SpearBio

Distributed by: bio-techne®

APPLICATION NOTE

Breaking barriers in Alzheimer's research: SPEAR pTau 217 outperforms leading ultra-sensitive technology

Abstract

Phosphorylated tau at threonine 217 (pTau 217) has emerged as a critical biomarker, distinguishing Alzheimer's disease (AD) from control with a high degree of specificity. Spear Bio has developed an ultra-sensitive immunoassay for the detection of pTau 217 (SPEAR pTau 217), demonstrating excellent accuracy and specificity distinguishing AD from control (n=124 clinical samples) with an AUC of 98.8% and a 5.3-fold increase between the means. Establishing SPEAR pTau 217 performance to a well-characterized commercial assay with a subset of samples illustrated good agreement, while demonstrating a higher level of predictability with an AUC of 98.6% vs. 92.4%. In addition, Spear Bio's proprietary technology affords several practical advantages, including low sample volume, lack of expensive proprietary equipment, and ease of use with an automatable simple workflow thereby accelerating advancements in AD research, clinical trial patient selection, drug response monitoring, and earlier intervention.

Introduction

Alzheimer's Disease (AD) is characterized by amyloid pathology leading to tau tangles and eventual neurodegeneration. A critical step preceding neurofibrillary tangles (NFT) is hyperphosphorylation of tau and the development of paired helical filaments (PHF). While PHFs are not neurotoxic on their own, with subsequent tau truncation they aggregate into neurodegenerative NFTs. Research into tau phosphorylation provides early insights into the emergence of tau pathology in AD.

Among various phosphorylated tau candidates, pTau 217 has emerged as a biomarker that differentiates dementias caused by other neurodegenerative diseases with the highest accuracy and the greatest fold difference.[1,2]

Importantly, only small increases in pTau 217 levels are necessary to be categorized as abnormal, with abnormal levels of the biomarker becoming present earlier in the AD pathogenesis.[3,4] pTau 217 levels also demonstrated longitudinal increases in amyloid positive compared to amyloid negative cases, making pTau 217 an excellent candidate for early AD screening.[4] Lack of access to and costs of cerebral spinal fluid (CSF) biomarker analyses have made blood-based assays another avenue of detection for diseases such as AD.[4] Early detection and intervention of AD is critical to the successful

Introduction, continued

development and treatment of this insidious disease and requires access to high performance blood-based biomarkers for research, clinical trial patient selection, treatment monitoring, and eventual diagnosis before symptoms emerge.

Materials and Methods

Clinical EDTA plasma samples[5] were provided in two batches by a hospital-based academic research center specializing in neurodegenerative diseases. Samples provided were from patients with the following diagnoses: apparently healthy control (HC), other neurological disease (OND), and AD; see Table 1 for additional information.

Group	Batch #1	Batch #2	Total
Healthy control (**HC**) *Cognitively unimpaired (**CUI**)*	8	12	**20**
Other neurological disease (**OND**) *Cognitively unimpaired (**CUI**)*	9	13	**22**
Other neurological disease (**OND**) *Cognitive status unknown*		1	**1**
Other neurological disease (**OND**) *Cognitively impaired (***CI**)*		22	**22**
Alzheimer's Disease (**AD**) *Cognitively impaired (***CI**)*	19	40	**59**
Total	36	88	**124**

Table 1. Clinical sample diagnosis and cognitive status provided in batch 1 and 2. *Cognitive impairment included patients with mild cognitive decline (MCI) and dementia. OND group 20/22 samples were identified with MCI. AD group, batch #1 7/19 MCI and batch #2 18/40 MCI.

Clinical diagnoses were established according to the 2011 National Institute of Aging – Alzheimer's Association diagnostic criteria for dementia and mild cognitive decline (MCI) due to AD and AD status was verified by CSF AD biomarker analysis. Results from a commercially available immunoassay, Simoa® ALZpath p-Tau 217 Assay (item 104570) were available for 34 of the 36 samples in batch 1; 17 from the AD group, and 17 from the control group. The Simoa ALZpath assay lost 2 out of 36 samples tested, this comparisons between assays focused on samples with data on both platforms.

