Biomarkers as Monitors of Drug Effect, Diagnostic Tools and Predictors of Deterioration Rate in Alzheimer’s Disease

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Dissertation presented at Uppsala University to be publicly examined in Enghoffsalen, Ingång 50, bv, Akademiska sjukhuset, Uppsala, Thursday, May 16, 2013 at 13:00 for the degree of Doctor of Philosophy (Faculty of Medicine). The examination will be conducted in Swedish.

Abstract

Decreased amyloid-ß42 (Aß42), increased total tau (t-tau) and phosphorylated tau (p-tau) in cerebrospinal fluid (CSF) reflect histopathological core changes in the most common dementia disorder, Alzheimer’s disease (AD). They discriminate AD from healthy controls and predict conversion to AD with a relatively high accuracy. Memantine, an uncompetitive NMDA-receptor antagonist, is indicated for symptomatic treatment of AD. The first aim of this thesis was to investigate effects of memantine on CSF concentrations of Aß42, tau and p-tau. Secondly, the aim was to explore the relation between these CSF biomarkers and retention of the amyloid biomarker Pittsburgh compound B using positron emission tomography (PIB PET), regional glucose metabolism measured with 18Fluoro-2-deoxy-d-glucose (FDG) PET and neuropsychological test performance. The third aim was to investigate their possible utility as predictors of future rate of AD dementia deterioration. All patients in the studies were recruited from the Memory Clinic, Uppsala University Hospital. In study I CSF p-tau concentrations in 11 AD patients were reduced after twelve months treatment with memantine, indicating that this compound may affect a key pathological process in AD. Results from study II showed that the concentrations of CSF Aß42 are lower in PIB+ patients than in PIB- patients, and that the PIB retention was stable during 12 months. In study III 10 patients with the diagnoses AD (6 PIB+/4 PIB-) and 8 subjects (1 PIB+/7 PIB-) with frontotemporal dementia were included. PIB+ patients had lower psychomotor speed measured by performance on the Trail Making Test A and impaired visual episodic memory compared to the PIB- patients. The initial clinical diagnoses were changed in 33% of the patients (6/18) during follow-up. Study IV is the first-ever report of an association between high CSF tau and dying in severe dementia. These 196 AD patients were followed up to nine years after baseline lumbar puncture. Moreover, CSF t-tau concentrations above median was associated with an increased risk of rapid cognitive decline (OR 3.31 (95% CI 1.53-7.16), independently of baseline functional stage. Thus, a clear association between high levels of CSF t-tau and p-tau and a more aggressive course of the disease was shown.

Keywords: Alzheimer's disease, biomarkers, CSF, PIB PET, amyloid-beta, tau, rapid cognitive decline, dying in severe dementia, mortality, neuropsychological tests

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urn:nbn:se:uu:diva-196965 (http://urn.kb.se/resolve?urn=nbn:se:uu:diva-196965)
To my varied talent, able and beautiful grandmothers Lahja Bergquist and Hilma Degerman who both slowly faded away in dementia

and for the future of my children Thyra and August
List of papers

This thesis is based on the following four papers, which are referred to in the text by their Roman numerals.


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Abbreviations

Aβ  Amyloid-β
AD  Alzheimer’s disease
ADCS-ADL Alzheimer's Disease Cooperative Study Activities of Daily Living Inventory
APOE Apolipoprotein E
APP Amyloid precursor protein
BACE1 β-site amyloid precursor protein cleaving enzyme
BPSD Behavioural and Psychological Symptoms of Dementia
bvFTD Frontotemporal dementia, behavioural variant
ChEIs Cholinesterase inhibitors
CJD Creutzfeldt-Jakob’s disease
CMRglc Cerebral metabolic rate of glucose
CNS Central nervous system
CSF Cerebrospinal fluid
Cdk5 Cyclin-dependent protein kinase 5
CT Computed Tomography
DLB Dementia with Lewy Bodies
DSM-IV Diagnostic and Statistical Manual of Mental Disorders, Fourth Edition
ELISA Enzyme-linked immunosorbent assay
FAS A verbal fluency task
FDG 18-Fluoro-2-deoxy-d-glucose
FTD Frontotemporal dementia
GFAP Glial fibrillary acidic protein
GSK3 Glycogen synthase kinase 3
I2 PP2A Inhibitor of PP2A
IL Interleukin
LP Lumbar puncture
MCI Mild cognitive impairment
MMSE Mini Mental State Examination
MRI Magnetic Resonance Imaging
MTA Medial temporal lobe atrophy
<table>
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<td>NFT</td>
<td>Neurofibrillary tangles</td>
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<td>NINCDS-ADRDA</td>
<td>National Institute of Neurological and Communicative Disorders and Stroke and Alzheimer’s disease and Related Disorders Association</td>
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<td>NMDA</td>
<td>N-methyl-D-aspartate</td>
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<td>NPI</td>
<td>Neuropsychiatric Inventory</td>
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<td>PET</td>
<td>Positron emission tomography</td>
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<td>PIB</td>
<td>Pittsburgh compound B</td>
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<td>PNFA</td>
<td>Progressive non-fluent aphasia</td>
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<td>PP2A</td>
<td>Protein phosphatase 2A</td>
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<tr>
<td>p-tau</td>
<td>Phosphorylated tau</td>
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<tr>
<td>rCMRglu</td>
<td>Regional cerebral metabolic rate of glucose</td>
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<tr>
<td>ROI</td>
<td>Regions of interest</td>
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<tr>
<td>SCI</td>
<td>Subjective cognitive impairment</td>
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<td>SPECT</td>
<td>Single-photon emission computed tomography</td>
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<td>TMT</td>
<td>Trail Making Test</td>
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<td>t-tau</td>
<td>Total tau</td>
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Introduction

Alzheimer’s disease

Background
Dementia is a syndrome characterised by loss of function in multiple cognitive domains. Dementia implies by definition a loss severe enough to cause impairment in activities of daily living including social and occupational functioning. Importantly, the condition must represent a significant decline from a previously higher level of functioning. To exclude delirium, the cognitive deficits must have persisted at least 6 months. Dementia is a common and age-related syndrome that is estimated to affect 1% of the population aged 65-70 years and up to 50% of those aged over 95. Dementia syndromes have a huge impact on both those who suffer from it and their caregivers, with substantial health, societal and economic consequences and they often lead to a premature death.

Alzheimer’s disease (AD) causes the majority of all cases of dementia. It is a chronic, progressive neurodegenerative disorder and was first described by the German psychiatrist and neuropathologist Alois Alzheimer in 1906. Histopathological core changes that characterise AD are neurodegeneration and accumulation of senile plaques and neurofibrillary tangles, but also inflammation and gliosis. In the beginning of the 1980s Glenner & Wong managed to purify and characterise the amyloid-beta protein (Aβ), the central content of the senile plaques. The Aβ peptide is a metabolite of a larger membrane bound precursor protein called the amyloid precursor protein (APP). In 1991, the amyloid hypothesis postulated that Aβ is the fundamental cause of the disease. Support for this postulate came from the discovery of a pathogenic mutation in the gene coding for APP on chromosome 21, together with the fact that people with trisomy 21 (Down’s Syndrome) who thus have an extra gene copy, almost universally exhibit an Alzheimer encephalopathy by 40 years of age. Almost at the same time it was shown that tangles are composed of abnormally hyperphosphorylated tau protein. These key findings marked the start of modern AD research.
Most cases of Alzheimer’s disease are sporadic, and only less than 1% has known mutations in the APP or presenilin genes. Nevertheless, a large number of susceptibility genes have been reported to associate with sporadic AD. Most firmly identified as a genetic risk factor is the ε4 allele of the apolipoprotein E (APOE) gene. The frequencies of APOE ε4 varies among ethnic groups (5.2%-31%), but are higher among AD patients (37%-64%). Compared to individuals with no ε4 allele, the risk for AD is 2- to 3-fold in subjects with one ε4 allele and about 12-fold in those with two ε4 alleles. Probably, several susceptibility and protective genes interact with the aging process and the environment. The most common risk factors for Alzheimer's disease are high age, heredity and head trauma. Cerebrovascular disease is a main contributor to dementia in very old age.

Besides the two different hallmark types of AD pathology, i.e. extracellular senile plaques and intracellular neurofibrillary tangles, AD is characterised by loss of neurons and synapses in the cerebral cortex. This loss results in progressive atrophy of the affected regions. In 1991 Braak & Braak classified the neuropathological AD into 3 stages of Aβ deposits and 6 stages of neurofibrillary changes. The senile plaques are first encountered in the basal portions of the frontal, temporal and occipital lobes. Then the Aβ deposits spread to other cortical areas and finally to the primary sensory and motor areas. Outside the cerebral cortex the final stage also involves subcortical structures as striatum, thalamus and hypothalamus. The tau pathology spreads from the transentorhinal and entorhinal cortex to the hippocampus, and then to the temporal, parietal and frontal cortices,
followed by the primary motor and somatosensory cortices and finally the occipital cortex. There are recent reports of neuron-to-neuron transmission of both Aβ and tau pathology\textsuperscript{17,18}. There seems to be a strong correlation between the severity of the cognitive decline and the density of neurofibrillary tangles and soluble Aβ\textsuperscript{19,20}, and to some extent with Aβ plaques, especially with mature plaques. Cognitive impairment in patients with AD plaque pathology is detectible only in the presence of neurofibrillary tangles. Finally, concomitant pathological abnormalities, most importantly cerebrovascular disease, lower the threshold for detectible cognitive changes and dampen the association between density of AD pathologies and severity of cognitive impairment. The Aβ changes are proposed to precede the diagnosis of AD by about 15-20 years\textsuperscript{21}, and the long-term disease survival varies from a few years to about two decades\textsuperscript{22}.

