INTRODUCTION 1.1
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Dementia
Dementia is a syndrome characterized by progressive deterioration of cognitive function interfering with daily life activities. Most causes of dementia are of cerebral origin and progressive in nature. There are many causes of dementia, this thesis is focused on a disease named after the German neurologist and pathologist Alois Alzheimer.

Alzheimer’s disease
Alzheimer’s disease (AD) is the most prevalent form of dementia. In its most typical form, impairment in episodic memory is the first complaint. As the disease progresses memory impairment is followed by a combination of disturbances of language, praxis, visuospatial and executive functions.

A post-mortem diagnosis is based on the accumulation of extracellular amyloid and intraneuronal neurofibrillary tangles. During life, a probable diagnosis is made according to clinical criteria such as the NINCDS-ADRDA or DMS–IV criteria.1,2 According to these criteria there should be evidence for impairment in two or more cognitive domains, that interfere with activities of daily living, which are progressive, and are a decline from a previous level of functioning. Ancillary investigations like brain MRI and CSF analysis are increasingly having a prominent role (see below) in establishing a more reliable diagnosis.3

Pathology of AD
Neuropathologically AD is characterized by two types of abnormalities, the accumulation of amyloid beta (Aβ) in senile plaques and (hyperphosphorylated) tau in neurofibrillary tangles.

Senile plaques – Amyloid
Plaques are located extracellularly and consist mainly of the protein amyloid beta (Aβ). Aβ is continuously produced through proteolytic cleavage of amyloid precursor protein (APP), in multiple steps.4 Several amyloid proteins can stick together to form oligomers and subsequently fibrils, that in turn aggregate in plaques. There are several species of Aβ, with different numbers of amino-acids (e.g. Aβ37, Aβ38, Aβ39, Aβ40, Aβ42). Although the concentration of Aβ40 in CSF is considerably higher than that of Aβ42, the latter is the predominant component of the plaques.5 Plaque formation may be the result of either overproduction or failure to clear Aβ42. For this reason focus has been on the importance of Aβ42, while the role of Aβ40 should be explored further.

Neurofibrillary tangles - Tau
Tangles are located intracellularly, and abnormally hyperphosphorylated tau is their main component. Tau protein is needed for axonal integrity, by binding to microtubules it increases the rate of polymerization of the microtubule.6 In addition, it acts as a
promoter of tubulin polymerization, and the protein is involved in axonal transport. The tau protein can be phosphorylated, however the role of phosphorylation is not completely clear. It probably influences microtubule assembly, since phosphorylated tau is less effective in microtubule polymerization. In AD, hyperphosphorylated forms of tau are the principal component of the paired helical filaments. These abnormal cytoskeletal fibrils accumulate as neurofibrillary tangles. Overall it is clear that there is a direct relationship between tau phosphorylation and tangle formation, however it is not yet clear whether phosphorylation is a cause or a consequence.

RISK FACTORS FOR AD

Age

Age is the risk factor that is most consistently found for AD, presumably due to the fact that during life the brain is exposed to different forms of damage such as minor vascular events, white matter disease and inflammation. Furthermore, the increasing risk of AD is probably a reflection of increasing amyloid plaque formation with age. Clinical presentation is different between age groups. Young patients, at post-mortem examination, typically have pure AD without other types of pathology. In these patients the most characteristic type of AD is seen with pronounced memory deficits, however there are also young patients presenting without memory complaints, but with mainly language and orientation problems. Older patients often have a more mixed type of dementia with also vascular damage and Lewy bodies, in addition to plaques and tangles. Possibly there is a link in the pathogenesis of the different pathologies. For instance vascular damages may promote the aggravation of AD pathology. This is supported by autopsy studies which reported that vascular risk factors like hypertension were associated with greater numbers of amyloid plaques and neurofibrillary tangles in the brain.
**APOE genotype**

The polymorphism of apolipoprotein E (APOE) is the most important genetic risk factor for AD. APOE exists in three common polymorphisms (ε2, ε3 and ε4). APOE ε4 heterozygotes have a 2-3 times, and ε4 homozygotes even have a 6-8 times higher risk of developing AD than non ε4 carriers.\(^{12}\)

