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RESEARCH

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Clinical performance and head-to-head comparison of CSF p-tau235 with p-tau181, p-tau217 and p-tau231 in two memory clinic cohorts

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Abstract

Background Cerebrospinal fluid (CSF) p-tau235 is a novel biomarker highly specific of Alzheimer's disease (AD). However, CSF p-tau235 has only been studied in well-characterized research cohorts, which do not fully reflect the patient landscape found in clinical settings. Therefore, in this multicentre study, we investigated the performance of CSF p-tau235 to detect symptomatic AD in clinical settings and compared it with CSF p-tau181, p-tau217 and p-tau231.

Methods CSF p-tau235 was measured using an in-house single molecule array (Simoa) assay in two independent memory clinic cohorts: Paris cohort (Lariboisière Fernand-Widal University Hospital Paris, France; $n=212$) and BIODEGMAR cohort (Hospital del Mar, Barcelona, Spain; $n=175$). Patients were classified by the syndromic diagnosis (cognitively unimpaired [CU], mild cognitive impairment [MCI] or dementia) and their biological diagnosis (amyloid-beta [A β]+ or A β -). Both cohorts included detailed cognitive assessments and CSF biomarker measurements (clinically validated core AD biomarkers [Lumipulse CSF A $\beta_{1-42/40}$ ratio, p-tau181 and t-tau] and in-house developed Simoa CSF p-tau181, p-tau217 and p-tau231).

Results High CSF p-tau235 levels were strongly associated with CSF amyloidosis regardless of the clinical diagnosis, being significantly increased in MCI A β + and dementia A β + when compared with all other A β - groups (Paris cohort: $P < 0.0001$ for all; BIODEGMAR cohort: $P < 0.05$ for all). CSF p-tau235 was pronouncedly increased in the A+T+ profile group compared with A-T- and A+T- groups ($P < 0.0001$ for all). Moreover, CSF p-tau235 demonstrated high diagnostic accuracies identifying CSF amyloidosis in symptomatic cases (AUCs=0.86 to 0.96) and discriminating AT groups (AUCs=0.79 to 0.98). Overall, CSF p-tau235 showed similar performances to CSF p-tau181 and CSF p-tau231

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when discriminating CSF amyloidosis in various scenarios, but lower than CSF p-tau217. Finally, CSF p-tau235 associated with global cognition and memory domain in both cohorts.

Conclusions CSF p-tau235 was increased with the presence of CSF amyloidosis in two independent memory clinic cohorts. CSF p-tau235 accurately identified AD in both MCI and dementia patients. Overall, the diagnostic performance of CSF p-tau235 was comparable to that of other CSF p-tau measurements, indicating its suitability to support a biomarker-based AD diagnosis in clinical settings.

Keywords Alzheimer's disease, CSF, Biomarkers, p-tau235, p-tau181, p-tau217, p-tau231, Memory clinic

Background

Alzheimer's disease (AD) is characterized by the accumulation of aggregated amyloid-beta ($A\beta$) and hyperphosphorylated tau protein into extracellular $A\beta$ plaques and intracellular neurofibrillary tangles (NFTs), respectively [1, 2]. These two immunohistochemical findings, together with gross atrophy and synaptic and neuronal loss, represent the hallmarks of AD and post-mortem examination demonstrating the presence of these proteinopathies is required for definitive diagnosis [3]. Validated imaging and fluid biomarkers increasingly support the clinical assessment and diagnosis of AD during life. Imaging biomarkers include positron emission tomography (PET) using radio-ligands capable of specifically binding $A\beta$ and tau aggregates, whereas widely validated fluid biomarkers currently include $A\beta_{1-42}$ (or $A\beta_{1-42/40}$ ratio), total tau (t-tau) and phosphorylated tau at threonine 181 (p-tau181), all measured in cerebrospinal fluid (CSF) [4–7] often by full-automated instruments.

Recently, the field of fluid biomarkers in neurodegeneration has undergone a remarkable expansion, particularly with regard to biomarkers measuring different variants of tau protein. This has resulted in a wide range of novel p-tau biomarkers, measured with different methods and platforms (fundamentally immunoassays and mass spectrometry) [8–11]. Different p-tau residues may change in different stages of the Alzheimer's continuum. For example, p-tau231 is the first p-tau residue increasing in preclinical AD, confirming the earliest underlying AD processes [11, 12] whereas p-tau217, with its pronounced fold changes and strong association with AD pathological hallmarks, appears to be the most suitable p-tau biomarker for AD diagnosis and patient monitoring [13, 14]. Moreover, different p-tau epitopes may provide different information about the disease and some of them be more suited as a state, stage, or prognostic biomarkers. The most recent p-tau biomarker to demonstrate potential utility in relation to AD is p-tau235 [15], a phosphorylation site that has been found to be a prominent feature in paired helical filaments (PHFs) [16–19]. Recently, using a targeted mass spectrometry method, our group has demonstrated that p-tau235 is highly increased in neuropathologically confirmed AD brain when compared

with control cases [15]. These findings align with previous studies, indicating that phosphorylation at serine 235 hampers tubulin assembly, compromising normal microtubules dynamics [20, 21]. We developed a single molecule array (Simoa) assay capable of measuring p-tau235 in CSF, demonstrating that this novel biomarker is highly specific for AD and that it increases during preclinical stages, when only subtle abnormalities in CSF $A\beta_{1-42/40}$ can be detected [15]. Interestingly, when examining post-mortem brain samples, phosphorylation at serine 235 appears to occur only when preceded by nearby phosphorylation at threonine 231, in what appears to be a sequential phosphorylation event. Due to the translatability of this sequential phosphorylation event from the brain into CSF, p-tau235 has been previously proposed as a potentially useful staging biomarker of preclinical AD (CSF p-tau235 positivity being indicative of late asymptomatic AD stage) [15]. With clinical trials increasingly focusing their intervention at the earliest stages of AD, CSF p-tau235 may be useful to discriminate early from late preclinical AD cases and to evaluate if novel compounds effectively prevent disease progression.

In order to further characterize this novel p-tau biomarker and contextualize its performance with other CSF p-tau species, we previously compared CSF p-tau235 with CSF p-tau217 and p-tau231 in a well-characterized research cohort including the AD *continuum* and a preclinical AD cohort. We demonstrated that CSF p-tau235 displays a statistically equal performance to that of CSF p-tau217 and p-tau231 when discriminating cognitively impaired CSF $A\beta$ -positive cases from cognitively impaired CSF $A\beta$ -negative cases. However, the cases enrolled in that study belonged to a well-characterized research cohort, which does not fully reflect the patients from daily clinical practice including atypical AD, other dementia and comorbidities. Therefore, evaluating the performance of CSF p-tau235 in the routine practice of a memory clinic is much needed, since in these settings patients present with a higher heterogeneity in demographics, comorbidities and disease presentations [22–25]. As a secondary aim, we tested whether CSF p-tau235 was associated with cognitive performance.

To this end, we measured this novel p-tau biomarker in two independent memory clinic cohorts, compared its performance with N-terminal directed Simoa immunoassays targeting CSF p-tau181, p-tau217 and p-tau231, and assessed its relation with cognitive status.

Methods

Study participants

Paris cohort

Paris cohort enrolled a total of 212 patients who had undergone CSF analysis at the Centre of Cognitive Neurology at Lariboisière Fernand-Widal University Hospital between March 2014 and December 2019, including participants with subjective cognitive decline (SCD, $n=21$), non-AD mild cognitive impairment (non-AD MCI, $n=45$), AD-MCI ($n=40$), AD dementia ($n=75$) and non-AD dementia ($n=31$). Non-AD dementia patients encompassed patients with dementia with Lewy bodies (DLB, $n=12$), frontotemporal dementia (FTD, $n=15$), vascular cognitive impairment and dementia (VCID, $n=3$) and Creutzfeldt Jakob disease ($n=1$). Patients underwent a thorough clinical examination involving personal medical and family histories, treatment, neurological examination, extensive neuropsychological assessment, APOE genotyping, brain magnetic resonance imaging (MRI) extensive neuropsychological evaluation, MRI, APOE genotyping, blood and CSF analysis and fluid sampling for collection (blood and CSF). The diagnosis for each patient was made during multidisciplinary consensus meetings (including neurologists, neuropsychologists, gerontologists, neuroradiologist and biochemists) considering results of validated CSF biomarkers and according to clinical diagnostic criteria for AD dementia [3], MCI due to AD (AD-MCI) [26], DLB [27] and FTD [28]. AD patients displayed CSF biomarkers on the AD *continuum* [3]. MCI of other causes (non-AD MCI) included patients with psychiatric disorder, sleep apnea, or systemic disease. Non-AD MCI presented with normal CSF biomarkers or suspected non-Alzheimer pathophysiology (normal $A\beta_{1-42/40}$, high p-tau and/or high t-tau). Included SCD participants were individuals with several years of clinical follow-up for a clinical complaint, presenting with normal cognitive testing and no abnormalities at imaging and CSF examinations [29, 30].

