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SANNA-KAISA HERUKKA

Usefulness of Cerebrospinal Fluid Biomarkers in Diagnosis of Early Alzheimer's Disease

Doctoral dissertation

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**Department of Neurology
University of Kuopio**

**Department of Neurology
Kuopio University Hospital**

Distributor: Department of Neurology
University of Kuopio
P.O. Box 1627
FI-70211 KUOPIO
FINLAND
Tel. +358 17 162 682
Fax +358 17 162 048

Author's address: Department of Neurology
University of Kuopio
P.O. Box 1627
FI-70211 KUOPIO
FINLAND
Tel. +358 17 163 213
Fax +358 17 162 048

Supervisors: Professor Tuula Pirttilä, M.D., Ph.D.
Department of Neurology
Kuopio University Hospital

Professor Heikki Tanila, M.D., Ph.D.
Department of Neurobiology
University of Kuopio

Reviewers: Professor Irina Elovaara, M.D., Ph.D.
Department of Neurology
University of Tampere

Professor Juha Rinne, M.D., Ph.D.
Turku PET Centre
University of Turku

Opponent: Professor Gunhild Waldemar, M.D., D.M.Sc
Department of Neurology
Copenhagen University Hospital
Denmark

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ABSTRACT

Alzheimer's disease (AD) is the most common dementing disease and the number of patients with AD is increasing as the population is aged. AD not only has a devastating effect on individual patients and their families, but it also poses an enormous socioeconomic burden to society. AD is a disease that develops slowly over a time range of two decades before the appearance of first symptoms. The neuropathology starts to develop during the preclinical period of the disease, so that at first symptomatic stage the pathology is already advanced. This is a major obstacle in treating the disease because at present there is no way known to repair nerve damage once it has occurred. Previous studies indicated that levels of CSF biomarkers A β 42, tau and phospho-tau are altered in AD patients in comparison to healthy controls or patients with other neurological diseases and they can be used as a diagnostic aid, especially in clinically challenging conditions, for example in the differentiation between mild AD and depression.

At the beginning of this study, little was known about these biomarkers before the onset of clinical AD. Since AD neuropathology starts to develop decades before symptoms, the original hypothesis was that AD associated changes in CSF biomarkers could be detected before the appearance of clinical AD. The main aim of the study was to examine whether these changes would predict the development of AD in patients with mild cognitive impairment (MCI) who have a high risk for suffering AD within a period of a few years. The association between AD associated pathological changes in brain and the CSF biomarker levels were also examined.

The results of this study revealed that the AD associated changes in CSF biomarkers, i.e. decrease in A β 42 and increase in tau and phospho-tau occurred during the predementia stage of AD before the clinical AD diagnosis. CSF A β 42, tau and phospho-tau values were also associated with medial temporal lobe atrophy. The combination of decreased CSF A β 42 and increased tau or phospho-tau could differentiate MCI patients who would develop AD from those who remained stable with high specificity even several years before the clinical diagnosis of AD. These results suggest that the abnormal CSF biomarker levels can be used for confirmation of a clinical diagnosis of possible early AD. However, the sensitivity was low and therefore a negative result cannot exclude presence of AD. A large proportion of MCI patients did not exhibit memory impairment. Executive dysfunction without amnesic symptoms was common, and these patients developed AD almost as frequently as MCI patients with memory impairment.

In conclusion, the pathological changes of the biomarker levels occur early during the course of the disease, and can predict development of AD in patients with cognitive impairment. In the clinical context, patients with cognitive impairment without memory complaint should also be considered to be at high risk for developing AD.

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To dare is to lose one's footing momentarily.

To not dare is to lose oneself.

-Soren Kierkegaard-

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Sanna-Kaisa Herukka

ABBREVIATIONS

AACD	age-associated cognitive decline
AAMI	age-associated memory impairment
A β	amyloid-beta peptide
AD	Alzheimer's disease
ApoE	apolipoprotein E
APP	amyloid precursor protein
AUC	area under curve
BBB	blood brain barrier
CDR	clinical dementia rating
CIND	cognitive impairment no dementia
CJD	Creutzfeldt-Jacob disease
DLB	dementia with Lewy bodies
DS	Down syndrome
EC	entorhinal cortex
FD	frontal degeneration
FTD	frontotemporal dementia
HC	hippocampus
LR	likelihood ratio
MCI	mild cognitive impairment
MMSE	mini mental state examination
MTL	medial temporal lobe
NAS	non aliter specificatus
NFT	neurofibrillary tangle
NINCDS-ADRDA	National Institute of Neurologic and Communicative Disorders and Stroke and Alzheimer's Disease and Related Disorders Association
NSAID	non-steroidal anti-inflammatory drug
OR	odds ratio
PET	positron emission tomography
PIB	Pittsburgh Compound-B
PS	presenilin
ROC	receiver operator characteristics
SPECT	single photon emission computed tomography
TE	time of echo
TR	time of repetition
VaD	vascular dementia
VCI	vascular cognitive impairment

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-IV**.

- I** Liu L, Herukka S-K, Minkeviciene R, van Groen T, Tanila H (2004). Longitudinal observation on CSF A β 42 levels in young to middle-aged amyloid precursor protein/presenilin-1 doubly transgenic mice. *Neurobiology of Disease* 17, 516-523
- II** Herukka S-K, Hallikainen M, Soininen H, Pirttilä T (2005). CSF A β 42 and Tau or phosphorylated Tau and prediction of progressive mild cognitive impairment. *Neurology* 64, 1294-1297
- IIIa** Herukka S-K, Helisalmi S, Hallikainen M, Tervo S, Soininen H, Pirttilä T (2007). CSF A β 42, Tau and phosphorylated Tau, APOE ϵ 4 allele and MCI type in progressive MCI. *Neurobiology of Aging* 28, 507-514
- IIIb** Herukka S-K, Helisalmi S, Hallikainen M, Tervo S, Soininen H, Pirttilä T (2007). Response to: "Heterogeneity of mild cognitive impairment and other predementia syndromes in progression to dementia". *Neurobiology of Aging* (in press)
- IV** Herukka S-K, Pennanen C, Soininen H, Pirttilä T. CSF A β 42, Tau and phosphorylated Tau correlate with medial temporal lobe atrophy. Submitted for publication

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1. INTRODUCTION

The proportion of the elderly is growing steadily as the longevity of the population increases. This leads to greater numbers of patients suffering age-associated neurodegenerative diseases, including dementia. Dementia not only has a devastating effect on the individual patients and their families but it also imposes enormous socioeconomic burden on society. It is estimated that in 2005 there were over 29 million patients with dementia and the worldwide societal costs of dementia were US\$315 billion (Wimo et al. 2007). Over half of the dementia cases are caused by Alzheimer's disease (AD). As the treatments for AD evolve, it will become increasingly important to diagnose AD patients as early and as accurately as possible.

Diagnosis of possible or probable AD is based on the clinical criteria and exclusion of other possible causes for dementia. The criteria include an insidious onset and progressive impairment in memory and other cognitive functions severe enough to cause a dementia syndrome. This is a significant problem, since AD has a preclinical period which may last over two decades. At the time of diagnosis using current criteria, the AD associated neuropathology is already well advanced. Therefore the focus of research on AD diagnostics has shifted to the time when the patient exhibits the first clinical symptoms of the disease, usually memory impairment, but the symptoms are not severe enough to validate a diagnosis of AD. These studies led to development of the concept of mild cognitive impairment (MCI), which refers to a transitional stage between cognitively healthy individuals to patients with clinically diagnosable AD (Flicker et al. 1991). Numerous different diagnostic criteria for MCI have been suggested, but it has become evident that MCI patients represent a very heterogeneous group with different etiologies and prognoses.

A definite diagnosis of AD requires a *post mortem* neuropathological examination of brain. The neuropathological hallmarks of AD include the neurofibrillary tangles (NFT) consisting of hyperphosphorylated tau protein and amyloid plaques consisting of amyloid-beta ($A\beta$) peptide. At the advanced stage of AD, the clinical diagnosis is relatively accurate, but the early diagnosis of AD is challenging. AD associated pathology is also common in patients with other dementing diseases. It is difficult to identify patients with mixed pathology using the current clinical methods. The hypothesis was that since both $A\beta$ and tau can be detected in CSF these proteins hold potential significance as biomarkers for AD. After numerous studies

determining the levels of A β 42 (the more amyloidogenic form of A β), tau and hyperphosphorylated tau (phospho-tau) in AD patients and comparing these values with healthy controls and patients with other neurological diseases, it became evident that CSF levels of A β 42 are decreased whereas those of tau and phospho-tau are increased in AD (Blennow and Hampel 2003).

Even though the CSF A β 42, tau and phospho-tau were established as the most promising biomarkers for AD, there were still many unanswered questions. Most studies have been cross-sectional studies performed in clinical patient series. Therefore the actual association between the neuropathologically confirmed diagnosis and CSF biomarkers was uncertain. Also the mechanisms responsible for the changes in CSF and the association between other AD associated pathological changes for example medial temporal lobe (MTL) atrophy were unclear. Furthermore, the AD patients in these studies had relatively advanced disease and there was no data available on CSF A β 42, tau and phospho-tau from the preclinical period of AD. A few small studies including MCI patients had been published, but the heterogeneity of MCI complicates the interpretation of the results of cross-sectional studies. In addition, the follow-up period needs to be several years to reliably detect those MCI patients who develop AD. In this context the present series of studies was initiated, to study the predictive value of CSF biomarkers for the development of dementia in patients with MCI and to examine the association between the CSF biomarkers and AD associated pathological changes.

2. REVIEW OF THE LITERATURE

2.1 ALZHEIMER'S DISEASE IS A STAGE CONCURRENT DISORDER

When Alois Alzheimer described his patient, Auguste D, about 100 years ago, the syndrome that was later named AD was thought to be a rare disease causing presenile dementia. Subsequently, it was revealed that AD is in fact the most common form of dementia, responsible for over half of all dementia cases. The rare early onset AD is caused by dominant mutations and this form runs in families, whereas in most of the cases, the onset of the disease occurs later, after the age of 65 years. There are genetic risk factors also for late onset AD but the late onset AD genes are not causative factors, they only increase the risk for AD.

Dementia is a syndrome that is defined by a decline in multiple cognitive domains so severe to cause impairment in functional abilities and difficulties in activities in daily living. These symptoms may be caused by different etiologies, but neuropsychiatric causes such as depression and delirium must be excluded. The diagnosis of dementia is currently based on DSM-IV (American Psychiatric Association 1994). AD is a slowly progressing disease that is currently diagnosed based on the clinical criteria proposed by the National Institute of Neurologic and Communicative Disorders and Stroke and Alzheimer's Disease and Related Disorders Association (NINCDS-ADRDA) (McKhann et al. 1984). The criteria include insidious onset, progressive impairment in memory and other cognitive functions plus exclusion of other possible causes for dementia. Clinical work up includes taking medical history and performing medical, neurological and neuropsychological examination. Routine blood tests are done to exclude some treatable causes of dementia, for example B12 vitamin deficiency. Brain imaging, usually CT scan, is done to exclude for example vascular disease and normal-pressure hydrocephalus. The objective of the clinical assessment is to exclude the possibility of clinical conditions other than AD.

The final clinical diagnosis can be either possible or probable AD, since the definite AD diagnosis is made by histopathological analysis of brain tissue obtained either by biopsy or during autopsy. The neuropathological changes in AD brain include amyloid plaques and NFTs, accompanied by neuronal death and synaptic loss in the affected areas. The initial

neuropathological changes may occur as early as two decades before the onset of clinical symptoms (Selkoe 2001).

2.1.1 Neuropathological changes

2.1.1.1 Amyloid pathology

The quintessential feature of AD pathology is the presence of amyloid plaques in the cortical areas of the brain. The main component of the amyloid plaques is A β peptide (Masters et al. 1985). The A β peptide is a derivative of a larger cell membrane spanning protein, amyloid precursor protein (APP). The A β peptide is formed by sequential cleavages of the APP, and it occurs as peptides with different numbers of amino acid residues. The most extensively studied species of A β peptides are the peptides containing 40 (A β 40) and 42 (A β 42) amino acid residues. Both forms occur in amyloid plaques, but the A β 42 is deemed to be more amyloidogenic and therefore more damaging to the brain.

The formation of amyloid plaques is thought to be initiated by A β 42 but subsequently also A β 40 starts to accumulate (Iwatsubo et al. 1994). The diffuse plaques occur early in the course of the disease. They can be detected only by antibodies staining A β and they are not surrounded by other neuropathological changes. Dense amyloid plaques contain fibrillar A β and also several other proteins, for example apolipoprotein E (ApoE). They can be detected by stains that are used for all amyloids found in the body, for example thioflavin-S. The dense plaques are also associated with dystrophic neurites and inflammatory processes.

The search for the AD causing gene was facilitated by the finding that patients with Down syndrome (DS), who have a third copy of chromosome 21, develop AD type amyloid pathology at an early age. This association located the APP gene into chromosome 21 and in time, it was characterised and mutations in the APP gene were confirmed to cause early onset AD (Goate et al. 1991). There are several isoforms of APP, but the main isoform found in neurones is APP₆₉₅ and this includes the A β sequence. It has been suggested that there are physiological and pathological metabolic routes for APP. When APP is cleaved by α -secretase, which cleaves APP from the middle of the A β sequence, production of A β peptide is prevented (Haass 2004). During the formation of A β peptide, the APP is cleaved by β - and

γ -secretases (Haass 2004, Vassar et al. 1999, Wolfe 2006). All of the currently known mutations that cause familial AD are found in the APP gene or the genes encoding presenilin 1 or 2. The presenilin 1 and 2 are essential parts of the γ -secretase complex and are thus crucial in determining the length of the generated A β peptide (Wolfe 2006). All familial AD mutations, except the Arctic APP mutation, selectively increase the formation of A β 42 and therefore may promote fibrillation of the A β into amyloid plaques (Scheuner et al. 1996). The Arctic mutation lowers secreted levels of both A β 40 and A β 42 (Nilsberth et al. 2001). The intracellular location of APP is altered so that the generated A β remains in the intracellular compartment and there is less available APP for production of secreted A β (Sahlin et al. 2007).

