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Original Article

Plasma and neurostructural biomarkers in the clinical-biological characterization of early stages of the Alzheimer's disease continuum: findings from the Compostela Aging Study

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ABSTRACT

Recent technical advances in peripheral blood analysis have enabled precise quantification of Alzheimer's Disease (AD) biomarkers in the early stages of the AD continuum, in an economical, non-invasive and safe manner. The main objective of this study was to contribute to the clinical-biological characterization of the initial stages of cognitive impairment by measurement of blood and neurostructural AD biomarkers in groups of participants classified according to their cognitive clinical phenotype.

Plasma concentrations of p-tau217, p-tau181, total tau, neurofilament light chain and amyloid-β 42/40 ratio biomarkers were measured along with APOE gene variants, hippocampal volume and cortical thickness of the AD signature regions. The cohort of 329 participants included Cognitively Unimpaired (CU), Subjective Cognitive Decline (SCD), single-domain amnestic Mild Cognitive Impairment (sd-aMCI), multidomain aMCI (md-aMCI), and single-domain non-amnestic MCI (sd-naMCI) groups.

P-tau217 concentrations were significantly higher in the md-aMCI and sd-aMCI groups than in the CU, SCD and sd-naMCI groups. P-tau181 concentrations were significantly higher in md-aMCI group than in CU, SCD and sd-naMCI groups. Hippocampal volume and AD signature cortical thickness were significantly lower in the md-aMCI group than in the CU, SCD and sd-naMCI groups. No across group differences were found in the distribution of carriers/non-carriers of APOE-ε4. Mediation analysis revealed that hippocampal volume and AD signature cortical thickness mediated the relationship between p-tau217 and p-tau181 levels and cognitive performance.

Sd-aMCI and md-aMCI represent two distinct and sequential clinical-biological stages of the AD continuum. Conversely, sd-naMCI does not appear to be associated with AD pathology. Finally, the SCD group does not seem to display a higher risk of progression along the AD continuum than the CU group.

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1. Introduction

Alzheimer's disease (AD) is a progressive neurodegenerative disorder that is histopathologically characterized by the deposition of extracellular amyloid-β (Aβ) plaques and intraneuronal neurofibrillary tangles composed of hyperphosphorylated tau (p-tau). It is clinically diagnosed by progressive cognitive impairment, functional deficits, and behavioural changes [1]. The World Health Organization has reported that AD is the most common cause of dementia in older individuals worldwide, with an increasing prevalence leading to significant public health and social burdens.

Although the precise etiopathogenic mechanisms of AD are not completely understood [2], neuropathological events in AD begin before the clinical stage, with the p-tau pathology extending from the brainstem into the transentorhinal and entorhinal cortices; to the hippocampus, amygdala, and perirhinal cortices; and to neocortical areas (Braak stages I-II; III; IV-VI, respectively) [3]. P-tau spreading is closely correlated with neurodegeneration patterns and the manifestations of clinical symptoms of AD [4]. Pathology due to p-tau usually precedes Aβ plaques [5] and is accompanied by neuroinflammation, synaptic dysfunction, mitochondrial and bioenergetic disturbances and/or vascular abnormalities, which can ultimately lead to neuronal death [2, 6]

In the AD continuum, the cognitive status typically ranges from a preclinical stage with Subjective Cognitive Decline (SCD) to Mild Cognitive Impairment (MCI) and dementia. SCD is a heterogeneous category that includes participants with cognitive complaints in the absence of objective cognitive decline [7]. MCI is a complex construct with different clinical phenotypes depending on cognitive status [8,9]: either with or without memory impairment (amnestic vs. non-amnestic), and with impairment in one or several cognitive domains (single vs. multi-domain). The risk of developing AD dementia has been found to differ in the various MCI subgroups, being higher in amnestic MCI (aMCI) than in non-amnestic MCI (naMCI) [9–11] and also higher in multidomain (md-) than in single domain (sd-) [12,13]. The different MCI subgroups also present specific neurostructural [14,15] and neurofunctional characteristics [16,17]. Therefore, they may also exhibit different characteristics in biomarkers sensitive to the molecular neuropathology of the AD continuum.

SCD or MCI does not necessarily progress to dementia. Progression along the cognitive impairment spectrum depends on several factors including age, sex, lifestyle, and apolipoprotein E (ApoE) status, and occurs around six to ten years after the onset of subjective symptoms, although timeframe varies across different studies [18]. Therefore, it is crucial to focus on the early AD stages, when early intervention, treatment and management of modifiable risk factors could potentially lower the risk of onset or delay disease progression.

Recent advance in analytical techniques have enabled precise quantification of AD biomarkers in blood samples, making them promising scalable tools for non-invasive and cost-effective screening, diagnosis, prognosis, and monitoring of disease progression [19,20]. Thus, p-tau217, p-tau181, Aβ40, and Aβ42 are considered sensitive, specific AD plasma biomarkers, with p-tau217 performing best for detecting the presence of brain amyloid and tau pathology in early stages of the AD continuum [19,21–23]. These advances have contributed to revision of the criteria for diagnosis and staging AD [24,25].

Currently available plasma analytes provide valuable information for the AD biological diagnosis of SCD and MCI participants [26–28], although the biological diagnosis of AD determined by neuroimaging and fluid biomarkers is not always accompanied by the same degree of cognitive decline [25]. Thus, a multimodal approach integrating the information provided by plasma AD biomarkers with volumetric and morphometric data provided by structural MRI [29], and with information on cognitive functions would be more appropriate for earlier, more reliable and cost-effective clinical-biological characterization in SCD and MCI stages of the AD continuum. This integrative research strategy would reveal new insights that can be relevant for improving early detection and differential diagnosis, ultimately contributing to more personalized and timely interventions along the AD continuum.

The main objective of the present study was to determine the value of including AD blood biomarkers, neurostructural MRI indexes and cognitive status in the clinical-biological characterization of early stages of the AD continuum. To this end, we examined the relationships between plasma AD biomarkers, structural MRI indexes of AD neurodegeneration, APOE status, and cognitive functioning in a cohort of the Compostela Aging Study (CompAS), classified into five groups: cognitively unimpaired (CU), SCD, sd-aMCI, md-aMCI and sd-naMCI. We also sought to determine the potential mediating effect of the structural integrity of brain areas sensitive to AD neurodegeneration on the relationship between AD blood biomarkers and cognitive status.

