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**Biological Markers for
Alzheimer's Disease**
**With Special Emphasis on Cerebrospinal
FLUID β -AMYLOID AND TAU**

Doctoral dissertation

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ABSTRACT

Dementia is related to aging, and with the increasing numbers of elderly people in the population, the number of patients with dementia is growing rapidly. The major cause of dementia is Alzheimer's disease (AD). Currently there exists no simple test or biological markers that could detect all early AD cases, and the definite diagnosis of AD is based on histopathological evidence obtained from autopsy. An ideal biomarker should be related to the neuropathological changes, such as β -amyloid plaques or neurofibrillary tangles, which are the major changes observed in the AD brain. In addition to improving the diagnosis of AD, a biological marker would be valuable in monitoring the progression of the disease and in evaluating the efficacy of therapies.

The present study characterizes the usefulness of four biological markers in the diagnosis and follow-up of patients with AD. The concentrations of β -amyloid 1-42 ($A\beta_{42}$), 1-40 ($A\beta_{40}$), tau protein and aspartate aminotransferase activity in cerebrospinal fluid (CSF) were analysed from patients with AD, other dementias and non-demented controls. The studies included neuropathologically confirmed AD cases, permitting an investigation of the relationships between neuropathological changes and the biomarkers. Tau protein and β -amyloid concentrations in CSF were quantified using enzyme linked immunosorbent assays (ELISA).

The CSF β -amyloid 1-42 concentrations were decreased and CSF tau levels increased in early AD. Combination of these biomarkers possessed a sensitivity of 50.4 % for AD and specificities of 94.8 % for controls and 85.2 % for other dementias. Ninety-one percent of the patients with abnormal CSF $A\beta_{42}$ and tau concentrations had AD. CSF tau and $A\beta_{42}$ were related to the patients' apolipoprotein E (ApoE) genotype. AD patients carrying the apoE $\epsilon 4$ allele, a well-characterized risk factor for AD, had the lowest CSF $A\beta_{42}$ levels and the highest tau concentrations. Eighty-two percent of AD patients with normal levels of tau and $A\beta_{42}$ did not carry the $\epsilon 4$ allele. The sensitivity of the $A\beta_{42}$ test to identify AD among individuals carrying the apoE $\epsilon 4$ allele was 83.6 %. CSF $A\beta_{40}$ concentrations were decreased at the end-stage of AD, but not in the early stage of the disease. During the long-term follow-up of AD patients, CSF $A\beta_{42}$ and $A\beta_{40}$ levels decreased, and the most pronounced decrease in $A\beta_{42}$ levels was observed in patients with the duration of the disease less than two years. CSF tau concentrations showed a positive correlation with the number of neurofibrillary tangles in brain. Aspartate aminotransferase activity was increased in AD, but there was a significant overlap in values between AD and other patient groups.

Taken together, low $A\beta_{42}$ and high tau concentration in CSF support the diagnosis of AD even during early stages of the disease. These biomarkers may be useful in confirming the diagnosis, in selecting AD patients for treatment trials, and they may assist in the early diagnosis of AD in individuals at risk for the disease, such as patients with mild cognitive impairment and apoE $\epsilon 4$ carriers. Since CSF β -amyloid concentrations decreased during the long-term follow-up period, and CSF tau and $A\beta_{42}$ were related to the neuropathological changes in AD brain, these biomarkers may be useful in monitoring the progression of AD, and in screening the efficacy of treatments of AD directed at inhibiting the formation of β -amyloid plaques and neurofibrillary tangles.

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To Mia and Atte

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ABBREVIATIONS

AAMI	Age-associated memory impairment
A β 40	β -amyloid 1-40
A β 42	β -amyloid 1-42
AD	Alzheimer's disease
ALS	Amyotrophic lateral sclerosis
ApoE	Apolipoprotein E
APP	Amyloid precursor protein
AST	Aspartate aminotransferase
CERAD	The Consortium to Establish a Registry for Alzheimer's Disease
CSF	Cerebrospinal fluid
CT	Computed tomography
DSM-IV	Diagnostic and Statistical Manual of Mental Disorders, 4th edition
EEG	Electroencephalography
ELISA	Enzyme linked immunosorbent assay
GFAP	Glial fibrillary acidic protein
ICD-10	International classification of diseases, 10th revision
MCI	Mild cognitive impairment
MMSE	Mini-Mental State Examination
MRI	Magnetic resonance imaging
NFT	Neurofibrillary tangle
NIA-RI	National Institute on Aging-Reagan Institute
NINCDS-ADRDA	National Institute of Neurological and Communicative Disorders and Stroke - Alzheimer's Disease and Related Disorders Association
PCR	Polymerase chain reaction
ROC	Receiver operating characteristics
SPECT	Single photon emission computed tomography
SPSS	Statistical Package for Social Sciences

LIST OF ORIGINAL PUBLICATIONS

This thesis is based on the following original publications that are referred to in the text by the Roman numerals **I-V**.

- I** Tapiola T, Lehtovirta M, Ramberg J, Helisalml S, Linnaranta K, Riekkinen PJ Sr, Soininen H. CSF tau is related to apolipoprotein E genotype in early Alzheimer's disease. *Neurology* 1998;50:169-174.
- II** Tapiola T, Overmyer M, Lehtovirta M, Helisalml S, Ramberg J, Alafuzoff I, Riekkinen PJ Sr, Soininen H. The level of cerebrospinal fluid tau correlates with neurofibrillary tangles in Alzheimer's disease. *Neuroreport* 1997;8:3961-3963 and *Alzheimer's Research* 1998;4:45-48.
- III** Tapiola T, Pirttilä T, Mehta PD, Alafuzoff I, Lehtovirta M, Soininen H. Relationship between apoE genotype and CSF β -amyloid (1-42) and tau in patients with probable and definite Alzheimer's disease. *Neurobiology of Aging* 2000;21:735-740.
- IV** Tapiola T, Pirttilä T, Mikkonen M, Mehta PD, Alafuzoff I, Koivisto K, Soininen H Three-year follow-up of cerebrospinal fluid tau, β -amyloid 42 and 40 concentrations in Alzheimer's disease. *Neuroscience Letters* 2000;280:119-122.
- Va** Tapiola T, Lehtovirta M, Pirttilä T, Alafuzoff I, Riekkinen P Sr, and Soininen H. Increased aspartate aminotransferase activity in cerebrospinal fluid and Alzheimer's disease. *Lancet* 1998;352:287.
- Vb** Tapiola T, Lehtovirta M, Pirttilä T, Alafuzoff I, Riekkinen P Sr, Soininen H Cerebrospinal fluid aspartate aminotransferase activity is increased in Alzheimer's disease. In: *Alzheimer's disease and Related disorders*. Eds. Iqbal K, Swaab DF, Winblad B, Wisniewski HM. John Wiley & Sons Ltd, Chichester, England, 1999;193-197.

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1. INTRODUCTION

Alzheimer's disease (AD) is the leading cause of dementia. The typical neuropathological changes occurring in this degenerative disease were first described nearly one hundred years ago (Maurer et al. 1997). However, still today detection and analysis of these histopathological changes must be done from an autopsied brain before one can come to a definite diagnosis of Alzheimer's disease. The clinical diagnosis, probable Alzheimer's disease, is set after an extensive clinical and neuropsychological evaluation. This can have a diagnostic accuracy of more than 80% at centres specialised in the evaluation of dementia. In particular, the clinical diagnosis of Alzheimer's disease in its very early stage is complicated, but important, because the current symptomatic therapy and most likely future therapies would be predicted to have their best efficacy during the early stages of the disease. Also the follow-up of the progression of the brain pathology in patients with Alzheimer's disease, or people at risk for the disease, is difficult. Thus, a reliable method for the diagnosis and follow-up of Alzheimer's disease is needed.

One possibility to improve the antemortem diagnosis, follow-up, and screening of the efficacy of therapies of Alzheimer's disease would be to develop a specific biological marker. An antemortem biomarker for Alzheimer's disease could include for example a biochemical analysis of cerebrospinal fluid or blood sample, histological analysis of a biopsy from the patient, or a genetic test. The Ronald and Nancy Reagan Research Institute of the Alzheimer's Association and the National Institute on Aging Working Group (1998) published guidelines for biomarker studies and criteria for an ideal antemortem biomarker. According to this report, an ideal biomarker should detect a fundamental feature of the neuropathology of Alzheimer's disease, be able to detect Alzheimer's disease early in the course of the disease with high sensitivity, distinguish it from other dementias, and be validated in neuropathologically confirmed Alzheimer's disease cases. The suitable test should also be reliable, simple to perform, noninvasive, and inexpensive. In the evaluation of the usefulness of a biomarker sensitivity, specificity, and positive and negative predictive values should be reported, and the follow-up data should be collected. In addition to carefully diagnosed patients, a control group with similar age and gender distributions should be included in the studies. At least two independent studies conducted by investigators with expertise in biomarkers should specify the usefulness of a proposed biological marker.

Many studies of putative biomarkers have been conducted, but none of the antemortem tests has fulfilled all the criteria for an ideal biomarker. Currently, two of the most promising biomarkers are the analysis of tau and β -amyloid 42 concentrations in cerebrospinal fluid samples (Trojanowski and Growdon 1998). Cerebrospinal fluid can be considered as a window into the brain, and thus many studies have concentrated on changes in its composition. In addition to tau protein and β -amyloid 42, results of various other CSF analyses, such as mediators of inflammation (Hampel et al. 1999), neurotransmitters and their metabolites (Hartikainen et al. 1992), amyloid precursor protein (Lannfelt et al. 1995), and neuronal thread protein (Kahle et al. 2000) have been reported. Other approaches to find an ideal biomarker include analysis of β -amyloid from plasma (Mayeux et al. 1999), tropicamide eye test (Scinto et al. 1994), dystrophic neurites in olfactory epithelium (Talamo et al. 1991), and apolipoprotein E genotyping (Mayeux et al. 1998).

In the present series of studies, four putative biomarkers were analysed from CSF samples. The analyses included detection of total tau protein, β -amyloid 40 and 42 peptides, all of which are closely related to the typical neuropathological changes of Alzheimer's disease, as well as the measurement of the activity of aspartate aminotransferase, an enzyme that may be associated with the cell death in brain. To increase the diagnostic accuracy, the combined use of the biomarkers was also studied. Moreover, the relationships between CSF analyses and the duration or severity of AD and changes in the concentrations of tau protein and β -amyloid peptides during follow-up were investigated. The apolipoprotein E genotype of the patients was determined, and a risk factor for AD, the apolipoprotein E ϵ 4 allele, was taken into account in the interpretation of the results.

The study included a large number of clinically diagnosed probable AD patients, carefully evaluated patients with other dementias and control groups. Moreover, neuropathologically confirmed definite AD cases were included in the study. In these cases neuropathological changes were quantified and correlated with the results of CSF analyses. To investigate long-term changes in the levels of biomarkers, a follow-up study was performed, which lasted for three years. The statistical analyses evaluated the sensitivity, specificity, and ROC curves, which are parameters needed in the interpretation of the usefulness of any potential biomarker.

The proportion of aged people is increasing, and in the future the number of patients having memory problems and dementing disorders will inevitably increase. This will pose a challenge for the health-care system. Many therapeutic agents that may attenuate or stop the progressive development of the neurodegenerative changes in AD are under development. These facts emphasize the need for studies directed at improving the diagnosis and follow-up of patients with AD. This study describes one possible approach, i.e. assessment of the reliability of CSF levels of β -amyloid, tau and aspartate aminotransferase activity as biomarkers for AD.

2. REVIEW OF THE LITERATURE

2.1 ALZHEIMER'S DISEASE

2.1.1 Epidemiology and risk factors

Dementia is one of the major public health problems, and the increasing numbers of patients with dementia will impose a major financial burden on health care systems. More than half of the patients with dementia have Alzheimer's disease. The proportion of elderly people is growing, and the prevalence and incidence of AD have been shown to increase exponentially with age. The prevalence for AD in Europe is 0.3 % for ages 60-69 years, 3.2 % for ages 70-79 years, and 10.8 % for ages 80-89 years (Rocca et al. 1991). The incidence rate of mild AD in Europe for individuals 65-69 years old is 2.5, for 75-79 years old 10.7, and for 85-89 years old 46.1 per 1 000 person years (Jorm and Jolley 1998). The survival time after the onset of AD is approximately from 5 to 12 years (Friedland 1993).

AD is an etiologically heterogeneous, multifactorial disease. A small proportion of AD cases show an autosomal dominant transmission of the disease, and currently mutations in the genes encoding amyloid precursor protein, presenilin I and presenilin II have been characterised in early onset familial AD cases (Tandon et al. 2000). The best described risk factors for AD are age and a positive family history of dementia, since more than one-third of AD patients have one or more affected first degree relatives (Van Duijn et al. 1991a). Other risk factors that may be associated with the development of AD include severe head trauma, low levels of education, female gender, previous depression, and vascular factors (Van Duijn et al. 1991b, Kivipelto et al. 2001).

At present the most important genetic risk factor for AD is the presence of the allele $\epsilon 4$ of apolipoprotein E (apoE). ApoE is a polymorphic protein encoded by a gene on chromosome 19. The three alleles, $\epsilon 2$, $\epsilon 3$, and $\epsilon 4$, determine the polymorphism leading to the occurrence of six phenotypes, E2/2, E3/3, and E4/4 in homozygotes and E2/3, E2/4, and E3/4 in heterozygotes. The most common phenotype is E3/3. The apoE $\epsilon 4$ allele frequency is approximately 15% in control material, but in AD patients its frequency may be as high as 36 % (Kuusisto et al. 1994, Farrer et al. 1997). The allele $\epsilon 4$ has been associated with sporadic and familial forms of late-onset AD in both sexes and in all ethnic groups (Farrer et al. 1997). Individuals carrying the $\epsilon 4$ allele have a 1.4-2.9 times higher risk to become cognitively impaired compared to homozygotes for $\epsilon 3$ allele, and homozygotes for $\epsilon 4$ allele can have as high as 15 times greater risk to become cognitively impaired compared to homozygotes for the $\epsilon 3$ allele (Henderson et al. 1995, Hyman et al. 1996). It is now well-established that the presence of the $\epsilon 4$ allele lowers the age of onset of AD (Corder et al. 1993, Gomez-Isla et al. 1996b, Farrer et al. 1997), but has no effect on the progression of the disease (Gomez-Isla et al. 1996b). The relationship between the allele $\epsilon 2$ and AD has remained unclear, it is claimed that the $\epsilon 2$ allele may even be protective against AD (Talbot et al. 1994, Farrer et al. 1997).