The SPEAR pTau 217 immunoassay was developed utilizing an antibody specific to tau phosphorylated at threonine 217 in conjunction with an antibody recognizing total tau at another epitope. Unique DNA probes were conjugated to each antibody to perform the proprietary two-factor authentication. Phosphorylated 217 peptide conjugate, including both antibody epitopes, was used to determine protein concentration in samples, and diluents were optimized for spike recovery and parallelism between 80-120% and mean concentration CVs under 10%. Samples were diluted 4-fold with 1 µL of diluted sample being used in the assay.

Results

SPEAR pTau 217 demonstrated exceptional accuracy in distinguishing AD from control with an AUC of 98.8% (Figure 1) in 124 samples with clinical diagnoses (Table 1), and an average 5.3-fold increase in AD over the control group.

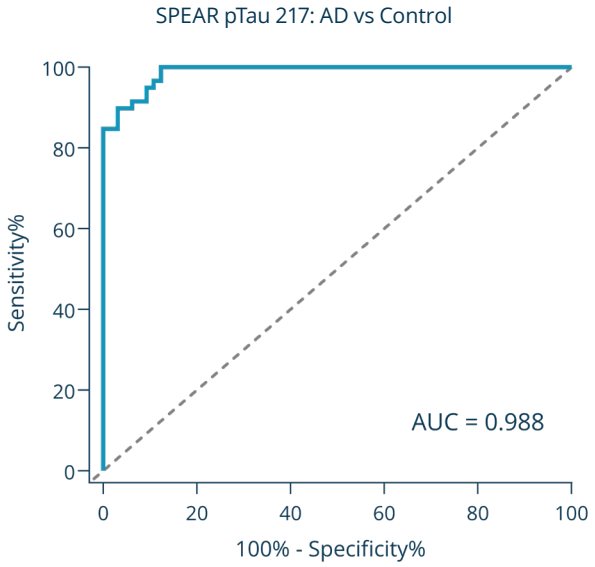


Figure 1. SPEAR pTau 217 ROC analysis for 59 diagnosed with AD with MCI or dementia and 65 controls, including cognitively unimpaired healthy, other neurological diseases without cognitive impairment and other neurological diseases with MCI or dementia. AUC calculated.

Results, continued

Control group parsed further by diagnosis and cognitive status were statistically indistinguishable from each other, exemplifying the specificity of the SPEAR assay to differentiate cognitive impairment due to AD over other neurological disorders (Figure 2).

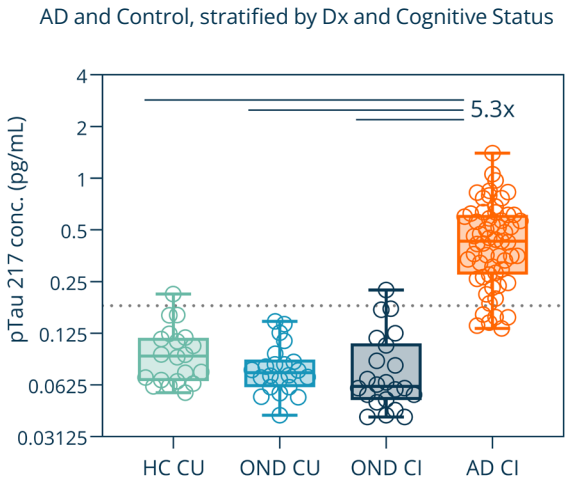


Figure 2. SPEAR pTau 217 concentrations (pg/mL) in 59 diagnosed AD with MCI or dementia vs. control (20 CU HC, 22 CU OND, and 22 CI OND). Box plot with all data and min and max bars. Fold difference calculated between AD CI group and the collective of HC CU, OND CU, and OND CI. Dotted line indicates estimated single cutoff. Note, exclusion of one OND sample with cognitive status unknown.

With an estimated single cutoff (>0.1813 pg/mL) this cohort resulted in 89.8% sensitivity and 96.9% specificity; positive predictive value (PPV) of 96.4% and negative predictive value (NPV) of 91.3%.