BIODEGMAR cohort

The BIODEGMAR cohort is an observational longitudinal study that enrolls patients with neurodegenerative diseases visiting the Cognitive Decline and Movement Disorders Unit of Hospital del Mar (Barcelona, Spain). The procedures of the BIODEGMAR study include extensive neuropsychological evaluation, MRI, APOE $\epsilon 4$ genotyping, lumbar puncture for CSF collection and

blood sampling. Clinical evaluation was performed by a neurologist, including anamnesis, physical examination and clinical diagnosis. Neuropsychological evaluation was performed by a neuropsychologist and consisted of a series of standardized cognitive tests and functional scales. A comprehensive description of the BIODEGMAR cohort has been previously published, with further details on the inclusion and exclusion criteria and on core AD CSF biomarker procedures, including assay details and cutoff determination [31, 32]. In this study, 175 patients of the BIODEGMAR cohort were included from 27 April 2017 to 24 July 2020. The clinical diagnoses included SCD ($n=18$, similar diagnostic criteria as for Paris cohort), MCI ($n=74$) and, for subjects with moderate to severe cognitive impairment, possible AD dementia ($n=11$), probable AD dementia ($n=43$), LBD ($n=2$), extrapyramidal syndrome ($n=2$), vascular cognitive impairment and dementia (VCID; $n=4$), progressive supranuclear palsy (PSP; $n=2$), corticobasal syndrome ($n=4$), behavioral variant of FTD (bvFTD; $n=4$), primary progressive aphasia (PPA; $n=8$) and cerebral amyloid angiopathy (CAA; $n=3$).

Cohort stratification

Patient data from the Paris cohort and the BIODEGMAR cohort was examined using two stratification criteria: (i) according to clinical syndrome (cognitively unimpaired [CU], MCI, or dementia) and CSF amyloid status ($A\beta^-/A\beta^+$, as defined by Lumipulse CSF $A\beta_{1-42/40}$, Supplementary Table 3), resulting in six groups: CU $A\beta^-$, CU $A\beta^+$, MCI $A\beta^-$, MCI $A\beta^+$, dementia $A\beta^-$ and dementia $A\beta^+$ (clinical diagnosis included in each group are available in Supplementary Tables 1 and 2); (ii) based on the $A\beta$ (A) and tau (T) status defined using CSF $A\beta_{1-42/40}$ and p-tau181, respectively (Lumipulse®) into $A-T^-$, $A+T^-$ and $A+T^+$ (the $A-T^+$ group considered suspected non-AD pathology [SNAP] was not included in the statistical analysis, but is depicted in the boxplots). The clinical diagnoses included in each group are available in Supplementary Tables 5 and 6). Additionally, CSF p-tau235 levels across clinical diagnostic groups for both cohorts are available in Supplementary Figure 1.

Neuropsychological assessment

All included participants were evaluated using the Mini-Mental State Examination (MMSE). Detailed cognitive assessment was available for a subsample of participants in both the Paris cohort (total $n=135$: CU [$n=14$], MCI patients [$n=64$], dementia patients [$n=57$]) and the BIODEGMAR cohort (total $n=139$: CU [$n=18$], MCI [$n=59$], dementia [$n=62$]). In the Paris cohort, memory domain scores were evaluated using total immediate and delayed recall of Free and Cued Selective Reminding Test

(FCSRT), and the delayed matching-to-samples 48 test (DMS 48) for visual memory testing. Executive function was assessed using forward and backward digit span, frontal assessment battery and letter and animal fluencies. The language domain was evaluated using the Dénomination Orale 80 test, a naming test. In BIODEGMAR cohort, memory domain was evaluated using total immediate and delayed recall of FCSRT and the Memory Impairment Screen; executive functions, with backward digit span, TMT A and B; language domain, with the Boston naming test. *Z*-scores were computed from the control group scores as a reference. Domain scores were obtained by averaging *z*-scores of the individual tests available results within that domain and the global cognition score by averaging the 3 domains' *z*-scores.

Biomarker measurements

In the Paris cohort, core AD CSF biomarkers ($A\beta_{1-42/40}$ ratio, p-tau181 and t-tau) were measured using the clinically validated Lumipulse® G1200 assay (Fujirebio) [33]. Biomarker measurements for CSF p-tau181, p-tau217 and p-tau231 were performed using *in-house* Simoa assays. CSF p-tau181 and p-tau217 measurements were previously reported [34] and were included here in order to allow the comparison with novel CSF p-tau235. In the BIODEGMAR cohort, core AD CSF biomarkers ($A\beta_{1-42/40}$ ratio, p-tau181 and t-tau) were measured at Laboratori de Referència de Catalunya (LRC) with Lumipulse G600II (Fujirebio). Cutoff values for biomarkers and their ratios ($A\beta_{1-42/40}$, p-tau181, t-tau) were previously defined in the CORCOBIA study [31]. Biomarker measurements for CSF p-tau181 and p-tau231 were performed using *in-house* Simoa assays and were previously reported [32].

CSF p-tau235 measurements

CSF p-tau235 measurements were performed at the Clinical Neurochemistry Laboratory at the University of Gothenburg using a Simoa HD-X instrument (Quanterix), and all samples were blinded. Prior to the analysis, samples were allowed to thaw at room temperature for 45 min. Thawed samples were subsequently vortexed for 15 s, after which they were plated and diluted 1:2 using Tau2.0 sample assay diluent (Quanterix). Due to sample volume availability, CSF samples from the Paris cohort were run in singlicates, while BIODEGMAR cohort samples were run in duplicates. An eight-point calibration curve was generated using recombinant full-length GSK-3 β -phosphorylated tau-441 (SignalChem) and run in duplicates. Two internal quality controls (iQC, low and high) were run at the beginning and end of each plate, also in duplicates. Repeatability (%CV_r) and intermediate precision (%CV_{Rw}) values for both cohorts were <15%.

Further details on assay specifications and validation can be found elsewhere [15].

Statistical analysis

Statistics were performed using SPSS (v26, IBM, Armonk, NY, USA) and Scistat (Ostend, Belgium). Graphs were generated using GraphPad PRISM (v7.03, San Diego, CA, USA) and R version 4.1 (<https://www.r-project.org/>). Box-plots show the median and interquartile range (IQR), with individual data points for all participants always shown. Statistical analyses were performed using parametric or non-parametric methods when appropriate, based on the normality or not of the data. Group comparisons between two categories were performed using a *t*-test and Mann-Whitney *U* test. All comparisons of multiple groups were tested with a one-way ANOVA adjusted for age and sex followed by Bonferroni-corrected post-hoc pairwise comparisons. Adjusted *P*-values for covariates are indicated as *P*_{ADJ}. For each of the CSF p-tau biomarkers, accuracy for binary outcomes was determined using receiver operating characteristics (ROC) analysis and area under the curve (AUC). AUC values are accompanied by the 95% confidence interval, abbreviated as CI_{95%}. Performance comparison between different p-tau biomarkers was analysed using DeLong test (MedCalc). Spearman's rank correlation (*r*_s) was used to determine associations between biomarkers. Association of CSF p-tau biomarkers with global and domain-specific cognitive *z*-scores was studied with linear regression models adjusted for age, sex and level of education.

Results

Cohort characteristics

A total number of 387 participants were included in this study, which belong to two independent clinical cohorts (Paris cohort [*n*=212]; BIODEGMAR cohort [*n*=175]). Demographics and stratification in syndromic groups (CU $A\beta^-/A\beta^+$, MCI $A\beta^-/A\beta^+$ and dementia $A\beta^-/A\beta^+$) are presented in Table 1 for both cohorts. In the Paris cohort, MCI $A\beta^+$ and dementia $A\beta^+$ were older than CU $A\beta^-$ group (*P*<0.0001). There was a weak yet significant association with age in both cohorts (Paris cohort: *r*_s=0.32, *P*<0.0001; BIODEGMAR cohort: *r*_s=0.17, *P*<0.05). There were no sex differences between groups (*P*≥0.05; χ^2 test). In both cohorts, CSF p-tau levels were significantly higher in *APOE* $\epsilon 4$ carriers (overall *P*<0.0001). As expected, there were significant differences in MMSE scores across groups in both cohorts (*P*<0.0001), which, as compared with CU, gradually decreased in MCI and further in patients with dementia.

