Alzheimer’s disease is the most common cause of dementia. When the first symptoms of memory deficit take place, neuronal damage has already irreversibly happened. Thus, better ways to achieve an earlier diagnosis will need to be devised. The aim of this study was to explain the relationship of the plasma and cerebrospinal fluid biomarker levels to cognition and brain tissue pathology. The results demonstrated that low levels of CSF $\text{A}_\beta$ are associated with a high amyloid plaque load of brain biopsies. In addition, increased CSF tau and p-tau levels are associated with the presence of tau in the cortical biopsy.
TONI SEPPÄLÄ

Blood and Cerebrospinal Fluid Biomarkers of Alzheimer’s Disease

Application in the Evaluation of Progression and Differential Diagnosis

To be presented by permission of the Faculty of Health Sciences, University of Eastern Finland for public examination in Mediteknia Auditorium (MET), Kuopio, on Friday, August 10th 2012, at 12 noon

Publications of the University of Eastern Finland
Dissertations in Health Sciences
Number 122

Department of Neurology, Institute of Clinical Medicine
School of Medicine, Faculty of Health Sciences
University of Eastern Finland
Department of Neurology, Kuopio University Hospital
Kuopio
2012
Author's address: School of Medicine
University of Eastern Finland
KUOPIO
FINLAND

Supervisors: Professor Hilkka Soininen, M.D., Ph.D.
School of Medicine
University of Eastern Finland
KUOPIO
FINLAND

Dr. Sanna-Kaisa Herukka, M.Sc, M.D. Ph.D.
School of Medicine
University of Eastern Finland
KUOPIO
FINLAND

Docent Ville Leinonen, M.D., Ph.D.
School of Medicine
University of Eastern Finland
KUOPIO
FINLAND

Professor Tuula Pirttilä, M.D., Ph.D. †
School of Medicine
University of Eastern Finland
KUOPIO
FINLAND

Reviewers: Associate Professor Martin Ingelsson, M.D., Ph.D.
Department of Public Health and Caring Sciences
University of Uppsala
Uppsala
SWEDEN

Docent Marc Baumann, M.D., Ph.D.
Protein Chemistry/Proteomics and Peptide Synthesis and Array Laboratory
University of Helsinki
HELSINKI
FINLAND

Opponent: Associate Professor Wiesje M. van der Flier, Ph.D.
Alzheimer Center, Department of Neurology
VU University Medical Center
AMSTERDAM
NETHERLANDS
ABSTRACT

Alzheimer’s disease (AD) is the most common cause of dementia; it is becoming more and more frequent in conjunction with the greying of the population. In addition to individual suffering, AD imposes a socioeconomical burden in western countries that is beyond comparison to any other disease.

AD begins in the brain about two decades before the clinical symptoms occur. When an individual experiences the first symptoms of memory deficit or other cognitive failures, the neuronal damage has already irreversibly happened to a great extent. Currently, the acetylcholinesterase inhibitor treatment only decelerates the rate of the cognitive deterioration and even in this respect is not very efficient and certainly does not influence the disease process. If one wishes to study new therapeutics or to bring forward the use of current drugs, better ways to achieve an earlier diagnosis will need to be devised. Earlier studies had reported cerebrospinal fluid (CSF) levels of biomarkers i.e. Aβ42, tau and p-tau were able to increase the accuracy of the underlying AD diagnosis at earlier stages of the disease, when only mild cognitive impairment (MCI) was present. However, at the beginning of the present study, relatively little was known about the longitudinal relationship of these biomarkers to AD stage.

The aims of the present study were to clarify the longitudinal characteristics of the plasma Aβ and CSF Aβ42, tau and p-tau for more successful interpretation as well as for clinical use, and also to explain the relationship of the plasma and CSF biomarker levels to cognition, differential diagnosis and brain tissue pathology in several clinical settings.

The results of this study detected changes of plasma Aβ and CSF biomarkers over time, and the changes were related to the cognitive decline in AD progression and the disease stage. Low levels of CSF Aβ42 were associated with a high amyloid plaque load of brain biopsies of living patients with presumed normal pressure hydrocephalus (NPH) that in follow-up were subsequently diagnosed to have AD. In addition, increased CSF tau and p-tau levels are associated with the increased presence of hyperphosphorylated tau in the cortical biopsy. There is also a concentration gradient between ventricular and lumbar CSF Aβ42, tau and p-tau i.e. the Aβ42 level is higher in lumbar samples whereas tau and p-tau concentrations are elevated in ventricular samples. The CSF biomarkers conferred a significant benefit in the differential diagnosis of patients with atypical dementing that may be confused between AD and neuropsychiatric disorders or NPH.

The CSF biomarkers offer the potential for detecting longitudinal changes in AD, but they require clarification before being useful for monitoring the disease progression. The CSF biomarkers represent well the neuropathological changes of a biopsy of prefrontal cortex and therefore provide considerable assistance in the differential diagnosis of atypically symptomatic AD patients.
Seppälä, Toni.
Venen ja selkäydinseteen biologiset merkkitekijät Alzheimerin tauussa – Soveltaminen etenemisen arviointiin ja erotusdiagnostiikkaan.
Publications of the University of Eastern Finland. Dissertations in Health Sciences Numero 122. 2012. 116 s.

TIIVISTELMÄ

Alzheimerin tauut (AT) on tavallisin dementiaa aiheuttava sairaus ja yleistyy väestön ikääntyessä. Potilaiden ja perheiden kokeman sosiaalisen käräjymyksen lisäksi sairaus aiheuttaa vahvasti sosioekonomiset ja yhteiskunnalliset vahingot

AT alkaa aivoissa jopa parikymmentä vuotta ennen ensimmäisenkään klinikisen oireen havaitsemista, mikä tuottaa edelleen ongelmaa. Tämä ongelmia kuitenkin hoitoavain toimien tutkimuksessa, kun potilaat on kyllä ollut määrä eikä niitä voida enää palauttaa. Nykyisellä lääkekäytöllä voidaan vahvasti taata oireiden ja toimintakyvyn kunnostamisen ja peittää

Tutkimuksen tavoitteena oli selvittää, miten selkäydinseteen merkkiaiempien potilaiden tapahtumat klinikassa ja selvitää, miten sairauden aiheuttamat potilaiden kasvattaa.

Esimman tutkimuksen perusteella tiedetään, että selkäydinseteen merkkiaiempien potilaiden tiedot

Tutkimuksen tavoitteena oli selvittää, miten selkäydinseteen merkkiaiempien potilaiden tapahtumat klinikassa ja selvitää, miten sairauden aiheuttamat potilaiden kasvattaa.

Esimman tutkimuksen perusteella tiedetään, että selkäydinseteen merkkiaiempien potilaiden tiedot

Selkäydinseteen merkkiaiempien potilaiden tapahtumat klinikassa ja selvitää, miten sairauden aiheuttamat potilaiden kasvattaa.

Tutkimuksen perusteella voit pian selvittää, miten selkäydinseteen merkkiaiempien potilaiden tapahtumat klinikassa ja selvitää, miten sairauden aiheuttamat potilaiden kasvattaa.

Yleinen Suomalainen asiasanasto: Alzheimerin tauut, dementia, neurologia, markkerit, proteiinit, aivo- selkäydinsetten, muistihäiriöt
Acknowledgements

The study was carried out in the Department of Neurology and Neurosciene, School of Medicine, Faculty of Health Sciences, University of Eastern Finland (former Faculty of Medicine, University of Kuopio) and the Neurocenter of Kuopio University Hospital during the years 2008-2012.

I want to express my deepest gratitude to Professor Hilika Soininen, my supervisor and the Head of the Department of Neurology and Neuroscience. Thank you for the opportunity to carry out the study with such fine facilities and for encouraging me to contact Tuula about the biomarkers seven years ago. Thank you for your careful commentary on my work and your accessible presence whenever there was something to ask or to be solved.

My warmest gratitude belong to my supervisor Dr. Sanna-Kaisa Herukka, who has been carefully guiding me in the world of medical research since the very beginning. Even though we went through medical school together, you have been light years ahead in neuroscience and I am proud of having had the chance to work with you. You have always been available also in practice, you have been kind and collegial to me and I hope these guidelines have been passed on to me. Thank you also for giving me the space and the independence to learn the things I needed to figure out myself. You have a long and successful career ahead of you as a scientist, physician and academic.

I am deeply grateful to my supervisor Professor Tuula Pirtilä who was in charge of the general study design and instructed me for the first two parts of this study. Tuula’s demise after years of illness was a great loss to everybody in the university as well as the entire field of neuroscience. Tuula was a passionate scientist and a great clinical physician, from whom I am proud to have learned a great deal about this specialized field and clinical neurology in general. Meeting Tuula for the last time is one of the memorable moments of my life and has given me the stamina to pass through some difficult times later in the study. Her legacy will pass on through this work as well as Sanna-Kaisa’s and many others'.

I wish to express my sincere appreciation to Docent Ville Leinonen who became my supervisor after Tuula. Ville has shown me an example of an enthusiastic combination of clinical physician and scientist. His ability to run multiple projects and to be able to be there for his colleagues has been something to look up to. I hope I have learned at least something from his positive attitude and the belief that anything is possible.

I am thankful to the official reviewers of this thesis, Associate professor Martin Ingelsson and Docent Marc Baumann, for their careful and constructive review and valuable comments to help me improve the outcome.

I wish to thank my key co-authors Docent Kati Juva, Dr. Anne Koivisto, M. Pharm. Lakshman Puli, Professor Juha E. Jääskeläinen, Dr. Henrik Zetterberg, Dr. Tuomo Hänninen and Dr. Seppo Helisalmi for their collaboration as well as reviews and constructive commentary on my manuscripts.

I am deeply grateful to Päivi Räsänen and Tarja Kauppinen for the priceless technical assistance in the laboratory. Thank you also to Seija Hynynen for valuable information on the history of the study projects and Marjo Laitinen for teaching me the basics of careful ELISA laboratory techniques. In addition, I am thankful to Esa Koivisto, Sari Palviainen, Tuija Parsons, Mari Tikkanen and Tuula Toivanen for their help in departmental matters.

I want to thank Ewen MacDonald for excellent language reviews he has provided during these years.

Thank you to my fellow PhD students Tuomas Paimela, Matti Kärkkäinen and Anne Lammi for the peer support in different occasions. Thank you to all those about a hundred students and colleagues for the countless hours spent in Mikrovitriini during the study
breaks of our medical training and this project. Special thanks belong also to Harri Hyppölä for his comments as a senior colleague several years ago, which encouraged me to begin this study in the first place. I am also grateful to Rita Sorvari and Professor Heikki Helminen for educating me on the essentials of medical research. Thank you to professor Jukka Pelkonen for his leadership in the medical school and standing up for the students.

I express my warmest thanks to Runks: Petja Orre, Veikko Schepel, Petrus Sonnininen, Pekka Lammi, Matti Iso-Mustajärvi, Samu Räsänen, Ari Kaski and Jukka Kuokkanen for your friendship and for being there for me every day for the last eight years. You are like family to me and I will remember it. I am also thankful to my other dear friends that I am not able to mention here but who have been there for me in some fields of my personal life, work or science.

I owe my deep gratitude to my mom Kirsi and my dad Timo for making me understand the meaning of books, education and responsibility. Thank you to my sisters Emmi and Assi. I am also grateful to my grandpa Veikko, grandma Tuula and my other granma Inga for enormous human capital and humane discussions with which they have always provided me.

I also wish to express my gratitude to KuoLO, Finnish Medical Students’ Association, Junior Doctors’ Association in Finland, Finnish Medical Association, Finnish Medical Society Duodecim and people with whom I have worked and become friends in recent years. Thank you for keeping me busy, teaching me to form a critical opinion and to think practically. Special thanks to my friend Hannu Lyysa for pushing me along the path of professional ambition which I’m still on by making me a supervisor for the first time in my life eleven years ago.

And finally. Thank you, Laura. Thank you for being my companion and part of my life, for supporting me at my busiest and most stressful moments, letting me dominate the home office and patiently understanding my drive even it wasn’t always easy. I love you from the bottom of my heart.

The study has been supported by the the strategic funding of the University of Eastern Finland, the EVO grants of Kuopio University Hospital, The Academy of Finland, The Nordic Center of Excellence in Neurodegeneration, The Graduate School of Molecular Medicine, The Finnish Cultural Foundation’s North Savo and Kymenlaakso regional funds, The Finnish Medical Foundation and The Foundation of Maire Taponen.

Kuopio, August 2012

Toni Seppälä
List of the original publications

This dissertation is based on the following original publications:


IV Seppälä TT, Louhija UM, Herukka SK, Appelberg B, Juva K: Comparison between clinical diagnosis and CSF biomarkers of Alzheimer’s disease in elderly patients with late onset psychosis (Helsinki Old Age Psychosis Study). *Submitted.*

The publications were adapted with the permission of the copyright owners.
Contents

1 INTRODUCTION ............................................................................................................. 1

2 REVIEW OF THE LITERATURE .................................................................................. 3
  2.1 Alzheimer’s disease as a clinical entity ................................................................. 3
  2.1.1 Clinical course of Alzheimer’s disease ............................................................. 3
  2.1.2 Criteria and definitions ...................................................................................... 4
  2.1.3 Before clinical Alzheimer’s disease – mild cognitive impairment and prodromal Alzheimer’s disease ................................................................. 6
  2.1.4 Neuropsychological changes .......................................................................... 7
  2.1.5 Neuropathological changes .......................................................................... 7
  2.1.6 Risk factors and prevention ............................................................................ 11
  2.1.7 Epidemiology and prognosis .......................................................................... 12
  2.2 Biomarkers of Alzheimer’s disease ...................................................................... 13
    2.2.1 Laboratory biomarkers ................................................................................. 13
    2.2.2 Neuroimaging ............................................................................................... 20
  2.3 Other relevant differential diagnoses of Alzheimer’s disease ......................... 23
    2.3.1 Normal pressure hydrocephalus .................................................................. 23
    2.3.2 Psychogeriatric disorders .............................................................................. 23

3 AIMS OF THE STUDY ......................................................................................... 25

4 GENERAL EXPERIMENTAL PROCEDURES ......................................................... 26
  4.1 Patients ................................................................................................................. 26
  4.2 Lumbar puncture .................................................................................................. 26
  4.3 CSF handling and storage .................................................................................... 26
  4.4 ELISA for measuring amyloid beta and tau .......................................................... 27

5 ETHICAL ASPECTS ........................................................................................... 28

6 PLASMA Aβ42 AND Aβ40 AS MARKERS OF COGNITIVE CHANGE IN FOLLOW-UP — A PROSPECTIVE, LONGITUDINAL, POPULATION-BASED COHORT STUDY ........................................................................ 29
  6.1 Introduction ........................................................................................................ 30
  6.2 Subjects and methods .......................................................................................... 31
    6.2.1 Subjects ......................................................................................................... 31
    6.2.2 Clinical evaluation ......................................................................................... 32
    6.2.3 Measurement of Aβ40 and Aβ42 .................................................................. 32
    6.2.4 APOE genotyping ......................................................................................... 33
    6.2.5 Statistics ....................................................................................................... 34
  6.3 Results ................................................................................................................ 34
    6.3.1 Baseline Aβ levels and cognitive decline during the follow-up .................. 34
    6.3.2 Relationship between changing plasma Aβ levels and cognitive decline.... 34
    6.3.3 Plasma Aβ and general health ....................................................................... 35
7 LONGITUDINAL CHANGES OF CSF BIOMARKERS IN ALZHEIMER’S DISEASE ................................................................. 39
7.1 Introduction .......................................................................................................................................................... 40
7.2 Methods ......................................................................................................................................................... 41
  7.2.1 Subjects .................................................................................................................................................. 41
  7.2.2 Diagnosis of mild cognitive impairment, Alzheimer’s disease and other dementias ......................... 41
  7.2.3 CSF analysis ...................................................................................................................................... 42
  7.2.4 APOE genotyping ............................................................................................................................. 42
  7.2.5 Statistics ........................................................................................................................................... 42
7.3 Results ......................................................................................................................................................... 43
  7.3.1 Cross-sectional data at the baseline ................................................................................................. 43
  7.3.2 Longitudinal data ............................................................................................................................. 44
7.4 Discussion .................................................................................................................................................. 51

8 CSF BIOMARKERS FOR ALZHEIMER’S DISEASE CORRELATE WITH CORTICAL BRAIN BIOPSY FINDINGS ................................................................. 54
8.1 Introduction .................................................................................................................................................. 55
8.2 Material and methods ................................................................................................................................. 55
  8.2.1 Patients .............................................................................................................................................. 55
  8.2.2 APOE genotyping ............................................................................................................................. 56
  8.2.3 Shunt response and final clinical diagnosis of AD ............................................................................. 56
  8.2.4 Cortical brain biopsy and CSF samples ............................................................................................ 57
  8.2.5 AD biomarkers in CSF ...................................................................................................................... 57
  8.2.6 Histology and immunohistochemistry ............................................................................................ 57
  8.2.7 Statistical analysis ............................................................................................................................. 58
  8.2.8 Ethical aspects ................................................................................................................................... 58
8.3 Results ......................................................................................................................................................... 59
  8.3.1 Aβ42, total tau, and P-tau-181 in CSF ............................................................................................... 59
  8.3.2 Aβ and tau proteins in cortical biopsy .............................................................................................. 60
  8.3.3 Correlation of CSF and cortical biopsy findings .............................................................................. 61
  8.3.4 Correlation of CSF findings to final clinical diagnosis of AD ............................................................. 61
  8.3.5 Multivariate analysis .......................................................................................................................... 61
8.4 Discussion .................................................................................................................................................. 66

9 COMPARISON BETWEEN CLINICAL DIAGNOSIS AND CSF BIOMARKERS OF ALZHEIMER’S DISEASE IN ELDERLY PATIENTS WITH LATE ONSET PSYCHOSIS – HELSINKI OLD AGE PSYCHOSIS STUDY .......... 68
9.1 Introduction .................................................................................................................................................. 69
9.2 Methods ..................................................................................................................................................... 70
  9.2.1 Participants ...................................................................................................................................... 70
  9.2.2 Clinical diagnosis ............................................................................................................................. 70
  9.2.3 CSF analysis ................................................................................................................................... 71
  9.2.4 Statistics ........................................................................................................................................ 71
9.2.5 Ethical aspects ................................................................. 71
9.3 Results .............................................................................. 71
9.4 Discussion ........................................................................ 78

10 GENERAL DISCUSSION AND FUTURE PROSPECTS ................. 80

11 CONCLUSIONS ..................................................................... 84

12 REFERENCES ........................................................................ 85
# Abbreviations

<table>
<thead>
<tr>
<th>Abbreviation</th>
<th>Definition</th>
</tr>
</thead>
<tbody>
<tr>
<td>Abeta</td>
<td>Amyloid beta protein</td>
</tr>
<tr>
<td>Aβ</td>
<td>Amyloid beta protein</td>
</tr>
<tr>
<td>Aβ40</td>
<td>Amyloid beta, length 40 amino acids</td>
</tr>
<tr>
<td>Aβ42</td>
<td>Amyloid beta, length 42 amino acids</td>
</tr>
<tr>
<td>AD</td>
<td>Alzheimer's disease</td>
</tr>
<tr>
<td>ADAS-cog</td>
<td>Alzheimer's Disease Assessment Scale-cognitive subscale</td>
</tr>
<tr>
<td>ADL</td>
<td>Activities of daily living</td>
</tr>
<tr>
<td>ADNI</td>
<td>Alzheimer's disease neuroimaging initiative</td>
</tr>
<tr>
<td>ALS</td>
<td>Amyotrophic lateral sclerosis</td>
</tr>
<tr>
<td>aMCI</td>
<td>Amnestic mild cognitive impairment</td>
</tr>
<tr>
<td>ANOVA</td>
<td>Analysis of variance</td>
</tr>
<tr>
<td>ApoE</td>
<td>Apolipoprotein E</td>
</tr>
<tr>
<td>APP</td>
<td>Amyloid precursor protein</td>
</tr>
<tr>
<td>ASA</td>
<td>Acetylsalicylic acid</td>
</tr>
<tr>
<td>BACE1</td>
<td>Beta-amyloid cleaving enzyme 1</td>
</tr>
<tr>
<td>CBD</td>
<td>Corticobasal degeneration</td>
</tr>
<tr>
<td>CDR</td>
<td>Clinical dementia rating</td>
</tr>
<tr>
<td>CERAD</td>
<td>Consortium to Establish A Registry for Alzheimer's Disease</td>
</tr>
<tr>
<td>CH</td>
<td>Cognitively healthy</td>
</tr>
<tr>
<td>CHD</td>
<td>Coronary heart disease</td>
</tr>
<tr>
<td>ChEI</td>
<td>Acetylcholine esterase inhibitor</td>
</tr>
<tr>
<td>CJD</td>
<td>Creutzfeldt-Jakob disease</td>
</tr>
<tr>
<td>CNS</td>
<td>Central nervous system</td>
</tr>
<tr>
<td>CSF</td>
<td>Cerebrospinal fluid</td>
</tr>
<tr>
<td>CT</td>
<td>Computer tomography</td>
</tr>
<tr>
<td>CV</td>
<td>Coefficient of variances</td>
</tr>
<tr>
<td>CVD</td>
<td>Cardiovascular disease</td>
</tr>
<tr>
<td>DLB</td>
<td>Dementia of Lewy bodies</td>
</tr>
<tr>
<td>DSM-IV</td>
<td>Diagnostic and Statistical Manual of Mental Disorders n:o 4</td>
</tr>
<tr>
<td>EC</td>
<td>Entorhinal cortex</td>
</tr>
<tr>
<td>ELISA</td>
<td>Enzyme-linked immunosorbent assay</td>
</tr>
<tr>
<td>EOAD</td>
<td>Early-onset Alzheimer's disease</td>
</tr>
<tr>
<td>FDG</td>
<td>Fluorodeoxyglucose</td>
</tr>
<tr>
<td>FTD</td>
<td>Frontotemporal dementia</td>
</tr>
<tr>
<td>FTLD</td>
<td>Frontotemporal lobar degeneration</td>
</tr>
<tr>
<td>FUT</td>
<td>Follow-up time</td>
</tr>
<tr>
<td>FYN</td>
<td>a type of non-receptor tyrosine kinases</td>
</tr>
<tr>
<td>GSK3</td>
<td>Glycogen synthase kinase 3</td>
</tr>
<tr>
<td>HC</td>
<td>Hippocampus</td>
</tr>
<tr>
<td>HOPS</td>
<td>Helsinki old age psychosis study</td>
</tr>
<tr>
<td>ICP</td>
<td>Intracranial pressure</td>
</tr>
<tr>
<td>iNPH</td>
<td>Idiopathic normal pressure hydrocephalus</td>
</tr>
<tr>
<td>IQ</td>
<td>Interquartile range</td>
</tr>
<tr>
<td>KUH</td>
<td>Kuopio university hospital</td>
</tr>
<tr>
<td>LDL</td>
<td>Low-density lipoprotein</td>
</tr>
<tr>
<td>LOAD</td>
<td>Late-onset Alzheimer's disease</td>
</tr>
<tr>
<td>LOP</td>
<td>Late-onset psychosis</td>
</tr>
<tr>
<td>LOSD</td>
<td>Late-onset schizophrenia spectrum disorder</td>
</tr>
<tr>
<td>LP</td>
<td>Lumbar puncture</td>
</tr>
<tr>
<td>MCI</td>
<td>Mild cognitive impairment</td>
</tr>
<tr>
<td>MCBP</td>
<td>Mean cortical binding potential</td>
</tr>
<tr>
<td>MMSE</td>
<td>Mini mental state examination</td>
</tr>
<tr>
<td>MRI</td>
<td>Magnetic resonance imaging</td>
</tr>
<tr>
<td>MTA</td>
<td>Medial temporal lobe atrophy</td>
</tr>
<tr>
<td>MTL</td>
<td>Medial temporal lobe</td>
</tr>
<tr>
<td>NFT</td>
<td>Neurofibrillary tangle</td>
</tr>
<tr>
<td>NINCDS-ADRDA</td>
<td>National Institute of Neurological Disorders and Stroke – Alzheimer’s Disease and Related Disorders Association</td>
</tr>
<tr>
<td>NMDA</td>
<td>N-methyl-D-aspartate</td>
</tr>
<tr>
<td>NPH</td>
<td>Normal pressure hydrocephalus</td>
</tr>
</tbody>
</table>
NSAID  Non-steroidal anti-inflammatory drug
OCD    Other cognitive disorder
PHF    Paired helical filament
p-tau  Hyperphosphorylated tau protein
PCR    Polymerase chain reaction
PET    Positron emission tomography
PIB    Pittsburgh compound B
PLHP   Post-lumbar puncture headache
PSEN1  Presenilin 1
PSEN2  Presenilin 2
PSP    Progressive supranuclear palsy
sAPP   Soluble amyloid precursor protein
SCA    Spinocerebellar ataxia
SD     Standard deviation
SPECT  Single photon emission tomography
VaD    Vascular dementia
VITA   Vienna Transdanube Aging
1 Introduction

The prevalence of memory-impairing neurodegenerative diseases is between 5-9% in people over age of 65. The most common form of these illnesses is Alzheimer’s disease (AD) with a total prevalence of 4.4% in individuals over age of 65, constituting over half of all the demented patients (Ferri et al. 2005, Lobo et al. 2000) and there is a progressive incidence by age. The prevalence doubles by every five years after the age of 65 (Hebert et al. 2003). The incidence of dementia is 0.1% at the age of 60-65 but increases to 5% per year at the age of 85-89 (Fratiglioni et al. 2000). Age-associated memory disorders consume a large proportion of the world’s healthcare expenses. The total worldwide costs of dementia were estimated at US$315 billion for 29 million patients in 2005 (Wimo, Winblad & Jönsson 2007). The total cost of illness (Col) for dementia in the European Union was assessed to be as much as €160 billion per year including formal and informal care (Wimo et al. 2011). AD is the 5th most common cause of death in the US in people over 65 years old (Heron 2011).

New disease-modifying therapies for AD are constantly being investigated but the final breakthrough has eluded scientists. The clinical trials have often treated many patients who do not have AD or are not going to progress to AD for a long time. Any drug development aimed at treatment requires both accurate and early diagnosis of AD in order to identify symptomless preclinical AD patients and to enrich the intervention cohorts.

The clinical diagnosis is based on criteria of possible or probable AD. These criteria were defined in 1984 and were primarily focused on differentiating the clinical dementia syndrome from other causes of dementia with laboratory tests and neuroimaging. The diagnosis of definite AD was based only on post mortem neuropathological examination of brain. The problem is that pathological process of AD probably begins as early as 20 years before the onset of any clinical symptoms. The diagnostics regarding dementia were far too late both for research as well as for clinical treatment. The need for earlier classification of symptoms led to classification of pre-dementia memory deficiencies forming the concept of mild cognitive impairment (MCI), a transitional stage between cognitively healthy and AD patients (Flicker, Ferris & Reisberg 1991).

As the focus of the AD study has been constantly shifting towards earlier diagnosis, the research criteria of AD were redefined by an international group of neuroscientists in 2007 aiming at identifying the disease already before the clinical dementia (Dubois et al. 2007). The new criteria define probable AD as a progressive impairment of episodic memory supported by at least one biomarker finding in neuroimaging, cerebrospinal fluid (CSF) analysis or confirmed autosomal mutation in family history together with exclusion of other causes. The criteria of definite AD were completed with the possibility of brain biopsy confirmation or genetic mutation in addition to the criteria of probable AD opening a window to definite ante mortem diagnosis.

The neuropathological hallmarks of AD are extracellular space amyloid plaques that are composed of amyloid beta peptide (Aβ) and the intracellular neurofibrillary tangles consisting of hyperphosphorylated tau protein. These proteins were identified in the CSF of AD patients in different concentrations than in healthy controls or most other neurological disorders and thus their biomarker potential was revealed. The CSF Aβ42 concentration was found to be lower and the tau protein and its hyperphosphorylated form phospho-tau (p-tau) to be increased in AD patients (Blinnow & Hampel 2003). Since amyloid and tau had been found also in the brains of the patients without a diagnosis of AD in earlier autopsy studies, the predictive potential of abnormal CSF Aβ42 and p-tau to conversion from MCI to AD was soon being debated (Hampel et al. 2004, Herukka et al. 2005).

The need for an ideal biomarker of AD is still evident. The ideal biomarker would be sensitive, specific, cost-effective and harmless and easy to perform as a test procedure
(Consensus report 1998) but brain biopsy or even lumbar puncture could not clearly fulfill these expectations because of the difficulties in collection. On the other hand, the most promising biomarker proteins of CSF, Aβ42 and tau, had been stated as diagnostically worthless if only available as a single measurement from plasma (Irizarry 2004).

Despite intensive study in the field of the AD biomarkers, numerous questions have remained unanswered. Most of the plasma and CSF studies were still made in cross-sectional settings, which complicated drawing conclusions about biomarker dynamics. On the other hand, possible longitudinal changes of biomarkers would provide a useful tool for assessing the progression of AD and increase knowledge about the temporal order of the pathological process events that take place in the body. The hypothesis was that the changes in these AD biomarker concentrations take place over time both in plasma and CSF and could be in association with clinical measurements such as cognitive change. We also had access to a window to the in vivo human brain as a form of a brain biopsy, which was hypothesized to reflect the presence of the AD process and the assumption was that the CSF findings would correlate with the corresponding changes in the brain biopsy. The present series of studies was initiated in order to clarify these relations and search for the novel usage of plasma and CSF biomarkers in primary and differential diagnostics of AD.
2 Review of the literature

2.1 ALZHEIMER’S DISEASE AS A CLINICAL ENTITY

Currently the most common cause of dementia, Alzheimer’s disease, was first described by Alois Alzheimer in 1907 when he reviewed the case of a former patient, Auguste D. Auguste D had deteriorated by the age of 51, suffering from paranoid jealousy, progressive memory loss, hearing delusions and aphasic symptoms. After years of follow-up, Alzheimer was able to associate the rare symptoms to post mortem findings that later formed the basis of the pathological hallmarks of AD in the brain tissue. For years, AD was considered as a rare form of dementing illness causing presenile dementia in the relatively young without any understanding of the true epidemiologic meaning of the pathology behind the clinical syndrome.

Dementia is not an individual disease but a syndrome defined by DSM-IV as a wide-ranging decline in several mental abilities including memory deficiency (ability to learn new or recall previously learned) combined with aphasia, apraxia, agnosia or a disturbance in executive functioning that together lead to significant impairment in social or occupational functioning and represent a significant decline from a previous level of functioning (American Psychiatric Association 1994). Dementia can be caused by several different etiologies and can thus be transitory, progressive or permanent. Dementia resulting from AD is a typical example of a slowly progressive disorder that represents approximately 60% of all cases of dementia in the developing countries (Kalaria et al. 2008) and as many as 70% in Western countries (Lobo et al. 2000).

2.1.1 Clinical course of Alzheimer’s disease

AD is a stage concurrent disease that is characterized by a temporal order of damage in neuroanatomical structures and consequent neurophysiological systems. It is commonly divided into early-onset and late-onset (sporadic) forms of the disease, but both forms basically exhibit a similar result in neuropathological findings and clinical symptoms differing only in the age when the symptoms appear. The early onset forms of AD (EOAD) are caused by relatively rare genetic mutations in APP, PSEN1 or PSEN2 that constitute only 1-2% of all AD cases. However, it is believed that up to 60 – 80% of late-onset AD are genetically determined (Gatz et al. 2006). EOAD always begins before the age of 65, usually before 60 and sometimes even as early as 30 (Rossor et al. 2010). LOAD begins to exhibit symptoms after the age of 65 or slightly earlier, although most often in the age range 60 – 70, when the APOE ε4 allele is present (Gomez-Isla et al. 1996).

In LOAD, the pathological process of AD begins possibly 20 – 30 years and definitely 10 years before the appearance of any clinical symptoms. An average person dying at the age of 78 will have a 5 to 10% likelihood of having an antemortem diagnosis of AD but at least 20 to 40% probability of having significant AD pathology in the brain in post mortem analysis (Nelson, Braak & Markesbery 2009). The Braak model subdivides the development of the neuropathological changes in the brain into six stages and the proceeding clinical signs of typical amnestic type AD follow this pathological process (Braak, Braak 1991). The preclinical phase is usually similar to Braak stages I-II. The symptomless period is often ended by an insidious onset of episodic memory impairment, which is neuropathologically localized to the severe damage in the entorhinal cortex (EC) and hippocampus (HC) neurons that relate to Braak stages III-IV called the limbic phase. The patient cannot still be diagnosed as demented and the impairment of social functioning is not so severe, but memory problems begin to increase and the learning of anything new becomes more
difficult. Working requires greater effort and time than before because of impaired learning skills, which can lead to stress, irritation and depression. The recall of recently heard or seen items is impaired, and the patient may be referred to a medical consultation by a friend or a relative. In clinical terms, the patient is suffering from MCI, but the etiology of the symptoms may still remain uncertain as they could be caused by several other factors. If the patient is suffering from an ongoing AD process, the stage may be called prodromal AD.

