Blood tests for Alzheimer disease are moving toward clinical use.

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Introduction

Alzheimer’s disease (AD) is a progressive neurodegenerative disease for which disease-modifying therapies are currently lacking. The first detectable pathology, which occurs decades before clinical symptom onset, is the accumulation of extracellular amyloid plaques in the brain, which have a 42 amino acid-long amyloid β (Aβ) protein as their core.\(^1\) Biomarker studies suggest that Aβ accumulation is followed by increased phosphorylation and secretion of tau,\(^2\) a microtubule-associated axonal protein that is highly expressed in cortical neurons.\(^3\) Dysfunctional tau metabolism leads to AD-type neurodegeneration with the development of dystrophic neurites and intraneuronal neurofibrillary tangles that are composed of hyperphosphorylated and truncated tau proteins.\(^4\) Neurodegeneration translates into the AD clinical syndrome, with neurodegeneration being spatially and temporally associated with the pattern of cognitive deficits that worsen as the disease progresses.\(^5\)

Blood-based biomarkers proposed as biomarkers for AD pathophysiology include the ratio of the 42- to 40-amino acid-long Aβ proteins (Aβ42:Aβ40), as a marker of plaque pathology, and phosphorylated tau, as a marker of AD-related tau phosphorylation and secretion. Additionally, serum neurofilament light (NfL) can be used as a general (nonspecific) marker of neurodegeneration. (See Neurofilament Light Chain as a Dementia Biomarker in this issue). Initially, these biomarkers could only be measured in cerebrospinal fluid (CSF) and their effectiveness led to their incorporation into contemporary diagnostic criteria for AD.\(^6\) Technologic progress has resulted in improved analytic sensitivity of the assays, however, making it possible to measure these biomarkers in blood samples. This increases their accessibility, and opens the prospect of much wider use, potentially extending into primary care.\(^7\) This is a rapidly evolving field; results so far largely come from large research cohorts and these assays still need to be validated in more routine clinical settings to understand better the appropriate contexts of use.

In this review, we provide an overview of the biomarkers that reflect the core components of AD pathophysiology, including biomarkers for Aβ and tau pathology and neurodegeneration, in line with a proposed amyloid, tau, and neurodegeneration (ATN) research classification scheme for AD biomarkers (Table).\(^8\) We describe the work that led to clinical implementation of the CSF biomarkers and discuss recent developments aimed at creation of clinically implementable and easy-to-use blood tests for AD.
Fluid Biomarkers for Aβ Pathology

Extracellular deposition of Aβ into plaques is the key pathologic feature of AD, and has been proposed as a major pathogenic event in the early disease process. The development of CSF biomarker tools to measure Aβ pathology in vivo and prior to autopsy started in the 1990s, but it was not until 2020 that full standardization of CSF Aβ42 measurement was achieved, through the use of certified reference materials and methods.

CSF Aβ42 concentration is reduced by approximately 50% in AD. Aβ42 is a secreted cleavage product of amyloid precursor protein (APP), normally mobilized from the brain into the CSF and blood, probably via the glymphatic system. In AD, Aβ42 aggregates in the brain parenchyma, resulting in decreased Aβ42 in CSF. The diagnostic accuracy of amyloid protein measurement for Aβ pathology can be increased by dividing the concentration of aggregation-prone Aβ42 by the concentration of soluble Aβ40 (Aβ42:Aβ40) to account for inter-individual differences in Aβ production. CSF Aβ42:Aβ40 is close to 100% concordant with amyloid positivity assessed using positron emission tomography (PET); individuals with discordant levels usually have positive CSF findings and negative PET findings, but often develop positive PET findings within a few years.

For many years, there was not much hope for a reliable blood test for cerebral Aβ pathology, in part because levels of Aβ42:Aβ40 decrease by 14% to 20% in blood compared with the 50% reduction seen in CSF. Recent findings, however, suggest measurement of plasma Aβ42:Aβ40 with immunoprecipitation mass spectrometry or ultrasensitive enzyme-linked immunosorbent assays (ELISAs) reflects cerebral Aβ pathology with relatively high accuracy correlating with both amyloid PET and CSF Aβ42:Aβ40, which are both validated measures of neuropathology. The relatively smaller reduction in plasma Aβ42:Aβ40 the reflects the weak correlation between absolute concentrations in plasma and CSF, which could be explained by the production of Aβ peptides in platelets and other extracerebral tissues. Paving the way for use in routine clinical practice, a recent validation study used a fully automated immunoassay to measure plasma Aβ42:Aβ40. Easy-to-use protocols for preanalytic sample handling that can be used across all plasma biomarkers for AD, have also been published. Taken together, the concordant research findings using high-precision analytical tools represent important progress towards future clinical implementation, perhaps using staged testing (eg, an Aβ test in blood favoring sensitivity over specificity, followed by a more
specific CSF- or imaging-based test in memory clinics). The potential importance of blood biomarkers is increasingly recognized by regulatory authorities.

