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# CSF Total tau, Aβ42 and Phosphorylated tau Protein as Biomarkers for Alzheimer's Disease

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## Abstract

With the arrival of effective symptomatic treatments and the promise of drugs that may delay progression, we now need to identify Alzheimer's disease (AD) at an early stage of the disease. To diagnose AD earlier and more accurately, attention has been directed toward peripheral biochemical markers. This article reviews promising potential cerebrospinal fluid (CSF) biomarkers for AD focussing on their role in clinical diagnosis. In particular, two biochemical markers, CSF total tau (t-tau) protein and the 42 amino acid form of β-amyloid (Aβ42), perform satisfactorily enough to achieve a role in the clinical diagnostic settings of patients with dementia together with the cumulative information from basic clinical work-up, genetic screening, and brain imaging. These CSF markers are particularly useful to discriminate early or incipient AD from age-associated memory impairment, depression, and some secondary dementias. In order to discriminate AD from other primary dementia disorders, however, more accurate and specific markers are needed. Preliminary evidence strongly suggests that quantification of tau phosphorylated at specific sites in CSF improves early detection, differential diagnosis, and tracking of disease progression in AD.

**Index Entries:** Alzheimer's disease (AD), β-amyloid (Aβ), tau, phosphorylated tau, biochemical markers, cerebrospinal fluid (CSF), diagnosis.

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## Introduction

Early and accurate diagnosis of Alzheimer's disease (AD) is important, particularly after the introduction of acetylcholine esterase (AChE) inhibitors for effective symptomatic treatment of AD. Moreover, a number of promising drugs are currently under development that may have beneficial effects on the disease process, e.g. γ-secretase inhibitors and β-amyloid (Aβ) vaccination. These options for specific therapeutic intervention further highlight the importance of identifying and diagnosing AD.

Current criteria for the clinical diagnosis of AD are largely based on the exclusion of other dementing disorders *(1).* Although a relatively high accuracy rate of 80–90% applying clinical criteria are reported *(2–4),* these studies emanate from specialized expert research academic centers. Data are mostly based on diagnoses in patients in later stages of the disease who were followed up longitudinally before autopsy. Diagnostic accuracy is probably considerably lower in general hospitals and particularly in the earlier clinical, as well as preclinical, stages of the disease when specific symptoms lack or are often vague and indistinct. Because pharmacological therapy is, however, probably most effective early in the course of disease, early diagnosis is highly desirable before neurodegeneration is too severe and widespread. Thus, there is a great need for biomarkers that could substantially aid early diagnosis of AD.

Criteria for an ideal biomarker of AD have been proposed by the consensus group on molecular and biochemical markers of AD *(5).* A biomarker for AD should detect a fundamental feature of neuropathology and be validated in neuropathologically confirmed cases, and it should have a sensitivity >80% for detecting AD and a specificity >80% for distinguishing other dementias. Further, a biomarker should also be reliable, reproducible, noninvasive, simple to perform, and inexpensive.

Because the cerebrospinal fluid (CSF) is in direct contact with the extracellular space of the brain, biochemical changes in the brain are reflected in the CSF. AD pathology is restricted to the brain. Therefore, CSF is an obvious source of biomarkers for AD. Biochemical markers for AD should reflect the central pathogenic processes of the disorder, i.e., neuronal degeneration, disturbance in the metabolism of β-amyloid (Aβ) and its subsequent deposition in senile plaques (SP), as well as hyperphosphorylation of tau with subsequent formation of neurofibrillary tangles (NFT). Suggested biomarkers for these pathogenic processes are, respectively, total tau protein (t-tau), Aβ42, and tau phosphorylated at AD-specific epitopes (p-tau).

## CSF Biomarkers for AD

Potential CSF biomarkers for the central pathogenic processes in AD have been extensively studied. In the following section of this article, we review these papers focusing on presenting the overall diagnostic potential of the biomarkers.

### *CSF Total-tau in the Differential Diagnosis of AD*

Tau is a microtubule-associated protein located in the neuronal axons. There are six different isoforms, and numerous phosphorylation sites of tau in the human brain *(6).* Using monoclonal antibodies that detect all isoforms of tau independent of phosphorylation, enzyme-linked immunosorbent assays (ELISA) have been developed that measure the "total" tau levels in CSF *(7–9).*

An increase in CSF t-tau in AD compared to elderly controls has consistently been found, with a high sensitivity and specificity (*see* Table 1). Around 2000 AD patients and 1000 controls have been investigated so far (*see* Table 1). The mean degree of increase in AD compared with elderly controls approaches 300%.