2. Materials and methods

2.1. Participants and clinical evaluation

The study participants were 329 adults over fifty years old, recruited to participate in the CompAS by their general practitioners in Health Centers in Santiago de Compostela (Galicia, northwestern Spain) (see Table 1). CompAS [30] is an ongoing longitudinal project composed by sequential cohorts, with three evaluations per cohort, separated by 24-36 months, with the general objective being the early detection and progression of cognitive impairment

The present cross-sectional study involves a cohort whose recruitment, neuropsychological screening, blood sampling and MRI recording took place between November 2021 and October 2023. The study was approved by the Galician Clinical Research Ethics Committee (Xunta de Galicia, Spain, Code: 2022/116) and complied with the ethical standards established in the 1964 Declaration of Helsinki [31]. Participants provided written informed consent prior to taking part in the study.

None of the participants reported prior diagnosis of (1) psychiatric conditions (except those related to mood or anxiety disorders), according to the Diagnostic and Statistical Manual of Mental Disorders criteria, Fifth Edition of the American Psychiatric Association (DSM-5) [32] or (2) neurological disease, including probable Alzheimer's Disease (AD) or other types of dementia (or mayor neurocognitive disorder), based on the National Institute of Neurological and Communicative Disorders and Stroke and the Alzheimer's Disease and Related Disorders Association (NINCDS-ADRDA) [33] and DMS-5 criteria. In addition, participants did not report any prior brain injury, brain surgery or chemotherapy, uncontrolled type II diabetes or substance use or abuse. Participants' sensory and motor difficulties were grounds for exclusion only when, even when corrected, they could interfere with the interpretation of neuropsychological instruments.

The participants completed clinical, neurological and neuropsychological examinations, which were conducted respectively by general practitioners, neurologists, and neuropsychologists specialized in aging and dementia. The Spanish version [34] of the Mini Mental State Examination (MMSE) [35] was administered to all participants to assess their general cognitive functioning.

The presence/absence of impairment in several cognitive domains was evaluated by the Spanish version [36,37] of the Cambridge Cognitive Assessment-Revised (CAMCOG-R) subscales and other scales. The following items were assessed: (a) attention, with Trail Making Test A [38] and the Attention and Calculation CAMCOG-R subscale; (b) executive functioning, with the Trail Making Test B [38], Phonological verbal fluency [39] (say words starting with "p" in one minute) and the Executive Function CAMCOG-R subscale; (c) memory, with the Spanish version [40] of the California Verbal Learning Test (CVLT), which measures A List Total Recall and Long-Delay Free Recall and the Memory CAMCOG-R subscale and (d) language, with the Spanish version of the Boston Naming Test (BNT) [41], Semantic Verbal Fluency (animals) [39] and the Language CAMCOG-R subscale. The Lawton and Brody

Index (maximum possible scoring = 8) was used to evaluate Instrumental Activities of Daily Living (IADL) [42].

The Spanish validation [43] of short form of the Geriatric Depression Scale (GDS-15) was used to evaluate depressive symptomatology. The Spanishś Version [44] of 12-Item General Health Questionnaire (GHQ-12) was used to assess psychological welfare and detect nonpsychotic psychiatric problems. The Generalized Anxiety Disorder (GAD-7) scale was used to assess generalized anxiety (Spanishś Version [45]), and the Mild Behavioral Impairment Checklist (MBI-C) was used to measure neuropsychiatric symptoms (Spanish version [46]).

MCI was diagnosed in accordance with Petersen's criteria [8,9]: a) evidence of concern corroborated by an informant about a change in cognition, relative to the previous level; b) evidence of poorer performance in one or more cognitive domains that is greater than expected for the patient's age and educational background; c) preservation of independence in functional abilities; and d) non-fulfilment of diagnostic criteria for dementia considering the NINCDS-ADRDA and DSM-5 criteria. For criterion b), we considered evidence of impaired domain when poorer performance was detected in two different tests for that domain [47], scoring 1–2 standard deviation range (between the 3rd and 16th percentiles) below norms by age and education [32]. Participants were therefore diagnosed with sd-aMCI when the impairment only affected episodic memory, with md-aMCI when other domains (in addition to memory) were affected, and with sd-naMCI when only one

Table 1
Means, standard deviations (SD) and group differences for demographic, cognitive, emotional, genetic, plasmatic, and neurostructural data; and distribution of participants in each group with the dual cut point strategy for p-tau217 concentration.

