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# Plasma A $\beta$ 42/40 ratio alone or combined with FDG-PET can accurately predict amyloid-PET positivity: a cross-sectional analysis from the AB255 Study

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## Abstract

**Background:** To facilitate population screening and clinical trials of disease-modifying therapies for Alzheimer's disease, supportive biomarker information is necessary. This study was aimed to investigate the association of plasma amyloid-beta (A $\beta$ ) levels with the presence of pathological accumulation of A $\beta$  in the brain measured by amyloid-PET. Both plasma A $\beta$ 42/40 ratio alone or combined with an FDG-PET-based biomarker of neurodegeneration were assessed as potential AD biomarkers.

**Methods:** We included 39 cognitively normal subjects and 20 patients with mild cognitive impairment from the AB255 Study who had undergone PiB-PET scans. Total A $\beta$ 40 and A $\beta$ 42 levels in plasma (TP42/40) were quantified using ABtest kits. Subjects were dichotomized as A $\beta$ -PET positive or negative, and the ability of TP42/40 to detect A $\beta$ -PET positivity was assessed by logistic regression and receiver operating characteristic analyses. Combination of plasma A $\beta$  biomarkers and FDG-PET was further assessed as an improvement for brain amyloidosis detection and diagnosis classification.

**Results:** Eighteen (30.5%) subjects were A $\beta$ -PET positive. TP42/40 ratio alone identified A $\beta$ -PET status with an area under the curve (AUC) of 0.881 (95% confidence interval [CI] = 0.779–0.982). Discriminating performance of TP42/40 to detect A $\beta$ -PET-positive subjects yielded sensitivity and specificity values at Youden's cutoff of 77.8% and 87.5%, respectively, with a positive predictive value of 0.732 and negative predictive value of 0.900. All these parameters improved after adjusting the model for significant covariates. Applying TP42/40 as the first screening tool in a sequential diagnostic work-up would reduce the number of A $\beta$ -PET scans by 64%. Combination of both FDG-PET scores and plasma A $\beta$  biomarkers was found to be the most accurate A $\beta$ -PET predictor, with an AUC of 0.965 (95% CI = 0.913–0.100).

**Conclusions:** Plasma TP42/40 ratio showed a relevant and significant potential as a screening tool to identify brain A $\beta$  positivity in preclinical and prodromal stages of Alzheimer's disease.

**Keywords:** Plasma, Amyloid-beta, FDG-PET, Biomarker, Preclinical Alzheimer's disease, Amyloid-PET, Mild cognitive impairment

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## Background

Alzheimer's disease (AD) is a progressive neurodegenerative disorder and the most common cause of dementia. Accumulating data from clinical research support that the AD pathophysiologic process starts decades before the onset of clinical symptoms [1–3]. The disease develops in a continuum from a preclinical stage in which amyloid pathology has been defined as the earliest Alzheimer's pathological changes [3–5]. Thus, individuals at the preclinical or prodromal AD stages represent an important target group in the context of clinical trials and population screening. New diagnostic procedures can identify measurable brain changes by positron emission tomography (PET) and cerebrospinal fluid (CSF) analysis, even at the preclinical stage. These procedures have been incorporated in the National Institute of Aging–Alzheimer's Association A/T/(N) research framework for the biological definition of AD [5].

A large number of clinical studies very consistently show that these neuroimaging or CSF biomarkers provide relevant information for the diagnosis. Yet, blood-based biomarkers are the desirable tool for large-scale assessments of patients either in clinical research or, eventually, in primary clinical settings, due to their cost-effectiveness and easiness of procedures [6].

Despite previous studies yielding contradictory results [7–9], recent works have shown an association of low plasma A $\beta$ 42/40 ratio with AD [10–13]. Plasma A $\beta$ 42/40 ratio has also demonstrated value in detecting brain A $\beta$  pathological changes [14–16], even using different methodological approaches as mass spectrometry (MS) and new-generation immunological methods [17–19]. Our group has been largely working on the development of reliable and informative plasma biomarkers based on A $\beta$  measurements [20]. We have previously reported a consistent association between a low TP42/40 ratio and both clinical MCI diagnosis [21] and A $\beta$  accumulation in the brain [22, 23]. Reduced TP42/40 levels were also found to predict higher rates of A $\beta$  accumulation in the brain [22, 24]. Furthermore, it has been recently reported that lower TP42/40 ratio levels were also associated with increased cortical uptake of the [18F]Flortaucipir tau-PET marker in AD-related regions [25].

