ATN Profile: For and beyond Alzheimer's disease

Alzheimer's disease (AD) is the most common form of dementia, accounting for 60%-70% of dementia cases.[1] Assays for blood-based biomarkers (BBMs) specific to AD pathology, such as beta amyloid 42/40 and pTau217, are now available to clinicians to help support assessment and monitoring of patients with dementia symptoms. But this still leaves up to 40% of patients with clinical signs of mild cognitive impairment (MCI) or possible dementia without answers.

With new therapies available for AD, appropriate triaging of patients who need a neurologist for treatment and monitoring is critical. BBMs can help with that triaging process, and pTau217 has emerged as a lead candidate for a triaging tool. Indeed, a single biomarker with high sensitivity and specificity to rule-in patients likely of having AD is promising. However, using this marker alone leaves the other 30%-40% of patients, who often present with ambiguous dementia symptoms and may perform less than optimally on cognitive exams, with limited options and answers, leaving clinicians with little else to go on.

When a patient with possible MCI or dementia first presents, the primary clinical question for the clinician is, "What is going on with this patient?" The question of whether a patient may have AD is a secondary clinical question, alongside questions regarding polypharmacy, vitamin deficiencies, hormonal imbalances or metabolic problems. Using pTau217 only addresses one of the many secondary clinical questions, making it a less effective clinical approach when compared to a BBM panel, which provides additional insight and evidence toward the primary clinical question and as an initial step before diagnostic tools such as testing of cerebrospinal fluid (CSF) or a positron emission tomography (PET) scan.

This was the impetus behind Labcorp's ATN Profile, which employs three well-studied BBMs in a single assay. Designed to help rule-in or rule-out evidence of AD pathology, the profile also provides additional biological evidence to help guide clinicians for those patients with MCI or dementia that may not be the result of AD. The three biomarkers in the ATN Profile include:

- **A:** Plasma Aβ42/40 immunoassay. Ovod et al[2] demonstrated that accurate amyloid-beta assessments for AD from blood were possible using mass spectrometry techniques. More recently, studies have demonstrated that plasma Aβ42/40 assessments could achieve levels similar to mass spectrometry-based methods for detecting AD pathology.[3,4] Labcorp's own internal validation of this Aβ42/40 immunoassay measured 200 plasma specimens acquired from the Australian Imaging, Biomarker and Lifestyle (AIBL) Study of Ageing.[5] Specimens were obtained from amyloid PET-negative subjects classified as cognitively unimpaired and amyloid PET-positive subjects classified as having no cognitive impairment, MCI or AD dementia. These same samples were run through Labcorp's pTau217 assay. Performance for the Aβ42/40 immunoassay was nearly equivalent to pTau217 (Figure 1). Receiver operating characteristic (ROC) analysis of clinical specimen results from validated assays produced an area under the curve (AUC) of 0.94 for both Aβ42/40 and pTau217. The sensitivity (96%) and specificity (87%) observed for Aβ42/40 measurements meets current recommendations for triage testing and was slightly better, although not statistically significant, compared to pTau217 (Figure 1). Notably, the same Aβ42/40 measurement system and assays utilized herein produced similar AUC results reported for different clinical cohorts with the same measurement system, making this assay one of the best-performing Aβ42/40 immunoassays to be validated with two independent cohorts on two different continents.[3,4,6]
- **T:** Plasma pTau181 immunoassay. pTau181 was discovered prior to pTau217. In time, pTau217 has been demonstrated to be the better phosphorylated tau marker for AD because it serves as a specific surrogate for beta-amyloid pathology with high correlation to PET. Given the performance of the Aβ42/40 assay and the design goals of the ATN Profile, a second high-precision marker for detecting amyloid pathology was not necessary. pTau181 is used intentionally because it may help differentiate between AD and suspected non-AD pathophysiology (SNAP), which would rule a patient out of anti-amyloid therapy.[7] Elevated pTau181 levels, in the absence of evidence of beta-amyloid

