ACCURATE DISCRIMINATION OF BRAIN AMYLOID STATUS IN THE MULTI-CENTRIC A4 STUDY BY PLASMA Aβ42/Aβ40 MEASURED WITH A NOVEL HPLC-MS/MS METHOD

Leticia Sarasa[1], María Pascual-Lucas[1], José A. Allué[1], Sergio Castillo[1], María Eugenia Sáez[2], Sara Abdel-Latif[3], Robert A. Rissman[3,4], Jose Terencio[1] and Manuel Sarasa[1†]

[1]Araclon Biotech-Grifols, Zaragoza, Spain. [2]CAEBI (Centro Andaluz de Estudios Bioinformáticos), Sevilla, Spain. [3]Alzheimer´s Disease Therapeutic Institute. University of Southern California, San Diego, CA, USA. [4]Department of Neurosciences. University of California, San Diego. La Jolla, CA, USA. †Deceased May 27, 2020

BACKGROUND

Previous studies have demonstrated that plasma Aβ42/Aβ40 ratio could be a valuable screening tool for Alzheimer's disease (AD)(1). In this work, a novel antibody-free HPLC-MS/MS method (ABtest-MS, Araclon Biotech) has been used to explore the ability of plasma Aβ42/Aβ40 ratio to predict the brain amyloid status in cognitively unimpaired individuals (CU) in a subset of the A4 study (screening visit)(2).

METHODS

Plasma samples from 731 CU individuals were obtained in 59 recruitment sites (A4 Trial, screening visit) across USA and Canada. A standardized uptake value ratio (SUVR) ≥ 1.15 was used to define amyloid-PET positivity based on ^{18}F-Florbetapir PET data.

***Table 1**. Demographic characteristics of the 731 CU individuals from the A4 study at baseline.*

		Aβ PET (-)	Aβ PET (+)	*p* value
Participants	*n (%)*	490 (67.0 %)	241 (33 %)	
Age, years	*median (IQR)*	70.3 (67.5-74.3)	70.6 (67.8-75.2)	0.068
Female	*n (%)*	283 (57.8 %)	133 (57.3 %)	0.899
APOE ε4	*n (%)*			**< 0.0001**
0 alleles		371 (75.7 %)	99 (41.1 %)	
1 alleles		110 (22.5 %)	120 (49.8 %)	
2 alleles		9 (1.8 %)	22 (9.1 %)	

Aβ40 and Aβ42 plasma levels were quantitated using ABtest-MS. Briefly, analytes were extracted directly from plasma and no immnunoprecipitation procedure was followed. Intact Aβ1-40 and Aβ1-42 species were measured as no enzymatic digestion was performed(3).

Receiver operating characteristic (ROC) curve analysis were performed to evaluate the ability of plasma Aβ42/Aβ40 to identify amyloid PET status.

References:

(1) Alzheimers Dement. doi:10.1016/j.dadm.2017.07.004

(2) JAMA Neurology. doi:10.1001/jamaneurol.2020.0387

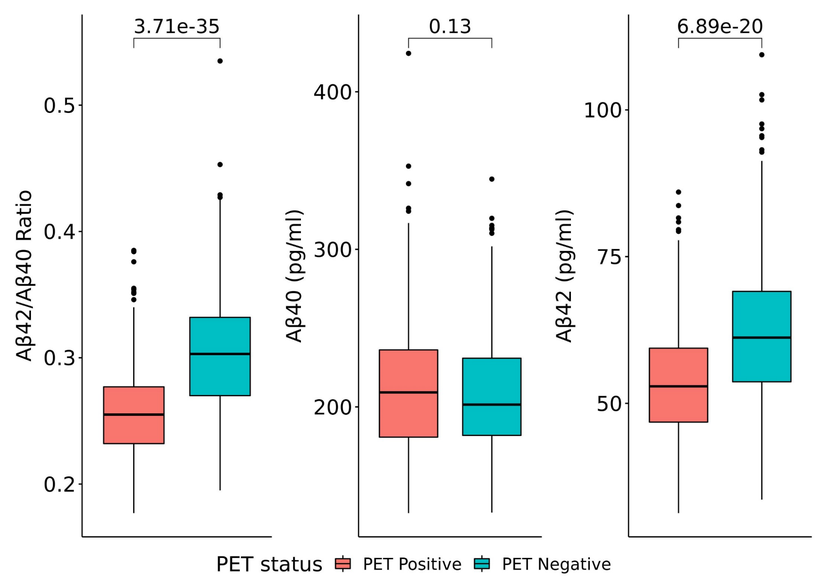
(3) Oral communication on March 19th at Symposium Fluid Biomarkers 50

RESULTS

1. Distribution of plasma Aβ levels between Aβ PET groups

Plasma Aβ42 and Aβ42/Aβ40 values were significantly lower in the Aβ PET positive group than in the Aβ PET negative group (p<0.0001, Mann-Whitney test).

