Alzheimer disease (AD) is the leading cause of dementia globally with a profound impact on individuals, families, and health care systems. Roughly 150 million cases of cognitive impairment worldwide are anticipated by 2050, with the majority being caused by AD. In the United States, this number is projected to be around 11 million, in part attributable to increasing life expectancy. The estimated health care costs for people in the United States with AD totaled $172 billion USD in 2020, and that number is projected to increase. Such escalating figures underscore the urgent need for improved clinical understanding, early detection, and effective management of this debilitating neurodegenerative disorder. In addition, because there are only a limited number of dementia specialists in practice as well as other barriers related to access of diagnostic and therapeutic resources, the US health care system is unprepared to meet the projected demand of treatment-eligible individuals with mild cognitive impairment (MCI) or mild dementia caused by AD (collectively called "early AD").

To initiate monoclonal antibody therapy for early AD, such as Leqembi (lecanemab; Eisai, Nutley, NJ), which recently received approval from the Food and Drug Administration (FDA), clinicians need to confirm amyloid beta (Aβ) status through biomarker testing. In addition, a focus on prevention or treatment of AD in its preclinical stages reflects a major shift in the field. Over the past 2 decades, cerebrospinal fluid (CSF) and imaging biomarkers (Table 1, available online) have been studied as potential tests to assist with the evaluation and early detection of neuropathologic changes associated with AD. Table 1 (available online) lists those biomarkers which have received FDA approval for AD-related evaluation. However, lumbar puncture for the collection of CSF biomarkers is costly, consumes substantial time, and has issues related to access, owing to fear of the procedure in addition to a limited number of practitioners. Although rare, complications ranging from mild headaches to CSF leaks and more severe adverse effects may occur.

Amyloid and tau ligands for positron emission tomography (PET) scans are also approved by the FDA, but PET imaging is costly and limited to specialized centers. Given the prevalence of AD, the growing demand for therapies, and the need for clinical trials, a new model for connecting individuals with resources is needed.

Using a routine blood draw as a triage tool in primary care for anti-amyloid therapy has the potential to accelerate diagnosis, access to therapy, clinical trial enrollment, and advanced care planning. This approach is an active area of research, with potential for broad implementation in the near future. However, barriers remain.

AD Neuropathologic Changes

The AT(N) classification system for AD has proven useful in the research setting to identify individuals with specific biomarker profiles who may be more likely to have AD. This classification system was largely built on results of the Alzheimer’s Disease Neuroimaging Initiative using infrastructure supported by the National Institute on Aging and Alzheimer’s Disease Research Centers. In the research set-
ung, individuals are defined as having AD neuropathologic change and placed on the AD spectrum of preclinical disease based on the presence of amyloid positivity (A+) even if cognitively unimpaired (CU). CSF biomarkers and amyloid PET have proven valuable in the recruitment of participants for clinical trials and are used as a surrogate reference standard given their concordance with pathology. Tau positivity (T+) and neurodegeneration (N+) are considered later stages of the disease and considered necessary for cognitive decline.

The Geneva roadmap for biomarker development, which is modeled after cancer biomarker development, describes a 5-phase framework. The amyloid and tau biomarkers discussed in this article are all approaching the end of phase 3 development, where these biomarkers are studied in retrospective and longitudinal cohorts. A few have some prospective validation studies (phase 4) underway. Phase 5 is focused on clinical implementation and guidelines for use. Our clinical care model must incorporate these tools to accelerate diagnosis and expand access.

Despite the need for readily available biomarkers, challenges and questions remain. Obtaining an AD diagnosis is often a lengthy process of exclusion even with a typical clinical syndrome and may involve years of anxiety and strained family relations before an individual reaches a neurologist or subspecialty memory clinic for a diagnosis. The reference standard for diagnosis of AD remains brain autopsy with characteristic amyloid plaques and hyperphosphorylated neocortical tau neurofibrillary tangles (NFTs). A traditional AD diagnosis based on clinical criteria has inadequate sensitivity (70.9% to 87.3%) and specificity (44.3% to 70.8%), leading to high misdiagnosis rates when compared with the pathology reference standard. The quest for the development of sensitive, specific, precise, and reproducible assays for detecting AD pathology in life is a worthy goal. Challenges include the prevalence of amyloid in cognitively normal individuals, the long duration before onset of symptoms, shifting biomarker profiles throughout the disease course, heterogeneous progression of AD, low concentration of biomarkers in the blood, copathologies, and the expertise required for the methods employed.

