Plasma Phosphorylated Tau 231 Increases at One-Year Intervals in Cognitively Unimpaired Subjects

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Abstract

Background: Plasma biomarkers of Alzheimer's disease (AD) constitute a non-invasive tool for diagnosing and classifying subjects. They change even in preclinical stages, but it is necessary to understand their properties so they can be helpful in a clinical context.

Objective: With this work we want to study the evolution of p-tau231 plasma levels in the preclinical stages of AD and its relationship with both cognitive and imaging parameters.

Methods: We evaluated plasma phosphorylated (p)-tau231 levels in 146 cognitively unimpaired subjects in sequential visits. We performed a Linear Mixed-effects Model to analyze their rate of change. We also correlated their baseline levels with cognitive tests and structural and functional image values. ATN status was defined based on cerebrospinal fluid biomarkers.

Results: Plasma p-tau231 showed a significant rate of change over time. It correlated negatively with memory tests only in amyloid-positive subjects. No significant correlations were found with any imaging measures.

Conclusions: Increases in plasma p-tau231 can be detected at one-year intervals in cognitively healthy subjects. It could constitute a sensitive marker for detecting early signs of neuronal network impairment by amyloid.

INTRODUCTION

Alzheimer's disease (AD) is a neurodegenerative process characterized by the brain’s accumulation of extracellular plaques of amyloid-β (Aβ) and intracellular neurofibrillary tangles of phosphorylated tau (p-tau). These pathological changes occur decades before the first symptoms appear and are reflected in the concentration of these proteins in the cerebrospinal fluid (CSF). During the AD continuum, including the preclinical stages, total tau (t-tau) and p-tau levels are elevated compared with non-AD concentrations, and the opposite is found for

Aβ42 (decreased CSF concentration in Aβ-positive people) [1]. This provides a valuable therapeutic window for future disease-modifying treatments (DMTs), but cost-effective, non-invasive, and population-level useful biomarkers are needed to identify suitable patients.

In the last few years, the diagnosis of AD has undergone a revolution with the development of high-sensitivity assays that identify markers in plasma such as Aβ42, Aβ 40, phosphorylated tau at Thr181 (p-tau181), Thr217 (p-tau217), Thr231 (p-tau231), neurofilament light (NfL), t-tau and glial fibrillary acid protein (GFAP) [2-6], opening up the possibility of avoiding other invasive, or more expensive and less available methods such as lumbar puncture (LP) or positron emission tomography (PET) scans.

With DMTs on the verge of approval, it becomes necessary to give clinical and biological significance to these markers, so they provide us with accurate and reliable information in the asymptomatic phases of AD. In this regard, plasma markers offer valuable opportunities. Their accessibility allows sequential assessment, which may increase their diagnostic potential [7]. Thus, we can compare the evolution of each subject intra-individually and not with population values. However, these markers also present challenges, and many questions remain to be answered. It is not yet known which marker is most useful at which stage of the disease, what time intervals are necessary to detect representative changes, nor the relationship of these temporal increments with the phenotype.

One of the AD blood biomarkers that has generated the most interest in recent months is p-tau231, as it seems to alter very early, at the time the first Aβ changes occur [5,8]. Although its performance has been evaluated in patients with mild cognitive impairment and AD in cross-sectional studies, the longitudinal changes of this marker in the preclinical stages have not been characterized in detail. In this longitudinal study, we aim to provide information about the short-term evolution of p-tau231 in the preclinical stages of AD to evaluate its clinical utility and relationship with other parameters, such as neuropsychological findings or imaging markers.

MATERIALS AND METHODS

Participants

We conducted this analysis with volunteers of the 'Valdecilla Cohort for the study of memory and brain aging' from the Memory Unit of the Marqués de Valdecilla University Hospital [9,10]. The Valdecilla cohort is designed to better understand the preclinical phases of AD. It is composed of cognitively unimpaired (CU) volunteers who responded to an open call in the local media. Inclusion criteria are 1) age ≥55 years; 2) signed consent for the collection and storage of biological samples and the performance of invasive techniques. Exclusion criteria are 1) cognitive impairment (determined by Clinical Dementia Rating (CDR) >0; 2) major psychiatric pathology; 3) major systemic disease or sensory deprivation impairing the performance of the cognitive tests; 4) any contraindication to perform the complementary tests such as anticoagulation or claustrophobia.

At baseline, all participants were assessed with a comprehensive neuropsychological battery, a brain magnetic resonance imaging (MRI), and an 18-fluorodeoxyglucose positron emission tomography (FDG-PET); DNA samples were extracted for genetic analyses, and CSF AD markers were determined (Aβ42, Aβ40, p-tau181 and t-tau). Plasma samples were collected at baseline

and annual follow-ups, including neuropsychological assessments. One hundred and forty-six participants were studied for baseline plasma p-tau231, one hundred and twenty-three had data from the follow-up visit, and sixteen for a second follow-up visit.