ATN framework

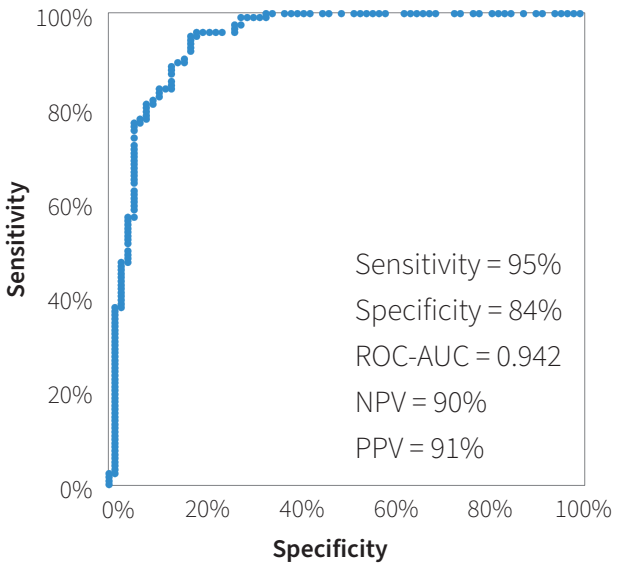
Category	Biological Changes → Biomarkers	
A amyloid	Amyloid plaques	amyloid PET CSF Aβ42/40 Blood Aβ42/40
T tau	Tau tangles	tau PET CSF pTau Blood pTau
N neurodegeneration	Damage to nerve cells in the brain	MRI CSF tTau Blood NfL

labcorp

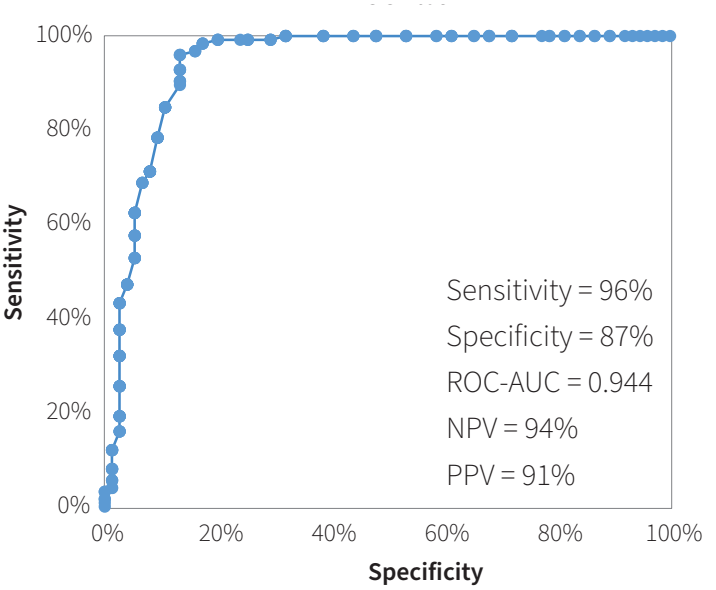
Figure 1: pTau217 and Beta-Amyloid 42/40 Ratio Performance

The beta amyloid 42/40 ratio and pTau217 tests were clinically validated using 200 samples from a well-studied cohort in which all samples were characterized with patient age, sex, amyloid PET status, and clinical diagnosis.

pTau217 ROC Plot



Beta-Amyloid 42/40 ROC Plot



pathology, is associated with memory impairments and atrophy in the medial temporal lobe.[8] As an initial BBM-based triaging test that goes beyond the binary question of AD or not, pTau181 may be a more useful marker.

- **N:** Plasma neurofilament light chain (NfL). NfL is an indicator of neurodegeneration not specific to any particular cause, as neurofilaments in blood are simply the result of axonal damage, regardless of cause. For AD, NfL can be useful as an indicator of disease progression and severity.[9] In the absence of beta-amyloid pathology, higher NfL levels can be indicative of other neurodegenerative diseases and conditions that a physician should investigate, particularly if pTau181 is elevated.