Older adults commonly have memory complaints (Bolla et al. 1991). Koivisto et al. (1995b) have shown that 38 % of 60-78 years old individuals fulfil the criteria for age associated memory impairment (AAMI), and in the follow-up 9% of AAMI patients became demented (Hänninen et al. 1995). In addition, during a follow-up period of four and a half years 55 % of individuals with the diagnosis of Mild Cognitive Impairment (MCI) developed dementia (Petersen et al. 1995).

2.1.2 Neuropathological changes

Macroscopic changes found in AD brain include shrinkage of the gyri and widening of the sulci especially in frontotemporal areas, thickening of leptomeninges and enlargement of the ventricles. The two major microscopical lesions are amyloid plaques and neurofibrillary tangles (NFT), which are found significantly more often in AD compared to normal aging. Other lesions include neuronal loss, synaptic alterations, neuropil threads, amyloid angiopathy, Hirano bodies, granulovacuolar degeneration, Lewy bodies, and inflammation (Esiri 1996). These changes, and the proteins related to these lesions, provide the starting point in the search for biological markers for AD. However, many of these lesions can also be found in normal aging brain, in cases without clinical signs of dementia, and in other neurodegenerative diseases.

The exact mechanism of cell death in AD is unknown, but it may be linked to the neurotoxicity of β -amyloid, the development of NFTs, synaptic degeneration or apoptosis as evidenced by the expression of proteins related apoptotic pathways (Anderson et al. 1996, Su et al. 1997). Extensive loss of synapses has been shown using immunocytochemistry for synaptophysin (Davies et al. 1987, Honer et al. 1992) or spectrin (Masliah et al. 1990). Also neuropil threads, short degenerating or aberrantly sprouting dendrites or axons, which contain tau, ubiquitin, and neuropil thread protein, are found in AD brain (Perry et al. 1991, De la Monte et al. 1996). In addition to neuronal cell death, changes in expression levels of proteins related to plasticity or regeneration, for example GAP-43 (De la Monte et al. 1995) and PSA-NCAM (Mikkonen et al. 1999) have been described in AD brain. In contrast to neurons, there is an increase in the number of astrocytes found especially near amyloid plaques (Schechter et al. 1981). Inflammation in AD brain is characterized by activated microglia associated with β -amyloid deposits (Sasaki et al. 1997) and up-regulation of many mediators of inflammatory responses (McGeer and McGeer 1995).

The earliest neuronal cell loss is detected in the entorhinal cortex (Gomez-Isla et al. 1996a), hippocampus (West et al. 1994) and in the basal nucleus of Meynert (Vogels et al. 1990). Braak and Braak (1995) have proposed a model for the progression of AD. This model emphasizes the role of the NFTs in AD. NFTs develop first in the entorhinal region during the preclinical stage, followed by NFTs in the hippocampus in the patients with mild symptoms and neocortical accumulation of NFTs during the later stages of AD. However, there are plaque-only AD cases who have a high number of β -amyloid plaques, but absent or very sparse number of NFTs, and clinically observed dementia.

2.1.3 β -amyloid in the pathogenesis of Alzheimer's disease

There are two major types of amyloid plaques in AD brain, neuritic plaques and diffuse plaques. Neuritic plaques contain dense bundles of amyloid fibrils and are surrounded by dystrophic neurites, astrocytes, and microglia. Diffuse plaques contain nonstructured amyloid and are not surrounded by dystrophic neurites. Neuritic plaques may develop from diffuse plaques. In addition to antibodies for β -amyloid, neuritic plaques can be stained using other antibodies, such as those against α_1 -antichymotrypsin (Abraham et al. 1988), protein kinase C (Masliah et al. 1991), apoE (Namba et al. 1991), and heparan sulphate proteoglycans (Snow et al. 1988). The number of neuritic plaques has not shown a clear correlation with the severity of dementia in AD (Terry et al. 1991), and some cognitively preserved aged individuals have such a high density of neuritic plaques in brain that the

diagnosis of AD would be made if the individual exhibited any clinical signs of dementia (Katzman et al. 1988, Price et al. 1999). However, these cases may represent preclinical AD.

The 4 kDa β -amyloid peptides that are deposited as amyloid plaques in AD brain are formed by the proteolytical processing of amyloid precursor protein (APP). APP is a transmembrane protein encoded by the chromosome 21. It has several isoforms resulting from alternative splicing of the transcript (Kitaguchi et al. 1988). The isoform APP₆₉₅ is expressed predominantly in neurons. Other isoforms include APP₇₅₁ and APP₇₇₀, both of which contain a Kunitz serine protease inhibitor protein domain. After its synthesis, APP is translocated to the endoplasmic reticulum. APP matures through the secretory pathway, and undergoes glycosylation and phosphorylation in the Golgi apparatus. Little is known about the function of APP. It has been proposed to be involved in neurite outgrowth, cell proliferation and adhesion (Saitoh et al. 1989, Milward et al. 1992), in signal transduction (Nishimoto et al. 1993) and in inhibition of serine proteases (isoforms 770 and 751) (Sinha et al. 1990).

The proteases involved in the proteolytical processing of APP are the α -, β - and γ -secretases. The β -secretases were recently characterized as transmembrane aspartic proteases (BACE, the beta-site amyloid precursor protein cleaving enzyme) (Nunan and Small 2000). Several proteases have been suggested to have α -secretase activity. The presenilins could be the γ -secretases, although there is no general agreement about these secretases (Nunan and Small 2000). The α -secretase cuts the mature APP on the plasma membrane or the APP molecule in the secretory pathway between the residues 16 and 17 of the A β -region just before the beginning of the transmembrane domain (between residues 687 and 688 of APP₇₇₀) and thus prevents the formation of A β peptide. This α -secretase cleavage releases a large soluble APP ectodomain (APP α) and retains a 10 kDa carboxy terminal fragment in the plasma membrane. The formation of β -amyloid peptides involves the cleavage of APP by β - and γ -secretases, a process which occurs constitutively also in normal metabolism (Haass et al. 1992, Shoji et al. 1992). β -secretase cleaves APP just before the β -amyloid region (between residues 671 and 672 of APP₇₇₀), which releases a large amino-terminal part of APP and creates a 12 kDa carboxy terminal fragment. The cleavage of this 12 kDa fragment by the γ -secretase generates the 4 kDa β -amyloid peptide. The γ -secretase can generate β -amyloid peptides ending at different residues, valine₄₀ or alanine₄₂ (A β ₄₀ and A β ₄₂). The γ -secretase cleavage can occur in endosomes following internalization of APP from plasma membrane, or in the secretory pathway, in Golgi apparatus or in endoplasmic reticulum. It is thought that A β ₄₂ is the most abundant form in endoplasmic reticulum, A β ₄₀ is found in endosomes, and both peptides are found in the Golgi complex (Hartmann 1999).

β -amyloid peptides are released into the extracellular fluid, but recent studies have shown that A β ₄₂ also accumulates inside the cells (Tienari et al. 1997, Wild-Bode et al. 1997, Gouras et al. 2000). The extracellular β -amyloid can be degraded by extracellular proteases (Qiu et al. 1997, 1998), taken up by cells (Knauer et al. 1992, Burdick et al. 1997) and become degraded intracellularly (Paresce et al. 1997). However, the clearance mechanisms of the secreted β -amyloid are poorly known. Excessive amounts of β -amyloid can lead to the formation of intracellular and extracellular amyloid aggregates.

Many studies indicate that the aggregation of A β ₄₂ may be the critical event in the pathogenesis of AD (Younkin 1995), particularly in the formation of plaques, but possibly also in cell death after intracellular β -amyloid accumulation. A β ₄₂ contains two more hydrophobic amino acid residues compared to A β ₄₀, and aggregates and forms oligomers more rapidly than A β ₄₀ (Burdick et al.

1992). Also, the initial extracellular A β deposits are composed mainly of non-filamentous A β 42 (Iwatsubo et al. 1994, Kuo et al. 1996). The mature plaques, the neuritic plaques, contain both aggregated A β 40 and A β 42 (Iwatsubo et al. 1994).

The important role of amyloid in the pathogenesis of AD is further supported by studies showing that mutations causing AD increase the production of β -amyloid, or disturb the normal proteolytical processing of APP (Selkoe 1997). APP mutations at codons 716 and 717 lead to increased production of A β 42 (Suzuki et al. 1994, Eckman et al. 1997), mutations at codons 670/671 (Swedish mutation) increase A β 42 and A β 40 production (Scheuner et al. 1996), and presenilin I and II mutations increase the production of A β 42 (Borchelt et al. 1996, Duff et al. 1996, Scheuner et al. 1996). In Down syndrome (trisomy 21), the overexpression of chromosome 21-linked genes, including the APP gene, is related to the development of the neuropathological changes similar to AD at the age of 40 years (Wisniewski et al. 1985, Rumble et al. 1989, Iwatsubo et al. 1995).

In addition to brain parenchyma, β -amyloid is deposited in intracranial blood vessels in more than 80% of AD cases (Premkumar et al. 1996, Zarow et al. 1999). Usually this cerebral amyloid angiopathy affects medium-sized vessels in the leptomeninges and in the cortex of cerebellar and occipital regions. In parenchymal arterioles and capillaries or in white matter, amyloid angiopathy is less frequently found. Also the hippocampal region is often relatively spared and in the basal ganglia there is no amyloid angiopathy. There is no evident correlation between the amyloid angiopathy and the frequency of parenchymal plaques, and approximately one in three non-demented elderly individuals exhibit cerebral amyloid angiopathy (Vonsattel et al. 1991). It has been proposed that A β 42, produced probably by vascular smooth muscle cells or derived from extracellular fluid of the brain, is initially deposited onto vessel walls followed by massive accumulation of A β 40 and the development of the final stage, congophilic angiopathy (Shinkai et al. 1995).

2.1.4 Neurofibrillary tangles and tau protein

Neurofibrillary tangles (NFT) affect primarily large neurons in which they form abnormal fiber masses in the cytoplasm. When this amorphous material is deposited in axons and dendrites, it is termed the neuropil thread (NT). In the hippocampus and entorhinal cortex also extraneuronal tangles can be found. NFTs are composed of 8-20 nm wide paired helical filaments (PHF), and to a lesser extent, straight filaments. The main component of PHFs is the abnormally phosphorylated microtubule associated tau protein (Lee et al. 1991). NFTs are immunoreactive also for antibodies directed against the microtubule associated protein 5 (MAP5) (Hasegawa et al. 1990), ubiquitin (Perry et al. 1987), protease nexin I (Rosenblatt et al. 1989), and β -amyloid peptide (Perry et al. 1992). It has been suggested that NFTs are related to the progression of AD (Braak and Braak 1995), and a correlation between the number of NFTs and cognitive decline in AD has also been reported (Wilcock and Esiri 1982, Samuel et al. 1994). In addition to AD, NFTs can also be found in individuals without dementia (Price et al. 1999) and in a variety of degenerative disorders, such as Down syndrome, corticobasal degeneration, progressive supranuclear palsy, Pick's disease, dementia pugilistica, amyotrophic lateral sclerosis/parkinsonism-dementia complex of Guam, postencephalitic parkinsonism, Gerstmann-Sträussler-Scheinker syndrome, Hallervorden-Spatz disease, myotonic dystrophy, Niemann-Pick disease and subacute sclerosing panencephalitis (Buee et al. 2000).

Microtubules are part of the neuronal cytoskeleton involved in cell motility, transport, shape, and mitosis. In neurons, the cytoskeleton undergoes changes continuously, which requires the

polymerisation of microtubule monomers. Tau protein regulates this polymerisation, it stabilizes the microtubules, and increases the polymerisation process. The phosphorylation of tau reduces binding of tau to microtubules, which decreases the microtubule polymerisation.

Tau exists in six isoforms coded by a gene located on chromosome 17 (Buee et al. 2000). Tau isoforms are derived by alternative splicing which lead to differences in the number of microtubule binding regions and 29 amino acid inserts in the amino terminal region. Only the shortest form with three microtubule binding regions and without the 29 amino acid inserts is present in the immature brain. All six tau isoforms are expressed in the adult human central nervous system (Goedert et al. 1989). Tau is mainly synthesized in neurons (Kosik et al. 1989), but it is present also in astrocytes (Papasozomenos 1989), oligodendrocytes (LoPresti et al. 1995), and in peripheral tissues (Lubke et al. 1994). More than ten mutations have been described in the tau gene among families with cases diagnosed as frontotemporal dementia with parkinsonism linked to chromosome 17 (FTDP-17) (Buee et al. 2000). Neuropathologically, FTDP-17 patients exhibit frontal and temporal lobe atrophy, filamentous tau pathology in neurons, gliosis, and neuronal loss.

Tau is a phosphoprotein, and it contains many proline-directed serine/threonine phosphorylation sites and nonproline directed phosphate acceptor residues (Trojanowski and Lee 1994, Morishima-Kawashima et al. 1995). Fetal tau and PHF-tau are more extensively phosphorylated compared to the normal adult tau protein. Many kinases and phosphatases, including mitogen-activated protein kinase (MAPK) (Drewes et al. 1992), cyclin-dependent kinase 5 (cdk5) (Baumann et al. 1993), glycogen synthase kinase 3 (GSK3) (Mandelkow et al. 1992) and phosphatase 2A (PP2A) (Sontag et al. 1996), have been shown to regulate the phosphorylation state of tau *in vitro*. The abnormally phosphorylated tau in AD does not bind microtubules (Bramblett et al. 1993), and the cellular trafficking system can be destroyed. The hyperphosphorylated free tau may associate with some chaperones, aggregate, and form PHFs and finally NFTs. It is not known, however, whether these processes are key features in neuronal cell death in AD.

2.1.5 Apolipoprotein E

Circulating ApoE is synthesized mainly in liver and it is associated to lipoproteins, but astrocytes and possibly microglia are important sources for apoE in brain (Pitas et al. 1987, Nakai et al. 1996). ApoE has an important function as a regulator of lipid metabolism during development and is involved in the growth and regeneration of injured neurons (Poirier 1994). The levels of apoE increase after brain lesions (Poirier et al. 1991, Page et al. 1998), which may contribute to the regeneration of neurons.