One proposed mechanism for development of AD neuropathology is the so-called amyloid cascade hypothesis (Hardy and Selkoe 2002). Not only the above mentioned findings but also the vast number of subsequent studies on APP metabolism in AD explains why this theory is the most popular (but not the only) mechanism to explain the primary pathogenesis of AD. The pathological cascade begins when some factor, for example a genetic risk factor, aging or an environmental factor, increases the formation of A β 42 or decreases its clearance from the brain. Should the initiating factor be a causative dominant genetic mutation, then the A β 42 load increases rapidly and the onset of symptoms happens at an early age. In late onset, sporadic AD, the progression of the pathology is slower and the symptomatic stage is reached later. Surprisingly, it was revealed that neither A β itself nor the accumulated A β plaques were particularly toxic to neurones. Instead, A β becomes toxic only after monomeric A β accumulates into oligomers (Walsh et al. 2002). These oligomers then induce an immunological response and mediate oxidative stress and impair the function of the affected neurones. Once a threshold in the pathology is passed, neuronal dysfunction and loss cause the first cognitive symptoms. Recently, a specific oligomer that includes twelve A β 42 units was found to be the main culprit in causing the toxic effects of A β in transgenic mice (Lesne et al. 2006). If this finding is corroborated by studies on humans, this might offer a new target molecule for both diagnosis and treatment of AD. The significance of intracellular A β in the pathological process is somewhat unclear, but it is possible that it also contributes to the pathology.

2.1.1.2 Tau pathology

In neurones, the tremendous length of the axon when compared to cell body complicates the maintenance of normal function of synapse. Therefore, neurones have very sophisticated machinery to guarantee a functional gateway between the body and periphery of the cell. This consists of microtubules that run along the axon and several different proteins that are involved in the trafficking of essential particles from the cell body to the synapse and back from synapse to body. These particles include cell organelles such as mitochondria and vesicles containing proteins derived from golgi, e.g. tau is one of these microtubule-associated proteins. The main function of tau is to stabilize the microtubules in axons but it also reduces binding of a motor protein called kinesin to microtubules, which in turn obstructs axonal transport (Ebner et al. 1998). Impaired axonal transport by tau hinders the growth of the neurites and makes them more vulnerable to oxidative stress (Stamer et al. 2002). These conflicting effects of tau on the wellbeing of neurone mean that tau is a metabolically dynamic protein. Under normal conditions, tau is constantly being phosphorylated and dephosphorylated and there is a physiological equilibrium between these two processes.

Tau is expressed in several isoforms of different lengths (Goedert et al. 1989). Phosphorylation of tau is possible at 79 serine and threonine residues, at least thirty of which have been shown to be associated with abnormal phosphorylation in AD (Gong et al. 2005). Phosphorylation of tau inhibits its binding to microtubules and hyperphosphorylation of tau makes it neurotoxic (Fath et al. 2002). Hyperphosphorylated tau is also more resistant to proteases and this makes it more prone to aggregate (Alonso et al. 2001, Johnson et al. 1989). Eventually, hyperphosphorylated tau forms intraneuronal bundles of fibrils called paired helical filaments (Grundke-Iqbal et al. 1986) and after the affected neurone dies, only NFTs are left behind and the second characteristic hallmark of AD neuropathology has been generated.

NFT pathology in the brain occurs in a well documented sequential manner (Braak and Braak 1991). The first changes can be seen in entorhinal cortex (EC) and at that stage the patients do not have any cognitive impairment. When the disease progresses to a stage at which the patient starts to exhibit cognitive impairment, the pathology has spread to hippocampus (HC). Later on, the density of NFTs in these areas increases and the pathology spreads to amygdala and limbic nuclei of the thalamus. Finally, the pathology can be detected in neocortical areas and at this point the patients usually fulfil the clinical criteria for AD. The progression in the

pathology is accompanied by loss of cholinergic neurones in a similar sequential manner. The loss of cholinergic innervation starts from MTL, progresses to adjacent limbic areas and finally to cerebral cortex (Mesulam 2004).

There has been an intense debate about which of these pathological processes, amyloid or tau associated neuropathology, is the initiator of AD pathology. The amyloid cascade hypothesis states that A β 42 oligomerization and accumulation occur first, and the tau pathology and neuronal dysfunction are downstream events. Previous studies have shown that A β accumulation induces phosphorylation of tau (Alvarez et al. 1999) and mice carrying both mutant tau and APP develop more abundant NFT pathology in those brain areas that express both APP and tau in comparison to those areas which express only tau (Lewis et al. 2001). Furthermore, the injection of A β fibrils into the brains of mutant tau transgenic mice induces the formation of NFTs (Götz et al. 2001). Support for the hypothesis also includes the fact that all dominant mutations that cause AD are found in genes that are associated with APP metabolism (Haass 2004) whereas a mutation in the tau gene causes frontotemporal dementia (FTD) with parkinsonism, not AD (Hutton et al. 1998, Poorkaj et al. 1998, Spillantini et al. 1998). Moreover, in one FAD patient, amyloid pathology was shown to appear before NFTs (Smith et al. 2001). Patients with the tau mutation display severe NFT pathology and cognitive impairment, but do not exhibit amyloid plaques. These findings indicate that pathological changes in tau are not alone sufficient enough to provoke amyloid pathology. Furthermore, PS1, which is linked to amyloid pathology, may also protect tau from hyperphosphorylation, and therefore PS1 mutations may promote AD pathology also through tau (Baki et al. 2004). A PS1 mutation can also cause tauopathy without the presence of amyloid plaques (Dermaut et al. 2004). Another serious argument against the amyloid cascade hypothesis is the classical staging of AD neuropathology depicted by Braak & Braak, which states that NFT pathology precedes cortical amyloid pathology and the extent of NFT pathology correlates better with the gradual cognitive impairment (Braak and Braak 1991). However, NFTs may develop as part of the normal aging since they are found in all aged individuals regardless of their cognitive status (Price and Morris 1999). However, not only NFTs but also amyloid pathology can be detected in many asymptomatic elderly subjects (Bennett et al. 2006, Galvin et al. 2005, Neuropathology Group. Medical Research Council Cognitive Function and Aging Study 2001).

2.1.2 Clinical symptoms

When AD was first described, it was thought to be a rare condition that affected only a small number of relatively young patients. The cognitive impairment of the elderly was considered to be part of the normal aging process, caused by atherosclerosis of brain arteries. Later on, the neuropathological studies changed that view by revealing that AD associated pathology was a common feature in the elderly (Tomlinson et al. 1968). Although the onset of dementia varied greatly, the early onset and late onset dementia were considered to represent the same disease with uniform pathology.

From the diagnostic perspective this classification presents some difficulties. In early onset AD, the symptoms appear at an early age and usually progress quickly. This form of the disease is relatively easy to diagnose, because the symptoms are so distinct from most other diseases occurring at that age. However, in late onset AD, the symptoms develop later and progression is much slower. The pathological process of AD may begin as much as two decades before the clinical symptoms. During this preclinical period, it is not possible to detect patients who have AD pathology in their brain even with the most sophisticated neuropsychological tests (Driscoll et al. 2006). Even when the first cognitive symptoms occur, detecting the patients who have these symptoms due to AD is difficult because of the very insidious onset of symptoms. Similar symptoms may also be evoked by other causes such as depression or they can be regarded as normal cognitive changes associated with aging.

The salient feature of AD is the impairment in episodic memory, which is usually the first clinically evident symptom. Memory impairment is often the first clinical symptom because the AD pathology first occurs in MTL structures; a region that is essential for memory (Squire and Zola-Morgan 1991). However, a slight decline in memory occurs frequently as a part of normal aging. Later on in AD, patients exhibit impairment in other cognitive domains, for example executive function. This stage is often called "preclinical" AD in the literature, but it is highly questionable whether this term should be used, because these patients clearly have symptoms of the disease, although their symptoms are not severe enough to fulfil the diagnostic criteria for AD. During this earliest stage of the disease, these deficits do not prevent patients from carrying out normal activities of daily living, and therefore these patients cannot be yet diagnosed as having dementia. In clinically overt AD, patients exhibit severe and progressive deficits in other cognitive domains.

2.1.3 Concept of mild cognitive impairment

The belief that the cognitive symptoms of developing AD occur before the onset of clinical AD criteria fulfilling disease inspired researchers to try to find methods to discriminate these patients from healthy individuals. The starting point for most of the studies was memory impairment, because this is often considered as the first clinical sign of AD. However, memory impairment was not sufficient to be used alone, because it is associated with normal aging and other etiologies as well. Therefore more extensive criteria that aimed for more accurate distinction of AD patients were developed.(Table 1.) Several different criteria were introduced, but the fundamental tenet was that the patient would exhibit impairment beyond normal age related process but nonetheless would not be considered as being demented.

Table 1. Clinical criteria proposed for diagnosis of cognitive impairment

CIND	Objective impairment in one or more cognitive domains No dementia
AAMI	Subjective memory impairment Objective memory impairment compared to normative values
AACD	Subjective memory impairment Objective memory impairment compared to age-adjusted normative values
CDR 0.5	Subjective memory impairment CDR score 0.5
Amnestic MCI	Subjective memory impairment Normal activities of daily living Normal general cognitive functioning Objective memory impairment compared to age-adjusted normative values No dementia

CIND, cognitive impairment, no dementia; AAMI, age-associated memory impairment; AACD, aging-associated cognitive decline; CDR, clinical dementia rating; MCI, mild cognitive impairment.

2.2 MILD COGNITIVE IMPAIRMENT AND EARLY DIAGNOSIS OF AD

2.2.1 Mild cognitive impairment is a heterogeneous disorder

Initially, the term MCI was considered as a transitional state between cognitively healthy and clinically diagnosable AD. Currently, this view has broadened to encompass a state in which a patient exhibits cognitive impairment that is measurable in some objective way but the patient is not demented. One of the most extensively used set of criteria is based on that proposed by Petersen and co-workers (Petersen et al. 2001). The criteria divide the clinical presentation of MCI into four categories; MCI amnesic, MCI amnesic multiple domains, MCI single non-memory domain and MCI multiple non-memory domain categories. The criteria also acknowledge other possible causes of MCI than AD. Since AD is the most common dementing disease, it is also the most common etiology of MCI, either alone or as a concomitant pathology. AD is associated especially with amnesic MCI. It must be noted though that many patients with MCI do not have any underlying progressive disease.

Another popular definition of MCI is simply using the CDR score of 0.5 as a clinical criterion for MCI (Morris et al. 2001). The main outcome of these patients is AD, but also vascular dementia (VaD) and dementia with Lewy bodies (DLB) pathologies may develop. In many studies, this criterion for MCI is divided into domain specific subgroup depending on what cognitive domain is affected, for example amnesic or executive subtype. The problem with this definition is that the group of patients with a CDR score of 0.5 include both patients with MCI and patients with very mild dementia.

Cognitive impairment before the onset of clinically overt dementia is associated with other dementing diseases in addition to AD. The progression to other dementias was suggested to be associated with a decline in non-memory domains (Petersen et al. 2001). Indeed, a recent study reported that all of the MCI patients who progressed to FTD exhibited a decline in a single non-memory domain (Yaffe et al. 2006). Furthermore, another study reported that behavioural and affective symptoms together with executive dysfunction without decline in memory are associated with the development of FTD (de Mendonca et al. 2004). Vascular cognitive impairment (VCI) is proposed to be a prodromal condition of VaD (Bowler 2005). However, VCI patients include a very heterogeneous group of patients who progress to AD almost as often as to VaD and on the other hand, patients who fulfil the more general criteria for MCI often develop VaD frequently (Meyer et al. 2002b, Wentzel et al. 2001). A recent

study showed that both amnesic and non-amnesic MCI patients frequently develop dementia and also amnesic MCI patients developed VaD and DLB (Fischer et al. 2007). Furthermore, a neuropathological assessment of amnesic MCI patients who progressed to clinical dementia revealed that cognitive features cannot predict the development of AD associated neuropathology and most patients exhibit two or more different pathological features concomitantly (Jicha et al. 2006).

No matter what definition is used at the present, those patients diagnosed as having MCI include also patients who have no progressive neurodegenerative disorder. Psychiatric disorders, especially depression, may cause a cognitive decline without any progression to dementia (Ganguli et al. 2006). Depression is also an important condition to consider in the differential diagnosis of AD. Furthermore, anticholinergic drugs could contribute to cognitive impairment to such an extent that the patient may be diagnosed as having MCI (Ancelin et al. 2006). Therefore, it is not surprising that the group of patients diagnosed as having MCI will include some patients who eventually develop dementia, some who remain cognitively stable at MCI stage and even some who improve to become cognitively normal.

2.2.2 Pathological findings

The neuropathology of AD develops slowly during a long preclinical phase of the disease. Gradually the amount of neuropathology reaches a threshold to evoke cognitive symptoms that fulfil the clinical criteria for AD. Therefore it would be logical to presume that patients with MCI would display a substantial amount of AD associated neuropathology in their brain, though less than that found in patients with AD. Indeed, patients with MCI display marked AD neuropathology and its degree lies between the situation in cognitively healthy and AD patients with both CERAD and Braak staging (Bennett et al. 2005, Petersen et al. 2006). The NFT pathology of AD develops in a highly hierarchical sequential manner (Braak and Braak 1991). Consistent with this model, MCI patients have elevated number of NFTs in MTL structures when compared to healthy controls (Guillozet et al. 2003, Markesbery et al. 2006). The number of NFTs in MTL correlates with memory function (Guillozet et al. 2003). Furthermore, the early AD patients have an elevated number of NFTs in MTL and other brain areas as well (Markesbery et al. 2006). The extent of amyloid pathology falls also between the healthy controls and AD patients (Bennett et al. 2005, Markesbery et al. 2006). However, the number of NFTs correlate better with dementia severity, the number of amyloid plaques do

not display such a clear correlation (Berg et al. 1998). Since AD associated neuropathology is so prominent in MCI patients, some investigators have proposed that MCI is in fact a preclinical or early phase of AD (Markesbery et al. 2006, Morris et al. 2001). The picture is not quite so straightforward since both plaques and tangles are consistently found in the brains of cognitively healthy subjects, (Price and Morris 1999) even to an extent that should they have been examined simply in histopathological terms, the individuals would have been described as exhibiting AD pathology (Neuropathology Group. Medical Research Council Cognitive Function and Aging Study 2001).