Despite these challenges, the need for AD blood-based biomarkers (BBBs) that can be used for screening, diagnosis, prognosis, copathology identification, treatment monitoring, disease staging, and progression assessment persists. Many BBBs are in development, with all of these goals in mind. However, most cohorts in which these biomarkers are studied are cross-sectional and have strict inclusion and exclusion criteria, preventing more generalized conclusions about their broader application. To reach clinical practice, biomarkers must be studied and validated in large, prospective, longitudinal multicenter cohorts with heterogeneous populations, and a reference protocol, sample material, and prespecified cut point. Prospectively validating a cut point or cutoff will establish normal values on blood-based assays.

A Framework for Early AD Diagnosis

Over the past decade, 2 expert working groups—the International Working Group for New Research Criteria for the Diagnosis of AD and the National Institute on Aging–Alzheimer’s Association (NIA-AA) International Working Group—have been working to align research efforts and facilitate communications by developing new criteria and a new vocabulary for the stages of AD. The NIA-AA has the goal of separating the clinical syndrome (clinically identified impairment) from biology (etiology and neuropathology). This goal has some scientific validity. However, the difficulty with this task is that we do not fully understand the underlying pathology of the clinical syndrome. A purely biomarker-based diagnosis demands robust evidence of biomarker positivity leading to an extremely high likelihood of subsequent clinical progression. However, data on the longitudinal follow-up of CU individuals who are amyloid biomarker–positive indicate that having evidence of amyloid in the brain does not necessarily mean that a person will develop dementia or even cognitive impairment in their lifetime—the presence of amyloid is only associated with an increased risk of developing dementia due to AD.

The NIA-AA working group ascribes to a dichotomous model where amyloid equates to AD being present and places individuals on a spectrum or continuum of disease (Figure 1A). This model incorporates the notion of individuals with neuropathologic changes but no clinical symptoms as asymptomatic or resilient to subsequent neuropathologies. This is a strength in their proposal, which typifies neuropathologic progression in autosomal dominant AD and sporadic AD (Figure 1A; red shading) as well as deviations from this pathway. These deviations are useful to illustrate those individuals who decline faster than expected for the level of pathology present, likely secondary to copathologies (Figure 1A; red shading [i.e., stage 4A through 6A]). Deviations also highlight individuals who have resilient, compensatory, or protective mechanisms in play to delay clinical decline (Figure 1A; green shading [i.e., stages 1B through 1D]). The researchers also assert that AD may be defined by testing positive for a “core biomarker” of amyloid positivity. The most recent recommendations of the International Working Group for New Research Criteria for the Diagnosis of AD (Figure 1B) suggest that a positive AD biomarker does not label an individual as being on the AD continuum or spectrum. Instead, amyloid positivity is treated as a risk factor. Even people with MCI are considered prodromal or at risk for AD, as denoted by the red line in Figure 1.

This distinction highlights a division between many physi-
Figure 1. Clinical and neuropathologic staging for individuals on the Alzheimer disease (AD) spectrum. The National Institute on Aging–Alzheimer’s Association (NIA-AA) spectrum of AD neuropathologic change (in development), using integrated biologic and clinical staging, is illustrated in (A). The dark purple rectangle represents individuals with known autosomal dominant AD gene sequence variations (PSEN1/2, APP, SORLA/SORL1) without evidence of biomarker changes or clinical symptoms. The red arrows indicate the most common progression of clinical stages from amyloid biomarker positive and asymptomatic (1A) to high amyloid and tau burden with dementia (4–6D). Green shading represents deviations from the expected progression of individuals with advanced pathologic changes who are cognitively resilient or compensating for these changes (ie, individuals with advanced pathology but who are clinically less symptomatic). The darker the green, the greater the clinical deviation, with 1D having advanced AD pathology but remaining asymptomatic. Red shading represents more severe clinical pathology than expected, and may indicate that other pathology, such as Lewy body disease, is driving the syndrome (4–6A) (A). The International Working Group for New Research Criteria for the Diagnosis of AD (IWG) clinical–biologic definition of AD, characterized by biologic changes and syndromic phenotype, is illustrated in (B). In this conceptual model, there is a clear delineation between people with and those without cognitive symptoms, indicated by the red line. Having positive AD biomarkers does not label an individual as being on the AD continuum or spectrum; rather, the presence of biomarkers is treated as a risk factor (B).

