

CSF markers for incipient Alzheimer's disease

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Early diagnosis of Alzheimer's disease (AD) is needed to initiate symptomatic treatment with acetylcholinesterase inhibitors, and will be of even greater significance if drugs aimed at slowing down the degenerative process, such as vaccination regimes and β -secretase and γ -secretase inhibitors, prove to affect AD pathology and to have clinical effect. However, there is no clinical method to determine in which patients mild cognitive impairment (MCI) will progress to AD with dementia, and in which patients MCI is benign. Hence, there is a great clinical need for biomarkers to identify incipient AD in patients with MCI. The CSF biomarkers total tau protein, phosphorylated tau protein, and the 42 amino-acid residue form of amyloid- β may, if put in the right clinical context, prove to have high enough diagnostic accuracy to meet this challenge.

Lancet Neurol 2003; **2**: 605–13

Mild cognitive impairment (MCI) is an age-related syndrome that has gained increasing attention in the research community and also in the general population. MCI is characterised by memory impairment that can be verified by objective measures adjusted for age and education in the absence of dementia.¹

MCI is aetiologically heterogeneous, and many patients with the disorder have incipient Alzheimer's disease (AD), whereas others have a benign form of MCI. Cerebrovascular disease may also contribute to memory impairment but can be identified by the presence of cerebrovascular risk factors (eg, hypertension) and findings of vascular pathology (eg, infarcts, white-matter lesions) by use of brain-imaging techniques. The definition and pathophysiology of MCI were recently reviewed in *The Lancet Neurology*.²

In many cases, MCI is a transition between normal ageing and AD, with a conversion rate reaching as high as 15% a year.² Research efforts have been directed to find methods to identify in which patients MCI will progress to AD and in which patients it will not. In this paper, we review the potential of CSF analyses as diagnostic markers for AD, specifically their usefulness in the identification of incipient AD, and their role in the diagnosis of MCI in clinical practice. We also give suggestions for how to use CSF markers in clinical practice.

Diagnostic markers

The introduction of effective symptomatic treatment of AD with acetylcholinesterase inhibitors has highlighted the importance of early and accurate diagnosis of AD. However, the most commonly used criteria for the clinical diagnosis of

AD was outlined almost 20 years ago by the National Institute of Neurological and Disorders and Stroke and the Alzheimer Disease and Related Disorders (NINCDS-ADRDA) Work Group.³ These criteria largely depend on the exclusion of other dementias. Although the NINCDS-ADRDA criteria have been reported to have a high accuracy rate (80–90%),^{4,5} studies of the diagnostic accuracy come from specialised expert research academic centres, and most data are from patients in later stages of the disease who were studied for several years before death and autopsy. The clinical diagnostic accuracy is probably lower in patients in the earlier stages of AD, especially in those with MCI, when specific symptoms other than memory disturbances are absent or indistinct.

The increasing knowledge and awareness of possible drug treatments for AD have made patients seek medical advice at an earlier stage of the disease. Many patients do not present with characteristic symptoms of moderate to severe AD, but have only mild memory disturbances without dementia. There is no clinical method to determine which patients will progress to AD with dementia, except for an extended clinical follow-up. Thus, there is a great clinical need for biomarkers to identify incipient AD in patients with MCI. This need will be much larger if new drugs to slow or stop the degenerative process (eg, vaccination regimes, β -secretase and γ -secretase inhibitors, and β -sheet breakers) prove to have a beneficial clinical effect. Such compounds are probably most effective early in the disease course, before neurodegeneration is too severe and widespread.

State and stage markers for AD

The degenerative process in AD probably starts 20–30 years before the clinical onset of the disease.⁶ During this preclinical phase of AD, plaque and tangle loads increase, and at a certain threshold the first symptoms appear. There are no data to suggest that the intensity of the disease process (ie, the rate of neuronal degeneration and plaque and tangle formation) changes during the disease process. Diagnostic markers for AD can be divided into two groups: state markers and stage markers.

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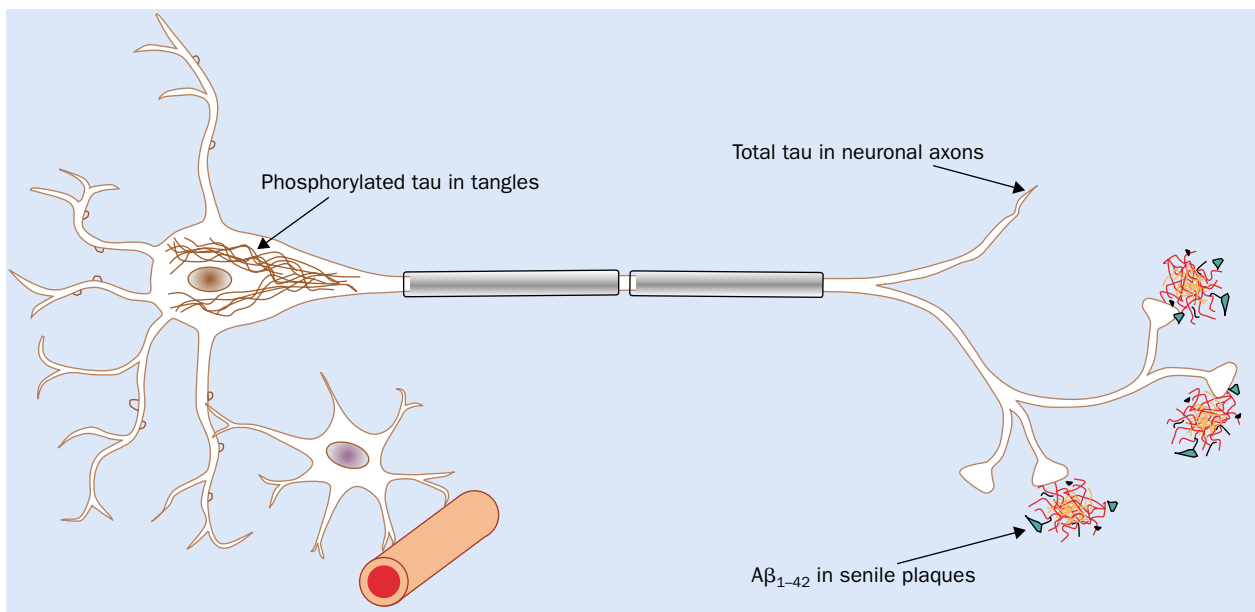