2.2.3 Neuropsychological findings

Since memory impairment is a key feature in the most widely used MCI criteria, it has also been the most extensively studied cognitive domain in MCI (Petersen et al. 2001). Many recently published, longitudinal studies have used the earlier criteria in which a memory complaint was required for MCI diagnosis and impairment in other domains was prohibited (Petersen et al. 1999). However, several other cognitive domains have been studied, for example executive function, attention, praxis and language and visuospatial skills.

Although memory impairment is the most widely studied feature of MCI, isolated amnesic MCI is rare (Rasquin et al. 2005, Ribeiro et al. 2006, Ritchie et al. 2001, Tabert et al. 2006). In one study that included a consecutive sample of MCI patients, only 1.8% of the patients exhibited an isolated amnesic deficit. A total of 64.2% had multiple domains impaired and 17.0% of the MCI patients had a single non-memory domain impairment (Nordlund et al. 2005). In another study, 41.5% of the MCI patients in a memory clinic cohort did not have memory impairment (Rasquin et al. 2005). Even when relatively strict criteria for amnesic MCI are used, patients may also exhibit impairment in other cognitive domains if a detailed assessment of these other cognitive domains is performed (Kramer et al. 2006).

Many MCI patients have impairment in executive functions on conjunction with memory impairment (Lopez et al. 2006, Tabert et al. 2006) but executive dysfunction occurs also in patients without memory impairment (Lopez et al. 2006). A slight executive dysfunction may appear before any disturbances in functions of activities of daily living (Ready et al. 2003). Impairment in executive functions and language skills in MCI patients may be even more common than memory impairment (Nordlund et al. 2005). These findings challenge the

hypothesis that an isolated memory deficit is the first symptom of developing dementia and the symptoms then extend to other cognitive domains only with disease progression (Ribeiro et al. 2006).

2.2.4 Other features in mild cognitive impairment

Neuropsychiatric symptoms are relatively common in MCI patients, as many as 59% of patients have at least one neuropsychiatric symptom and up to 36% have three or more symptoms (Feldman et al. 2004). The most common symptoms noted in that study were depression, irritability, anxiety, agitation and apathy and the appearance of neuropsychiatric symptoms was associated with more severe cognitive impairment (Feldman et al. 2004). Another study reported that MCI patients had increased agitation, dysphoria, anxiety, apathy and irritability when compared to healthy controls. AD patients had similar symptoms, only the extent of delusional behaviour was further increased in AD when compared to MCI patients (Hwang et al. 2004). On the contrary, a large population-based study found that even though MCI patients have an elevated number of neuropsychiatric symptoms when compared to healthy controls, the AD patients exhibit still significantly more symptoms than MCI patients (Lyketsos et al. 2002). Nonetheless, neuropsychiatric symptoms, especially mood disturbances are common in MCI patients and are detrimental to the well-being of both patients and their families.

Although the decline in activities of daily living is restricted in the MCI criteria, a slight decline in functional capabilities is common in MCI patients (Farias et al. 2006). Those patients with a slight impairment in activities of daily living progress to dementia at a higher rate than patients with no functional decline and inclusion of decline in complex activities of daily living in MCI criteria might improve the sensitivity for development dementia (Peres et al. 2006). Motor dysfunction, particularly the parkinsonian signs, has also been associated with MCI and developing dementia (Aggarwal et al. 2006, Boyle et al. 2005).

2.2.5 Epidemiology and prognosis

Given the heterogeneity of the different criteria for MCI, it is not surprising that the incidence and prevalence rates of MCI in different studies vary greatly. The incidence, prevalence and progression rates to dementia are generally higher in clinic based studies than in population

based studies. When the cognitive impairment is defined according to broad criteria, the prevalence in the elderly population is high. The prevalence rate for age-associated cognitive decline (AACD) varies between 19.8% to 26.6% (Hanninen et al. 1996, Ritchie et al. 2001), but prevalence rates as low as 3.1% have been reported (Kumar et al. 2005). The prevalence of age-associated memory impairment (AAMI) has been reported to be 29% to 39% (Goldman and Morris 2001, Koivisto et al. 1995) or as low as 1.0% (Kumar et al. 2005). The prevalence of cognitive impairment no dementia (CIND) is close to the prevalence of AACD and AAMI, 16.8% (Graham et al. 1997).

The prevalence for MCI, defined with the Petersen criteria (Petersen et al. 1999, Petersen et al. 2001) is generally lower but also varies in the different studies. One of the reasons for these varying results is that even though the Petersen criteria are used, there have been numerous small modifications to the criteria between the studies. The prevalence and incidence rates vary also between different populations. In the population-based studies the prevalence rates vary between 2.8% and up to 28.3% (Hanninen et al. 2002, Larrieu et al. 2002, Lopez et al. 2003a, Manly et al. 2005). The prevalence of amnesic MCI is lower than the prevalence of MCI that also permits inclusion of other cognitive deficits. When both criteria for MCI were assessed in the same population, the prevalence for amnesic MCI was 6% and for the multiple cognitive deficits MCI 16% (Lopez et al. 2003a). In a Canadian population, the prevalence for amnesic MCI was only 1.0% (Fisk et al. 2003). The combination of memory impairment with impairment in another cognitive domain seems to be the most prevalent MCI type (Lopez et al. 2006, Ribeiro et al. 2006). The reported incidence rates in turn are between 9.9/1 000 and 25.9/1 000 person years among the non-demented elderly (Larrieu et al. 2002, Solfrizzi et al. 2004, Tervo et al. 2004).

The prognosis of MCI is heterogeneous possibly due to differences in etiology, studied population and inclusion criteria. Although MCI is viewed as the transitional state between the healthy aging and clinically overt dementia, i.e. an indication of a dementing disease in progress, as many as 40% of MCI patients revert to normal (Larrieu et al. 2002). Some MCI patients remain stable and are still classified as MCI at the follow-up as well. The most prevalent dementing disease as the outcome of progressive MCI is AD. Clinic based studies usually report higher conversion rates than the population based studies. The clinic based studies generally report conversion rates of 10-15% per year (Bozoki et al. 2001, Petersen et al. 1999, Rasquin et al. 2005). Conversion rates in population based studies are usually about

2-10% (Fisk et al. 2003, Larrieu et al. 2002, Solfrizzi et al. 2004). The reasons for this might be that the clinic based studies include more selected samples and possibly patients with slightly more advanced disease (Arnaiz et al. 2004). In a clinic based study that included only patients who were referred to the clinic by primary care physician after at least a three month history of memory complaint, the conversion rate was as high as 41% after one year and 64% after two year follow-up (Geslani et al. 2005). Furthermore, population based studies may include patients with reversible causes for cognitive impairment, for example anticholinergic drug use (Ancelin et al. 2006). The type of cognitive impairment also affects conversion rate. In a clinic based study that included patients with isolated amnesic MCI symptoms, the yearly conversion rate was 21.4% which is higher than that found in most clinic based studies (Perri et al. 2007). It seems that those MCI patients who have underlying progressing disease tend to convert to dementia within a few years time because the yearly conversion rates decrease in longer follow up from 10.8% in the first year to a mere 2.5% in the fifth and following years (Visser et al. 2006).

The risk factors for MCI are essentially similar to those for AD. The most important risk factor is increasing age. The strongest genetic risk factor for MCI and progression of MCI to dementia is the presence of at least one ApoE 4 allele (Hsiung et al. 2004, Manly et al. 2005, Petersen et al. 1995, Tervo et al. 2004). The ApoE 4 allele is especially detrimental to episodic memory, but it is also associated with accelerated decline also in other cognitive domains in the follow-up (Wilson et al. 2002). People with lower levels of formal education are at higher risk for MCI (Kivipelto et al. 2001, Lopez et al. 2003b, Manly et al. 2005, Tervo et al. 2004). Vascular risk factors, cardiovascular disease and elevated blood pressure, increase the risk of MCI and conversion to AD (Lopez et al. 2003b, Ravaglia et al. 2006, Solfrizzi et al. 2004, Tervo et al. 2004). An elevated blood cholesterol at midlife results in increased susceptibility for MCI later in life. Subjects with elevated blood pressure and high blood cholesterol at midlife are 2.7 times more likely to develop MCI two decades later than those subjects with normal values (Kivipelto et al. 2001). Cerebrovascular disease determined by MRI-identified infarcts also increases MCI risk (Lopez et al. 2003b). However, the association between the hypertension and MCI has not been found in other studies (Manly et al. 2005). Nonetheless, vascular risk factors seem to be associated with the development of MCI at least in some populations. Whether this association is causative is yet to be determined. The use of anticholinergic drugs may contribute to the cognitive impairment, such that after cessation of drug use the cognitive status returns to normal.

2.2.6 Towards a consensus - current concept of mild cognitive impairment

The heterogeneity of MCI criteria, epidemiology, prognosis and clinical outcome has raised some inevitable questions. First, is the syndrome currently called MCI in reality a uniform clinical entity? The etiology has been shown to be caused by several different pathological conditions or be even iatrogenic. Secondly, if the concept of MCI is accurate enough to identify patients with incipient AD, should the AD diagnosis itself be modified so that it includes these currently so called preclinical AD patients? Many researchers already consider MCI as early AD.

International Working Group on Mild Cognitive Impairment presented recommendations for general criteria for MCI after a symposium attended by specialists in different fields of dementia research (Winblad et al. 2004). Figure 1 represents the stepwise diagnostic process of MCI recommended by the group. The main difference with the former criteria is that there must be evidence of decline over time, the cognition of the patients is not compared to age adjusted normative values only. Also the MCI Working Group of the European Consortium on Alzheimer's Disease has recently proposed a new three stage diagnostic procedure for MCI (Portet et al. 2006). These criteria are essentially the same as those in the previous consensus report. In brief, to receive a MCI diagnosis, the patient needs to have a cognitive complaint, not necessarily a memory complaint, expressed by either the patient or a relative. There needs to be decline in cognitive functioning within the past year and the cognitive impairment should be corroborated by objective testing. Slight impairment in complex tasks of daily living are allowed but dementia must be excluded. The MCI subtype is then determined and additional examinations are initiated for detecting the underlying pathology.

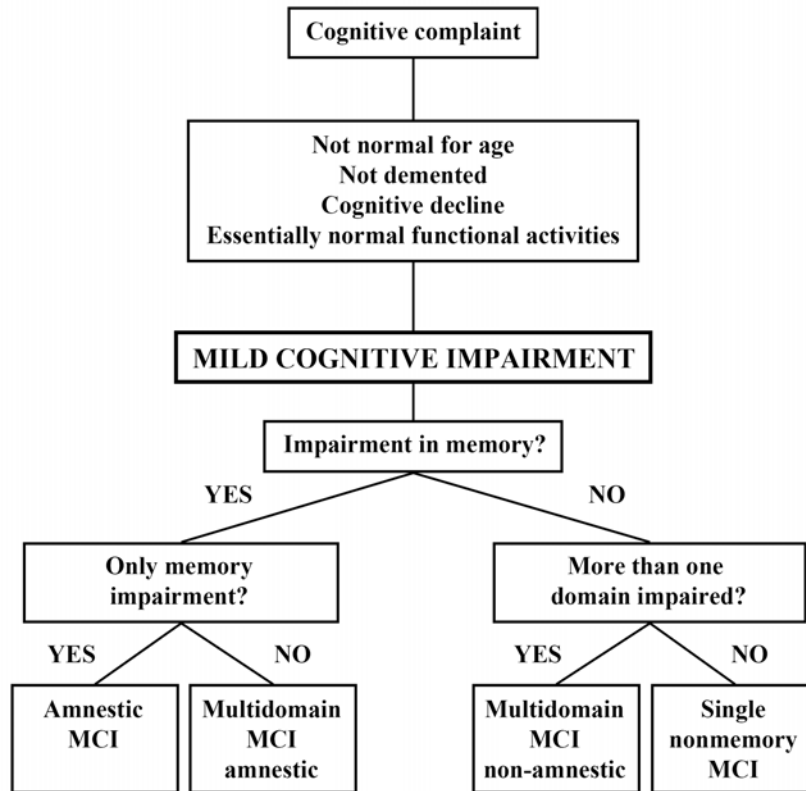


Figure 1. The recommendations of diagnostic classification process for MCI patients from the International Working Group on Mild Cognitive Impairment. Adapted from Winblad et al. 2004.

2.3 DEVELOPMENT OF DIAGNOSTIC TOOLS FOR EARLY DIAGNOSIS OF ALZHEIMER'S DISEASE

2.3.1 Search for a biomarker of early AD

When the diagnosis of AD is possible using current methods, the disease process is already far advanced. Even the most sophisticated neuropsychological testing and clinical examination cannot reliably pinpoint those MCI patients who will eventually develop AD, much less those subjects who are still cognitively healthy but already have on-going neuropathology processes in the brain (Driscoll et al. 2006). Also, current methods are not sensitive enough to observe the possible, disease-modifying effects of different treatments for AD, either current or still experimental therapies. Therefore, there is a dire need for an

objective biomarker for AD.

The Working group on "Molecular and Biochemical Markers of Alzheimer's Disease" stated in 1998 that a biomarker should be able to detect a fundamental feature of AD neuropathology, be validated in neuropathologically confirmed AD cases, and it should have sensitivity and specificity of >80%. It should also be precise, reliable, non-invasive, simple to perform and inexpensive (Consensus report of the Working Group on: "Molecular and Biochemical Markers of Alzheimer's Disease". The Ronald and Nancy Reagan Research Institute of the Alzheimer's Association and the National Institute on Aging Working Group. 1998). Since AD has a long preclinical stage of the disease, the biomarker should also be predictive. In AD, naturally, the biomarker should be relevantly linked to either amyloid- or tau pathology.

There are two approaches for searching possible biomarkers. The discovery type of approach searches new biomarkers from plasma or CSF from a limited number of cases using methods that analyse many different molecules simultaneously, for example proteomics. When it is believed that there is a molecule that differs between the AD patients and controls, this molecule is identified and then examined further in larger study population. Another approach is the hypothesis driven search for a biomarker. Some already known feature of AD associated pathology is studied in a group of AD patients and healthy controls, and the accuracy of this feature in distinguishing patients with AD is calculated. For example atrophy of MTL structures is a well known characteristic of AD, and MRI imaging of atrophy has been assessed as a potential biomarker.

