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Cerebrospinal fluid biomarkers for Alzheimer's disease: their role in Clinical Chemistry

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In this article

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SUMMARY

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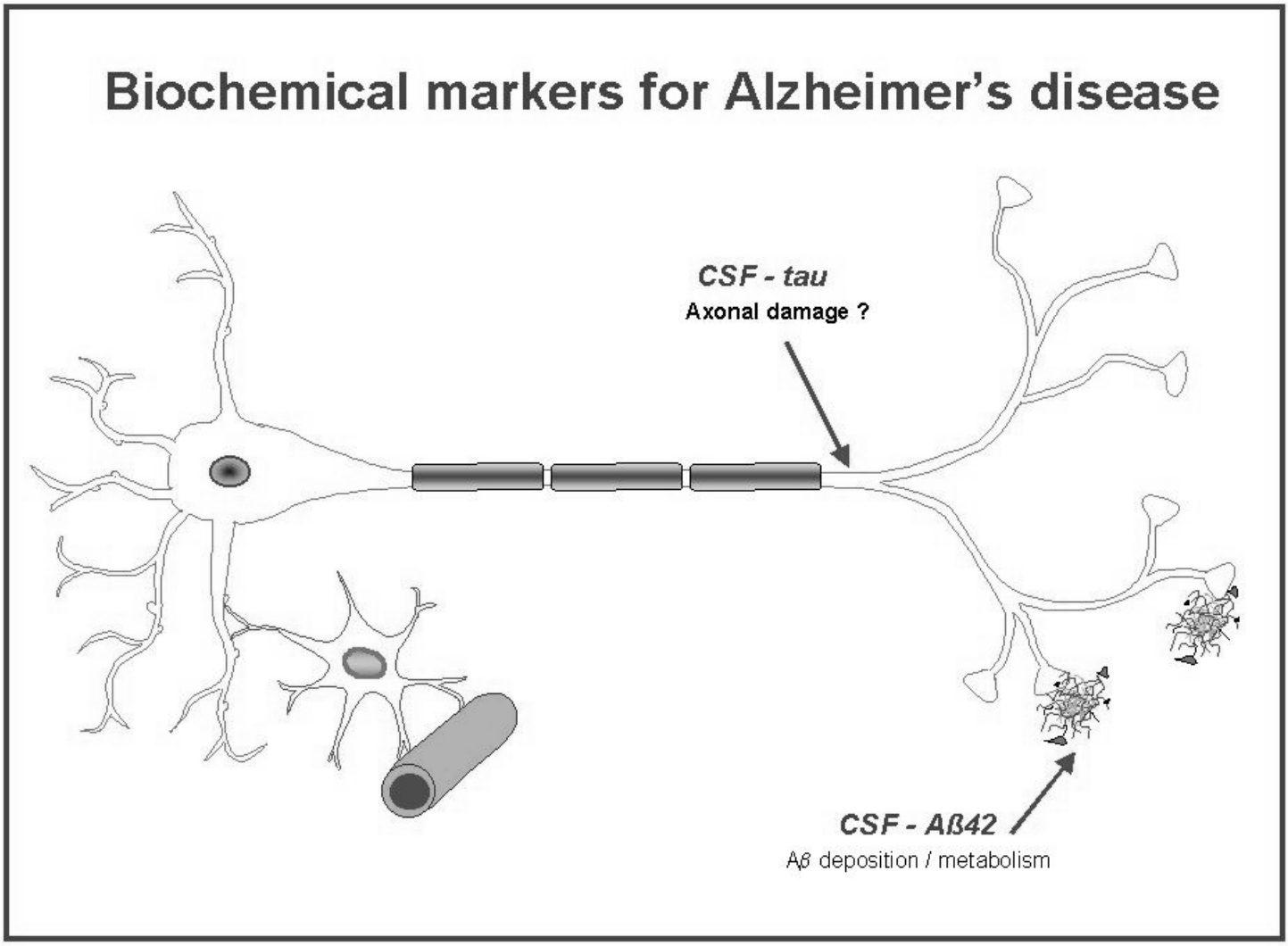
ABSTRACT

