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The role of biomarkers in preclinical and prodromal Alzheimer's disease

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THE ROLE OF BIOMARKERS IN

PRECLINICAL AND PRODROMAL ALZHEIMER'S DISEASE

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THE ROLE OF BIOMARKERS IN PRECLINICAL AND PRODROMAL ALZHEIMER'S DISEASE

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Promotor:
Prof. dr. F.R.J. Verhey

Co-promotor:
Dr. P.J. Visser

Beoordelingscommissie:
Prof. dr. R.W.H.M. Ponds (voorzitter)
Prof. dr. F. Jessen (University Hospital Bonn, Duitsland)
Dr. S. Köhler
Prof. dr. R.J. van Oostenbrugge
Dr. N.S. Schoonenboom (Kennemer Gasthuis, Haarlem)

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alzheimer nederland

INTERNATIONALE STICHTING ALZHEIMER ONDERZOEK

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Voor oma

“In some ways disease does not exist until we have agreed that it does - by perceiving, naming, and responding to it.”

Charles E. Rosenberg

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CHAPTER 1

GENERAL INTRODUCTION

AIM

OUTLINE OF THESIS

GENERAL INTRODUCTION

Alzheimer's disease

Alzheimer's disease (AD) is a progressive neurodegenerative disease consisting of a long asymptomatic stage, a stage of mild cognitive impairment and finally a dementia stage in which impaired cognition leads to interference in daily life activities. AD accounts for the majority of dementia cases among people over the age of 65[1,2] and is worldwide one of the fastest growing threats to health with no cure available so far.[3] The global prevalence of dementia is now 35.6 million[4] and is expected to affect more than 80 million people worldwide by 2040.[5,6] The cost of dementia care is likely to double by that time.[7] Advances in early diagnosis of AD as well as in therapy are needed to anticipate and manage future societal burden and costs.[2,8]

According to the original criteria for AD that were published in 1984,[9] a clinical diagnosis was only possible when someone is already demented. At that stage extensive neuropathological damage has occurred and treatment has limited effects.[10] The last decade, tremendous progress has been made in the area of biomarkers for AD neuropathology. This makes it possible to identify subjects with AD before they are demented, which opens new opportunities for treatment and prevention of the disease.[11] At present, one of the major challenges is to investigate how biomarkers or biomarker combinations can be used for diagnosis and prognosis of AD for clinical and research purposes. In this thesis we will study the use of AD biomarkers in the stage before dementia, i.e. the predementia stages.

Biomarkers for Alzheimer's disease neuropathology

The neuropathological hallmarks of AD include extracellular amyloid plaques, composed of aggregated amyloid-ß, and intracellular neurofibrillary tangles, consisting of hyperphosphorylated tau proteins.[12,13] Other important pathological features are neuroinflammation and neurodegeneration, which is characterized by synaptic loss, neuron loss, and overall brain atrophy.[14] AD biomarkers should be surrogate markers of these neuropathological changes.[15] We can differentiate between biomarkers for amyloid pathology and biomarkers of neuronal injury. Previous studies have shown that decreased amyloid-ß in cerebrospinal fluid (CSF)[16-18] and increased binding of positron emission tomography (PET)-amyloid tracers[19,20] are good markers of amyloid pathology, whereas increased total tau and phosphorylated tau in CSF,[16-18] hippocampal atrophy on magnetic resonance imaging (MRI)[12,21,22] and hypometabolism as measured by PET[23,24] have shown to be good markers of neuronal injury.

Diagnostic criteria for Alzheimer's disease

Advances in the AD biomarker field have changed our view on AD. This, in turn, has recently led to the development of new diagnostic criteria for AD.[25-29] Currently, these criteria are considered research criteria although they will likely be applied in clinical practice in the

future. Below we describe the old and new classification of AD and the role of biomarkers in the new criteria.

Old classification

According to the original criteria for AD,[9] a clinical diagnosis was primarily based on clinical symptoms and was only possible when clinical symptoms surpass the threshold of dementia. These criteria for AD-type dementia required cognitive impairments in memory and at least one other domain that lead to interference in daily living.

Subjects with cognitive impairments that are still able to function independently in daily life were diagnosed with mild cognitive impairment (MCI).[30] These subjects are at increased risk of developing dementia, with annual progression rates from MCI to a clinical diagnosis of AD-type dementia of 5-15%.[31,32] Subjects with amnestic MCI are most likely to progress to AD-type dementia, although AD may also initially present as non-amnestic MCI.[33-37] Several subjects with MCI will however remain stable or revert back to normal cognition.[38] The challenge is to identify those subjects that will develop AD-type dementia as early as possible. Table 1 summarizes these stages of AD.

Table 1. Glossary of AD concepts according to the old and new classification

OLD CLASSICIATION	
Cognitively healthy	No cognitive impairments regardless of underlying AD pathology
Amnestic MCI	Impairments in memory (and possibly other domains) regardless of underlying AD pathology
Non-amnestic MCI	Impairments in non-memory domain(s) regardless of underlying AD pathology
AD-type dementia	Clinical diagnosis of AD-type dementia regardless of underlying pathology
NEW CLASSIFICATION	
Preclinical AD / asymptomatic AD	Cognitively healthy with underlying AD pathology
Prodromal AD / MCI due to AD	Mild cognitive impairment(s) with underlying AD pathology
Dementia due to AD	Clinical diagnosis of AD-type dementia with underlying AD pathology

AD=Alzheimer's disease, MCI=mild cognitive impairment

New classification

The key differences of the new AD criteria from the original criteria are the incorporation of biomarkers and the distinction between different stages of the disease, which allows a diagnosis of AD before the dementia stage, i.e. in the predementia stages.

Alzheimer's disease is now subdivided in different clinical stages depending on cognitive status. Preclinical or asymptomatic AD is the earliest stage in which AD neuropathology begins to accumulate but cognitive performance is still normal. Prodromal AD or MCI due to AD is the next disease stage in which individuals experience MCI. Dementia due to AD is the last stage with AD pathology and a clinical diagnosis of AD-type

dementia. Table 1 summarizes these stages of AD and Figure 1 shows how these stages relate to the old classification.

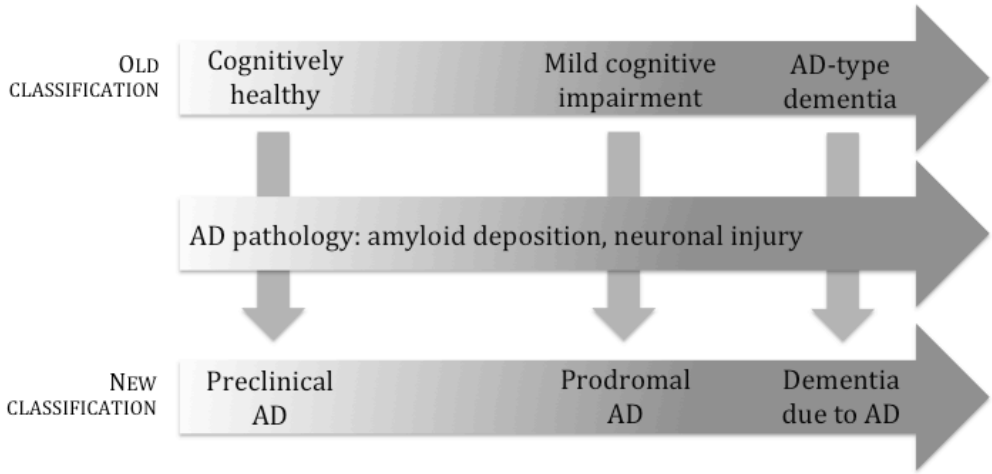

Figure 1. The relation between several AD stages of the old and new classification.

The international working group (IWG) of Dubois and colleagues was the first to present revised AD criteria.[25,26] The IWG referred to subjects with normal cognition and amyloid pathology as asymptomatic at risk for AD. Furthermore, they introduced the concept of prodromal AD, defined by at least one abnormal well-established AD biomarker in the presence of episodic memory impairment without interference in daily functioning. In presence of a clinical diagnosis of AD-type dementia, this latter group is referred to as AD dementia. While memory impairment is highlighted in the IWG criteria, other clinical phenotypes may be associated with atypical forms of AD. Later on, the National Institute on Aging and Alzheimer's Association (NIA-AA) workgroup proposed new revised criteria for AD.[39] These criteria divide the preclinical AD stage in 3 ordered substages: abnormal amyloid markers (stage 1), abnormal amyloid and neuronal injury markers (stage 2) and abnormal amyloid and neuronal injury markers and subtle cognitive changes (stage 3).[27] For the diagnosis of MCI due to AD or dementia due to AD, the number of positive biomarkers are supposed to be related to the likelihood that the diagnosis is due to AD: both a positive amyloid and injury marker is high likelihood, one positive marker is intermediate likelihood, and both a negative amyloid and injury marker is unlikely that the cognitive/clinical impairments are due to AD.[28,29] Conflicting amyloid and injury marker results are considered uninformative. The NIA-AA criteria emphasize that subjects can also present initially with non-memory problems. Before implementation of these proposed sets of criteria in clinical routine, the criteria need to be thoroughly validated to see whether they refer to a solid disease construct and whether they reflect different stages of disease severity with different progression rates to AD-type dementia. Figure 2 summarizes the proposed criteria for subjects with predementia AD.

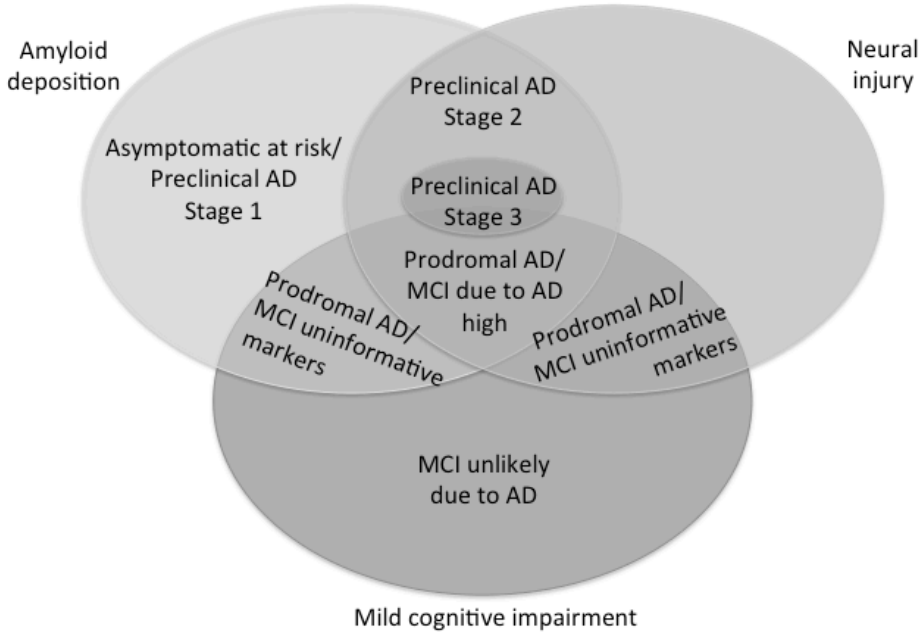

Figure 2. Summary of the proposed IWG and NIA-AA criteria for subjects with predementia AD

Use of biomarkers in the new diagnostic criteria

Appropriate biomarkers are needed to apply the recently proposed diagnostic criteria for AD. The last decades, AD research has been dominated by the amyloid cascade hypothesis.[40,41] This hypothesis states that AD pathophysiology starts with amyloid deposition 10 to 15 years before clinical symptoms appear, which in turn initiates multiple downstream events leading to neuronal injury and eventually to clinical symptoms. Based on the position in the amyloid cascade, amyloid markers are considered early AD markers, while neuronal injury markers may better predict future cognitive decline, since they become abnormal at a later stage, closer to clinical symptoms.[42,43]

As biomarkers are starting to find their way into clinical practice, knowledge about how they should be applied in clinical settings is urgently needed. It is not yet clear which biomarker or which combination of biomarkers we should use for optimal prediction of AD-type dementia at different stages of the disease, since the exact temporal order of AD biomarker abnormality is not known yet. Moreover, it is difficult to understand the relative importance of different biomarkers when used together and to interpret biomarker results when they are conflicting.[28] These issues need to be addressed before biomarkers can be widely applied in clinical practice.

There are also some problems with the CSF analysis itself. Previous studies showed considerable variability between analyses in the same laboratory as well as in different laboratories.[44-48] The impact of CSF variability on the diagnostic CSF-based AD classification of subjects is not examined yet. Together with the lack of standardized CSF cut-offs to define markers as normal vs. abnormal,[49] this may have a major influence on diagnosis or treatment decisions in clinical practice.

AIM OF THE THESIS

The general aim of this thesis was to investigate the role of biomarkers for diagnosis and prognosis of Alzheimer's disease in cognitively normal subjects, subjects with amnestic MCI, and subjects with non-amnestic MCI.

Our study addressed the following questions:

[1] What is the predictive ability of CSF biomarkers for AD-type dementia in subjects with MCI?

[2] What is the best combination of CSF and MRI biomarkers for predicting AD-type dementia in subjects with MCI?

[3] Is the predictive ability of biomarkers for AD-type dementia different in subjects with amnestic and non-amnestic MCI?

[4] Which biomarkers can predict clinical progression in subjects with abnormal amyloid-ß in CSF?

[5] What is the influence of CSF variability within and between centers on CSF-based AD diagnosis?

[6] What is the prevalence and prognosis of preclinical AD based on the NIA-AA criteria?

[7] What is the prevalence and prognosis of prodromal AD based on the IWG and NIA-AA criteria?

OUTLINE OF THE THESIS

The thesis consists of two parts. In part I, we describe the diagnostic and prognostic ability of single and combined AD biomarkers in subjects with MCI. In part II, we focus on the recently proposed diagnostic criteria for preclinical and prodromal AD. These criteria highlight the use of biomarkers to increase the certainty of whether a subject has underlying AD pathology or not.

The outline is as follows:

<u>PART I</u>

Chapter 2 provides a review and meta-analysis of the use of CSF and plasma AD biomarkers for the prediction of AD-type dementia in subjects with MCI.

Chapter 3 compares the predictive ability of CSF and MRI biomarkers for AD-type dementia in subjects with MCI of the European multi-center memory clinic-based DESCRIPA study. We determined the optimal test sequence of these biomarkers.

In **Chapter 4** the predictive ability of CSF markers, MRI markers and APOE genotype for AD-type dementia is compared in subjects with amnestic and non-amnestic MCI of the DESCRIPA study.

Chapter 5 assesses the progression rate to dementia and identifies prognostic markers in subjects with MCI and evidence of CSF amyloid pathology recruited from the DESCRIPA study.

In **Chapter 6** we report the variability between CSF analyses in the same laboratory and in different laboratories and the influence of this variability on CSF-based AD diagnosis in a memory-clinic population of the Dutch multi-center LeARN study.

PART II

In **Chapter 7** we compare the theoretical framework of the International Working Group (IWG) criteria and National Institute on Aging–Alzheimer's Association (NIA-AA) criteria for Alzheimer's disease

Chapter 8 describes the prevalence and prognosis of subjects with preclinical AD according to CSF biomarkers and the proposed NIA-AA criteria. Subjects were recruited from longitudinal studies of memory and aging in cognitively normal community-dwelling volunteers at the Knight Alzheimer's Disease Research Center (ADRC) of the Washington University School of Medicine in St. Louis (USA).

In **Chapter 9** the prevalence and prognosis of prodromal AD is examined based on well-established biomarkers and the proposed NIA-AA and IWG criteria. Subjects were recruited from multiple centers of the European Alzheimer's Disease Consortium (EADC) and the Alzheimer's Disease Neuroimaging Initiative (ADNI).

Last, **Chapter 10** provides a general discussion, summary of the main findings, list of author affiliations, list of publications, information about the author, and acknowledgements.

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PART I

BIOMARKERS FOR ALZHEIMER'S DISEASE IN SUBJECTS WITH MCI

CHAPTER 2

BIOMARKERS AS PREDICTOR FOR CONVERSION FROM MILD COGNITIVE IMPAIRMENT TO ALZHEIMER-TYPE DEMENTIA

IMPLICATIONS FOR TRIAL DESIGN

Journal of Alzheimer's Disease 2010;20:881-891

Ineke A. van Rossum, Stephanie Vos, Ron Handels, Pieter Jelle Visser

ABSTRACT

Disease modifying drugs for Alzheimer's disease (AD) are likely to be most effective when given in non-demented subjects. In this review we summarized biomarkers in cerebrospinal fluid (CSF) and blood that can predict AD-type dementia in subjects with mild cognitive impairment (MCI). In addition we investigated whether these markers could reduce sample size and costs if used to select subjects for trials on the prevention of AD in subjects with MCI. A meta-analysis of markers that had been investigated in multiple studies showed that the combination of Aβ1-42 and tau in CSF had the best predictive accuracy for AD (odds ratio (OR) 18.1, 95% confidence interval (CI) 9.6-32.4). Aβ1-42, total tau and phosphorylated tau in CSF also predicted conversion, but with lower accuracy (OR 7.5 to 8.1). Plasma level of Aβ1-40 tended to predict conversion to AD (Cohen's delta 0.23, 95% CI -0.01-0.48, p 0.07). Plasma levels of Aβ1-42, the ratio Aβ1-42/Aβ1-40 and homocysteine did not predict outcome. In a fictive trial design, the use of the combination of Aβ1-42 and tau in CSF in the selection of subjects could reduce sample size by 67% and trial costs by 60% compared to a trial in which unselected subjects with MCI would be enrolled. In conclusion, the combination of Aβ1-42 and tau in CSF is useful to select subjects for trials that aim to slow down the progression from MCI to AD-type dementia.

INTRODUCTION

Disease modifying drugs that may slow down the progression of Alzheimer's disease (AD) are currently under development. These drugs are likely to be most effective when given early in the course of the disease, but diagnostic criteria for AD in non-demented subjects are currently lacking. Predementia AD can not be accurately diagnosed by clinical criteria alone, such as criteria for mild cognitive impairment (MCI).[1,2] Biomarkers may help to increase the diagnostic accuracy compared to clinical criteria,[3] because pathological changes of AD can already be found in non-demented subjects.[4] Which biomarkers would be most useful as inclusion criterion in trials with disease modifying drugs and to what extent these markers could reduce sample size and costs of such trials is however unknown.

The aim of the present study was to investigate which biomarkers could predict AD-type dementia in subjects with MCI and how the use of these biomarkers as an inclusion criterion for trials with drugs that aim to slow down the progression from MCI to AD-type dementia, could reduce sample size and costs. We limited our study to biomarkers in cerebrospinal fluid (CSF) and blood.

First, we will briefly summarize the pathophysiology in AD, since appropriate biomarkers should reflect AD pathology.[5] Next, we will discuss biomarkers for the key pathological processes in AD in CSF and blood and discuss their use as predictor for progression to AD-type dementia in subjects with MCI. Of CSF and blood markers that have been investigated in at least 4 studies, we will calculate pooled measures of predictive accuracy. Finally, we will use the predictive accuracy of the best markers to calculate for a fictive trial how the use of these markers as an inclusion criterion will influence sample size and costs.

PATHOPHYSIOLOGY OF AD

Abnormalities in amyloid beta (Aβ) processing play a central role in the pathophysiology of AD. In AD, Aβ peptides aggregate and form oligomers, fibrils and eventually plaques, a pathological hallmark of AD. Aggregated Aβ is supposed to be neurotoxic and to initiate a cascade of events including oxidative stress, inflammation and dysregulation of lipid metabolism. This cascade eventually leads to loss of synapses and cell death, as shown in figure 1.[6] Different isoforms of the Aβ peptide are generated from the amyloid beta precursor protein (AβPP). In AD, especially Aβ1-42 seems to be important as it is more prone to aggregate compared to other isoforms. Cumulating evidence suggests that mainly the oligomer form of Aβ is toxic.[7] The increase in aggregated Aβ may be caused by either increased production, disturbance of the post-translational modification or reduced clearance of Aβ. Another pathological hallmark of AD are neurofibrillary tangles. These are aggregates of hyperphosphorylated tau and may contribute to neuronal dysfunction. Inflammatory processes, oxidative stress and alteration of lipid metabolism are though to occur as a consequence of Aβ pathology, but may in turn further dysregulate Aβ processing.

The primary cause for dysregulation of the Aβ metabolism is unknown and multiple factors may be involved, for example genetic or environmental factors.

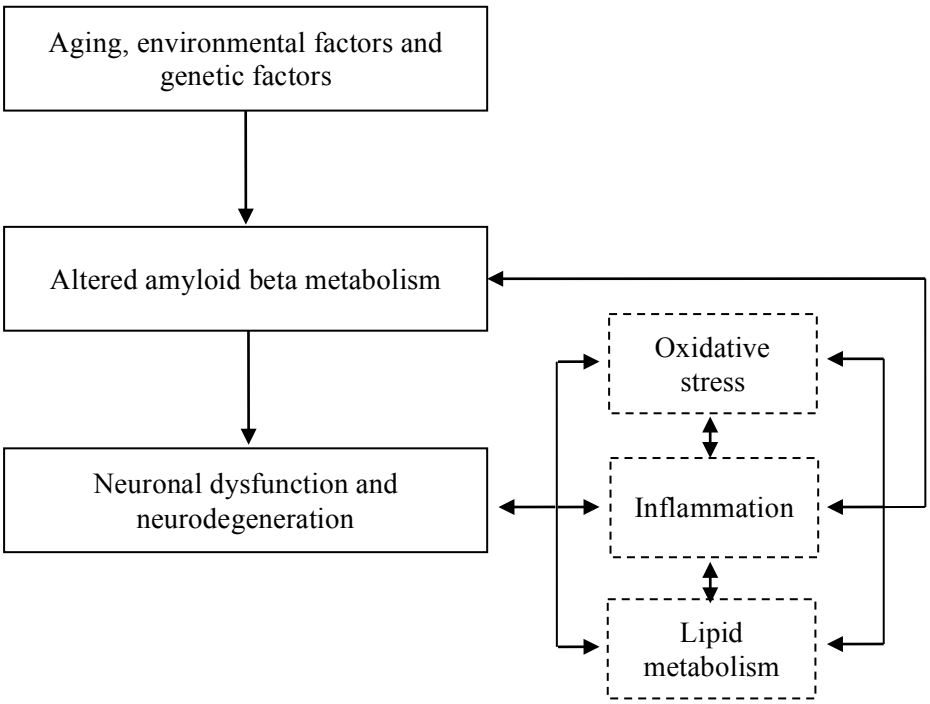

Figure 1. Pathophysiology of Alzheimer's disease

Biomarkers for AD in subjects with MCI

Many of the biochemical changes related to the pathophysiology of AD can be measured in vivo in body fluids.

Markers for Aβ processing in CSF

Biomarkers for Aβ processing in CSF include isoforms of the peptide itself as well as markers for its production and clearance. Aβ peptides can be measured with assays that measure monomers or aggregated species, or with assays that are not specific for aggregation status.[8-10] The concentration Aβ1-42 correlates with the presence of amyloid plaques in the brain.[11] Many studies have shown that in MCI subjects a low concentration of Aβ1-42 in CSF can predict conversion to AD-type dementia, as will be discussed in more detail below. Also a combination of different Aβ isoforms in CSF may predict AD-type dementia in subjects with MCI.[12] Production of Aβ1-42 from AβPP is dependent on the β site AβPP cleaving enzyme (BACE-1). One study showed that increased BACE1 activity could predict AD-type dementia in MCI subjects.[13] Of the peptides that may be involved in the clearance of Aβ only few have been investigated in CSF of subjects with MCI. Levels of neprilysin were decreased in subjects with progressive MCI and mild AD-type dementia compared to controls.[14] Aβ binding proteins may also influence Aβ aggregation and a lower level of serum amyloid P component predicted AD-type dementia in MCI subjects.[15]

Markers for Aβ processing in plasma

In plasma, abnormal Aβ concentrations have been associated with AD, although they do not correlate with the occurrence of Aβ plaques in the brain.[16,17] Four longitudinal studies have assessed predictive value of plasma Aβ levels in MCI subjects as will be discussed in more detail below. In a small study, the AβPP isoform ratio in platelets could predict conversion to AD-type dementia in MCI subjects with a sensitivity and specificity of 0.83.[18]

Markers for neurodegeneration

Many markers of neurodegeneration are available, but most of them are not specific for AD pathology. Concentrations of total tau (t-tau) and phosphorylated tau (p-tau) can be measured in CSF and correlate with the presence of neurofibrillary tangles.[11,19] In MCI subjects increased CSF level of t-tau and p-tau were associated with an increased risk of progression to AD-type dementia as will be discussed below. In plasma, levels of tau are very low and no reliable assay is available yet. Other markers for neurodegeneration, like visinin-like protein, neurofilaments and GAP43 (neuromodulin) are increased in CSF of AD subjects compared to controls,[20] but have not been tested as predictor for AD-type dementia in subjects with MCI.

Markers for inflammation

A wide range of inflammatory markers have been studied in blood and CSF of AD subjects, including cytokines, acute-phase proteins and complement factors, but none of these markers could discriminate between subjects with AD and healthy controls.[21-24] In MCI, CSF tumor necrosis factor α (TNF-α) was increased and transforming growth factor beta (TGFβ) was decreased in subjects progressing to AD-type dementia compared to healthy controls, but not compared to subjects with stable MCI.[25] Another study showed higher levels of TNF receptors in CSF as well as in plasma in MCI subjects that converted to AD-type dementia.[26,27] Several other cytokines did not predict progression to AD-type dementia.[25,28] Antibodies against Aβ are found in CSF and plasma of AD subjects. Studies comparing levels of antibodies of AD subjects and healthy controls showed conflicting results.[21] The possible use of antibodies as marker in MCI has not been studied yet.