The mechanisms by which the $\epsilon 4$ allele convey the development of AD are largely unclear but many studies have revealed isoform specific effects on neurodegeneration, formation of NFTs and β -amyloid. Arendt et al. (1997) have shown that plastic responses in brain are impaired in $\epsilon 4$ carriers, and *in vitro* studies have shown that apoE3 promotes neurite outgrowth more than apoE4 (Nathan et al. 1994). ApoE $\epsilon 4$ carriers have also more severe astrogliosis compared to $\epsilon 4$ non-carriers (Overmyer et al. 1999).

Accumulating data suggest that apoE plays a role in the development of neurofibrillary and amyloid pathology. ApoE3 has been shown to bind avidly to microtubule-associated proteins (Huang et al. 1994) and to promote their polymerization, and apoE4 is claimed to depolymerize microtubules (Nathan et al. 1995). The stabilizing effect of apoE3 may prevent the abnormal phosphorylation of

the tau protein (Strittmatter et al. 1994). In addition to the *in vitro* data, neuropathological studies have shown that the apoE ϵ 4 allele is linked to increased density of NFTs in brain (Nagy et al. 1995, Ohm et al. 1995). On the other hand, ApoE is bound strongly to β -amyloid plaques (Namba et al. 1991). *In vitro* studies have indicated that apoE4 promotes β -amyloid aggregation (Ma et al. 1994, Sanan et al. 1994, Wisniewski et al. 1994), and apoE3 inhibits the aggregation of β -amyloid (Evans et al. 1995). The accumulation of β -amyloid in brain is increased in controls and AD patients carrying the ϵ 4 allele (Rebeck et al. 1993, Schmechel et al. 1993, Gomez-Isla et al. 1996b). This effect may be based on the augmentation of A β 40 deposition (Gearing et al. 1996, Ishii et al. 1997). In contrast, AD patients carrying the ϵ 2 allele have a decreased amyloid burden (Polvikoski et al. 1995). It has also been suggested that the apoE ϵ 4 allele increases vascular β -amyloid deposition (Zarow et al. 1999) and amyloid accumulation after a traumatic brain injury (Nicoll et al. 1995).

Clinical studies support the significance of apoE in the development of AD. MRI studies have shown that AD patients with at least one ϵ 4 allele have decreased volume in their entorhinal cortex (Juottonen et al. 1998) and hippocampus (Lehtovirta et al. 1996). In single photon emission computed tomography (SPECT), AD patients homozygous for ϵ 4 allele exhibit the most severe cerebral hypoperfusion (Lehtovirta et al. 1998), and in positron emission tomography (PET) the low baseline metabolism in non-demented ϵ 4 carriers predicts a cognitive decline (Small et al. 2000). In neuropsychological tests, the ϵ 4 allele has been associated with the cognitive decline in subjects with cognitive impairment, but not in cognitively normal individuals (Dik et al. 2000).

Despite the strong association between AD and apoE ϵ 4 allele, apoE genotyping is not recommended in the routine diagnosis of AD. The presence of the ϵ 4 allele by itself does not confirm AD, and the disease cannot be excluded if an individual does not carry the risk allele. In the population based study of Hyman et al. (1996) over half of the individuals over 80 years of age carrying two ϵ 4 alleles were cognitively normal. Therefore, apoE genotyping provides only a marginal increase in the confidence of the diagnosis and cannot be used as a diagnostic test for AD (Mayeux et al. 1998).

2.1.6 Diagnosis

2.1.6.1 Clinical diagnosis

There are several guidelines for the clinical diagnosis of AD: the National Institute of Neurological and Communicative Disorders and Stroke - Alzheimer's Disease and Related Disorders Association Work Group (NINCDS-ADRDA) criteria (McKhann et al. 1984), the Diagnostic and Statistical Manual of Mental Disorders, 4th edition (DSM-IV) (American Psychiatric Association 1994) and the International classification of diseases, 10th revision (ICD-10). The NINCDS-ADRDA criteria have been most widely used in research because they are well validated, provide high diagnostic accuracy and allow comparison between studies (Blacker et al. 1994, Gearing et al. 1995). The NINCDS-ADRDA criteria divide AD into three categories with increasing reliability of the diagnosis (*possible AD*, *probable AD* and *definite AD*). The patient has *probable AD* when dementia is characterized by gradual onset and progression, when deficits are documented by examination and testing in two or more cognitive areas, and when other disorders that could cause dementia are absent. The onset should be between the ages from 40 to 90 years, and no disturbances of consciousness should be present. The *probable AD* diagnosis is strengthened by a positive family history of dementia, normal findings in routine cerebrospinal fluid analysis, atrophy in brain imaging,

impaired activities in daily life and a change in behaviour. Histopathological AD changes in autopsy or biopsy of a *probable* AD patient confirms the diagnosis of *definite* AD. *Possible* AD is diagnosed when the patient has another potentially dementing disorder that is not considered to be the primary cause of dementia, or when the patient has variations in the presentation of dementia. The accuracy of the clinical diagnosis of AD using NINCDS-ADRDA criteria is over 80% (Blacker et al. 1994, Galasko et al. 1994, Gearing et al. 1995, Lopez et al. 1999). In these studies, the sensitivity has been better than the specificity, and the follow-up of patients has improved the diagnostic accuracy. Disorders that may mimic or overlap with AD include depression, normal pressure hydrocephalus, dementia with Lewy bodies, vascular dementia, and frontotemporal dementias.

2.1.6.2 Neuropsychological tests

Neuropsychological deficits in AD include changes in a variety of cognitive functions, such as episodic memory, language, semantic memory, executive abilities, attention, and visuospatial and visuoperceptual processes. Neuropsychological tests are often influenced by education (Doraiswamy et al. 1995), practice (Galasko et al. 1993), and sociocultural or ethnic factors (Manly et al. 1998). For example, the performance in the widely used Mini-Mental Status Examination (MMSE) test, is dependent on age and education (Ylikoski et al. 1992), and it is not suitable for the evaluation of the progression of AD in individuals (Clark et al. 1999). As a screening test for dementia, the sensitivity of MMSE test has been 56-90 % and specificity has varied from 85 % to 95 % (Tombaugh and McIntyre 1992, Koivisto 1995a).

Many studies have shown that impairment in episodic memory and learning or retaining new information are the best ways for the differentiation of patients with early AD from normal elderly people. Measures of delayed recall, particularly word lists, have shown a sensitivity of 86 % and specificity of 94 % in distinguishing patients with mild AD from controls (Welsh et al. 1991, Welsh et al. 1992). Furthermore, the word list delayed recall test has shown a better accuracy than MMSE and many other neuropsychological tests in discriminating between individuals with presymptomatic AD and those who remain nondemented (Chen et al. 2000). The discrimination of AD from other dementing disorders usually requires resorting to additional neuropsychological tests.

2.1.6.3 Brain imaging

The diagnostic usefulness of different imaging methods in AD has been widely studied. Computed tomography (CT) does not differentiate early AD from normal aging with high diagnostic accuracy (DeCarli et al. 1990, 1992), but it is useful in detecting some causes of dementia. Similarly, single photon emission computed tomography (SPECT) may be useful in the differential diagnosis of dementia (Talbot et al. 1998). Magnetic resonance imaging (MRI) offers a superior anatomic discrimination power and permits accurate imaging of the affected regions. Many studies have shown that volumetric MRI of hippocampus and entorhinal cortex may be helpful in the diagnosis of AD (Juottonen et al. 1999). The decrease in hippocampal volume has a diagnostic accuracy from 85 % to 100 % in the differentiation of AD patients from non-demented controls (Jack et al. 1997, Laakso et al. 1998). Hippocampal atrophy may also predict the development of dementia in patients with mild cognitive impairment (Jack et al. 1999). The differentiation of AD patients from other dementias using hippocampal volumetry has not, however, achieved a high diagnostic accuracy (Laakso et al. 1996). The use of MRI volumetry as a routine method is quite time consuming and requires normalization and calibrations in departments specialized in the field (Jack et al. 1995). However,

Wahlund and colleagues (1999) have found a good correlation between visual estimation of hippocampus from coronal sections and volumetry.

2.1.6.4 Neuropathological diagnosis

The presence of both β -amyloid plaques and NFTs in normal aging and in other dementing disorders complicates the neuropathological diagnosis of AD. The widely accepted criteria for neuropathological diagnosis of AD has been established by the Neuropathology Task Force of the Consortium to Establish a Registry for Alzheimer's Disease (CERAD) (Mirra et al. 1991). The analysis includes macroscopic examination of brain, spinal cord, meninges and cerebral blood vessels and semiquantitative microscopic analysis of neuritic plaques characterized by conventional stainings in different brain regions. The frequency of plaques is then combined with the age of the patients to obtain an age related plaque score (0, A, B, C) that is then integrated with the clinical information regarding the absence or presence of dementia. The CERAD -criteria define the presence of *possible*, *probable* or *definite* AD. The *definite* AD diagnosis requires the age-related plaque score C and clinical history of dementia, *probable* AD requires plaque score B and clinical history of dementia, and the diagnosis of *possible* AD is set if the patient has a clinical history of dementia and a plaque score A or absence of clinical manifestations of dementia but age-related plaque score B or C. Neurofibrillary lesions are not taken into account in the CERAD criteria. Other guidelines for the neuropathological diagnosis of AD include those described by Khachaturian (1985), and the guidelines proposed by the National Institute on Aging-Reagan Institute (NIA-RI) (1997). The criteria of Khachaturian (1985) use quantitation of senile plaques of any type plus some NFTs, and the NIA-RI criteria focus on neuritic plaques and NFTs.

2.2 BIOMARKERS IN THE DIAGNOSIS OF DEMENTIA

A well-characterized biological marker that fulfils the requirements for the diagnostic test for AD would have several advantages. An ideal biological marker would identify AD cases at a very early stage of the disease, before the cognitive symptoms are found in neuropsychological tests, and before there is degeneration in brain imaging studies. A biomarker reflecting neuropathological changes at the molecular level in AD brain would be very useful in the differential diagnosis of dementia, and in distinguishing AD patients from those individuals with mild cognitive impairment who do not develop AD, and from patients with depression. The therapies for AD are improving all the time. A biomarker could be the first indicator for starting the treatment as early as possible, and very valuable in screening the efficacy of new therapies, especially those directed to stop or prevent the development of neuropathological changes. A biological marker would also be useful in the follow-up of the progression of the disease or the evaluation of people at risk for the disease. Antemortem biochemical markers for AD have been sought for years. For example, analysis of plasma, erythrocytes (Bosman et al. 1991), lymphocytes (Pirttilä et al. 1992), urine (Lindner et al. 1993, Ghanbari et al. 1998), hair (De Berker et al. 1997, Bonafe et al. 1998) and skin (Soininen et al. 1992, Heinonen et al. 1994) have been performed. However, currently the most promising biomarkers have been found in the analysis of CSF.

2.2.1 Cerebrospinal fluid (CSF) as a window to brain metabolism

There is approximately 100-150 ml of cerebrospinal fluid circulating in ventricles and subarachnoidal space. Every day 400-500 ml of cerebrospinal fluid is formed, and thus the total volume of CSF is turned over 4-5 times a day. Most of the CSF is secreted in ventricles by the choroid plexus where CSF is formed from blood by capillary filtration and active epithelial secretion. Ninety-nine percent of CSF is water. The concentrations of most proteins (CSF/plasma ratio 0.005), glucose, bicarbonate, K^+ , and Ca^{2+} are lower compared to plasma. CSF is absorbed mainly to the venous sinuses by the arachnoid villi. Other sites of absorption are ependyma and the lymphatic drainage system that includes the absorption of CSF from subarachnoidal space into the cervical lymphatic system (Weller et al. 1992).

However, a considerable fraction of CSF is derived from brain extracellular fluid, and approximately 20% of proteins in CSF are synthesized in brain. Extracellular fluid from brain tissue is drained to the postcapillary venules, veins as well as to the CSF. Extracellular fluid can diffuse across ependyma of ventricles into the CSF. Brain extracellular fluid passes also across pial mater and from the perivascular spaces into CSF in the subarachnoidal space. Perivascular spaces (Virchow-Robin spaces) around intracerebral arteries are formed by thin perivascular sheaths consisting of pia mater cells. This pial sheath is present around subarachnoid and intracerebral arteries and arterioles extending as far as capillaries. There are no perivascular sheaths around intracerebral or subpial veins. The fluid in perivascular spaces around the subarachnoidal arteries can diffuse to the CSF and thus facilitate the movement of metabolites from deep parts of the cortex.

CSF performs several important functions. In addition to protecting the brain from impact and reducing the effective weight of the brain, it is important in maintaining a constant environment for neurons. It also conveys substances secreted into CSF to many brain regions and drains metabolites that have passed from extracellular fluid to CSF into the venous circulation. As a window to brain, CSF analysis is useful in the diagnosis of infections and inflammations of the central nervous system. In neoplasias, malignant cells can be found in CSF, and analysis of CSF can be used in the diagnosis of trauma, hemorrhage, and intracranial hypertension (Thompson 1995). However, the development of imaging techniques, such as MRI, have diminished the value of CSF analysis as a primary diagnostic tool in the latter conditions.

Disease-specific changes in CSF composition have been sought in many neurological diseases. However, several confounding factors must be taken into account in the interpretation of CSF analysis. Patient-related parameters such as age, diet, medication, height, weight, physical and psychic conditions and other factors like circadian variation, changes in blood-brain-barrier and CSF dynamics may affect the results of any CSF analysis. The concentration of a particular agent in CSF is a net effect of diffusion from the blood, its synthesis in the brain, its clearance and degradation in the extracellular space, and finally dilution in the total CSF volume. These parameters are often unknown for the agents to be evaluated.

2.2.2 CSF measurements in dementias

Early CSF analyses in dementing disorders concentrated mostly to investigations of different neurotransmitters and their metabolites. The establishment of the cholinergic hypothesis in AD prompted the analysis of acetylcholine and acetylcholinesterase activity in CSF. However, these analyses showed inconsistent results and none of the measured metabolites were useful in the diagnosis of AD.