	Overall	CU	SCD	sd-aMCI	md-aMCI	sd-naMCI	Group differences
n	329	98	122	39	46	24	
Age, years	65.38	62.70	64.91	67.54	70.65	65.08	(1) md-aMCI>CU***, SCD**;
	(8.82)	(8.12)	(8.13)	(8.79)	(9.17)	(9.86)	sd-aMCI>CU*
Sex, women	239	75	93	19	28	24	(2) ***
n, %	72.6 %	76.5 %	76.2 %	48.7 %	60.9 %	100 %	
Education,	12.91	13.95	13.52	11.54	10.72	11.92	(1) md-aMCI<CU*, SCD*
years	(5.56)	(5.45)	(5.6)	(4.45)	(5.63)	(5.93)	
APOE, n	325	98	121	38	44	24	(2) p=.18
No-ε4, n (%)	256 (78.8 %)	78 (79.6 %)	98 (81 %)	28(73.3 %)	30 (68.2 %)	22(91.7 %)	
ε4-Heteroz.	66 (20.3 %)	20 (20.4 %)	22 (18.2 %)	10 (26.3 %)	12 (27.3 %)	2 (8.3 %)	
ε4-Homoz.	3 (0.9 %)	0	1 (0.8 %)	0	2 (4.5 %)	0	
MMSE	27.97	28.65	28.50	27.50	25.59	27.83	(1) md-aMCI<CU***, SCD***,
	(2.15)	(1.54)	(1.50)	(1.96)	(3.04)	(2.12)	sd-aMCI***, sd-naMCI***
CAMCOG-R (total score)	91.00	95.98	93.83	88.58	77.13	86.61	(1) md-aMCI<CU***, SCD***,
	(10.13)	(5.84)	(7.29)	(7.02)	(12.53)	(9.29)	sd-aMCI***, sd-naMCI***;
							sd-naMCI<CU***, SCD***;
							sd-aMCI<CU***, SCD*
GHQ-12	9.72	8.16	10.66	9.74	10.50	9.78	(1) md-aMCI>CU*;
	(4.77)	(4.32)	(4.48)	(4.45)	(5.58)	(5.55)	SCD>CU**
GDS-15	3.35	2.23	4.03	3.61	3.59	3.61	(1) md-aMCI>CU*;
	(3.10)	(2.63)	(3.10)	(3.47)	(2.70)	(3.92)	sd-aMCI>CU*;
							SCD>CU***
GAD-7	4.56	3.20	5.55	4.26	4.74	5.26	(1) SCD>CU**
	(5.08)	(4.19)	(5.77)	(4.65)	(5.04)	(4.41)	
MBI-C	4.44	2.66	4.17	4.37	9.39	3.61	(1) md-aMCI>CU**, SCD*,
	(7.82)	(5.78)	(6.64)	(6.13)	(12.05)	(9.43)	sd-aMCI*
Plasma Biomarkers (pg/mL)							
p-tau217	.35	.26	.29	.48	.66	.24	(1) md-aMCI>CU**, SCD**,
	(0.31)	(0.18)	(0.16)	(0.41)	(0.52)	(0.12)	sd-naMCI***;
							sd-aMCI>CU*, SCD*, sd-naMCI**
p-tau181	21.66	18.74	20.69	23.95	29.54	19.46	(1) md-aMCI>CU*, SCD*, sd-naMCI*
	(13.59)	(10.41)	(13.02)	(13.79)	(18.4)	(11.01)	
Total-tau	3.41	3.34	3.42	3.29	3.49	3.61	(1) p=.98
	(2.00)	(1.86)	(1.99)	(1.61)	(1.94)	(3.20)	
NfL	13.35	11.51	13.14	13.42	16.59	15.81	(1) p=.24
	(8.73)	(6.82)	(7.89)	(6.84)	(10.96)	(14.51)	
Aβ 42/40	.060	.060	.062	.062	.055	.060	(1) p=.50
	(0.023)	(0.019)	(0.025)	(0.027)	(0.021)	(0.019)	
MRI: n	247	75	97	31	36	8	
Hippocampus volume mm^3	3580.26	3707.99	3619.09	3560.73	3231.49	3557.03	(1) md-aMCI<CU**, sd-naMCI*, SCD *
	(476.07)	(375.98)	(383.21)	(542.81)	(660.41)	(435.35)	
AD Signature	2.54	2.57	2.56	2.51	2.45	2.54	1) md-aMCI<CU**, sd-naMCI*, SCD***
Thickness mm	(0.12)	(0.10)	(0.11)	(0.13)	(0.14)	(0.08)	
p-tau217 (pg/mL). Dual cut point strategy [19](3)							
N: < 0.4	76.2 %	88.8 %	81.8 %	59.0 %	41.3 %	91,7 %	(2) ***
I:0.4–0.63	11.6 %	6.1 %	13.2 %	15.4 %	19.6 %	4,2 %	
P:> 0.63	12.2 %	5.1 %	5.0 %	25.6 %	39.1 %	4.2 %	

* $p<.05$;
** $p<.01$;
*** $p<.001$
(1) ANCOVA;
(2) Chi-square;
(3) cut-off [19]: neuropathology AD negative (N), neuropathology AD intermediate, confirmation is necessary (I), neuropathology AD positive (P). CU: cognitively unimpaired. SCD: subjective cognitive decline. sd-naMCI: single domain non amnestic mild cognitive impairment. sd-aMCI: single domain amnestic mild cognitive impairment. md-aMCI: multidomain amnestic mild cognitive impairment. MMSE: mini mental state examination. CAMCOG-R: Cambridge cognitive assessment-revised. CHQ-12:12-Item General Health Questionnaire.GDS-15: short form of the geriatric depression scale. GAD-7: generalized anxiety disorder scale. MBI-C: mild behavioural impairment checklist. ε4-Heteroz: APOE-ε4 heterozygotic; ε4-Homoz: APOE-ε4 homozygous.

domain other than memory was affected.

Diagnosis of SCD was made following the two main criteria proposed by the SCD-initiative (SCD-I) working group [48]: 1) self-experienced persistent decline in cognitive capacity, especially in memory, relative to a previously normal cognitive status, which is unrelated to an acute event; and 2) normal cognitive performance in standardized tests adjusted for age and education. Considering the presence of cognitive complaints in normative aging [49], compliance with the first criterion was verified in two steps: a) the response to a question that inquired about the persistence of cognitive complaints that were a source of concern; and b) by assessing the severity of the complaints using a brief version of the Questionnaire d'autoévaluation de la Mémoire (QAM) [50]. Participants were identified as SCD if they answered affirmatively to the question and also obtained a QAM score above the age- and education-adjusted 5th percentile, a cut-off that has been shown to be valid for assessing the severity of subjective cognitive complaints and predicting progression from SCD to MCI and dementia [51].

Furthermore, participants identified as SCD could not meet the objective cognitive impairment criterion established for mild cognitive impairment (MCI).

CU participants were identified by elimination when, in addition to being free of objective cognitive impairment, they did not meet the first criterion regarding cognitive complaints established for SCD.

In the present study, the MMSE and CAMCOG-R total score data are shown because they are related to part of the current objectives. The scores for the GDS-15, GHQ-12, GAD-7 and MBI-C scales are also shown, because these facilitate composition of the emotional state of the participants in the diagnostic groups (Table 1). The other scales and questionnaires were used to classify the participants into the five groups and are not related to the study objectives.

2.2. Blood sample collection and processing

For each participant, two peripheral blood samples were collected in two independent ethylene diamine tetraacetic acid (EDTA) tubes (BD -Becton, Dickinson- Vacutainer® K2EDTA 10 ML with BD Hemogard™ closure. -REF BD367525-). Peripheral blood samples contained in one of these tubes were centrifuged at 1,300 g for 10 min at 4°C in a tabletop centrifuge to obtain the plasma sample and were used for determination of protein biomarkers. Blood collection and plasma sampling were immediately aliquoted, and stored at –80°C according to standardized biobank protocols. Genomic DNA was obtained from the second tube of peripheral blood and isolated using the Chemagic DNA Blood 100 kit (PerkinElmer Chemagen Technologies GmbH), following the manufacturer's recommendations.

2.3. SNP array genotyping and haplotype of APOE

DNA samples were genotyped with the Axiom Spain Biobank Array v2 (Thermo Fisher Scientific) following the manufacturer's instructions in the Santiago de Compostela Node of the National Genotyping Center (CeGen-FPGMX). This array includes probes for genotyping a total of 756,834 single nucleotide polymorphisms (SNPs) across the genome. Clustering and genotype calling were performed using Axiom Analysis Suite v5.3.0.45 software. The haplotype of the APOE locus region was inferred from SNPs rs429358 and rs7412 [52]. Genotypes associated with these SNPs were extracted in a procedure applied using PLINK 1.9 [53].