Markers for oxidative stress

Some markers of lipid peroxidation, protein oxidation and nitrosylation and DNA and RNA oxidation have shown to be abnormal in CSF, plasma or peripheral blood cells of subjects with AD-type dementia.[29] In MCI subjects, an increased level of F2-isoprostane, a marker of lipid peroxidation was associated with cognitive decline and progression to AD-type dementia.[30]

Markers for lipid metabolism

Serum cholesterol is suggested to be associated with an increased risk for AD, but results have been conflicting.[31] In CSF, increased levels of 24OHC and 27-hydroxycholestrol

(27OHC) were found in AD and MCI subjects compared to controls.[32-35] In plasma, results have been conflicting.[31] One study in MCI found that CSF levels of sulfatide and the ratio of sulfatide to phosphatidylinositol were decreased in very mildly demented subjects.[36] However, the predictive accuracy for AD-type dementia in subjects with MCI has not yet been tested.

Other markers

Homocysteine

Increased plasma homocysteine may be associated with an increased risk for AD, although the exact pathophysiological mechanism behind this is not clear.[37] Two studies found an association between homocysteine level and incidence of AD in the general population,[38,39] another study found no association of plasma homocysteine level and the incidence of MCI.[40] The predictive accuracy of homocysteine for AD-type dementia in subjects with MCI will be discussed in more detail below.

Proteomics

Unbiased approaches have been useful to select biomarkers in CSF or plasma that may differentiate between AD subjects and healthy controls.[23] In CSF, a panel of 17 proteins and peptides in CSF could distinguish between subjects with stable MCI and subjects with MCI who progressed to AD-type dementia.[41] In plasma, a combination of 18 different proteins in plasma predicted progression to AD-type dementia in MCI subjects with over 90% accuracy.[42] In a subsequent study that tested for reproducibility of these markers diagnostic accuracy was however lower.[43]

POOLED ANALYSIS OF PREDICTORS FOR AD IN SUBJECTS WITH MCI

We performed a meta-analysis of markers that had been tested in at least 4 studies to obtain pooled estimates of the predictive accuracy for progression to AD-type dementia in subjects with MCI. These markers were Aβ1-42, t-tau, and p-tau in CSF and Aβ1-40, Aβ1-42 and homocysteine in plasma. We identified studies through a systematic search in PubMed, Medline and Psychinfo (search terms available on request) and from the references of retrieved studies. If multiple studies reported on the same sample, we included the study with the longest follow up or the largest sample size. Data were pooled in a random effects model with STATA (version 9.2; Stata Corp, College Station, TX). For continuous variables we calculated Cohen's delta as outcome measure.[44] For dichotomized scores we calculated the sensitivity, specificity, positive predictive value and odds ratio (OR). These dichotomized scores were based on cut-offs provided in the study. If different types of cut-off levels were used within one study, we chose predefined cut-offs over data-driven cut-offs.

Aβ1-42, t-tau and p-tau in CSF

We identified 19 studies[4,25,45-61] which provided unique data of CSF levels of Aβ1-42, t-tau, p-tau as predictor for AD-type dementia in subjects with MCI (table 1). One multi-centre study may have included data that had been presented in other studies as well,[56] but exclusion of this study did not change the outcome substantially and therefore the study was included in the analyses.

The mean conversion rate to AD-type dementia in the studies was 37% during a mean follow up of 2.5 years. Cohen's delta showed that Aβ1-42, t-tau and p-tau were significant predictors of outcome (table 2). The highest odds ratio (OR) for AD-type dementia was found for the combination of Aβ1-42 and t-tau with or without p-tau (OR 18.1, 95% confidence interval (CI) 9.6-34.2). An abnormal combination was defined as an abnormal ratio of these markers or as an abnormal score for at least 2 markers. The combination Aβ1-42 with only p-tau had a slightly lower OR (17.5, 95% CI 10-30.6). For the individual markers the OR ranged from 7.54 (Aβ1-42) to 8.05 (p-tau). For further analysis we selected the combination of Aβ1-42 and t-tau, which was studied in 8 studies with a total of 1236 MCI subjects of which 454 converted to AD-type dementia. The sensitivity of this combination to predict AD-type dementia was 0.87 (95% CI 0.80-0.95), the specificity 0.70 (95% CI 0.57-0.83) and the positive predictive value 0.65 (95% CI 0.53-0.77).

Aβ1-40 and Aβ1-42 in plasma

We selected 4 studies on the association of Aβ plasma levels with conversion to AD-type dementia in subjects with MCI[62-64] in which a total of 244 subjects with MCI was included (table 3). The mean conversion rate to AD-type dementia was 51% during a mean follow up of 4.0 years. The pooled effect sizes for Aβ1-40, Aβ 1-42 and the ratio Aβ 1-42/Aβ1-40 and conversion to AD-type dementia are shown in table 2. None of the effect sizes was statistically significant, although increased levels of Aβ1-40 tended to be associated with conversion to AD-type dementia (Cohen's delta 0.23, p=0.07 (table 3, figure 2)). No dichotomized scores were available.

Homocysteine in plasma

Four studies[55,65-67] with in total 369 subjects investigated the predictive value of homocysteine for AD-type dementia in subjects with MCI (table 3). Mean conversion rate to AD-type dementia was 37% during a mean follow up of 3.3 years. Homocysteine did not predict AD-type dementia (Cohen's delta 0.21, table 2). No dichotomized scores were available.

Table 1. Baseline characteristics of studies included in the meta-analysis of CSF biomarkers in subjects with MCI.

Study	N	Age	MMSE	Follow up (year)	Markers from each study included in the meta-analysis*	
					Dichotomous outcome measure	Cohen's delta
Riemenschneider (2002)[59]	28	70.1 (8.1)	27.4 (1.5)	1.5	4	-
Hampel (2003)[50]	52	72.5 (8.3)	28.9 (1)	0.7 (0.4)	1,2	1,2
Tarowski (2003)[25]	56	74.0 (7.5)	28.6 (1.5)	0.8 (range 0.2-3.0)	-	1,2
Zetterberg (2003)[61]	53	range 50-83	27.0 (1.7)	1.7 (0.8)	2,4	-
Maruyama (2004)[55]	57	73.9 (6.2)	25.7 (1.3)	2.0 (0.4)	2	2
Herukka (2005)[53]	78	70.4 (7.4)	-	3.0 (range 0.5-12)	1-4	1,2,3
Hansson (2006)[52]	134	71.8 (range 50-86)	27.0 (1.6)	4.7 (range 1.1-6.8)	3,4	1,2,3
Parnetti (2006)[58]	55	-	-	1	1-3	1,2,3
Ewers (2007)[47]	88	70.9 (7.3)	27.0 (2.0)	1.8 (0.8)	3	-
Fellgiebel (2007)[48]	16	68.6 (7.9)	25.7 (2.7)	1.6 (0.8)	3	2,3
Hansson (2007)[52]	134	71.8 (range 50-86)	27.1 (1.6)	4.7 (range 1.1-6.8)	1	-
Schonknecht (2007)[60]	80	69.3 (7.8)	26.6 (1.6)	≥ 1	-	2,3
Forsberg (2008)[49]	21	63.3 (7.8)	28.2 (1.4)	0.7 (0.5)	1-3	1,2,3
Blom (2009)[45]	58	62.9 (8.2)	-	4.5 (2.4)	4	1,2,3
Brys (2009)[46]	65	71.8 (7.9)	27.8 (1.8)	2.0 (0.6)	2-4	2,3
Kester (2009)[54]	100	67.8 (8.2)	26.6 (2.4)	1.5	1,2	-
Mattsson (2009)[56]	750	69.0 (range 43-89)	27.0 (range 16-30)	3.0 (range 2-11)	1-4	-
Papaliagkas (2009)[57]	53	67.7 (7.8)	27.8 (range 24-29)	0.9 (0.5)	1	-
Visser (2009)[4]	108	70 (7.7)	26.5 (2.6)	2.1 (0.7)	4	-

Data are mean (SD), unless otherwise specified. MMSE=Mini-Mental State Examination

*1=Aβ1-42, 2=Total tau, 3=Phosphorylated tau, 4=Combination of Aβ1-42 with total tau or phosphorylated tau.

Table 2. Effect sizes of biomarkers in CSF or plasma for prediction of Alzheimer's disease in subjects with MCI

	Number of studies	Number of subjects (AD at follow-up/ no-AD at follow-up)	Cohen's delta (95% CI)	P-value
CSF				
Aβ1-42	7	172/252	0.84 (0.45-1.24)	<0.001
T-tau	11	238/404	0.90 (0.62-1.17)	<0.001
P-tau	8	161/316	1.21 (0.85-1.57)	<0.001
Plasma				
Aβ1-40	4	133/190	0.23 (-0.01-0.48)	0.07
Aβ1-42	4	133/190	0.03 (-0.31-0.38)	0.55
Ratio Aβ1-42/Aβ1-40	4	133/190	-0.13 (-0.37-0.12)	0.25
Homocysteine	4	138/231	0.21 (-0.08-0.50)	0.15

AD=Alzheimer type dementia; CSF=Cerebrospinal fluid; Aβ1-42= Amyloid beta peptide 1-42; Aβ1-40=Amyloid beta peptide 1-40; T-tau=Total tau; P=tau=Phosphorylated tau.

Table 3. Characteristics of studies included in the meta-analysis of plasma markers in subjects with MCI

Study	N	Age	MMSE	Follow up (year)
Aβ1-42 and Aβ1-40				
Hansson (2008)[63]	117	69.7, range 50-85	27.0 (1.6)	5.2
Hansson (2008)[63]	110	62.9, range 47-79	27.0 (1.6)	Range 2-4
Lopez (2008)[64]	42	79.8 (4.3)	-	4.5
Cammarata (2009)[62]	79	75.2 (5.2)	> 26	2.0
Homocysteine				
Maruyama (2004)[55]	57	73.9 (5.7)	25.6 (1.3)	2.0
Annerbo (2006)[65]	93	65.0 (8.5)	26.4 (2.6)	6.0
Gabryelewicz (2007)[66]	105	69.3 (6.9)	27.3 (1.8)	3.0
Siuda (2009)[67]	55	70.8 (5.7)	-	1.0

Data are mean (SD), unless otherwise specified. MMSE=Mini Mental State Examination; Aβ1-42= Amyloid beta peptide 1-42; Aβ1-40=Amyloid beta peptide 1-40.

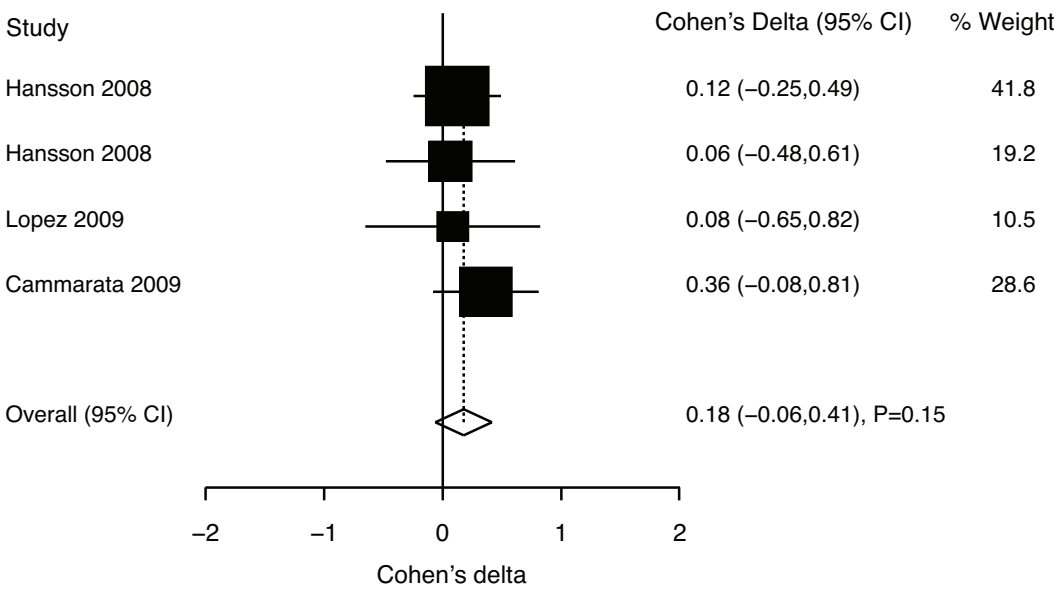

Figure 2. Pooled Cohen's delta of Aβ-40 plasma levels for the prediction of AD-type dementia in subjects with MCI.

A positive Cohen's delta indicates that increased plasma levels are associated with an increased risk for AD.

USE OF CSF BIOMARKERS IN SELECTION SUBJECTS FOR DRUG TRIALS

In the previous section we showed that the combination of Aβ1-42 and tau in CSF had the best predictive accuracy to predict conversion to AD-type dementia in subjects with MCI. In this section we will investigate how the use of these markers for the selection of subjects can reduce sample size and costs of a trial with a drug that could slow down the progression from MCI to AD-type dementia. We compare the costs of 2 strategies. The first strategy is a trial in which subjects with MCI are included. The second strategy is a trial in which subjects are enrolled with MCI and an abnormal combination of Aβ1-42 and tau in CSF. Calculations will be performed from the perspective of the performer of the trial and input estimates are mainly based on expert opinion.

The hypothetical trial is a 3-year placebo-controlled trial with 50% on active treatment to examine the effectiveness of an oral drug on the delay of progression to AD-type dementia in subjects with MCI. Subjects would be selected from a memory clinic setting, with MCI defined as an abnormal performance on neuropsychological tests in the absence of dementia.[68] Costs needed to apply the trial inclusion and exclusion criteria (including cognitive tests and imaging) are estimated on €700 per patient. Costs for the collection and analysis of CSF are estimated on €324. The costs of the treatment phase of the trial were estimated on €5800 per included patient. This included costs for 7 assessments with clinical and cognitive testing and blood analysis and costs related to drugs, drug supply, approval of the medical ethical committee and subject reimbursement. Costs related to side effects and adverse events were not included in the analysis.

The number of subjects was chosen such that the study could detect a relative decrease of 20% in conversion rate to AD-type dementia over the 3-year period, with a power of 90%, a 2-sided alpha of 5%, and a drop-out rate of 30%. The number of subjects to be included would depend on the conversion rate to AD-type dementia in each strategy. Based on the meta-analysis above, we estimated that the 3-year conversion rate would be 40% in subjects with MCI and 70% in subjects with MCI and an abnormal Aβ and tau in CSF. Based on these conversion rates, the number of subjects to be included in the trial would be 2160 for a study with only subjects with MCI (strategy 1) and 714 for a study with subjects with MCI and an abnormal combination of Aβ1-42 and tau in CSF (strategy 2). Based on a sensitivity of 0.87 and a specificity of 0.70 of the CSF test (see previous section) and a failure rate of lumbar puncture of 10%, 1503 subjects with MCI should undergo lumbar puncture in order to include 714 subjects with an abnormal combination of Aβ1-42 and tau in CSF. The strategies are visualized in figure 3. This figure shows the rounded number of subjects for each arm, while costs were calculated based on the unrounded number of subjects.

The trial costs of strategy 1 with only subjects with MCI included would be € 14,040,000 (pre-trial screening costs and trial costs for 2160 subjects, figure 3). The trial costs of strategy 2 in which subjects with MCI and an abnormal combination of Aβ1-42 and tau in CSF are included would be € 5,681,580 (pre-trial screening costs and CSF collection and analysis costs for 1503 subjects, and trial costs for 714 subjects, figure 3). This is a reduction in costs of 60% compared to strategy 1.

A one-way sensitivity analysis using the lower bound of the confidence interval of the test-sensitivity (0.80 instead of 0.87) mentioned above would increase the number of subjects with 208 and trial costs with € 536,194 (reduction costs compared to strategy 1 of 56% instead of 60%). Using the lower bound of the specificity (0.57 instead of 0.70) would increase the number of subjects with 261 and trial costs with € 1,704,852 (reduction costs compared to strategy 1 of 47%). If the lower bound of positive predictive value was used (0.58 instead of 0.70), 839 extra subjects needed to be included and trial costs would increase with 3,171,554 (reduction costs compared to strategy 1 of 37%). A CSF test-price increase of 25% resulted in an increase of € 121,743 of the total trial costs (reduction costs compared to strategy 1 of 59%). If trial costs would double to € 11,600 per patient strategy 2 would reduce costs by 63% compared to strategy 1.

				Costs			
			Number of subjects	*Pre-trial*	*Test*	*Trial*	*Total*
Strategy 1 2160 subjects with MCI	AD positive (40%)		864#	€ 700	€ 0	€ 5,800	
	AD negative (60%)		1296#	€ 700	€ 0	€ 5,800	
							€ 14,040,000
Strategy 2 1503 subjects tested for CSF marker*	AD positive (40%)	Test positive	471#	€ 700	€ 324	€ 5,800	
		Test negative	70	€ 700	€ 324		
	AD negative (60%)	Test positive	243#	€ 700	€ 324	€ 5,800	
		Test negative	568	€ 700	€ 324		
							€ 5,681,580 *
Strategy 3 3013 subjects tested for plasma marker	AD positive (40%)	Test positive	699#	€ 700	€ 100	€ 5,800	
		Test negative	506	€ 700	€ 100		
	AD negative (60%)	Test positive	633#	€ 700	€ 100	€ 5,800	
		Test negative	1175	€ 700	€ 100		
							€ 10,134,527

Figure 3. Costs related to different recruitment strategies in a hypothetical trial.

Strategy 1 refers to a trial in which subjects with MCI are included and strategy 2 to a trial in which subjects with MCI and an abnormal amyloid beta peptide 1-42/tau ratio in CSF are included. It is assumed that 40% of the subjects with MCI would convert to AD at follow-up. The number of subjects with a positive and negative test result are based on a sensitivity of 0.87 and a specificity of 0.70 as explained in the text. The rounded number of subjects in each arm is shown, while costs were calculated based on the unrounded numbers of subjects. Test positive=number of subjects with abnormal CSF ratio; test negative=number of subjects with normal CSF ratio; test failed=number of subjects in which lumber puncture failed (10% of sample). #Subjects included in trial

DISCUSSION

Our main findings are that the combination of Aβ1-42 with total tau in CSF is the best predictor for conversion from MCI to AD-type dementia and that use of these markers in the selection of subjects for trials with drugs that aim to prevent the progression from MCI to AD trials can substantially reduce the sample size and trial costs.

Although a large variety of biomarkers for AD can quantify dysregulation of Aβ metabolism or secondary processes in AD like neurodegeneration, inflammation, oxidative stress and altered lipid metabolism in CSF and blood, only few of them have been tested as predictor for AD-type dementia in subjects with MCI in more than 4 independent studies. Among the markers tested in multiple studies, the combination Aβ1-42 with t-tau in CSF had the best predictive accuracy. CSF levels of Aβ1-42, t-tau and p-tau could also predict conversion to AD-type dementia but the accuracy was lower. In plasma, increased levels of Aβ1-40 tended to be associated with an increased risk for AD-type dementia, although the effect size of 0.23 was small. Aβ1-42, the ratio of Aβ1-42/Aβ1-40, and homocysteine levels did not predict AD-type dementia.

In our hypothetical trial on the prevention of progression from MCI to AD-type dementia, use of Aβ1-42 and tau in CSF for the selection of subjects could reduce trial costs

up to 60% relative to a trial that would include subjects with MCI that were not selected by CSF markers. This reduction largely resulted from the fact that fewer subjects were needed to be enrolled in order to obtain the required statistical power. The sample size could be smaller because the conversion rate in subjects with MCI and abnormal Aβ1-42 and tau levels (70%) was much higher compared to that in unselected subjects with MCI (40%). The sensitivity analysis confirmed the strong effect of conversion rate on costs, as the cost reduction of a model with a conversion rate of 58% in subjects with abnormal Aβ1-42 and tau levels would be much lower (37%). The sensitivity analyses also showed that the costs of the CSF test had only a minor influence on the cost reduction and that benefits would be larger if the costs for conducting the trial increased.

Our study has several limitations. We may have overestimated the predictive accuracy of the CSF markers as some studies included in the meta-analysis dichotomized scores according to the best cut off in their study. There were differences in study design between the pooled studies but it was outside the scope of the present paper to explore the effect of this variability on the predictive accuracy. Our meta-analysis was based on relatively few studies, which reduced the accuracy of the parameter estimates. Therefore, we used lower bound estimates in the sensitivity analyses of the cost calculations. Still, these were one-way sensitivity analyses and not full sensitivity analyses. Our cost-calculation did not take into account costs associated with side effects of lumbar puncture. The most common side effect is post-lumbar headache in less than 3% of the subjects, which occasionally needs intervention.[69]

We calculated the effect on costs when CSF markers were used to select subjects for treatment trials. However, when CSF markers are used to select subjects for treatment outside the clinical trial setting, the cost-benefit could be markedly different. In that case, costs need to be calculated from a societal perspective, which includes all costs and effects related to the disease, such as medication use, caregiver time, patient and caregiver quality of life and institutionalization. The cost reduction of the use of CSF tests in the selection of subjects for treatment in such a setting is likely to be much lower as subjects with a false-negative diagnosis or subjects that could not undergo the diagnostic test would not receive treatment and could not benefit from the reduction in societal costs associated with treatment.

Clinical implications

Our findings suggest that the design of trials on the prevention of progression from MCI to AD-type dementia could have a higher cost-benefit ratio if subjects are included based on abnormal CSF markers. A possible disadvantage of the use of CSF markers, however, is that the logistics of such trial could become more complex. CSF collection may not be available in all settings and subjects may refuse to undergo lumbar puncture or have a contraindication for it. In addition, facilities should be set-up for central analysis of CSF samples, which can provide a standardized cut-off.[70] If lumbar puncture is performed on a large scale, rare side effects may become apparent.

Future perspectives

Since lumbar puncture is relatively invasive, a plasma biomarker for AD would be more attractive. So far attempts to develop an accurate plasma AD marker have been unsuccessful. It seems unlikely that new biomarkers in either CSF or blood may reach or improve the diagnostic accuracy of that of Aβ1-42 and tau in CSF in the near future. New markers that have been tested in subjects with AD-type dementia versus healthy control have generally yielded a lower diagnostic accuracy compared to Aβ1-42 and tau in CSF. A promising alternative may be the combination of different plasma or CSF markers or gene expression profiles, but so far replication of such multiparameter approaches has been difficult.[43] Development of diagnostic markers from proof of concept to large-scale application may take up to 15 years, as was the case for Aβ1-42 and tau in CSF. Future studies should compare the use of CSF makers in trials with that of other diagnostic modalities such as PET or MRI imaging. Finally, it should be investigated whether biomarkers could increase the cost-benefit ratio in the selection of subjects in prevention trials in subjects without MCI or outside a clinical setting, in which the conversion rate to AD-type dementia is much lower.

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CHAPTER 3

TEST SEQUENCE OF CSF AND MRI BIOMARKERS FOR PREDICTION OF AD IN SUBJECTS WITH MCI

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Stephanie Vos, Ineke van Rossum, Leah Burns, Dirk Knol, Philip Scheltens, Hilkka Soininen, Lars-Olof Wahlund, Harald Hampel, Magda Tsolaki, Lennart Minthon, Ron Handels, Gilbert L'Italien, Wiesje van der Flier, Pauline Aalten, Charlotte Teunissen, Frederik Barkhof, Kaj Blennow, Robin Wolz, Daniel Rueckert, Frans Verhey, Pieter Jelle Visser

ABSTRACT

Objective

Our aim was to identify the best diagnostic test sequence for predicting Alzheimer's disease (AD)-type dementia in subjects with mild cognitive impairment (MCI) using cerebrospinal fluid (CSF) and MRI biomarkers.

Methods

We selected 153 subjects with MCI from a multicenter memory-clinic-based cohort. We tested the CSF Aβ1-42/tau ratio using ELISA and hippocampal volumes (HCVs) using the atlas-based LEAP method. Outcome measure was progression to AD-type dementia in 2 years.

Results

At follow-up, 48 (31%) subjects converted to AD-type dementia. In multivariable analyses, CSF Aβ1-42/tau and HCV predicted AD-type dementia regardless of APOE genotype and cognitive scores. Test sequence analyses showed that CSF Aβ1-42/tau increased predictive accuracy in subjects with normal HCV ($p<0.001$) and abnormal HCV ($p=0.025$). HCV increased predictive accuracy only in subjects with normal CSF Aβ1-42/tau ($p=0.014$). Slope analyses for annual cognitive decline yielded similar results. For selection of subjects for a prodromal AD trial, the best balance between sample size and number of subjects needed to screen was obtained with CSF markers.

Conclusions

These results provide further support for the use of CSF and MRI biomarkers to identify prodromal AD.