Plasma and CSF collection

Baseline plasma and CSF extractions were performed on the same day, between 9 and 10 AM, with a difference of fewer than 30 minutes between them. Our institution is part of the Alzheimer's Association quality control program, and we follow international recommendations for plasma and CSF collection and storage [11,12]. LP is performed with the subject fasting, in lateral decubitus, between the L3-S1 spaces and with a standard 22G needle. The CSF is deposited into a 15 ml polypropylene tube and centrifuged at room temperature at 2000 g for 10 minutes. The resultant is aliquoted in 500 µl volumes into 1 ml tubes and frozen at -80 °C until analysis. Aβ, p-tau, and t-tau values were determined using Fujirebio's automated immunoassay analyzer Lumipulse G600 II [13].

Plasma samples were obtained following the standardized operating procedure described elsewhere [14]. In short, the blood is stored in 10 ml EDTA tubes and kept cold until processing within the next three hours. Then, it is centrifuged at 1800 g for 10 minutes. The supernatant is stored in polypropylene tubes in volumes of 500 µl and frozen at -80 °C until analyzed at the Clinical Neurochemistry Laboratory at the University of Gothenburg, Sweden. Plasma P-tau231 concentration was measured using an in-house ultrasensitive Single molecule array (Simoa) assay on an HD-X Analyzer (Quanterix, Billerica, MA, USA), as previously described in detail.

Magnetic Resonance and PET Imaging

All MRI scans were performed on the same 3T Philips Medical Systems MRI scanner with an 8-channel head coil. To determine the volume of the different structures, a sagittal T1-weighted MPRAGE sequence was used (170 slices, 1,2 mm voxel size, 9º of flip angle and shortest echo and repetition times) with subsequent processing as described in previous publications [9].

The production of ^{18}F-FDG and PET images was carried out in our hospital's Department of Nuclear Medicine. ^{18}F was obtained using the 18O(p,n)18F nuclear reaction in a cyclotron PET Trace (General Electric Healthcare, Wisconsin, USA) and ^{18}F-FDG scans were acquired in a Siemens Biograph LSO Pico 3D equipment (Siemens Healthcare Molecular Imaging, Illinois, USA). Subjects were administered intravenously with 3–4MBq/kg ^{18}F-FDG. Image acquisition consisted of a static image acquired 30-45 min after tracer injection. Images were reconstructed on a 128 x 128 matrix using the ordered subset expectation maximization method.

CSF biomarkers

Participants were classified according to ATN classification [15] based on their CSF biomarkers. The cut-off points were established using an unbiased Gaussian mixture modeling [16]**.** We have dichotomized these variables and considered Aβ-positive (A+) when CSF Aβ42/40 ratio <0.076, tau-positive (T+) when CSF p-tau181 >73.2 pg/ml, and neurodegeneration-positive (N+) if CSF t-tau >543 pg/ml.

***APOE* testing**

APOE genotype was determined by TaqMan SNP genotyping assays (Applied Biosystems, Foster City, CA, United States). Subjects carrying ≥1 copy of the ε4 allele were considered ε4+ and the rest were considered ε4-.

Neuropsychological tests

Participants in our cohort performed a comprehensive neuropsychological battery designed to detect early signs of AD. It includes several tests to evaluate the different cognitive domains. For memory assessment, we used the validated Spanish version of the Free and Cued Selective Reminding Test (FCSRT) [17] and we have considered different items such as the total free recall (TFR), total recall (TR), delayed free recall (DFR) and delayed total recall (DTR). We also measured memory through the logical memory (LM) subtest of the Weschler Memory Scale-III [18], taking into account the total delayed units (LMDU); and through the Spanish version of the Face Name Associative Memory Exam (FNAME) [19]. It consists of asking subjects to recall sixteen faces associated with a name and sixteen faces associated with an occupation. The first section includes initial recall of face-occupation and face-name pairs, followed by immediate recall of the name and occupation associated with each face. After thirty minutes, the subject is asked to recall as much of the information associated with each face as possible. The total value (TFNAME) is the number of all names and occupations recalled throughout the test and it is what we have measured. The executive functions were assessed by means of the two parts of the Trail Making Test (TMTA and TMTB) [20].

Statistics.

We used Shapiro test to check the normality assumption. The baseline p-tau231 values deviated from normality; however, the follow-up visit and the difference between basal levels and the follow-up visit showed a normal distribution. We used Student's test-test for paired samples to analyze the differences between visits for p-tau231 values. We also used Student's test-test for paired samples after stratifying subjects according to their ATN group.