**Fluid Biomarkers for Tau Pathology**
Both CSF total tau (t-tau; measured using assays directed at the protein midregion that do not discriminate between different isoforms) and phosphorylated tau (p-tau) concentrations reflect AD-related tau pathophysiology but do not reliably reflect tau pathology in nonAD tauopathies.\(^{24,25}\) The most likely explanation for this is that the increased concentrations of CSF tau seen in AD are owing to increased phosphorylation and secretion of tau from neurons, in response to Aβ exposure.\(^ {26,27}\) Thus, CSF t-tau may best be regarded a predictive, although not entirely specific, marker of AD-type neurodegeneration, and CSF p-tau a marker of AD-related tau phosphorylation that may result in tangle formation. It is important, however, to note that CSF t-tau increases are also seen in certain disorders with rapid neurodegeneration without amyloid or tau pathology, notably Creutzfeldt-Jakob disease (CJD),\(^ {28}\) and in acute conditions (eg, stroke and brain trauma).\(^ {29,30}\) Fully automated t-tau and p-tau assays are available for clinical use,\(^ {31,32}\) and work to standardize these is ongoing in collaborative efforts between the International Federation of Clinical Chemistry and Laboratory Medicine (IFCC) and the Global Biomarker Standardization Consortium (GBSC).

Although ultrasensitive plasma t-tau assays can detect neuronal injury in acute brain disorders (eg, stroke and traumatic brain injury),\(^ {33,34}\) they perform relatively poorly for AD,\(^ {35}\) with only a weak correlation with CSF tau levels.\(^ {36}\) A potential explanation for this is the short half-life of tau (~10 hours),\(^ {37}\) compared with around 20 days in CSF.\(^ {27}\) A more likely explanation, considering the promising p-tau data reviewed below, is that currently available t-tau assays in blood may partly measure tau produced by peripheral tissues. Quantifying a phosphorylated-form of tau might make the test more central nervous system (CNS)-specific. Measuring t-tau using antibodies directed to the N-terminal region of the protein may also make the result more CNS-specific with stronger correlation between CSF and plasma concentrations.\(^ {38,39}\)

There are 85 potential phosphorylation sites in tau,\(^ {40}\) and in AD brain tissue phosphorylation has been demonstrated at 49 vs only 17 in controls. The main diagnostic candidates, p-tau181, p-tau217 and p-tau231, which are all found to some extent in AD and controls, can be measured in blood and CSF. P-tau181 has long been established as a CSF biomarker for AD,
and p-tau231 is suggested for differentiating AD from frontotemporal dementia (FTD), although they now seem to perform similarly in this regard. Recent data suggest that CSF p-tau217 may correlate more strongly with clinical AD and tau pathology determined by PET, and change earlier than CSF p-tau181.42,43 These observations warrant additional research not only to establish the optimal diagnostic method, but also to provide insights into the progression of tau phosphorylation in vivo.

Recently, a Single molecule array (Simoa)-based ultrasensitive method for p-tau181 quantification was published.46 In a discovery and 2 validation cohorts (n=1,131), the ultrasensitive single molecule array (Simoa) assay was used to measure plasma p-tau181 levels and showed differences between AD dementia from other neurodegenerative diseases and controls with 80% to 100% accuracy as measured by the area under the curve (AUC). Plasma p-tau181 correlated with both tau PET (AUC=83-93%) and amyloid PET (AUC=76-88%), as well as 1-year cognitive decline (P=0.0015) and hippocampal atrophy rate (P =0.015). Large validation studies show very similar results,47,48 confirming plasma p-tau181 as a robust blood biomarker for AD pathology, using techniques with potential to be standardized and implemented in clinical laboratory practice.

Although both plasma p-tau217 and p-tau181 levels are highly correlated with one another and with other biomarkers for AD dementia (ie, MRI, NfL, and tau-PET), plasma p-tau217 has significantly higher accuracy for differentiating clinical AD dementia from other neurodegenerative diseases.49 It is presently difficult to recommend one p-tau assay over another for clinical use; more research is needed in populations more representative of those seen in routine clinical practice, and efforts are required to improved assay availability and ensure appropriate standardization.