Table 1 Demographics and biomarkers levels

	BIODEGMAR cohort (n = 175)												
	Paris cohort (n = 212)												
	CU Aβ- n = 21	MCI Aβ- n = 47	Dementia Aβ- n = 27	MCI Aβ+ n = 40	Dementia Aβ+ n = 77	P-value	CU Aβ- n = 11	MCI Aβ- n = 28	Dementia Aβ- n = 19	CU Aβ+ n = 7	MCI Aβ+ n = 46	Dementia Aβ+ n = 64	P-value
Age, years	64.6 (9.42)	66.30 (9.58)	66.04 (7.73)	72.40 (7.96) ^a	71.90 (8.61) ^a	< 0.0001	70.4 (5.8)	71.4 (6.1)	70.0 (6.1)	70.1 (4.5)	74.4 (4.8)	73.5 (4.5)	0.009
Male (% male)	8 (38%)	16 (34%)	15 (56%)	16 (40%)	30 (39%)	0.338	3 (27%)	16 (57%)	6 (32%)	2 (29%)	19 (41%)	25 (39%)	0.141
APOEε4 carriers	7/21 (33%)	5/47 (11%) ^a	7/26 (27%)	22/40 (55%)	51/76 (67%) ^a	< 0.0001	1/10 (10%)	4/24 (17%)	3/18 (17%)	3/7 (43%)	24/38 (63%) ^a	39/62 (61%) ^a	< 0.02
Level of education, years	11.5 (2.4)	10.7 (2.2)	11.0 (2.1)	10.0 (2.5)	10.6 (2.4)	0.299	10.4 (4.9)	8.5 (4.6)	7.75 (4.7)	11.4 (4.0)	8.4 (4.6)	7.7 (3.6)	0.0267
MMSE score	27.14 (2.45)	24.00 (4.01) ^a	23.85 (4.87) ^a	23.82 (3.84) ^a	19.09 (5.74) ^a	< 0.0001	28.4 (2.3)	25.6 (2.7)	22.2 (5.8) ^a	28.7 (1.5)	23.3 (3.9) ^a	20.4 (4.8) ^a	< 0.0001
Core biomarkers Lumipulse													
CSF Aβ₁₋₄₂	1060.95 (245.23)	1090.66 (468.75)	1027.78 (354.02)	553.00 (209.36) ^a	497.88 (155.27) ^a	< 0.0001	1186 (357)	1164 (549)	986 (449)	634 (206) ^a	556 (178) ^a	522 (153) ^a	< 0.0001
CSF Aβ_{1-42/40}	0.093 (0.010)	0.088 (0.012)	0.09 (0.01)	0.044 (0.009) ^a	0.042 (0.009) ^a	< 0.0001	0.091 (0.017)	0.0879 (0.012)	0.091 (0.017)	0.049 (0.008) ^a	0.043 (0.009) ^a	0.043 (0.008) ^a	< 0.0001
CSF pTau, pg/mL	32.25 (8.16)	40.97 (20.81)	33.59 (10.50)	94.80 (47.31) ^a	113.53 (60.02) ^a	< 0.0001	56.8 (31.4)	50.2 (30.9)	41.0 (24.4)	59.1 (14.3)	112 (59.6) ^a	121.4 (57.9) ^a	< 0.0001
CSF t-tau, pg/mL	236.62 (63.69)	333.085 (172.630)	362.26 (341.90)	594.83 (285.45) ^a	722.01 (393.87) ^a	< 0.0001	307 (141)	357 (230)	311 (166)	407 (107)	669 (323) ^a	759 (342) ^a	< 0.0001
Biomarker profile													
Normal profile	21 (100%)	39 (83%)	25 (92%)	0 (0%)	0 (0%)		9 (82%)	24 (86%)	17 (89%)	0 (0%)	0 (0%)	0 (0%)	
AD continuum	0 (0%)	0 (0%)	0 (0%)	40 (100%)	77 (100%)		0 (0%)	0 (0%)	0 (0%)	7 (100%)	46 (100%)	64 (100%)	
A+T-	/	/	/	7 (18%)	10 (13%)		/	/	/	6 (86%)	11 (24%)	3 (5%)	
A+T+	/	/	/	33 (82%)	67 (87%)		/	/	/	1 (14%)	35 (76%)	61 (95%)	
SNAP	0 (0%)	8 (17%)	2 (8%)	0 (0%)	0 (0%)		2 (18%)	4 (14%)	2 (11%)	0 (0%)	0 (0%)	0 (0%)	
CSF p-tau biomarkers													
CSF N-term p-tau181, pg/mL	224.72 (66.33)	300.708 (245.52)	242.23 (110.58)	965.91 (628.08) ^a	1223.94 (802.66) ^a	< 0.0001	427 (110)	410 (164)	426 (168)	494 (129)	771 (257) ^a	871 (343) ^a	< 0.0001

Table 1 (continued)

	BIODEGMAR cohort (n = 175)												
	Paris cohort (n = 212)					BIODEGMAR cohort (n = 175)							
	CU Aβ- n = 21	MCI Aβ- n = 47	Dementia Aβ- n = 27	MCI Aβ+ n = 40	Dementia Aβ+ n = 77	P-value	CU Aβ- n = 11	MCI Aβ- n = 28	Dementia Aβ- n = 19	CU Aβ+ n = 7	MCI Aβ+ n = 46	Dementia Aβ+ n = 64	P-value
CSF N-term p-tau217, pg/mL	1.84 (0.94)	2.90 (2.50)	2.14 (1.67)	13.88 (9.48) ^a	16.62 (9.74) ^a	< 0.0001	N/A	N/A	N/A	N/A	N/A	N/A	N/A
CSF N-term p-tau231, pg/mL	271.93 (64.74)	325.05 (148.44)	286.62 (114.72)	657.64 (205.53) ^a	800.58 (640.95) ^a	< 0.0001	313 (211)	348 (201)	331 (236)	541 (144)	788 (399) ^a	893 (469) ^a	< 0.0001
CSF N-term p-tau235, pg/mL	11.19 (2.94)	12.74 (5.67)	11.56 (4.30)	24.75 (8.11) ^a	28.03 (11.52) ^a	< 0.0001	17.6 (8.10)	18.5 (11.3)	17.4 (7.23)	23.1 (3.65)	34.8 (12.9) ^a	41.6 (20.0) ^a	< 0.0001

Data is shown as mean (SD) or n (%), as appropriate. We used Kruskal-Wallis test to compare age between groups and Pearson's chi-square to compare sex and APOE ε4 frequencies between groups. Fluid biomarkers levels and MMSE were compared with a one-way ANOVA adjusted by age and sex. ^aP < 0.0001 versus controls. ^bP < 0.05 versus CU Aβ-.

Aβ₁₋₄₂ β-amyloid 42, Aβ₁₋₄₀ β-amyloid 40, CSF Cerebrospinal fluid, CU Cognitively unimpaired, MCI Mild cognitive impairment, MMSE Mini-Mental State Examination, N-term N-terminal, p-tau181 Tau phosphorylated at threonine 181, p-tau217 Tau phosphorylated at threonine 217, p-tau231 Tau phosphorylated at threonine 231, p-tau235 Tau phosphorylated at threonine 235, p-tau231, p-tau235 Tau phosphorylated at threonine 231, p-tau235 Tau phosphorylated at threonine 235, p-tau181 Total tau

CSF p-tau235 across syndromic and CSF A β -positive and CSF A β -negative groups

All comparisons of multiple groups were adjusted for age and sex. In the Paris cohort, CSF p-tau235 was significantly higher in MCI A β + and dementia A β + when compared with CU A β -, MCI A β - and dementia A β - groups (all $P_{ADJ} < 0.0001$) (Fig. 1a). Similarly, in the BIODEGMAR cohort, CSF p-tau235 was significantly increased in MCI A β + and dementia A β + when compared with CU A β -, MCI A β - and dementia A β - (all $P_{ADJ} < 0.001$) (Fig. 1b). CSF p-tau235 was also significantly higher in dementia A β + participants when compared with CU A β + (despite this group being only comprised by seven subjects, $P_{ADJ} = 0.038$), but no statistical differences were found between MCI A β + and CU A β +. No significant differences were found between CU A β - and CU A β +, likely due to small sample sizes (eleven and seven cases, respectively), although higher CSF p-tau235 levels in CU A β + were noticeable (Fig. 1b).

CSF p-tau235 discriminatory accuracy identifying CSF amyloidosis in syndromic groups

We then investigated the discriminatory accuracies of CSF p-tau235 to detect CSF amyloidosis between each of the syndromic groups and compared its performance with other novel N-terminal directed CSF p-tau biomarkers, specifically p-tau181, p-tau217 and p-tau231 (Fig. 2).

