

Comparative Analysis of Different Definitions of Amyloid- β Positivity to Detect Early Downstream Pathophysiological Alterations in Preclinical Alzheimer

M. Milà-Alomà^{1,2,3,4}, G. Salvadó^{1,2}, M. Shekari^{1,2,3}, O. Grau-Rivera^{1,2,4,5}, A. Sala-Vila^{1,2}, G. Sánchez-Benavides^{1,2,4}, E.M. Arenaza-Urquijo^{1,2,4}, J.M. González-de-Echávarri^{1,2}, M. Simon⁶, G. Kollmorgen⁷, H. Zetterberg^{8,9,10,11}, K. Blennow^{8,9}, J.D. Gispert^{1,2,3,12}, M. Suárez-Calvet^{1,2,4,5}, J.L. Molinuevo^{1,2,3,4} for the ALFA study†

1. Barcelona β Brain Research Center (BBRC), Pasqual Maragall Foundation. Barcelona, Spain; 2. IMIM (Hospital del Mar Medical Research Institute), Barcelona, Spain; 3. Universitat Pompeu Fabra, Barcelona, Spain; 4. Centro de Investigación Biomédica en Red de Fragilidad y Envejecimiento Saludable (CIBERFES), Madrid, Spain; 5. Servei de Neurologia, Hospital del Mar, Barcelona, Spain; 6. Roche Diagnostics International Ltd, Rotkreuz, Switzerland; 7. Roche Diagnostics GmbH, Penzberg, Germany; 8. Department of Psychiatry and Neurochemistry, Institute of Neuroscience and Physiology, Sahlgrenska Academy, University of Gothenburg, Sweden; 9. Clinical Neurochemistry Laboratory, Sahlgrenska University Hospital, Mölndal, Sweden; 10. Department of Neurodegenerative Disease, UCL Institute of Neurology, Queen Square, London, United Kingdom; 11. UK Dementia Research Institute at UCL, London, United Kingdom; 12. Centro de Investigación Biomédica en Red Bioingeniería, Biomateriales y Nanomedicina, Madrid, Spain; †The complete list of collaborators of the ALFA Study can be found in the acknowledgements section.

Corresponding Author: José Luis Molinuevo, Alzheimer Prevention Program - Barcelona β Brain Research Center, Wellington 30, 08005, Barcelona, Spain, +34933160990, E-mail: jlmolinuevo@barcelonabeta.org; Marc Suárez-Calvet, Alzheimer Prevention Program - Barcelona β Brain Research Center, Wellington 30, 08005, Barcelona, Spain, +34933160990, E-mail: msuarez@barcelonabeta.org

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Abstract

Amyloid- β (A β) positivity is defined using different biomarkers and different criteria. Criteria used in symptomatic patients may conceal meaningful early A β pathology in preclinical Alzheimer. Therefore, the description of sensitive cutoffs to study the pathophysiological changes in early stages of the Alzheimer's *continuum* is critical. Here, we compare different A β classification approaches and we show their performance in detecting pathophysiological changes downstream A β pathology.

We studied 368 cognitively unimpaired individuals of the ALFA+ study, many of whom in the preclinical stage of the Alzheimer's *continuum*. Participants underwent A β PET and CSF biomarkers assessment. We classified participants as A β -positive using five approaches: (1) CSF A β 42 < 1098 pg/ml; (2) CSF A β 42/40 < 0.071; (3) A β PET Centiloid > 12; (4) A β PET Centiloid > 30 or (5) A β PET Positive visual read. We assessed the correlations between A β biomarkers and compared the prevalence of A β positivity. We determined which approach significantly detected associations between A β pathology and tau/neurodegeneration CSF biomarkers.

We found that CSF-based approaches result in a higher A β -positive prevalence than PET-based ones. There was a higher number of discordant participants classified as CSF A β -positive but PET A β -negative than CSF A β -negative but PET A β -positive. The CSF A β 42/40 approach allowed optimal detection of significant associations with CSF p-tau and t-tau in the A β -positive group.

Altogether, we highlight the need for sensitive A β -classifications to study the preclinical Alzheimer's *continuum*. Approaches that define A β positivity based on optimal discrimination of symptomatic Alzheimer's disease patients may be suboptimal for the detection of early pathophysiological alterations in preclinical Alzheimer.

Key words: Alzheimer's disease, biomarkers, cerebrospinal fluid, positron emission tomography, preclinical Alzheimer.

Introduction

Alzheimer's disease (AD) is preceded by a long preclinical phase, which is characterized by the emergence of pathological brain changes in the absence of evident clinical symptoms. Cognitive symptoms may arise decades after the first pathological brain changes and can eventually progress to dementia. AD is therefore understood as a *continuum* (the Alzheimer's *continuum*) entailing a preclinical (*i.e.*, asymptomatic) and a clinical (*i.e.*, symptomatic) phase (1, 2). Following the latest NIA-AA guidelines, the term 'Alzheimer's disease' should be applied when there is biomarker evidence of both amyloid- β (A β) and tau pathologies (2, 3).

A key aspect in preclinical Alzheimer's studies is the biomarker-based definition of A β pathology. Currently established A β pathology biomarkers can be divided in two main categories: fluid [mainly cerebrospinal fluid (CSF) and, more recently, also blood] and neuroimaging (A β PET) biomarkers (4, 5). Although all these biomarkers may be used on a continuous scale, cutoffs are needed to dichotomize biomarker values for diagnosis or research individual categorization purposes. Different approaches may be used to establish biomarker cutoffs. These mainly include: (1) comparison against a gold standard (neuropathology, other validated biomarkers reflecting pathology), (2) optimal discrimination between

a control and a pathological group (case-control studies), (3) prediction of disease progression, or (4) description of abnormality based on population percentiles in a 'normal' or reference population (e.g., 95% of the unaffected population have lower/higher values) (2, 6, 7).

Regarding CSF A β biomarkers, a decrease in CSF A β 42 is the widely known pattern in AD, which also correlates with increased A β PET uptake (8, 9). In addition, CSF A β 42 cutoffs have been validated against A β PET as gold standard (10, 11). Nevertheless, the use of CSF A β 42 may be limited by interindividual differences in A β 42 production, and by the impact of pre-analytical and assay-related factors. To overcome some of these limitations, normalization of A β 42 with A β 40, by using the A β 42/40 ratio, was proposed (12). Studies have shown that the CSF A β 42/40 ratio is a better predictor of abnormal A β PET and that the ratio allows a more accurate identification of AD patients than CSF A β 42 (13, 14).

