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Blood-Based Biomarkers

Plasma amyloid β 42/40 ratios as biomarkers for amyloid β cerebral deposition in cognitively normal individuals

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Abstract

Introduction: Plasma amyloid β (Aβ) peptides have been previously studied as candidate biomarkers to increase recruitment efficiency in secondary prevention clinical trials for Alzheimer's disease.

Methods: Free and total Aβ42/40 plasma ratios (FP42/40 and TP42/40, respectively) were determined using ABtest assays in cognitively normal subjects from the Australian Imaging, Biomarker and Lifestyle Flagship Study. This population was followed-up for 72 months and their cortical Aβ burden was assessed with positron emission tomography.

Results: Cross-sectional and longitudinal analyses showed an inverse association of Aβ42/40 plasma ratios and cortical Aβ burden. Optimized as a screening tool, TP42/40 reached 81% positive predictive value of high cortical Aβ burden, which represents 110% increase over the population prevalence of cortical Aβ positivity.

Discussion: These findings support the use of plasma Aβ42/40 ratios as surrogate biomarkers of cortical Aβ deposition and enrichment tools, reducing the number of subjects submitted to invasive tests and, consequently, recruitment costs in clinical trials targeting cognitively normal individuals.

Keywords: Amyloid β; Plasma amyloid β ratio; Biomarker; Preclinical Alzheimer's disease; β-Amyloid imaging; Positron emission tomography; Clinical trials

1. Introduction

Dementia is a major public health problem worldwide, which currently affects 46 million people, a number estimated to increase up to 131.5 million by 2050, entailing an enormous social and financial burden [1]. Alzheimer's disease (AD) accounts for 60% to 70% of all cases of dementia; thus, the benefits of a successful therapeutic intervention that could stop or, ideally, prevent the development of AD are undeniable. However, therapeutic trials have had limited success so far, partly because of the advanced neurodegenerative stage of individuals typically targeted in clinical trials over the last two decades. Considering that the efficacy of potential AD treatments would likely depend on an early intervention, there is a growing need for accurate identification of asymptomatic (preclinical) individuals with underlying pathology for inclusion in the current and more favored secondary prevention trials [2–4].

Conflicts of interest: The authors have no conflicts of interest related to this work.

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Consequently, considerable investigational effort has been devoted in recent years to identify biological markers indicative of preclinical and/or prodromal AD before the onset of dementia [5,6]. This new concept of AD has also emphasized the importance of biomarkers as eligibility criteria to enrich clinical trial cohorts with subjects at increased risk of suffering more rapid cognitive decline. Amyloid β (Aβ) is the most likely cause of the pathophysiological process leading to AD dementia and, consequently, Aβ-related biomarkers should be ideal for the identification of the earliest stages of the disease [7,8].

Aβ peptides are most frequently measured in the cerebrospinal fluid (CSF) or through brain imaging of Aβ deposition with positron emission tomography (Aβ-PET). Both biomarkers have demonstrated high diagnostic and prognostic value [9–12], and they might start changing decades before the clinical onset of AD [8,13,14]. However, despite the robustness of these biomarkers, they are not suitable for a broad screening of the population, either because of invasiveness or high cost and low availability of the technology in primary care clinical settings. Given the greater accessibility of blood sampling, there is considerable interest in examining whether circulating Aβ levels correlate with brain Aβ levels and, therefore, with risk of developing AD dementia. A blood-based biomarker would be a less invasive and cost-effective screening method to identify individuals at-risk who could be subsequently confirmed by neuroimaging or CSF analysis. In this context, several large studies have consistently reported that a lower Aβ42/Aβ40 ratio in plasma is associated with higher risk of dementia [15–19], and greater cognitive decline in healthy control subjects at follow-up [20]. Nevertheless, some studies have reported weak or no association of the Aβ42/Aβ40 plasma ratio with AD [21–23].

Some of these contradictory findings likely reflect the complexity of measuring Aβ in plasma and the preanalytical and analytical differences between quantitative methods [24,25]. Differences in study populations with regard to factors such as age or disease stage [26] are also confounding factors. Moreover, to date, assessment of the accuracy of blood Aβ biomarkers relies essentially on the use of clinical diagnosis as the gold standard, despite the fact that it has shown sensitivities ranging from 70.9% to 87.3%, and specificities from 44.3 to 70.8 [27], which could seriously skew the results of any test and is almost certainly a relevant source of variability between studies.

