Antibody free, Mass Spectrometric procedure for the determination of Aβ40 and Aβ42 in human plasma.

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BACKGROUND

With the potential development of new β-amyloid targeted treatments for AD, screening tests that can be widely and inexpensively deployed to identify among cognitively normal people those presenting Aβ pathological change, are urgently needed. The assessment of Aβ42/Aβ40 plasma ratio by different immunoassays have shown satisfactory clinical performance as surrogate biomarkers of cerebral amyloid burden as determined by Aβ-PET or CSF Aβ42 analysis. However, robustness and reliability of immunoassays seem to be still hampered by the largely unknown interactions between the biochemical properties of Aβ peptides and the extremely complex composition of the plasma matrix.
Mass Spectrometric (MS) procedures have become an alternative to immunoassays due to their high specificity and sensitivity. However, most of the currently available MS procedures still rely on a preliminary immunoprecipitation step, that not only increases the cost, but could be also subject to plasma matrix interactions, particularly in samples coming from patients treated with anti-amyloid peptides monoclonal antibodies.

OBJECTIVES

We aimed to develop an antibody-free MS assay for the determination of intact Aβ40 and Aβ42 in human plasma (ABtestMS) that reduces drastically sample preparation time. In addition, as no analyte digestion is carried out, intact Aβ species are quantified. Clinical performance was also tested with a subset of samples from AB255 Study[1,2].

METHODS

Calibration curves and quality control samples were prepared in human plasma, after spiking with ^{15}N-Aβ40 and ^{15}N-Aβ42. Deuterated analogues ^{2}H-Aβ40 and ^{2}H-Aβ42 were used as internal standards for quantitation. Analytes are extracted from plasma without any immunoprecipitation procedure and no subsequent enzymatic digestion is carried out. Aβ species are separated in a Micro-LC system and analyzed in a hybrid Triple Quadrupole-Linear Ion Trap mass spectrometer (ABSciex 6500+ Q-TRAP) fitted with a DMS interface (SelexIon).A comprehensive analytical validation including precision, accuracy, sensitivity, selectivity and linearity was performed following FDA´s recommendations[3].

RESULTS

1. Analytical performance

ABtestMS allowed quantification of intact Aβ40 and Aβ42 species in human plasma samples in the ranges of 50-1000 and 10-200 pg/ml respectively, without preliminary immunoprecipitation nor enzymatic digestion.
Intra-assay precision and accuracy, expressed as coefficient of variation (CV in %) and %Error respectively, ranged from 1.9 to 9% and -8.9 to 5.8% for ^{15}N-Aβ40. For ^{15}N-Aβ42, %CV and %Error ranged from 3.6 to 13.2% and -5.5 to 5.6% respectively. Inter-assay precision and accuracy, ranged from 6.2 to 7.0% and -0.6 to 0.3% for ^{15}N-Aβ40. For ^{15}N-Aβ42, %CV and %Error ranged from 6.1 to 11.0% and -1.5 to 1.3% respectively. Stability studies according to FDA´s bioanalytical guidelines were also carried out, yielding satisfactory results.

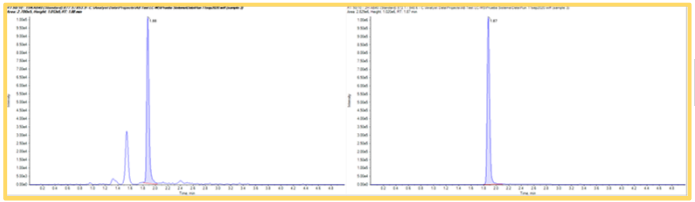


Figure 1. Left pane, lower limit of quantitation for ^{15}N-Aβ40, 50 pg/ml. Right pane, ^{2}H-Aβ40 (Internal Standard)