By the time that the AD diagnosis is set, the neuropathology has usually progressed to Braak stages V-VI called the neocortical phase. EC and HC are severely damaged and the prefrontal and temporoparietal cortex are also affected. As the disease proceeds and the neurons continue to be destroyed, there is a general neocortical atrophy, which leads to a further decline in the cognitive domains, especially executive functioning. Activities of daily living (ADL) are impaired, which slowly creates the need for more intensive social care.

However, in EOAD, the progression through the stages may be more rapid. As the symptoms are also easier to distinguish and are considered abnormal because of the younger age of the patients, the diagnosis is usually set earlier in the disease.

![Figure 1](image_url)

**Figure 1.** Typical clinical course of early Alzheimer’s disease according to disease stage. (Based on Erkinjuntti et al. 2010).

### 2.1.2 Criteria and definitions

In everyday work, the clinical diagnosis of AD is still made according to the criteria of the National Institute of Neurological Disorders and Stroke – Alzheimer’s Disease and Related Disorders (NINCDS-ADRDA) working group published in 1984 (McKhann et al. 1984). However since that time, the knowledge about the neurobiological basis of AD has increased and new, sophisticated medical technology has made it possible to create distinctive markers of the disease. The need for evolving criteria of AD was spurred by the many studies into these markers in attempts to identify those patients with prodromal AD from within the heterogeneous group of patients with cognitive functioning that falls outside normal ageing (Dubois & Albert 2004). The NINCDS-ADRDA criteria were not able
to sufficiently distinguish AD from the other dementing disorders as the specificity against the other dementias remained only in the range 23 – 88 % (Varma et al. 1999, Kazee et al. 1993). Furthermore, the NINCDS-ADRDA criteria were not useful for diagnosing individuals with only mild problems in memory, because of primarily high cognitive functioning when still at working age. It was becoming clear that with the new methods based on imaging, neuropsychology and CSF analysis the ongoing AD pathology would make it possible to identify in patients having merely a mild memory deficit. Finally, the criteria published in 2007 enabled possibility of a diagnosis of AD before the appearance of clinical dementia, preferably at the earliest possible stage before any global deterioration (Dubois et al. 2007) (Table 1). However, these research criteria only apply to the typical presentation of amnestic AD and the atypical and mixed pathology forms must be dealt in the clinical practice according to older guidelines. It should also be noted, that the 2007 criteria propose that the distinction between MCI and dementia should be eliminated. The term dementia is widely accepted in the clinical practice and provides a clear indication of a serious illness to both the patient and the family (Jack et al. 2010).

Table 1. Research criteria for diagnosis of Alzheimer’s disease (adapted from Dubois, 2007)

**Probable AD: Core criteria A and at least one supportive feature (B, C, D or E)**

**Core criteria**
A. Presence of an early episodic memory impairment with following features:
1. Gradual and progressive change in memory function over more than 6 months reported by patient or an informant
2. Objective evidence of significantly impaired episodic memory function on testing (recall deficit that does not improve with cueing or recognition testing and after effective encoding)
3. Aβ2 can be isolated or associated with other cognitive changes at the onset of AD or as AD advances

**Supportive features**
B. Presence of medial temporal lobe atrophy
• Volume loss of HC, EC, amygdala on MRI (qualitative rating or volumetric rating)
C. Abnormal concentration of CSF biomarker
• Low Aβ42, high tau or p-tau
• Other well validated biomarker
D. Specific pattern on functional neuroimaging with PET
• Reduced glucose metabolism in bilateral temporoparietal regions
• PIB PET or other well validated ligand
E. Proven AD autosomal dominant mutation within the immediate family

**Exclusion criteria**
Exclusion of other causes by history and clinical features

**Definite AD**
• Both clinical AND histopathological (using brain biopsy or autopsy) evidence of AD by NIA-Reagan criteria OR
• Both clinical AND genetic (mutation on chromosome 1, 14 or 21) evidence of AD.

AD = Alzheimer’s disease, HC = hippocampus, EC = entorhinal cortex, MRI = magnetic resonance imaging, CSF = cerebrospinal fluid, PET = positron emission tomography, PIB = Pittsburgh compound B.

Before the 2007 guidelines, mild cognitive impairment was the most generally accepted term that described the transitional stage between normal aging and dementia. However, MCI was and still is a quite variably defined concept in practice. Depending on its suspected etiology it can include also other progressive, stable and even reversible causes of cognitive problems in addition to AD. Therefore, the terminology was also specified in
2007 to differentiate between MCI, amnestic MCI, preclinical AD and prodromal AD (Dubois et al. 2007).

2.1.3 Before clinical Alzheimer’s disease – mild cognitive impairment and prodromal Alzheimer’s disease

There is a long asymptomatic period of time between the first brain lesions that could be detected at autopsy and the first appearance of symptoms. These individuals with normal cognitive abilities who later fulfill the diagnostic criteria of AD are classified as having preclinical AD (Dubois et al. 2007). On the other hand, the classification for the time from the first symptoms to diagnosis of AD has been been very variable.

More than fifteen years ago, the concepts for mild cognitive problems were varied. Terms such as benign senescent forgetfulness (Kral 1962), aging-associated cognitive decline (Levy 1994), age-related cognitive decline (American Psychiatric Association 1994) and age-associated memory impairment (Crook, Bahar & Sudilovsky 1987) were all used to depict the cognitive changes that relate to normal aging. Even though the term MCI was initially intended to represent the transitional stage between healthy aging and AD, it was broadened to describe a state between the cognition of normal aging and any mild dementia. MCI is defined in multiple ways, but the most widely used criteria are that the patient has a cognitive impairment that is objectively measurable but not extensive enough to be defined as dementia. The impairment can be in memory and/or some other cognitive domain depending on the subtype of MCI but the ADL functions are generally unaffected (Petersen et al. 2001). Sometimes MCI has been defined only using the CDR score of 0.5 as the criterion for MCI, which leads to inclusion of cases of mild dementia (Morris et al. 2001). When it was observed that not all forms of MCI evolve into AD, and a broader conceptualization was necessary, a consensus conference subdivided MCI to four subtypes (MCI amnestic, MCI multiple domains, MCI single non-memory domain and MCI multiple non-memory domain) (Winblad et al. 2004). The amnestic type MCI (aMCI) was intended to represent the transition to AD, but the criteria for other subtypes were also intended to cover other possible causes of cognitive impairment. Criteria for aMCI are 1) memory complaint, preferably corroborated by an informant 2) impaired memory function for age and education 3) preserved general cognitive function 4) intact activities of daily living 5) not demented (Petersen 2004). In a recent latent class analysis of 1655 patients, as many as seven subgroups of MCI could be identified (Hanfelt et al. 2011).

In a study of Mayo Clinic, the prevalence of MCI was 16 % with the prevalence of amnestic MCI being 11 %. MCI seems to be 1.5 times higher in men at the age of 70-89 years (Petersen et al. 2010). Conversion rates of MCI to AD have been 10 – 15 % per year in clinical setting follow-up studies, which is explained by the high prevalence of AD causing dementia. In the community based studies, the annual conversion rate has remained rather low, only 4.2 % per year (Mitchell, Shiri-Feshki 2008). One of the main arguments defending the new guidelines has been the problematic definition of MCI. When a selected population-based cohort of amnestic MCI was followed up until dementia, only 71 % had AD in the neuropathological examination and 29 % had non-AD primary pathologic abnormalities responsible for the symptoms (Jicha et al. 2006). A classification of this specificity without any objective evidence of AD such as imaging or CSF analysis would be crucial to patient selection for inclusion in clinical trials. The binary outcome of conversion to AD according to NINCDS-ADRDA is eliminated using the 2007 criteria, which leads to higher specificity in patient selection for clinical research. The viewpoint of the possibility of early intervention especially in clinical trials of disease-modifying drug development played an important role when justifying the new research criteria. Exposure to potentially harmful therapies should be limited only to those individuals with ongoing AD pathology and reliably exclude the others. If clinically applied guidelines still require dementia as a basis for AD diagnosis, the term prodromal AD would be suitable to describe the symptomatic predementia phase. The practical goal would be to identify a precisely
defined group within the amnestic MCI, using the 2007 criteria including at least one of the AD supporting features of MTL atrophy, abnormal CSF markers, functional imaging or family genetics (Dubois et al. 2007).

2.1.4 Neuropsychological changes

The most widely studied cognitive domain in MCI and AD is memory and this is even emphasized in the new criteria. The core diagnostic criterion for AD in 2007 guidelines is early episodic memory impairment that lasts for at least six months and for which there is objective evidence in memory testing. The memory impairment can be the patient’s only cognitive deficit although this isolated amnestic MCI seems to be rare within MCI; the MCI patients usually also have other cognitive domains affected (Ribeiro, de Mendonca & Guerreiro 2006, Tabert et al. 2006). The neuropsychological delayed recall test (Morris et al. 1989) discriminates AD patients from healthy controls better than other memory measures (Welsh et al. 1991) and predicts AD conversion from MCI (Devanand et al. 1997) and from the non-demented population-based sample (Tierney et al. 2005). Although memory recall slows down and becomes more difficult also in normal aging, the more automatic systems that use for example, cueing or recognition, remain intact and recall using hints still functions. Therefore, cued recall test and coordinated encoding paradigms improve specificity of neuropsychological memory testing as the results in these tests do not improve in prodromal AD patients and thus they discriminate them effectively from healthy patients (Buschke et al. 1997). Episodic memory testing with cuing may also have differentiating potential against other memory disorders as the loss of cued recall ability is rather specific for AD (Traykov et al. 2005, Pillon et al. 1993, Grober et al. 2008).

Other relevant cognitive domains that are impaired in AD are working memory, attention and executive functions, which are key contributors to impairment in ADL functions and everyday tasks. Executive dysfunction is often associated with underlying VaD, which poses differential diagnostic problems. Executive dysfunction and ADL impairment are related in the multivariate model in MCI patients (Marshall et al. 2011) and may be even more common than memory impairment (Nordlund et al. 2005). The executive dysfunction is associated with greater severity of common neuropsychiatric symptoms such as depression, anxiety, agitation, disinhibition, irritability and sleep problems in MCI patients (Rosenberg et al. 2011). Despite the frequency of the multiple domain impact on cognition or neuropsychiatric symptoms in MCI, they are not sufficiently specific to provide an early diagnosis of AD on their own and therefore the episodic memory impairment should manifest before one can make a diagnosis. If these symptoms do occur, there should be signs of a more widespread disease (Braak & Braak 1991), which should be identified by supportive features e.g. biomarkers (Dubois et al. 2007).

2.1.5 Neuropathological changes

Amyloid pathology

Amyloid plaques are extracellular, often roughly spherical, proteinaceous deposits that are composed of fibrillar polymers of Aβ. Neuritic plaques are amyloid deposits surrounded by swollen, degenerating neurons that represent the neuropathological hallmarks of AD. Diffuse plaques are found also in the brain autopsy of the asymptomatic elderly patients, usually appearing after 60 years of age, but they lack the nearby degenerating neurons. All amyloid deposits are histologically nonspecifically stainable with Congo red, silver stains and thioflavin. (Nelson, Braak & Markesbery 2009). The appearance of neuritic plaques in large amounts is considered to be specific for AD. Amyloid plaques are not a nonspecific result of neurofibrillary pathology, because several non-AD tauopathies do not display amyloid deposits (McKee et al. 2009).

The amyloid plaques were first shown to consist of amyloid β, a peptide 40 (Aβ40) or 42 (Aβ42) amino acids in length (Glenner & Wong 1984), which was later found to be derived
as a cleavage product from a cell-surface protein, amyloid precursor protein (APP) (Kang et al. 1987). The diffuse plaques appear early in AD and contain mostly Aβ42 whereas neuritic plaques also contain Aβ40, which accumulates later in the disease course (Iwatsubo et al. 1994, Iwatsubo et al. 1995). Even though Aβ42 is more hydrophobic and prone to form fibrils, it represents only 10% of all the Aβ that is produced (Burdick et al. 1992). In PSEN 1 and PSEN 2 mutations causing familial AD, the amounts of Aβ42 are increased compared to those of Aβ40, which highlights the potential toxicity of Aβ42 (Borchelt et al. 1996). It also seems that even minor increases in cellular Aβ42 production can be crucial in AD pathogenesis and neuronal death since they can trigger caspase activation and further upregulation of amyloidogenic Aβ production (Gervais et al. 1999). The mature neuritic plaques are mostly fibrillar Aβ but are also combined with several other proteins such as APOE.

The triplication of chromosome 21 leads to dementia and AD neuropathology at an early age in patients with Down syndrome, which is due to the location of the APP gene in the long arm of the chromosome (Rumble et al. 1989). This finding soon led to the identification of the APP gene mutations that cause familial EOAD (Goate et al. 1991). The precise physiological function of APP is not yet clear, but it seems to play a role in protein trafficking in most studies (Zhang et al. 2011). APP is generated in the endoplasmic reticulum and placed by Golgi apparatus and trans-Golgi-network to the cell membrane as a type I transmembrane protein or to an endosomal compartment (Greenfield et al. 1999). On the cell surface, it is normally cleaved by α-secretase (Sisodia 1992, Roberts et al. 1994) and γ-secretase, which do not create Aβ but instead produce soluble APP (sAPP) and p3. In the endosomal compartment however, beta-amyloid cleaving enzyme (BACE1) and γ-secretase cleavage result in the formation of Aβ40 and Aβ42 (Sinha et al. 1999, Vassar et al. 1999) and the APP intracellular domain. The γ-secretase is a multiprotein complex that consists of PSEN1 or PSEN2, a type I transmembrane glycoprotein nicastrin, and two transmembrane proteins Aph-1 and Pen-2 (De Strooper 2003). The γ-secretase activation requires PSEN1 or PSEN2 cleavage, which is essential for activating the protein complex (Takasugi et al. 2003). The γ-secretase formation determines the cleavage site of APP and is responsible to which length of Aβ peptide is produced. Almost all familial AD mutations have an influence on this balance and cause a minor 1.5 to 2-fold relative overproduction of the more amyloidogenic species of Aβ such as Aβ42 over the more benign Aβ40. There seems to be a dynamic equilibrium between Aβ42 and Aβ40, because even a slight alteration, e.g. from a ratio 1:9 to 3:7, induces an increase in synaptotoxic oligomers that directly impair learning when administered to mice (Kuperstein et al. 2010). An overview of the known and studied mutations of PSEN encoding genes leads to the conclusion that the absolute amount of Aβ peptides generated seems to be less important than the type of Aβ peptide, at least in familial AD associated to presenilin mutations (Bergmans & De Strooper 2010). The only exception to the rule is the Swedish mutation, which is known to increase both Aβ42 and Aβ40 without changing their ratio (Citron et al. 1992) but it still causes AD by overproduction favoring the intracellular compartment and BACE1 route (Sahlin et al. 2007).

It seems that the monomers of Aβ and the amyloid plaques are not as toxic as they are thought to be and that the oligomers of Aβ would cause the neurotoxic and neuroinflammatory reactivity of Aβ (Walsh et al. 2002, Lesne et al. 2006). The postsynaptic compartment is thought to be the primary target of the neurotoxicity in the cell (Selkoe 2002). In the study of Shankar et al., Aβ oligomers were detected in the brain extracts of LOAD patients, injected to mice and observed to acutely perturb the synapse plasticity and memory, a property not shared by the monomers alone (Shankar et al. 2008). In addition, direct neurotoxicity of Aβ oligomers has been shown in vitro even at nanolevel concentrations (Lambert et al. 1998). A mutation of APP693Delta that produces Aβ lacking glutamate-22 (Aβ E22Δ) was found in Japan 2008. This deletion produces a dementia of AD type and inner temporal lobe atrophy but the individual is virtually lacking the amyloid
plaque formation in PIB-PET scan, which was assumed to result from an intensive property of their Aβ to form oligomers but not fibrils and plaques (Tomiyama et al. 2008). Later, another group reported that the Aβ42 E22Δ aggregated actually faster than the wild type Aβ42 and that it bound the PIB similarly, questioning the previous result of missing Aβ plaques (Ovchinnikova et al. 2011).

The amyloid cascade hypothesis is based on findings of known familial AD mutations and mouse models. The amyloid hypothesis states that it is the deposition of the Aβ in the brain parenchyma which is the crucial step that leads to AD (Hardy & Selkoe 2002). The hypothesis has been updated in recent years, but the general concept has remained: some factor, internal or external or both, initiates the pathological cascade by increasing or altering the relative Aβ42 production or decreasing its clearance from the brain. At present, it has become evident that the correlation between cognitive changes and brain amyloid load is not linear (Price et al. 2009) and the association between amyloid and NFT formation must be more complex. For example, mutations in the tau gene (chromosome 17, long arm) can cause autosomal dominant frontotemporal dementia (FTD). The tau pathology in FTD is similar to that seen in AD, but without the appearance of Aβ plaques. Thus, tau pathology itself can cause neuronal loss, which places tau pathology downstream to the Aβ pathology (Karran, Mercken & De Strooper 2011, Hutton et al. 1998).

**Tau pathology**

Another pathological hallmark of AD pathology in the brain, neurofibrillary tangles (NFT), are composed of intracellular, insoluble and protease-resistant fibrillary polymers of hyperphosphorylated microtubule-associated protein tau. NFTs consist of aggregated straight or paired helical filaments (PHF) or other conformations of aberrantly phosphorylated forms of tau (Buee et al. 2000). The ‘spreading’ of NFT pathology throughout the brain correlates with the progression of AD in a sequential manner (Braak & Braak 1995) leading to the clinical presentation as described earlier in the paragraph “Clinical course of AD”.

On the other hand, there are some indications from the neuropathological study that do not support amyloid cascade hypothesis and the assumption that tau pathology is a downstream phenomenon. Tau pathology precedes Aβ accumulation by decades in the entorhinal region, which is believed to result in the sporadic form of AD later in life. Fresh NFTs indicate that this is a continuous process which may be present in MTL as early as third and fourth decades of life i.e. stages I and II of tau pathology in the brain (Braak & Braak 1997). When these relatively young cases have not had amyloid plaques preceding these changes, it has been postulated that NFTs may be present first in the entorhinal area whereas amyloid pathology precedes tau pathology in the basal neocortex (Braak & Braak 1997). The neuropathological findings do not exactly overrule the amyloid cascade hypothesis. Instead, they emphasize the importance of oligomer toxicity that may influence the initiation of tau pathology even before there is any plaque accumulation (Duyckaerts 2011).

Physiologically, tau takes part in stabilization of microtubules and the regulation of axonal transport, but it may have multiple additional functions as a result of its interactions with other structures and enzymes such as the plasma membrane, the actin cytoskeleton and tyrosine kinases (Ballatore, Lee & Trojanowski 2007). Tau proteins are present in the brain as six isoforms, which range from 352 to 441 amino acids in length and each of these have their own particular physiological roles. Overall, they bind to spectrin and actin filaments and interconnect microtubules to cytoskeletal structures, thus restricting the flexibility of microtubules, stabilizing them (Buee et al. 2000). Tau proteins are also known to promote tubulin and microtubule polymerization from monomers (Higuchi, Lee & Trojanowski 2002). Microtubules act as ‘railway tracks’ of axonal transport, which is not an easy task as the axons can stretch as far as one meter from the neuronal cell body. Every transport mechanism requires a ‘motor’, which in the axon are the motor proteins that
accompany the microtubules translocating the protein vesicles and organelles from the cell body via axon to synapse and vice versa (Roy et al. 2005). In addition to mechanically stabilizing the ‘railway’ and forming the morphologic structure of the neurons, tau also takes part in protecting the microtubules from traffic obstructions, regulating the motor-driven axonal transport (Gotz, Ittner & Kins 2006). Tau seems to be involved in multiple phases of axonal transport and quite a ready binder. Therefore, it may be prone to interactions that cause its disengaging from microtubules, which leads to protein misfolding and aggregation (Ballatore, Lee & Trojanowski 2007).

Tau has 84 putative phosphorylation sites in serine, threonine and tyrosine along its amino acid structure (Ittner & Gotz 2011). The reason that tau phosphorylation takes place in the brain is not yet fully understood. Transient and normal phosphorylation occurs in the brain, for example in fetal development and during anesthesia, but this does not lead to polymerization and is less extensive than in AD (Iqbal et al. 2010). However, in a pathological situation, excess phosphorylation at abnormal sites of tau causes it to detach from the microtubules (Ballatore, Lee & Trojanowski 2007), which is followed by its accumulation in the somatodendritic compartment and aggregation to paired helical filaments (Buee et al. 2000). In addition, filamentous tau seems to have the ability to spread to other regions of the brain. In an animal work, an injection of brain extract from tau-expressing mutant mice into the brain of transgenic wild-type tau-expressing mice can induce the assembly of wild-type human tau into filaments and the spreading of NFT pathology from the site of injection to the neighbouring brain regions (Clavaguera et al. 2009). Tau oligomers possess abilities to trigger neurotoxic actions that lead to deterioration of the cytoskeleton (Maeda et al. 2007). The resulting NFTs are detected in the brain as a hallmark of AD (and other tauopathies) after neuronal death.

For years, the supporters of amyloid cascade hypothesis and those who think amyloid is only a nonspecific result of tau pathology have attempted to find a way of merging these two theories. The amyloid hypothesis could not simply be overlooked; it is still hard to bypass this theory for several reasons. First, every known FAD mutation causes changes in APP metabolism that lead to amyloid overproduction and the same applies also to Down syndrome.

The hierarchical hypothesis suggests that Aβ drives the NFT pathology through hyperphosphorylation of tau; this theory is supported by mouse model findings according to which synthetic Aβ fibrils exacerbate the NFTs when they are injected into the brain of a mutant tau transgenic mice (Gotz et al. 2001).

The dual pathway hypothesis was introduced to explain why LOAD occurs differently from EOAD and would explain separately both amyloid and tau pathologies as being attributable to a common upstream driver (Small & Duff 2008), which remains to be identified. Whereas the serial model of causality linked tau as a downstream effect of amyloid, the dual pathway hypothesis suggested that this common driver would not be only plausible but also mechanistically interconnected through three molecular pathways i.e. APOE, glycogen synthase kinase 3 (GSK3) and retromere deficiency, which may all be strengthened by each other.

The integrative explanation model of AD considers several damage signals such as Aβ oligomers, oxygen free radicals, iron overload, homocysteine, cholesterol and LDL species which function as microglia cell activators leading to the release of proinflammatory cytokines and pathological modifications to tau and ultimately causing neuronal degeneration (Maccioni et al. 2010).

A recent model links amyloid and tau together in the same cascade; there is evidence that intracellular tau can mediate the toxic effects of Aβ in the postsynaptic compartment. When the excess accumulation of tau takes place in an APP transgenic mouse by double transgenic APP/tau mutation in the soma, the interaction with tyrosine protein kinase FYN is increased and the NMDA receptor is hyperphosphorylated, which leads to increased Aβ induced toxicity in the neuron and early mortality as compared to tgAPP mice and tgAPP
mice with the tau gene knocked out. According to this hypothesis, tau would play only a secondary role in Aβ toxicity (Ittner et al. 2010). Consequently, so called tau axis hypothesis states that tau dependent Aβ toxicity would causatively lead to the neurons being continuously exposed to toxic Aβ inside the cell, which would increase the hyperphosphorylation of tau and create a vicious circle to axonal transport failure and aggregation of tau (Ittner & Gotz 2011). The finding in 3xTg-AD mice according to which Aβ oligomerization appears intraneuronally and is reversible by a specific antibody, clearing also tau pathology, could be viewed as support for this hypothesis (Oddo et al. 2006).

2.1.6 Risk factors and prevention
Only 1-2 % of all cases are familial AD explained by autosomal dominant mutations of APP, PSEN1 or PSEN2. LOAD or sporadic AD, however, is a multifactorial entity that has risk factors of its own. The two most relevant risk factors for LOAD are age and an APOE ε4 allele (Dartigues, Feart 2011). There are genetic risk factors for LOAD, but the major component of the risk consists of environmental factors, as observed in a large Swedish twin study (Pedersen et al. 2004). However, hereditary factors explained 58 to 78 % of LOAD (Gatz et al. 2006), which means that family history is always an important factor to be ascertained. A well-known risk factor for AD is also trisomy 21 (Rumble et al. 1989).

Apolipoprotein E (APOE) is expressed in most organs, mainly in liver and brain, and its main physiological function is as a lipid transporter. It does not cross the blood-brain barrier, which means that neuronal and peripheral APOE are different compartments. The brain APOE is produced by astrocytes and microglia, but probably also by neurons in minor amounts (Kim, Basak & Holtzman 2009). After the ε4 allele of the APOE gene situating at the chromosome 19 was discovered to be a risk factor for AD (Strittmatter et al. 1993), its potential has been elucidated in recent years. One copy of ε4 allele increases the risk for AD by about fourfold and being homozygotic i.e. two alleles raises the risk by about tenfold, which means that the significance of APOE is probably equivalent to all the rest of the potential risk genes combined (Eisenstein 2011). The APOE ε4 is associated with an earlier age of AD onset (Gomez-Isla et al. 1996), but there is variation between ethnic groups (Kim, Basak & Holtzman 2009). There is debate over the influence of the effect of APOE on the rate of cognitive decline after the onset of dementia, which is why most of the results need to be discussed by dividing the groups according to APOE genotype. It is not clear how and why APOE increase the risk for AD, although there are several possible explanations. APOE binds to Aβ and it is found inside the senile plaques (Naslund et al. 1995), a higher APOE ε4 allele dosage is associated with more neuritic plaques and NFTs and APOE ε2 may have protective role for reduced plaque burden (Tiraboschi et al. 2004). In addition, fibrillar Aβ load was significantly associated with APOE ε4 carrier status and ε4 gene dose of cognitively normal patients in PIB-PET imaging (Reiman et al. 2009), which indicates that at least amyloid pathology and APOE are strongly linked. APOE is also known to be able to form amyloid itself (Wisniewski et al. 1995). However, it is also possible that APOE is a neuroprotective player and the effect on AD risk is a result of APOE ε4’s weaker ability to execute this function. The protein produced by APOE ε4 is the least stable form and may impair the movement of cholesterol and amyloid-β within the brain compared to those encoded by ε2 and ε3 (Kim, Basak & Holtzman 2009, Eisenstein 2011).

It remains unresolved whether the mechanistic connection between APOE and AD derives from lipid transportation or some other function, for example the ability to assist in neuronal regeneration, but the lipid transportation definitely links cardiovascular effects and diabetes to AD risk. APOE ε4 carriers with diabetes (DM) have an increased risk (risk ratio of 5.5 (CI 2.2-13.7)) of developing AD as compared to those with neither of these risk factors (Peila et al. 2002).
Seven potentially modifiable risk factors for AD have been identified: midlife hypertension, DM, smoking, depression, midlife obesity, or low educational attainment along with cognitive and physical inactivity. A 10–25% reduction in these risk factors could, theoretically, potentially prevent 1.1 to 3 million AD cases worldwide (Barnes & Yaffe 2011).

2.1.7 Epidemiology and prognosis
The prevalence for MCI varies depending on the criteria used and the setting of the study. In general, the prevalence figures converge in the 14 % to 18 % range for patients aged 70 years and older (Petersen et al. 2009). In a population-based study of Mayo clinic of 2700 subjects from 70 to 89 years old, the prevalence of aMCI was 11.1 % and that of nonamnestic MCI was 4.9 % (Petersen et al. 2010).

In another community-based setting of nearly 2400 nondemented individuals, the annual incidence rate of MCI was 5.1 % (95% confidence interval, 4.6-5.6 %) and of those with MCI, annually 5.4 % (95 % confidence interval, 4.7-6.3 %) progressed to AD (Manly et al. 2008). The general annual incidence of dementia in the normal population is 1 to 2 %, whereas among patients with MCI, the annual conversion rate to dementia is 5 to 10 % in community-based settings and 10 to 15 % in memory clinic settings (Petersen 2011). This results from a higher probability of advanced neurodegenerative disease in those who seek medical assistance (Petersen et al. 2009). Subjects with early non-amnestic MCI or multi-domain amnestic MCI may be more prone to develop dementia with Lewy bodies (DLB) or frontotemporal dementia (FTD) than AD (Molano et al. 2010, Busse et al. 2006), whereas those with aMCI usually convert to AD if they progress to dementia (Petersen 2011). Patients with aMCI are also less likely to revert to normal cognition in follow-up than other subtypes, even though some improvement may occur from MCI to normal cognition in general (Manly et al. 2008). Longer follow-up periods are needed especially in community-based studies in order to clarify the true improvement rate.

The annual incidence of AD has been reported as being 8.4% (95% CI 3.7 % to 13.1 %) for persons aged 85 years and older and 0.6% (95% CI 0.3 % to 0.9 %) for persons aged 65 to 69 years (Hebert et al. 1995). The percentage of the total population in USA with the onset of new AD each year will nearly double in 2050 (0.24%) compared to 1995 (0.14%) because of the increasing numbers of people still alive at the high-risk ages (Hebert et al. 2001). The prevalence of AD will increase by almost 3-fold to 13 million in the USA in 2050 compared to year 2000 (Hebert et al. 2003). The AD prevalence of women is higher than that of men, which is explained by the fact that women live longer; otherwise there seems to be no difference in the age-specific incidence (Alzheimer’s Association, Thies & Bleiler 2011).

The increased use of symptomatic treatment of AD (approximately 90 %) by cholinesterase inhibitors (ChEIs) and memantine, N-methyl-D-aspartate (NMDA) receptor antagonist, has decelerated the cognitive decline related to AD compared to the time before these drugs were available (Gillette-Guyonnet et al. 2011). With standard care, patients will lose 2.4 mini-mental state exam (MMSE) points per year and the points on the ADAS-cog test increased by 4.5 per year in a 4-year follow-up study, but the dropout rate from the study was high (Gillette-Guyonnet et al. 2011). The increase rate of ADAS-cog seems to be in line with a meta-analysis of drug trials, in which the average ADAS-cog increase was 5.5 points per year (Ito et al. 2010). The general mortality of AD patients is about 1.5-fold higher than those without AD. If one examines individuals with AD at the age of 70, 61 % are expected to die before the age of 80; this is much higher than the corresponding value of only 30% of subjects aged 70 in the general population (with and without AD) (Arrighi et al. 2010).
2.2 BIOMARKERS OF ALZHEIMER’S DISEASE

A biomarker is an objective measure of a disease process that is used to assist in making the diagnosis, to evaluate the risk and prognosis, or to monitor the therapy effect. An ideal AD biomarker should therefore reflect the underlying molecular pathology of the disease (Consensus report 1998).

2.2.1 Laboratory biomarkers

Cerebrospinal fluid amyloid beta peptide
CSF was not considered useful for AD diagnostics in the NINCDS-ADRDA criteria but beneficial only in excluding inflammatory reasons for non-AD dementia (Dubois et al. 2007, McKhann et al. 1984). Later findings of decreased CSF APP and total amyloid triggered an interest in clarifying the use of CSF further (Van Nostrand et al. 1992, Farlow et al. 1992). Since it is in direct contact with the brain, it was thought to well reflect the pathological changes of the brain in AD. However, as many negative results were reported (van Gool et al. 1995), it was soon realized that Aβ consists of two main isoforms Aβ40 and Aβ42 of which the latter is more prone to form plaques (Miller et al. 1993) and so the Aβ42 levels would be expected to decrease in CSF AD patients (Motter et al. 1995). There was a debate over whether the low Aβ levels were the result from Aβ deposition into plaques or only a parallel finding reflecting some other process. Some diseases, such as Creutzfeld-Jakob disease (CJD) and amyotrophic lateral sclerosis (ALS) were found to exhibit very low CSF Aβ42 levels without any plaques in the brain but a population-based study showed a strong inverse correlation between CSF Aβ42 and brain amyloid plaque count in the neocortex and hippocampus (Strozyk et al. 2003). Nonetheless, the decrease in the level of CSF Aβ42 depends on reduced availability of the brain parenchyma to diffuse Aβ into the CSF (Blennow et al. 2010). This could well result in reduced clearance, which has been modeled by applying the 13C6-leucine labelled Aβ method (Bateman et al. 2006). In a study of 12 AD patients and 12 controls, the clearance rate of the labeled Aβ to CSF was reduced by 30% in AD patients compared to controls whereas there was no difference between Aβ42 and Aβ40 production (Mawuenyega et al. 2010). As a result of the AD process, there is approximately 50% decrease in CSF Aβ42 levels seen in AD and the deposition of the amyloid in plaques remains as a strong hypothesis, although not undisputed (Mattsson, Blennow & Zetterberg 2009).