INTRODUCTION

Diagnosis of Alzheimer's disease (AD) in the stage of mild cognitive impairment (MCI) is important in order to give patients a prognosis. Moreover, disease-modifying drugs for AD might be most effective in this early course of the disease. Several biomarkers of AD are now available that can help to identify AD pathology including abnormal levels of beta amyloid (Aβ)1-42 and tau in cerebrospinal fluid (CSF)[1-5] and medial temporal lobe atrophy on magnetic resonance imaging (MRI).[6-9]

For clinical practice and for selection of subjects for prodromal AD trials, it is of major importance to investigate which combination of CSF and MRI biomarkers could best predict AD-type dementia in subjects with MCI. Some studies found that CSF biomarkers could best predict AD-type dementia,[10-13] but others studies found that MRI biomarkers were the best predictor.[14,15] Moreover, several studies showed that a combination of these biomarkers could predict AD-type dementia in subjects with MCI more accurately than each biomarker alone.[10-12,14-16]

These previous studies, however, had several limitations. They had a small sample size or were performed in a highly selected research sample.[10-12,14-16] Moreover, some studies included only subjects with aMCI while subjects with MCI due to AD may also present as non-amnestic MCI (naMCI).[17] The use of a broad definition of MCI is also recommended in the new criteria for MCI due to AD of the National Institute on Aging and Alzheimer's Association workgroup.[18]

Our aim was to identify the best combination of CSF and MRI biomarkers to predict conversion to AD-type dementia after 2 years in a large clinically representative sample of subjects with MCI, such that the number of tests could be reduced and as a consequence reduce patients' burden and costs.[19] We performed test sequence analyses of CSF and MRI measures and examined how the use of different combinations of biomarkers as inclusion criterion influenced the sample size and number of subjects required for screening for a fictive trial in prodromal AD. Although conversion to AD-type dementia after a 2-year follow-up is a clinically relevant outcome, the potential of CSF and MRI biomarkers to identify prodromal AD may be underestimated as subjects may convert at longer follow-up intervals. Therefore, we additionally performed slope analyses with annual cognitive decline as outcome measure.[20]

METHODS

Subjects

Subjects were recruited from the DESCRIPA cohort and the Alzheimer Center of the VU University medical center (VUmc) cohort. For the present study, inclusion criteria were baseline diagnosis of MCI, age ≥55 years, availability of data on the CSF ratio of Aβ1-42 to total tau (t-tau) and automatically measured hippocampal volumes (HCVs) on MRI, and being newly referred for assessment of cognitive complaints. Exclusion criteria were

diagnosis of dementia at baseline or any other somatic, psychiatric or neurological disorder that might have caused the cognitive impairment.

DESCRIPA is a European multicenter study performed in a memory clinic setting.[21] Subjects were recruited from 6 centers, as both MRI and CSF data were only available at these centers. Of the 207 eligible subjects enrolled between 2003 and 2005 at these centers, 74 had data for both measures. The VUmc center was one of the DESCRIPA centers and contributed an additional sample of subjects that were seen outside the DESCRIPA inclusion period. Of the 217 additional eligible subjects that were included between 1998 and 2007 at this center, 91 had baseline data for both MRI and CSF.

Subjects for whom both MRI and CSF data were available differed from subjects for whom MRI and CSF data were not both available with regard to age (69.3 vs. 71.2 years; $p<0.001$), MMSE score (26.6 vs. 27, $p=0.019$), and MCI type (68 vs. 59% aMCI; $p=0.016$) at baseline. The CSF Aβ1-42/tau ratio and HCV were similar in both groups. The total sample size was 165. The medical ethics committee at each center approved the study. All patients provided informed consent.

Clinical and cognitive assessment

Assessment of the subjects included a clinical history, medical and neurological examination, laboratory tests, functional evaluation with the clinical dementia rating scale, rating scales for neuropsychiatric symptoms, scoring on the MMSE, and neuropsychological assessment.[10,21] All data were collected by investigators who were blinded to the results of biomarker analyses. Follow-up assessment was performed annually up to 5 years. Primary outcome measure was conversion to AD-type dementia after 2 years according to the DSM-IV[22] and NINCDS-ADRDA criteria.[23] Secondary outcome measure was annual cognitive decline on the MMSE and a cognitive composite score.

Baseline diagnosis of MCI was made according to the criteria of Petersen et al.[24] Raw scores on neuropsychological tests were corrected for age, education, and sex, in accordance with locally collected or published normative data and expressed as z-scores; the z-scores were used for further analysis.[21] Subjects with a z-score <-1.5 SD on the learning measure or delayed recall of a word list learning test or equivalent memory test were classified as having aMCI (n=102). Subjects with a z-score <-1.5 SD on the trail making test (TMT) part A, TMT part B, verbal fluency, Rey figure copy test or equivalent test but no memory impairment were classified as having naMCI (n=48; see appendix S1).[21] The MCI subtype diagnosis was missing for 3 subjects, as they did not undergo a neuropsychological assessment. We calculated a cognitive composite score as the average z-score of 5 tests (learning and delayed recall score of the word learning list, TMT A and B, verbal fluency, copy figures), if scores were available for at least 3 cognitive tests.[17]

CSF analyses

CSF was collected by lumbar puncture, centrifuged, and stored at -80°C in polypropylene tubes. One sample was thawed twice but analyses without this sample revealed similar

results. CSF Aβ1-42 and t-tau were measured using commercially available sandwich ELISAs (Innotest β-amyloid 1-42; Innotest hTAU-Ag; Innogenetics, Ghent, Belgium), specially constructed to measure Aβ1-42 and t-tau[25,26] by experienced technicians at the lab in Gothenburg for the DESCRIPA cohort and in Amsterdam for the VUmc cohort. We corrected for interlaboratory ELISA differences by analyzing 33 samples at both labs and we adjusted VUmc values to those of DESCRIPA using the following formula: Gothenborg=(SD Gothenborg/SD VUmc)*VUmc+average Gothenborg–((SD Gothenborg /SD VUmc)*average VUmc).[27]

As CSF measure, we used the ratio of Aβ1-42 to t-tau, which is the best CSF biomarker according to a recent meta-analysis[28] and accepted by the European Medical Agency for inclusion of subjects for prodromal AD trials.[29] The CSF ratio was dichotomized by defining a cut-off point (1.14) that could best predict AD-type dementia after 2 years based on the Youden index from a time-dependent ROC curve in R,[30] including all subjects with CSF data in both cohorts (n=219). In 5 subjects, CSF was obtained at follow-up. As these subjects had a normal CSF ratio score, they were considered to have a normal CSF score at baseline as well.

MRI analyses

For the DESCRIPA cohort, subjects were scanned according to the routine MRI protocol at each site (appendix S2). Scanning was performed at 1.0 or 1.5 T and included a three-dimensional T1-weighted gradient echo sequence and a fast fluid attenuated inversion recovery (FLAIR) sequence.[31]

As measure of medial temporal lobe atrophy, we used HCV measured centrally at the Department of Computing at Imperial College London by experienced technicians, using LEAP.[32] LEAP is an automated structural segmentation, performed by propagating multiple manually segmented atlas images to a diverse image set in a stepwise fashion, ensuring that image registration needed to be performed between similar images only. All labels propagated to a target image were considered together with a structures intensity distribution to estimate the final segmentation (e.g. supplemental figure S1). Quality control was performed by visual inspection of the segmentation on transverse, coronal and sagittal slices. HCVs were normalized to intracranial volume using MNI scaling. The sum of left and right HCV was dichotomized by defining a cut-off (5.3 cm^3) that could best predict AD-type dementia after 2 years based on the Youden index from a time-dependent ROC curve in R,[33] including all subjects with HCV data in both cohorts (n=339). In 6 subjects, MRI was performed at follow-up. As these subjects had a normal HCV score, they were considered to have a normal HCV score at baseline as well.

APOE genotype

APOE genotype was determined by polymerase chain reaction of genomic DNA extracted from EDTA anticoagulated blood in 138 subjects. Subjects were classified as APOE-ε4 carriers or non-carriers.

Statistical analyses

Statistical analyses were done with SPSS version 16.0 (Chicago, IL, USA) and statistical software package R version 2.10.1.[33] Significance was set at $p<0.05$ and 95% confidence intervals (CI) were calculated. Differences between groups were analyzed using a t-test for continuous variables and chi-square test for categorical variables.

Univariable and multivariable logistic regression analyses were performed for dichotomized CSF and MRI markers to assess whether a combination of markers was better predictive for AD-type dementia. Sensitivity, specificity, positive predictive value, negative predictive value, Youden index (sensitivity+specificity-1) and odds ratio (OR) were calculated. Multivariable analyses with correction for center yielded similar findings. Therefore, we did not correct for center in the final analyses.

Change on the MMSE and cognitive composite score were assessed by use of slope analyses with mixed models. The analyses included the baseline score and available follow-up scores up to 5 years after baseline. We used an unstructured covariance structure with center as a random effect as this model provided the best -2 log likelihood compared to models with simpler covariance structures.[17] We examined whether the slopes of cognition were different for subjects with and without abnormal CSF or MRI biomarker scores and whether the combination of both biomarkers increased predictive accuracy for cognitive decline.

Test sequence analyses were performed to investigate the added predictive value of a second biomarker for AD-type dementia or cognitive decline in specific subgroups. We identified the best test sequence of CSF Aβ1-42/tau ratio and HCV assessment and visualized it using probability-modifying plots.[34]

The relation between biomarkers, the sample size, and number of subjects required for screening, was tested for a fictive placebo-controlled AD drug trial targeting amyloid pathology in subjects with MCI. The number of subjects was calculated in such a way that the study could detect a relative decrease of 25% in conversion rate to AD-type dementia over a 2-year period in subjects with AD pathology, which was defined as an abnormal CSF Aβ1-42/tau ratio, with a power of 90%, a 2-sided alpha of 5%, a drop-out rate of 30% and equally sized treatment groups.[28] The prevalence of abnormal biomarkers and conversion rate were based on the observed data in our study.

RESULTS

Sample characteristics

153 subjects had at least one follow-up assessment (average 3.7 years (SD=1.4)). 48 (31%) subjects converted to AD-type dementia after 2 years, 6 (4%) subjects converted to other types of dementia and were included in the non-AD group. Subjects with at least one follow-up had similar baseline characteristics as those without follow-up (supplemental table S1).

Predictors of AD-type dementia

Univariable predictors

Subjects with AD-type dementia at 2-year follow-up had lower MMSE scores (p=0.014), lower cognitive composite score (p=0.009), lower CSF levels of Aβ1-42 (p<0.001), higher CSF levels of t-tau (p<0.001), a lower CSF Aβ1-42/tau ratio (p<0.001), smaller HCV (p<0.001), a higher frequency of APOE-ε4 alleles (p=0.011), and tended to have more often aMCI (75 vs. 65%, p>0.10), compared to subjects without AD-type dementia (table 1). Scores on verbal fluency (p=0.052) and delayed recall (p=0.069) tended to be lower for subjects who progressed to AD-type dementia compared to those who did not progress. The overall predictive accuracy (OR) was more than twice as high for the CSF Aβ1-42/tau ratio (OR=9.2, 95% CI 3.9-22, p<0.001) than for HCV (OR=3.7, 95% CI 1.8-7.6, p<0.001, table 2). Of the subjects without dementia at follow-up, 14 had a follow-up shorter than 1.5 years. When we repeated analyses after exclusion of these subjects, results were similar.

Table 1. Baseline characteristics of subjects with MCI by outcome at 2-year follow-up

	MCI-non AD	MCI-AD
N‡	105	48
Age	68.8 (7.3)	70.4 (7.2)
Female, n (%)	43 (41.0)	24 (50.0)
aMCI, n (%)	67 (65.0)	35 (74.5)
Years of education	10.7 (3.2)	11.2 (3.0)
MMSE	26.9 (2.6)	25.8 (2.8)*
Delayed recall, z-score	-1.34 (1.1)	-1.76 (1.0)
Learning, z-score	-1.15 (1.1)	-1.44 (1.1)
Verbal fluency, z-score	-0.66 (1.1)	-1.04 (0.9)
TMT A, z-score	-0.44 (1.6)	-0.87 (1.8)
TMT B, z-score	-0.60 (1.3)	-0.85 (1.3)
Visuo-construction, z-score	0.26 (1.2)	0.18 (1.2)
Cognitive composite score, z-score	-0.63 (0.7)	-0.95 (0.7)†
APOE ε4 carrier, n (%)	42 (44.2)	29 (67.4)*
Hippocampal volume, cm^3	5.7 (0.7)	5.3 (0.6)‡
Aβ1-42, pg/ml	682 (309)	470 (145)‡
T-tau, pg/ml	409 (219)	686 (377)‡
Ratio Aβ1-42/t-tau	2.3 (1.7)	0.8 (0.5)‡

Data are mean (SD), unless specified otherwise. *P<0.05; †p<0.01; ‡p<0.001 compared to MCI-non AD. Group size is different for cognitive tests, APOE, hippocampal volume, and CSF measures. MCI-nonAD=subjects with MCI who did not convert to AD-type dementia at follow-up; MCI-AD=subjects with MCI who converted to AD-type dementia at follow-up; aMCI=amnestic mild cognitive impairment; MMSE=Mini-Mental State Examination; TMT=trail making test; APOE=apolipoprotein E genotype; Aβ1-42=beta amyloid 1-42; t-tau=total tau.

Multivariable predictors

When both the CSF Aβ1-42/tau ratio and HCV were entered in the same model, the markers predicted AD-type dementia independently from each other (OR CSF ratio=7.9, 95% CI 3.3-

19, p<0.001; OR HCV=2.8, 95% CI 1.3-6.2, p=0.01). The combination increased the predictive accuracy relative to the model with only the CSF Aβ1-42/tau ratio (χ^2=6.6, df=1, p=0.01). Next, we tested the added diagnostic value of CSF Aβ1-42/tau ratio and HCV compared to models with other markers that could predict AD-type dementia in the univariable analyses and age. When age, MMSE score, and APOE genotype were entered in the first step, CSF Aβ1-42/tau ratio and HCV increased the predictive accuracy in the second step (χ^2=24.1, df=2, p<0.001). Both markers were significant predictors (OR CSF ratio=7.9, 95% CI 2.8-22, p<0.001; OR HCV=2.8, 95% CI 1.1-6.9, p=0.03), whereas age, MMSE score, and APOE genotype no longer predicted outcome (p>0.42). When we tested a multivariable model in which the cognitive composite score was entered in the first step (p=0.012) and CSF Aβ1-42/tau ratio and HCV in the second step, both biomarkers predicted AD-type dementia (p<0.05) but the cognitive composite score not (p=0.13).

Table 2. Predictive accuracy of CSF and MRI markers for AD-type dementia after 2 years

Markers	Sensitivity	Specificity	PPV	NPV	Youden Index	Odds ratio*
CSF Aβ1-42/tau	0.83 (0.73-0.94)	0.65 (0.56-0.74)	0.52 (0.41-0.63)	0.89 (0.83-0.96)	0.48 (0.34-0.62)	9.2 (3.9-22)
HCV	0.56 (0.42-0.70)	0.74 (0.66-0.83)	0.50 (0.37-0.63)	0.79 (0.71-0.87)	0.31 (0.14-0.47)	3.7 (1.8-7.6)
CSF Aβ1-42/tau or HCV abnormal	0.94 (0.87-1.00)	0.52 (0.43-0.62)	0.47 (0.37-0.57)	0.95 (0.89-1.00)	0.46 (0.34-0.58)	17 (4.8-56)
CSF Aβ1-42/tau and HCV abnormal	0.46 (0.32-0.60)	0.87 (0.80-0.93)	0.61 (0.45-0.77)	0.78 (0.70-0.85)	0.33 (0.17-0.48)	5.5 (2.5-12)

*All p-values <0.001. Results are presented with 95 % confidence intervals.

Youden index=(sensitivity+specificity-1). CSF=cerebrospinal fluid; MRI=magnetic resonance imaging; MCI=mild cognitive impairment; HCV=hippocampal volume; PPV=positive predictive value; NPV=negative predictive value.

Test sequence

In further analyses, we only included the CSF and HCV measures because other covariates did not contribute to the overall predictive accuracy.The added predictive value of the CSF Aβ1-42/tau ratio and HCV to the other marker is presented in supplemental S2 and figure 1. If the CSF Aβ1-42/tau ratio was analyzed first, assessment of HCV increased predictive accuracy for AD-type dementia in subjects with a normal CSF Aβ1-42/tau ratio (OR=7.1, p=0.014), but not in subjects with an abnormal CSF Aβ1-42/tau ratio (OR=2.0, p=0.13). If HCV was analyzed first, assessment of CSF Aβ1-42/tau ratio increased predictive accuracy both in subjects with normal HCV (OR=14.4, p<0.001) and with abnormal HCV (OR=4.1, p=0.025). Figure 1 shows probability plots of AD-type dementia for different sequences of CSF Aβ1-42/tau ratio and HCV assessment, thereby visualizing the change in AD probability after adding the results for each biomarker.

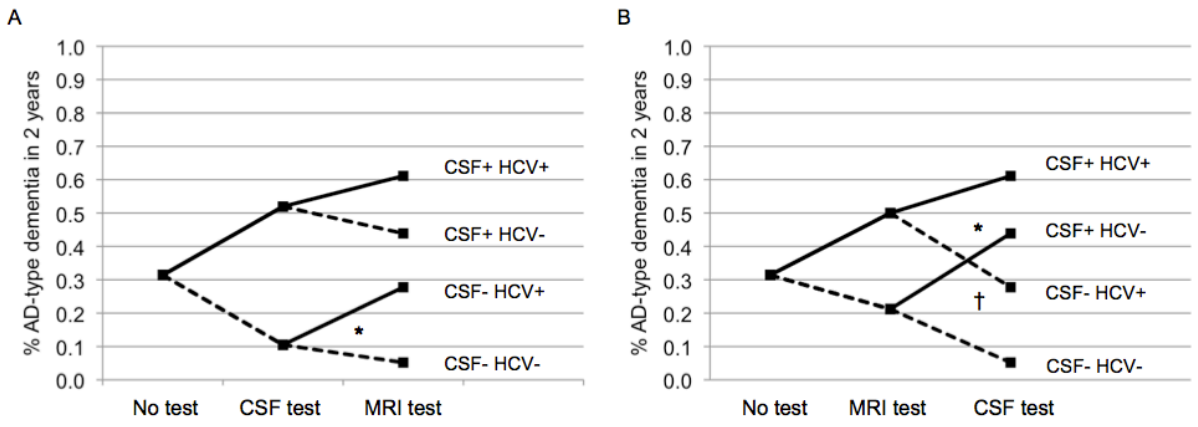

Figure 1. Probability of AD-type dementia in 2 years related to the sequence of CSF and HCV assessment.

The graph shows the change in AD probability according to biomarker scores. The starting point represents the AD conversion rate in the total MCI population. Dotted lines represent negative (normal) test results and full lines represent positive (abnormal) test results. (A) First CSF assessment then HCV assessment; (B) First HCV assessment then CSF assessment. 36 subjects had both and abnormal CSF ratio and HCV (with 22 converters to AD-type dementia), 41 subjects had an abnormal CSF ratio and a normal HCV (18 converters), 18 subjects had a normal CSF ratio and an abnormal HCV (5 converters), 58 subjects had both a normal CSF ratio and HCV (3 converters). AD=Alzheimer's disease; CSF=cerebrospinal fluid; HCV=hippocampal volume. *P<0.05; †p<0.001: difference in AD probability in 2 years between subjects with normal and abnormal biomarkers after second biomarker assessment.

Predictors of cognitive decline

Although conversion to AD-type dementia after 2-year follow-up is a clinically relevant outcome measure, the potential of CSF and MRI biomarkers to identify prodromal AD may be underestimated as subjects may convert at longer follow-up intervals. Therefore, we additionally performed slope analyses for annual cognitive decline over the 5-year follow-up period.

Univariable predictors

The CSF Ab1-42/tau ratio at baseline predicted cognitive decline on the MMSE and cognitive composite score (all p<0.001), while HCV only predicted decline on the MMSE (p<0.008; table 3). The CSF Ab1-42/tau ratio was a better predictor for decline on the MMSE compared to HCV (difference -2 log likelihood (LL)=15.4; CSF F=27.6, p<0.001; MRI F=7.5, p=0.008).

Multivariable predictors

Multivariable analyses were performed with decline on the MMSE as outcome, since only for this outcome measure both biomarkers predicted decline in univariable analyses. When the CSF Ab1-42/tau ratio was entered first, addition of HCV significantly increased the overall predictive accuracy (decrease in -2LL=6.5, F=4.72, p=0.011).

Test sequence

The added predictive value for decline on the MMSE of the CSF Aβ1-42/tau ratio and HCV to the other marker is presented in supplemental table S3 and figure 2. If the CSF Aβ1-42/tau

ratio was analyzed first, assessment of HCV increased predictive accuracy for cognitive decline in subjects with a normal CSF Aβ1-42/tau ratio (p=0.001), but not in subjects with an abnormal CSF Aβ1-42/tau ratio (p>0.3). If HCV was analyzed first, assessment of CSF Aβ1-42/tau ratio increased predictive accuracy both in subjects with normal HCV (p<0.001) and with abnormal HCV (p=0.038).

Table 3. Annual cognitive decline over 5 years of follow-up according to CSF and MRI biomarker results at baseline

		MMSE		Cognitive composite score	
		Baseline score	Slope	Baseline score	Slope
CSF Aβ1-42/tau	Normal	27.5 (0.82)§	-0.28*‡ (0.13)	-0.70 (0.10)§	0.001‡ (0.02)
	Abnormal	26.6 (0.83)	-1.24† (0.12)	-0.93 (0.11)	-0.25† (0.04)
HCV	Normal	27.3 (0.73)¥	-0.61†¥ (0.13)	-0.76 (0.09)	-0.05 (0.02)
	Abnormal	26.1 (0.80)	-1.17† (0.16)	-0.89 (0.11)	-0.14* (0.04)

Data are mean (SE). Slope refers to annual change on the test. A negative slope indicates cognitive decline. CSF=Cerebrospinal fluid, HCV=Hippocampal volume, MMSE=Mini Mental State Examination. *P<0.05, †p<0.001 for slope different from zero (this means a statistically significant change over time in test score); ‡P<0.001, ¥p<0.01, §p<0.05 for baseline score or slope compared to baseline score or slope in abnormal biomarker group.

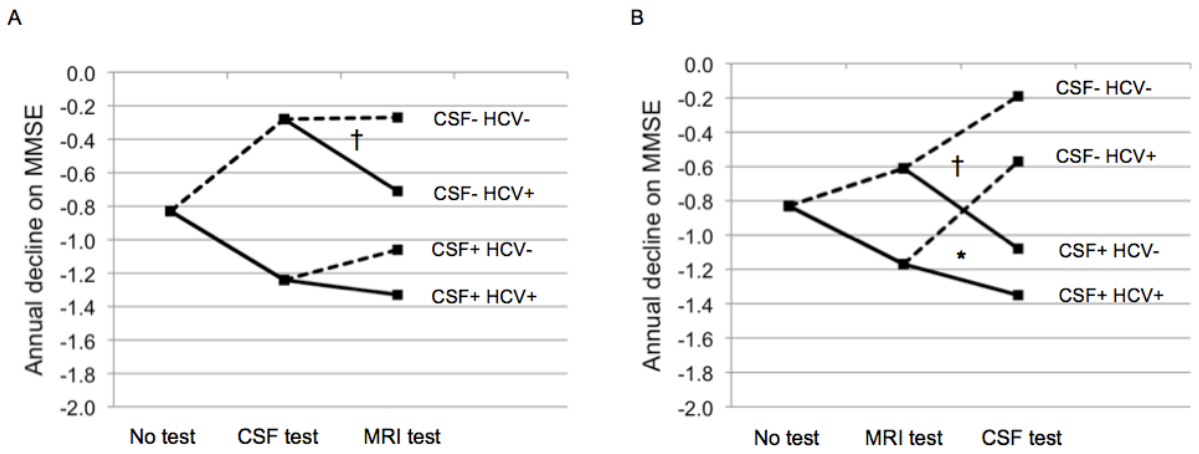

Figure 2. Average annual cognitive decline on MMSE related to the sequence of CSF and HCV assessment.

The graph shows change in annual cognitive decline according to biomarker scores. The starting point represents the decline on MMSE in the total MCI population regardless of biomarker results. Dotted lines represent negative (normal) test results and full lines represent positive (abnormal) test results. (A) First CSF assessment then HCV assessment; (B) First HCV assessment then CSF assessment. MMSE=Mini Mental State Examination; CSF=cerebrospinal fluid; HCV=hippocampal volume. As the sample size of the subgroups was different, different slopes may be presented for the same final groups. *P<0.05; †p<0.001: difference in annual cognitive decline on MMSE between subjects with normal and abnormal biomarkers after second biomarker assessment.

Biomarkers as inclusion criterion for prodromal AD trial

We used the different combinations of the CSF Aβ1-42/tau ratio and HCV shown in table 2 to select subjects with MCI for a fictive amyloid targeting AD trial with conversion to AD-type dementia after 2 years as outcome. The sample sizes, numbers required for screening, and screening failure rates varied considerably between the different combinations (table 4). The sample size was smallest for the strategy that required both an abnormal CSF Aβ1-42/tau ratio and abnormal HCV at baseline (642 vs. 880-1381). The number required for screening was lowest if only the CSF Aβ1-42/tau ratio was required to be abnormal (1761 vs. 2228-3931). The screening failure rate was lowest if either the CSF Aβ1-42/tau ratio or HCV was required to be abnormal (38% vs. 50-76%). The strategy that required only abnormal CSF markers had the best trade-off between sample size (n=880) and number required for screening (n=1761), as numbers were best (screening) or second best (sample size).

Table 4. Number of subjects required for AD trial design based on biomarker inclusion criteria

Biomarkers	Number required for screening	Sample size for trial	Screening failure rate
CSF Aβ1-42/tau abnormal	1761	880	0.50
HCV abnormal	3931	1376	0.65
CSF Aβ1-42/tau or HCV abnormal	2228	1381	0.38
CSF Aβ1-42/tau and HCV abnormal	2675	642	0.76

The assumptions with regard to trial design are described in the methods section. Screening failure rate=1-sample size divided by number required for screening, i.e. the proportion of subjects that were screened but not included in the AD trial, as they did not have abnormal biomarkers. Note that this failure rate does not take into account failure rates of CSF collection. AD=Alzheimer's disease; CSF=cerebrospinal fluid; HCV=hippocampal volume.