PET imaging, using the most widely available radio-tracer ([18F]fluorodeoxyglucose PET, FDG-PET) as a measure of cerebral glucose metabolism, is a marker of neurodegeneration that has been established as a sensitive tool for detecting neuronal dysfunction [26]. FDG-PET diagnostic performance could be upgraded by the use of quantitative indices that have been developed to account for inter-observer variability and to support challenging evaluation [27–29]. Arbizu et al. have previously described FDG-based automated quantitative scores, such as the AD conversion score (AD-Conv

score), which has proved to predict and detect AD dementia with good diagnostic performance [30].

This work was primarily aimed to investigate the potential value of the TP42/40 ratio as a screening tool for brain A $\beta$ -PET positivity in a population of 59 CN and amnesic MCI (a-MCI) patients. This population was partially explored in a previous work in which clinical performance of TP42/40 was evaluated (considering clinical diagnosis as the gold standard), together with a general assessment of the correlation of TP42/40 with CSF and A $\beta$ -PET biomarkers. With this study, we pursued confirming previous results from our own group in an independent AIBL population [22], focusing in the discriminating performance of TP42/40 in detecting A $\beta$  positivity in the brain. Secondly, we have explored the potential value added by the combination of A $\beta$  plasma levels and AD-Conv score to improve prediction of A $\beta$ -PET status at early disease stages. Furthermore, in the framework of the new biological concept of AD based in the A/T/(N) system [5], we also explored if combination of both the TP42/40 plasma ratio, as a  $\beta$ -amyloid marker (A), and AD-Conv score, reflecting neurodegeneration (N), might help in clinical assessment at early stages.

## Methods

### Participants

The AB255 Study is a multicenter longitudinal study with evaluations of the cognitive status of individuals at 0, 12, and 24 months, including 83 cognitively normal (CN) and 145 age-paired subjects with probable a-MCI [31], all over 64 years of age. The study was designed to evaluate the potential of blood-based A $\beta$  biomarkers to detect AD. A complete description of the AB255 Study protocols and population details have been previously referred [21]. Clinical diagnosis of each participant was performed using an extensive neurological examination [32] and a battery of neuropsychological tests, including evaluation of global cognition using the Mini-Mental State Examination [33, 34] and verbal learning and memory by The Word List Learning test from the Wechsler Memory Scale-Third Edition (WMS-III) [35], delayed recall and a recognition task without list of interference [36], and the Free and Cued Selective Reminding Test (FCSRT) [37]. A subpopulation of 39 cognitively normal subjects and 20 patients with a-MCI who had undergone PiB-PET scans and fulfilled criteria for inclusion were considered for the present work. Participants were all recruited and assessed from 2010 to 2013 at *The Memory Clinic* from Fundació ACE (Barcelona, Spain) [38].

### Plasma analyses

EDTA blood was obtained after overnight fasting, and samples were immediately cooled to 2–8 °C until processed within 30 h of collection by centrifugation at

2500×g for 15 min at 4 °C. Plasma was appropriately aliquoted in polypropylene tubes and stored at –80 °C until analysis, avoiding freeze-thaw cycles. ABtest40 and ABtest42 ELISA kits (Araclon Biotech Ltd., Spain) were used for the quantification of total Aβ40 and Aβ42 in plasma after proprietary treatment of the sample. The specific analytical procedures and performance characteristics of these tests are described elsewhere [20]. Samples were randomized and encoded by an external CRO to guarantee the validity of results.

#### PiB-PET analysis

Cortical Aβ burden was assessed by PET using 11C-Pittsburg compound B (PiB-PET). Detailed procedures of neuroimaging acquisition and analysis are described by Espinosa et al. [31]. Imaging data were analyzed using the Fundació ACE Pipeline for Neuroimaging Analysis, available at <http://detrinitus.fundacioace.com/>. Participants were classified as β-amyloid positive (PET-Aβ(+)) or β-amyloid negative (PET-Aβ(-)) with relation to a cutoff of 1.4 SUVR in PiB-PET scans [39].

#### FDG-PET analysis

PET acquisition was performed after 4 h of fasting and once it was confirmed that blood glucose levels were below 110 mg/dl. The dose of <sup>18</sup>FDG was established according to weight (150 μCi/kg), being the standard adult dose 10 mCi (370 MBq) in a volume of 1–10 ml saline. Dosimetry was established in accordance with ICRP 53. For adults, critical organ dose (bladder) was 0.16 mGy/MBq and effective dose was 0.019 mSv/MBq. Acquisition started 40 min after <sup>18</sup>FDG administration and lasted for 20 min. FDG-PET scans were analyzed following the procedure previously described by Arbizu et al. [30]. Using this automated voxel-based analytical method, quantitative indices were developed to compute the hypometabolic pattern of each subject. The AD-Conv score integrates the information provided by PET into a multivariate model including age, gender, Mini-Mental State Examination (MMSE) score, and APOE ε4 genotype.