The aim of this study was to examine the potential of the plasma Aβ42/Aβ40 ratio as a marker of cortical Aβ deposition and its use as a screening tool for clinical trial enrichment of cognitively normal (CN) subjects with high brain Aβ levels. With this in mind, we focused on the CN group of the Australian Imaging, Biomarker and Lifestyle Flagship (AIBL) Study using Aβ-PET as the gold standard, supported by previous results showing an association between Aβ42/Aβ40 plasma ratio and brain Aβ levels [28–31]. Moreover, we have taken a comprehensive approach for the evaluation of Aβ42/40 plasma ratio, differentiating the peptide fractions that are found free in plasma (FP42/40) from the total Aβ peptides in plasma (TP42/40) and the amount of Aβ that is bound to other plasma components (BP42/40), by means of validated enzyme-linked immunosorbent assays (ELISAs) [32]. In this study, the cross-sectional and longitudinal association of these plasma markers with brain Aβ-PET results was evaluated, together with an assessment of their diagnostic performance and ability to predict brain Aβ deposition trajectories, evaluating the potential of plasma Aβ ratios as enrichment tools for secondary prevention clinical trials.

2. Methods

2.1. Study population

CN subjects from the AIBL cohort included in this study were selected from those who underwent Aβ imaging with PET. Complete description of the clinical classification procedures in this study was described previously [33]. Subjects were followed-up for 72 months with visits at baseline (bl) and 18-month intervals (visits m18, m36, m54, and m72).

2.2. Amyloid PET imaging

At each of these time points, cortical Aβ burden was assessed using PET with either ^{11}C-Pittsburgh Compound-B (PiB) or ^{18}F-flutemetamol. The PET methodology for each tracer has been previously described [34,35] (see Supplementary material, Imaging Methods for detailed description). To use the results of both PET tracers as a single continuous variable, flutemetamol results were transformed into PiB-like standardized uptake value ratios (SUVR) termed BeCKeT [36]. The SUVR/BeCKeT was then dichotomized into high (Aβ+) or low (Aβ−) Aβ burden using a cutoff value of 1.5 [36].

Those individuals with both a valid Aβ-PET measurement and a valid corresponding plasma measurement at visits m18, m36, and/or m54 were considered in the cross-sectional and in the discriminating performance analysis. For longitudinal analysis, subjects with both valid plasma and Aβ-PET data at bl and at least a valid Aβ-PET measurement during the whole follow-up were considered.

2.3. Plasma Aβ40 and Aβ42 quantification

Plasma samples were obtained using ethylenediaminetetraacetic acid (EDTA) as anticoagulant and following AIBL procedures [30], and were conserved at −70°C until analysis without undergoing any extra freezing/thaw cycles. Only plasma samples from visits m18, m36, and m54 were available for Aβ plasma analysis in this study. Aβ40 and Aβ42 peptides were quantified using ABtest40 and ABtest42, respectively (Araclon Biotech Ltd. Zaragoza, Spain), being blinded to all participant characteristics at the time of

Table 1
Descriptive statistics of demography, Aβ-PET data, and plasma biomarkers at each visit and amyloid PET status

	bl		m18		m36		m54		m72	
	PET Aβ−	PET Aβ+	PET Aβ−	PET Aβ+	PET Aβ−	PET Aβ+	PET Aβ−	PET Aβ+	PET Aβ−	PET Aβ+
Participants, *n*	49	27	49	30	42	30	52	36	44	20
Age (mean ± SD)	72.0 ± 7.3	74.4 ± 7.4	73.7 ± 7.3	74.9 ± 7.4	74.9 ± 7.5	76.4 ± 7.2	76.7 ± 6.8	77.2 ± 7.7	77.3 ± 6.7	78.9 ± 7.1
Female, *n* (%)	28 (57.1)	11 (40.7)	30 (61.2)	11 (36.7)*	28 (66.7)	12 (40.0)*	33 (63.5)	17 (47.2)	26 (59.1)	9 (45.0)
APOE ε4 carriers, *n* (%)	15 (30.6)	17 (63.0)*	17 (34.7)	22 (73.3)*	11 (26.2)	20 (66.7)†	15 (28.8)	24 (66.7)†	9 (20.5)	9 (45.0)
Years of education (mean ± SD)	12.5 ± 2.3	12.2 ± 2.7	12.4 ± 2.3	12.1 ± 2.9	12.4 ± 2.4	12.1 ± 2.7	12.6 ± 2.7	12.0 ± 2.5	12.5 ± 2.8	12.0 ± 2.5
TP42/40; median (IQR)	—	—	0.083 (0.028)	0.068 (0.020)†	0.088 (0.024)	0.071 (0.027)*	0.085 (0.034)	0.067 (0.015)†	—	—
BP42/40; median (IQR)	—	—	0.089 (0.046)	0.067 (0.038)*	0.094 (0.042)	0.070 (0.032)†	0.095 (0.041)	0.069 (0.030)†	—	—
FP42/40; median (IQR)	—	—	0.074 (0.040)	0.066 (0.016)*	0.081 (0.029)	0.074 (0.016)	0.077 (0.037)	0.069 (0.015)*	—	—