2.4. Measurement of plasma biomarkers

Plasma biomarker measurements were conducted using the ultrasensitive Single Molecule Array (Simoa) technology, in the Simoa SR-X platform (Quanterix Corp, Billerica, Massachusetts, USA). Serum Neurofilament light chain (NfL) levels were quantified with the Simoa NF-light Advantage Kit (Cat. No: 104364). The concentrations of total-tau, Aβ40, and Aβ42 were measured using the Simoa Human Neurology 3-Plex A Advantage Kit (Cat. No: 101995). Plasma levels of p-tau 217 and p-tau 181 were determined using the ALZpath Simoa® p-tau 217 v2 Assay (Cat. No: 104371) and the ptau-181 Advantage V2.1 Kit (Cat. No: 104111), respectively.

All samples were processed and analyzed following the manufacturer's instructions, using the same batch of reagents to ensure consistency. A four-parameter logistic curve-fitting method was applied to generate the calibration curve. For quality control, two control samples with known analyte concentrations (high-control and low-control) were included. The operators performing the analyses were blinded to the participants' disease status and clinical information.

2.5. MRI processing

For structural MRI analysis, a sagittal T1-weighted 3D-MPRAGE sequence (repetition time/echo time = 7.45 ms/3.40 ms, flip angle = 8°; 180 slices, voxel size = 1 × 1 × 1 mm, field of view = 240 × 240 mm^2, matrix size = 240 × 240 mm) was obtained, in 247 participants, using a Philips 3T Achieva scanner (Philips Medical Systems, Best, The Netherlands) at the University Hospital Complex, Santiago de Compostela, Galicia, Spain. To mitigate scanner noise, participants wore foam earplugs, while head motion was minimized through a head restraint system incorporating foam padding around the subject's head.

Structural MRI data were pre-processed using the default pipeline for automated cortical reconstruction implemented in FreeSurfer (v6.0.0) (https://surfer.nmr.mgh.harvard.edu/). Briefly, this protocol includes motion correction, skull stripping, transformation to Talairach space, segmentation of cortical and subcortical grey matter (GM) and white matter (WM) structures, intensity normalization, tessellation of the GM-WM boundary, and topology correction [54,55]. A quality control protocol was implemented using the Freeview program to verify the FreeSurfer segmentations. A trained technician visually inspected the segmentations slice by slice to improve the accuracy of cortical thickness measurements. Misplacement errors of the pial surface, including those affecting the meninges and skull, were manually corrected in all subjects. Final segmentations were reviewed by a senior researcher.

Hippocampus volume and AD signature cortical thickness were obtained as measurements of neurostructural integrity. Estimates of the hippocampus volume were based on an atlas including probabilistic information on the location of the structures [56]. These volumetric measurements were adjusted for total intracranial volume (eTIV) using the following formula: adjusted volume = observed volume − b×(eTIV – mean-eTIV), where mean-eTIV is the average eTIV of all subjects and b is the regression coefficient between the observed volume and eTIV. To obtain a general marker of hippocampal volume, the mean hippocampal volume was estimated by averaging the adjusted volumetric hippocampal estimates of left and right hemispheres. Moreover, cortical thickness measurements were extracted on the basis of the Desikan-Killiany cortical parcellation atlas [57]. The AD signature index was computed by averaging thickness measurements of the following brain regions: parahippocampal gyrus, entorhinal cortex, inferior temporal gyrus, middle temporal gyrus, inferior parietal lobe, fusiform gyrus, and praecuneus [58].

2.6. Statistical analysis

Chi-squared tests were used to evaluate the distribution of males/females and of carriers (homozygous and heterozygous) / non-carriers of the APOE-ε4 variant, as well as the number of participants with positive, negative or intermediate plasma p-tau217 concentrations, used in the AD pathology classification established by Ashton et al. (2024). Fisher's exact test was used to assess the significance of any differences between pairs of groups in this classification.

Analysis of covariance (ANCOVA) was conducted to assess the effect of group (CU, SCD, sd-aMCI, sd-naMCI, md-aMCI) on cognitive

performance (CAMCOG-R total score and MMSE), GHQ-12, GDS-15, GAD-7 and MBI-C scales, plasma biomarkers (total-tau, p-tau217, p-tau181, NfL, Aβ 42/40 ratio) and neurostructural MRI measures (hippocampal volume and AD signature). The Bonferroni method was applied to post-hoc pair-wise multiple comparisons, and effect sizes were calculated from the partial Eta squared value.

Additionally, multivariate linear regression analysis was performed to examine the effects of plasma biomarkers on cognitive and neurostructural measures, as well as of the neurostructural indixes on cognitive performance. Tolerance and VIF indices were obtained to control for multicollinearity. No significant multicollinearity effects were observed in the analyses performed.

Age, sex and years of education were included as covariates in ANCOVAS and regression analysis, and the Bootstrap method was used for neurostructural and plasma measures.

Mediation analysis was conducted to examine whether the neurostructural index mediates the relationship between the plasma AD biomarker and cognitive performance.

The mediation model included age, years of education and sex as covariates to control for potential confounding effects. Mediation analysis was applied to plasma variables for which significant group effects were found. First, an ordinary least squares (OLS) regression was used to estimate the effect of the plasma AD biomarker on the neurostructural index (path a). A second OLS regression was then conducted to assess the direct effect of the plasma biomarker on cognitive performance (adjusted for neurostructural index, path c') and to assess effects of hippocampal volume or AD signature on cognition (adjusted for plasma biomarker, path b). The indirect effect, or mediation effect of neurostructural index, was estimated using the product of coefficients approach (path a*b), and the significance was assessed by nonparametric bootstrapping with 5000 resamplings to generate bias-corrected confidence intervals. The proportion of mediation was calculated as the ratio of the indirect effect to the total effect. The significance of mediation was determined on the basis of whether or not the bootstrapped confidence intervals excluded zero.

ANCOVA and linear regression analyses were conducted with SPSS, and mediation analysis was performed using the mediation package in R (version 4.4.2), following the causal mediation framework [59]. Statistical significance was set at $\alpha = 0.05$

3. Results

Sex, APOE-ε4 and mean age (and SD), education years, MMSE, CAMCOG-R-Total, GAD-7, GHQ-12, MBI-C and GDS-15 Yesavage scales, hippocampal volume (mm^3), AD signature cortical thickness (mm) and plasma biomarkers concentrations (pg/mL) from the five groups of participants and the significant differences between groups are shown in Table 1. For each diagnostic group, the percentage of participants exceeding the cut-off points for p-tau271 concentrations [19] are also showed.

