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Plasma p-tau181 as a promising non-invasive biomarker of Alzheimer's Disease pathology in Subjective Cognitive Decline and Mild Cognitive Impairment

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ABSTRACT

Introduction: The aim of this study is to investigate the role of plasma phosphorylated tau (p-tau) 181 as a potential biomarker for Alzheimer's Disease (AD) pathology in the early stages of the disease, as a valuable marker for tauopathy.

Materials and methods: Thirty-three Subjective Cognitive Decline (SCD), 32 Mild Cognitive Impairment (MCI) and 14 AD demented (AD-d) patients underwent plasma p-tau181 analysis with SiMoA assay. Twenty-six SCD, 32 MCI and 14 AD-d patients also underwent CSF biomarkers analysis (Aβ1–42, Aβ1–42/1–40, p-tau, t-tau) and were classified as carriers of AD pathology (AP+) when A+ was associated with T+ (regardless of N), or non-carriers (AP-) when they were A- (regardless of T and N), or A+/T-/N-, or A+/T-/N+ according to the A/T (N) system.

Results: Plasma p-tau181 levels were higher in SCD AP+ than in SCD AP- (2.85 ± 0.53 vs 1.73 ± 0.64, $p < 0.001$), and in MCI AP+ than in MCI AP- (4.03 ± 1.07 vs 2.04 ± 0.87, $p < 0.001$). In a multivariate linear regression analysis, AP status was the only variable that influenced plasma p-tau181 (B = 1.670 [95% CI 1.097:2.244], $p < 0.001$). Plasma p-tau181 was highly accurate for discriminating between AP+ and AP- patients (AUC = 0.910). We identified a cut-off level of 2.69 pg/mL to distinguish between AP+ and AP- (sensibility 0.86, specificity 0.82, PPV 75.00% NPV 90.32%).

Conclusions: Plasma p-tau181 levels were influenced by the presence of underlying AD pathology, independently from the cognitive status and were highly accurate in differentiating SCD-MCI patients who were carriers of AD pathology from non-carriers. Plasma p-tau181 might be a promising non-invasive biomarker of AD pathology at a very early stage.

1. Introduction

Alzheimer's Disease (AD) is a progressive debilitating neurodegenerative disease, with a prevalence estimated at 50 million people worldwide and projected to triple by 2050 [1]. The definition of AD moved from a pure clinical entity [2] to a clinic-biological construct through the International Working Group (IWG) [3] and the NIA-AA criteria [4–6]. Indeed, the new clinic-biological definition of AD was anchored to the positivity of the in vivo biomarkers, which may document the underlying pathologic processes showing the deposition of β-amyloid plaques and neurofibrillary tau.

In this perspective, the description of AD as a biological entity will allow a more accurate characterization of the cognitive impairment typically due to AD, also enabling a more precise approach as far as

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therapeutic strategies are concerned [7]. The evolution of AD unfolds in several phases before the well-known clinical picture [4], presenting presymptomatic period lasting from several years to decades [5,6]. In fact, early stages of AD have been identified: mild cognitive impairment (MCI) describes subjects with objective cognitive impairment without impact on instrumental activities of daily living and it is considered transitional between normal cognition and dementia.

In recent times, increasing attention has been paid to an even potential earlier stage in the disease's progression that is Subjective Cognitive Decline (SCD). SCD was defined as a as a self-experienced persistent decline in cognitive capacity in comparison with the subject's previously normal status, during which the subject has normal age-, sex-, and education-adjusted performance on standardized cognitive tests [8]. Despite SCD is a heterogeneous entity and with many potential underlying causes and different trajectories, SCD increases the risk of progression to plain cognitive impairment considering both the clinical [9] and the biological perspective, particularly by the use of biomarkers [10,11]. Indeed, patients with SCD showed a higher incidence of progression to AD and higher prevalence of AD biomarkers as compared to individuals without [12,13].

The recently approved new disease-modifying therapies have focused attention towards the early detection of AD through the use of biomarkers [14]. Indeed, the identification of subjects with a higher risk to progress to overt dementia appears to have the outmost importance. In fact, subjects in early phases of AD seems to be the ideal group in which to intervene with a specific treatment in order to stop or slow down neurodegeneration. Current biomarkers of AD pathology are obtained through lumbar puncture (cerebrospinal fluid Aβ1–42, Aβ1–42/1–40, hyperphosphorylated tau and total tau) [15,16] and positron emission tomography (PET) imaging (amyloid PET and tau PET) [17–19]. Nevertheless, CSF collection requires an invasive procedure, while PET imaging is expensive and not widely available. For this reason, the search for biomarkers has progressively shifted towards a more accessible substrate, that is peripheral blood.

Among the possible peripheral biomarkers, growing attention is being directed towards isoforms 181 and 237 of phosphorylated tau protein as earlier and more specific markers of the typical degeneration due to AD [20]. Indeed, withing the complex interplay with β-amyloid, the aggregation of phosphorylated tau has a key role in the pathogenesis of AD [21–23]. Moreover, several studies demonstrated a strong correspondence between the onset and the type of cognitive deficits with the time and the site of tau pathology accumulation [24,25]. Thus accurate biomarkers of tau pathology offer great opportunities to improve diagnosis and to early detect AD both in clinical practice and in clinical trials [26]. Plasma p-tau may potentially provide a tool for a non-invasive screening of the disease's pathophysiology in order to identify individuals at greatest risk of developing AD dementia even up to many years before neuropathological confirmation, clearly distinguishing between AD and non-AD pathologies [27]. Further studies have confirmed that increased plasma p-tau181 levels could be detected even at the preclinical and prodromal stage of AD, besides full-blown dementia [28], making this protein an useful dynamic biomarker along the course of the disease that strongly associates with cognitive decline [29]. Nevertheless, only few studies explored the potential use of this biomarker in SCD population, and results were not clearly conclusive [30,31].