Abbreviations: APOE, apolipoprotein E; Aβ, amyloid β; Aβ+ or Aβ−, Aβ positive or negative subjects regarding categorical Aβ-PET measurements; bl, baseline; IQR, interquartile range; median, median value of plasma Aβ ratios in individuals classified as Aβ+ or Aβ−; PET, positron emission tomography; SD, standard deviation.

NOTE. Aβ plasma analysis was only performed at visits month 18 (m18), m36, and m54; participants included had both valid plasma and PET measurements at these visits. Visits bl (baseline) and m72 include subjects with a valid Aβ-PET measurement at these visits and a valid plasma measurement at visit m18. Fisher exact test was performed for categorical variables and Mann-Whitney test for quantitative variables.

*Significant differences between PET amyloid negative/positive groups at the *P* value level of .05.

†Significant differences between PET amyloid negative/positive groups at the *P* value level of .001.

analysis. Analytical procedures and performance characteristics of these tests are described elsewhere [32].

Samples were assayed both undiluted and after treatment by a three-fold dilution in sample/standard diluent, specifically formulated to disrupt Aβ interactions with other plasma components. This previously validated approach allows the quantification of Aβ peptides (either Aβ40 or Aβ42) in two plasma fractions: free Aβ in plasma (FP), which is measured in the undiluted sample, and the total Aβ in plasma (TP) fraction measured in the diluted sample [37]. The difference between the concentrations of TP and FP fractions corresponds to the amyloid peptide bound to plasma (BP) components. The Aβ42/Aβ40 ratios in each of these plasma fractions (FP42/40, TP42/40, and BP42/40) were calculated as the plasma markers to be evaluated.

2.4. Statistical analysis

As an initial exploratory analysis for assessing the association between plasma Aβ42/40 ratios and PET measurements, a Mann-Whitney *U* test was performed at visits m18, m36, and m54 to evaluate plasma ratio differences between subject groups defined by the dichotomized SUVR/BeCKeT (Aβ+ and Aβ−).

To explore the effects of covariates (age, apolipoprotein E (*APOE*) ε4 genotype, and gender) on the association between the SUVR/BeCKeT and plasma ratios, a linear regression model using the SUVR/BeCKeT as a quantitative response variable was performed at each visit from m18 to m54. In each model, a single plasma marker was used in log-units. Log transformation of ratio variables provided better-behaved statistics.

Linear mixed-effects models were used to assess the association between the first plasma measurement (at visit m18) and the trajectories of the SUVR/BeCKeT at follow-up (visits bl-m72). The fixed-effects terms were the contribution of the plasma variable to the intercept and slope of the SUVR/BeCKeT trajectories, as well as the demographic covariates. The random-effects terms included intercept and slope at the individual level.

A Receiver Operating Characteristic (ROC) curve analysis was used to assess the prediction performance of the dichotomic SUVR/BeCKeT from a single plasma marker measurement. In addition to sensitivity/specificity performance measures, positive predictive value (PPV) is of special interest in this study. The criterion for choosing the operating point along the ROC curve was Youden's index maximum. Classification performance was assessed by means of a repeated random subsampling cross-validation (CV) experiment with 10,000 rounds and a data split distribution of about 50% to 50% for training and testing sets, respectively. More details of the CV experiment are given in the Supplementary material.

Statistical analysis was performed with MATLAB (The MathWorks, Inc, Natick, MA, USA).