3.1. Group differences on demographic variables, APOE-ε4, global cognitive functioning and emotional state

A significant main effect of Group factor was obtained for sex ($\chi^2(4)$= 25.02; p<.001), age (F(4,324)=7.6; p<.001; ηp^2=0.09), years of education (F (4,324)=3.94; p=.004; ηp^2=0.05), MMSE (F(4, 318)=16.65; p<.001; ηp^2=0.17), CAMCOG-R-Total (F(4,318)=56.43; p<.001; ηp^2=0.42), GAD-7 (F(4,318)=3.82; p=.005; ηp^2=0.046), GHQ-12 (F (4,318)=4.29; p=.002; ηp^2=0.05), MBI-C (F(4,318)=3.98; p=.004; ηp^2=0.05), and GDS-15 (F(4,318)=5.84; p<.001; ηp^2=0.07).

In the whole sample and in each group, the percentage of women was higher than that of men, except in group sd-aMCI, in which the proportions were balanced. Age differences between groups showed that md-aMCI group was significantly older than the CU (p<.001) and SCD (p=.001) groups, and sd-aMCI significantly older than CU (p<.03). In addition, the CU and SCD groups had significantly more years of education than md-aMCI (p=.01 and p=.03, respectively) (see Table 1).

Cognitive performance assessed by MMSE and CAMCOG-R-Total was significantly lower in the md-aMCI group than in all other groups (p<.001 for all between group comparisons). Furthermore, for the CAMCOG-R-Total, the sd-naMCI group obtained significantly lower scores than CU and SCD groups (p<.001 in both comparisons), as well as in the sd-aMCI group, who scored significantly lower than the CU and SCD participants (p<.001and p<.02, respectively).

In GHQ-12, GAD-7 and GDS-15, the SCD group scored significantly higher than the CU group (p=.001, p=.002, p<.001, respectively); moreover, in GHQ-12 and GDS-15 scales, the md-aMCI group also scored significantly higher than the CU group (p=.04, p=.025, respectively); in the GDS-15 scale, the sd-aMCI group scored significantly higher than CU group (p=.037). Finally, in the MBI-C scale, md-aMCI group scored significantly higher than CU (p=.001), SCD (p=.02) and sd-aMCI (p=.032) groups.

The distribution of APOE-ε4 carriers across the diagnostic groups was not significantly different (chi-square test).

3.2. Group differences in plasma and in neurostructural biomarkers

A significant main effect of group on p-tau217 plasma biomarker (F (4, 320), =13.25, p<.001, ηp^2=0.14) was observed. Specifically, the p-tau217 levels were significantly higher in the md-aMCI than in the CU (p=.002), SCD (p=.002) and sd-naMCI groups (p<.001), and they were higher in the sd-aMCI than in sd-naMCI(p=.008), SCD (p=.047) and CU (p <.039) groups (see Fig. 1).

Statistically significant differences among the diagnostic groups ($\chi^2(8) = 62.77$, $p < .001$) were detected in relation to the proportion of participants classified as positive (>0.63 pg/mL), intermediate (0.40–0.63 pg/mL, requiring further confirmation) or negative (<0.40 pg/mL) according to the dual cut-off point strategy of AD pathology derived from plasma p-tau217 levels [19]. The distribution of levels was significantly different in the CU and SCD groups than in the single and multiple aMCI groups ($p \leq .01$ in all pairs of comparisons): the proportion of participants classified with negative AD pathology was higher (and that of participants classified with positive and intermediate AD pathology was lower) in the CU and SCD groups than in the md-aMCI and sd-aMCI groups. Furthermore, the proportion of participants classified with positive and intermediate AD pathology was higher in the md-aMCI group than in the sd-naMCI group ($p = .001$) (see Fig. 2).

A significant group effect was detected for the concentrations of p-tau181 biomarker (F(4,319)=2.51, p=.042, ηp^2=0.03), which were significantly higher in the md-aMCI group than in the CU (p=.012), SCD (p=.034) and sd-naMCI (p=.034) groups (see Fig. 1).

No other group effects were obtained for the other plasma biomarkers

For the neurostructural indexes, a statistically significant group effect was detected for hippocampal volume (F(4,239)=3.73; p=.01; ηp^2=0.05), as the volume was lower in the md-aMCI group than in the sd-naMCI (p=.03), SCD (p=.018) and CU (p=.005) groups; and for AD signature (F(4,239)=4.15; p=.003; ηp^2=0.065), with the thickness being lower in the md-aMCI group than in the sd-naMCI (p=.02), SCD (p<.001) and CU (p=.003) groups.

3.3. Relationships between plasma biomarkers and cognitive functioning and neurostructural indexes

Multivariate linear regression analyses of plasma biomarkers values on cognitive functioning showed that only p-tau217 had a significant effect on MMSE (β= -0.199; 95 % CI: -2.96 to -0.381; p=.042) and CAMCOG-R-Total (β= -0.228; 95 % CI: -13.805 to -3.165; p<.003), as higher levels of p-tau217 were associated with lower cognition.

Furthermore, p-tau217 had a significant effect on AD signature cortical thickness (β= -0.295; 95 % CI: -0.166 to -0.065; p=.002), and on