Figure 2. Progression of neuropathological core changes in AD i.e. senile plaques, neurofibrillary tangles and atrophy (modified from Svensk geriatrik nr 3:2012).

The clinical course of Alzheimer’s disease

The first subjective cognitive impairment (SCI) symptoms are subtle and not possible to distinguish from normal aging. The period of gradual cognitive decline in episodic memory as well as non-memory domains is probably progressing up to a decade before the onset of dementia. In the stage of mild cognitive impairment (MCI), performance in psychometric tests is affected
but the patient is still independent in all daily activities\textsuperscript{23}. Decline in recent memory is an early symptom in AD and reflects hippocampal dysfunction. As the illness advances, the functional impairment and dependence increases substantially. There is a progressive deterioration of cognitive functions such as prominent loss of episodic memory, anoma, constructional apraxia and disturbances in executive functioning, perceptual skills, logical thinking, attention and orientation, mirroring neurodegeneration in the parietotemporal cortices. Associated symptoms are mood and behavioural changes. Mild dementia is by definition interfering significantly with work and normal social activities\textsuperscript{24,25}. Over time, mild dementia progresses to moderate dementia and dependency in self-care activities such as bathing and dressing.

\begin{figure}
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\includegraphics[width=\textwidth]{figure3.png}
\caption{Schematic model of the pathological AD processes in the brain in relation to cognitive decline and clinical stage of the disease.}
\end{figure}

In the final stage of severe dementia, the patient will lose the ability to perform all activities of daily living. End-stage AD is also associated with loss of gait and communicative abilities, physical rigidity and the appearance of primitive reflexes\textsuperscript{26}. Common terminal death causes are bronchopneumonia and ischemic heart disease\textsuperscript{27}. Alzheimer’s disease varies widely both in its clinical manifestations and rate of cognitive decline, from an aggressive course with death within a few years to a disease which can progress slowly over decades\textsuperscript{28-30}. 
Diagnosis of Alzheimer’s disease

In clinical routine the diagnosis of AD is based on a careful description of onset and symptoms, neuropsychological test results, neuroimaging, identification of typical symptoms of AD and exclusion of other known causes of dementia. The NINCDS-ADRDA and the DSM IV criteria have been the most commonly used diagnostic criteria for AD. These criteria describe a two-step diagnostic process where there is an initial identification of a dementia syndrome, and secondly is characterized by an insidious onset with slowly progressive cognitive deterioration over several years. Eventually, AD reaches the end-stage with severe dementia, impossible to distinguish from other dementia disorders. Disease severity can be estimated by cognitive tests and scales based on interviews of caregivers measuring daily functioning, e.g. Alzheimer’s Disease Cooperative Study Activities of Daily Living (ADSC-ADL). One common tool for assessing behavioral and psychological symptoms is the Neuropsychiatric Inventory (NPI). The most commonly used screening test for cognitive impairment in the world is the Mini-Mental State Examination (MMSE), which was introduced in 1975. The MMSE is a series of questions and tests, with a maximum score of 30 points. It assesses several areas of cognitive function including orientation, registration, memory, attention, calculation, recall, language and visuospatial and constructional abilities. Computed tomography (CT) has since its introduction in the 1970s become an important imaging method to detect structural changes in the brain such as tumors, atrophy, hemorrhages and infarctions. A well-established technique used for visual rating assessment is the Scheltens method, which uses a five point scale to grade atrophy in the medial temporal lobes (MTA). As a diagnostic tool, MTA scores differentiate between AD patients, with moderate to severe dementia, and cognitive healthy controls with a sensitivity of 70-100% and almost as high specificity. In the 1980s magnetic resonance imaging (MRI) was introduced which provides a better contrast between normal and diseased tissue compared to CT scans, but with more medical restrictions. Functional imaging as single-photon emission computed tomography (SPECT) has been an additional diagnostic tool at many memory clinics. Furthermore, in the last decade, lumbar puncture is a routine examination and analyses of three cerebrospinal fluid (CSF) biomarkers Aß42, total tau (t-tau) and phosphorylated-tau (p-tau) have improved the diagnostic accuracy. Another functional imaging method, the 2-deoxy-2[F-18] fluoro-D-glucose positron emission tomography (FDG PET) is available at some specialist memory clinics and for research purpose. The accuracy of the clinical diagnosis of AD compared to the neuropathologically confirmed diagnosis varies between 65-90% in different studies.
Revised diagnostic criteria for probable AD were proposed in 2007\textsuperscript{37} as a response to the increased research demand for earlier and more specific diagnosis. They were based on new distinctive markers of the disease including structural brain changes on MRI, molecular neuroimaging changes visualised with PET, analyses of cerebrospinal fluid biomarkers and genetic evidence. Recommendations and another new set of AD criteria, including the preclinical, MCI and dementia stages, were published in 2011 by the National Institute on Aging and the Alzheimer’s Association. This new framework for staging preclinical AD is aimed to define future study cohorts at risk of developing AD dementia, and is divided into three stages representing preclinical phases towards MCI AD\textsuperscript{38}. For MCI due to AD there are two sets of criteria. The first is core clinical criteria based on evidence of decline in one or more cognitive domain compared with the person’s previous level. Moreover, the patients should have preserved independence of function in daily life, with minimal aids or assistance. The second criteria are for research purpose and incorporate biomarkers\textsuperscript{39}. The update of the AD dementia criteria classifies individuals with dementia caused by AD in ‘probable AD dementia’, ‘possible AD dementia’ and ‘probable/possible AD dementia with evidence of AD pathophysiological process’. The first two definitions are intended for use in clinical settings and the third for research purpose. All patients who met criteria for “probable AD” by the old criteria would meet the new criteria, but not all of those who fulfilled the criteria for “possible AD” by the 1984 NINCDS-ADRDA criteria\textsuperscript{40}. Notably, in real life there are no sharp demarcations between normal cognition and MCI or between MCI and dementia. Furthermore, there is a large overlap in the clinical features, and in structural and functional imaging between different dementia disorders, and sometimes a challenge for the clinician to ante mortem differentiate AD from other dementia diagnosis\textsuperscript{36}.

Current therapeutic strategies for Alzheimer’s disease

In the mid-1990s cholinesterase inhibitors (ChEIs) were approved for pharmacological treatment of mild-to-moderate AD. The approval of memantine in 2002 for treatment of moderate-to-severe AD is the latest in Sweden. Memantine is an uncompetitive N-methyl-D-aspartate (NMDA) receptor antagonist. Glutamate is the principal excitatory neurotransmitter in the brain and stimulates a number of postsynaptic receptors including the NMDA receptor. Many studies suggest it plays an important role in the pathophysiology of neurodegenerative diseases, including AD\textsuperscript{41}. Treatment with memantine has positive effects in both cognitive and functional capacities of patients with AD\textsuperscript{42,43}. The neuroprotective properties of memantine have been studied in a large number of \textit{in vitro} and \textit{in vivo} animal models by several laboratories. Prolonged glutamatergic stimulation of NMDA receptors can result in degeneration and death of cortical and
subcortical neurones. Memantine has been shown to block the pathologically sustained activation of the receptor, hypothesised to occur in AD, and may protect neurons from the neurotoxic effects of glutamate\textsuperscript{44}.

The effects of ChEIs and memantine are limited and only symptomatic. The current treatments of AD provide no cure or proven reduction of the disease progression rate. One main area of clinical research is focusing on reduction of Aβ levels. Different possible strategies are reducing Aβ production, enhancing Aβ clearance or inhibiting Aβ aggregation. Immunotherapies for the Aβ protein are treatments under investigation at several sites all over the world. Despite initial negative results, with no effects on cognitive or ADL functions, reduction of monomeric and aggregated forms of Aβ in the brain is still a main topic. Inhibiting tau aggregation is another field of enhanced interest, and several immunotherapies targeting tau are under development. Other approaches are neuroprotective agents and drugs that may inhibit tau phosphorylation or inflammation. Still the best and most effective treatment of AD and associated mood and behavioural changes is non-pharmacologic, including structured social activities, physical activity, environmental strategies and good and professional care.

Other dementia disorders

Next to AD, the second most common dementia disorder is cerebrovascular diseases as ischemic/haemorrhagic stroke and small vessel brain disease\textsuperscript{45}. Other common neurodegenerative dementia disorders are frontotemporal dementia (FTD) and dementia with Lewy bodies (DLB). FTD is characterised by slowly progressing impairment in personality, behaviour and language\textsuperscript{46}. Clinical subgroups of FTD have been recognised, including progressive non-fluent aphasia (PNFA), semantic dementia (SD), and frontotemporal dementia, behavioural variant (bvFTD)\textsuperscript{47}. The clinical diagnosis of DLB is based on progressive cognitive decline combined with at least two of three core features: visual hallucinations, spontaneous parkinsonism and/or fluctuating attention and alertness\textsuperscript{48}. More unusual, but not exceptionally rare are neurodegenerative dementia disorders as progressive supranuclear palsy, multiple system atrophy and corticobasal degeneration. Mixed pathologies are common, especially in the oldest-old, with coexistence of AD and/or cerebrovascular disease and/or DLB pathology\textsuperscript{49}. Furthermore, the proportion of non-AD dementia disorders is higher compared to younger ages. Among the oldest-old argyrophilic grain disease and neurofibrillary tangle-predominant dementia are common and count for 5-10 % of all dementia cases\textsuperscript{50-52}. 
Lumbar puncture

A lumbar puncture (LP) is a method used to collect the CSF surrounding the brain and the spinal cord. The first technique for accessing the dural space was developed in the late 1880s, and lumbar puncture is today a routine procedure in Europe. After local anesthesia a needle is carefully inserted into the spinal canal between the lumbar vertebrae L3/L4 or L4/L5, and 10-15 ml CSF is collected. In dementia assessment the CSF samples are commonly analysed for Aβ42, t-tau, p-tau, antibodies against borreliosis, CSF/serum ratio of albumin and intrathecal immunoglobulin G synthesis. Post spinal headache is the most common complication (less than 2% in demented patients), but more serious complications as infections are extremely rare. Other disadvantages are that sampling requires an invasive LP with associated costs and in some medical conditions LP is contraindicated. The diagnostic value of measuring CSF biomarkers in patients over 80 years of age is markedly lower than in younger patients since low concentrations of CSF Aβ42 is common even in elderly without cognitive complaints, probably indicating presymptomatic AD. Consequently, most dementia diagnostic work-ups are made without analysis of CSF biomarkers.

