

Plasma Biomarkers for Alzheimer's Disease

BD pTau-217 and eMTBR-tau243 for Amyloid and Tau PET Classification in Alzheimer's Disease

Introduction

Alzheimer's disease (AD) is a progressive neurodegenerative disorder defined by extracellular amyloid- β (A β) plaques and tau-containing tangles.¹ Both amyloid and tau pathologies are clinically relevant: the early accumulation of A β plaques aids AD diagnosis,² while tau deposition tracks cognitive decline (**Figure 1**).³

Positron emission tomography (PET) can image amyloid and tau burden; unfortunately, PET scans are expensive and have limited global availability.⁴

A more scalable and cost-effective diagnostic solution is to quantify specific biomarkers in plasma.⁵ Recently identified biomarkers, such as phosphorylated tau-217 (pTau-217)⁶ and its brain-derived isoform (BD pTau-217),⁷ strongly correlate with amyloid PET.

Levels of pTau-217 rise with amyloid pathology;⁸ however, kidney dysfunction or other non-AD disorders can confound the results by increasing peripherally expressed tau.^{9,10} BD pTau-217 is a cleaner marker of AD-related changes, as it measures only tau originating from the brain.⁷

Assessing tau pathology with plasma biomarkers has been more elusive. eMTBR-tau243 (endogenously cleaved, microtubule-binding region of tau containing residue 243), measured by mass spectrometry, was found to correlate with AD tauopathy.¹¹

Measuring both BD pTau-217 and eMTBR-tau243 in a scalable and cost-effective assay could support more comprehensive biological characterization and improved screening for AD.

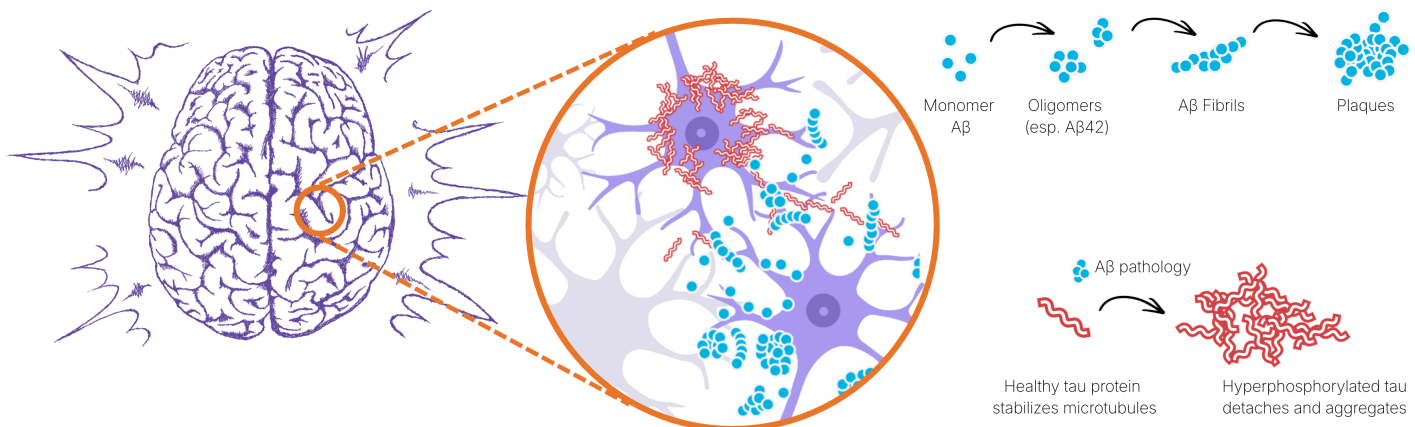


Figure 1. Cartoon of amyloid and tau pathology in Alzheimer's disease (AD). Amyloid monomers of various lengths are created by cleavage of the amyloid precursor protein (APP). Amyloid monomers aggregate into oligomers, fibrils, and plaques. Levels of BD pTau-217 rise with amyloid pathology. Amyloid accumulation may increase phosphorylation on tau proteins. Healthy tau proteins stabilize microtubules; however, hyperphosphorylated tau detaches and aggregates. The eMTBR-tau243 biomarker has been found to correlate with tau pathology.