The potential of CSF t-tau to discriminate AD from relevant other dementia disorders is, however, limited. At a sensitivity level of 81%, CSF

Table 1
CSF Total tau (t-tau) to Discriminate AD Patients from Elderly Controls[a]

| Assay specificity | Number of AD cases | Mean sensitivity (%) | Mean change in AD (%) | Number of controls | Mean specificity (%) | References |
|---|---|---|---|---|---|---|
| t-tau (phospho-independent) | 1758 | 82 | 320 | 784 | 86 | *(7,8,10–12, 14–18,22, 23,26,32, 33,51–61)* |
| t-tau (phospho-independent) | 250 | 55 | 197 | 167 | 92 | *(9,21,62–64)* |
| t-tau (phospho-independent, repeat sequence) | 14 | 100 | 216 | 36 | 94 | *(65)* |
| Total | 2022 | | | 987 | | |

[a] Sensitivity and specificity figures were given in papers, or were determined from scatterplots. The mean sensitivity was calculated as: (total positive AD cases/total studied AD cases). The mean specificity was calculated as: (total negative control cases/total studied control cases). Abbreviations: AD, Alzheimer's disease.

t-tau yields a specificity level of 57% to distinguish other dementias *(10).* Increased CSF t-tau is found in a proportion of patients with relevant differential diagnoses of AD as well. In vascular dementia (VAD) elevated CSF t-tau has been found in a relatively high proportion of cases in some studies *(8,11–13),* or only occasionally in other studies *(14,15).* The same is true for frontotemporal dementia (FTD) and Lewy-body dementia (LBD). Some authors reported CSF t-tau levels equal to AD in FTD and LBD, respectively *(16–20),* whereas others found no increase compared to controls *(9,10,18,21–23).* An elevation of CSF t-tau has further been shown in normal pressure hydrocephalus *(24).* In contrast, patients with alcoholic dementia and chronic neurological disorders (e.g., Parkinson's disease [PD], progressive supranuclear palsy [PSP]) show elevated CSF t-tau levels only occasionally *(8,18,25–27).*

The level of CSF t-tau probably reflects the degree of neuronal degeneration and damage *(8).* This suggestion is supported by the findings that a marked transient increase in CSF t-tau is found after acute stroke, with a positive correlation between CSF t-tau and infarct size measured by cranial computerized tomography *(28).* Furthermore, the degree of increase in CSF t-tau is higher in disorders with more extensive and/or rapid neuronal degeneration. A very marked increase is found in Creutzfeldt-Jakob disease (CJD), along with rapid clinical progression and neurodegeneration *(29,30);* a moderate/marked increase is found in AD, with widespread neurodegeneration *(8–10),* while normal levels are found in patients with PD, with rather limited degeneration *(22).*

A highly relevant differential diagnosis of AD particularly in mild to moderate dementia is depression since clinical symptoms may overlap *(31).* With regard to therapy, however, a correct diagnosis is essential. Therefore, CSF t-tau has been investigated as a potential tool to differentiate AD from depression. Andreasen and coworkers *(32)* studied a relatively young group (mean age 49 yr) of patients with depression and dysthymia who did not develop dementia within 1 yr follow-up. CSF-t-tau was not different from cognitively normal controls. In an age-matched sample, however, it has been shown that dis-

Table 2
CSF Phosphorylated tau (p-tau) to Discriminate AD Patients from Elderly Controls[a]

| Assay specificity | Number of AD cases | Mean sensitivity (%) | Mean change in AD (%) | Number of controls | Mean specificity (%) | References | Comments |
|---|---|---|---|---|---|---|---|
| p-tau$_{181 + 231}$ | 44 | 88 | 348 | 31 | 97 | *(8)* | |
| p-tau$_{231 + 235}$ | 36 | 53 | n.g. | 20 | 100 | *(60)* | "Non-AD" controls |
| p-tau$_{199}$ | 36 | 94 | n.g. | 20 | 80 | *(60)* | "Non-AD" controls |
| p-tau$_{231}$ | 27 | 85 | n.g. | 31 | 97 | *(66)* | "Non-AD" controls |
| p-tau$_{181}$ | 41 | 44 | 148 | 17 | 94 | *(67)* | |
| Total | 184 | | | 119 | | | |