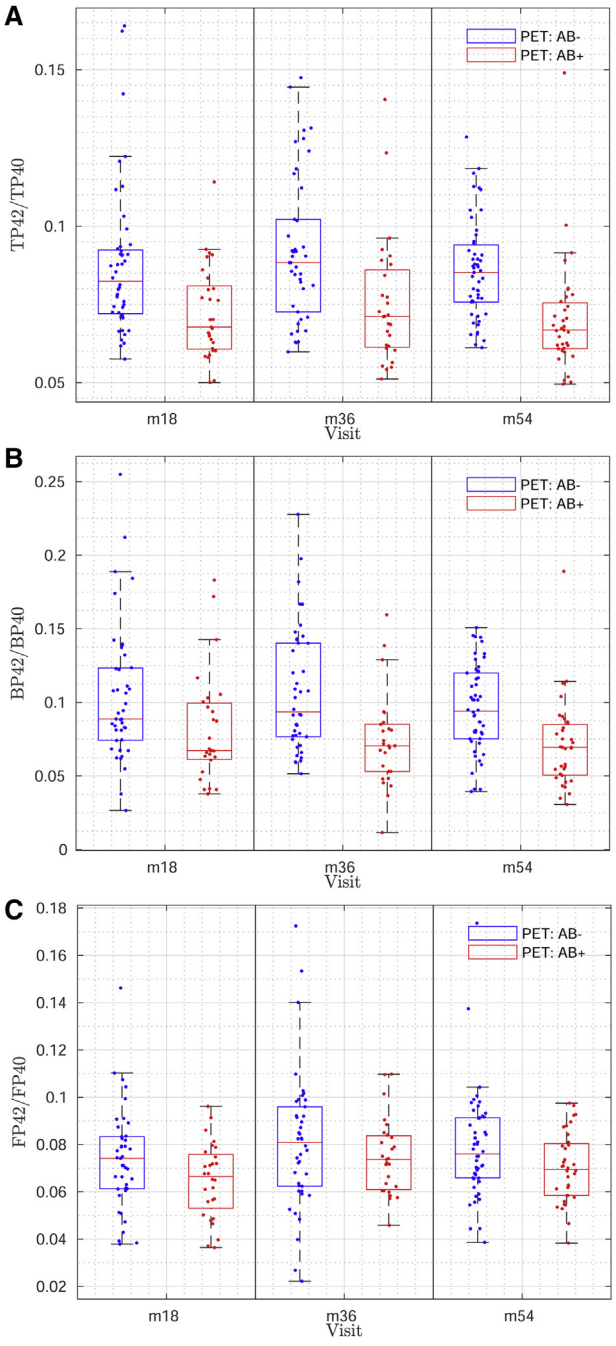

Fig. 1. Box-and-whisker plots of plasma markers with regards to categorical Aβ-PET status (Aβ+ or Aβ−) measurements at each visit. (A) TP42/40, for visualization purposes three outliers (of the 236 valid measurements) with TP42/40 values between 0.19 and 0.54 were discarded. (B) BP42/40. (C) FP42/40, for visualization purposes three outliers (of the 217 valid measurements) with FP42/40 values between 0.19 and 0.29 are not shown. See data in Table 1. Abbreviations: BP, amyloid peptide bound to plasma; FP, free in plasma; TP, total amyloid β in plasma.

3. Results

The demographic characteristics of individuals included in this study at each visit are presented in the upper rows of Table 1. The bottom rows provide descriptive statistics of plasma Aβ ratios, which were lower in the group of subjects with positive Aβ-PET scans than in those with negative Aβ-PET scans. This association reached statistical significance in all the three analyzed visits for TP42/40 and BP42/40 ($P < .001$ in two of the three visits) and only in two visits for FP42/40 (see also Fig. 1).

The cross-sectional linear regression modeling of the SUVR/BeCKeT as a function of each plasma ratio, adjusting for demographic covariates, is summarized in Table 2. The linear association between the SUVR/BeCKeT and plasma ratios (TP42/40, BP42/40, and FP42/40) was consistent with the previous exploratory analysis, although statistical significance was reached at a lower number of visits. The negative sign of the coefficients is in agreement with the previous hypothesis testing: lower plasma Aβ ratios were associated with higher SUVR/BeCKeT values. Regarding covariates, *APOE* genotype effects on the SUVR/BeCKeT were statistically significant at all visits and for all the plasma ratios (see Supplementary Table 1). Gender effects were not significant.

In the longitudinal analysis, linear mixed-effects models showed a significant association between the slope of the SUVR/BeCKeT trajectories and the first available plasma measurement at m18 (Table 3; complete information is provided in Supplementary Table 2). The negative sign of the models' coefficients indicated that the lower the plasma Aβ ratios at visit m18, the steeper the SUVR/BeCKeT trajectory. The inverse association between plasma Aβ ratios and SUVR/BeCKeT was also observed at bl (intercept), although with a weaker or borderline significance, which was in line with the results of the cross-sectional study. Among the three plasma ratios, TP42/40 showed the most significant association with SUVR/BeCKeT slope. This inverse association between the Aβ42/40 plasma ratio and the SUVR/BeCKeT slope is in correspondence with the fact that in those individuals with low plasma Aβ42/40 ratio, the change in SUVR/BeCKeT from bl increased along the follow-up, whereas on average it did not change in those subjects with a high Aβ42/40 plasma ratio (Fig. 2). *APOE* genotype also had significant effects on both the intercept and slope of the SUVR/BeCKeT trajectories, whereas age had only significant effects on the intercept (see Supplementary Table 2). The direction of the association was as expected: *APOE* ε4 carriers had SUVR/BeCKeT trajectories with both a larger amplitude and a steeper slope than noncarriers. Regarding age, older subjects had trajectories with larger amplitude.