#### APOE genotyping

Genomic DNA was isolated from EDTA blood. Target DNA was amplified by PCR and digested with the restriction enzyme HhaI. APOE genotyping was carried out by subsequent restriction analysis of the pattern of fragments obtained after electrophoresis in a polyacrylamide gel [40].

#### Statistical analysis

Statistical analysis was performed using SPSS version 22 for Windows (SPSS Inc., Chicago, IL). A probability level of  $p < 0.05$  was considered statistically significant. Total in plasma Aβ42/40 ratio (TP42/40) and AD-Conv score

were used as the predictive variables. Demographic characteristics and biomarker levels were first compared between groups using chi-squared and Mann-Whitney tests as appropriate. Statistically significant differences in TP42/40 levels between Aβ-PET(+) and Aβ-PET(-) individuals were evaluated using generalized linear regression models (GLM) adjusted for significant demographic covariates (age, APOE genotype, and clinical group). Correlation of quantitative measures of both plasma TP42/40 ratio and AD-Conv score with Aβ-PET SUVR was assessed by Spearman rank correlation analyses.

The association of TP42/40 and AD-Conv score with PET-based abnormal amyloid status was further investigated using logistic regression followed by receiver operating characteristic (ROC) curve analyses. Predicted values of binary logistic regression models were used to combine variables in ROC analysis. All TP42/40 models were adjusted for age, APOE genotype (APOEε4 allele carriers versus non-carriers), and clinical diagnosis. AD-Conv score was determined by a multivariate model already including age and APOE ε4 genotype, so it was not readjusted for these covariates in the analysis. Plasma and FDG-PET biomarkers were evaluated alone or in combination, so that improvement in classification performance using both amyloid and neurodegeneration information derived from them was assessed. Youden's index maximizing cutoffs were considered for evaluation of biomarker performance in detecting brain Aβ positivity, either individually or combined. This same procedure was applied to the total AB255 population to assess TP42/40 and AD-Conv score clinical discrimination ability (CN versus a-MCI). For visualization purposes, heat maps representing predicted probability of brain Aβ positivity depending on the chosen TP42/40 cutoff were depicted with regard to age and APOE genotype.

#### Results

Demographic characteristics of the population included in this study are summarized in Table 1. Eighteen (30.5%) individuals were classified as Aβ-PET(+), and most of them were clinically diagnosed as a-MCI (14 a-MCI Aβ-PET(+)) versus 6 a-MCI Aβ-PET(-)). Likewise, 35 out of the 39 CN individuals were classified as Aβ-PET(-). Comparing Aβ-PET(+) and Aβ-PET(-) groups, subjects with abnormal Aβ-PET status were on average older and were more frequently APOE ε4 carriers. Age, APOE ε4, and clinical diagnosis were significantly different between groups, whereas no statistically significant differences were found with regard to gender or education level (Table 1). Both AD-Conv score and TP42/40 were significantly different between Aβ-PET groups (Mann-Whitney  $p < 0.001$ ) and correlated with Aβ-PET SUVR measures (TP42/40  $r_s = -0.464$ ; AD-Conv score  $r_s = 0.581$ ; both  $p < 0.001$ ). Figure 1 shows

**Table 1** Demographic and biomarker features of this population

	Aβ-PET (-)	Aβ-PET (+)	p value
N (%)	41 (69.5%)	18 (30.5%)	
Age, mean (SD)	71.6 (4.11)	75.2 (5.65)	p 0.014
Gender Female, n (%)	18 (43.9%)	9 (50.0%)	p 0.665
APOE4 carriers, n (%)	6 (14.6%)	12 (66.7%)	p < 0.001
Education level, mean years (SD)	12.33 (3.96)	10.56 (4.44)	p 0.113
CN/a-MCI, n (% a-MCI)	35/6 (14.6%)	4/14 (77.8%)	p < 0.001
TP42/40, mean (SD)	0.1329 (0.0208)	0.0997 (0.0197)	p < 0.001
AD-Conv score, mean (SD)	0.133 (0.162)	0.384 (0.259)	p < 0.001

