Alzheimer's Pathology Detection Made Simple

A Blood Test for p-Tau 217 to Aid in the Diagnostic Evaluation for Alzheimer's Disease

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Introduction

The recent emergence of promising new Alzheimer's therapies has brought to light an urgent need for improved tools to help diagnose the disease in its early stages when therapeutic intervention is most likely to deliver clinical benefit. While Alzheimer's disease diagnostics often rely on a clinically based diagnosis of exclusion, biomarkers are playing an increasingly important role. In recognition of this evolution, the National Institute on Aging—Alzheimer's Association (NIA-AA) has recently released a draft document that attempts to define biomarker based criteria for diagnosis and staging of Alzheimer's Disease (AD).[1] As with NIA-AA's previously published research framework defining Alzheimer's as a biological process characterized by associated biomarkers,[2] the new criteria document recognizes amyloid and tau pathology as the defining biological-based signature of the disease. Both amyloid and phosphorylated tau have been shown in many clinical studies to be highly predictive biomarkers of the presence of amyloid pathology, whether measured by positron emission tomography (PET), in cerebrospinal spinal fluid (CSF), or in blood. Extending this framework, the new NIA-AA document is aimed at more specifically addressing different use-cases and performance criteria for fluid-based biomarkers, including blood-based tests.

The Challenge: Inaccessibility of Alzheimer's Diagnostics

A Rand Corporation study on the readiness of the U.S. healthcare system infrastructure for an Alzheimer's treatment highlighted a dire situation of unpreparedness and where key bottlenecks lie.[3] These bottlenecks are associated with the current diagnostic modalities that are impractical for the broad needs of determining disease status as an entry criteria for potential drug trial and/or the therapeutic candidacy. Currently the most established biomarker-based approaches to diagnostic workup include PET imaging and lumbar puncture-derived CSF biomarkers for amyloid and tau/pTau, both of which are invasive, expensive, and may not be widely available.

The Solution: Non-invasive Blood Tests

Fortunately, in tandem with progress in Alzheimer's therapeutics, there has been rapid progress on the development of tests for blood-based biomarkers that have the potential to enable non-invasive alternatives to CSF and PET biomarker modalities for assessing amyloid and tau pathology status. This progress has been enabled in large part by technical advances in mass spectrometry and ultrasensitive immunoassay techniques such as single-molecule array (Simoa). Blood-based biomarkers hold the promise of expanding access by reducing the number expensive and invasive procedures and could enable screening, improve clinical diagnosis, and allow for repeated sampling as possible monitoring markers for disease progression and therapeutics

LucentAD p-Tau 217 Test and Intended Population

The LucentAD p-Tau 217 test helps identify whether an individual with cognitive symptoms is likely or unlikely to have amyloid plaques in the brain, a hallmark of Alzheimer's disease. The test relies on quantitation of the tau isoform that is phosphorylated at the 217 amino acid residue (p-Tau 217) in plasma using proprietary single molecule array (Simoa) technology[4] which provides unprecedented sensitivity and precision for measuring low abundance proteins, such as brain-derived proteins in blood. This readily accessible, non-invasive test leverages a simple blood draw to provide a result to help rule out or rule in patients with cognitive symptoms for the presence of amyloid pathology and aid in obtaining a final diagnosis.

Blood p-Tau vs. Amyloid

Both amyloid beta isoforms (peptides Aβ40 and Aβ42, typically measured as a ratio) and phosphorylated tau isoforms are correlated with amyloid PET status, but even the best amyloid ratio methods are insufficient as a standalone diagnostic for Alzheimer's disease, providing a general clinical accuracy of approximately 85% for discerning amyloid positive individuals from amyloid negative individuals. A weakness of blood-based amyloid measurements is that the biomarker in blood comes mostly from peripheral sources, with only a small fraction thought to be from the central nervous system compartment. Thus, blood amyloid measures mostly background noise, and amyloid signal differences (as Aß42/Aß40 ratio) of only about 10-15% can be detected between Alzheimer's subjects and healthy controls. This creates an analytical challenge that renders the test difficult to implement in real-world settings where measurement variation from different sources can severely compromise the reliability and accuracy of the test.[5]

Phosphorylated tau, on the other hand, exhibits a much larger signal difference between Alzheimer's subjects and healthy controls, up to several fold and more. This permits more reliable discrimination between disease state and non-disease state, including subjects in the early stages of the disease. A recent study has highlighted that p-Tau accuracy is robust to random variation up to 20%, well above typical expected variation in a clinically implemented test.[5] While the interrelationship between amyloid and pTau in the early stages of incipient Alzheimer's pathology is still being elucidated, soluble pTau elevation and amyloid deposition are closely linked, and blood pTau has been shown to be highly predictive of amyloid PET as well as tau PET.[6] Thus, blood pTau can serve as a surrogate for predicting amyloid positivity. An additional attractive characteristic of pTau is its high specificity for Alzheimer's pathology. This makes the test insensitive to potential neurological co-morbidities, including dementias cause by diseases other than Alzheimer's.