Abbreviations: ADL, activities of daily living; CN, cognitively normal; CU, cognitively unimpaired; IADL, instrumental activities of daily living; MCI, mild cognitive impairment; SCD, subjective cognitive decline; SCI, subjective cognitive impairment.
BIOMARKERS of current AD BBBs as a triage tool in a research partnership

Large at the individual level. Although definitive, prospective, use of BBBs as a screening test in the primary care setting. concerns, even a small false-positive rate may not justify the potential harm in defining a disease by a single biomarker, even if the biomarker is accurate and precise. The magnitude of the probability of developing cognitive decline from AD neuropathologic changes is of primary importance. Labeling a person as “on the spectrum” of AD because of having a positive outcome on amyloid biomarker testing is problematic, as this risk factor only partially translates to clinical outcome. Individuals may have abundant amyloid pathology without clinical disease, and tau correlates much better with cognitive decline. The missing link connecting these pathologies remains to be defined.

What is at the root of AD neuropathologic changes is hotly debated. What is not debated is that high CNS amyloid burden and corresponding low soluble amyloid in the CSF are markers of risk for cognitive decline, NFTs, and AD dementia. What is also clear is that a positive amyloid BBB test does not indicate a definitive diagnosis of AD; rather, positive results in an individual with cognitive symptoms imply an increased probability that AD may be present. Validation of BBBs as a screening test is in progress.

**General Clinical Implementation**

Given the high rate of amyloid positivity in the older population as well as the number of people with cognitive concerns, even a small false-positive rate may not justify the use of BBBs as a screening test in the primary care setting. The potential harm of missing an easily treatable diagnosis is large at the individual level. Although definitive, prospective, multicenter trials are lacking, it is reasonable to attempt use of current AD BBBs as a triage tool in a research partnership with primary care clinics for accelerated diagnosis and entry into a treatment pathway or clinical trial. These tests may be used to determine which individuals will undergo an existing or “reference” test. This may be accomplished in partnership with a subspecialty group, which can connect individuals with confirmatory testing, counseling, and therapy. The goal would be to maximize the benefit of early detection by connecting the individual to community resources, education, treatment, and clinical trials, while minimizing the potential harm of misdiagnosis and psychologic harm. Model trials for this rule-in triage approach are in progress. A Swedish group proposes using p-tau217 as a first test for clinical trial or treatment enrollment and pursuing confirmatory testing in uncertain cases. At the same time, this group cautions against interpreting AD BBBs based on a single test result as serial sampling reveals unexpectedly high values in healthy individuals. However, until more results become available, a new clinical organization that identifies individuals at early stages for both rule-in and rule-out triage is essential. The approach described in Figure 2 should accelerate individual access to both monoclonal antibody therapies and clinical trials while prospectively testing AD BBB validity and workflows in the real world.

In this approach, amyloid and tau BBB testing are available for triage in primary care clinics. BBB results are used to place individuals into different clinical pathways based on prespecified cutoffs. Individuals who are clearly amyloid-positive but CU (stage 1) or classified as experiencing subjective cognitive decline (stage 2) will remain with their primary care provider. Serial sampling may better determine factors affecting variation in individual biomarker levels. Individuals can be referred to a brain health clinic for enrollment in clinical trials for prevention or monitoring. Analyzing data from this population would be useful to identify potential risk factors and BBBs that may predict progression from CU to dementia.