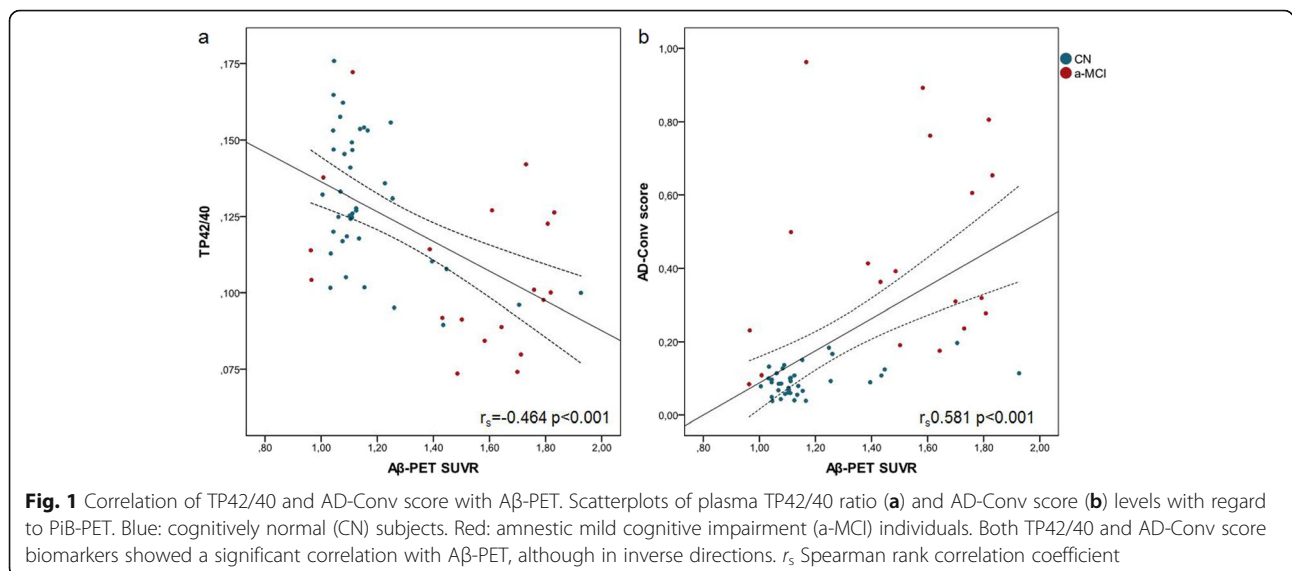
p value was obtained from Mann-Whitney or chi-squared tests as appropriate. SD standard deviation, CN cognitively normal, a-MCI amnesic mild cognitive impairment, Aβ amyloid-beta, TP42/40 total plasma Aβ42/40 ratio, AD-Conv score AD conversion score based on FDG-PET

these associations including information of the clinical status of each participant.

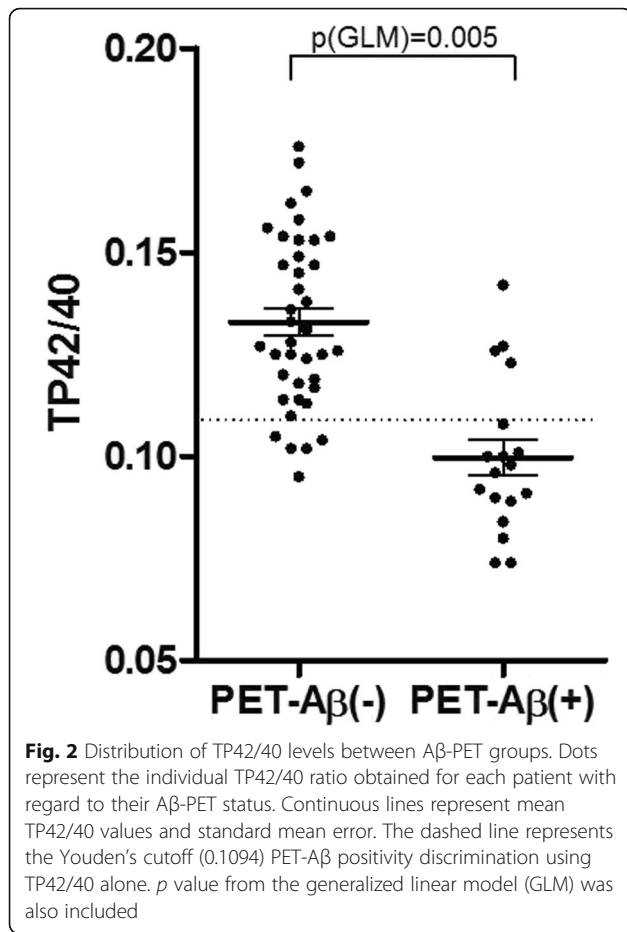
Plasma TP42/40 ratio was in average 25% lower in PET-Aβ(+) subjects compared to PET-Aβ(-) individuals (Table 1 and Fig. 2). After adjusting for significant demographic covariates (age, APOE 4, and clinical diagnosis), association of TP42/40 with Aβ-PET status remained statistically significant (GLM  $\beta = 87.67$ ,  $p = 0.005$ ). ROC analysis revealed an AUC of 0.881 (95% confidence interval [CI] 0.779–0.982) for the prediction of Aβ-PET positivity using TP42/40 alone (Fig. 3a). The Youden’s cutoff of plasma TP42/40 ratio was 0.1049 and yielded a sensitivity of 77.8% and a specificity of 87.5% in detecting PET-Aβ(+) subjects (Table 2). When studying independently CN and a-MCI subjects, we found excellent performance of TP42/40 in the CN group (AUC unadjusted TP42/40 0.957, 95% CI 0.890–1.000), although it declined in the a-MCI group (AUC unadjusted TP42/40 0.814, 95% CI 0.617–1.000) (Table 2).