DISCUSSION

We showed that the CSF Aβ1-42/tau ratio was the best predictor for AD-type dementia at follow-up and for cognitive decline in subjects with MCI. HCV could increase the predictive accuracy of the CSF Aβ1-42/tau ratio. This increase in predictive accuracy for AD-type dementia and cognitive decline was because HCV predicted AD-type dementia and cognitive decline in subjects with a normal CSF Aβ1-42/tau ratio.

Test sequence analyses suggested that CSF assessment should be performed first for prediction of AD-type dementia at follow-up. HCV assessment may be considered in subjects who have a normal CSF Aβ1-42/tau ratio. A strategy in which HCV assessment is performed first and CSF assessment next seems less efficient as CSF assessment would further increase predictive accuracy regardless of the outcome of the HCV analysis. However, with respect to feasibility, MRI scanning may be more acceptable than CSF collection in daily practice. MRI

is often already routinely done to exclude other diseases. Still, volumetric assessment of the hippocampus is not widely available.

Furthermore, the current study showed that for a fictive AD trial targeting amyloid pathology in subjects with MCI, subjects could be best selected based on CSF biomarkers because this provided the best balance between sample size and number of subjects required for screening. Still, the sample size was smaller if both CSF Aβ1-42/tau ratio and HCV were required to be abnormal. However, the screening failure rate was very high for this combination, which may limit its clinical utility. The screening failure rate was lowest if subjects were required to have either an abnormal CSF Aβ1-42/tau ratio or an abnormal HCV. A disadvantage of this approach was the much higher sample size compared to that of the selection based on an abnormal CSF Aβ1-42/tau ratio alone. Selection based on an abnormal HCV alone was least efficient because both the number required for screening and sample size were among the highest of the four approaches. This may be partly because the fictive trial was designed for targeting amyloid pathology such that only subjects with an abnormal CSF Aβ1-42/tau ratio were supposed to respond to therapy.

This is the first large-scale multicenter study conducted in a memory clinic setting of subjects classified according to a broad definition of MCI, including aMCI (68%) and naMCI that compared CSF and MRI markers for prediction of cognitive decline. Our study corroborates the findings of studies of subjects with aMCI,[14-16] that the combination of the CSF ratio and measure of medial temporal lobe atrophy predicted AD-type dementia better than each biomarker alone. This also confirms the findings of smaller single-center studies performed in subjects with MCI.[10-12] It validates the use of biomarkers for prediction of AD-type dementia in subjects who meet the criteria of a broad definition of MCI, including aMCI and naMCI. This supports the new NIA-AA criteria for MCI due to AD, which uses a broad definition of MCI as well.[18] The conversion rate of 31% to AD-type dementia after 2 years in the present study is comparable to conversion rates in previous studies with a similar follow-up period.[11,12,15]

The overall predictive accuracy (OR) for AD-type dementia of the CSF Aβ1-42/tau ratio was twice as high as that of the HCV. This was mainly due to the higher sensitivity of the CSF Aβ1-42/tau ratio compared to that of the HCV (table 2). This is in line with the hypothetical dynamic model of AD biomarkers,[35] stating that CSF biomarkers become abnormal at an earlier stage than MRI biomarkers. We also found that the specificity of the HCV was higher than that of the CSF Aβ1-42/tau ratio. This may be considered unexpected as medial temporal lobe atrophy is not specific for AD and also observed in other neurodegenerative and vascular disorders. However, the higher specificity of the HCV could also be explained by the hypothetical dynamic model of AD biomarkers. As CSF markers are expected to become abnormal before the HCV,[35] there will be more MCI subjects with prodromal AD with an abnormal CSF Aβ1-42/tau ratio who have not yet converted to AD-type dementia within 2 years than there will be MCI subjects with prodromal AD with an abnormal HCV who have not yet converted to AD-type dementia within 2 years.

Test sequence analyses revealed the presence of a small group of subjects (n=18, 12% of the total sample) with a normal CSF Aβ1-42/tau ratio but abnormal HCV and a conversion rate of 28% to AD-type dementia after 2 years. This is an interesting group because it is not in line with the hypothetical dynamic model of AD biomarkers,[35] as abnormal CSF biomarkers did not precede medial temporal lobe atrophy. Further research is needed to investigate whether these subjects have an atypical presentation of AD or whether they are misclassified cases either because of the applied cut-offs or because AD is not the cause of the dementia.

MMSE score, a cognitive composite score, and APOE genotype predicted AD-type dementia in univariable analyses, however, in the combined model with CSF and MRI markers, these variables were no longer significant predictors. This indicates that CSF and MRI can be used independent of cognitive scores and APOE genotype for short-term prediction of AD-type dementia. Nevertheless, our findings are inconsistent with some previous studies of subjects with aMCI. One study showed that MRI and APOE genotype did not significantly add to the predictive accuracy of neuropsychological tests.[36] Another study found that the optimal biomarker combination and neuropsychological tests were equally predictive for AD-type dementia.[14] The more severely impaired cohort that was used in the former study and the inclusion of only subjects with aMCI in both studies could explain the inconsistent findings.

Although a 2-year follow-up is clinically relevant, the potential of CSF and MRI biomarkers to identify prodromal AD may be underestimated.[20] Therefore, we also performed slope analyses for annual cognitive decline over 5 years. These analyses yielded very similar findings as our analysis on conversion to AD-type dementia after 2 years. The CSF Aβ1-42/tau ratio predicted annual cognitive decline better than HCV. In the sequence analysis with decline on MMSE as outcome, HCV increased predictive accuracy only in subjects with a normal CSF Aβ1-42/tau ratio but not in subjects with an abnormal ratio, while the CSF Aβ1-42/tau ratio improved predictive accuracy regardless of HCV. The lower predictive accuracy of HCV can again in part be explained by a lower sensitivity of this marker for prodromal AD. This is supported by the observation that decline in subjects with a normal HCV is higher than in subjects with a normal CSF ratio in table 3. This higher decline is probably driven by subjects with an abnormal CSF ratio among subjects with a normal HCV.

This study had several limitations. As the findings were based on memory clinic populations, they may not be generalized to other settings, including the general population. Furthermore, the cut-offs of the CSF Aβ1-42/tau ratio and HCV were determined within a study population that also included the subjects from the present analyses. Although this may have led to an overestimation of the predictive accuracy of the biomarkers, it is unlikely that it influences our findings with respect to the differences in predictive accuracy between the CSF Aβ1-42/tau ratio and HCV, as we used the same method to define the cut-point for each biomarker. We defined cut-offs regardless of age, although biomarkers may change with age. However, when we repeated analyses using age-adjusted cut-offs (≤70 vs. >70

years) analyses yielded similar findings. In addition, there was variability in scanners used, which may have influenced the volumetric measurements and predictive accuracy of the HCV. However, the LEAP approach has been shown to be robust for scanner variability.[32] The follow-up period to AD-type dementia was relatively short but for clinical trials short-term prognosis may be important. However, we used a longer follow-up period to investigate the predictive accuracy of CSF and MRI biomarkers for annual cognitive decline over 5 years. Moreover, the diagnosis of AD-type dementia at follow-up was not neuropathologically validated. This may have led to the misclassification of some cases.

A major strength of this study was the large sample size. The overall multicenter design favors generalizability to other memory clinic settings, although a considerable amount of subjects was recruited from one single center. Furthermore, this is the first study of predictive accuracy of the combination of CSF and MRI markers for AD-type dementia and cognitive decline that investigated the test sequence of CSF and HCV assessments in an unselected population with subjects classified according to a broad definition of MCI.

In sum, test sequence analyses may lead to cost reduction and to a decrease in patients' burden. Future research is needed to evaluate the ratio of costs and effectiveness for patients regarding biomarker assessment, as this may eventually require adjustments to patient policy. Also the combination of CSF and MRI biomarkers with PIB-PET, and FDG-PET should be investigated, since this may further aid to define an algorithm of markers for the accurate prediction of AD-type dementia in subjects with MCI. While hippocampal atrophy and CSF markers are given equal diagnostic accuracy in recent criteria for prodromal AD or MCI-due to AD, our findings suggest that this may not be the case and a CSF Aβ1-42/tau ratio may be preferred over HCV assessment because of its higher sensitivity.

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SUPPLEMENTAL DATA

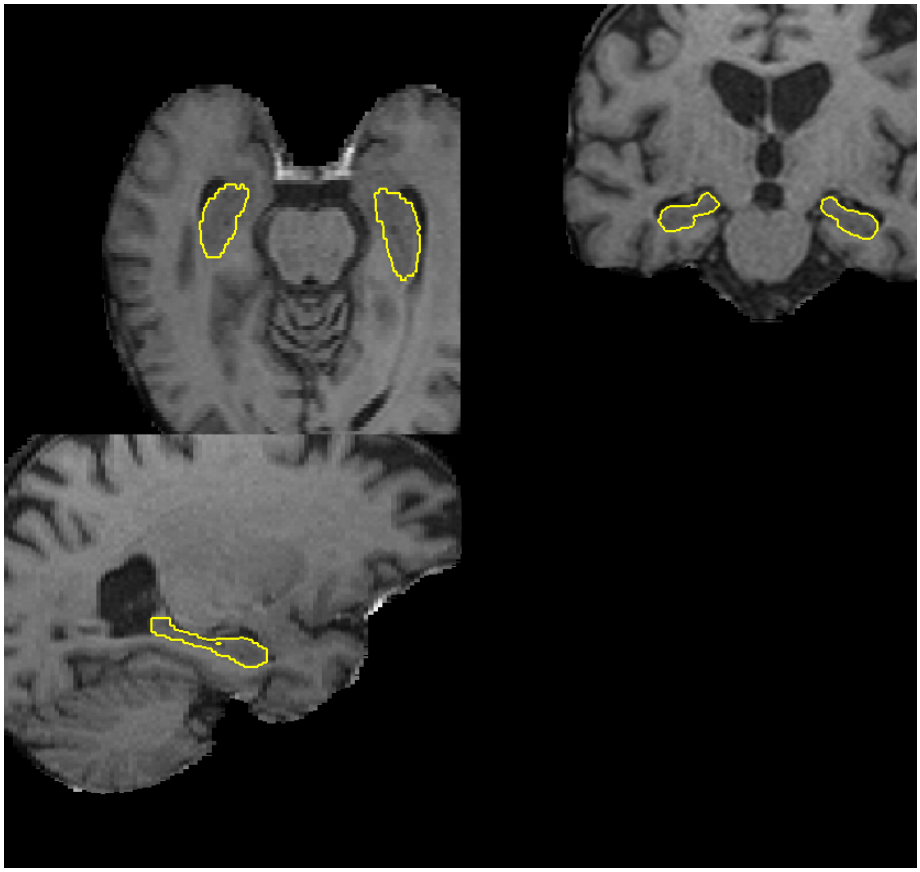
Supplemental Table S1. Baseline characteristics for subjects with and without follow-up

	MCI all	MCI FU	MCI no FU
N*	165	153	12
Age	69.3 (7.3)	69.3 (7.3)	70.3 (6.8)
Female, n (%)	73 (44.2)	67 (43.8)	6 (50.0)
aMCI, n (%)	112 (67.9)	102 (66.7)	10 (83.3)
Years of education	10.8 (3.1)	10.9 (3.2)	10.2 (2.8)
MMSE	26.6 (2.6)	26.6 (2.7)	26.6 (2.1)
APOE ε4 carrier, n (%)	75 (50.3)	71 (51.4)	4 (36.4)
Hippocampal volume, cm^3	5.6 (0.7)	5.6 (0.7)	5.8 (0.1)
Aβ1-42, pg/ml	609 (282)	616 (285)	527 (236)
T-tau, pg/ml	487 (301)	496 (305)	377 (219)
Ratio Aβ1-42/t-tau	1.9 (1.6)	1.8 (1.6)	2.0 (1.6)

Data are mean (SD), unless specified otherwise. *Group size is different for APOE, hippocampal volume and CSF measures. No significant statistical differences were found between subjects with and without follow-up. FU=follow-up; aMCI=amnestic mild cognitive impairment; MMSE=Mini-Mental State Examination; APOE=apolipoprotein E genotype; Aβ1-42=beta amyloid 1-42; t-tau=total tau.

Supplemental Figure S1. Image of hippocampal LEAP segmentation.

This figure shows an example of an image of hippocampal volume segmentation for horizontal, coronal, and sagittal sections using the atlas-based LEAP method.



Supplemental Table S2. Added predictive value of a second biomarker for AD-type dementia after 2-year follow-up

Biomarker status after first test	Second biomarker tested	N	LR+	LR-	Odds ratio
CSF Aβ1-42/tau ratio normal	HCV	76	3.3 (1.6-6.8)	0.46 (0.19-1.14)	7.1* (1.5-33)
CSF Aβ1-42/tau ratio abnormal	HCV	77	1.5 (0.88-2.4)	0.72 (0.47-1.11)	2 (0.8-5.0)
HCV normal	CSF Aβ1-42/tau ratio	99	2.9 (2.0-4.3)	0.2 (0.07-0.58)	14† (3.9-54)
HCV abnormal	CSF Aβ1-42/tau ratio	54	1.6 (1.1-2.4)	0.38 (0.16-0.93)	4.1* (1.2-14)

Results are presented with 95% confidence intervals. AD=Alzheimer's disease; MCI=mild cognitive impairment; CSF=cerebrospinal fluid; HCV=hippocampal volume; LR+=positive likelihood ratio; LR-=negative likelihood ratio. *P<0.05; †p<0.001.

Supplemental Table S3. Added predictive value of a second biomarker for cognitive decline on MMSE

Biomarker status after first test	Second biomarker tested	Biomarker status	Slope	P-value difference
CSF Aβ1-42/tau ratio normal	HCV	Normal	-0.27‡ (0.1)	0.001
		Abnormal	-0.71‡ (0.1)	
CSF Aβ1-42/tau ratio abnormal	HCV	Normal	-1.06‡ (0.2)	0.333
		Abnormal	-1.33‡ (0.2)	
HCV normal	CSF Aβ1-42/tau ratio	Normal	-0.19 (0.1)	<0.001
		Abnormal	-1.08‡ (0.1)	
HCV abnormal	CSF Aβ1-42/tau ratio	Normal	-0.57 (0.3)	0.038
		Abnormal	-1.35‡ (0.2)	

Data are slopes (SE) for annual decline on MMSE. A negative slope indicates cognitive decline. As the sample size of the subgroups was different, different slopes may be presented for the same final groups. CSF=Cerebrospinal fluid, HCV=Hippocampal volume, MMSE=Mini Mental State Examination. ‡P<0.001.

Appendix S1. Neuropsychological tests used to diagnose MCI

Neuropsychological tests were used to assess cognitive performance in the domains of memory, language, executive function and attention, and visuoconstruction. Because the DESCRIPA study followed the routine practice at each centre, the tests used to assess each domain varied among centers.

At each center, we selected a primary test for each cognitive domain that was the same as or similar to the tests that were used at the other centers (Visser et al 2008). The primary tests to assess memory were the learning and delayed recall measure of the Rey auditory verbal learning test (4 centers) and the word list of the consortium to establish a registry for AD (CERAD) test battery (2 centers). The primary test to assess language was the 1-min verbal fluency for animals (all centers). The primary test to assess executive function and attention was the trail making test (TMT) parts A and B (all centers). The primary tests to assess visuoconstruction were the copy subtest of the Rey–Osterrieth complex figure (4 centers) or the copy of the CERAD figures (2 centers). If patients could not be classified on the basis of these primary tests because of missing data, we used alternative tests, qualitative rating by a neuropsychologist, or a subscore on the MMSE.

Appendix S2. Scan parameters and MRI protocols used at each center

CENTER AMSTERDAM VUMC: Siemens Magnetom Impact Expert 1.0 T, 3D scan, 168 slices, FOV 250 mm, matrix 256 × 256; slice thickness 1.5 mm, TE: 7 ms, TR: 15 ms, TI 300 ms, flip angle 15◦.

CENTER HUDDINGE: Siemens Avanto 1.5 T, 21 slices, FOV 220 mm, FOV phase 87.1, distance factor 30, phase R>L, slice thickness 5.0 mm, TE: 96 ms, TR: 4000 ms, flip angle 150◦, number of averages 1; and Siemens Symphony 1.5 T, 21 slices, FOV 220 mm, FOV phase 75.0, distance factor 30, phase R>L, slice thickness 5.0 mm, TE: 99 ms, TR: 4100 ms, flip angle 150◦, number of averages 2.

CENTER KUOPIO: Siemens Vision 1.5 T, T1 3D-scan, MPRAGE OBL; COR>TRA, FOV 250, mat 256x256, 128 slices, TR 9.7ms, TE 4 ms, Slice th 2.0mm, no slice gap, flip angle 12◦

CENTER MALMO: Siemens Sonata 1.5 T, MPRAGE + lmpr-cor, 144 slices, FOV 250 mm, phase R>L, TR 1970, TE 3.93, distance factor 50, slice thickness 1.5 mm, flip angle 15◦.

CENTER MUNICH: Siemens Magnetom Vision; 1.5 T; MPRAGE; Slice thickness 1.05 mm, TR 11.4, TE 4.4, TI 300; FOV 256*256; Flip angle 8graden; number of averages 1.

CENTER THESSALONIKI: Siemens Expert Plus unit 1.0 T, 3D-MPR: 15 (TR) ,7 TE ,8 FLIP ANG., 250 (Slabth), 1,49Ef thick, 168 Partitions, 250 FOV,256x192 Matrix, 1 Aquis. ,ACQ TIME 10,21min.

CHAPTER 4

PREDICTION OF ALZHEIMER'S DISEASE IN SUBJECTS WITH AMNESTIC AND NON-AMNESTIC MCI

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Stephanie J.B. Vos, Ineke A. van Rossum, Frans Verhey, Dirk L. Knol, Hilkka Soininen, Lars-Olof Wahlund, Harald Hampel, Magda Tsolaki, Lennart Minthon, Giovanni B. Frisoni, Lutz Froelich, Flavio Nobili, Wiesje van der Flier, Kaj Blennow, Robin Wolz, Philip Scheltens, Pieter Jelle Visser

ABSTRACT

Objective

To compare the predictive accuracy of beta amyloid (Aβ)1-42 and total tau in cerebrospinal fluid (CSF), hippocampal volume (HCV), and apolipoprotein E (APOE) genotype for AD-type dementia in subjects with amnestic MCI (aMCI) and non-amnestic MCI (naMCI).

Methods

We selected 399 subjects with aMCI and 226 subjects with naMCI from a multicenter memory clinic-based cohort. We measured CSF Aβ1-42 and tau by ELISA (n=231), HCV on MRI (n=388), and APOE-ε4 (n=523). Follow-up was performed annually up to 5 years. Outcome measures were progression to AD-type dementia and cognitive decline.

Results

At least 1 follow-up was available for 538 subjects (86%). 132 (38%) subjects with aMCI and 39 (20%) subjects with naMCI progressed to AD-type dementia after an average follow-up of 2.5 years. CSF Aβ1-42, tau and Aβ1-42/tau ratio, HCV, and APOE-ε4 predicted AD-type dementia in each MCI subgroup with the same overall diagnostic accuracy. However, CSF Aβ1-42 concentration was higher and hippocampal atrophy less severe in subjects with naMCI compared to aMCI. This reduced the sensitivity but increased the specificity of these markers for AD-type dementia in subjects with naMCI.

Conclusions

AD biomarkers are useful to predict AD-type dementia in subjects with aMCI and naMCI. Biomarkers may, however, not be as sensitive for early diagnosis of AD in naMCI compared to aMCI. This may have implications for clinical implementation of the NIA-AA criteria.

INTRODUCTION

The new research criteria for mild cognitive impairment (MCI) due to Alzheimer's disease (AD) proposed by the National Institute on Aging and Alzheimer's association (NIA-AA) consider both amnestic MCI (aMCI) and non-amnestic MCI (naMCI) as possible prodromal stages of AD-type dementia.[1] While previous studies on aMCI showed that biomarkers in cerebrospinal fluid (CSF), hippocampal volume (HCV) and apolipoprotein E (APOE) genotype could predict AD-type dementia in subjects with aMCI,[2-5] the predictive accuracy of these markers in subjects with naMCI remains to be established.

Recent studies showed that AD pathology is common in subjects with naMCI.[6] AD may, however, present differently in naMCI than in aMCI[7] and AD biomarkers may therefore have a different predictive accuracy for AD-type dementia. Information on the predictive accuracy of AD biomarkers in naMCI will be important both for clinical practice and AD trial design.[8]

We previously reported the predictive accuracy of HCV and the CSF Aβ1-42/tau ratio for AD-type dementia after 2 years in a subset of our subjects with MCI[9] and reported the predictive accuracy of neuronal injury markers for cognitive decline in subjects with CSF amyloid pathology[10]. The aim of the present study was to compare the predictive accuracy of biomarkers for AD-type dementia and cognitive decline after up to 5 years in subjects with aMCI with that in subjects with naMCI. As predictors we used CSF beta amyloid (Aβ)1-42, tau, Aβ1-42/tau ratio, HCV, and APOE genotype. We also tested short-term AD prediction after 2 years.

METHODS

Subjects

Subjects were recruited from the DESCRIPA cohort[11] and Alzheimer Center of the VU University medical center (VUmc).[12] Inclusion criteria were baseline diagnosis of aMCI or naMCI, age ≥55 years, data on either CSF Aβ1-42 and tau, HCV, or APOE genotype, and being newly referred for the assessment of cognitive complaints. Exclusion criteria were diagnosis of dementia at baseline or any other somatic, psychiatric or neurological disorder that might have caused the cognitive impairment.

DESCRIPA is a European multicenter study performed in a memory-clinic setting and enrolled subjects between 2003 and 2005.[11] For this study, 431 eligible subjects were selected from 16 participating centers in which CSF was collected, MRI was performed, or APOE genotype was determined. The VUmc center was one of the DESCRIPA centers and contributed an additional sample of 194 subjects that were seen outside the DESCRIPA inclusion period with data on CSF, MRI, or APOE measures. No differences in biomarkers were found between subjects from the VUmc center in the DESCRIPA study and those from the additional VUmc sample. Subjects with baseline data on CSF, MRI or APOE genotype (n=625) differed from subjects in whom CSF, MRI, and APOE genotype data were not

available (n=126) with regard to gender (female 54 vs. 67%;p=0.034), and MCI type (aMCI 64 vs. 52%;p=0.003) at baseline.

Standard Protocol Approvals, Registrations, and Patient Consents

The medical ethics committee at each center approved the study. All subjects provided informed consent.

Clinical and cognitive assessment

We performed a clinical history, medical and neurological examination, laboratory tests, functional evaluation with the clinical dementia rating scale, rating scales for neuropsychiatric symptoms, scoring on the Mini-Mental State Examination (MMSE), and a neuropsychological assessment.[11,12] Data was collected blinded to results of biomarker analyses.

Baseline diagnosis of MCI was made according to the criteria of Petersen et al.[13] Raw scores on neuropsychological tests were corrected for age, gender, and education, and expressed as z-scores.[11] Subjects with a z-score <-1.5 SD on the learning measure or delayed recall of a word list learning test or equivalent memory test were classified as having aMCI. Subjects with a z-score <-1.5 SD on the trail making test (TMT) part A, TMT part B, verbal fluency, Rey figure copy test or equivalent test but no memory impairment were classified as having naMCI. Subjects with aMCI were subclassified as aMCI-single domain (SD) if they only had an impairment in memory and as aMCI-multi domain (MD) if they had also impairments in non-memory domains. We calculated a memory composite score as the average z-score of the learning and delayed recall score of the word list learning test and a cognitive composite score as the average z-score of all 6 cognitive tests, if scores were available for at least 3 tests.

Cerebrovascular measures were hypertension (n=486; systolic blood pressure ≥140 mmHG or diastolic blood pressure ≥90 mmHG), hypercholesterol (n=483), and white matter lesions (WML; n=361). WML were scored by the PVL Scheltens scale[14] for the Descripa cohort and by the Fazekas scale[15] for the additional VUmc sample. We scored the PVL scores as Fazekas score: PVL 0=Fazekas 0, PVL 1-2=Fazekas 1, PVL 3=Fazekas 2, and PVL ≥4 =Fazekas 3. Final WML scores were dichotomized as 0-1 vs. 2-3.

Follow-up assessment was performed annually up to 5 years. Primary outcome measures were conversion to AD-type dementia at follow-up according to the DSM-IV[16] and NINCDS-ADRDA criteria[17] and annual cognitive decline on MMSE, memory and overall cognition. Secondary outcome measure was short-term conversion to AD-type dementia after 2 years.

CSF analyses

CSF data were available for 231 subjects. These subjects showed minor differences in age (69.7 vs. 71.3;p=0.035), MMSE (26.6 vs. 27.1;p=0.042), and gender (female 44 vs.

59%;p=0.02) compared to subjects without CSF data. 214 subjects with CSF data had a least one follow-up assessment.