In this scenario, the aims of our study were:

- to assess quantitative differences in plasma p-tau181 levels between SCD, MCI and AD demented patients;
- to assess diagnostic accuracy, sensitivity, specificity of plasma p-tau in differentiating SCD and MCI patients carrying AD pathology from non-carriers;
- to investigate the role of plasma p-tau181 as a biomarker of AD pathology in the early stages of the disease.

2. Materials and methods

2.1. Patients

Between March 2019 and September 2022, we consecutively collected 81 plasma samples from patients referred to the Centre for Alzheimer's Disease and Adult Cognitive Disorders of Careggi Hospital in Florence. Blood samples were collected at the first evaluations for patients who come to our centre for the first time, or at the check-up visit for patients who were regularly followed-up at our centre. Patients met the following inclusion criteria: (1) patients who received a clinical diagnosis of AD dementia according to the NIA-AA criteria, including the atypical variant [4], (2) patients who received a clinical diagnosis of MCI according to NIA-AA criteria [5], (3) patients who received a clinical diagnosis of SCD [8]. Two patients were excluded because they received a diagnosis of psychiatric disturb and non-fluent/agrammatic variant of Primary Progressive Aphasia [32]. Finally, we included 79 patients: 33 SCD, 32 MCI, 14 AD demented (AD-d).

All patients underwent a comprehensive family and clinical history, neurological examination and extensive neuropsychological investigation. Age at baseline corresponded to the age at the time of plasma collection. A positive family history was defined as one or more first-degree relatives with documented cognitive decline.

Seventy-two (26 SCD, 32 MCI, 14 AD-d) gave consent for lumber puncture and underwent CSF biomarker analysis (Aβ1–42, Aβ1–42/1–40 ratio, t-tau, p-tau). Eighteen patients also underwent amyloid PET (14 SCD and 4 MCI).

Study procedures and data analysis were performed in accordance with the Declaration of Helsinki and with the ethical standards of the Committee on Human Experimentation of our Institute. The study was approved by the local Institutional Review Board (reference 15691oss). All individuals involved in this research agreed to participate and agreed to have details and results of the research about them published.

2.2. Neuropsychological assessment

All subjects were evaluated by an extensive neuropsychological battery consisting of: global measurements (Mini-Mental State Examination [MMSE]), tasks exploring verbal and spatial short-working and long-term memory (Digit and Visuo-spatial Span forward and backword [33], Rey auditory Verbal Learning test immediate recall RVLT-I and delayed recall RVLT-D [34]; Babcock Short Story Immediate and Delayed Recall [35], Rey-Osterrieth complex figure recall [36]), attention (Trail Making Test A [37], attentional matrices [38]), language (Category Fluency Task [39], Phonemic Fluency Test [40]), constructional praxis (Rey-Osterrieth Complex Figure copy [41]), and executive functions (Trail Making Test B [37], Stroop Test [42]). In patients with SCD, we estimated cognitive complaints using a survey based on the Memory Assessment Clinics-Questionnaire (MAC-Q) [43]. We defined the presence of cognitive complaints if participants perceived decline in cognitive capacity than in the past or if they reported difficulties in carrying out at least four of the following activities: remembering the name of a person just introduced to them; recalling telephone numbers or zip-codes used on a daily or weekly basis; recalling where they put objects in their home or office; remembering specific facts from a newspaper or magazine article just read; remembering the item(s) they intend to buy when they arrive at the grocery store or pharmacy.

2.3. Collection of AD biomarker

The CSF samples were collected by lumbar puncture, then immediately centrifuged and stored at −80 °C until performing the analysis. Aβ1–42, Aβ42/40 ratio, t-tau, and p-tau were measured using a chemiluminescent enzyme immunoassay (CLEIA) analyzer LUMIPULSE G600 (Lumipulse Beta Amyloid1–40, Lumipulse Beta Amyloid1–42, Lumipulse GTotal Tau, and Lumipulse GPhospho Tau (181)). Cut-offs for

normal values were: for Aβ1–42, > 670 pg/mL; Aβ42/40 ratio, > 0.062; t-tau, < 400 pg/mL; and p-tau, < 60 pg/mL [44]. Reagent kits were obtained from Fujirebio.

Amyloid PET imaging was performed according to national and international standards [45], with any of the available fluorine18-labeled tracers (18Florbetaben [FBB]-Bayer-Pyramal, 18Flutemetamol [FMM]-General Electric). Images were rated as either positive or negative according to criteria defined by the manufacturers.

2.4. Classification of patients according to ATN system

Based on biomarker results, patients were classified according to the A/T/N classification: patients were rated as A+ if at least one of the amyloid biomarkers (CSF or amyloid PET) revealed the presence of Aβ pathology and as A- if none of the biomarkers revealed the presence of Aβ pathology. In case of discordant CSF and Amyloid PET results, we considered only the pathologic result. Patients were classified as T+ or T– if CSF p-tau concentrations were higher or lower than cut-off values respectively. Patients were classified as and N+ if CSF t-tau was higher than cut-off values. Patients were further classified as carrier of AD pathology (AP+) when A+ was associated with T+ (regardless of N classification), or non-carriers (AP-) when they were classified as A- (regardless of T and N classification), or A+/T-/N-, or A+/T-/N+.

2.5. Apolipoprotein E ε4 genotyping

A standard automated method (QIAcube, QIAGEN) was used to isolate DNA from peripheral blood samples. *APOE* genotypes were investigated by high-resolution melting analysis (HRMA) [46]. Two sets of PCR primers were designed to amplify the regions encompassing rs7412 [NC_000019.9:g[M13] [GG14] .45412079C>T] and rs429358 (NC_000019.9:g.45411941T>C). The samples with known *APOE* genotypes, which had been validated by DNA sequencing, were used as standard references.

APOE genotype was coded as *APOE* ε4- (no *APOE* ε4 alleles) and *APOE* ε4+ (presence of one or two *APOE* ε4 alleles).