In the clinical setting, A β PET imaging is used to detect A β pathology in patients with cognitive impairment suspected to be AD-related. The definition of a positive or negative scan is done through visual read (VR) by trained specialists, who categorize images according to well-established criteria. The VR method has been validated against neuropathology and has shown high concordance with quantitative methods (15–17). A β PET images can be quantified using standardized uptake value ratios (SUVR), as well as with the "Centiloid" (CL) scale. The CL scale is a method for standardizing measurements to a uniform scale, thus facilitating comparisons across radiotracers and also across studies (18), and has a robust association with pathology (19, 20).

It is important to note that, although binary cutoffs allow us to stratify patients or research participants according to their biomarker profile, there is a *continuum* of A β pathology changes, that is a dynamic transition from A β -negative to A β -positive states. Therefore, binarization may leave emerging A β pathology undetected. This is especially important in the study of preclinical Alzheimer, in which the use of cutoffs that have been defined for a clinical use possibly conceals meaningful amounts of early A β pathology. Thus, the description of cutoffs that are sensitive enough to detect subtle pathological changes in the very early stage of the Alzheimer's *continuum* is of great importance (21, 22).

The aim of this study is to describe and compare different A β classification approaches, derived through both CSF and PET measures, and to show their performance in the detection of other early pathophysiological changes in the Alzheimer's *continuum*. We hypothesize that an optimal A β classification approach at this early stage would set the point where A β -downstream pathophysiological changes start.

Methods

Participants

The ALFA+ cohort is a nested longitudinal study of the ALFA (for ALzheimer and FAmilies) study, which aims at characterizing the preclinical stage of the Alzheimer's *continuum* (23). The ALFA study includes 2,743 cognitively unimpaired individuals, aged between 45 and 75 years old, which are enriched for family history of AD and *APOE- ϵ 4* carriership. In the ALFA+ cohort, participants undergo a more detailed characterization, which entails acquisition of CSF samples for biomarker levels determination, neuroimaging biomarkers (MRI and FDG and A β PET), *APOE* genotyping and cognitive assessments. Among the ALFA+ participants, 381 had available CSF biomarker measurements. Since we aimed at studying participants in the Alzheimer's *continuum*, we excluded 13 participants that had a CSF biomarker of suspected non-Alzheimer's pathology (2), as defined by CSF A β 42/40 \geq 0.071 and CSF p-tau $>$ 24 pg/ml. None of them was A β -positive using A β PET in any of the approaches defined below. Thus, 368 participants were finally included in the present study. Of those, a subset of 303 and 301 participants also had CL quantification or VR assessment of A β PET imaging, respectively. For all participants, the time difference between CSF collection and A β PET assessments was less than one year.

CSF biomarkers assessment

CSF samples were obtained by lumbar puncture and collected, processed and stored following standard procedures (24). Total tau (t-tau) and phosphorylated tau (p-tau) measurements were performed using the electrochemiluminescence Elecsys® Total-Tau CSF and Phospho-Tau(181P) CSF immunoassays, respectively. CSF A β 42 and A β 40 were measured with the prototype NeuroToolKit (Roche Diagnostics International Ltd.). All assays were performed on a fully automated cobas e 601 or e 401 instrument (Roche Diagnostics International Ltd.) at the Clinical Neurochemistry Laboratory, Sahlgrenska University Hospital, Mölndal, Sweden.

PET imaging acquisition and processing

Imaging acquisition and preprocessing protocols have been described previously (20). In brief, a T1-weighted MRI and an [18F] Flutemetamol PET scan was acquired in all participants. The T1-weighted 3D-TFE sequence was acquired in a Philips 3 T Ingenia CX scanner. PET imaging was conducted in a Siemens Biograph mCT, following a cranial CT scan for attenuation correction. Participants were injected with 185 MBq (range 166.5–203.5 Mbq) of [18F] Flutemetamol, and 4 frames of 5 min each were acquired 90 min post-injection.

PET processing was performed following the standard CL pipeline (18) using SPM12. In brief, PET images are first coregistered to their respective T1-weighted images and, afterwards, moved to MNI space using the normalization transformation derived from the segmentation of the T1-weighted image. PET images were intensity normalized using whole cerebellum as reference region. CL values were calculated from the mean values of the standard CL target region (<http://www.gaain.org/centiloid-project>) using the transformation previously calibrated (20).

A nuclear medicine physician visually rated the scans as A β -positive or A β -negative using standard clinical criteria as specified in the Summary of Product Characteristics of the tracer (https://www.ema.europa.eu/en/documents/product-information/vizamyl-epar-product-information_en.pdf).

A β pathology biomarkers and cutoffs

We classified the participants as A β -positive or A β -negative using five different A β biomarker/cutoff combinations (A β classification approaches; Table 1). Each of the approaches were determined as follows. In approach 1, participants with a CSF A β 42 < 1098 pg/ml were considered as A β -positive. This cutoff had been previously established in concordance with A β PET in a similar cohort and had area under the ROC curve (AUC) of 0.85 to discriminate between A β PET-positive or negative (10). In approach 2, we used CSF A β 42/40 as a biomarker of A β pathology and we determined a cutoff using a Gaussian mixture model (GMM). The cutoff was defined as the mean minus 2 standard deviations (SD) of the non-pathologic Gaussian distribution. As the fitting of the GMM can vary depending on initial conditions, the process was repeated 1000 times with random initial conditions. The final cutoff for CSF A β 42/40, derived as the median value of the 1000 repetitions, was 0.071. In both approaches 3 and 4, we used the A β PET CL scale as biomarker of A β pathology, but with different cutoffs. In approach 3, we defined A β positivity when CL > 12; this cutoff has been previously proposed to detect subtle A β pathology in a comparison against CSF A β 42 (20). In approach 4, we defined A β positivity when CL > 30, a cutoff that falls within the optimal range of agreement

with VR method in clinical populations (24 - 35 CL), and has been proposed to detect established A β pathology (19,20,25–27). Finally, in approach 5, we categorized participants as A β -positive or A β -negative using the A β PET VR, the approach most commonly used in the clinical setting.

Statistical analysis

CSF biomarker distributions were tested for normality with the Kolmogorov-Smirnov test and histograms visual inspection. CSF A β 42, p-tau and t-tau did not follow a normal distribution and were thus log₁₀-transformed. In contrast, CSF A β 42/40 ratio followed a normal distribution and was not transformed. CSF biomarker extreme values were identified following Tukey criterion (*i.e.*, values falling at over three times the interquartile range below the first quartile or above the third quartile) and excluded from the analyses.