Little by little, the biomarker data has accumulated and today there is considerable evidence linking the low CSF Aβ42 to high amyloid load in the brain. In vivo CSF Aβ42 and a definite AD diagnosis in autopsy were associated (Clark et al. 2003). CSF Aβ42 also correlated to amyloid quantification (Tapiola et al. 2009) and the low CSF Aβ42 level was independently related to the clinical diagnosis of AD dementia in one work (Schoonenboom et al. 2008). Patients that have undergone both PIB amyloid imaging and CSF analysis have revealed the rather good concordance between low CSF Aβ42 and high cortical amyloid binding potential in the brain (Fagan et al. 2006, Jagust et al. 2009, Grimmer et al. 2009, Tolboom et al. 2009). In a study of 241 cognitively normal individuals, the APOE ε4 allele had a dose-dependent effect to cause reduced CSF Aβ42 level and higher mean cortical binding potential of PIB in PET (Morris et al. 2010). Thus, both PIB PET and CSF Aβ42 are considered as valid biomarkers of brain Aβ load. However, all data on associations between in vivo CSF biomarkers with brain amyloid have been conducted with post mortem pathology or in vivo imaging, and correlations between biopsy values of amyloid in the brain and corresponding CSF Aβ42 concentrations have not been reported.

The increased rate of hippocampal volume loss was associated with decreased CSF Aβ42 in a study of Alzheimer’s neuroimaging initiative (ADNI) patients with early AD (Schuff et al. 2009) even though another group did not find an association after adjustment
for age, sex and diagnosis (Sluimer et al. 2010). The decreased Aβ42 levels correlated with the hippocampal volume loss in MCI-AD converters as well (Herukka et al. 2008). The low CSF levels of Aβ42 are also associated with brain structure and medial temporal lobe atrophy even without diagnosed dementia or cognitive deficit. The decreased CSF levels of Aβ42 correlated to whole brain atrophy in cognitively healthy controls in a study of Fagan et al. (Fagan et al. 2009). In another study of ADNI, healthy elderslies with low CSF Aβ42 values had higher levels of CSF tau, higher whole brain loss, larger ventricles and hippocampal atrophy rate compared to those with high Aβ42 in the cohort (Schott et al. 2010).

The CSF Aβ40 isoform remains stable or increases in AD, which is why it has been stated that measurement of the CSF Aβ42/Aβ40 ratio might be more useful than that of Aβ42 alone, even in an early stage of disease (Hansson et al. 2007). The CSF Aβ42/Aβ40 ratio has also been proposed to improve differentiation of AD patients from non-AD dementias compared to Aβ42 alone (Spies et al. 2010). In another study, different CSF Aβ peptide isoforms from Aβ1–37 to Aβ1–42 were measured from 11 neuropathologically confirmed subjects and 71 clinical AD dementia subjects. All isoforms were decreased in the definite AD patients compared to those with clinical diagnoses but the percentage proportions of the isoforms were the same (Mollenhauer et al. 2011). As some diseases exhibit low CSF Aβ without plaques there may be some other reasons for that apart from deposition to aggregates. It has been speculated that the highly fibrillogenic Aβ42 forms oligomers that may well escape ELISA detection (Stenh et al. 2005) but may not aggregate at the same level as in AD. Circulating CSF levels of Aβ40 oligomers were recently proposed as biomarkers to identify AD with good accuracy (Gao et al. 2010).

The use of CSF Aβ42 in research for enriching the MCI study populations emerged with evidence on differentiating the patients with AD dementia from controls. In a meta-analyses of 13 studies, the pooled sensitivity and specificity of CSF Aβ42 values for discriminating patients with AD dementia from normal ageing were 86 % and 90 %, respectively (Blennow, Hampel 2003). However, the clinical relevance was not exactly on identifying those with AD dementia from completely cognitively healthy, but identifying which of the healthy or mildly impaired subjects would subsequently develop AD dementia. There are a few studies to support the idea that a decrease in the CSF Aβ42 level in the presymptomatic elderly could predict cognitive decline (Skoog et al. 2003, Gustafson et al. 2007, Stomrud et al. 2007).

One aim would be to differentiate prodromal AD and possibly even presymptomatic patients from those with other dementias, which had seemed problematic, at least regarding patients with FTD or VaD who could also present slightly decreased levels of CSF Aβ42 (Hulstaert et al. 1999, Riemenschneider et al. 2002). Since it was known that the ongoing AD pathological process in the brain is initiated even decades before the AD dementia diagnosis, the research was focused on identifying individuals with early AD and AD converters among those with simply stable MCI or MCI leading to some other dementia by using the CSF amyloid, tau and p-tau in combination. By adding more biomarkers into the research battery, it could be speculated that the specificity would be increased.

Cerebrospinal fluid tau
After the development of the monoclonal antibody for ELISA to detect human tau protein from CSF (Vandemeeren et al. 1993), increased tau concentrations in AD have been frequently reported (Blennow & Hampel 2003, Jensen, Basun & Lannfelt 1995, Blennow et al. 1995) with a meta-analyzed single-assay sensitivity of 90 % and specificity of 81 % to discriminate AD patients from controls (Blennow & Hampel 2003). The high tau concentration in the CSF of AD patients is believed to result from axonal injury in those disorders in which there is cortical damage accompanied with degeneration, as also seen for example in CJD (Otto et al. 1997), which is the main reason why the AD specificity for
tau alone against other dementing or cognitive-impairing disorders is not optimal enough. In addition, patients with ischemic and traumatic brain injury have increased CSF tau (Hesse et al. 2001, Ost et al. 2006). In a recent meta-analysis, CSF tau concentrations were moderately elevated in DLB, FTLD and VaD as compared to controls, but they were still lower in these dementias as compared to AD yielding a sensitivity of 73 % - 74 % and specificity of 74 % - 90 % (van Harten et al. 2011).

The level of CSF tau is increased in MCI as compared to healthy controls and elevated more in those subjects that develop AD dementia in the follow-up (Herukka et al. 2005, Hansson et al. 2006). The increase in the tau level in CSF because of AD is around threefold of that of controls (Blennow & Zetterberg 2009). A more rapid decline of cognition is seen in patients with highest quartile of tau concentrations in CSF (Samgard et al. 2010). One probable explanation for the measurable increase of the level of tau in CSF in AD is that it is released from inside those neurons harbouring NFTs and dystrophic neurons in response to their destruction (Shaw et al. 2007). The in vivo CSF tau level correlates with both presence and the magnitude of tau pathology in the post mortem brain (Tapiola et al. 2009, Tapiola et al. 1997) but a reliable method for neuroimaging or quantifying in vivo tau pathology in the brain has not yet been validated.

Cerebrospinal fluid hyperphosphorylated tau
Whereas CSF tau is considered as a nonspecific marker of axonal injury, its hyperphosphorylated form p-tau is thought to be a specific biomarker of AD pathology. The NFTs in AD consist of tau aggregates and p-tau is more prone to create this form (Buee et al. 2000). The CSF p-tau level has also been shown to correlate with brain pathology at autopsy (Buerger et al. 2006). This is at least the case with epitope p-tau-231 as the studies done with p-tau-181 have reported negative results in a similar setting (Engelborghs et al. 2007, Buerger et al. 2007) and thus different epitopes (i.e. phosphorylated at different sites) may have different roles in the pathophysiology. Unlike tau, p-tau levels do not increase after stroke (Hesse et al. 2001). A high level of CSF p-tau predicts a faster cognitive decline in MCI (Buerger et al. 2005, Blom et al. 2009) and is related to more rapid hippocampal atrophy (Hampel et al. 2005).

There are nearly 80 putative phosphorylation sites of protein tau, but most CSF studies have been conducted using two assays that detect p-tau epitopes threonine 181 and threonine 231; these seem to be equally effective in detecting AD (Blennow & Hampel 2003, Mitchell 2009). In a meta-analysis of 11 studies, the average sensitivity of CSF p-tau to discriminate AD from controls was 80 % when the specificity was determined as 92 % (Blennow & Hampel 2003). Sensitivity varies among studies with different ELISA assays, but the p-tau-181, p-tau-199 and p-tau-231 have displayed similar diagnostic performance with sensitivities of about 85 % (Blennow & Hampel 2003). In another meta-analysis, the sensitivity was 78 % and specificity 88 % for p-tau alone to identify AD patients from stable MCI patients (Mitchell 2009).

Despite the sensitivity problems, the CSF p-tau level was expected to provide a potential for differentiating AD from other dementing disorders. However, for AD relative to FTLD, p-tau alone provides sensitivity of 79 % and specificity of 83 % to differentiate AD by higher concentration. For AD against VaD the sensitivity is 88 % and specificity is 78 % (van Harten et al. 2011). The separating feature against other dementias was considered as inadequate also by a second meta-analysis (Mitchell 2009). However, it seems that CSF p-tau provides the best usefulness for both diagnostic and differentiating purpose when combined to Aβ42.

Combination of CSF amyloid, tau and p-tau
In a study of 137 MCI patients and 39 controls, a combined test consisting of CSF Aβ42 less than 530 pg/ml and tau more than 350 pg/ml achieved sensitivity of 95 % and specificity of 83 % for conversion to AD from MCI in 4 – 6 years of follow-up (Hansson et al. 2006). A
CSF value of Aβ42 less than 530 pg/ml together with p-tau more than 60 pg/ml yielded sensitivity of 95 % and specificity of 81 % for progression from MCI to AD (Hansson et al. 2006). One work was conducted with only a one-year follow-up time but showed similarly pathological CSF biomarkers in those who converted from MCI to AD (Parnetti et al. 2006). Later, a multi-center initiative studied the sensitivity and specificity of a combination of Aβ42/p-tau ratio and total tau yielding to 83% (95% CI, 78%-88%) and 72% (95% CI, 68%-76%), respectively (Mattsson et al. 2009). Both sensitivity and specificity were considered as being less accurate compared to corresponding results of 95 % and 87 % by the former meta-analysis of the reported single studies (Mattsson et al. 2009). Once more, the usefulness of Aβ42, tau and p-tau to discriminate progressive AD from cognitively healthy was confirmed in ADNI patient material (Shaw et al. 2009). The typical AD profile of CSF in patients with aMCI related to an increased risk for AD dementia in a large multi-center study (OR 26.8, 95% CI 1.6–456.4) (Visser et al. 2009).

The combinations of two or more CSF biomarkers provide better distinguishing potential of AD from other dementias. The p-tau/Aβ42 ratio discriminated AD from FTD with a sensitivity of 91.7% and a specificity of 92.6% in a French study (de Souza et al. 2011b). In addition, biomarker combinations seem to describe well the clinical course of AD. AD patients with low Aβ42 and high tau and p-tau have also been demonstrated to deteriorate more quickly over time and their mortality rate is increased (Kester et al. 2009, Wallin et al. 2010).

The role of follow-up time is crucial in the studies that observe the conversion from cognitively healthy or MCI to AD. An adequate follow-up time is needed if one wishes to confirm that the group of ‘stable’ does not include patients who will later convert to AD dementia, which could readily influence the result. In addition, as most of the studies have been ascertained by clinical diagnoses (mostly NINCDS-ADRDA) and not neuropathologically confirmed, there is a risk of circular evidence. The diagnostic performance of the biomarker combinations cannot exceed the accuracy of the clinical diagnostic criteria (Blennow & Hampel 2003). One limitation of the CSF biomarkers is also that inter-laboratory variability complicates the possibility of creating optimal cut-off values between AD and control subjects. At present, the cut-off values used need to be based on reference values, which are distinct for each individual laboratory (Prvulovic & Hampel 2011).

**Longitudinal changes of CSF amyloid, tau and p-tau**

The CSF biomarkers of AD were thought to remain rather stable longitudinally and the changes to occur at an early stage of the disease. However, the biomarker studies that have been conducted in longitudinal settings have reported very inconsistent results i.e. varying from no change to changes in either direction for each biomarker type (Table 2).
Table 2. Studies on longitudinal changes of the CSF biomarkers.

<table>
<thead>
<tr>
<th>Author</th>
<th>Year</th>
<th>N</th>
<th>AD</th>
<th>MCI</th>
<th>CH</th>
<th>OCD</th>
<th>FUT</th>
<th>AB42</th>
<th>Tau</th>
<th>P-Tau</th>
</tr>
</thead>
<tbody>
<tr>
<td>Blomberg et al.</td>
<td>1996</td>
<td>18</td>
<td>18</td>
<td></td>
<td></td>
<td></td>
<td>14</td>
<td>-</td>
<td>↑</td>
<td>-</td>
</tr>
<tr>
<td>Isoe et al.</td>
<td>1996</td>
<td>9</td>
<td>9</td>
<td></td>
<td></td>
<td></td>
<td>31</td>
<td>-</td>
<td>↑</td>
<td>-</td>
</tr>
<tr>
<td>Kanai et al.</td>
<td>1998</td>
<td>32</td>
<td>32</td>
<td></td>
<td></td>
<td></td>
<td>19</td>
<td>↓</td>
<td>↑</td>
<td>-</td>
</tr>
<tr>
<td>Kanai et al.</td>
<td>1999</td>
<td>33</td>
<td>33</td>
<td></td>
<td></td>
<td></td>
<td>20</td>
<td>-</td>
<td>↑</td>
<td>-</td>
</tr>
<tr>
<td>Andreasen et al.</td>
<td>1998</td>
<td>44</td>
<td>19</td>
<td></td>
<td></td>
<td>21 VaD</td>
<td>12</td>
<td>-</td>
<td>↔</td>
<td>-</td>
</tr>
<tr>
<td>Andreasen et al.</td>
<td>1999</td>
<td>31</td>
<td>16</td>
<td>15</td>
<td></td>
<td></td>
<td>6-27</td>
<td>↔</td>
<td>↔</td>
<td>-</td>
</tr>
<tr>
<td>Andreasen et al.</td>
<td>1999</td>
<td>53</td>
<td>53</td>
<td></td>
<td></td>
<td></td>
<td>10</td>
<td>↔</td>
<td>-</td>
<td>-</td>
</tr>
<tr>
<td>Sunderland et al.</td>
<td>1999</td>
<td>29</td>
<td>29</td>
<td></td>
<td></td>
<td></td>
<td>24</td>
<td>-</td>
<td>↔</td>
<td>-</td>
</tr>
<tr>
<td>Tapiola et al.</td>
<td>2000</td>
<td>17</td>
<td>17</td>
<td></td>
<td></td>
<td></td>
<td>36</td>
<td>↓</td>
<td>↔</td>
<td>-</td>
</tr>
<tr>
<td>Wahlund et al.</td>
<td>2003</td>
<td>47</td>
<td>24</td>
<td>23</td>
<td></td>
<td></td>
<td>16</td>
<td>↓</td>
<td>↑</td>
<td>↑</td>
</tr>
<tr>
<td>Mollenhauer et al.</td>
<td>2005</td>
<td>40</td>
<td>19</td>
<td></td>
<td>21</td>
<td></td>
<td>1,5</td>
<td>↓</td>
<td>↔</td>
<td>↓</td>
</tr>
<tr>
<td>Höglund et al.</td>
<td>2005</td>
<td>19</td>
<td>19</td>
<td></td>
<td></td>
<td></td>
<td>12</td>
<td>↔</td>
<td>↔</td>
<td>↑</td>
</tr>
<tr>
<td>De Leon et al.</td>
<td>2006</td>
<td>16</td>
<td>7</td>
<td>9</td>
<td></td>
<td></td>
<td>21</td>
<td>↔</td>
<td>-</td>
<td>↔</td>
</tr>
<tr>
<td>Bouwman et al.</td>
<td>2007</td>
<td>105</td>
<td>50</td>
<td>38</td>
<td>17</td>
<td></td>
<td>21</td>
<td>↑</td>
<td>↑</td>
<td>↔</td>
</tr>
<tr>
<td>Blennow et al.</td>
<td>2007</td>
<td>53</td>
<td>53</td>
<td></td>
<td></td>
<td></td>
<td>6</td>
<td>↔</td>
<td>↔</td>
<td>↔</td>
</tr>
<tr>
<td>Zetterberg et al.</td>
<td>2007</td>
<td>100</td>
<td>83</td>
<td>17</td>
<td></td>
<td></td>
<td>24</td>
<td>↔</td>
<td>↔</td>
<td>↔</td>
</tr>
<tr>
<td>Andersson et al.</td>
<td>2008</td>
<td>39</td>
<td>25</td>
<td>14</td>
<td></td>
<td></td>
<td>34</td>
<td>↔</td>
<td>↔</td>
<td>↑</td>
</tr>
<tr>
<td>Brys et al.</td>
<td>2009</td>
<td>76</td>
<td>55</td>
<td>21</td>
<td></td>
<td></td>
<td>24</td>
<td>↔</td>
<td>↔</td>
<td>↔</td>
</tr>
<tr>
<td>Buchave et al.</td>
<td>2009</td>
<td>89</td>
<td>45</td>
<td></td>
<td>34</td>
<td></td>
<td>24</td>
<td>↔</td>
<td>↑</td>
<td>-</td>
</tr>
<tr>
<td>Stomrud et al.</td>
<td>2010</td>
<td>37</td>
<td>37</td>
<td></td>
<td></td>
<td></td>
<td>46</td>
<td>↔</td>
<td>↔</td>
<td>↔</td>
</tr>
<tr>
<td>Kester et al.</td>
<td>2011</td>
<td>130</td>
<td>68</td>
<td>62</td>
<td>24</td>
<td></td>
<td>24</td>
<td>↔</td>
<td>↑</td>
<td>↔</td>
</tr>
<tr>
<td>Lo et al. (ADNI)</td>
<td>2011</td>
<td>106</td>
<td>16</td>
<td>54</td>
<td>36</td>
<td></td>
<td>36</td>
<td>↓</td>
<td>↔</td>
<td>↔</td>
</tr>
</tbody>
</table>

AD = Alzheimer’s disease dementia, MCI = mild cognitive impairment, CH = cognitively healthy, OCD = other cognitive disorder, VaD = vascular dementia, FUT = follow-up time (months), ADNI = Alzheimer’s disease neuroimaging initiative. ↑ = increase, ↓ = decrease, ↔ = no change.

Even though the previous data still lacks the definitive evidence on when the pathological changes in the CSF take place, it is obvious that they must occur at some point. In case of CSF Aβ42, the decline would be expected to occur rather early in the course of the disease and all the study groups have simply been examined too late to detect this change or else the change is too slow to be detected in such small patient groups. In 2010, Jack et al. proposed a hypothesis of the temporal order of the biomarkers including the CSF proteins (Jack et al. 2010, Jack et al. 2009). The hypothesis is based on the dual assumption that Aβ biomarkers deviate from normal to abnormal early during the symptomless phase and the neurodegenerative biomarkers follow behind, correlating with the clinical symptoms. According to previous data, the Aβ biomarker pathology (including CSF Aβ42) precedes clinical symptoms and also biomarkers of neuronal destruction (including tau and p-tau) (Jack et al. 2010). The biomarkers of neurodegeneration are temporally ordered and correlate with the cognitive stage but it seems that CSF tau does not change sufficiently over time to be detected with short follow-ups of already impaired patients (Bouwman et al. 2007b) even though it shows different levels for normal cognition, MCI and AD if measured in the same setting. The hypothetical model assumes that the maximum rate of
change alters from one biomarker to other, and finally all biomarkers begin to change to more abnormal levels simultaneously (Jack et al. 2011). The group tested their hypothesis later by examining CSF biomarkers and hippocampal volume in 298 patients with a 12-month follow-up. In the healthy subjects with low CSF Aβ42, the number of those having high CSF tau, increased in follow-up, but they remained cognitively normal. In addition, in those individuals with MCI and low CSF values of Aβ42, the hippocampal volume decreased without an increase in CSF tau value (Jack et al. 2009). They concluded that their findings supported the hypothesis in several respects, for example that the CSF tau increase was a downstream effect to the Aβ42 level decline and deposition (Jack et al. 2009). The hypothesis of Jack et al. fits well to previous data about biomarkers except for the earlier discussed neuropathological finding that NFTs can be present in the brain independently of amyloid (Duyckaerts 2011).

![Dynamic biomarkers of the Alzheimer's disease pathological cascade](image)

Figure 2. Dynamic biomarkers of the Alzheimer's disease pathological cascade (Based on Jack et al. 2010). Brain Aβ is identified by CSF Aβ42 or PIB-PET. Tau-mediated neuronal injury and dysfunction is identified by CSF tau and p-tau. Brain structure is measured by structural MRI. Aβ = amyloid beta, MCI = mild cognitive impairment, AD = Alzheimer's disease.

There are also other hypotheses related to the order of the biomarker changes. For example, one study group used a statistical point of view with which to simulate nonlinear associations and a change point of the CSF alterations. After a certain ‘point of no return’, the development of biomarker concentrations in CSF would occur differently than before. However, the data was cross-sectional and thus will need to be confirmed in a longitudinal setting (Williams et al. 2011).

**Plasma amyloid beta**

During the hunt for the ideal AD biomarker, potential factors in blood have also been studied. The concentrations of tau in plasma are mostly below detection limits but
measuring Aβ has been possible, although there have been problems encountered in the validation of the methods. Aβ can be transported across the blood-brain barrier but probably most of the Aβ in the amyloid plaques is produced within the brain (Zlokovic 2004). However, an equilibrium between the central and peripheral compartments is considered by the postulated clearance from brain to blood alongside with CSF (Shibata et al. 2000). With respect to the peripheral Aβ, probably most is secreted by platelets, after which Aβ is bound to albumin during transportation (Biere et al. 1996). Subsequently, there is both uptake and degradation of Aβ in liver and excretion by kidneys (Ghiso et al. 2004), which is the reason why the plasma Aβ correlates with serum creatinine levels (Arvanitakis et al. 2002). The cycle of peripheral Aβ40 and Aβ42 is short, only about 3.5 minutes from brain ventricle via transportation to secretion/localization (Ghersi-Egea et al. 1996) or < 3 min from blood (Ghiso et al. 2004) in mice.

Previous study showed inconsistent results of Aβ40 and Aβ42, but did conclude that single measurements of Aβ in plasma were not useful for diagnosis of AD and instead favoured longitudinal studies (Irizarry 2004). As in some cross-sectional studies, the most common result in the follow-up studies has been a decline in Aβ42 level and an increase or stability of Aβ40 level in association with the cognitive decline, MCI-AD conversion or AD, increasing the credibility of the potential of plasma Aβ42/Aβ40 ratio as an AD biomarker (van Oijen et al. 2006, Pesaresi et al. 2006, Graff-Radford et al. 2007, Okereke et al. 2009, Lewczuk et al. 2010). In contrast, a high plasma Aβ42/Aβ40 ratio was associated with lower risk of dementia (van Oijen et al. 2006, Lambert et al. 2009). On the other hand, also other findings have been reported, mostly no change or association or even increase of plasma Aβ42 level in association with future AD (Lopez et al. 2008, Hansson et al. 2010). The conflicts in the results may be attributable to differences in the study populations, follow-up times and variations in the laboratory assays. One common finding both in cross-sectional studies and longitudinal studies has been that high baseline values of plasma Aβ42 predict a cognitive decline (Cosentino et al. 2010). This represents something of a discrepancy in the former study but may result from peaking of peripheral Aβ42 before cognitive decline and subsequently decrease in parallel with the impairment, which seemed to occur also in the study of 880 subjects and 4.5-year follow-up (Cosentino et al. 2010). If this were to be the case, plasma Aβ42 levels would alter very differently from their CSF counterpart, which seems to decrease very early in the course of the disease.

Very recent results of the value of plasma Aβ42/Aβ40 helped to understand plasma Aβ dynamics and their relation to cognition. In particular, the large studies have corroborated each other. A study of 1125 non-demented subjects, declining Aβ42 and low Aβ42/Aβ40 ratio were associated with the onset of cognitive decline (Schupf et al. 2008). In a study of 481 healthy nurses with a 10-year follow-up, high baseline plasma Aβ40/Aβ42 ratio (i.e. low Aβ42/Aβ40) was associated with worse outcome of late-life cognitive decline, when the nurses were about 75 years old at the end of the follow-up (Okereke et al. 2009). In another large community-dwelling study of 997 non-demented subjects, a low baseline plasma Aβ42/Aβ40 ratio was associated with a greater cognitive decline during 9-year follow-up, at least in those with low educational level (Yaffe et al. 2011). These results are all in agreement with the first and the largest (n=1756) study with a 8.6 year follow-up, which reported that low Aβ42 and high plasma Aβ40 were indicative of an increased risk of dementia (van Oijen et al. 2006).

The evidence for the connection between plasma Aβ and brain Aβ accumulation is only starting to accumulate. In a small case-control study of 20 MCI patients and 19 controls, the plasma Aβ42/Aβ40 ratio was associated with greater PIB binding in PET with the relationship being strongest in MCI subjects (Devanand et al. 2011). In a cross-sectional study of 1032 patients, a lower Aβ42/Aβ40 ratio was found in those with MCI and AD as compared to cognitively healthy. In addition, 255 of these subjects were investigated by MRI and PET scanned with PIB and an inverse correlation was observed between Aβ42/Aβ40 ratio and the PIB binding (Lui et al. 2010).
**Other possible CSF and plasma biomarkers**

In addition to the well-validated CSF biomarkers of AD, Aβ42, tau and p-tau, also potential candidate molecules are under investigation. The β-site APP cleaving enzyme 1 (BACE1) that mediates β-secretase activity, can be measured from CSF and has been found in AD patients (Holsinger et al. 2004). The APP isoforms have been proposed as AD biomarkers in both CSF and blood. In CSF, they may correlate with BACE1 activity (Zetterberg et al. 2008) and in blood platelets their ratio is shifted towards the lower molecular weight particles (Padovani et al. 2001).

The Aβ oligomers are a promising biomarker candidate, but a valid method is needed to reliably detect them from their low concentration in the CSF as compared to those of the monomers. The group of Fukumoto et al. developed a novel ELISA specific for high molecular weight oligomers (40-200 kDa, mainly 10-20 mers of synthetic Aβ) and reported that their concentrations were elevated in CSF of AD patients compared to controls. The method does not measure low molecular weight oligomers such as dimers or tetramers. Surprisingly, the APOE ε4 allele did not influence the concentration of these large Aβ oligomers (Fukumoto et al. 2010). There are also amplification methods that may be developed for oligomer detecting purposes. (Georganopoulou et al. 2005).

Aβ autoantibodies can be quantified from CSF by ELISA. AD patients have been shown to have fewer anti-Aβ antibodies than controls (Du et al. 2001) and they are also measurable in blood (Hyman et al. 2001). Other biomarkers candidates include markers of oxidative stress and inflammation (Mattsson, Blennow & Zetterberg 2009).

In a recent study of 143 MCI patients, 47 AD patients and 46 controls, AD patients were characterized at baseline by diminished ether phospholipids, sphingomyelins, phosphatidylcholines and sterols in serum (Orešič et al. 2011). A molecular signature of AD progression in follow-up was identified based on 2,4-dihydroxybutanoic acid, which was upregulated in converters and may be associated with hypoxia in AD pathogenesis (Orešič et al. 2011). In another study, the abundance 120 different known signaling proteins from plasma samples was measured and 18 proteins identified as predicting AD. By using certain algorithms, all non-AD MCI patients (eight subjects) were identified and 20 of 22 MCI-AD converters identified with 91% positive agreement by analyzing a baseline plasma sample (Ray et al. 2007).

### 2.2.2 Neuroimaging

The primary function of neuroimaging in AD diagnostics has been to exclude intracranial reasons for memory disorder such as tumors, hydrocephalus and hemorrhages. However, the developing techniques and better-quality images have provided new methods to use the imaging results as biomarkers of AD. Neuroimaging of the brain should be conducted on every patient with memory impairment.

**Magnetic resonance imaging**

Magnetic resonance imaging (MRI) is the most widely used method to image the neurodegenerative changes in the brain and it has also been the most studied imaging method in attempts to devise an accurate biomarker of AD. MRI is more sensitive and specific for use in memory impairment patients than computer tomography (CT) (Waldemar et al. 2007). The initial tissue loss changes in AD are present in the medial temporal lobe (MTL), focusing on entorhinal cortex (EC) and hippocampus (HC). Cortical and general atrophy occur in later stages according to Braak stages (Braak & Braak 1991). The atrophy in the MTL is common in AD but it is not disease specific (Jack et al. 2002). The HC volume correlates with the density of neurofibrillary tangles at autopsy but not with amyloid load (Jack et al. 2002, Csernansky et al. 2004). Since the HC volume and tangles are correlated, it is natural that also CSF tau and p-tau correlate with HC volume (de Souza et al. 2011a). In another study, the levels of MTL atrophy and CSF biomarkers did not
correlate but instead they both had an independent association with AD (Schoonenboom et al. 2008).

The HC atrophy intensity is assessed in MRI planes by different methods: visual (qualitative), linear measurements, volumetric measurement and digital voxel based techniques. The visual approach is based on comparing the image planes to predefined comparative images and is suitable for clinical work since it correlates well with the linear measurement (Scheltens et al. 1992). There are computer-based volumetric tools to determine the volumes of the structures that are useful as well, but the more complex digital measures are still used only in research because they are so labor intensive. It is also possible that automated volumetric techniques yield larger volume losses in longitudinal settings (Moula, Duchesn & Alzheimer’s Disease Neuroimaging Initiative 2011). However, very recently, a fast 2-minute volumetric automated method has been introduced with 80% accuracy for AD in comparison with healthy controls and MCI-AD converters with accuracy of 65% against stable MCI (Löjtönen et al. 2011). Combining global and regional MRI measures together may achieve better accuracy than MTA measures alone (Westman et al. 2011). A promising way to predict MCI-AD conversion could be EC volumetry since EC is affected before HC in AD, but the method has not shown any truly significant benefits compared to HC volumetry and is complicated by laborious measurement procedure (Xu et al. 2000, Pennanen et al. 2004, Teipel et al. 2006).

Hippocampal atrophy predicts future conversion from MCI to AD (Wang et al. 2006, Jack et al. 1999). The visually abnormal MTA assessment associates with increased risk of progression from MCI to dementia (Korf et al. 2004, Bouwman et al. 2007a) and the presence of MTA in volumetric MRI predicts faster AD conversion from MCI (Heister et al. 2011). The hippocampal volume loss also predicted a cognitive decline from normal cognition to the level of mild dementia (Csernansky et al. 2005).

Hippocampal atrophy starts to accelerate years before one can make any clinical diagnosis of AD, which means that there may always be also preclinical AD patients in the control groups of cross-sectional or short follow-up studies (Fox & Schott 2004). MRI has rather good longitudinal power in identifying the change in AD stage as compared to clinical measures (Jack et al. 2010). In a meta-analysis of nine studies and 595 AD patients and 212 controls, the MTA rate was markedly faster in AD patients compared to controls (Barnes et al. 2009). It has been shown that MTA rate predicts the future cognitive decline in cognitively healthy patients, as was demonstrated in a six-year longitudinal study (Rusinek et al. 2003). In addition, HC volume loss in cognitively normal subjects is greater in APOE ε4 carriers than in non-carriers (Schuff et al. 2009).

Functional imaging

The functional imaging of the brain aims to describe the activation or metabolism in the areas of interest. Functional MRI provides a way to study the level of activity for example by measuring blood flow difference between rest and cognitive tasking (Greicius et al. 2004). It is possible to investigate changes in the functional connectivity, which reflects the linear association between the regions of activated network (Bokde et al. 2006). The possible prodromal AD in MCI subjects seems to affect the functional connectivity from the fusiform gyrus to other visual processing areas and medial frontal areas, which may act as a biomarker for AD (Bokde et al. 2006).

A more straightforward way to image the metabolism in the area of interest is to use positron emission tomography (PET) with 18fluorodeoxyglucose (18FDG), which is able to detect the decreased glucose uptake by the neurons. In addition to cerebral glucose metabolism, PET can be used to investigate neuroinflammation, various neurotransmitter systems and the protein aggregates, which are characteristic of AD (Nordberg et al. 2010). In MCI and AD, the FDG uptake is reduced in the temporal and parietal association cortex (posterior cingulum) and at the prefrontal association areas in AD (Hampel et al. 2008). In two studies, the sensitivity and specificity for FDG-PET to predict the conversion from MCI
to AD were 38% and 97% in the first area (temporal cortex) (Drzezga et al. 2005) and 92% and 89% in the second area (parietal cortex) (Mosconi et al. 2004), respectively. FDG-PET may also be useful in differentiating AD from FTD (Jagust 2006). On the other hand, a recent study concluded that FDG-PET is not more sensitive than structural MRI, which makes MRI more useful in AD detection (Karow et al. 2010).

Single photon emission computer tomography (SPECT) is another method to image brain metabolism. However, the usefulness of SPECT has remained low because of its low sensitivity, interpretation variation and lack of standardization (Modrego 2006). Some AD patients have normal blood flow in SPECT (McKelvey et al. 1999), which limits the sensitivity, although encouraging results have also been reported (Hirao et al. 2005). In a large study of 85 AD and 78 MCI patients with 34 controls, the sensitivity was 75% (Stauffen et al. 2006). In a systematic review of 27 studies, the sensitivity to distinguish AD from VaD was 71.3% with specificity 75.9% with the corresponding levels to distinguish AD from FTD being 71.5% and 78.2% (Dougall, Bruggink & Ebmeier 2004).