The APOE ε4 genotype has been found to be associated with an increased rate and extent of amyloid beta deposition and neurofibrillary tau pathology in post-mortem studies.\(^{13-15}\) Furthermore, non-demented subjects carrying the APOE ε4 allele were more likely to have amyloid plaques and neurofibrillary tangles than non-carriers.\(^{16}\) It is not yet clarified which mechanisms, and to what extent, play a role for APOE ε4 carriers in the development of AD. Theories relate increased amyloid production to increased accumulation in plaques,\(^{13,15,17}\) but there are also hypotheses related to the APOE ε4 genotype as vascular risk factor,\(^{18,19}\) increased inflammation\(^{20,21}\) and impaired repair mechanisms in APOE ε4 carriers.\(^{22}\) Furthermore, studies have shown that the APOE genotype with the clinical phenotype of AD. The typical amnestic phenotype, presenting mainly with memory problems seems to be promoted by the APOE ε4 allele, whereas the atypical non-memory phenotype (presenting with language and orientation difficulties) occurs in the absence of the APOE ε4 allele.\(^{23}\) Interestingly, these patients, with an atypical clinical presentation lacking the APOE ε4 allele, are also often younger than the APOE ε4 carriers.\(^{24}\)

**Biomarkers for AD**

Cerebrospinal fluid (CSF) provides a ‘reflection of the brain’, since it is in direct contact with the brain, where the pathological processes like senile plaque and neurofibrillary tangle formation take place. In the CSF Aβ42, as marker for amyloid, and tau and ptau-181, as markers for tangle pathology, can be measured.

**Aβ42**

The concentration of amyloid beta 1-42 (Aβ42) in CSF is reduced by about 50% in AD.\(^{25}\) Sensitivity is about 86% at a specificity of 90% for the discrimination of AD and healthy aging.\(^{26,27}\) Decreased levels of Aβ42 are thought to reflect amyloid pathology in the brain, which is supported by post-mortem studies which showed that CSF Aβ42 levels were well related with amyloid pathology.\(^{28,29}\) In addition, in-vivo amyloid imaging studies using PET, showed that increased amyloid deposition is reflected by reduced levels of CSF Aβ42.\(^{30,31}\) However, it is as yet unclear why levels of CSF Aβ42 are lower (as opposed to higher) in AD patients. It has been hypothesized that due to deposition of amyloid, less amyloid is diffused to the CSF. Aβ42 could also form oligomers in the CSF of AD patients, which results in less available monomers for measurement in an ELISA setting.\(^{32}\)
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Tau
CSF tau levels are increased 2-3 times in AD patients in comparison to non-demented controls. Sensitivity is about 81% at a specificity of 90% for the differentiation of AD and healthy aging.\textsuperscript{26} Tau levels can however also be raised due other causes. For example in Creutzfeldt Jakob disease and after acute stroke, CSF tau levels are extremely high. In these disorders there is a high rate of neuronal cell death. For this reason, CSF tau has been suggested to be a more general marker for neuronal damage which reflects the degree of neuronal degeneration.\textsuperscript{33-38}

Ptau-181
CSF levels of tau phosphorylated at threonine 181 (ptau-181) in AD can be increased by one or two orders of magnitude compared with non-demented controls. Sensitivity is about 80% at a specificity of 92% for the discrimination of AD from healthy aging.\textsuperscript{26} For the differentiation of AD from other types of dementia, ptau-181 has been considered to be more specific than Aβ42 and tau, although there are also contradictory reports.\textsuperscript{26} In the literature it has been assumed that CSF ptau reflects phosphorylation.\textsuperscript{36,39,40} In accordance, phosphorylated tau is considered to be a specific biomarker for AD as it is related to the process of neurofibrillary tangle formation.\textsuperscript{39-41}

Plasma biomarker
Until now there are no reliable biomarkers for AD that can be measured in plasma. Methods for measurement of Aβ40 and Aβ42 in plasma have however been developed. Plasma Aβ40 and Aβ42 levels were found to be predictive for the development of AD in healthy elderly,\textsuperscript{42,43} but have not yet been evaluated for the diagnosis of AD.

Aside from plasma biomarkers that are directly related to AD pathology, a recent promising study showed that a combination of signalling proteins could distinguish AD patients from controls.\textsuperscript{44} This study implicated that there was an overall reduction in the abundance of factors associated with hematopoiesis and inflammation during AD, as well as to deficits in neuroprotection, neurotrophic activity, phagocytosis and energy homeostasis. Further, independent, studies are needed to confirm this hypothesis.