Differences in demographic characteristics and CSF biomarker values as a function of A β classification schemes were assessed by means of chi-squared or one-way analysis of variance (ANOVA) tests, as appropriate. Correlations between A β biomarkers were tested using partial Spearman rank correlation, corrected by age. We used a chi-squared analysis in order to test significant differences in A β -positive prevalence using different A β classification approaches.

We tested the association between tau and neurodegeneration CSF biomarkers (*i.e.*, CSF p-tau, t-tau) and the A β biomarker (*i.e.*, CSF A β 42, A β 42/40 or CL scale) in a linear model, adjusting by the effect of age and sex. These analyses were performed separately in A β -positive or A β -negative groups as defined by each approach. Moreover, in order to determine which of the approaches was more sensitive to detect early pathophysiological changes, we conducted the same analyses in the whole cohort but additionally introducing in the model the interaction term 'A β biomarker x A β group'. A significant interaction term denotes that the association of the A β biomarker with CSF p-tau and t-tau differs between the A β -negative and A β -positive groups, as defined by that approach. These analyses were performed in each of the five A β classification approaches.

Table 1. Summary of A β biomarkers/cutoffs combinations used in each A β classification approach

	Aβ biomarker	Aβ positivity cutoff
Approach 1	CSF A β 42	CSF A β 42 < 1098 pg/ml
Approach 2	CSF A β 42/40	CSF A β 42/40 < 0.071
Approach 3	A β PET	CL > 12
Approach 4	A β PET	CL > 30
Approach 5	A β PET	Visual Read

Abbreviations: A β , amyloid- β ; CSF, cerebrospinal fluid; CL: Centiloids.

Table 2. Participants' demographic characteristics and biomarker levels by five Aβ classification approaches

	Aβ-negative							Aβ-positive					
	Total	Approach					P	Approach					
		1	2	3	4	5		1	2	3	4	5	P
Age, years	61.2 (4.69)	60.8 (4.44)	60.6 (4.44)	60.5 (4.59)	60.7 (4.59)	60.6 (4.59)	0.97	61.7 (4.96)	62.3 (4.93)	63.8 (4.13)*	65.7 (2.64)†,‡	64.5 (3.56)§	<0.0001
Female, n (%)	223 (64.9)	133 (64.7)	146 (61.6)	158 (62.2)	169 (60.8)	161 (61.7)	0.92	90 (55.2)	77 (58.8)	29 (59.2)	18 (72.0)	24 (60.0)	0.62
APOE-ε4 carriers, n (%)	196 (53.3)	87 (42.4)	97 (40.9)	122 (48.0)	143 (51.4)	128 (49.0)	0.094	109 (66.9)	99 (75.6)	38 (77.6)	17 (68.0)	30 (75.0)	0.40
Education, years	13.4 (3.50)	13.1 (3.52)	13.5 (3.46)	13.5 (3.42)	13.5 (3.41)	13.6 (3.35)	0.72	13.7 (3.46)	13.2 (3.57)	12.8 (3.71)	12.2 (3.95)	12.3 (4.02)	0.10
Biomarkers													
CSF Aβ42 (pg/ml)	1062 (326)	1624 (431)	1469 (514)§	1362 (523)	1318 (529) ,‡	1333 (528) ,‡	<0.0001	814 (179)	896 (307)	826 (274)	801 (196)	925 (386)	0.14
CSF Aβ42/40 ratio	0.069 (0.018)	0.084 (0.013)	0.086 (0.009)	0.079 (0.016)§,	0.070 (0.017) ,	0.078 (0.017)†,	<0.0001	0.061 (0.018)	0.051 (0.012)	0.047 (0.011)	0.040 (0.008)	0.048 (0.016)†	<0.0001
Centiloid scale	3.01 (17.3)	-2.76 (10.6)	-4.69 (6.48)	-3.28 (6.85)	-1.29 (9.32)#	-2.07 (8.65)**	0.0004	10.3 (21.0)	16.9 (21.5)	35.6 (18.4) ,	50.8 (12.6) ,	34.3 (23.3)‡, , ††	<0.0001
CSF p-tau (pg/ml)	15.5 (5.93)	16.3 (4.99)	13.8 (4.23)	14.5 (4.87)†	14.7 (5.05)†	14.5 (4.95)†	<0.0001	14.42 (6.82)	18.6 (7.27)	21.3 (7.95)	25.9 (6.94) , **	22.8 (7.56)	<0.0001
CSF t-tau (pg/ml)	192 (64.7)	203 (53.9)	175 (48.6)	182 (55.1)†	184 (56.5)†	182 (55.7)†	<0.0001	178 (74.1)	224 (77.3)	249 (81.2)	293 (71.6) , **	264 (76.2)	<0.0001

Data are expressed as mean (M) and standard deviation (SD) or number of participants (n) and percentage (%), as appropriate. One-way ANOVA followed by Tukey corrected *post hoc* comparisons was used to compare age and education and Pearson's Chi-square test to compare sex and APOE-ε4 status between approaches within each Aβ-group. CSF biomarkers and centiloid scale were compared with an ANCOVA adjusted by age and sex followed by Tukey corrected *post hoc* comparison. The P-values indicated in the last column refer to the approach main effect. All P-values are corrected for multiple comparisons using the FDR method; Abbreviations: Aβ42, amyloid-β 42; p-tau, phosphorylated tau; t-tau, total tau; * P<0.05 vs approach 1; † P<0.001 vs approach 1; ‡ P<0.01 vs approach 2; § P<0.01 vs approach 1; | P<0.0001 vs approach 1; | P<0.0001 vs approach 2; # P<0.001 vs approach 2; ** P<0.05 vs approach 2; †† P<0.05 vs approach 4

All statistical models were adjusted for the effects of age and sex. For all analysis, we applied a false discovery rate (FDR) multiple comparison correction following the Benjamini-Hochberg procedure (28). All tests were 2-tailed, with a significance level of $\alpha = 0.05$.

Results

Participants' demographics

Participants' characteristics are depicted in Table 2. We compared the demographical characteristics of the Aβ-positive and Aβ-negative groups defined by the different Aβ classification approaches. We observed that Aβ-positive participants were significantly older using PET-based versus CSF-based approaches. There were not significant differences in years of education, frequency of the APOE-ε4 allele or sex between the different Aβ-classification approaches.