Figure 1. Schematic drawing of a neuron with an adjacent astrocyte and capillary. The central pathogenetic processes in AD and their corresponding biochemical markers are depicted. Total concentration of tau protein is a marker of neuronal and axonal degeneration, $A\beta_{1-42}$ concentration is a marker of plaque formation, and concentration of phosphorylated tau is a marker for hyperphosphorylation of tau and formation of tangles.

State markers

State markers reflect the intensity of the disease process. One example of a state marker is the total amount of tau protein. The total concentration of tau protein in the CSF probably indicates the intensity of the neuronal damage and degeneration. In acute disorders (such as ischaemic stroke) there is a transient increase in tau protein concentration in the CSF that correlates with infarct size measured by CT.⁷ Furthermore, the degree of increase in tau protein concentrations in the CSF is highest in disorders with the most intense neuronal degeneration, such as Creutzfeldt-Jakob disease,⁸ whereas a moderate increase is found in AD, in which the degeneration is less intense, and a normal concentration is found in patients with depression and no or limited degeneration.⁹

Stage markers

Stage markers give a measure of how far the degenerative process has proceeded. An example of a stage marker is atrophy of the hippocampus as measured with CT or MRI. Clinical rating scales (which measure the stage of the cognitive decline) are also stage markers.

Because the disease process starts several years before the onset of the first symptoms, both state and stage markers are probably positive in the early phase of AD. Indeed, patients with MCI have both high concentrations of tau protein in their CSF and hippocampal atrophy.¹⁰ However, the increase in tau protein concentrations is larger (210–290% increase)^{11–15} than the degree of hippocampal atrophy (9–15% reduction of hippocampal volume).¹⁰ State markers, such as tau protein, probably have a larger potential than stage markers, such as hippocampal atrophy on CT or MRI, as prognostic markers to identify incipient AD in patients with MCI.

CSF markers for AD

The CSF is in direct contact with the extracellular space of the brain, and hence biochemical changes in the brain affect the CSF. Because AD pathology is restricted to the brain, CSF is an obvious source of biomarkers for AD. Biochemical markers for AD should reflect the central pathogenetic processes (ie, the neuronal degeneration, the deposition of amyloid- β peptide [$A\beta$] in plaques, and the hyperphosphorylation of tau with subsequent formation of tangles; figure 1). Possible biomarkers for these pathogenetic processes are the concentrations in the CSF of total tau protein, the 42 amino acid form of $A\beta$ ($A\beta_{1-42}$), and phosphorylated tau protein.

Tau protein

Tau protein is located in the neuronal axons. There are six different isoforms and numerous phosphorylation sites of tau protein in the human brain (figure 2). The first report on tau protein concentration in the CSF as a biomarker for AD—in which ELISA with a polyclonal antibody was used—was published in 1993.¹⁶ After this, ELISA methods based on monoclonal antibodies that detect all isoforms of tau protein, independent of their phosphorylation, have been developed (figure 2).^{9,17}

The concentration of tau protein in the CSF probably reflects the intensity of neuronal degeneration in chronic neurodegenerative disorders.

$A\beta_{1-42}$

$A\beta$ is the major component of plaques.¹⁸ It is a proteolytic cleavage product from the amyloid precursor protein: APP is cleaved by β -secretase to release a large N-terminal derivative called β -sAPP, which is cleaved by γ -secretase to release free $A\beta$.