To study the longitudinal changes of p-tau231 levels, we performed a Lineal Mixed Model (LMM), with p-tau231 value as the dependent variable and sample point as the main predictor. We included age, sex, *APOE* ε4 status and ATN group as covariates.

We used Spearman's Rho to study the correlation between the plasma biomarker level and the phenotype of the subjects. Thus, we correlated baseline p-tau231 values with MRI measures (right and left hippocampal volume), PET-FDG (hypometabolism in right and left hippocampus, right and left precuneus, and globally) and with the main outputs of FCSRT, FNAME, LM and TMT test. We also correlated baseline p-tau231 with cognitive tests stratified by the ATN group. Afterward, we selected the significant results and adjusted for covariates using a univariate general linear model with cognitive measures as the dependent variable. P-tau231 basal levels were transformed using box cox to resemble a normal distribution.

Statistical analyses were performed using SPSS Statistics V.20.0 (IBM, NY, USA) and R Statistical Software (version 2.14.0; R Foundation for Statistical Computing, Vienna, Austria).

RESULTS

The sample includes 146 cognitively unimpaired subjects (average Mini-Mental State Examination (MMSE) score of 28.8±1.50 and average DTR of 14.73±1.95), with a mean age of 64.69±8.33 years. 71.91% were women. The proportion of carriers of *APOE* ε4 was 29.45%; and 37.67% of subjects had at least one positive AD marker in the CSF (Table 1). The average

time between the collection of the baseline plasma samples and the first follow-up was 267.34±86.5 days, and between the first and second follow-up was 343.68±62.15 days.

Longitudinal analysis

Plasma p-tau231 was analyzed in 146 subjects at baseline and 123 at follow-up. We had a second follow-up determination only for 16 individuals. The mean baseline p-tau231 was 11.40 pg/ml ± 5.98 and 13.28 pg/ml ± 6.04 at follow-up. In the limited sample of the second follow-up visit p-tau231 was 16.36 pg/ml ± 7.39. The difference between the baseline levels and the first follow-up mean was statistically significant (-1.20 pg/ml; 95%CI -1.22 to -2.77; p-value 0.0000013). Compared with the second follow-up, baseline levels were also significantly different (-3.50 pg/ml; 95%CI -6.10 to -0.93; p-value 0.011). The difference between the first follow-up determination and the second was statistically significant as well (-1.81 pg/ml; 95%CI -3.59 to -0.03; p-value 0.046) (Figure 1).

When stratifying by ATN group, in the A-T-N-, the mean baseline p-tau231 was 10.07 pg/ml (± 5.00) and at follow-up it was 11.86 pg/ml (± 4.51). In this group, the mean difference was -1.79 pg/ml (95% CI=-2.82 to -0.76; p-value=0.0009). In the A+T-N- group, the mean baseline p-tau231 value was 11.81 pg/ml (± 5.24) and at follow-up 14.17 pg/ml (±6.4). The difference was also significant in this group, with a value of -2.36 pg/ml (95% CI=-3.56 to -1.16; p-value=0.0003). Finally, in the A+T+N+ group, the baseline p-tau231 mean was 17.4 pg/ml (± 8.00) and at follow-up 19.64 pg/ml (±8.88). In the A+T+N+ group, the average difference was not significant, with a value of -2.24 pg/ml (95% CI= -5.83 to -1.34; p-value=0.20) (Figure 2).

Next, to study longitudinal changes in p-tau231 values considering the influence of other variables such as age, sex, presence of *APOE* ε4 and ATN group we used a lineal mixed model. We estimated an increase of 1.93 p-tau231 units per follow-up visit (p-value=3.94E-7). Sex did not significantly influence the p-tau231 growth slope between visits, but the effect of age was borderline significant (β=0.14 p-value=0.10 and β=-1.60 p-value=0.052, respectively). *APOE* ε4 carriers showed a predicted increase in p-tau231 of 2.79 units over non-carriers (p-value=0.0067), and the A+T+N+ group presented an estimated 3.91 units higher than the A-T-N- group (p-value=0.016). The ATN groups showed no interactions with the different follow-up visits in the growth rate of p-tau231 (Figure 3).

Phenotypic correlations

We correlated p-tau231 plasma levels and their rate of change between baseline and the first follow-up visit with a wide range of phenotypic traits (Table 2).