Individuals in early clinical stages (MCI or mild dementia; stage 3 or 4) with positive BBB testing results and a classic amnestic syndrome without signs of copathology will be routed to the anti-amyloid treatment clinic for confirmatory CSF or PET testing as well as APOE genotyping for risk assessment. Once confirmation with CSF or PET testing has been made, the individual may be started on acetylcholinesterase inhibitors and an approved monoclonal antibody therapy or join a clinical trial. The traditional memory clinic, when available, will continue to serve as a diagnostic hub for evaluating and treating individuals with complex or atypical presentations, copathologies, or unclear BBB results.

Prevention studies in cognitively normal individuals with amyloid positivity are ongoing and more are planned. The proper use of any new therapy in the context of prevention must take into account the high prevalence of amyloid posi-
Molecular Biomarkers and Genetics

Blood-Based $\beta_42/\beta_40$

$\beta$ is the primary neuropathologic hallmark of AD. CSF
and amyloid PET biomarkers may be abnormal 20 years before symptom onset. CSF Aβ42 and Aβ40 levels are also the best-known biomarkers of AD. According to the amyloid cascade hypothesis, proteolytic cleavage of the amyloid precursor protein (APP) produces multiple soluble peptides, including Aβ40 and Aβ42, which form soluble oligomers, and then protofibrils, fibrils, and fibrillar plaques. The production of Aβ stimulates the development of NFTs and neuroinflammation, which leads to a further increase in amyloid. The accumulation of Aβ42 into plaques is signaled by the decrease in soluble Aβ42 in the CSF as well as a change in the Aβ42/Aβ40 ratio: the first sign of a pathologic process.26

There are difficulties with sampling Aβ concentrations in the blood. Aβ is also made peripherally,66 and concentrations may not always reflect central amyloid accumulation.66 The total analytical error associated with any diagnostic assay must be much lower than the magnitude of change among the levels of individuals with the disease of interest, controls, and individuals with other diseases.27,28 Aβ measurements in the blood do not meet these criteria.27,28 The Global Biomarker Standardization Consortium, an initiative by the Alzheimer’s Association to standardize and validate biomarker assays, reported poor correlation among 11 different plasma Aβ42/40 assays conducted at 11 different sites.29 A head-to-head comparison of 8 plasma Aβ42/40 assays showed that mass spectrometry-based methods were more accurate than most of the immunoassays in detecting brain Aβ pathology.30

Combining Aβ42/Aβ40 ratio with other biomarkers, especially using high-resolution liquid chromatography–tandem mass spectrometry, in an algorithm to generate an amyloid probability score with high and low cutoffs (eg, PrecivityAD test [C2N, St. Louis, MO]), leads to an area under the receiver operating characteristic curve (AUC) of 0.90 and accuracy of 86% with adjustments for age and APOE status.31 The PrecivityAD2 test has yielded encouraging results as well with inclusion of p-tau217/non–p-tau217, Aβ42/Aβ40, age, and apolipoprotein E (APOE), improving the AUC to 0.95 for a positive amyloid PET.32 Leading candidates for AD BBB tests a US clinician may encounter are listed in Table 2 (available online).

The Elecsys Amyloid Plasma Panel (Roche, Indianapolis, IN) and Amyloid-Tau-Neurodegeneration (ATN) Profile (Labcorp, Burlington, NC) use different technologic platforms to analyze blood samples but have similar use when triaging for amyloid positivity.33 The Elecsys Amyloid Plasma Panel reports APOE4 status, which is of additional clinical value especially in those being considered for anti-amyloid monoclonal antibody therapies. The ATN profile also reports neurofilament light chain (NfL), which is a general marker of neurodegeneration.

Quest AD-Detect (Quest Diagnostics, Secaucus, NJ) is a direct-to-consumer test measuring the Aβ42/Aβ40 ratio in plasma that lacks validation or clear interpretive value as no peer-reviewed literature on this method exists. Even with substantial support in place, delivery of a positive Aβ biomarker result may cause stress and lead to significant psychologic harm. This type of unregulated testing should be discouraged.