**Molecular positron emission tomography**
Pittsburgh compound P (PIB) was developed from thioflavin and is one of the first and most extensively studied radioligand for amyloid in PET (Nordberg et al. 2010). PIB binds to fibrillar arrays of Aβ with high affinity but not to amorphous amyloid plaques or neurofibrillary tangles. The binding of PIB imaged with PET correlates well with the AD brain tissue amyloid load in brain biopsy (Leinonen et al. 2008) and autopsy (Ikonomovic et al. 2008). In addition, the PIB binding in brain tissue correlates well with the true insoluble Aβ40 and Aβ42 in the post mortem brain tissue (Svedberg et al. 2009). The PIB retention in PET in AD is most intense in frontal cortex but it is also increased in parietal, temporal and occipital cortex and striatum (Klunk et al. 2004), which are the known sites of amyloid deposition in neuropathological studies. The cerebellum, subcortical white matter and pons are usually unaffected and therefore the cerebellum is often used as a reference site to assess non-loading tissue.

The PIB retention in PET effectively distinguishes AD patients from healthy controls (Kemppainen et al. 2006, Mintun et al. 2006). However, the PIB retention in healthy individuals is not uncommon, almost 30-40% at the age of 80 and relating more frequently to the presence of APOE ε4 (Rowe et al. 2010). Five out of six subjects over 85 years of age have PIB retention indicative of a high amyloid load (Rowe et al. 2010). There is also a bimodal retention of PIB in MCI i.e. some have very high retention and some have very low retention even though the clinical phenotype is aMCI, and in some studies the proportion of patients assessed as being unlikely to have prodromal AD based on no retention of PIB may be as high as one third of an entire clinical aMCI cohort (Jack et al. 2009, Rowe et al. 2010, Rowe et al. 2007, Kemppainen et al. 2007, Pike et al. 2007, Forsberg et al. 2008). Nonetheless, those individuals with high PIB retention in PET in MCI exhibit a high risk of developing dementia of AD whereas those with no retention have a very small risk of conversion (Forsberg et al. 2008, Okello et al. 2009, Wolk et al. 2009, Jack et al. 2010), which indicates that even the diagnosis of aMCI may be heterogeneous and result in very different outcomes. The amyloid imaging supported by other biomarkers is a way to achieve more accuracy in the cognitive subgroups in attempts to predict those with concomitant AD.

The group of Jack et al. tested their hypothesis that there is a temporal order of the biomarkers by one-year follow-up study done with PIB PET before publishing the hypothesis. In their PIB PET study, they found that the amyloid accumulates rather slowly when memory impairment is present and the accumulation of amyloid is not related to clinically observed decline of cognition (Jack et al. 2009). The neurodegeneration detected with MRI on the other hand is clearly coupled to clinical symptoms and therefore amyloid accumulation in PIB PET must occur prior to neurodegeneration (Jack et al. 2009), which led them to propose their hypothesis of the temporal order of the biomarkers (Jack et al. 2010) (Figure 2). This also means that a biomarker of neuronal degeneration that correlates
well with the symptoms, such as 18FDG PET might be better at predicting the onset of clinical AD dementia (Landau et al. 2011).

The limitation of the clinical use of $^{14}$C-labelled PIB is that the physical half-life is very short (20 min), which sets the geographic area of use to near to a cyclotron. Other limitations of the method are the price of PET, availability and radiation burden on the subjects. New radioligands for Aβ with longer half-lives are under investigation, for example flurbetaben and florbetapir (Herholz & Ebmeier 2011). In addition, a ligand for imaging neurofibrillary tangles of tau ($^{18}$F-FDDNP) has been introduced which appears to exhibit a high correlation to cognitive functioning (Braskie et al. 2010) but it has not yet been thoroughly validated.

2.3 OTHER RELEVANT DIFFERENTIAL DIAGNOSES OF ALZHEIMER’S DISEASE

2.3.1 Normal pressure hydrocephalus
Normal pressure hydrocephalus (NPH) is a rare cognitive disorder characterized by progressive cognitive and mental impairment, gait disturbance and urinary incontinence. Despite the name, the intracranial pressure (ICP) in NPH is not always normal and peaks sometimes. In about half of the cases, an exposing factor to NPH can be identified and the rest are idiopathic (iNPH). Above all, NPH is one of the few causes of dementia that is potentially reversible, which means that a treated individual may recover to normal cognition.

The incidence of iNPH in the USA is 1/100 000, in Finland 5/100 000 and in Norway 5.5/100 000 (Breen, Eide 2008) and comorbidity with other diseases such as cerebrovascular disease (60 %) (Bech-Azeddine et al. 2007) or AD (22 %) is common (Leinonen et al. 2010). The pathophysiology of iNPH is believed to result from impaired CSF pulsation and reduced CSF flow and absorption through arachnoid granulations presumably due to poor venous compliance (Bateman 2000), but in reality both the pathophysiology and neuropathology are poorly understood. The cognitive symptoms of iNPH include psychomotor slowing, impaired attention and executive and visuospatial dysfunction (Iddon et al. 1999). If the most apparent first symptom is a progressive memory deficit, then diagnoses other than NPH are more probable. In MRI, enlarged brain ventricles and periventricular hypodensity can be seen. The relevant differential diagnoses of iNPH are AD, VaD, Parkinson’s disease, intravascular attacks, brain tumor, subdural hemorrhage and depression.

The diagnostic procedures for identifying NPH include CSF drainage and possible ICP-monitoring overnight. The CSF biomarkers may be useful in identifying iNPH and are easy to perform, as the diagnostics usually will involve LP. Since tau levels are generally elevated in CSF following axonal injury independent of cause, the ratio between p-tau and total tau is especially helpful for identifying AD tau pathology and for differentiating AD from pure iNPH (Kapaki et al. 2007), though the common comorbidity with AD can limit the benefit.

2.3.2 Psychogeriatric disorders
Sometimes, in about 10 % of the AD cases, the symptoms begin with some other characteristic than progressive memory impairment, for example with behavioral changes. These changes may be present for years before the cognitive symptoms become apparent, which emphasizes the importance of the differential diagnosis of AD from psychogeriatric disorders, such as delusional disorder or late-onset schizophrenia spectrum disorders (LOSD).

In a study of one hundred AD patients, 72 % of the subjects had experienced mood changes, depression, suicidal thoughts or social withdrawal for more than 2 years before
the clinical diagnosis of AD dementia. Hallucinations, accusations and paranoia were seen more closely to the diagnosis of AD (Jost & Grossberg 1996). In a large study of 228 MCI patients and 427 mild AD patients, it was concluded that late behavioral changes and psychosis in MCI should be considered as an indication of neurodegenerative disorder (Lopez, Becker & Sweet 2005). However, according to the earlier literature, this interpretation of the data is controversial.

Some chronic patients with LOSD exhibit a progressive cognitive decline in follow-up (Harvey et al. 1999) but in a community-based study, the data provided no evidence of a progressive deterioration in cognitive functioning among LOSD patients (Palmer et al. 2003). One review concluded that cognitive deficits are non-progressive in most patients with late-onset psychosis (LOP) (Reeves & Brister 2008) and another report stated that cognitive deficits encountered in LOP and very late-onset schizophrenia are distinct from those seen in dementia (Howard et al. 2000). In a French systematic review of the literature, it was considered improbable that late-onset schizophrenia would be a prodromal form of AD but it could be possible that these subjects were more prone to develop dementia (Lagodka & Robert 2009). One review concluded that there is not enough evidence to decide whether LOSD is related to prodromal dementia (Kerssens et al. 2006).

However, 60% of LOSD patients experience AD type neuropathological changes in autopsy (Braak III and IV) and differ from controls (Casanova et al. 2002). A prospective study of LOSD showed that almost half of patients became demented within five years of follow-up (Brodaty et al. 2003). In a study of 15 patients with LOP, 17 elderly patients with early-onset psychosis and their 22 controls, significant cognitive deficits were encountered in the LOP subjects. Forty percent of the late-onset subjects were diagnosed as having MCI or vascular cognitive impairment and 20% as suffering from dementia (Girard et al. 2011).
3 Aims of the Study

AD is a disease process that begins in the brain perhaps decades before the diagnosis or prior to the appearance of any clinical symptoms. By measuring changes in concentrations of amyloid and tau in body fluids like plasma and CSF, new information on biomarker dynamics can be achieved.

The aims of the study were:

1. To study the usefulness of plasma amyloid β proteins for following or predicting cognitive change and for identifying MCI patients at risk of developing AD during follow-up.
2. To study the applicability of longitudinal changes of the CSF biomarkers (Aβ42, tau and p-tau) for monitoring the cognitive decline and clinical progression of the disease process.
3. To study the concentration and concentration gradients of ventricular and lumbar CSF biomarkers and their association to the in vivo neuropathological changes in cortical brain tissue biopsy.
4. To study, which proportion of elderly people with a first psychotic episode actually suffer from a dementing illness, especially AD.
4 General Experimental Procedures

4.1 PATIENTS

The patients in the present series of studies were recruited from a community-based population (plasma biomarkers) and from ongoing clinical projects (clinic setting and ward setting). The exact recruitment methods for each part of the study are described in each section. All patients were examined using standard clinical routines.

4.2 LUMBAR PUNCTURE

Lumbar puncture (LP) is a general method to collect CSF from the spinal canal. The procedure is usually conducted with a 22 Gauge (G) or smaller needle in vertebrae LIII-IV or LIV-V interspace. The most common complication of LP is post-lumbar puncture headache (PLPH), which typically lasts for 1 – 2 days and is usually rather incapacitating. PLHP is treated with analgesics, bed rest and increased fluid intake, which usually improve the condition. If none of these help and the PLPH has lasted for 1-2 days, an epidural blood patch should be considered and is almost invariably effective. Age is the most important risk factor for PLHP as young patients are more prone to suffer from this symptom. The most important determinant that can be affected is the needle size. Small needles such as 27 G are used typically in spinal anesthesia and therefore the incidence of PLPH afterwards is half of that seen with diagnostic LP (Lavi, Rowe & Avivi 2010). In a study of 395 demented patients, the incidence of PLPH was low, occurring in only eight patients (2.0%). Most cases were mild, and the duration was less than 2 days in all cases but one (Blennow, Wallin & Hager 1993).

4.3 CSF HANDLING AND STORAGE

There are numerous pre-analytical factors that influence the CSF biomarker analysis. It is known that the inter-laboratory variation is great even if the same assay techniques are in use and therefore it is important to minimize all the factors that could influence the results. However, at present, the results are comparable only within the same laboratory.

The time interval between collecting the CSF and analyzing it is usually long because of extensive follow-up times or even simply the time needed for collection of the specimen. Requirements are set for long-term storage and CSF handling in order to result with valid measurements. The CSF samples are stored at -80 °C. It has been shown that CSF Aβ42, Aβ40 and tau do not change during storage if it is done properly (Schipke et al. 2011, Schoonenboom et al. 2005). Instead, storage in non-frozen conditions in +4 to +37 °C decreases the measurable CSF Aβ42 by 20 % during the first 2 days (Schoonenboom et al. 2005). The freeze/thaw cycles influence only the Aβ42 levels (Schoonenboom et al. 2005) but not tau, which is the reason why Aβ42 should be analyzed first after the long-time storage at – 80 °C and then tau and p-tau subsequently. The CSF specimen should be centrifuged before storage as cell lysis may increase Aβ42 concentration (Bjerke et al. 2010). Polypropylene tubes should always be used as other tubes may absorb Aβ42 and result in apparently decreased levels (Bjerke et al. 2010).

The standard protocol in use in our laboratory takes all of these factors into account.
4.4 ELISA FOR MEASURING AMYLOID BETA AND TAU

Commercially available enzyme-linked immunosorbent assay (ELISA) was used for measuring biomarkers in plasma and CSF. For plasma Aβ40, a non-commercial but previously described method was used. The commercially available ELISA kits were manufactured by Innogenetics (Ghent, Belgium). All biomarker analyses were made in duplicate.

Shortly, the basic principle of ELISA is as follows: the studied protein (amyloid, tau or p-tau) is captured by a monoclonal antibody bound on a solid phase. The CSF samples are added and incubated with biotinylated protein-specific antibody. Then the antibodies are detected by a peroxidase-labeled streptavidin. When substrate is added, the samples develop a blue color and after the reaction is stopped with sulphuric acid, the absorption of the subsequently produced yellow color is measured at 450 nm.

Innogenetics uses monoclonal antibody AT120 for human tau detection with two biotinylated tau-specific antibodies (HT7 and BT2). For p-tau, the monoclonal antibody is HT7 and the biotinylated antibody is AT270Bio. For Aβ42, the monoclonal antibody is 21F12 and the biotinylated antibody is 3D6.
5 Ethical Aspects

All parts of the present study were evaluated by the local ethical committee and were provided with a favourable statement.

Patients with severe memory deficits represent a group of study subjects whose capability to understand the information prior to giving informed consent may sometimes be compromised. Consent is essential to ensure that the ethics of the study are immaculate. If the patient cannot provide consent, then consent of a close relative should be obtained. Written informed consent was obtained from all patients and if the patient was more than slightly impaired, also consent from a close relative was obtained.

ICP measurement and a brain biopsy are invasive procedures that require special circumstances and an operating theatre setting. They may also result in complications. However, the neurosurgical procedures were indicated because the subjects were suspected of suffering from NPH, which is one of the rare potentially treatable causes of memory deficit and therefore the invasive procedure was expected to be beneficial.

LP is also an invasive procedure. LP is conducted in a polyclinic setting and is usually free of serious complications. However, it is uncomfortable and may sometimes result in treatable but annoying post-lumbar puncture headache. In the present study, the patients were old and therefore the incidence of PLPH was rare.

Alzheimer’s disease is an incurable disease with a rather dismal prognosis. Devising new methods to increase the certainty of AD diagnoses even in the preclinical phase when there are no symptoms create an ethical problem. The use of CSF or any other biomarkers to predict AD in asymptomatic people with letting them know the results is not warranted until effective drugs with distinct disease-modifying effects, and few adverse effects, become available (Blinnow et al. 2010). The patients of this study were not supplied with the results of their CSF analysis unless they were used in clinical differential diagnostics.
6 Plasma Aβ42 and Aβ40 as markers of cognitive change in follow-up — a prospective, longitudinal, population-based cohort study *

ABSTRACT

Background: Single measurements of plasma Aβ are not useful in the diagnostics of Alzheimer’s disease (AD). However, changes in plasma Aβ levels during repeated testing may be helpful in the prediction and evaluation of progression of the incipient AD or mild cognitive impairment.

Objective: To examine the relation of baseline and serial plasma Aβ levels to cognitive change in follow-up.

Methods: 269 subjects (52 cognitively impaired and 217 controls) from a population-based cohort were clinically followed up from three to six years. Serial plasma samples were available from 70 subjects who were followed up for three years and 43 subjects followed for six years. The plasma Aβ levels were measured using ELISA.

Results: Subjects who declined cognitively during the follow-up had lower levels of plasma Aβ42 at the baseline. Plasma Aβ42 and the Aβ42/Aβ40 ratio decreased (-2.4 pg/ml for Aβ42 in six years) in those who declined in follow-up whereas Aβ42 and the Aβ42/Aβ40 ratio increased in the subjects who remained cognitively stable or improved in follow-up. Subjects using acetylsalicylic acid, dipyridamole, antidiabetic or anticoagulant drugs as well as subjects with coronary heart disease had higher levels of Aβ40.

Conclusions: Low or decreasing plasma Aβ42 during the follow-up is associated with cognitive decline. Serial measurement of plasma Aβ42 may be useful in the detection of the subjects who are at risk for cognitive decline.

6.1 INTRODUCTION

Alzheimer’s disease (AD) is the most common cause of dementia. At present, its diagnosis is based on clinical criteria and the exclusion of other causes. However, objective biomarkers for the early diagnosis and monitoring of the disease process are clearly needed because symptomatic treatments are available and disease-modifying drugs are already in phase III trials. The presence of amyloid β deposition in senile plaques is one pathological hallmark of Alzheimer’s disease (AD) together with neurofibrillary tangles (Selkoe 1994). The amyloid β is a peptide secreted by neurons (Selkoe 1994) and platelets (Chen et al. 1995), derived from amyloid precursor protein APP via the activity of proteases β and γ secretase (Selkoe 2001). Most of the Aβ deposited in the brain is constituted of 42 amino acids (Aβ42) form (Gravina et al. 1995). Aβ42 has also been shown to be the first amyloid form to accumulate with Aβ40 being deposited later in the process of the AD pathogenesis (Iwatsubo et al. 1995). The level of Aβ42 in cerebrospinal fluid (CSF) is reduced in patients with mild cognitive impairment (MCI) and AD (Blinnnow & Hampel 2003, Herukka et al. 2007) and a combination test of CSF Aβ42 and tau or phospho-tau has been claimed to be helpful in the early diagnosis of AD (Dubois et al. 2007).

Aβ is present in plasma but it is still unknown whether it originates from peripheral sources or from the brain. In Tg2576 transgenic mouse, plasma Aβ levels decline in parallel with their increasing accumulation in the brain. (Kawarabayashi et al. 2001) Since Aβ can be transported bidirectionally across the blood-brain-barrier, it has been hypothesized that there may be an equilibrium between CSF and plasma pools of Aβ (Shibata et al. 2000, Gheris-Egea et al. 1996). Seeing that it is well established that CSF Aβ42 levels decrease in conjunction with the cognitive decline, it has been postulated that plasma Aβ42 may decrease similarly (Graft-Radford et al. 2007). If so, plasma Aβ would offer a straightforward, non-invasive and economical biomarker for AD. However, patients with known mutations in chromosome 21 causing early-onset familial AD as well as patients with trisomy 21 have increased plasma Aβ42 levels which are detectable before the onset of the symptoms of dementia (Scheuner et al. 1996, Schupf et al. 2007). Also the first-degree relatives of late-onset AD patients exhibit elevated Aβ levels measured in plasma (Ertekin-Taner et al. 2008).

Previous studies have suggested that the levels of plasma Aβ40 are increased before the onset of sporadic AD (van Ojien et al. 2006, Mehta et al. 2000). However, one recent study concluded that low plasma Aβ40 level predicted AD in elderly men (Sundelof et al. 2008). Other studies have found elevated Aβ42 levels in patients who later develop dementia (Mayeux et al. 2003), particularly in MCI amnestic type (aMCI) (Sobow et al. 2005). Finally, several studies have not been able to detect any significant difference of Aβ levels between AD converters and cognitively stable controls (Tamaoka et al. 1996, Vanderstichelen et al. 2000).

It seems that a single measurement of plasma Aβ is not useful whereas the change in plasma Aβ levels observed in repeated testing may be of help in the prediction and evaluation of progression of incipient AD or MCI (Irizarry 2004). However, only a limited number of longitudinal studies have been performed (Schupf et al. 2008, Graft-Radford et al. 2004, Blasko et al. 2008a). Our aim was to examine whether the change in plasma Aβ levels during follow-up would be more predictive of cognitive decline than straightforward baseline plasma Aβ levels in a population-based cohort of MCI and cognitively intact controls.
6.2 SUBJECTS AND METHODS

6.2.1 Subjects

Study subjects were participants in the population-based study (n=806, aged 60-76 years) examining the risk factors and predictors of dementia in the elderly (Hänninen et al. 2002, Tervo et al. 2004) (Table 3). At baseline (years 1997-1998), 52 subjects were cognitively impaired (CDR 0.5 n=51 and CDR 1 n=1). Group 1 of this study included all of the cognitively impaired subjects from the original cohort who provided a plasma sample (n=52). For each of them, we randomly selected 4 - 5 cognitively unimpaired (n=217) age- and sex-matched controls from the same cohort. These subjects were clinically re-evaluated after three (n=197) and six years (n=60).

Table 3. Baseline demographic information of the subjects.

<table>
<thead>
<tr>
<th></th>
<th>Population-based cohort</th>
<th>Group 1 (cognitive follow-up)</th>
<th>Group 2 (plasma follow-up)</th>
</tr>
</thead>
<tbody>
<tr>
<td>n</td>
<td>806</td>
<td>269</td>
<td>70</td>
</tr>
<tr>
<td>Age</td>
<td>68 (60 - 76)</td>
<td>70 (60 - 77) p &gt; 0.1</td>
<td>71 (61 - 77) p &gt; 0.1</td>
</tr>
<tr>
<td>Men / Women</td>
<td>321 / 485</td>
<td>121 / 148 p &gt; 0.1</td>
<td>25 / 45 p &gt; 0.1</td>
</tr>
<tr>
<td>APOE ε4 - / +</td>
<td>414 / 207*</td>
<td>169 / 99† p &gt; 0.1</td>
<td>37 / 33 p &gt; 0.05</td>
</tr>
<tr>
<td>MMSE</td>
<td>26 (7 - 30)</td>
<td>27 (13 - 30) p &gt; 0.1</td>
<td>26 (17 - 30)</td>
</tr>
<tr>
<td>CDR = 0</td>
<td>731</td>
<td>217</td>
<td>59</td>
</tr>
<tr>
<td>CDR = 0.5</td>
<td>70</td>
<td>51</td>
<td>11</td>
</tr>
<tr>
<td>CDR = 1</td>
<td>3</td>
<td>1</td>
<td>0</td>
</tr>
</tbody>
</table>

The data is given as median values (range) or as number of subjects with the percentage of all subjects in the group.

*APOE data missing from 185 subjects.
†APOE data missing from one subject.
‡One subject had CDR 2. Data missing from one subject.

The longitudinal marker group (group 2) included 70 subjects of the original cohort of 269 subjects who provided a 3-year follow-up plasma sample and 43 of them a 6-year follow-up sample. The group 2 included 11 cognitively impaired (at baseline), non-demented subjects (CDR 0.5) who provided at least one follow-up plasma sample and 59 cognitively intact (at baseline) age- and sex-matched controls with at least one follow-up plasma sample (Figure 3). Drop-outs occurred mainly due to refusal of the participants to continue the study. Written informed consent was obtained from all the subjects and the study was approved by the local Ethical Committee.
6.2.2 Clinical evaluation
The evaluation included a structured detailed interview including demographic information, medical history, medication, smoking habits and alcohol consumption, and a subjective assessment of memory disturbances and depression. The evaluation also included clinical examination as well as an assessment of cognitive impairment by applying the Clinical Dementia rating (CDR) and using a battery of neuropsychological tests. Memory: Visual Reproduction Test from Wechsler Memory Scale (Russe 1975), Word List Recall from the CERAD Neuropsychological Assessment Battery (Morris et al. 1989), Logical Memory Test from Wechsler Memory Scale-Revised (Wechsler 1987), NYU Paragraph Recall (Kluger et al. 1999), Delayed Recall of the Constructional Praxis from CERAD (Morris et al. 1989); Language: vocabulary subtest from the Wechsler Adult Intelligence Scale-Revised (Wechsler 1981), Abbreviated (15 items) Boston Naming Test (Kaplan, Goodglass & Wintrob 1991); Attention and executive function: Trail Making Test (Reitan 1958) parts A and B, Verbal Fluency Test (Borkowski, Benton & Spreen 1967, Butters et al. 1987); Visuospatial skills: Block Design from the WAIS-R(Wechsler 1981), Constructional Praxis from CERAD (Morris et al. 1989); Global functioning: Mini-Mental State Examination (Folstein, Folstein & McHugh 1975) (MMSE) Clock Drawing Test (Morris et al. 1989).

Cognitive decline was defined by the CDR change from 0 to 0.5 or 0.5 to 1.

6.2.3 Measurement of \(\text{A}\beta_{40}\) and \(\text{A}\beta_{42}\)
The 269 baseline samples (group 1) were measured in year 2002 – 2003. After completing the six-year follow-up, we re-analysed the baseline samples of 70 subjects (group 2) together with their follow-up samples in year 2006 – 2007 (group 2).

A venous blood sample was obtained into heparine tubes and plasma was separated using the standard methods. The samples were aliquotted and stored in polypropylene
tubes at -70°C until analyses. Aβ40 was measured by the ELISA method modified from a well established method (Mehta et al. 2000). The capture antibody was 6E10 (Sigma, Saint Louis, MO) and the detection antibody was a biotin labelled G2-10 antibody (The Genetics Company, Schlieren, Switzerland). The synthetic Aβ1-40 peptide (Bachem, Bubendorf, Switzerland) was used as the standard. Before the analyses, 0.05% Tween 20 - 0.5% BSA was added to the samples. Aβ42 was measured by a high sensitivity method of a commercially available ELISA (Innogenetics, Gent, Belgium), which we modified to be suitable for the measurements of more than 7 pg/ml concentrations. Before the analyses, 0.5M guanidine chloride was added to the standards and the samples. The detergents were used to avoid coagulation of samples and to release Aβ peptides from plasma proteins.

In longitudinal analysis, baseline and follow-up samples from one individual were placed on the same plate to prevent inter-assay variation. Thus, we measured baseline samples from 70 subjects twice (4 years apart). The absolute concentrations differed between these two measurements; median level for Aβ40 174.5 (year 2003) and 198 pg/ml (74) (year 2007), and Aβ42 17 (year 2003) and 49 pg/ml (110) (year 2007). However, there was a moderately good correlation between these measurements (Aβ40 r=0.674, p<0.001 and Aβ42 r=0.824, p<0.001). The correlations are presented in figure 4.

![Figure 4. Correlations between the Aβ measurements in 2002-2003 and 2006-2007. The outliers (arrows) have been removed for the pictures on the right.](image)

The inter-assay variation for Aβ40 assay was 23.8% and for Aβ42 assay 19.1%. The inter-assay CVs were measured by using reference samples of medium concentration (~250 pg/ml for Aβ40 and ~400 pg/ml for Aβ42). The intra-assay variations for Aβ40 were 0.71% for high (~ 1200 pg/ml), 0.95% for medium and 5.9% for low concentrations (~150 pg/ml). The intra-assay CVs (median) for Aβ42 were 1.6% (~1000 pg/ml), 2.5% and 9.8% (~15 pg/ml), respectively.

6.2.4 APOE genotyping
The APOE allele genotyping was done by a PCR based method (Tsukamoto et al. 1993). The subjects were subdivided into the APOE ε4 negative and APOE ε4 positive subjects.
6.2.5 Statistics
The statistical analyses were conducted by the SPSS for Windows release 14.0.1. (SPSS Inc., Chicago, IL, USA). Due to the non-normal distribution of data, Kruskal Wallis, Mann Whitney and Spearman’s correlation tests were used. The categorical data was analysed by χ² test. The odds ratios (OR) for cognitive decline of patients in different groups were calculated by logistic regression analysis. We fitted a linear regression slope by Microsoft Excel to analyse the alteration trend of Aβ levels.

6.3 RESULTS

Table 3 presents the demographic information about the subjects. The baseline Aβ40 and Aβ42 levels of 269 individuals were generally low, although some subjects exhibited extremely high Aβ42 levels. The limit for the 90th percentile was 101 pg/ml, but the highest measured level was 1341 pg/ml. The Aβ40 levels showed a weak correlation with age (r=186, p=0.002), but this was not the case with the Aβ42 levels. There were no differences in Aβ40 and Aβ42 levels between the sexes or between the APOE ε4 carriers and non-carriers. Aβ42 levels did not correlate with Aβ40 concentrations.

6.3.1 Baseline Aβ levels and cognitive decline during the follow-up
No significant differences were found in plasma Aβ40, Aβ42 or the Aβ42/Aβ40 ratio between cognitively impaired (n=52) and cognitively intact subjects (n=217) at baseline.

However, 197 of these subjects were clinically assessed after three years and 60 were clinically assessed after six years. The baseline Aβ42 levels were significantly lower in the subjects who showed cognitive decline after three years of follow-up (cognitively stable, n=147: 19 pg/ml (0-1341), cognitive decline, n= 50: 12 pg/ml (0-276), p=0.001). Baseline Aβ42 levels were also lower in subjects who had declined cognitively after six years (10 pg/ml, n=36) compared to those who remained cognitively stable (18 pg/ml, n=24), p=0.013.

Subjects who had baseline Aβ42 levels in the lowest quartile displayed an OR of 3.12 (95% CI 1.25 - 7.79, p=0.015) for cognitive decline after three years and 4.77 (95% CI 1.14 - 19.98, p=0.033) after six years in comparison to subjects who had Aβ42 levels in the highest quartile. Similarly, subjects who had Aβ42/Aβ40 ratio in the lowest quartile had an OR of 3.26 (1.31 - 8.11, p=0.011) for cognitive decline after three years and 8.40 (1.83 - 83.568, p=0.006) after six years of follow-up when compared to the subjects in the highest quartile.

6.3.2 Relationship between changing plasma Aβ levels and cognitive decline
The follow-up plasma samples were available from 70 subjects after six years and 43 subjects after six years of follow-up. The median levels of Aβ42 did not change or decreased in subjects with cognitive decline (n=27 after three years and n=14 after six years) whereas they increased in those who remained cognitively stable (Table 4). No statistically significant changes were found in Aβ40 levels. The Aβ42/Aβ40 ratio decreased significantly in the subjects who experienced a cognitive decline.

A trend analysis was undertaken by calculating a slope for each subject and this was used to assess the change of Aβ42 between cognitively stable and cognitively declining subjects. During the follow-up of 3 to 6 years, the cognition of 28 out of the total 70 subjects had declined and there was a decreasing trend of the level of Aβ42 in 24 subjects during the follow-up. The Aβ42 level remained the same in the declining subjects and increased in the cognitively stable subjects (0.0 (4.9) pg/ml/year and 2.1 (21) pg/ml/year; p=0.009 for slope difference). The corresponding changes of Aβ42/Aβ40 ratio were also significant (0.0 (0.029) per year and 0.0036 (0.055) per year; p=0.02 for slope difference).
*Table 4. Cognition and changes of Aβ between baseline and follow-ups in subjects.*

The data are given as medians (interquartile range), unit pg/ml when applicable. The values of p reflect the significance against the cognitively stable subgroup. *p < 0.05 against "stable or improved".

<table>
<thead>
<tr>
<th>Comparison</th>
<th>Baseline</th>
<th>Cognitively healthy</th>
<th>Cognitively impaired</th>
</tr>
</thead>
<tbody>
<tr>
<td>n</td>
<td>59</td>
<td>11</td>
<td></td>
</tr>
<tr>
<td>40</td>
<td>195 (88)</td>
<td>201 (60)</td>
<td></td>
</tr>
<tr>
<td>42</td>
<td>50 (121)</td>
<td>46 (92)</td>
<td></td>
</tr>
<tr>
<td>42/Aβ40</td>
<td>0.236 (0.464)</td>
<td>0.291 (0.487)</td>
<td></td>
</tr>
</tbody>
</table>

<table>
<thead>
<tr>
<th>Follow-up</th>
<th>3 years</th>
<th>6 years</th>
<th>3 years</th>
<th>6 years</th>
</tr>
</thead>
<tbody>
<tr>
<td>n</td>
<td>43†</td>
<td>29‡</td>
<td>27</td>
<td>14</td>
</tr>
<tr>
<td>change of 40</td>
<td>18 (55)</td>
<td>33 (45)</td>
<td>21 (24)</td>
<td>33 (75)</td>
</tr>
<tr>
<td>change of 42</td>
<td>3.7 (29)</td>
<td>12 (54)</td>
<td>0.0 (14)*</td>
<td>-2.4(50)*</td>
</tr>
<tr>
<td>change of 42/Aβ40</td>
<td>0.0093 (0.169)</td>
<td>0.0166 (0.451)</td>
<td>-0.0027 (0.084)*</td>
<td>-0.0486 (0.251)*</td>
</tr>
</tbody>
</table>

†Aβ data of one subject is from a plasma sample of 4 years of follow-up. ‡Cognition data of two subjects is from the previous year.

### 6.3.3 Plasma Aβ and general health

Table 5 shows the relationship between medication as well as certain diseases on the plasma Aβ levels. The Aβ40 levels were not associated with the use of lipid lowering drugs or NSAIDs at baseline. Hormone replacement therapy was not related to Aβ values in women. The Aβ40 levels were higher in those subjects using ASA (n = 62, p = 0.004) or dipyridamole (n = 12, p = 0.017). The Aβ40 values were lowest in subjects using neither of the drugs, intermediate in subjects using either ASA or dipyridamole and highest in the subjects taking both drugs. The Aβ40 levels were also higher in subjects using insulin alone (n = 5, p = 0.009) or insulin in combination with oral antidiabetic drugs (n = 13, p = 0.003). The Aβ42 levels were not associated with use of any of the drugs. Coronary heart disease (CHD) was associated with a high plasma Aβ40 level (p=0.035). There was no statistically significant difference in medication use between the cognitively stable and the cognitive decliners.
Table 5. Relation of Aβ40 level to medication at baseline

<table>
<thead>
<tr>
<th>Group 1</th>
<th>Aβ40</th>
<th>pg/ml Users</th>
<th>pg/ml Non-users</th>
</tr>
</thead>
<tbody>
<tr>
<td>Sex, female</td>
<td>148</td>
<td>-</td>
<td></td>
</tr>
<tr>
<td>Smoking</td>
<td>21</td>
<td>-</td>
<td></td>
</tr>
<tr>
<td>CHD</td>
<td>61</td>
<td>↑ p=0.035 188 (105 - 360)</td>
<td>176 (0 - 780)</td>
</tr>
<tr>
<td>Diabetes</td>
<td>22</td>
<td>-</td>
<td></td>
</tr>
<tr>
<td>Anticoagulants</td>
<td>16</td>
<td>↑ p=0.038 198 (144 - 360)</td>
<td>176 (0 - 780)</td>
</tr>
<tr>
<td>NSAIDs</td>
<td>22</td>
<td>-</td>
<td></td>
</tr>
<tr>
<td>ASA</td>
<td>62</td>
<td>↑ p=0.004 194 (0 - 278)</td>
<td>175 (0 - 780)</td>
</tr>
<tr>
<td>Dipyridamole</td>
<td>12</td>
<td>↑ p=0.016 208 (156 - 278)</td>
<td>176 (0 - 780)</td>
</tr>
<tr>
<td>Antidiabetics*</td>
<td>13</td>
<td>↑ p=0.003 219 (161 - 426)</td>
<td>179 (0 - 780)</td>
</tr>
<tr>
<td>Lipid lowering agents</td>
<td>24</td>
<td>-</td>
<td></td>
</tr>
</tbody>
</table>

*insulin and oral antidiabetics

6.4 DISCUSSION

Our results indicated that low or decreasing plasma Aβ42 levels and Aβ42/Aβ40 ratio were related with cognitive decline during the follow-up. Moreover, possible age-related increase in plasma Aβ42 levels in serial measurements was associated with stable cognitive performance.