[a] Sensitivity and specificity figures were given in papers, or were determined from scatterplots. Abbreviations: AD, Alzheimer's disease; n.g., not given.

criminative power of CSF t-tau between AD and major depression is affected by age *(33)*. Subgrouping a sample of AD-patients, healthy controls and patients with major depression according to age led to a correct classification rate of 94.5% in the "young old" subjects (<70 yr of age) compared to only 68.4% in the "old old" (≥70 yr of age). Thus, elevated CSF t-tau in patients younger than 70 yr of age strongly points out a neurodegenerative disorder.

### *Phosphorylated tau in the CSF*

Using monoclonal antibodies (MAbs) specific for phosphorylated epitopes of tau, ELISAs have been developed that measure levels of phosphorylated tau-protein. An increase in CSF p-tau in AD has been found using assays specific for several different phosphorylated epitopes (*see* Table 2). Today, four different studies have been published, with varying data on diagnostic sensitivity and specificity, and also varying degrees of increased concentrations in CSF of AD patients compared with controls (*see* Table 2).

Data on the specificity of CSF p-tau is still sparse. CSF tau-protein phosphorylated at threonine 181 (p-tau$_{181}$), however, is normal in VAD and in FTD *(61)*. It has been shown that CSF tau-protein phosphorylated at threonine 231 (p-tau$_{231}$) is superior to t-tau in differentiating AD from its most relevant differential diagnoses, i.e., FTD, VD, and LBD *(34)*.

Further, after acute stroke, there is a marked increase in CSF t-tau, while CSF p-tau$_{181}$ does not change *(35)*. This finding suggests that CSF p-tau$_{181}$ is not simply a marker for neuronal damage, as CSF t-tau, but specifically reflects PHF/NFT pathophysiology.

Another line of recent evidence suggests that CSF p-tau$_{231}$ declines during the natural course of AD *(36)*. Seventeen pharmacologically untreated patients with probable AD were followed up over 6 yr with repeated serial CSF measurements. CSF p-tau$_{231}$ concentrations, but not of t-tau, decreased over time in AD independent of age. Rate of change of p-tau$_{231}$ was inversely correlated with the Mini-Mental State Examination (MMSE) score at baseline. These results suggest that CSF p-tau$_{231}$ may have the potential to track AD progression and may be a valuable tool to map effects of disease modifying drugs on AD specific neurodegeneration. International multicenter efforts are currently underway to further explore the value of p-tau in early and differential diagnosis, as well as in tracking disease progression.

Table 3
CSF β-Amyloid (Aβ42) to Discriminate AD Patients from Elderly Controls[a]

| Assay specificity | Number of AD cases | Mean sensitivity (%) | Mean change in AD (%) | Number of controls | Mean specificity (%) | References | Comments |
|---|---|---|---|---|---|---|---|
| Aβ 1-42 | 562 | 85 | 56 | 273 | 84 | *(10,22,23,38–40, 59,68)* | |
| Aβ x-42 | 119 | 85 | 58 | 80 | 82 | *(21,64)* | |
| Aβ 1-42 | 113 | n.g. | 48 | 88 | n.g. | *(56,69)* | |
| Aβ x-42 | 80 | n.g. | 161 | 74 | n.g. | *(37)* | |
| Aβ x-42 | 39 | 64 | 43 | 11 | 91 | *(70)* | Aβ42 deter mined by Western-blot analysis |
| Aβ x-42 | 36 | n.g. | 32 | 32 | n.g. | *(71)* | |
| Total | 949 | | | 558 | | | |

[a] Sensitivity and specificity figures were given in papers, or were determined from scatterplots. The mean sensitivity was calculated as: (total positive AD cases/total studied AD cases). The mean specificity was calculated as: (total negative control cases/total studied control cases). Abbreviations: AD, Alzheimer's disease: n.g., not given.