**Phosphorylated Tau**

Hyperphosphorylated tau is a hallmark of AD neuro-pathologic change, and NFT formation as evident in PET imaging tracks well with [18F]fluorodeoxyglucose (FDG)–PET and cognitive changes.34,35 P-tau biomarkers are effective BBBs, both in isolation and in combination panels, for predicting positive amyloid status. More than 80 p-tau targets are under investigation, with p-tau217 and p-tau181 being the most promising.36,37 P-tau231 may have use as a state marker, but this is yet to be verified.38 Janelidze et al39 demonstrated that p-tau217 levels, as measured by the Lilly Research Laboratories (Indianapolis, IN) electrochemiluminescence method outperformed plasma Aβ42/Aβ40 and plasma NfL in predicting CSF Aβ pathology, with AUC of 0.73 in clinically unimpaired individuals and 0.86 in people with AD. Preliminary evidence suggests that p-tau217 levels increase in preclinical stages of AD, and correlate with brain Aβ burden.40 Recent studies suggest a strong correlation with NFT burden with the test (AUC, 0.92; 95% CI, 0.86–0.97) and clinical utility in helping clinicians determine a patient’s eligibility for anti-amyloid treatment. In a prospective cohort, plasma p-tau217 was more strongly associated with Aβ positivity (AUC, 0.94; 95% CI, 0.90–0.97) than p-tau181, p-tau231, N-terminal tau, glial fibrillary acidic protein (GFAP), or NfL, placing it as a frontrunner for a blood-based diagnostic screening test.10 The ALZpath plasma p-Tau217 Simoa assay (Carlsbad, CA) accurately identified elevated Aβ (AUC, 0.92; 95% CI, 0.89–0.99) and tau pathology (AUC, 0.93; 95% CI, 0.84–0.99) across all cohorts,31 had reproducible cutoffs across cohorts, and detected longitudinal changes, even in preclinical stages. P-tau217 levels trend down with anti-amyloid treatment, making it a potential target for pharmacodynamic monitoring.40

Multiple p-tau217 assays by 11 different manufacturers were compared in a similar blinded Global Biomarker Standardization Consortium evaluation as mentioned previously, and performed very well.41 They all were associated with high AUCs, although the study was limited by small sample size, and results have not been fully released. Compared with p-tau217, serum p-tau181 and p-tau231 performed less well. When differentiating types of neurodegenerative pathology, p-tau181 and p-tau217 both performed well, but p-tau181 appeared less accurate in differentiating AD from other forms of neurodegeneration than p-tau217.42
In other studies, plasma p-tau181 predicted amyloid status with high accuracy and could have potentially helped an estimated 60% of individuals in a memory care setting avoid CSF testing or amyloid PET test.25 Prospectively, plasma p-tau181 correlates with the presence or absence of AD neuropathologic change.25 In a prospective cohort, GFAP levels also increased in advance of increases in p-tau 81 or NFL concentrations and may be a useful biomarker in predicting cognitive decline.25

**APOE Testing**

While our understanding of how different APOE variants influence AD risk is evolving, with known protective and detrimental variants, APOE variant detection is being used in clinics to counsel individuals regarding the risk of side effects associated with anti-amyloid therapy. Individuals who are ε4 carriers, especially those homozygous for the ε4 allele, who are at a much higher risk for amyloid-related imaging abnormalities.43 Some data support that both spontaneous and therapy-induced hemorrhagic amyloid-related imaging abnormalities reflect underlying amyloid angiopathy, which is associated with ε4 allele status.44