***Figure 1**. Box and whiskers plots of plasma Aβ42/Aβ40 ratio (left graph), Aβ40 (middle graph) and Aβ42 (right graph) between PET groups.*



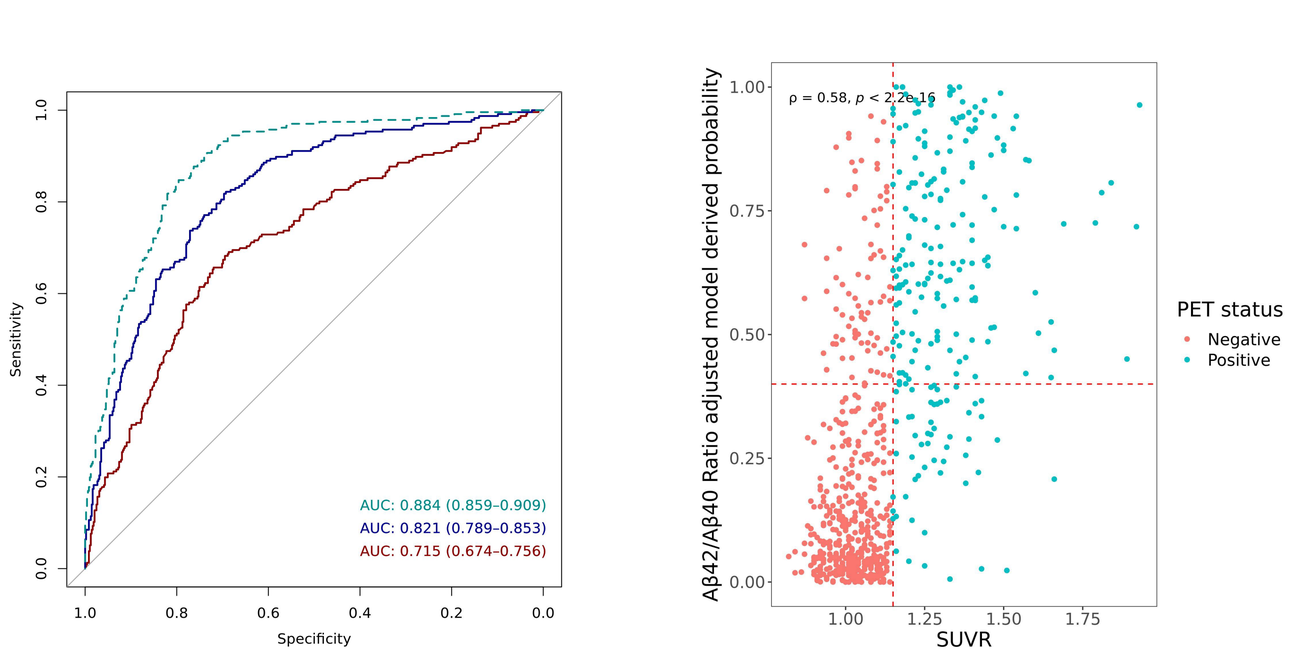
Plasma Aβ42 and Aβ42/Aβ40 values showed significant negative correlations with SUVR PET measures (rho=-0.3 and -0.44 respectively; p<0.0001).

2. Discriminative ability of Aβ PET status

A logistic regression model including demographic covariates (age, sex and APOE; base model) yielded an AUC of 0.71 (95% confidence interval [CI] 0.67-0.75). Inclusion of Aβ42/Aβ40 ratio (ratio model) outperformed the base model, with an AUC of 0.82 (0.79-0.85; p<0.0001 DeLong test).

The effect of the recruitment site on Aβ determinations was also investigated. Significant differences were found for plasma Aβ42/Aβ40 values among the 59 sites involved (p<0.05, Kruskal-Wallis test). Inclusion of the recruitment site in the ratio model increased the predictive ability up to AUC=0.88 (0.86-0.91; p<0.0001 DeLong test; Sensitivity 84.7%, Specificity 79.6%, Accuracy 81.3%).

***Figure 2**. ROC curve analysis for discriminating Aβ PET status. ROC curve of the base, ratio and full models, (left graph); concordance graph between model probability scores (full model) and SUVR values (right graph).*



CONCLUSIONS

ABtest-MS accurately identified amyloid brain deposition in this subset of 731 CU individuals from the A4 study. Although pre-analytical variables were standardized, these data show that recruitment site variable should be taken into account. An extensively validated, robust and centralized sample analysis would be highly desirable in these large and multi-centric clinical trials.