Introduction, *continued*

In this work, we developed and evaluated a novel plasma duplex assay, requiring only a standard qPCR instrument.

The assay simultaneously quantifies the two targets with only 12.5 μ L of plasma sample using Taudia's SPLASH™ (Solid Phase Ligation Assay with Single wasH) technology (Figure 2).¹²

SPLASH is a highly sensitive proximity-based workflow leveraging antibody pair binding and qPCR read-out.¹³ The assay can achieve sub-pg/mL sensitivity in under four hours, using widely available qPCR instrumentation.

The assay has three conceptual steps: (1) **Target Binding**, (2) **Capture and Wash**, and (3) **Ligate and Quantify**.

During **target binding**, proprietary antibody pairs bind to the target protein in solution to form an immunocomplex. One of the antibodies in the pair is functionalized with biotin, enabling **capture** by streptavidin-coated magnetic beads. The magnetic beads with attached immunocomplexes are held in place by a magnetic rack. A single **wash** step removes unbound antibodies and irrelevant sample matrix material. Signal read-out is accomplished by **ligating** the unique oligonucleotide sequences connected to each antibody, then amplifying each ligated product by PCR and **quantifying** it with a target-specific dye.

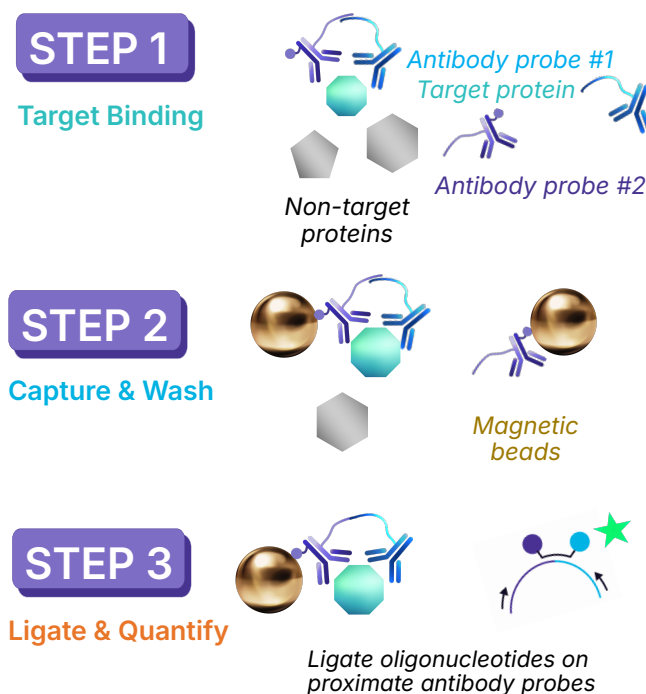


Figure 2. Cartoon illustrating the three conceptual steps in SPLASH assays: (1) **Target Binding**, (2) **Capture and Wash**, and (3) **Ligate and Quantify**.

Results

Human plasma samples were sourced from the Bio-Hermes-002 study (ClinicalTrials.gov identifier: NCT06584357), organized by the Global Alzheimer's Platform Foundation (GAP). Samples were classified by amyloid (A) and tau (T) PET status, with "+" denoting a positive scan and "-" a negative scan. The analysis group contained A-/T-, A+/T-, and A+/T+ (n=25 per group).

Sample concentrations were determined using an in-run calibration standard curve (7-point, 4-fold serial dilution), which converts the Cq (quantification cycle) from the qPCR instrument into absolute protein concentration. Both proteins achieved R^2 values greater than 0.999 across a wide dynamic range (Figure 3).

The mean intra-assay coefficient of variation (%CV) across duplicate measurements of 75 plasma samples was 4%.

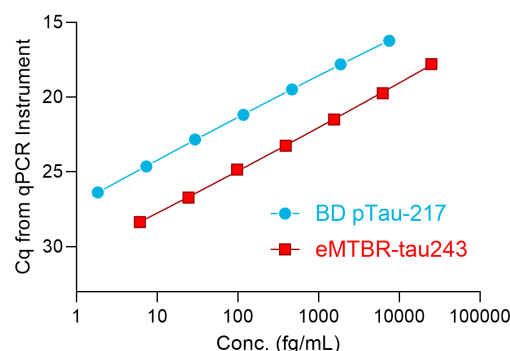


Figure 3. Standard curves for BD pTau-217 and eMTBR-tau243.