The plasma marker with the most significant association with the SUVR/BeCKeT in both cross-sectional and longitudinal analyses (TP42/40) was selected for a subsequent ROC curve analysis. The inclusion of TP42/40 in the classifier yielded a statistically significant improvement (DeLong test P value .0017) in the area under the ROC curve with only demographic covariates (age and APOE) from 74% to 79%. Mean difference (95% confidence interval [95% CI]) was 5.4% (2.0% to 8.8%) (see Supplementary Section

Table 2
Association between log-transformed plasma ratio levels and the SUVR/BeCKeT score in the cross-sectional study

Biomarker	Coefficient	m18	m36	m54
log(TP42/40)	Estimate (95% CI)	−0.40 (−0.76, −0.027)	−0.10 (−0.43, 0.22)	−0.59 (−0.94, −0.24)
	P value	.036	.52	.0013
log(BP42/40)	Estimate (95% CI)	−0.086 (−0.32, 0.14)	−0.29 (−0.51, −0.066)	−0.30 (−0.54, −0.065)
	P value	.46	.012	.013
log(FP42/40)	Estimate (95% CI)	−0.35 (−0.63, −0.067)	−0.055 (−0.36, 0.25)	−0.32 (−0.64, 0.0042)
	P value	.016	.72	.053

Abbreviations: APOE, apolipoprotein E; BP, amyloid peptide bound to plasma; 95% CI, 95% confidence interval; FP, free in plasma; m18, month 18; m36, month 36; m54, month 54; TP, total amyloid β in plasma.

NOTE. Estimate: coefficient of the plasma ratio as the explanatory variable in a generalized linear regression model adjusted for relevant demographic variables (age, gender, and *APOE* genotype) at each visit.

III and Supplementary Fig. 3). Gender was not included in this analysis because of the lack of significant associations found in previous analyses. The average performance for the classifier including TP42/40 at Youden's index maximum (indicative of its best expected performance) was 71% sensitivity with 78% specificity. Interestingly, the distribution of the SUVR/BeCKeT threshold values that maximized Youden's index had a median value of 1.57 with a 95% CI of 1.40 to 1.73. As expected, these threshold values are close to the cutoff selected for the definition of the dichotomic SUVR/BeCKeT variable.

For trial enrichment purposes, a biomarker with a large value of PPV is desired, which can be obtained by selecting a large value of the threshold in the classifier so that the false-positive rate is reduced. Fig. 3 shows the distribution of the PPV measure across CV rounds as a function of the threshold value. The classifier including TP42/40 plus demographic covariates provided a median PPV performance of 81% for a model threshold value of 1.73. In concordance with the improvement in the area under the ROC curve mentioned previously, the inclusion of TP42/40 in the model together with the demographic covariates provided a statistically significant improvement in the classifier PPV (see also Supplementary Material, Section III). Note that this threshold value is slightly higher than the one that maximized Youden's index. This median PPV performance represented over a 110% increase over the prevalence of Aβ-PET positivity in the study population, which was about 37.5%.

Table 3
Association between log-transformed plasma ratio levels and the SUVR/BeCKeT score in the longitudinal study

Biomarker	Coefficient	Estimate (95% CI)	*P* Value
log(TP42/40)	Intercept	−0.34 (−0.62, −0.064)	.016
	Interaction with time	−0.034 (−0.054, −0.015)	.0006
log(BP42/40)	Intercept	−0,13 (−0.31, 0.047)	.15
	Interaction with time	−0.014 (−0.028, −0.00094)	.036
log(FP42/40)	Intercept	−0.22 (−0.43, 0.0045)	.055
	Interaction with time	−0.026 (−0.42, −0.0098)	.0017

Abbreviations: APOE, apolipoprotein E; BP, amyloid peptide bound to plasma; 95% CI, 95% confidence interval; FP, free in plasma; TP, total amyloid β in plasma.

NOTE. Estimate: coefficient of the plasma ratio as the explanatory variable in a generalized linear mixed-effects model adjusted for relevant demographic variables (age, gender, and *APOE* genotype).