Basal levels of p-tau231 showed no correlation with the total right (r=-0.01; p-value=0.85) or left (r=-0.12; p-value=0.23) hippocampal volume. Nor did the difference in p-tau231 between visits correlate significantly with left (r=0.007; p-value=0.94) or right (r=0.014; p-value=0.88) hippocampal volume. Uptake of FDG-PET did not correlate significantly with baseline p-tau231 values in different regions of interest, such as right precuneus (r=-0.035; p-value=0.68), left precuneus (r=-0.026; p-value=0.76), right hippocampus (r=-0.08; p-value=0.31), left hippocampus (r=-0.11; p-value=0.16) and neither with global average uptake (r=-0.04; p-value=0.59).

However, baseline p-tau231 values did show a correlation with different neuropsychological tests, such as the DTR (r=-0.25; p-value=0.002) and with the TFNAME (r=-0.25; p-value=0.002).

These tests did not correlate with the variation of p-tau231 between visits (r=-0.026; p-value=0.77 and r=0.027; p-value=0.76 respectively).

To better understand our results, we stratified the baseline correlations of p-tau231 with neuropsychological tests by the ATN groups. P-tau231 levels did not correlate significantly with TFNAME in the A-T-N- group (r=-0.034; p-value=0.75), but it did in the amyloid-positive groups, in which the magnitude of the correlation increased progressively: A+T-N- (r=-0.37; p-value=0.022) and A+T+N+ (r=-0.67; p-value=0.009) (Table 3). We further examined the association between baseline p-tau231 and TFNAME accounting by ATN and other covariates by a general lineal model. Using transformed p-tau231 as a dependent variant, we found that in the A+T-N- group the correlation with TFNAME remained significant (p-value=0.044) after adjusting for age and sex. This did not occur in the A+T+N+ group, though the number of individuals included in this analysis was limited (N=14).

DISCUSSION

Novel plasma biomarkers for AD are a promising tool for diagnosing and classifying patients in hospital and primary care settings. Among them, p-tau231 is one of the most interesting ones, as it rises in plasma very early and allows the detection of subjects at risk for AD [5,8]. Previous studies in cognitively unimpaired individuals modelling the trajectory of p-tau231 showed that it increased significantly with a range of only 26.4 centiloids on Aβ PET [5]. In the same study, p-tau217 levels also raised very early but required a higher threshold of amyloid pathology [5]. On the other hand, p-tau181 is a very specific marker of AD and predicts cognitive impairment and hippocampal atrophy within one year, but it only increases significantly in plasma with much higher amyloid loads [5,21]. The results of our study support the hypothesis that plasma p-tau231 changes occur very early, in the preclinical phase of AD, and are detectable even in individuals that, based on CSF biomarker results, would be considered as not in the AD continuum.

In a cognitively healthy population of, on average, 64.79 years, we have seen that p-tau231 increases sequentially in samples obtained with time differences of approximately one year. This increase was significantly detectable even in the A-T-N- group. Although it could constitute an ultra-early sign of amyloid dysmetabolism, its significance is still unclear and does not correlate with any cognitive, functional, or structural trait. To clarify this aspect of our findings, longer-term studies determining p-tau231 in A-T-N- subjects are necessary. A similar trend of increase of p-tau231 from baseline to the first follow-up visit in A+T-N- and A+T+N+ was found, though this was only significant in A+T-N-, which could be explained by the low numbers of the A+T+N+ group (N=12). This is a limitation of our study as some of the strata has low numbers, therefore, we might be underpowered for some of the analysis.

The linear mixed model adjusting, by ATN and other relevant covariates, estimated that the increase from sample point to sample point was of 1.93 units of p-tau231, and we did not find an interaction with the ATN groups; therefore, the slopes of the increase that we observed in the three ATN groups were not significantly different. This would point toward a lineal increase in our population of subjects around 65 years of age on average independent of their ATN status; however, some of our subgroups (especially the A+T+N+) had very low numbers, and we might be underpowered to detect interactions. A relevant aspect of our cohort is that it encompasses the age spectrum in which AD is known to begin to increase in incidence [22]. Even though age was only borderline significantly associated with p-tau231, we speculate that

our population might be well-powered to detect early p-tau231 levels increase, reflecting an early stage in the neurodegeneration process.

Longitudinal studies with post-mortem anatomopathological examination mention that p-tau231 levels also increase in the late stages of AD and are higher than those of subjects with mild amyloid pathology [23]. This gradual correlation with the main pathological signatures of AD in clinicopathological studies suggests that the p-tau231 increase might be related to early AD changes. In this sense, p-tau231 has shown to be able to discriminate between Braak 0 and Braak I–II stages [24]. However, recent evidence indicates that, although p-tau217 increases progressively along the AD continuum, p-tau231 reaches a plateau and may not be useful for monitoring disease progression or long-term treatments efficacy [23]. In the case of p-tau181, it appears to act similarly to p-tau217, as it increases progressively along the AD continuum, including the preclinical stages, and its levels correlate well with amyloid pathology [25,26].