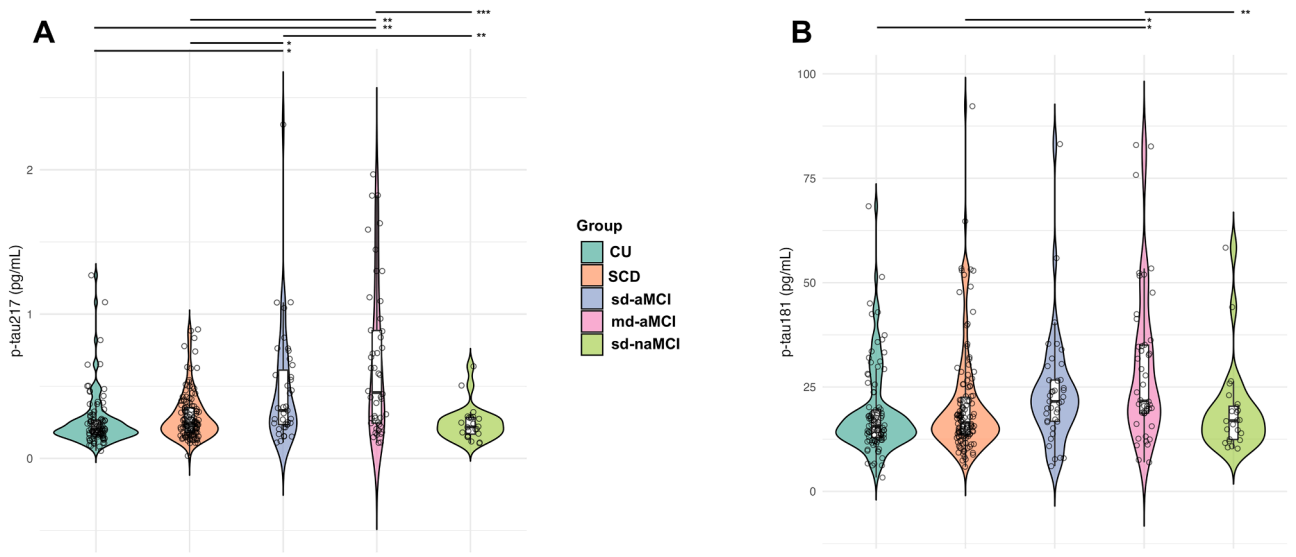

Fig. 1. Comparison of plasma p-tau217 (A) and p-tau181 (B) concentrations among the different phenotypes (diagnostic groups).
CU: Cognitively Unimpaired. SCD: Subjective Cognitive Decline. sd-aMCI: single domain amnestic Mild Cognitive Impairment. md-aMCI: multidomain aMCI. sd-naMCI: single domain non-aMCI. The black horizontal lines represent statistically significant comparisons obtained from the p-values of the pairwise contrasts. *p<.05; **p<.01; ***p<.001.

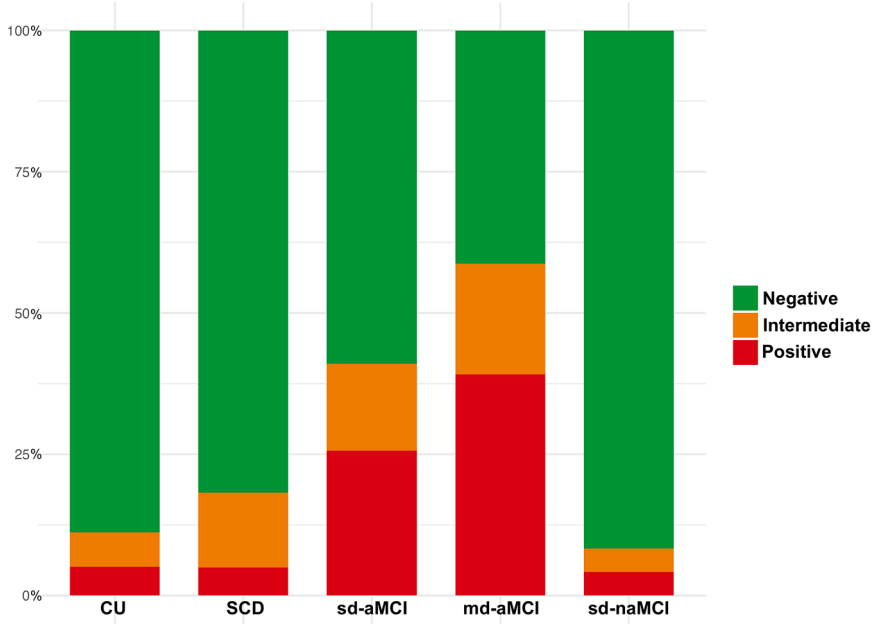

Fig. 2. Proportion of participants in each diagnostic group with Positive (>0.63 pg/mL, Red), Intermediate (0.40–0.63 pg/mL, Orange) and Negative (<0.40 pg/mL, Green) levels of plasma p-tau217 concentrations, classified according to the dual cut point strategy [19]. CU: Cognitively Unimpaired. SCD: Subjective Cognitive Decline. sd-aMCI: single domain amnestic Mild Cognitive Impairment. md-aMCI: multidomain aMCI. sd-naMCI: single domain non-aMCI.

hippocampal volume (β= -0.254; 95 % CI: -734.05 to -127.21; p=.011); thus, higher concentrations of p-tau217 were associated with lower neurostructural integrity.

No other relationships were detected.

3.4. *Associations between neurostructural indices and cognitive functioning*

AD signature cortical thickness was associated a significant impact on CAMCOG-R-Total (β= 0.157; 95 % CI: 3.16 to 22.49; p=.014), and hippocampal volume was significantly related to scores in the CAMCOG-R-Total (β=0.177; 95 % CI: 0.001 to 0.006; p<.001) and MMSE (β= 0.251; 95 % CI: 0.000 to 0.002; p<.001). Higher levels of neurostructural integrity were associated with higher cognition scores.

3.5. *Mediation analyses of neurostructural indices on the relationship between p-tau217 and p-tau181 and cognitive performance*

The mediation analyses revealed significantly mediated proportions and significant direct (path c′) and indirect (path a*b), total (direct + indirect) effects of p-tau217 and p-tau181 on cognitive outcomes (MMSE and CAMCOG-R-Total) via hippocampal volume and AD signature cortical thickness as mediators (Table 2, Fig. 3). The significant indirect effects indicated that the hippocampus and AD signature

Table 2
Mediation analysis of neurostructural indexes (hippocampal volume, AD signature cortical thickness) on the relationship between p-tau217 and p-tau181 and cognitive performance (CAMCOG-R, MMSE). Unstandardised regression coefficients, along with lower and upper 95 % confidence intervals and p-values are presented for Indirect, Direct and Total effects and mediated proportions. Direct effect= path c′. Indirect effect= path a*b. Total effect: direct + indirect effects.