CSF was collected by lumbar puncture, centrifuged, and stored at -80°C in polypropylene tubes. Three samples were thawed twice but analyses without these samples revealed similar results. CSF Aβ1-42 and total tau (t-tau) were measured by experienced technicians using commercially available sandwich ELISAs (Innotest β-amyloid 1-42; Innotest hTAU-Ag; Innogenetics, Ghent, Belgium), specially constructed to measure Aβ1-42 and t-tau,[18,19] at the lab in Gothenburg for the DESCRIPA cohort and in Amsterdam for the additional subjects of the VUmc cohort. We corrected for interlaboratory ELISA differences by analyzing 33 samples at both labs and we adjusted VUmc values to those of DESCRIPA using the following formula: Gothenborg=(SD Gothenborg/SD VUmc)*VUmc+average Gothenborg–((SD Gothenborg /SD VUmc)*average VUmc).[20]

The CSF measures were dichotomized based on prespecified cut-offs.[21,22] The cut-off was <500 pg/ml for Aβ1-42, >450 pg/ml for t-tau in subjects below 70 years and >500 pg/ml for t-tau in subjects above 70 years, and Aβ1-42/(240+[1.18*t-tau]) < 1.0 for Aβ1-42/t-tau ratio.

MRI analyses

MRI scans that allowed automated measurements of HCV were available for 388 subjects. These subjects did not differ from subjects without data of automatically measured HCVs on demographical variables. 329 subjects with MRI data had a least one follow-up assessment.

For the DESCRIPA cohort, at each site, subjects were scanned according to the routine MRI protocol. All scanning was performed at 1.0 or 1.5 T and included a three-dimensional T1-weighted gradient echo sequence and a fast fluid attenuated inversion recovery (FLAIR) sequence.[9] HCV was measured centrally at the Department of Computing at Imperial College London, using learning embeddings for atlas propagation (LEAP), an automated segmentation technique based on atlas registration.[9, 23]

The sum of the left and right HCV was dichotomized by defining a cut-off (5.389 cm^3) that could best differentiate healthy controls from subjects with AD-type dementia in the ADNI cohort, based on the Youden index from a time-dependent ROC curve in R.[24] The cut-off was similar to the optimal cut-off in our own cohort.[9]

APOE genotype

APOE data were available for 523 subjects. These subjects did not differ from subjects without APOE data on demographical variables. 464 subjects with APOE data had a least one follow-up assessment. APOE genotype was determined by polymerase chain reaction of genomic DNA extracted from EDTA anticoagulated blood. Subjects were classified as APOE-e4 carriers or non-carriers.

Statistical analyses

Statistical analyses were done with SPSS version 19.0 (Chicago, IL, USA) and statistical software package R version 2.10.1,[25] with significance set at $p<0.05$. All analyses were corrected for age, gender, and education.

We calculated hazard ratios (HRs) using Cox regression to investigate the predictive accuracy for AD-type dementia of CSF Aβ1-42, tau, Aβ1-42/tau ratio, HCV, and APOE genotype in both MCI groups. For analyses with continuous variables, variables were log transformed and expressed as z-scores.

The relation between biomarkers and change on the MMSE, memory, and overall cognition in each MCI subgroup was assessed by slope analyses with mixed models. The analyses included the baseline score and available follow-up scores up to 5 years. The model was specified with center as a random effect, as this model provided the best -2 log likelihood compared to models with simpler covariance structures. The median number of measurements per subject was 3 (range 1-6) for the MMSE and 2 (range 1-6) for memory with minor differences between MCI subgroups and between predictor variables.

We calculated odds ratios (ORs) to investigate predictive accuracy for AD-type dementia after 2 years. Additionally, we calculated sensitivity, specificity, positive predictive value, and negative predictive value.

RESULTS

Sample characteristics

399 (64%) subjects were classified as aMCI and 226 (36%) as naMCI. Of the aMCI subjects, 174 (44%) had aMCI-SD and 225 (56%) aMCI-MD. Subjects with aMCI had poorer MMSE scores and memory scores, better TMT part A scores, lower levels of CSF Aβ1-42, higher levels of CSF tau, a lower CSF Aβ1-42/tau ratio, and a smaller HCV than subjects with naMCI at baseline (table 1). Subjects with aMCI-MD had poorer cognitive scores than subjects with aMCI-SD but they did not differ in biomarker scores. 538 (86%) subjects had at least one follow-up assessment (average 2.5 years (SD=1.0)). Progression rate to AD-type dementia was higher in aMCI (38%) than in naMCI (20%), and higher in aMCI-MD (46%) than in aMCI-SD (29%). 17 (5%) of the subjects with aMCI progressed to other dementias (7 frontotemporal dementia, 7 Lewy body dementia, 1 Parkinson dementia, 1 alcohol dementia, and 1 vascular dementia) and 15 (8%) of the subjects with naMCI progressed to other dementias (3 frontotemporal dementia, 6 Lewy body dementia, 5 vascular dementia, 1 normal pressure hydrocephalus). These subjects were included in the no-AD dementia group.

Table 1. Baseline characteristics of subjects with aMCI and naMCI

	naMCI (n=226)	aMCI (n=399)	aMCI single domain (n=174)	aMCI multi domain (n=225)
Age	70.7 (7.6)	70.7 (7.8)	70.3 (7.6)	71.0 (8.0)
Female, n	111 (49%)	224 (56%)	76 (44%)‡	148 (66%)**
Years of education	10.0 (4.1)	10.2 (3.9)	10.8 (3.5)¶	9.8 (4.1)
CDR-SOB	1.1 (1.0)	1.3 (1.0)	1.2 (1.0)	1.4 (1.0)*
MMSE	27.5 (2.1)	26.5 (2.5)***	27.1 (2.2)‡	26.1 (2.6)***
Memory score (z-score)	-0.3 (0.7)	-2.0 (0.6)***	-1.8 (0.5)***‡	-2.1 (0.6)***
Verbal fluency (z-score)	-0.6 (1.2)	-0.8 (1.2)	-0.3 (1.0)**‡	-1.2 (1.2)***
TMT part A (z-score)	-1.2 (1.7)	-0.8 (1.6)*	0.1 (0.8)***‡	-1.5 (1.8)
TMT part B (z-score)	-1.5 (1.9)	-1.2 (1.9)	0.04 (0.8)***‡	-2.2 (1.9)***
Visuoconstruction (z-score)	-0.1 (1.3)	0.1 (1.1)	0.6 (0.7)***‡	- 0.2 (1.2)
Aβ1-42, pg/ml	624 (283)	550 (267)**	589 (270)	512 (261)*
Tau, pg/ml	427 (261)	524 (322)*	533 (300)*	488 (241)
Ratio Aβ1-42 /tau	0.96 (0.6)	0.78 (0.5)*	0.84 (0.5)	0.73 (0.5)*
Hippocampal volume, cm^3	5.89 (0.7)	5.55 (0.8)***	5.59 (0.8)**	5.53 (0.8)***
APOE-ε4, n	73 (40%)	158 (47%)	79 (54%)*¶	79 (41%)
Hypertension, n	97 (47%)	144 (41%)	50 (34%)*¶	94 (46%)
Hypercholesterol, n	56 (27%)	116 (33%)	39 (27%)¶	77 (38%)*
White matter hyperintensities, n	60 (37%)	79 (30%)	37 (29%)	42 (31%)
Follow-up, n	192 (85%)	346 (87%)	155 (89%)	191 (85%)
AD conversion rate, n	39 (20%)	132 (38%)***	45 (29%)†	87 (46%)***
No-AD dementia conversion rate, n	15 (8%)	17 (5%)	3 (2%)	14 (7%)
Time to AD, y	1.9 (1.1)	1.9 (1.0)	2.2 (1.2)	1.8 (1.0)
Follow-up time non-converters and no-AD dementia converters, y	2.6 (1.0)	2.4 (1.0)	2.4 (1.0)	2.3 (1.0)

Data are mean (SD), unless specified otherwise. Sample size APOE: aMCI n=339, naMCI n=184; hippocampal volume: aMCI n=241, naMCI n=147; CSF: aMCI n=156, naMCI n=75; blood pressure: aMCI n= 353, naMCI n=205; cholesterol: aMCI n=351, naMCI n=204; white matter lesions: aMCI n=260, naMCI n=164. aMCI=amnestic mild cognitive impairment, naMCI=non-amnestic mild cognitive impairment, single=single domain impaired (memory), multi=multiple domains impaired, CDR-SOB=Clinical Dementia Rating Scale Sum of Boxes, MMSE=Mini-Mental State Examination, TMT=trail making test, Aβ1-42=beta amyloid 1-42, APOE=apolipoprotein E, AD=Alzheimer's disease. *P<0.05, **p<0.01, ***p<0.001 for aMCI all, single aMCI or multi aMCI compared to naMCI; ¶p<0.05, †p<0.01, ‡p<0.001 for aMCI single domain compared to aMCI multi domain.

Table 2. Predictive accuracy of biomarkers for AD-type dementia

Biomarkers	Group	HR for continuous predictor variable	P-value	HR for dichotomous predictor variable	P-value
CSF Aβ1-42	naMCI	1.8 (1.0-3.1)	0.034	3.3 (1.4-7.9)	0.008
	aMCI	1.7 (1.3-2.2)	<0.001	3.5 (2.0-6.3)	<0.001
	aMCI single	2.0 (1.4-3.0)	<0.001	3.8 (1.6-9.0)	0.002
	aMCI multi	1.7 (1.1-2.4)	0.007	3.2 (1.4-7.4)	0.005
CSF t-tau	naMCI	2.7 (1.6-4.6)	<0.001	5.2 (2.1-13)	<0.001
	aMCI	2.1 (1.5-2.9)	<0.001	3.0 (1.7-5.3)	<0.001
	aMCI single	2.6 (1.5-4.5)	0.001	3.9 (1.6-9.9)	0.004
	aMCI multi	2.0 (1.2-3.4)	0.006	2.2 (1.0-7.4)	0.034
CSF ratio	naMCI	2.6 (1.6-4.4)	<0.001	7.1 (1.6-31)	0.009
	aMCI	2.0 (1.6-2.6)	<0.001	14 (3.4-58)	<0.001
	aMCI single	2.1 (1.5-3.0)	<0.001	17 (2.3-127)	0.006
	aMCI multi	2.5 (1.6-3.8)	<0.001	12 (1.5-87)	0.018
HCV	naMCI	2.1 (1.1-3.8)	0.016	2.3 (0.9-6.0)	0.085
	aMCI	1.6 (1.3-2.1)	<0.001	3.0 (1.8-4.9)	<0001
	aMCI single	1.4 (0.9-2.1)	0.107	2.2 (1.0-4.8)	0.045
	aMCI multi	1.8 (1.3-2.5)	<0.001	3.1 (1.6-6.0)	0.001
APOE-e4	naMCI	-		1.9 (0.9-3.9)	0.085
	aMCI	-		1.8 (1.2-2.6)	0.003
	aMCI single	-		5.6 (2.2-14)	<0.001
	aMCI multi	-		1.3 (0.8-2.1)	0.215

Data are hazard ratios (95%CI), corrected for age, gender, and education. Scores were dichotomized based on prespecified cut-offs (see methods). AD=Alzheimer's disease, aMCI=amnestic mild cognitive impairment, naMCI=non-amnestic mild cognitive impairment, CSF=cerebrospinal fluid, Aβ1-42=beta amyloid 1-42, HCV=hippocampal volume, APOE=apolipoprotein E. Hazard ratio aMCI vs naMCI: all p>0.12.

Prediction of AD-type dementia

CSF Aβ1-42, tau, Aβ1-42/tau ratio, and HCV all predicted AD-type dementia during the 5-year follow-up both in subjects with aMCI and naMCI (table 2). When scores were dichotomized, similar results were obtained. Also presence of the APOE-ε4 allele predicted AD-type dementia in each MCI group, but with only a trend in naMCI (p=0.085). The HRs for AD-type dementia did not differ between aMCI and naMCI (all p>0.12). They did also not differ between aMCI-SD and aMCI-MD, except for APOE-ε4. However, the HR of APOE-e4 was not different between naMCI and aMCI subtypes.

The ability of the biomarkers to predict outcomes after 2 years was generally similar to what was seen at the longer time point for aMCI vs. naMCI (table 3) and for aMCI-SD vs. aMCI-MD (data not shown). However, CSF and MRI markers showed a higher sensitivity and lower specificity in aMCI than naMCI (table 3) because both aMCI subjects with and without AD-type dementia at follow-up had more abnormal biomarker scores compared to subjects with naMCI (supplemental table e-1).

Table 3. Predictive accuracy for AD-type dementia after 2 years

Markers	Group[a]	Odds ratio[b]	Sensitivity	Specificity	PPV	NPV
CSF Aβ1-42	naMCI	5.8 (1.3-25)*	0.55 (0.33-0.77)	0.71 (0.57-0.85)	0.48 (0.27-0.68)	0.76 (0.63-0.90)
	aMCI	4.6 (2.1-10)‡	0.75 (0.64-0.87)	0.58 (0.47-0.69)	0.56 (0.44-0.67)	0.78 (0.67-0.88)
CSF tau	naMCI	5.4 (1.4-21)*	0.60 (0.39-0.81)	0.78 (0.65-0.91)	0.57 (0.36-0.78)	0.80 (0.68-0.92)
	aMCI	4.5 (2.1-9.9)‡	0.74 (0.62-0.85)	0.61 (0.50-0.72)¥	0.57 (0.45-0.68)	0.77 (0.66-0.88)
CSF Aβ1-42/tau ratio	naMCI	9.9 (1.6-60)*	0.90 (0.77-1.00)	0.54 (0.38-0.69)	0.49 (0.33-0.65)	0.92 (0.81-1.00)
	aMCI	33 (4.3-257)†	0.98 (0.94-1.00)	0.38 (0.27-0.48)	0.52 (0.42-0.62)	0.97 (0.90-1.00)
HCV	naMCI	3.6 (1.1-12)*	0.39 (0.16-0.61)	0.88 (0.82-0.95)	0.39 (0.16-0.61)	0.88 (0.82-0.95)
	aMCI	4.1 (2.1 -8.0)‡	0.63 (0.51-0.74)	0.72 (0.64-0.80)¥	0.55 (0.43-0.66)	0.78 (0.70-0.86)
APOE-e4	naMCI	1.8 (0.7-4.7)	0.56 (0.37-0.74)	0.62 (0.53-0.71)	0.25 (0.14-0.35)	0.86 (0.79-0.93)
	aMCI	1.8 (1.1-3.0)*	0.56 (0.46-0.66)	0.57 (0.49-0.64)	0.40 (0.32-0.49)	0.71 (0.64-0.79)

Results are presented with 95% confidence intervals. Scores were dichotomized based on prespecified cut-offs (see methods). Subjects with a follow-up less than 1.5 years were excluded for these analyses. [a]Group size is different for all measures: CSF: aMCI n=130, naMCI n=61; HCV: aMCI n=181, naMCI n=111; APOE: aMCI n=274, naMCI n=148. [b]Odds ratios are corrected for age, gender, and education. AD=Alzheimer's disease, aMCI=amnestic mild cognitive impairment, naMCI=non-amnestic mild cognitive impairment, PPV=positive predictive value, NPV=negative predictive value, CSF=cerebrospinal fluid, Aβ1-42=beta amyloid 1-42, HCV=hippocampal volume, APOE=apolipoprotein E genotype. *P<0.05. †p<0.01, ‡p<0.001. ¥P<0.05 compared to naMCI.

Table 4. Annual decline on the MMSE according to biomarker scores

Biomarker		naMCI			aMCI		
		Normal biomarker	Abnormal biomarker	P-value	Normal biomarker	Abnormal biomarker	P-value
CSF Aβ1-42	Baseline	27.6 (0.37)	27.3 (0.45)	0.6	26.5 (0.37)	26.0 (0.35)	0.29
	Slope	-0.29 (0.16)	-0.30 (0.19)	0.96	-0.70 (0.14)‡	-1.12 (0.16)‡	0.05
CSF t-tau	Baseline	27.6 (0.31)	27.2 (0.48)	0.5	26.8 (0.35)	25.5 (0.33)	0.009
	Slope	0.01 (0.13)	-1.12 (0.22)‡	<0.001	-0.54 (0.16)†	-1.14 (0.14)‡	0.005
CSF ratio	Baseline	27.6 (0.44)	27.3 (0.38)	0.64	27.1 (0.49)	25.9 (0.28)	0.03
	Slope	0.04 (0.17)	-0.59 (0.16)‡	0.007	-0.24 (0.22)	-1.07 (0.12)‡	0.001
HCV	Baseline	27.7 (0.23)	27.4 (0.45)	0.59	27.0 (0.26)	26.0 (0.31)	0.02
	Slope	-0.17 (0.09)	-0.70 (0.22)†	0.03	-0.65 (0.12)‡	-1.12 (0.14)‡	0.01
APOE-e4	Baseline	27.9 (0.22)	27.6 (0.27)	0.46	27.0 (0.22)	26.2 (0.23)	0.01
	Slope	-0.17 (0.09)	-0.42 (0.10)‡	0.06	-0.60 (0.09)‡	-0.95 (0.08)‡	0.005

Data are slopes (SE) of MMSE corrected for age, gender, and education. Scores were dichotomized based on prespecified cut-offs (see methods). Sample size: CSF: aMCI n=145, naMCI n=69; HCV: aMCI n=206, naMCI n=123; APOE: aMCI n=300; naMCI n=164. MMSE=Mini Mental State Examination, aMCI=amnestic mild cognitive impairment, naMCI=non-amnestic mild cognitive impairment, CSF=cerebrospinal fluid, Aβ1-42=beta amyloid 1-42, HCV=hippocampal volume, APOE=apolipoprotein E. †P<0.01, ‡p<0.001.

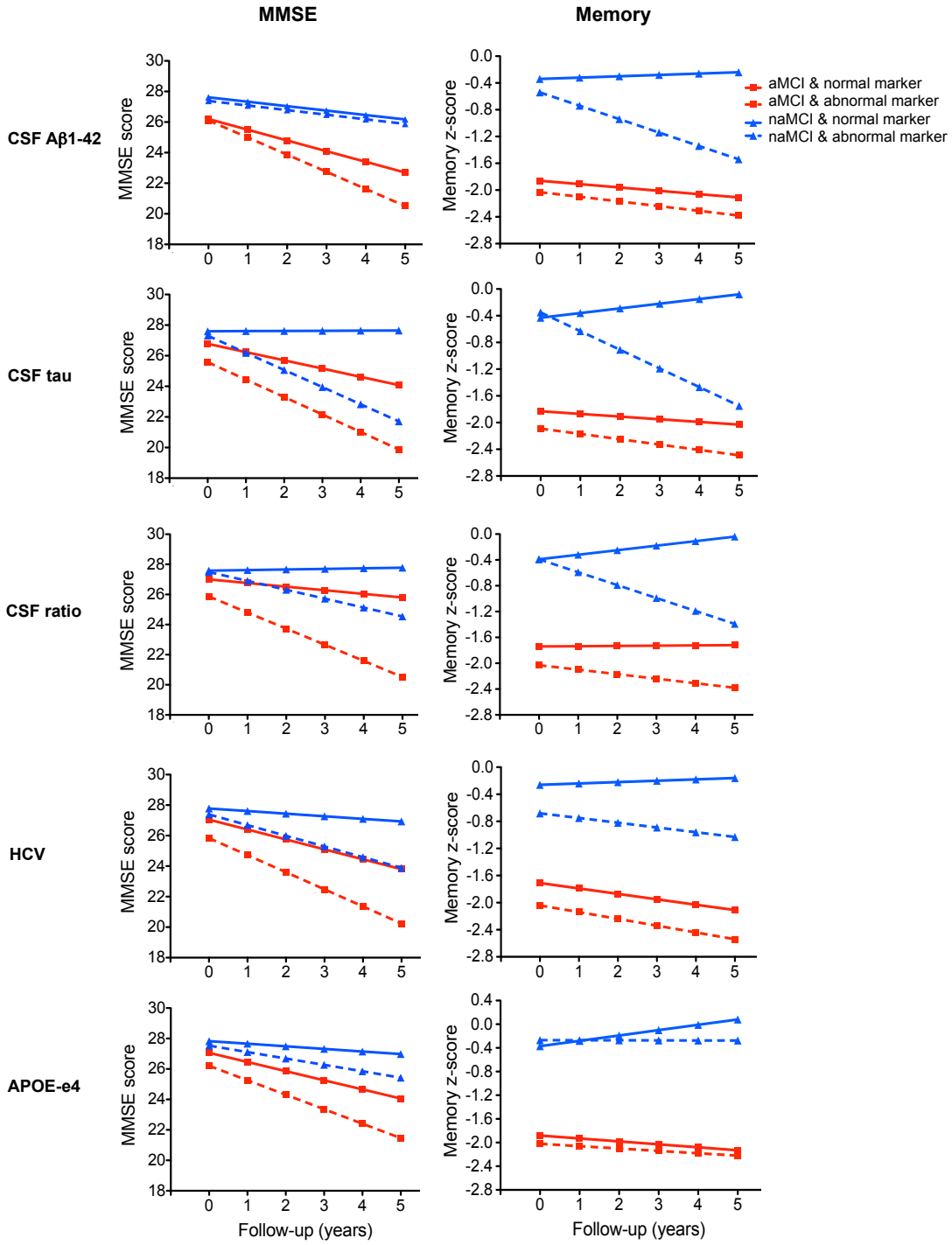

Figure 1. Annual decline on MMSE and memory scores in subjects with aMCI and naMCI by biomarker scores.

The figure shows slopes of MMSE scores (left) and memory z-scores (right) for subjects with aMCI and naMCI according to baseline biomarker profiles of CSF Aβ1-42, CSF tau, CSF Aβ1-42/tau ratio, HCV, and APOE genotype. Red solid lines (squares) represent subjects with aMCI and a normal biomarker score; Red dotted lines (squares) represent subjects with aMCI and an abnormal biomarker score; Blue solid lines (triangles) represent subjects with naMCI and a normal biomarker score; Blue dotted lines (triangles) represent subjects with naMCI and an abnormal biomarker score. aMCI=amnestic mild cognitive impairment; naMCI=non-amnestic mild cognitive impairment; MMSE=Mini-Mental State Examination; Aβ1-42=beta amyloid 1-42; HCV=hippocampal volume; APOE=apolipoprotein E.

Prediction of annual cognitive decline

All biomarkers predicted decline on the MMSE in subjects with aMCI and naMCI, except for CSF Aβ1-42 in naMCI (table 4, figure 1). In subjects with aMCI, none of the biomarkers predicted change in memory score or overall cognition (figure 1, supplemental table e-2). In subjects with naMCI, CSF Aβ1-42, tau, and Aβ1-42/tau ratio predicted decline in memory and overall cognition, with only a trend for CSF Aβ1-42 (figure 1, supplemental table e-2). Results remained similar after exclusion of subjects who progressed to no-AD dementias. Slopes did not differ between aMCI-SD and aMCI-MD, except for APOE-ε4 and decline on the MMSE and the CSF ratio and decline in memory (data not shown).

Post-hoc analyses

Since CSF and MRI biomarker scores differed between subjects with aMCI and naMCI, we calculated subgroup specific cut-offs. In each MCI group, we defined a cut-off point that maximized the Youden index for the prediction of AD-type dementia after 2 years. For subjects with aMCI, the cut-offs were similar to the prespecified cut-offs, except for the CSF Aβ1-42/tau ratio for which a lower cut-off was selected. For subjects with naMCI, the optimal cut-offs for CSF Aβ1-42 and HCV were substantially different from the prespecified cut-offs (supplemental table e-3). Using these cut-off points, the odds ratio remained similar to the predictive accuracy using the prespecified cut-offs. The sensitivity increased but at the cost of a decrease in specificity (supplemental table e-3).

DISCUSSION

This study tested the predictive accuracy of CSF markers, MRI markers and genotype for AD-type dementia in subjects with naMCI. We found that the predictive accuracy was similar to that in subjects with aMCI. Biomarkers also predicted decline on the MMSE in both MCI groups and decline in memory and overall cognition in subjects with naMCI. While there are differences in the relationship of biomarkers to outcome in aMCI and naMCI, our findings suggest that the symptomatic manifestations of the AD pathophysiological process is broader than the amnestic presentation at the MCI stage and also includes non-amnestic MCI.

Although the overall predictive accuracy for AD-type dementia was similar for subjects with aMCI and naMCI, sensitivity of CSF and MRI markers was higher and specificity lower in subjects with aMCI. While most of the differences in sensitivity and specificity between MCI subtypes were not statistically significant, they may be clinically relevant when biomarker cut-offs are applied to define abnormal AD markers and predict clinical progression. Differences were observed because subjects with aMCI had more abnormal biomarker scores than subjects with naMCI. The finding that subjects with naMCI and AD-type dementia at follow-up had less abnormal amyloid markers than subjects with aMCI and AD-type dementia is remarkable. This suggests that subjects with naMCI may have comorbidity, for example vascular lesions, such that less Alzheimer pathology is needed to

reach the dementia threshold.[26] However, post-hoc analyses showed no difference in white matter hyperintensities, blood pressure, and cholesterol levels between subjects with naMCI and aMCI with AD-dementia at follow-up (supplemental table e-2). Alternatively, subjects with naMCI may be in an earlier stage of the disease. This would be supported by higher MMSE and cognitive scores, and larger HCV in subjects with naMCI at baseline. However, the time to dementia was the same in subjects with naMCI and aMCI. It is also possible that naMCI is an atypical presentation of AD with a different expression of amyloid pathology in CSF.

The biomarkers predicted decline on MMSE scores generally equally well in subjects with aMCI and naMCI. However, biomarkers predicted decline in memory and overall cognition only in subjects with naMCI but not in subjects with aMCI. This was probably because of floor effects on the memory tests in subjects with aMCI, which reduced the ability to detect further cognitive decline.

