IMPORTANT REMINDER

Medical Policies are developed to provide guidance for members and providers regarding coverage in accordance with contract terms. Benefit determinations are based in all cases on the applicable contract language. To the extent there may be any conflict between the Medical Policy and contract language, the contract language takes precedence.

PLEASE NOTE: Contracts exclude from coverage, among other things, services or procedures that are considered investigational or cosmetic. Providers may bill members for services or procedures that are considered investigational or cosmetic. Providers are encouraged to inform members before rendering such services that the members are likely to be financially responsible for the cost of these services.

DESCRIPTION

Biochemical and cellular biomarkers may predict risk of Alzheimer’s disease (AD); however, since there is currently no treatment to delay or prevent development or progression if AD, early detection is not useful in treatment planning.

MEDICAL POLICY CRITERIA

I. Measurement of cerebrospinal fluid biomarkers of Alzheimer's disease, including but not limited to tau protein, amyloid beta peptides, or neural thread proteins, is considered investigational.

II. Measurement of urinary biomarkers of Alzheimer's disease, including but not limited to neural thread proteins, is considered investigational.

III. Skin cell (fibroblast) testing for biomarkers of Alzheimer’s disease, including but not limited to the DISCERN™ test, is considered investigational.

NOTE: A summary of the supporting rationale for the policy criteria is at the end of the policy.

CROSS REFERENCES

1. Genetic Testing for Familial Alzheimer’s Disease, Genetic Testing, Policy No. 01
Currently the diagnosis of Alzheimer's disease (AD) is a clinical diagnosis, focusing on the exclusion of other causes of dementia. In 1984 the National Institute of Neurological and Communicative Disorders and Stroke (NINCDS) and the Alzheimer's and Related Disorders Association (ADRDA) published clinical criteria for the diagnosis of AD. These organizations defined three categories: possible, probable, and definite AD. The only difference between probable and definite AD is that the definite category requires a brain biopsy confirming the presence of characteristic neurofibrillary tangles. Therefore, definite AD is typically identified only at autopsy. The categories are defined as follows:

I. Possible Alzheimer's Disease
   A. May be made on the basis of the dementia syndrome in the absence of other neurological, psychiatric, or systemic disorders sufficient to cause dementia, and in the presence of variations in the onset, in the presentation, or in the clinical course
   B. May be made in the presence of a second systemic or brain disorder sufficient to produce dementia, which is not considered to be the cause of the dementia
   C. Should be used in research studies when a single gradually progressive severe cognitive deficit is identified in the absence of other identifiable cause

II. Probable Alzheimer's Disease
   A. The criteria for the clinical diagnosis of probable AD include:
      1. Dementia, established by clinical examination and documented by the Mini-Mental State Examination, the Blessed Dementia Scale, or some similar examination and confirmed by neuropsychological tests
      2. Deficits in two or more areas of cognition
      3. Progressive worsening of memory and other cognitive functions
      4. No disturbance of consciousness
      5. Onset between ages 40 and 90, most often after the age of 65
      6. Absence of systemic disorders or other brain diseases that in and of themselves could account for the progressive deficits in memory and cognition
   B. The diagnosis of probable AD is supported by:
      1. Progressive deterioration of specific cognitive functions such as language (aphasia), motor skills (apraxia), and perception (agnosia)
      2. Impaired activities of daily living and altered patterns of behavior
      3. Family history of similar disorders, particularly if confirmed neuropathologically
      4. Laboratory results: normal lumbar puncture as evaluated by standard techniques, normal pattern or non-specific changes in the EEG, and evidence of cerebral atrophy on CT scanning with progression documented by serial observation
C. Other clinical features consistent with the diagnosis of probable AD, after exclusion of causes of dementia other than AD, include:

1. Plateaus in the course of progression of the illness;

2. Associated symptoms of depression, insomnia, incontinence, delusions, illusions, hallucinations, sexual disorders, weight loss, and catastrophic verbal, emotional, or physical outbursts

3. Other neurologic abnormalities in some patients, especially with more advanced disease and including motor signs such as increased muscle tone, myoclonus, or gait disorder

4. Seizures in advanced disease CT normal for age

D. Features that make the diagnosis of probable AD uncertain or unlikely include:

1. Sudden apoplectic onset

2. Focal neurological findings such as hemiparesis, sensory loss, visual field deficits, and incoordination early in the course of the illness

3. Seizures or gait disturbances at the onset or very early in the course of the illness

III. Definite Alzheimer's Disease

A. Clinical criteria for probable Alzheimer's disease AND

B. Histopathologic evidence obtained from a biopsy or autopsy

In 2011, the National Institute on Aging and the Alzheimer’s Association workgroup revised diagnostic criteria for diagnosis of dementia due to Alzheimer’s disease. All probable AD by NINCDS-ADRDA criteria are subsumed in the revised probable AD criteria, which is now defined by the following:

"Meets criteria for dementia [...] and in addition, has the following characteristics:

A. Insidious onset. Symptoms have a gradual onset over months to years, not sudden over hours or days;

B. Clear-cut history of worsening of cognition by report or observation; and

C. The initial and most prominent cognitive deficits are evident on history and examination in one of the following categories.

1. Amnestic presentation: It is the most common syndromic presentation of AD dementia. The deficits should include impairment in learning and recall of recently learned information. There should also be evidence of cognitive dysfunction in at least one other cognitive domain, as defined earlier in the text.

2. Nonamnestic presentations: Language presentation: The most prominent deficits are in word-finding, but deficits in other cognitive domains should be present. Visuospatial presentation: The most prominent deficits are in spatial cognition, including object agnosia, impaired face recognition, simultanagnosia, and alexia.
Deficits in other cognitive domains should be present. Executive dysfunction: The most prominent deficits are impaired reasoning, judgment, and problem solving. Deficits in other cognitive domains should be present.