**Fluid biomarkers for neurodegeneration**

Neurofilament light (NfL) has emerged as a strong biomarker candidate for neurodegeneration,53 (See Neurofilament Light Chain as a Dementia Biomarker in this issue) and can be measured in CSF, plasma, or serum. The correlation between CSF and blood concentrations is good to excellent (r values of 0.70 to 0.97).54 In familial AD, mutation carriers have rapid changes in blood NfL concentration approximately 10 to 15 years before their expected clinical onset in close association with neuroimaging and cognitive measures,56,57 perhaps marking early stages of the neurodegenerative process. In sporadic AD,
there is also association of increased plasma NfL with Aβ and tau PET positivity and neurodegeneration, although with a larger overlap across groups (including controls) than in familial AD, which may reflect well-documented changes in NfL that occur with aging.⁵⁸,⁵⁹

**Clinical Laboratory Implementation**

In Europe, CSF biomarkers have been used as an integral part of the diagnostic evaluation of patients with suspected AD in some memory clinics since the early 2000s, although they have not been formally approved or recommended by many regulatory authorities. In 2018, the UK National Institute for Health and Care Excellence (NICE) recommended the use of CSF testing in specialist centers to confirm a diagnosis of AD in certain settings (https://www.nice.org.uk/guidance/ng97/chapter/Recommendations#diagnosis); both the Food and Drug Administration (FDA) and the European Medicines Agency (EMA) have encouraged the further study of CSF biomarkers in the context of clinical AD diagnosis. The Coalition Against Major Diseases (CAMD) CSF Biomarker Team is working toward formal qualification from the FDA for CSF biomarkers for clinical trial enrichment at the predementia stage of the disease.⁶⁰ Additionally, the Alzheimer’s Association has published Appropriate Use Criteria, providing specific clinical indications for persons with suspected AD.⁶¹ Standard operating procedures for preanalytical sample handling have been agreed upon and published for both CSF⁶² and plasma.²³ Certified reference methods and materials for CSF Aβ42 assay standardization,¹¹ and high-precision clinical chemistry tests on fully automated instruments, are in place,⁶³ which bodes well for full implementation of these biomarkers in clinical laboratory practice with uniform reference limits around the globe. Work on the reference measurement procedures for CSF Aβ40, t-tau and p-tau181 is ongoing under the auspices of the IFCC CSF Proteins working group; the Aβ40 part of this work should be concluded during 2020. Several working groups are now initiating standardization work for the blood tests along similar lines. Plasma NfL is already an available test in clinical laboratory practice in Sweden, the Netherlands, and France, and many clinical laboratories are now working towards validating plasma p-tau tests for clinical use.

**Interpretation in Clinical Practice**

AD-related pathologies appear many years before the clinical onset of the disease. This has ethical and clinical implications for the interpretation of biomarker results. Although positive Aβ and tau biomarkers provide strong evidence that an individual has plaque and tangle pathology, the challenge will be to determine if these pathologies explain an individual's
symptoms or not. A depressed person with some cognitive problems may have preclinical AD pathology that could develop into AD in several years, but it may be difficult to know which, if any, current symptoms are related to those pathologies or not. A further challenge is communicating the risk of symptomatic AD associated with abnormal biomarkers within a specific time frame; clinically, a high risk of symptoms in the next year is very different to a risk of symptoms sometime in the next 10 years. The role of comorbid AD in other neurodegenerative diseases represents another challenge. For example, an individual with a typical FTD syndrome who has positive AD biomarkers may have an atypical AD phenotype, or they may have FTD with comorbid AD pathology that may or may not be contributing to the clinical syndrome at all. An important practical consequence of the long presymptomatic period of amyloid accumulation is that markers of amyloid pathology must be interpreted in the context of a person’s age. Amyloid “positivity” (blood, CSF, or PET) is found in approximately 20% of people at age 70, increases rapidly thereafter with a 10% increase in the proportion of people with positive AD biomarkers for every additional decade of life. For a person with early-onset AD, amyloid markers have both good sensitivity and good specificity for AD as the likely cause of cognitive decline, whereas in older patients there is less specificity (ie, could be coincidental) with age, but the markers still have good value in “ruling out” AD. At all ages, interpretation of these markers should be in the context of other investigations (eg, blood or imaging) as part of an overall clinical assessment. Among those over age 70 who are cognitively unimpaired, the presence of AD biomarkers does not necessarily mean an individual will develop cognitive impairment during life. For example, a woman age 65 without cognitive impairment but with positive amyloid findings has approximately a 30% lifetime risk of developing AD dementia but a woman age 85 with these same findings has approximately a 14% risk. Likewise, the relationship between pathologic brain lesions and clinical status appears to attenuate at advanced ages. Postmortem evaluation of tissue from almost 300 elders without neurologic impairment showed that approximately half had some Aβ deposition, whereas some degree of tau pathology could be seen in almost all brains. Accordingly, the specificities of tau and Aβ biomarkers for people without AD and the areas under the receiver-operating characteristics curves for distinguishing AD from nonAD patients decrease with age.