**Potential Confounders and Mitigation Strategy**

Assay-related variability is the most studied variation in the research setting, and most studies account for this well. Intraindividual and test–retest variabilities are important considerations in the real-world implementation of BBBs. Based on recent studies, demographic characteristics (eg, age, race and ethnicity, sex) affect AD BBB levels, but they also may be dependent on other factors, such as chronic kidney disease.45,46 These variations likely can be accounted for with development of cut points by prospectively enrolling a diverse population. However, there are certain medications and comorbid conditions for which clinical intervention, an adjustment factor, or exclusion may be necessary when using this triage approach (see Table 3, online). Quality control measures and standardization of collection procedures should be instituted to minimize these confounders. Biotin and supplements are known to interact with immunoassays and a number of other diagnostic tests. All blood draws should be performed at least 72 hours after supplement discontinuation. It is unclear how fasting affects these markers, but preliminary data are encouraging.47 Medications that affect renal clearance, chronic kidney disease, and vascular disease are known to affect Aβ42, p-tau217, and p-tau181 levels. Reference ranges for these individuals must be validated.48 Sleep apnea and disordered breathing are also suspected to affect AD BBBs.49 Screening for obstructive sleep apnea in the primary care clinic in individuals with cognitive concerns is warranted. If a sleep study has positive results, consider 6 months of treatment before biomarker testing in the subjective cognitive impairment or MCI population. Genetic disorders, such as tuberous sclerosis, that are known to be associated with elevated p-tau levels26 should be screened for as part of a detailed family history or a statistical adjustment factor needs to be developed for use in this population.

**Conclusions**

Although AD BBBs do not meet standards for a stand-alone screening test, they are available in research and in the clinic. Their use as a triage test, especially as a panel, could be implemented with quality control measures for acceleration toward confirmatory testing, treatment, and clinical trial enrollment. Broad primary care and APP education about the limitations of such tests and the potential harms of false-positive test results can prevent improper use and minimize potential harm. AD BBBs can identify individuals with a high likelihood of brain amyloidosis, thus reducing the need for invasive confirmatory CSF and expensive amyloid PET testing. Phase 4 and 5 studies can provide prospective validation of the clinical use of AD BBBs as a triage or diagnostic screening test. One possible model for this process is presented in Figure 2. Using AD BBBs to rule in or rule out the need for referral to a memory clinic/specialist consult could help reduce bottlenecks and the potential for inappropriate prescribing. The considerable cost, modest delay in progression, and high prevalence of amyloid in cognitively normal individuals, as well as risks associated with anti-amyloid therapies, are all reasons to require evidence of clinical decline as well as confirmatory testing with CSF biomarkers or an amyloid or tau PET scan before the initiation of therapy in an amyloid treatment clinic.51 APOE testing for risk stratification and genetic counseling should also be performed routinely in people being evaluated for anti-amyloid therapy.


NOTE: The complete article including tables and references is available online at practicalneurology.com.

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<table>
<thead>
<tr>
<th>Test name (developer)</th>
<th>Biomarkers</th>
<th>Intended population and clinical stage</th>
<th>Suggested clinical management if positive</th>
<th>Suggested clinical management if indeterminant or negative</th>
</tr>
</thead>
<tbody>
<tr>
<td>Elecsys AD (Roche Diagnostics, Indianapolis, IN)</td>
<td>Aβ42, Aβ40, p-tau 181, total tau/Aβ42 ratio, p-tau 181/Aβ42 ratio</td>
<td>Individuals with MCI or mild dementia in the neurology clinic</td>
<td>If positive for amyloid and p-tau 181/Aβ42 ratio and no other signs of copathology, initiate AChEi in the morning and refer to amyloid treatment clinic for consideration of further therapy</td>
<td>Further diagnostic testing as indicated or referral to subspecialty memory clinic</td>
</tr>
<tr>
<td>β-Amyloid Ratio (Fujirebio, Malvern, PA)</td>
<td>Aβ42/Aβ40</td>
<td>Individuals with MCI or mild dementia in the neurology clinic</td>
<td>Amyloid positivity is common and does not correlate with symptoms; further history and diagnostic testing as indicated for other copathology; referral if necessary</td>
<td>Not likely to have AD neuropathologic changes driving symptoms; further diagnostic testing as indicated or referral to subspecialty memory clinic</td>
</tr>
<tr>
<td>Amyvid (florbetapir; Eli Lilly, Indianapolis, IN)</td>
<td>Amyloid PET</td>
<td>Individuals with MCI or mild dementia in the neurology clinic</td>
<td>Amyloid positivity is common and does not correlate with symptoms; further history and diagnostic testing as indicated to rule out other copathology</td>
<td>Not likely to have AD neuropathologic changes driving symptoms; further diagnostic testing as indicated</td>
</tr>
<tr>
<td>Neuraceq (florbetaben; Life Molecular Imaging, Boston, MA)</td>
<td>Amyloid PET</td>
<td>Individuals with MCI or mild dementia in the subspecialty memory clinic</td>
<td>Amyloid positivity is common and does not correlate with symptoms; further history and diagnostic testing as indicated to rule out other copathology</td>
<td>Not likely to have AD neuropathologic changes driving symptoms; further diagnostic testing as indicated</td>
</tr>
<tr>
<td>Vizamyl (flutemetamol; GE Healthcare, Chicago, IL)</td>
<td>Amyloid PET</td>
<td>Individuals with MCI or mild dementia in the subspecialty memory clinic</td>
<td>Amyloid positivity is common and does not correlate with symptoms; further history and diagnostic testing as indicated to rule out other copathology</td>
<td>Not likely to have AD neuropathologic changes driving symptoms; further diagnostic testing as indicated</td>
</tr>
<tr>
<td>Tauvid (flortaucipir; Eli Lilly, Indianapolis, IN)</td>
<td>Tau PET</td>
<td>Individuals with MCI or mild dementia in the subspecialty memory clinic</td>
<td>Tau positivity typically correlates well with symptoms; if positive for CSF or blood biomarkers, consider anti-amyloid therapy</td>
<td>Not likely to have AD neuropathologic changes driving symptoms; further diagnostic testing as indicated</td>
</tr>
</tbody>
</table>