To better characterize the early preclinical phase of AD, we compared p-tau231 levels with phenotype. We found that the increase from baseline to the first follow-up did not correlate with any phenotypic trait. However, baseline levels were associated with cognitive measurements. In contrast to what appears to happen with p-tau217 and p-tau181, which associates with cortical atrophy in CU subjects [27,28], we did not detect any significant correlation with structural measures such as hippocampal volume or glucose metabolism of different regions of interest for AD. This hints that p-tau231 levels rise even before clinically significant signs of neurodegeneration occur in neuroimaging.

However, basal levels of p-tau231 correlated with cognitive decline, measured by sensitive neuropsychological memory tests such as the FNAME and FCSRT. Interestingly, when we stratified by ATN, this happened only from the A+T-N- group onwards and progressively increased along the AD continuum. Moreover, this correlation remained significant after adjusting for covariates. These findings are in line with another previous study that has shown a correlation between baseline p-tau231 levels and longitudinal changes in cognition, determined by the Preclinical Alzheimer's Cognitive Composite (PACC)) at three years, only in Aβ+ subjects [5]. Our interpretation of this finding is that only in individuals with substantial amyloid deposition (positive CSF Aβ42/40 ratio) do we observe an association between tau pathology (p-tau231 plasma levels) and cognitive impairment.

For its part, in more recent studies, basal levels of plasma p-tau217 have been shown to correlate with a worsening of memory (word list delayed recall) in a group of CU presenilin-1 E280A carriers [29], and increases in this biomarker also correlate with a worsening in modified PACC and MMSE over as long as six years in Aβ+ CU subjects [26]. P-tau181 is not altered as early as p-tau217 and p-tau231 but has shown to be a good predictor of progression to AD and is longitudinally related to cognitive decline through parameters such as the MMSE, CDR and PACC [30].

Our findings indicate that plasma p-tau231 could be a promising tool for detecting early changes in preclinical AD. Our results also suggest that time intervals as short as one year could be meaningful in detecting significant changes. Sequential testing of plasma biomarkers could play an important role when selecting people for clinical trials and, in the near future, for initiation of DMT in an efficient and non-invasive way. Still, long-term longitudinal studies are needed to give us a better understanding of its temporal evolution.

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FIGURE LEGENDS

Figure 1: Longitudinal changes in p-tau231. The ordinate axis represents plasma p-tau231 concentration expressed in pg/ml and the abscissa axis, the sequential visits. In the boxplot, the boxes show the interquartile range (the upper boundary is the Q3, and the lower boundary is the Q1). The line inside the box corresponds to the median of the sample and the whiskers represent the maximum (upper) and minimum (lower) values. 146 subjects were studied at baseline, 123 at first follow-up and 16 in a second follow-up. The mean baseline was 11.40 pg/ml ± 5.98, at first follow-up it was 13.28 pg/ml ± 6.04, and 16.36 pg/ml ± 7.39 at the second one. The mean difference between baseline and first visit was significant (p-value=0.0000013), and so was the difference between baseline and second visit (p-value=0.011) and between first and second visit (p-value=0.046).

Figure 2: Changes in p-tau231 stratified by ATN group. The ordinate axis represents plasma p-tau231 concentration expressed in pg/ml and the abscissa axis, the baseline and first visit. The boxes show the interquartile range (the upper boundary is the Q3, and the lower boundary is the Q1). The line inside the box corresponds to the median of the sample and the whiskers represent the maximum (upper) and minimum (lower) values. The dots outside the boxes indicate outliers. White boxes correspond to A-T-N- group, grey boxes to A+T-N- and the black ones to the A+T+N+ group. An increase in p-tau231 values is observed between visits. The mean differences in the A-T-N- and A+T-N- groups were significant (p-value=0.0009 and p-value=0.0003 respectively, asterisks).

Figure 3: Influence of ATN group on the increase rate of p-tau231. In this linear mixed model plot the dots represent each individual value and the shaded area around the regression line represents the confidence interval. The ordinate axis represents the plasma p-tau231 units. In the abscissa axis we show the different visits stratified by ATN group. There are no differences between slopes, but there are differences in the general level. The A+T+N+ group showed an estimated 3.91 units higher than the A-T-N- group (p-value=0.016), suggesting that the change has happened earlier. The ATN groups showed no interactions with the different follow-up visits in the growth rate of p-tau231.

TABLES

Table 1 – Characteristics of the subjects.