For dichotomized measures, CSF Aβ1-42/tau ratio was the best predictor for AD-type dementia in each MCI subgroup. This is consistent with recent meta-analyses that showed that the combination of these markers is a better predictor than each separate CSF marker and that the odds ratio of APOE genotype for AD-type dementia is lower than that of CSF and MRI measures.[27,28] The odds ratio for AD-type dementia of the CSF ratio in subjects with aMCI was very high (>30). However, because only one subject with AD at follow-up had a normal ratio, the 95% confidence interval was very wide, which limits the interpretation of this finding.

The observation that APOE-ε4 frequency was similar between subjects with aMCI and naMCI is inconsistent with previous studies showing that the absence of APOE-ε4 is related to less memory impairment.[29] It is possible that subjects with naMCI in our study have had a late onset of memory impairment as we found that subjects with naMCI and abnormal AD biomarkers showed a rapid decline in memory at follow-up.

Progression rate to AD-type dementia was higher in aMCI than in naMCI while progression to no-AD dementia was similar for MCI subtypes, which is in line with a previous study.[30] This made also the ratio of AD dementia converters to no-AD dementia converters different between the groups (p=0.005).

Our study had several limitations. As the findings were based on memory clinic populations, they may not be generalized to other settings. Biomarkers were analyzed in different subgroups which optimized statistical power for each analysis but which limited the opportunity to compare the predictive accuracy between biomarkers since differences in accuracy could result form differences in subject characteristics. For the main objective of our study, however, this was not a limitation as we aimed to compare the predictive accuracy between MCI subtypes for each biomarker. Moreover, post-hoc analysis in subjects with all biomarkers available (aMCI n=91, naMCI n=42) showed that biomarkers had a generally similar hazard ratio for AD-type dementia compared to the analysis in the total sample. There was variability in scanners used, which may have influenced the volumetric measurements and predictive accuracy of the HCV. Nevertheless, the LEAP approach has

been shown to be robust for scanner variability.[20] In addition, the diagnosis of AD-type dementia at follow-up was not neuropathologically validated. This may have led to misclassification. The major strengths of this study were the large sample size and relatively long follow-up period. However, not all subjects had a 5-year follow-up. Furthermore, the multicenter design favors generalization to other memory clinic settings.

Our findings suggest that AD biomarkers are useful to predict AD-type dementia both in subjects with aMCI and naMCI. The differences in sensitivity and specificity for AD-type dementia may have important implications for clinical implementation of the NIA-AA criteria and AD trial design. AD biomarkers, and especially CSF Aβ1-42 levels, may not be as sensitive for early diagnosis of AD in subjects with naMCI compared to aMCI when the same cut-offs are applied in each subgroup. More lenient cut-offs may optimize the sensitivity in subjects with naMCI, however, at the expense of a decrease in specificity. Longitudinal biomarker studies are needed to investigate the change in biomarkers over time to clarify the pathology and prognosis of subjects with naMCI.

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SUPPLEMENTAL DATA

Supplemental Table e-1. Baseline characteristics of subjects with aMCI and naMCI by outcome after 2 years

	naMCI			aMCI		
	No AD (n=161)	AD (n=31)	P-value No AD vs. AD	No AD (n=236)	AD (n=110)	P-value No AD vs. AD
Age	69.2 (7.3)	75.2 (6.3)	<0.001	69.8 (8.0)	71.9 (7.2)†	0.03
Female, n	80 (50%)	13 (42%)	NS	127 (54%)	68 (62%)	NS
Years of education	9.8 (4.1)	10.8 (4.6)	NS	10.4 (3.9)	10.4 (3.7)	NS
CDR-SOB	1.1 (0.9)	1.3 (1.0)	NS	1.2 (0.9)	1.5 (1.1)	0.02
MMSE	27.8 (1.9)	26.4 (2.2)	0.001	27.0 (2.3)‡	25.6 (2.7)	<0.001
Memory score (z-score)	-0.2 (0.8)	-0.6 (0.4)	0.001	-1.9 (0.6)‡	-2.1 (0.6)‡	0.04
Verbal fluency (z-score)	-0.5 (1.3)	-0.9 (0.9)	0.04	-0.6 (1.2)	-1.1 (1.2)	<0.001
TMT part A (z-score)	-1.2 (1.6)	-1.2 (1.4)	NS	-0.6 (0.5)‡	-1.0 (1.7)	NS
TMT part B (z-score)	-1.4 (1.8)	-1.7 (2.1)	NS	-1.0 (1.8)*	-1.4 (1.8)	NS
Visuoconstruction (z-score)	-0.1 (1.3)	0.2 (1.2)	NS	0.2 (1.1)*	0.1 (1.2)	NS
Aβ1-42, pg/ml	685 (308)	526 (166)	NS	630 (294)	415 (140)‡	<0.001
Tau, pg/ml	359 (216)	632 (287)	<0.001	437 (246)*	694 (385)	<0.001
Ratio Aβ1-42/tau	1.15 (0.6)	0.61 (0.3)	<0.001	0.97 (0.6)*	0.45 (0.2)*	<0.001
Hippocampal volume, cm^3	6.03 (0.7)	5.43 (0.4)	0.002	5.71 (0.8)‡	5.23 (0.7)	<0.001
APOE-e4, n	50 (37%)	15 (56%)	0.03	86 (42%)	53 (56%)	0.008
Hypertension, n	73 (48%)	11 (42%)	NS	84 (39%)	42 (45%)	NS
Hypercholesterol, n	47 (31%)	1 (4%)	0.003	81 (38%)	22 (24%)*	0.025
White matter hyperintensities, n	47 (40%)	5 (23%)	NS	48 (32%)	18 (26%)	NS

Data are mean (SD), unless specified otherwise. Results were corrected for center. P-values are reported for subjects with no AD versus AD at follow-up within each MCI group. Sample size: CSF: aMCI n=145, naMCI n=69; hippocampal volume: aMCI n=206, naMCI n=123; APOE: aMCI n=300, naMCI n=164; blood pressure: aMCI n= 309, naMCI n=177; cholesterol: aMCI n=307, naMCI n=176; white matter lesions: aMCI n=222, naMCI n=139. aMCI=amnestic mild cognitive impairment; naMCI=non-amnestic mild cognitive impairment; AD=Alzheimer's disease, CDR-SOB=Clinical Dementia Rating Sum of Boxes; MMSE=Mini-Mental State Examination; TMT=trail making test; Aβ1-42=beta amyloid 1-42; APOE=apolipoprotein E genotype. NS=not statistically significant with alpha=0.05. *P<0.05, †p<0.01 ‡p<0.001 for aMCI-no AD vs. naMCI-no AD or for aMCI-AD vs. naMCI-AD.

Supplemental Table-e2. Annual decline in memory and overall cognition according to biomarker scores

	Biomarker		naMCI			aMCI		
			Normal biomarker	Abnormal biomarker	P-value	Normal biomarker	Abnormal biomarker	P-value
a. Memory	CSF Aβ1-42	Baseline	-0.44 (0.17)	-0.57 (0.20)	0.63	-1.90 (0.08)	-2.06 (0.08)	0.16
		Slope	0.02 (0.07)	-0.20 (0.10)	0.07	-0.05 (0.04)	-0.07 (0.03)*	0.59
	CSF t-tau	Baseline	-0.54 (0.16)	-0.44 (0.23)	0.73	-1.85 (0.07)	-2.13 (0.08)	0.01
		Slope	0.07 (0.06)	-0.28 (0.10)†	0.004	-0.04 (0.03)	-0.08 (0.03)*	0.42
	CSF ratio	Baseline	-0.47 (0.20)	-0.45 (0.17)	0.94	-1.75 (0.11)	-2.06 (0.07)	0.02
		Slope	0.07 (0.08)	-0.20 (0.08)†	0.01	0.004 (0.05)	-0.07 (0.03)†	0.16
	HCV	Baseline	-0.35 (0.09)	-0.69 (0.18)	0.09	-1.73 (0.05)	-2.06 (0.07)	<0.001
		Slope	0.02 (0.04)	-0.07 (0.07)	0.27	-0.08 (0.03)†	-0.10 (0.04)†	0.66
	APOE-e4	Baseline	-0.52 (0.09)	-0.37 (0.11)	0.30	-1.91 (0.05)	-2.03 (0.06)	0.11
		Slope	0.09 (0.04)*	-0.001 (0.04)	0.13	-0.05 (0.03)*	-0.04 (0.03)	0.67
b. Overall Cognition	CSF Aβ1-42	Baseline	-0.52 (0.11)	-0.70 (0.13)	0.26	-0.77 (0.09)	-1.14 (0.09)	0.004
		Slope	-0.08 (0.05)	-0.23 (0.07)†	0.09	-0.14 (0.04)‡	-0.15 (0.03)‡	0.85
	CSF t-tau	Baseline	-0.58 (0.10)	-0.64 (0.15)	0.72	-0.89 (0.09)	-1.03 (0.09)	0.28
		Slope	-0.05 (0.05)	-0.28 (0.07)‡	0.01	-0.10 (0.04)†	-0.19 (0.03)‡	0.07
	CSF ratio	Baseline	-0.61 (0.14)	-0.57 (0.11)	0.81	-0.71 (0.13)	-1.04 (0.08)	0.03
		Slope	-0.01 (0.06)	-0.22 (0.05)‡	0.007	-0.12 (0.05)*	-0.15 (0.03)‡	0.52
	HCV	Baseline	-0.84 (0.07)	-0.67 (0.14)	0.31	-1.02 (0.07)	-1.03 (0.08)	0.91
		Slope	-0.05 (0.03)	-0.15 (0.05)†	0.09	-0.16 (0.03)‡	-0.16 (0.04)‡	0.98
	APOE-e4	Baseline	-0.80 (0.06)	-0.64 (0.08)	0.13	-1.20 (0.06)	-1.05 (0.06)	0.09
		Slope	-0.01 (0.03)	-0.03 (0.03)	0.66	-0.10 (0.03)‡	-0.12 (0.02)‡	0.59

Data are slopes (SE) of memory (a) and cognitive composite score (b), corrected for age, gender, and education. Scores were dichotomized based on prespecified cut-offs (see methods). Group size is different for biomarkers: CSF: aMCI n=145, naMCI n=69; HCV: aMCI n=206, naMCI n=123; APOE: aMCI n=300, naMCI n=164. CSF=Cerebrospinal fluid, HCV=Hippocampal volume, aMCI=Amnestic mild cognitive impairment, naMCI=non-amnestic mild cognitive impairment, APOE=apolipoprotein E. *P<0.05, †p<0.01, ‡p<0.001.

Supplemental Table e-3. Predictive accuracy of AD markers after 2 years with subgroup-specific cut-offs in subjects with aMCI and naMCI

Biomarker	Group[a]	Cut-off	Odds ratio[b]	Sensitivity	Specificity	PPV	NPV
CSF Aβ1-42	naMCI	630	5.1 (1.2-21)*	0.75 (0.56-0.94)	0.59 (0.43-0.74)	0.47 (0.30-0.64)	0.83 (0.69-0.97)
	aMCI	496	4.2 (1.9-9.0)‡	0.74 (0.62-0.85)	0.58 (0.47-0.69)	0.55 (0.43-0.67)	0.76 (0.65-0.87)
CSF tau	naMCI	459	6.8 (1.7-28)†	0.65 (0.44-0.86)	0.78 (0.65-0.91)	0.59 (0.39-0.80)	0.82 (0.70-0.94)
	aMCI	491	5.2 (2.4-11)‡	0.72 (0.60-0.84)	0.66 (0.56-0.77)	0.59 (0.47-0.71)	0.77 (0.67-0.87)
CSF Aβ1-42/tau ratio	naMCI	0.91	10 (2.1-48)†	0.85 (0.69-1.00)	0.66 (0.51-0.80)	0.55 (0.37-0.72)	0.90 (0.79-1.00)
	aMCI	0.52	8.2 (3.6-19)‡	0.75 (0.64-0.87)	0.71 (0.61-0.82)	0.65 (0.53-0.76)	0.81 (0.72-0.90)
HCV	naMCI	5.81	3.8 (1.1-13)*	0.78 (0.59-0.97)	0.59 (0.49-0.69)	0.27 (0.15-0.39)	0.93 (0.87-1.00)
	aMCI	5.30	3.6 (1.8-7.0)‡	0.59 (0.47-0.71)	0.72 (0.64-0.80)	0.54 (0.42-0.65)	0.76 (0.68-0.84)

Results are presented with 95% confidence intervals. Subjects with a follow-up less than 1.5 years were excluded for these analyses. [a]Sample size: CSF: aMCI n=130, naMCI n=61; HCV: aMCI n=181, naMCI n=111; APOE: aMCI n=274, naMCI n=148. [b]Odds ratios are corrected for age, gender, and education. AD=Alzheimer's disease, aMCI=amnestic mild cognitive impairment, naMCI=non-amnestic mild cognitive impairment, PPV=positive predictive value, NPV=negative predictive value, CSF=cerebrospinal fluid, Aβ1-42=beta amyloid 1-42, HCV=hippocampal volume.*P<0.01, †p<0.01, ‡p<0.001.

CHAPTER 5

INJURY MARKERS PREDICT TIME TO DEMENTIA IN SUBJECTS WITH MCI AND AMYLOID PATHOLOGY

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Ineke A. van Rossum, Stephanie J.B. Vos, Leah Burns, Dirk L. Knol, Philip Scheltens, Hilkka Soininen, Lars-Olof Wahlund, Harald Hampel, Magda Tsolaki, Lennart Minthon, Gilbert L'Italien, Wiesje M. van der Flier, Charlotte E. Teunissen, Kaj Blennow, Frederik Barkhof, Daniel Rueckert, Robin Wolz, Frans Verhey, Pieter Jelle Visser

ABSTRACT

Objectives

Alzheimer's disease (AD) can now be diagnosed in subjects with mild cognitive impairment (MCI) using biomarkers. However, little is known about the rate of decline in those subjects. In this cohort study we aimed to assess the conversion rate to dementia and identify prognostic markers in subjects with MCI and evidence of amyloid pathology.

Methods

We pooled subjects from the VUmc Alzheimer Center and the DESCRIPA study. We included subjects with MCI, an abnormal level of Aß1-42 in the cerebrospinal fluid (CSF) and at least one diagnostic follow-up visit. We assessed the effect of APOE-genotype, CSF t-tau and p-tau and hippocampal volume on time to AD-type dementia using Cox proportional hazard models and on decline on the MMSE using linear mixed models.

Results

We included 110 MCI subjects with abnormal CSF Aß1-42 and a mean MMSE of 26.3±2.8. During a mean follow up of 2.2±1.0 (range 0.4-5.0) years, 63 subjects (57%) progressed to AD-type dementia. Abnormal CSF t-tau (hazard ratio (HR) 2.3, 95% CI 1.1-4.6, p=0.03) and CSF p-tau (HR 3.5, 1.3-9.2, p=0.01) concentration and hippocampal atrophy (HR 2.5, 1.1-5.6, p=0.02) predicted time to dementia. For subjects with both abnormal t-tau concentration and hippocampal atrophy HR was 7.3 (1.0-55.9, p=0.06). Furthermore, abnormal CSF t-tau and p-tau concentrations and hippocampal atrophy predicted decline in MMSE score.

Conclusions

In subjects with MCI and evidence of amyloid pathology, the injury markers CSF t-tau and p-tau and hippocampal atrophy can predict further cognitive decline.

INTRODUCTION

Recently, two sets of research criteria[1,2], were established allowing a diagnosis of Alzheimer's disease (AD) in subjects with mild cognitive impairment (MCI) and biomarker evidence of AD pathology. An international working group defined criteria for 'prodromal AD' in 2007[2] and in 2011 the National Institute of Aging and the Alzheimer Association published criteria for 'MCI due to AD'.[1] However, at this moment, prognosis of subjects fulfilling these criteria is largely unknown, which limits the use of the criteria in clinical practice. Prognostic markers for cognitive decline in subjects with MCI due to AD[1] or prodromal AD[2] are therefore urgently needed.

Subjects can be diagnosed with MCI due to AD[1] or prodromal AD[2] when they have a clinical diagnosis of MCI and biomarker evidence of either amyloid-beta pathology, AD-related neuronal injury or both. Abnormal amyloid markers may already be present at the earliest stage of the disease and reach a plateau in a very early stage of the disease and can therefore be useful as an early diagnostic marker.[3-5] Markers of the subsequent neuronal injury on the other hand, such as cerebrospinal fluid (CSF) tau and hippocampal atrophy on MRI, may reflect more advanced pathology and might be useful as prognostic markers.[3-5]

For the present study we selected subjects with MCI and evidence of amyloid pathology, defined by an abnormal level of amyloid-beta 1-42 (Aß1-42) in the CSF. We hypothesized that the injury markers tau (t-tau) and phopshorylated tau (p-tau)[6-8] in CSF and hippocampal atrophy on MRI[9,10] would be associated with progression to AD-type dementia and cognitive decline.

METHODS

Subjects

We selected subjects from the DESCRIPA cohort and the memory clinic of the Alzheimer Center of the VU University medical center (VUmc). DESCRIPA is a European multicenter study performed in a memory clinic setting.[11] The VUmc was one of the DESCRIPA partners and contributed an additional sample of subjects that were seen outside the DESCRIPA inclusion period. Inclusion criteria were a clinical diagnosis of MCI, an abnormal level of CSF Aß1-42, based on a clinically validated cut-off (≤ 550 pg/ml),[12] and at least one follow up diagnosis. Subjects with obvious causes for MCI other than AD, such as alcohol abuse or severe depression, were excluded. In 10 of the participating centers CSF was collected. Of the subjects enrolled at these centers between 2003 and 2005, 64 subjects fulfilled inclusion criteria. From the VUmc 46 additional subjects were included.

Standard protocol approvals, registrations and patient consents

The medical ethics committee at each center approved the study. All patients provided written informed consent.

Clinical assessment

Diagnosis of MCI was made according to the criteria of Petersen.[13] Raw scores on neuropsychological tests were corrected for age, gender and educational level in accordance with locally collected or published normative data and expressed as z-scores. MCI was defined as a z-score below -1.5 SD on any of the following tests: the learning measure or delayed recall of a verbal memory task, trail making test (TMT) part A, TMT part B, verbal fluency or Rey figure copy or equivalent test, as described in more detail previously.[11,14] Follow-up assessment was performed annually up to 5 years. For subjects from the Alzheimer center of the VUmc, follow up was part of regular patient care. Diagnosis of AD-type dementia was made according to the DSM-IV[15] and NINCDS-ADRDA criteria.[16] Time to dementia was defined as the time between baseline visit and the date AD-type dementia was diagnosed.

CSF analyses

CSF was collected by lumbar puncture, centrifuged, and stored at -80°C in polypropylene tubes. One sample was thawed twice but analyses without this sample revealed similar results. CSF Aβ1-42, t-tau and tau phosphorylated at threonine 181 (p-tau) were measured with Innotest sandwich ELISA (Innogenetics, Ghent, Belgium) in Gothenburg for the DESCRIPA cohort and in Amsterdam for the VUmc cohort. We corrected for interlaboratory ELISA differences by means of 33 samples that were analysed at both labs and adjusted VUmc values to those of DESCRIPA using the equating formula: Gothenborg=(SD Gothenborg/SD VUmc)*VUmc+average Gothenborg–((SD Gothenborg/SD VUmc)*average VUmc).[17]

MRI analyses

For the DESCRIPA cohort, subjects were scanned according to the routine MRI protocol at each site. Scanning was performed at 1.0 or 1.5 T and included a three-dimensional T1 weighted gradient echo sequence with near-isotropic voxels and a fast fluid attenuated inversion recovery (FLAIR) sequence.[14,18]

Hippocampal volume (HCV) was measured at the Department of Computing of Imperial College London, using LEAP, a segmentation technique based on atlas registration.[19] We tested whether the MRI field strength influenced the LEAP scores in 348 subjects with MCI from the Descripa cohort. Field strength did not affect the LEAP score (difference of 0.07%, p-value=0.8 after correction for age, gender, educational level, baseline MMSE score and follow up diagnosis) and therefore we used data from both field strengths without correction.

MRI data were available in 35 of the 64 subjects (55%) from the DESCRIPA cohort and in 30 of the 46 subjects (65%) from VUmc. Subjects with and without MRI data available did not differ with respect to age, gender, educational level, APOE status, CSF markers or score on the Mini-Mental State Examination (MMSE)[20] at baseline.

APOE genotyping

DNA was isolated from 10 ml EDTA blood for apolipoprotein E (APOE) genotyping, using the light cycler APOE mutation detection kit (Roche Diagnostics GmbH, Mannheim, Germany). APOE genotype was determined in 99 subjects (90%). Subjects in whom no APOE status was determined scored higher on the MMSE at baseline (27.7 vs 26.1, p=0.005). There were no differences with respect to age, gender, educational level, medial temporal lobe atrophy or CSF markers between subjects with and without APOE data available. Subjects were classified as APOE-ε4 positive when having one or two APOE-ε4 alleles.

Statistical analyses

Analyses were performed with SPSS 18.0 for the Macintosh.
For group comparisons of subjects with and without AD-type dementia at follow up we used chi-squared tests for categorical variables and Student's t-tests for continuous variables. Data of the CSF markers were log transformed in order to obtain an approximately normal distribution. For further analyses we used dichotomized values of the respective markers. We used clinically validated cut-off points for CSF t-tau (>375 pg/ml) and p-tau (>52 pg/ml).[12] For hippocampal volume we used a summed volume of the left and right hippocampus of 5.39 cm^3 as cut-off point. This cut-off point could best differentiate between healthy controls and subjects with AD-type dementia in the ADNI cohort (Vos et al., submitted), based on the Youden index using R.[21,22] This cut-off point was similar to the cut-off point of 5.34 cm^3 that could best predict AD-type dementia in our own dataset.[23]

We assessed the effect of APOE genotype, CSF levels of t-tau and p-tau, hippocampal atrophy on time to dementia using Cox proportional hazards with correction for age, gender, education and MMSE score at baseline. Analyses were performed for each variable alone and with all variables together using a stepforward model to select the variables that could best predict AD-type dementia.

We also assessed the association of CSF t-tau and p-tau and hippocampal volume with decline in MMSE score. We performed mixed models analyses with an unstructured co-variance structure with correction for age, gender, educational level and center.[24]

RESULTS

Baseline characteristics

We included 110 subjects with MCI and abnormal CSF Aß1-42. Subjects were on average±standard deviation (SD) 70.8±7.7 years old, 46% was female and 62% had at least 1 APOE-ε4 allele. Mean MMSE score was 26.3±2.8. Baseline characteristics of the subjects are shown in Table 1. Two subjects progressed to other types of dementia (one subject with vascular dementia and one subject with Parkinsons disease dementia). They were included in the group of subjects that did not progress to AD-type dementia. Excluding those two subjects from the analyses did not change the results (data not shown).

Table 1. Baseline characteristics according to diagnosis at follow up

	All subjects	No AD-type dementia at follow up	AD-type dementia at follow up
N	110	47	63
Age	70.8±7.7	70.1±8.1	71.3±7.4
Female, n (%)	51 (46)	20 (43)	31 (49)
Education, years	10.8 ±3.5	10.5±3.5	11.1±3.4
Follow up, years	2.2±1.0	2.3±1.1	2.0±0.9
APOE-ε4 positive, n (%)$	61 (62)	23 (54)	38 (68)
Aβ1-42, pg/ml	382±98	369±100	392±97
T-tau, pg/ml	564±345	421±252	670±368#
T-tau, abnormal*, n (%)	81 (74)	28 (60)	53 (84)#
P-tau, pg/ml	89±49	71±35	103±54#
P-tau, abnormal*, n (%)	90 (82)	32 (68)	58 (92)#
Hippocampal volume, cm^3†	5.4±0.7	5.8±0.8	5.2±0.6#
Hippocampal atrophy&, n (%)	35 (54)	8 (31)	27 (69)#
MMSE score	26.3±2.8	26.8±2.6	25.9±2.8
Verbal memory, learning (z-score)	-1.5±1.0	-1.4±1.1	-1.6±0.9
Verbal memory, delayed recall (z-score)	-1.6±1.0	-1.3±1.1	-1.9±0.8#
Verbal fluency (z-score)	-0.8±1.1	-0.7±1.3	-1.0±0.9
TMT part A (z-score)	-0.8±1.8	-0.7±1.6	-0.9±2.0
TMT part B (z-score)	-1.1±1.6	-1.0±1.6	-1.2±1.6
Visuoconstruction (z-score)	0.2±1.1	0.03±1.2	0.3±1.0

Data are mean±standard deviation unless otherwise indicated. $APOE genotype was determined in 99 subjects, †hippocampal volume was determined in 65 subjects, *abnormal values were defined as >375 pg/ml for CSF t-tau and >52 pg/ml for CSF p-tau, &hippocampal atrophy was defined as a summed volume of left and right hippocampus of <5.39 cm^3. #p<0.005 compared to no dementia at follow up, ‡p < 0.05 compared to no dementia at follow up. APOE=apolipoprotein E, CSF=cerebrospinal fluid, Aβ1-42=beta amyloid1-42, t-tau=total tau, p-tau=tau phosphorylated at threonine 181, MMSE=Mini-Mental State Examination.

Predictors of progression to AD-type dementia

During a mean follow up of 2.2±1.0 years (median 2.0 years, range 0.4-5.0 years) 63 subjects (57%) progressed to AD-type dementia. These subjects had higher levels of CSF t-tau (mean±SD 670±368 vs 421±252 pg/ml, p<0.001) and p-tau (103±54 vs 71±35 pg/ml, p<0.001), a smaller hippocampal volume (5.2±0.6 cm^3 vs 5.8±0.8 cm^3, p=0.002) and a lower score on the delayed recall of a verbal memory task (z-score -1.9±0.8 vs -1.3±1.1, p=0.004) than subjects that did not progress to AD-type dementia (Table 1).