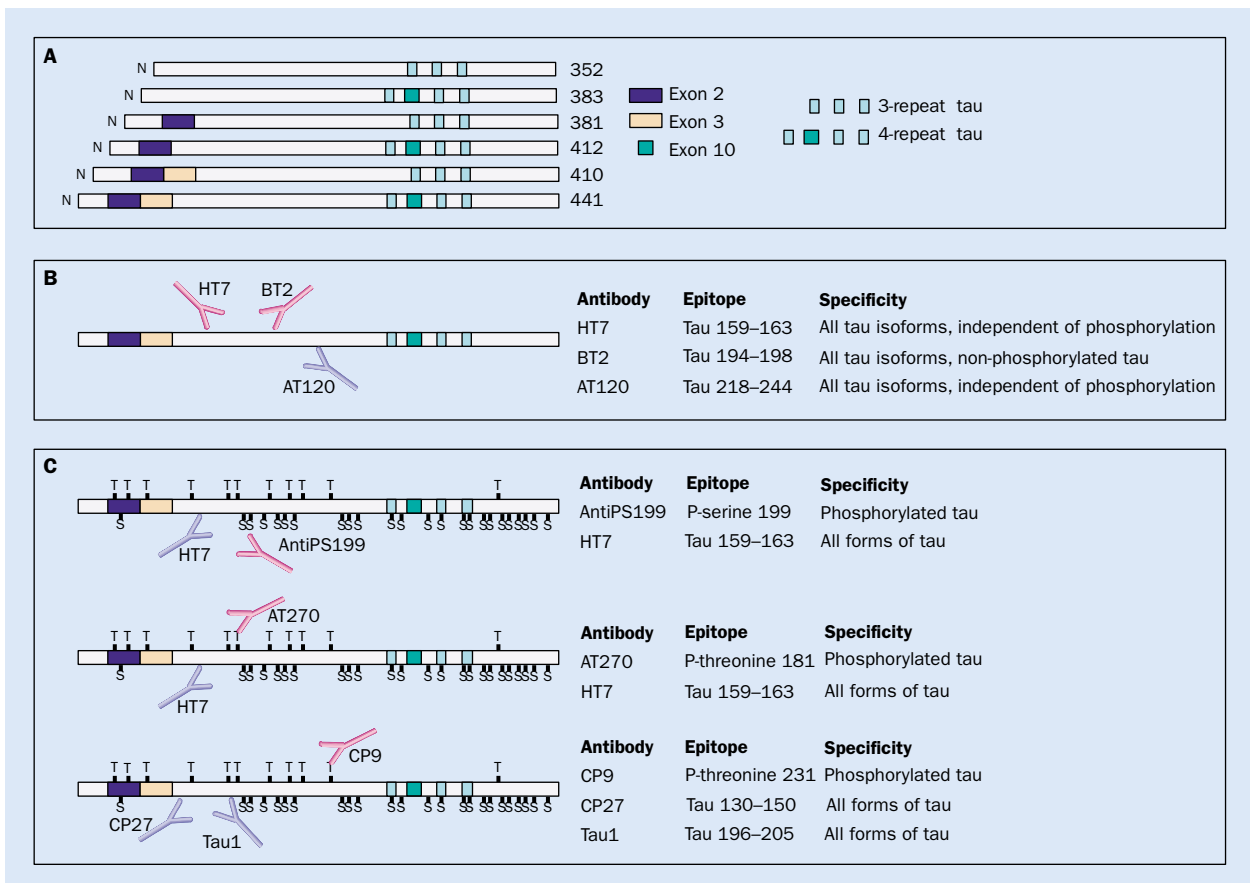


Figure 2. The six isoforms of human tau protein, with alternatively spliced exons marked (A). The smallest contains 352 amino-acid residues and three repeat (microtubule-binding) domains. The largest tau isoform has 441 residues (τ_{441}) and four repeat domains with exon 10 spliced in and two extra domains from exons 2 and 3. Schematic drawing of an ELISA for total tau (B).⁹ The antibodies were selected to react with tau irrespective of phosphorylation state, and are specific for epitopes outside the alternatively spliced exons. Thus, ELISA recognises all forms of tau. Three ELISAs specific for phosphorylated tau (C). Phosphorylation sites on τ_{441} , either threonine (T) or serine (S), are marked. All ELISAs are based on the combination of an antibody that recognises all isoforms of tau irrespective of phosphorylation state together with an antibody specific to different phosphorylated amino acids. Top: ELISA specific for the serine 199 isoform of phosphorylated tau protein.³¹ Middle: ELISA specific for the threonine 181 isoform.³⁰ Bottom: ELISA specific for P-threonine 231.³²

The first reports on CSF A β as a biomarker for AD were disappointing, and results ranged from a slight decrease in AD to no change.^{19–21} In these studies total A β in the CSF was measured. After research showed that there are two major C-terminal variants of A β , with either 40 (A β_{1-40}) or 42 (A β_{1-42}) amino-acid residues, and that A β_{1-42} has the highest tendency for aggregation and is the quickest A β to form plaques,^{22,23} ELISA methods were developed with antibodies specific for this form (figure 3).^{24,25}

The decrease in concentrations of A β_{1-42} in the CSF was first thought to be caused by a deposition of the peptide in plaques.²⁵ However, later studies showed a substantial decrease in CSF A β_{1-42} in disorders without A β plaques, such as Creutzfeldt-Jakob disease,²⁶ amyotrophic lateral sclerosis,²⁷ and multiple system atrophy.²⁸ Although a recent population-based autopsy study found a strong association between low concentrations of A β_{1-42} in the CSF and high numbers of plaques in the neocortex and hippocampus.²⁹ Therefore, the decrease in CSF A β_{1-42} in AD may be due, at least in part, to deposition of A β in plaques.

Phosphorylated tau protein

The most recently discovered biomarker for AD is phosphorylated tau protein. Several ELISA methods have been developed for different phosphorylated epitopes of tau protein—including threonine 181 and 231,⁹ threonine 181,³⁰ threonine 231 and serine 235,³¹ serine 199,³¹ threonine 231,³² and serine 396 and 404.³³

The concentration of phosphorylated tau protein in the CSF probably reflects the phosphorylation state of tau in the brain. In contrast to the total concentration of tau protein, there is no change in the concentration of phosphorylated tau protein after acute stroke.³⁴ Furthermore, despite a very pronounced increase in total tau protein concentrations in Creutzfeldt-Jakob disease, there is no increase in the concentration of the phosphorylated protein.³⁵ These findings suggest that the concentration of phosphorylated tau protein in the CSF is not simply a marker for neuronal damage, like total tau protein, but that it specifically reflects the phosphorylation state of tau and, thus, the formation of tangles in AD.