2.6. Plasma phosphorylated tau 181 analysis

Blood samples were collected by venipuncture into standard polypropylene EDTA test tubes (Sarstedt, Nümbrecht, Germany). Plasma was isolated from peripheral blood sample within 2 h of collection. Blood sample was centrifugated at 1300 rcf for 10 min and plasma was stored at −80°C until tested. The Simoa Human p-tau181 Advantage V2 kit (item #103714, provided by Quanterix Corp. - Billerica, MA, USA) was used for the quantitative determination of p-tau181 in plasma sample on the automated Simoa SR-X instrument (Quanterix Corp. - GBIO, Hangzhou, China). The kit Analytical Lower Limit of Quantification (LLOQ) value was 0.085 pg/mL, instead the kit Limit of Detection (LOD) was 0.041 pg/mL (range 0.018–0.060 pg/mL). For the run setup, 7 calibrators and 2 controls, provided by Quanterix, were required for the analysis. Calibrators were used to set a calibration curve of serially measurements, controls were the lower and higher target concentration. Plasma samples and controls were diluted 4x. Calibrators, controls and samples were run in duplicate, detected in a single run basis [47].

2.7. Statistical analysis

All statistical analysis were performed via IBM SPSS Statistics Software Version 25 (SPSS Inc., Chicago, USA) and the computing environment R 4.0.3 (R Foundation for Statistical Computing, Vienna, 2013). All *p*-values were two-tailed and significance level for all analyses was set at $\alpha = 5\%$, corresponding to a threshold p of 0.05. Distribution of all variables was assessed through Shapiro-Wilk test. Patient groups were characterized by using means and standard deviations, frequencies or percentages and 95% confidence intervals (95%C.I.) for continuous distributed variables, continuous non-normally distributed variables and categorical variables, respectively. Depending on the distribution of our data, we used *t*-tests or non-parametric Mann-Whitney-*U* tests for between-groups comparisons and Pearson's r or Spearman's ρ for correlations. We used chi-square tests to compare categorical data. We calculated the size effect by the Cohen's d for normally distributed numeric measures, η2 for Mann-Whitney-*U* Test and the Cramer's V for categorical data. Differences among groups in continuous variables were assessed through one-way ANOVA followed by Bonferroni post-hoc test. A multiple regression analysis was run in order to assess which variables independently influenced plasma p-tau181 levels.

Receiver operating characteristic (ROC) analyses were performed to evaluate the ability of plasma p-tau181 to distinguish between AP+ and AP- patients. The Youden's method was used to detect the best cut-off value and accuracy, sensitivity, specificity, positive predictive value and negative predictive value for plasma p-tau181.

3. Results

3.1. Comparisons among groups

Demographic features are summarized in Table 1. MMSE was significantly different among the groups (F [2,89] = 41.62, $p < 0.001$) with poorer scores in AD-d (23.08 ± 3.40) compared to SCD (28.73 ± 1.46, $p < 0.001$) and MCI (27.19 ± 2.30, $p < 0.001$). Thirty-two patients (43.24%) were *APOE* ε4 carriers: no differences in the frequency of *APOE* ε4 allele were found among the three groups.

Plasma p-tau181 levels were significantly different among the three groups (F [2,76] = 12.303, $p < 0.001$). In more details, plasma p-tau181 levels were significantly lower in SCD patients (2.15 ± 0.88) as compared to AD-d (4.03 ± 1.39, $p < 0.001$) (Table 1, Fig. 1).

Plasma p-tau181 levels were correlated with age at plasma collection only in the SCD group (Spearman's ρ 0.520, $p = 0.002$). Plasma p-tau181 levels were different between *APOE* ε4+ and ε4- in the MCI group (3.67 ± 1.36 vs 2.52 ± 0.16, $p = 0.025$, $\eta^2 = 0.17$), but not in SCD and AD-d subgroups. We did not find any other correlations between p-tau181 levels and other variables. There were no differences between women and men in p-tau181 concentration.

Table 1
Demographic features in Subjective Cognitive Decline (SCD), Mild Cognitive Impairment (MCI) and Alzheimer's Disease dementia (AD-d) groups (79 patients).

	SCD	MCI	AD-d
	N° 33	N° 32	N° 14
Age at onset in years	57.10 (±9.92)	63.22 (±11.00)	67.00 (±4.84)
Age at plasma collection	66.72 (±8.74)	69.89 (±8.06)	70.86 (±5.15)
Family history of AD	73.33%	60.71%	41.66%
Sex (M – F)	10–23	12–20	5–9
Years of education	12.28 (±3.87)	12.28 (±4.45)	10.14 (±5.60)
MMSE	28.73 (±1.46)*	27.19 (±2.30)**	23.08 (±3.40)*,**
APOE ε4+	33.33%	43.33%	64.28%
Plasma p-tau181 (pg/ml)	2.15 (±0.88)***	2.91 (±1.38)	4.03 (±1.39)***

Values are reported as mean and standard deviation or frequencies or percentages for continuous variables and categorical variables respectively. Statistically significantly different values between the groups are reported as *underlined character*. M: males; F: females; MMSE: Mini Mental State Examination. The sample size for AP+ status is reported into brackets. Statistically significance after Bonferroni Correction: $p = 0.006$.

* $p < 0.001$.