The diagnosis of probable AD dementia should not be applied when there is evidence of (a) substantial concomitant cerebrovascular disease, defined by a history of a stroke temporally related to the onset or worsening of cognitive impairment; or the presence of multiple or extensive infarcts or severe white matter hyperintensity burden; or (b) core features of Dementia with Lewy bodies other than dementia itself; or (c) prominent features of behavioral variant frontotemporal dementia; or (d) prominent features of semantic variant primary progressive aphasia or nonfluent/agrammatic variant primary progressive aphasia; or (e) evidence for another concurrent, active neurological disease, or a non-neurological medical comorbidity or use of medication that could have a substantial effect on cognition.”

Diagnosis by exclusion is frustrating for physicians, patients and families, and there has been considerable research interest in identifying an inclusive laboratory test for AD, particularly for use early in the disease process. Abnormal levels in cerebrospinal fluid (CSF) of the tau protein (total tau [T-tau] or phosphorylated [P-tau] ) or an amyloid beta (Aβ) peptide such as Aβ42, have been found in patients with AD, and thus these proteins have been investigated for their diagnostic utility. The tau protein is a microtubule-associated molecule that is found in the neurofibrillary tangles that are typical of Alzheimer's disease. This protein is thought to be related to degenerating and dying neurons, and high levels of tau proteins in the CSF have been associated with AD. Aβ42 is a subtype of amyloid beta peptide that is produced following the metabolism of an amyloid precursor protein. Aβ42 is the key peptide deposited in the amyloid plaques that are characteristic of AD. Low levels of Aβ42 in the CSF have been associated with AD, perhaps because the Aβ42 is deposited in the amyloid plaques instead of remaining in solution.

Neural thread protein (NTP) is another protein that is associated with neurofibrillary tangles of Alzheimer's disease. Both CSF and urine levels of this protein have been investigated as a biochemical marker of Alzheimer's disease. Urine and CSF tests for neural thread protein may be referred to as the AD7CTM test.

**REGULATORY STATUS**

No biomarker tests for AD are currently approved by the U.S. Food and Drug Administration (FDA). Commercially available tests include:

- AlzheimAlert™ (Nymox Pharmaceutical Corp.)
- Innotest® assays for T-tau, P-tau, and AB-42 (Fujirebio [previously Innogenetics])
- AdMark® CSF analysis
- DISCERN™ (Neurodiagnostics) skin sample fibroblast testing

These are laboratory-developed tests (LDTs). Clinical laboratories may develop and validate tests inhouse and market them as a laboratory service; LDTs must meet the general regulatory standards of the Clinical Laboratory Improvement Act (CLIA). AlzheimAlert™ and AdMark® CSF analysis are available under the auspices of CLIA. Laboratories that offer LDTs must be licensed by CLIA for high-complexity testing. To date, FDA has chosen not to require any regulatory review of these tests.
Nymox Pharmaceutical Corp. previously offered AD7C testing as an LDT but no longer lists the test on its website.

**EVIDENCE SUMMARY**

Validation of the clinical use of any prognostic test focuses on three main principles:

1. The analytic validity of the test, which refers to the technical accuracy of the test in detecting a disease marker of interest that is present or in excluding a disease marker that is absent;
2. The clinical validity of the test, which refers to the diagnostic performance of the test (sensitivity, specificity, positive and negative predictive values) in detecting clinical disease; and
3. The clinical utility of the test, i.e., how the results of the diagnostic test will be used to change management of the patient and whether these changes in management lead to clinically important improvements in health outcomes. The clinical utility of both positive and negative tests must be established.

This evidence review focuses on clinical validity and utility.

Evaluation of evidence of clinical utility requires consideration of the following:

- **Reference/Criterion Standard.** The gold standard for definitive diagnosis of Alzheimer’s disease (AD) is autopsy. The accuracy of testing for AD is best established by comparison with this gold standard; therefore, the gold standard must be employed to accurately assess incremental diagnostic improvement.

- **Predicting Conversion from mild cognitive impairment (MCI) to AD.** Predicting conversion from MCI to AD may rely on a clinical diagnosis, albeit with some attendant error and misclassification, because the prediction of interest is conversion and not the gold standard diagnosis.

- **Incremental Diagnostic Improvement.** Incremental diagnostic or prognostic improvement is best demonstrated through evidence that the proposed predictor can correctly reclassify individuals with and without AD, or those with MCI who will and will not progress to AD. Alternative approaches such as classical receiver operating characteristic (ROC) analyses, while providing some insight, do not allow directly translating improvements in diagnostic or prognostic accuracy to changes in health outcomes.

- **Improved Health Outcomes (Clinical Utility).** In order to establish clinical utility, AD biomarkers would need to provide information which improves treatment decisions and health outcomes beyond that of clinical diagnosis.

- **Test Cutoffs.** Almost all studies employ optimal (data-driven) test cutoffs to define test accuracy (sensitivity and specificity). This approach is typically accompanied by a degree of optimism and potentially overstates test accuracy.

- **Sample Definition.** Clear description of whether samples included consecutive patients or were selective is required to evaluate potential bias—including verification bias—and generalizability but almost absent in this literature.
• Validation. Validation in independent samples is required to establish generalizability of markers but has been scant.