Predictors of time to AD-type dementia

Survival analyses using Cox proportional hazards model with correction for age, gender and education showed that time to dementia was predicted by abnormal CSF t-tau (hazard ratio

(HR) 2.3, 95% CI 1.1-4.6, p=0.03), abnormal CSF p-tau (HR 3.5, 1.3-9.2, p=0.01) and hippocampal atrophy (HR 2.5, 1.1-5.6, p=0.02, Figure 1, Table e-1). After correction for baseline MMSE score results remained essentially the same, with an HR of 2.0 (1.0-4.2, p=0.06) for CSF t-tau, 3.1 (1.2-8.4, p=0.03) for CSF p-tau and 2.2 (1.0-5.0, p=0.06) for hippocampal atrophy. Of the neuropsychological measures only delayed recall predicted AD-type dementia (HR 2.1 (1.0-4.3), p=0.05, Table e-1). The APOE-ε4 genotype, age, gender and education did not predict time to dementia (Table e-1). Cox multivariate analyses with forward-step selection and biomarkers entered as log-transformed continuous variables selected only CSF p-tau (β 1.2, HR 3.3 (1.4-7.5) p=0.005). In the multivariate analysis we did not find a significant interaction between CSF p-tau or t-tau with hippocampal atrophy (p=0.8).

MMSE slope analyses

Subjects with abnormal CSF t-tau more rapidly declined on the MMSE, with an annual decline of -1.1, compared to -0.4 for subjects with normal CSF t-tau (Table 2). At baseline there were no differences in MMSE score between subjects with normal and abnormal CSF t-tau (26.5 and 26.3 respectively). For CSF p-tau results were similar (Table 2). Subjects with hippocampal atrophy showed more rapid decline in MMSE score compared to subjects without hippocampal atrophy (average annual decline -1.2 vs -0.5, p=0.09) (Table 2). At baseline, subjects with hippocampal atrophy had lower MMSE score than subjects without hippocampal atrophy (25.6 vs 27.0, p=0.02).

Biomarker subgroup analyses

In order to investigate the effect of the combination of abnormal CSF t-tau and hippocampal atrophy on progression to AD-type dementia and cognitive decline, we subdivided subjects with both CSF and MRI available (N=65) into three groups, depending on their biomarker status at baseline (Figure e-1): 1. normal CSF t-tau and no hippocampal atrophy (N=9, of whom 1 progressed to AD-type dementia), 2. either abnormal CSF t-tau or hippocampal atrophy (N=28, of whom 16 progressed to AD-type dementia), 3. both abnormal CSF t-tau and hippocampal atrophy (N=28, of whom 22 progressed to AD-type dementia). Compared to subjects with normal CSF t-tau and no hippocampal atrophy, subjects with either abnormal CSF t-tau or hippocampal atrophy had an HR (95% CI) of 5.2 (0.7-40.3, p=0.1) for progression to AD-type dementia. For subjects with both abnormal CSF t-tau and hippocampal atrophy the HR was 7.3 (1.0-55.9, p=0.06) (Table 3).

Table 2. Predictors for decline in MMSE score

		N	Baseline MMSE	p-value±	Slope	p-value±
CSF t-tau	≥ 375 pg/ml	81	26.2 (25.1-27.4)	0.6	-1.1 (-1.4- -0.8)	0.02
	< 375 pg/ml	29	26.5 (25.3-27.9)		-0.4 (-0.9- 0.2)	
CSF p-tau	≥ 52 pg/ml	90	26.2 (25.1-27.3)	0.4	-1.1 (-1.3- -0.8)	0.005
	< 52 pg/ml	20	26.7 (25.3-28.2)		-0.04 (-0.7- 0.6)	
Hippocampal	< 5.39 cm^3	35	25.6 (23.8-27.5)	0.02	-1.2 (-1.5- -0.8)	0.01
volume	≥ 5.39 cm^3	30	27.0 (25.2-28.8)		-0.5 (-0.9- -0.1)	

Baseline MMSE-scores and slope values of annual change in MMSE-score estimated using mixed models with correction for age, gender, educational level and center. Values are estimated assuming subjects are 50% female, 70 years of age and with 11 years of education. Data are mean (95% confidence interval). ±p-value of the difference between subjects with normal and abnormal values for each biomarker. CSF=cerebrospinal fluid, t-tau=total tau, p-tau=tau phosphorylated at threonine 181, MMSE=Mini-Mental State Examination.

Table 3. Progression to AD-type dementia and rate of cognitive decline with respect to biomarker status at baseline

CSF t-tau and hippocampal volume*	N	Dementia free survival after 4 years	HR dementia	Baseline MMSE$	Slope
Both normal	9	0.73±0.06	reference	27.4 (25.2-29.6)	-0.1 (-0.9 - 0.7)
One abnormal	28	0.19±0.08	5.2 (0.7-40.3)	26.6 (24.7-28.5)	-0.8 (-1.2 - -0.4)#
Both abnormal	28	0.09±0.03	7.3 (1.0-55.9)	25.5 (23.6-27.4)	-1.1 (-1.5- -0.7)±

Dementia free survival and the hazard ratio were calculated using Cox regression analyses with correction for age, gender and educational level. Baseline MMSE-scores and slope values of annual change in MMSE-score estimated using mixed models with correction for age, gender, educational level and center. Values are estimated assuming subjects are 50% female, 71 years of age and with 11 years of education. Data are mean (95% confidence interval) or mean±SE. *Abnormal CSF t-tau was defined as a value >375 pg/ml, Hippocampal atrophy was defined as a volume of both left and right hippocampus of < 5.39 cm^3. $ Differences in baseline MMSE between the groups were not statistically significant. #p-value compared to both markers normal=0.1, ± p-value compared to both markers normal=0.02. CSF=cerebrospinal fluid, t-tau=total tau, HR=hazard ratio, MMSE=Mini-Mental State Examination, SE=standard error.

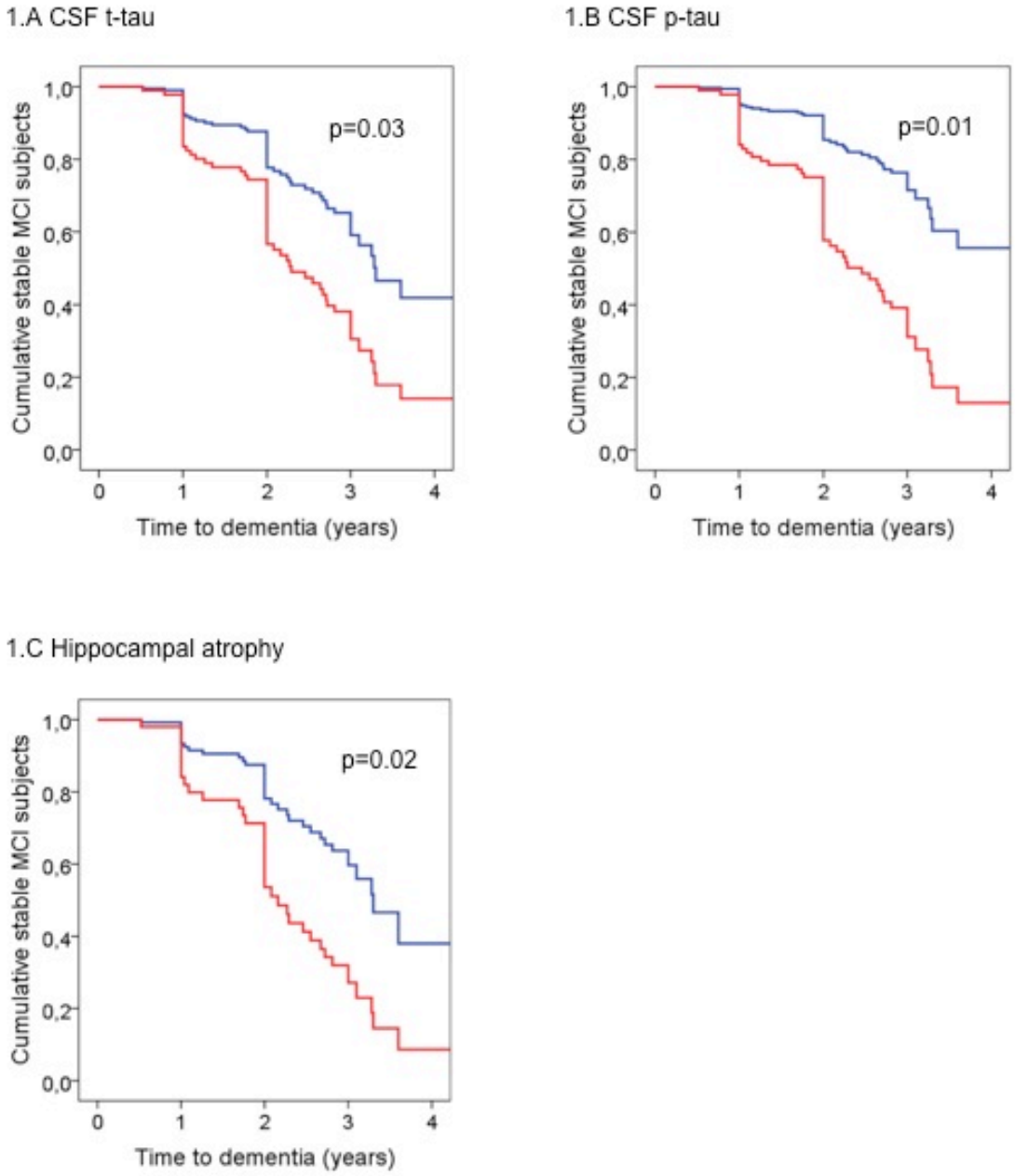
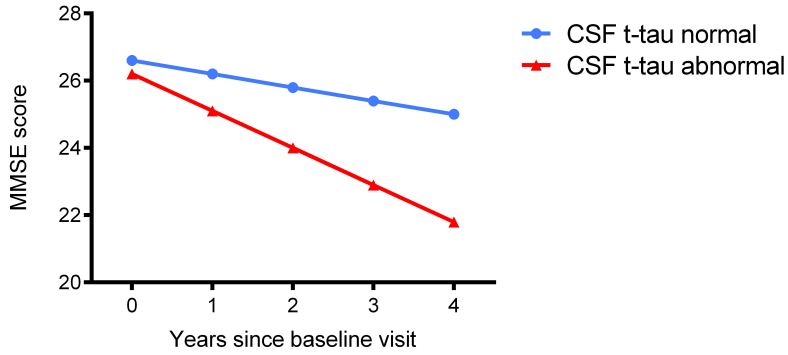


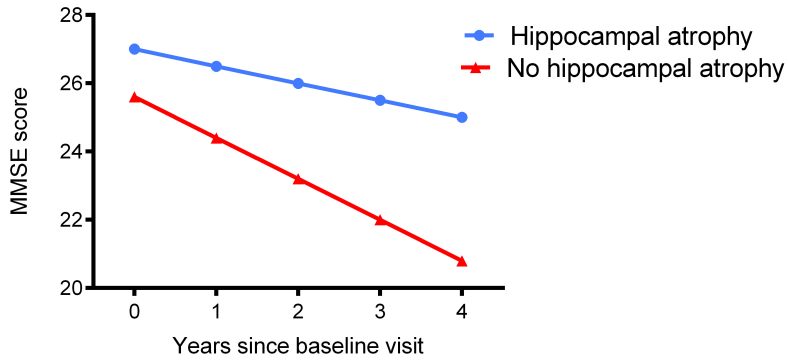
Figure 1. Survival curves for time to dementia in subjects with MCI and abnormal CSF Aß1-42, corrected for age, gender and education

Red lines indicate the subjects with an abnormal value of each respective marker, defined as CSF t-tau >375 pg/ml (A), CSF p-tau >52 pg/ml (B) and hippocampal volume < 5.39 cm^3 (C). Blue lines indicate the subjects with normal values of each marker. MCI=mild cognitive impairment, CSF=cerebrospinal fluid, Aβ1-42=beta amyloid1-42, t-tau=total tau, p-tau=tau phosphorylated at threonine 181.

A. CSF t-tau



B. Hippocampal volume



C. Combination of CSF t-tau and hippocampal volume

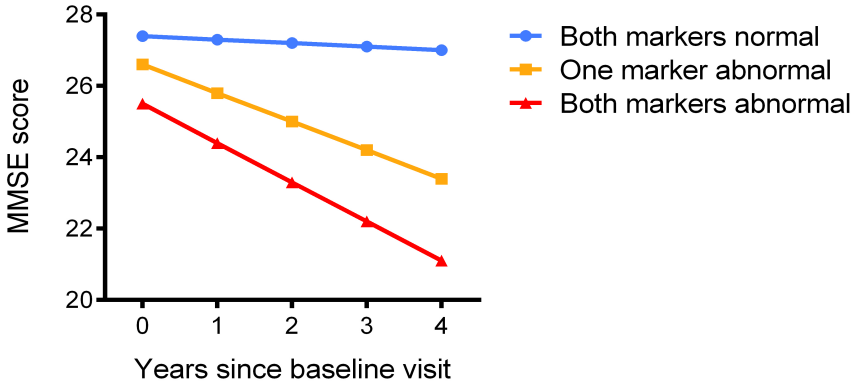


Figure 2. Decline in MMSE score in subjects with MCI and abnormal CSF Aß1-42 according to CSF t-tau and hippocampal volume

Slopes of decline in MMSE score in subjects with MCI and abnormal CSF Aß1-42. Subjects were classified according to their CSF t-tau levels and hippocampal volume at baseline. Abnormal values were defined as CSF tau >375 pg/ml and hippocampal volume < 5.39 cm^3. Figure A+B: the blue (dot) line indicates the subjects with normal values. The red (triangle) line indicates subjects with abnormal values. Figure C: the blue (dot) line indicates the subjects with normal values of both. The red (triangle) line indicates subjects with both markers abnormal. The orange (square) line indicates subjects with either abnormal CSF t-tau or hippocampal atrophy. CSF=cerebrospinal fluid, t-tau=total tau, MMSE=Mini-Mental State Examination.

The annual decline in MMSE score was -0.1 (p-value slope=0.8) for subjects with normal CSF t-tau and no hippocampal atrophy, -0.8 (p=0.001) for subjects with either abnormal CSF t-tau or hippocampal atrophy and -1.1 (p<0.001) for subjects with both abnormal CSF t-tau and hippocampal atrophy (Table 3, Figure 2). The slopes of decline of subjects with one or two abnormal markers differed from the slope of subjects with both markers normal, but not from each other. For subjects with only abnormal CSF t-tau (N=21) the annual decline in MMSE score was -0.6 (-1.0- -0.2, p=0.006). For subjects with only hippocampal atrophy, no slope analyses could be performed, due to small sample size (N=7).

DISCUSSION

In this prospective study of subjects who fulfilled the criteria for 'MCI due to AD'[1] and 'prodromal AD'[2] based on abnormal CSF Aß1-42, we found that during a mean follow up of 2.2 years 63 subjects (57%) progressed to AD-type dementia. High CSF levels of t-tau and p-tau and hippocampal atrophy predicted progression to dementia and decline in MMSE score.

The overall annual conversion rate to dementia of around 20% in this study was higher than the conversion rate typically observed in subjects with MCI unselected for biomarkers status.[25] For comparison, subjects with MCI and a normal concentration of CSF Aß1-42 in our dataset had an annual conversion rate of less than 10% (data not shown). Still, a considerable percentage of our subjects did not develop AD-type dementia within the follow up period. Since abnormal beta amyloid is suggested to be an early marker for AD[3], higher progression rates to AD-type dementia might be expected with a longer follow up period.

The rapid decline to dementia in subjects with high CSF levels of t-tau and p-tau and hippocampal atrophy could mean that these subjects either suffered from a more aggressive course of the disease or were already in a more advanced stage when assessed at baseline. Slope analyses suggested that they suffered from a more aggressive course of the disease since they showed a more rapid decline in MMSE score compared to subjects with normal values of these markers at baseline. This is in line with previous studies that found a more rapid cognitive decline in subjects with AD-type dementia with high levels of CSF tau.[26,27] Subjects with hippocampal atrophy may have also been already in a more advanced stage of the disease at baseline as they had lower MMSE scores at baseline compared to subjects without hippocampal atrophy. This is consistent with the previously suggested order of events in the amyloid cascade,[3,4] with hippocampal atrophy being a relatively late feature of AD pathology. In a previous study in subjects with MCI and biomarker evidence of beta amyloid pathology, hippocampal atrophy also predicted time to dementia.[28] In another study in subjects with MCI who all progressed to AD-type dementia, CSF t-tau, CSF p-tau and hippocampal atrophy were also associated with rapid progression from MCI to AD-type dementia, while CSF Aß1-42 was not.[5] Our finding that the predictive value of the respective CSF and MRI markers for progression to AD-type dementia remained after correction for

baseline MMSE score indicates that AD-biomarkers can have prognostic value in addition to clinical measures alone.

The predictive accuracy of CSF t-tau and p-tau and hippocampal atrophy we observed in our MCI subjects with abnormal CSF Aß1-42 was lower than that reported in studies conducted in subjects with MCI regardless of amyloid biomarker status[7-9,23] Most likely this is because in our analyses only the additional predictive effect relative to abnormal amyloid was tested, although differences could partly also be due to differences in setting and other study characteristics.

We found no differences in age, gender and APOE status between subjects with and without dementia at follow up, although age, gender and APOE genotype are known risk factors for AD in the general population. A possible explanation for this finding could be that advanced age and APOE-ε4 genotype are risk factors for development of abnormal beta amyloid processing, but do not influence clinical progression once abnormal beta amyloid processing is established.

We included subjects with MCI and abnormal amyloid. According to the criteria of the National Institute of Aging and the Alzheimer Association,[1] these subjects would meet criteria of 'MCI due to AD-intermediate likelihood'. Of the 65 subjects with both CSF and MRI available 9 subjects (14%) had both normal CSF t-tau and normal hippocampal volume and met criteria of 'MCI, biomarker evidence uninformative'. The course of the disease in these subjects was relatively benign with a 27% conversion rate to AD-type dementia after 4 years, although the interpretation is limited by the small sample size. 28 subjects (43%) had both abnormal CSF t-tau and hippocampal atrophy and fulfilled criteria for 'MCI due to AD, high likelihood'[1]. Their prognosis was poor, with 91% progressing to AD-type dementia after 4 years. In 28 subjects (43%), the injury markers were conflicting, with either CSF t-tau abnormal or hippocampal volume abnormal. According to the NIA-AA criteria it is not clear whether these subjects should be diagnosed as 'MCI, biomarker evidence uninformative' or 'MCI due to AD high likelihood'.[1] Our data suggest that these subjects should be considered as 'MCI due to AD-high likelihood' since the decline in MMSE score and progression rate to AD-type dementia (81%) was similar to that of subjects with both markers abnormal while the rate of decline on the MMSE was worse than that of subjects with both markers normal, although group comparisons are hampered by the small sample size.

Two subjects included in the study progressed to other types of dementia, despite abnormal CSF Aβ1-42 levels ate baseline. One subject, aged 75, had extrapyramidal signs at baseline and was later diagnosed with Parkinson's disease dementia. CSF Aß1-42 was 326 pg/ml, CSF t-tau and CSF p-tau were normal and hippocampal volume was not available. Decreased CSF Aß1-42 has been described before in subjects with alphasynucleiopathies.[29] This highlights the importance of ruling out causes for the cognitive symptoms other than AD before the criteria for MCI due to AD can be applied.[1] The other subject, aged 61, was diagnosed with vascular dementia at follow up. She had a CSF Aß1-42 concentration of 357 pg/ml, and abnormal CSF t-tau and p-tau concentrations. On the MRI scan she had multiple

vascular white matter lesions and parietal atrophy, in the absence of hippocampal atrophy. In retrospect this subject may have suffered from mixed dementia with both vascular and Alzheimer's pathology.

A major limitation of our study is that we did not have MRI data available of all subjects, which limited possibilities for multivariate analyses. Another limitation is the limited follow up. Studies with longer clinical follow up are needed to assess whether all subjects with MCI due to AD will indeed develop dementia eventually.

Our results implicate that markers of AD-related neuronal injury as CSF level of t-tau, p-tau and hippocampal atrophy, could help to identify those subjects with MCI due to AD that will more rapidly progress to dementia. Subjects with both abnormal CSF Aβ1-42 and abnormal injury markers, thereby fulfilling criteria for 'MCI due to AD-high likelihood', showed most rapid cognitive decline and a high progression rate to AD-type dementia, even within our limited follow up period.

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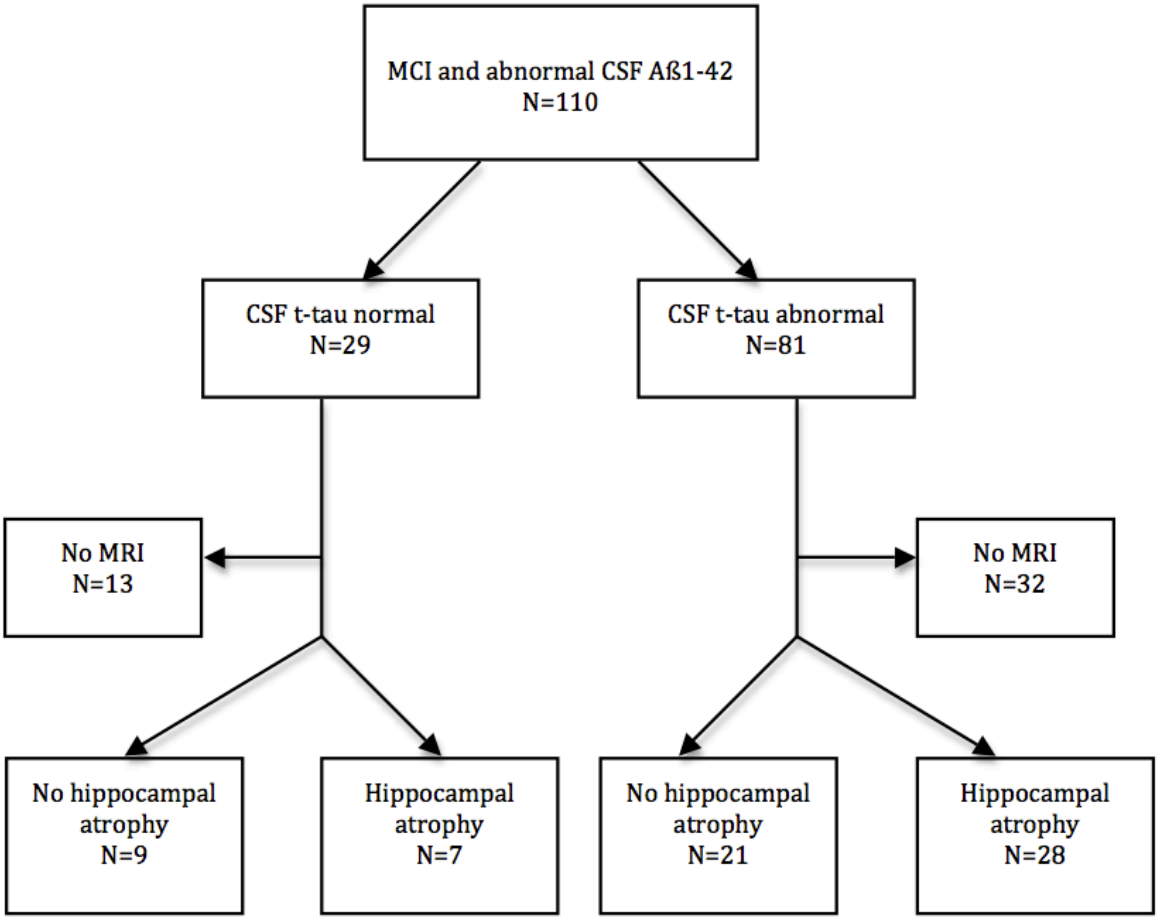
SUPPLEMENTAL DATA

Supplemental Table e-1. Predictors for AD-type dementia

	Hazard ratio uncorrected	p-value	Hazard ratio corrected*	p-value
CSF t-tau	2.3 (1.2-4.6)	0.02	2.3 (1.1-4.6)	0.03
CSF p-tau	3.4 (1.4-8.8)	0.009	3.5 (1.3-9.2)	0.01
Hippocampal atrophy	1.7 (0.9-3.4)	0.1	2.5 (1.1-5.6)	0.02
MMSE score	1.8 (1.1-3.0)	0.02	2.3 (1.3-4.0)	0.003
APOE genotype	1.1 (0.6-1.9)	0.7	1.0 (0.6-1.8)	0.9
Memory, learning	1.1 (0.6-2.1)	0.8	1.1 (0.6-2.2)	0.7
Memory, delayed recall	2.2 (1.1-4.3)	0.03	2.1 (1.0-4.3)	0.05
Verbal fluency	1.7 (1.0-2.9)	0.06	1.5 (0.8-2.9)	0.2
TMT part A	1.1 (0.6-2.0)	0.7	1.1 (0.6-2.0)	0.8
TMT part B	1.4 (0.7-2.5)	0.3	1.5 (0.8-2.9)	0.3
Visuoconstruction	0.9 (0.3-2.5)	0.8	1.0 (0.4-3.0)	0.9

*Analyses corrected for age, gender and educational level. All predictor variable were dichotomized scores. Hazard ratio's are provided with 95% confidence intervals. Abnormal values were defined as: CSF t-tau >375 pg/ml, CSF p-tau >52 pg/ml, hippocampal volume volume of < 5.39 cm^3 (sum left and right), MMSE score ≤ 26 at baseline or at least one APOE- ε4 allele and a z-score of ≤ -1.5 standard deviation on the neuropsychological tests. Abbreviations: CSF=cerebrospinal fluid, Aβ1-42=beta amyloid1-42, t-tau=total tau, p-tau=tau phosphorylated at threonine 181, MMSE=Mini-Mental State Examination, APOE=apolipoprotein E, TMT=trail making test.