In view of current (AChE inhibitors) and future (e.g. anti-Aβ aggregators), development and evaluation of cerebrospinal fluid (CSF) biomarkers for Alzheimer's disease (AD) has become a rapidly growing research field. Diagnostic biomarkers for AD would be especially valuable as aids in the diagnosis early in the course of the disease, when correct diagnosis is difficult, and when therapeutic compounds have the greatest potential of being effective. This paper reviews CSF biomarkers for AD, with emphasis on their role in the clinical diagnosis, and methodological aspects of importance for developing such analyses. Today, two biochemical markers, CSF-tau and CSF-Aβ42, perform satisfactory enough to have a role in the clinical work-up of patients dementia, if used together with the cumulative information from clinical information and brain-imaging techniques. These markers are especially useful to discriminate early or incipient AD from age-associated memory impairment, depression, and some secondary dementias.

INTRODUCTION

Alzheimer's disease (AD) is the major cause of dementia in the elderly. Although rare genetic (autosomal dominant) forms of AD exist, most patients have no obvious family history and are classified as having sporadic AD. The neuropathology of AD shows neuronal and synaptic degeneration, and an increased number of senile plaques (SP) and neurofibrillary tangles (NFT) compared to non-demented individuals of comparable age. Synaptic degeneration in AD is found in widespread cortical areas (Masliah et al, 1991). SP are composed of a central core of aggregated β-amyloid (Aβ) (Masters et al, 1985), a breakdown product derived from the amyloid precursor protein (APP) (Kang et al, 1987). NFT are insoluble intracellular thread-like structures made up of a hyperphosphorylated form of the microtubule-associated protein tau, called phospho-tau (Goedert et al, 1993).

Today, several acetylcholine esterase inhibitors are available for symptomatic treatment of AD. Drugs that also may have beneficial effects on the disease process, e.g. compounds affecting the deposition of Aβ, are under development. These possibilities for therapeutic intervention have heightened awareness of the impor-



tance of early and accurate diagnosis of AD.

However, current clinical criteria for the diagnosis of AD are relatively vague, and are largely based on the exclusion of other dementing illnesses (McKhann et al, 1984). A relatively high accuracy rate with regard to the clinical diagnosis of AD (80-90%) has been reported (Tierney *et al.* 1988; Jellinger 1996; Galasko *et al.* 1994). However, these reports emanate from expert research academic centers and are often based on patients in the later stages of the disease who were followed for several years before the confirming autopsy. The diagnostic accuracy rate is probably considerably lower in general hospitals, and especially in the earlier stages of the disease when the symptoms are often silent or indistinct and clinical diagnosis is more difficult. This is unfortunate, as pharmaceutical therapy is probably most effective early in the course of disease, before neurodegeneration is too severe and widespread. Thus, there is a great need for biochemical diagnostic markers (biomarkers) that could aid in the diagnosis of AD early in the course of the disease.

The cerebrospinal fluid (CSF) is in direct contact with the extracellular space of the brain, and its constituents reflect many biochemical changes in the brain. Since AD pathology is restricted to the brain, CSF is an obvious source of biomarkers for AD. During the last years, CSF biomarkers for AD have also gained increased attention. In this paper, we review the two CSF biomarkers that have been most extensively studied by different research groups and have proved to have the highest clinical diagnostic potential, i.e. CSF-tau and CSF-Aβ42 (Fig. 1). We also focus on neurochemical factors of importance for making these biomarkers useful in clinical chemistry.