CEREBRAL SPINAL FLUID MARKER TESTING

Clinical Validity

Diagnosis of AD

Systematic Reviews

Olsson (2016) published a systematic review and meta-analysis on the diagnostic performance of the three core CSF biomarkers for the diagnosis of Alzheimer's disease (Aβ42, T-tau, and P-tau).\(^5\) The investigators included 231 cross-sectional cohort and longitudinal studies that contained a cohort with Alzheimer's disease and a control cohort, or a cohort with mild cognitive impairment due to Alzheimer's disease and a stable mild cognitive impairment cohort (n=15,699 Alzheimer’s patients and 13,018 controls). Biomarker performance was reported as the ratio between biomarker concentration in patients with Alzheimer's disease and controls (fold change) or the ratio between biomarker concentration in those with mild cognitive impairment due to Alzheimer's disease and those with stable mild cognitive impairment who had no further cognitive decline in minimum of two years. In the CSF, T-tau was able to differentiate clinically diagnosed Alzheimer’s disease from controls with good performance (average ratio 2.54, 95% confidence interval [CI] 2.44 to 2.64, p<0.0001), with similar effect sizes reported for the emerging biomarker neurofilament light chain protein (NFL) (2.35, 1.90 to 2.91, p<0.0001). CSF P-tau (1.88, 1.79 to 1.97, p<0.0001) and plasma T-tau (1.95, 1.12 to 3.38, p=0.02) also had large effect sizes when differentiating between controls and patients with Alzheimer's disease. All other markers, including CSF Aβ42, had only marginal effect sizes. Limitations of this review include the fact that only five of the included studies were considered to be of good quality; and substantial publication bias for all three core biomarkers.

| Table 1. CSF Biomarkers Performance for Distinguishing AD from Controls |
|-------------------------------------------------|----------------|----------------|----------------|
|                                                | Nondemented Controls | Controls with Dementia\(^a\) |                  |
|                                                | Sensitivity, %        | Specificity, %       | Sensitivity, % | Specificity, % |
| Aβ42                                           |                      |                    |
| Ferreira (2014)\(^6\) SR                       | 80 (73 to 85)        | 82 (74 to 88)      | 73 (67 to 78) | 67 (62 to 72) |
| Ferreira (2014)\(^6\) IS                       | 63 to 97%            | 67 to 92           | 82 to 95      | 22 to 80       |
| Rosa (2014)\(^7\)                             | 84 (81 to 85)        | 79 (77 to 81)      | NR            | NR             |
| Bloudek (2011)\(^8\)                          | 80 (73 to 85)        | 82 (74 to 88)      | 73 (67 to 78) | 67 (62 to 72) |
| Formichi (2006)\(^9\)                         | NR                   | NR                 | NR            | NR             |
| Vogelsgang (2018)\(^10\)                      | NR                   | NR                 | NR            | NR             |
| T-tau                                          |                      |                    |
| Ferreira (2014)\(^8\) SR                       | 82 (76 to 87)        | 90 (86 to 93)      | 73 to 91     | 75 to 98       |
| Ferreira (2014)\(^8\) IS                       | 61 to 91             | 53 to 97           | 61 to 92     | 40 to 93       |
| Bloudek (2011)\(^8\)                          | 82 (76 to 87)        | 90 (86 to 93)      | 78 (72 to 83) | 75 (68 to 81) |
| Formichi (2006)\(^9\)                         | NR                   | NR                 | 52 to 100    | 50 to 100      |
| P-tau                                          |                      |                    |
| Ferreira (2014)\(^8\) SR                       | 78 to 80             | 83 to 88           | 72 to 88     | 78 to 83       |
| Ferreira (2014)\(^8\) IS                       | 61 to 89             | 37 to 92           | 77 to 88     | 56 to 88       |
| Bloudek (2011)\(^8\)                          | 80 (70 to 87)        | 83 (75 to 88)      | 79 (72 to 84) | 80 (71 to 86) |
| Formichi (2006)\(^9\)                         | NR                   | NR                 | 37 to 100    | 80 to 100      |
| BACE1                                           |                      |                    |
Ferreira (2014) published a meta-review of systematic reviews with meta-analyses to assess the use of CSF biomarker tests for AD after publication of revised AD diagnostic criteria[1] in 2011.[6] Literature was searched in September 2013, and seven systematic reviews were included. None was published after introduction of the revised AD diagnostic criteria, so primary studies were searched. Twenty-six prospective or retrospective case-control, cross-sectional, or longitudinal studies were included. Most included studies used clinical criteria for AD diagnosis or did not specify. For differentiating AD from nondemented controls, positive and negative likelihood ratios for all three biomarkers ranged from 4 to 8 and from 0.1 to 0.3, respectively.

Rosa (2014) conducted a systematic review with meta-analysis of studies of CSF Aβ42 in patients with clinically diagnosed AD.[7] Literature was searched to May 2013, and 41 prospective or retrospective, cohort, case-control, and cross-sectional studies were included (total n=5,086: 2,932 AD, and 2,154 nondemented controls). Patients with MCI were excluded. Seventy-six percent of studies satisfied all quality domains of the Quality Assessment of Diagnostic Accuracy Studies (QUADAS-2) tool. Publication bias was detected. A summary ROC curve was generated from all reported thresholds. Pooled sensitivity and specificity were 84% (95% CI 81 to 85) and 79% (95% CI 77 to 81), respectively. Positive and negative likelihood ratios were 4.5 (95% CI 3.7 to 5.4) and 0.18 (95% CI 0.14 to 0.22), respectively, and their ratio, the diagnostic odds ratio, was 29 (95% CI 21 to 40). Statistical heterogeneity was substantial (I²=68%); studies varied in test cutoffs used and severity of AD across patient samples. Eleven studies (total n=1,459: 830 AD, 629 controls) reported AB42 CSF levels. Mean (standard deviation [SD]) CSF AB42 was 467 (189) pg/mL in patients with AD and 925 (414) pg/mL in controls (weighted mean difference, 450 pg/mL, 95% CI -600 to -289, p<0.001). However, statistical heterogeneity was considerable (I²=99%).

Cure (2014) published a systematic review with meta-analysis of CSF and imaging studies for the diagnosis of definite AD (autopsy-confirmed).[13] Literature was searched in January 2012, and three studies of CSF markers (P-tau, T-tau, Aβ42, Aβ40) were identified (total n=337). Pooled sensitivity of all CSF tests was 82% (95% CI, 72 to 92), and pooled specificity was 75% (95% CI 60 to 90). Statistical heterogeneity was not reported, but studies varied in AD definitions, controls (nondemented patients or patients with dementia due to other causes), and test thresholds. Area under the summary ROC curve constructed using multiple test thresholds was 0.84.