** $p < 0.001$.

*** $p < 0.001$.

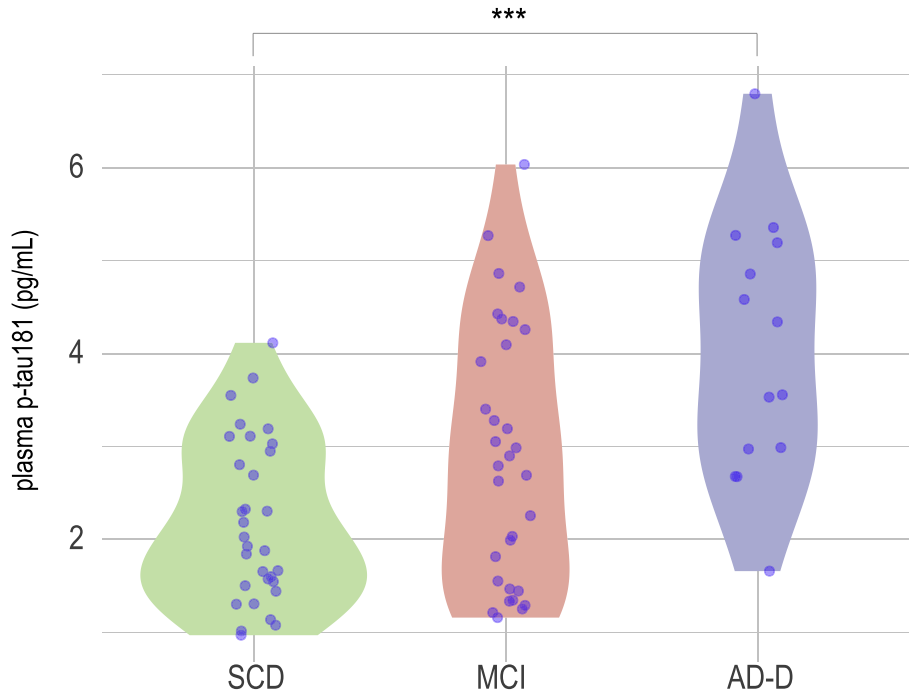

Fig. 1. Plasma p-tau181 levels in SCD, MCI and AD demented patients.

3.2. Correlations between plasma p-tau181 and CSF biomarkers

In SCD patients, plasma p-tau181 levels were significantly correlated with Aβ1–42 (Spearman's $\rho = -0.516$, $p = 0.008$), Aβ1–42/1–40 ratio (Spearman's $\rho = -0.748$, $p < 0.001$) and p-tau (Spearman's $\rho = 0.779$, $p < 0.001$). Similarly, in MCI patients, plasma p-tau181 levels were significantly correlated with Aβ1–42 (Spearman's $\rho = -0.618$, $p < 0.001$), Aβ1–42/1–40 ratio (Spearman's $\rho = -0.692$, $p < 0.001$), p-tau (Spearman's $\rho = 0.674$, $p < 0.001$) and t-tau (Spearman's $\rho = 0.693$, $p < 0.001$). No correlations with CSF biomarkers were detected in AD-d patients (Fig. 2).

3.3. Comparison of plasma p-tau181 levels according to AP status

According to CSF biomarkers and amyloid-PET, 36 (8 SCD, 14 MCI, 14 CE-d) out of 72 patients (50.00%) were classified AP+, while the other 36 (18 SCD, 18 MCI) were classifies as non-carriers AP. All AD-d patients were AP+. Percentage of AP+ patients were different among groups, in more details between AD-d and MCI (χ^2 12.93, $p < 0.001$, Cramer's V 0.530) and between AD-d and SCD (χ^2 17.62, $p < 0.001$, Cramer's V 0.664), while no differences were detected between SCD and MCI patients (χ^2 1.07, $p = 0.311$, Cramer's V 0.133) (Table 2).

Age at onset was significantly higher in MCI AP+ (69.43 ± 5.14) than in SCD AP- (53.59 ± 8.95, $p < 0.001$, $\eta^2 = 0.62$). Age at plasma collection was lower in SCD AP- (62.35 ± 8.82) than in SCD AP+ (73.63 ± 6.05, $p = 0.003$, $\eta^2 = 0.33$) and in MCI AP+ (72.87 ± 5.36, $p < 0.001$, $\eta^2 = 0.38$). SCD AP- had higher score at MMSE (29.22 ± 0.73) as compared to MCI AP- (27.28 ± 2.32, $p = 0.001$, $\eta^2 = 0.29$) and to MCI AP+ (26.08 ± 2.36, $p = 0.003$, $\eta^2 = 0.30$).

No difference in sex, prevalence of *APOE* ε4 allele and family history were detected among groups, except for a higher prevalence of *APOE* ε4 allele in MCI AP+ than in MCI AP- (78.57% vs 12.50, χ^2 13.27, $p < 0.001$, Cramer's V 0.665) (Table 3).

We compared plasma p-tau181 levels between groups defined according to the AP status (SCD AP+, SCD AP-, MCI AP+, MCI AP- and AD-d). Plasma p-tau181 levels were significantly different between SCD AP+ and SCD AP- (2.85 ± 0.53 vs 1.72 ± 0.63, $p < 0.001$, $\eta^2 = 0.44$), between MCI AP+ and MCI AP- (4.03 ± 1.07 vs 2.04 ± 0.87, $p < 0.001$, $\eta^2 = 0.53$), and between SCD AP- and MCI AP+ ($p < 0.001$, $\eta^2 = 0.64$). No differences were detected between SCD AP+ and MCI AP- ($p = 0.039$, $\eta^2 = 0.16$), between SCD AP- and MCI AP- ($p = 0.258$, $\eta^2 = 0.04$) and between MCI AP+ and AD-d ($p = 0.981$, $\eta^2 = 0.00$ (Fig. 3).

In order to analyze which factors might influence plasma p-tau181 levels, we ran a multiple regression analysis. We considered plasma p-tau181 levels as dependent variable, and diagnosis (SCD, MCI or AD dementia), age at plasma collection, AP status and *APOE* genotypes as covariates. The multiple regression model significantly predicted plasma p-tau181 levels (F [4,64] = 16.76, $p < 0.001$, adj. $R^2 = 0.512$). Among the covariates, only AP status (B = 1.627 [95% CI 1.006:2.248], $p < 0.001$) added statistically significantly to the prediction (Table 4).

3.4. Plasma p-tau181 accuracy in distinguishing AP+ and AP-

We performed a ROC curve analysis in order to evaluate the diagnostic accuracy of plasma p-tau181 in distinguishing between AP- and AP+ patients. For the purpose of this analysis, we did not consider AD-d group (as 100% of these patients were AP+) and we merged SCD and MCI, as there were no differences between these groups in p-tau181 concentrations when adjusted for confounding variables. The analysis showed that plasma p-tau181 was highly accurate for discriminating between AP+ and AP- patients (AUC = 0.910) (Fig. 4). A cut-off value of 2.69 pg/mL had the maximum Youden Index and showed to be able to discriminate AP+ and AP- patients with good accuracy (84.21% [95% CI 75.28–93.14]), sensibility (86.36% [95% CI 77.29–95.34]) and specificity of 82.5% [95% CI 72.28–92.43], a fair PPV 79.17% ([95% CI 68.43–89.90]) and an excellent NPV (90.32% [95% CI 82.51–98.14]).