Most of the AD biomarkers significantly differed between the five Aβ classification approaches within the Aβ positive or Aβ-negative group (Table 2). PET-based Aβ classification approaches (namely, approach 3, 4 and 5) had a more pathologic biomarker profile (that is, lower CSF Aβ42 and CSF Aβ42/40 ratio, but higher CL scale and CSF p-tau and t-tau), than the CSF-based Aβ classification approaches (namely, approach 1 and 2). These results suggest that CSF-based Aβ biomarkers are capturing Aβ accumulation at an earlier stage of the Alzheimer's *continuum*. This idea is supported by the fact that Aβ-negative groups defined by PET-based approaches had higher levels of CSF p-tau and t-tau (Table 2).

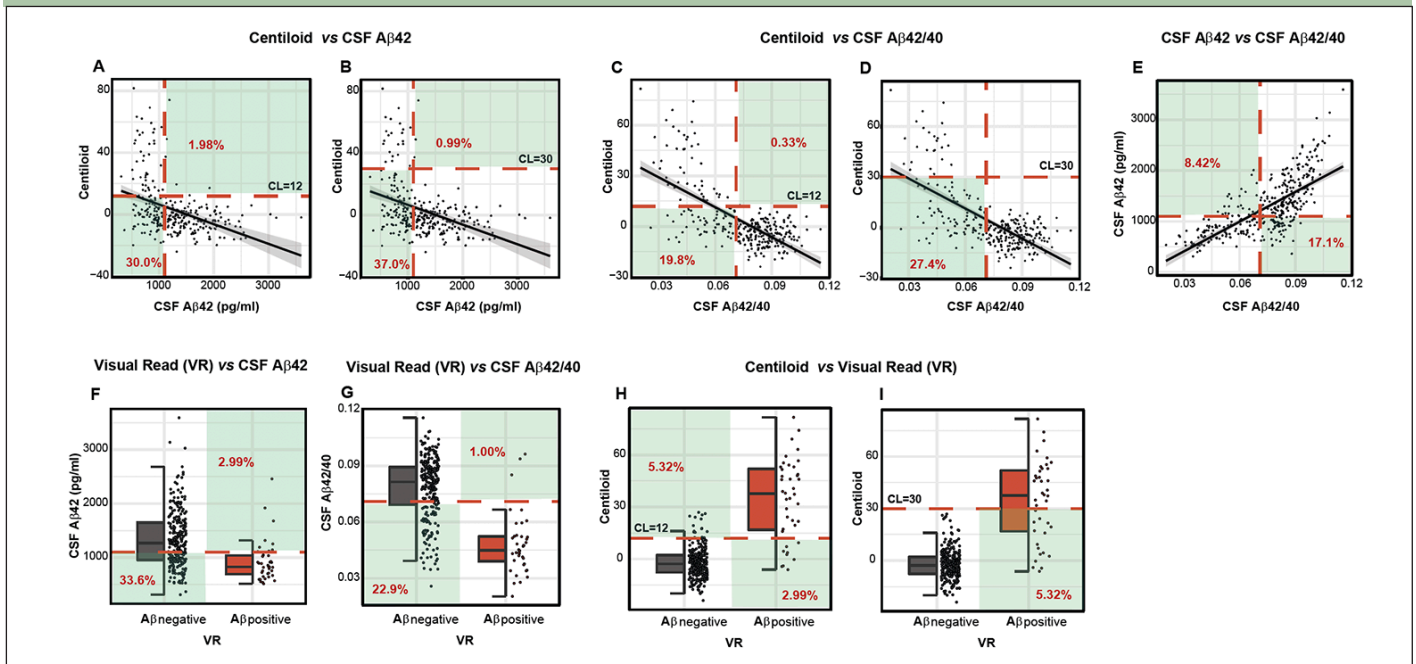
Correlations between Aβ biomarkers

We tested the correlations between CSF and PET Aβ biomarkers, corrected by age. There was a moderate correlation between CSF Aβ42 and CL values (Spearman's rho = -0.41; P<0.0001; Figure 1A and B) and CSF Aβ42/40 and CL values (Spearman's rho = -0.54; P<0.0001; Figure 1C and D). There was also a high correlation between CSF Aβ42 and CSF Aβ42/40 ratio (Spearman's rho = -0.75; P<0.0001; Figure 1E).

Prevalence of Aβ positivity using different Aβ classification approaches

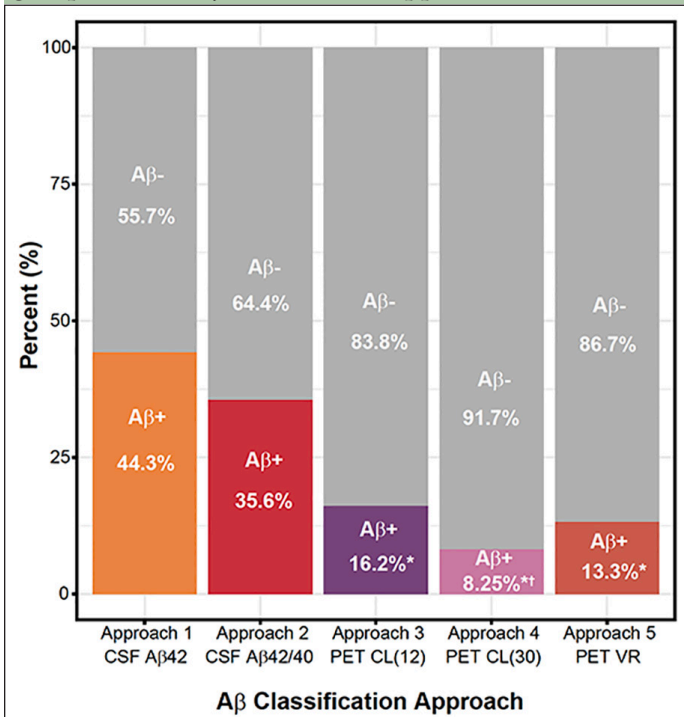
We next determined the prevalence of Aβ-positive and Aβ-negative participants according to each Aβ classification approach and, remarkably, we found a wide range of Aβ-positive prevalences ranging from 8.25 to 44.3% (Figure 2; Table 3). Approach 1 (*i.e.*, CSF Aβ42) resulted in the highest prevalence of Aβ-positive participants (N = 163, 44.3%), followed by approach 2 (CSF Aβ42/40 ratio; Aβ-positive: N = 131, 35.6%), approach 3 (CL > 12; Aβ-positive: N = 49, 16.2%), approach 5 (VR; Aβ-positive: N = 40, 13.3%) and, finally, approach 4 (CL > 30; Aβ-positive: N = 25, 8.25%) (Figure 2). The prevalence of Aβ-positive participants in CSF-based classification approaches (approach 1 and 2) was significantly higher than that in PET-based classification approaches (approach 3, 4 and 5, P < 0.0001; Figure 2). Notably, approach 3 (CL > 12) showed a statistically significant higher prevalence of Aβ-positive participants compared to approach 4 (CL > 30, P = 0.043, Figure 2).