Abbreviations: Aβ, amyloid-beta; AChEi, acetylcholinesterase inhibitor; AD, Alzheimer disease; CSF, cerebrospinal fluid; FDA, Food and Drug Administration; MCI, mild cognitive impairment; p-tau, phosphorylated tau; PET, positron emission tomography.
### TABLE 2. EARLY CANDIDATES FOR AD BBB CLINICAL TRIAGE

<table>
<thead>
<tr>
<th>Test name (developer)</th>
<th>Biomarker</th>
<th>Assay platform</th>
<th>Availability</th>
<th>Important considerations</th>
</tr>
</thead>
<tbody>
<tr>
<td>AD-Detect (Quest Diagnostics, Secaucus, NJ)</td>
<td>Aβ42/40</td>
<td>IP-LC-MS/MS</td>
<td>Direct to consumer or clinical order</td>
<td>No validation data available for review</td>
</tr>
<tr>
<td>Amyloid Plasma Panel (Roche, Indianapolis, IN)</td>
<td>Aβ42/Aβ40, p-tau 181, APOE4 status</td>
<td>Elecsys (electrochemiluminescence)</td>
<td>Clinical order</td>
<td>Prediction of amyloid PET status; predicted 6-year progression; longitudinal evaluation using predefined cutoffs ongoing</td>
</tr>
<tr>
<td>ATN Profile (Labcorp, Burlington, NC)</td>
<td>Aβ42/40, p-Tau 181, NfL</td>
<td>Simoa</td>
<td>Clinical order</td>
<td>Prediction of amyloid PET status and some evidence of neurodegeneration; longitudinal evaluation using predefined cutoffs ongoing</td>
</tr>
<tr>
<td>AD BBB testing (Fujirebio, Malvern, PA)</td>
<td>Aβ42/Aβ40, p-Tau 181, p-tau 217</td>
<td>Lumipulse G (chemiluminescence)</td>
<td>Research use only</td>
<td>Strong AUC and mean fold change</td>
</tr>
<tr>
<td>Lucent-AD (Lucent Diagnostics, Billerica, MA)</td>
<td>P-tau 181</td>
<td>Simoa</td>
<td>Clinical order</td>
<td>Aβ pathology positivity, does not reflect tau pathology</td>
</tr>
<tr>
<td>PrecivityAD (C2N, St. Louis, MO)</td>
<td>Aβ42/40, APOE4 status, age</td>
<td>IP-LC-MS/MS</td>
<td>Clinical order</td>
<td>Prediction of amyloid PET status is strong</td>
</tr>
<tr>
<td>PrecivityAD2 (C2N, St. Louis, MO)</td>
<td>Aβ42/40, p-tau 217/np-tau 217</td>
<td>IP-LC-MS/MS</td>
<td>Clinical order</td>
<td>Prediction of amyloid PET status is strong; assay appears accurate and robust thus far</td>
</tr>
<tr>
<td>Quanterix (multiple partners; eg, ALZpath, Eli Lilly, Labcorp, Jannsen)</td>
<td>p-tau 217, p-tau 181, p-tau 231, YKL-40, GFAP</td>
<td>Simoa</td>
<td>Research use only</td>
<td>Prediction of amyloid PET status; p-tau 217 may detect tau changes as well; available, accessible, and scalable</td>
</tr>
<tr>
<td>Multiplex assays (Luminex, Brooklyn, NY)</td>
<td>21-protein panel</td>
<td>Luminex (multiplex bead immunoassay)</td>
<td>Research use only</td>
<td>Consideration for rule-out testing; does not use amyloid or tau; being tested in primary care</td>
</tr>
<tr>
<td>Single-plex and large multiplex assays (Alamar Biosciences, Fremont, CA)</td>
<td>Single-plex (p-tau 217) and large multiplex assays (120 targets)</td>
<td>NULISA (immuno-complex capture and release)</td>
<td>Research use only</td>
<td>Research use only; but strong AUC and mean fold change for individual markers; high sensitivity with low sample volumes; can give information on multiple pathways simultaneously</td>
</tr>
<tr>
<td>P-tau testing (Meso Scale Discovery, Rockville, MA)</td>
<td>P-tau 217, p-tau 181, p-tau 231</td>
<td>S-PLEX (electrochemiluminescence)</td>
<td>Research use only</td>
<td>Research use only, but strong AUC and mean fold change; larger cohort with longitudinal follow-up is ongoing</td>
</tr>
</tbody>
</table>