We found no differences in plasma Aβ levels between cognitively intact and impaired subjects in the cross-sectional analysis at baseline. Previous cross-sectional studies comparing plasma Aβ levels in patients with sporadic AD and controls have reported contradictory results. Elevations of plasma Aβ40 (Mehta et al. 2000) or Aβ42 (Mayeux et al. 2003) have been described whereas other studies have found no differences between AD and controls (Tamaoka et al. 1996). One recent study found increased levels of plasma Aβ42 in women with amnestic MCI compared to healthy controls or affected men (Assini et al. 2004). However, low Aβ42 levels at baseline were associated with a cognitive decline occurring during the follow-up. In line with our results, a recent study detected an association between a low Aβ42/Aβ40 ratio and cognitive decline (Graff-Radford et al. 2007). Respectively, a prospective three-city study of 257 dementia patients found an association of high Aβ42/Aβ40 ratio with a lower risk of dementia in follow-up (Lambert et al. 2009). Another population-based case-cohort study claimed that individuals with a combination of low Aβ42 and high Aβ40 measured from plasma at baseline had an over 10-fold risk of dementia, but found no association between the Aβ42 or Aβ40 levels alone with cognitive decline (van Oijen et al. 2006). Other studies have reported different results. The VITA study found no association between baseline Aβ levels and cognitive decline during the follow-up. Other studies have found elevated concentrations of Aβ42 at the baseline in subjects who developed AD during the follow-up (Lopez et al. 2008, Mayeux et al. 2003) although plasma Aβ levels were not associated with AD in the fully adjusted multivariate model (Lopez et al. 2008).

Differences in study cohorts, for example timing with respect to cognitive decline, assessment of cognitive functioning (different tests) and presence of confounding factors
such as medication and other diseases, are all factors that can influence the results. The outcome in some studies has been conversion to dementia (van Oijen et al. 2006, Mayeux et al. 2003) whereas the outcome in our study as well as in some other studies (Graff-Radford et al. 2007) was cognitive decline. The difference in the selected outcome and the timing of the sample collection may partially explain differences in the results.

Single measurement of plasma Aβ may not be a suitable marker for AD due to many confounding factors. The timing of the Aβ measurement in terms of the natural history of AD may be critical. Experimental studies on transgenic animals suggest that plasma Aβ levels decrease at the time when accumulation of Aβ begins in the brain (Kawarabayashi et al. 2001). It is possible that the increased plasma Aβ concentration is related to the development of AD as suggested by the findings of elevated plasma Aβ levels in AD gene mutation carriers (Citron et al. 1997) and in the first degree relatives of the patients with late onset AD (Ertekin-Taner et al. 2008). However, since amyloid pathology in the brain begins years before the appearance of the first symptoms, the possible increase of plasma Aβ may not be detected in the symptomatic individuals. This hypothesis can only be addressed in longitudinal studies. In line with our results, previous studies have suggested that decreasing Aβ levels are associated with cognitive decline (Mayeux et al. 2003, Graff-Radford et al. 2004) and Aβ42 levels were lower in patients diagnosed having AD than in those with MCI (Pesaresi et al. 2006). Many studies have shown that plasma Aβ levels increase with age, as was found in the cognitively intact subjects in our study (Lopez et al. 2008, Mayeux et al. 2003, Blasko et al. 2008a). In one study, the age-related increase was smallest in those subjects who converted to AD from MCI (Blasko et al. 2008a). It is possible that age-related changes of plasma levels of Aβ40 and Aβ42 differ in subjects with AD. In this respect, the Aβ42/Aβ40 ratio may be a better predictor for AD than the single markers.

Differences in study cohorts make the comparison between different studies difficult. There are many confounding factors that may influence plasma Aβ levels. Renal dysfunction may increase plasma since plasma Aβ is excreted through the kidneys (Arvanitakis et al. 2002). Many studies have suggested an association between vascular disease and plasma Aβ levels (van Dijk et al. 2004, Lee et al. 2005, Gurol et al. 2006). The levels of plasma homocysteine, a possible marker for vascular disease, correlate positively with plasma Aβ40 and Aβ42 levels (Irizarry et al. 2005). Previous studies have also suggested that certain drugs may influence plasma Aβ (Blasko et al. 2008b). We found elevated levels of Aβ40 in the subjects who were using ASA and dipyridamole, i.e. drugs that directly influence platelet function and activation, and subjects who used anticoagulation and antiangiobiotic drugs, whereas there was no association between the use of NSAIDs or lipid lowering agents and plasma Aβ levels. The association between ASA and plasma Aβ40 was seen also in subjects without cardiovascular diseases. In line with previous studies (Blasko et al. 2008b, Tokuda et al. 2001), no relationship was found between Aβ levels and lipid lowering agents has been found in previous studies.

Differences in methodology and experimental conditions may also influence results. Erythrocytes and plasma proteins, for example albumin and lipoproteins, bind Aβ and denaturing conditions liberate Aβ into the free pool of plasma (Kuo et al. 2000). Also, the different antibodies used in the immunological assays may detect different fractions of Aβ. Previous studies suggested that the absolute levels of Aβ vary across different ELISA batches (Golde, Eckman & Younkin 2000). We also noticed a difference between absolute levels in 70 samples that were measured twice four years apart. Due to these methodological difficulties, the diagnostic value of a single measurement is limited.

The significance of the standardization of the conditions in storing and handling the samples is reported in a work by Vanderstichele et al. (Vanderstichele et al. 2000). To better utilize these analyses, the assays used should be commercially available, well standardized and thoroughly validated.
We conclude that plasma Aβ is not a diagnostic marker for AD but the decreasing levels of Aβ42 in serial measurements may associate with cognitive decline and indicate the development of AD.
7 Longitudinal Changes of CSF Biomarkers in Alzheimer’s Disease *

ABSTRACT

Longitudinal changes of Alzheimer’s disease CSF biomarkers have been studied but there are few consistent conclusions and even less is known about their variation during the different stages of the disease. We hypothesized that changes in CSF biomarker values would correlate with the progression of the cognitive decline in AD.

One hundred and thirty-one memory clinic patients (56 AD, 57 MCI, 10 other neurological disorders, eight unimpaired subjects) underwent a clinical follow-up with repeated MMSE tests and two lumbar punctures with a median interval of 3 years. Levels of CSF Aβ42, tau and p-tau-181 were measured using commercially available ELISA.

Twenty-one of the MCI subjects progressed to AD whereas 26 subjects remained stable and 56 subjects had AD already at the baseline. The subjects displaying the most rapid MMSE decline rate had the lowest baseline Aβ42, highest tau and highest p-tau-181 CSF concentrations. An annual decrease of 2.20 pg/ml/year in the CSF p-tau-181 concentration was seen in AD-AD patients (p=0.001). The difference was significant compared to stable MCI-MCI (increase of 1.24 pg/ml/year, p=0.001) and cognitively healthy (increase of 0.84 pg/ml/year, p=0.013) subjects (p for group difference 0.004). The decrease rate of p-tau-181 correlated with the MMSE decrease rate in AD subjects (r=0.579, p<0.001). The CSF Aβ42 level decreased in the AD-AD group (decrease 11.9 pg/ml/year, p<0.001).

Concentrations of hyperphosphorylated tau decline in the late stages of the AD process. The decrease of p-tau-181 appears to correlate with cognitive functioning and probably reflects neuronal loss. More longitudinal studies of CSF biomarker dynamics are needed, especially in patients during the preclinical stage of the disease.

7.1 INTRODUCTION

Alzheimer’s disease (AD) is the most common cause of dementia in the elderly. The pathological process in the brain begins decades before the appearance of any clinical symptoms of dementia; this is referred to as preclinical AD (Price & Morris 1999). The pathologic hallmarks of AD in the brain tissue are extracellular amyloid plaques consisting of beta-amyloid Aβ1-42 and Aβ1-40 as well as intracellular neurofibrillary tangles containing tau protein, especially its hyperphosphorylated form, p-tau (Glenner & Wong 1984, Grundke-Iqbal et al. 1986a, Grundke-Iqbal et al. 1986b) These proteins are also present in human cerebrospinal fluid (CSF) and pathological changes of their levels have been claimed to be predictive for detecting the conversion of mild cognitive impairment (MCI) into AD (Hampel et al. 2004, Herukka et al. 2005). Abnormal levels of CSF biomarkers have been included in the NINCDS-ADRDA (National Institute of Neurological Disorders and Stroke – Alzheimer’s Disease and Related Disorders Association) revised research criteria of AD as one of the supporting features of the disease (Dubois et al. 2007).

Most of the CSF biomarker studies have been conducted in cross-sectional settings or with one available CSF sample and a longitudinal follow-up of cognition. Over the last 15 years, there have been a few small studies reporting changes in biomarker levels with repeated measurements, mainly in AD patients (Sluimer et al. 2010, Andreasen et al. 1999a, Andreasen et al. 1999b, Sunderland et al. 1999, Tapiola et al. 2000, Mollenhauer et al. 2005, Hoglund et al. 2005, de Leon et al. 2006). More recently, some data have emerged from studies with MCI subjects and healthy elderly individuals with larger sample sizes (Bouwman et al. 2007b, Andersson et al. 2008, Brys et al. 2009, Stomrud et al. 2010, Beckett et al. 2010).

The results have been inconsistent in that most of the studies have described small or statistically insignificant longitudinal changes in the biomarkers. For example, the concentration of Aβ42 has been found to decrease in some studies (Tapiola et al. 2000, Mollenhauer et al. 2005, Beckett et al. 2010, Kanai et al. 1998, Wahlund & Blennow 2003) but to increase in one study (Bouwman et al. 2007b) while other studies did not detect statistically significant change during follow-up (Andreasen et al. 1999a, Hoglund et al. 2005, de Leon et al. 2006, Andersson et al. 2008, Brys et al. 2009, Stomrud et al. 2010, Andreasen et al. 1998). Six studies have reported an increase in total tau levels during follow-up (Bouwman et al. 2007b, Beckett et al. 2010, Wahlund, Blennow 2003, Blomberg et al. 1996, Isoe et al. 1996, Kanai et al. 1999) whereas there are almost as many which have found no significant change (Andreasen et al. 1999a, Sunderland et al. 1999, Tapiola et al. 2000, Mollenhauer et al. 2005, Hoglund et al. 2005, Andersson et al. 2008, Brys et al. 2009, Stomrud et al. 2010, Andreasen et al. 1998). Three studies described an increase in the levels of phosphorylated tau during follow-up (Hoglund et al. 2005, Andersson et al. 2008, Wahlund, Blennow 2003), one study reported a decrease (Mollenhauer et al. 2005) and four studies detected no change (Bouwman et al. 2007b, de Leon et al. 2006, Brys et al. 2009, Stomrud et al. 2010).

Furthermore, longitudinal CSF biomarker changes have not correlated with either the change in the performance in cognitive testing (Sluimer et al. 2010, Sunderland et al. 1999, Tapiola et al. 2000) or with MMSE results in AD (Andreasen et al. 1998) though they did seem to be linked to poorer performance in cognitive testing in healthy elderly (Stomrud et al. 2010).

The APOE genotype has been reported to influence the biomarker changes in nondemented (Stomrud et al. 2010) and AD patients (Andersson et al. 2008, Kanai et al. 1998, Blomberg et al. 1996). The strongest evidence of an APOE effect on biomarkers was shown in a recent study that found a gene dose effect for APOE e4 with greater reductions in CSF Aβ42 and greater increases in Aβ mean cortical binding potential (MCBP) (Morris et al. 2010). However, also negative results in this respect have been published (Sunderland et al. 2010).

We hypothesized that longitudinal changes might occur in CSF biomarker levels during the course of AD, also after the preclinical phase and furthermore that the changes in biomarker levels would correlate with the progression of cognitive decline. Therefore, we studied the changes in the levels of CSF Aβ42, tau and p-tau-181 in a total of 131 subjects including 57 MCI subjects, 56 AD patients, 10 subjects with other cognitive disorders and 8 controls over time and correlated these changes with the progression of the cognitive decline assessed in a memory clinic setting.

7.2 METHODS

7.2.1 Subjects
The subjects of the study were selected from the biobank of previous studies of MCI and AD performed in the memory clinic of the University of Eastern Finland, Kuopio, Finland. All subjects with at least two CSF samples from the previous projects were included in the present study regardless of their diagnoses.

A total of 131 subjects were followed up in the memory clinic. The subjects were referred to University of Eastern Finland Clinical Research Unit or Neurology Department of Kuopio University Hospital by general physicians or neurologists for a diagnostic evaluation or they were participants in population studies on memory disorders.

The group of 10 patients with other cognitive disorders was heterogeneous and included patients with amyotrophic lateral sclerosis (ALS) (one), spinocerebellar ataxia (SCA) (one), progressive supranuclear palsy (PSP) (one), Parkinson’s dementia (PD) (one), corticobasal degeneration (CBD) (one), dementia with Lewy bodies (DLB) (one), vascular dementia (VaD) (one) and frontotemporal dementia (FTD) (three).

At baseline, the subjects in the MCI group evaluated in this study showed cognitive impairment with a subjective memory complaint and an objective decline in at least one cognitive domain (>-1.5 SD) at the time of the first CSF sample collection. Thus, the group included both amnestic type of MCI and other types of mild cognitive impairment. The baseline AD group included all patients diagnosed as having AD at the collection time of the first CSF sample. The subjects were allocated into the study groups on the basis of the clinical diagnosis i.e. blinded to the CSF data.

Written informed consent was obtained from all subjects and the study was approved by the local ethical committee.

Follow-up results of 20 patients have been partly published by Tapiola et al. (Tapiola et al. 2000) and the CSF cross-sectional data with clinical follow-up of 17 patients by Herukka et al (Herukka et al. 2007).

7.2.2 Diagnosis of mild cognitive impairment, Alzheimer’s disease and other dementias
All subjects underwent a routine dementia screening including history, medical and neurological examination and imaging of the brain. The cognitive evaluation included clinical examination by a physician specializing in neurodegenerative disorders, blinded to the CSF results. The assessment of cognitive impairment was performed using a battery of neuropsychological tests which have been described in detail earlier (Herukka et al. 2007).

Mild cognitive impairment was diagnosed using the revised Petersen Criteria (Petersen et al. 2001). The diagnosis of probable AD was made according to the NINCDS-ADRDA criteria at baseline and follow-up (McKhan et al. 1984). A multidisciplinary team confirmed the clinical diagnoses.

The neuropathological confirmation of definite AD using the CERAD criteria was available for 15 patients. The median delay between last CSF sampling and death of the
definite AD subjects was 5.7 years (3.6). Four subjects were probable AD and two subjects had possible AD according to post-mortem analysis.

Two subjects with MCI converted to frontotemporal dementia and two subjects with MCI converted to multiple system atrophy (MSA) in the follow-up on the basis of clinical criteria.

All diagnoses were established independently of the CSF analysis.

Subjects underwent at least two MMSE tests during the follow-up. The follow-up change of MMSE was calculated using the first and latest available MMSE result. The median MMSE follow-up time was 3.08 years (range 0.67-10.57). The first MMSE was usually tested on the same day as the baseline lumbar puncture. If not available, an evaluation taken within one month before or after the tap was accepted.

7.2.3 CSF analysis
The lumbar puncture was performed in vertebrae LIII-IV or LIV-V interspace. No serious complications were encountered. The CSF samples, which were handled using a standard protocol in the clinic, were collected into polypropylene tubes and stored at −80 °C or lower until analysis.

The median follow-up time between sample collections was 2.98 years (range 0.48-8.15). Three or more CSF samples were available for 33 subjects.

The CSF levels of Aβ42, total tau, and P-tau-181 were measured by commercial ELISAs (Innotest beta-amyloid-1-42, Innotest hTau-Ag, Innotest Phosphotau(181P), Innogenetics, Ghent, Belgium) according to the manufacturer’s protocol. The ELISA analyses were done blinded to clinical data. All analyses were conducted in our own quality controlled and validated laboratory.

In order to examine the intra-assay variation, we produced reference standards by pooling CSF samples to acquire the concentration levels of upper and higher limits for each biomarker. The reference concentration levels were repeatedly tested in advance.

In order to avoid inter-assay variation and to minimize intra-assay variation, the baseline and follow-up samples of the same individual were always placed on the same plate of a particular ELISA assay in wells next to each other. The inter-assay coefficient of variation was 4.5 % ± 4.7 % for Aβ42, 5.6 % ± 3.7 % for tau and 1.8 % ± 1.7 % for p-tau-181. The intra-assay coefficient of variation was 7.3 % ± 9.2 % for Aβ42, 3.2 % ± 3.6 % for total tau and 2.2 % ± 3.2 % for p-tau-181.

7.2.4 APOE genotyping
The APOE genotype of 121 subjects was determined as described earlier (Tsukamoto et al. 1993).

7.2.5 Statistics
Since the values were not normally distributed, data is presented in the text and the tables as medians (interquartile range) unless stated otherwise. Statistical analyses were conducted with SPSS for Windows (release 14.0; SPSS, Chicago, IL) and for Mac (release 16.0). With respect to the changes in biomarkers and MMSE scores, random slope with time was assumed and the changes were annualized by dividing by the number of years in the time interval. Random slope was determined using MS Excel. We used Kolmogorov-Smirnov test for checking for normality, Kruskall-Wallis test for multiple comparisons, and Mann-Whitney U for pairwise comparisons. Wilcoxon signed rank test was used for within-group significances of the change between baseline and follow-up (non-normally distributed data). When assessing normally distributed data, we applied Levene’s test for the assessment of the equality of variances and independent sample’s T-test for group differences. Multiple group differences of continuous variables were tested using one-way analysis of variances (ANOVA) with Bonferroni post hoc correction. Correlations were
analyzed using the Spearman correlation coefficient. The level of statistical significance was set at p<0.05.

7.3 RESULTS

Table 6 presents the demographic data for the subjects and the baseline levels of CSF Aβ42, tau and p-tau-181. At baseline, the groups did not differ in terms of age or gender. The APOE ε4 allele was more frequently present among the AD and MCI patients compared to controls. The time interval between the first and second CSF collections did not differ significantly between cognitively intact, MCI and AD groups, but was shorter for subjects with other dementias. The follow-up period for cognitive performance was longer for the MCI group compared to the other groups, though the difference was not statistically significant. By definition, AD patients had lower scores in the MMSE assessment at the baseline compared to MCI subjects or controls.

**Table 6.** Demographic information and baseline biomarker concentrations.

<table>
<thead>
<tr>
<th></th>
<th>All</th>
<th>CH</th>
<th>OCD</th>
<th>MCI</th>
<th>AD</th>
<th>p</th>
</tr>
</thead>
<tbody>
<tr>
<td>N</td>
<td>131</td>
<td>8</td>
<td>10</td>
<td>57</td>
<td>56</td>
<td>n.s.</td>
</tr>
<tr>
<td>Men/Women, No.</td>
<td>55 / 76</td>
<td>2 / 6</td>
<td>5 / 5</td>
<td>25 / 32</td>
<td>23 / 33</td>
<td>n.s.</td>
</tr>
<tr>
<td>Age, Years †</td>
<td>70 (7)</td>
<td>70 (4)</td>
<td>66 (6)</td>
<td>71 (6)</td>
<td>70 (7)</td>
<td>n.s.</td>
</tr>
<tr>
<td>APOE ε4 heterozygote (homozygote), No. *</td>
<td>43 (24)</td>
<td>3 (0)</td>
<td>5 (0)</td>
<td>14 (5)</td>
<td>21 (19)</td>
<td>0.005</td>
</tr>
<tr>
<td>MMSE score *</td>
<td>23 (6)</td>
<td>28 (3)</td>
<td>22 (7)</td>
<td>26 (4)</td>
<td>20 (5)</td>
<td>&lt;0.001</td>
</tr>
<tr>
<td>Time between CSF collection, Months</td>
<td>36 (12)</td>
<td>35 (13)</td>
<td>30 (27)</td>
<td>35 (23)</td>
<td>37 (0,9)</td>
<td>0.002</td>
</tr>
<tr>
<td>Follow-up time of cognition, Months</td>
<td>37 (18)</td>
<td>34 (14)</td>
<td>35 (15)</td>
<td>48 (25)</td>
<td>37 (13)</td>
<td>n.s.</td>
</tr>
<tr>
<td>CSF Aβ42, pg/ml</td>
<td>439 (296)</td>
<td>560 (274)</td>
<td>507 (334)</td>
<td>578 (377)</td>
<td>379 (144)</td>
<td>&lt;0.001</td>
</tr>
<tr>
<td>CSF total tau, pg/ml</td>
<td>373 (342)</td>
<td>306 (175)</td>
<td>214 (225)</td>
<td>283 (263)</td>
<td>480 (475)</td>
<td>&lt;0.001</td>
</tr>
<tr>
<td>CSF p-tau-181, pg/ml</td>
<td>72 (48)</td>
<td>58 (37)</td>
<td>41 (35)</td>
<td>59 (31)</td>
<td>90 (53)</td>
<td>&lt;0.001</td>
</tr>
<tr>
<td>CSF Aβ42/ptau ratio</td>
<td>6.10 (9.59)</td>
<td>8.80 (8.41)</td>
<td>14.0 (7.10)</td>
<td>10.67 (10.15)</td>
<td>3.96 (2.40)</td>
<td>&lt;0.001</td>
</tr>
<tr>
<td>CSF Aβ42/tau ratio</td>
<td>1.13 (2.12)</td>
<td>1.76 (1.54)</td>
<td>3.13 (2.40)</td>
<td>2.10 (2.70)</td>
<td>0.663 (0.647)</td>
<td>&lt;0.001</td>
</tr>
</tbody>
</table>

CH = cognitively healthy, OCD = other cognitive disorder.
Significances are determined using Kruskall-Wallis test. Chi-square test was used for APOE and gender.
The results are presented as median (IQR range). Age is presented as mean (SD).
†Anova was used due to normal distribution of the variable.
* Missing one AD and four OCD subjects.
APOE status is missing for nine MCI and one AD subjects.

7.3.1 Cross-sectional data at the baseline

There was a strong positive correlation between total tau and p-tau-181 baseline levels (r=0.927, p<0.001) and a moderate negative correlation between baseline Aβ42 and tau/p-tau-181 levels (for all: r=-0.306 and r=-0.363, respectively; p<0.001; for CH r=0.095, p=0.823 and r=0.071, p=0.867; for MCI r=-0.399, p<0.001 and r=-0.195, p=0.145; for AD r=-0.44, p=0.746 and r=0.019, p=0.892; for OCD r=0.491, p=0.150 and r=0.552, p=0.098). CSF Aβ42, tau or p-tau-181 levels did not correlate with age or gender in the whole cohort or any of the subgroups.

Those subjects who had received an AD diagnosis at the baseline had significantly lower levels of Aβ42 and higher levels of tau and p-tau-181 at the baseline than those in the...
baseline MCI or control groups. Accordingly, the Aβ42/tau and Aβ42/p-tau-181 ratios were also lower. The difference of Aβ42 and tau levels between MCI subjects and cognitively healthy did not reach significance at the baseline as is seen in the median values in Table 6.

At baseline, all the three measured CSF biomarkers differed significantly between APOE ε4 positive and negative subjects in the entire cohort (n=131) as well as in the baseline MCI and AD subgroups separately. The levels of Aβ42 were markedly lower and total tau and p-tau-181 concentrations were higher in the APOE ε4 allele carriers. Subjects with 4:4, 3:4 and 2:4 genotypes had decreased Aβ42 and increased tau and p-tau-181 levels compared to those with genotypes 3:3 and 2:3, but in the baseline groups of MCI and AD, the difference between genotypes remained non-significant. However, there was a trend towards lower Aβ42 and higher tau and p-tau-181 for genotypes 4:4, 34 and 2:4.

The groups of cognitively healthy and patients with other cognitive disorders were too small to permit any statistically reliable analysis at the baseline. As the group of other cognitive disorders remained small and also heterogeneous and thus uninformative, we have focused on the results of clinically relevant cognitive subgroups. (Table 7)

7.3.2 Longitudinal data
During the three-year follow-up, a total of 21 subjects progressed to AD, i.e. 20 subjects with MCI and one subject who had normal cognition at baseline. Twenty-six subjects remained in stable MCI, and 56 subjects had AD already at the baseline. Four subjects progressed from MCI to other dementias (one to dementia of Parkinson’s disease, two to frontotemporal dementia and one to dementia with Lewy bodies). Seven subjects with MCI displayed an improvement in their cognitive performance and were considered as being cognitively normal at the follow-up.

Baseline CSF biomarkers and cognitive change
The diagnostic groups at the follow-up with the corresponding biomarker values are presented in Table 8. The baseline levels of all measured biomarkers differed significantly between the diagnostic follow-up groups (p<0.001 for all markers).

The baseline Aβ42 was lowest in those subjects who had AD already at the baseline (AD-AD; n=56). MCI-AD converters (n=21) had significantly lower levels of Aβ42 at the baseline than those who remained stable with MCI (MCI-MCI; n=26) or cognitively healthy subjects (CH; n=14) after the follow-up (p=0.003 against CH and p=0.01 against MCI-MCI). There was no difference between the stable MCI group and the cognitively healthy subjects.

Those who converted to AD had higher baseline total tau concentrations than those who remained stable in the MCI or the control group. The MCI–AD converters had significantly higher total tau levels at the baseline than the stable MCI and cognitively healthy.

The p-tau-181 level at the baseline was highest in the AD–AD group. The difference of the baseline p-tau-181 levels between MCI–AD converters and stable MCI group did not quite reach significance (p=0.066).

The baseline Aβ42/tau and Aβ42/p-tau-181 ratio were lowest in the AD–AD group. The MCI–AD converters had significantly lower baseline ratios of Aβ42/tau and Aβ42/p-tau-181 than the stable MCI group (p=0.001 and p=0.005, respectively).
Table 7. The effect of APOE genotype and carrier status on biomarker levels at the baseline.

<table>
<thead>
<tr>
<th>APOE genotype</th>
<th>All subjects</th>
<th>MCI</th>
<th>AD</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>n</td>
<td>AB42</td>
<td>Tau</td>
</tr>
<tr>
<td>4:4</td>
<td>24</td>
<td>360 (105)</td>
<td>608 (550)</td>
</tr>
<tr>
<td>3:4</td>
<td>42</td>
<td>419 (155)</td>
<td>452 (230)</td>
</tr>
<tr>
<td>2:4</td>
<td>1</td>
<td>409</td>
<td>684</td>
</tr>
<tr>
<td>3:3</td>
<td>49</td>
<td>546 (379)</td>
<td>253 (249)</td>
</tr>
<tr>
<td>2:3</td>
<td>5</td>
<td>729 (224)</td>
<td>252 (374)</td>
</tr>
<tr>
<td>p-value</td>
<td>&lt; 0.001</td>
<td>&lt; 0.001</td>
<td>&lt; 0.001</td>
</tr>
<tr>
<td>ε4 +</td>
<td>67</td>
<td>391 (147)</td>
<td>480 (376)</td>
</tr>
<tr>
<td>ε4 -</td>
<td>54</td>
<td>613 (355)</td>
<td>252 (232)</td>
</tr>
<tr>
<td>p-value</td>
<td>&lt; 0.001</td>
<td>&lt; 0.001</td>
<td>&lt; 0.001</td>
</tr>
</tbody>
</table>

The effect of APOE genotype and carrier status on biomarker levels at the baseline. The values are given as median (IQ-range), unit pg/ml. The significance was tested by the Kruskall-Wallis (genotypes) and Mann-Whitney tests (carrier vs. non-carrier). N = 121. APOE data is missing for 10 subjects (nine MCI and one AD at baseline).
All the measured baseline biomarkers differed significantly between groups formed by dividing the cohort into quintiles of the MMSE score annual change. The lowest quintile (i.e., those individuals with the most rapid MMSE score decline) had the highest baseline total tau (535 pg/ml) and the lowest Aβ42 (393 pg/ml) concentrations in their CSF. The lowest two quintiles had the highest CSF p-tau-181 concentrations (93 and 95 pg/ml). The corresponding levels of the highest quintile of MMSE score changing rate were 654 pg/ml for Aβ42, 246 pg/ml for total tau and 54 pg/ml for p-tau-181. The lowest quintile had significantly lower Aβ42/tau and Aβ42/p-tau-181 ratios compared to the highest quintile. The lower the quintile of MMSE decline rate, the lower were biomarker ratios measured. This was also seen separately in the AD group (n=56). The lowest quintile concentrations were 393 pg/ml for Aβ42, 764 pg/ml for tau and 113 pg/ml for p-tau. The highest quintile levels were 385 pg/ml for Aβ42, 325 pg/ml for tau and 73 pg/ml for p-tau. (Figure 5)

![Graph showing MMSE score changing rate in 3-year follow-up (quintiles)](image)

**Figure 5.** Baseline biomarker ratios subdivided into MMSE changing rate quintiles.
1. quintile = fastest decrease; 5. quintile = slowest decrease
A) N=131, p < 0.001 (Aβ42/ptau) and p < 0.001 (Aβ42/tau). Kruskall-Wallis test.
B) N=56, p = 0.049 (Aβ42/ptau) and p = 0.036 (Aβ42/tau), lowest against highest quintile.

**Longitudinal CSF biomarkers and cognitive change**
The measured biomarker levels at follow-up and their average annual change are presented numerically in Table 8. The CSF biomarker levels at follow-up correlated strongly with the levels at baseline. Correlation coefficient between baseline and follow-up levels was r=0.911 for Aβ42, r=0.914 for tau and r=0.937 for p-tau-181 (p<0.001 for all).

The Aβ42 level decreased significantly in the AD-AD group (p<0.001). The changes of Aβ42 between baseline and follow-up were not significant within the groups of cognitively healthy, stable MCI or MCI-AD converters. The significances of the changes within the follow-up cognition groups are also presented in Table 8.
Table 8. Cognitive groups and their CSF biomarker levels at the 3-year follow-up.