Results, continued

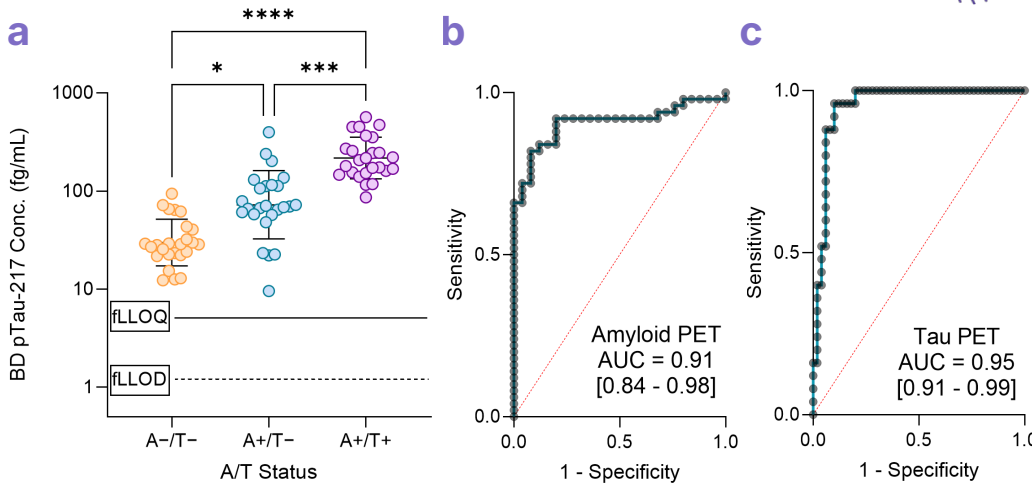


Figure 4. (a) BD pTau-217 levels are significantly different between the A-/T- (orange), A+/T- (blue), and A+/T+ (purple) populations, and all samples were above the functional Lower Limit of Quantitation (fLLOQ) (solid line) and the functional Lower Limit of Detection (fLLOD) (dashed line). (b) Receiver Operating Characteristic (ROC) curve showing predictive power of BD pTau-217 to amyloid PET status, with AUC of 0.91. (c) ROC curve of BD pTau-217 to predict tau PET status, with AUC of 0.95.

BD pTau-217 was detectable in 100% of plasma samples. The levels were significantly different between the A-/T-, A+/T-, and A+/T+ groups (Figure 4a). Both amyloid and tau PET status could be predicted from BD pTau-217 levels, with area under the curve (AUC) of 0.91 for amyloid and 0.95 for tau (Figure 4b, c).

eMTBR-tau243 is hypothesized to reflect tau pathology more closely than amyloid pathology. Consistent with this hypothesis, we observed statistically significant differences between the T- and T+ samples, but no significant difference between the A-/T- and A+/T- samples (Figure 5a).

Further, eMTBR-tau243 was detectable in 100% of the samples (all samples above the fLLOD). The biomarker was quantifiable (above the fLLOQ) in 100% of the tau PET-positive samples and 96% quantifiable in the tau PET-negative samples. The AUC for tau PET status was 0.83 (Figure 5b).

Overall, BD pTau-217 had a higher AUC for tau pathology. However, since the T- group includes the A-/T- samples, BD pTau-217's tau AUC partly reflects amyloid status.

