognitive assessments show significant difference per P-TAU concentration in CSF or plasma, see Table 2. Scatterplots of all cognitive assessments were created with subjects pooled by Aβ+ and above median P-TAU

concentrations for all P-TAU isoforms. Visual check of the scatterplots resulted into no apparent differences between the groups therefore no statistical analyses were performed. Analyses on age (years) and total MMSE scores were performed for the total subject group and subjects with Aβ+ and P-TAU, see table 3. Age was significantly higher in the total subject group. Age was not correlated in subjects with Aβ+ alone. There was no difference in any of the comparisons for total MMSE score.

Table 1 Cross-sectional demographics and clinical characteristics of the studied population based on Aβ+/ Aβ- groups.

	Aβ+ (n=50)	**Aβ- (n=50)**	**p**
Aβ level (mean, SD)	706.0 (174.36)	>1700	
Sex (male/female)	33/17	29/21	0.41
BMI (mean, SD)	26.07 (3.95)	25.17 (3.44)	0.225
Age (years, mean, SD)	73.40 (4.72)	71.88 (4.45)	0.101
ApoE ε4 carrier (n, %)	25 (50%)	7 (14.6%)	0.003
MMSE (mean, SD)	28.60 (1.41)	28.82 (1.37)	0.431
CDR (mean, SD)	0 (0)	0 (0)	
P-TAU181 CSF PG/mL (mean, SD)	N=50 318.8 ± 180.6	N=50 220.2 ± 62.30	0.221
P-TAU181 Plasma PG/mL (mean, SD)	N=50 16.99 ± 6.84	N=50 15.46 ± 5.98	0.254
P-TAU231 CSF PG/mL (mean, SD)	N=50 419.46 ± 208.21	N=50 288.05 ± 81.12	0.110
P-TAU231 Plasma PG/mL (mean, SD)	N=50 14.20 ± 4.73	N=50 13.67 ± 5.05	0.897
P-TAU217 CSF PG/mL (mean, SD)	N=46 4.58 ± 3.42	N=50 1.70 ± 0.83	0.001

P values in bold font were considered significant (p<0.05). Independent T-Test and Pearson Chi-Square test were applied as appropriate.

Figure 1 Significant violin plot for CSF P-TAU217 in healthy elderly subjects (Abeta-, n=50) and subjects with preclinical AD (Abeta+, n=46).

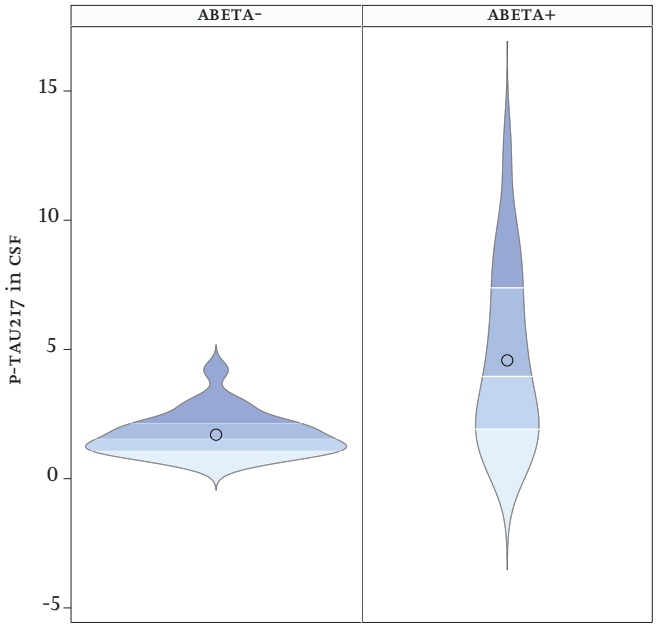


Figure 2 Boxplots of CSF P-TAU181 and CSF P-TAU231 in healthy elderly subjects (Abeta-, n=50) and subjects with preclinical AD (Abeta+, n=50).