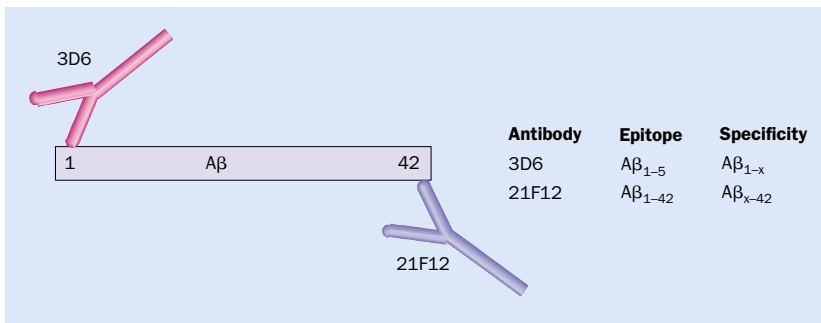


Figure 3. ELISA specific for Aβ₁₋₄₂. The ELISA does not cross-react with either of Aβ₁₋₄₀ or Aβ₁₋₄₉ or with N-terminally truncated Aβ peptides.²⁴

Specificity and sensitivity of CSF markers for AD Tau protein

An increase in the total concentration of tau protein in the CSF has been found in many studies of AD; concentrations are about three times higher in patients with AD than in control individuals. For the most commonly used method, the “Innogenetics ELISA”,⁹ sensitivity and specificity figures are available from 36 different studies, which include about 2500 AD patients and 1400 controls (figure 4).^{9,12,24,27,30,33,36-66} The specificity of this assay is 90% and the mean sensitivity to AD is 81% (figure 4). In the five studies with the “Athena” assay,¹⁷ the mean sensitivity was lower, with similar specificity (figure 4).^{17,25,67-69}

Importantly, normal total concentrations of tau protein in the CSF are found in several important differential diagnoses—including depression, alcoholic dementia, and chronic neurological disorders, such as Parkinson’s disease and progressive supranuclear palsy.^{9,36,40,47,60,61}

The specificity for tau protein is not optimum, because high concentrations are also found in some other dementia

disorders. In vascular dementia, a high concentration of tau protein has been found in a large percentage of patients in some studies,^{9,46,61} although in other studies the concentration of tau protein was raised in only a few patients.^{17,36,52} High total tau protein concentrations in clinically diagnosed vascular dementia may be caused by the presence of concomitant AD pathology, which is a common finding at autopsy.^{70,71} The same explanation was also suggested in a CSF study with longitudinal MRI in patients with vascular dementia.⁶¹ A mild to moderate increase in the total concentration of tau protein in the CSF has also been found in frontotemporal dementia in some,^{9,44} but not in all,^{30,52} studies.

Aβ₁₋₄₂

In patients with AD, a decrease in CSF concentration of Aβ₁₋₄₂ to about 50% of that in control individuals has been recorded. For the most commonly used method,^{24,72} the “Innogenetics ELISA”, sensitivity and specificity figures are available from 13 different studies, which include about 600 patients with AD and 450 control individuals (figure 5).^{24,26,27,46,47,50,54,57-59,63,72,73} With specificity at 90%, the mean sensitivity of CSF Aβ₁₋₄₂ to discriminate between AD and normal ageing is 86%. In the two studies with the “Athena” assay,²⁵ sensitivity and specificity figures were similar (figure 5).^{25,68}

There are not many data on the ability of CSF Aβ₁₋₄₂ to distinguish AD from other dementias and neurological disorders. Normal CSF Aβ₁₋₄₂ concentrations are found in psychiatric disorders such as depression and in chronic

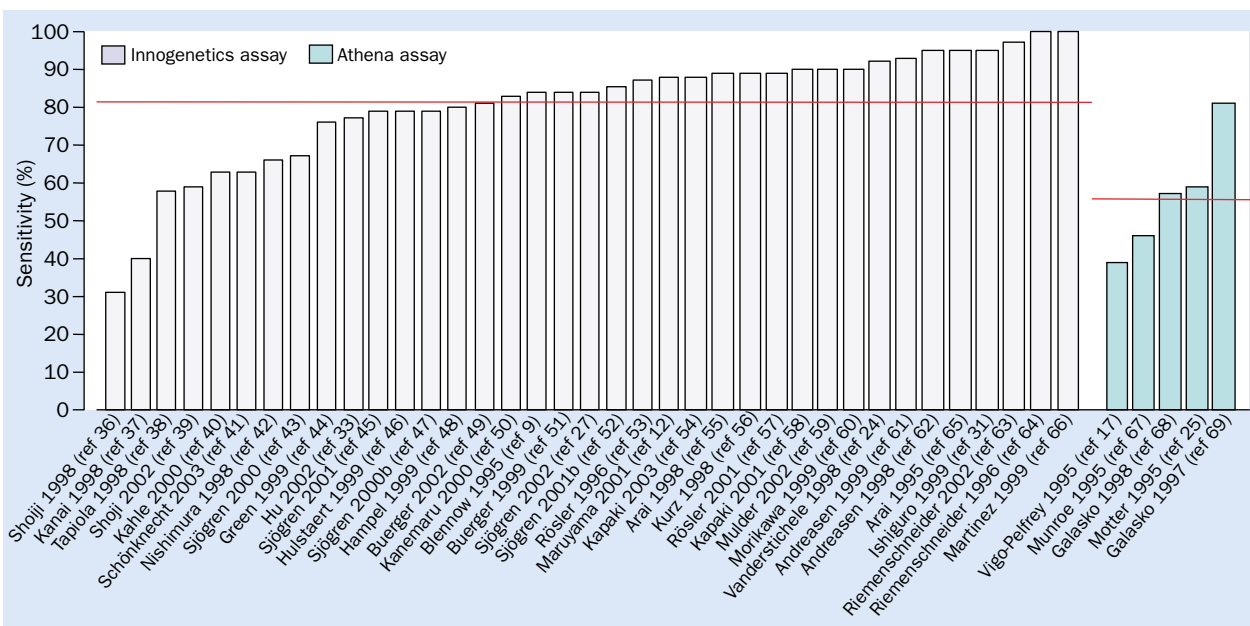


Figure 4. CSF total tau (T-tau) as a diagnostic marker for AD. Bars represent sensitivity figures for the two most commonly used ELISAs, the Innogenetics ELISA⁹ and the Athena ELISA.¹⁷ Red lines represent the mean sensitivity for AD versus controls. Reference number, first author, and year of publication are given for each study.