Besides their diagnostic potential, CSF biomarkers may also be useful to monitor the biochemical effect of therapeutic compounds. In AD, a drug that slows or ar-

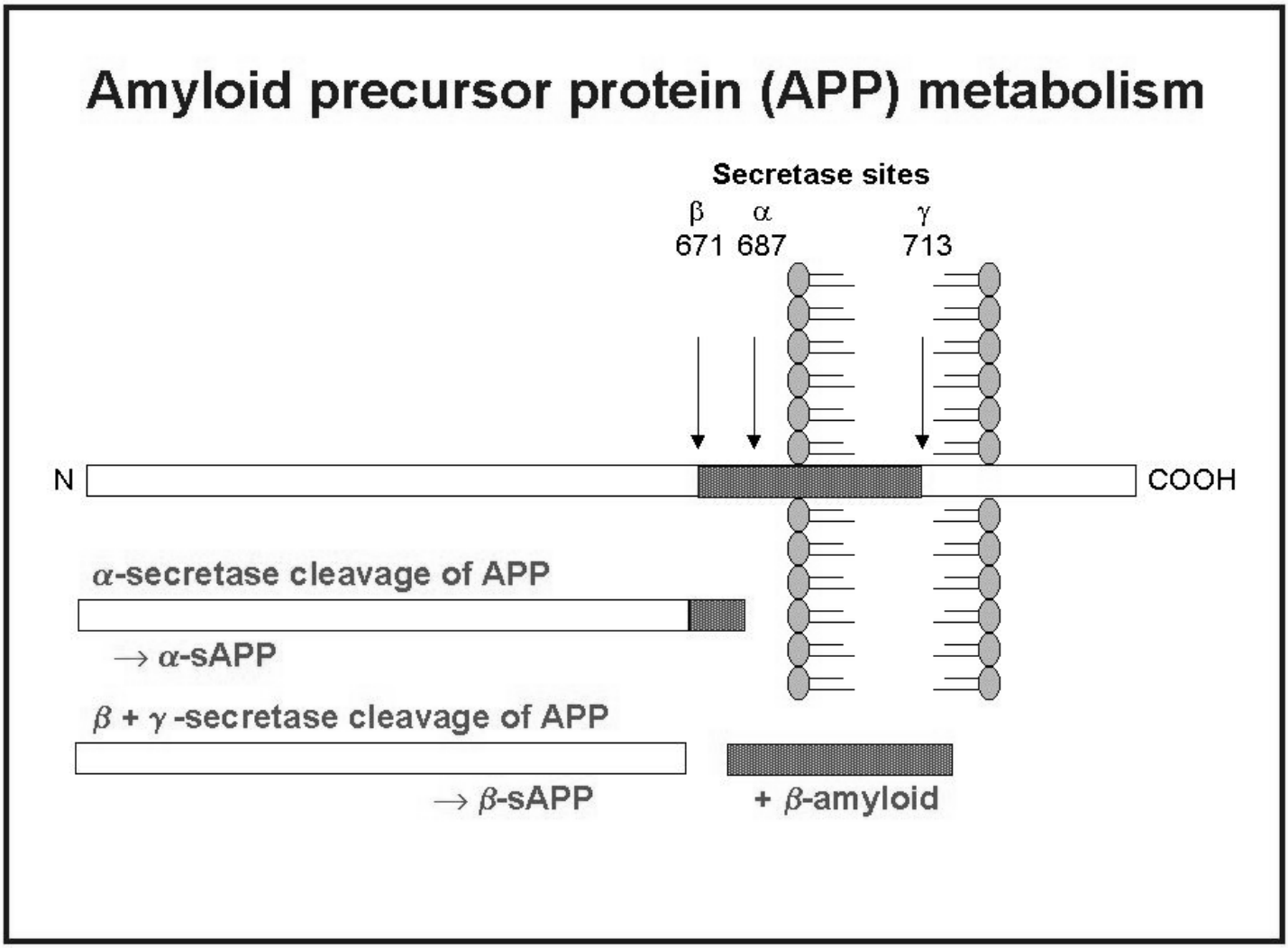
rests neurodegeneration might lower the level of a marker of active neuronal damage, such as CSF-tau. Similarly, a drug that acts by decreasing the deposition of Aβ might increase the level of CSF Aβ42 in repeated samples.