When adjusting TP42/40 with relevant covariates (age, APOE genotype, and clinical group), discrimination was

improved yielding an AUC of 0.963 (95% CI 0.917–1.000). Sensitivity, specificity, and NPV were all over 0.920 (Fig. 3b, Table 2). We used the logistic regression model to predict probabilities of Aβ-PET positivity with regard to age in both APOE  $\epsilon 4$  non-carriers (Fig. 4a) and carriers (Fig. 4b). This figure represents a statistical inference since the actual study population does not cover all the age ranges represented. As expected, the predicted probability increased with age and was always higher in APOE  $\epsilon 4$  carriers compared to non-carriers. Low TP42/40 ratio considerably augmented likelihood of abnormal Aβ-PET status for every genotype and age condition. Indeed, the high NPV and low LR- characterized TP42/40 as an ideal marker for pre-screening. Considering a hypothetical clinical trial targeting Aβ-PET-positive subjects, a recruitment procedure based in Aβ-PET alone would require 3.3 PET scans per successful recruitment. However, following a sequential strategy including a TP42/40 pre-screening stage would require 1.2 Aβ-PET scans per successful recruitment, implying a 64% saving in PET scans.







AD-Conv score was also evaluated as a predictor of Aβ-PET positivity in this cohort, showing good diagnostic performance (AUC 0.898, 95% CI 0.817–0.979) (Fig. 3b), and sensitivity and specificity values at maximum Youden's cutoff of 100% and 67.5%, respectively

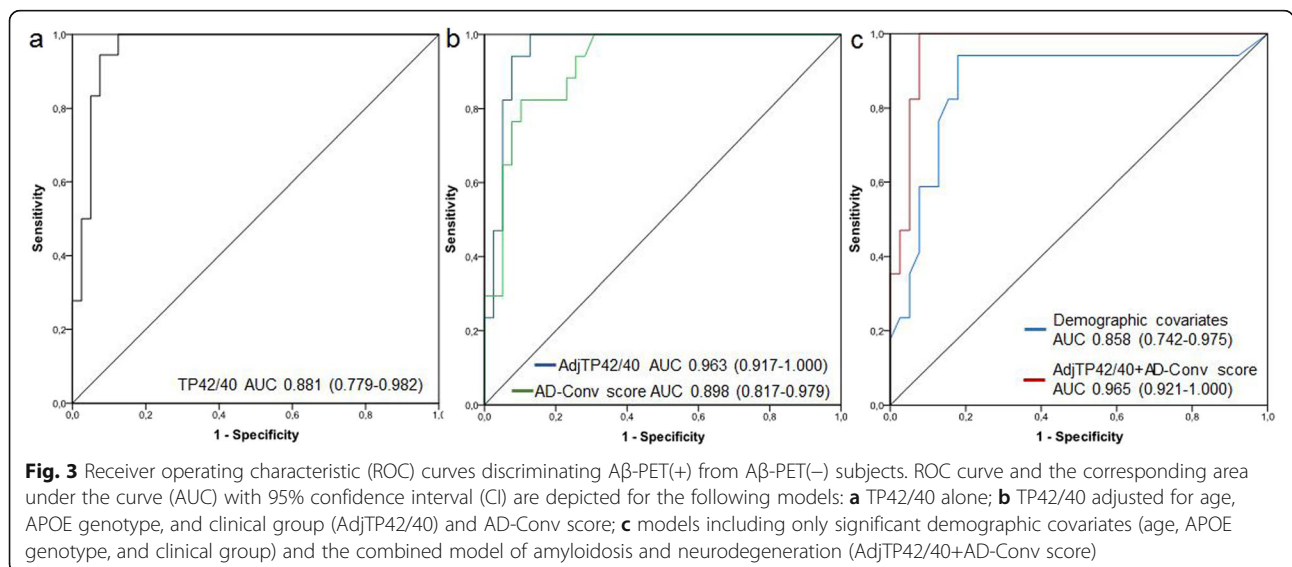
(Table 2). Thus, we hypothesized that a combination of both plasma TP42/40 and neurodegeneration (FDG-PET) biomarkers could outperform the single-predictor models in detecting Aβ-PET(+) subjects.