Simoa ALZpath p-Tau 217 results were available for a subset of the samples tested (n=34, 17 AD and 17 control) and further analyzed to better understand the performance of the SPEAR pTau 217 assay. Correlation between Simoa ALZpath and SPEAR pTau 217 (Figure 3) demonstrated good agreement between the assays with Spearman r of 0.905. Further analysis illustrated a higher predictability of the SPEAR immunoassay with an AUC of 98.6% vs.

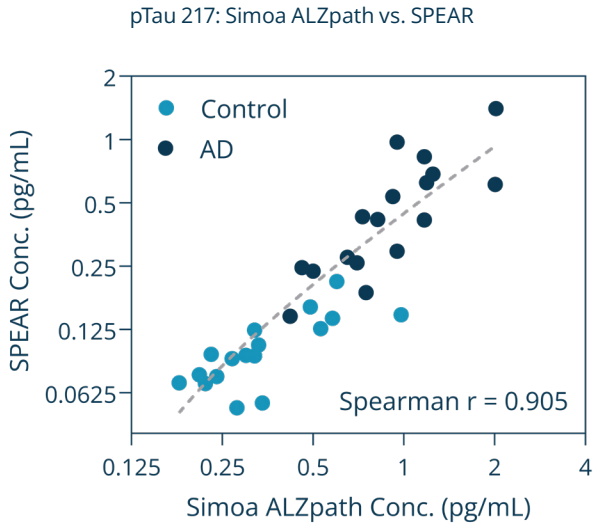


Figure 3. pTau 217 concentrations for 17 AD and 17 control samples for Simoa ALZpath (x-axis) vs. SPEAR (y-axis).

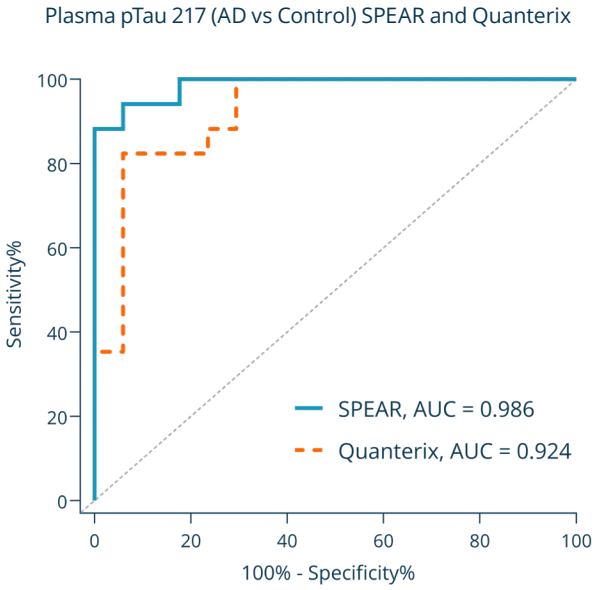
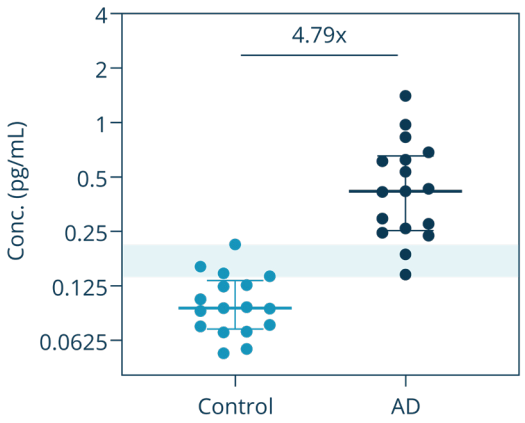


Figure 4. ROC analyses, comparing SPEAR vs. Quanterix ALZpath assays for pTau 217 in 34 EDTA plasma samples; 17 control (9 HC, 8 OND, all CU) and 17 AD (11 dementia, 6 MCI). Area under the curve (AUC) calculated for each assay.