Interpretation

Hampel et al[10] published a review article summarizing how to interpret and understand the eight possible outcomes from an ATN-like test. Each assay within the ATN Profile has a cutoff that provides an indication of whether a patient's measured value is considered normal or abnormal. For NfL, cutoffs are based on age ranges recognizing that baseline measurable NfL levels increase with age.[11] Each of the three assays is then given a "-" or "+" indicator corresponding to a normal or abnormal result, respectively. The eight possible combinations of results from the three markers: A, T and N, group into three possible clinical interpretations: normal, AD continuum ("AD pathology" and "probable AD") and non-AD pathology or neurodegenerative disease (Figure 2). A normal result, where there is no evidence of disease, occurs when each of the three markers is negative. Any result where A is positive indicates that the patient is on the AD continuum. If these patients are not already under the care of a neurologist, they are candidates for immediate referral, where these findings can then be confirmed and potential treatment options can be assessed. Any result where A is negative, but T and/or N are positive, indicates non-AD pathology or neurodegeneration. This additional set of A negative results provides additional value to a clinician trying to address the primary clinical question of what might be going on with the patient.

Clinical use for patient triaging

Based on the demonstrated performance of the plasma Aβ42/40 component of the ATN Profile, the assay meets the newly published recommendations for sensitivity (≥90.0%) and specificity (≥85.0%) for a triage test performed in a primary care setting.[12] For neurologists familiar with the various markers and the type of information they convey about disease, pTau217 is a single marker that may become the default assay for assessing AD. However, in primary care settings, the ATN Profile has notable benefits over pTau217. Providing important clinical information about the patient's health beyond just AD makes this a more useful assay for assessment and triage. In terms of interpretation, the results of the three biomarkers are summarized using language that abstracts the complexity of the individual biomarker results to help primary care physicians better understand and assess their patient's status. Ultimately, the ATN Profile provides the physician with a clear summary of likely AD status and whether there is evidence of other non-AD pathologies that should be investigated through a neurology referral and other potential assessment and diagnostic tools.

Figure 2

Summary comments are based on a consensus between National Institute for Aging and the International Working Group recommendations for ATN Profile interpretation published by Jack et al 2018[8] and updated in Hampel et al 2021.[10]

Profile	Clinical Summary	
A- T- N-	A normal beta-amyloid 42/40 ratio and normal concentrations of pTau181 and NfL were observed at this time. These results are not consistent with the presence of Alzheimer's-related pathology. Plasma findings may be less precise than CSF or PET. Additional assessments may be necessary. These tests are intended to be used only in the context of clinical care.	
A+ T- N-	A low beta-amyloid 42/40 ratio was observed. Normal concentrations of pTau181 and NfL were observed at this time. These results may be consistent with the presence of Alzheimer's-related pathology. Plasma findings may be less precise than CSF or PET. Additional assessments may be necessary. These tests are intended to be used only in the context of clinical care.	**AD Continuum**
A+ T+ N-	A low beta-amyloid 42/40 ratio and a high pTau181 concentration were observed. A normal NfL concentration was observed at this time. These results are consistent with the presence of Alzheimer's-related pathology. Plasma findings may be less precise than CSF or PET. Additional assessments may be necessary. These tests are intended to be used only in the context of clinical care.	
A+ T+ N+	A low beta-amyloid 42/40 ratio and a high pTau181 and NfL concentrations were observed at this time. These results are consistent with the presence of Alzheimer's-related pathology. Plasma findings may be less precise than CSF or PET. Additional assessments may be necessary. These tests are intended to be used only in the context of clinical care.	
A+ T- N+	A low beta-amyloid 42/40 ratio and a high NfL concentration were observed. A normal pTau181 concentration was observed at this time. These results may be consistent with the presence of Alzheimer's-related pathology and concomitant suspected non-AD pathological change. Plasma findings may be less precise than CSF or PET. Additional assessments may be necessary. These tests are intended to be used only in the context of clinical care.	
A- T+ N-	A high pTau181 concentration was observed. A normal beta-amyloid 42/40 ratio and normal concentration of NfL were observed at this time. These results are not consistent with the presence of Alzheimer's-related pathology, but may indicate pathology of another condition. Additional assessments may be necessary. These tests are intended to be used only in the context of clinical care.	
A- T+ N+	High pTau181 and NfL concentrations were observed. A normal beta-amyloid 42/40 ratio was observed at this time. These results are not consistent with the presence of Alzheimer's-related pathology, but may indicate pathology of another condition. Additional assessments may be necessary. These tests are intended to be used only in the context of clinical care.	
A- T- N+	A high NfL concentration was observed. A normal beta-amyloid 42/40 ratio and normal pTau181 concentration were observed at this time. These results are not consistent with the presence of Alzheimer's-related pathology, but may indicate pathology of another condition. Additional assessments may be necessary. These tests are intended to be used only in the context of clinical care.	