Along with the increasing understanding of the pathogenesis of AD, the search for a biomarker for AD focused on the examination of disease-characteristic changes in CSF. The possibility that brain pathology could be reflected in CSF, guided the selection of the putative markers that were examined in the studies. For example, inflammatory responses are one pathological feature in AD brain, and many studies measured the levels of inflammatory mediators in CSF. Changes in the levels of interleukin-1 and interleukin-6, as well as their receptors have been found in CSF of AD patients in some (Blum-Degen et al. 1995, Hampel et al. 1998) but not in all (Pirttilä et al. 1994b, Marz et al. 1997) studies. Proteins associated with senile plaques, such as apoE, have also been detected in CSF, but studies have shown conflicting results in AD (Blennow et al. 1994, Lefranc et al. 1996, Rösler et al. 1996a, Merched et al. 1997). Other approaches include measurements of CSF proteins that may reflect synaptic pathology and neuronal degeneration, such as gangliosides (Blennow et al. 1991), chromogranin A (Blennow et al. 1995a), or the neuronal thread protein (De la Monte et al. 1992, Kahle et al. 2000).

In conclusion, most tests have shown a marked overlap of values between patients with dementia and controls, and in many cases there have been variable results between research groups, or the results need to be confirmed in further studies. Currently, proteins linked to the main pathological changes in AD, CSF A β 42 and tau, are viewed as the most promising biological markers in AD.

2.2.3 CSF tau protein

Under normal conditions, a detectable amount of tau is secreted to the CSF (Wolozin and Davies 1987) although the source of this CSF tau is unknown. Changes in the CSF tau concentration may reflect the neurofibrillar pathology in brain or a more general phenomenon of neuronal cell death. Some studies have shown an increase in CSF tau with aging (Arai et al. 1995, Kanai et al. 1998, Kurz et al. 1998), while other studies have shown no significant change (Blennow et al. 1995b, Vigo-Pelfrey et al. 1995, Galasko et al. 1998).

Vandermeeren et al. (1993) first showed increased CSF tau concentration in AD. Since then many studies have confirmed the increase of total CSF tau protein concentration in AD patients compared to controls (Trojanowski et al. 1996, Boss 2000). The specificity to distinguish patients with AD from controls has varied from 64 % to 100 % and the sensitivity for the CSF tau as a diagnostic test has varied from 39 % to 98 %.

The relationship between CSF tau and the progression of the disease has remained unresolved. Some studies have shown a correlation between the CSF tau concentration and the severity of dementia (Hock et al. 1995, Tato et al. 1995, Rösler et al. 1996b), whereas others have found no association (Blennow et al. 1995b, Motter et al. 1995, Nitsch et al. 1995, Vigo-Pelfrey et al. 1995, Andreasen et al. 1998, Andreasen et al. 1999b). Isoe et al. (1996) collected serial CSF samples from nine patients with AD and their results suggested that the CSF tau concentration increased to the mid-stage of AD and then decreased during later stages. In other longitudinal studies of AD with mean follow-up period of approximately one year, CSF tau values remained stable (Andreasen et al. 1998, 1999b) or increased (Kanai et al. 1998). It has also been suggested that CSF tau concentrations increase with time in AD patients carrying at least one apoE ϵ 4 allele, but not in AD patients without the ϵ 4 allele (Blomberg et al. 1996).

An increase of CSF tau levels is not specific for AD and has been found in patients with head trauma (Zemlan et al. 1999), Creutzfeldt-Jacob's disease (Arai et al. 1997a, Otto et al. 1997), normal pressure hydrocephalus (Kudo et al. 2000), and neuro-AIDS (Andersson et al. 1999). These findings suggest that CSF tau may be a marker of neuronal cell death and axonal degeneration (Zemlan et al. 1999).

CSF tau consists of variable tau protein fragments with molecular weights from 14 kDa to 68 kDa (Wolozin and Davies 1987, Arai et al. 1995, Vigo-Pelfrey et al. 1995, Johnson et al. 1997, Ishiguro et al. 1999, Zemlan et al. 1999). Phosphorylated forms of CSF tau include at least N-terminal fragments partially phosphorylated on Ser199, Thr231, and Ser225 (Ishiguro et al. 1999, Kohnken et al. 2000). Most studies have used ELISA tests that detect and measure the total amount of tau including different isoforms, cleavage products, as well as phosphorylated and unphosphorylated forms of the protein. Blennow and colleagues (1995b) have shown that the amount of PHF-tau in CSF correlates with the amount of total tau in CSF of patients with AD and controls. Interestingly, Hesse et al. (2001) reported that after stroke the total CSF tau level increases, but the phosphorylated tau remains stable.

In conclusion, the increase of CSF tau in AD is well-established, but the diagnostic accuracy of the test has varied between studies, and there is a clear overlap in the values between patients with AD, neurological controls and other dementia patients. There are very few studies of neuropathologically confirmed AD and other dementia patients, and the possible role of CSF tau in the long-term follow-up remains unresolved.

2.2.4 Amyloid precursor protein and β -amyloid in CSF and plasma

Due to their central role in the pathogenesis of AD, APP and β -amyloid peptide concentrations have been extensively studied in body fluids in addition to brain parenchyma. APP and β -amyloid are secreted during the normal cellular metabolism (Haass et al. 1992, Seubert et al. 1992), and small amounts of APP and β -amyloid peptides are found in CSF and blood. β -amyloid in CSF and plasma is associated with high density lipoproteins (Koudinov et al. 1994, 1996) apoE, apoJ (Zlokovic et al. 1994), and other plasma proteins (Kuo et al. 2000), which may help to maintain the solubility of A β . However, the binding of A β with carrier proteins may also hinder its detection in immunoassays.

2.2.4.1 Amyloid precursor protein in CSF and plasma

Immunoblot analyses have shown soluble APP (α - and β -secretase cleaved APP) including isoforms with or without the Kunitz type protease inhibitor domains in plasma and CSF (Weidemann et al. 1989). No correlation between age and CSF APP levels has been found in humans (Carroll et al. 1995), but in rats the APP levels decrease with age (Anderson et al. 1999). Many studies have shown that levels of APP in the brain are upregulated as a result of a brain injury (Gentleman et al. 1993). For example, increased CSF γ -secretase cleaved APP concentrations have been reported after subcortical lesions in rats (Wallace et al. 1995), and ischemia in rat brain induces the expression of APP mRNAs containing the Kunitz type serine protease inhibitor domain (Koistinaho et al. 1996).

The usefulness of CSF APP analysis as a biomarker for AD is not established, and studies have shown conflicting results. Some studies have shown elevated levels of soluble APP in CSF (Weidemann et al. 1989, Kitaguchi et al. 1990), but others have reported decreased levels (Van Nostrand et al. 1992). APP isoforms in plasma and especially in platelets have been detected, and

in western blot studies it has been suggested that the ratio of 130 kDa/110 kDa APP proteins is decreased in platelets of AD patients (Rosenberg et al. 1997, Di Luca et al. 2000).

AD-causing mutations in the APP gene lead to a decrease in CSF APP levels. Low APP levels have been detected in patients with the 717-APP mutation (Farlow et al. 1992), the APP670/671 mutation (Lannfelt et al. 1995), and in patients with cerebral haemorrhage amyloidosis of the Dutch type (Van Nostrand et al. 1992).

2.2.4.2 β -amyloid peptides in CSF

The source of β -amyloid peptides in CSF and their relationship to brain pathology is unclear. CSF concentrations reflect the net result of the production and clearance of β -amyloid into CSF. The β -amyloid from CSF consists mainly of A β 40, although there are also detectable levels of A β 42 (Vigo-Pelfrey et al. 1993). Soluble β -amyloid peptides from brain parenchyma enter blood, CSF, or become phagocytosed by glial cells (Pluta et al. 1999). Studies with an animal model of AD, the APP23 mouse strain, suggest that β -amyloid produced by neuronal cells is the source of the high levels of β -amyloid in CSF and the cerebrovascular amyloid deposition (Calhoun et al. 1999). On the other hand, it is also possible that part of the β -amyloid in brain and CSF originates from blood, because β -amyloid injected intravenously to blood can enter brain through the blood-brain-barrier (Maness et al. 1994). Histological studies have shown that β -amyloid accumulates in periarterial interstitial fluid drainage pathways of the brain (Weller et al. 1998), suggesting that a proportion of β -amyloid in brain parenchyma can be eliminated through the perivascular spaces, and thus be involved in the pathogenesis of cerebral amyloid angiopathy. In a transgenic mouse model of AD, the Tg2567 strain, there is a decline of CSF β -amyloid levels, coincident with the marked deposition of β -amyloid in brain (Kawarabayashi et al. 2001). In addition to forming aggregates in brain parenchyma, clumps of β -amyloid can be detected in CSF. Using fluorescence correlation spectroscopy Pitschke et al. (1998) detected aggregates of β -amyloid peptides in CSF of patients with AD.

Some factors that influence the concentration of β -amyloid peptides in CSF in non-demented individuals have been characterised. Early studies detected no changes of total β -amyloid with age (Pirttilä et al. 1994a, Carroll et al. 1995), but more recent studies have indicated that CSF A β 40 levels decrease in aging whereas the A β 42 levels remain unchanged (Kunicki et al. 1998). Also, the β -amyloid concentration is increased transiently in brain tissue and in CSF after traumatic brain injury (Raby et al. 1998, Emmerling et al. 2000). The A β 40/A β 42 ratio is decreased in both CSF and brain, reflecting a more pronounced increase in A β 42 levels (Gentleman et al. 1997, Raby et al. 1998).

The changes of levels of total β -amyloid, A β 40, and A β 42 in CSF have been extensively studied in AD. Initially, total β -amyloid concentrations were measured. The results were variable showing a decrease (Pirttilä et al. 1994a), an increase (Nakamura et al. 1994) or no change (Motter et al. 1995, Nitsch et al. 1995, Van Gool et al. 1995, Southwick et al. 1996) in AD patients. Due to the great overlap between the groups in all studies measurement of total β -amyloid in CSF proved not a suitable biomarker of AD, and most studies have not shown any correlation between the level of total CSF β -amyloid and the severity of dementia (Nakamura et al. 1994, Pirttilä et al. 1994a, Southwick et al. 1996). There are few studies, which have examined the relationship between CSF A β and the progression of the disease. In one study CSF was collected from AD patients during a three-year period and levels of total β -amyloid showed a decrease in the follow-up, particularly in

patients with moderate dementia (Pirttilä et al. 1998). Previous studies have also shown that CSF levels of total β -amyloid are lower in patients with severe congophilic angiopathy compared to those with mild to moderate angiopathy (Pirttilä et al. 1996). Also, there was an inverse correlation between concentrations of β -amyloid in the frontal cortex and levels of β -amyloid in CSF (Pirttilä et al. 1996). These results suggest that CSF β -amyloid may reflect brain pathology.

More recent studies have focused on measurements of different isoforms of $A\beta$ in CSF, particularly $A\beta_{42}$, due to its critical role in the early pathogenesis of AD. No differences in CSF $A\beta_{40}$ concentrations have been found in AD compared to controls (Ida et al. 1996, Tamaoka et al. 1997, Kanai et al. 1998, Shoji et al. 1998). There are two exceptions, studies that reported decreased concentrations in AD compared to patients with depression (Schröder et al. 1997) or healthy controls (Jensen et al. 1999). A significant correlation between $A\beta_{40}$ and MMSE scores in AD has been described in the study of Samuels and colleagues (1999) but not in all studies (Kanai et al. 1998, Shoji et al. 1998). In frontotemporal dementia, recent studies reported that CSF $A\beta_{40}$ concentrations increase with the degree of frontal lobe atrophy (Andersen et al. 2000). The concentration of $A\beta_{40}$ has not been shown to be modulated by the apoE genotype in AD (Kunicki et al. 1998, Mehta et al. 2000).

Motter et al. (1995) first reported that $A\beta_{42}$ concentrations are decreased in CSF of AD patients. Subsequently most studies have confirmed these findings (Galasko et al. 1998, Kanai et al. 1998, Shoji et al. 1998, Hulstaert et al. 1999). However, the study of Jensen and colleagues (1999) showed increased CSF $A\beta_{42}$ levels in mild AD and thereafter a decline with the disease progression. $A\beta_{42}$ levels have also shown an inversed correlation with the severity of dementia (Samuels et al. 1999) and with brain atrophy (Schröder et al. 1997). In addition to AD, decreased $A\beta_{42}$ levels in CSF have been reported also in patients with other dementias, Creutzfeldt-Jakob disease (Otto et al. 2000) and in Down syndrome (Tamaoka et al. 1999). The specificity to distinguish patients with AD from controls has varied from 55 % to 90 % and the sensitivity for the CSF $A\beta_{42}$ as a diagnostic test has varied from 78 % to 100 %.

2.2.4.3 β -amyloid peptides in plasma

β -amyloid can normally be detected in plasma but the levels are 100-fold lower than in CSF. As with CSF β -amyloid, the source of β -amyloid in plasma is unknown. Previous studies have shown that β -amyloid injected into the ventricular CSF of rat is rapidly cleared into blood, most likely by subependymal capillaries and the choroid plexuses (Gherzi-Egea et al. 1996). Other studies have suggested peripheral sources, such as platelets, for β -amyloid in plasma (Chen et al. 1995).

Increased levels of $A\beta_{42}$ have been reported in the plasma of AD patients and presymptomatic carriers of mutations in APP (Scheuner et al. 1996, Kosaka et al. 1997), or presenilins (Scheuner et al. 1996). De Jonghe et al. (1999) suggested, however, that all presenilin 1 mutations do not increase plasma $A\beta_{42}$ levels. In Down syndrome, the levels both $A\beta_{42}$ and $A\beta_{40}$ are increased in plasma (Tokuda et al. 1997, Mehta et al. 1998, Matsubara et al. 1999). Mayeux and co-workers (1999) have reported that plasma $A\beta_{42}$ may be elevated several years before the onset of sporadic AD. However, sporadic AD cases do not exhibit increased soluble $A\beta_{42}$ concentrations in plasma (Tamaoka et al. 1996, Mehta et al. 2000), though an increase in lipoprotein-free $A\beta_{42}$ levels have been reported (Matsubara et al. 1999). The increase in plasma levels have been suggested to be related to the abnormal processing of APP in non-neuronal cells and in platelets, or it may reflect changes in the aggregation properties of β -amyloid. The significance of β -amyloid levels in plasma in

relation to the β -amyloid accumulation in brain is unclear. A recent study of transgenic mice expressing the Swedish mutation of APP has suggested that plasma β -amyloid levels decrease with the marked deposition of A β in brain (Kawarabayashi et al. 2001).