4. Discussion

Our study aimed to explore differences in plasma p-tau181 levels in SCD, MCI and AD demented patients, in order to assess diagnostic accuracy of this non-invasive biomarker in early phases of Alzheimer's Disease. First of all, we found that plasma p-tau181 levels were different among patients depending on the underlying pathology (i.e., the presence or absence of Alzheimer's pathology) and not on the cognitive status. Moreover, plasma p-tau181 was highly accurate for

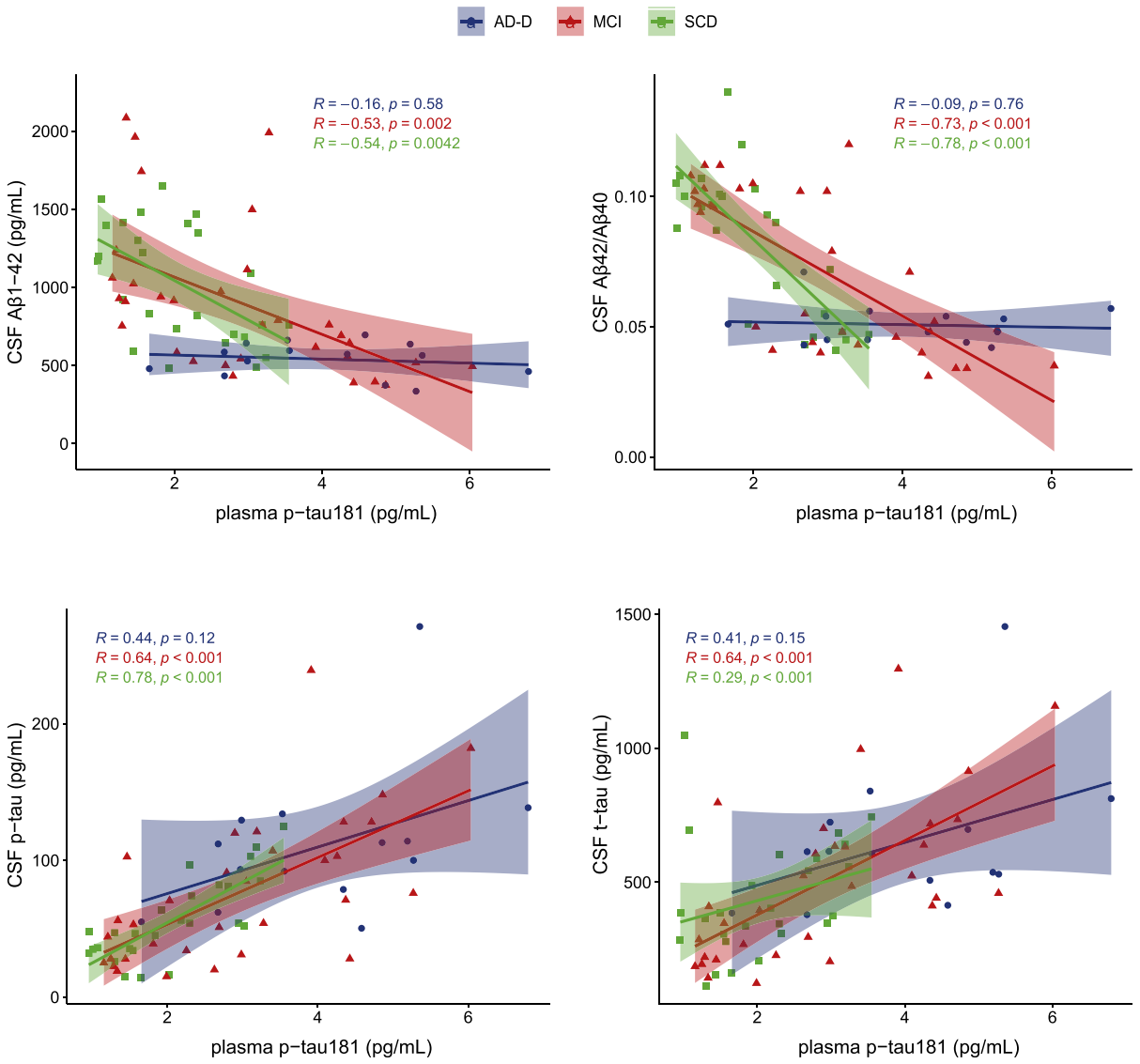

Fig. 2. Correlation between plasma p-tau181 levels and CSF biomarkers in SCD, MCI and AD demented patients.
Scatter plots with lines of best fit (95% C.I.) show the relationship between plasma p-tau181 levels and CSF Aβ1–42, Aβ1–42/1–40, p-tau and t-tau.

discriminating between patients carrying AD pathology from non-carriers in early stages of AD.

In more details, considering comparisons according to clinical diagnosis, plasma p-tau181 levels were lower in SCD as compared to AD demented patients, while no differences were detected between SCD and MCI and between MCI and AD demented patients. Previous works have already described that plasma p-tau181 is increased along the AD continuum, with higher levels in AD demented as compared to MCI patients and healthy controls [20,28,48,49]. However, little is known about this biomarker in SCD populations, since other authors performed comparisons with cognitively unimpaired individuals or healthy controls [20,29,48,49]. Moreover, SCD patients are sometimes not well-defined or classified as healthy controls, thus leading to difficulties in exploring the role of biomarkers in this specific population [20]. Only few studies found that plasma p-tau181 levels were increased in what the authors described as "objectively defined SCD" as compared to healthy controls [31,50].