Characteristic	**N=146**
Females, n. (%)	105 (71.91%)
Age, mean (SD)	64.69 (8.33)
APOE ε4 carrier, n. (%)	43 (29.45%)
MMSE (0–30), mean (SD)	28.8 (1.50)
DTR in FCSRT (0–16), mean (SD)	14.80 (2.45)
CSF Biomarkers	
Aβ40, mean (SD), pg/ml	10577.56 (3256.50)
Aβ42, mean (SD), pg/ml	796.05 (327.44)
Ratio Aβ42/40, Mean (SD)	0.076 (0.021)
Total-tau, mean (SD), pg/ml	336.36 (147.33)
Phosphorylated-tau, mean (SD), pg/ml	45.2 (28.13)
ATN group, n. (%)	
A-T-N-	91 (62.33%)
A+T-N-	40 (27.4%)
A+T+N+	15 (28.13%)

Table 1 abbreviations: n, number of subjects. SD, standard deviation. MMSE, mini-mental state examination. DTR, delayed total recall. FCSRT, Free and Cued Selective Reminding Test. CSF, cerebrospinal fluid. Aβ, amyloid beta. A, amyloid. T, tau. N, neurodegeneration.

Table 2 – Correlation between p-tau231 and phenotype

				Basal p-tau231 (pg/ml)	p-tau231 change (pg/ml)
		N		Spearman's Correlation coefficient	Spearman's Correlation coefficient
NPS	LMDU	146	Rho	-0.157	-0.152
			p-value	0.057	0.09
	DFR	146	Rho	-0.122	-0.088
			p-value	0.14	0.33
	DTR	144	Rho	-0.255	-0.026
			p-value	**0.002**	0.77
	TMTA	146	Rho	0.161	0.074
			p-value	0.051	0.41
	TMTB	139	Rho	0.103	0.073
			p-value	0.22	0.43
	TFNAME	146	Rho	-0.25	0.027
			p-value	**0.002**	0.76
MRI	Left hippocampus	107	Rho	0.124	0.007
			p-value	0.20	0.94
	Right hippocampus	107	Rho	-0.018	0.014
			p-value	0.85	0.88
FDG PET	Average	136	Rho	-0.046	-0.017
			p-value	0.59	0.84
	Right precuneus	136	Rho	-0.035	-0.073
			p-value	0.68	0.42
	Left precuneus	136	Rho	-0.026	-0.039
			p-value	0.76	0.66
	Right hippocampus	136	Rho	-0.088	0.057
			p-value	0.31	0.52
	Left hippocampus	136	Rho	-0.011	0.083
			p-value	0.16	0.36

Table 2 abbreviations: NPS, neuropsychological. MRI, magnetic resonance imaging. FDG PET, fluorodeoxyglucose positron emission tomography. N, number of subjects. p value, statistical significance. Rho, correlation coefficient. LMDU, total delayed units in Logical Memory test. DFR, delayed free recall in Free and Cued Selective Reminding test. DTR, Delayed Total Recall. TMTA, Trail Making Test part A. TMTB, Trail Making Test part B. TFNAME, total value of Face Name Associative Memory Exam

Table 3 – Correlation between baseline p-tau231 and cognitive tests stratified by ATN group.

	Cognitive test	A-T-N-		A+T-N-		A+T+N+	
		Rho	p-value	Rho	p-value	Rho	p-value
p-tau231	LMDU	0.01	0.88	-0.16	0.32	-0.33	0.25
	DFR	-0.03	0.77	-0.04	0.8	-0.43	0.12
	DTR	-0.12	0.27	-0.18	0.27	-0.43	0.12
	TMTA	0.02	0.86	0.02	0.9	0.47	0.08
	TMTB	-0.05	0.67	0.006	0.96	0.53	0.75
	TFNAME	-0.03	0.75	-0.37	**0.022**	-0.67	**0.009**

Table 3 abbreviations: p value, statistical significance. Rho, correlation coefficient. LMDU, total delayed units in Logical Memory test. DFR, delayed free recall in Free and Cued Selective Reminding test. DTR, Delayed Total Recall. TMTA, Trail Making Test part A. TMTB, Trail Making Test part B. TFNAME, total value of Face Name Associative Memory Exam. A, amyloid. T, tau. N, neurodegeneration.

FIGURES

FIGURE 1: P-tau231 longitudinal changes.