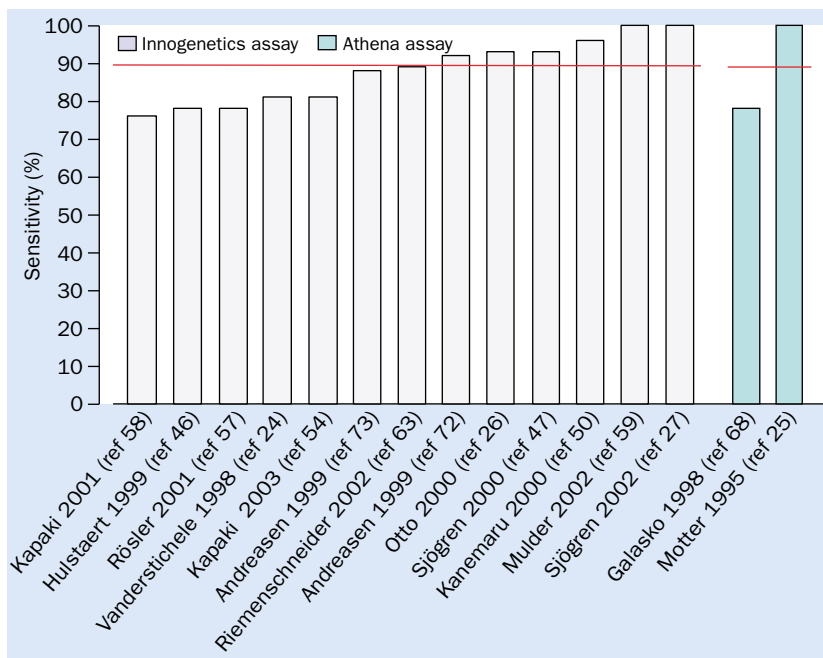


Figure 5. CSF A β_{1-42} in the diagnosis of Alzheimer's disease. Bars represent sensitivity figures for the two most commonly used ELISAs, the Innogenetics ELISA^{24,72} and the Athena ELISA.²⁵ Red lines represent the mean sensitivity for AD versus controls.

neurological disorders such as Parkinson's disease and progressive supranuclear palsy.^{27,28,47,50} However, the specificity of CSF A β_{1-42} for AD is not optimum, because low concentrations are also found in a proportion of cases with other dementia disorders. Moderately low concentrations are also found in Lewy-body dementia,^{50,72} a disorder also characterised by senile plaques, but a mild to moderate decrease in CSF A β_{1-42} is also found in some patients with frontotemporal and vascular dementias.^{27,46,47}

Phosphorylated tau protein

Six different ELISA methods showed a high concentration of phosphorylated tau protein in the CSF of patients with AD.^{9,30-33} 11 different studies of sensitivity and specificity have been published, which include about 800 patients with AD and 370 control individuals (figure 6). At a specificity of 92%, the mean sensitivity of assessment of phosphorylated tau protein to discriminate between AD and normal ageing is 80% (figure 6). Sensitivity varies widely among studies both with different and the same ELISA methods. Thus, further studies are needed to determine if there is a difference in sensitivity and specificity figures for the different phosphorylated tau protein methods. The phosphorylated-tau protein epitopes

threonine 181, serine 199, and threonine 231 have similar diagnostic results, with sensitivity figures of about 85% (unpublished).

Importantly, the specificity of CSF phosphorylated tau protein for AD is higher than for total tau protein and A β_{1-42} . High concentrations of tau protein have only been shown in patients with AD, and normal concentrations have been found in patients with psychiatric disorders such as depression;^{9,76} in chronic neurological disorders such as amyotrophic lateral sclerosis and Parkinson's disease,^{9,27,52} and in vascular, frontotemporal, and Lewy-body dementias.^{27,30,49,52,74,75} Thus, phosphorylated tau protein will increase the specificity of CSF biomarkers in the discrimination between AD and other dementias.

Use of CSF markers to identify incipient AD

Several studies have examined the performance of CSF markers in patients with AD with mild dementia (Mini-Mental State Examination⁷⁷ >23; figure 7) and high concentrations of total and phosphorylated tau protein, and low concentrations of CSF A β_{1-42} are present, with sensitivity figures similar to those found in later stages of the disease (figure 7).