Concerning the correlations between plasma p-tau181 levels and CSF biomarkers, interestingly we found that plasma p-tau181 negatively correlated with CSF Aβ1–42 and Aβ1–42/1–40 ratio and directly correlated with p-tau and t-tau in the MCI group. The same correlations were also found in SCD patients, except for the correlation between plasma p-tau181 with t-tau. Interestingly, no correlations were detected in AD demented patients.

These findings are in line with previous works, which highlighted the strong correlation between CSF and plasma p-tau levels [20,29,49,51]. Indeed, Moscoso et al. showed that CSF and plasma p-tau181 levels were correlated, even if this correlation was present only up to relatively high CSF p-tau181 levels (50 pg/ml) in a cohort of cognitively unimpaired subjects, MCI and AD demented patients [29]. Moreover, Janelidze et al. described a significant correlation between CSF and plasma p-tau181 levels in patients with positive amyloid biomarkers, even in cognitive unimpaired subjects [20], thus suggesting that plasma p-tau181 reflects changes in hyperphosphorylated tau in the central nervous system that occurs in Aβ + individuals.

The correlations between plasma p-tau181 and CSF Aβ1–42 and Aβ1–42/1–40 ratio might be explained by the fact that plasma p-tau181 is a marker of phosphorylated tau protein and so it is extremely specific for the typical degeneration of AD. Karikari et al. described a high association of plasma p-tau181 with measures of amyloid pathology (both CSF Aβ1–42 and amyloid PET), suggesting a potential biological relationships between Aβ pathology and the secretion of brain-specific p-

Table 2
Differences in CSF biomarkers and Alzheimer's Pathology Status in Subjective Cognitive Decline (SCD), Mild Cognitive Impairment (MCI) and Alzheimer's Disease dementia (AD-d) groups (72 patients).

	SCD N° 26	MCI N° 32	AD-d N° 14
Aβ1–42	1026.58 (±372.73)*	897.59 (±482.54)	541.00 (±109.77)*
Aβ1–42/1–40	0.081 (±0.028)[a]	0.071 (±0.030)	0.050 (±0.00)[a]
p-tau	56.42 (±30.14)[b]	75.59 (±52.78)	110.22 (±54.27) [b]
t-tau	436.46 (±214.59)	505.53 (±298.54)	650.93 (±272.87)
A+	53.33%[c]	50.00%[d]	100%[c,d]
T+	34.61%[e]	50.00%[f]	100%[e,f]
N+	42.30%[g]	59.37%[h]	100%[g,h]
AP+	30.76%[i]	43.75%[j]	100%[i,j]

Values are reported as mean and standard deviation. Statistically significantly different values between the groups are reported as underlined character. Statistically significance after Bonferroni Correction: $p = 0.006$.
* $p = 0.001$.
[a] $p = 0.003$.
[b] $p = 0.002$.
[c] χ^2 9.58, $p = 0.002$.
[d] χ^2 10.73, $p = 0.001$.
[e] χ^2 15.92, $p < 0.001$.
[f] χ^2 10.73, $p = 0.001$.
[g] χ^2 12.93, $p < 0.001$.
[h] χ^2 7.92, $p = 0.005$.
[i] χ^2 17.62, $p < 0.001$.
[j] χ^2 12.93, $p < 0.001$.

Table 3
Demographic features in Subjective Cognitive Decline (SCD) and Mild Cognitive Impairment (MCI), classified according to Alzheimer's Pathology Status.

	SCD		MCI	
	AP- N° 18	AP+ N° 8	AP- N° 19	AP+ N° 13
Age at onset in years	53.59 (±8.95)*	62.50 (±10.99)	58.39 (±12.00)	69.43 ± (5.14)*
Age at plasma collection	62.35 (±8.82)[a,b]	73.63 (±6.05)[a]	67.58 (±9.15)	72.87 (±5.36)[b]
Family history of AD	81.25%	71.42%	66.66%	50.00%
Sex (M – F)	4–14	4–4	7–11	5–9
Years of education	12.88 (±3.44)	12.88 (±4.88)	11.83 (±4.42)	12.86 (±4.60)
MMSE	29.22 (±0.73)[c,d]	28.63 (±1.18)	27.28 (±2.32)[c]	27.08 (±2.36)[d]
APOE ε4+	35.29%	37.50%	12.50%[e]	78.57%[e]
Plasma p-tau181 (pg/ml)	1.72 (±0.63)[f,g]	2.85 ± 0.53[f]	2.04 ± 0.87[g]	4.03 ± 1.07[g,h]

Values are reported as mean and standard deviation. Statistically significantly different values between the groups are reported as underlined character. Statistically significance after Bonferroni Correction: $p = 0.006$.
* $p < 0.001$.
[a] $p = 0.003$.
[b] $p < 0.001$.
[c] $p = 0.001$.
[d] $p = 0.003$.
[e] χ^2 13.27, $p < 0.001$.
[f] $p < 0.001$.
[g] $p < 0.001$.
[h] $p < 0.001$.

tau181 into blood, in support of the amyloid cascade hypothesis [49]. Consequently, it has been hypothesized that prominent changes in plasma p-tau181 coincide with the presence of established Aβ pathology, thus indicating that the increase of plasma p-tau181 levels is highly specific for Alzheimer's pathology [29].

Little is known about correlations between plasma p-tau181 and CSF biomarkers in SCD. The strong correlations with CSF p-tau, Aβ1–42 and Aβ1–42/1–40 ratio may lead to the hypothesis that this peripheral biomarker might be predictive of Alzheimer's pathology also in SCD population. On the other hand, the lack of correlation with CSF t-tau could be explained by the fact that neurodegeneration might not be so prominent in this early phase of cognitive decline.

The absence of correlations with CSF biomarkers in AD demented patients might be quite surprising. Indeed, it has been previously stated that plasma p-tau181 can be used to indicate Tau status due to the strong correlations with CSF p-tau181, Tau PET and AD neuropathology [20]. On the other hand, other studies hypothesized that AD biomarkers might show different dynamic changes along the AD continuum, such as brain β-amyloid accumulation, which shows a sigmoid-shaped trajectory [52], and plasma neurofilament light chains (NfL), which reaches a a plateau in AD dementia [53]. Taking together this previous evidence, we might speculate that the lack of correlations observed in AD demented patients might reflect dynamic of change of AD biomarkers along the AD continuum. However, this hypothesis needs to be confirmed due to the small number of AD demented patients in our cohort.