Figure 1. Correlation between Aβ classification approaches



Scatter plots representing the associations between each Aβ biomarker. Each point depicts the value of the Aβ biomarker of an individual. Green areas depict discordant subjects and percentage of them is shown in red. The red dashed lines indicate the Aβ biomarker cutoffs used in each Aβ classification approach: CSF Aβ42 = 1098 pg/ml, CSF Aβ42/40 = 0.071, CL = 12 and CL = 30. A, B. Correlation and comparison between CSF Aβ42 and CL; cutoff CL = 12 (A) and CL = 30 (B). C, D. Correlation and comparison between CSF Aβ42/40 and CL; cutoff CL = 12 (C) and CL = 30 (D). E. Correlation and comparison between CSF Aβ42 and CSF Aβ42/40. F. Comparison between CSF Aβ42 and VR. G. Comparison between CSF Aβ42/40 and VR. H, I. Comparison between VR and CL; cutoff CL = 12 (H) and CL = 30 (I). Abbreviations: Aβ, amyloid β; CL, Centiloids; CSF, Cerebrospinal fluid; PET, Positron Emission Tomography

Figure 2. Prevalence of Aβ-negative and Aβ-positive groups in each Aβ classification approach



The graph depicts the prevalence of the Aβ-positive (Aβ+; color) and Aβ-negative (Aβ-; grey) individuals in each Aβ classification approach. We conducted a pair wise chi squared comparison between all the approaches and we found significant statistical differences between them ($P < 0.0001$). Bonferroni-corrected pair-wise *post hoc* comparisons: * $P < 0.0001$ vs approach 1 and approach 2, † $P < 0.05$ vs approach 3. Abbreviations: Aβ, amyloid-β; CL, Centiloids; CSF, Cerebrospinal fluid; PET, Positron Emission Tomography; VR, Visual Reads.

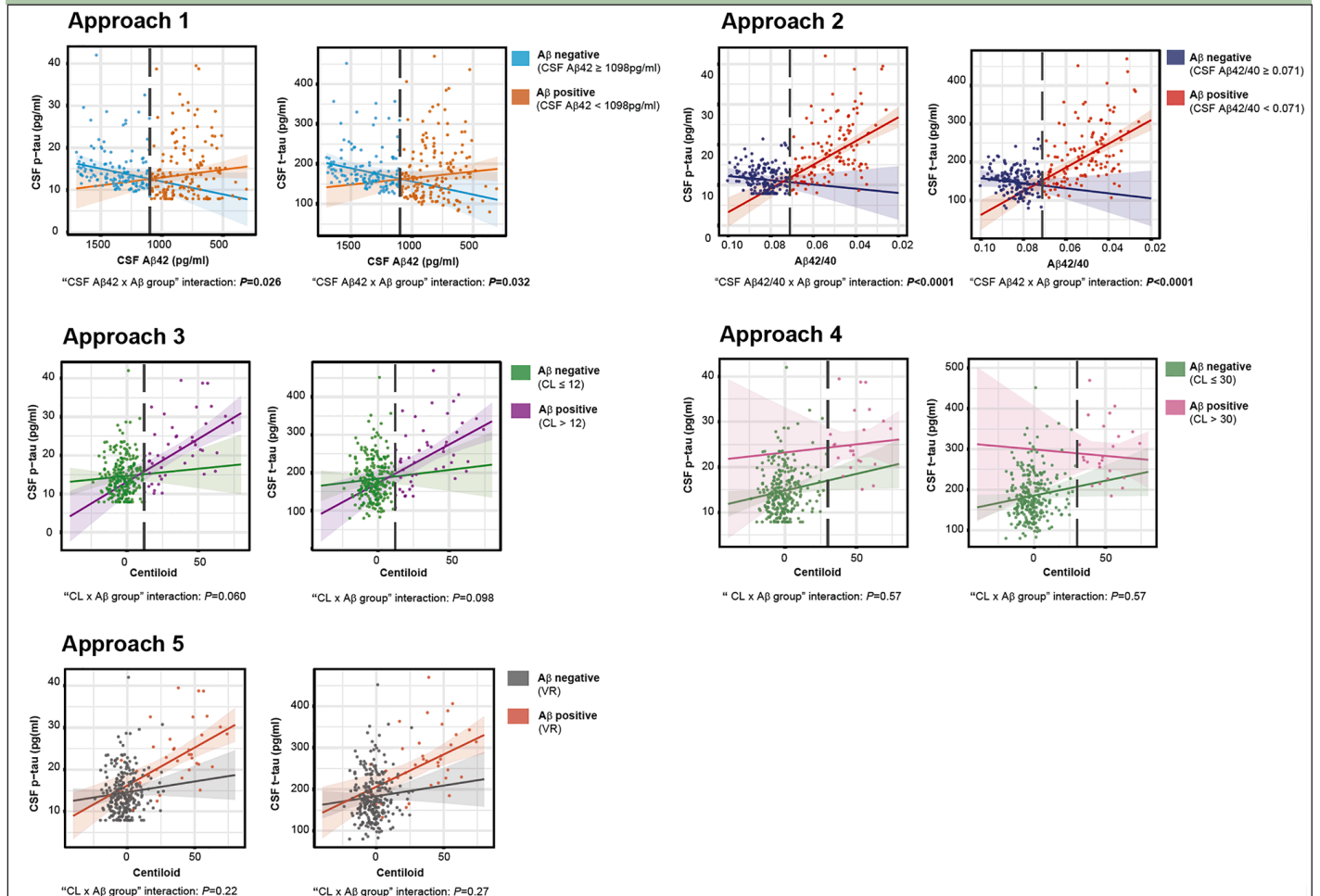
We assessed the differences between the CSF- and PET-defined Aβ-positive participants (*i.e.*, CSF/PET-discordant participants) within each Aβ classification approach (shown in Table 3 and Figure 1). The participants that were classified as CSF Aβ-positive but PET Aβ-negative range from 19.8 to 37.0% (Table 3; green areas in Figure 1). The highest discordance occurred between Aβ classification approach 4 (CL < 30) and approach 1 (CSF Aβ42; 37.0% discordance; Figure 1B), and the lowest occurred between approach 3 (CL < 12) and approach 2 (CSF Aβ42/40; 19.8% discordance, Figure 1C).