<table>
<thead>
<tr>
<th>N</th>
<th>CH</th>
<th>MCI - MCI (stable)</th>
<th>MCI - AD (conv.)</th>
<th>AD - AD (AD)</th>
<th>OCD</th>
<th>(definite AD)</th>
<th>group diff. p</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>14</td>
<td>26</td>
<td>21</td>
<td>56</td>
<td>14</td>
<td>(15)</td>
<td></td>
</tr>
<tr>
<td>Follow-up time (CSF)</td>
<td>2.98 (0.61)</td>
<td>2.98 (1.99)</td>
<td>2.03 (1.87)</td>
<td>3.00 (0.08)</td>
<td>2.63 (1.96)</td>
<td>2.99 (0.80)</td>
<td>0.007</td>
</tr>
<tr>
<td>Follow-up time (MMSE)</td>
<td>3.17 (1.75)</td>
<td>4.00 (2.64)</td>
<td>3.17 (2.53)</td>
<td>3.08 (1.08)</td>
<td>2.87 (1.85)</td>
<td>3.08 (0.42)</td>
<td>n.s.</td>
</tr>
<tr>
<td>MMSE at baseline</td>
<td>26 (5)</td>
<td>26 (3)</td>
<td>26 (6)</td>
<td>20 (5)</td>
<td>22 (7)</td>
<td>20 (7)</td>
<td>&lt;0.001</td>
</tr>
<tr>
<td>MMSE change between samples</td>
<td>1.14 ±2.31</td>
<td>-0.04 ±2.76</td>
<td>-3.68 ±3.79</td>
<td>-9.73 ±6.12</td>
<td>-1.50 ±5.67</td>
<td>-12.7 ±5.33</td>
<td>&lt;0.001</td>
</tr>
<tr>
<td>MMSE annual change p/year</td>
<td>0.083 (1.27)</td>
<td>-0.083 (1.24)</td>
<td>-1.187 (2.96)</td>
<td>-3.00 (2,11)</td>
<td>-0.500 (1.76)</td>
<td>-3.88 (2.23)</td>
<td>&lt;0.001</td>
</tr>
<tr>
<td>Aβ42 at baseline</td>
<td>709 (455)</td>
<td>674 (439)</td>
<td>464 (167)</td>
<td>379 (144)</td>
<td>507 (306)</td>
<td>391 (112)</td>
<td>&lt;0.001</td>
</tr>
<tr>
<td>Aβ42 at follow-up</td>
<td>677 (456)</td>
<td>673 (446)</td>
<td>431 (175)</td>
<td>327 (142)</td>
<td>566 (170)</td>
<td>300 (151)</td>
<td>&lt;0.001</td>
</tr>
<tr>
<td>Aβ42 annual change</td>
<td>-20.6 (28.5)</td>
<td>3.42 (45.3)</td>
<td>0.781 (33.7)</td>
<td>-11.9 (28.0)</td>
<td>18.6 (97.3)</td>
<td>-20.0 (37.0)</td>
<td>0.032</td>
</tr>
<tr>
<td>tau at baseline</td>
<td>297 (163)</td>
<td>231 (96)</td>
<td>512 (458)</td>
<td>480 (475)</td>
<td>229 (200)</td>
<td>613 (626)</td>
<td>&lt;0.001</td>
</tr>
<tr>
<td>tau at follow-up</td>
<td>324 (205)</td>
<td>258 (217)</td>
<td>543 (533)</td>
<td>481 (324)</td>
<td>217 (197)</td>
<td>583 (319)</td>
<td>&lt;0.001</td>
</tr>
<tr>
<td>tau annual change</td>
<td>5.66 (18.9)</td>
<td>19.7 (32.0)</td>
<td>17.4 (42.4)</td>
<td>0.55 (55.0)</td>
<td>8.69 (19.8)</td>
<td>-30.4 (115.1)</td>
<td>0.047</td>
</tr>
<tr>
<td>p-tau at baseline</td>
<td>57 (28)</td>
<td>55 (26)</td>
<td>76 (65)</td>
<td>90 (53)</td>
<td>40 (32)</td>
<td>110 (68)</td>
<td>&lt;0.001</td>
</tr>
<tr>
<td>p-tau at follow-up</td>
<td>58 (33)</td>
<td>56 (29)</td>
<td>71 (66)</td>
<td>84 (44)</td>
<td>38 (38)</td>
<td>76 (43)</td>
<td>&lt;0.001</td>
</tr>
<tr>
<td>p-tau annual change</td>
<td>0.844 (1.94)</td>
<td>1.24 (3.42)</td>
<td>-0.21 (4.50)</td>
<td>-2.20 (0.804)</td>
<td>0.40 (3.32)</td>
<td>-6.86 (11.1)</td>
<td>&lt;0.001</td>
</tr>
<tr>
<td>Aβ42/tau at baseline</td>
<td>2.71 (2.74)</td>
<td>2.91 (1.95)</td>
<td>1.00 (1.51)</td>
<td>0.66 (0.65)</td>
<td>2.50 (2.40)</td>
<td>0.60 (0.78)</td>
<td>&lt;0.001</td>
</tr>
<tr>
<td>Aβ42/tau at follow-up</td>
<td>2.45 (2.76)</td>
<td>2.80 (2.76)</td>
<td>0.65 (1.09)</td>
<td>0.70 (0.66)</td>
<td>2.35 (2.60)</td>
<td>0.69 (0.50)</td>
<td>&lt;0.001</td>
</tr>
<tr>
<td>Aβ42/tau annual change</td>
<td>-0.0698 (0.190)</td>
<td>-0.540 (0.283)</td>
<td>-0.419 (0.102)</td>
<td>-0.0085 (0.084)</td>
<td>-0.432 (0.334)</td>
<td>0.0098 (0.104)</td>
<td>0.09</td>
</tr>
<tr>
<td>Aβ42/p-tau at baseline</td>
<td>14.7 (9.45)</td>
<td>12.8 (9.23)</td>
<td>5.27 (6.41)</td>
<td>3.96 (2.40)</td>
<td>13.1 (7.1)</td>
<td>3.57 (2.30)</td>
<td>&lt;0.001</td>
</tr>
<tr>
<td>Aβ42/p-tau at follow-up</td>
<td>14.2 (10.9)</td>
<td>13.1 (9.60)</td>
<td>5.03 (6.40)</td>
<td>4.09 (2.74)</td>
<td>14.5 (8.71)</td>
<td>3.62 (2.59)</td>
<td>&lt;0.001</td>
</tr>
<tr>
<td>Aβ42/p-tau annual change</td>
<td>-0.284 (0.493)</td>
<td>-0.191 (0.874)</td>
<td>-0.069 (0.516)</td>
<td>0.180 (0.381)</td>
<td>0.384 (2.13)</td>
<td>0.020 (0.343)</td>
<td>n.s.</td>
</tr>
</tbody>
</table>
(Table 8) The values are given as median (IQ range). The biomarker concentration unit is pg/ml. The group difference significance is obtained with Kruskall-Wallis test. N.s. = not significant. Within group significance between baseline and follow-up: ** < 0.02 level; *** < 0.01 level; † < 0.001 level. Wilcoxon signed rank test. The definite AD group: autopsy confirmed definite AD cases of subgroups MCI-AD and AD-AD.

There was a significant group difference in the annual change of the total tau level (p=0.047) despite the fact that the level increased in every group. In the AD-AD group, there was only a minor median increase, i.e. the increase was not significant within this group. The increment was significantly higher in the stable MCI-MCI group compared to AD-AD group. However, there was no change between AD-AD and the controls or between the MCI-AD converters and the stable MCI.

There was a significant difference in the annual change of p-tau-181 between the groups (p<0.001). The p-tau-181 decline was fastest in the AD-AD group (p=0.001) whereas the level increased in the stable MCI-MCI group (p=0.001) as well as in the cognitively healthy (p=0.013).

When studying separately the 15 autopsy-confirmed AD subjects, the median Aβ42 decreased at the rate of 19.9 (37) pg/ml/year from baseline 391 (112) pg/ml to 300 (151) pg/ml (p=0.001). Total tau decreased at the rate of 30.3 (115) pg/ml/year from baseline 613 (626) pg/ml to 583 (319) pg/ml (p=0.100), but the change was not statistically significant. P-tau-181 decreased at the rate 6.86 (11.1) pg/ml/year from baseline 110 (68) pg/ml to 76 (43) pg/ml (p=0.002).

There was a correlation between the annual change in p-tau-181 and the corresponding change in MMSE (r=0.389, p<0.001) in the entire cohort, but when divided into subgroups according to the diagnoses, only the AD-AD group showed a significant correlation in this respect (r=0.579, p<0.001). The correlation coefficient for the definite AD subjects (n=15) was r=0.617, p=0.014. (Figure 6)

**APOE genotype and longitudinal CSF biomarker changes**

The median Aβ42 level of APOE ε4 carriers decreased by 0.34 pg/ml/year (6.24) (p=0.05). The APOE genotype 4:4 displayed a significant decrease in the Aβ42 level during follow-up. The Aβ42 of genotype 3:4 also decreased, but the change did not reach significance (p=0.073). With respect to genotypes 3:3 and 2:3, there was no significant change. (Table 9)

The total tau concentration increased more in the APOE ε4 negative (9.5 pg/ml/year, IQ range 20,9) than in APOE ε4 positive subjects (3.2 pg/ml/year, IQ range 55.5, p=0.046 for difference). There was a significant increase of 9.32 pg/ml/year in genotype 3:3 (p=0.002) and a minor increase in genotype 23 (p=0.043) in total tau levels during the follow-up, but no significant change in genotypes 4:4 and 3:4.

The longitudinal decline of p-tau-181 was greatest in genotype 4:4 (1.84 pg/ml/year, p=0.002). The changes of p-tau-181 in genotype 3:4, 3:3 and 2:3 did not reach statistical significance and there was only one subject with genotype 2:4 (-11.9 pg/ml/year).
Figure 6. Correlations between p-tau-181 changing rate and MMSE score changing rate. Correlations without the one lowest outlier:
A) n=130, r=0.376, p<0.001  B) n=55, r=0.562, p<0.001  C) n=14, r=0.552, p=0.041
Table 9. CSF biomarker levels and follow-up diagnoses by APOE genotype.

<table>
<thead>
<tr>
<th></th>
<th></th>
<th></th>
<th></th>
<th></th>
<th></th>
<th></th>
<th></th>
<th></th>
<th></th>
<th></th>
</tr>
</thead>
<tbody>
<tr>
<td>N</td>
<td>24</td>
<td>42</td>
<td>49</td>
<td>1</td>
<td>5</td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>CH at follow-up (n)</td>
<td>-</td>
<td>5</td>
<td>9</td>
<td>-</td>
<td>-</td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>OCD at follow-up (n)</td>
<td>-</td>
<td>5</td>
<td>8</td>
<td>-</td>
<td>1</td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>MCI-MCI (n)</td>
<td>-</td>
<td>6</td>
<td>14</td>
<td>-</td>
<td>3</td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>MCI-AD (n)</td>
<td>5</td>
<td>6</td>
<td>3</td>
<td>-</td>
<td>1</td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>AD-AD (n)</td>
<td>19</td>
<td>20</td>
<td>15</td>
<td>1</td>
<td>-</td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Aβ42 at baseline</td>
<td>360 (105)</td>
<td>0.028</td>
<td>419 (155)</td>
<td>0.073</td>
<td>546 (379)</td>
<td>n.s.</td>
<td>409</td>
<td>729 (224)</td>
<td>n.s.</td>
<td>&lt; 0.001</td>
</tr>
<tr>
<td>Aβ42 at follow-up</td>
<td>321 (140)</td>
<td>0.002</td>
<td>395 (184)</td>
<td>0.073</td>
<td>576 (395)</td>
<td>n.s.</td>
<td>275</td>
<td>775 (422)</td>
<td>n.s.</td>
<td>&lt; 0.001</td>
</tr>
<tr>
<td>Aβ42 annual change</td>
<td>-7.54 (24)</td>
<td>0.002</td>
<td>-7.44 (30)</td>
<td>-1.04 (43)</td>
<td>-40</td>
<td>n.s.</td>
<td>1.55 (56)</td>
<td>n.s.</td>
<td></td>
<td></td>
</tr>
<tr>
<td>tau at baseline</td>
<td>608 (550)</td>
<td>n.s.</td>
<td>452 (230)</td>
<td>n.s.</td>
<td>253 (249)</td>
<td>0.002</td>
<td>684</td>
<td>252 (374)</td>
<td>0.043</td>
<td>&lt; 0.001</td>
</tr>
<tr>
<td>tau at follow-up</td>
<td>597 (322)</td>
<td>n.s.</td>
<td>457 (288)</td>
<td>n.s.</td>
<td>258 (244)</td>
<td>0.002</td>
<td>678</td>
<td>307 (842)</td>
<td>0.001</td>
<td></td>
</tr>
<tr>
<td>tau annual change</td>
<td>-5.28 (92)</td>
<td>0.002</td>
<td>8.08 (55)</td>
<td>9.32 (24)</td>
<td>-1.9</td>
<td>n.s.</td>
<td>1.21 (157)</td>
<td>n.s.</td>
<td></td>
<td></td>
</tr>
<tr>
<td>p-tau at baseline</td>
<td>112 (69)</td>
<td>0.002</td>
<td>76 (34)</td>
<td>n.s.</td>
<td>54 (37)</td>
<td>n.s.</td>
<td>116</td>
<td>59 (59)</td>
<td>0.08</td>
<td>&lt; 0.001</td>
</tr>
<tr>
<td>p-tau at follow-up</td>
<td>88 (43)</td>
<td>0.002</td>
<td>73 (29)</td>
<td>n.s.</td>
<td>55 (38)</td>
<td>n.s.</td>
<td>76</td>
<td>62 (78)</td>
<td>0.001</td>
<td></td>
</tr>
<tr>
<td>p-tau annual change</td>
<td>-1.84 (9.92)</td>
<td>0.003</td>
<td>0.46 (5.26)</td>
<td>0.53 (3.23)</td>
<td>-11.9</td>
<td>n.s.</td>
<td>0.97 (7.73)</td>
<td>0.003</td>
<td></td>
<td></td>
</tr>
</tbody>
</table>

* Wilcoxon signed rank test for baseline vs. follow-up results within group.

** Kruskall-Wallis test

N=121 (Eight cognitively healthy, 10 subjects with other cognitive disorder, 48 MCI and 55 AD at baseline). APOE data is missing for 10 subjects (nine MCI and one AD at baseline).
7.4 DISCUSSION

Based on earlier studies, we hypothesized there would be an association between the change of CSF biomarkers levels and the cognitive decline over time. Therefore we examined the relationship between the CSF biomarker levels and cognitive decline in both cross-sectional and longitudinal settings.

The medians of the biomarker levels differed between the cognitive follow-up groups in most of the cross-sectional measurements. As in many previous studies, the results of this study revealed that subjects with advanced dementia of Alzheimer type had lower levels of Ab42 and higher concentrations of tau and p-tau than subjects with stable mild cognitive impairment or patients in the earlier phase of AD i.e. MCI patients who later converted to AD.

We found that the CSF Aβ42 levels decreased during the follow-up in AD patients whereas on the contrary, the levels slightly increased in stable MCI. The largest earlier study of 105 patients reported that the levels of Aβ42 appeared to increase over time (Bouwman et al. 2007b). On the other hand, most of the prior studies that have examined changes in Ab levels have reported a decrease of Aβ42 during their follow-up periods (Tapiola et al. 2000, Mollenhauer et al. 2005, Beckett et al. 2010, Kanai et al. 1998, Wahlund, Blennow 2003). The contradictory results may be attributable to differences in patient selection. It has been proposed earlier that CSF Aβ42 levels decline already during the preclinical stage of AD as a consequence of the accumulation of amyloid in plaques. Thereafter the levels remain relatively stable until there is a further decrease during the late phase of the disease due to extensive neuronal loss and therefore decreased production of Aβ. Our results support this hypothesis as the non-significant decrease in the levels of CSF Aβ42 in cognitively healthy group may have been attributable to some subjects suffering a preclinical stage of AD. Furthermore, in the present study, there was a large number of late-phase AD patients and the decrease of Aβ42 was most rapid in those subjects having AD already at the baseline.

We observed a decrease of p-tau levels in AD patients and an increase in stable MCI patients. Most of the earlier studies have not detected evidence of any changes in the levels of p-tau during follow-up. Two studies reported an increase in p-tau concentrations (Andersson et al. 2008, Wahlund & Blennow 2003) whereas one study observed a decline (Mollenhauer et al. 2005).

The annual rate of change of Aβ42 or total tau could not clearly discriminate stable MCI from MCI–AD converters. The group of AD patients already diagnosed at the baseline differed from those with stable MCI in terms of their annual change of every CSF biomarker. The converter group showed a biomarker profile closer to stable MCI than AD, which may indicate that there were still progressive subjects in the stable MCI group that had not yet converted. The wide difference between AD–AD and converter groups probably results from the stage of the disease. When we studied the 15 autopsy-confirmed definite AD subjects separately, the changes of the CSF biomarkers were more prominent. Aβ42 and p-tau decreased even more than in the clinically diagnosed AD–AD group.

The APOE ε4 allele appeared to influence the baseline biomarker levels. I.e. APOE ε4 carriers had lower Aβ42 and higher tau and p-tau levels. There seemed to be a dose dependent effect of the APOE ε4 allele on CSF Aβ42. A significant difference of CSF Aβ42 levels between specific APOE genotypes was seen in the entire cohort (n=131). The difference did not reach significance in the baseline cognition groups. The finding of a lower Aβ42 concentration in connection with APOE ε4 is in accordance with previous studies (Galasko et al. 1998, Prince et al. 2004). It seems that APOE ε4 leads to increasing cerebral accumulation of Aβ as a phenotype of the allele even in symptomless aging (Morris et al. 2010). As noted in an earlier study, there is little to be gained from using CSF
Aβ42 as a surrogate of amyloid load since it is more closely linked to the presence of APOE ε4 than to clinical cognitive status (Vemuri et al. 2010a).

However, very few longitudinal studies have examined the association between APOE ε4 and CSF biomarker levels. A recent study reported that the presence of the APOE ε4 allele resulted in a temporal decrease in Aβ42 (Stomrud et al. 2010). Our study also detected a more rapid decline of CSF Aβ42 levels for subjects with the APOE genotype 4:4 and there was a similar trend for subjects with genotype 3:4.

Even though APOE genotype is not known to affect the tau pathology, we found that the levels of total and hyperphosphorylated tau increased more in the APOE ε4 negative individuals than in the APOE ε4 positive subjects. Instead, earlier studies had claimed that there would be a trend towards increasing levels of tau and p-tau when APOE ε4 was present (Andersson et al. 2008, Blomberg et al. 1996, Kanai et al. 1999).

These contradictory results might be due to our study population, as the p-tau levels decreased in the AD group and this has most of the APOE ε4 positive subjects. When the CSF Aβ42, tau and p-tau changes were analyzed for each genotype, APOE 4:4 exhibited with the most rapid and statistically significant decline in the levels of p-tau and Aβ42 as well as the greatest relative proportion of AD cases.

A recent study indicated that pathological baseline CSF levels predicted higher mortality and a faster cognitive decline in AD (Wallin et al. 2010, Snider et al. 2009). We analyzed whether there was an association between the rate of cognitive decline and the baseline CSF biomarkers. In our cohort, the subjects with the lowest levels of Aβ42 and the highest levels of tau and p-tau at the baseline exhibited the most rapid decline in the MMSE scores during the follow-up. Interestingly, the same difference in biomarker ratios between fastest and slowest cognitive decliners was seen also within the AD–AD group. However, the difference between the ratios within the AD–AD group was mainly due to the decrease of tau and p-tau rather than to any decline of Aβ42. It seems that cross-sectional levels of the CSF biomarkers may display an association with the further decrease rate of cognitive abilities. As Aβ42 probably does not seem to be linked to cognition, caution is warranted when interpreting the biomarker ratios. (Figure 5)

The longitudinal changes in the CSF biomarkers did not correlate with cognitive functioning in one study (Vemuri et al. 2010b) but an increase of 20 % in p-tau-181 correlated with slower results of cognitive speed (quick test) in a retrospective study (Stomrud et al. 2010). We found a correlation between the annual change in the p-tau level and the MMSE change per year. In contrast to previous studies (Stomrud et al. 2010, Andreasen et al. 1998), the faster MMSE decline was associated with a decrease of p-tau-181 and not with any increase. This correlation was seen only in the AD–AD group when the groups were analyzed separately, but the definite AD group (n=15) did show a similar correlation.

If p-tau-181 were to decrease earlier in the disease process, then an inverse correlation with performance on cognitive tests should have been seen in cross-sectional studies. However, it is possible that the p-tau decrease remains at such a limited scale or occurs at such late stage of the disease that it can only be detected in longitudinal setting.

Different subgroups of AD with characteristic biomarker profiles have been proposed earlier (Iqbal et al. 2005). Iqbal et al. define one the subgroups of AD as ATEO, which consists of subjects with low Aβ42, increased tau (+1.5 SD) and a relatively early onset of dementia. It is possible that the proportion of different subtypes (and biomarker profiles) varies between the study populations. The AD-AD group of this study resembles the defined ATEO subgroup and also had the same proportion of APOE ε4 carriers.

One limitation of the study is the measurement of cognitive decline by MMSE. In the very late stage of the disease, MMSE no longer functions as a continuing variable as the result declines to zero and shows a floor effect in the non-co-operative subjects. We also had nine subjects with progressive and stable MCI and one subject with AD for whom there was lacking APOE genotype information. Due to the small number of APOE ε2
carriers, we could not properly examine the possible effect of the ε2 allele on the Aβ42 levels.

In a cross-sectional study of 241 healthy volunteers, 50% of the 80-88 year-old subjects had reduced levels of CSF Aβ42 and 30% had increased MCBP for PIB. (Morris et al. 2010) The CSF Aβ42 abnormalities appear to begin even earlier than reported with PIB-PET and the severity of amyloid load does not alone predict the risk of progression (Jack et al. 2010). A recently published hypothetical model of dynamic biomarkers of AD proposed that the cognitive symptoms were directly related to biomarkers of neurodegeneration rather than to biomarkers of Aβ42 deposition (Jack et al. 2010). The same hypothesis also emphasized the temporal order of the biomarker abnormalities starting from symptomless amyloid deposition continuing with the initial tau increase followed by the plateau which occurred in parallel with cognitive changes and anatomical atrophy (Jack et al. 2010).

Most of the present longitudinal findings of amyloid and tau seem to support this hypothesis. If the follow-up groups are simplified to represent the continuum of the disease process (healthy – MCI – converters – AD), then the annual changing rates support the concept that there is a temporal order in the biomarker changes: Aβ42 decreases in non-symptomatic healthy individuals, remains virtually unchanged in stable MCI and MCI-AD converters and decreases again in the late AD process. Similarly, tau changes only slightly in healthy individuals, increases in stable MCI and MCI-AD converters and finally remains rather stable during the late stage of AD. If the increase of CSF tau and p-tau were to begin in the MCI phase and reach a maximum, it would be predicted to increase less rapidly or even to start to decline in a later stage, e.g. late AD.

In the present study, the most rapid cognitive decline in AD patients occurred in relation to the decrease in the level of p-tau. We interpret this finding to mean that decreasing levels of tau or p-tau in the follow-up are related to the extensive neuronal loss in end-stage AD i.e. these are markers of neuronal injury. When a certain critical point is passed, the number of neurons that still remain start to decline and this could be monitored via the decrease in levels of p-tau over time. The clinically most relevant timing concerning the AD biomarker dynamics is the preclinical phase and undoubtedly more studies will be needed in order to test this hypothesis.
8 CSF biomarkers for Alzheimer’s disease correlate with cortical brain biopsy findings *

ABSTRACT

Objective: To assess the relationship between AD-related pathological changes in frontal cortical brain biopsy and AD-biomarkers in ventricular vs. lumbar CSF, and to evaluate the relationships of AD-biomarkers in CSF and cortical biopsy with the final clinical diagnosis of AD.

Methods: In 182 patients with presumed normal pressure hydrocephalus (152 with known APOE carrier status), Aβ plaques and tau in the cortical brain biopsies were correlated with the ventricular and lumbar CSF Aβ42, total tau and p-tau levels measured by ELISA. In a median follow-up of 2.0 years, 51 patients developed AD dementia.

Results: The patients with Aβ plaques in the cortical biopsy had lower (p=0.009) CSF Aβ42 levels than those with no Aβ plaques. The patients with tau in the cortical biopsy had lower (p=0.014) Aβ42 but higher (p=0.015) p-tau-181 in CSF as compared to those with no tau in the cortical biopsy. The patients with amyloid+ tau+ biopsies had the lowest Aβ42 and highest tau and p-tau-181 levels in CSF. The Aβ42 levels were lower and the tau and p-tau-181 higher in the ventricular vs. corresponding lumbar CSF samples. In multivariate analysis, the presence of cortical Aβ was independently predicted by the APOE ε4 carrier status and age but not by CSF Aβ42 or tau levels.

Conclusions: Amyloid plaques and hyperphosphorylated tau in cortical brain biopsies are reflected by low CSF Aβ42 and high CSF tau and p-tau levels, respectively.

8.1 INTRODUCTION

The increasing understanding of the pathological timeline of Alzheimer’s disease (AD) and the possibilities for disease-modifying interventions have evoked a need for earlier and more specific diagnosis. Biomarkers have made it possible to relate AD pathology to clinical presentation and disease progression in living patients. The pathological process in AD initiates several years prior to the first clinical symptoms, emerging with neurofibrillary tangles (NFT) in the transentorhinal area (Duyckaerts 2011). The amyloid build-up precedes neuritic plaques and NFTs in the neocortex (Braak & Braak 1997). These neuropathological hallmarks of AD are marked by cerebrospinal fluid (CSF) levels of total tau (t-tau) that reflects the intensity of the neuronal degeneration, p-tau that reflects tangle pathology and the 42 amino acid isoform of amyloid β (Aβ42) that is inversely correlated with Aβ plaque counts and retention of the amyloid tracer Pittsburgh compound B (PIB) in positron emission tomography (PET) studies (Blennow et al. 2010). In a hypothetical model, CSF Aβ42 level decreases before CSF tau level increases (Jack et al. 2010).

Another possibility to obtain these types of data is brain biopsy. Naturally, this is rarely performed in the diagnosis of dementia (Warren et al. 2005) but, when available, it may give important information on the diagnosis of the patient (Leinonen et al. 2010).

In the current study, we take advantage of the possibility to compare biochemical and pathological data in specimens obtained during evaluation of patients with suspected normal pressure hydrocephalus (NPH). The objectives were to assess the relation between AD-related pathological changes in CSF and frontal cortical brain biopsy, to study possible biomarker gradients between the ventricular and lumbar CSF, and to evaluate the relationship of CSF biomarkers and cortical biopsy with final clinical diagnoses.

8.2 MATERIAL AND METHODS

8.2.1 Patients

Neurosurgery of Kuopio University Hospital (KUH) has solely served the defined catchment population in Eastern Finland. Since 1993, the diagnostic work-up of KUH Neurosurgery for presumed NPH has included a clinical examination by a neurologist and a neurosurgeon, a computed tomography (CT) or a magnetic resonance imaging (MRI) scan, and a 24-hour intraventricular ICP monitoring together with a right frontal cortical biopsy (Leinonen et al. 2010).

Until the end of 2010, the Kuopio NPH Registry (www.uef.fi/nph) consisted of 587 consecutive patients evaluated for presumed NPH fulfilling the following criteria: 1) one to three symptoms related to NPH: impaired cognition, gait or urinary continence; 2) enlarged brain ventricles disproportionate to the size of the sulci of cerebral convexities in computed tomography (CT) or magnetic resonance imaging (MRI); 3) brain biopsy available (Leinonen et al. 2010).

Altogether 188 patients had a cortical biopsy together with a ventricular CSF sample, but six were excluded due to insufficient clinical follow-up data. The formation of the study population is presented in figure 7. Of the 182 patients in the study cohort, 101 developed a final clinical diagnosis of idiopathic NPH and 51 that of AD.
8.2.2 APOE genotyping
Blood sample for APOE analysis was available for 152 subjects. A PCR method was used in the APOE analysis as described earlier (Tsukamoto et al. 1993).

8.2.3 Shunt response and final clinical diagnosis of AD
Of the 182 patients, 121 patients were shunted based on the results obtained by the ICP monitoring. The indications for the ventriculoperitoneal shunt treatment were: during 24-hour ICP monitoring 1) basal ICP pressure continuously between 10 mmHg and 20 mmHg, or 2) the presence of any A-waves or more than 30% B-waves when basal pressure was between 5 and 10 mmHg (Savoilainen et al. 2002). The clinical response to shunt was evaluated at 2 to 3 months at KUH Neurosurgery outpatient clinic as an improvement in patients' gait, memory, or urinary continence (Leinonen et al. 2010).

The 182 patients were followed up until death or the end of 2010 for a median follow-up time of 2.0 years (range 0.19-12.3 years). For the final clinical diagnosis, all available clinical data from the hospitals in the KUH catchment area were retrospectively reviewed by a neurologist (AMK or ON) (Leinonen et al. 2010). Altogether 31 cases (biopsied between 2005-2006) overlapped the patients included in our previous paper (Leinonen et al. 2010). The selection criteria for biopsy/ICP-monitoring was identical in the current and in the previous paper.

Severity of memory disorder was estimated by using the clinical dementia rating (CDR) based on the clinical data. Possible or probable AD dementia was diagnosed in 51 patients according to the NINCDS-ADRDA and DSM-IV criteria (McKhann et al. 1984, Leinonen et al. 2010, Runeson, Rich 1994). Idiopathic NPH (iNPH) was diagnosed in 101 patients according to the following criteria: abnormal ICP findings indicating a shunt; no known cause for secondary NPH; and no clinical AD in the end of follow-up (Leinonen et al. 2010). The shunt response was positive for 88 patients.
8.2.4 Cortical brain biopsy and CSF samples

The biopsy procedure has been described in detail previously (Leinonen et al. 2010). Briefly, a right frontal 12 mm burr hole was made approximately three cm laterally from the midline and close to the coronal suture of the skull under local anesthesia and sedation. Prior to the insertion of an intraventricular catheter for 24-hour ICP monitoring, one to three cylindrical cortical brain biopsies of two to five mm in diameter and three to seven mm in length were obtained with biopsy forceps.

The ventricular CSF samples were collected immediately after placing the catheter and discarding the first one ml. A lumbar CSF sample was available for 55 patients. The lumbar CSF was collected immediately after puncture in vertebrae LIII-IV or LIV-V interspace prior to the ventriculostomy procedure (13 patients) or 24-28 hours after removing the 24-hour ICP catheter (42 patients). The CSF samples were handled using a standard protocol in the clinic: collected into polypropylene tubes and stored at –80 ºC until the analysis.

8.2.5 AD biomarkers in CSF

All CSF analyses were performed blinded to the clinical data in the quality controlled and validated laboratory in Neurology and Neuroscience (www.uef.fi/neuro), University of Eastern Finland (Mattsson et al. 2009, Herukka et al. 2007). The CSF levels of Aβ42, total tau, and P-tau-181 were measured by commercial ELISA kits (Innotest beta-amyloid1-42, Innotest Tau-Ag, Innotest Phosphotau181P, Innogenetics, Ghent, Belgium) according to the manufacturer’s protocol. In order to examine the intra-assay variation, reference standards were used (Seppälä et al. 2011). To minimize inter-assay and intra-assay variation, the ventricular and lumbar samples of the same individual were always placed in adjacent wells on the same plate of the particular ELISA assay. Possible biomarker gradients between the corresponding ventricular and lumbar CSF samples were analyzed as paired samples in the same assay. The inter assay coefficients of variation were 17.8 % ±8.5 % for Aβ42, 6.6 % ±10.4 % for tau and 10.8 % ±11.7 % for p-tau-181. The intra assay coefficients of variation were 5.0 % ±5.3 % for Aβ42, 1.9 % ±2.5 % for total tau and 2.2 % ±2.3 % for p-tau-181.

8.2.6 Histology and immunohistochemistry

The biopsy samples were fixed in buffered formalin overnight and embedded in paraffin. The 182 paraffin-embedded biopsy samples were sectioned (7 µm) and stained with hematoxylin-eosin, and immunostained with monoclonal antibodies directed to Aβ (6F3D, M0872; Dako; dilution 1:100; pre-treatment 80% formic acid 1 hour) and p-tau (AT8, 3Br-3; Innogenetics; dilution 1:30) (Leinonen et al. 2010).

Aβ was semi-quantified by counting plaques in the biopsy under light microscopy and dividing the total number of plaques by the area of the gray matter (mm²). Cellular or neuritic immunoreactivity for p-tau was evaluated in light microscopy in all samples and was graded as present or absent by a neuropathologist (IA or JR) (Alafuzafoff et al. 2008).

In addition, Aβ was quantified by the following method. Representative high-resolution images spanning the cortical regions of interest were acquired at 2X magnification (Plan N2X/0.60) using an upright Olympus optical microscope (OLYMPUS BX40) with Olympus optical DP50 camera. A flatfield image was also acquired under similar settings for correcting uneven illumination. Subsequently, image processing and analysis was performed by using Photoshop CS3 Extended version 10 software (Adobe Systems Incorporated, San Jose CA). On the grey-scaled images, cortical regions of interest were outlined and selected using Lasso tools. Images were then thresholded to segregate plaques from the background (Figure 8). The number of pixels counted within selections, after calibration, gave corresponding areas in mm². Percentage of cortical area covered with Aβ immunostain was reported for the available biopsy samples.
Figure 8. Semi-quantification of Aβ deposits from brain biopsy tissue.
a) Color image before analysis. Aβ deposits are shown in brown colored immunostain. This image has been flat field corrected for uneven illumination.
b) Threshold image showing Aβ deposits (black) that were selected by software for quantification. A single optimal threshold value irrespective of size, shape and intensity of Aβ deposits was used. Vascular Aβ was excluded from the analysis.

8.2.7 Statistical analysis
For normally distributed data, the results are presented as mean ±SD. Kolmogorov-Smirnov test was used as a test of normality. Mann-Whitney or Chi-square tests were used to test difference between two groups. Differences between several groups were analyzed using analyses of variances (ANOVA) or Kruskal-Wallis tests with Bonferroni post hoc corrections. Correlations between continuous variables were analyzed using Pearson’s correlation coefficient. Multivariate logistic regression analyses were used to assess the association of the covariates to the end variables. Statistical analyses were conducted with PASW Statistics for Mac (release 18.0, SPSS Inc, Chicago, IL).

8.2.8 Ethical aspects
Written informed consent was obtained from all subjects. The study was approved by the Kuopio University Hospital ethical committee, the Finnish National Supervisory Authority and the Finnish Ministry of Social Affairs and Health.
8.3 RESULTS

Clinical characteristics of the subjects are presented in table 10.