2.2.5 CSF aspartate aminotransferase activity

Aspartate aminotransferase (AST) is an intracellular enzyme expressed in most cells including neurons. It catalyzes the transfer of α -amino groups from aspartic acid to α -ketoglutaric acid to yield glutamic acid and oxalacetic acid. AST activity in serum increases for example following a myocardial infarction. In CSF, increased AST activity has been reported after brain injury in animal models (Maas 1977), and in patients with stroke (Vaagenes et al. 1986), which presumably results from the release of enzymes from necrotic and damaged brain cells. Another possibility is that the use of glucogenic amino acids, as an alternative source of energy due to the impairment of glucose metabolism, might lead to increased activity of aminotransferases (Hoyer and Nitsch 1989).

Riemenschneider and co-workers (1997) studied CSF AST activity and tau concentrations in 30 control subjects, 39 patients with AD and 12 patients with other dementing disorders. They found the highest AST activity in the AD group, and suggested that the analysis of AST activity could be useful in distinguishing AD from other dementias. Their results showed no statistically significant increase of AST in the other dementias group compared to controls, and the combination of AST and tau analyses increased the specificity of the test to 83%. Jensen-Steur et al. (1998) studied the CSF AST activity in controls and in two groups of patients with Parkinson's disease (patients with or without dementia). Their results showed no significant differences in AST activity between any of the three groups. AST activity analyses are a promising candidate for a biomarker, because the method is inexpensive and routinely used in the analyses of serum samples in many laboratories.

3. AIMS OF THE STUDY

A biological marker would improve the accuracy of the early diagnosis of AD. An ideal biomarker should reflect the neuropathology of AD, and thus be valuable in monitoring the progression of the disease and in evaluating the efficacy of therapy for AD. The aim of this study was to determine the levels of four putative biomarkers in cerebrospinal fluid, and to characterize their role in the diagnosis and follow-up of AD patients.

The specific aims of the present study were:

- 1) To investigate the role of CSF tau and CSF β -amyloid (A β 42 and A β 40) in diagnosing AD and in monitoring the progression of AD. **(I, III, IV)**
- 2) To study the effect of apoE ϵ 4 allele, a well-characterized risk factor for AD, on CSF levels of tau and β -amyloid. **(I, III)**
- 3) To characterize the relation of the levels of CSF biomarkers to neuropathological changes in AD brain. **(II)**
- 4) To evaluate the usefulness of CSF AST activity in the diagnosis of AD. **(V)**

4. MATERIALS AND METHODS

4.1 SUBJECTS

The study population consisted of a total of 323 subjects: 168 AD patients, 52 patients with other dementias, 3 patients with mixed dementia (AD/Vascular dementia; included only in study **I**), 12 Down syndrome patients, and 88 patients in control groups. In studies **I**, **II**, **III**, and **V** the material was partially overlapping. Clinically diagnosed patients with probable AD, and patients with other dementias, were recruited from diagnostic investigations in the Department of Neurology of the Kuopio University Hospital. The neuropathologically verified *definite* AD group consisted of patients derived from a follow-up study of hospitalized patients in the geriatric department of Harjula Hospital in Kuopio. Down syndrome subjects were derived from the Vaalijala Hospital in Pieksämäki. Patients in control groups were recruited from diagnostic investigations in the Departments of Neurology of the University Hospitals of Kuopio and Tampere. The study was approved by the local ethics committee of the Universities and University Hospitals of Kuopio and Tampere, and informed consent for participation in the study was obtained from all subjects and caregivers of demented patients. Patient demographics are shown in Table 1.

4.1.1 Clinically diagnosed Alzheimer's disease patients

The study included 120 patients with probable AD. These patients were drawn from diagnostic examinations or had been recently diagnosed. The diagnosis of probable AD was made according to the NINCDS-ADRDA criteria (McKhann et al. 1984). Patients underwent extensive clinical neurologic examination including neuropsychological tests, Mini-Mental State Examination (MMSE) (Folstein et al. 1975), EEG, brain CT or MRI, routine laboratory tests, and CSF analysis.

4.1.2 Neuropathologically confirmed Alzheimer's disease cases

Brain tissue from autopsy was available from 48 AD cases. The autopsies were performed in the Department of Pathology of the Kuopio University Hospital. The brains were weighed and visually evaluated for gross lesions and vessel abnormalities and fixed in 10% formalin for at least one week. After fixation the brains were cut in coronal slices, and specimens were taken from six cortical, four subcortical grey matter and five infratentorial regions and embedded in paraffin. The tissue samples were cut into 5 μm -thick sections, and processed for diagnostic purposes using hematoxylin eosin counterstain combined with a modified version of Bielschowsky's silver impregnation method or thioflavin-S stain. The extent of histological Alzheimer's degenerative changes was graded according to the CERAD classification (Mirra et al. 1991). According to these criteria, 35 cases had definite AD, 10 cases had probable and 3 had possible AD.

4.1.3 Patients with other dementias

The patients underwent extensive clinical neurologic examination including neuropsychological tests, MMSE (Folstein et al. 1975), EEG, brain CT or MRI, routine laboratory tests, and CSF analysis. The diagnoses of other dementias were based on the guidelines of Diagnostic and Statistical Manual of Mental Disorders, fourth edition (DSM-IV) (American Psychiatric Association 1994). This study group included patients with other dementias such as vascular dementia (N=20), frontotemporal

dementia (N=7), Lewy body dementia (N=6), Parkinson's disease with dementia (N=3), unclassified dementia (N=16). Neuropathological data of patients in this group is not available.

4.1.4 Control patients

The control groups consisted of patients with different neurological diseases without any signs of dementia and from patients with psychosomatic disorders, mainly with depression. Control patients with neurological diseases had for example polyneuropathy, amyotrophic lateral sclerosis (ALS) (N=13, included only in study **V**), hypothyreosis, or they had come to the neurologic examination because of some neurological symptom, such as ataxia, vertigo or chronic pain. The diagnoses of these patients were based on DSM-IV -criteria (American Psychiatric Association 1994).

Table 1. Patient demographics.

	N	Women/ Men	Age	Age at onset	Duration of the disease	MMSE	study
Probable AD	120	74/46	71±8	69±8	2±2	20±5	I, III, IV, V
Definite AD	48	43/5	83±9	75±9	8±4	4±7	II, III, IV, V
Other dementias	52	29/23	73±8	71±9	2±2	19±6	III, V
Controls	88	41/47	61±9	-	-	-	I,III,V
Down syndrome	12	6/6	41±11	-	-	-	I

Values are mean ± SD. The number in the last column represents the study where the patient group or part of it is included.

4.2 CEREBROSPINAL FLUID ANALYSIS

CSF samples were obtained using a standardized protocol. Lumbar punctures were performed at L3/L4 or L4/L5 interspace, and a total of 10-15 ml of CSF was collected after the patients had rested overnight. The 1-ml aliquots were immediately frozen and stored at -70 °C until biochemical assays. The analyses were carried out in duplicate without knowledge about the clinical data. All samples in the 3-year follow-up study (**IV**) were analysed simultaneously. In the clinical-neuropathological correlation study (**II**), the mean ± SD interval between lumbar puncture and autopsy was 19 ± 15 months.

4.2.1 Analysis of CSF tau

Tau concentrations in CSF were quantified using the Innostest hTAU-Antigen sandwich enzyme linked immunoassay (ELISA) (Innogenetics, Zwijndrecht, Belgium) according to the manufacturer's protocol. Volumes of 75 μ l of conjugate consisting of two biotinylated monoclonal anti-hTAU antibodies (HT7 against both phosphorylated and non-phosphorylated tau and BT2 against unphosphorylated tau protein) and 25 μ l of CSF samples or standards were added to polystyrene wells coated with anti-human tau monoclonal antibody (AT120 against all isoforms of tau) and incubated overnight at room temperature. Recombinant hTAU in six different concentrations ranging from 75 to 1200 pg/ml were used as standards. After washing, the plates were incubated with peroxidase conjugated streptavidin (100 μ l/well) for 30 min at room temperature. After washing, tau immunoreactivity was visualized by adding 100 μ l of tetramethylbenzidine in phosphate-citrate buffer containing 0.006% hydrogen peroxide to each well. After incubation of 30 min at room temperature, the reaction was stopped by adding sulphuric acid, and absorbance was read at 450 nm using Microplate Reader, Model 550, with Microplate Manager V4.0 software (Bio-Rad Laboratories Inc., Hercules, CA, USA).

4.2.2 Analysis of CSF β -amyloid

β -amyloid concentrations ($A\beta$ 40 and $A\beta$ 42 in study **III**; $A\beta$ 40 in study **IV**) were quantified using the method developed by Mehta and colleagues (1998). Three well-characterised antibodies (gifts from Dr. Mehta) were used in an in-house ELISA; mouse monoclonal anti β -amyloid 6E10 (mAb 6E10) (Kim et al. 1990) and two rabbit polyclonal antibodies R162 and R164 have been previously described (Mehta et al. 1998, Potempska et al. 1999, Mehta et al. 2000). 100 μ l of mAb 6E10 (2.5 μ g/ml) antibody diluted in carbonate-bicarbonate buffer, pH 9.6, was coated in microplates and incubated at 4° C overnight. Thereafter the plates were washed four times with phosphate buffer containing 0.05 % Tween-20. After blocking for two hours with 200 μ l of 2 % bovine serum albumin (BSA fraction V, Boehringer Mannheim, Mannheim, Germany) in phosphate buffer in each well, plates were washed, and 100 μ l of standards $A\beta$ 42 (a gift from Dr. Mehta) or $A\beta$ 40 (Bachem, Bubendorf, Switzerland) at 8 concentrations from 78 pg/ml to 10 ng/ml diluted in phosphate buffer containing 0.05 % Tween-20 and 0.5 % bovine serum albumin, and CSF samples diluted 3:1 in 4x phosphate buffer containing 0.2% Tween-20 and 2 % bovine serum albumin were added. Standard and CSF sample solutions were first incubated for 2 hours at room temperature and then at 4° C overnight. Next, the plates were washed, and 100 μ l of biotinylated rabbit polyclonal antibody R162 (to detect $A\beta$ 40) or R164 (to detect $A\beta$ 42) diluted 1:400 and 1:200, respectively, in phosphate buffer containing 0.05 % Tween-20 were added to the wells. Plates were incubated in this detector antibody solution for 1 h 15 min at room temperature and washed. Thereafter, 100 μ l of horseradish peroxidase conjugated neutravidin (Pierce, Rockford, IL, USA) in phosphate buffer containing 0.05 % Tween-20 was added to each well, and the plates were incubated for one hour at room temperature. Finally, after washing, $A\beta$ 42 or $A\beta$ 40 concentrations were assayed by adding 100 μ l of o-phenylenediamine dihydrochloride (Sigma Chemical Co. St. Louis, MO, USA) in 50 mM citric acid/100 mM sodium phosphate buffer (pH 5.0) containing 0.015 % hydrogen peroxide into each well. The color reaction was stopped by adding 100 μ l of 1 M sulphuric acid and the absorbance was measured at 490 nm using a Microplate Reader, Model 550, with Microplate Manager V4.0 software (Bio-Rad Laboratories Inc., Hercules, CA, USA). The detection limit of the assay was 20 pg/ml for $A\beta$ 40 and 40 pg/ml for $A\beta$ 42. The interassay coefficient of variation was 9.9 % for $A\beta$ 42 ELISA and 7.8 % for $A\beta$ 40 ELISA.

A β 42 concentrations in CSF samples of the follow-up study (study **IV**) were quantified using the Innostest™ β -amyloid (1-42) ELISA method (Innogenetics, Zwijndrecht, Belgium) according to the manufacturer's protocol. A volume of 75 μ l of conjugate solution (mouse anti- β -amyloid (1-42) IgG 3D6 labelled with biotin) was added to each well of the plate coated with monoclonal β -amyloid 42 antibody 21F12. Thereafter 25 μ l of CSF or standards (β -amyloid 42 peptide at six different concentrations from 125 pg/ml to 2000 pg/ml) were added to each well. After one hour of incubation at room temperature, the plate was washed 5 times, and 100 μ l of peroxidase-labelled streptavidin was added. After 30 min incubation, the plate was washed, and 100 μ l of substrate solution (tetramethylbenzidine in phosphate-citrate buffer containing hydrogen peroxide) was added to each well. The color reaction was stopped after 30 min by adding 50 μ l of 0.9 N sulphuric acid and the absorbance was measured at 450 nm using a Microplate Reader, Model 550, with Microplate Manager V4.0 software (Bio-Rad Laboratories Inc., Hercules, CA, USA).

4.2.3 Analysis of CSF aspartate aminotransferase activity

A colorimetric determination kit for transaminases (Sigma Diagnostics, St Louis, MO, USA) was used for the quantification of the aspartate aminotransferase (AST) activity in CSF. The method is based on the colorimetric quantification of oxalacetic acid that is the end product of the reaction between aspartic acid and α -ketoglutaric acid. This reaction is catalysed by AST and the formation of oxalacetic is proportional to the transaminase activity. The reaction was performed according to the manufacturer's protocol with the exception that the total volume was reduced to 610 μ l. A Calibration series for transaminase at 9 different concentrations from 0 to 14 U/l was used as standards. Aliquots of 50 μ l of Sigma Prepared Substrate (0.2 mol/l DL-aspartic acid and 1.8 mmol/l α -ketoglutaric in phosphate buffer pH 7.5) was pipetted into a test tube. The substrate was warmed to 37° C and 10 μ l of CSF sample was added. Exactly after one hour, 50 μ l of Sigma Color reagent (2,4-dinitrophenylhydrazine in 1 N hydrochloric acid) was added to stop the activity and to start the color reaction. After 20 min incubation at room temperature, 0.4 N sodium hydroxide solution was added, and absorbances were read at 490 nm using a photometer (Microplate Reader, Model 550, with Microplate Manager V4.0 software, Bio-Rad Laboratories Inc., Hercules, CA, USA).

4.3 QUANTIFICATION OF NEUROFIBRILLARY TANGLES AND ASTROCYTOSIS IN BRAIN

NFTs and immunostaining for glial fibrillary acidic protein (GFAP) as a marker for astrocytosis in AD brain were analysed, and the relationship between these neuropathological changes and CSF tau levels was studied (study **II**). Neurofibrillary tangles and GFAP immunoreactivity were analysed in the frontal, temporal, and parietal cortices and the hippocampus.