By contrast, fewer participants showed discordance in the other direction, *i.e.*, a CSF Aβ-negative but a PET Aβ-positive biomarker (range: 0.33% to 2.99%; Figure 1; Table 3). Of note, the proportion of discordant participants was higher for CSF Aβ42 (approach 1) than for CSF Aβ42/40 ratio (approach 2) in all comparisons.

Association of Aβ biomarkers with tau pathology and neurodegeneration CSF biomarkers

We next assessed whether changes in tau pathology and neurodegeneration CSF biomarkers as a function of Aβ pathology differed before and after the computed Aβ cutoffs. We hypothesized that an optimal Aβ biomarker cutoff would discriminate between an Aβ-negative state (where Aβ pathology is not associated with downstream

Figure 3. Association of each Aβ biomarker with CSF p-tau and t-tau by Aβ classification approach



Scatter plots representing the associations of CSF p-tau and t-tau with each Aβ biomarker in Aβ-positive and Aβ-negative groups. Each point depicts the value of the CSF biomarker of an individual and the solid lines indicate the regression line for each group. The P-values shown for the 'Aβ biomarker x Aβ group' interaction were computed using a linear model adjusting for age and sex. All P-values are corrected for multiple comparisons using the FDR method. The dashed lines indicate the Aβ biomarker cutoffs used in each Aβ classification approach: CSF Aβ42 = 1098 pg/ml, CSF Aβ42/40 = 0.071, CL = 12 and CL = 30; Abbreviations: Aβ, amyloid β; CL, Centiloids; CSF, Cerebrospinal fluid; PET, Positron Emission Tomography; p-tau, phosphorylated tau; t-tau, total tau; VR, Visual Reads.

Table 3. Contingency table with the percentage of agreement and discordance between the different Aβ classification approaches

		Approach 2 CSF Aβ42/40		Approach 3 CL (12)		Approach 4 CL (30)		Approach 5 VR	
		Aβ -	Aβ +	Aβ -	Aβ +	Aβ -	Aβ +	Aβ -	Aβ +
Approach 1 CSF Aβ42	Aβ -	47.3	8.42	53.8	1.98	54.8	0.99	53.2	2.99
	Aβ +	17.1	27.2	30.0	14.2	37.0	7.26	33.6	10.3
Approach 2 CSF Aβ42/40	Aβ -			64.0	0.33	64.3	-	63.8	1.00
	Aβ +			19.8	15.8	27.4	8.25	22.9	12.3
Approach 3 CL (12)	Aβ -					83.8	-	81.4	2.99
	Aβ +					7.92	8.25	5.32	10.3
Approach 4 CL (30)	Aβ -							86.7	5.32
	Aβ +							-	7.97

Data are expressed as percentage (%) of participants in each category. Bold letters indicate discordance. We performed a chi-square test of independence to examine the relation between Aβ positivity and the Aβ classification approach used and we observed that the percentage of Aβ positivity was not equally distributed between Aβ-classification approaches $\chi^2(4, N = 301) = 176, P < 0.0001$. A Bonferroni corrected pair-wise *post hoc* comparisons showed significant differences between the approach 1 and approach 3, 4 and 5 ($P < 0.0001$), between the approach 2 and approach 3, 4 and 5 ($P < 0.0001$), and between approach 3 and 4 ($P < 0.05$). Abbreviations: Aβ, amyloid-β; Aβ+, amyloid-β positive; Aβ-, amyloid-β negative; CL, Centiloids; VR, Visual Read.

Table 4. CSF p-tau and t-tau associations with A β biomarkers by A β classification approach

	Approach 1				
	CSF A β 42 negative		CSF A β 42 positive		CSF A β 42 x A β group
	β (SE)	Adj <i>P</i>	β (SE)	Adj <i>P</i>	Adj <i>P</i>
CSF p-tau	0.029 (0.010)	0.007*	-0.011 (0.014)	0.84	0.026*
CSF t-tau	0.025 (0.009)	0.007*	-0.002 (0.012)	0.86	0.032*
	Approach 2				
	CSF A β 42/40 negative		CSF A β 42/40 positive		CSF A β 42/40 x A β group
	β (SE)	Adj <i>P</i>	β (SE)	Adj <i>P</i>	Adj <i>P</i>
CSF p-tau	0.012 (0.008)	0.13	-0.077 (0.013)	<0.0001*	<0.0001*
CSF t-tau	0.013 (0.008)	0.13	-0.067 (0.011)	<0.0001*	<0.0001*
	Approach 3				
	CL (12) negative		CL (12) positive		CL x A β group
	β (SE)	Adj <i>P</i>	β (SE)	Adj <i>P</i>	Adj <i>P</i>
CSF p-tau	0.007 (0.009)	0.40	0.073 (0.023)	0.007*	0.060
CSF t-tau	0.008 (0.008)	0.40	0.057 (0.021)	0.008*	0.098
	Approach 4				
	CL (30) negative		CL (30) positive		CL x A β group
	β (SE)	Adj <i>P</i>	β (SE)	Adj <i>P</i>	Adj <i>P</i>
CSF p-tau	0.018 (0.008)	0.046*	0.006 (0.026)	0.86	0.57
CSF t-tau	0.016 (0.008)	0.046*	-0.005 (0.025)	0.86	0.57
	Approach 5				
	VR negative		VR positive		CL x A β group
	β (SE)	Adj <i>P</i>	β (SE)	Adj <i>P</i>	Adj <i>P</i>
CSF p-tau	0.062 (0.009)	<0.0001*	0.065 (0.022)	0.010*	0.22
CSF t-tau	0.050 (0.008)	<0.0001*	0.050 (0.020)	0.022*	0.27

CSF p-tau or t-tau were assessed by a linear model with the A β biomarker (CSF A β 42, CSF A β 42/40 or CL) as main effect and age and sex as covariates. This analysis was performed separately in A β -negative or A β -positive groups as defined by each approach. Moreover, we added the interaction term 'A β biomarker x A β group' in order to test statistical differences in the regression slopes between A β -negative and -positive groups. The standardized regression coefficients (β) and standard errors (SE) are depicted. *P*-values are corrected for multiple comparisons using FDR method. *Significant values; Abbreviations: A β 42, amyloid- β 42; CL, Centiloids; p-tau, phosphorylated tau; t-tau, total tau; VR, Visual Read.

tau pathology and neurodegeneration changes) and an A β -positive state (where those downstream changes arise). For these purposes, we computed the association between each A β biomarker and CSF p-tau and t-tau in A β -negative and A β -positive groups defined by the different A β classification approaches. We also computed the interaction term 'A β biomarker x A β group'.

