ADNI Alzheimer's Disease Neuroimaging Initiative

Plasma NT1-tau in ADNI

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Introduction

Our group recently developed a high-sensitivity plasma immunoassay for a novel N-terminal tau fragment termed NT1-tau on the Quanterix Simoa HD-1 platform.[1–4] This assay succeeded at discriminating AD and mild cognitive impairment due to AD from normal controls. We have recently moved this assay to the Quanterix HD-X platform.

Summary (or Abstract)

We sought a set of plasma samples from ADNI participants across the full range of AD pathology development. Thus, we requested 200 baseline plasma samples from participants from each category of health control, mild cognitive impairment, and Alzheimer disease, who all had at least one amyloid PET. The following sample size was identified in the ADNI repository: 275 healthy control, 379 mild cognitive impairment, and 131 Alzheimer disease.

We ultimately received 829 deidentified plasma samples. The NT1-tau assay was run between 3/18/2022 and 4/1/2022. We obtained NT1-tau values for all plasmas, though some with fewer than the standard three replicates (see below). There was no significant variation from day to day. Of the 829 plasmas, 27 (3%) were below the lower limit of quantitation (LLoQ). Five (1%) had a coefficient of variation (CV) greater than or equal to 20%. We would consider values with CV<20% to be acceptable.

Methodology and Quality Control

NT1-tau

Consumables and reagents other than antibodies were obtained from Quanterix (Lexington, MA). The tau capture antibody BT2 (MN1010 194–198, ThermoFisher) was conjugated onto paramagnetic beads at 1.8 mg/mL. Detector antibody Tau12 (MAB2241, EMD-Millipore) was biotinylated according to the manufacturer using a ratio of 40 parts biotinylation reagent to 1-part antibody. Plasmas were centrifuged at 14,000 × *g* for 4 min and then diluted 1:4 with Tau 2.0 sample diluent reagent (Quanterix). Tau210 untagged standard was diluted linearly with Tau 2.0 sample diluent to a concentration range of 270–0.02 pg/mL. Sample, standards and blanks were prepared in 1.5 mL low-binding Eppendorf tubes and were analyzed in triplicate.

The NT1 Simoa assay used a 3-step protocol and was performed at ambient temperature on a Simoa HD-X analyzer (Quanterix Corp.). In step 1, 100 µl of standard, blank, or sample were added to beads coated with capture antibody and mixed for 30 min. The beads were then harvested and washed with wash buffer. In step 2, biotinylated detection antibody (0.6 µg/ml) was added and incubated for 10 min 30 s, and the beads were then washed three times. In step 3, 150 pM streptavidin-β-galactosidase was added, and following a further wash step, enzyme substrate (resorufin β-D-galactopyranoside) was added. The bead-bearing complexes were then resuspended and loaded into Simoa arrays, each containing 216,000 femtoliter-sized wells. The average enzyme unit per bead (AEB) was determined as described previously (3). Standard curves of AEB vs. Tau210 concentration were fitted to a four-parameter logistic function with 1/Y2 weighting.

The samples were analyzed over ten runs spanning fifteen days, with pooled-plasma internal controls run on each plate to ensure reproducibility across runs. All patient plasma samples were run in triplicate at a 1:4 dilution. The average CV for all samples was 7% (range 0-32%; with 5 above the ideal 20%). The average CV for the internal control was 8%. The LLOQ for all runs was between 0.741 and 2.222 pg/ml. Technical issues with the Simoa HD-X instrument cause occasional random sample drops, in which no data are retrieved from certain wells. So out of the 829 samples, 86% had the expected 3 replicates, 3% had 2 replicates and 2% had a single data point (due to drops, high CV or low volume outliers). There was a final count of 27 (3%) which were below the detection limit.

References

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