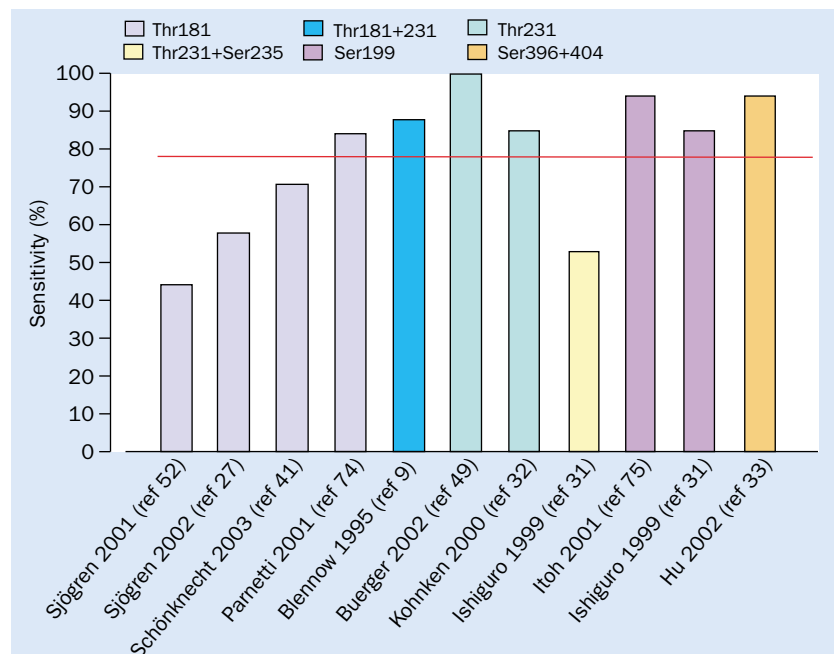


Figure 6. CSF phosphorylated tau in the diagnosis of Alzheimer's disease. Bars represent sensitivity figures for the ELISAs, including the phosphorylation epitopes threonine 181,³⁰ threonine 181 and 231,⁹ threonine 231,³² threonine 231 and serine 235,³¹ serine 199,³¹ and serine 396 and 404.³³ The red line represents the mean sensitivity for AD versus controls.

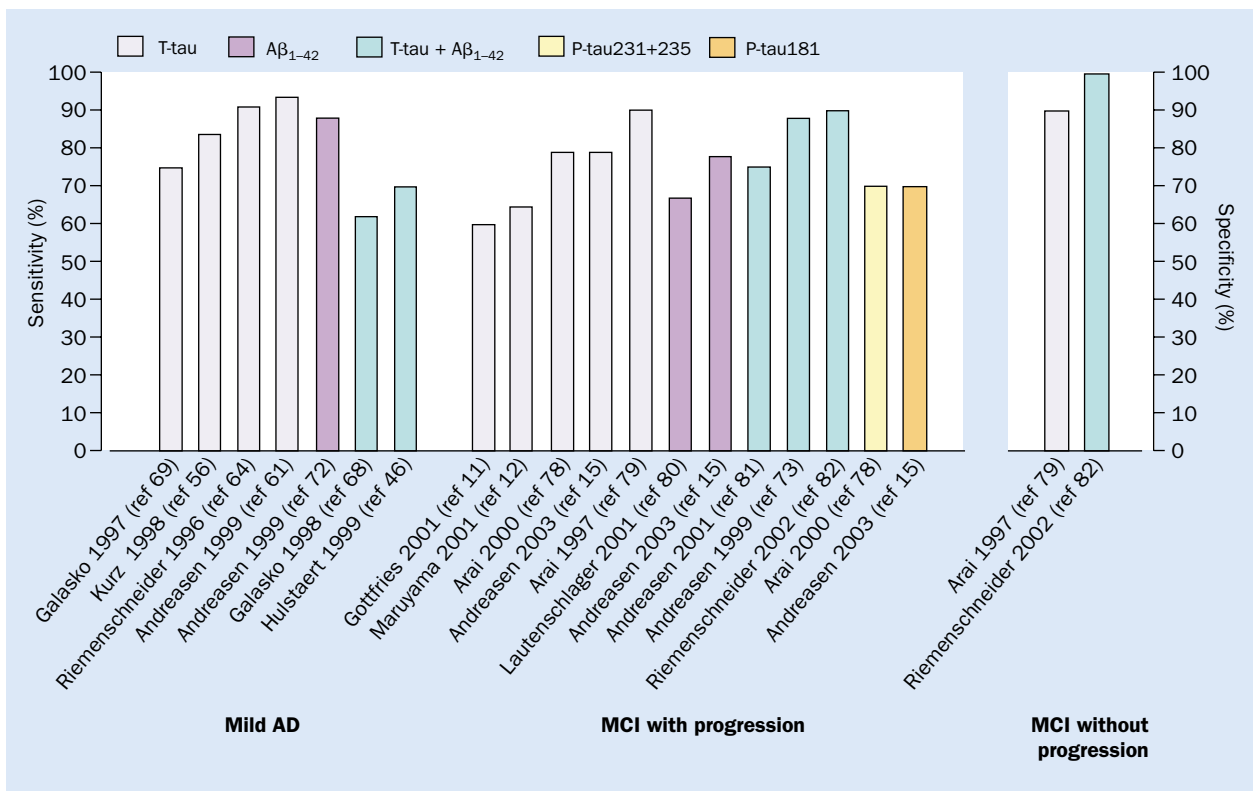


Figure 7. Sensitivity figures for CSF markers in the diagnosis of mild AD with mini-mental state examination score⁷⁷ above 23, MCI with progression to AD and specificity figures for MCI with progression to AD.

Several groups of researchers have examined patients with MCI who developed AD during a clinical follow-up period of 1–2 years. All studies have found high total and phosphorylated tau protein and low CSF concentrations of Aβ₁₋₄₂ already in this early phase of AD, with sensitivity figures similar to, or slightly lower than, those found in AD cases with dementia (figure 7).