The combined model of plasma Aβ and neurodegeneration biomarkers (AdjTP42/40+AD-Conv score) improved performance of both individual biomarkers and covariates alone (Fig. 3c) to discriminate Aβ-PET status. AUC for this model was found to be 0.965 (95% CI 0.921–1.000), with very high sensitivity and specificity (> 92%) and NPV of 1.00 (Table 2).

On the other hand, TP42/40 performed poorly at discriminating between CN and a-MCI patients in the whole AB255 study population (AUC = 0.639, 95% CI 0.564–0.714). Nevertheless, combining TP42/40 with AD-Conv score improved subjects' clinical classification up to an AUC of 0.915 (95% CI 0.875–0.955). The effect is mainly driven by AD-Con score which by itself alone presented an AUC of 0.893 (95% CI 0.849–0.937).

### Discussion

In this study, we aimed at assessing the potential value of TP42/40 ratio in the prediction of brain Aβ pathology measured by Aβ-PET in a preclinical and prodromal AD cohort of 59 patients from the AB255 Study. In addition, the present study evaluated the potential benefit of the combination of amyloid and neurodegeneration biomarkers, reflected by plasma TP42/40 and the FDG-PET-derived AD-Conv score [30]. Both individually and combined, these biomarkers significantly detected Aβ pathology in the brain. Combination of adjusted TP42/40 ratio and AD-Conv score was found to be the most accurate Aβ-PET predictor, with sensitivity of 100% and specificity of 92.3%.



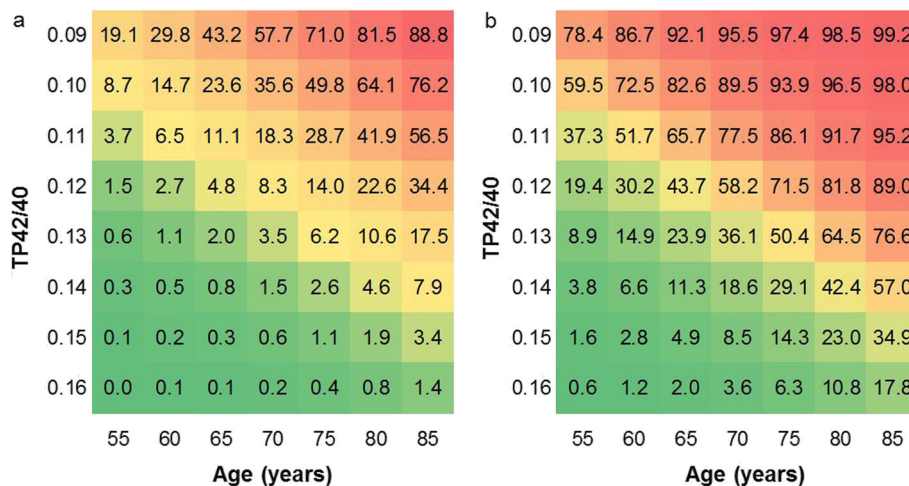
**Table 2** Diagnostic performance of individual and combined models in detecting Aβ-PET positivity at Youden's cutoff