The key requirement for an AD biomarker is that it should be validated in neuropathologically confirmed AD cases. Neuropathological confirmation could be reached either by biopsy of the brain, which is not in practice in AD diagnostics, or by neuropathological examination after death. Therefore the majority of the studies on biomarkers are conducted on clinical cohorts and this leads to some problems. The clinical diagnosis of AD reaches relatively high accuracy in experienced clinical units, but nonetheless, sensitivity remains at about 85% of the AD cases. Furthermore, the accuracy of clinical AD diagnosis is poor especially in the early stages of the disease, so the follow-up should be long enough to detect true AD patients. This problem is of course even more severe when studying MCI patients, who do not fulfil the criteria for even possible AD. Therefore, when studying AD biomarkers for prediction of

AD in MCI patients, the study population needs to be large and the follow-up has to last several years at the minimum. Another significant problem is that some cognitively healthy subjects exhibit substantial AD type neuropathology (Price and Morris 1999). On the other hand, some patients with severe cognitive impairment display very little neuropathology. It is also common that patients with other dementias, for example VaD or DLB, have concomitant AD neuropathology or AD patients have vascular or LB pathology (Neuropathology Group. Medical Research Council Cognitive Function and Aging Study 2001). The presence of different pathologies cannot be detected by the current clinical methods.

2.3.2 Imaging

At present in AD diagnosis, the brain imaging is used mainly for the exclusion of other possible causes of dementia, for example vascular disease. In MRI and CT it is also possible to see general brain atrophy in AD patients, but this atrophy is in no means either sensitive or specific to AD. Since imaging techniques are non-invasive, there has been great interest in developing imaging based methods on an early diagnosis of AD.

2.3.2.1 Magnetic resonance imaging

In addition to general brain atrophy, ongoing neurodegeneration of AD causes atrophy of MTL structures, especially HC and EC. The MTL atrophy occurs early in development of AD, which is in accordance with the Braak model of AD pathology (Braak and Braak 1991). This atrophy can be seen in CT, but MRI has proved to be better and more sensitive especially in detecting slight changes. Since the AD neuropathology begins from MTL, the HC and EC atrophy has been studied extensively in the hope of finding a possible preclinical imaging marker for AD. Several studies have shown that EC and HC atrophy is a typical feature of AD (Bobinski et al. 2000, Jack et al. 2002, Pennanen et al. 2004) and it is associated with decreased number of neurones of the region (Bobinski et al. 2000, Simic et al. 1997). However, MTL atrophy is not specific to AD but it also occurs in other neurodegenerative diseases (Jack et al. 2002). In MCI patients, the atrophy seems to lie between the healthy subjects and AD patients (Pennanen et al. 2004). Individual variability is large, but there is evidence that MTL atrophy may be useful in detecting those MCI patients who are at greater risk for development of AD. Since EC is affected before HC by AD pathology, especially during MCI stage of the disease, measurement of EC and HC separately may have better

diagnostic value than evaluating overall MTL atrophy (Pennanen et al. 2004). Nonetheless it is possible that because the EC is a rather small structure, the technical and anatomical limitations may nullify this theoretical benefit (Xu et al. 2000).

A more practical clinical way of assessing MTL atrophy is to use a qualitative approach, because it enables grading of MTL atrophy from one imaging plane. The grading system can be easily learned, and acceptable inter-rater accuracy can be achieved (Scheltens et al. 1992). This type of rating can distinguish AD patients from healthy controls with an accuracy of 96% when used in a clinically rational context (Wahlund et al. 2000). It can also be used as an aid to predict AD in MCI patients (Bouwman et al. 2006, Korf et al. 2004). Volumetric analysis is more detailed and may have better predictive accuracy. However, volumetric analysis is time consuming and laborious and it places great demands on the skills of the analyst. Moreover, HC and EC atrophy may not provide significant added value for a diagnosis between AD and other dementias when compared to neuropsychological testing. In a study comparing the diagnostic value of mini mental state examination (MMSE) and MTL atrophy, the overall accuracy of MMSE alone was 88% whereas MMSE in conjunction with volumetric or visual MTL atrophy rating was only 90% (Wahlund et al. 2000). Another study examined the predictive value of neuropsychiatric assessment and MTL atrophy for development of dementia and found that the accuracy of MMSE together with two other neuropsychiatric tests was 79.6% and accuracy of neuropsychiatric tests together with MTL atrophy 86.8% (Devanand et al. 2007). Therefore, at least for the time being, inclusion of volumetric MRI is not appropriate as a component of the clinical work-up. Functional MRI has also been assessed as a possible diagnostic aid for early AD, but the results are varying and the method is very laborious for both patient and analyst.

2.3.2.2 Functional imaging

Positron emission tomography (PET) and single photon emission computed tomography (SPECT) have been studied as possible biomarkers for AD. The most commonly used method in PET is the examination of regional glucose metabolism of different brain areas by using radio-labelled [¹⁸F] fluorodeoxyglucose. SPECT is used for imaging of regional blood flow. In neuropathologically confirmed patients, the hypoperfusion in parietal and temporal lobes yielded a sensitivity of 89% and a specificity of 80% against healthy controls and patients with other dementias (Jobst et al. 1998). Another study showed that the severity of

hypoperfusion was associated with the severity of cognitive impairment in AD patients (Waldemar et al. 1994). Temporal lobe hypoperfusion occurs already in patients with amnesic symptoms and hypoperfusion of posterior cingulate cortex has been reported to differentiate the MCI patients with a cognitive decline at the five year follow-up from stable MCI patients with 79% sensitivity and 67% specificity (Hogh et al. 2004, Johnson et al. 2007). The accuracy of PET imaging seems to be better than the accuracy of SPECT (Mosconi 2005). The hypometabolism in MTL structures and posterior cingulate cortex has been shown to occur already in mild AD (Mosconi et al. 2007). Hypometabolism in parietal and temporal regions was claimed to differentiate AD patients from controls with 93% sensitivity and specificity and MCI patients who develop dementia from stable MCI patients with 93% sensitivity and 82% specificity (Anchisi et al. 2005, Herholz et al. 2002).

2.3.2.3 Molecular imaging

However, it is another PET based technique that has been raised the greatest hopes of improving AD diagnostics. Thioflavin compounds are traditionally used in histopathological examination of many tissues because they bind to the amyloids formed by different amyloidogenic proteins. In AD, the thioflavin compounds are routinely used for staining amyloid plaques that contain fibrillar A β . Pittsburgh Compound-B (PIB) is a thioflavin T derivative that can cross the blood brain barrier (BBB). In living AD and amnesic MCI patients, the PIB load as detected in PET is significantly higher than in healthy controls (Kemppainen et al. 2007, Klunk et al. 2004), and PIB binding correlates with the extent of brain atrophy (Archer et al. 2006). PIB binding also correlates with hypometabolism in temporal and parietal cortices of brain, although this association is lacking in frontal cortex (Edison et al. 2007). The distribution of PIB binding in brain is in accordance with the distribution of amyloid pathology found in autopsy studies on AD patients (Kemppainen et al. 2006). Furthermore, in a recent study, all of the examined AD patients had PIB positive scans. However, that study also reported that four of 12 frontotemporal lobar degeneration patients and one out eight controls displayed positive PIB binding (Rabinovici et al. 2007). It is possible that these false positives were subjects with underlying amyloid pathology, because no neuropathological validation was available. One obstacle to using PIB as a AD biomarker is that even though PIB binding accurately confirms the presence of amyloid, it seems that it cannot distinguish between the amyloid in plaques and as cerebral amyloid angiopathy in blood vessels (Bacskai et al. 2007). No large follow-up studies are available, but it does seem

plausible that PIB could be used as an imaging biomarker for amyloid pathology in AD and even for progressive MCI.

2.3.3 Laboratory tests for Alzheimer's disease

2.3.3.1 Plasma A β peptide

A biomarker that could be found in plasma would be ideal because a blood sample is easy to obtain and routinely used in clinical practice, minimally invasive and safe. The credibility of plasma biomarker is supported by the fact that the BBB damage is associated with AD pathology. Since APP and its metabolites, including A β peptide can be found in plasma, their feasibility for use in AD diagnosis has been studied. A large variety of other types of molecules have also been studied, but the results have been variable and at least for the time being, none of them have any validity in AD diagnosis (Irizarry 2004).

APP, PS1 and PS2 mutation carriers as well as patients with DS who possess three copies of the APP gene were shown to have elevated plasma A β 42 levels when compared to control subjects (Mehta et al. 1998, Scheuner et al. 1996). Also AD patients were reported to have increased A β 42 levels when compared to healthy controls (Younkin et al. 2000). Furthermore, AD patients and those patients who developed AD during the follow-up had increased A β 42 levels when compared to controls (Mayeux et al. 2003). Another study reported elevated plasma A β 40 but no difference in plasma A β 42 levels between AD patients and controls (Mehta et al. 2000). In addition, in a third study, neither A β 40 nor A β 42 were different between AD patients and controls, but they correlated with age in each group (Fukumoto et al. 2003). The picture is as diverse in patients with MCI; A β 42 levels have been shown to be increased in women with MCI when compared to controls or men with MCI (Assini et al. 2004) but in another study there was no difference between groups (Fukumoto et al. 2003). Other amyloid related molecules studied as potential biomarker include A β directed autoantibodies found in plasma and the ratio of platelet derived APP isoforms (Di Luca et al. 1998).

2.3.3.2 Cerebrospinal fluid amyloid beta peptide

CSF is in direct contact with brain and thus reflects brain associated biochemical events better

than any other biological fluid. Therefore CSF is a more likely source for finding a clinically usable biomarker than plasma. A β , tau and phospho-tau are linked to AD associated neuropathological changes, and they have been the most widely studied potential biomarkers for AD. Although most of the CSF is produced in the choroid plexus by capillary filtration, a substantial proportion is derived from extracellular fluid of brain parenchyma and about 20% of CSF proteins are synthesized in brain. A β peptide is secreted during physiological metabolism and it is a normal constituent of human CSF (Haass et al. 1992, Seubert et al. 1992). The first studies on total CSF A β as a biomarker for AD gave disappointing results. Studies showed that CSF total A β levels were decreased (Pirttila et al. 1994), increased (Nakamura et al. 1994) or not changed at all in AD patients (Motter et al. 1995, van Gool et al. 1995). After the discovery of the two main types of A β peptides in CSF, A β 40 and A β 42, it was found that CSF A β 42 levels were consistently lower in AD (Motter et al. 1995). Subsequently it was established that not only CSF A β 42 was lower in AD, but it also could distinguish patients with AD from healthy controls with reasonable accuracy, even in patients with mild AD (Blennow and Hampel 2003).

In MCI patients the results have been somewhat inconsistent. In a cross-sectional study, a small group of subjects with mild memory impairment exhibited low CSF A β 42 levels (Hulstaert et al. 1999), whereas another study on a larger group of MCI patients reported significantly elevated A β 42 levels in MCI patients when compared to healthy controls (Jensen et al. 1999). In a follow-up study on 19 MCI patients, the baseline levels of CSF A β 42 were similar between the MCI group and the healthy control group, but when the MCI patients developed clinically overt AD, then the A β 42 levels decreased (Maruyama et al. 2001).

There is huge interest in differentiating those MCI patients who will develop dementia from those who remain stable or even improve to cognitively healthy. One study included 15 cognitively healthy controls and 16 MCI patients who developed dementia within one year follow-up, and it was found that MCI patients had significantly lower CSF A β 42 levels at the baseline (Andreasen et al. 1999). In another study, the baseline A β 42 levels were lower in MCI patients who developed AD or had some other progressive cognitive impairment but did not develop dementia within an 18 month follow-up when compared to those MCI patients whose cognition remained stable (Riemenschneider et al. 2000). Furthermore, a study that included 52 MCI patients found that those 23 patients who developed probable AD during a

seven month follow-up had lower A β 42 levels when compared to nonconverters (Hampel et al. 2004). However, in a study including 53 MCI patients, of whom 22 developed AD during an average 20 month follow-up, the baseline A β 42 levels did not reveal any statistically significant difference between converters and non-converters (Zetterberg et al. 2003).

In studies on MCI patients, the follow-up period should be much longer than used in these studies, because the development of dementia in MCI patients may take several years. In a study with too short a follow-up time, the stable MCI group is likely to include patients who would develop dementia during longer follow-up. The results of studies with longer follow-up are starting to emerge. A large study with 137 MCI patients reported that those MCI patients who progress to dementia within an average of five years follow-up had decreased CSF A β 42 levels at the baseline (Hansson et al. 2006). Therefore, the reduced A β levels seem to be evident even several years before the onset of dementia. Furthermore, CSF A β 42 levels were shown to be lower in cognitively healthy subjects who developed dementia after three years follow-up (Skoog et al. 2003). The same group have extended their study and observed that decreased A β 42 levels are associated with decreased MMSE score after eight years follow-up (Gustafson et al. 2006).

CSF A β levels are thought to be associated with the amyloid pathology of the brain, but few studies have compared CSF A β levels and brain pathology. One study correlated *post mortem* ventricular CSF A β 42 levels to the amount of brain amyloid pathology and found that more pronounced amyloid pathology was associated with lower A β 42 levels in CSF (Strozyk et al. 2003). Other studies have reported decreased CSF A β 42 levels in neuropathologically confirmed AD patients, but did not report data on the quantitative amount of amyloid pathology (Clark et al. 2003, Engelborghs et al. 2007). The reasons for the decreased CSF A β 42 levels in AD are not known yet, but it is possibly due decreased production resulting from cell death or abnormal clearance from brain to CSF as amyloid plaques sequester the A β peptides.

2.3.3.3 Cerebrospinal fluid tau

CSF tau has been consistently shown to be increased in AD when compared to healthy subjects relatively soon after the measurement of CSF tau became possible (Jensen et al.

1995, Vandermeeren et al. 1993, Vigo-Pelfrey et al. 1995). The association between AD and increased tau levels has been corroborated also in neuropathologically confirmed patients (Clark et al. 2003). These results have been replicated in several studies, and CSF tau was established as one of the most promising biomarkers for AD (Blennow and Hampel 2003). CSF tau levels were found to be increased even in patients with very mild AD (Kurz et al. 1998, Riemenschneider et al. 1996). In the cross-sectional setting, the patients with minimal memory impairment or MCI had higher CSF tau levels than the healthy controls (Hulstaert et al. 1999, Maruyama et al. 2001). Baseline tau levels were also associated with developing dementia in the follow-up (Andreasen et al. 1999, Maruyama et al. 2001, Riemenschneider et al. 2000, Zetterberg et al. 2003). There is evidence that in MCI patients, the CSF tau levels are increased in those patients who develop AD compared to those who develop some other form of dementia (Zetterberg et al. 2003). However, the association between increased tau and developing AD has not been confirmed in all studies (Buerger et al. 2002a).