NFTs were quantified as described by Mölsä et al. (1987). Scoring of NFTs from 0 to 10 was performed on five random fields under light microscopy with magnification X100 (area 0.92 mm²). The neocortical score of NFT lesions was the sum of scores in frontal, temporal and parietal cortices.

Immunohistochemistry for GFAP expression was performed as described by Overmyer et al. (1999). The sections were deparaffinized, rehydrated and blocked with normal goat serum (HistoMark, Kirkegaard & Perry Laboratories, Inc, Gaithersburg, MD, USA). Polyclonal rabbit anti-cow GFAP antibody (DAKO, Glostrup, Denmark) was used as a primary antibody. GFAP

immunostaining was visualized by using biotinylated secondary antibody, alkaline-phosphatase conjugated streptavidin and Vector-Red stain (Vector Laboratories, Inc, Burlingame, CA, USA). The GFAP expression was estimated on five randomly selected fields using Quantimet 570 Image analysis system (Leica Cambridge Ltd., Cambridge, England). The staining was analysed per standard unit field (0.5 mm²) in two parameters: stained area and stained cell counts. For area measurement (fibrillary astrocytosis), all features were included in the estimation, and for cell counts, a size limitation (>30 µm²) for each individual feature quantified (protoplasmic astrocytosis) was used.

4.4 APOLIPOPROTEIN E GENOTYPING

Determination of apoE genotype was performed as described by Helisalimi (1998). Genomic DNA was extracted from peripheral a venous blood sample collected in EDTA tubes using a phenol-chloroform-isoamyl alcohol method. ApoE DNA was amplified by the polymerase chain reaction (PCR). The PCR product was digested with HhaI (New England Biolabs, Beverly, MA), which generated characteristic DNA fragments for each apoE genotype. Digested PCR products were subjected to polyacrylamide gel electrophoresis and the separated DNA fragments were visualized with ethidium bromide staining.

4.5 STATISTICAL ANALYSIS

The data were analyzed using SPSS for Windows V.6.0.1 or V.8.0.1 software (SPSS Inc., Chicago, IL.). In study **I**, to test the effects of apoE allele and diagnostic group on CSF tau, a MANOVA was performed. The univariate ANOVA with Scheffe *post hoc* analysis was used to compare differences in means between factor levels. In studies **III**, and **V** Oneway ANOVA with Bonferroni *post hoc* analysis was used to compare differences in means. The Student's *t*-test for independent samples or the Mann-Whitney *U*-test, when assumptions were not met, were used to compare differences between two patient groups. Correlations were calculated using a two-tailed Pearson's correlation test or Spearman's correlation test (study **IV**). For the analysis of the longitudinal data (study **IV**), Wilcoxon signed-rank test with exact statistics was used. A p-value of less than 0.05 was considered statistically significant. The relationship between sensitivity and specificity was described using the Receiver Operating Characteristics (ROC) curve. The areas under the ROC curves as measures of diagnostic accuracy were calculated using the ROC Curve Analyzer V.6 software (R.M. Centor, Richmond, VA)

5. RESULTS

5.1 CSF TAU IS INCREASED IN ALZHEIMER'S DISEASE

The CSF tau levels were significantly increased in patients with probable and definite AD as compared to neurological control subjects. The mean CSF tau concentrations in the non-AD dementia group was higher than in controls, but lower when compared to the AD patients. These differences did not, however, reach statistical significance.

Increased levels of the tau protein were found early in the course of AD (**I**) and in AD patients with severe dementia (**II**) (Figure 1). However, many patients with definite AD and severe dementia had normal CSF tau concentrations. During the three-year-follow-up, CSF tau concentrations increased in 53 % of AD patients whereas a decrease was observed in 47 % of AD patients (study **IV**).

CSF tau was not related to gender, age, age of onset, duration or severity of dementia (**I**, **II**, **III**). In the follow-up study (**IV**) of 17 AD patients, there was a positive correlation between CSF tau and duration of the disease (baseline: $r=0.644$, $p<0.001$; end of the follow-up: $r=0.541$, $p<0.05$).

The cutoff value of 400 pg/ml distinguished the AD patients from neurological controls with a sensitivity of 58 % and a specificity of 88 % (study **I**). To separate patients with AD from other dementias, this cutoff value resulted in a specificity of 60 %. In study **III** the cutoff value of 380 pg/ml resulted a sensitivity of 63 % for clinically diagnosed AD and 56 % for definite AD, and specificities of 92 % for controls and 67 % for other dementias.

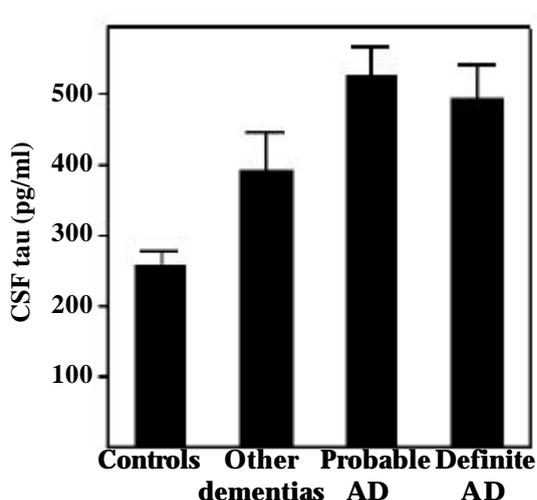


Figure 1. Mean \pm SE CSF tau concentration is increased in AD groups compared to controls.

5.2 CSF β -AMYLOID 42 IS DECREASED IN ALZHEIMER'S DISEASE

Two ELISA methods were used for detection of $A\beta_{42}$ in CSF samples. The Innostest™ β -amyloid (1-42) ELISA method used in the longitudinal study (**IV**) resulted higher $A\beta_{42}$ concentrations in CSF of AD patients compared to the method developed by Mehta and colleagues (1998) .

CSF A β 42 concentrations were decreased in AD patients and in patients with other dementias compared to neurological controls (III). The lowest A β 42 concentrations were found in definite AD cases. The mean A β 42 level was lower in definite and probable AD compared to clinically diagnosed other dementia patients, but only the difference between the definite AD and other dementias achieved statistical significance. (Figure 2.)

The cutoff value of 340 pg/ml distinguished probable AD patients with a sensitivity of 69 % and definite AD patients with a sensitivity of 78 %. The specificities of the test were 85 % to separate patients with AD from neurological controls and 59 % to separate patients with AD from other dementias.

There was a significant reduction of CSF A β 42 concentrations with time in the follow-up study (IV). The A β 42 levels decreased in 82 % of AD patients, and the decrease was more pronounced in AD patients with a disease duration of two years or less at baseline as compared to those who had a the duration of more than two years at baseline. In the cross-sectional study (III), decreased A β 42 concentrations were found already in AD patients with mild dementia, and there was a positive correlation between A β 42 levels and MMSE scores.

In the analysis of CSF A β 40, similar levels were observed in probable AD patients, other dementia patients, and controls. The lowest A β 40 concentrations were found in severely demented definite AD cases (study III) (Figure 2). CSF A β 40 levels showed a decrease in 87 % of AD patients in the follow-up of three years (study IV).

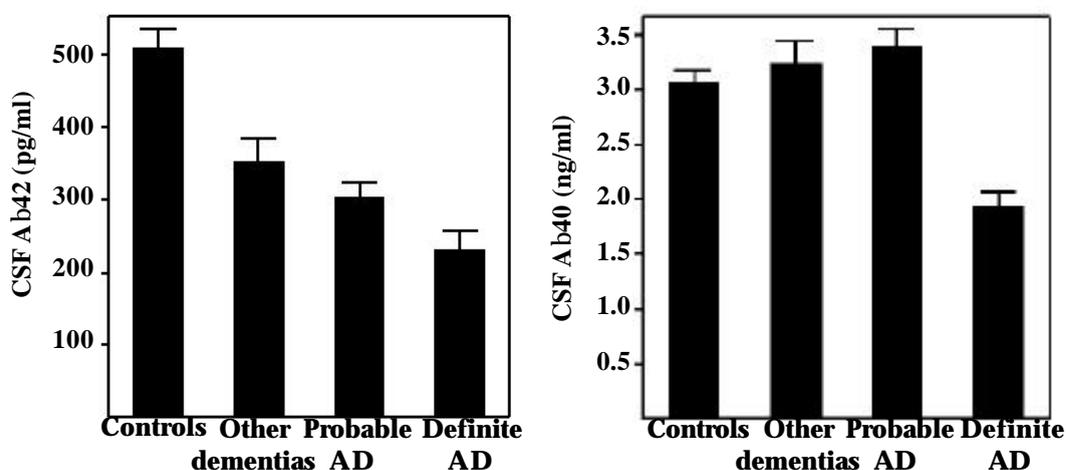


Figure 2. Mean \pm SE CSF A β 42 and A β 40 in the study groups. CSF A β 42 levels are decreased in patients with AD and other dementias as compared to controls. A β 40 is decreased only in severely demented definite AD cases.

5.3 COMBINATION OF CSF b-AMYLOID 1-42 AND TAU

Combination of CSF A β 42 and tau increased the specificity of the test (III). Using the cutoff values 340 pg/ml for A β 42 and 380 pg/ml for tau, the specificities were 95 % for controls, and 85 % for other dementias. Ninety-one percent of the patients with low A β 42 and high tau had AD. However, the sensitivities were low, 53 % for probable AD and 46 % for definite AD cases. (Figure 3)

The AD index ($\tau \times A\beta_{40}/A\beta_{42}$) and A β -ratio ($A\beta_{40}/A\beta_{42}$) did not improve the diagnostic accuracy of the tests. The AD index resulted in a sensitivity of 68 % and specificities of 89 % for controls and 63 % for other dementias. Using the A β -ratio, the sensitivity was 62 % and the specificities were 86 % for controls and 63 % for other dementias.

5.4 RELATION OF CSF BIOMARKERS TO APOLIPOPROTEIN E GENOTYPE

CSF tau and A β_{42} concentrations were related to patients' apoE genotype (**I**, **III**). CSF tau was higher in AD patients carrying at least one apoE $\epsilon 4$ allele as compared to $\epsilon 4$ non-carriers. The highest tau concentrations were found in patients with two $\epsilon 4$ alleles. AD patients carrying at least one apoE $\epsilon 4$ allele had also lower CSF A β_{42} concentrations than AD patients without the $\epsilon 4$ allele, and the lowest levels were observed in patients with two $\epsilon 4$ alleles. CSF tau levels showed a tendency to increase in patients with at least one $\epsilon 4$ allele in neurological control and other dementia groups, but the differences were, however, not statistically significant (**I**). ApoE genotype was not related to CSF A β_{40} concentrations.

Combined analysis of CSF tau and A β_{42} (Figure 3) showed that 22 AD patients had normal levels of both markers (**III**). Eighteen of these patients (82 %) were apoE $\epsilon 4$ allele non-carriers. The A β_{42} analysis resulted in a sensitivity of 84 % among $\epsilon 4$ carriers, and a sensitivity of 54 % among patients without $\epsilon 4$ allele. The corresponding values for CSF tau measurement were 71 % ($\epsilon 4$ carriers) and 44 % ($\epsilon 4$ non-carriers).

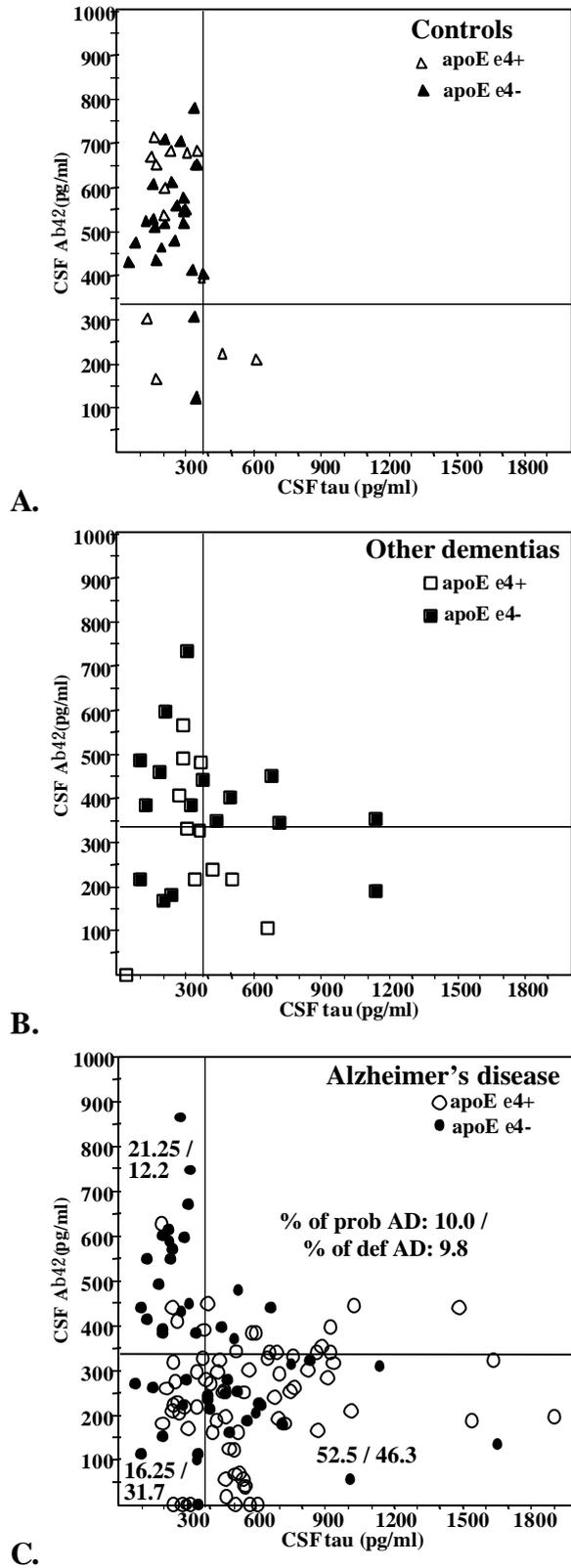


Figure 3. Combination of CSF tau and A β 42 analysis using the cutoff values 380 pg/ml for tau and 340 pg/ml for A β 42. **A.** Control patients. **B.** Other dementias. **C.** Alzheimer's disease Percentages of probable and definite AD patients in each quadrant are shown.