In a systematic review by Noel-Storr (2013), authors assessed the weight and quality of the evidence available from primary diagnostic test accuracy studies for Alzheimer's disease.[14] Authors identified 142 longitudinal studies relating to the biomarkers of interest, which included subjects who had objective cognitive impairment but no dementia at baseline. Authors concluded the body of evidence for biomarkers was not large and was variable across the different types of biomarkers. Authors suggest that important information is missing from many
study reports, highlighting the need for standardization of methodology and reporting to improve the rigor of biomarker validation.

A meta-analysis by Bloudek (2011) included 119 studies on biomarkers and diagnostic imaging in AD.\[^8\] Sensitivity and specificity were calculated for distinguishing AD from nondemented controls, and for distinguishing AD from non-AD dementias with and without MCI, if available. Selected studies of CSF biomarkers used a variety of thresholds, with clinical diagnosis or autopsy as the reference standard. Twenty studies of the Aβ42 CSF marker were included with nondemented and demented controls; pooled analysis resulted in a sensitivity of 76% (95% CI 72% to 80%) and a specificity of 77% (95% CI 72% to 82%). CSF total tau was evaluated in 30 studies with a resulting sensitivity of 79% (95% CI 75% to 83%) and specificity of 85% (95% CI 81% to 89%). CSF P-tau was evaluated in 24 studies, resulting in a pooled sensitivity of 78% (95% CI, 73% to 83%) and specificity of 81% (95% CI 76% to 85%). Six studies evaluated CSF P-tau as a biomarker to distinguish patients with AD from patients with MCI, with a pooled sensitivity of 73% (95% CI 54% to 86%) and specificity of 69% (95% CI 53% to 82%). The combination of total tau and Aβ42 was evaluated in 12 studies, with a pooled sensitivity of 80% (95% CI 72% to 85%) and specificity of 76% (95% CI 57% to 88%). Comparison of CSF biomarkers, area under the receiver operating characteristic curve was highest for P-tau alone (0.85, 95% CI 82 to 88). Study heterogeneity was due to the use of different test thresholds and different assay kits. Sensitivity analysis including studies that used autopsy as the reference standard for P-tau resulted in slightly higher sensitivity (82%, 95% CI 75% to 87%) and lower specificity (57%, 95% CI 37% to 75%).

A systematic review by van Harten (2011) of seven studies using CSF biomarkers to differentiate AD from other dementias, reporting positive and negative likelihood ratios of 46 and 0.09, respectively, for differentiating AD (n=175) from Creutzfeldt-Jakob disease (n=110).\[^15\] With this systematic review excluded, positive and negative likelihood ratios ranged from 2 to 7 and from 0.15 to 0.4, respectively.

**Nonrandomized Studies**

Alexopoulos (2018) conducted a retrospective study of data from the Alzheimer Disease Neuroimaging Initiative databank to evaluate the utility of measuring β-site amyloid-β precursor protein (AβPP) cleaving enzyme 1 (BACE1) activity and soluble AβPP β (sAβPPβ) levels in CSF as predictors for AD.\[^11\] In the study, data from 56 patients with AD dementia, 76 patients with mild cognitive impairment from AD, 39 patients with mild cognitive impairment with normal CSF markers, and 48 control patients without preclinical AD were analyzed using several statistical tests. There were no differences in sAβPPβ levels among any of the groups, and the AD-dementia group did not show a difference in BACE1 activity compared with the other groups. However, BACE1 activity was significantly higher in MCI-AD patients compared with both MCI-nonAD (p=0.02) and control groups (p<0.001). Limitations included a relatively small sample size, the retrospective design, and patients recruited at specialized centers.

Wang (2018) conducted a longitudinal study whether the addition of total and phosphorylated αsynuclein to the AD biomarker panel improves the panel’s performance.\[^12\] The researchers analyzed 792 baseline and longitudinal CSF samples from 87 AD patients, 177 MCI patients, and 104 age-matched healthy controls across up to seven years as part of the AD Neuroimaging Initiative. Statistical analysis showed that α-synuclein predicted AD Assessment Scale-Cognitive (p=0.0015), memory (p=0.00025) and executive-function (p<0.0001)
composite scores and progression from MCI to AD (p=0.0011). Limitations include cohort heterogeneity and the longitudinal design.

Trombetta (2018) conducted an observational study to identify biomarkers with good to excellent reliability at predicting AD.\textsuperscript{[16]} The researchers analyzed baseline CSF samples from 20 patients with MCI or mild dementia due to AD who were enrolled in a clinical drug trial. The researchers identified 32 biomarker candidates that consistently and reliably were associated with incidence of AD. Limitations included the observational design and small sample size.

Vogelsgang (2018) conducted an analysis of CSF from 114 patients to determine the reproducibility of using Aβ40 and Aβ42 in AD screenings.\textsuperscript{[10]} CSF samples for each patient were collected under routine clinical conditions at two different sites, and the samples for each patient were compared for discrepancies. Statistical analysis showed that inclusion of Aβ42/Aβ40, compared with Aβ42 alone, leads to 16.8\% fewer discordant results. Limitations included the sample size and the observational design.

Howell (2017) evaluated the clinical validity of CSF biomarkers in diverse populations by prospectively recruiting 135 older Americans to undergo detailed clinical, neuropsychological, genetic, magnetic resonance imaging (MRI), and CSF analysis.\textsuperscript{[17]} Despite finding comparable levels of CSF Aβ42 and Aβ42/Aβ40, cognitive impairment in African Americans was noted to be associated with smaller changes in CSF tau markers but greater impact from similar MRI white matter hyperintensity burden than whites, leading to the conclusion that race-associated differences in CSF tau markers and ratios may lead to underdiagnosis of AD in African Americans.