### *CSF Aβ42*

Several different assays have been developed that are specific to Aβ42, with minimal cross-reactivity against peptides ending at residues 43 or shorter peptides. The most consistent finding is a marked (≈50% of control levels) decrease in Aβ42 in AD. Aβ42 alone showed a sensitivity of 78% and a specificity of 81% to distinguish AD from elderly controls *(10).* Several other studies using different assays also investigated Aβ42 in AD (*see* Table 3). However, one study found an increase in Aβ42 in AD *(37),* which may be due to methodological differences (e.g., assay specificity for mono- vs oligomers) or differences in patient and control materials. Indeed, in that study, increased CSF-Aβ42 was also found in patients with depression *(37),* while two other studies showed normal Aβ42 CSF-levels in depression *(22,38).*

Data on the ability of CSF-Aβ42 to distinguish AD from other dementias and neurological disorders is relatively limited. Low levels are also found in LBD *(23,39),* a disorder also characterized by the presence of senile plaques. Furthermore, low CSF-Aβ42 is found in a relatively large percentage of patients with FTD and vascular dementia *(10,22).*

The reduction in CSF-Aβ42 in AD has been hypothesized to reflect a deposition of the peptide in SP, with lower levels diffusing to the CSF. A marked reduction in CSF-Aβ42, however, is also found in CJD, also in cases without Aβ positive plaques *(30,40),* and in Amyotrophic Lateral Sclerosis (ALS) *(40a),* a disorder without Aβ positive plaques. These findings question the putative relationship between low CSF-Aβ42 and the formation of SP.

## CSF Biomarkers in Early AD

Several studies have found high CSF t-tau, and/or low CSF-Aβ42 in early AD, i.e., in AD patients with MMSE scores *(41)* above 23–25 (*see* Table 4). The potential of CSF t-tau to discriminate between AD even in mild dementia and normal aging is high, with a mean sensitivity of about 75% and specificity of about 85%.

Table 4
CSF t-tau, Aβ42, and p-tau in Early AD and MCI[a]

| Biomarker | Criteria | Number of cases | Mean sensitivity (%) | References | Comment |
|---|---|---|---|---|---|
| **t-tau** | Early AD, MMSE >25 | 11 | 91 | *(54)* | Approximate sensitivity |
| | Early AD, MMSE >20 | 36 | 81 | *(63)* | |
| | Early AD, MMSE >25 | 12 | 75 | *(63)* | |
| | Early AD, MMSE >25 | 19 | 89 | *(57)* | |
| | Early AD, MMSE >23 | 205 | 94 | *(32)* | |
| | MCI, mean MMSE = 25 | 10 | 90 | *(43)* | MCI cases with progression |
| **Aβ42** | Early AD, MMSE >25 | 24 | 88 | *(38)* | Approximate sensitivity |
| | Early AD, MMSE >25 | 25 | n.g. | *(68)* | Sensitivity not given. Decrease to 63% of controls. |
| **t-tau + Aβ42** | Early AD, MMSE >23 | 24 | 62 | *(64)* | |
| | Early AD, MMSE >23 | 23 | 70 | *(10)* | |
| | MCI, MMSE >28 | 16 | 88 | *(72)* | MCI cases with progression. |
| | MCI, MMSE >28 | 20 | 75 | *(39)* | MCI cases with and without progression. |
| **p-tau$_{181}$** | MCI, MMSE >28 | 15 | 40 | *(44)* | |
| **p-tau$_{231 + 235}$** | MCI, MMSE 19–27 | 20 | 65 | *(45)* | MCI cases with low MMSE scores, in the range of early AD cases. |

[a] Sensitivity and specificity figures were given in papers, or were determined from scatterplots. Abbreviations: AD, Alzheimer's disease; MCI, mild cognitive impairment; MMSE Mini-Mental State Examination; n.g., not given.

It has been shown that patients with mild cognitive impairment (MCI) are at higher risk to develop dementia and therefore are at risk of AD. A conversion rate to dementia of about 40% within 3 yr was suggested *(42).* Some studies addressed the question whether CSF biomarkers could detect incipient AD in MCI patients. Interestingly, high CSF t-tau discriminated memory impaired patients that later progressed to AD from those that did not convert with 90% sensitivity and 100% specificity *(43).* A high sensitivity for the combination of high CSF t-tau and low CSF-Aβ42 to predict the progression from MCI to AD with clinical dementia has also been found (*see* Table 4). One study on CSF p-tau$_{181}$ in MCI cases with MMSE scores above 28 also found an increase, although sensitivity was relatively low *(44).* In another study on CSF p-tau$_{231 + 235}$ in MCI cases, the sensitivity was 65% *(45),* but the MMSE scores of the MCI patients in that study were low, in the range seen in patients with early AD. These findings show that these CSF markers have the potential to detect the disease process before the development of clinically overt dementia.