4. Discussion

In this study, we report that in CN individuals, low levels of FP42/40, BP42/40, and TP42/40 plasma ratios are associated with higher levels of cerebral fibrillary Aβ deposition as determined by PET. This inverse association was found to be significant for BP42/40 and TP42/40 at each of the three visits analyzed and in two of them for FP42/40. The association between plasma Aβ42/40 ratios and Aβ-PET SUVR/BeCKeT was still significant in two of the three visits after adjusting the comparisons for the most relevant demographic covariates (age, APOE genotype, and gender) providing robustness to the findings. Furthermore, our results showed that low levels of Aβ42/40 ratios (particularly TP42/40) at bl are associated with faster increase (slope) of Aβ-PET SUVR/BeCKeT over time, establishing the potential of Aβ42/40 plasma ratios as predictors of Aβ-PET individual trajectories.

These results are in concordance with previous studies [28–31,38]. In particular, present results are consistent with the inverse association between plasma Aβ42/40 and cortical Aβ burden determined by PiB-PET previously observed in the AIBL population including CN, mild cognitive impairment (MCI), and AD patients, as shown by Rembach et al. [31]. In this study, we have extended the follow-up from 18 to 72 months and have used Aβ-PET SUVR/BeCKeT scores obtained with either PiB or flutemetamol, confirming this inverse association and its ability to predict faster cortical Aβ deposition over time. Remarkably, this study provides added value because, to the best of our knowledge, it is the first study in which the association between Aβ plasma ratios and cortical Aβ burden is explored within a population of stable CN individuals. Moreover, our results are congruent with the association of low Aβ42/40 plasma ratio with greater cognitive decline in

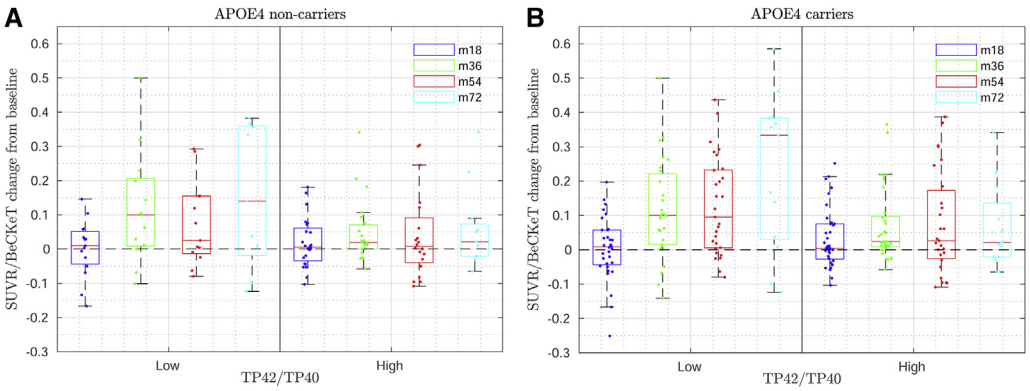

Fig. 2. Box-and-whisker plot of changes in the SUVR/BeCKeT along the follow-up for subjects with low or high baseline TP42/40 plasma ratio. Each point represents the change in each individual in SUVR/BeCKeT at visit month 18 (m18, blue), m36 (green), m54 (red), or m72 (cyan), with respect to the baseline SUVR/BeCKeT in *APOE* ε4 noncarriers (A) and *APOE* ε4 carriers (B). In (A or B), left panel contains the individuals with low (<the population median) TP42/40 levels at baseline (m18) and right panel the individuals with high (≥the population median) TP42/40 levels at baseline (m18). Note that in those individuals with low baseline TP42/40, average change in SUVR/BeCKeT significantly increased over visits, whereas in those with high baseline TP42/40 there was almost no change in the SUVR/BeCKeT during the follow-up. Similar figures are also obtained for the other markers FP42/40 and BP42/40 (see Table 3). Abbreviations: APOE, apolipoprotein E; BP, amyloid peptide bound to plasma; FP, free in plasma; TP, total amyloid β in plasma.

elderly people without dementia [20] and the increased risk of developing AD observed by others [15,19,39]. The robustness of this association has been reinforced over the last 10 years by reports from well-known large population-based studies such as the Framingham [17], the Rotterdam [18], the Three-City [16], and BioFINDER Studies [38], as well as the findings of a meta-analysis including more than 10,000 subjects [40].