Park (2017) examined the AD biomarkers T-tau, P-tau, and Aβ42 with the primary aim of finding accurate cutoff values for CSF biomarkers to distinguish between AD and either control or other neurological disorders with cognitive decline (OND). Of the total of 194 patients, 71 were matched control subjects, 76 were patients with AD dementia (ADD), and 47 were patients with OND.\textsuperscript{[18]} CSF biomarkers differentiated between the ADD and control groups (p<0.001 for all), and between the ADD and OND groups (p<0.001 for all). The areas under the curve in differentiation of ADD from control subjects were 0.97, 0.93, 0.86, and 0.99 for Aβ42, 0.93 for T-tau, P-tau, and T-tau/Aβ42 and P-tau/Aβ42 ratios, respectively. Based on the results, the authors suggest a revised cutoff value for Aβ42, higher than the previous one. The T-tau/Aβ42 ratio had the highest accuracy, 97\%. While study limitations included a younger-than-average group of AD patients and a small comparison group with several neurologic disorders, the authors concluded that the combined biomarker ratio was superior to individual markers at accurately predicting AD. They based this conclusion on the comparability of cutoff values between this study and previous studies.

Janelidze (2016) also found that the CSF Aβ42/Aβ40 ratio was significantly better than Aβ42 alone in detecting brain amyloid deposition in prodromal AD and in differentiating AD dementia from non-AD dementias across three different immunoassays and three patient cohorts.\textsuperscript{[19]} Alexopoulos (2016) analyzed a monocentric cohort with healthy (n=41) and disease (n=22) controls and patients with AD dementia (n=119), and a multicentric sample with healthy controls (n=116) and patients with AD dementia (n=102).\textsuperscript{[20]} The CSF biomarkers Aβ42, T-tau, and phosphorylated tau at threonine 181 were measured. Only 40.3\% to 52.9\% of patients with AD dementia exhibited a typical CSF profile for AD. In addition, up to 8.6\% of controls had abnormal CSF biomarkers. The authors concluded that there is a discordance between CSF biomarkers and AD symptomatology.
Mattsson (2016) examined 93 patients with AD, 187 patients with MCI, and 109 controls from the ADNI cohort. Diagnostic accuracy for AD diagnosis was found to be 80.8%, 71.4%, and 77.7% (area under the curve [AUC]) for T-tau, neurogranin, and NFL. Combinations of the three biomarkers improved diagnostic accuracy (AUC 85.5%) over the individuals. T-tau and neurogranin are primarily correlate with degeneration in the presence of Aβ pathology while neurofilament light correlates with degeneration independent of Aβ pathology.

Palmqvist (2015) compared the diagnostic accuracy of CSF biomarkers with amyloid PET for diagnosing early-stage AD, including 122 healthy elderly and 34 patients with mild cognitive impairment who developed AD dementia within three years (MCI-AD). The best CSF measures for identifying MCI-AD were Aβ42/T-tau and Aβ42/P-tau (AUC 0.93 to 0.94). Although the best PET measures performed similarly (AUC 0.92 to 0.93; the investigators reported that CSF Aβ42/T-tau had the highest accuracy of all CSF/PET biomarkers (sensitivity 97%, specificity 83%). The combination of CSF and PET was not better than using either biomarker separately.

Sauvee (2014) examined the Aβ42/Aβ40 ratio in 122 patients with atypical dementia who had discordant CSF biomarker results (i.e., tau, P-tau, Aβ42). Using 0.05 as the ratio threshold, biological profiles were clarified in 72 (59%) of 122 patients with the addition of the Aβ42/Aβ40 ratio. However, of 35 patients diagnosed with AD by biological profile, nine (26%) did not meet clinical criteria for AD or mixed dementia.

Lowe (2013) evaluated CSF Aβ42, amyloid PET, FDG-PET, and MRI in 211 ADNI patients with at least one detected amyloid biomarker. Using the most recent diagnostic criteria, in the 92 patients undergoing all tests, Aβ42 had a 94% sensitivity for a positive FDG-PET or MRI. The authors concluded, “[m]ore correlation and validation studies of biomarkers in the AD population will be essential to understand biomarker performance and correlation with autopsy data.”

Schmand (2011) evaluated the value of neuropsychologic tests, neuroimaging, and biomarkers (Aβ and tau in CSF) for diagnosing AD in all participants in the ADNI database who had a lumbar puncture. The study included 105 normal controls, 179 individuals with MCI, and 91 with AD. Neuropsychologic tests and magnetic resonance imaging (MRI) were found to be the most informative techniques, with 84% and 82% correct classifications, respectively. CSF assessments had 73% correct classifications, respectively, and did not add diagnostic information when all the techniques were combined. CSF assessments were less informative in patients aged 75 years and older.

As previously noted, among patients with clinically diagnosed AD some have suggested the tau/Aβ42 ratio a more accurate measure than either alone. For example, using optimal cutoffs de Jong (2008) reported sensitivities and specificities for the ratio of 95% and 90% in a sample with clinically diagnosed AD (n=61) and vascular dementia (VaD, n=61). In contrast, a number of earlier studies concluded that CSF biomarkers did not provide incremental diagnostic value.

Prognosis for Progression of MCI to AD

Systematic Reviews

Ritchie (2014, 2017) published Cochrane reviews assessing the evidence to determine the accuracy of CSF biomarkers for detecting which patients with MCI would progress to AD or
other dementias. The two systematic reviews analyzed many of the same studies and reached the same conclusion.\[31,32\] In the 2017 review, literature was searched in January 2013 and 15 prospective and retrospective studies (1,172 participants with MCI had analyzable data) were included. Only studies that applied a reference standard for Alzheimer’s disease dementia diagnosis were included. There were 430 patients that converted to Alzheimer’s disease dementia and 130 that converted to other forms of dementia. The sensitivity of T-tau values ranged from 51% to 90% and the specificity from 48% to 88%. Sensitivities for P-tau ranged from 40% to 100% and specificities ranged from 22% to 86%. In the five studies that evaluated the CSF P-tau/Aβ ratio, the sensitivities were between 80% and 96% and the specificities were between 33% and 95%. Eight of 15 studies were of poor methodological quality and, in the majority of studies, there was an unclear risk of bias. The authors conclude that the biomarkers analyzed lack the accuracy to identify patients who will progress from MCI to AD.