Labcorp offers

Test Name	Test No.
ATN Profile	484400
Phosphorylated Tau 217 (pTau-217), Plasma	484390

*For the most current information regarding test options, including specimen requirements and CPT codes, please consult the online Test Menu at www.labcorp.com.

References

1. Dementia. World Health Organization website: https://www.who.int/news-room/fact-sheets/detail/dementia. Updated March 15, 2023. Accessed August 19, 2024.
2. Ovod V, Ramsey KN, Mawuenyega KG, et al. Amyloid β concentrations and stable isotope labeling kinetics of human plasma specific to central nervous system amyloidosis [published correction appears in Alzheimers Dement. 2017 Oct;13(10):1185]. *Alzheimers Dement.* 2017 Aug;13(8):841-849.
3. Yamashita K, Miura M, Watanabe S, et al. Fully automated and highly specific plasma β-amyloid immunoassays predict β-amyloid status defined by amyloid positron emission tomography with high accuracy. *Alzheimers Res Ther*. 2022 Jun 23;14(1):86.
4. Yamashita K, Watanabe S, Ishiki K, et al. Fully automated chemiluminescence enzyme immunoassays showing high correlation with immunoprecipitation mass spectrometry assays for β-amyloid (1-40) and (1-42) in plasma samples. *Biochem Biophys Res Commun*. 2021 Oct 22;576:22-26.
5. Fowler C, Rainey-Smith SR, Bird S, et al. Fifteen years of the Australian Imaging, Biomarkers and Lifestyle (AIBL) Study: progress and observations from 2,359 older adults spanning the spectrum from cognitive normality to Alzheimer's disease. *J Alzheimers Dis Rep.* 2021 Jun 3;5(1):443-468.
6. Bun S, Ito D, Tezuka T, et al. Performance of plasma Aβ42/40, measured using a fully automated immunoassay, across a broad patient population in identifying amyloid status. *Alzheimers Res Ther.* 2023 Sep 4;15(1):149.
7. Oberstein TJ, Schmidt MA, Florvaag A, et al. Amyloid-β levels and cognitive trajectories in non-demented pTau181-positive subjects without amyloidopathy. *Brain.* 2022 Nov 21;145(11):4032-4041.
8. Jack CR. PART and SNAP. *Acta Neuropathol.* 2014 Dec;128(6):773-776. Epub 2014 Nov 8.
9. Janelidze S, Palmqvist S, Leuzy A, et al. Detecting amyloid positivity in early Alzheimer's disease using combinations of plasma Aβ42/Aβ40 and p-tau. *Alzheimers Dement*. 2022 Feb;18(2):283-293.
10. Hampel H, Cummings J, Blennow K, Gao P, Jack CR, Vergallo A. Developing the ATX(N) classification for use across the Alzheimer disease continuum. *Nat Rev Neurol.* 2021 Sep;17(9):580-589.
11. Khalil M, Pirpamer L, Hofer E, et al. Serum neurofilament light levels in normal aging and their association with morphologic brain changes. *Nat Commun*. 2020 Feb 10;11(1):812.
12. Schindler SE, Galasko D, Pereira AC, et al. Acceptable performance of blood biomarker tests of amyloid pathology — recommendations from the Global CEO Initiative on Alzheimer's Disease. *Nat Rev Neurol.* 2024 Jul;20(7):426-439.

Visit the online test menu at **Labcorp.com** for additional test options and full test information, including CPT codes and specimen collection instructions.

labcorp

L546951_WP. 0125-5