What is the importance of ATN in Alzheimer's disease?

Alzheimer's disease (AD) is a progressive neurodegenerative condition that slowly damages brain cells, causing cognitive and memory impairment and, eventually, death. AD is characterized by a build-up of protein plaques and tangles, made up of amyloid-beta 42(Aβ42)[1] and tau proteins, respectively.[2] These biological changes generally occur over years, disrupting typical neuronal function and thereby causing neurodegeneration. Continued neurodegeneration leads to the observable memory and cognitive impairment symptoms characteristic of AD.

Until recently, a definitive diagnosis of AD could only be established by autopsy. The 2023 draft Revised Clinical Criteria for Alzheimer's Disease by the National Institute on Aging and the Alzheimer's Association (NIA-AA) working group, designed for both research and clinical care, have set out to now define AD "biologically, not based on a clinical syndrome(s)" and as such "can be diagnosed by the presence of any abnormal core AD biomarker – i.e., fluid Aβ42/40, ptau, amyloid PET, or neocortical tau PET".[3]

Various cerebral spinal fluid (CSF) and blood biomarkers (BBMs) have been identified and studied as possible markers to facilitate evaluation, diagnosis or disease severity staging for Alzheimer's disease.[4] The definition of a "core biomarker" was initially established in 2018 by an international working group that established the AT(N) classification system (where parentheses were included to highlight that 'N' biomarkers were not specific for AD) to group both fluid and imaging biomarkers by the type of Alzheimer's-associated biological change for which they provide evidence.[5]

ATN groups these various biomarkers into three categories that signal pathologic changes typical of AD, irrespective of patient symptomology (Figure 1):

- **A** represents amyloid and categorizes markers associated with detecting amyloid plaques, including amyloid PET and CSF and BBM Aβ42 assays
- **T** represents tau and categorizes markers associated with detecting tau pathology (tangles), including tau PET and phosphorylated tau isoforms in CSF and BBMs, such as pTau181, pTau217 and pTau231
- **N** represents neurodegeneration and categorizes markers associated with determining neurodegenerative pathology in AD patients. Unlike **A** and **T** markers, **N** markers facilitate disease staging. Markers included in this category are MRI scans, CSF total Tau and plasma neurofilament light chain (NfL)

Figure 1: 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

Advancements in CSF and BBM biomarker platforms, which enable more accurate identification of disease pathology at lower levels of detection, led to the extension of the ATN framework to support the clinical diagnosis of AD.[6] The ATN framework provides an objective approach to determine the status of biological changes that are indicative of Alzheimer's disease. For primary care providers, an ATN test can support the triage of patients whose initial lab results and/or cognitive exams merit further evaluation and, if necessary, facilitate referral to a neurologist. For neurologists, availability of an ATN test -or- diagnostic would allow for a simple, blood-based initial test to provide pathological evidence of AD, which could then be confirmed with either a CSF test or PET scan. In other words, the promise of such a test could help identify AD patients much faster in either the primary care or specialized setting. This would potentially shorten the overall AD patient diagnostic journey, enabling earlier treatment, improved outcomes and peace of mind for families who often do not have answers. In every case, a full clinical workup is still necessary; as with any biomarker, simply detecting biological changes is insufficient for definitive diagnosis.[6]

Overall, the ATN framework can complement imaging and CSF analysis and create an opportunity for a more effective patient journey. Providing both viable and clinically useful diagnostic information early in the patient presentation means focusing on markers that are readily available, easily accessible, cost-effective and minimally invasive. A complete BBM-based ATN panel test would bring both providers and patients one step closer to the "holy grail" of earlier AD diagnosis through blood-based testing.[7]

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Labcorp's solution

Labcorp is the first laboratory to offer a complete blood-based ATN panel and make it widely accessible to clinicians nationwide. Though some markers are individually available from other labs, Labcorp is the first lab to offer all relevant ATN markers, available as a panel or independently. Labcorp's ATN panel solution employs biomarkers that have been well-studied in clinical research:

- **A**: plasma Aβ42/40 immunoassay based on Sysmex reagents and technology.