EffecT	Coefficient	CI Lower	CI Upper	P value
Mediator: Hippocampal volume				
P-tau217->Camcog-R-TOTAL				
Indirect Effect	-1.27	-2.43	-0.40	0.004
Direct Effect	-6.01	-10.91	-2.94	<0.001
Total Effect	-7.28	-12.34	-3.78	<0.001
Mediated Proportion	0.17	0.05	0.32	0.004
P-tau217->MMSE				
Indirect Effect	-0.37	-0.72	-0.12	0.001
Direct Effect	-0.98	-2.38	-0.09	0.030
Total Effect	-1.35	-2.83	-0.37	0.002
Mediated Proportion	0.27	0.09	0.77	0.004
P-tau181->Camcog-R-TOTAL				
Indirect Effect	-0.02	-0.05	-0.01	0.004
Direct Effect	-0.11	-0.18	-0.04	<0.001
Total Effect	-0.13	-0.21	-0.06	<0.001
Mediated Proportion	0.19	0.05	0.41	0.004
P-tau181->MMSE				
Indirect Effect	-0.01	-0.01	0.00	0.004
Direct Effect	-0.03	-0.05	-0.01	0.003
Total Effect	-0.03	-0.06	-0.01	<0.001
Mediated Proportion	0.19	-0.06	0.49	0.005
Mediator: AD Signature				
P-tau217->Camcog-R-TOTAL				
Indirect Effect	-1.47	-2.84	-0.31	0.011
Direct Effect	-5.80	-10.86	-2.48	<0.001
Total Effect	-7.27	-12.40	-3.79	<0.001
Mediated Proportion	0.20	0.04	0.41	0.011
P-tau217->MMSE				
Indirect Effect	-0.32	-0.67	-0.02	0.041
Direct Effect	-1.02	-2.52	-0.07	0.034
Total Effect	-1.34	-2.87	-0.34	0.004
Mediated Proportion	0.24	-0.01	0.80	0.045
P-tau181->Camcog-R-TOTAL				
Indirect Effect	-0.03	-0.06	-0.01	0.002
Direct Effect	-0.10	-0.17	-0.04	0.004
Total Effect	-0.13	-0.21	-0.06	<0.001
Mediated Proportion	0.22	0.06	0.52	0.002
P-Tau181->MMSE				
Indirect Effect	-0.01	-0.01	0.00	0.017
Direct Effect	-0.03	-0.05	-0.01	0.004
Total Effect	-0.03	-0.06	-0.01	<0.001
Mediated Proportion	0.17	0.02	0.45	0.018

regions significantly mediated the effects of p-tau217 and p-tau181 on MMSE and CAMCOG-R-Total. Significant direct effects demonstrated that both p-tau217 and p-tau181 were negatively associated with cognitive performance after adjustment for neurostructural indeces. Finally, the mediated proportions showed that hippocampal volume and AD signature cortical thickness explained between 17 % and 27 % (all significant) of the effects of p-tau217 and p-tau181 on cognitive performance.

4. Discussion

The present study aimed to provide clinical-biological characterisation of early stages of the AD continuum by using plasma biomarkers and neurostructural indices in participants classified in five clinical groups according to their cognitive status. Thus, the overall cognitive performance of participants in the CAMCOG-R and the MMSE was as expected. Global cognitive functioning was lower in all three groups of participants with MCI than in the CU and SCD groups, and the md-aMCI group performed worse than single domain participants, regardless of whether they were classified amnesic or non-amnesic. No differences in the cognitive level of the SCD group and the CU group were detected. Therefore, CU and SCD participants showed normal cognitive functioning for their age and education, while the MCI groups performed lower than expected for their age and education, especially multidomain aMCI [7–9].

The SCD group displayed higher levels of anxiety and depression and lower psychological well-being than the CU group, although without reaching the clinical disorder stage. These results are consistent with the self-perception of decreased cognitive performance and preoccupation with this perception that are characteristic of SCD, and they are also consistent with previous findings [7]. The two aMCI groups also reported higher levels of depressive symptoms than the CU group, and the md-aMCI group displayed lower levels of psychological well-being than the CU group and obtained higher scores in the neuropsychiatric symptom questionnaire than the other diagnostic groups. Both depressive and neuropsychiatric symptoms have been associated with an increased risk of developing dementia, and it is not uncommon for dementias to debut with a depressive condition or the appearance of neuropsychiatric symptoms [60]. These data support the importance of scales and tests that report on emotional state and mental health when assessing the risk of developing dementia by participants older than fifty years, especially those with md-aMCI.

In the present study no group-related differences in the distribution of APOE-ε4 carriers / non-carriers were found. Carriers of the APOE-ε4 variant have a higher risk of developing AD dementia than non-carriers [61]. Therefore, it would be appropriate to follow up all participants in our cohort who are carriers of this variant, regardless of their current cognitive status.

The plasma p-tau217 and p-tau181 biomarkers showed main Group effects. The plasmatic p-tau217 concentrations were higher in the amnestic MCI groups (sd-aMCI and md-aMCI) than in the CU, SCD and sd-naMCI groups. Plasma p-tau181 concentrations were also higher in the md-aMCI than in CU, SCD and sd-naMCI groups, although there were no differences between sd-aMCI and CU, SDC or sd-naMC groups. Plasma p-tau217 and p-tau181 concentrations have been shown to be reliable biomarkers for determining the presence of both hallmarks of AD neuropathology, i.e. brain amyloidosis and tauopathy, although p-tau217 does so more accurately than p-tau181 [6,19,20,25]. Thus, the differences in p-tau217 and p-tau181 between amnestic MCIs and other groups in the present study support linkage between amnestic MCI diagnosis and the AD continuum. P-tau217 performed better than p-tau181 as a biomarker for detecting AD pathology and for discriminating the study groups; consequently, p-tau217 appears to be a reliable AD biomarker with potentially important clinical utility [20].

However, the plasma Aß 42/40 ratio, total-tau and NfL did not differ among groups, and they were not related to cognitive performance or to the two measures of neurostructural integrity, contrary to what has been observed in others research [62]. This may suggest that the neuropathological burden associated with amyloidosis and tauopathy indicated by p-tau217 of the aMCI groups were not sufficient to be detected by the plasma Aβ 42/40 ratio, total-tau or NfL concentrations. The plasma Aβ 42/40 ratio has limitations for detecting amyloid pathology [20], and there is no clear consensus regarding the efficacy of plasma total-tau for detecting AD neuropathology [63]. Furthermore, several studies have not observed any relationship between plasma Aβ 42/40 ratio or NfL concentrations and cognitive performance [26], and concentrations of NfL biomolecule were found to be related to neurostructural measures when involved participants with dementia [29]. Thus, p-tau217 showing the highest diagnostic value in the prodromal and asymptomatic early stages of AD [19,21,22].