5.5 RELATION OF CSF BIOMARKERS TO BRAIN PATHOLOGY

CSF tau concentrations correlated with the NFTs in brain tissue (II). The NFT scores were available from 43 AD cases. This group included 30 definite, 10 probable, and 3 possible AD cases. A significant positive correlation was noted between tau values and NFT scores in temporal, frontal and parietal cortex, but not in CA1 area of hippocampus. Among definite AD cases, a statistically significant correlation was found only in the temporal cortex. Astrocytosis in brain as evidenced by GFAP immunoreactivity was analysed from the same patients, but no correlations were noted between CSF tau and GFAP immunoreactivity.

Studies on the relationship between CSF β -amyloid levels and brain pathology are ongoing. Preliminary analyses showed that CSF A β 42 or A β 40 levels did not correlate with cortical amyloid plaque counts. Negative correlations were noted between neuritic plaques in CA1 area of hippocampus and CSF A β 42 and CSF A β 40. Immunohistochemistry of β -amyloid in parietal, temporal and frontal cortices showed a negative correlation with A β 42, but not with A β 40 (unpublished data).

5.6 CSF TAU AND Ab42 IN DOWN SYNDROME

There was a marked inter-individual variation in CSF tau and A β 42 values in Down syndrome patients, and a positive correlation between age and CSF tau and a negative correlation between age and CSF A β 42 levels (unpublished data). Older Down syndrome patients (age > 40 years) had significantly lower CSF A β 42 levels compared to controls (unpublished data).

5.7 ASPARTATE AMINOTRANSFERASE ACTIVITY IN ALZHEIMER'S DISEASE

CSF AST activity was increased in AD patients compared to neurological control patients or patients with psychosomatic disorders, but there was a considerable overlap of values in these groups (V). Also patients with other dementias had higher AST activity than patients with psychosomatic disorders. However, AST activity did not differ significantly between AD patients and the other dementias.

AST was increased early in the course of AD, but there was no correlation between AST activity and MMSE scores or duration of the disease. No correlations were found between age and CSF AST activity in any of the groups. ApoE genotype was not related to AST activity in any of the study groups.

6. DISCUSSION

The present series of studies describes the usefulness of CSF tau (**I, II, IV**), A β 42 (**III, IV**), A β 40 (**III, IV**) and AST activity (**V**) analyses in the diagnosis and follow-up of patients with AD. CSF A β 42 and tau concentrations were significantly changed in AD patients. These changes were found already during the early stages of the disease, and they were related to the patients' apoE genotype and to the neuropathological changes in brain. In the long-term follow-up of AD patients, A β 40 and A β 42 levels in CSF decreased.

6.1 METHODOLOGICAL CONSIDERATIONS

All patients and controls participating in the study underwent careful clinical examinations. They were recruited in the university hospitals, and as in all non-epidemiological studies, a possible selection bias should be noted. Neuropathological confirmation of AD was available for 48 AD cases, other patients had a clinical diagnosis. In clinically diagnosed AD patients, there exists the possibility for misdiagnosis though the accuracy of the clinical AD diagnosis is high. The presence of AD changes in the brains of controls and especially in patients with other dementias cannot be excluded due to the lack of neuropathological data from these patients.

The lumbar punctures were obtained using a standardized protocol, and repeated freezing and thawing of the samples was avoided. Metabolic changes in brain are well-reflected in CSF, but the lumbar puncture is an invasive method and carries a small risk for the patient. Therefore a blood test for AD would be very valuable, but currently available methods for plasma analysis have not proven useful in the diagnosis of sporadic AD. Increased A β 42 concentrations in plasma of AD patients and presymptomatic individuals carrying some rare APP or presenilin mutations have been reported (Scheuner et al. 1996), but no significant changes in soluble A β 42 levels have been observed between sporadic AD cases and controls (Mehta et al. 2000). However, some studies imply that plasma β -amyloid concentration may be increased in presymptomatic AD cases (Mayeux et al. 1999). The ongoing studies on patients with mild cognitive impairment will reveal the value of plasma β -amyloid measurements in the diagnosis of AD.

The ELISA methods and the detection method for AST activity that were used in this study have been characterized in earlier studies. All analyses were carried out in duplicate, and in the β -amyloid analyses an internal control sample was included. The tau ELISA used in this study detects the total tau protein or tau fragments in CSF. The abnormal phosphorylation of tau is widely accepted as a key feature in the formation of neurofibrillary tangles, and the detection of the abnormally phosphorylated tau in CSF (Ishiguro et al. 1999, Vanmechelen et al. 2000) might improve the diagnostic use of the test. The β -amyloid ELISAs detect the soluble β -amyloid in CSF, and the aggregation and binding of β -amyloid to some carrier proteins might hinder their detection in these ELISA-assays. One possible improvement for the β -amyloid assays might be the detection of aggregated forms of β -amyloid in the CSF of AD patients (Pitschke et al. 1998). In the analysis of AST activity in CSF we used a colorimetric measurement system, while Riemenschneider et al. (1997) used a detection method based on kinetic ultraviolet techniques, which should be taken into account in the interpretation of the results. However, the results of colorimetric assays have been found to correlate well with other methods.

6.2 SPECIFICITY OF CSF TAU AND Ab42 ANALYSES

According to the criteria published by the Ronald and Nancy Reagan Research Institute of the Alzheimer's Association and the National Institute on Aging Working Group (1998), the specificity (percentage of individuals without AD with a negative test result from all individuals without AD) of an ideal diagnostic test should be more than 85 %.

In our study, the specificity for the cutoff value of 400 pg/ml for CSF tau was 88% for controls. In all studies, CSF tau has been shown to separate with high probability healthy controls or neurological control patients without dementia from patients with AD (Table 2). The specificity to differentiate AD from other dementias in our study was 60 %, indicating that increased CSF tau levels are also found in other dementias. Specificities for CSF tau to separate AD from other dementias in other studies are shown in Table 2. Studies reporting CSF tau levels in some specific clinically diagnosed neurological disorders other than AD are also available, and these studies have shown increased CSF tau concentrations in frontotemporal dementia (Arai et al. 1997b, Green et al. 1999), Creutzfeld-Jacob's disease (Otto et al. 1997), in patients with head trauma (Zemlan et al. 1999), normal pressure hydrocephalus (Kudo et al. 2000), and neuro-AIDS (Andersson et al. 1999). On the contrary, there are also reports of normal CSF tau levels in vascular dementia (Mori et al. 1995, Arai et al. 1998), frontotemporal dementia (Sjögren et al. 2000), in alcoholics with or without dementia (Morikawa et al. 1999), and in dementia with Lewy bodies (Kanemaru et al. 2000).

Table 2. Sensitivity and specificity of CSF tau (AD=Alzheimer's disease, OD=other dementias, C=controls). *Healthy controls. #Patients with degenerative diseases included.

CSF tau	AD N	OD N	C N	Sens. (%)	Spec. (%)	cutoff
Vandermeeren et al. (1993)	81	-	51 129#	81	96 (AD vrs. C) 64 (AD vrs. C)	1.11 pg/ml
Arai et al. (1995)	70	-	19*	98.6	100 (AD vrs. C)	22.6 pg/ml
Jensen et al. (1995)	48	-	22* 31	95	93 (AD vrs. C)* 87 (AD vrs. C)	180 ng tau/ g prot
Motter et al. (1995)	37	-	20* 32	59	95 (AD vrs. C) 97 (AD vrs. C)	312 pg/ml
Vigo-Pelfrey et al. (1995)	71	25	59	39	100 (AD vrs. C) 96 (AD vrs. OD)	400 pg/ml
Blennow et al. (1995b)	44	28	31*	84	97 (AD vrs. C) 39 (AD vrs. OD)	258 pg/ml
Galasko et al. (1997)	36	9	14* 10	80.5		312 pg/ml
Andreasen et al. (1998)	43	21	18* 18	95	94 (AD vrs. C)* 56 (AD vrs. C) 14 (AD vrs. OD)	306 pg/ml
Kurz et al. (1998)	40	-	36	89	97 (AD vrs. C)	260 pg/ml
Kanai et al. (1998)	93	33	41* 56	40	86 (AD vrs. OD + C)	474 pg/ml
Galasko et al. (1998)	82	74	60*	57	83 (AD vrs. C)	502.5 pg/ml
Andreasen et al. (1999b)	407	-	65* 28	93	86 (AD vrs. C)	302 pg/ml
Hulstaert et al. (1999)	150	79	100* 84	85	65 (AD vrs C)	
Kahle et al. (2000)	30	-	16* 29	63	75 (AD vrs. C)* 96 (AD vrs. C)	530 pg/ml
Our study (I)	81	40	33	58	88 (AD vrs. C) 60 (AD vrs. OD)	400 pg/ml

The specificity for CSF A β 42 analysis in our study was 85 % for controls and 59 % for other dementias. Similarly, a high specificity of A β 42 for controls, but lower specificity for other dementing disorders, has also been reported in other studies (Table 3). Hulstaert et al. (1999) showed that nearly half of the clinically diagnosed patients with vascular dementia had decreased A β 42, and low A β 42 levels has been reported in Lewy body dementia (Kanemaru et al. 2000) and in Creutzfeldt-Jakobs disease (Otto et al. 2000). However, normal A β 42 levels have been reported in frontotemporal dementias (Sjögren et al. 2000), and A β 42 levels may increase after traumatic brain injuries (Raby et al. 1998, Emmerling et al. 2000).

Table 3. Sensitivity and specificity of CSF A β 42 analysis (AD=Alzheimer's disease, OD=other dementias, C=controls). *Healthy controls, #patients with degenerative diseases included.

Ab42	AD N	OD N	C N	Sens. (%)	Spec. (%)	cutoff
Motter et al. (1995)	37		20* 32	100	63 (AD vrs. C)	505 pg/ml
Kanai et al. (1998)	93	33	41* 56	94	47 (AD vrs. OD + C)	256 fmol/ml
Galasko et al. (1998)	82	74	60*	78	83 (AD vrs. C)	1031.5 pg/ml
Andreasen et al. (1999a)	53		21	92	90 (AD vrs. C)	1130 pg/ml
Hulstaert et al. (1999)	150	79	100* 84	85	55 (AD vrs C)	
Our study (III)	121	27	39	72	86.4 (AD vrs. C) 59.3 (AD vrs. OD)	340 pg/ml

In our study, the combination of CSF tau and A β 42 resulted in specificities of 95 % for controls and 85 % for other dementias. Currently there are six studies, including the present one, which have reported the combination of CSF A β 42 and tau levels in AD (Table 4), and in all studies the combination of these two biomarkers have resulted in a specificity of at least 85 % for controls. The specificities for other dementias have varied from 58% to 85 % (Table 4).

Table 4. Combination of CSF tau and A β 42 analysis (AD=Alzheimer's disease, OD=other dementias, C=controls). *healthy controls

Combination of Ab42 and tau	AD N	OD N	C N	Sens. (%)	Spec. (%)
Motter et al. (1995)	37	-	20* 32	59	96 (AD vrs. C)
Kanai et al. (1998)	93	33	41* 56	71	83 (AD vrs.OD + C)
Galasko et al. (1998)	82	74	60*	77	93 (AD vrs. C) 65 (AD vrs OD)
Shoji et al. (1998)	55	23	34* 45	69	88 (AD vrs OD+C)
Hulstaert et al. (1999)	150	79	100* 84	85	86 (AD vrs. C) 58 (AD vrs. OD)
Our study (III)	121	27	39	50	95 (AD vrs. C) 85 (AD vrs. C)

There are, however, some aspects related to the study populations and the origin of β -amyloid and tau in CSF that need to be taken into account when the specificity of an antemortem test for AD is determined. In clinically diagnosed patients, the co-existence of AD pathology in other dementing disorders cannot be excluded. Neither in our study nor in any other biomarker study, has it been possible to carry out a follow-up and exclusion of AD changes after neuropathological analysis. This has a confounding effect on the specificity of CSF tau and A β 42 as diagnostic tests. Jellinger and colleagues (1990) have shown that co-existence of AD pathology is a common finding in brains of patients with other dementias. Also, in demented patients with Parkinson's disease, neuropathological changes related to AD are not rare findings (Mattila et al. 1998). Thus it is possible that low levels of CSF A β 42 and high tau concentrations in a patient in non-AD dementia group are related to the co-existence of AD pathology. On the other hand, it is important to include a group of patients with neurodegenerative diseases other than AD in the studies to characterise whether or not the test is related specifically to AD, instead of general neurodegeneration. Although CSF A β 42 and tau theoretically reflect only AD pathology, there is no direct evidence for a relationship linking these CSF biomarkers and the formation of plaques and tangles. In addition to non-AD dementia groups, it is important to have well-characterized control groups. For example, the possibility that there are patients with mild cognitive impairment, or young individuals in the control group, may result in underestimation or overestimation of the specificity of the test.

6.3 SENSITIVITY OF CSF TAU AND Ab42: CONFOUNDING FACTORS

In our study, the sensitivity (percentage of AD patients with the test result indicating the presence of the disease from all AD patients) for CSF tau, using the cutoff value of 400 pg/ml, was 58 %, indicating that a group of AD patients have a CSF tau concentration, which is at the same level as neurological controls. For definite AD, the sensitivity for CSF tau was 46.3 %. In other studies, sensitivities have ranged from 39 % to 98 % (Table 2). The sensitivity for A β 42 using the cutoff value of 340 pg/ml to find AD patients was 69 % for clinically diagnosed probable AD and 78 % for definite AD. Similarly, other studies have shown that the decrease of A β 42 is not observed in all clinically diagnosed AD patients (Table 3). Combination of CSF A β 42 and tau resulted in sensitivities of 53 % for probable AD, and 46 % for definite AD. Interestingly, in the combined analysis of A β 42 and tau, most patients with false negative results in the probable AD group (only clinical diagnosis) had both normal tau and A β 42 levels, whereas in definite AD (neuropathological confirmation) most false negative values resulted from normal tau but decreased A β 42 levels (see results, figure 3). The sensitivities of the combined analysis in other studies are shown in Table 4.

Factors that affect the levels of the biomarkers, and thus resulted in low sensitivity of the tests, are unclear. One possible explanation for the variation in the levels of CSF A β 42 and tau within the AD group is the heterogeneity of AD. This includes individual variations in the duration and progression of the disease that may result in longitudinal changes in CSF biomarkers, differences in the extent of neuropathological changes in brain, and genetic heterogeneity, such as apoE genotypes. Furthermore, in most studies only clinically diagnosed AD patients have been included, and thus it is possible that some of the patients diagnosed as having probable AD, have instead some other dementing disorder that does not result in changes of CSF tau or A β 42 levels.