  In general, Aβ42 proteins are known to have a high adhesion index and are typically referred to as "sticky," thus complicating their assessment in blood or CSF samples. New technologies—including low binding tubes and high sensitivity immunoassay platforms that can detect down to picogram levels—have enabled better assessments. Ovod et al demonstrated that accurate amyloid beta assessments for AD from blood were possible using mass spectrometry techniques.[8] More recently, studies using Sysmex-based technology showed that plasma Aβ42/40 assessments could achieve levels similar to mass spectrometry-based methods for detecting AD pathology.[9,10]

- **T**: plasma pTau181 immunoassay based on Roche reagents and technology.

  Karikari et al showed the diagnostic utility of plasma pTau181 in AD,[11] and additional studies that same year demonstrated that plasma pTau181 correlates with tau PET,[12,13] thereby validating it as an important and useful BBM for AD pathology determination. Subsequent studies have demonstrated the prognostic value of combining Aβ42/40 testing with plasma pTau181 on a Roche platform.[14]

- **N**: plasma NfL immunoassay based on Roche reagents and technology.

  NfL is an indicator of neurodegeneration, but is not specific to any particular disease, as neurofilaments in blood are simply the result of axonal damage, regardless of cause. In CSF, total Tau is a key biomarker for neurodegeneration. However, for a complete blood-based solution, NfL is the more suitable biomarker[15] for determining neurodegenerative disease staging.[16,17] The new updated draft guidelines demote NfL from a core biomarker to a secondary one. Labcorp has chosen to leave the marker in the profile for clinical purposes since, even in the setting of normal beta-amyloid results, a raised NfL result may help alert the clinician to another non-Alzheimer's condition.

Performance

The ATN Profile was 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. The beta-amyloid 42/40 ratio assay had a ROC analysis area under the curve (AUC) of 0.944, with a sensitivity of 96% and specificity of 86.7% (Figure 2). The pTau181 assay had an AUC of 0.847, with a sensitivity of 89.6% and specificity of 68%. NfL is an indicator test, and in the context of AD, serves as an indicator of disease severity based on the levels of neurodegeneration, and therefore sensitivity and specificity are not applicable for this marker.

Figure 2: Beta-Amyloid 42/40 Ratio Perfomance

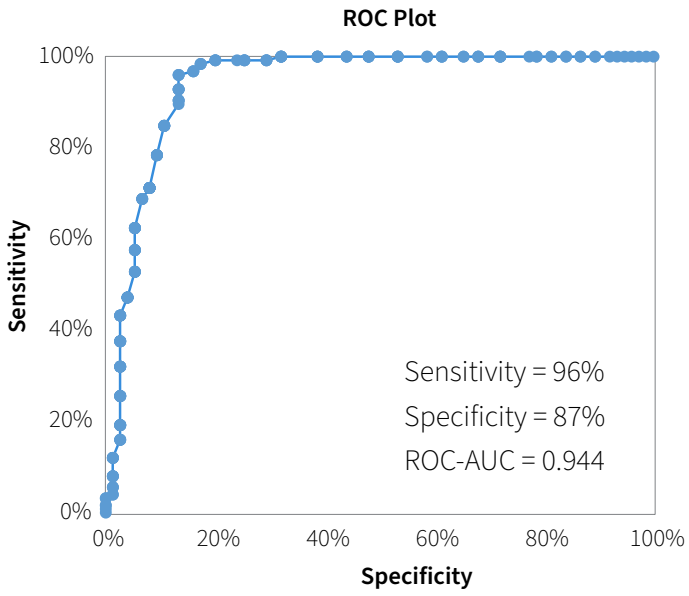

Figure 3

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

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.	

Assay Results Summary

Each assay within the ATN panel has a cutoff that provides an indication of whether a patient's measured value is consistent with what is observed in amyloid PET positive Alzheimer's patients. For NfL, cutoffs are based on age ranges recognizing that baseline measurable NfL levels increase with age.[18,19] Each of the three assays is then given an indicator corresponding to consistent with (+) or not consistent with (-) Alzheimer's disease, respectively. There are eight possible combinations of results, and these combinations group into three possible clinical interpretations: normal, AD continuum ("AD Pathology" and "Probable AD") and non-AD pathology or other neurodegenerative disease (Figure 3). A normal result, where there is no evidence of disease, occurs when each of the three markers is negative: A-, T- and N-. Any result where A is negative, but T and/or N are positive, indicates non-AD pathology or other neurodegeneration. 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 with amyloid PET and/or CSF testing, and possible treatment options can be assessed.

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