The 2014 meta-review of systematic reviews by Ferreira summarized above included studies of CSF biomarkers for differentiating patients with MCI who progress to AD from those who do not.\[6\] In systematic reviews with meta-analyses, sensitivity and specificity of Aβ42 were 67% (95% CI 59 to 75) and 71% (95% CI 65 to 78), respectively; for T-tau, 82% (95% CI 76 to 86) and 70% (95% CI 65 to 85), respectively; and for P-tau, 81% (95% CI 69% to 91%) and 65% to 76%, respectively. Positive and negative likelihood ratios for all three tests ranged from 2 to 3 and from 0.3 to 0.5, respectively.

**Nonrandomized Studies**

Liu (2017) conducted an observational study of 94 patients (17 potential AD patients, 35 patients with mild cognitive impairment, and 41 control patients with subjective memory complaints) who received extensive dementia screenings.\[33\] Samples from the patients were tested for levels of let-7b miRNA. The results were analyzed using numerous statistical tests. Analysis found that when let-7b is added to predicted parameters in CSF screening, the predicted probability of the occurrence of AD increases from 75.9% to 89.7% (CI 0.844 to 1.000, p<0.001). Limitations include the small sample size and lack of further validation.

Hansson (2018) compared the performance of the Elecsys CSF immunoassay with positron emission tomography (PET) to predict clinical progression of AD. Immunoassay cutoffs were determined using PET results in the Swedish BioFINDER cohort (n=277), and then validated using PET results in the Alzheimer’s Disease Neuroimaging Initiative cohort (n=646). The CSF T-tau/Aβ42 and P-tau/Aβ42 ratio results showed an overall concordance of 90% with the PET classification in the BioFINDER calibration cohort (AUC 94%). In the validation cohort, the overall percent agreement between the immunoassay and PET was 89% to 90% with an AUC of 96% and were associated with more severe two-year decline in patients with MCI.

van Maurik (2017) published a study evaluating biomarker-based prognostic models to predict progression from MCI to AD.\[34\] This retrospective study included 525 patients with MCI as part of the Alzheimer’s Biomarkers in Daily Practice (ABIDE) project. Baseline measurements of CSF Aβ42 and tau were taken as well as patient characteristics and MRI biomarkers. Clinical endpoints were AD dementia and any type of dementia after one and three years. Risk of progression based on abnormal MRI results was calculated to be 27% (95% CI 17% to 41%) at one year and 86% (95% CI 71% to 95%) at three years. Risk of progression based on normal MRI results was calculated to be 3% (95% CI 2% to 5%) at one year and 18% (95% CI 13% to 27%) at three years. When CSF test results were abnormal one- and three-year progression risks were 26% (95% CI 20% to 33%) and 82% (95% CI 73% to 89%). When CSF
test results were normal, one- and three-year progression risks were 1% (95% CI 0.5% to 2%) and 6% (95% CI 3% to 9%). When both results were abnormal, one- and three-year progression risks were 26% (95% CI 18% to 36%) and 89% (95% CI 79% to 95%). When both results were normal, one- and three-year progression risks were 0.5% (95% CI 0.2% to 1%) and 4% (95% CI 2% to 7%). The authors concluded that these results support the use of biomarker-based prognostic models.

Frölich (2017) analyzed data from a German multicenter cohort study to investigate which indicators best predicted a short-term conversion from MCI to AD.[35] Indicators included the Mini-Mental State Examination (MMSE), Clinical Dementia Rating (CDR)-sum-of-boxes, the word list delayed free recall from the Consortium to Establish a Registry of Dementia (CERAD) test battery, hippocampal volume (HCV), Aβ42, Aβ40 levels, the ratio of Aβ42/Aβ40, P-tau, and T-tau levels. At baseline there was no difference between progressing and non-progressing patients in age and gender distribution, but progressing patients had lower educational attainment. Each biomarker individually was significantly different between groups at baseline and Aβ40 was statistically inferior to the other individual biomarkers as a predictor of conversion. ROC curves gave AUCs between 0.66 and 0.77 for individual predictors, 0.77 to 0.81 for the two-parameter combinations, 0.80 to 0.83 for the three-parameter combinations, and 0.81 to 0.82 for the four-parameter combinations. The authors concluded that none of the biomarker combinations assessed in this study is a superior predictor of progression to AD dementia compared to the individual biomarkers.

Schjønning (2016) retrospectively evaluated 348 subjects, including healthy controls, AD patients and diagnostically unresolved patients, which included patients with MCI or dementia of unknown etiology.[36] CSF was collected at the initial study and diagnoses were reevaluated at follow-up visits with at least one-year intervals. Aβ42 concentration and Aβ42/p-tau and T-tau x P-tau/Aβ42 ratios were significantly different between patients that clinically progressed and healthy controls. Additionally, Aβ42, T-tau, P-tau concentrations and Aβ42/P-tau and T-tau x P-tau/Aβ42 ratios were all significantly different between patients with progressive disease versus stable diagnostically unresolved patients. Limitations of this study include variability in follow-up periods and circularity due to the use of the CSF analyses in the initial diagnosis.

Alexopoulos (2016) examined how biomarker constellations predict progression risk of MCI patients.[37] They performed a multicenter study including 469 patients with MCI and compared T-tau, P-tau, and Aβ42 with progression to dementia. Patients with all biomarkers positive for AD (n=145) had the highest risk of progression to dementia due to AD and patients with no positive biomarkers (n=111) had the lowest risk. Patients with mixed biomarker results had significantly lower risk than patients with all biomarkers positive.

Mattsson (2015) studied whether Aβ positivity could be predicted in 35 healthy subjects with normal baseline Aβ42 levels.[38] The CSF Aβ42 levels declined in 11 subjects and the CSF became Aβ positive. Baseline CSF Aβ42 level was reported to be a strong predictor of future positivity (accuracy 79% [95% CI, 70% to 87%]), with 10 of the 11 subjects having baseline levels in the lower tertile of the reference range and 22 of the 24 nondecliners with levels in the upper two tertiles. A high CSF P-tau level was also associated with decline (accuracy 68%; 95% CI, 55% to 81%).