The results of the analyses are summarised in Table 4 and Figure 3. Remarkably, robust statistically significant interactions occurred using approach 2. In other words, the cutoff derived from the CSF A β 42/40 ratio significantly discriminated between two states: (1) an A β -negative state where tau and neurodegeneration CSF biomarkers were not associated with CSF A β 42/40 levels and (2) an A β -positive state where CSF p-tau and t-tau significantly increase as a function of higher A β pathology (*i.e.*, decreased CSF A β 42/40 ratio).

In approach 1 (*i.e.*, CSF A β 42), we also observed statistical significance in the interaction term

'A β biomarker x A β group' for CSF p-tau and t-tau. Nevertheless, both CSF p-tau and t-tau were positively associated with CSF A β 42 in the A β -negative group.

None of the A β -positive and A β -negative groups defined with A β PET-based approaches differed in their association between CL scale and CSF p-tau and t-tau. However, there was a tendency to statistical significance in the interaction term 'A β biomarker x A β group' for CSF p-tau in approach 3 (*i.e.*, CL > 12). Moreover, there was a significant association between CSF p-tau and t-tau with CL scale in the A β -positive group using approach 3 and approach 5 (*i.e.*, VR).

Discussion

In this study, we compared five different approaches to classify cognitively unimpaired individuals as A β -positive or A β -negative, and we found considerable

differences between them. Specifically, our results show that: (1) A β -positive prevalence vary from 8 to 44% depending on the approach used; (2) A β CSF-based approaches result in the highest prevalence of A β -positive subjects compared with PET-based approaches; (3) Discordant classification appears more frequently as a CSF A β -positive but PET A β -negative (19.8 - 37.0%), while only a few individuals were CSF A β -negative but PET A β -positive (0.33 - 2.99%); (4) In A β PET, the use of a lower CL cutoff (12 CL instead of 30 CL) increases the detection of A β -positive individuals based on CSF levels; (5) among all A β classification approaches assessed, the CSF A β 42/40 ratio cutoff shows the greater concordance with A β PET imaging (using the 12 CL cutoff), and the most significant difference of regression slopes between the A β -positive and A β -negative groups in the association with A β downstream pathology (e.g., tau pathology and neurodegeneration).

The ATN classification is widely used in AD research to classify patients or study participants based on biomarker evidence of A β pathology (A), tau pathology (T) or neurodegeneration/neuronal injury (N) (7). Each biomarker group can be defined with different biomarkers. For A β pathology (A), the most accepted biomarkers are CSF A β 42, the CSF A β 42/40 ratio and A β PET.

In regard to CSF A β biomarkers, CSF A β 42 has been the most widely used, and cutoffs have been established against A β PET (10, 11). However, increasing evidence favors the use of CSF A β 42/40 ratio to normalize both preanalytical and interindividual differences, which provides a higher reproducibility and specificity for AD pathology (13, 14). We show that the CSF A β 42 approach results in a higher number of A β -positive participants than any other approach. However, CSF A β 42 approach also results in a higher frequency of discrepancies with the PET-based approaches compared to the CSF A β 42/40 ratio approach. This observation is in line with the idea that the CSF A β 42/40 ratio predicts more accurately A β PET than CSF A β 42. Furthermore, a high concordance between A β PET VR and ratios of CSF biomarkers have been shown to best distinguish A β -positive from negative individuals in both cognitively unimpaired and impaired populations. The CSF p-tau/A β 42 ratio has a high diagnostic clinical utility as its cutoff has been derived using A β PET VR concordance (10). However, we have not used this ratio in this setting because it represents a mixed pathology of A β and tau, and tau biomarkers become abnormal later in the disease stage. For CSF A β 42, we used the cutoff of 1098 pg/ml, that was previously defined in a study where CSF A β 42 was measured by a Roche Elecsys assay and a ROC curve was built in comparison against A β PET. This cutoff is also very similar to that obtained in the BioFINDER study (1100 pg/ml) which is anchored to a positive A β PET VR (11).

For CSF A β 42/40, we rendered a cutoff with a different

approach. Instead of comparing the CSF values with A β PET as the gold standard, we applied a data-driven approach, namely a Gaussian Mixture Modelling (GMM). This method allows to describe a normal range of CSF A β 42/40 ratios in the non-pathological group and derive a cutoff defined as the mean minus 2 standard deviations of this Gaussian distribution. By definition, under this criterion, 97.7% of A β -negative subjects are expected to display high CSF A β 42/40 values. In other words, this criterion is highly specific by design. We hypothesised that this method would be more sensitive to detect incipient A β pathology in cognitively unimpaired individuals than criteria based on discriminating asymptomatic from symptomatic AD populations. In fact, GMM has already been used to derive cohort-specific CSF A β cutoffs, showing a robust ability to discriminate normal and pathologic distributions of A β 42 (29–31). Our results confirm the suitability of both using the CSF A β 42/40 ratio and deriving its cutoff with a GMM method. In fact, approach 2 shows the lowest number of discordant values compared with any other approach. According with our hypothesis, we observed that this approach provides optimal discrimination between A β -negative participants, showing no associations with A β -downstream pathologies (as measured by CSF p-tau and t-tau), and A β -positive participants, which display significant association with them. This is shown by the fact that this approach results in a significant difference of regression slopes between the A β -positive and A β -negative groups in the association between CSF p-tau or t-tau as a function of A β pathology. In fact, visual inspection of Figure 4 shows that the CSF A β 42/40 cutoff we have computed by GMM falls close to the intersection of the regression lines in the A β -positive and A β -negative groups. Remarkably, this result is not seen when using the CSF A β 42 approach. In this approach, we found unexpected significant positive correlations between CSF A β 42 and both CSF p-tau and t-tau in the A β -negative group. Without the A β 40 normalization, CSF A β 42 may unspecifically correlate with other proteins because of the abovementioned limitations in terms of interindividual differences (high and low A β producers) and preanalytical variability.