Based on the superior performance of plasma p-Tau, and specifically p-Tau 217, for determining amyloid status, it is the only blood-based biomarker recommended by the NIA-AA criteria for Alzheimer's disease for diagnosis.

Background Evidence for Clinical Validity of LucentAD p-Tau 217 Assay Design

The advantages of tau phosphorylated at the threonine 217 site over other phosphorylated tau isoforms for detection of amyloid pathology was first described in CSF by Janelidze et al,[7] and then in plasma by Palmvist and coworkers,[8] both reports appearing in 2020. The interest in plasma p-Tau 217 grew rapidly as a blood-based diagnostic biomarker with applications that include prognostics and disease progression monitoring.[10]

The LucentAD p-Tau 217 test is based on a well-studied Simoa assay design and antibodies licensed from J&J Innovative Medicine (formally Janssen R&D). The assay design and development for measuring p-Tau 217 in plasma was first described in 2021 by Triana-Baltzer et al.[10] Subsequently, the efficacy of the plasma assay for detection of amyloid pathology was extensively studied in published comparisons with amyloid and tau PET,[11,12] as well as CSF biomarkers.[13] In the later study, the ability of the assay to correctly detect amyloid positivity in a real-world memory clinic setting was compared with an FDA-cleared CSF test for Aß42/p-tau ratio. Across 197 patients presenting with memory concerns, the Janssen/LucentAD p-Tau 217 test achieved very high accuracy vs. the CSF reference method, with a Receiver Operator Characteristics area under the curve (AUC) of 0.96, where an AUC of 1.0 represents perfect agreement. This level of performance in plasma matched the accuracy of detecting p-Tau 217 in CSF, and was the highest of all the plasma-based p-Tau assays tested. Subsequent work has further highlighted the equivalency of this p-Tau 217 plasma assay to CSF biomarkers for diagnostic accuracy,[14] the utility of the assay as a state biomarker for amyloid accumulation in cognitively unimpaired individuals, and to inform on downstream tau tangle accumulation.[15] The diagnostic performance of the assay for identifying amyloid-PET positivity in cognitively unimpaired individuals, as well as identifying biological AD (amyloid-PET and tau-PET positivity) in cognitively impaired individuals was recently studied in 294 subjects from the TRIAD cohort that spanned the disease spectrum.[16] Among cognitively unimpaired individuals, the assay demonstrated an AUC of 0.92 for identifying amyloid PET positivity, and an AUC of 0.96 for identifying biological AD in cognitively

impaired patients. Measured p-Tau 217 was also observed to increase significantly according to AD severity as assessed with PET-based Braak staging. As exhibited in **Figure 1**, p-Tau 217 results increased sequentially from cognitively unimpaired (CU) Aß- individuals to CU Aß+individuals, to mild cognitive impairment (MCI) Aß+ to Aß+ individuals with AD dementia. The low concentrations of plasma p-Tau 217 in young adults, as well as Aß- individuals highlight the specificity of p-Tau 217 for AD.

Figure 1

p-Tau 217 assay results across clinical categories

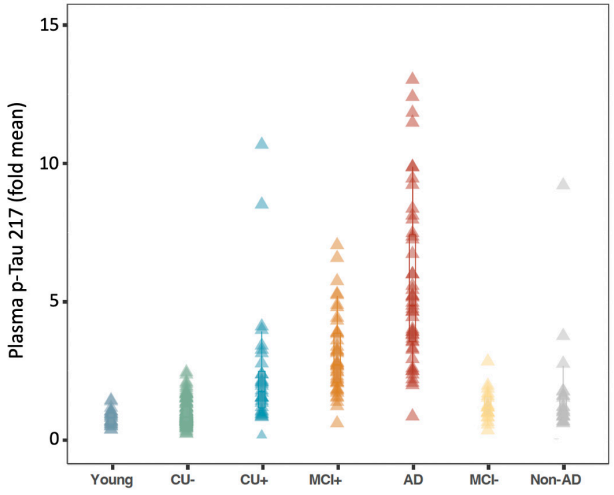

Fig 1. Plasma p-Tau217 (as fold change) across diagnostic groups and amyloid-PET status. CU=cognitively unimpaired, MCI=mild cognitive impairment, AD=Alzheimer's disease with dementia. +/- refers to amyloid status. Non-AD= non Alzheimer's dementia. Reproduced from Therriault et al.[16]

Fig 2. Highlights the strong relationship between plasma p-Tau 217 and amyloid PET.