Fluid biomarkers do not reflect anatomic distribution of brain region-specific molecular or degenerative changes, which may limit use for staging of disease severity and monitoring progression. In contrast, MRI, tau PET, and Aβ PET provide both global and regional
information that may help direct assessment of disease stage and subtype in select clinical cases and clinical trials (See *Imaging Biomarkers for Dementia* in this issue). For plasma p-tau181, a stepwise increase with disease severity has been reported, but this is less clear for the other biomarkers. CSF Aβ42:Aβ40, for example, appears to be a binary marker (normal or abnormal) without a clear relationship between the degree of change and the extent of the pathology. Although changes of different p-tau isoforms over the time course of familial AD have recently been elucidated, similar work is needed to provide more detailed staging in sporadic AD.

**When Disease-Modifying Treatments Are Available**

Regulatory agencies are currently considering Aβ-targeting drugs for AD. If approved, treatments of this kind will likely be expensive and mandate documentation of molecular evidence of underlying AD pathology. Synthesizing the recent biomarker breakthroughs above, it is clear that plasma Aβ42:Aβ40 and plasma p-tau are very promising candidates for this purpose. Exactly how these would best be used in clinical practice is the subject of much debate and depends largely on the results of larger and more representative studies. Although blood-based biomarkers may prove to be as sensitive and specific as more costly and complex CSF or PET diagnostics, an approach that may be particularly useful for evaluating large populations is to use blood-based tests as screening tools, using cut-points tuned for sensitivity to minimize false negatives. Assuming that the ethical issues of identifying cohorts of presymptomatic or unaffected patients in primary care can be overcome and managed, those testing positive could then be referred to a specialized memory clinic for more detailed assessment, with confirmation of pathology using PET imaging or CSF where available, followed by treatment with disease-modifying therapy(ies), if Aβ positivity is verified. Plasma p-tau, representing a neuronal reaction to Aβ, and NfL levels, representing neurodegeneration, may be particularly useful for monitoring response to treatments. For antiAβ antibodies, repeat MRIs would be needed, at least initially, to monitor amyloid-related imaging abnormalities (ARIA), but in the future, increases in plasma NfL concentration could possibly substitute for MRI for this purpose, but this potential use needs to be formally examined. Posttreatment, a patient could be followed with annual plasma p-tau and NfL measurements to gauge response and the potential need for additional therapy. For this to be possible, future clinical trials should incorporate both imaging and fluid biomarker approaches to allow development of effective biomarker algorithms for treatment selection, dose optimization, drug monitoring, and assessment of treatment response.
Conclusions
In summary, having been dismissed as unfeasible only a few years ago, blood-based biomarkers for the core AD pathologies are now a reality poised to enter and have a major effect on clinical practice. Considerable efforts are underway to standardize collection and quantification, and ongoing studies are determining which methodologies and, particularly with p-tau, which moieties provide optimal diagnostic and prognostic information. It is likely that these measures will initially be used in clinical trials alongside more established CSF and PET biomarkers. Once validated and licensed, the ease by which samples can be collected (including serially) means they are likely to be rapidly taken up in clinical practice. Careful planning is required to establish where in the clinical pathway they should be deployed and how best they should be interpreted.

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| TABLE. SUMMARY OF ALZHEIMER DISEASE BLOOD BIOMARKERS |
|---------------------------------|-----------------|----------------|------------------|
| **Aβ42/Aβ40**<sup>a,b</sup> & **Total-tau**<sup>b</sup> & **Phosphorylated tau**<sup>a,b</sup> & **Neurofilament light**<sup>b</sup> |
| Biological significance | Produced by amyloid precursor protein metabolism in brain | Secreted from neurons, in response to Aβ exposure or released by damaged neurons | 85 potential phosphorylation sites; several promising candidates | Reflects rate of neurodegeneration |
| Clinical relevance | Reduced Aβ42 reflects brain amyloidosis | Elevation in AD; reflects tauopathy and tangle formation | Presymptomatic elevation in AD reflects AD tauopathy and tangles | Reflects rate of neurodegeneration/disease progression |
| | Aβ42/40 ratio corrects for interindividual variation in amyloid metabolism | Indirect marker of amyloidosis | Indirect marker of amyloidosis | Correlated with amyloid PET, tau PET, and MRI brain atrophy rates |
| | Reduced Aβ42/40 ratio highly concordant with amyloid PET | Blood and CSF Aβ42/40 highly concordant | Correlates with amyloid and tau PET | Blood and CSF p-tau highly concordant |
| Limitations | Smaller reduction of Aβ42 in blood than CSF | Poor correlation with CSF tau | Not widely available or internationally standardized | Nonspecific |

Abbreviations: AD, Alzheimer disease; CJD, Creutzfeldt-Jakob disease; CSF, cerebrospinal fluid; PET, positron emission tomography.

<sup>a</sup>Measured by immunoprecipitation mass spectrometry.

<sup>b</sup>Measured by ultrasensitive enzyme-linked immunosorbent assay (ELISA).