Richard (2013), found neither MRI nor CSF biomarkers improved classification of patients developing AD over a brief memory test in 181 ADNI patients with MCI.[39] The net reclassification improvement obtained by adding MRI results to the memory test was 1.1% and
for CSF Aβ42/P-tau -2.2%. The authors concluded that after administration of a brief test of memory, MRI or CSF do not substantially affect diagnostic accuracy for predicting progression to Alzheimer's disease in patients with MCI.

Schmand (2012) evaluated the value of neuropsychologic tests, neuroimaging, and biomarkers (Aβ and tau in CSF) for predicting the conversion to AD in 175 patients with MCI. With a mean follow-up of 2.7 years, 81 patients (46%) had converted to AD. Neuropsychologic assessment and MRI variables predicted conversion with 63% to 67% classification success both in patients younger and older than 75 years. CSF biomarkers correctly classified 64% of patients younger than 75 years and 60% of patients >75 years. The difference in prediction for the combined markers (70%) was not significantly better than the individual markers.

Okonkwo (2011) reported an association of Aβ42 abnormalities in CSF with increased rate of cognitive decline, disease progression, and risk of conversion to AD in 195 patients with MCI. This association was not found for tau abnormalities in CSF.

In the largest case series to date, Mattsson (2009) studied sensitivity, specificity, positive and negative likelihood ratios (LRs) of CSF Aβ42, and higher T-tau and P-tau for identifying incipient AD in patients with MCI. A total of 750 consecutive patients with MCI, 529 with AD, and 304 healthy controls were included in the study. Individuals with MCI were followed up for at least two years or until symptoms had progressed to clinical dementia. Reported sensitivity was 83% (95% CI, 78% to 88%), specificity 72% (95% CI, 68% to 76%), positive predictive value 62%, and negative predictive value 88%, which is less accurate than reported in prior smaller studies. While this was reported as good accuracy, the authors noted that this testing is not appropriate for routine clinical use because there is currently not a treatment that alters the development of AD. Therefore, early detection of risk would not impact treatment planning or health outcomes. Other limitations of the study included considerable variability in biomarker levels between the 12 centers participating in the study and short follow-up period. The authors also note that, “if these biomarkers are to be used throughout the world, external control programs that help laboratories harmonize their measurements with each other will be essential.”

Clinical Utility

Although not without controversy because of modest efficacy, cholinesterase inhibitors are used to treat mild-to-moderate Alzheimer’s disease. Memantine, an NMDA receptor antagonist, appears to provide a small benefit in those with moderate-to-advanced disease. Given available therapies, in principle more accurate diagnosis might allow targeting treatment to those most likely to benefit. However, clinical trial entry criteria and benefit have been based on clinical diagnosis. While the possibility that more accurate diagnosis might lead to improved outcomes is plausible, it is not based on current evidence. Pharmacologic interventions for MCI have not demonstrated benefit in reducing progression to Alzheimer’s disease.

Section Summary

The evidence for testing for AD-related CSF biomarkers for diagnosis in patients who have dementia or mild cognitive impairment consists of systematic reviews, meta-analyses and case series. However, most of the studies focus on select patient populations and define optimal test cutoffs without validation, thereby limiting generalizability. Further, there is limited existing evidence examining incremental diagnostic accuracy of CSF biomarkers for AD diagnosis employing autopsy as a referent standard. The evidence does not demonstrate improvement
over a clinical diagnosis, or whether diagnosis using CSF biomarkers would lead to improved net health outcomes. For predicting conversion from mild cognitive impairment (MCI) to AD, limited evidence suggests testing might define increased risk; however, further validation studies are needed. Whether earlier diagnosis leads to improved health outcomes through delay of AD onset or quality of life is also unknown.

**URINARY MARKER TESTING**

**Analytic Validity**

Levy (2007) described components of analytic validity for a competitive enzyme-linked immunosorbent assay (ELISA) format affinity assay to measure neural thread protein (NTP) in urine samples was found.[49] Seven-hundred-twenty replicates were assayed at four different clinical laboratories by four different trained personnel, on three different days each, consisting of high, medium, and low NTP urines in 20 replicates each per day. The CVs were reported to vary from 2.3% to 7.1% in high-NTP urine, 1.5% to 8.5% in medium-NTP urine, and 2.5% to 15% in low-NTP urine. Between and within laboratory variation was not given. Three lots of high-, medium-, and low-NTP controls were tested in four replicates each for three days. The CVs varied from 4.3 to 8.6%. Twenty replicates of low-NTP urine samples were spiked with known concentrations of NTP to 18.9, 23.9, 28.9, 33.9, and 38.9 mg/mL; mean recovery was 105.5%.

**Clinical Validity**

**Nonrandomized Studies**

Data have been limited on neural thread protein as a marker for AD and consist mainly of non-randomized observational studies. Examples of the current published literature include:

Zhang (2014) conducted a systematic review and meta-analysis of urinary AD–associated neural thread protein for diagnosing AD in patients with suspected AD.[50] Nine studies were included (total n=841 patients with probable or possible AD, 37 patients with MCI, 992 non-AD demented or nondemented controls). For probable AD, pooled sensitivity and specificity were 89% (95% CI 86 to 92) and 90% (95% CI 88 to 92), respectively. Pooled positive and negative likelihood ratios were 8.9 (95% CI 7.11 to 11.1) and 0.12 (95% CI 0.09 to 0.16), respectively.

In a prospective multicenter study conducted at eight sites, Goodman (2007) enrolled 168 patients with recent referral to memory clinics.[51] The urinary neural thread test was 91.4% sensitive for a diagnosis of probable AD (32/35) and 90.1% specific among healthy subjects. However, it was unclear whether the marker changed management or what the potential consequences of a 9.9% false-positive rate might be.

