ADNI Alzheimer's Disease Neuroimaging Initiative

Aβ40 and Aβ42 quantification in plasma from ADNI cohort using ABtest40 and ABtest42

Virginia Pérez, Noelia Fandos, Pedro Pesini, Manuel Sarasa, Ian Sherriff.
Araclon Biotech

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Summary

Characterization of preclinical Alzheimer's disease (AD) on the basis of biomarkers is absolutely vital for the development of early diagnostic tools and new therapeutic strategies. For practical reasons, peripheral biomarkers, particularly those based on the quantification of plasma Aβ peptides, are seen as the most desirable first-step screening tools.

Results from numerous experimental studies and meta-analyses strongly suggest that the association between Aβ plasma levels, particularly the Aβ42/Aβ40 ratio, and AD diagnosis goes beyond what could be attributable to pure chance, although the sense of this association is still controversial.[1-3]

The primary goal of this work is to analyze whether plasma Aβ peptide (Aβ42, Aβ40) levels are associated with the longitudinal trajectories of CSF biomarkers (Aβ42, total-tau and p-tau) and aPET in an ADNI cohort of 305 individuals with follow-up. This would allow the use of the non-invasive plasma Aβ levels as a first-step screening tool to search for people with cortical amyloid positivity and eventually at increased risk of developing AD. Secondarily, we will explore whether plasma Aβ peptide levels are associated with the longitudinal trajectories of hippocampal atrophy (HA) and cognitive decline (CD).

Method

The ABtest service includes the determination of the levels of Aβ40 and Aβ42 free in plasma (FP40 and FP42), which are assessed in undiluted plasma samples, and the determination of the total levels of Aβ40 and Aβ42 in plasma (TP40 and TP42), which are assessed in plasma samples diluted in our proprietary buffer. Therefore, 4 determinations (two for Aβ40 and two for Aβ42) were carried out per individual and time point, always in duplicate. Araclon was blinded to the cognitive status and all other characteristics of the participants.

Determinations

As a whole 3136 determinations were carried out in 784 plasma samples 1568 with ABtest40 and 1568 with ABtest42, for the undiluted and diluted plasma matrix in each case.

Experimental design

The optimized ABtest methodology for a final ADNI agreed plasma sample volume of 500µl lead to the use of 100µl sample per well for Aβ42 determination and 50µl sample per well for Aβ40 quantification. In both cases, the reliability of the results was guaranteed by a battery of previous control assays.

The time points from the same individual were assayed in the same plate and, additionally, both Aβ40 and Aβ42 in diluted and undiluted plasma from the same participant were assayed in the same run.

Three control samples (always the same and different from the ADNI collection) were analyzed in each plate with the purpose of assessing the reproducibility within and throughout the runs. The control samples were located in the plate areas more susceptible to suffer from external effects.

Possible matrix interferences were avoided by pretreating the samples using a blocking procedure that guarantees the specificity of the signal obtained. In order to be included in the final report, each run had to fulfill the previously established quality criteria for ABtest (see below). In this sense, none of the assays needed to be rejected.

A non-linear regression with 4 parameters and 1/y weighting was used in every assay. The dynamic range goes from 210pg/ml to 3.13pg/ml for ABtest40 and from 105pg/ml to 1.56pg/ml for ABtest42. Samples which do not fall into this quantification range are defined as "above the ULOQ" (>ULOQ) if their concentration is above the upper limit of the quantification range or "non-detectable" (ND) in case their concentration is below the lowest point of the range. Therefore, samples with Aβ40 levels above the quantification range will have an undetermined concentration higher than 210pg/ml for the undiluted plasma and 630pg/ml for the diluted plasma. Regarding Aβ42, samples defined as >ULOQ would have Aβ42 levels higher the 105pg/ml in undiluted plasma and 315pg/ml for the diluted sample. Non-detectable levels will be lower than 3.13pg/ml for Aβ40 and 1.56pg/ml for Aβ42.

Control and reliability parameters

The reliability of the results was assessed by evaluating and monitoring the accuracy and reproducibility of each assay. The average data obtained are summarized in the table below.

	ABtest40	ABtest42
Mean percentage calibration error	7.5%	7.2%
Intra-plate variability (CV)	6.2%	5.3%
Intra-assay variability (CV)	6.1%	9.2%
Inter-assay variability (CV)	7.4%	5.5%

Table 1. *Quality control results.*

The intra-plate variability refers to the plasma sample duplicate reproducibility. The intra-assay variability corresponds to the reproducibility of the quantification of the same three control samples assayed in the different plates of each run. The variability in the concentration of these three control samples among different runs is defined as the inter-assay variability. The mean percentage calibration error reflects the accuracy of the quantification using the calibration curve. All these data are within the acceptance criteria for ABtest.

Version Information

This is the first version of this document.

Dataset Information

This methods document applies to the following dataset(s) available from the ADNI repository:

Dataset Name	Date Submitted
Araclon Biotech S.L. ABtest40 and 42 Plasma Analysis Version 1.0	11 February 2016

References

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About the Authors

This document was prepared by Virginia Pérez, Araclon Biotech S.L. For more information please contact Ian Sherriff at +34 976 796 562 or by email at isherriff@araclon.com