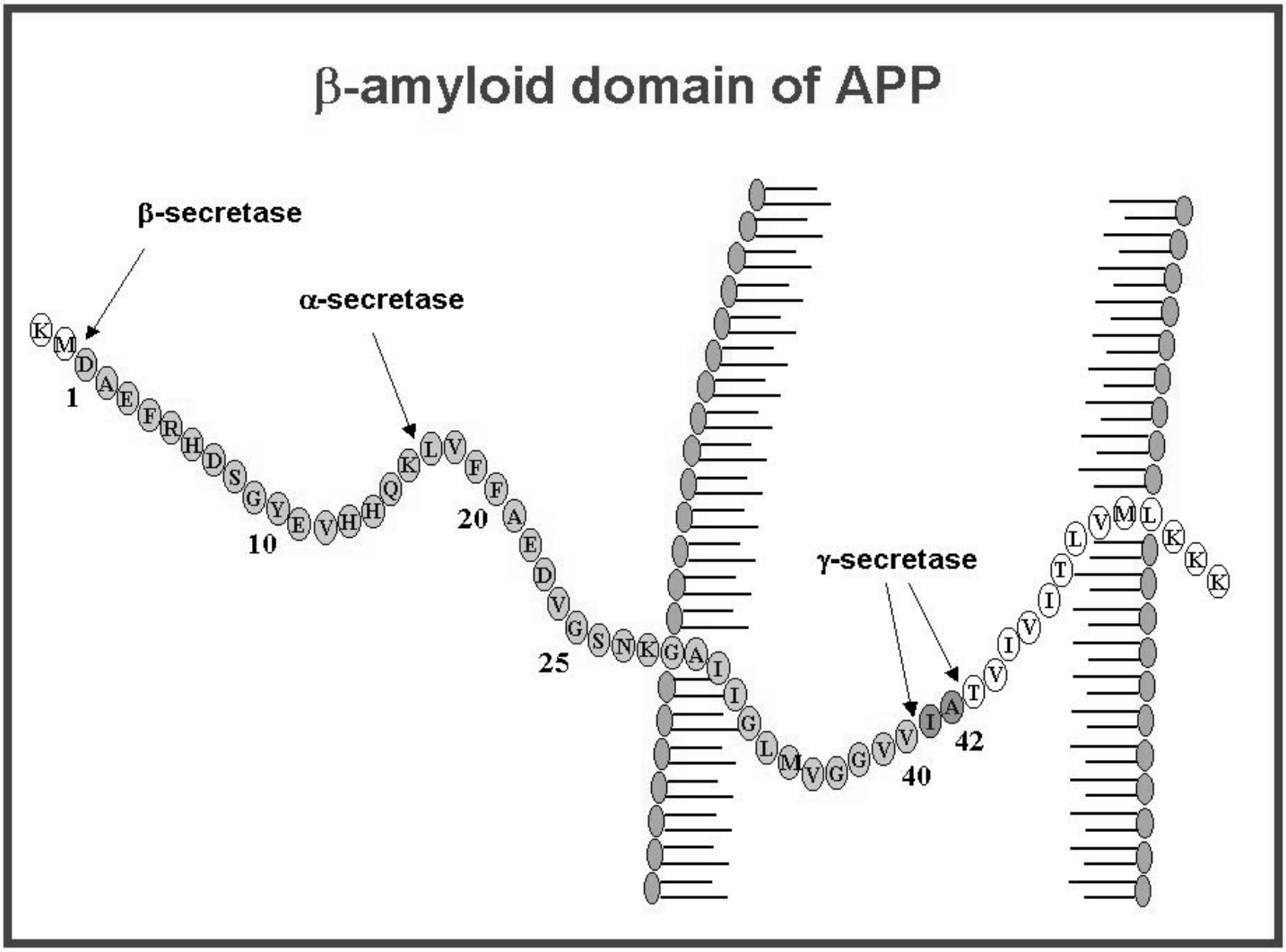
CURRENT CSF biomarkers for AD

CSF - (total) tau protein

Tau is a microtubule-associated protein located in the neuronal axons, while it is not present in dendrites (Goedert, 1993). There are six different isoforms, and numerous phosphorylation sites, of tau in the human brain (Goedert 1993).