Kahle (2000) reported on the diagnostic potential of CSF levels of total tau protein and neural thread protein in a group of 35 patients with dementia (30 with probable or definite AD), five patients with Lewy body disease, 29 patients with Parkinson’s disease, and 16 elderly healthy control patients.[52] Levels of both tau and neural thread protein were elevated in patients with AD compared to controls—sensitivities and specificities for tau (63% and 93%) and neural thread protein (70% and 80%).

**Clinical Utility**
As above, there is no direct evidence or indirect chain of evidence to support the clinical utility of urinary markers for diagnosing AD.

Section Summary

At present, the diagnostic accuracy of neural thread protein for diagnosis of AD has not been established. Neither have studies of clinical utility been identified. Additional research on both diagnostic and clinical validity of this biomarker is needed before conclusions can be made about the effectiveness of its use.

SKIN CELL (FIBROBLAST) TESTING

Two small case-control studies have linked various characteristics of fibroblast cell cultures to AD. Chirila (2013) compared skin fibroblast samples between individuals diagnosed with AD (n=10), individuals diagnosed with Parkinson’s disease and/or Huntington disease (n=7) and age-matched non-demented controls (n=11), and reported significant differences in various markers of cell culture network formation, aggregation, and migration.[53] Chirila (2014) reported on differences in cellular aggregation measures between fibroblast samples from AD patients and non-demented controls in samples that overlapped substantially with the previous study (29/38).[54] It is not clear how the measures in these studies compare to the DISCERN™ test that is commercially available. No studies evaluating the performance of this test were identified.

PRACTICE GUIDELINE SUMMARY

Several clinical practice guidelines address the use of biomarkers in the diagnosis of Alzheimer’s disease (AD). Among those which are proponents of their use, support is conditioned on further study, or use within research settings alone.

AMERICAN ACADEMY OF NEUROLOGY

The American Academy of Neurology (AAN) does not address laboratory testing for the clinical evaluation of dementia in their Practice Parameter for the diagnosis of dementia, including AD.[55] In their practice guideline “Update: Mild Cognitive Impairment,” they state that “there are no biomarkers clearly shown to predict progression in patients with MCI.”

NATIONAL INSTITUTE ON AGING AND THE ALZHEIMER’S ASSOCIATION

Recommendations from the National Institute on Aging-Alzheimer's Association (NIA-AA) workgroup on diagnostic guidelines for Alzheimer's disease include a category entitled, “Probable AD dementia with evidence of the AD pathophysiological process.”[1] Evidence of the AD pathophysiologic process is supported by detection of low CSF Aβ-42, positive positron emission tomography (PET) amyloid imaging, or elevated CSF tau, and decreased 18-F fluorodeoxyglucose uptake on PET in the temporo-parietal cortex with accompanying atrophy by magnetic resonance imaging (MRI) in relevant structures. This recommendation is tempered by the following statement from the NIA-AA workgroup:

However, we do not advocate the use of AD biomarker tests for routine diagnostic purposes at the present time. There are several reasons for this limitation: 1) the core clinical criteria provide very good diagnostic accuracy and utility in most patients; 2) more research needs to be done to ensure that criteria that include the use of biomarkers have been appropriately designed, 3) there is limited standardization of
biomarkers from one locale to another, and 4) access to biomarkers is limited to varying degrees in community settings. Presently, the use of biomarkers to enhance certainty of AD pathophysiological process may be useful in three circumstances: investigational studies, clinical trials, and as optional clinical tools for use where available and when deemed appropriate by the clinician.

Therefore, although biomarkers are included in these guidelines for diagnosis, their use is not routinely recommended to aid in the diagnosis of probable AD.

AMERICAN PSYCHIATRIC ASSOCIATION

A 2007 guideline on the treatment of patients with AD and other dementias by the American Psychiatric Association (APA) workgroup on AD stated, “Except in rare circumstances (notably the use of CSF-14-3-3 protein when Creutzfeldt-Jakob disease is suspected and recent stroke or viral encephalitis can be excluded), these techniques remain investigational, and there is insufficient evidence for their utility in routine clinical practice.”

ALZHEIMER’S ASSOCIATION

In 2013, recommendations from the Alzheimer’s Association (AA) for operationalizing the detection of cognitive impairment during the Medicare annual wellness visit in primary care settings. The recommended algorithm for cognitive assessment was based on “current validated tools and commonly used rule-out assessments.” Guideline authors noted that use of biomarkers (e.g., CSF tau and β-amyloid proteins) “was not considered as these measures are not currently approved or widely available for clinical use.”[58]

SUMMARY

There is not enough research to show that testing for Alzheimer disease (AD)-related biomarkers improves health outcomes for people who have AD, dementia, or mild cognitive impairment (MCI). No clinical guidelines based on research recommend the use of AD biomarkers. Therefore, the use of cerebral spinal fluid, urinary, and skin cell biomarkers for diagnosis of AD, or for prediction of conversion from MCI to AD, is considered investigational.

REFERENCES


Schjonning Nielsen, M, Simonsen, AH, Siersma, V, Hasselbalch, SG, Hogh, P. Are CSF Biomarkers Useful as Prognostic Indicators in Diagnostically Unresolved Cognitively Impaired Patients in a Normal Clinical Setting. *Dementia and geriatric cognitive disorders extra.* 2016 Sep-Dec;6(3):465-76. PMID: 27843444


### CODES

**NOTE:** Some of the following CPT codes may be used to identify the steps in testing for tau protein and amyloid beta peptides. There are no specific codes used for testing for neural thread protein.

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<td>0207U</td>
<td>Neurology (Alzheimer disease); quantitative imaging of phosphorylated ERK1 and ERK2 in response to bradykinin treatment by in situ immunofluorescence,</td>
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using cultured skin fibroblasts, reported as a probability index for Alzheimer disease (List separately in addition to code for primary procedure)

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**Date of Origin**: October 1999