In the Paris cohort, CSF p-tau235 displayed high accuracies when discriminating dementia A β - from dementia A β + ($AUC_{\text{Dementia A}\beta^- \text{ vs A}\beta^+} = 0.96$, $CI_{95\%} = 0.90-0.99$) and MCI A β + from MCI A β - ($AUC_{\text{MCI A}\beta^- \text{ vs A}\beta^+} = 0.90$, $CI_{95\%} = 0.82-0.96$) (Fig. 2a). In these two scenarios, CSF p-tau235 performance showed no statistical differences when compared with CSF p-tau181 ($AUC_{\text{Dementia A}\beta^- \text{ vs A}\beta^+} = 0.98$, $CI_{95\%} = 0.93-1.00$; $AUC_{\text{MCI A}\beta^- \text{ vs A}\beta^+} = 0.94$; $CI_{95\%} = 0.86-0.98$) and CSF p-tau231 ($AUC_{\text{Dementia A}\beta^- \text{ vs A}\beta^+} = 0.97$, $CI_{95\%} = 0.92-0.99$, $AUC_{\text{MCI A}\beta^- \text{ vs A}\beta^+} = 0.92$, $CI_{95\%} = 0.85-0.97$). In contrast, lower accuracies were observed when compared with CSF p-tau217 ($AUC_{\text{Dementia A}\beta^- \text{ vs A}\beta^+} = 0.99$, $CI_{95\%} = 0.94-1.00$; $AUC_{\text{MCI A}\beta^- \text{ vs A}\beta^+} = 0.95$, $CI_{95\%} = 0.88-0.99$; both $P < 0.05$) (Fig. 2a). In the BIODEGMAR cohort, CSF p-tau235 showed high performance discriminating dementia A β + from dementia A β - ($AUC_{\text{Dementia A}\beta^- \text{ vs A}\beta^+} = 0.93$, $CI_{95\%} = 0.86-0.98$) and MCI A β + from MCI A β - ($AUC_{\text{MCI A}\beta^- \text{ vs A}\beta^+} = 0.86$, $CI_{95\%} = 0.76-0.93$) (Fig. 2b). When compared with other p-tau species, CSF p-tau235 performance discriminating dementia A β + from dementia A β - was equal to CSF p-tau181 ($AUC_{\text{Dementia A}\beta^- \text{ vs A}\beta^+} = 0.92$, $CI_{95\%} = 0.84-0.97$) and CSF p-tau231 ($AUC_{\text{Dementia A}\beta^- \text{ vs A}\beta^+} = 0.88$, $CI_{95\%} = 0.79-0.94$). On the other hand, the accuracy of CSF p-tau235 distinguishing MCI A β + from MCI A β - approached significance compared with CSF p-tau181 ($AUC_{\text{MCI A}\beta^- \text{ vs A}\beta^+} = 0.91$, $CI_{95\%} = 0.82-0.96$; $P = 0.0513$),

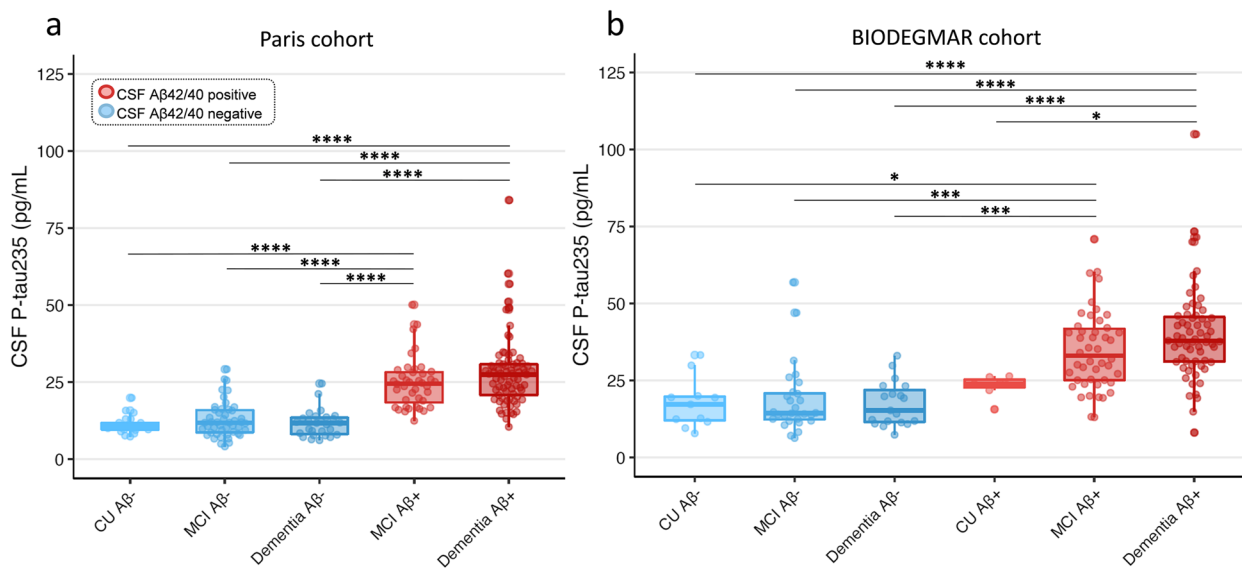


Fig. 1 CSF levels of p-tau235 across syndromic groups stratified by CSF amyloidosis. **a** In the Paris cohort, CSF p-tau235 was significantly increased in both dementia A β + and MCI A β + when compared with CU A β -, MCI A β - and dementia A β -. **b** In the BIODEGMAR cohort, CSF p-tau235 was significantly higher in both dementia A β + and MCI A β + when compared with CU A β -, MCI A β - and dementia A β -. Additionally, CSF p-tau235 was significantly increased in dementia A β + when compared with CU A β +. *Data information:* Boxplots show the median, IQR, and all the participants colour-coded based on the presence (red) or absence (blue) of CSF amyloidosis measured with Lumipulse CSF A β A $\beta_{1-42/40}$. *P*-values were determined using one-way ANOVA adjusted by age and sex, followed by Bonferroni-corrected post hoc comparison (* $P < 0.05$, ** $P < 0.01$, *** $P < 0.001$, **** $P < 0.0001$)