An increase in CSF-(total)tau in AD has been recorded in numerous studies (for review see e.g. Andreasen *et al.* 1998; Galasko 1998). The ability of CSF-tau to discriminate between AD and normal aging has been relatively good, above 80%, in most studies. High CSF-tau levels are, however, also found in a proportion of cases with other dementia disorders. This applies particularly to vascular dementia, in which high CSF-tau levels have been found in a relatively high proportion of cases in some studies (Blennow *et al.* 1995; Andreasen *et al.* 1998), while only in occasional cases in other studies (Tato *et al.* 1995; Mori *et al.* 1995; Arai *et al.* 1998; Mecocci *et al.* 1998; Nishimura *et al.* 1998; Hulstaert *et al.* 1999). It has been suggested that vascular dementia patients with high CSF-tau levels may constitute a subgroup with concomitant AD pathology (Andreasen *et al.* 1998), which implies that





CSF-tau might be of use in identifying vascular dementia cases where AD is a contributory factor to the dementia.

In contrast, in patients with other types of de mentia (e.g. alcoholic dementia), chronic neurological disorders (e.g. Parkinson's disease, progressive supranuclear palsy) and psychiatric disorders (e.g. depression), elevated CSF-tau levels are found only in occasional cases (Blennow *et al.* 1995; Molina *et al.* 1997; Ellis *et al.* 1998; Mitani *et al.* 1998; Morikawa *et al.* 1999; Urakami *et al.* 1999).

The major clinical usefulness of CSF-tau seems to be in the discrimination of AD from normal aging. It might also be of use in some other differential diagnoses (e.g. depression, alcoholic dementia and Parkinson's disease), which sometimes may be difficult to differentiate form AD on clinical grounds. The fact that an increase in CSF-tau can be found in acute destructive conditions, such as stroke (Arai et al, 1995), does not really reduce the clinical usefulness of CSF-tau, since these disorders are not differential diagnoses from AD.

Most likely, the level of CSF-tau reflects the degree of neuronal/axonal degeneration, regardless of cause. Longitudinal data on CSF tau shows that levels remain stably elevated over 12–24 months of follow-up (Andreasen et al, 1999a). This

would enable CSF-tau levels to be used as an outcome measure to assess treatment aimed at neuroprotection.

CSF-Aβ42

The β-amyloid protein (Aβ or β/A4 protein) is a cleavage-product from the amyloid precursor protein (APP), encoded by a single gene on chromosome 21 (Kang et al, 1987). APP is a transmembrane protein with a single transmembrane domain, a long N-terminal segment and a shorter cytoplasmic C-terminus (Fig 2). The Aβ part of APP encompasses the first 28 extracellular and the following 12-14 transmembrane amino acids (Fig 2). Non-amyloidogenic secretory forms of APP is normally generated by cleavage within the Aβ region by an unidentified enzyme termed α-secretase, while Aβ is produced by an alternative metabolic pathway, in which two proteases, termed β- and γ-secretase cleave APP on each side of the Aβ sequence (Fig 2).

There are two major C-terminal variants of Aβ, a shorter form ending at Val-40 (Aβ40), and a longer form ending at Ala-42 (Aβ42) (Fig 3). Aβ42 aggregates more rapidly than Aβ40 and is also the predominating form of amyloid in diffuse plaques and in SP (for review see Dickson *et al.* 1997).

Aβ is generated as a soluble peptide during normal cellular metabolism, and is secreted into the extracellular space and biological fluids, including CSF (Haass et *al.* 1992). A marked decrease in CSF-Aβ42 is found in a high percentage of patients with AD (Motter *et al.* 1995; Galasko *et al.* 1998; Andreasen *et al.* 1999b; Hulstaert *et al.* 1999). The sensitivity is above 80-90%, resulting in a relatively good ability for CSF-Aβ42 to distinguish AD from normal aging and depression. However, the specificity for CSF-Aβ42 for the diagnosis of AD compared to other dementias has been less extensively studied than for CSF-tau, and needs to be further evaluated.