CLINICAL CHALLENGES IN AD

Differential diagnosis
Other prevalent types of dementia are vascular dementia (VaD), frontotemporal lobar degeneration (FTLD) and dementia with Lewy bodies (DLB). CSF biomarkers amyloid beta 1-42 (Aβ42), total tau (tau) and tau phosphorylated at threonine 181 (ptau-181) can be used as biomarkers for a more reliable clinical diagnosis, in research settings sensitivity and specificity of 80-90% were shown for differentiation of AD patients from controls.\textsuperscript{26,45} However, the value of these biomarkers in the differentiation of AD from other dementias is less clear.\textsuperscript{46-48} In addition, it is important to note that most studies on the diagnostic value of CSF biomarkers have been done in specialized tertiary
referral settings that differ in many ways from local hospital memory clinics where a large part of demented patients are diagnosed.

MCI
A recent development has been the recognition of a state before dementia, called Mild Cognitive Impairment (MCI). MCI is defined by memory complaints of the patient, preferably supported by an informant, in combination with an objective memory deficit without interference with activities of daily living. The relevance of recognizing this state is that 15%-20% progress to dementia (mostly Alzheimer’s) per year, as compared to 1-2% of the elderly population. Earlier studies have shown that identification of MCI patients that will convert to AD is feasible with relatively high accuracy using CSF biomarkers Aβ42, tau and ptau-181. In addition the APOE genotype was associated with an elevated risk for AD in MCI patients. The combined effect of the APOE genotype and the CSF biomarkers as predictors for progression is however not yet clear.

Disease progression
AD, by definition, is a progressive disease, though the rate of progression is highly variable among individuals. Little is known about determinants for the rate of progression. Predicting progression and outcome in AD is clinically important, since it provides important information for patients and caregivers and could improve patient management. Results could for instance be used in treatment trials, studies could focus on patients with expected rapid decline and thereby increase their power. In addition, it has scientific importance by giving insight in the possible processes involved in progression of the disease. CSF biomarkers are associated with progression of MCI to AD. Results from studies to further cognitive decline in relation to the CSF biomarkers, however, are conflicting, and often have limited statistical power. It would be equally important to find a biomarker that reflects the course of AD, in order to monitor disease progression and effects of possible future treatment options. There have been several studies evaluating biomarkers as disease stage markers, however until now these biomarkers showed little effects in longitudinal settings. Further research is needed to find one or more biomarkers that reflect the stage of disease.
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REFERENCES


1.1


AIMS AND OUTLINE

1.2
The overall objective of this thesis was to extend the insight in the clinical applicability of body fluid biomarkers for AD. To this end, we evaluated the biomarkers in relation to several other factors:

» To explore the effects of APOE genotype, in relation to other risk factors, on CSF biomarker levels
» To explore the clinical applicability of CSF biomarkers in differential diagnosis
» To explore the value of CSF biomarkers in relation to AD disease progression
» To develop novel plasma biomarkers for AD

These four aims were pursued and the results of the studies resulted in the four following chapters:

Alzheimer’s disease and APOE genotype
In this chapter we explored the role of the APOE genotype in relation to CSF biomarker levels. In chapter 2.1 we looked at the relation of CSF biomarkers levels in relation to age and the APOE genotype, in AD patients and controls. In chapter 2.2 we analyzed the combined effect of hypertension and APOE genotype on CSF biomarker levels. In chapter 2.3 we followed-up MCI patients, and investigated the effect of APOE genotype in the prediction models of CSF biomarkers for progression of MCI to AD. In chapter 2.4 we compared AD patients with multiple microbleeds with those lacking this pathology on CSF biomarker levels and APOE genotype.

The value of CSF biomarkers in differential diagnosis of Alzheimer's disease
We subsequently explored the value of CSF biomarkers in the differential diagnosis of AD. In chapter 3.1 we explored the value of CSF biomarker testing in differential diagnosis for a clinician in a local hospital memory clinic. In chapter 3.2 we investigated the additional value of determination of cerebrospinal fluid Aβ40 levels in the differentiation of AD from controls, and frontotemporal lobar degeneration.

Progression of Alzheimer's disease and CSF biomarkers
In chapter 4.1 we investigated the value of CSF Aβ42, tau and ptau-181 in prediction models for cognitive decline in AD over time. In chapter 4.2 we investigated the use of several biomarkers (Aβ42, tau, ptau-181, Aβ40, neurofilaments and isoprostanone) in a longitudinal follow-up study, to find out whether these markers are usable to monitor disease progression.

Development of novel plasma biomarkers for Alzheimer's disease
In this chapter we described the use of newly developed plasma biomarkers for AD. In chapter 5.1 we described plasma Aβ42 and Aβ40 in the differentiation of AD patients from controls. In chapter 5.2 we described the use of messenger RNA expression for several proteins related to inflammation, hematopoiesis and apoptosis.

In chapter 6 the main findings of this thesis are summarized and discussed and recommendations for future research are given.