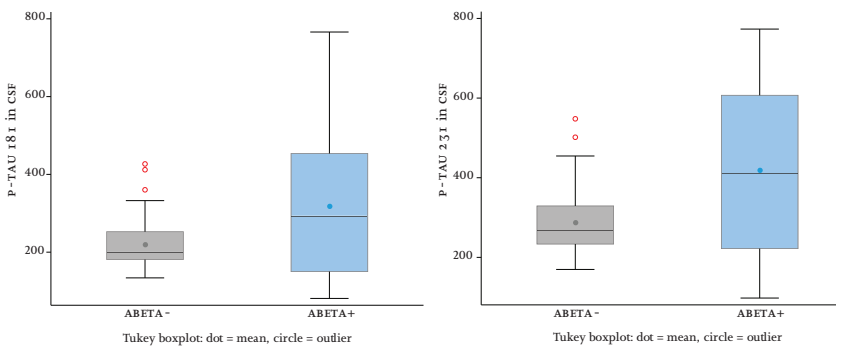


Table 2 Correlation for cognitive assessments and P-TAU concentrations in CSF and plasma calculated with Spearman r.

	P-TAU181 CSF	P-TAU181 plasma	P-TAU 231 CSF	P-TAU231 plasma	P-TAU 217 CSF
Saccadic inaccuracy (%)	r:-0.01 p=0.94 n=100	r:-0.08 p=0.43 n=100	r:-0.04 p=0.72 n=100	r:-0.12 p=0.25 n=100	r:0.01 p=0.91 n=96
Smooth pursuit (%)	r:-0.05 p=0.62 n=100	r:-0.03 p=0.81 n=100	r:-0.11 p=0.26 n=100	r:-0.01 p=0.93 n=100	r:-0.15 p=0.15 n=96
Tapping (taps/10s)	r:0.01 p=0.90 n=100	r:-0.04 p=0.68 n=100	r:-0.01 p=0.95 n=100	r:0.03 p=0.79 n=100	r:-0.04 p=0.74 n=96
Adaptive tracking (%)	r:0.06 P=0.56 n=100	r:-0.01 p=0.27 n=100	r:0.04 p=0.72 n=100	r:-0.07 p=0.47 n=100	r:0.02 p=0.83 n=96
VVLT delayed word recognition (# correct)	r:-0.08 p=0.43 n=99	r:-0.10 p=0.31 n=99	r:-0.08 p=0.46 n=99	r:-0.03 p=0.79 n=99	r:-0.14 p=0.17 n=95
VVLT delayed word recall (# correct)	r:-0.05 p=0.61 n=100	r:-0.10 p=0.30 n=100	r:-0.05 p=0.59 n=100	r:-0.11 p=0.29 n=100	r:-0.07 p=0.51 n=96
N-Back 2-back (correct)	r:-0.11 p=0.28 n=100	r:-0.05 p=0.63 n=100	r:-0.14 p=0.17 n=100	r:-0.11 p=0.28 n=100	r:-0.12 p=0.24 n=96
FACE (# correct)	r:-0.19 p=0.06 n=100	r:-0.07 p=0.48 n=100	r:-0.13 p=0.20 n=100	r:-0.04 p=0.68 n=100	R:-0.05 p=0.62 n=96

Table 3 Correlations for age (years) and MMSE (total) score per total group (n=100), per Aβ positive group (n-50) and Aβ positive group with above median P-TAU concentrations.

	Spearman r correlations total group	Spearman r correlations Aβ+ and P-TAU
Age (yrs)		
P-TAU181 CSF	r=0.21 p=0.04 n=100	r=0.22 p=0.13 n=50
P-TAU181 plasma	r=0.14 p=0.17 n=100	r=0.04 p=0.78 n=50
P-TAU231 CSF	r=0.24 p=0.02 n=100	r=0.21 p=0.14 n=50
P-TAU231 plasma	r=0.14 p=0.16 n=100	r=0.16 p=0.27 n=50
P-TAU217 CSF	r=0.22 p=0.03 n=96	r=0.14 p=0.35 n=46
MMSE (total)		
P-TAU181 CSF	r=-0.06 p=0.55 n=100	r=-0.05 p=0.71 n=50
P-TAU181 plasma	r=-0.02 p=0.81 n=100	r=0.11 p=0.47 n=50
P-TAU231 CSF	r=-0.08 p=0.42 n=100	r=-0.04 p=0.77 n=50
P-TAU231 PLASMA	r=0.03 p=0.77 n=100	r=-0.05 p=0.72 n=50
P-TAU217 CSF	r=-0.04 p=0.74 n=96	r=0.12 p=0.44 n=46

P values in bold font were considered significant (p<0.05).