**Abbreviations:** Aβ, amyloid-beta; Aβ42/40, ratio of Aβ1-42 to Aβ1-40; AD, Alzheimer disease; AUC, area under the receiver operating characteristic curve; BBB, blood-based biomarker; GFAP, glial fibrillary acidic protein; IP-LC-MS/MS, immunoprecipitation liquid chromatography–tandem mass spectrometry; NfL, neurofilament light chain; p-tau, phosphorylated tau; p-tau 217/np-tau 217, ratio of tau phosphorylation at 217 to nonphosphorylated tau at 217; PET, positron emission tomography; Simoa, single molecule array (microbead-based sandwich enzyme-linked immunosorbent assay); YKL-40, chitinase 3-like 1.

### TABLE 3. POSSIBLE CONFOUNDERS THAT MAY AFFECT AD BBB AND PROPOSED MITIGATION

<table>
<thead>
<tr>
<th>Possible confounder</th>
<th>Mitigation</th>
</tr>
</thead>
<tbody>
<tr>
<td>Biotin and supplements</td>
<td>Stop all supplements 72 hours before blood draw.</td>
</tr>
<tr>
<td>Chronic kidney disease</td>
<td>Establish adjustment factor or prospective cut point validation for renal function.</td>
</tr>
<tr>
<td>Genetic disorders (eg, tuberous sclerosis)</td>
<td>Establish adjustment factor or prospective cut point validation for genetic subpopulations and compare with confirmatory CSF findings prospectively.</td>
</tr>
<tr>
<td>Intraindividual and test–retest variation</td>
<td>Perform serial sampling at regular intervals in the fasted and fed state in clinic to assess the validity of testing before broader implementation.</td>
</tr>
<tr>
<td>Obstructive sleep apnea, obstructive sleep-disordered breathing</td>
<td>Screen for obstructive sleep apnea and other respiratory illness causing hypoxemia. If positive, consider 6 months treatment before biomarker testing in the SCI/SCD/MCI population.</td>
</tr>
</tbody>
</table>

**Abbreviations:** AD, Alzheimer disease; BBB, blood-based biomarker; CSF, cerebrospinal fluid; MCI, mild cognitive impairment; SCD, subjective cognitive decline; SCI, subjective cognitive impairment.
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