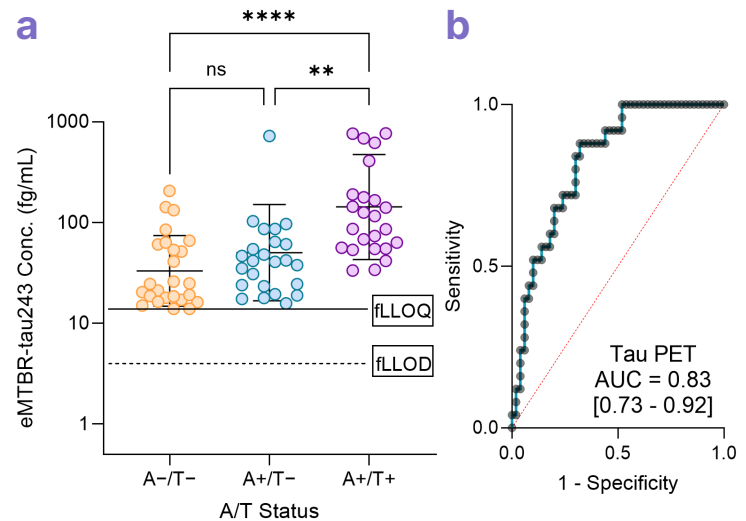
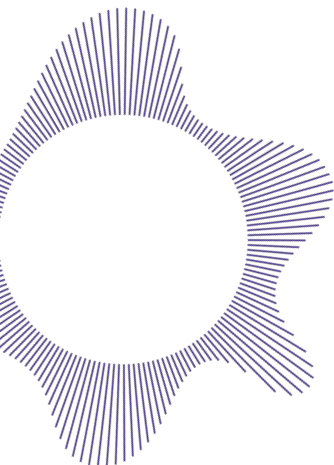


Figure 5. (a) eMTBR-tau243 levels are significantly different between the T- (orange and blue) and T+ (purple) populations, but not between A-/T- and A+/T-. In the A-/T- population, two samples are below the fLLOQ. (b) ROC curve showing predictive power of eMTBR-tau243 to tau PET status, AUC of 0.83.

Discussion



In this PET-annotated cohort, plasma BD pTau-217 demonstrated excellent detectability and strong classification of both amyloid and tau PET status. eMTBR-tau243 showed more modest discrimination of tau PET status, but its potential association with more advanced tau pathology warrants further investigation. The results suggest that a combination of the two biomarkers, BD pTau-217 and eMTBR-tau243, may provide complementary insights for biological screening in some AD clinical trial settings. BD pTau-217 may reflect the overall AD continuum, while eMTBR-tau243 is selective for tauopathy.

An important consideration is that this cohort consisted primarily of individuals across early clinical stages of AD, potentially limiting representation of advanced tau pathology. The relatively modest performance of eMTBR-tau243 compared with BD pTau-217 may therefore reflect the stage dependence of this biomarker. eMTBR-tau243 may become more informative as tau pathology advances, consistent with its potential utility for identifying later-stage disease.

Rather than providing redundant information, the two biomarkers may ultimately serve complementary roles: BD pTau-217 for sensitive identification of AD-related pathology and eMTBR-tau243 for identifying individuals with more advanced tau accumulation. Larger cohorts spanning the full spectrum of tau pathology are needed to determine whether combining these biomarkers can improve biological staging and clinical trial participant selection.

Materials and Methods

Cohort Acquisition

Plasma samples were sourced from the Bio-Hermes-002 study (ClinicalTrials.gov identifier: NCT06584357), organized by the Global Alzheimer's Platform Foundation (GAP). All participants provided written informed consent prior to sample collection, and study protocols were administered by approved clinical trial sites. All data access and experimental methods complied with institutional review board (IRB) standards. The plasma samples were thawed and aliquoted upon receipt, then stored at -80°C until analysis.

SPLASH Workflow

Plasma samples and standards were measured in duplicate following the standard SPLASH protocol, as described in reference 12. The samples were run on a benchtop qPCR instrument (QuantStudio 3 or QuantStudio 5 96-well 0.2 mL, Thermo Fisher Scientific, Waltham, MA, USA) for ligation, amplification, and signal read-out. Data were collected in the FAM (BD pTau-217) and Cy5 (eMTBR-tau243) channels, with ROX normalization enabled.

Data Analysis

Cq (quantification cycle) values from the qPCR curves were obtained from the instrument manufacturer's analysis software. The calibration standard curves were fit with four-parameter logistic (4PL) regression, then used to convert plasma Cq values to concentrations in fg/mL. The ROC curves were fit with logistic regression, using the PET status as the dependent variable and protein concentration as the predictor. Group differences were assessed by Kruskal–Wallis with Dunn's post hoc multiple comparisons (* $p < 0.05$, ** $p < 0.01$, *** $p < 0.001$, **** $p < 0.0001$; ns, not significant).

References

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