Table 4 refers to the correlations between the biomarkers of the total group (n=100). CSF p-tau181 is strongly correlated with CSF p-tau 217 and p-tau231 (P=<0.0001) but also with plasma p-tau181 (p=0.0184) and p-tau231 (p=0.0189). CSF p-tau217 and p-tau231 are also strongly correlated (p=<0.0001). CSF p-tau217 correlates with plasma p-tau181 (P=0.0042) and p-tau231 (p=0.0358). CSF p-tau231

correlates with plasma p-tau181 (P=0.0054) and p-tau231 (p=0.0170). Plasma p-tau181 correlates strongly with plasma p-tau231 (p=<0.0001). None of the p-tau biomarkers correlates with $A\beta_{1-42}$.

Table 4 Correlation table with p values for correlations between CSF p-tau181, p-tau217, p-tau231 and plasma p-tau181, p-tau231 and CSF $A\beta_{1-42}$.

	CSF p-tau181	CSF p-tau217	CSF p-tau231	Plasma p-tau181	Plasma p-tau231	$A\beta_{1-42}$
CSF p-tau181	-					
CSF p-tau217	r:0.855 p=<0.0001	-				
CSF p-tau231	r:0.959 p=<0.0001	r:0.899 p=<0.0001	-			
Plasma p-tau181	r:0.235 p=0.0184	r:0.289 p=0.0042	r:0.276 p=0.0054	-		
Plasma p-tau231	r:0.234 p=0.0189	r:0.215 p=0.0358	r:0.238 p=0.0170	r:0.603 p=<0.0001	-	
$A\beta_{1-42}$	r:-0.111 p=0.4425	r:-0.207 p=0.1675	r:-0.161 p=0.2638	r:-0.191 p=0.1832	r:-0.095 p=0.5128	-

P values in bold font were considered significant (p<0.05).

DISCUSSION

This exploratory study investigated p-tau181, p-tau217 and p-tau231 isoforms in CSF and p-tau181 and p-tau231 in plasma in subjects with preclinical AD (Aβ+) and healthy elderly (Aβ-), to investigate whether phosphor-tau can differentiate healthy elderly from preclinical AD subjects. Cognitive performance was also studied in subjects with preclinical AD based on CSF Aβ in combination with higher levels of p-tau isoforms. CSF p-tau217 was significantly different between Aβ+ and Aβ- subjects. CSF p-tau181 and CSF p-tau231 were increased at higher age, there was no group difference between Aβ+ and Aβ-. Plasma p-tau181 and p-tau231 were not significantly different between Aβ+ and Aβ- subjects or any of the covariates. Cognitive performance did not differ in subjects with different p-tau concentrations. A positive correlation was found between age and CSF p-tau181, p-tau231 and p-tau217. All p-tau isoforms in CSF and plasma show high correlations.

CSF phosphorylated tau and total tau together with CSF amyloid beta 42 represent the core biomarkers for AD. Research shows that even in the preclinical

stage of AD, with only slight Aβ pathology, changes in tau metabolism are already measurable.[12] When referring to P-TAU in literature, P-TAU at threonine-181 is usually meant in AD research as this isoform has been studied most and accurately distinguishes AD patients from mild cognitive impairment (MCI) and healthy subjects.[26] With new analyses methods making it possible to measure P-TAU in plasma, P-TAU181 has been studied extensively and results show that P-TAU181 in plasma has the ability to discriminate AD from other neurological diseases. Also, P-TAU181 starts to increase in preclinical AD with further increases in MCI and dementia stages.[27] In our study, P-TAU181 in CSF and plasma was not different between Aβ+ and Aβ+- subjects.