A pitfall with the studies of CSF markers in patients with MCI is that because only around 15% of MCI cases progress to AD each year,² a very extensive follow-up period (>5 years) is needed to be certain that patients with MCI have stable disorder and do not develop dementia. In the first study with this approach, a high total concentration of tau protein in the CSF was found to discriminate patients with MCI that later progressed to AD from those that did not progress, with 90% sensitivity and 100% specificity.⁷⁹ Another study also found high total concentrations of tau protein and low concentrations of Aβ₁₋₄₂ in 90% of patients with MCI that later progressed to AD with dementia, compared with 10% of patients with stable MCI.⁸² Similarly, a recent study also found a substantially higher concentration of phosphorylated tau protein in patients with MCI that progressed to AD than in those with stable MCI.¹³ Taken together, these studies suggest that CSF markers are positive very early in the disease process in AD, and may be of clinical value to differentiate MCI cases with incipient AD—which will progress to AD—from benign MCI cases.

Limitations of CSF studies in clinically diagnosed patients

Almost all data on the diagnostic capacity of CSF markers come from studies of clinically diagnosed patients. This introduces a risk of circular evidence—ie, the diagnostic performance of CSF markers cannot be higher than the accuracy of the clinical diagnostic criteria used. Some patients who fulfil the NINCDS-ADRDA criteria³ for AD have other disorders. Further, there is an overlap between AD and other dementias. Neuropathological studies have shown that a high proportion (40–80%) of clinically diagnosed patients with vascular dementia have notable concomitant AD pathology,^{5,71} and that there is a large overlap between AD and Lewy-body dementia.⁸³

Asymptomatic elderly people may have presymptomatic AD lesions in their brains;⁸⁴ therefore, extensive follow-up is needed to account for those individuals who have preclinical AD. These limitations with clinical CSF studies make it difficult to judge whether suboptimal sensitivity and specificity figures are caused by shortcomings of the CSF markers or by the patients and controls studied. Indeed, although the differences did not reach statistical significance, AD cases confirmed by autopsy showed a tendency for lower concentrations of Aβ₁₋₄₂ than clinically diagnosed cases (170 pg/mL vs 187 pg/mL) and a trend for higher concentrations of total tau was also found (677 pg/mL vs 559 pg/mL), even after exclusion of five misdiagnosed patients.⁸⁵

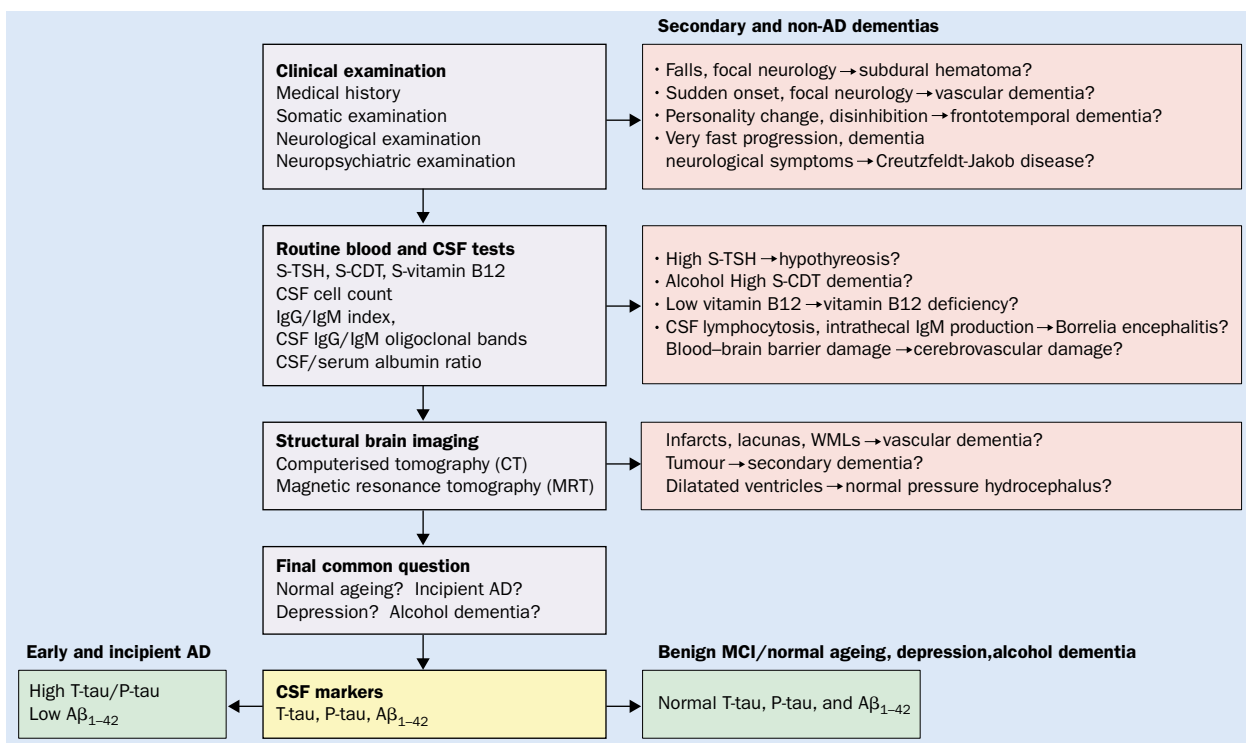


Figure 8. Flow chart for the position of CSF markers in the clinical assessment of patients with MCI and early AD. The suggested investigation is simplified and the secondary and non-AD dementias are only examples, intended to show the position of CSF markers in the clinical investigation. TSH=thyroid stimulating hormone, CDT=carbohydrate deficient transferrin.

Another limitation in the interpretation of the performance of CSF markers is the lack of standardisation of these CSF assays. Concentrations of the CSF markers have been found to vary among studies from different centres with the same ELISA method and among studies with different methods. For example, in studies with the same ELISA method,⁹ there is a 354% variation (from 106 pg/mL⁶⁶ to 375 pg/mL) variation in total concentrations of tau in the CSF between the control groups.⁴³ Thus, future availability of external control CSF samples for standardisation of methods will be a prerequisite for direct comparisons of results from different academic centres.