The association between p-tau217 and amnestic phenotypes was supported by differences in the distribution of participants according to the dual cut-point strategy [19]. The proportion of participants with positive and intermediate p-tau217 levels was higher in both aMCI groups than in the other groups. These results are consistent with an increased risk of developing dementia due to AD in aMCI participants

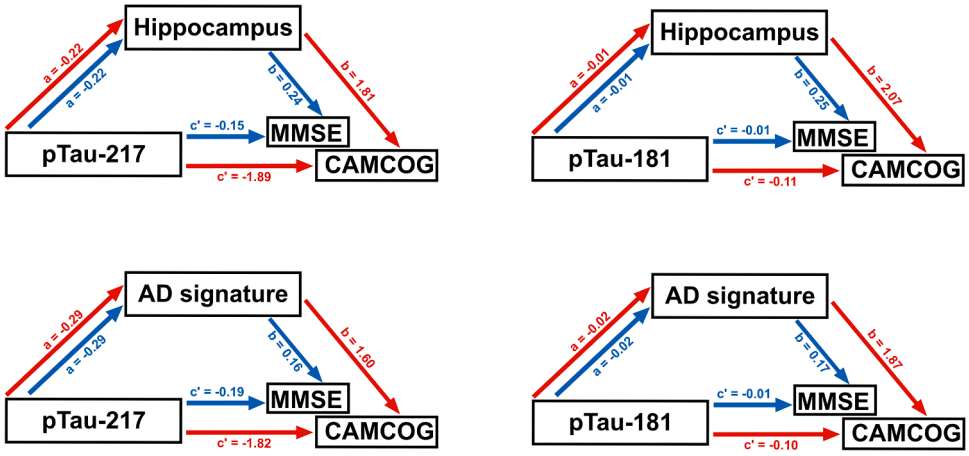

Fig. 3. Conceptual diagrams of the mediation analyses with standardized regression coefficients for paths "a" (effect of the plasma biomarker on the neurostructural index), "b" (effect of neurostructural index on cognition, adjusted for plasma biomarker) and "c′ " (direct effect of the plasma biomarker on cognitive performance adjusted for neurostructural index).
Indirect effect= path a*b.

[10–12]

Hippocampal volume and AD signature cortical thickness were lower in the md-aMCI group than in CU, SCD and sd-naMCI groups. Following the criteria for AD diagnosis proposed by Jack et al [25], these neurostructural results reinforce the presence of neuropathological changes compatible with AD in the md-aMCI group. Furthermore, it is possible that md-aMCI group may have reached at least stage III of the Braak and Braak model of progression of brain atrophy characteristic of AD [3]. By contrast, the sd-aMCI group did not show any differences in hippocampal volume and AD signature thickness relative to the other groups. This is consistent with the higher risk of md-aMCI progressing to AD dementia than sd-aMCI [12,13], with the idea [62] that throughout the AD continuum, the alteration in plasma biomarkers precedes the alteration in MRI indices, and it supports separating the aMCI into two clinical-biological stages within the AD continuum, with md-aMCI as a more advanced stage than sd-aMCI.

In the sd-naMCI group, in which deficits in a cognitive domain other than memory were detected, the levels of p-tau217, p-tau18, total-tau, NfL and Aß 42/40 ratio plasma biomarkers did not differ from those in the CU and SCD groups. In addition, in 92 % of sd-naMCI participants, the p-tau217 concentrations were indicative of negative AD pathology. These results are consistent with those reported for CSF biomarkers [10]. The authors observed that tau/Aβ42 ratios in CSF of non-amnestic MCI participants did not reach the cut-off threshold for positive diagnosis of amyloidosis. Furthermore, hippocampal volume and AD signature cortical thickness of sd-naMCI did not differ from those in the CU and SCD groups. However, the MRI results in the sd-naMCI group should be viewed with caution due to the small number of participants in this group for which MRI data are available. On the other hand, only 2 of a total of 24 participants with sd-naMCI were heterozygous carriers of the APOE-ε4. These results, despite their limitations, led us to hypothesize that with the possible exception of these two carriers, the cognitive impairment of the sd-naMCI participants in the present study may be related to some neuropathology other than AD. Future follow-up research in these participants will clarify the relevance of this hypothesis.

The SCD group did not differ from CU group in relation to plasma and neurostructural biomarkers. SCD has mainly been studied in the context of the preclinical phase of AD [7], to determine characteristics of risk of progression to dementia. Thus, it has been found that the likelihood of SCD participants progressing to MCI and dementia is higher in participants with amyloid neuropathology [64]. In the present study, plasma biomarkers, hippocampal volume, AD signature cortical thickness and APOE-ε4 data did not show that the SCD group had a higher risk than CU group to progress cognitive impairment of the AD continuum

The interdependence between neurostructural integrity, AD plasma biomarkers and cognitive functioning was highlighted by the observed relationships between the variables. Plasma p-tau217 concentrations were negatively related to global cognitive performance and to neurostructural integrity, as observed in previous studies [26,28,29]. In addition, neurostructural integrity was positively related to global cognitive functioning. Furthermore, mediation analyses indicated that the effects of p-tau217 and p-tau181 on cognitive functioning were mediated by hippocampal volume and AD signature cortical thickness. These results are consistent with those of previous studies showing that hippocampal volume mediated the relationship between both plasma p-tau217 and p-tau181 and cognitive functioning in the AD continuum [65,66], and that hippocampal volume and cortical thickness mediated the relationship between Aβ-PET and memory in participants with aMCI [67].

The close relationship between cognitive functioning and neurostructural characteristics and plasma AD hallmarks supports an integrated clinical-biological diagnosis. Thus, clinical-biological characterization at the early stages of cognitive decline has enabled us to link aMCI with AD neuropathology and to define sd-aMCI and md-aMCI as two stages along the AD continuum. By contrast, sd-naMCI does not appear to be associated with AD pathology. Finally, SCD participants of the present cohort do not seem to display a greater risk of development of cognitive impairment than the CU group.

In conclusion, use of AD plasma biomarkers such as p-tau217 concentrations and of indices of neurostructural integrity such as hippocampal volume and AD signature cortical thickness complete cognitive-clinical diagnoses in the early stages of cognitive impairment, in a cost-effective and minimally invasive way. This approach also enables the identification of risk factors for future development of AD dementia and, consequently, the possibility of longitudinal monitoring and addressing these risk factors through preventive programmes.

This study has several limitations that we hope to address in future research. The number of participants is not large for this type of study, and it is also smaller for MRI data; the sample is not representative of the population because it was recruited incidentally in primary care centres; and sex was not analysed as a primary factor. These limitations hamper generalization of the results. Furthermore, the cross-sectional nature of this study limits the assessment of clinical trajectory.

Declaration of generative AI and AI-assisted technologies in the writing process

I declare that we have used DeepL Translate as an aid to writing in English, at a rate of 5 %.

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CRediT authorship contribution statement

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Declaration of competing interest

The authors declare that they have no known competing financial interests or personal relationships that could have appeared to influence the work reported in this paper.

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