Biomarkers for Alzheimer’s disease

Amyloid-β

In the mid-1980s the Aβ protein was purified and characterised to be the central protein in senile plaques. Aβ is formed from the transmembrane amyloid precursor protein (APP). APP is cleaved by α- or β-secretases into soluble APP (sAPPα or sAPPβ) and C-terminal fragments (αCTFs and βCTFs). The major β-secretase, β-site amyloid precursor protein cleaving enzyme (BACE1), is the enzyme responsible for initiating Aβ generation. Subsequent cleavage of βCTFs by γ-secretase yields different Aβx –38, Aβx–40 and Aβx–42 species. Aβ42 (Aβ 1-42 and Aβx-42) is most prone to aggregate to deposition in senile plaque.
Aβ can be detected and quantified in CSF as well as in plasma. In CSF, low levels of Aβ42 are strongly associated both with manifest AD and an increased risk of future development of AD. Numerous studies on AD patients have shown a moderate to marked decrease of Aβ42 in CSF \(^{55,56}\). The mean concentration in AD patients compared with healthy controls is about 50% lower on a group-level \(^{57}\). However, the specificity for discrimination of AD from other disorders is moderate. Low levels of Aβ in CSF have, for example, been found in DLB \(^{58}\), in normal pressure hydrocephalus \(^{59}\), in a small percentage of patients with FTD, vascular dementia, Creutzfeldt-Jakob’s disease (CJD) and amyotrophic lateral sclerosis \(^{60}\). The CSF Aβ42 levels decrease very early during the pathogenesis of AD and reach a plateau several years before the conversion to AD dementia \(^{61,62}\). Low Aβ42 has a high sensitivity in predicting conversion to AD in MCI \(^ {63}\). Furthermore, in SCI, a CSF AD profile with low Aβ42 is more common than in HC, indicating that they might be in a prodromal stage of AD \(^ {64}\). Soluble Aβ oligomers in CSF have been reported to exert neurotoxic effects and to impair synaptic function. An aggregation into oligomers in CSF may partially explain the lowering of CSF Aβ42 in AD \(^ {65}\). However, the most likely main cause of the decreased CSF Aβ42 concentration is that the aggregated state inhibits Aβ2 from being transported from the interstitial
fluid to the CSF\textsuperscript{66}. The concentration of Aβ oligomers is compared with Aβ42 very low in CSF, and measurement of such oligomers is afflicted with difficulties\textsuperscript{67}. CSF Aβ levels can be measured by different enzyme-linked immunosorbent assay (ELISA) methods.

The results from different studies investigating whether plasma Aβ may be a useful AD biomarker are contradictory. In familial AD with mutations in presenilin 1 or 2 and APP genes as well as in people with Down syndrome plasma Aβ is increased\textsuperscript{68,69}. In prospective and cross-sectional studies on sporadic AD, studies report conflicting results regarding plasma Aβ levels\textsuperscript{70-72}.

**Tau**

In the mid-1970s tau was discovered as a heat stable protein that facilitates \textit{in vitro} microtubule assembly\textsuperscript{73}. Tau is a hydrophilic protein, which is mainly located in axons. Apart from initiating and stabilising the formation of microtubules, tau also plays a role in regulating neuritic outgrowth and axonal transport. The tau protein has six isoforms. The most commonly used ELISA method for t-tau is based on monoclonal antibodies that detect all isoforms of tau independently of phosphorylation state. Many studies have consistently demonstrated a moderate to marked increase in CSF of total tau (t-tau) levels in AD\textsuperscript{74,75}. However, very high levels of t-tau in the CSF have also been found in CJD. Further, increased levels of CSF tau are present in a proportion of cases with other dementia disorders such as FTD and DLB\textsuperscript{76}. It is suggested that the CSF t-tau levels reflect the neuronal (especially axonal) degeneration and damage, and a transient increase in CSF t-tau has been found after acute stroke\textsuperscript{77}. CSF t-tau and p-tau levels begin to increase gradually during the MCI/mild AD dementia stages\textsuperscript{62,78,79} and are also able to predict conversion from MCI to mild AD dementia on a group level\textsuperscript{80}. Alzheimer’s disease varies widely in its clinical course and rate of cognitive decline\textsuperscript{28-30}. High CSF t-tau has in some longitudinal studies been associated with rapid cognitive decline\textsuperscript{81-83}, increased mortality as well as a more pronounced hippocampal atrophy and ventricular widening\textsuperscript{84-85}. However, other studies have not found any association between CSF tau in AD patients and an aggressive course of the disease\textsuperscript{86,87}. 
Figure 5. The physiological balance between phosphorylation and dephosphorylating on the left side, and pathological hyperphosphorylation of tau on the right side (from J Cell Sci. 2004; 117, 5721-5729).

Phosphorylated tau

All six isoforms of tau have serine, threonine and some tyrosine residues that have the potential to be phosphorylated. More than 30 phosphorylation sites have been identified in the hyperphosphorylated tau isolated from AD brain. A dynamic, site-specific phosphorylation of tau is essential for its proper functioning. The phosphorylated status of tau is assumed to be a result of a balanced action between kinases (phosphorylating enzymes as i.e. glucogen synthase kinase 3 (GSK3) and cyclin-dependent protein kinase 5 (Cdk5)), and phosphatases (dephosphorylating enzymes e.g. protein phosphatase 2A (PP2A))\(^\text{88,89}\). PP2A is maybe the major tau dephosphatase. A decrease of about 20% in the activity of PP2A has been reported in AD brain\(^\text{90}\). In AD all six isoforms of tau are abnormally phosphorylated, causing tau to dissociate from microtubules and form paired helical filaments that eventually deposit as neurofibrillary tangles (NFT). The density of NFTs is correlated with the degree of dementia in AD\(^\text{91}\). The most commonly used ELISA methods for measuring p-tau in CSF use antibodies that are specific for phosphorylation at either threonine 181 (p-tau181) or threonine 231 (p-tau 231). Increased CSF p-tau181 seems to have higher
specificity and lower sensitivity than CSF Aβ42 for separating AD from non-AD dementia92.

Other potential AD biomarkers

Today there is no reliable biological marker that unequivocally and easy can detect AD early in its course nor distinguish it from other causes of dementia and normal ageing. In clinical routine CSF Aβ42, t-tau and p-tau 181 levels are well established AD biomarkers. By combining these, the diagnostic sensitivity is approximately 80% and the specificity around 90% compared to non-AD dementia93. Apart from Aβ, t-tau and p-tau a number of AD biomarkers have been suggested. The presence of the APOE ε4 allele has been associated with lower levels of ApoE protein in both serum and brain tissue from healthy controls as well as AD subjects94. However, results from studies measuring CSF ApoE protein levels are conflicting13,95. Other proposed AD biomarkers in CSF, plasma or urine are markers of inflammation, such as interleukins (IL) e.g. IL-6, IL-1B and sIL-1RII96-98, markers of astrocytic activation as S100B99 glial fibrillary acidic protein (GFAP)100, markers of oxidative stress as F₂-isoprostanes101, factors involved in amyloid processing and aggregation such as sAPP-β and sAPP-α102, cystatin C103, BACE1104 and Aβ oligomers67,105. None of these enumerated AD biomarkers are currently available for clinical purposes. During the last couple of years, a lot of effort has been made to find reliable biomarkers for AD in peripheral blood. In the Alzheimer's Disease Neuroimaging Initiative (ADNI), several studies have explored large panels of potential blood AD biomarkers. By using broad sets of different biomarkers they have had some success in distinguishing AD from controls, but less success in adequately predicting MCI to AD conversion106,107.

The arrival of a disease-modifying therapy will increase the need of an accurate biomarker able to detect presymptomatic AD. According to a proposal of a consensus group on molecular and biochemical markers of AD108, an ideal marker of AD should be able to detect a fundamental feature of neuropathology and should be validated against neuropathologically confirmed cases. Furthermore, its sensitivity for detection of AD as well as its specificity for discrimination of AD from other dementia disorders should exceed 80%. A marker for AD should also be reliable, reproducible, non-invasive, simple to perform in clinical routine, inexpensive, able to measure the progress of disease or the effects of treatment, and reflect the underlying pathogenesis.
Positron emission tomography

Positron emission tomography (PET) is an imaging technique that produces a three-dimensional image or picture of functional processes in the body. The system detects pairs of gamma rays emitted indirectly by a positron-emitting radionuclide (tracer), which is introduced into the body on a biologically active molecule. The first steps towards the concept of emission and transmission tomography were taken in the late 1950s. In the 1970s, 18 Fluoro-2-deoxy-d-glucose (FDG), an analogue of glucose, was introduced and is the most commonly used marker of cerebral glucose metabolism. Reduced FDG uptake reflects affected and less functioning areas of the brain. The patterns of cerebral glucose hypometabolism differ between different dementia diseases, and AD is typically associated with hypometabolism in the temporoparietal regions. However, there are overlaps in the patterns of hypometabolic regions between different dementia disorders, and FDG PET has a diagnostic accuracy around 70-80%.