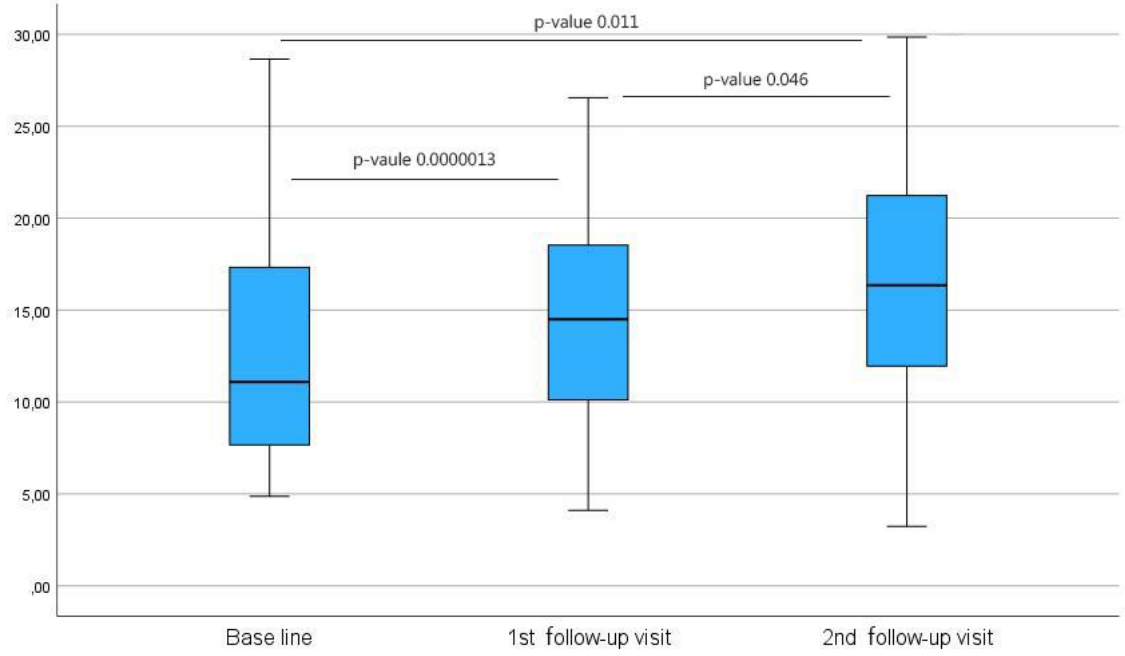

Figure 1: Longitudinal changes in p-tau231.The ordinate axis represents the plasma p-tau231 value expressed in pg/ml and the abscissa axis, the sequential visits. In the boxplot, the boxes show the interquartile range (the upper boundary is the Q3, and the lower boundary is the Q1). The line inside the box corresponds to the median of the sample and the whiskers represent the maximum (upper) and minimum (lower) values. 146 subjects were studied at baseline, 123 at first follow-up and 16 in a second follow-up. The mean baseline was 11.40 pg/ml ± 5.98, at first follow-up it was 13.28 pg/ml ± 6.04, and 16.36 pg/ml ± 7.39 at the second one. The mean difference between baseline and first visit was significant (p-value=0.0000013), and so was the difference between baseline and second visit (p-value=0.011) and between first and second visit (p-value=0.046).