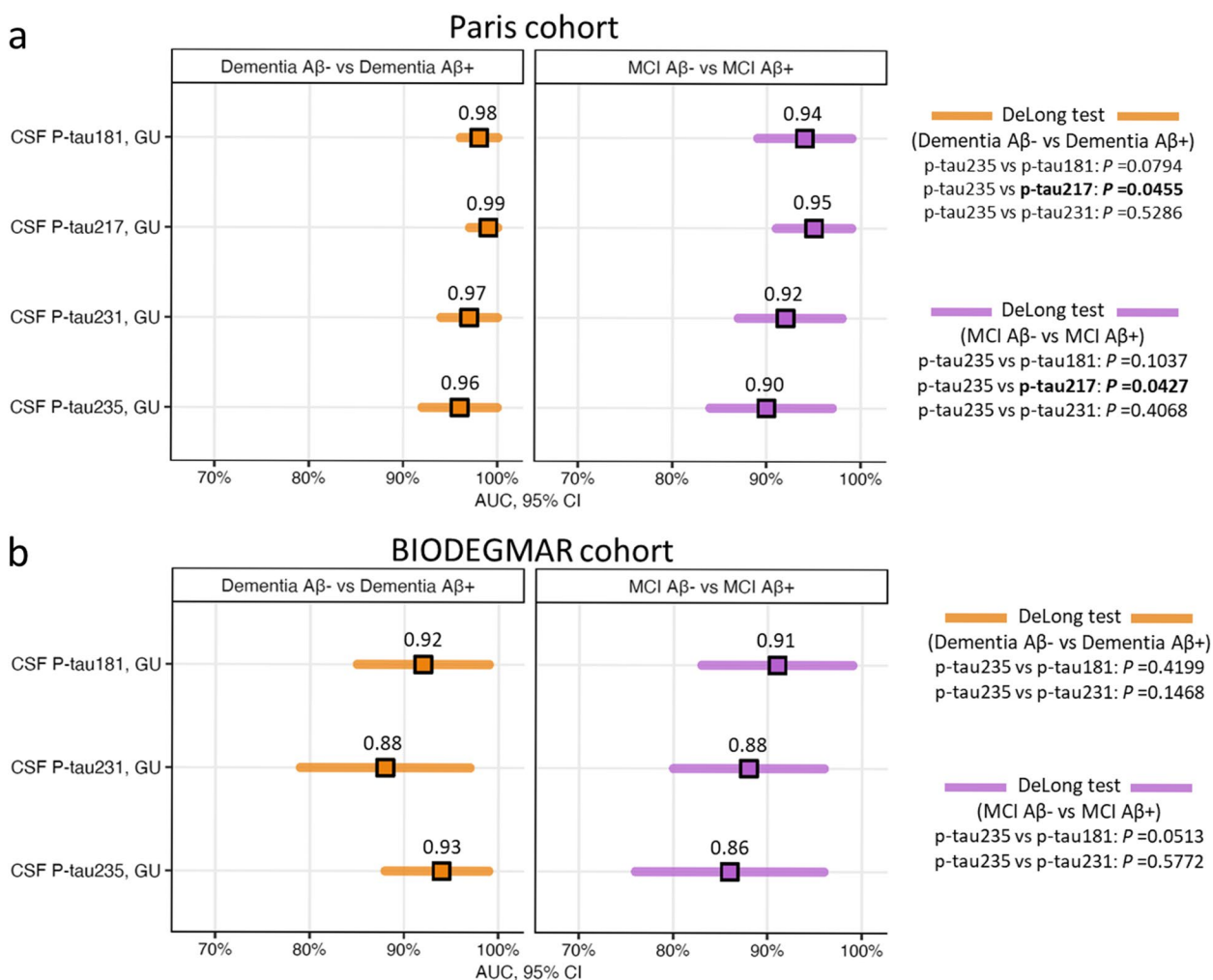


Fig. 2 Diagnostic performance of CSF p-tau235 when discriminating syndromic groups stratified by CSF amyloidosis. Both in **a** the Paris cohort and **b** the BIDEGBMAR cohort CSF p-tau235 displayed high accuracies differentiating dementia Aβ+ from dementia Aβ- and MCI Aβ+ from MCI Aβ-, similar to CSF p-tau181 and p-tau231, and lower than CSF p-tau217. *Data information:* forest plots showing AUC values from ROC analysis (CI_{95%} available in Supplementary Table 4). Comparisons of AUC values between CSF p-tau235 and other available CSF p-tau biomarkers were determined using DeLong test (significance is indicated in bold)

while matched CSF p-tau231 (AUC_{MCI Aβ- vs Aβ+}=0.88, CI_{95%}=0.78–0.94) (Fig. 2b).

CSF p-tau235 across AT groups

All comparisons of multiple groups were adjusted for age and sex. In the Paris cohort, CSF p-tau235 levels progressively increased across the AD continuum, that is, from A-T- to A+T- and from A+T- to A+T+ (Fig. 3a). A borderline significant increase in CSF p-tau235 levels was observed between A-T- and A+T- (P_{ADJ}=0.054). This was followed by a prominent increase between A+T- and A+T+ (P_{ADJ}<0.0001). CSF p-tau235 concentration was also significantly

higher in A+T+ when compared with A-T- (P_{ADJ}<0.0001) (Fig. 3a). In the BIDEGBMAR cohort, CSF p-tau235 also followed an increasing trajectory across the AD continuum, although no significant differences were found between A-T- and A+T- subjects when performing multiple comparisons (Fig. 3b). On the other hand, the levels of CSF p-tau235 were significantly higher in A+T+ when compared with A-T- and A+T- (P_{ADJ}<0.0001 for both) (Fig. 3b). Finally, we stratified patients based on exclusively CSF Aβ, which resulted in CSF p-tau235 being highly increased in A+ cases when compared with A- in both cohorts (P<0.0001 for all) (Supplementary Figure 2).

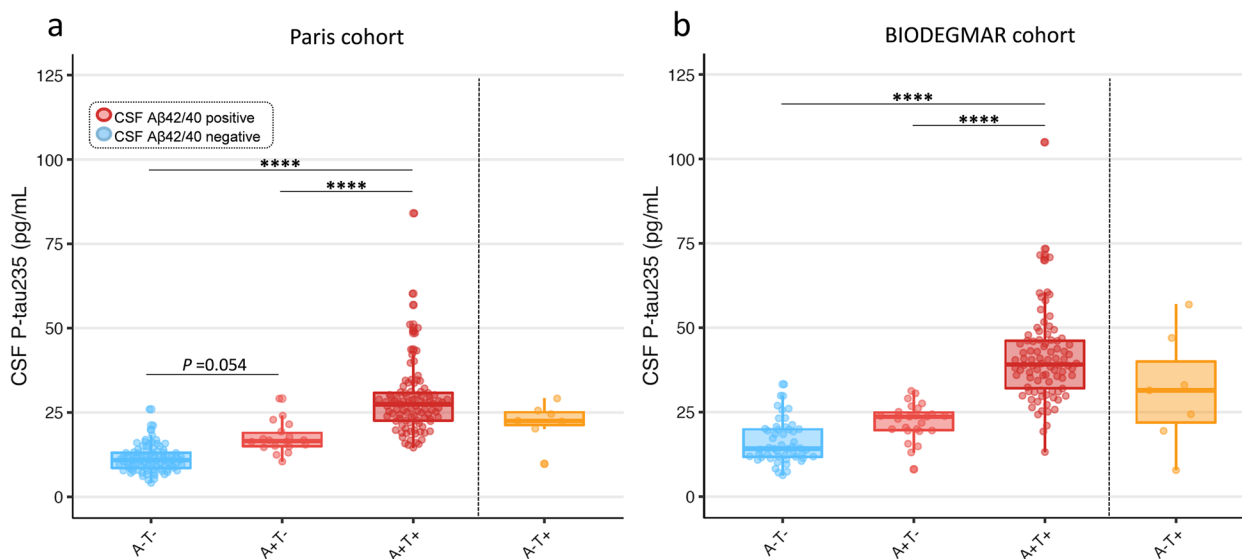


Fig. 3 CSF levels of p-tau235 across AT groups. In both **a** the Paris cohort and **b** the BIODEGMAR cohort, CSF p-tau235 followed an increasing trajectory across the AT continuum. CSF p-tau235 was significantly increased in A+T+ when compared with A-T- and A+T-, and approached significance between A-T- and A+T- in the Paris cohort. *Data information:* Boxplots show the median, IQR, and all the participants colour-coded based on the presence (red) or absence (blue) of CSF amyloidosis measured with Lumipulse CSF $\beta_{1-42/40}$. *P*-values were determined using one-way ANOVA adjusted by age and sex, followed by Bonferroni-corrected post hoc comparison (* $P < 0.05$, ** $P < 0.01$, *** $P < 0.001$, **** $P < 0.0001$)

CSF p-tau235 discriminatory accuracy identifying CSF amyloidosis in AT groups

We then examined the performance of CSF p-tau235 for discriminating the AT groups which comprise the AD continuum, that is A-T-, A+T- and A+T+ (Fig. 4). In the Paris cohort, CSF p-tau235 displayed high accuracy discriminating A-T- from A+T- ($AUC_{A-T- vs A+T-} = 0.88$, $CI_{95\%} = 0.80-0.93$), similar to CSF p-tau231 ($AUC_{A-T- vs A+T-} = 0.93$, $CI_{95\%} = 0.87-0.97$), but lower than both CSF p-tau181 ($AUC_{A-T- vs A+T-} = 0.95$, $CI_{95\%} = 0.89-0.98$, $P < 0.05$) and CSF p-tau217 ($AUC_{A-T- vs A+T-} = 0.95$, $CI_{95\%} = 0.89-0.98$, $P < 0.05$) (Fig. 4a). CSF p-tau235 showed high accuracy discriminating A+T- from A+T+ participants ($AUC_{A+T- vs A+T+} = 0.89$, $CI_{95\%} = 0.82-0.94$) and A-T- from A+T+ ($AUC_{A-T- vs A+T+} = 0.98$, $CI_{95\%} = 0.95-1.00$). In both scenarios, CSF p-tau235 performance was statistically equal to that of CSF p-tau181 ($AUC_{A+T- vs A+T+} = 0.93$, $CI_{95\%} = 0.87-0.97$; $AUC_{A-T- vs A+T+} = 0.99$, $CI_{95\%} = 0.97-1.00$), CSF p-tau217 ($AUC_{A+T- vs A+T+} = 0.91$, $CI_{95\%} = 0.84-0.96$; $AUC_{A-T- vs A+T+} = 0.99$, $CI_{95\%} = 0.97-1.00$) and CSF p-tau231 ($AUC_{A+T- vs A+T+} = 0.88$, $CI_{95\%} = 0.81-0.93$; $AUC_{A-T- vs A+T+} = 0.98$, $CI_{95\%} = 0.95-1.00$) (Fig. 4a).