Table 10. Demographic characteristics of the patients

<table>
<thead>
<tr>
<th></th>
<th>All patients (n = 182)</th>
<th>AD dementia present (n = 51)</th>
<th>AD dementia not present (n = 130)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Age, years</td>
<td>72.7 ± 8.8</td>
<td>75.4 ± 7.6</td>
<td>71.7 ± 9.0</td>
</tr>
<tr>
<td>Females, n (%)</td>
<td>90 (46.6)</td>
<td>24 (47.1)</td>
<td>65 (50.0)</td>
</tr>
<tr>
<td>Follow-up time, y</td>
<td>2.01 (1.75)</td>
<td>1.73 (2.11)</td>
<td>2.12 (1.55)</td>
</tr>
<tr>
<td>Immunoreactivity in biopsy</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Aβ- Hτ-</td>
<td>85</td>
<td>10</td>
<td>75*</td>
</tr>
<tr>
<td>Aβ+ Hτ-</td>
<td>58</td>
<td>18</td>
<td>40</td>
</tr>
<tr>
<td>Aβ+ Hτ+</td>
<td>33</td>
<td>22</td>
<td>10*</td>
</tr>
<tr>
<td>Aβ- Hτ+</td>
<td>6</td>
<td>1</td>
<td>5</td>
</tr>
<tr>
<td>APOE genotype</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>ε2/ε3</td>
<td>10</td>
<td>1</td>
<td>9</td>
</tr>
<tr>
<td>ε2/ε4</td>
<td>1</td>
<td>1</td>
<td>0</td>
</tr>
<tr>
<td>ε3/ε3</td>
<td>97</td>
<td>17</td>
<td>79†</td>
</tr>
<tr>
<td>ε3/ε4</td>
<td>38</td>
<td>19</td>
<td>19†</td>
</tr>
<tr>
<td>ε4/ε4</td>
<td>6</td>
<td>4</td>
<td>2</td>
</tr>
<tr>
<td>ε4 carriers, n (%)</td>
<td>45 (23)</td>
<td>24 (47)</td>
<td>21 (16)*</td>
</tr>
</tbody>
</table>

Data are presented as mean ±SD and median (IQ range) unless indicated otherwise. APOE ε4 status was missing for 30 patients. AD diagnosis was missing for one patient.

* p < 0.001
† p < 0.01

AD = Alzheimer’s disease, Aβ = beta amyloid in the biopsy, Hτ = hyperphosphorylated tau in the biopsy.

8.3.1 Aβ42, total tau, and P-tau-181 in CSF

The mean Aβ42 level in all ventricular CSF samples (n=182) was 492±236 pg/ml. Aβ42 levels in ventricular CSF were lower but correlated with the corresponding levels in lumbar samples (r=0.607, p<0.001; figure 9). The mean Aβ42, total tau and p-tau-181 levels in ventricular and lumbar CSF of the 55 patients are presented in table 11.
Figure 9. Correlation of ventricular and lumbar CSF Aβ42 (n = 55)
Pearson correlation coefficient was used. CSF = cerebrospinal fluid, Aβ42 = amyloid beta 1-42. Patients with both ventricular and lumbar CSF (n = 55) are included.

Table 11. AD biomarker levels in ventricular and lumbar CSF

<table>
<thead>
<tr>
<th></th>
<th>Ventricular CSF (n = 55)</th>
<th>Lumbar CSF (n = 55)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Aβ42</td>
<td>497 (226)</td>
<td>584 (179)</td>
</tr>
<tr>
<td>Total tau</td>
<td>1370 (2231)</td>
<td>268 (223)</td>
</tr>
<tr>
<td>P-tau-181</td>
<td>80.3 (90.6)</td>
<td>40.5 (15.5)</td>
</tr>
</tbody>
</table>

Data are presented as mean (SD). The unit is pg/ml.
Both ventricular and lumbar CSF samples were available for 55 patients.
p < 0.001 for all biomarkers; ventricular vs. lumbar CSF.
CSF = cerebrospinal fluid, Aβ42 = amyloid beta 1-42, p-tau-181 = tau phosphorylated at threonine 181.

CSF total tau and p-tau-181 levels were lower in lumbar samples than in ventricular samples and there was no correlation between the levels in the ventricular and lumbar space. T-tau and p-tau-181 correlated strongly in both ventricular and the lumbar samples (r=0.910 and r=0.926, respectively; p<0.001 for both). The tau and p-tau-181 levels were higher in lumbar samples collected after the placement of the ventricular catheter, but correlation between ventricular and lumbar CSF samples did not occur even if the lumbar CSF was collected before the brain procedure. Aβ42 levels did not differ whether the lumbar CSF was collected before or after the catheter placement.

8.3.2 Aβ and tau proteins in cortical biopsy
Of the 182 patients, 58 had only Aβ plaques and 33 Aβ plaques with hyperphosphorylated tau in their cortical biopsy.

Patients with AD dementia at follow-up had a median of 11.8 amyloid plaques per mm² (26.4) in their cortical brain biopsy, whereas those with other clinical diagnoses had a median of 0.0 plaques per mm² (2.5). The corresponding percentages of amyloid load were 1.13% (3.06) and 0.00% (0.35). Patients who had CDRs of 1 or more at follow-up had 0.25%
(2.01) of amyloid in their cortical biopsies whereas those with CDR 0.5 had 0.00% (0.55) and those with CDR 0 had 0.00 (0.51).

There was a dose-dependent APOE ε4 effect for lower CSF Aβ42 and higher amyloid load in the biopsy. CSF Aβ42 was 337±161 pg/ml in APOE ε4 positive patients compared to patients who were APOE ε4 negative (461±234 pg/ml, p<0.001). Subjects carrying the ε4 allele had a median of 1.87% amyloid (2.73) and 9.49 plaques/mm² (17.42) in their biopsies, whereas the non-carriers had <0.01% (0.237) and <0.01 (0.237) 1/mm², respectively (p<0.001).

### 8.3.3 Correlation of CSF and cortical biopsy findings

The Aβ42 levels were markedly lower in patients who had amyloid plaques in their cortical brain biopsy than in patients who did not have amyloid plaques at all. Patients with amyloid plaques also had a lower Aβ42/p-tau-181 ratio in the ventricular CSF than those without amyloid in the biopsy.

The CSF tau and p-tau-181 levels in the ventricular sample were related to the presence of tau in the cortical brain biopsy. Patients with tau present in their cortical biopsy had lower Aβ42 and higher p-tau-181 levels in the CSF compared to tau-negative patients. Total CSF tau was also higher in tau-positive patients but the difference did not reach statistical significance (p=0.066). CSF Aβ42 was lowest and tau and p-tau-181 highest in patients who had both tau- and amyloid-positive brain biopsies (table 12).

Ventricular (n=182) and lumbar (n=55) CSF levels of Aβ42 were low if the amyloid load in the cortical biopsy sample was high (figure 10). The results were verified by semi-quantitation (1/mm²) of the amyloid plaques (r=0.213, p=0.004 for ventricular CSF; r=0.397, p=0.003 for lumbar CSF). In patients with AD dementia during follow-up, both ventricular (n=51) and lumbar (n=16) CSF Aβ42 levels were low if the biopsy amyloid load was high (figure 10). This did not occur in patients who did not develop AD dementia. As expected, the biopsy amyloid load correlated to the semi-quantitation of the plaques (r=0.695, p<0.001).

### 8.3.4 Correlation of CSF findings to final clinical diagnosis of AD

The 51 patients who developed AD dementia in the follow-up had lower ventricular CSF Aβ42 levels than the 130 patients with other diagnoses. However, the difference did not reach significance. The ventricular CSF tau or p-tau-181 concentrations did not differ significantly between the patients that developed AD dementia and those who did not. The Aβ42/p-tau-181 ratio was lower in patients who were diagnosed to have AD dementia than the subjects with other clinical diagnoses. The levels of the ventricular CSF biomarkers did not differ between 101 patients with iNPH or 81 patients with no iNPH in the follow-up. Neither did the cognitive functioning measured by CDR influence the CSF levels (table 13).

### 8.3.5 Multivariate analysis

In the multivariate logistic regression (table 14), the APOE ε4 carrier status and age per year were the independent predictive covariates for cortical Aβ as the endpoint. For AD dementia as the endpoint, Aβ plaques and hyperphosphorylated tau in the cortical biopsy as well as age, APOE ε4 and the absence of iNPH were the independent predictive covariates. The ventricular CSF Aβ42 level alone had no predictive value for Aβ plaques or AD dementia.
Figure 10. Correlation of ventricular and lumbar CSF Aβ42 with cortical brain biopsy Aβ area. All patients and AD subgroups. Pearson correlation coefficient was used.
AD = Dementia of Alzheimer's disease, CSF = cerebrospinal fluid, Aβ42 = amyloid beta 1-42.
Table 12. Ventricular CSF biomarker levels by cortical brain biopsy histology.

<table>
<thead>
<tr>
<th></th>
<th>Aβ+ (n=91)</th>
<th>Aβ- (n = 91)</th>
<th>Hτ+ (n = 40)</th>
<th>Hτ- (n = 142)</th>
<th>Aβ- Hτ- (n = 85)</th>
<th>Aβ+ Hτ- (n = 58)</th>
<th>Aβ+ Hτ+ (n = 33)</th>
<th>Aβ- Hτ+ (n = 6)</th>
</tr>
</thead>
<tbody>
<tr>
<td>CSF Aβ42</td>
<td>360 (188)</td>
<td>444 (239)†</td>
<td>328 (191)</td>
<td>423 (222)*</td>
<td>441 (239)</td>
<td>403 (198)</td>
<td>284 (142)†§</td>
<td>485 (257)</td>
</tr>
<tr>
<td>CSF total tau</td>
<td>1393 (1820)</td>
<td>1297 (1915)</td>
<td>1813 (2363)</td>
<td>1215 (1688)</td>
<td>1285 (1973)</td>
<td>1093 (1132)</td>
<td>1928 (2575)</td>
<td>1459 (787)</td>
</tr>
<tr>
<td>CSF p-tau-181</td>
<td>83.2 (53.8)</td>
<td>78.7 (77.8)</td>
<td>97.8 (64.2)</td>
<td>76.3 (67.0)*</td>
<td>78.0 (79.8)</td>
<td>73.2 (41.1)</td>
<td>100.9 (68.2)</td>
<td>89.3 (42.5)</td>
</tr>
<tr>
<td>CSF Aβ42/p-tau</td>
<td>5.79 (4.27)</td>
<td>8.07 (5.27)†</td>
<td>4.49 (3.45)</td>
<td>7.62 (5.06)‡</td>
<td>8.21 (5.33)</td>
<td>6.92 (4.68)</td>
<td>3.79 (2.39)§</td>
<td>6.12 (4.08)</td>
</tr>
</tbody>
</table>

Data are presented as mean (SD). The unit is pg/ml.
P-tau-181 level was missing for three patients.
ANOVA was used for CSF Aβ42 and Aβ42/p-tau and Kruskall-Wallis test for tau and p-tau-181.

*p < 0.05
†p < 0.01
‡p < 0.001
§Aβ+ Hτ+ vs. Aβ- Hτ-, ANOVA with Bonferroni post hoc correction.

Aβ = beta amyloid in the biopsy, Hτ = hyperphosphorylated tau in the biopsy, CSF = cerebrospinal fluid, Aβ42 = amyloid beta 1-42, p-tau-181 = tau phosphorylated at threonine 181.
Table 13. Ventricular CSF biomarker levels in diagnostic and cognitive groups.

<table>
<thead>
<tr>
<th></th>
<th>AD (n = 51)</th>
<th>not AD (n = 130)</th>
<th>iNPH (n = 101)</th>
<th>not iNPH (n = 81)</th>
<th>CDR 0 (n = 20)</th>
<th>CDR 0.5 (n = 79)</th>
<th>CDR ≥1.0 (n = 83)</th>
</tr>
</thead>
<tbody>
<tr>
<td>CSF Aβ42</td>
<td>360 (202)</td>
<td>419 (224)</td>
<td>399 (188)</td>
<td>405 (252)</td>
<td>348 (195)</td>
<td>416 (226)</td>
<td>402 (217)</td>
</tr>
<tr>
<td>CSF total tau</td>
<td>1432 (1990)</td>
<td>1275 (1781)</td>
<td>1261 (1354)</td>
<td>1450 (2357)</td>
<td>1430 (1644)</td>
<td>1253 (1293)</td>
<td>1410 (2333)</td>
</tr>
<tr>
<td>CSF p-tau-181</td>
<td>83.9 (50.1)</td>
<td>78.3 (70.3)</td>
<td>78.1 (51.5)</td>
<td>84.5 (82.4)</td>
<td>79.4 (64.8)</td>
<td>77.2 (47.9)</td>
<td>84.9 (81.8)</td>
</tr>
<tr>
<td>CSF Aβ42/p-tau</td>
<td>5.57 (3.67)</td>
<td>7.49 (5.23)*</td>
<td>6.99 (4.74)</td>
<td>6.86 (5.16)</td>
<td>7.13 (6.78)</td>
<td>7.39 (5.30)</td>
<td>6.45 (3.93)</td>
</tr>
</tbody>
</table>

Data are presented as mean (SD). The unit is pg/ml.

CSF tau level was missing for 2 patients (1 AD/iNPH/CDR≥1 and 1 non-AD/non-iNPH/CDR 0.5).
CSF p-tau-181 level was missing for 3 patients (1 AD/iNPH/CDR≥1, 1 AD/non-iNPH/CDR≥1 and 1 non-AD/non-iNPH/CDR 0.5). AD data was missing for one patient (iNPH/CDR 0).

*p < 0.01 for Aβ42/p-tau AD vs. not AD
iNPH = idiopathic normal pressure hydrocephalus, CDR = Clinical dementia rating, AD = Dementia of Alzheimer’s disease, CSF = cerebrospinal fluid, Aβ42 = amyloid beta 1-42, p-tau-181 = tau phosphorylated at threonine 181.
Table 14. Logistic regression analysis of the association of age, sex, CSF biomarkers and APOE ε4 carrier status to the cortical brain biopsy amyloid and final clinical diagnosis.

<table>
<thead>
<tr>
<th>Covariates</th>
<th>Aβ as end variable (n=151)</th>
<th>AD as end variable (n=151)</th>
<th>iNPH as end variable (n=151)</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>n</td>
<td>OR (95% CI)</td>
<td>n</td>
</tr>
<tr>
<td>Age per year</td>
<td></td>
<td>1.09 (1.03-1.15)†</td>
<td>1.06 (1.00-1.12)*</td>
</tr>
<tr>
<td>Male</td>
<td>75</td>
<td>1</td>
<td>75</td>
</tr>
<tr>
<td>Female</td>
<td>76</td>
<td>0.98 (0.44-2.20)</td>
<td>76</td>
</tr>
<tr>
<td>APOE ε4 noncarrier</td>
<td>106</td>
<td>1</td>
<td>106</td>
</tr>
<tr>
<td>APOE ε4 carrier</td>
<td>45</td>
<td>9.95 (3.37-29.36)‡</td>
<td>45</td>
</tr>
<tr>
<td>Immunoreactivity</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Aβ- Hτ-</td>
<td>68</td>
<td>1</td>
<td>68</td>
</tr>
<tr>
<td>Aβ+ Hτ-</td>
<td>52</td>
<td>1.41 (0.45-4.40)</td>
<td>52</td>
</tr>
<tr>
<td>Aβ+ Hτ+</td>
<td>25</td>
<td>6.41 (1.62-25.41)†</td>
<td>25</td>
</tr>
<tr>
<td>Aβ- Hτ+</td>
<td>6</td>
<td>1.13 (0.10-13.27)</td>
<td>6</td>
</tr>
<tr>
<td>CSF Aβ42</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>1st quartile (highest)</td>
<td>43</td>
<td>1</td>
<td>43</td>
</tr>
<tr>
<td>2nd quartile</td>
<td>40</td>
<td>1.35 (0.47-3.87)</td>
<td>40</td>
</tr>
<tr>
<td>3rd quartile</td>
<td>34</td>
<td>1.45 (0.46-4.57)</td>
<td>34</td>
</tr>
<tr>
<td>4th quartile (lowest)</td>
<td>34</td>
<td>2.37 (0.78-7.17)</td>
<td>34</td>
</tr>
<tr>
<td>Alzheimer’s disease</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Not present</td>
<td>109</td>
<td>1</td>
<td>109</td>
</tr>
<tr>
<td>Present</td>
<td>42</td>
<td>2.20 (0.77-6.25)</td>
<td>42</td>
</tr>
<tr>
<td>iNPH</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Not present</td>
<td>61</td>
<td>1</td>
<td>61</td>
</tr>
<tr>
<td>Present</td>
<td>90</td>
<td>1.16 (0.47-2.85)</td>
<td>90</td>
</tr>
</tbody>
</table>

APOE ε4 status was missing for 30 patients. AD data was missing for one patient (iNPH/CDR 0).

*p < 0.05; †p < 0.01; ‡p < 0.001

Aβ = beta amyloid in the biopsy, Hτ = hyperphosphorylated tau in the biopsy, CSF = cerebrospinal fluid, Aβ42 = amyloid beta 1-42, p-tau-181 = tau phosphorylated at threonine 181, iNPH = idiopathic normal pressure hydrocephalus. AD = Dementia of Alzheimer’s disease.
8.4 DISCUSSION

We believe our study to be the first to evaluate the association of CSF biomarkers of AD to amyloid and tau pathologies in brain biopsies of living patients. We found that the concentration of Aβ42 in ventricular CSF differentiates the groups between the presence of amyloid and tau in cortical brain biopsy. The accumulated brain amyloid was high when the ventricular CSF amyloid was low, which is in line with the data shown earlier by post-mortem brain (Strozyk et al. 2003, Tapiola et al. 2009) and PIB PET retention (Fagan et al. 2006, Grimmer et al. 2009, Tolboom et al. 2009, Forsberg et al. 2008). The low CSF Aβ42 together with high amyloid load in the biopsy was seen also with lumbar CSF and was most emphasized in the subgroup with AD dementia as the final clinical diagnosis.

The patients with hyperphosphorylated tau in the cortical biopsy had lower levels of ventricular CSF Aβ42 and higher levels of p-tau compared to tau-negative. The differentiating potential for presence of Aβ and hyperphosphorylated tau in the biopsy increased when CSF biomarkers were used together as the CSF Aβ42/p-tau ratio, which has been shown to be accurate also in respect to clinical diagnoses of AD (Mattsson et al. 2009). Since the low concentration of ventricular CSF Aβ42 as a covariate did not show independent risk potential for Aβ accumulation or predict AD dementia alone in the multivariate model, it may not have a direct causality to the presence of tau in the biopsy. More likely, the low Aβ42 reflects the presence of Aβ and consequently the tau in the brain. Our result that an increment of CSF p-tau associates with the tau-positive biopsy is supported by the previous finding that the CSF p-tau reflects the high densities of NFTs seen in the post-mortem AD brain (Buerger et al. 2006).

The ventricular CSF concentrations of our study showed substantially higher levels of tau and p-tau than in the large studies performed with lumbar CSF samples (Mattsson et al. 2009). The subjects of our study were patients with presumed NPH, in which increased CSF tau levels have been detected earlier (Kudo et al. 2000, Silverberg et al. 2008). However, the levels of CSF tau and p-tau were higher than usual regardless of the diagnoses, and cannot be explained solely by the presence of NPH. It seems evident that the neuronal trauma caused by placing the ventricular catheter increases CSF tau and p-tau, also in the lumbar space (Agren-Wilsson et al. 2007). The previous study has shown the CSF total tau to correlate with the presence of NFTs at autopsy (Tapiola et al. 2009). The generally high concentration of CSF total tau may have been the reason why CSF tau did not differ significantly in our present study between those with tau in the cortical biopsy and those without.

Our work is one of the few studies to evaluate the gradients in AD biomarker concentrations between the ventricular and lumbar CSF. We found a higher concentration of Aβ42 in the lumbar CSF than in the ventricular CSF, which has been noted previously (Talab et al. 2009). Most studies have found low concentrations but no gradient between the ventricle and lumbar CSF Aβ42 levels (Serot et al. 2011, Tamaris, Watkins & Kitchen 2006). In general, the brain proteins are considered to exhibit a higher level in ventricular CSF, whereas blood proteins are more concentrated in lumbar CSF (Reiber 2003).

The strongest independent predictor for AD dementia was the simultaneous immunohistochemical presence of amyloid together with tau in cortical biopsy, which has been shown earlier (Leinonen et al. 2010). Low ventricular CSF Aβ42 was not a statistically significant covariate for brain Aβ or AD dementia, but the presence of the APOE ε4 allele was markedly associated with both end variables.

Our subjects were suspected NPH patients whose symptoms did not represent typical AD cases, which is a limitation, but the diagnosis of iNPH was rather accurate based on the proportion of shunt responders. Also, the comorbidity of NPH and AD is known to be high. The lumbar CSF was collected mainly after the ventriculostomy, which may have had
an effect on CSF dynamics and hence on CSF biomarker levels. The procedure did not alter the Aβ42 levels but increased tau and p-tau concentrations compared to LP prior the ventriculostomy. Moreover, we did not have inter-rater comparative data for image analysis methodology we employed here. However, we used manual semi-quantitation for confirmation and the two methods came out equivalent in correlation analysis.

Our results show a decrease of CSF Aβ42 (ventricular and lumbar) when cortical brain biopsy amyloid load increases. The levels of CSF Aβ42, t-tau and p-tau associate also with the presence of hyperphosphorylated tau in cortical brain biopsy. These findings support the role of CSF biomarkers as reflectors of pathological changes of AD in the living brain and encourage studying further the usefulness of cortical biopsy in research.
9 Comparison between clinical diagnosis and CSF biomarkers of Alzheimer’s disease in elderly patients with late onset psychosis – Helsinki Old Age Psychosis Study (HOPS)*

ABSTRACT

Objectives: To determine the proportion of elderly people with a first psychotic episode actually suffering from a dementing illness, especially Alzheimer’s disease (AD), by using CSF biomarkers.

Design: Prospective case-control study.

Setting and participants: Sixty-six patients age 65 years and older with recent psychotic symptoms and 12 control subjects with chronic schizophrenia over 10 years that were referred to geriatric neuropsychiatry in-ward treatment.

Measurements: Concentration levels of CSF Aβ42, tau and p-tau-181 measured by ELISA compared to clinical final clinical diagnosis made by a multiprofessional team of one neurologist and several psychiatrists.

Results: The CSF specimen was obtained from 51 (65.4%) of the patients. In five subjects out of 13 with a final clinical diagnosis of AD dementia, all the CSF biomarkers (Aβ42, tau and p-tau) were normal. Only one patient out of 25 with a psychiatric diagnosis and none of the controls with schizophrenia showed a CSF profile typical of AD. Three patients with an AD dementia diagnosis, four patients with a psychiatric diagnosis and one control with schizophrenia had a low Aβ42 concentration with normal levels of tau or p-tau. The patients with AD dementia had lower CSF Aβ42 levels than other patients.

Conclusions: The CSF biomarkers are important and useful as part of the diagnostic procedure for detecting AD and other dementing illnesses in elderly patients displaying psychotic symptoms. The accuracy of AD diagnosis encounters problems due to atypical behavioural symptoms in psychiatric settings and thus the differential diagnostics can be improved by using CSF biomarkers of AD more frequently.

* Adapted with permission of [name of the Publisher] from: Bibliographic information (authors, title, journal, volume: pages, year) of the original article.
9.1 INTRODUCTION

Alzheimer’s disease (AD) is the most common cause of dementia. It is estimated that 5.4% of people over 65 years old have AD (Ferri et al. 2005), with the prevalence doubling every five years after the age of 65 (Hebert et al. 2003) e.g. the prevalence of AD after the age of 85 years is 43% in USA (Alzheimer's Association, Thies & Bleiler 2011). The typical early symptoms of Alzheimer’s disease are problems in short-term memory and difficulties to learn new tasks. These symptoms can be assessed by cognitive screening tests and by neuropsychological examinations (Welsh et al. 1991). Hippocampal atrophy seen in magnetic resonance imaging (MRI) is correlated with AD (Apostolova et al. 2006), but a normal MRI finding does not exclude AD. In addition, severe depression can cause hippocampal atrophy (O’Brien et al. 2004). The research criteria for AD diagnosis include several possible biomarkers as supporting features but are specifically designed to identify a typical amnestic form of preclinical or prodromal AD that contains approximately 85% of all AD (Dubois et al. 2007). However, in some cases AD can begin with neuropsychiatric symptoms, which can be prominent long before there are any clear cognitive symptoms – alternatively the cognitive symptoms may be interpreted as being secondary to mental illness.

In a study of one hundred neuropathologically verified AD patients, a large proportion of patients had paranoid ideation before the diagnosis of AD and at the time of the diagnosis, 45% had displayed some kind of psychotic symptom (hallucinations, paranoia, other delusions). The average time from first paranoid ideations to AD diagnosis was 18 months (Jost, Grossberg 1996). In addition, diffuse Lewy body disease (DLB) tends to begin with symptoms often interpreted as functional. Two of the three core features are recurrent visual hallucinations and fluctuating cognition (McKeith et al. 2004), which can be a reason why the patient needs to be admitted into a psychiatric hospital. Fortunately, DLB can be quite reliably diagnosed with beta-CIT-SPECT. Although frontotemporal degenerations are quite rare in the population aged 65 and over, they may present with psychotic symptoms, e.g. mania (Woolley et al. 2011).

There are very few studies of the prevalence of neurodegenerative diseases in the elderly with recent psychotic symptoms. In Post’s study (1966) 15% of paranoid patients in a psychogeriatric hospital developed dementia (Post 1966), and in Holden’s study (Holden 1987) 35% of patients with paraphrenia had become demented after three years.

There are changes in cerebrospinal fluid (CSF) that can be identified in AD. The concentration of 42 amino acid length amyloid-β (Aβ42) diminishes whereas those of protein tau and its hyperphosphorylated form p-tau increase in AD many years before the appearance of clinical dementia. If both changes are observed, there is marked likelihood of AD i.e. the pooled 86% sensitivity and 90% specificity for CSF Aβ42 have been reported to discriminate the AD patients from cognitively healthy controls (Blennow & Hampel 2003). The corresponding figures for total tau were 81% and 90% and for p-tau 80% and 92%, respectively (Blennow & Hampel 2003). By combining the abnormal CSF biomarkers, a sensitivity of >90 \% and a specificity of >85 \% has been achieved (Hansson et al. 2006). The biomarker profile of elderly schizophrenia patients is reported to show a normal CSF tau level and a slightly decreased Aβ42 concentration (Frisoni et al. 2011).

Cholinergic medication has changed the treatment of AD and several other neurodegenerative illnesses, and because it seems that the early intervention is the best way to support the independent daily living and delay hospitalization, it has become extremely important to diagnose these diseases at an early stage, preferably before the onset of dementia.

The aim of the Helsinki Old Age Psychosis Study is to identify, what proportion of elderly people with a first psychotic episode are actually suffering from a dementing illness, especially AD.
9.2 METHODS

9.2.1 Participants
The psychogeriatrics department of Helsinki University Central Hospital serves a population of 1 071 000 inhabitants in the capital area of Finland. The population aged 65 and over in the area is 145 000. The department consists of four wards with a total of 68 beds, and is the only hospital offering psychogeriatric in-ward treatment in the area.

Ninety-three patients aged 65 years and over were included in the study after ten patients refused to participate. All but one of the refused were women (90%), they did not differ by age (mean 79.2 years) but AD (40%) was more frequent than in the study group. The recruitment began on 1.12.2009 and ended on 3.10.2011, but during the study there were some pauses (from 1-3 months) because of other commitments of the main researcher.

All study patients were psychotic at the time of admission to the hospital, but their psychotic symptoms had not lasted more than two years before their admission. The control group consisted of patients with chronic schizophrenia (disease history over 10 years) experiencing acute psychotic symptoms. All patients with a known diagnosis of a dementing illness (AD, DLB etc.) were excluded, as well as patients with other diseases of central nervous system (e.g. Parkinson’s disease (PD) or major stroke).

9.2.2 Clinical diagnosis
All patients were evaluated clinically by a psychiatrist. An MRI examination of the brain was taken when possible, and if not (e.g. presence of a pacemaker), then a CT-scan was carried out. In the MRI examinations, the hippocampuses were assessed by the Sheltens grade (Scheltens et al. 1992) and vascular lesions by Fazekas grade (Fazekas et al. 2000).

All patients were neuropsychologically examined. The CERAD –battery (Sotaniemi et al. 2011) was performed by nurses with a wider testing conducted by a trained neuropsychologist. Routine blood tests were carried out.

All patients with a suspicion of a memory disease (according to clinical signs, neuropsychological tests or imaging) were examined and evaluated also by a neurologist. The diagnosis of AD was made according to the guidelines of the new EFNS research criteria (Hort et al. 2010) excluding the use of CSF biomarkers. An exception was made to the criteria, which normally demand a dementia-stage cognitive decline, because here we wanted to diagnose AD as early as possible, i.e. before any signs of cognitive problems. AD begins with a mild cognitive impairment, and when neuropsychiatric symptoms, especially delusions, precede the more disruptive cognitive symptoms, these psychiatric symptoms often significantly interfere with usual social activities and relationship with others even without the presence of a severe cognitive decline. So when the neuropsychological tests showed an amnestic memory disorder and imaging supported the diagnosis, and there was no other explanation for the state, we diagnosed the patient as having AD even when the cognitive decline did not fulfil the full criteria of dementia.

DLB was diagnosed according to the McKeith criteria (McKeith et al. 2004), and the 123I-FP-Spect was conducted to verify the diagnosis (O’Brien et al. 2009). The diagnoses of VaD and psychiatric disorders were made according to the DSM-IV criteria. When the patients fulfilled both the diagnoses of degenerative or vascular memory illness and that of a psychiatric illness, the diagnosis of memory illness was used.

The final clinical diagnosis was made according to all clinical (psychiatric and neurological), neuropsychological and imaging data. The diagnosis was based on the information from the first ward period and was made as a consensus in the research team of two psychiatrists and one neurologist. The results of the CSF analysis or follow-up did not influence the diagnosis.
9.2.3 CSF analysis
A CSF sample was obtained from 51 patients. Some patients refused to participate in this part of the study, some had contraindications (warfarin treatment) and in some the lumbar puncture failed. Lumbar puncture was performed in L3/L4 or L4/L5 vertebrae interspace with a 0.7 mm needle and CSF was collected into a polypropylene tube. The samples were stored at ~80 °C or lower until analysis.

The common biomarkers for AD (Aβ42, tau, p-tau-181) were analysed using commercial ELISAs (Innotest beta-amyloidβ42, Innotest Tau-Ag, Innotest Phosphotau(181P), Innogenetics, Ghent, Belgium) according to the manufacturer’s protocol. The CSF analyses were made after the ward period and the results did not influence the clinical diagnosis. The analyses were conducted in our quality controlled and validated laboratory in the University of Eastern Finland.

Reference standards from pooled CSF samples were used in order to examine the intra-assay variation. The inter-assay coefficient of variation (CV%) for control samples with their concentration near the cut-off limit were 8.6% for Aβ42, 8.9% for tau, and 5.2% for p-tau-181. The regular cut-off limits in our laboratory were used (Herukka et al. 2005). These cut-offs referring to AD were Aβ42 < 450 pg/ml, tau > 400 pg/ml and p-tau > 70 pg/ml.

9.2.4 Statistics
The statistical analyses were conducted with SPSS 19.0.0 (IBM, USA). The Kolmogorov-Smirnov test was used to test the normality of the data. The student t-test was used for normally distributed continuous data and χ²-test for ordinal and nominal data. ANOVA with Bonferroni post hoc correction was used for group comparisons of normally distributed continuous data.

9.2.5 Ethical aspects
All subjects and in most cases also a relative signed an informed consent form. The study was approved by the ethics committee of the Helsinki and Uusimaa hospital district. After the analysis and comparison of the clinical diagnoses and the CSF findings, we re-examined the data of those patients in whom there was a discrepancy between diagnosis and the biomarkers in an attempt to discern the reasons for this paradox.

9.3 RESULTS

Demographic data and the diagnosis of the entire study sample and those with CSF sample obtained are presented in table 15.

Of the 93 subjects in the study, 15 were excluded because in the final analysis they did not fulfil the inclusion criteria: three were not clearly psychotic, seven were found to have had psychotic symptoms for more than two years (e.g. episodes of psychotic depression, bipolar disease or late-onset schizophrenia), one had PD, two had previously diagnosed AD and two had suffered a major stroke. Thus, the study sample consisted of 78 subjects. Twelve were controls who had suffered from schizophrenia for over 10 years and 66 had recent psychotic symptoms.

A CSF specimen was obtained from 51 (65.4%) of the patients. Table 16 compares the sample of patients from whom a CSF sample was obtained with those from whom CSF was not available (n=27).