## CSF Biomarkers in Clinical Practice

Most studies on CSF biomarkers for AD have been performed in research settings, with highly selected patients samples and CSF analyses run simultaneously, i.e., under conditions providing data on optimal assay performance. Two studies have been conducted on prospective samples of AD patients *(32)* or on all patients admitted for investigation of dementia symptoms during 1 yr *(39).* In these studies, the patients have been examined con-

tinuously, and CSF samples taken that were sent directly to the laboratory and assayed the following week in clinical neurochemical routine. These studies may give figures closer to the true performance of CSF t-tau and CSF Aβ42. The analytical variation (examined by re-analyzing CSF samples previously run on multiple occasions during 1 yr) for these CSF analyses were adequate. Also in these studies, the ability of CSF t-tau *(32)* and the combination of CSF t-tau and CSF-Aβ42 *(39)* to differentiate AD from normal aging, depression, and PD was high, while the specificity compared other dementias, especially VAD, was poorer.

CSF markers may not be ideal biomarkers since spinal taps often are avoided because of fear of complications, particularly post-lumbar puncture headache (PLPH). Yet, the incidence of PLPH is very low in older patients admitted for evaluation of dementia, around 2–4%, in most cases with only minimal discomfort *(39,46).* Thus spinal taps are generally safe procedure under trained neurologists or geriatricians.

In daily practice CSF studies are most often performed on clinically diagnosed patients. Although the positive predictive value for the clinical diagnosis of AD (i.e., the probability that AD is present when the criteria are met) has been relatively high, at about 85%, the negative predictive value (i.e., the probability that AD is not present when the diagnostic criteria are not met) are considerably lower *(2–4,47).* This is particularly troublesome for some of the non-AD dementias (e.g., vascular dementia and frontotemporal dementia). In fact, neuropathological studies have found that high proportions (40–80%) of clinically diagnosed patients with vascular dementia have notable concomitant AD pathology *(3,47).* Thus, in clinically diagnosed patient samples, it seems difficult to get high specificity figures for CSF biomarkers. Further, even if they are asymptomatic, age-matched control subjects may harbor presymptomatic AD lesions in their brains *(48–50),* which also reduces the sensitivity figures for CSF biomarkers for AD.

Much effort has focused on finding a single neurochemical marker for AD. This may be elusive unless the marker is related to a pathogenic step that is unique to AD. For example, neuronal degeneration is not only found in AD but also in most chronic degenerative disorders of the brain. Consequently, increased CSF t-tau is not specific for AD. Similarly, deposition of Aβ is not specific to AD, but is also found in normal aging and LBD, and consequently, reduced CSF-Aβ42 is not specific to AD. Therefore, combination of several CSF biochemical markers may increase specificity. For example, at a sensitivity level of 85%, the combined test yielded a specificity of 86% to discriminate AD from elderly controls. At the same sensitivity level, specificity to distinguish non-AD dementias was 58% *(10).* Combinations of t-tau with other CSF markers, e.g., neuronal thread protein (NTP), *(51)* or the soluble interleukin-6 (IL-6) receptor complex (sIL-6RC) *(52)* have been investigated as well. These studies suggest that not only the combination of t-tau and Aβ42, but also the addition of other CSF markers, may further increase the sensitivity and specificity.

Further, the overall accuracy of the clinical diagnosis of AD may increase if the diagnosis is based on cumulative information gained from the clinical examination, brain-imaging techniques (e.g., single photon emission tomography [SPECT] and magnetic resonance tomography [MRT] scans), and CSF biochemical markers. As an analogy, the clinical diagnosis of myocardial infarction is based on the combination of clinical examination, electrocardiogram, and biochemical markers (e.g., creatine kinase).

Commercially available bioassays for the CSF markers t-tau and Aβ42 have already reached clinical value as adjuncts to clinical diagnosis, in differentiating AD from some problematic differential diagnoses, particularly age-associated memory impairment, depressive pseudodementia, PD, and alcoholic dementia.

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