In the BIODEGMAR cohort (Fig. 4b), CSF p-tau235 displayed equal accuracies when discriminating A-T- from A+T- ($AUC_{A-T- vs A+T-} = 0.79$, $CI_{95\%} = 0.68-0.87$) as CSF p-tau181 ($AUC_{A-T- vs A+T-} = 0.80$, $CI_{95\%} = 0.69-0.88$) and CSF p-tau231 ($AUC_{A-T- vs A+T-} = 0.80$, $CI_{95\%} = 0.70-0.88$). The accuracy

of CSF p-tau235 when discriminating A+T- and A+T+ ($AUC_{A+T- vs A+T+} = 0.95$, $CI_{95\%} = 0.88-0.98$) statistically matched that of CSF p-tau181 ($AUC_{A+T- vs A+T+} = 0.94$, $CI_{95\%} = 0.88-0.98$) and slightly outperformed that of CSF p-tau231 ($AUC_{A+T- vs A+T+} = 0.86$, $CI_{95\%} = 0.79-0.92$, $P = 0.0448$). Finally, CSF p-tau235 showed a nearly perfect accuracy discriminating A-T- and A+T+ ($AUC_{A-T- vs A+T+} = 0.97$, $CI_{95\%} = 0.93-0.99$), same as that observed for CSF p-tau181 ($AUC_{A-T- vs A+T+} = 0.98$, $CI_{95\%} = 0.94-1.00$) and CSF p-tau231 ($AUC_{A-T- vs A+T+} = 0.95$, $CI_{95\%} = 0.90-0.98$) (Fig. 4b).

Finally, we evaluated the performance of CSF p-tau235 when discriminating $A\beta+$ from $A\beta-$ individuals (Supplementary Figure 3, Supplementary Table 8). In the Paris cohort, CSF p-tau235 showed high accuracy distinguishing these two groups ($AUC_{A\beta- vs A\beta+} = 0.94$, $CI_{95\%} = 0.90-0.97$). When compared with other p-tau species (Supplementary Table 8), CSF p-tau235 performance was significantly lower than CSF p-tau181 ($AUC_{A\beta- vs A\beta+} = 0.97$, $CI_{95\%} = 0.93-0.99$, $P < 0.05$) and CSF p-tau 217 ($AUC_{A\beta- vs A\beta+} = 0.98$, $CI_{95\%} = 0.95-0.99$, $P < 0.01$), but equal to CSF p-tau231 ($AUC_{A\beta- vs A\beta+} = 0.96$, $CI_{95\%} = 0.92-0.98$) (Supplementary Figure 3a). In the BIODEGMAR cohort, CSF p-tau235 ($AUC_{A\beta- vs A\beta+} = 0.89$, $CI_{95\%} = 0.83-0.93$) matched the performance of both CSF p-tau181 ($AUC_{A\beta- vs A\beta+} = 0.91$, $CI_{95\%} = 0.85-0.94$) and CSF p-tau231 ($AUC_{A\beta- vs A\beta+} = 0.88$, $CI_{95\%} = 0.82-0.92$) (Supplementary Figure 3b).

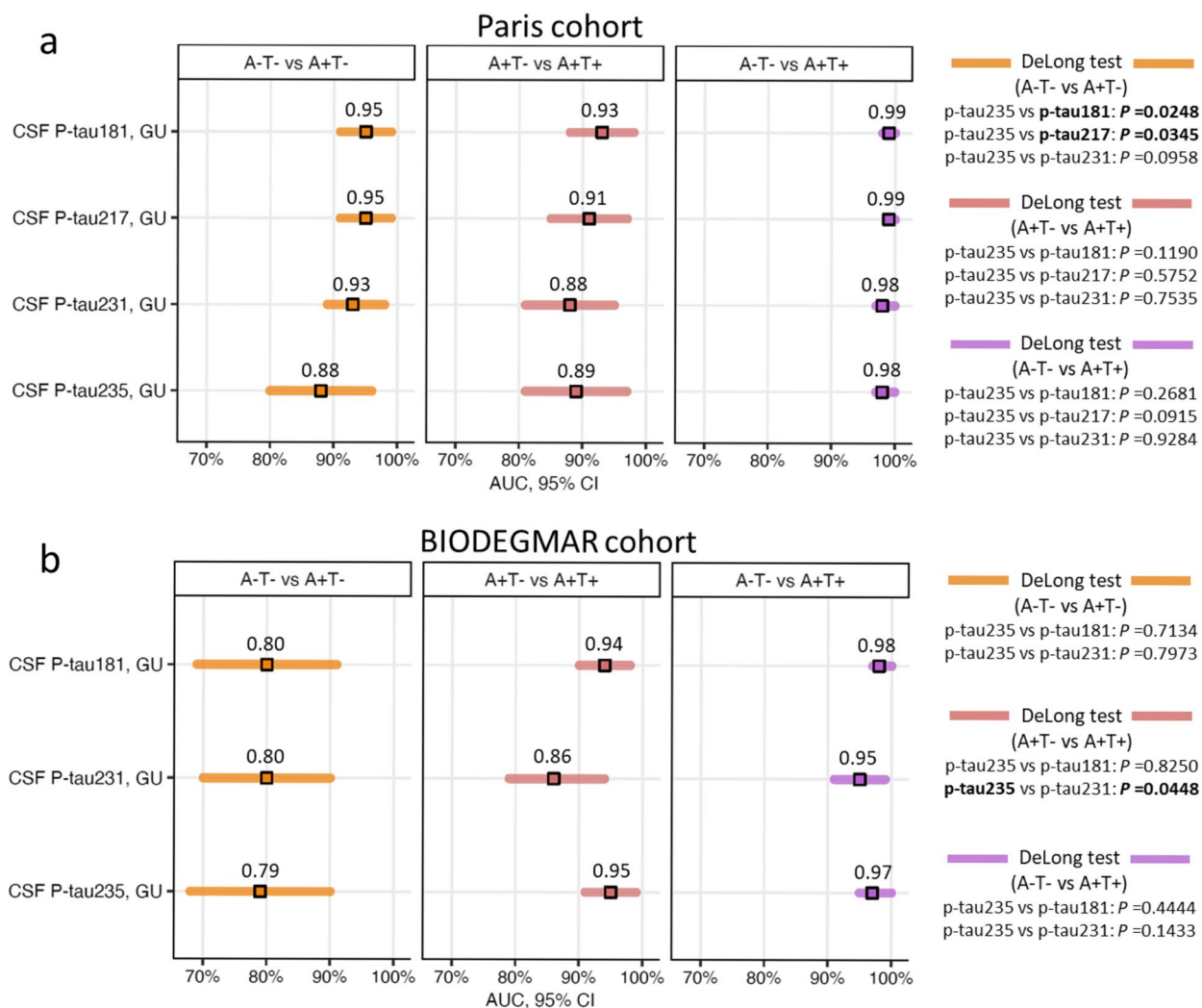


Fig. 4 Diagnostic performance of CSF p-tau235 when discriminating AT groups. **a** In the Paris cohort, CSF p-tau235 showed high accuracies in all three scenarios. CSF p-tau235 performance discriminating A–T– from A+T– was lower than CSF p-tau181 and p-tau217, but matched that of CSF p-tau231. When differentiating A+T– from A+T+ and A–T– from A+T+, CSF p-tau235 matched the performance of CSF p-tau181, p-tau217 and p-tau231. **b** In the BIODEGMAR cohort, CSF p-tau235 displayed high accuracies in all three scenarios, equal to those of CSF p-tau181 and p-tau231, slightly outperforming the latter when discriminating A+T– from A+T+. *Data information:* Forest plots showing AUC values from ROC analysis ($CI_{95\%}$ available in Supplementary Table 7). Comparisons of AUC values between CSF p-tau235 and other available CSF p-tau biomarkers were determined using DeLong test (significance is indicated in bold)

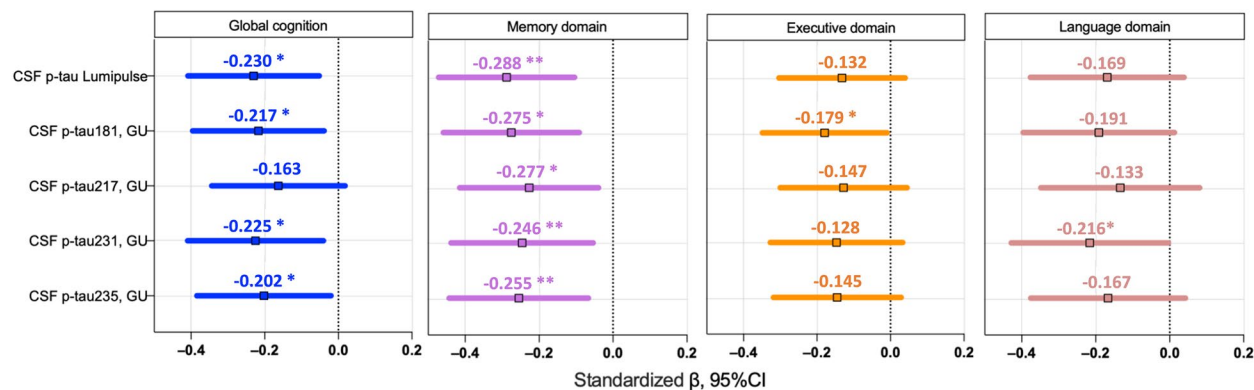
CSF p-tau235 association with cognition

In both cohorts, MMSE score was associated with CSF p-tau235 after adjustment on age, sex and level of education (Paris: $\beta=-0.304$, $P_{ADJ}<0.0001$; BIODEGMAR: $\beta=-0.247$, $P_{ADJ}=0.0009$). The association remained significant after further adjustment on $A\beta_{1-42}/40$ ratio (Paris Cohort: $\beta=-0.243$, $P_{ADJ}=0.0012$; BIODEGMAR cohort: $\beta=-0.225$, $P_{ADJ}=0.004$).