N-methyl [11C]2-(40-methylaminophenyl)-6-hydroxybenzothiazole (also referred to as Pittsburgh compound B or PIB) was introduced in the beginning of the 2000s and is an amyloid binding PET tracer used to detect amyloid depositions in vivo in the human brain. PIB is a derivative of thioflavin T and labels senile plaques and cerebral amyloid angiopathy in tissue sections from AD patients. This compound also crosses the blood-brain barrier, allowing peripheral administration and is readily labelled with carbon-11 for PET scanning. PIB enters CNS rapidly, targets fibrillar Aβ deposits specifically, and is cleared from the brain quickly when not bound to Aβ. AD patients show a typically marked retention of PIB in the areas of association cortex known to contain large amount of amyloid deposits in AD. In areas known to be relatively unaffected by amyloid deposition, such as subcortical white matter, pons and cerebellum, PIB retention does not differ between AD patients and controls. Increased PIB retention is not specific for AD. Patients with DLB sometimes have high cortical PIB binding, since senile plaques are present in many DLB cases. Other neurodegenerative dementia disorders as FTD are not associated with increased PIB retention. It has been reported that some rare genetic forms of AD have low PIB retention, probably due to enhanced formation of Aβ oligomers without Aβ fibril formation.
Previous observations indicate that PIB retention reaches a plateau early in the course of AD\textsuperscript{21,118}, and remains stable when the cognition deteriorates and brain atrophy advances\textsuperscript{119}. Large studies have shown elevated PIB binding in a high proportion of the MCI\textsuperscript{120,121} patients. Moreover, a substantial share of HC subjects has increased PIB retention\textsuperscript{122}, which probably mirrors an asymptomatic AD pathology commonly seen in autopsy studies on elderly not-demented individuals\textsuperscript{19}. There is some evidence that the decrease of CSF A\(\beta\) may appear some years before the increase in PIB retention\textsuperscript{123-125}. ApoE4 genotype is associated with higher PIB retention in cognitively normal elderly in a dose-dependent manner\textsuperscript{126}. ApoE4 carriers are also more likely to convert from PIB- (negative) to PIB+ (positive) over time\textsuperscript{127}. Furthermore, older cognitively normal individuals with subjective cognitive complaints are more likely to be PIB+\textsuperscript{128}, probably reflecting prodromal AD. MCI PIB+ patients progress to AD at an estimated rate of 25 % per year\textsuperscript{129,130}. PIB+ healthy controls show a medial temporal lobe volume decline at follow-up. In addition, PIB+ MCI patients have faster cognitive decline and a faster decline in glucose metabolism and progression of grey matter atrophy within temporal and parietal brain regions\textsuperscript{131}. In patients with clinically manifest AD dementia, PIB retention does not correlate to glucose metabolism or the severity of the disease\textsuperscript{132,133}. 
The work at many institutions has over the latest years focused on developing \textsuperscript{18}F-labeled PET radiotracers for more widespread availability and routine clinical usefulness than \textsuperscript{11}C-labeled PIB. The first \textsuperscript{18}F-labeled agent for human clinical A\textbeta\ imaging, florbetapir, was approved by the FDA in April 2012\textsuperscript{134}, and other similar agents are currently in phase II or III clinical trials. In contrast to the achievements of \textit{in vivo} A\textbeta\ imaging, progress in developing a selective PET radioligand to quantify neurofibrillary tangles in in living human brain has lagged, but some recent advances are encouraging\textsuperscript{135,136}.

**Neuropsychological assessment**

A neuropsychological examination includes an administration of a test battery and observations of dysfunctional behaviours, and provides a comprehensive evaluation of cognitive domains associated with various brain regions. The cognitive domains typically assessed include language, attention/concentration, executive functions, visuospatial thinking and verbal and visual learning and memory. The tests are standardised, well-validated and normed for age and education. A neuropsychological evaluation is an essential component in the diagnosis of early stages of different dementia.
disorders. The neuropsychological profile is useful to differentiate cognitive dysfunction due to other causes like psychiatric disorders or substance abuse.

Rey-Osterrieth Complex figure, a test measuring visual episodic memory by copying and recall.
Aims

The overall aims of this thesis were to investigate established AD biomarkers concerning their relations to medical treatment with memantine, their relation to other potential CSF AD biomarkers, neuropsychological test performance, PIB retention and regional cerebral glucose metabolism and further their possible utility as predictors of future rate of AD dementia deterioration.

The specific aims were:

**Paper I:** To investigate effects of memantine on levels of t-tau, p-tau and A\(\beta\)42 in CSF. The hypothesis was that memantine has neuroprotective effects and that this may be reflected by a normalisation of these markers.

**Paper II:** To explore the relations between PIB retention and different CSF, plasma and urine biomarkers in AD patients, who were characterised regarding neuropsychological test performance and regional CMRglc.

**Paper III:** To study whether there are any differences between PIB+ and PIB- patients with mild neurodegenerative, non-vascular dementia regarding neuropsychological test performance and regional cerebral glucose metabolism (rCMRglu) measured with FDG PET. Furthermore, we also aimed to re-evaluate the clinical diagnoses after long-term follow-up.

**Paper IV:** To investigate whether high CSF levels of tau, p-tau and low CSF levels of A\(\beta\)42 predict rapid decline and death in severe AD dementia. Further, we also aimed to study other possible predictors of a rapid deterioration; such as age, education and co-morbidity.
Subjects and methods

Study population and investigations

All patients were recruited at the Memory Clinic at Uppsala University Hospital. The diagnosis of probable AD dementia was made according to the NINCDS-ADRDA criteria and the DSM-IV criteria. DLB was diagnosed according to the McKeith criteria. The FTD diagnoses were made according to the Neary criteria. All patients had CT scans consistent with their clinical diagnosis. Unspecified dementia (dementia UNS) was defined as dementia, without fulfilling any specific dementia diagnosis despite a comprehensive evaluation. All participants gave their informed consent. The studies were approved by the local Ethical review board.

**FDG and PIB PET (studies II-III)**

All PET investigations were analysed using identical standardized regions of interest (ROI:s) in the brain and each subject had its set of ROIs individually delineated. The CMRglu values were normalized to the pons value (ROI/ref). For PIB the mean uptake values of the ROI:s were normalized to the corresponding uptake in the cerebellar cortex, which was chosen as reference region (ROI/ref). Scans were characterised as “PIB positive” both on visual inspection and by a mean ratio > 1.6, obtained by calculating a mean value of following areas: the frontal, parietal, temporal and posterior cingulum (ROI/ref). PIB retention “negative” scans were also characterised both on visual inspection and by a ratio mean < 1.6 of the same areas (ROI/ref). Mean PIB retention was also calculated as a mean value of the frontal, parietal, temporal and posterior cingulum areas (ROI/ref).

**CSF analyses (studies I-II+IV)**

The CSF values of tau, p-tau and Aβ42 were determined using sandwich ELISAs.

**Neuropsychological assessment (studies II-III)**

A neuropsychological protocol consisting of 14 psychometric tests was applied to assess the following abilities; logical thinking: Arithmetics (Wechsler Adult Intelligence Scale – Revised (WAIS-R) and Wechsler
Adult Intelligence Scale 3rd Ed (WAIS-III), verbal function: word fluency test (FAS), object naming (Boston Naming Test), Similiarites (WAIS-R, WAIS-III) and Information (WAIS-R, WAIS-III), visuospatial function: Clock Drawing with pre-drawn clock faces according to Luria and Block Design (WAIS-R, WAIS-III), psychomotor speed /attention: Trail Making Test, part A (TMT A)\(^{140}\) and Digit Span (WAIS-R, WAIS III) and memory: episodic verbal memory: Claeson-Dahl Test for Learning and Memory and visual episodic memory: Rey-Osterrieth Complex figure, immediate recall\(^{141}\).

**Paper I**

Thirteen outpatients with mild-to-moderate probable AD were included. They did not tolerate or respond to ChEIs, or were on stable doses of ChEIs with progressive worsening after more than one year’s treatment. After a basic investigation memantine was titrated up to 20 mg daily, and then they were followed up after 3, 6 and 12 months. LP was performed twice, both before starting medication with memantine and at the 12 months’ follow-up. The CSF concentrations of t-tau, p-tau 181 and A\(\beta\)42 were analysed. MMSE was performed and daily functioning was measured by Alzheimer’s Disease Cooperative Study Activities of Daily Living (ADSC-ADL)\(^{31}\) and behavioural and psychological symptoms by The Neuropsychiatric Inventory (NPI)\(^{142}\).

*Statistical analyses:*

Wilcoxon matched pairs test was used to compare baseline concentrations of t-tau, p-tau and A\(\beta\)42 with values after one year’s medication with memantine. To detect correlations between changes in CSF biomarkers and changes of MMSE, NPI and ADSC-ADL, Spearman Rank Order Correlations was applied.

**Paper II**

Ten outpatients with a clinical AD diagnosis of mild to moderate severity were recruited. All had neuropsychological test results consistent with the diagnosis of probable AD. All patients were on stable doses with ChEIs, and one was on additional treatment with memantine.

The patients were examined at baseline and after 12 months with PIB and FDG PET, MMSE, CSF and plasma samples. Concentrations of A\(\beta\)1-42, A\(\beta\)1-40, A\(\beta\)x-42, Apo E protein, cystatin C, IL-6, IL-1B and sIL-1RII were analysed in CSF and plasma. Further, A\(\beta\)38, t-tau, p-tau 181, sAPP-\(\beta\), sAPP-\(\alpha\) and GFAP were measured in CSF. Concentrations of A\(\beta\)x40 and S100B
were analysed in plasma. F₂-isoprostane was measured in urine and adjusted for the urinary creatinine concentrations and thus given as pmol/mmol creatinine. APOE genotyping was performed.