The regular cut-off values of our validated laboratory were used. Twenty-five (49.0%) patients had completely normal CSF biomarkers of AD. Eleven patients (21.6%) had elevated tau or p-tau or both and 16 patients (31.4%) had only diminished CSF Aβ42 concentrations. Six patients (11.8%) had both elevated p-tau levels and decreased Aβ42 concentrations. In two patients, all three biomarker levels were abnormal. Table 17 shows the distribution of the biomarkers with respect to diagnoses.
The mean biomarker concentrations in all 51 patients were Aβ42: 636 ±283 pg/ml, total tau: 276 ±199 pg/ml and p-tau: 55 ±37 pg/ml. The clinically diagnosed AD dementia patients (without cerebrovascular disease, CVD) had a higher CSF p-tau concentration (66.7 ±19.6 pg/ml compared to all other subjects (47.4 ±22.4 pg/ml; p=0.016). The patients with diagnoses of AD or AD+CVD had lower Aβ42 levels than patients with psychiatric diagnoses or schizophrenia. Otherwise, the mean biomarker levels did not differ statistically between diagnostic groups. Table 18 presents the biomarker concentrations by simplified diagnostic groups and table 19 by all diagnostic groups.
**Table 15.** Demographic information and the diagnoses in the samples

<table>
<thead>
<tr>
<th></th>
<th>Study sample</th>
<th>CSF sample</th>
<th>No CSF</th>
<th>p</th>
</tr>
</thead>
<tbody>
<tr>
<td><strong>n</strong></td>
<td>78</td>
<td>51</td>
<td>27</td>
<td>p</td>
</tr>
<tr>
<td><strong>Age, years</strong></td>
<td>76.7</td>
<td>77.3</td>
<td>75.4</td>
<td>n.s.</td>
</tr>
<tr>
<td>Female</td>
<td>63 (81)</td>
<td>40 (78)</td>
<td>23 (85)</td>
<td>n.s.</td>
</tr>
<tr>
<td>Psychiatric diagnosis total (%)</td>
<td>41 (53)</td>
<td>25 (49)</td>
<td>16 (59)</td>
<td>n.s.</td>
</tr>
<tr>
<td>Delusion disorder</td>
<td>16</td>
<td>10</td>
<td>6</td>
<td></td>
</tr>
<tr>
<td>VLOS LP</td>
<td>17</td>
<td>10</td>
<td>7</td>
<td></td>
</tr>
<tr>
<td>Psychotic depression</td>
<td>8</td>
<td>5</td>
<td>3</td>
<td></td>
</tr>
<tr>
<td>Organic diagnosis total (%)</td>
<td>25 (32)</td>
<td>19 (37)</td>
<td>6 (22)</td>
<td>n.s.</td>
</tr>
<tr>
<td>AD</td>
<td>14</td>
<td>10</td>
<td>4</td>
<td></td>
</tr>
<tr>
<td>AD with CVD</td>
<td>3</td>
<td>3</td>
<td></td>
<td></td>
</tr>
<tr>
<td>VaD</td>
<td>2</td>
<td>1</td>
<td>1</td>
<td></td>
</tr>
<tr>
<td>DLB</td>
<td>1</td>
<td>1</td>
<td></td>
<td></td>
</tr>
<tr>
<td>FTLD</td>
<td>3</td>
<td>3</td>
<td></td>
<td></td>
</tr>
<tr>
<td>Alcohol dementia</td>
<td>1</td>
<td>1</td>
<td></td>
<td></td>
</tr>
<tr>
<td>Delirium</td>
<td>1</td>
<td>1</td>
<td></td>
<td></td>
</tr>
<tr>
<td>Schizophrenia &gt; 10 years (%)</td>
<td>12 (15)</td>
<td>7 (14)</td>
<td>5 (19)</td>
<td>n.s.</td>
</tr>
</tbody>
</table>

T-test (age) and $\chi^2$-test were used. CSF = cerebrospinal fluid. VLOS LP = very late-onset schizophrenia-like psychosis. AD = Alzheimer’s disease dementia. CVD = cerebrovascular disease. VaD = vascular dementia. DLB = Dementia with Lewy bodies. FTLD = frontotemporal lobar degeneration (includes one patient with primary progressive aphasia (PPA). Schizophrenia over 10 years = control group.
Table 16. The reasons for missing CSF sample and the comparison between those with and those without a CSF sample for analysis.

<table>
<thead>
<tr>
<th>Age (y)</th>
<th>Females</th>
<th>Clinical diagnosis (%)</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td></td>
<td>Psychiatric</td>
</tr>
<tr>
<td>CSF obtained (n = 51)</td>
<td>77.3 (63.5-91.7)</td>
<td>40 (78.4%)</td>
</tr>
<tr>
<td>CSF not obtained (n = 27)</td>
<td></td>
<td></td>
</tr>
<tr>
<td>Patient refused (n= 17)</td>
<td>73.5 (65.5-82.6)</td>
<td>14 (82.4%)</td>
</tr>
<tr>
<td>LP failure (n = 7)</td>
<td>77.8 (66.5-86.9)</td>
<td>7 (100%)</td>
</tr>
<tr>
<td>Warfarin treatment (n = 3)</td>
<td>80.7 (72.5-89.0)</td>
<td>2 (66.6%)</td>
</tr>
</tbody>
</table>

Age presented as mean (range). LP = Lumbar puncture. CSF = cerebrospinal fluid.
Table 17. Normal and abnormal CSF biomarker levels subdivided according to diagnoses.

<table>
<thead>
<tr>
<th>Clinical diagnosis</th>
<th>Delusion disorder</th>
<th>VLO</th>
<th>SLP</th>
<th>Depression</th>
<th>AD</th>
<th>AD+ CVD</th>
<th>VaD</th>
<th>FTLD (PPA*)</th>
<th>Other organic dg</th>
<th>Schizophrenia &gt;10 y</th>
</tr>
</thead>
<tbody>
<tr>
<td>Normal CSF biomarker levels</td>
<td>6</td>
<td>5</td>
<td>4</td>
<td>3</td>
<td>2</td>
<td>1</td>
<td></td>
<td>1</td>
<td>1</td>
<td>3</td>
</tr>
<tr>
<td>CSF Aβ42 level decreased, but normal tau and p-tau concentrations</td>
<td>1</td>
<td>2</td>
<td>1</td>
<td>2</td>
<td>1</td>
<td>2</td>
<td></td>
<td>1*</td>
<td>1</td>
<td>3</td>
</tr>
<tr>
<td>CSF tau or p-tau (or both) levels increased, but normal Aβ42 level</td>
<td>1 (2)</td>
<td>2</td>
<td></td>
<td>(1)</td>
<td></td>
<td></td>
<td>1*</td>
<td>1</td>
<td>3</td>
<td></td>
</tr>
<tr>
<td>CSF Aβ42 level decreased and tau or p-tau (or both) levels increased</td>
<td>(1)</td>
<td></td>
<td>3 (1)</td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
</tbody>
</table>

CSF = cerebrospinal fluid. Aβ42 = amyloid beta 42. P-tau = hyperphosphorylated tau. VLOSLP = very late-onset schizophrenia-like psychosis. AD = Alzheimer's disease dementia. CVD = cerebrovascular disease. VaD = vascular dementia. FTLD = frontotemporal lobar degeneration (includes one patient with primary progressive aphasia (PPA)). Schizophrenia over 10 years = control group. The cut-offs were set to: Aβ42 < 450 pg/ml, tau > 400 pg/ml and p-tau > 70 pg/ml (indicative to AD).
Table 18. Biomarker concentrations in the diagnostic groups of AD, psychiatric diagnoses and controls (schizophrenia)

<table>
<thead>
<tr>
<th>Clinical diagnosis</th>
<th>Psychiatric diagnoses</th>
<th>AD or AD+CVD</th>
<th>Schizophrenia &gt; 10 years</th>
<th>p</th>
</tr>
</thead>
<tbody>
<tr>
<td>n</td>
<td>25</td>
<td>13</td>
<td>7</td>
<td></td>
</tr>
<tr>
<td>CSF Aβ42</td>
<td>662 ±208</td>
<td>515 ±329</td>
<td>865 ±323</td>
<td>0.026</td>
</tr>
<tr>
<td>CSF tau</td>
<td>252 ±166</td>
<td>256 ±159</td>
<td>322 ±146</td>
<td>0.590</td>
</tr>
<tr>
<td>CSF p-tau</td>
<td>45 ±23</td>
<td>59 ±24</td>
<td>56 ±17</td>
<td>0.166</td>
</tr>
<tr>
<td>CSF Aβ42/p-tau</td>
<td>21.0 ±18.2</td>
<td>11.3 ±9.1</td>
<td>16.6 ±7.6</td>
<td>0.168</td>
</tr>
</tbody>
</table>

CSF = cerebrospinal fluid. Psychiatric diagnoses include: very late-onset schizophrenia-like psychosis (10), delusion disorder (10) and psychotic depression (5). AD = Alzheimer’s disease dementia (10 patients with AD and 3 patients with AD+CVD). CVD = cerebrovascular disease. A patient suffering pure vascular dementia, two patients with frontotemporal lobar degeneration, one patient with primary progressive aphasia and two patients with other organic diagnoses were left out of the analysis. Schizophrenia over 10 years = control group. The data are presented as mean ± SD, unit pg/ml. The group difference for statistical significance is tested with ANOVA.
Table 19. The biomarker concentrations by diagnoses.

<table>
<thead>
<tr>
<th>Clinical diagnosis</th>
<th>The CSF sample</th>
<th>Delusion disorder</th>
<th>VLOSLP</th>
<th>Depression</th>
<th>AD</th>
<th>AD+CVD</th>
<th>VaD</th>
<th>FTLD (PPA*)</th>
<th>Other organic diagnosis</th>
<th>Schizophrenia &gt; 10 years</th>
</tr>
</thead>
<tbody>
<tr>
<td>n</td>
<td>51</td>
<td>10</td>
<td>10</td>
<td>5</td>
<td>10</td>
<td>3</td>
<td>1</td>
<td>3</td>
<td>2</td>
<td>7</td>
</tr>
<tr>
<td>CSF Aβ42</td>
<td>636 ±283</td>
<td>603 ±195</td>
<td>704 ±268</td>
<td>631 ±163</td>
<td>550 ±367</td>
<td>397 ±133</td>
<td>642</td>
<td>549 ±427</td>
<td>491 ±23</td>
<td>865 ±323</td>
</tr>
<tr>
<td>CSF tau</td>
<td>276 ±199</td>
<td>266 ±160</td>
<td>274 ±199</td>
<td>158 ±39</td>
<td>284 ±162</td>
<td>160 ±125</td>
<td>336</td>
<td>265 ±112</td>
<td>180 ±46</td>
<td>322 ±146</td>
</tr>
<tr>
<td>CSF p-tau</td>
<td>55 ±37</td>
<td>52 ±25</td>
<td>48 ±27</td>
<td>30 ±7.1</td>
<td>67 ±22</td>
<td>18 ±14</td>
<td>62</td>
<td>50 ±17</td>
<td>56 ±47</td>
<td>56 ±17</td>
</tr>
<tr>
<td>CSF Aβ42/p-tau</td>
<td>16.7 ±14.6</td>
<td>21.1 ±26.5</td>
<td>18.8 ±10.3</td>
<td>21.9 ±7.8</td>
<td>9.1 ±6.6</td>
<td>18.5 ±14.2</td>
<td>10.4</td>
<td>10.1 ±4.7</td>
<td>13.2 ±10.6</td>
<td>16.6 ±7.6</td>
</tr>
</tbody>
</table>

CSF = cerebrospinal fluid. VLOSLP = very late-onset schizophrenia-like psychosis. AD = Alzheimer’s disease dementia. CVD = cerebrovascular disease. VaD = vascular dementia. FTLD = frontotemporal lobar degeneration (includes one patient with primary progressive aphasia (PPA)). Schizophrenia over 10 years = control group. Other organic diagnosis includes two subjects with alcohol dementia and one with delirium. The data are presented as mean ± SD, unit pg/ml. The group differences do not reach statistical significance by ANOVA after post hoc correction.
9.3.1 Analysis of those with a discrepancy between clinical diagnosis and AD biomarker findings

There were a total of five patients with a diagnosis of AD dementia having all three CSF biomarkers (Aβ42, tau and p-tau) at the normal level. Two of those with a combined diagnosis of AD + CVD displayed a clear cognitive decline, both hippocampuses were small in MRI (Scheltens 2) and severe vascular degeneration was observed (Fazekas 3). Accordingly, they seemed to have had pure vascular dementia and not AD according to the CSF biomarkers. One of those patients with a pure AD dementia diagnosis had small hippocampus (Scheltens 2-3) and also vascular degeneration (Fazekas 2), so she probably also had vascular dementia based on the normal CSF biomarkers.

The other two with an AD dementia diagnosis and normal CSF biomarkers did not seem to have had an organic brain disease at all. One had experienced a bipolar disorder with psychotic depression (no psychotic episodes before) based on the follow-up information, clear amnestic memory problems and hippocampal atrophy (Scheltens 2-3) in MRI but no vascular changes. The other had an amnestic cognitive decline but a normal MRI. These two patients seem to have been clearly misdiagnosed.

The two individuals with an AD diagnosis and a low Aβ42 level but normal tau and p-tau concentrations may well have had very early stage of AD. Both displayed evidence of hippocampal atrophy (Scheltens 2), and in the follow-up of 1.5 years, the cognitive decline had a typical Alzheimer-like progression.

The one patient with the diagnosis of AD+CVD showing low Aβ42 level but normal tau and p-tau concentrations did exhibit hippocampal atrophy (Scheltens 3) and severe vascular degeneration (Fazekas 3) so she may well have had both disorders.

The subject with a diagnosis of VLOSLP with both a low levels of Aβ42 and elevated tau and p-tau concentrations did not have major memory problems. Her MMSE was 28/30 and in CERAD only in the recognition of the wordlist she was slightly below normal and her score in the clock-drawing test was 4/6. Her MRI did not show any signs of hippocampal atrophy or vascular degeneration. Therefore, it would have been very difficult to assess her as having AD without the CSF biomarkers.

9.4 DISCUSSION

As expected, the psychotic patients who had suffered schizophrenia for decades did not display a profile of CSF biomarkers typical for Alzheimer’s disease. In one earlier study examining elderly early-onset schizophrenia patients, several had normal CSF tau or p-tau levels, but Aβ42 concentration was lower than that found in healthy controls (Frisoni et al. 2011). In our study, three out of seven patients with early onset schizophrenia had normal AD biomarkers, one had a low Aβ42 level but normal tau and p-tau levels, and three had a normal Aβ42 concentration but elevated tau or p-tau levels so the results are rather inconclusive.

Five out of thirteen clinically diagnosed AD patients had completely normal concentrations of AD biomarkers in CSF. Three of these seemed to have had pure vascular dementia and two were clear misdiagnoses (no organic brain disease at all). Three patients had a low Aβ42 level with normal tau or p-tau values.

Two patients with a low Aβ42 level but a normal tau or p-tau concentrations were probably at a very early stage of AD. Aβ42 decreases before one sees any elevations in tau or p-tau concentrations, so this profile can well be indicative of early AD pathology (Jack et al. 2010).

One reason for the tendency for overdiagnosing AD in memory patients may be that in Finland the patient only receives reimbursement for medication (acetylcholinesterase
inhibitors or memantine) by having a verified diagnosis of AD or PD dementia, so when there is a doubt of the cause, the clinicians tend to ensure the availability of subsidized medication.

Among the subjects with psychiatric diagnoses there was only one subject with a clear Alzheimer-like biomarker profile. However, she was not demented and had a normal MRI. One reason for this finding may be that the biomarkers may become abnormal before there are any prominent clinical symptoms of AD as described earlier in the literature (Herukka et al. 2007).

The mean biomarker concentrations in the different diagnostic groups did not show the statistical differences as clear as would be expected in these types of studies. For example, the group of ten AD patients had a relatively high mean concentration of CSF Aβ42 and both AD and AD+CVD groups showed a low mean CSF tau level. When combined, patients with AD and AD+CVD displayed a lower mean CSF Aβ42 level than those in other groups, but the mean concentration was still rather high. This probably results from the proportion of VaD and psychiatric disorders being misdiagnosed as AD and thus presenting more normal concentrations. Certainly, the diagnostic subgroups were too small for single biomarkers to achieve statistical significance after post hoc corrections. However, the AD group did exhibit with highest p-tau levels leading to lowest CSF Aβ42/p-tau ratio, which is a common finding in AD subgroups in cross-sectional measurements.

It is interesting that in this sample of psychotic elderly people, four out of the 17 subjects (23.6%) with a diagnosis of an organic brain disease (in the final analysis, after the CSF biomarker analysis) seemed to have had vascular dementia. In population based studies the proportion of vascular dementia has been significantly lower, e.g. 15.8% in the study by Lobo and co-workers (Lobo et al. 2000). One explanation can be that vascular dementia tends to cause psychotic symptoms more often in the early stages and the patients are thus more likely to be referred to psychiatry, or that classical AD is more easily diagnosed in primary care or in geriatric settings, and the patients are not referred to psychiatry even when psychotic symptoms are present. Whatever the reason, it is very important to bear in mind the possibility of vascular small vessel disease as a reason for psychotic symptoms.

Two of the FTD patients had low CSF Aβ42 levels, as has been reported previously (de Souza et al. 2011b). Usually the most effective way to distinguish FTD from AD is the lower CSF Aβ42/p-tau ratio in AD (de Souza et al. 2011b), but it did not reveal a significant difference to FTD in the present study, possibly due to potentially misdiagnosed patients in the AD group or the small number of FTDs.

The limitation of this study was the relatively high number of patients refusing or otherwise being excluded that did not provide a CSF sample. The majority of non-participating patients were those with a psychiatric diagnosis, which may have been due to their symptoms i.e. suspiciousness about the LP and paranoid behavior towards the figures of authority. The women were more likely to refuse to undergo the LP and all whose LP failed were women. The reasons for failure of the LP were usually related to obesity and some of the women were also very sensitive to puncture, and they were not willing to allow a next attempt if the previous failed.

This study shows the importance and the usefulness of the CSF biomarkers as part of the diagnostic procedure for detecting AD and other dementing illnesses. Especially in a psychiatric setting, it seems to be very difficult even with good availability of MRI imaging, neuropsychological examinations and multiprofessional team, to make an exact diagnosis of memory disorders without access to the CSF biomarkers of AD. The study strongly recommends that the patients with AD dementia diagnosis based on very atypical or psychotic symptoms should not be included in AD groups of clinical studies unless this is confirmed by CSF biomarkers of AD or some other additional biomarker.
10 General discussion and future prospects

Plasma amyloid beta and other blood biomarkers

Based on the present knowledge, the single measurements of plasma Aβ42 or Aβ40 are not useful biomarkers for AD, mostly because of the very large overlapping of Aβ concentrations in blood. However, in population samples, a difference is often seen between groups of MCI patients, AD patients and the cognitively healthy. In the present study, Aβ42 in plasma appeared to be reduced over time in conjunction with the cognition-imparing process, mostly AD. In contrast, the level of Aβ40 did not decrease, or it even increased. This may indicate that serial measurements of Aβ reveal the longitudinal change of Aβ42 in the periphery in addition to that seen in the CSF. The decrease over time is probably due to decreased clearance or increased accumulation to the CNS, but the design of the present study is not able to clarify this pathophysiology.

The low ratio of Aβ42 and Aβ40 in a single measurement, indicating a decreased concentration of Aβ42 and stable or increased Aβ40, is believed to be the most informative plasma measure indicating future cognitive decline or conversion to AD (van Oijen et al. 2006, Okereke et al. 2009, Schupf et al. 2008, Yaffe et al. 2011). It has recently been linked directly to brain fibrillar amyloid accumulation with PIB PET examination (Devanand et al. 2011, Lui et al. 2010). Based on a prior study on peripheral Aβ, it may well reflect the change of Aβ in the central compartment. Aβ is very rapidly transported from the CNS to periphery (Ghis et al. 2004, Gherei-Egea et al. 1996) and because secretion through kindey and liver is rapid as well, an imbalance in the clearance mechanism of CNS may therefore produce a constant flow of less Aβ42 and relatively more Aβ40 to the periphery that is subsequently seen as constantly altered ratio of Aβ42/Aβ40 in plasma. It is also possible that changes in the plasma Aβ are seen later than the changes in CSF Aβ as the concentrations in the latter have been reported as being rather stable during the symptomatic stages of AD.

The results in the present study are similar to the large single measurement settings but provide further support for the theory that there is a longitudinal decrease in the Aβ42/Aβ40 ratio. The impact of this finding is related to understanding the disease progression and developing tools for patient follow-up. Clearly, the disease stage affects the final amount of Aβ present in the periphery. However, much larger longitudinal studies with multiple time points and long follow-up will be needed in order to study and understand the impacts of the multiple confounding factors. The renal and liver function may affect the results because of the binding of Aβ in the plasma proteins. On the other hand, they influence the longitudinal change only if impairment in these organ functions takes place during follow-up. Renal function should be reported in the future studies, which was one limitation in the present setting. In addition, large collaborations like ADNI provide the possibility to confirm the AD diagnosis with other biomarkers. A study of plasma biomarkers with more certain diagnostic groups using CSF biomarkers and more complex imaging could benefit also the plasma biomarker study.

New and very promising biomarkers of plasma are being developed or still waiting to be found. There may not be only one plasma biomarker but a battery of individual molecules that together serve as an accurate biomarker. It is anticipated that in the future, the AD process may possibly be diagnosed by an ideal biomarker, perhaps a blood test, earlier than the appearance of any symptoms. In the case that the diagnostical methods develop more rapidly than the therapeutics, the clinical field faces an ethical problem. The information of an imminent AD diagnosis must not be provided to asymptomatic patients until a disease-modifying treatment with at least tolerable adverse effects becomes available.
Longitudinal biomarker changes

With a deeper understanding of longitudinal CSF biomarker changes, AD diagnostics can be improved by advancing early patient selection in clinical trials studying the disease-modifying treatments. The use of current impairment-decelerating therapeutics can be expected to benefit from early and accurate diagnosis. By backing up diagnostic procedures such as the biomarkers, the long-term prognosis of the AD patients can be improved and the independence of the patients can be supported so that they may avoid institutionalization.

The hypothetical model on the temporal order of the AD biomarkers during the disease process (Jack et al. 2010) is supported by the findings in the present study. It was found that there was a longitudinal decrease in CSF Aβ42 levels in the group of healthy control individuals, which may indicate the common problem that the control group is contaminated by the presence of future MCI and AD patients that do not yet display cognitive symptoms. The CSF Aβ42 level remained rather stable after the beginning of the symptoms, but during the very late stage AD there was a longitudinal decrease of the Aβ42 level. Earlier studies have reported the CSF Aβ42 levels to decrease in AD (Tapiola et al. 2000, Mollenhauer et al. 2005, Kanai et al. 1998, Wahlund & Blennow 2003, Lo et al. 2011) but an increase has been reported only once (Bouwman et al. 2007b). The rest of the longitudinal studies have reported no change (Kester et al. 2009, Andreasen et al. 1999a, Andreasen et al. 1999b, Hoglund et al. 2005, de Leon et al. 2006, Andersson et al. 2008, Brys et al. 2009, Blennow et al. 2007, Zetterberg et al. 2007, Buchhave et al. 2009). One study reported no change in the CSF Aβ42 level over time, but this was conducted on asymptomatic individuals and subjects with decreasing CSF Aβ42 levels were identified as those exhibiting worse performance in cognitive testing (Stomrud et al. 2010). The present study may be the largest reported serial measurement of CSF biomarkers so far (Table 2).

The discrepancy between the findings that the levels of CSF Aβ42 either increase or show no change may be due to differences in the patient selection and clinical diagnosis. In addition, the methods of Aβ measuring have varied substantially. If one excludes the single result of an increasing CSF level of Aβ42, it seems probable that Aβ42 does not increase. However, this only emphasizes the sensitivity of the setting to bias. It has been proposed that perhaps the change in the CSF Aβ42 level takes place at such an early stage that the minimal change occurring after the onset of symptoms cannot be properly identified (Tapiola et al. 2000, de Leon et al. 2006, Buchhave et al. 2009) but there is also a view that two or more follow-up measurements of CSF Aβ42 could help to detect even this slight change and form a reliable slope of decline (Lo et al. 2011). The fact is that it was not possible to provide more than two time points for some patients and therefore the results had to be based on one follow-up only, which is a limitation of the present study in addition to the heterogenous study population. However, a recent ADNI study with almost the same number of patients corroborated these present results of a longitudinal decline in the CSF Aβ42 concentration (Lo et al. 2011).

The longitudinal changes in biomarkers of neurodegeneration such as the increase of CSF tau and p-tau take place several years after the amyloid pathology has begun in typical cases of sporadic AD. The finding that CSF p-tau would actually begin to decrease at the later stage of AD has not been reported earlier except for the results of one very small study (Mollenhauer et al. 2005). In the present study, the decrease of CSF p-tau level was associated with the cognitive decline, which means that it refers to a change taking place at very late stages of the disease, which could be the time there is a rapid loss of neurons. Nonetheless, the finding is not directly contradictory to the hypothesis of Jack et al. although speculation will need to be tested in subsequent studies.

If research were to be conducted to try to confirm Jack’s hypothesis, i.e. one wishes to study reliably very early longitudinal changes for CSF Aβ42 pathology, it would be necessary to include healthy individuals without any knowledge about whether they represent normal or abnormal ageing and large numbers of subjects would be needed. At
present, the moment when CSF Aβ42 levels start to decline in the course of AD has not been exactly determined.

**Correlation of CSF biomarkers to AD pathology**

In the present study, the hypothesis was that the CSF biomarkers would reflect the AD neuropathological findings from cortical brain biopsy. An earlier study has shown that low CSF Aβ42 levels correlate inversely to post mortem Aβ plaque count at autopsy (Strozyk et al. 2003) and that a high CSF tau concentrations correlate to the presence and magnitude of neurofibrillary tangles at autopsy (Tapiola et al. 2009, Tapiola et al. 1997). It was found that the low CSF Aβ42 level correlated inversely also to the plaque count and percentual area of amyloid plaques in the cortical biopsy in AD as well as all subjects in the study sample of patients with suspected NPH. This result is logical considering the earlier findings but previously, it has been difficult to confirm these findings due to methodological difficulties related to collection of the biopsy samples. The finding is also in accordance with data revealed by amyloid imaging studies.

These findings also indicate that CSF tau and p-tau reflect the presence of neurofibrillary tangles in the biopsy samples consisting of hyperphosphorylated tau, which is a result similar to that emerging from the autopsy data (Tapiola et al. 2009, Tapiola et al. 1997).

Patients with presumed NPH very often display neuropathological signs of a progressive neurodegenerative disease such as AD that cannot be properly diagnosed with neuroimaging, neuropsychological methods and clinical status. In these cases, CSF biomarkers can provide additional assistance in the diagnosis.

The results confirm the proposed benefits of using CSF biomarkers as reflectors of AD pathological hallmarks in the brain tissue also before the autopsy. When available, a brain biopsy may provide additional information on a patient’s AD pathology and confirm the differential diagnosis, at least in atypically symptomatic patients.

**CSF biomarker gradients**

It has been suggested that there may be a gradient between ventricular and lumbar concentrations of CSF biomarkers. The present study is one of the few works comparing the biomarkers between the ventricular CSF space and those of CSF collected by lumbar puncture. It seems that the neuronal trauma resulting from placing the ICP catheter increases the amount of tau liberated into the CSF, which also increases the concentration in the lumbar sample. However, there is no difference between the lumbar CSF Aβ42 concentration whether the neuronal trauma in the brain is present or not and therefore the CSF Aβ42 correlates inversely to the brain tissue amyloid plaque proportion. In general, the concentration of CSF Aβ42 taken from the brain ventricle is lower than that collected by LP and concentrations of tau and p-tau are higher in the brain ventricle than in the samples taken by LP.

**Amyloid cascade hypothesis**

A common assumption has been that CSF Aβ42 level is not directly associated with cognitive decline in MCI or AD because of the early decrease of Aβ42 concentration and it has already reached a plateau before any measurement is conducted (i.e. when symptoms appear). Therefore, the cognitive symptoms are believed to relate more to abnormal biomarkers of neurodegeneration than to those of amyloid pathology (Jack 2010). However, recent studies have concluded that a low CSF Aβ42 level could predict worse performance in episodic, semantic and working memory testing in potentially early stages of AD or even healthy individuals (Stomrud et al. 2010, Rolstad et al. 2011).

The present findings are in agreement with earlier studies, i.e. abnormal levels of Aβ42 together with a high p-tau level are related to worse outcome in the later memory function

However, given that also the theory of tau pathology begins rather early, there is no certainty that the cognitive changes seen in the present study are related to changes in the CSF amyloid concentrations and amyloid pathology; instead they may quite as well result from the ongoing AD neurodegeneration process. In order to clarify the distinctive roles of amyloid cascade and tau pathology separately, a future study should be designed to focus on those with very early AD processes which exist even though only few signs of neurodegeneration are present but there may be some indications of abnormal amyloid processing.

**Accuracy of clinical AD diagnosis and the role of CSF biomarkers in atypical early AD**

This study showed that there might be some inaccuracy of diagnosing patients with late-onset psychotic symptoms for AD and other dementing disorders in Finland for various reasons. First of all, the medical reimbursement system encourages physicians to overdiagnose AD in order provide them with approved medication. Secondly, it seems that the proportion of VaD patients is increased among in-ward late-onset psychosis patients and these patients may be mistakenly diagnosed as having AD. The use of CSF biomarkers may increase the accuracy of the AD diagnosis in psychiatric settings. In addition, caution is necessary when including patients with atypical AD symptoms into AD groups of clinical studies without confirming the diagnosis with CSF biomarkers, PIB PET or some other supportive feature of AD criteria.
11 Conclusions

1. Low plasma levels of Aβ42 and a reduced Aβ42/Aβ40 ratio at baseline predict later cognitive decline in patients with MCI or AD. Plasma Aβ42 levels and Aβ42/Aβ40 ratio decrease during the cognitive decline in patients with MCI or AD.

2. The longitudinal changes of the CSF biomarkers of AD appear to occur in a temporal order and longitudinal observation may provide information of the disease stage. CSF hyperphosphorylated tau decreases in late AD and the rate of decline is connected to the rate of cognitive decline.

3. The CSF Aβ42 concentration is decreased together with high amyloid load in cortical brain biopsy of patients with AD and suspected normal pressure hydrocephalus. The CSF Aβ42 level is inversely correlated with the area of accumulated Aβ plaques in the biopsy. Increased CSF tau and p-tau levels are associated with the increased presence of hyperphosphorylated tau in cortical brain biopsy. Comorbidity of NPH and AD is common and CSF biomarkers represent a useful a tool for differential diagnostics.

4. There is a concentration gradient between ventricular and lumbar CSF biomarkers of AD. The Aβ42 level is higher in lumbar samples whereas tau and p-tau concentrations are higher in ventricular samples.

5. AD may be overdiagnosed in patients with late-onset psychosis in Finland. CSF biomarkers are useful for distinguishing AD from other dementias and psychiatric conditions with behavioral symptoms.
12 References


American Psychiatric Association 1994, Diagnostic and statistical manual of mental disorders.


Arrighi, H.M., Neumann, P.J., Lieberburg, I.M. & Townsend, R.J. 2010, "Lethality of


Blasko, I., Jellinger, K., Kemmler, G., Krampla, W., Jungwirth, S., Wichart, I., Tragl, K.H. & Fischer, P. 2008a, "Conversion from cognitive health to mild cognitive impairment and Alzheimer’s disease: prediction by plasma amyloid beta 42, medial temporal lobe atrophy


Buerger, K., Ewers, M., Pirtilta, T., Zinkowski, R., Alafuzoff, I., Teipel, S.J., DeBernardis, J.,


Forsberg, A., Engler, H., Almkvist, O., Blomquist, G., Hagman, G., Wall, A., Ringheim, A.,


Hansson, O., Zetterberg, H., Buchhave, P., Andreasson, U., Londos, E., Minthon, L. &


Alzheimer's & Dementia, vol. 6, no. 3, pp. 212-220.


Kalaria, R.N., Maestre, G.E., Arizaga, R., Friedland, R.P., Galasko, D., Hall, K., Luchsinger,


Schipke, C.G., Jessen, F., Teipel, S., Luckhaus, C., Wiltfang, J., Esselmann, H., Frolich, L.,


Shaw, L.M., Korecka, M., Clark, C.M., Lee, V.M. & Trojanowski, J.Q. 2007, "Biomarkers of
neurodegeneration for diagnosis and monitoring therapeutics”, Nature Reviews Drug Discovery, vol. 6, no. 4, pp. 295-303.


Westman, E., Simmons, A., Zhang, Y., Muehlboeck, J.S., Tunnard, C., Liu, Y., Collins, L.,


Zhang, Y.W., Thompson, R., Zhang, H. & Xu, H. 2011, "APP processing in Alzheimer's
disease", Molecular Brain, vol. 4, pp. 3.

Alzheimer’s disease is the most common cause of dementia. When the first symptoms of memory deficit take place, neuronal damage has already irreversibly happened. Thus, better ways to achieve an earlier diagnosis will need to be devised. The aim of this study was to explain the relationship of the plasma and cerebrospinal fluid biomarker levels to cognition and brain tissue pathology. The results demonstrated that low levels of CSF Aβ42 are associated with a high amyloid plaque load of brain biopsies. In addition, increased CSF tau and p-tau levels are associated with the presence of tau in the cortical biopsy.