Although there is a body of evidence that increased CSF tau levels are associated with AD, even during an early stage of the disease, problems in using tau as a biomarker soon emerged. An increased tau level was not specific to AD, but seemed to be associated with many neurodegenerative states. In a population based study, the patients with VaD or mixed AD and VaD had similar tau values to AD patients (Andreasen et al. 1998). Increased tau levels were also reported from patients suffering from DLB or FTD (Arai et al. 1997). Extremely high tau values are found in patients with Creutzfeldt-Jakob disease (CJD) (Otto et al. 1997) and patients with recent stroke (Hesse et al. 2001). Although some of these findings proved inconsistent, it became obvious that tau mirrors neuronal and axonal damage in general rather than the neurodegenerative process of AD specifically. Thereafter, the search for a reliable biomarker moved on to find a more AD specific form of tau.

Table 2. The characteristics of CSF A β 42 and tau in predicting dementia in MCI patients

		Stable MCI Specificity	Progressive MCI Sensitivity	Overall Accuracy	Positive LR	Negative LR
Riemenschneider et al. 2002 ¹	A β 42 + tau	90% 9 / 10	90% 9 / 10	90% 18 / 20	9.00 (1.35-58.44)	0.11 (0.02-0.71)
Hampel et al. 2004 ²	A β 42	57% 13 / 23	83% 24 / 29	71% 37 / 52	1.90 (1.61-3.12)	0.31 (0.13-0.73)
	Tau	48% 11 / 23	90% 26 / 29	71% 37 / 52	1.72 (1.14-2.59)	0.22 (0.07-0.69)
Maruyama et al. 2004 ³	Tau	87.5% 14 / 16	88% 36 / 41	88% 50 / 57	7.02 (1.91-25.81)	0.14 (0.06-0.32)
Hansson et al. 2006 ⁴	A β 42 + tau	82% 46 / 56	95% 54 / 57	88% 100 / 113	5.31 (3.01-9.34)	0.06 (0.02-0.19)

The table contains all the studies that have provided sufficient information concerning stable and progressive MCI patients to make it possible to calculate diagnostic accuracy figures. Data presented as percentage and number of correctly allocated subjects in the group or as LR (95% CI). MCI, mild cognitive impairment; LR, likelihood ratio.

1. Endpoint Alzheimer's disease, cut-off: $A\beta 42 = 240 + 1.18 \times \text{tau}$
2. Endpoint Alzheimer's disease, cut-off: A β 42 679 pg/ml and tau 479 pg/ml
3. Endpoint dementia, cut-off: tau 320 pg/ml
4. Endpoint Alzheimer's disease, cut-off: A β 42 530 pg/ml and tau 350 pg/ml

2.3.3.4 Cerebrospinal fluid phospho-tau

Phosphorylation of tau is a normal physiological event, and it may occur in several dozens of amino acid residues of tau. In AD, as well as in some other neurodegenerative diseases associated with tau pathology, tau is phosphorylated beyond the normal functional level. The result is hyperphosphorylated tau which impairs axonal transport and forms intracellular aggregates (Avila 2006). There are several assays for measurement of CSF phospho-tau, but the most widely utilized are the assays for phosphorylated at threonine 181 (phospho-tau181) and threonine 231 (phospho-tau231) both of which seem to perform equally well in discriminating AD patients from controls and patients with other neurological diseases (Hampel et al. 2004).

Measurement of phospho-tau instead of total tau especially improves the discrimination of AD patients from patients with DLB or FTD (Hampel et al. 2004, Sjogren et al. 2001). The

benefits of phospho-tau are also that unlike total tau, phospho-tau is not markedly increased in CJD (Buerger et al. 2006b, Riemenschneider et al. 2003) or after a stroke (Hesse et al. 2001). Elevated phospho-tau levels have been found in progressive MCI patients (Arai et al. 2000) and phospho-tau levels have been reported to display a correlation to annual points lost in MMSE (Buerger et al. 2002a, Buerger et al. 2002b).

2.3.3.5 Other possible CSF biomarkers

Numerous different proteins that are present in CSF have been studied as potential biomarkers for AD in the past decade. Many of the studied proteins are related to different aspects of AD pathology, for example the presence of glycosylated acetylcholinesterase may reflect the changes of cholinergic system in AD brain (Saez-Valero et al. 2000). Apart from tau proteins, other molecules that accompany neuronal cell death have been examined; one example of these is tissue transglutaminase (Bonelli et al. 2002). There are also studies into a large number of possible biomarkers for inflammation of AD brain (Jia et al. 2005). Molecules that reflect homeostasis of the brain have been proposed as representing possible biomarkers, these include 24S-hydroxycholesterol which is a brain specific metabolite of cholesterol (Leoni et al. 2006) and isoprostane which is a marker for oxidative damage (Montine et al. 2001). It is also possible that the most accurate diagnosis of early AD can be achieved by using a panel of several different biomarkers (Simonsen et al. 2007). Even though the above mentioned and numerous other molecules including neurofilament proteins, neuromodulin and neuronal thread protein have been studied (Blennow 2004), at the present, the only usable biomarkers are the CSF A β 42, tau and phospho-tau levels in CSF.

3. AIMS OF THE STUDY

AD is a disease that has been suggested to develop slowly over a time range of two decades before the first symptoms make their appearance. Neuropathology starts to develop early during a presymptomatic period of the disease, and at the first symptomatic stage, the pathology is already advanced. This is a great obstacle in treating the disease because repairing the already existing damage is currently impossible. Previous studies have suggested that changes in CSF biomarkers A β 42, tau and phospho-tau can be used as a diagnostic aid especially in clinically challenging conditions, for example in the differentiation between mild AD and depression.

At the onset of this study, little was known about these markers before the onset of clinical AD. Since AD neuropathology starts to develop decades before the appearance of symptoms, the original hypothesis was that AD associated changes in CSF biomarkers could be detected before these clinical signs of AD. The main aim of the study was to examine whether these changes would predict the development of AD in patients with MCI and whether there was an association between AD associated pathological changes in brain and the CSF biomarker levels.

The specific aims of the study were:

1. To study whether the CSF A β levels are related to amyloid pathology in the brain of the transgenic mouse model of AD.
2. To study whether the AD associated changes in CSF biomarker are detectable before the clinical diagnosis of AD.
3. To study whether these changes are predictive for AD when used as a diagnostic test.
4. To study the association between the CSF biomarkers and changes in medial temporal lobe structures.

4. MATERIALS AND METHODS

4.1 SUBJECTS

4.1.1 Study population

The study population was derived from two different sources. The majority, 107 of the subjects, were recruited from the neurological department of the Kuopio University Hospital to which they had been referred by their primary care physician due to neurological symptoms. The reasons for referral included headaches, dizziness and cognitive symptoms. Those patients who agreed to undergo lumbar puncture for research purposes were included in the study. The patients who were found to be neurologically intact or whose symptoms were caused by depression were designated as controls. The diagnosis of depression was based on the clinical examination and patients with depression were further referred to psychiatrist. The MCI patients included both amnesic MCI patients and patients with some other type of cognitive decline, for example executive dysfunction or impaired visuospatial or language skills. One subject with fibromyalgic pains, who entered the study as a control subject, was excluded due to receiving a diagnosis of AD ten years later. A total of 33 subjects included in the study were part of a large population-based follow-up study of cognitive impairment in the elderly population of Kuopio region. Those patients who agreed to undergo lumbar puncture for research purposes were included in the study. All subjects gave informed consent and the study was approved at the Kuopio University Hospital Ethical Committee.

In study **II** all of the MCI patients were analysed as a single group without any more detailed division into diagnostic groups. For study **III**, the MCI patients were divided into three different groups based on the MCI type: amnesic MCI (with or without any other cognitive impairment), executive MCI (with or without any other cognitive impairment, no amnesic symptoms) and other MCI (subjects with impairment in one or more cognitive domains other than memory or executive function). In study **III**, the control group was divided into patients with depression and other controls, since depression is one of the most important diseases to consider in the differential diagnosis of early AD. The study **IV** included all the MCI patients from the larger neuroimaging study (Pennanen et al. 2004) who had also agreed to a lumbar

puncture and therefore for whom biomarker data were available. All of these patients except one were also included in study **III**. In all of the studies **II-IV**, the MCI patients were further divided into those who remained cognitively stable and those who developed dementia during the follow-up.

Baseline examination of all subjects included a neurological and neuropsychological examination, imaging of the brain (mainly computed tomography), and laboratory tests for exclusion of the secondary causes of cognitive decline according to Finnish guidelines. The subjects from whom the follow-up information was available formed the final cohort of the study. The follow-up examinations were performed yearly for the population based group. The patients from the clinic group were either seen again at the clinic or the follow-up data were later derived from the medical charts of the patients.

4.1.2 Neuropsychological testing

The large variety of neuropsychological tests was used to assess impairment in different cognitive domains. In the clinical group, the tests that were used varied because the baseline examination of patients was conducted over a period of several years. However, all of the domains that were studied in the population based group were assessed also in the clinical group.

In the population based group, the following tests were used: *Memory*: Visual Reproduction Test (VR, immediate and delayed recall) from Wechsler Memory Scale (WMS) (Russel 1975), Logical Memory Test (LM, immediate and delayed recall) from Wechsler Memory Scale-Revised (WMS-R) (Wechsler 1987), Word List Recall (immediate and delayed recall) from the CERAD Neuropsychological Assessment Battery (Morris et al. 1989), Delayed Recall of the Constructional Praxis from CERAD (Morris et al. 1989); *Language*: Abbreviated (15 items) Boston Naming Test (Kaplan et al. 1991), vocabulary subtest from the Wechsler Adult Intelligence Scale-Revised (WAIS-R) (Wechsler 1981); *Attention and executive function*: Verbal Fluency Test (Borkowski et al. 1967, Butters et al. 1987), Trail Making Test (Reitan 1958) parts A and B; *Visuospatial skills*: Constructional Praxis from CERAD (Morris et al. 1989), Block Design from the WAIS-R (Wechsler 1981); *Global functioning*: Mini-Mental State Examination (Folstein et al. 1975) (MMSE), Clock Drawing Test. (Morris et al. 1989) Subjects performing below the age-adjusted norms in at least one

cognitive domain were diagnosed as having MCI if they also had a score of 0.5 on the CDR scale (Morris 1993). The assessment of CDR score was based on a non-structured clinical interview on patient and caregiver.

4.1.3 Diagnosis of dementia

The diagnosis of dementia was based on the criteria of the Diagnostic and Statistical Manual of Mental Disorders, Fourth Edition (DSM-IV) (American Psychiatric Association 1994) and AD was diagnosed using the National Institute of Neurological and Communicative Disorders and Stroke and Alzheimer's Disease and Related Disorders Association (NINCDS-ADRDA) criteria (McKhann et al. 1984). The diagnosis was made by consensus decision by two neurologists and two neuropsychologists. The diagnosis was made based on the clinical symptoms and neuropsychological test results. The diagnostic workup included the standard laboratory testing and imaging, mainly computed tomography, to exclude any other possible causes for dementia than AD. The diagnosis of dementia was done independently and blinded to the CSF biomarker data and the ApoE genotype.

4.2 TRANSGENIC MICE

4.2.1 Transgenic mice

Two different doubly transgenic mouse lines were used in the study. Both lines carry an APP695 gene that includes the human A β domain with mutations K595N and M596L that are linked to Swedish familial AD pedigrees (APP^{swe}). The first mouse line also carries a human PS1 gene with AD linked mutation A246E (APP^{swe}/PS1 mice) (Borchelt et al. 1997). The second line carries a PS1-dE9 mutation (APP^{swe}/PS1dE9) (Jankowsky et al. 2004).

4.2.2 Surgical procedures

The sampling method for mouse CSF was adapted from a previously described method (Meyding-Lamade et al. 1996). CSF was taken from 22 APP^{swe}/PS1 mice at the age of 5 and 7 months and from 39 mice at the age of 8, 10 and 13 months. From 36 APP^{swe}/PS1dE9 mice, the CSF sample was taken at the age of 6 and 9 months. CSF was collected by penetrating the dura in the cisterna magna with a 6-cm-long glass capillary that had a tip with an outer diameter of 0.5 mm. The sample was immediately transferred to a polypropylene

tube and stored at -70°C until analyses. The average collected volume of CSF was approximately 7 μl .

After the last round of CSF sampling, the mice were transcardially perfused with 50 ml heparinized ice-cold 0.9% saline (10 ml/min), and the brains were rapidly removed. One hemibrain was immediately immersed in 4% paraformaldehyde in 0.1 M Na-phosphate buffer for 4 h and then transferred overnight to 30% sucrose solution. These samples were then stored in a cryoprotectant at -20°C until histological analyses. The other hemibrain was dissected on ice into selected brain areas (including HC and cerebellum) that were stored at -70°C until ELISA analyses.

4.2.3 Sample preparation for ELISA

The analysis focused on the HC which was evaluated from all mice that were included in the study. The cerebellar $\text{A}\beta_{42}$ was also studied from a subset of mice because the cisterna magna lies next to the cerebellum, and therefore it is possible that CSF $\text{A}\beta_{42}$ originates from cerebellum instead of the main plaque forming areas of the brain, including HC. The tissue sample was first homogenized in phosphate buffered saline, pH 7.2, which contained a mixture of protease inhibitors (CompleteTM, Boehringer Mannheim, Germany). Tissue homogenates were then centrifuged at $218\,000 \times g$ for 2 h at $+4^{\circ}\text{C}$. The supernatants that contained the soluble $\text{A}\beta_{42}$ were collected and stored at -70°C until analyses. The remaining pellet containing the insoluble $\text{A}\beta_{42}$ was further homogenized in guanidine buffer (5.0 M guanidine-HCl in 50 mM Tris-HCl, pH 8.0), incubated at room temperature for 3 h, centrifuged, and diluted to reduce the guanidine concentration to 0.5 M.