CSF markers in clinical practice

Complications after lumbar puncture

Lumbar puncture is often avoided because of fear of post-lumbar-puncture headache. However, the incidence of this headache is strictly age-related and less common in individuals over 60 years of age than in younger individuals.⁸⁶ Furthermore, the incidence of post-lumbar-puncture headache is even lower (<2%) in patients admitted for assessment of cognitive symptoms, most of whom have only minimal discomfort.^{81,87} Thus, a spinal tap in the geriatric populations is a safe procedure when done by a trained physician.

Analysis and confounding factors

There are several preanalytical and biological confounding factors (ie, factors that may influence the analytical outcome) that need to be accounted for before a CSF marker can be introduced in clinical routine. The only preanalytical

confounding factor is that both tau protein and Aβ have a tendency to stick to the walls of test tubes made of glass and hard plastic, which results in falsely low measurements.⁷² Therefore, CSF should be drained into non-absorbent test tubes made of polypropylene. The CSF sample can be sent to the laboratory either at room temperature or at 4°C, and storage for up to 3 days does not influence concentrations of these proteins. In the laboratory, all CSF samples are frozen before assay. Reproducibility of ELISA results are confirmed by use of two internal controls, stored at -80°C, on each ELISA plate. The analytical variation for these ELISA methods (10–15%) is acceptable.⁷²

A biological factor that has to be taken into account is variation in CSF concentrations of biomarkers with disease progression. Large longitudinal studies on total tau protein and Aβ₁₋₄₂ show no significant change at 1–2 year follow-up.^{61,72} Similarly, there is no significant change in concentrations of either total tau, Aβ₁₋₄₂, or threonine-181 phosphorylated tau in longitudinal studies of patients with MCI that progress to AD with dementia.^{15,73} In contrast, in a 6 year longitudinal study, phosphorylated tau epitope threonine 231 concentration decreased linearly with time in patients with AD,⁸⁸ and a 1 year longitudinal study of MCI showed progressive increase in the concentration of this epitope in patients with MCI at follow-up.⁸⁹

Clinical routine

Although many studies have assessed the diagnostic potential of these CSF markers, almost all have been done in research settings with selected patient samples and CSF analyses run on

Search strategy and selection criteria

Data were identified by searches on MEDLINE and references from relevant articles. The search terms “Alzheimer”, “CSF”, and either “tau” or “amyloid” were used. CSF markers that have been assessed in more than ten studies, by independent research groups using different methods, were considered. Only studies that include sensitivity and specificity figures, or in which such figures could be calculated from graphs, were included. Studies were excluded if they included mixed control groups, did not report CSF proteins in concentration units (ie, mass or mole per volume CSF), or included less than ten patients with AD. Only papers published in English were reviewed.

one occasion (ie, under conditions providing figures on the optimum capability of the markers). Two studies have, however, been done in prospective patient samples, with ELISA assays run each week in clinical neurochemical routine. Also, in these studies the ability of total tau and the combination of total tau and $A\beta_{1-42}$ to differentiate AD from normal ageing, depression, and Parkinson’s disease was high, with sensitivity figures similar to, or higher than, other studies.^{62,81} However, the specificity against other dementias was less than optimum.

As in other areas of medicine, CSF markers for AD should not be used as isolated tests. A possible analogy is myocardial infarction, for which the clinical diagnosis is based on the combined information from the clinical examination, electrocardiogram, and biomarkers (eg, creatine kinase and troponin T). Likewise, the clinical diagnosis of AD should be based on cumulative information from the clinical examination, brain-imaging techniques (eg, single photon emission CT and MRI), and CSF biochemical markers.

Patients with cognitive disturbances are now seeking medical advice at a very early stage, in many cases when mild memory impairment is the only objective symptom. In these cases there is no clinical method to determine which patients

will progress to AD and which patients will not. Biomarkers may be included in the assessment of these patients to help identify incipient AD (figure 8). After the clinical examination and standard auxiliary investigations, secondary dementias (eg, hypothyreosis and subdural haematoma) and also dementias with differing history, symptoms, and findings on brain imaging (eg, frontotemporal or vascular dementia) can be identified. A common final question is whether a patient with MCI has incipient AD or benign MCI. Other possible differential diagnoses that may be hard to differentiate from incipient AD are depression and variants thereof, and alcohol-related cognitive dysfunction. Although more studies are needed to determine the diagnostic capability of CSF markers to identify incipient and early AD, we suggest that CSF markers have a great clinical potential to help to resolve this diagnostic challenge. Early diagnosis of AD is of importance to be able to initiate symptomatic treatment with acetylcholinesterase inhibitors, and ongoing studies will tell us whether these drugs are also effective in patients with MCI or incipient AD. Furthermore, identification of incipient AD will be the basis for initiation of treatment with drugs that might slow or stop the degenerative process, such as inhibitors of β -secretase and γ -secretase.

Authors' contributions

Both authors contributed equally to this review.

Conflict of interest

We have no conflicts of interest.

Role of the funding source

This work was supported by grants from the Sahlgrenska Academy at Göteborg University, Sweden (KB), the Medical Faculty of the Ludwig-Maximilian University, Munich, Germany (HH), the Volkswagen Foundation, Hannover, Germany (HH), the Hirnliga e. V. Nürnberg, Germany (HH), and by the German Competence Network on Dementias funded by the German Federal Department of Education and Research, Germany (HH). None of these funding sources had any role in the preparation of this review or in the decision to submit it for publication.

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