Some studies (McKee et al. 1991, Hansen et al. 1993) have reported that approximately 20 % of AD cases are plaque-only or plaque-predominant AD cases with few neurofibrillary tangles, which might result in normal CSF tau level. Furthermore, in brains of AD patients, the total levels of A β 42,

A β 40 (Wang et al. 1999) and tau (Khatoun et al. 1994) show large individual variations, which may be reflected in variations in the CSF levels of these biomarkers.

In our study, the apoE genotype was significantly related to the levels of biomarkers, which resulted in low sensitivity of the tests. We found that AD patients who did not carry the apoE ϵ 4 allele had the lowest CSF tau concentrations with considerable overlap between the values of control patients. In contrast, 94 % of the AD patients with two ϵ 4 alleles had a CSF tau concentration above the cutoff level of 400 pg/ml. If only ϵ 4 carriers were included, the sensitivity of CSF tau increased to 71 %. CSF A β 42 was higher in AD patients without the ϵ 4 allele compared to those with an ϵ 4 allele, and the lowest concentrations were observed in those patients with two ϵ 4 alleles. We found that the sensitivity of A β 42 was 83.6% for AD patients carrying an ϵ 4 allele (90.5% for definite AD), whereas in AD patients without an ϵ 4 allele it was 54.2%.

Our results are in agreement with other studies that have shown higher CSF A β 42 concentrations in AD patients who do not carry the ϵ 4 allele (Galasko et al. 1998, Hulstaert et al. 1999). Other studies (Golombowski et al. 1997, Molina et al. 1999) have also found this dose dependent effect of the apoE ϵ 4 allele on CSF tau levels in AD patients, and ϵ 4 allele has been linked to increased CSF levels of tau in the follow-up of AD patients (Blomberg et al. 1996, Kanai et al. 1999). In contrast to our results, there are studies that have not detected any effect of the apoE genotype on CSF tau values (Arai et al. 1995, Motter et al. 1995, Nitsch et al. 1995, Skoog et al. 1995, Galasko et al. 1998). Furthermore, if only neuropathologically confirmed AD cases were included in our study, patients carrying the ϵ 4 allele had higher mean CSF tau concentrations and lower A β 42 levels than ϵ 4 non-carriers, although the differences were not statistically significant.

The divergent results of the effect of apoE genotype on the levels of biomarkers may be related to the differences in patient populations or in the number of patients in each group, and to the neuropathological changes. However, in addition to apoE it is also possible that there are some other yet unknown genetic factors that influence the levels of the biomarkers. In our study there were no significant differences in age, age at onset, duration of dementia or severity of dementia between patients with at least one ϵ 4 allele and those without the ϵ 4 allele. Furthermore, in other studies of AD patients in our department, the apoE genotype has been found to influence other parameters such as MRI-findings (Lehtovirta et al. 2000) and brain pathology (Alafuzoff et al. 1999, Overmyer et al. 1999). The CSF findings are consistent with the neuropathological data that have revealed more severe AD pathology in AD patients carrying the ϵ 4 allele. Some studies have shown that AD patients with the ϵ 4 allele have increased amounts of NFTs compared to those without the ϵ 4 allele (Nagy et al. 1995, Polvikoski et al. 1995), and there is much evidence that β -amyloid deposits are more numerous in AD patients with at least one apoE ϵ 4 allele (Rebeck et al. 1993, Schmechel et al. 1993), which might be related to the decrease in CSF A β 42 levels in these patients.

6.4 RELATIONSHIP BETWEEN CSF MARKERS AND BRAIN PATHOLOGY AND PROGRESSION OF THE DISEASE

The relationship between CSF markers and brain pathology is unknown, and it is not clear whether the AD changes in brain are directly related to β -amyloid and tau concentrations in CSF. Our study included neuropathologically confirmed cases, and thus it was also possible to study the relationship between CSF findings and neuropathology in brain. It is not known whether CSF tau reflects the degeneration of neurons in general or the neurofibrillary changes occurring in AD brain. The β -

amyloid levels in CSF may reflect the net effect of the production and clearance of the β -amyloid peptides in brain.

The positive correlation between NFTs in brain and CSF tau concentration indicates that the elevated levels of CSF tau in AD are related to the severe neurofibrillar pathology. A correlation does not directly imply that there is a causal relationship between the two variables. NFTs are often associated with other neuropathological changes, such as neuronal cell death and axonal damage, which may result in leakage of tau into the extracellular space and CSF. The latter hypothesis is supported by the facts that there is an increased concentration of CSF tau in Creutzfeldt-Jacob's disease (Otto et al. 1997) and in patients with head trauma, whose CSF tau levels decrease with the clinical improvement (Zemlan et al. 1999). However, the reports of normal CSF tau levels in frontotemporal dementias (Sjögren et al. 2000) or vascular dementias (Arai et al. 1998), suggest that there are also other processes that are capable of increasing the CSF tau concentration.

Low A β 42 levels may be related to the change in the clearance of β -amyloid and accumulation into plaques, or reflect the reduction in β -amyloid producing neurons. Our preliminary data showed that the number of cortical senile plaques does not correlate with CSF A β 42 levels, but that the total β -amyloid immunoreactivity has a negative correlation with CSF A β 42 concentrations. In a transgenic mouse model of AD, Kawarabayashi and colleagues (2001) have identified that a marked increase of β -amyloid levels in brain results in decreased levels of A β 42 in CSF. These findings support the hypothesis that the increased accumulation of β -amyloid in brain is related to the decrease of CSF A β 42 levels. Recent studies showed that A β 42 forms the first aggregates in AD brain, and that the aggregation of A β 40 is related to the increased deposition of neuritic plaques. It is interesting to note that in our study, CSF A β 40 levels were decreased at the end stage of AD, in definite AD cases, which may be related to the formation of A β 40 positive plaques from diffuse A β 42 containing plaques, or to the loss of A β 40 producing neurons.

Our results are consistent with the hypothesis that these markers are closely related to the pathology in AD brain. Therefore, these biomarkers could be useful in evaluating the progression of the disease. However, we found that in the long-term follow-up CSF tau levels did not show any consistent changes, because in 47 % of the patients, CSF tau concentration decreased, whereas in 53 % of the patients CSF tau increased. Previous studies have reported that tau levels increase with time (Kanai et al. 1998), while other studies have shown that the levels remain stable during one or two years of follow-up (Andreasen et al. 1998, Andreasen et al. 1999b, Sunderland et al. 1999). Some studies have revealed a significant increase in CSF tau levels only in AD patients carrying the apoE ϵ 4 allele (Blomberg et al. 1996, Kanai et al. 1999). On the other hand, the study of Isoe et al. (1996) showed in a small series of patients that CSF tau increases during the early stages of the disease and decreases in patients with severe disease that might be related to severe degeneration of brain and subsequent lack of the sources of the tau protein. It is also shown that increased CSF tau levels are better at identifying young AD patients (<70 years) from controls, but are not so good for old AD patients (>70 years) (Burger nee Buch et al. 1999). In our study, low levels of CSF tau were found more frequently in severely demented definite AD patients than in probable AD (Figure 3 C).

In the cross-sectional studies, we showed that CSF tau levels were not related to the MMSE scores or to the duration of early AD, which suggests that CSF tau does not appear to be very useful in monitoring the progression of AD. Most of the studies (Blennow et al. 1995b, Nitsch et al. 1995, Andreasen et al. 1998, Andreasen et al. 1999b) have not found any significant correlations between CSF tau and duration of dementia. Also the lack of a correlation between CSF tau concentration

and MMSE scores in our study is consistent with other studies (Blennow et al. 1995b, Motter et al. 1995, Vigo-Pelfrey et al. 1995, Andreasen et al. 1998).

In contrast to CSF tau protein, our longitudinal study showed that CSF A β 42 levels decreased with time in 82 % and A β 40 levels in 87 % of patients with AD. However, previous follow-up studies have not shown significant changes of CSF A β 42 levels (Kanai et al. 1998, Andreasen et al. 1999a). The differences in the length of follow-up periods may explain the discrepancies in the results. The follow-up periods in these earlier studies were considerably shorter (mean 10-20 months) compared to the fixed follow-up time of three years in the present study. These results suggest that during the course of AD the changes in CSF β -amyloid develop slowly, and thus with an extended follow-up period, CSF A β levels will decrease. Therefore, CSF A β 42 levels may be useful in monitoring the long-term progression of AD.

In non-demented controls, A β 40 concentration may decline with aging, while no correlation between age and CSF A β 42 has been reported (Kunicki et al. 1998). The decreased A β 42 levels in CSF may be related to the progression of AD. In the cross-sectional study we found a positive correlation between MMSE scores and CSF A β 42 levels, which is consistent with previous studies (Jensen et al. 1999, Samuels et al. 1999). Our results of longitudinal analysis showed the most pronounced decrease in CSF A β 42 concentrations in patients with short duration of the disease, which is supported by the early accumulation of A β 42 in AD brain. Furthermore, it has been shown that decreased A β 42 concentrations are found already in those patients with mild cognitive impairment who later developed AD (Andreasen et al. 1999c). In addition to accumulation, it is possible that the production of A β 42 decreases with time due to the loss of neurons, and there may be a floor effect resulting in changes that are too small to be detected in longitudinal studies in patients with advanced forms of the disease.

6.5 CSF ASPARTATE AMINOTRANSFERASE ACTIVITY IN THE DIAGNOSIS OF DEMENTIA

We could not confirm the report which has shown that measurement of AST activity in CSF may be useful in distinguishing AD from other types of dementia (Riemenschneider et al. 1997). Our results showed that AST activity was increased in AD compared to controls, but no differences were observed in comparison with other dementias. Furthermore, there was a significant overlap in values between AD patients and neurological controls. The lowest AST activities were observed in patients with psychosomatic disorders. Compared to this group, patients with other neurological diseases, a group that included 13 patients with amyotrophic lateral sclerosis, had increased AST activity.

In addition to methodological differences, one possible explanation for the differences in the results is the group of patients with other dementias. The study of Riemenschneider and colleagues (1997) included 12 clinically diagnosed patients with other dementias, such as normal pressure hydrocephalus, frontal lobe degeneration, cerebrovascular dementia, progressive supranuclear palsy, and unclassified dementia. In our study, we had a total of 49 clinically diagnosed patients with other dementias including patients with vascular dementia, Lewy body dementia, frontal dementia or unspecified dementia. Our results of increased AST activity in patients with other neurological diseases suggest, that CSF AST activity reflects the general degeneration. Also earlier studies have suggested that increased AST activity in CSF reflects non-specific central-nervous-system damage (Maas 1977). However, Jansen-Steur et al. (1998) showed that there were no differences in CSF AST activities between demented or non-demented Parkinson's disease patients and controls.

6.6 THE ROLE OF CSF A β AND TAU IN DIAGNOSING ALZHEIMER'S DISEASE

Recent development of therapies for AD will increase the need for the early diagnosis, or better still the detection of presymptomatic cases of the disease. Currently, the time of the appearance of the first AD changes in CSF is not clear. We found that CSF tau is increased at an early stage of AD, which has been observed also by other investigators (Riemenschneider et al. 1996, Galasko et al. 1997, 1998, Kurz et al. 1998, Andreasen et al. 1999b). Furthermore, Andreasen and colleagues (1999c) have shown that increased CSF tau is found in patients with mild cognitive impairment, who are at risk for developing AD. The CSF A β 42 concentration was decreased in AD already during the early stages of the disease in our study. Many studies have shown similar results in patients with clinically diagnosed AD (Galasko et al. 1998, Andreasen et al. 1999a, Riemenschneider et al. 2000). In the study of Jensen et al. (1999) there was, however, an increase in the A β 42 level in the early stage of AD compared to healthy controls. Nonetheless, the role of decreased CSF A β 42 as a very early biomarker for AD is further supported by Andreasen et al. (1999c) who reported a decrease in A β 42 levels in patients with mild cognitive impairment who developed AD during the follow-up. However, follow-up studies of the CSF changes in patients with mild cognitive impairment or individuals at risk for AD are lacking.

At present, CSF A β 42 and tau represent the most promising biomarkers for AD. In patients with AD CSF tau and A β 42 analyses have shown high specificities. Using a combination of these markers, an AD type profile is characterised by increased CSF tau level and decreased A β 42. These findings support the clinical diagnosis, and CSF A β 42 and tau can be used as additional diagnostic tools. In contrast, negative test results cannot be said to exclude the presence of AD. Therefore, CSF A β 42 and tau are not suitable for diagnostic screening or as the only diagnostic tool. However, almost all of the published studies are based on selected patient populations, and the results need to be confirmed in unselected population based studies with neuropathologically confirmed AD, other dementia, and control cases.

7. CONCLUSIONS

The goals of this thesis were to study the usefulness of CSF A β 42, A β 40, tau protein and AST activity in the diagnosis of Alzheimer's disease. The study material included CSF samples from clinically diagnosed and neuropathologically confirmed AD cases, from neurological controls and from clinically diagnosed patients with dementing disorders other than AD. In addition to a diagnostic use, we wanted to study the long-term changes in CSF A β 40, A β 42, and tau, and to investigate their relation to the neuropathological changes in AD. From the results of these studies we can draw the following conclusions:

1. The specificity of the combined measurement of A β 42 and tau was high. Low levels of A β 42 and high levels of tau support the diagnosis of AD. The combination of these biomarkers may be useful in confirmation of the AD diagnosis, and for selecting AD patients for treatment trials. CSF A β 40 and A β 42 decreased during the long-term follow-up of the patients, suggesting that CSF β -amyloid may be useful in the evaluation of the progression of the disease.
2. CSF tau and A β 42 are early markers of Alzheimer's disease. CSF tau increases and CSF A β 42 decreases in the early stage of AD, and both changes are related to the patient's apoE genotype. This suggests that these biological markers may help to detect AD in individuals at risk for the disease, such as patients with mild cognitive impairment, and apoE ϵ 4 carriers.
3. CSF tau and β -amyloid correlated with the neuropathological changes in brain. This finding indicates that these biomarkers may be useful in screening for the efficacy of treatments directed at preventing the formation of β -amyloid plaques and neurofibrillary tangles.
4. Analysis of CSF AST activity is not very useful in the diagnosis of AD, because there is significant overlap in the values between AD and controls. CSF AST may be more useful in the evaluation of neuronal degeneration.

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