4.2.4 Histology

The brain was cut into 30 μm coronal sections, which were stained with W02 antibody (ZMBH, Heidelberg, Germany) that recognizes the human $\text{A}\beta$ as described earlier (van Groen et al. 2003). Three stained sections through the HC were digitized using a Nikon Coolpix 990 camera, and the images were converted to grey scale using the Photoshop 5 program. The percentage of area covered by the reaction product to $\text{A}\beta$ (i.e. $\text{A}\beta$ load) was measured using the ScionImage (NIH) program.

4.3 CSF BIOMARKER ANALYSES

4.3.1 A β 42

Human CSF A β 42 levels were measured by using an ELISA kit (Innogenetics, Ghent, Belgium). All analyses were done in duplicate. The mouse CSF A β 42 levels were measured by using the High Sensitivity method provided in the same kit. Due to the small amount of available sample, the analyses were done without duplicates. The brain A β 42 was measured by the High Sensitivity method according to the protocol, except that 0.5 M guanidine was added to the kit's standards when measuring the insoluble A β 42 from brain tissue samples. This was done because guanidine significantly decreases the strength of the signal in ELISA and if the guanidine was missing from standards the results would have been erroneously low. The brain A β 42 from the 7 month old mice was measured by using a Signal Select β -amyloid ELISA kit (BioSource International Inc.).

4.3.2 Tau and phospho-tau

CSF tau levels were measured by using an ELISA kit (Innogenetics, Ghent, Belgium). The CSF phospho-tau levels were measured by using an ELISA kit that measures specifically the Tau phosphorylated at residue 181. All analyses were made in duplicate.

4.4 APOE GENOTYPING

The ApoE genotype was determined by using a PCR based method (Tsukamoto et al. 1993). For the statistical analyses, the subjects were classified according their ApoE genotype; either by the presence (ApoE 4 positive) or absence (ApoE 4 negative) of ApoE 4 or the number of possessed ApoE 4 alleles (none, one or two ApoE 4 alleles).

4.5 MAGNETIC RESONANCE IMAGING

All subjects in study **IV** underwent a high resolution MRI in the Kuopio University Hospital. The images were acquired using a 1.5 Tesla Vision (Siemens, Erlangen, Germany), with a 3D magnetization prepared rapid acquisition gradient echo sequence (for the patients scanned in 1998/1999, the parameters were: TR (time of repetition) = 9.7 ms, TE (time of echo) = 4 ms, matrix 256 \times 256, 1 acquisition and in plane resolution = 0.98 mm, while for the patients

scanned in 1999-2001 the parameters were: TR = 13.5, TE = 7, matrix 256x256, 1 acquisition and in plane resolution = 0.94 mm). Standard neuroanatomical landmarks were used to correct for possible deviations in any of the orthogonal planes and the scans were reconstructed into 2.0 mm thick contiguous coronal slices, oriented perpendicular to the intercommissural line. The hippocampi and EC were manually traced by a single tracer (C.P.), using custom-made software for a standard Siemens work console (Pennanen et al. 2004). The boundaries of the regions of interest were outlined using a trackball driven cursor, always proceeding in an anterior to posterior direction. The coronal intracranial area at the level of the anterior commissure was measured and used for the normalization of the volumetric data according to the formula: (volume/intracranial area) x 100. The intraclass correlation coefficients for intrarater reliability were 0.96 for the HC and 0.95 for the EC measured from 10 subjects. All analyses were done blinded to the clinical data (Pennanen et al. 2004).

4.6 STATISTICAL ANALYSIS

All statistical analyses were performed by using SPSS software for Windows (SPSS Inc. Chicago, IL). The comparisons between the different groups of subjects were done by ANOVA with appropriate *post hoc* corrections. When the assumptions for normality were not met, the nonparametric tests were used. For the categorical data, the comparisons between different groups were made with Chi-square tests. The correlations between different variables were calculated by Pearson's correlation test or by Spearman's correlation test. The best cut-off values for development of dementia of different measures were determined by receiver operator characteristics (ROC) analysis. The odds ratios for different factors that might contribute to the conversion of MCI to dementia were determined by logistic regression analysis, so that only the patients with MCI at the baseline were included. Cox regression analysis was used for survival analyses.

4.7 ETHICAL CONSIDERATIONS

Elderly patients with cognitive impairment are a very special group to consider in clinical studies. Additional caution needs to be used when informed consent is obtained. All of the patients who entered the study were only slightly impaired at the time of inclusion into the study and thus capable of providing informed consent. Since CSF biomarkers and ApoE allele

analysis are not currently used in routine diagnostics, the results of these analyses were not revealed to the patients.

Lumbar puncture is an invasive method that may be uncomfortable for the patient and sometimes has side effects. However, in a study on 342 subjects, it was shown to be a safe procedure, and even the post puncture headache, the most frequent side effect, was rare especially in the patients with AD and MCI (Peskind et al. 2005). On the other hand, the dire need for the development of methods for earlier diagnosis of AD is increasing as the treatment possibilities progress. Since CSF is in direct contact with brain, it offers the best potential as a reliable source of biomarkers to be used in future diagnostic aids.

All of the animal experiments were conducted according to the Council of Europe and Finnish guidelines and approved by the State Provincial Office of Eastern Finland.

5. RESULTS

5.1 ASSOCIATION BETWEEN CSF A β 42 AND NEUROPATHOLOGY IN MICE

The CSF A β 42 levels increased significantly in APPswe/PS1 mice from age 5 months to 7 months ($p < 0.001$). None of the mice showed any amyloid deposits in the histology at this stage. Between the age of 8 to 13 months, the CSF A β 42 levels remained stable, and at the age of 13 months a few amyloid deposits could be detected in all mice. The APPswe/PS1dE9 mice had a few amyloid deposits already at the age of 6 months, but a severe amyloid pathology was observed only from the 9 months old mice. The CSF A β 42 levels of 6 months old APPswe/PS1dE9 mice was comparable to the 13 months old APPswe/PS1 mice. The CSF A β 42 levels decreased significantly during this time that the amyloid pathology advanced rapidly and the levels in 9 months old mice were significantly lower than the levels in 6 months old mice ($p < 0.001$). Taken together, the CSF A β levels increased before the development of amyloid pathology and reached a plateau where the levels remained until the levels decreased when full blown amyloid pathology developed.

The CSF A β 42 levels of 5 months old APPswe/PS1 mice correlated with the HC insoluble A β 42 at the age of 7 months ($r = 0.48$, $p = 0.03$). Also the CSF A β 42 levels of the 8 months old mice correlated with the HC insoluble A β 42 of the 13 months old mice ($r = 0.53$, $p < 0.001$). The CSF levels that were measured from mice at the 13 months when the tissue sample was obtained showed weaker correlation ($r = 0.33$, $p = 0.04$). In the APPswe/PS1dE9 mice there was no correlation between the HC insoluble A β 42 levels and the CSF A β 42 levels at either time point.

5.2 CLINICAL CHARACTERISTICS OF HUMAN SUBJECTS

A total of 60 controls and 79 patients with MCI of whom 33 progressed to dementia were included in the study. Study **II** included 46 control subjects and 78 MCI patients. During the follow-up, 23 MCI patients developed AD. The age and gender distribution were similar in all groups. Study **III** included all of the patients from study **II**, except one control subject who was suffering from fibromyalgic pains and was cognitively healthy at the baseline but progressed to AD by the time of the last follow-up of study **III**. Study **III** included also one

additional MCI patient and the follow-up period was extended for many of the subjects. At the end of study **III**, the control group included 19 subjects with depression and 41 subjects with other neurological symptoms at the baseline. The MCI group consisted of 46 stable MCI patients and 33 who progressed to dementia. The age and gender distribution was similar between the groups. The follow-up period for the progressive patients ended at the time of diagnosis of dementia, and therefore the follow-up was shorter in the progressive MCI group. The presence of the ApoE 4 allele was more frequent in progressive MCI group than in the control or stable MCI groups. Table 3 presents the baseline demographic details of subjects in the different groups.

At the baseline of study **III**, the MCI patients were divided into three groups according to the type of the cognitive impairment. The largest group was the amnesic MCI patients with a total of 47 patients. Eight had isolated amnesic MCI, 39 had impairment in some other cognitive domain as well. Seventeen patients had executive MCI and 15 patients had impairment in some other cognitive domain, for example language or visuospatial skills. In all, 51% of the amnesic MCI patients, 41% of the executive MCI patients and 13% of the other MCI patients progressed to dementia during the follow-up. Twelve of the stable MCI patients had cognitive impairment due to vascular reasons, the etiology of 32 stable MCI patients causing the cognitive impairment remained undetermined. The most frequent diagnosis in the progressive MCI group was AD, but the progressive MCI group included also five patients with dementia of mixed etiology and one VaD patient.

Table 3. The clinical characteristics of studied controls (n=60) and MCI patients (n= 79) at the baseline.

	Controls			Stable MCI				Progressive MCI			
	All	OND	Depression	All	Amnesic	Executive	Other	All	Amnesic	Executive	Other
n	60	41	19	46	23	10	13	33	24	7	2
Age	68.3±8.1	69.4±6.8	66.0±10.2	69.5±8.1	70.0±7.7	68.4±7.3	69.2±9.9	71.7±6.7	71.1±7.1	74.3±5.8	69.0±4.2
Sex, men/women	25 / 36 42%/58%	17 / 24 41%/59%	8 / 11 42%/58%	20 / 26 44%/56%	11 / 12 48%/52%	7 / 3 70%/30%	2 / 11 15%/85%	13 / 20 39%/61%	10 / 14 42%/58%	2 / 5 29%/71%	1 / 1 50%/50%
MMSE	26.2±2.9 missing 9	26.7±2.5 missing 6	25.1±3.4 missing 3	24.1±2.5	23.3±3.1	24.3±1.8	24.2±1.7	23.9±2.7	23.8±2.9	23.9±1.9	25.5±3.5
Follow-up, years	3.94±2.22	3.85±2.13	4.13±2.45	4.57±3.09	3.87±2.63	5.90±4.23	4.77±2.71	3.52±1.95	3.50±2.09	3.00±1.41	5.50±0.71
ApoE4 +/-	21 / 39 35%/65%	13 / 28 32%/68%	8 / 11 42%/58%	15 / 30 33%/67% missing 1	7 / 15 32%/68% missing 1	3 / 7 30%/70%	5 / 8 38%/62%	26 / 7 79%/21%	20 / 4 83%/17%	4 / 3 57%/43%	2 / 0 100%/0%
Etiology				12 VCI 2 FD 32 NAS	6 VCI 1 FD 16 NAS	4 VCI 6 NAS	2 VCI 1 FD 10 NAS	27 AD 5 Mixed dementia 1 VaD	21 AD 3 Mixed dementia	5 AD 2 Mixed dementia	1 AD, atypical 1 VaD

Data presented as mean ± SD or number of subjects and percentage of all subjects in the group. MCI, mild cognitive impairment; OND, other neurological diseases; VCI, vascular cognitive impairment; FD, suspected frontal degeneration; NAS, non aliter specificatus; AD, Alzheimer's disease; VaD, vascular dementia.

5.3 CEREBROSPINAL FLUID BIOMARKER LEVELS AT BASELINE

In studies **II** and **III**, the CSF A β 42, tau and phospho-tau levels were not statistically different between the controls and stable MCI patients. However, in progressive MCI patients, the CSF A β 42 levels did decrease and tau and phospho-tau levels increased when compared either controls or stable MCI patients.

The CSF biomarker levels were not different between men and women. In study **III**, tau levels correlated with age in the control group ($r=0.290$, $p=0.025$), but none of the measured biomarkers correlated with age in the whole study group or any other diagnostic group. However, the ApoE genotype was found to have an effect on all of the studied biomarkers in the control group and on A β 42 in the progressive MCI group.(Figure 2)

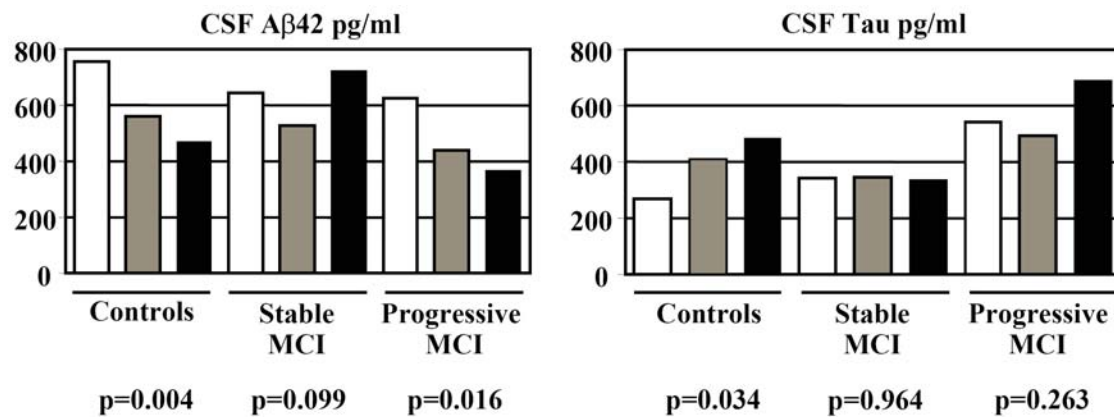


Figure 2. CSF levels of A β 42 and tau in different diagnostic groups. White bars: subjects with no ApoE 4 alleles (n=76), grey bars: subjects with one ApoE 4 allele (n=44) and black bars: subjects with two ApoE 4 alleles (n=18).

5.4 CUT-OFF VALUES OF DIFFERENT BIOMARKERS FOR DEVELOPING AD

The cut-off values for different biomarkers were determined by using a ROC analysis. The analysis included all controls subjects and progressive MCI subjects from study **II**. Figure 3 shows an example of the result of A β 42 ROC analysis in a visual form.

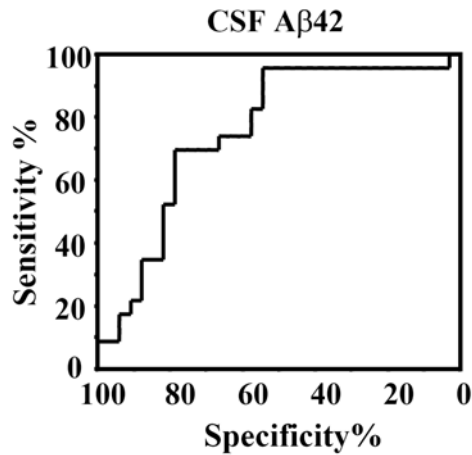


Figure 3. The ROC results for Aβ42. The analysis included 46 controls and 23 MCI patients who developed AD during the study.

