ADNI Alzheimer's Disease Neuroimaging Initiative

Plasma Aβ42/Aβ40 Ratio as a Predictor of Brain Amyloidosis

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Contents

Abstract

We hypothesize that plasma Aβ42/Aβ40 ratio, as measured by a high precision assay, can accurately predict central nervous system amyloidosis using amyloid PET as a reference standard.

Methodology

Upon intake, participant samples and analytical standards were stored at –80° C. The simultaneous immunoprecipitation of Aβ38, Aβ40, and Aβ42 from human plasma was performed as previously described with minor modifications (Schindler *et al.*, 2019). Briefly, samples were thawed at 22°C/1000 RPM for 10 minutes on a Thermomixer C (Thermo Fisher) and centrifuged at 10000 *rcf* for 5 minutes prior to processing on a MicroLab Star Automated Liquid Handler Workstation (Hamilton) with an on-deck KingFisher Presto immunoprecipitation module (Thermo Fisher) and MPE2 solid phase extraction unit (Hamilton). Relevant Aβ isoforms were immunoprecipitated from 0.45 mL of plasma via a monoclonal anti-Aβ mid-domain antibody (HJ5.1, anti-Aβ13-28) that was conjugated to M-270 Epoxy Dynabeads (Invitrogen). Prior to immunoprecipitation, samples were spiked with a known amount of $^{12}C^{15}N$-Aβ38, $^{12}C^{15}N$-Aβ40 and $^{12}C^{15}N$-Aβ42 for use as analytical reference standards. Enriched proteins were subsequently digested into peptides using LysN endoprotease (Seikagaku). Peptide digests were treated with performic acid to convert all methionine residues to their sulfone derivatives and subsequently purified using a mixed-mode cation exchange plate (MCX, Waters). Liquid chromatography-tandem mass spectrometry (LC-MS/MS) was performed as previously described (Ovod *et al.*, 2017). All analyses were performed on an Orbitrap Fusion Lumos Tribrid mass spectrometer (Thermo Fisher) operated in-line with an M-class nanoAcquity chromatography system (Waters). The precursor and product ion pairs utilized for parallel reaction monitoring (PRM) analysis of Aβ species were chosen as previously described (Mawuenyega *et al.*, 2013; Ovod *et al.*, 2017). The derived integrated peak areas were analyzed using the Skyline software package (Pino *et al.*, 2020). Data compiling and analysis was performed using Tableau Desktop Professional Edition (Tableau). Finally, data standardization and statistical analyses were

performed using the R statistical package (v. 3.6.1) as previously described (Schindler *et al.*, 2019).

References

1) Schindler SE, Bollinger JG, Ovod V, et al. High-precision plasma β-amyloid 42/40 predicts current and future brain amyloidosis. Neurology. 2019;93(17):e1647-e1659.
2) Ovod V, Ramsey K, Mawuenyega KG, et al. Amyloid beta concentrations and stable isotope labeling kinetics of human plasma specific to CNS amyloidosis. Alzheimers Dement. 2017;13(8):841-849.
3) Mawuenyega KG, Kasten T, Sigurdson W, Bateman RJ. Amyloid-beta isoform metabolism quantitation by stable isotope-labeled kinetics. Anal Biochem 2013;440:56–62.
4) Pino LK, Searle BC, Bollinger JG, Nunn B, MacLean B, MacCoss MJ. The Skyline ecosystem: informatics for quantitative mass spectrometry proteomics. Mass Spectrom Rev Epub 2017 Jul 9.

Version Information

This document is new and prepared on 2022, November 18 for ADNI data to be uploaded to LONI and will be assigned an initial revision version 1.0.

Dataset Information

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

Dataset Name	Date Submitted
BATEMANLAB_ADNI_Plasma_Abeta4240_20221118	18 November 2022

The data for this analysis was funded by a NIH R01 grant (AG061900) for the aim of determining the relationship between plasma amyloid-beta and other measures of amyloid plaques, including amyloid PET & CSF. We are planning a publication on this data and happy to discuss with others who are interested in seeing the results. If so, please contact the PI, Randy Bateman, at batemanr@wustl.edu

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