Measurement of Aß in CSF may reflect cerebral amyloid deposition. Hypothetically, in AD Aβ secreted from neurons, and possibly other cells in the brain, binds to existing aggregates of Aβ in extracellular SP, with lower levels remaining to circulate in the CSF (Andreasen et al, 1999b). Longitudinal data show that CSF-Aβ42 levels remain relatively stable over 12–24 months of follow-up (Andreasen et al, 1999b). This implies that CSF could be used to monitor the effects of anti-amyloid drugs in AD.

Combination of CSF-tau and CSF-Aβ42

The strategy of combining CSF-tau and CSF-Aβ42 as biomarkers for AD is appealing, since the concentrations of these substances are believed to reflect two of the central pathogenic processes in the disorder, and the combination might thus result in increased sensitivity and specificity. Indeed, some large studies have shown that both sensitivity and specificity increase for the combination compared with CSF-tau or CSF-Aβ42 alone (Galasko et al, 1998; Kanai et al, 1998; Hulstaert *et al.* 1999, Andreasen et al, 2000). These studies are summarized in Table 1.

Also when used as routine analyses in clinical chemistry, and the sensitivity and specificity figures are determined on all consecutive patients admitted for investigation of cognitive disturbances during one year in a community-based setting, the sensitivity to identify AD is above 90% (Andreasen et al, 2000).

Further, the combination of CSF-tau and CSF-Aβ42 also has a high sensitivity to predict progression to AD in patients with mild cognitive impairment (Andreasen et al, 1999c). This finding shows that these CSF markers show abnormal values very early in the disease process, already before the clinical dementia.

CSF-tau and CSF-Aβ42 In clini-

SENSITIVITY AND SPECIFICITY FIGURES FOR THE COMBINATION OF CSF-TAU AND CSF-Aβ42 AS BIOMARKERS FOR ALZHEIMER'S DISEASE.

Disorder	n	Sensitivity	Specificity	Comment	Reference
AD	82	90%		• Multi-center study in USA (6 centers)	*Galasko et al, 1998*
Controls	60		80%	• Clinical diagnosis	
OND including non-AD dementia	74		39-65%	• Stored CSF samples.	
AD	93	40%		• Multi-center study in Japan (3 centers)	*Kanai et al, 1998*
Controls	54		90%	• Clinical diagnosis	
OND including non-AD dementia	89			• Stored CSF samples.	
AD	150	85%		• nternational multi-center study (10 centers)	*Hulstaert et al, 1999*
Controls	100		87%	• Clinical diagnosis	
OND	84		86%	• Stored CSF samples	
Non-AD dementia	79		58%	• CSF analyses run at each research center	
AD	105	94%		• Prospective, community-based study	*Andreasen et al, 2000*
MCI	20	75%		• Clinical diagnosis	
Controls	100		87%	• CSF samples run continuously in routine clinical neurochemistry.	
Depression	5		100%		
Non-AD dementia	32		53%		

cal chemistry

As for all other test in clinical chemistry, methodological factors have to be evaluated when developing CSF biomarkers. CSF sampling, handling and storage can influence levels of many analytes. It has to be evaluated whether a molecule passes from serum to CSF across the blood-brain barrier, which occurs for many proteins that have higher levels in serum than in CSF (Tibblin et al, 1977). If so, the CSF levels will reflect the periphery more than the CNS. Neither CSF-tau nor CSF-Aβ42 are affected by this problem (Blennow et al, 1995; Vanderstichele et al, 1998). Further, CSF-tau or Aβ42 do not show concentration gradients in lumbar CSF (Vanderstichele et al, 1998), which, if present, complicates the interpretation of lumbar CSF levels, since these will vary with the volume and portion of CSF analyzed (Blennow et al, 1993).

However, the hydrophobic Aβ peptide may absorb to some types of test tubes commonly used for lumbar puncture or for centrifugation in the laboratory (Andreasen et al, 1999b). The level of Aβ42 decreases to about 65% in polystyrene or in glass tubes, as compared with polypropylene tubes (Andreasen et al, 1999b). Therefore, polypropylene tubes must be used both for CSF sampling and for centrifugation and storage.