Statistical analyses:
The analyses of correlations between PIB retention and biomarkers in CSF and plasma, FDG PET data, respectively, were conducted using Spearman coefficient of correlation. Wilcoxon Matched Pairs tests were applied to assess changes in PIB retention from baseline to the one year follow-up. Comparisons of CSF and plasma data between PIB positive and negative patients were performed by Mann-Whitney U test. Comparisons between numbers of copies of the APOE ε4 allele and PIB retention were performed by Kendall tau correlations. The α level was set to 0.05. Adjustments for multiple comparisons were not made since this was an exploratory study with a small number of patients.

Paper III

Methods:
Eighteen outpatients with mild, non-vascular dementia were included, out of which nine patients also participated in study II. At baseline, ten of the patients were diagnosed as probable AD. Six patients were diagnosed as bvFTD and two as SD. They were examined with PIB PET and FDG PET. An experienced neuropsychologist carried out and assessed all neuropsychological investigations. All patients were followed for 5 - 9 years, or to death, and the clinical diagnoses were re-evaluated.

Statistical analyses:
Comparisons of data from the psychometric tests and rCMRglu between PIB+ and PIB- patients were performed by the Mann-Whitney U test. The analyses of correlations between FDG PET data, PIB PET data and neuropsychological test results respectively, were conducted using Spearman coefficient of correlation. The α level was set to 0.05. Adjustments for multiple comparisons were not made since this was an exploratory study with a small number of patients.
Trail Making Test A (TMT A), a test measuring psychomotor speed and attention.

Paper IV

Four hundred and twenty nine individuals underwent LP as a part of the routine diagnostic work-up at the Memory Clinic between 2003 and 2009. Of all subjects, 196 were included in the study. These patients fulfilled the AD dementia criteria at baseline or converted to AD during the follow-up period (2-9 year). All patients with an AD diagnosis from study I-III were also included in study IV. Data on educational level, numbers of medications, cardiovascular disease (heart failure or coronary heart disease), treatment for hypertension and diabetes were collected from the medical records. Rapid decline was defined as $\geq 4$ points decline in MMSE/12 months. Dying of severe dementia was identified as death occurring in those subjects who died after they had experienced a prolonged decline over months in the end-stage of dementia with immobilization and dependency in all ADLs, as documented in the medical record.
Statistical analyses:

Mann-Whitney U-test was used when comparing not normally distributed variables between groups. Logistic regression analyses were performed to determine odds ratios of rapid cognitive decline. Cox proportional hazards models were applied to assess the hazard ratios to die of severe dementia. Analyses were performed in univariate and multivariate models, adjusted for age, educational level, coronary heart disease/heart failure and baseline AD stage. Separate analyses were also conducted in subjects with mild AD dementia and AD MCI at baseline. The level of statistical significance was set to $p = 0.05$.

*Pentagon copying in MMSE assesses visuospatial and constructional abilities*
Results

Paper I

Out of the 13 patients, two were lost to follow-up. The MMSE scores dropped from baseline to the 12-month follow-up with 3 [median] points. ADSC-ADL scores decreased from 40 (31-54) [median] points to 32 (8-47) points (p=0.006), indicating a significant deterioration of functions. At baseline, all patients had pathological changes of CSF Aβ42, t-tau or p-tau consistent with the diagnosis of probable AD, i.e. Aβ42 <450 ng/l and/or t-tau >400 ng/l and/or p-tau >80 ng/l. After twelve months treatment with memantine, mean CSF p-tau concentrations was significantly reduced from 126 (107-153) ng/L to 108 (88-133) ng/L (median [interquartile range]; p=0.018) (Figure 7). No statistically significant differences were found in mean CSF concentrations of t-tau and Aβ42 at baseline compared with the 12 months' follow-up. There were no correlations found between changes of CSF biomarkers and changes of MMSE, NPI or ADCS-ADL.

![Figure 7. Concentrations of CSF p-tau in each patient (n=11) at baseline and after 12 months treatment with memantine.](image-url)
PIB PET scans were positive in six patients and negative in four. Baseline FDG PET showed a hypometabolic pattern in accordance with AD, i.e. temporoparietal hypometabolism, in all patients except two. At the 12 months’ follow-up regional cerebral glucose metabolism was virtually unchanged in 9/10 subjects. One PIB+ patient with very mild AD at baseline had a marked deterioration in both glucose metabolism and cognition. Furthermore, his PIB retention increased between baseline and the one-year follow-up. All other patients had stable PIB retention over time in all brain regions. PIB uptake was not related to dementia severity according to MMSE or ADSC-ADL scores, or to FDG PET data.

CSF levels of Aβ1-42 and t-tau were constant over time. Concentrations of CSF Aβ1-42 (PIB+:266, PIB-:1090 (mean, ng/L), p=0.01) and CSF Aβx-42 (PIB+:483, PIB-:1488 (mean, ng/L) (p=0.01) levels differed significantly between PIB+ and PIB- patients. All PIB+ patients had low concentrations of Aβ1-42, i.e.<450 ng/l, whereas all PIB- patients had normal Aβ1-42 (Figure 8). Four out of the ten patients had one APOE ε4 allele and 3/10 were homozygous for APOE ε4. Numbers of the APOE ε4 allele were positively correlated to mean PIB retention (Kendall’s tau= 0.54, p=0.02). There were moderately strong, although not statistically significant, correlations between mean PIB retention and CSF ApoE protein (r= -0.59, p=0.07) and plasma cystatin C (r=-0.56, p=0.09).

Figure 8. CSF Aβ-42 (ng/L) concentrations and PIB retention (mean values of the frontal, parietal temporal and posterium cingulum areas (ROI/ref) of individual patients at baseline.
After this study was published, we have followed the PIB- patients with clinical assessments up to seven years. Two of the PIB- patients have developed parkinsonism and visual hallucinations and later fulfilled the McKeith criteria [46] for probable DLB. The other two PIB- patients have had a slow deterioration of memory and loss of executive functions. One PIB+ patient developed parkinsonism, but without visual hallucinations or fluctuations, and fulfilled the criteria for possible DLB. Later, autopsy confirmed the DLB diagnosis with additional senile plaques. Post-mortem examination has also confirmed the AD diagnosis in 2 PIB+ patients.

Paper III
The PIB+ and the PIB- groups were well matched concerning gender, age and performance on the MMSE. The median length of education was four years longer in the PIB+ subjects. Parietotemporal hypometabolism was present in 6/7 PIB+ and in 5/11 PIB- patients. During follow-up, the clinical diagnoses were changed in six patients out of whom three patients were re-diagnosed from AD to DLB. Autopsy confirmed the FTD diagnosis in one PIB- patient, AD diagnosis in two PIB+ patients and the DLB diagnosis combined with the presence of senile plaques in another PIB+ patient.

PIB+ patients had significantly lower psychomotor speed measured by time to completion on TMT A (PIB+: 95 ± 27 seconds vs. PIB-: 65 ± 24 seconds (mean ± SD), p=0.03) and more impaired visual episodic memory (p=0.04) compared to PIB- patients. Moreover, the median score on verbal episodic memory was lower in the PIB+ group compared to the PIB- group, although not significant. Otherwise the results did not differ between groups.

Regional CMRglu was approximately 30% lower in the parietal cortices in PIB+ patients compared to PIB- patients, although not significant. Regional glucose metabolism in the frontal and temporal cortices was similar in the two groups.

Paper IV
Sixty-one percent of the AD patients were in the MCI stage at the time of lumbar puncture, and twenty-nine percent were in the mild dementia stage. Concentrations of CSF t-tau, p-tau and Ab42 did not differ between patients in the MCI stage and patients with mild/moderate dementia at baseline. The mean deterioration in MMSE over twelve months was approximately 1.5 points and the mean interval between baseline and the last MMSE was around thirty months. At the last available MMSE testing, 21% of the
patients were classified as rapid decliners, i.e. they lost four points or more in MMSE during 12 months. Twenty-three percent of the patients performed equal or marginally better compared to baseline. During a median follow-up period of 5.9 years, 66 patients died out of whom 32 of severe dementia.

We found no associations between rapid decline and age, APOE genotype, education or baseline stage of AD. Further, rapid decliners did not differ in performance on MMSE at baseline compared to the non-rapid decliners. Split by medians, the odds ratio of rapid decline in patients with high CSF t-tau was 3.31 (95% CI 1.53-7.16), and OR in patients with CSF p-tau above median was 2.53 (95% CI 1.21-5.26), adjusted for age, education, heart disease and baseline stage. The risk of rapid decline 2-4 times higher in patients in the highest quartiles of t-tau and p-tau compared to the lowest quartile. There was a tendency to a U-shaped association between Aβ42 and rapid decline, with higher risk in both the lowest quartile as well as above the 87.5th percentiles. After adjusting for covariates the lowest quartile of CSF Aβ had an OR 2.23 (95% CI 1.04-4.68), using the second to fourth quartile as reference.

In Cox proportional hazard analyses, neither CSF t-tau nor p-tau levels were associated with mortality irrespective of cause. High educational level was associated with a lower risk of dying in severe dementia in crude Cox proportional hazard analysis. The risk was also increased in patients with heart disease and in those with moderate dementia at baseline, although not statistically significant. Subjects with CSF t-tau above median had a higher risk of dying in severe dementia, HR 2.29 (95% CI 1.02- 5.13) adjusted for age, education and baseline AD stage. Patients in the highest quartile of t-tau had an even higher hazard ratio of this outcome and the association remained significant in multivariable adjusted models, HR 4.67 (95% CI 1.16- 18.82) (Figure 9). There was a tendency to a U-shaped association between p-tau and HR of dying of severe dementia. Subjects in the highest quartile of p-tau had a multivariable adjusted HR 2.39 (95% CI 1.94-4.79) using quartile 1-3 as reference. No association was found between baseline CSF levels of Aβ42 and dying in advanced dementia.
Figure 9. Cumulative hazard ratios of death in severe dementia by baseline levels of CSF t-tau (highest quartile compared to quartile 1-3).
Discussion and future perspectives

The close relationship between CSF and the central nervous system enables unique possibilities to detect changes inside the brain reflected to the CSF. Decreased Aβ42, increased t-tau and p-tau in CSF mirror the core pathophysiological processes in AD i.e. the Aβ deposits in senile plaques and the hyperphosphorylated tau in neurofibrillary tangles. Measurements of CSF Aβ42, t-tau and p-tau levels have been used as a tool in the clinical work-up for several years67. Two important questions remain: can CSF biomarkers also be used to monitor biological effects of treatment and can they predict future AD deterioration rate?