In relation to A β PET biomarkers, A β PET classification can be performed through VR, widely used in the clinical practice, or using a quantitative approach such as the CL scale. This scale is expressed as universal units and allows the comparison among different tracers and studies (18). Different cutoffs to categorize participants according to CL might be used, depending on the sample and on the study objectives. Optimal cutoffs for CL against VR typically fall within the range of 24 - 35 CL in clinical populations (25–27). However, these VR-based CL cutoffs might not be optimal to study the preclinical stage of the Alzheimer's *continuum*, when the goal is to detect subtle A β accumulation. Therefore, here we compared three different PET-based approaches: CL > 12, which was

established as a cutoff with an optimal agreement against the standard cutoff of CSF A β 42 < 1098 pg/ml (20) and is expected to detect subtle early A β changes; CL > 30, a cutoff falling within the range proposed to robustly detect established A β pathology (19,20,25–27); and VR, expected to represent a cutoff closer to the clinical practice. As expected, a cutoff of CL > 12 showed a higher prevalence of A β -positive individuals than the other two PET-based approaches. The slopes of CSF p-tau or t-tau as a function of CL did not significantly differ between the A β -positive and A β -negative groups defined by the CL12 approach, although tendency to this direction is observed, especially for CSF p-tau. However, the CL12 approach allowed the detection of significant associations of CSF p-tau and t-tau in the A β -positive group, an association that is also observed with the VR approach, but not with the CL30 one. These results suggest that the CL12 approach may be detecting A β deposition that is already associated with increasing tau pathology and neurodegeneration biomarkers. On the contrary, classifying A β groups using CL > 30 as cutoff, resulted in the lowest prevalence of A β -positive individuals and did not allow the discrimination of changes in A β -downstream CSF biomarkers changes between the two A β -groups. This suggests that 30 CL is a late cutoff to apply in preclinical Alzheimer's studies, especially in cohort such as ALFA+, comprised by individuals in the very earliest phase of the Alzheimer's *continuum*. A β -positive subjects according to the CL30 criterion are probably in a more advanced stage of A β pathology. We did not find a significant association between either CSF t-tau or p-tau with CL in the A β -positive group defined by the CL30 approach. This might be explained by the reduced sample size in that A β -positive group (n = 25), but also because A β -pathology might start to plateau in this late stage of preclinical Alzheimer. Conversely, a positive association is observed in the A β -negative group, supporting the idea that CL30 is a late cutoff and that A β -downstream processes have arisen before this cutoff. Interestingly, the VR approach rendered an A β -positive prevalence closer to the CL12 approach than to the CL30 approach. Of note, the raters in our study are highly trained to detect early A β deposition, but may differ in other studies or in the clinical setting. Similarly to the CL30 approach, the VR approach does not discriminate between the associations of the CL scale with CSF t-tau or p-tau in the A β -positive and A β -negative groups, although in this case positive associations are found in the A β -positive group.

Finally, we found obvious differences between the CSF- and PET-based A β classification approaches. Our results show only a moderate correlation between A β CSF and A β PET, which is not surprising as they may reflect different aspects of A β pathology, *i.e.*, soluble A β and fibrillar A β aggregates, respectively (1). Noteworthy, the proportion of discordant values that were CSF A β -positive but PET A β -negative was considerably higher than those that were CSF A β -negative but PET

A β -positive. This is consistent with the idea that A β CSF biomarkers detect earlier stages of A β pathology than A β PET, that is A β dysregulation that precedes A β plaques formation (32). Furthermore, this earlier dysregulation seems to be already associated to changes in A β -downstream events (*i.e.*, tau pathology and neurodegeneration), thus proving the importance of studying this very early stage.

Our study has some limitations: (1) it is a cross sectional study and we could not track the progression of A β biomarkers, which would be particularly relevant in the A β -discordant biomarkers individuals; (2) it is a comparative analysis between different approaches, lacking a gold standard, so conclusions on sensitivity and specificity for each approach cannot be drawn. However, since comparisons among A β positivity criteria are made in the same dataset, conclusions on the relative sensitivity to detect associations with downstream pathophysiological events are valid; (3) results might not be generalizable to other cohorts because ALFA+ is a very specific cohort aimed at studying the preclinical stage of the Alzheimer's *continuum*.

Overall, in this study we show that, in the preclinical stage of the Alzheimer's *continuum*, there are important differences in the prevalence and the characteristics of A β -positive and A β -negative study participants depending on the A β classification approach used for the dichotomization. This emphasizes the importance of considering the sample and the study aims when deriving cutoffs for participant dichotomization. This is particularly important when studying the preclinical Alzheimer's *ccontinuum* stage, where the approaches usually used in symptomatic AD patients may be not suitable to detect subtle changes in A β pathology.

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Conflicts of Interest: JLM has served/serves as a consultant or at advisory boards for the following for-profit companies, or has given lectures in symposia sponsored by the following for-profit companies: Roche Diagnostics, Genentech, Novartis, Lundbeck, Oryzon, Biogen, Lilly, Janssen, Green Valley, MSD, Eisai, Alector, BioCross, GE Healthcare, ProMIS Neurosciences. JDG has given lectures in symposia sponsored by the following for-profit companies: General Electric, Philips and Biogen. KB has served as a consultant or at advisory boards for Abcam, Axon, Biogen, Lilly, MagQu, Novartis and Roche Diagnostics, 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 for Denali, Roche Diagnostics, Wave, Samumed and CogRx, has given lectures in symposia sponsored by Fujirebio, Alzecure and Biogen, and is a co-founder of Brain Biomarker Solutions in Gothenburg AB, a GU Ventures-based platform company at the University of Gothenburg. GK is a full-time employee of Roche Diagnostics GmbH. MS is a full-time employee of Roche Diagnostics International Ltd. The remaining authors declare that they have no conflict of interest.

Ethical Standards: The ALFA+ study (ALFA-FPM-0311) was approved by the Independent Ethics Committee "Parc de Salut Mar," Barcelona, and registered at ClinicalTrials.gov (Identifier: NCT02485730). All participants signed the study's informed consent form that had also been approved by the Independent Ethics Committee "Parc de Salut Mar," Barcelona.

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