F**IGURE 2: Changes in p-tau231 stratified by ATN group.**

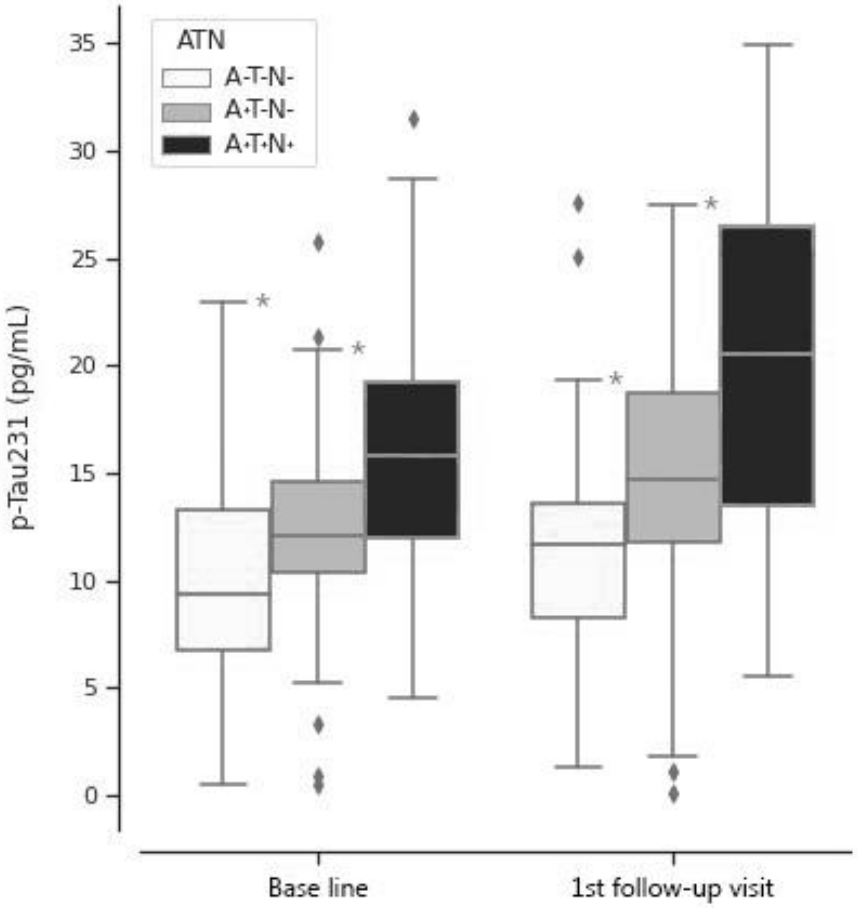

Figure 2: Changes in p-tau231 stratified by ATN group. The ordinate axis represents the plasma p-tau231 value expressed in pg/ml and the abscissa axis, the baseline and first visit. The boxes show the interquartile range (the upper boundary is the Q3, and the lower boundary is the Q1). The line inside the box corresponds to the median of the sample and the whiskers represent the maximum (upper) and minimum (lower) values. The dots outside the boxes indicate outliers. White boxes correspond to A-T-N- group, grey boxes to A+T-N- and the black ones to the A+T+N+ group. An increase in p-tau231 values is observed between visits. The mean differences in the A-T-N- and A+T-N- groups were significant (p-value=0.0009 and p-value=0.0003 respectively, asterisks).

FIGURE 3:

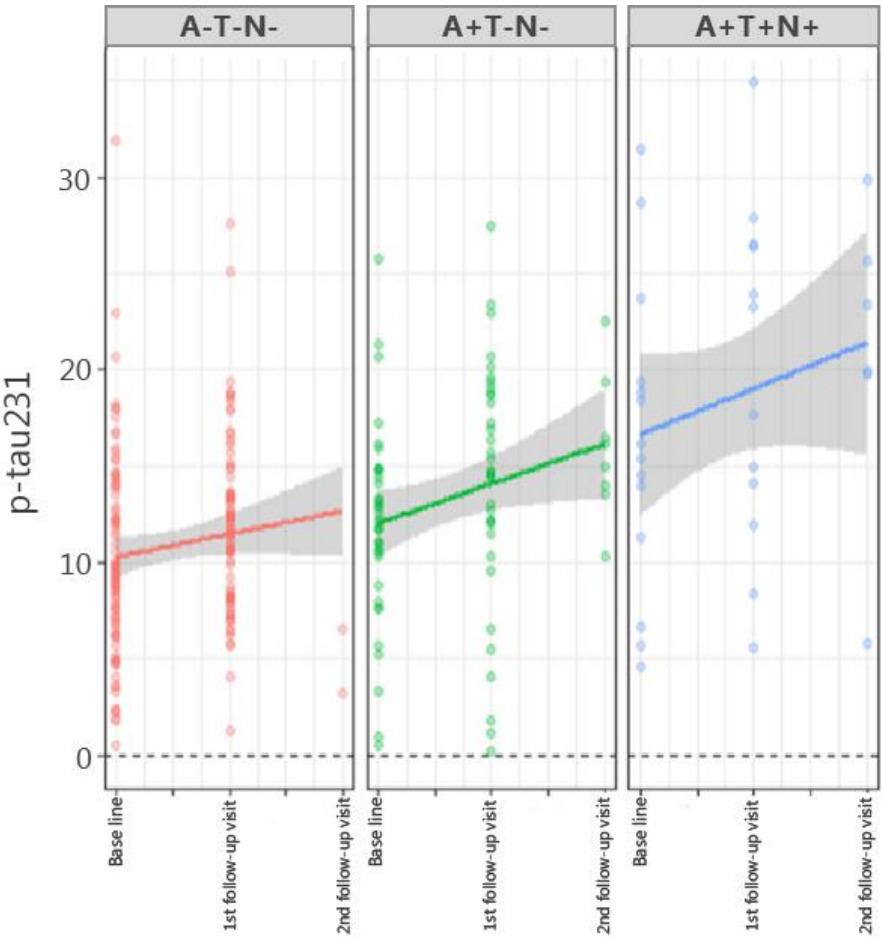

Figure 3: Influence of ATN group on the growth rate of p-tau231. In this linear mixed model plot the dots represent each individual value and the shaded area around the regression line represents the confidence interval. The ordinate axis represents the plasma p-tau231 units. In the abscissa axis we show the different visits stratified by ATN group. As it can be seen, there are no differences between slopes, but there are differences in the general level. The A+T+N+ group showed an estimated 3.91 units higher than the A-T-N- group (p-value=0.016). The ATN groups showed no interactions with the different follow-up visits in the growth rate of p-tau231.