Cerebrospinal fluid biomarkers as monitors of disease-modifying treatment efficacy

Still, the knowledge about the natural course of CSF biomarkers in AD is limited. Results from previous studies concerning CSF Aβ, tau and p-tau as stage markers are conflicting, but there seems to be an intra-individual stability between the late MCI and moderate AD stages143,144. The major limitation of study I is the lack of a placebo group. The interpretation of the results is based on the assumption from other studies with repeated measurements of p-tau showing stable concentrations over at least one year79,145. Studies with shorter follow up demonstrate that the levels of p-tau do not change significantly during ChEI medication146,147. There is some evidence that p-tau concentrations increase during cognitive decline148,149, but also contradictionary findings150. Study I is the first study on AD patients that supports the hypothesis that memantine may act as a modulator and restorer of abnormal tau hyperphosphorylation. To the best of our knowledge, there is one additional human study where treatment with memantine was associated with reduction of p-tau, but only in subjects with normal cognition151.
A great deal of evidence support that the NMDA receptors may play a significant role in the execution of synaptic dysfunction and neuronal death in AD. In the absence of corroborative results from human studies, there are several *in vitro* and *in vivo* non-human studies showing the effects of memantine on the pathological tau phosphorylation and Aβ processing occurring in AD. In one study, rat hippocampal slices treated with okadaic acid (OA) to inhibit PP2A activity. Additional treatment with memantine completely restored the activities of PP2A and tau was dephosphorylated at several sites, indicating that memantine maybe affects tau phosphorylation. Further, in another study Alzheimer-like alternations induced by intrahippocampal OA in rats were prevented with pretreatment of memantine. The authors suggested that the prevention of increased glutamate levels along with the reduced tau phosphorylation by Cdk5/p25 signalling pathway are the mechanisms of memantine’s prophylactic effects. In the AD brain, the transcription and expression of an inhibitor of PP2A (I₂PP2A) is increased, and it co-localises both with PP2A and abnormally hyperphosphorylated tau in the neuronal cytoplasm. One study shows that memantine modulates PP2A by directly affecting I₂PP2A inhibition in vitro at therapeutic concentrations. Other studies suggest effects of memantine treatment by reducing soluble Aβ and Aβ oligomers or by blocking the pathological tonic NMDA receptor activation caused by the soluble oligomers. In vitro studies on cultured neurons indicate that Aβ toxicity is, at least partly, mediated by increased phosphorylation of tau via induced activation of multiple kinases including GSK3. Memantine treatment of transgenic mice (3xTg-AD) significantly reduced the levels of tau phosphorylated at residues that are known targets for GSK3. Thus, memantine may reverse the I₂PP2A inhibition of PP2A and/or reduce Aβ induced GSK3 activity, leading to in vivo dephosphorylation of tau, as seen in study I.

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*Figure 10. Memantine reverses the pathological phosphorylation of tau in AD by restoring the activities of PP2A.*
Despite the fact that p-tau in CSF levels decreased in our study we couldn’t show any symptomatic benefits of memantine measured by the MMSE or the ADCS-ADL and NPI scales. The progression rate of AD measured by MMSE continued at an average rate despite memantine treatment. However, all included patients were rapid cognitive decliners and/or non-responders of ChEIs. Recent findings on transgenic mice (developing progressive tau-pathology) treated with methylene blue (an inhibitor of tau aggregation), showed no beneficial effect of the drug when the treatment started on mice who were old and already cognitively impaired. By contrast, preventive methylene blue treatment on young and still cognitive healthy mice preserved the cognitive and behaviour functions during their lifetime\textsuperscript{159}. Likewise, treatment with memantine would maybe have a more efficient effect on preventing cognitive decline if the patients were in the preclinical or MCI AD stages instead of moderate-to-severe AD stages.

Drug candidates with no proven effect on the molecular pathogenesis of AD, such as cholinesterase inhibitors, have no effect on CSF AD biomarkers. The effects of disease-modifying drugs on Aβ and tau pathologies are commonly evaluated in AD animal models but apart from study I, there are only some few other in vivo human studies reporting changes in CSF AD biomarkers as response of treatment\textsuperscript{151, 160-163}. In slowly progressive disorders such as AD which vary widely in rate of decline, the evaluation of the clinical effect of a drug requires large patient cohorts and extended treatment periods. A biomarker with the ability to monitor a specific action of a drug on a core pathogenic process would probably require relatively smaller patient materials and shorter treatment periods. Results from such studies may be especially valuable for decision if making a bid for larger and more expensive trials. Lastly, a claim for a disease-modifying effect can only be made when a drug has been proven to improve cognition, mood, behavioural changes and functioning in daily activities. Moreover, there should be a biomarker evidence of an effect on the central pathogenic process\textsuperscript{164}.

Cerebrospinal fluid Aβ and PIB PET in AD diagnostics

The CSF Aβ42 levels decrease very early during the pathogenesis of AD and are fully changed several years before any AD symptoms are clinically detectable and the conversion to AD dementia. The increase of CSF t-tau and p-tau levels is a later event and probably begins gradually during the early MCI/mild dementia stages\textsuperscript{62}. Senile plaques and hyperphosphorylated tau in cortical brain biopsies are reflected by low CSF Aβ42 and high CSF tau and p-tau levels, respectively\textsuperscript{165}. High PIB retention in vivo also mirrors the amount of Aβ in post-mortem tissue\textsuperscript{166}. The concordance between high PIB retention, low CSF Aβ1-42 levels and AD pathology is very strong, but
not complete\textsuperscript{116,167,168}. The findings in study II are in accordance with previous and later studies that PIB retention is stable over time; that a strong inverse relation between Aβ1-42 in CSF and PIB retention exists; and that there is a correlation between PIB retention and numbers of the APOE ε4 allele. Although not statistically significant, high PIB uptake was also linked to low concentrations in CSF of the ApoE protein and plasma cystatin C. There is some evidence for connections between AD and low levels of CSF ApoE protein\textsuperscript{169}, but stronger associations between cystatin C and AD\textsuperscript{170}.

An unexpected finding was that four out of the ten patients with a clinical AD diagnosis in study II were classified as PIB-. They also had higher concentrations of CSF Aβ1-42 and Aβx-42, and the APOE ε4 allele was less frequent. Otherwise, the clinical profile and FDG PET pattern did not differ. The percentage of PIB negative patients in Study II is higher than in previous AD studies. In two recent studies the proportion of PIB- AD patients was slightly more than 20\%\textsuperscript{171,172}. The most likely explanation is that the patients have other dementia disorders mimicking AD. Other possible contributing causes are different analysis methods of PIB PET data, different definitions of PIB +/- patients and varying threshold values make direct comparisons between studies difficult. The characterisation of PIB +/- in studies II+III is based on previous studies at Uppsala Imanet. Moreover, the same scanner protocol for transmission, emission and reconstructions and the same analysis methods of data have been applied.

There is a significant proportion of AD cases identified using strict clinical diagnostic criteria that show non-AD pathology. Most commonly DLB is misdiagnosed as AD\textsuperscript{173}. All ten patients in study II fulfilled the diagnostic criteria of AD (NINCDS-ADRDA and DSM-IV) at the time of the inclusion, but two PIB- patients and one PIB+ patient later developed symptoms indicating DLB. One PIB+ patient who was included both in study II and III, fulfilled the criteria of possible DBL at follow-up, and later the autopsy confirmed the DLB diagnosis. In study III, 1 patient out of 8 with baseline diagnosis FTD also had high PIB retention indicating the presence of AD pathology. Altogether, the diagnoses were changed for 6/18 patients after PIB PET, FDG PET and repeated clinical examinations. Both studies II+III clearly illustrate the clinician’s weekday where patients present with a mixture of clinical features, CSF biomarkers and pathology visualised by neuroimaging. Especially in the early stages of different dementia diseases, the assessment of the clinical diagnosis is a challenge. There are several subtypes and large variations in the clinical presentations of AD dementia. Based on large groups, the profile of neuropsychological performance differs distinctively from other neurodegenerative dementia disorders as FTD and DLB. However, the parkinsonistic features in DLB are not always prominent and can appear several years after the first symptoms of cognitive decline. Patients and their caregivers do not always report hallucinations or
fluctuating attention i.e. the other two core symptoms of DLB. Another common cause of the mix-up between DLB and AD patients is that a high percentage of DLB patients also have AD pathology with senile plaques\textsuperscript{114}. These patients have the same biomarker pattern as AD subject with high PIB retention and low levels of CSF A\textbeta\textsubscript{42}. Therefore the sensitivity for the DLB diagnosis is low and only approximately 30%, for “pure” DLB, and even lower for DLB in combination with AD-pathology. However, the specificity for the DLB diagnosis is high and about 95%\textsuperscript{174}. Another common mix-up is between AD and FTD, and this dementia disorder sometimes starts with impaired episodic memory and spatial disorientation, i.e. typical features of AD\textsuperscript{173,175}. Other differential diagnoses are argyrophilic grain disease and neurofibrillary tangle-predominant dementia. These disorders are common especially among the oldest-old\textsuperscript{50,51}. Their clinical presentations are similar to AD and they are not possible to diagnose ante mortem. Mixed pathologies are frequent in clinically diagnosed Alzheimer patients, mainly patients older than 80 years\textsuperscript{49}. Furthermore, the cognitive profiles are overlapping not only depending on the underlying dementia disorders, but also due to the disease severity, the patients’ premorbid abilities, personality, education and age.