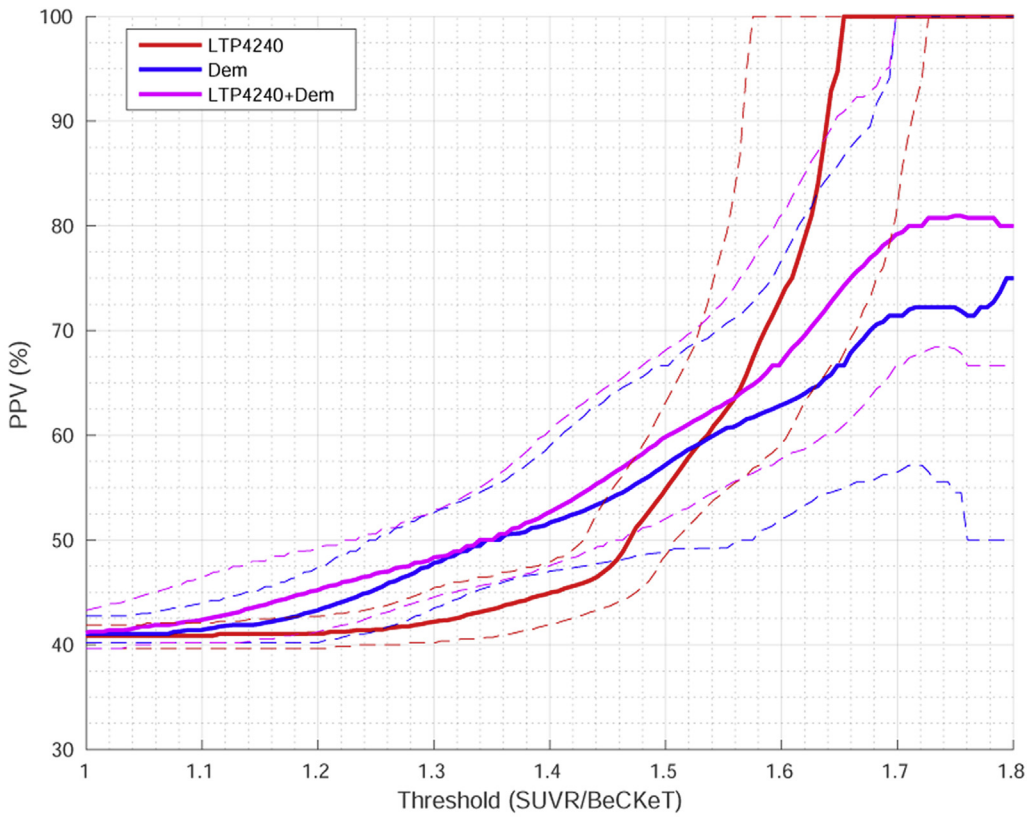

Fig. 3. Comparison of the performance on PPV for three different classifiers as a function of the threshold value. In red, classifier including only the plasma marker log(TP42/40). In blue, classifier including age + *APOE* ε4 variables. In pink, classifier with all previous variables. The thick line represents the median value of the performance, and the thin dashed lines represent the 5th and 95th percentile values. Abbreviations: APOE, apolipoprotein E; PPV, positive predictive value; TP, total Aβ in plasma.

Nevertheless, discrepant results from other studies [21–23] cannot be disregarded and emphasize the need for further substantial investigation on Aβ plasma levels as preclinical biomarkers. In this line, thorough preanalytical and analytical standardization is required to obtain reliable data that facilitate comparison between studies. The variety of assay formats used in different studies may have also contributed to these varying results. The use of Aβ42/40 ratios instead of single peptide measurements may attenuate possible bias in single Aβ peptide levels caused by inconsistencies in sample handling [41]. In line with this, recent studies in CSF have reported better diagnostic performance for the Aβ42/40 ratio than for Aβ42 alone [42]. Furthermore, our approach allows a deeper knowledge of Aβ behavior in blood [32], by the assessment of Aβ bound to plasma components [43–45]. It is becoming increasingly clearer that sensitivity to cortical Aβ burden varied substantially among individuals and that the rate of SUVR change over time could be more relevant to predict disease progression than single cross-sectional measurements [46]. Similarly, change in the relative abundance and distribution of Aβ peptides in plasma could be more effectively reflecting the progression of AD than absolute plasma levels. Having higher proportion of free Aβ, prone to aggregate or re-enter the brain could be more relevant pathologically than the absolute total Aβ levels [47]. In addition, the proportion of bound peptides in plasma might reflect Aβ clearance capacity at the individual's level [48]. Further experiments will be necessary to test these ideas, which in first place require a more comprehensive assessment of the different Aβ species in the different plasma fractions.