	Sensitivity	Specificity	PPV	NPV	LR+	LR-
Whole population (N = 59)						
TP42/40	0.778	0.875	0.732	0.900	6.22	0.25
Age+APOE4+Clinical Group	0.944	0.805	0.680	0.945	4.84	0.07
TP42/40+Age+APOE4+Clinical Group	0.944	0.925	0.847	0.974	12.59	0.06
AD-Conv score	1.000	0.675	0.706	1.000	3.08	0.00
AdjustedTP42/40+AD-Conv score	1.000	0.923	0.851	1.000	13.00	0.00
Only CN (N = 39)						
TP42/40	1.000	0.886	0.500	1.000	8.75	0.00
Age+APOE4	0.750	0.943	0.600	0.971	12.12	0.26
TP42/40+Age+APOE4	1.000	1.000	1.000	1.000	-	0.00
AD-Conv score	1.000	0.765	0.327	1.000	4.25	0.00
AdjustedTP42/40+AD-Conv score	1.000	1.000	1.000	1.000	-	0.00
Only a-MCI (N = 20)						
TP42/40	0.714	1.000	1.000	0.653	-	0.29
Age+APOE4	0.357	0.833	0.799	0.411	2.14	0.77
TP42/40+Age+APOE4	0.643	1.000	1.000	0.601	-	0.36
AD-Conv score	0.846	0.500	0.759	0.636	1.70	0.31
AdjustedTP42/40+AD-Conv score	0.615	1.000	1.000	0.583	-	0.38

Cases in which LR+ was not quantifiable were presented as -. Prevalence considered for each group calculation was corresponding to the present cohort: 30.5% in the whole population, 10.25% in the CN group, and 65% in the a-MCI group. PET PPV-positive predictive value, NPV negative predictive value, LR+ positive likelihood ratio, LR- negative likelihood ratio

Aβ-PET(+) patients showed significantly lower levels of TP42/40, with an average reduction of 25% compared to the Aβ-PET(-) group. This significant inverse association of plasma Aβ42/40 ratio with brain Aβ deposition is in accordance with previous studies [14–19]. These

findings also replicate our previous results showing the potential of TP42/40 as a surrogate biomarker of Aβ-PET status in an independent preclinical population [22]. TP42/40 ratio adjusted for significant covariates detected Aβ-PET positivity in the present cohort with



**Fig. 4** Heat maps showing predicted probability of being Aβ-PET(+) based on TP42/40 and age stratified for APOE genotype. Predicted probabilities in APOE ε4 non-carriers (a) and APOE ε4 carriers (b). Probabilities are displayed as percentages. Note that TP42/40 decreases upward in axis for optimal visualization purposes. It should be noted that probabilities were predicted from a logistic regression model modeling expected probabilities in the age range most likely present during pre-screening or clinical assessment. These predicted probabilities could be more accurately determined in a larger population including the complete age range

94.4% sensitivity and 92.5% specificity. This means that in a sequential screening scenario aiming to detect subjects with abnormal A $\beta$ -PET status, a first step assessing TP42/40 would allow a 64% reduction in the number of A $\beta$ -PET scans. Considering the restricted availability and costs of A $\beta$ -PET neuroimaging [41], this sequential strategy would imply a significant reduction of the patient burden and procedural costs, both for clinical trials and patient management at primary care settings.

Clinical performance of plasma A $\beta$ 42/A $\beta$ 40 in the present study is comparable to that obtained using MS-based methods [17, 18] or new-generation immunological assays [19] and are congruent with other studies demonstrating a consistent inverse association of the plasma A $\beta$ 42/A $\beta$ 40 with the disease [10–13, 42, 43]. Nevertheless, there still exists considerable overlap of plasma A $\beta$  ratio between diagnostic groups or amyloid status groups, which limits the use of A $\beta$  plasma ratios for clinical diagnosis at the individual level. On the other hand, clinical performance of plasma biomarkers is also hampered by the fact that A $\beta$ -PET classification, used as the gold standard, neither is utterly precise. In line with this, plasma TP42/40 false-positive results could correspond to a-MCI subjects who are already accumulating A $\beta$  in the brain, but have not reached the threshold for abnormal status yet. If this were the case, plasma A $\beta$  biomarkers would turn out to be abnormal before A $\beta$ -PET does. Alternatively, misclassification could be due to heterogeneity of the underlying disease in a-MCI patients. When using only CN to explore TP42/40 potential in detecting A $\beta$ -PET+ subjects, results are better compared to the a-MCI group or the mixed population. Adjusted TP42/40 can accurately detect the 4 AB-PET+ individuals in the cohort of 39 CN, which confirms the preclinical potential of this biomarker when detecting A $\beta$  abnormality in the brain, detected also in previous work from our group [22]. However, due to the small size of the CN and a-MCI groups in the present population, together with the different prevalence of A $\beta$ -PET positivity, these findings should be taken cautiously and further replicated.