The best cut-off value was the point on the curve that had the highest sensitivity and specificity, in the case of Aβ42 the value was 452 pg/ml, area under curve (AUC) 0.831 (0.726-0.935). The cut-off value for tau was 399 pg/ml, AUC 0.869 (0.786-0.951) and phospho-tau 70 pg/ml, AUC 0.839 (0.744-0.935). The test for Aβ42 was positive if the CSF Aβ42 level was below the cut-off value and for tau and phospho-tau if the CSF levels were above the cut-off value. These cut-off values were used to determine the sensitivity and specificity of these biomarkers for progressive MCI.(Table 4) Also the 5 mixed dementia subjects and the one VaD patient were included in the analysis.

5.5 PREDICTORS OF DEVELOPING DEMENTIA

The predictive value of different clinical measures was determined by logistic regression analysis on study III. The analysis included only MCI patients. The patients' age, sex or MMSE score at the baseline were not significant predictors for dementia. The patients carrying the ApoE 4 allele were about 7 times more likely to develop dementia during the study. The patients with two ApoE 4 alleles were also younger at the time of dementia diagnosis.

Table 4. The characteristics of different measures as a diagnostic test for developing dementia.

	Controls Specificity	Stable MCI Specificity	Progressive MCI Sensitivity	Overall Accuracy	Positive LR Controls Stable MCI	Negative LR Controls Stable MCI
Aβ42	90.0%	80.4%	57.6%	79.1%	5.76 (2.55 - 12.99)	0.47 (0.31 - 0.71)
	54 / 60	37 / 46	19 / 33	110 / 139	2.94 (1.53 - 5.67)	0.53 (0.35 - 0.81)
Tau	76.7%	63.0%	84.8%	74.1%	3.64 (2.25 - 5.88)	0.20 (0.09 - 0.45)
	46 / 60	29 / 46	28 / 33	103 / 139	2.30 (1.53 - 3.44)	0.24 (0.10 - 0.56)
Phospho-tau	66.7%	60.9%	84.8%	69.1%	2.55 (1.73 - 3.74)	0.23 (0.10 - 0.52)
	40 / 60	28 / 46	28 / 33	96 / 139	2.17 (1.47 - 3.20)	0.25 (0.11 - 0.58)
Aβ42 + tau	93.3%	89.1%	48.5%	82.0%	7.73 (2.83 - 21.07)	0.52 (0.36 - 0.74)
	56 / 60	41 / 46	17 / 33	114 / 139	4.74 (1.94 - 11.56)	0.54 (0.38 - 0.78)
Aβ42 + phospho-tau	93.3%	89.1%	48.5%	81.3%	7.27 (2.65 - 19.97)	0.55 (0.39 - 0.77)
	56 / 60	41 / 46	16 / 33	113 / 139	4.46 (1.81 - 10.96)	0.58 (0.41 - 0.82)
HC atrophy		61.5%	87.5%	71.4%	NA*	NA*
		8 / 13	7 / 8	15 / 21	2.28 (1.09 - 4.75)	0.20 (0.03 - 1.34)

*Data presented as percentage and correctly allocated subjects of all subjects in the group or as LR (95% CI). The table also includes these figures of MTL atrophy (at least one of the HC was atrophied) of the 21 patients from study IV. The first LR indicates the results when progressive MCI patients were compared to controls and the second LR indicates the results when the progressive MCI patients were compared to stable MCI patients. LR, likelihood ratio, MCI, mild cognitive impairment. *NA not applicable.*

The amnesic MCI and executive MCI patients were over 10 times more likely to develop dementia than patients with other types of cognitive impairment.(Figure 4) There was no significant difference in progression to dementia between the amnesic MCI patients and executive MCI patients.

All of the studied CSF biomarkers were significant predictors of dementia alone, but in the backward logistic regression CSF tau was the only biomarker that remained significant. In order to obtain more reliable results on the predictivity of the CSF biomarkers for development of dementia, the survival analysis of MCI patients with different numbers of abnormal biomarker values (A β 42 or tau) was conducted by including all of the patients from

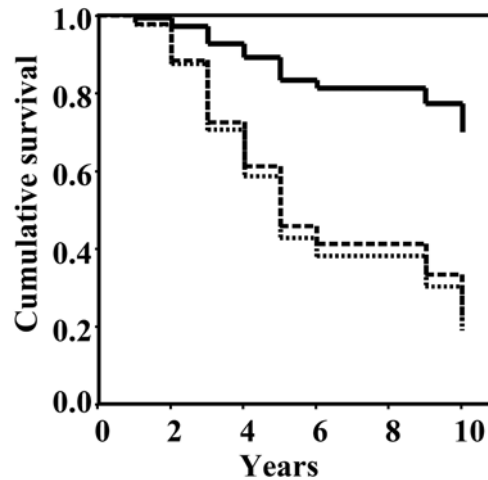


Figure 4. The survival curves of 79 MCI patients divided into groups according to the type of their cognitive impairment. The end point event was the development of dementia. The solid line represents the patients with other types of cognitive decline, the dashed line represents amnesic MCI patients and the dotted line represents executive MCI patients.

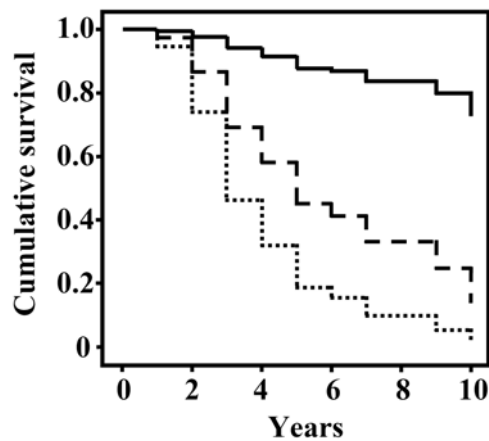


Figure 5. Survival curves of 80 MCI patients (79 from study **III** and one from study **IV**) divided into groups according to CSF A β 42 and tau values. The end point event was the development of dementia. The solid line represents the patients with normal biomarker values, the dashed line represents patients with either abnormal A β 42 or tau value and the dotted line represents patients with both abnormal A β 42 and tau values.

study **III** and one additional patient from study **IV**. The follow-up period was also one to two years longer in eight stable MCI patients from study **II**. Figure 5 shows that MCI patients who have normal CSF biomarker values progress to dementia less frequently than those MCI patients who have either abnormal CSF levels of A β 42 or tau. Almost all of the MCI patients who have both abnormal A β 42 and tau develop dementia, over half of them during the first five years after the sample collection.

5.6 ASSOCIATION BETWEEN CSF BIOMARKERS AND MEDIAL TEMPORAL LOBE ATROPHY

In the whole study group, CSF tau levels correlated inversely with both right and left HC and volume of left EC. The results of phospho-tau were virtually identical. CSF A β 42 correlated with the volume of left HC. When the groups were analysed separately, in the stable MCI patients tau correlated with volumes of both hippocampi and with left EC volume. The results of phospho-tau were otherwise similar, but the correlation with left EC did not reach statistical significance. In the progressive MCI patients, left HC volume correlated with CSF A β 42 and phospho-tau. Word list delayed recall correlated positively with both right and left HC volumes and inversely with CSF tau and phospho-tau. In the subgroup analyses, there were no significant correlations in the stable MCI patients whereas there was a significant correlation between delayed recall and left hippocampal volume in the progressive MCI patients.

6. DISCUSSION

6.1 CHANGES IN CSF A β 42, TAU AND PHOSPHO-TAU OCCUR EARLY IN THE COURSE OF THE DISEASE

Since the neuropathological changes of AD may start to develop as early as two decades before the onset of symptoms, we hypothesized that the AD associated changes in CSF biomarkers would be evident before the clinical diagnosis of AD and could be used to predict the development of AD in MCI patients. Based on the results of studies **II** and **III**, this hypothesis was proven to be true. CSF A β 42 levels were decreased and CSF tau and phospho-tau levels were increased in those MCI patients who received a diagnosis of AD during a follow-up period of approximately four years. It should be emphasized that these subjects could not be clinically separated from the stable MCI patients even though they underwent a thorough clinical and neuropsychological examination. The results indicate that the changes in CSF biomarkers are related to the presence of AD associated neuropathology in patients with progressing cognitive impairment before dementia. However, the variability in CSF biomarker levels between individual subjects in each group was large and there was a significant overlap between the groups.

6.2 BIOLOGICAL VALIDITY OF CSF BIOMARKERS

One of the most important criteria for a biomarker is that it should detect the fundamental feature of neuropathology of AD (Consensus report of the Working Group on: "Molecular and Biochemical Markers of Alzheimer's Disease". The Ronald and Nancy Reagan Research Institute of the Alzheimer's Association and the National Institute on Aging Working Group. 1998). Previous studies in transgenic mice have suggested that the on-going amyloid pathology is reflected in plasma and CSF (DeMattos et al. 2002, Kawarabayashi et al. 2001). The results from study **I** on mice confirmed these findings. CSF A β 42 levels in mice started to decline at the time when the progression of amyloid pathology accelerated in brain. Although it is not possible to directly extrapolate the results from the mice to human disease, these results imply that the amyloid plaques may well interfere with the clearance of A β 42 from brain to CSF.

Few human studies that have examined the association between CSF A β 42, tau and phospho-tau and AD neuropathology. One study examined *post mortem* ventricular CSF A β 42 levels and found that lower levels correlated with amyloid pathology in brain and cerebral vessels (Strozyk et al. 2003). Other studies have shown that CSF tau or phospho-tau231 correlate with NFT score or levels of hyperphosphorylated tau in neocortex (Buerger et al. 2006a, Tapiola et al. 1997). One study on *ante mortem* CSF found that increased tau levels were associated with AD and decreased A β 42 levels were associated with DLB and prion disease (Clark et al. 2003). However, the association was not assessed between the CSF biomarkers and quantitative amount of amyloid or tau pathology. One recent study examined the diagnostic accuracy of CSF A β 42, tau and phospho-tau in 100 neuropathologically confirmed AD patients (Engelborghs et al. 2007). The CSF biomarkers helped to distinguish seven out of nine of those AD patients whose clinical diagnosis had remained uncertain. Again, CSF A β 42 levels were lower and tau and phospho-tau levels higher in the AD patients when compared to controls, but the association of the CSF biomarkers to the quantitative amount of pathology was not reported. Even though the current data is sparse, it seems that these biomarkers reflect AD associated neuropathological changes. Our own preliminary data from a large clinicopathological study supports this belief, the *ante mortem* biomarkers correlated with quantitative amyloid and NFT pathology in the neuropathological examination.

Our own longitudinal study showed that the CSF A β 42 levels decreased during a three year period, possibly reflecting the aggravation of the amyloid pathology in the brain (Tapiola et al. 2000b). Other human data support also the association between the CSF A β 42 and amyloid pathology. Molecular imaging of amyloid using amyloid binding compound PIB in living subjects has been shown to reflect amyloid accumulation in the brain (Kemppainen et al. 2006). The reduced CSF A β levels are associated with increased PIB binding in brain, which supports the association of a decline in CSF A β 42 levels and extent of amyloid pathology (Fagan et al. 2006). Furthermore, in a study combining CSF biomarkers and PIB analysis, all of the subjects whose CSF A β 42 level was below 457 pg/ml displayed also positive PIB binding and those whose levels were above that level showed negative PIB binding (Fagan et al. 2007). This value is virtually the same as the calculated cut-off value for A β 42 in study II.

Thus the current data indicate that CSF biomarkers are strongly associated with the AD

associated neuropathology. However, the mechanisms involved in the development of CSF changes have remained unclear. Several mechanisms may influence CSF A β 42 levels during the development of AD. The reduced levels may be due to the decreased synthesis, increased elimination or decreased clearance into CSF from the brain. Metabolism of A β 42 is a very dynamic process. A recent study showed that A β 42 is rapidly produced and cleared from the brain (Bateman et al. 2006). Therefore even very slight changes in the balance between the synthesis and elimination might lead to accumulation of amyloid in brain as plaques. Many studies, including our study **III**, have shown that the ApoE 4 allele has a dose dependent effect on CSF A β 42 levels (Prince et al. 2004, Sunderland et al. 2004, Tapiola et al. 2000a). Furthermore, the CSF A β 42 levels decrease faster over an average of a four year period in ApoE 4 positive subjects than in the ApoE 4 negative subjects (Huey et al. 2006). The association between the ApoE and neuropathology of AD is complex but the neuropathological studies have suggested that ApoE 4 can enhance A β aggregation (Gomez-Isla et al. 1996, Moir et al. 1999, Polvikoski et al. 1995) which in turn might lead to decreased CSF A β levels. Recent data also indicate that CSF A β 42 levels show an hour-to-hour variation within individuals, with the trend for increasing levels starting from morning (Bateman et al. 2007). The changes seem large enough to have possible implications on diagnostic use and may require standardization of protocols with respect to time for collecting CSF samples.

6.3 CSF BIOMARKERS AS DIAGNOSTIC AIDS

Our results revealed that the combination of decreased CSF A β 42 and increased tau or phospho-tau predicted relatively accurately the development of AD during a follow-up of a few years. Another recent study also reported that the combination of CSF A β 42 and tau was a good predictor of developing AD (Hansson et al. 2006). The high positive likelihood ratio (LR) indicates that the abnormal result in the combination test confirms AD in mildly impaired patients with high accuracy. Only 7% of the controls and 11% of the stable MCI patients who had both abnormal A β 42 and tau did not develop dementia. However, these subjects may be false positives because it is impossible to exclude AD associated neuropathological changes even in clinically healthy controls, much less in the stable MCI patients. Many studies have shown that apparently asymptomatic subjects may display brain pathology that fulfils the neuropathological criteria for AD (Bennett et al. 2006, Galvin et al.