Figure 2

p-Tau 217 vs. amyloid PET across clinical categories

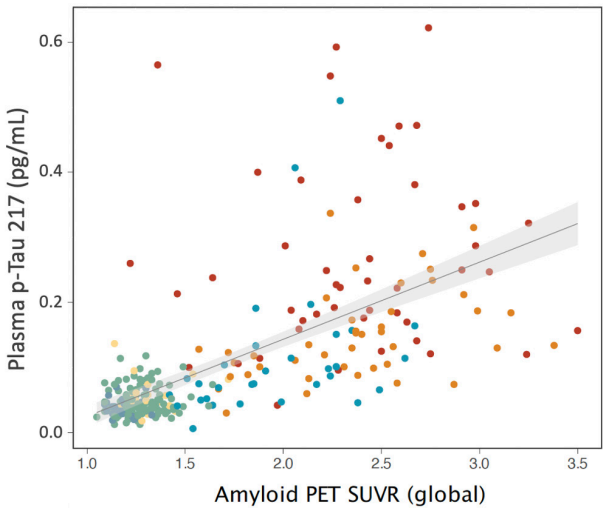

Fig 2. Relationship between plasma p-Tau217 assay concentrations vs. [18F]AZD4694 SUVR across the AD continuum (see Fig 1 for color key). The assay exhibited very strong correlation with amyloid-PET SUVR (rho(Þ)= 0.71, P<0.0001). Reproduced from Therriault et al.[16]

Summary. The data discussed in this section for the p-Tau 217 plasma assay which forms the basis of the LucentAD p-Tau 217 test, add to a growing body of evidence of the suitability of plasma p-Tau 217 for implementation in clinical settings to aid in the diagnostic evaluation of individuals suspected of AD. Clinical validation of the LucentAD p-Tau 217 test for this intended use is described in the following sections.

Summary of LucentAD p-Tau 217 Test and Intended Use Population

The LucentAD p-Tau 217 test helps identify whether an individual with cognitive symptoms is likely or unlikely to have amyloid plaques in the brain, a hallmark of Alzheimer's disease. The test relies on quantitation of p-Tau 217 in plasma using proprietary single molecule array (Simoa) technology[4] which provides unprecedented sensitivity and precision for measuring low abundance proteins, such as brain-derived proteins in blood. This readily accessible, non-invasive test enables leveraging a simple blood test for a more informed evaluation of patients with cognitive symptoms to help determine the likelihood of the presence of amyloid plaques as an aid toward a diagnosis.

Clinical Performance of the LucentAD p-Tau 217 Test

The LucentAD p-Tau 217 test was optimized to maximize clinical sensitivity and specificity for patients with cognitive symptoms. A 2-cutoff approach was utilized as recommended by the draft NIA-AA Revised Criteria for Diagnosis and Staging of Alzheimer's Disease,[1] and Brum et al.[17] The use of two cutoffs establishes a three-zone test reflecting low, intermediate, and high risk of amyloid pathology. Samples reading at or below the lower cutoff are unlikely to have amyloid pathology, and samples reading at or above the upper cutoff are likely to have amyloid pathology. Test results in the intermediate range between the lower and upper cutoffs are considered to have an intermediate risk of amyloid pathology and may require referral for evaluation by other methods, including CSF biomarker testing.

To establish the clinical performance of the LucentAD p-Tau 217 test, EDTA plasma samples were tested in duplicate in the Quanterix CLIA laboratory. The clinical sample sets included patients with MCI and AD dementia from the Amsterdam Dementia Cohort (ADC),[18] and recruited individuals with MCI and mild AD from the BioHermes trial.[19] The ADC cohort represents a memory clinic setting with CSF biomarkers as the method for determining amyloid status. The BioHermes trial included recruited participants from 17 US sites with emphasis on racial/ethnic diversity and utilized amyloid PET to determine amyloid status. These two clinical groups represent divergent settings, reference methods, and geographic/ethnic/racial samplings.