AD-Conv score has proven a significant correlation with A $\beta$ -PET and a good performance in detecting brain A $\beta$  pathology within this cohort, being the AUC values obtained within the range previously defined [30]. These results reinforce the suitability of quantitative indices derived from automated FDG-PET measures replacing standard qualitative brain FDG-PET imaging [27–29].

Previous studies evaluating the association of FDG-PET and A $\beta$ -PET measures have shown discrepant results. Several works did not find a correlation in AD [44–46], MCI [47], and CN individuals [48, 49], although it was found in other cohorts [50–54]. Another study including both CN and MCI individuals showed a

significant correlation between PiB-PET and FDG-PET [55], corresponding well to our findings.

Only few studies have explored combination of FDG-PET and A $\beta$  biomarkers, mostly in CSF and strictly focused on conversion to AD dementia [43, 46–48]. Interestingly, combination of TP42/40 and AD-Conv score improves subjects' clinical classification into CN or a-MCI. As could be expected, the effect is mainly driven by the AD-Conv score which itself integrates into a multivariate model FDG-PET neurodegeneration (downstream to  $\beta$ -amyloid changes as per the amyloid cascade hypothesis) with APOE genotype, MMSE score, age, and gender. This approach is in agreement with the recent research framework based on the A/T/(N) system [5], and our findings support the suitability of a biological definition of AD, measured by biomarkers that are reflecting different processes underlying AD clinical manifestations.

Larger and varied populations need to be explored in order to confirm the potential utility of FDG-PET and TP42/40 combination, which may vary depending on population characteristics and disease stage. In fact, the overall improvement of the adjusted TP42/40 model by AD-Conv score, although relevant, was relatively modest in our cohort. Another weakness of our study is the absence of a PiB-PET follow-up, which would be of great utility in ascertaining the potential prognostic value of plasma biomarkers to predict the rate of brain A $\beta$  deposition and could have provided relevant evidence for elucidating the possible source of bias in the classification performance.

## Conclusions

This study demonstrates a consistent inverse association of plasma TP42/40 and FDG-PET biomarkers with A $\beta$ -PET status. Findings from this work replicate our previous results in different cohorts, hence confirming TP42/40 as a useful biomarker to rule out brain  $\beta$ -amyloidosis in preclinical and early AD stages. Plasma TP42/40 tests may be used as an initial screening tool for patient management; only those resulting TP42/40 positives would have to undergo diagnostic confirmation by CSF analysis or PET neuroimaging. Integration of neurodegeneration and blood-based A $\beta$  biomarkers could contribute to early AD diagnosis in compliance with the A/T/(N) research framework, although it requires further validation.

## Abbreviations

AD: Alzheimer's disease; PET: Positron emission tomography; CSF: Cerebrospinal fluid; CN: Cognitively normal; MCI: Mild cognitive impairment; A $\beta$ -PET: Amyloid-beta PET; MS: Mass spectrometry; A $\beta$ : Amyloid-beta; TP42/40: Total in plasma A $\beta$ 42/40; AIBL: Australian Imaging Biomarkers and Lifestyle Study of Aging; FDG-PET: [18F]fluorodeoxyglucose PET; AD-Conv score: AD conversion score (FDG-PET); a-MCI: Amnesic MCI; CRO: Contract research organization; ROC: Receiver operating characteristic; CI: Confidence interval; GLM: Generalized linear regression models; PPV: Positive predictive value; NPV: Negative predictive value; LR: Likelihood ratio

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### Authors' contributions

VPG and PP carried out the data analysis and interpretation and prepared and wrote the manuscript. JR performed A $\beta$  quantification. LS contributed to the APOE analysis. IM and ISJ contributed to the study design, sample collection, and processing. JA contributed to the study design, recruitment, and FDG-PET acquisition and data analysis. IH, MA, and MB participated in the data collection and analysis. PML, JM, LT, AR, MB, and MS conceived and designed the study. All authors reviewed the manuscript. All authors read and approved the final manuscript.

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### Availability of data and materials

The datasets used and/or analyzed during the current study are available from the corresponding author on reasonable request.

### Ethics approval and consent to participate

The study was approved by the CEIC ethic committee 2009/5455, and all participants provided written informed consent prior to inclusion. Patient recruitment and collection protocols were in accordance with ethical standards according to WMA Declaration of Helsinki-Ethical Principles for Medical Research Involving Human Subjects.

### Consent for publication

Consent for publication was provided by every participant of the study.

### Competing interests

VPG, PP, JR, ISJ, LS, IM, and MS were employees of Araclon Biotech at the moment the study and data analysis were carried out. All other co-authors declare that they have no competing interests.

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