2005, Neuropathology Group. Medical Research Council Cognitive Function and Aging Study 2001, Price and Morris 1999). The lack of a golden standard, the neuropathological diagnosis, is a problem in most biomarker studies performed on living subjects. This may also result in low accuracy in the differentiation between AD and other dementias since AD pathology is a common finding in subjects with VaD and DLB (Holmes et al. 1999, Londos et al. 2001, Neuropathology Group. Medical Research Council Cognitive Function and Aging Study 2001). It is possible that changes in CSF A β 42 and tau reflect the presence of neuropathology of AD in subjects that receive another clinical diagnosis. One way to improve the accuracy of the clinical diagnosis is to use a long follow-up. The one control subject who entered the study as a healthy control but was later excluded from study due to development of AD emphasizes this problem in cross-sectional studies. Her measured biomarkers were clearly abnormal at the stage when her cognitive capability was normal, but after ten years follow-up she developed AD. Also others have reported decreased CSF A β 42 levels in cognitively healthy subjects who later developed AD (Skoog et al. 2003). Although the follow-up period in our study, approximately four years, was longer than in most of the previous MCI studies, it would need to be even longer to reliably exclude all patients with developing AD. Thus it is possible that our stable MCI group also includes a few patients who will ultimately progress to dementia.

The present clinical criteria for AD date from 1993 and progress in both clinical and biomedical research has implications for revising the criteria. Currently, symptomatic medications for AD are widely used, but also disease modifying treatments are under development. Numerous compounds have entered preclinical studies and phase I and II clinical trials, and there are as many as twelve different compounds undergoing phase III trials for either slowing or attenuating the disease progression (Alzheimer Research Forum). Many of these compounds are drugs that are already in clinical use for other indications, for example statins and non-steroidal anti-inflammatory drugs (NSAIDs). Two of these test compounds may influence metabolism of A β . Tramiprosate (AlzhemedTM) binds to A β and prevents the formation of amyloid fibrils in transgenic mice (Alzheimer Research Forum). R-flurbiprofen (FlurizanTM) belongs to the NSAID group, but it is the only member of that class of compounds that has proven effect on A β production since it selectively modulates γ -secretase activity, decreasing the production of A β 42 (Eriksen et al. 2003). Although the first A β vaccination trial was prematurely terminated due to side effects, less toxic A β

immunotherapeutic approaches are under development (Brendza and Holtzman 2006, Schenk 2002, Senior 2002). It is likely that any disease modifying treatment would be most helpful if it could be initiated at an early stage of the disease. However, although the current clinical criteria work relatively well in advanced AD, they are not suitable for providing an early diagnosis. The discussion on updating the criteria is already underway with the key objective being to improve the early diagnosis of AD. One issue in the debate is the role of CSF biomarkers and other paraclinical tests in AD diagnostics. Although there are still many unresolved issues in understanding the characteristics of CSF A β 42 and tau, a knowledge of their levels may be helpful for clinicians. However, due to their low sensitivity, CSF biomarkers will only serve as a supportive test for positive diagnosis, not as a crucial part of the standard diagnostic criteria.

6.4 ASSOCIATION BETWEEN CSF BIOMARKERS AND STRUCTURAL CHANGES IN MEDIAL TEMPORAL LOBE

MTL atrophy has also been intensively evaluated as a possible biomarker for AD and several studies have shown that the presence of MTL atrophy predicts progression of MCI and development of AD (Bouwman et al. 2006, Jack et al. 1999, Killiany et al. 2000). Study **IV** showed that CSF biomarkers, especially tau and phospho-tau were significantly correlated with MTL atrophy. The data in the literature is sparse and contradictory. One study reported a significant relationship between CSF phospho-tau₂₃₁ and HC volume in 22 AD patients, whereas another study on 88 patients found no correlation between these parameters (Hampel et al. 2005, Schonknecht et al. 2003). A study with 18 MCI patients and controls reported an inverse correlation between CSF phospho-tau₂₃₁ and HC volume, whereas a positive correlation in AD patients was found in another study (Hampel et al. 2005, de Leon et al. 2004). One recent study using a visual rating of MTL atrophy instead of volumetric analysis reported an inverse relationship between MTL atrophy and CSF A β 42, but no correlation to CSF tau in 39 MCI patients (Schoonenboom et al. 2005). The data from study **IV** should be considered very cautiously due to the small sample size. However, it is intriguing, that CSF tau levels correlated with MTL atrophy. CSF tau has been suggested to represent a marker for axonal and neuronal damage (Blennow et al. 1995). It is possible that CSF tau reflects the rate of neuronal cell death and axonal damage predominantly in MTL structures, which would fit well to the model of progression of NFT pathology in AD (Braak and Braak 1991). Another possibility is that the correlation arises from the presence of AD per se since both markers

reflect the disease.

In conclusion, these studies support the hypothesis that CSF tau may reflect neurodegeneration in AD. However, the number of patients in the studies is small and thus it is not possible to examine the added value of each of these biomarkers in the diagnosis of AD.

6.5 CLINICAL IMPLICATIONS

In addition to having devastating effects on life of the individual patients and their families, AD also poses enormous socioeconomic burden the society. It is estimated that in 2005 there were over 29 million people who had dementia and worldwide societal costs were US\$ 315 billion, over US\$ 100 billion in European Union alone (Wimo et al. 2007). As the population ages, the number of patients and costs of care will increase even further. The undiagnosed AD patient is more expensive for society than the patient with a diagnosis and appropriate treatment (Hill et al. 2002). Therefore it is essential to detect the AD patients as early as possible out of the vast pool of patients who may express concern about cognitive impairment. Predicting the prognosis of MCI based on neuropsychological features is not possible without follow-up examinations to reveal a decline. Progression to AD and other dementias varies greatly between studies due to different definitions of MCI, differences in populations studied and length of the follow-up period. Therefore, the design of this study was practical, based on a "real life" situation. The majority of the subjects were recruited from the memory clinic and the inclusion criteria were intentionally relatively wide. The MCI was not defined according to strict criteria of amnesic MCI but also patients with declines in other cognitive domains were included. Nonetheless, a detailed assessment of several cognitive domains was performed, and the effect of the type of cognitive impairment on progression was also examined.

Many studies have shown that the most consistent predictor for AD and other dementias is memory performance during a long follow-up. AD patients exhibit problems in both visual and verbal memory as early as ten years before the onset of dementia (Elias et al. 2000, Kawas et al. 2003, Tierney et al. 2005). The decline in other cognitive domains including fluency, speed and attention becomes apparent closer to the diagnosis (Saxton et al. 2004, Tierney et al. 2005). Studies with a short follow-up period have shown inconsistent results. Some studies have found that the amnesic symptoms are the best indicators for predicting

dementia (Busse et al. 2003, DeCarli et al. 2004, Perri et al. 2007). On the contrary, the predictive value of amnesic symptoms has been poor in many studies and patients with isolated memory impairment have shown low rates of progression to dementia (Bozoki et al. 2001, Meyer et al. 2002a, Sacuiu et al. 2005). A significant number of patients even improve during the follow-up (Ganguli et al. 2004). Furthermore, individual amnesic MCI patients progress to dementia with very distinct patterns of cognitive decline. It seems that the rate of progression varies and cognitive domains are affected in a different order (Hodges et al. 2006).

The results of study **III** indicated that executive MCI was frequent and these patients progressed to dementia even faster than amnesic MCI patients. This is probably due to the fact that a large proportion of the MCI patients was derived from the clinic and therefore might have been closer to the dementia stage of AD at the baseline. The progression rate to dementia was 51% in the amnesic MCI patients and 41% in the executive MCI patients. However, it needs to be noted that the follow-up period of the stable amnesic MCI patients was two years shorter than that of the executive MCI patients. Therefore the difference in progression rates to dementia might be higher if the amnesic MCI patients were followed as long as the executive MCI patients. Executive dysfunction has emerged as possible predictor of dementia also in other studies. Executive dysfunction is a common comorbidity factor in amnesic MCI patients (Loewenstein et al. 2006, Nordlund et al. 2005) and it may occur on its own without amnesic symptoms, as in our study (Royall et al. 2004, Tabert et al. 2006). Amnesic MCI patients with concomitant executive dysfunction also convert to dementia at higher a rate than patients with isolated amnesic symptoms (DeCarli et al. 2004).

The rarity of isolated amnesic MCI suggests that in the clinical setting the patients who complain of cognitive problems other than memory disturbance should also be considered to be at high risk for dementia, particularly AD. The high frequency of executive MCI without amnesic symptoms in this study and the high progression rate to dementia of these patients strongly supports this belief. It has also been suggested that executive dysfunction influences the activities of daily living and thus the development of the full-blown dementia syndrome more than memory decline (Royall et al. 2005). Furthermore, almost half of the subjects who develop dementia within a three year period do not report memory problems in the clinic even when they are asked directly (Palmer et al. 2003). Therefore the amnesic MCI criteria exclude a high number of MCI patients who are at a high risk of developing AD and other

dementias. In summary, clinicians should not concentrate only on memory impairment in their evaluation of the predementia stage of AD. Nonetheless, it seems that even the most detailed single neuropsychological assessment cannot distinguish MCI patients who will develop dementia from those who will remain unchanged. Also, the causes of executive MCI may be more heterogeneous than those of amnesic MCI. Therefore one will need to resort to other diagnostic instruments such as CSF biomarkers are when trying to clarify the diagnosis and prognosis of the typical MCI patient.

6.6 LIMITATIONS OF THE STUDY

The study population of this study included subjects from both a population based cohort and from the memory clinic. Therefore the neuropsychological tests that were used for assessing different cognitive domains, especially memory, varied between different patients. However, the normative values for the different memory tests were available. The inclusion of subjects from the memory clinic may lead to a potential selection bias. Although all patients with cognitive complaints were invited to participate in the study, many declined and it is possible that those with more severe symptoms were more willing to enter the study. Also the inclusion of patients with depression may be problematic. Many behavioural symptoms, especially depression, may precede memory impairment and dementia years before the onset of symptoms. Therefore it is possible that the control group included a few patients who may eventually progress to MCI and dementia. The etiology of MCI was not defined in large proportion of stable MCI patients. This may be partly due to the fact that most patients underwent CT imaging instead of MRI and therefore mild atrophy or vascular pathology could not be detected. Thus, many of the stable MCI patients may have been suffering from VCI. It is also impossible to exclude mild depression or temporary use of some medication that could slightly impair cognitive function. Furthermore, when the study was initiated, the criteria for MCI included the cognitive impairment beyond age-adjusted normative values, not impairment over a period of time. Thus, some stable MCI patients may have had a low basic level of cognitive functions for all their lives but the values would low enough that they were included in the study. The follow-up period was longer than in most previous studies, but an even longer time would be needed to reliably detect all patients who will eventually develop AD. Also, the final diagnosis of AD was based on clinical criteria, and no neuropathological confirmation of the diagnosis was undertaken. Although the number of studied subjects was quite substantial, it is too small to accurately assess the added value of CSF biomarkers as

diagnostic tests for AD.

There are also some methodological limitations. Considering the recent data on hour-to-hour variability of CSF A β 42 values within the same individual, (Bateman et al. 2007) one possible limitation of this study is that the time of the CSF sampling was not standardized. The CSF samples were collected mainly at midday or in the early afternoon, which is also the time that out-patient clinic CSF samples are taken in general. The standard polypropylene tubes were used to prevent the artefactually low A β 42 level due to binding to the tubes. The samples were immediately aliquoted and frozen, and they were stored at -70°C until analyses. Some of the samples were frozen for up to a decade but no association between the storage time and CSF A β 42, tau and phospho-tau was detected. The intra-assay variation was not assessed during the study. However, these assays are currently provided as a diagnostic service and run routinely in the same department where the study was conducted. The long term variability has remained good, median intra-assay CV% being 14.6% for A β 42, 8.3% for tau and 6.7% for phospho-tau.

In the context of the studies on mice it needs to be taken into account that the physiology of the mouse is different from the human and the results of these studies cannot be extrapolated to humans directly. The transgenic mice are even further divergent from humans, because their normal physiology is disturbed by the inclusion of two genes with different mutations. The resulting changes in different studied parameters are most likely due to the influence of these inserted genes, but it is also possible that the transgene construct is inserted in a way that it disturbs the function of some endogenous gene. However, both of the mouse lines that were used in this study have been well characterized, and the development of amyloid pathology as a result of the mutated APP and PS1 genes has been described in numerous other transgenic mouse lines as well (Borchelt et al. 1997, Jankowsky et al. 2004, Spire and Hyman 2005). The development of amyloid pathology in mice is different from the neuropathological process in human brains. In transgenic mice, the main reason for A β accumulation is the increased production of A β , whereas in human brain, especially in sporadic AD, the accumulation is a consequence of several factors, including decreased degradation and reduced clearance of A β . Furthermore, the mice do not exhibit abundant vascular amyloid pathology or age related changes in brain. It could be speculated that these common changes including atherosclerosis, are also important contributors to the

neurodegenerative process in humans but are not present in the mouse models. The amyloid pathology also develops very rapidly in the mice, during a 6-month time frame, whereas in humans the pathology appears over a period as long as two decades. Another limitation of the mice that were used in this study is the fact that they do not develop tau pathology. Therefore the study examined only the association between the CSF A β 42 and amyloid pathology in these mice. However, the results of study **I** supported the data on human studies; the CSF A β 42 levels decreased simultaneously with the increasing amyloid pathology.

7. CONCLUSIONS

The aim of the present study was to investigate whether the CSF A β 42, tau and phospho-tau can be used as diagnostic aid in diagnosis of early AD. The secondary aim was to examine the relationship between the CSF biomarkers and neuropathological processes associated with AD. The following conclusions can be drawn:

1. AD associated changes in CSF biomarkers, i.e. decrease in A β 42 and increase in tau and phospho-tau occur during a predementia stage of AD.
2. CSF A β 42, tau and phospho-tau values are associated with MTL atrophy. CSF tau levels may reflect the neuronal damage occurring in MTL.
3. ApoE 4 allele has a dose dependent effect on CSF biomarkers such that the A β 42 levels decrease and tau and phospho-tau levels increase with elevating numbers of ApoE 4 alleles.
4. A large proportion of MCI patients do not have memory impairment. Executive dysfunction without amnesic symptoms is common, and many of these patients progress to AD. In the clinical context, patients with cognitive impairment without memory complaint should be also considered to be at a high risk for developing AD.
5. Several years before the any clinical diagnosis can possibly be made, the combination of decreased CSF A β 42 and increased tau or phospho-tau can differentiate with high specificity those MCI patients who will eventually develop AD from those who remain stable. The positive test result can be used as a confirmation of a clinical diagnosis of possible early AD. However, the sensitivity of the test is low and a negative result cannot exclude the presence of AD.

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