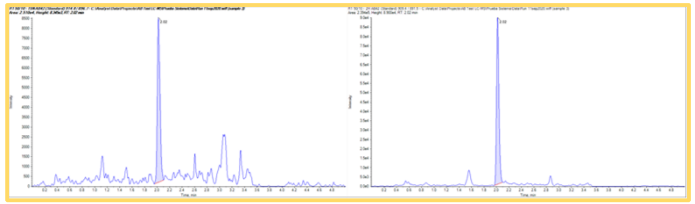
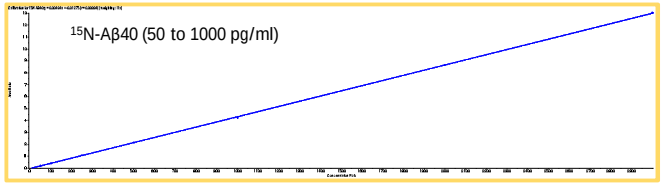


Figure 2. Left pane, lower limit of quantitation for ^{15}N-Aβ42, 10 pg/ml. Right pane, ^{2}H-Aβ42 (Internal Standard)



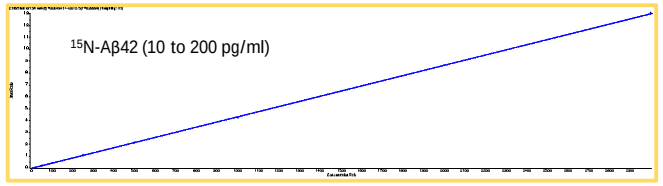


Figure 3. Examples of calibration curves in human plasma for ^{15}N-Aβ40 (upper pane, r = 0.99994) and ^{15}N-Aβ42 (lower pane, r = 0.99988).

2. Clinical performance

Clinical performance of ABtestMS was tested by ROC analysis to discriminate between Aβ-PET+ve and Aβ-PET-ve individuals in a pilot study using a subset of 36 samples from the AB255 study. The unadjusted Aβ42/Aβ40 ratio, as determined by ABtestMS, allowed the identification of Aβ-PET+ve subjects with an AUC of 0.84.

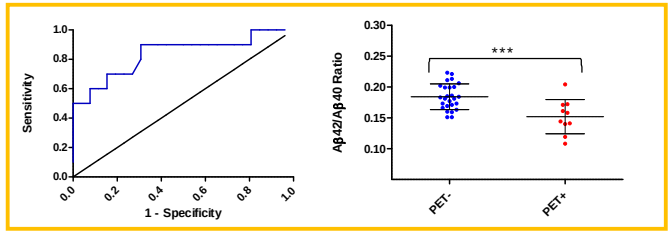


Figure 4. ROC curve (AUC = 0.84) and Aβ42/Aβ40 ratio distribution for 36 individuals from AB255 Study (Mean ± SD). *** P < 0.001 (Student´s t)

CONCLUSIONS

ABtestMS allows the robust and reliable quantification of Aβ42/Aβ40 ratio in plasma without requiring preliminary immunoprecipitation nor enzymatic digestion of the sample. The Aβ42/Aβ40 ratio in plasma as determined by this innovative ABtestMS assay, could be useful for the identification of cognitive normal people with cerebral β-amyloid preclinical Alzheimer's pathologic change, as demonstrated in a small subset of samples from AB255 Study.

(1). Perez-Grijalba V, Romero J, Pesini P, Sarasa L, Monleon I, San-Jose I, Arbizu J, Martinez-Lage P, Munuera J, Ruiz A, Tarraga L, Boada M, Sarasa M (2019) J Prev Alzheimers Dis 6, 34-41.
(2). Perez-Grijalba V, Arbizu J, Romero J, Prieto E, Pesini P, Sarasa L, Guillen F, Monleon I, San-Jose I, Martinez-Lage P, Munuera J, Hernandez I, Buendia M, Sotolongo-Grau O, Alegret M, Ruiz A, Tarraga L, Boada M, Sarasa M (2019) Alzheimers Res Ther 11, 96.
(3). Bioanalytical Method Validation. Guidance for Industry. US Department of Health and Human Services, FDA, CDER, CVM. May 2018.