Affected episodic memory is the most specific early symptom of AD. Not surprisingly, the PIB+ patients in study III had lower test results regarding visual episodic memory and a tendency to more impaired performance also in verbal episodic memory. Another expected finding in study III was the substantially lower CMRglu in the parietal cortices compared to the PIB- patients, however not statistically significant due to the small sample size. Notably is that the majority of the PIB- patients had parietotemporal hypometabolism, i.e. an AD-like hypometabolic pattern on FDG PET. Furthermore, one of the PIB+ patients had normal glucose metabolism in the parietotemporal areas.

The more impaired psychomotor speed among the PIB+ patients in study III was a more unexpected result. There are a lot of conditions and disorders that are known to influence the performance on the TMT A as high age, low educational level as well as in different psychiatric and non-AD dementia disorders\textsuperscript{176-178}. Despite the four years longer median length of education, the PIB+ patients had more impaired psychomotor speed compared to the PIB- dementia patients. The higher level of education probably affected their performance in TMT A, and with more equal education levels between the PIB+/- groups the difference would most likely been larger. The majority of patients fulfilled the criteria of either AD or FTD diagnosis at both baseline and follow-ups, and the results are probably due to slower psychomotor speed in AD patients compared to FTD patients. Previous studies have shown contradictory results concerning performance on psychomotor speed tests in AD patients compared to FTD\textsuperscript{179-183}. The performance on TMT A
differs between various subtypes of FTD, and patients with bvFTD seem to be faster than the temporal variants, i.e. SD and PNFA. Further, performance on TMT A is also depending on disease stage. In one study, the mildly demented (MMSE≥20) AD patients had more impaired psychomotor speed than FTD patients at the same disease stage. However, in the group with more severe dementia the AD patients had better performance on TMT A compared with FTD subjects.

Decreased CSF Aß42 levels reflect Aβ deposits in senile plaques and high PIB retention in cortex. Further, CSF Aß42 is useful as a diagnostic tool, but alone it is not optimal to discriminate AD from other dementias. There are several reasons for this. Firstly, the decrease begins many years before the first symptoms of AD and a significant percentage of the non-demented elderly have low levels of CSF Aß42. Secondly, there is a large overlap in pathology between AD and other dementias, such as DLB, FTD and vascular dementia. Thirdly, there are large both intracentre and intercentre assay variability for CSF Aß42, which complicate the interpretation of the values.

PIB PET has been used in many different human research studies throughout the world. It has demonstrated the usefulness of assessing the Aβ load of subjects many years before the clinical diagnosis of probable AD by identifying MCI patients with high risk to convert to AD. Several PIB PET studies on older healthy controls show that PIB retention is related to future cognitive decline and brain atrophy, indicating a pre-symptomatic AD stage. Amyloid PET also seems to be an effective tool to differentiate AD from non-AD dementia, particularly in early-onset patients. Being an early event in the pathogenesis of AD, PIB PET is not an optimal marker of progression during the clinical stage of the disease. To detect disease progression and to discriminate between different non-AD dementia disorders, clinical assessment, neuropsychological tests, MRI and FDG PET are more effective. In the last years more long-lived, potentially easier to use and cheaper (18)F-radiolabeled Aβ-selective radiopharmaceuticals have been developed, and in April 2012 the PET agent 18F-florbetapir was approved by FDA for clinical use. Immunotherapies for the Aβ protein are treatments under investigation at several sites all over the world. The possibilities of a specific Aβ load reducing treatment enhance the need for accurate diagnosis with possibilities to detect underlying AD pathology. In the future the full clinical impact of these imaging agents will be realised by identifying presymptomatic subjects who would benefit from early drug treatments. Further, amyloid PET will also be useful in monitoring new treatments targeting the Aβ-processes. Equally important is to identify PIB-patients with AD symptomatology, who only risk side effects of a specific Aβ load reducing treatment.
Cerebrospinal fluid biomarkers as predictors of deterioration rate in Alzheimer’s disease

To the best of our knowledge, there are only three studies which have investigated associations between AD CSF biomarkers and mortality irrespective of cause and none of these has had death in severe dementia as an outcome. Wallin et al (2006) showed that patients with low levels of Aβ42 and high CSF tau levels had an increased risk of dying during a 6 years follow-up. The same author has also identified a cluster of patients with extreme levels of CSF biomarkers and a higher mortality. Another large study found an association between high CSF tau levels and mortality among DLB patients, but not among the AD cases. Study IV is the first to report a strong association between high CSF t-tau concentrations and a future likelihood of dying in severe dementia. This was possible due to the detailed documentation in the digital medical records system, which is common to all health care including the primary care. The most likely explanation to why we could demonstrate an association between high levels of CSF t-tau and p-tau and death of severe dementia, but not with total mortality, is the competing risk by premature death of other causes. Alzheimer’s disease has a substantial influence on longevity and we found no association between age and total mortality. Common causes of death in terminal dementia in study IV were bronchopneumonia and ischemic heart disease, consistent with previous findings.

In study IV we found associations between high levels of CSF t-tau and p-tau, and low concentrations of Aβ42, and a more aggressive course of the disease, defined as deterioration in the MMSE of 4 points/year or more. Importantly, the concentrations of CSF t-tau, p-tau and Aβ42 did not differ between subjects in the MCI stage and patients with mild/moderate dementia. Further, the results were unchanged when stratifying for baseline stage. We have found six reports on an association between high values of t-tau and/or p-tau and deterioration as measured by the MMSE, whereas three other studies have failed to find any association between CSF biomarkers and an aggressive course of the disease. Differences in AD severity, length of follow-up, small patient cohorts and different statistical methods are possible explanations to the divergent results (Table 1).
Table 1. Longitudinal studies on AD patients measuring CSF tau as predictor of deterioration rate.

<table>
<thead>
<tr>
<th>First author, year</th>
<th>Number of AD patients</th>
<th>Follow-up period (years)</th>
<th>Outcome</th>
<th>CSF tau levels predict rate of deterioration No/Yes</th>
</tr>
</thead>
<tbody>
<tr>
<td>Sunderland -99</td>
<td>29</td>
<td>2</td>
<td>MMSE</td>
<td>N</td>
</tr>
<tr>
<td>Hampel -05</td>
<td>22</td>
<td>1-3.5</td>
<td>Athropy MR</td>
<td>N (Y p-tau)</td>
</tr>
<tr>
<td>Schuff -09</td>
<td>53</td>
<td>1</td>
<td>Athropy MR</td>
<td>N</td>
</tr>
<tr>
<td>Vemuri -09</td>
<td>98</td>
<td>1.5</td>
<td>CDR-SB, MMSE</td>
<td>N</td>
</tr>
<tr>
<td>Henneman -09</td>
<td>31</td>
<td>1-2</td>
<td>Athropy MR</td>
<td>N (Y p-tau)</td>
</tr>
<tr>
<td>Sluimer -10</td>
<td>47</td>
<td>1-4</td>
<td>Athropy MR, MMSE</td>
<td>N</td>
</tr>
<tr>
<td>Okonkwo -10</td>
<td>100</td>
<td>3</td>
<td>ADL</td>
<td>N</td>
</tr>
<tr>
<td>Walhovd -10</td>
<td>25</td>
<td>2</td>
<td>Athropy MR</td>
<td>N</td>
</tr>
<tr>
<td>Boström -09</td>
<td>159</td>
<td>0-7</td>
<td>Mortality</td>
<td>N</td>
</tr>
<tr>
<td>Tosun -11</td>
<td>54</td>
<td>1-2</td>
<td>Athropy MR</td>
<td>N</td>
</tr>
<tr>
<td>Kanai -98</td>
<td>93</td>
<td>0.2-3.5</td>
<td>MMSE</td>
<td>Y</td>
</tr>
<tr>
<td>Wahlund -03</td>
<td>24</td>
<td>0.5-2.6</td>
<td>Athropy MR</td>
<td>Y</td>
</tr>
<tr>
<td>Wallin -06</td>
<td>21</td>
<td>6</td>
<td>Mortality</td>
<td>Y</td>
</tr>
<tr>
<td>Ravaglia -08</td>
<td>31</td>
<td>1</td>
<td>MMSE</td>
<td>Y (p-tau)</td>
</tr>
<tr>
<td>Kester -09</td>
<td>151</td>
<td>1-5</td>
<td>MMSE</td>
<td>Y</td>
</tr>
<tr>
<td>Fjell -10</td>
<td>90</td>
<td>1-2</td>
<td>Athropy MR</td>
<td>Y</td>
</tr>
<tr>
<td>Sämgård -10</td>
<td>142</td>
<td>3</td>
<td>MMSE</td>
<td>Y</td>
</tr>
<tr>
<td>Wallin -10</td>
<td>151</td>
<td>5</td>
<td>MMSE, Mortality</td>
<td>Y (cluster with extreme levels of CSF biomarkers)</td>
</tr>
<tr>
<td>Seppälä -11</td>
<td>56</td>
<td>3</td>
<td>MMSE</td>
<td>Y</td>
</tr>
<tr>
<td>Degerman Gunnarsson -13</td>
<td>196</td>
<td>2-9</td>
<td>MMSE Mortality in severe dementia</td>
<td>Y</td>
</tr>
</tbody>
</table>

Low levels of CSF Aß42 have also been reported to be associated with rapid cognitive decline\textsuperscript{82,83,191}. In accordance with these studies, we found that the patients in the lowest quartile of CSF Aß42 levels had an increased risk of dropping in MMSE \( \geq 4 \) points/year. Several other predictors of rapid progression of AD have been suggested, e.g. cardiovascular risk, age and
education\cite{192,193}. We found no association between these variables and rapid decline. High educational level was associated with a lower risk of dying in severe dementia in crude Cox proportional hazard analysis. However, the association did not remain significant in the multivariable adjusted models. Neither rapid decline nor death in advanced dementia was associated with the APOE ε4 genotype, which is also in accordance with previous studies\cite{194}.