Neuropsychological assessment was available for a subset of patients in the Paris ($n=136$) and BIODEGMAR cohort ($n=139$). The cross-sectional associations of CSF p-tau235 levels and other CSF p-tau measurements

with global and domain-specific cognition are shown in Fig. 5, after adjustment on age, sex and level of education. CSF p-tau 235 associated with global cognition in both cohorts (Paris $\beta=-0.202$, $P_{ADJ}=0.046$; BIODEGMAR $\beta=-0.363$, $P_{ADJ}<0.0001$), similar to all CSF p-tau measurements but CSF p-tau217. Regarding specific cognitive domains, the strongest association was found with memory impairment, both for CSF p-tau235 and other p-tau species (CSF p-235: Paris $\beta=-0.255$, $P_{ADJ}=0.0031$; BIODEGMAR: $\beta=-0.454$, $P_{ADJ}<0.0001$). A weak association was also found with executive domain function but only in BIODEGMAR ($\beta=-0.229$, $P_{ADJ}=0.0051$).

a Paris Cohort



b BIODEGMAR Cohort

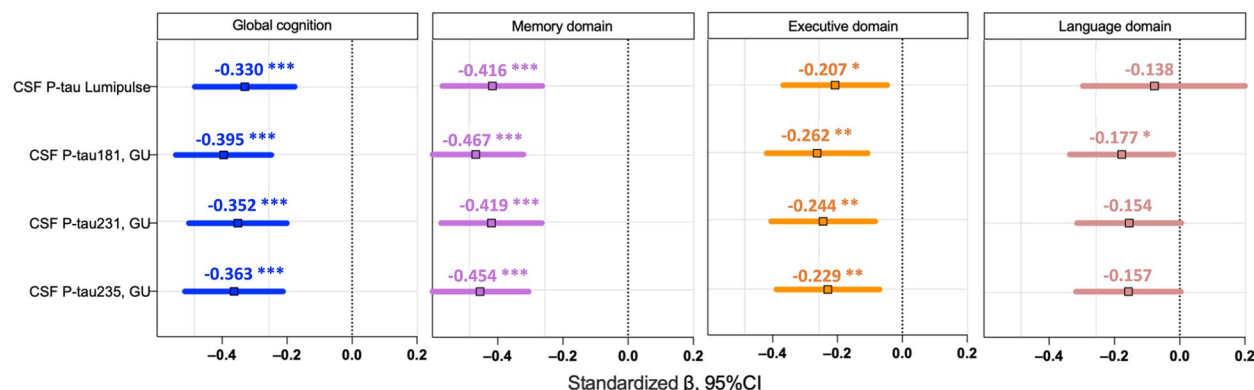


Fig. 5 CSF p-tau 235 association with global cognition and specific cognitive domains. Comparison of CSF p-tau235 and other p-tau markers (p-tau181, p-tau217 and p-tau231) for global and domain-specific cognition in **a** the Paris and **b** the BIODEGMAR cohort. *Data information:* Linear regressions were adjusted for age, sex and level of education. Estimates are presented standardized to allow for comparison in between the different p-tau biomarkers. (* $P < 0.05$, ** $P < 0.01$, *** $P < 0.001$, **** $P < 0.0001$)

Discussion

In this study, we investigated the novel CSF biomarker p-tau235 in two independent memory clinic cohorts and compared its diagnostic performance with other relevant p-tau species, specifically CSF p-tau181, p-tau217 and p-tau231. We found that (i) CSF p-tau235 is strongly associated with CSF amyloidosis, regardless of clinical presentation; (ii) high levels of CSF p-tau235 in cognitively impaired patients are highly indicative of MCI or dementia of the AD type; (iii) the main increase in CSF p-tau235 levels occurs between A+T- and A+T+, irrespective of the presence of symptoms or not; (iv) CSF p-tau235 displayed overall the same diagnostic performance as CSF p-tau181 and p-tau231, but a slightly lower diagnostic performance compared with CSF p-tau217; and (v) CSF p-tau235 levels associate with global and specific-domain cognitive decline.

In a previous study, we described CSF p-tau235 across the AD *continuum* using two research cohorts, demonstrating its high specificity for AD and its comparable

performance to other CSF p-tau species [15]. However, these findings cannot be directly translated into the real-world of memory clinics, which is characterized by higher heterogeneity in population (both in social-economic and ethnic terms), clinical presentations and co-pathologies, vastly contrasting with the stringent inclusion/exclusion criteria and detailed pathophysiological characterizations in research cohorts. Thus, the main goal of the present study was to investigate the translatability of previous CSF p-tau235 results into the routine practice of memory clinic. Consistent with our previous findings in selected research cohorts (similarly stratified using $A\beta_{1-42/40}$) [15], CSF p-tau235 was elevated across CSF $A\beta$ -positive groups in both clinical cohorts. CSF p-tau235 was increased in MCI $A\beta+$ and dementia $A\beta+$ showing similar levels between the two groups. This aligns well with previous results in a pre-clinical AD cohort in which CSF p-tau235 measurements seemingly plateau between MCI $A\beta+$ and AD $A\beta+$ [15]. Moreover, in the BIODEGMAR cohort, CSF p-tau235

was noticeably higher in CU A β ⁺ when compared with CU A β ⁻, and despite lacking sufficient statistical power (eleven and seven cases, respectively), it approached significance. Hence, these results from clinical practice are concordant with our previous results in research cohorts, where CSF p-tau235 was increased in A β ⁺ subjects both in preclinical and symptomatic AD [15]. Furthermore, these results indicate that high levels of CSF p-tau235 are strongly associated with CSF amyloidosis and highly indicative of MCI or dementia of an AD type. In terms of diagnostic accuracy identifying A β ⁺ in MCI and dementia, CSF p-tau235 showed equal performance to that of CSF p-tau181 and p-tau231, but slightly lower than CSF p-tau217. Because these differences were very marginal, with almost fully overlapping 95% confidence intervals, and together with the lack of CSF p-tau217 measurements in the BIODEGMAR cohort to corroborate these results, this should be cautiously interpreted.

CSF p-tau235 followed an increasing trajectory across AT groups, which was characterized by a minor increment between A-T- and A+T-, followed by a prominent increase from A+T- to A+T+. These differences across AT groups are similar to those previously reported in preclinical AD [15], despite the fact that the cohorts analysed here are memory clinic-based, and thus included proportionally more symptomatic cases. Therefore, this suggests that the increase in CSF p-tau235 is more prominent when abnormalities in CSF A β and p-tau are established (i.e. A+T+ cases), regardless of these cases being symptomatic, as shown here, or asymptomatic, like previously reported [15]. CSF p-tau235 showed high performance discriminating all three AT groups and was, for the most part, comparable to other CSF p-tau biomarkers. When compared with CSF p-tau181, CSF p-tau235 was outperformed when discriminating A-T- from A+T-, but only in the Paris cohort. This may suggest that N-terminal p-tau181 species emerge earlier in response to A β pathology than N-terminal p-tau235 species (which in turn start increasing earlier than mid-region p-tau181) [15, 35]. CSF p-tau217 also outperformed CSF p-tau235 in the same scenario (A-T- vs A+T-) in the Paris cohort, which aligns well with previous results indicating that CSF p-tau217 emerges prior to CSF p-tau235 [15]. On the other hand, no differences in performance could be found between CSF p-tau235 and CSF p-tau231, except when discriminating A+T- and A+T+ in the BIODEGMAR cohort, where CSF p-tau235 slightly outperformed CSF p-tau231. The marginal difference observed is likely due to the fact that CSF p-tau231 emerges very early in preclinical stages, and tends to plateau later on the *continuum* AD, thus rendering a lower discrimination between A+T- and A+T+ groups [12]. Finally, CSF p-tau235 showed a high

diagnostic accuracy discriminating A β ⁻ and A β ⁺, however, slightly lower than CSF p-tau181 and clearly lower than CSF p-tau217. Despite this, CSF p-tau235 exhibited the same performance as CSF p-tau231 in both the Paris and BIODEGMAR cohorts. In our previous publication, CSF p-tau235 started to increase in A+T- cases; however, the magnitude or extent of this increase was smaller than for other CSF p-tau species, whereas the increase was more pronounced between A+T- and A+T+ [15]. The same was observed here in this study, and the resulting overlap between the levels of CSF p-tau235 in A-T- and A+T- groups rendered the lower performance of this novel biomarker species identifying CSF amyloidosis. Thus, despite its high performance, other p-tau biomarkers emerge earlier than CSF p-tau235 in response to CSF amyloidosis and, therefore, provide a superior performance discriminating A-T- from A+T- and A β ⁻ from A β ⁺.