Yet, the use of Aβ plasma ratios for diagnostic purposes remains problematic and seriously limited because of the extensive overlapping of individual measurements among CN, MCI, and AD patients, previously discussed in [39]. This situation is reflected in the ROC curve analysis in which our classifier including TP42/40 (together with age and *APOE* genotype) reached 71% sensitivity with 78% specificity to discriminate cortical-Aβ positivity at the maximum of Youden's index, which would not be enough for a standalone diagnosis. However, beyond diagnostic purposes, noninvasive and cost-effective plasma biomarkers would be of great utility as a screening tool in secondary prevention clinical trials to preselect a cohort enriched for people with presumptive cortical amyloid pathology. The cortical Aβ burden of these subjects would have to be subsequently confirmed by Aβ-PET scans. In this scenario, the plasma classifier cut-point that maximizes the trade-off between sensitivity and specificity (at the maximum of Youden's index) may not be optimal. Instead, a higher cutoff may be a better choice to increase the PPV of the prescreening test, reducing the number of false positive decisions and, consequently, the number of subjects needed to receive confirmatory Aβ-PET scans. This strategy has already been explored by others in the Alzheimer's Disease Neuroimaging Initiative (ADNI) cohort [49].

With this in mind, we have evaluated the distribution of the PPV as a function of the classifier's threshold. At the optimal threshold, an average 81% PPV was obtained, which is more than two times higher than the prevalence of Aβ-PET positivity in this population (37.5%). Therefore, in a recruiting scenario targeting CN cortical-Aβ+ people, a prescreening step with our plasma classifier would reduce the number of individuals undergoing Aβ-PET scans by more than half. Remarkably, considering the 5% percentile, the PPV of the classifier including TP42/40 will be more than 68% with 95% certainty, which represents an improvement of 81% over the sample prevalence in the identification of preclinical subjects with a positive Aβ-PET scan (Fig. 3). The magnitude of this improvement can be adequately assessed if compared with the 60% increase over the prevalence of Aβ-PET positivity obtained in the ADNI cohort (including CN, MCI, and AD), with a classifier combining demographics (*APOE*) and cognitive decline [49]. Thus, considering the invasiveness, technical requirements and high costs of Aβ-PET scans (up to $5000; [49]), in an average phase III clinical trial including hundreds of subjects, the recruitment process could be simplified and the associated costs considerably reduced by preselecting the subjects with a classifier including the TP42/40 ratio. Nevertheless, it should be noted that sensitivity monotonically decreases for larger threshold values of the classifier. Thus, in a practical enrichment scenario the PPV must be "traded-off" with sensitivity and specificity to balance costs, logistical considerations, and general benefit to the clinical trial in question. This will be explored further in future work.

It also deserves to be emphasized that the inclusion of the TP42/40 ratio in the classifier significantly improved the identification of cortical-Aβ+ subjects regardless their *APOE* genotype. This would allow a recruitment strategy not restricted to the presence of a risk factor such as *APOE* ε4, which could lead to a biased cohort selection and, eventually, to uncertainties when translating clinical trial results (and claims) to the general population.

Our work has a weakness that must be noted in the relatively small sample size available for this study, particularly relevant for the CV approach of the ROC analysis, which required the partition of the sample population in two independent training and testing sets and obviously would have to be replicated in a larger sample population. However, we have assessed the 95% CI for this variability and present results were still relevant from a practical point of view at the 95% certainty.

In conclusion, our results show an inverse association between Aβ42/40 plasma ratios and fibrillary Aβ deposition in the brain. The population screening application of this blood-based biomarker, which is inexpensive, minimally invasive, and can easily be performed at multiple time points, could be translated into a more efficient use of neuroimaging resources in clinical trials. Present results warrant further research on the development of plasma

Aβ42/40 ratios as surrogate biomarkers of Aβ deposition in brain.

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Supplementary data

Supplementary data related to this article can be found at http://dx.doi.org/10.1016/j.dadm.2017.07.004.

RESEARCH IN CONTEXT

1. Systematic review: The literature was reviewed using PubMed database and bibliography included in target articles to identify previous research about the association of plasma amyloid β (Aβ) levels and Alzheimer's disease (AD). Most studies show that low levels of Aβ42/40 in plasma are associated with increased risk of AD or cortical fibrillary Aβ burden.
2. Interpretation: Our results are consistent with most of the previous research showing an association of low Aβ42/40 plasma ratios with high Aβ deposition in brain determined by Aβ imaging with positron emission tomography and considerable potential as an enrichment tool in clinical trials.
3. Future directions: These findings support and encourage further research in the development of plasma Aβ42/40 ratios as surrogate biomarkers of cortical Aβ deposition.

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