Clinical thresholds were established with a randomized sampling of approximately 50% of ADC and BioHermes cohorts with a focus on the ADC cohort. The thresholds were selected to provide sensitivity, specificity and overall assay accuracy vs. CSF and PET reference methods of at least 90%, with an intermediate range within approximately 30% of the total samples tested. "Accuracy" is defined per the NIA-AA criteria[1] as (True positive + True negative)/(True positive + True negative + False positive + False negative). The thresholds were then validated with the remaining ADC and BioHermes samples. With a lower diagnostic threshold of ≤0.040 pg/mL and an upper threshold of ≥0.090 pg/mL, the LucentAD p-Tau 217 test gave the performance characteristics exhibited in **Tables 1** and **2**.

Table 1: Diagnostic Performance with Amsterdam Dementia Cohort

Performance category	ADC training n=165	ADC validation n=187
AUC	0.96	**0.93**
Sensitivity†	92.4%	**90.2%**
Specificity†	100%	**97.8%**
Accuracy (NIA-AA) †	94.2%	**92.8%**
Interm. range	26.7%	**26.2%**

†Excluding samples in the intermediate range

Table 2: Diagnostic Performance with BioHermes cohort and combined cohorts

Performance category	BioHermes n=521	ADC + BioHermes n=873
AUC	0.87	**0.89**
Sensitivity†	89.3%	**90.3%**
Specificity†	88.0%	**91.3%**
Accuracy (NIA-AA) †	88.7%	**90.7%**
Interm. range	34.0%	**30.9%**

†Excluding samples in the intermediate range

Across both cohorts (n = 873 patients), the LucentAD p-Tau 217 test gave an overall accuracy of **90.7%** as compared with CSF biomarkers and amyloid PET imaging. 30.9% of the results fell within the intermediate range between 0.040 and 0.090 pg/mL. The overall diagnostic performance of the LucentAD p-Tau 217 test meets the NIA-AA recommendation for plasma test accuracy to support AD diagnosis and is aligned with a proposed 2-step workflow to screen for amyloid ß positivity with confirmatory testing only in uncertain cases (within the intermediate risk range).[17]

Using and Interpreting the LucentAD p-Tau 217 Test

The LucentAD p-Tau 217 test is intended to be used in patients with symptoms of cognitive impairment who are being evaluated for Alzheimer's disease (AD) to aid in diagnostic evaluation. A low result by the LucentAD p-Tau 217 test at or below 0.040 pg/mL indicates a low likelihood of the presence of amyloid pathology. Alternative causes for the patient's memory concerns should be investigated. An elevated result at or above 0.090 pg/mL indicates a high likelihood of the presence of amyloid pathology. An elevated result at or above 0.090 is consistent with the presence of Alzheimer's disease but does not in itself establish a diagnosis. Test results in the diagnostic gray zone between 0.040

and 0.090 pg/mL are associated with an intermediate likelihood of amyloid pathology. If clinically indicated, an intermediate result may require referral for evaluation by other methods such as CSF biomarker testing or PET imaging to confirm the absence or presence of amyloid pathology. **Table 3** summarizes the interpretation of the LucentAD p-Tau 217 test results.

Table 3: Test result interpretation

Test Result p-Tau 217 (pg/mL)	Interpretation
≤0.040	Low likelihood of amyloid pathology
0.041-0.089	Intermediate likelihood of amyloid pathology If clinically indicated, consider confirmatory testing
≥0.090	High likelihood of amyloid pathology

LucentAD p-Tau 217 is not a standalone diagnostic test. LucentAD p-Tau 217 results support a diagnostic assessment as an adjunct to other methods, such as clinical assessment, exclusionary blood workup, and cognitive evaluations. In uncertain cases, including an intermediate result from the LucentAD p-Tau 217 test, cerebrospinal fluid (CSF) biomarker tests or amyloid positron emission tomography (PET) may be indicated for further evaluation of amyloid pathology status to support a diagnosis.

Summary

The LucentAD p-Tau 217 blood test achieves an overall accuracy exceeding 90% which meets the stringent requirements set forth in the proposed NIA-AA Revised Criteria for Diagnosis and Staging of Alzheimer's Disease. The test provides a blood-based method for accurate amyloid risk stratification of patients exhibiting cognitive symptoms to aid in diagnostic evaluation.

Ordering Information

To order LucentAD p-Tau 217 collection materials go to **LucentDiagnostics.com**

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