92.4% for Simoa (Figure 4). This was further evidenced by a greater fold increase between the means of AD and control groups for SPEAR, 4.79, vs. Simoa, 2.60 (Figure 5).

A. SPEAR pTau 217 (1 µL of diluted sample)



B. Quanterix ALZpath (100 µL of diluted sample)

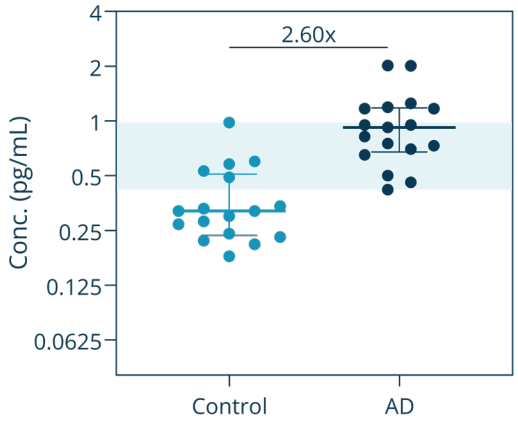


Figure 5. A. SPEAR and B. Quanterix ALZpath assays for pTau 217 in 34 EDTA plasma samples; 17 control (9 HC, 8 OND, all CU) and 17 AD (11 dementia, 6 MCI). Fold increase between control and AD means indicated for each assay in addition to shaded bands illustrating pTau 217 concentration overlap between groups.

Discussion

The SPEAR pTau 217 immunoassay stands as a powerful tool for the early detection and differentiation of AD. With remarkable clinical sensitivity and specificity, SPEAR achieved an AUC of 98.8%, including cognitively impaired patients from other neurological diseases. The impressive 5.3-fold separation between control and AD highlights SPEAR's potential capability to minimize indeterminate results, while maintaining exceptional accuracy, especially when considering PPV and NPV of 96.4% and 91.3% with a single proposed cutoff.

Increased levels of pTau 217 have been previously correlated with disease progression and severity, thus, the samples selected for evaluation of an assays predictive power to differentiate can have an impact on the results. Baseline establishment to existing methodologies provides deeper understanding for various assays measuring pTau 217.

A direct comparison of SPEAR pTau 217 to Quanterix Simoa ALZpath illustrated better predictability of SPEAR to differentiate AD from control with an AUC of 98.6% vs. 92.3%, which was further exemplified with nearly twice the fold increase between groups. SPEAR technology's advantages extend to requiring 100 times less sample volume than Quanterix, utilizing standard qPCR instrumentation, and streamlined automation with liquid handlers.

Taking robust performance and practical advantages together, SPEAR pTau 217 immunoassay holds the promise to accelerate the pace of discovery, improve patient outcomes, and bring us closer to conquering Alzheimer's Disease.

References

1. Palmqvist S, et al. (2020) Discriminative Accuracy of Plasma Phospho-tau217 for Alzheimer Disease vs Other Neurodegenerative Disorders. *JAMA.* 324(8):772–781.
2. Niklas Mattsson-Carlgren, et al. (2020) Longitudinal plasma p-tau217 is increased in early stages of Alzheimer's disease, *Brain,* Volume 143, Issue 11, Pages 3234–3241.
3. Milà-Alomà, et al. (2022) Plasma p-tau231 and p-tau217 as state markers of amyloid-β pathology in preclinical Alzheimer's disease. *Nat Med* 28, 1797–1801.
4. Gonzalez-Ortiz, F., et al. (2023) Plasma phospho-tau in Alzheimer's disease: towards diagnostic and therapeutic trial applications. *Mol Neurodegeneration* 18, 18.
5. Kivisäkk, P., et al. (2024) Clinical evaluation of a novel plasma pTau217 electrochemiluminescence immunoassay in Alzheimer's disease. *Sci Rep* 14, 629.

SpearBio
100 Tradecenter Dr. Suite 510
Woburn, MA 01801
spear.bio

SPR-DOC80001-V1

bio-techne®
614 McKinley Place NE
Minneapolis, MN 55413
bio-techne.com
1-800-343-7475