Both CSF-tau and CSF-Aβ42 are determined using sandwich ELISA techniques, using antibodies directed against different epitopes of the proteins, making the analyses very sensitive and specific. Current ELISA assays for CSF-tau use monoclonal antibodies that detect all isoforms of tau independent of phosphorylation, and thus measure the "total" CSF-tau level (Blennow et al, 1995), while Aβ ELISA assays are specific to Aβ42, with minimal cross-reactivity against peptides ending at residues 43 or 40 (Motter *et al.* 1995; Vanderstichele *et al.* 1998).

The biological variation is low for both CSF-tau (Andreasen et al, 1998) and CSF-Aβ42 (Andreasen et al, 1999b), i.e. very similar CSF levels are found when longitudinal CSF samples are analyzed from individual patients. Also the analytical variation is low, the coefficient of variance (CV) for internal control samples when CSF-tau and CSF-Aβ42 are run as a routine clinical neurochemical analyses during one year is approximately 10% (Andreasen et al, 2000).

Finally, it is also of importance to c onsider clinical confounding factors when evaluating CSF biomarkers for AD. One problematic issue is that studies are most often performed on clinically diagnosed patients, and data on neuropathologically confirmed cases are scarce. 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, about 85%, the negative predictive value (i.e. the probability that AD is not present when the diagnostic criteria are not met) has been considerably lower (Tierney *et al.* 1988; Jellinger 1996; Galasko *et al.* 1994). This is especially troublesome for some of the non-AD dementias (e.g. vascular dementia and fronto-temporal dementia). In fact, neuropathological studies have found that a high proportion (40-80%) of clinically diagnosed patients with vascular dementia have notable concomitant AD pathology (Jellinger 1996; Kosunen *et al.* 1996). The fact that current clinical diagnostic criteria cannot be considered to be of 'gold standard' quality results in that it is difficult to get high sensitivity and specificity figures for CSF biomarkers. Further, even if they are asymptomatic, age-matched control subjects may harbor presymptomatic AD lesions in their brains (Tomlinson & Henderson 1976; Davies et al. 1988; Price & Morris 1999), which also reduces the specificity figures of CSF biomarkers for AD.

TH E CL IN IC AL use of CSF bi omarkers for AD

Much effort has focused on finding a single neurochemical marker for AD. This may be elusive unless the marker is re-

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lated to a pathogenic step that is unique to AD. For example, neuronal and synaptic degeneration is not only found in AD but also in most chronic degenerative disorders of the brain. Similarly, deposition of Aβ is not specific to AD, but is also found in normal aging, dementia pugilistica, Lewy body dementia, and after acute brain trauma, while deposition of PHF to in inclusions such as tangles may form in normal aging, dementia pugilistica, myotonic dystrophy, and other forms of tau are found in inclusions in progressive supranuclear palsy and fronto-temporal dementia(Davies et al, 1988; Roberts et al, 1994; Mc Kenzie et al, 1996). Thus, since the central neuropathological findings in AD are not specific for AD, it is unlikely that one single biochemical marker will absolutely discriminate between AD and other dementia disorders.

Instead, the combination of several CSF biochemical markers (e.g. CSF-tau, CSF-Aβ42 and possibly other like phospho-tau and α/β-secretase cleaved APP) could be used in conjunction with other diagnostic methods. 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. SPECT and MRT scans), and CSF biochemical markers. As an analogy, the clinical diagnosis of myocardial infarction is based on the combination of clinical symptomatology, electrocardiogram, and biochemical markers (e.g. creatine kinase). Today, the CSF markers tau and Aβ42, when used as an adjunct to clinical diagnosis, have the potential to help to differentiate AD from some problematic differential diagnoses, especially age-associated memory impairment, depressive pseudo-dementia, Parkinson's disease, progressive supranuclear palsy and alcoholic dementia.

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