Remarkably, when we classified patients as carriers and non-carriers of AD pathology according to the A/T(N) system, we found that plasma p-tau181 levels were significantly higher in MCI AP+ than in MCI AP-, as well as in SCD AP+ than in SCD AP-. These findings are in lines with previous works, which highlighted that plasma p-tau181 levels were higher in Aβ + than in Aβ- MCI patients but also in Aβ + cognitively unimpaired subjects than in Aβ- ones, thus further supporting the hypothesis of the biological relationship between Aβ pathology and the release of brain-specific p-tau181 into blood [20,48,49].

Consequently, we run a multiple regression analysis to detect which variables influenced plasma p-tau181 levels. Only the presence or absence of Alzheimer's pathology (assessed by the positivity of CSF biomarkers) was associated to plasma p-tau181, while neither cognitive status (i.e. SCD, MCI, AD dementia), nor age at plasma collection, nor *APOE* genotyping were significantly associated. This suggest that plasma p-tau181 levels were not influenced by the stage or the severity of cognitive decline, but exclusively by the underlying neurodegenerative processes [49].

Finally, taking together SCD and MCI patients, plasma p-tau181 showed a high accuracy in discriminating patients who were carriers of Alzheimer's pathology from non-carriers, with an AUC of 0.910. Moreover, we defined a cut-off of 2.69 pg/mL which was able to discriminate Alzheimer's pathology carriers from non-carriers with a good accuracy, sensitivity and an excellent NPV.

Previous works detected a high accuracy of plasma p-tau181 in differentiating AD demented patients from healthy controls: indeed, both Thijssen et al. and Baiardi et al. found an AUC of 0.97 [51,54]. Similarly, plasma p-tau181 also showed a high accuracy in discriminating AD dementia from FTD [51,54]. Moreover, Karikari et al. described that plasma p-tau181 was able to discriminate Aβ + from Aβ- MCI (AUC of 79.9%) and also Aβ + from Aβ- cognitively unimpaired subjects (AUC of 70.4), [49]. To the best of our knowledge, only one previous study showed a high performance of plasma p-tau181 in discriminating Aβ- healthy controls from Aβ + "objectively defined" SCD (AUC of 0.814) [31]. The higher AUC that we found in our study may be due to the fact that we did not consider Aβ positivity alone, but the combination with other biomarkers of A/T(N) system in order to define the presence of Alzheimer's pathology in our SCD and MCI patients.

A major requirement for widespread use of plasma p-tau181 is the establishment of cut-off values. Indeed, previous studies tried to define cut off-values to differentiate patients with positive from negative amyloid PET [49], or to discriminate AD dementia from other neurodegenerative diseases with high sensitivity and specificity [54]. Obviously, to move the use of plasma p-tau181 from research setting to clinical practice, there is a great need to harmonize these cut-off values across laboratories and in different populations and to clearly establish what to discriminate (i.e. amyloid positivity alone, or the combination of A/T(N) biomarkers).

Taking together, this evidence leads to the hypothesis that plasma p-tau181 levels may predict the presence of CSF Alzheimer's pathology

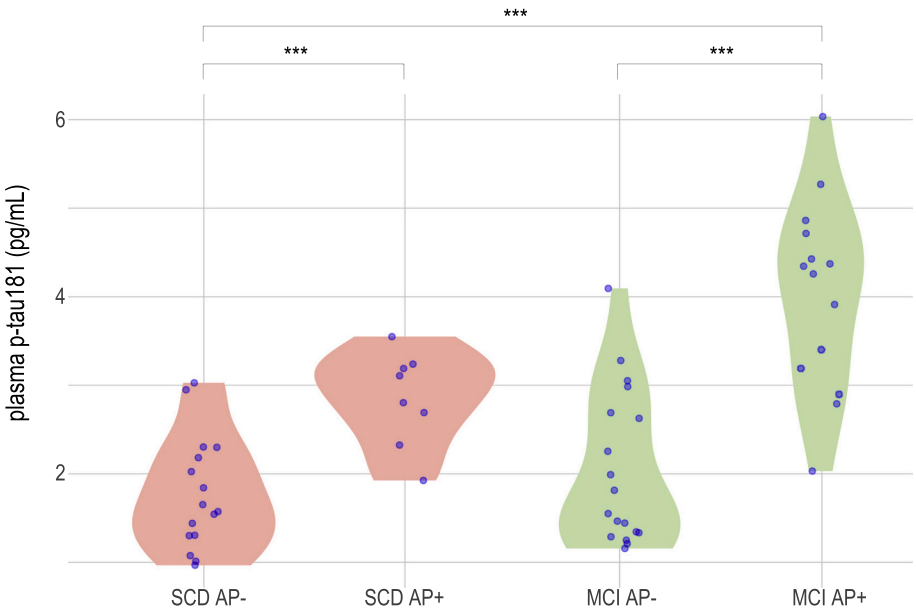

Fig. 3. Plasma p-tau181 levels in SCD and MCI patients, classified as Alzheimer's pathology carriers and non-carriers.

Table 4
Multiple regression model for plasma p-tau181.

	B	95% C.I. for B		β	*p*
		lower	upper		
(Costant)	1.373	−0.888	3.633		0.230
Diagnosis	0.415	0.051	0.778	0.224	0.026
Age at plasma collection	−0.001	−0.034	0.032	−0.007	0.944
APO ε4	−0.092	−0.617	0.433	−0.034	0.728
AP status	1.627	1.006	2.248	0.600	**<0.001**

Unstandardized Regression Coefficients (B) and 95% Confidence Intervals (95% C.I.), standardized coefficient (β) and *p*-value (*p*), are reported (significant differences at $p < 0.0125$).