Difference in results may be due to different subject populations, where Karikari (2020).[16] included subjects from independent cohorts, this current study included 100 cognitively healthy subjects above the age of 65 with 50 subjects known to have lowered CSF $A\beta_{1-42}$ levels consistent with AD. The subjects described in the Karikari et al., study were different in an important fashion from the subjects described here. The discovery cohort which included AD patients and age-matched controls with minor neurological or psychiatric symptoms, the TRIAD and BIOFINDER-2 studies, which included cognitively healthy elderly subjects and patients with MCI, AD and frontotemporal dementia. TRIAD also included young adults (20-30 years old). No preclinical AD was determined in subjects in these trials, which could explain the differences in our data set as the analytical sensitivity of the assays may be insufficient for the detection of preclinical AD.[16]

CSF P-TAU217 has gained interest as recent studies showed this isoform to be better at detecting AD than P-TAU181.[5,28] This was also shown in subjects with preclinical AD where higher levels of P-TAU217 were observed compared to P-TAU181.[12] which is in agreement with our study. In a longitudinal study P-TAU217 was found to be able to monitor disease progression from cognitive unimpaired subjects to MCI to AD.[29] Suarez-Calvet et al., (2020) also investigated P-TAU231 in CSF finding this to be a very promising biomarker for preclinical AD as P-TAU231 was more prominently increased in preclinical AD than CSF P-TAU217 and CSF P-TAU181. This current study could not replicate these findings. The preclinical AD subjects investigated by Suarez-Calvet et al., were younger than our population, but were otherwise comparable with regards to MMSE score and ApoE ε4 disposition.

Plasma P-TAU181, P-TAU217 and P-TAU231 shows to already be increased in subjects with Aβ positive PET scans while tau-PET is still negative.[30–32] No PET was performed in the current study and based on CSF $A\beta_{1-42}$ alone, we could not replicate these findings. Having a soluble assay for the detection of tau, especially

in plasma, is however far easier applicable and less costly in identifying otherwise healthy subjects with tauopathy. Apart from that, CSF P-TAU seems to precede detection with tau PET and measuring CSF or plasma P-TAU demonstrates to be indicative for early tau pathology closely related to Aβ.[32] This study measured CSF and plasma using Simoa HD-X using (in-house assays at the Department of Psychiatry and Neurochemistry, University of Gothenburg, Mölndal, Sweden as described by Karikari et al., (2020)[17]), which has been reported to be a robust analytical method.[33]

Subjects participating in this study were healthy elderly with no cognitive complaints. This was confirmed by a medical and cognitive prescreening before trial participation. This was again confirmed as data of the subjects was split into a group of subjects with CSF Aβ+ and Aβ- resulting in a group of subjects with preclinical AD. Data was further divided into subjects with Aβ+ and above median concentrations of P-TAU isoforms and cognitive performance still did not differ between these subjects, even in the preclinical stage of AD, even though lower concentration of Aβ and higher concentrations of P-TAU in CSF indicates that AD pathology is present to a greater extent. When comparing the results of some of the cognitive assessments performed in this study (Adaptive tracking, VVLT, N-Back test and saccadic and smooth eye movements) with previous literature, our population did perform below average compared to general healthy subjects. The NeuroCart scores however do not yet resemble scores of AD patients (Prins et al., 2022, submitted: Journal of the Neurological Sciences). The subjects with preclinical AD in this study might reflect a remarkably early stage of the preclinical phase in which not all P-TAU isoforms are yet increased. This studied population can therefore be referred to as cognitively healthy elderly who are likely to be enrolled in studies aimed at demonstrating disease modifying effects of a DMT in healthy elderly subjects.

Currently (2022), there are 13 DMTs in development aiming to reduce tauopathy in AD.[34] Mechanisms of action range from inhibition of tau aggregation to monoclonal antibodies promising to remove (extracellular) tau. As P-TAU seems to emerge in the preclinical phase of AD as a response to upcoming Aβ misfolding in the brain, this could be the earliest possible intervention window for treatment before neurofibrillary tangles arise. Measuring P-TAU in plasma can be used for the measurement of target engagement of these specific anti-tau DMT and early phase removal or lowering of P-TAU might lead to less subjects progressing from preclinical AD to AD. As this study does not confirm the discriminating power of P-TAU in preclinical AD, more (longitudinal) research is needed to provide more insight into the usefulness of plasma P-TAU biomarkers for distinction between preclinical AD and healthy subjects.

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