Higher CSF p-tau235 levels were associated with lower global cognition and memory function in both cohorts, which had not been previously investigated. CSF p-tau181 and CSF p-tau217 have been reported to associate with global cognition and memory impairment cross-sectional and longitudinally, with p-tau217 showing stronger associations with cognitive decline [36]. In our study, CSF p-tau235 performed overall similarly to other p-tau species. We observed modest differences between assays; however, we cannot determine if this reflects differences in the analytical performances of the assays or differences in the biology underlying tau phosphorylation association with cognition.

Limitations/strengths

The strengths of this study include the use of two independent memory clinic-based cohorts, allowing us to validate and confirm our findings in two different clinical settings. Secondly, both cohorts had clinically validated biomarker measurements available, which enabled the detailed stratification of participants and the subsequent investigation of CSF p-tau235 in syndromic or AT groups. Moreover, the studied cohorts included measurements of other novel p-tau species, specifically N-terminal directed CSF p-tau181, p-tau217 and p-tau231 (all measured in the same analytical platform), making possible a head-to-head comparison between different p-tau residues. However, this study does not go without limitations. CSF p-tau217 measurements were not available in the BIODEGMAR cohort, and therefore, the biomarker comparison with CSF p-tau235 could only be explored in the Paris cohort. Another limitation is that CU A β ⁺ cases were limited to the BIODEGMAR cohort, and their sample size was rather small. Considering that CSF p-tau235 increases late during preclinical AD [15], it is

expected that in cohorts richer in CU cases (especially A+T–), other CSF p-tau biomarkers that abnormally emerge earlier in the AD *continuum* (such as p-tau231 and p-tau217) [15] would provide a superior performance identifying CSF amyloidosis in asymptomatic individuals. This would subsequently affect the A–T– vs A+T– analysis, where CSF p-tau235 discriminatory accuracy would likely be lower than that of other CSF p-tau biomarkers. A limited number of patients in non-AD dementia group also warranted further studies.

Conclusions

In conclusion, our memory-clinic-based study including two well-characterized cohorts brings further evidence that p-tau235 is a novel and specific CSF biomarker for AD diagnosis, both at MCI and dementia stages. Comparison with other CSF p-tau species, including p-tau181, p-tau217 and p-tau231, supports that CSF p-tau235 should be suitable for use in clinical settings. In addition, significant association with cognitive decline adds an argument to its relevance as a clinical tool to identify and monitor CSF amyloidosis along the whole AD *continuum*. Future studies will aim to elucidate whether CSF p-tau235 can predict cognitive decline in longitudinal samples and attempt to measure p-tau235 in blood samples.

Abbreviations

AD	Alzheimer's disease
AD-MCI	Mild cognitive impairment due to AD
A β	Amyloid beta
ANOVA	Analysis of variance
AUC	Area under the curve
A β _{1–40}	A β peptide 1–40
A β _{1–42}	A β peptide 1–42
A β _{1–42/40}	Ratio A β _{1–42} /A β _{1–40}
bvFTD	Behavioural variant frontotemporal dementia
CAA	Cerebral amyloid angiopathy
CBS	Corticobasal syndrome
CJD	Creutzfeldt-Jakob disease
CSF	Cerebrospinal fluid
CU	Cognitively unimpaired
CU A β –	Cognitively unimpaired A β negative
CU A β +	Cognitively unimpaired A β positive
CI _{95%}	95% confidence interval
CV	Coefficient of variation
DLB	Dementia with Lewy bodies
FTD	Frontotemporal dementia
IP-MS	Immunoprecipitation-mass spectrometry
MCI	Mild cognitive impairment
MCI A β –	Mild cognitive impairment A β negative
MCI A β +	Mild cognitive impairment A β positive
MMSE	Mini-Mental State Examination
MS	Mass spectrometry
NFT	Neurofibrillary tangles
PPA	Primary progressive aphasia
PSP	Progressive supranuclear palsy
p-tau181	Tau phosphorylated at threonine 181
p-tau217	Tau phosphorylated at threonine 217
p-tau231	Tau phosphorylated at threonine 231
p-tau235	Tau phosphorylated at serine 235

ROC	Receiver operating characteristic curve
SCD	Subjective cognitive decline
r _s	Spearman's rank correlation coefficient
Simoa	Single molecule array
t-tau	Total tau
ULOQ	Upper limit of quantification
VCID	Vascular contributions to cognitive impairments and dementia

Supplementary Information

The online version contains supplementary material available at <https://doi.org/10.1186/s13195-023-01201-0>.

Additional file 1: Supplementary Figure 1. CSF levels of p-tau235 across clinical diagnosis. **Supplementary Figure 2.** CSF levels of p-tau235 in A β + and A β - cases. **Supplementary Figure 3.** CSF p-tau235 diagnostic performance discriminating A β + from A β - cases. **Supplementary Table 1.** Clinical diagnosis included in each syndrome group in Paris cohort. **Supplementary Table 2.** Clinical diagnosis included in each syndrome group in BIODEGMAR cohort. **Supplementary Table 3.** CSF AD biomarkers cut-offs for BIODEGMAR and Paris Cohort. **Supplementary Table 4.** Accuracies of CSF p-tau181, p-tau217, p-tau231 and p-tau235 when identifying CSF amyloidosis in dementia and MCI cases in Paris and BIODEGMAR cohort. **Supplementary Table 5.** Clinical diagnosis included in each AT group in Paris cohort. **Supplementary Table 6.** Clinical diagnosis included in each AT group in BIODEGMAR cohort. **Supplementary Table 7.** Accuracies of CSF p-tau181, p-tau217, p-tau231 and p-tau235 when discriminating AT groups in Paris and BIODEGMAR cohort. **Supplementary Table 8.** Accuracies of CSF p-tau181, p-tau217, p-tau231 and p-tau235 when discriminating A β - from A β + in Paris and BIODEGMAR cohort.

Authors' contributions

JLR, AV, AFL, HZ, MSC, CP, and KB created the concept and design. Data acquisition was performed by JLR, AFL, AV, EC and JD. JLR, AV and AFL performed data analysis. JLR, AV, AFL, POR, AES, LGM, WSB, EC, JD, APJ, ING, GGE, TK, EV, NJA, HZ, MSC, CP, KB contributed to sample selection and/or interpretation of data. JLR, AV and AFL drafted the manuscript and all authors revised. All authors read and approved the final manuscript.

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Availability of data and materials

Bulk anonymized data can be shared by request from qualified investigators, providing data transfer is in agreement with EU legislation and decisions by the institutional review board of each participating center.

Declarations

Ethics approval and consent to participate

All patients or their legal relatives in case of severe dementia gave written informed consent to their participation in this study. Collection and analysis of samples were approved by the ethic committee of Bichat University, Paris, France (CEERB GHU Nord n°10-037) for Paris cohort and by the local ethics committee (CEIC PSMAR, project code 2018/78051) for the BIODEGMAR cohort.

Consent for publication

Not applicable

Competing interests

KB has served as a consultant, at advisory boards, or at data monitoring committees for Abcam, Axon, Biogen, JOMDD/Shimadzu, Julius Clinical, Lilly, MagQu, Novartis, Prothena, Roche Diagnostics, and Siemens Healthineers, and is a co-founder of Brain Biomarker Solutions in Gothenburg AB (BBS), which is a part of the GU Ventures Incubator Program. HZ has served at scientific advisory boards and/or as a consultant for Abbvie, Acumen, Alector, Alzinova, ALZPath, Annexon, Apellis, Artery Therapeutics, AZTherapies, CogRx, Denali, Eisai, Nervgen, Novo Nordisk, Passage Bio, Pinteon Therapeutics, Prothena, Red Abbey Labs, reMYND, Roche, Samumed, Siemens Healthineers, Triplet Therapeutics, and Wave, has given lectures in symposia sponsored by Cellectricon, Fujirebio, Alzecure, Biogen, and Roche, and is a co-founder of Brain Biomarker Solutions in Gothenburg AB (BBS), which is a part of the GU Ventures Incubator Program (outside submitted work). MSC has served as a consultant and at advisory boards for Roche Diagnostics International Ltd and has given lectures in symposia sponsored by Roche Diagnostics, S.L.U and Roche Farma, S.A. MSC was granted with a project funded by Roche Diagnostics International Ltd; payments were made to the institution (BBRC). EVM is cofounder of ADx NeuroSciences.

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