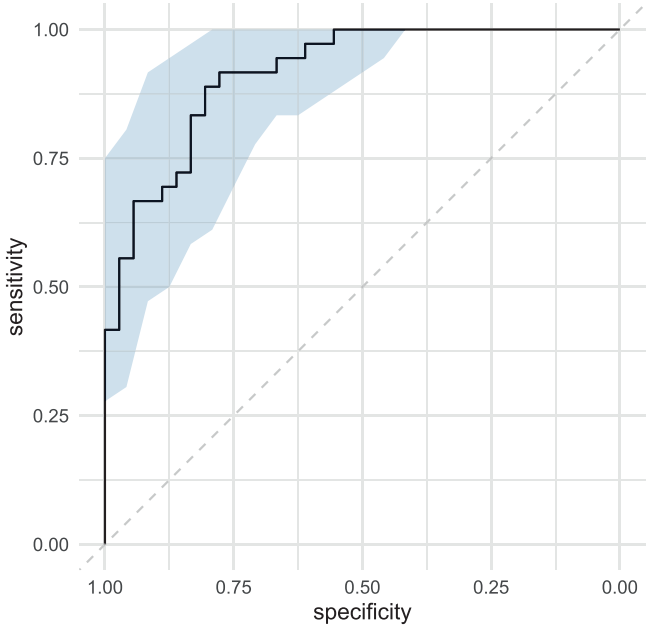

Fig. 4. ROC curves for accuracy of plasma p-tau181 in distinguishing AP+ from AP- in SCD and MCI patients.
Colored shapes indicate 95% C.I.

independently from the cognitive status. Thus, due to the good accuracy, sensitivity and the high negative predictive value, plasma p-tau181 may represent a promising non-invasive biomarker which could discriminate patients who are carriers of Alzheimer's pathology from non-carriers even in the earliest phases of the disease. Moreover, our findings support the use of plasma p-tau181 for the detection of Alzheimer's pathology in SCD population. Indeed, due to the heterogeneity of the causes which may lead to SCD, it is crucial to identify those patients in early stages of AD. The NIA-AA included SCD as a first manifestation of the symptomatic stages of AD [8], preceding MCI. Consequently, in this perspective, SCD patients may represent an optimal selected population to be screened for the early detection of AD, even before cognitive disturbs become objectively demonstrated by neuropsychological tests. Our study showed that plasma p-tau181 has a good accuracy in discriminating AD pathology carriers from non-carriers, independently from cognitive status, thus having a potential practical use in SCD patients.

Our study presents some limitations: first, the relatively small number of patients, in particular in SCD subgroup, which might reduce the power of our study. Secondly, the lack of a healthy control group: consequently, we could not verify if plasma p-tau181 levels in SCD were higher than in healthy controls. Third, the design of this study is cross-sectional: a longitudinal study should be performed in order to evaluate how plasma p-tau181 levels change over time.

On the other hand, this study has some remarkable strengths. First of all, to the best of our knowledge, this is one of the first studies that tried to explore the accuracy of plasma p-tau181 levels in well-characterized SCD defined according to consensus criteria SCD [8]. Secondly, patients were classified as carriers or non-carriers of Alzheimer's pathology considering not only A status, but also the positivity of T and/or N biomarkers, while previous studies have considered the positivity of amyloid biomarkers alone. Our approach will increase the probability that patients with mild objective or subjective cognitive decline are real carriers of Alzheimer's pathology. Indeed, despite A+/T-/N- patients are considered part of the Alzheimer's continuum, they are properly classified as carriers of "Alzheimer's pathological changes" and not Alzheimer's Disease patients [7]. Moreover, the presence of amyloid pathology alone in early stages of cognitive decline might not be specifically prognostic of conversion to dementia [55]. Third, we tried to define a cut-off of plasma p-tau181 levels which might be useful in order to discriminate carriers from non-carriers of Alzheimer's pathology.

In conclusion, our findings may have clear implication for the future use of plasma p-tau181, which might be a promising peripheral biomarker due to the good accuracy in discriminating patients carrying Alzheimer's pathology from non-carriers in early stages of the disease, such as SCD population. Considering previous evidence, plasma p-tau181 seems to directly reflects tau pathology that is intimately related to fibrillar Aβ pathology and that might be predictive of downstream aggregation of tau fibrils several years before established NFT pathology [20,56]. Consequently, as a specific marker of the typical degeneration due to AD, plasma p-tau181 might be an encouraging tool for the early detection of AD thus leading to the identification of those individuals at greatest risk of developing AD dementia, which seem to be the ideal group in which to intervene with a specific treatment in order to stop neurodegeneration.

Data accessibility

The data that support the findings of this study are available from the corresponding author upon reasonable request.

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CRediT authorship contribution statement

Giulia Giacomucci: Conceptualization, Data curation, Formal analysis, Investigation, Methodology, Supervision, Validation, Writing – original draft, Writing – review & editing. **Salvatore Mazzeo:** Conceptualization, Data curation, Formal analysis, Investigation, Methodology, Software, Writing – review & editing. **Chiara Crucitti:** Writing – original draft, Data curation, Validation, Visualization. **Assunta Ingannato:** Data curation, Validation, Visualization. **Silvia Bagnoli:** Data curation, Validation, Visualization. **Sonia Padiglioni:** Data curation, Validation, Visualization. **Giulia Galdo:** Data curation, Validation, Visualization. **Filippo Emiliani:** Data curation, Validation, Visualization. **Daniele Frigerio:** Data curation, Validation, Visualization. **Valentina Moschini:** Data curation, Validation, Visualization. **Carmen Morinelli:** Data curation, Validation, Visualization. **Sandro Sorbi:** Supervision, Validation, Visualization. **Valentina Bessi:** Project administration, Resources, Supervision, Validation, Visualization, Writing – review & editing. **Benedetta Nacmias:** Funding acquisition, Resources, Supervision, Validation, Visualization.

Declaration of Competing Interest

The authors have nothing to disclose.

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