Beside the large heterogeneity in the clinical presentations of AD, there is a great inter-individual variation of AD progression rate. Study IV strengthens previous research showing that high CSF t-tau and p-tau levels predict a poor prognosis in AD. Slightly more than one fifth of the cases suffered from a rapid cognitive decline. Clinical research should focus on these patients since they and their caregivers will experience a very low quality of life. CSF biomarkers aid in identifying the subjects who need prompt supportive interventions from a specialised Memory Clinic. Caregiver education, support from the community, contact with social workers, occupational therapists and physiotherapists, together with pharmacological treatment, can relieve some of the disease burden. Likewise, AD patients with CSF biomarkers indicating a mild course of the disease would benefit from this information. In study IV almost one fourth of the patients had not deteriorated at the last available follow-up MMSE, demonstrating a very mild disease progression. This favourable trajectory may be due to positive response to medical intervention and non-pharmacologic support to the patients and caregivers, as well as it indicates a natural mild course of AD\cite{30}. Furthermore, clinical trials trying to prove efficacy of future potential drugs should take into consideration the large inter-individual variation of AD disease progression. Maybe, a “normal disease deterioration rate” in patients with highly pathological CSF AD biomarkers could be interpreted as a response when evaluating effects of new potential drugs. The recent findings with a possible prion-like tau-driven neurodegeneration in AD have amplified the attention to the tau protein and its part in the pathophysiology\cite{18}. The results in study IV consolidate previous findings of tau pathology as a key process behind the clinical deterioration in AD.
Strengths and limitations

**Paper I:**
A strength was that all patients had pathologically elevated levels of CSF p-tau consistent with AD at baseline; the long interval between the two CSF samplings and that 11/13 patients completed the study. Weaknesses of this study include the lack of a control group and the small number of patients.

**Paper II:**
The very comprehensive measurements of potential biomarkers in CSF, plasma and urine, the extensive neuropsychological assessment and the FDG PET and PIB investigations, all repeated after one year, were the strengths of this study. A limitation was the small number of patients. A larger study would most likely identify more elusive differences between PIB+ and PIB- patients concerning concentrations of CSF, plasma and urine biomarkers.

**Paper III:**
A strength of this study was the mixed cohort of AD and non-AD patients, mimicking the weekday for physicians at memory clinics who often encounter a scenario of patients with overlapping clinical features, CSF biomarkers and structural and functional images. Additional strengths are the comprehensive neuropsychological test battery carried out and assessed by an experienced neuropsychologist and that all patients also were examined with both PIB PET and FDG PET. One obvious limitation of the study was the small number of patients. Larger studies would probably detect more subtle differences between PIB+ and PIB- patients concerning performance on psychometric tests and rCMRglu. Another limitation was that the design did not admit adjustment for differences in educational level and degree of cognitive impairment. Further, although all patients had a mild dementia at baseline, further adjustment for differences in the disease state was not possible.

**Paper IV:**
In contrast to the previous papers in this thesis, this paper investigates a large cohort of AD patients, which is a major strength. This is the first study that assessed death in severe dementia as an outcome. Other strengths include that we used clinically relevant end-points and that a high accuracy of the AD diagnosis was ensured. All patients received comprehensive
assessments, 98% had at least one pathological CSF biomarker and most patients were continuously re-evaluated at 6-12 months intervals during several years. However, only a few of the AD diagnoses are confirmed with post-mortem autopsy. Furthermore, the measures of co-morbidity were limited since only heart disease, number of drugs and diabetes were registered. In this cohort a large proportion of the patients was younger, highly educated and relatively healthy, with only a few patients suffering from diabetes and almost none with atrial fibrillation (since anticoagulant therapy is a contraindication of LP). Hence, the results are not readily applicable to a general AD population with concomitant cerebrovascular disease and multi-morbidity.
Conclusions

**Paper I:**
Today, a decade after memantine was approved as a pharmacological treatment of moderate-to-severe AD, we know that the clinical effects of the drug are limited. The results in this study, with a statistically significant reduction of CSF p-tau after one year of medication with memantine, are in concordance with previous *in vitro* findings. This effect indicates that memantine, despite the modest clinical benefits, may affect a key pathological process in AD. To date, to our knowledge, no study has tested this hypothesis in prodromal AD patients.

**Paper II**
The results confirm previous findings with PIB retention being stable over time; that a strong inverse relation between Aβ1-42 in CSF and PIB retention exists; and that PIB retention is positively correlated to the number of *APOE* ε4 alleles.

**Paper III**
The subtle differences in neuropsychological performance and the overlapping of hypometabolic patterns on FDG PET between patients clinically diagnosed as AD and non-AD dementia, highlight the need of amyloid biomarkers (amyloid PET or CSF Aβ) in the diagnostic work-up and the readiness to re-evaluate the initial diagnosis.

**Paper IV:**
In this large AD cohort we found a clear association between high levels of CSF t-tau and p-tau and a higher risk of dying in severe dementia. Further, low concentrations of CSF Aβ42 were associated with a rapid decline. Research investments should focus on those patients who will experience the most aggressive course of the disease. Our results consolidate previous findings of tau pathology as a key process behind the clinical deterioration in AD. Furthermore, CSF tau may be a possible target for AD therapy and also a possible tool to capture future treatment effects.
Alzheimers sjukdom är den vanligaste demenssjukdomen och står för drygt hälften av alla fall av demens. Sänkta koncentrationer i ryggvätskan (CSF) av proteinerna amyloid-beta samt förhöjda nivåer av total-tau (t-tau) och fosforylerat tau (p-tau) är värdefulla biomarkörer i diagnostiken av Alzheimers sjukdom. De kan även förutsäga vilka fall av lindrig kognitiv störning som har ökat risk att utveckla alzheimerdemens. FDG PET (positronemissionstomografi) mäter upptaget av en sockermolekyl i olika delar av hjärnan och kan i vissa fall vara ett viktigt komplement i demensutredningar. Vid Alzheimers sjukdom är upptaget typiskt nedsatt i de bakre delarna av tinning- och hjässlobarna. Ett annat sätt att använda PET-metoden är att mäta upptaget av PIB (Pittsburgh B), som är en markör för proteinet amyloid-beta. Inlagringen av fibrillärt amyloid-beta i hjärnan är en av grundläggande patofysiologiska processerna vid Alzheimers sjukdom. PIB PET har hittills endast använts i forskning. I dag finns ännu ingen botande behandling mot Alzheimers sjukdom, utan all medicin är symptomatiska. Utav fyra läkemedel som är registrerade mot alzheimerdemens är tre kolinesterashämmare och en är memantin (Ebixa®). Möjligt för denna avhandling var att studera Ebixas® effekt på de etablerade CSF alzheimerbiomarkörerna samt relationerna mellan olika potentiella alzheimerbiomarkörer i CSF, plasma och urin samt PIB PET, FDG PET samt neuropsykologiska testresultat. Vidare nämnade vi också undersöka huruvida CSF biomarkörer, utbildning, ålder och samsjuklighet predicerar för snabb progress i Alzheimers sjukdom samt död i avancerad demens.

I samtliga studier har patienter rekryterats från Minnesmottagningen vid Akademiska sjukhuset i Uppsala. I studie 1 följes 11 alzheimerpatienter med mild till måttlig demens under ett års behandling med Ebixa®. Kognitiva tester och lumbalpunktion utfördes dels före insättandet av läkemedlet samt dels efter 12 månaders behandling. De tio patienterna med klinisk diagnos Alzheimers sjukdom i studie II genomgick extensiva undersökningar vilka omfattade PIB PET, FDG PET, ett neuropsykologiskt testbatteri samt analys av alzheimerbiomarkörer i CSF, plasma och urin, där


Fynden i studie I överensstämmer med tidigare iakttagelser i studier på försöksdjur, och indikerar att Ebixa® påverkar fosforyleringsprocessen av tau vid Alzheimers sjukdom. Det stabila PIB upptaget över tid samt korrelationen mellan PIB retention och CSF amyloid-beta i studie II bekräftar tidigare studiers resultat. Sambandet mellan högt PIB upptag och försämrat visuellt episodminne i studie III var helt i linje med det förväntade, medan fyndet att de med höga PIB upptag hade signifikant sämre psykomotorisk hastighet var delvis oväntat. Det kan dock förklaras utifrån tidigare kunskap om skillnader och likheter mellan Alzheimers sjukdom, frontotemporal demens och Lewy body demens. De små skillnaderna vid kognitiv testning, de överlappande kliniska symtom och mönstren av nedsatt sockermetabolism mätt med FDG PET, tydliggör behovet av en amyloid-beta biomarkör (PIB PET alternativt CSF amyloid-beta) för att säkrare kunna diagnostisera Alzheimers sjukdom och icke-alzheimerdemens. Det tydliga sambandet i studie IV mellan CSF tau och ett mer aggressivt sjukdomsförlopp stämmer väl överens med det aktuella
kunskapsläget. Förutom en snabbare försämringstakt av resultaten på MMT, hade de patienter med höga CSF tau-koncentrationer ökad risk att dö i avancerad demens vilket inte tidigare visats i någon studie.
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