Supplemental Figure e-1. Selection of included subjects



Flowchart of subjects included in the analyses depending on their biomarker status at baseline.
CSF=cerebrospinal fluid, t-tau=total tau, MMSE=Mini-Mental State Examination.

CHAPTER 6

VARIABILITY OF CSF ALZHEIMER'S DISEASE BIOMARKERS: IMPLICATIONS FOR CLINICAL PRACTICE

Stephanie J.B. Vos, Pieter Jelle Visser, Frans Verhey, Pauline Aalten, Inez Ramakers, Philip Scheltens, Marcel G.M. Olde Rikkert, Marcel M. Verbeek, Charlotte E. Teunissen

ABSTRACT

Background

Cerebrospinal fluid (CSF) biomarkers are increasingly being used for diagnosis of Alzheimer's disease (AD).

Objective

We investigated the influence of CSF intralaboratory and interlaboratory variability on diagnostic CSF-based AD classification of subjects and identified causes of variation.

Methods

We measured CSF amyloid-β (Aβ)1-42, total tau (t-tau), and phosphorylated tau (p-tau) by INNOTEST enzyme-linked-immunosorbent assays (ELISA) in a memory clinic population (n=126). Samples were measured twice in a single or two laboratories that served as reference labs for CSF analyses in the Netherlands. Predefined cut-offs were used to classify CSF biomarkers as normal or abnormal/AD pattern.

Results

CSF intralaboratory variability was higher for Aβ1-42 than for t-tau and p-tau. Reanalysis led to a change in biomarker classification (normal vs. abnormal) of 26% of the subjects based on Aβ1-42, 10% based on t-tau, and 29% based on p-tau. The changes in absolute biomarker concentrations were paralleled by a similar change in levels of internal control samples between different assay lots. CSF interlaboratory variability was higher for p-tau than for Aβ1-42 and t-tau, and reanalysis led to a change in biomarker classification of 12% of the subjects based on Aβ1-42, 1% based on t-tau, and 22% based on p-tau.

Conclusions

Intralaboratory and interlaboratory CSF variability frequently led to change in diagnostic CSF-based AD classification for Aβ1-42 and p-tau. Lot-to-lot variation was a major cause of intralaboratory variability. This will have implications for the use of these biomarkers in clinical practice.

INTRODUCTION

Amyloid-β (Aβ)1-42, total tau (t-tau), and phosphorylated tau (p-tau) proteins in cerebrospinal fluid (CSF) are well-established biomarkers for Alzheimer's disease (AD) [1-3] and are increasingly being used for diagnosis in clinical practice. Previous studies reported considerable intra- or interlaboratory variability of CSF analyses,[4-7] which may influence the diagnostic classification. In this study, we performed a large-scale CSF multicenter study and investigated the exact influence of intra- and interlaboratory variability on CSF-based AD classification of subjects.

We hypothesized that the change of diagnosis would be largest for classification of subjects based on CSF Aβ1-42, as previous studies showed larger variability for CSF Aβ1-42 than for t-tau and p-tau.[5-7] It was also hypothesized that change of diagnosis would be lower for analyses performed in the same laboratory than for analyses performed in different laboratories because CSF intralaboratory variability has been reported to be lower than interlaboratory variability (2.3-25% vs. 13-38%).[5-7]

We investigated intralaboratory and interlaboratory variability of CSF Aβ1-42, t-tau, and p-tau analyses by INNOTEST enzyme-linked-immunosorbent assays (ELISA). Samples were measured twice in one or two laboratories that served as reference labs for AD CSF analyses in the Netherlands. We classified subjects based on validated cut-offs and examined how often CSF-based AD diagnosis changed after the second analysis.

MATERIALS AND METHODS

CSF samples

CSF samples were collected from subjects included in the Leiden Alzheimer Research Netherlands (LeARN) study.[8] LeARN is a Dutch multicenter study performed in a memory-clinic setting that included subjects between October 2009 and May 2011 that had been newly referred for the assessment of cognitive complaints. Inclusion criteria were baseline diagnosis of subjective cognitive impairment (SCI), mild cognitive impairment (MCI) or dementia, Mini-Mental State Examination (MMSE) ≥20, clinical dementia rating scale (CDR) of maximal 1. Exclusion criteria were somatic, psychiatric or neurological disorders that could have caused the cognitive impairment.

In 3 of the 4 participating centers (Amsterdam, Maastricht, and Nijmegen), 126 CSF samples were analyzed in twofold, the first time as part of clinical routine and secondly for the LeARN study (Supplemental Figure 1). For routine practice, samples were analyzed in Amsterdam and Nijmegen, which serve as national CSF AD biomarker centers. For the LeARN study, all samples were analyzed in a single batch in Amsterdam. The samples collected in Amsterdam (n=50) were measured twice in the same lab and were used to assess intralaboratory variability. Samples collected in Nijmegen (n=32) and Maastricht (n=44) were measured twice in different laboratories, i.e. in Nijmegen for clinical routine and in Amsterdam for the LeARN study, and were used to study interlaboratory variability

(total n=76). Supplemental Table 1 provides baseline patient demographics. The medical ethics committee at each center approved the study. All subjects provided informed consent.

CSF procedures and analyses

CSF was obtained by lumbar puncture between the L3/L4 or L4/L5 intervertebral space, and collected and aliquoted into polypropylene tubes. Samples for clinical routine of Maastricht were transported the same day on room temperature or stored at -20ºC and transported on dry ice within one week to the Nijmegen laboratory for analysis. Samples for clinical routine analysis were stored at -20ºC for up to 4 weeks (Amsterdam cohort) or at -80ºC for up to 2 weeks (Maastricht/Nijmegen cohort) before analysis. Research samples (i.e. samples of the LeARN study) were stored at -80ºC[9] at each center and samples of Maastricht and Nijmegen were transported on dry ice to Amsterdam for analysis after up to 2.5 years. Both laboratories used the commercially available INNOTEST enzyme-linked immunosorbent assays (ELISAs; Innogenetics, Ghent, Belgium) to quantify CSF Aβ1-42, CSF t-tau, and CSF p-tau, all performed by experienced laboratory technicians. For analysis of the research samples, the same lot number was used for all analyses, while for clinical routine analyses different lots were used in Nijmegen as well as in Amsterdam. Due to insufficient fluid material, CSF p-tau values were only available for 49 samples for intralaboratory analyses.

We also analyzed internal control samples from the Amsterdam lab to investigate the influence of lot-to-lot variation on measured CSF concentrations. One control sample had an AD typical profile and the other a normal CSF profile. The internal controls were aliquots for single use obtained by pooling surplus CSF. These aliquots were stored at -80ºC and all internal controls used in the current study were from the same batch of pools.

To study differences in biomarker classification as normal versus abnormal between CSF measurements, we dichotomized the CSF variables according to routinely used validated cut-offs of each lab. In Amsterdam, cut-offs were determined that could differentiate subjects with SCI from subjects with AD-type dementia with 85% sensitivity: CSF Aβ1-42 ≤550 pg/ml, t-tau >375 pg/ml, and p-tau >52 pg/ml.[10] In Nijmegen, cut-offs were determined that could differentiate cognitively normal controls from subjects with AD-type dementia with a specificity of 95%: CSF Aβ1-42 <500 pg/ml, t-tau >350 pg/ml, and p-tau >85 pg/ml.[11] Given that a different approach was used to define cut-offs in Amsterdam and Nijmegen, we performed interlaboratory analyses with the same cut-offs (Amsterdam cut-offs) as well as with lab-specific cut-offs (Amsterdam and Nijmegen cut-offs).

Statistical analyses

Statistical analyses were done with SPSS version 19.0 (Chicago, IL, USA) and GraphPad Prism 5, with significance set at $p<0.05$. Intralaboratory and interlaboratory coefficients of variation (CV) were calculated as the standard deviation (SD) divided by the mean of the measurements of each sample for each biomarker. Subsequently, a mean CV was calculated.

We performed paired t-tests to investigate the intralaboratory and interlaboratory variability between CSF analyses of CSF Aβ1-42, t-tau, and p-tau. In addition, we calculated Pearson correlations *r* and Intraclass Correlation Coefficients (ICC). An ICC score ranges from 0 to 1, representing virtually no (0.00-0.10), a slight (0.11-0.40), fair (0.41-0.60), moderate (0.61-0.80), or substantial (0.81-1.00) level of agreement between the analyses.[12] Confidence intervals were calculated for both the Pearson correlations and ICC scores. We made Bland-Altman plots to visualize the agreement between the CSF analyses[13] and calculated the percentage of subjects with change of AD marker classification after the second analysis using routine CSF cut-offs of each laboratory.

RESULTS

Intralaboratory variability

CSF Aβ1-42 and t-tau levels were higher and p-tau levels were lower after reanalysis in the same laboratory ($p<0.05$ for all analysis, Figure 1A, Supplemental Figure 2A). The mean intralaboratory CV was 14.4% for Aβ1-42, 8.5% for t-tau, and 12.6% for p-tau. For CSF Aβ1-42, the correlation (0.85) and ICC (0.76) were moderate and lower than that of CSF t-tau (r=0.98 and ICC=0.97) and p-tau (r =0.95 and ICC=0.90; Table 1, Figure 2A). Internal quality control samples that were analyzed at each measurement also showed higher Aβ1-42 levels, slightly higher t-tau levels, and lower p-tau levels in the second measurement compared to the first measurement (Figure 3).

Interlaboratory variability

CSF Aβ1-42 levels were lower, t-tau levels higher, and p-tau levels lower after reanalysis in the second laboratory ($p<0.05$ for all analysis, Figure 1B, Supplemental Figure 2B). The interlaboratory CV was 7.3% for Aβ1-42, 6.7% for t-tau, and 27.6% for p-tau. For CSF p-tau, the correlation (0.94) and the ICC (0.73) were high to moderate and lower than that of CSF Aβ1-42 (r=0.94 and ICC=0.92) and t-tau (r=0.98 and ICC=0.98; Table 1, Figure 2B).

Table 1. Agreement between CSF analyses

	Intralaboratory analyses		Interlaboratory analyses	
	Correlation*	ICC*	Correlation*	ICC*
CSF Aβ1-42	0.85 (0.74-0.91)	0.76 (0.43-0.89)	0.94 (0.91-0.96)	0.92 (0.85-0.96)
CSF t-tau	0.98 (0.97-0.99)	0.97 (0.92-0.99)	0.98 (0.96-0.99)	0.98 (0.96-0.99)
CSF p-tau	0.95 (0.92-0.97)	0.90 (0.53-0.97)	0.94 (0.90-0.96)	0.73 (0.07-0.92)

Results are Pearson correlation and ICC (95% CI) for intra- and interlaboratory CSF analyses. ICC=Intraclass coefficients, ratio= Aβ1-42/t-tau, CSF=cerebrospinal fluid, Aβ=amyloid beta, t-tau=total tau, p-tau=phosphorylated tau. *All $p<0.001$.

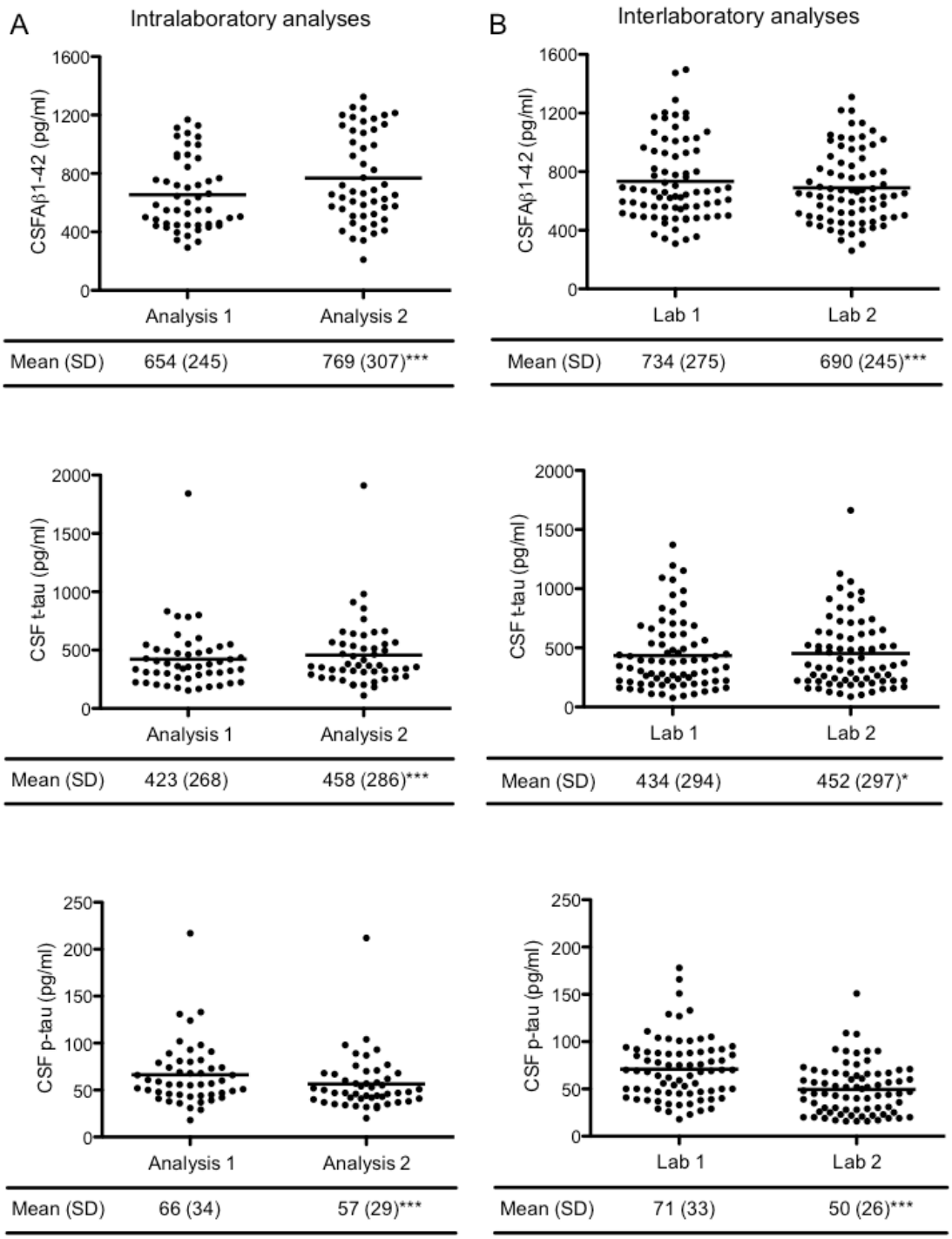


Figure 1. CSF levels by analysis and marker

Results are frequencies and mean (SD) for each CSF marker on the left (A) for CSF intralaboratory analyses and on the right (B) for CSF interlaboratory analyses. The solid line represents the mean CSF levels. Analysis 1 is routine practice and analysis 2 is performed as part of the LeARN study. CSF=cerebrospinal fluid, Aβ=amyloid beta, t-tau=total tau, p-tau=phosphorylated tau. $^{**}P<0.001$, $^{*}p<0.05$ compared to CSF analysis 1 or lab 1.

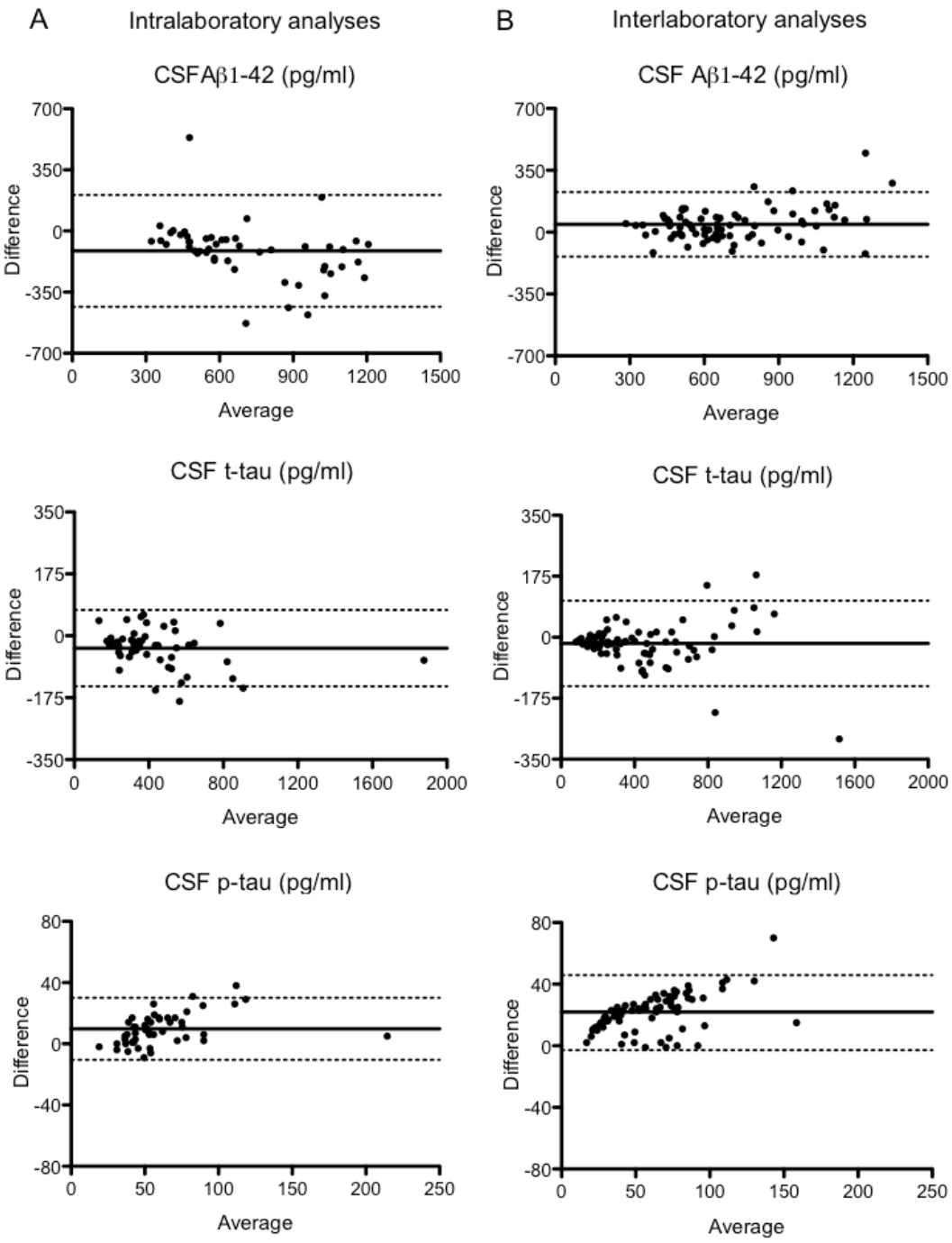


Figure 2. Bland-Altman plots of variability between CSF analyses

The average of CSF analysis 1 and 2 is plotted against the difference between both analyses, on the left (A) for CSF intralaboratory analyses and on the right (B) for CSF interlaboratory analyses. The solid line represents the mean and the dotted lines the upper and lower 1.95 SD. CSF=cerebrospinal fluid, Aβ=amyloid beta, t-tau=total tau, p-tau=phosphorylated tau.

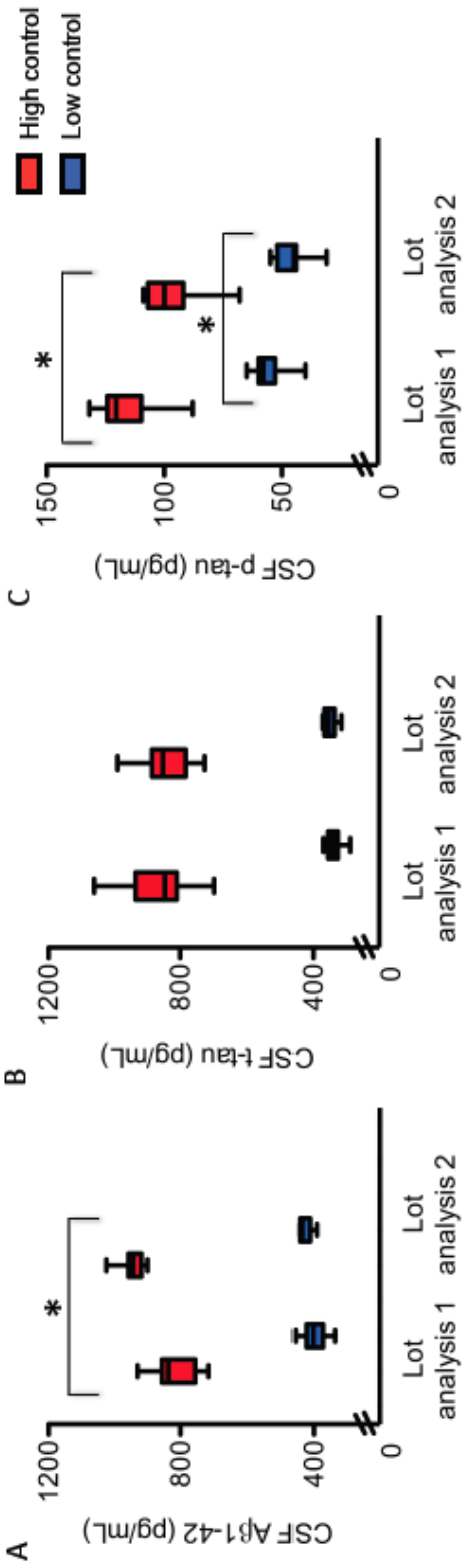


Figure 3. Internal control data of the Amsterdam laboratory

Results are changes in high and low CSF biomarker levels for intralaboratory reanalysis in two lots (routine lot and LeARN lot) as part of internal control of data at the Amsterdam laboratory. A) Aβ1-42: Lot 1 used from February 2010 to February 2011 (n=18) and lot 2 used from March to October 2012 (n=17). B) T-tau: Lot 1 used from February 2010 to April 2011 (n=24) and lot 2 used from March to June 2012 (n=11). C) P-tau: Lot 1 used from February 2010 to April 2011 (n=23) and lot 2 used from March to October 2012 (n=11). CSF=cerebrospinal fluid, Aβ=amyloid beta, t-tau=total tau, p-tau=phosphorylated tau. *P<0.001 for differences between lot of analysis 1 (routine lot) and lot of analysis 2 (LeARN lot).

Change in AD classification

We investigated how reanalysis changed the CSF AD classification based on individual CSF markers and based on the combination of CSF markers with an AD profile being defined as abnormal Aβ1-42 and abnormal t-tau or p-tau. Using predefined cutoffs, repeated CSF analyses in the same laboratory led to a change in biomarker classification (normal vs. abnormal) of 26% of subjects based on Aβ1-42, 10% based on t-tau, 29% based on p-tau, and 16% based on the AD profile (Table 2). Repeated CSF analyses in different laboratories using the cut-offs from the Amsterdam lab led to a change in biomarker classification of 12% of subjects based on CSF Aβ1-42, 1% based on t-tau, 22% based on p-tau, and 14% based on the AD profile (Table 2). When we applied lab-specific cut-offs to define an abnormal score, the repeated CSF analyses in different laboratories led to a change in biomarker classification of 17% of subjects based on CSF Aβ1-42, 1% based on t-tau, 12% based on p-tau, and 12% based on the AD profile (Table 2). Figure 4 shows the change in CSF levels for each biomarker for intra- and interlaboratory analyses of subjects in whom reanalysis led to a different biomarker classification as normal vs. abnormal when Amsterdam cut-offs were applied. Most of the subjects with a change in AD classification after reanalysis in the same laboratory as well as in a different laboratory had CSF biomarker values around the cut-off points.

Table 2. Numbers of subjects with normal and abnormal classified CSF marker values based on predefined cut-offs

	Intralaboratory analyses				Interlaboratory analyses							
	Amsterdam cut-offs				Amsterdam cut-offs*				Lab-specific cut-offs**			
Classification of biomarker(s)	Aβ1-42	T-tau	P-tau	AD profile	Aβ1-42	T-tau	P-tau	AD profile	Aβ1-42	T-tau	P-tau	AD profile
Analysis 1 & 2 same classification	74% (37)	90% (45)	71% (35)	84% (42)	88% (67)	99% (75)	78% (59)	86% (65)	83% (63)	99% (75)	88% (67)	88% (67)
Analysis 1 normal & 2 abnormal	2% (1)	4% (2)	6% (3)	-	11% (8)	-	-	9% (7)	16% (12)	-	11% (8)	12% (9)
Analysis 1 abnormal & 2 normal	24% (12)	6% (3)	23% (11)	16% (8)	1% (1)	1% (1)	22% (17)	5% (4)	1% (1)	1% (1)	1% (1)	-

Results are the percentage (number) of subjects with normal and abnormal classified CSF markers based on CSF analysis 1 (routine practice) and analysis 2 (LeARN study). The CSF AD profile was defined as abnormal Aβ1-42 and abnormal t-tau or p-tau, based on predefined cut-offs (see methods). CSF=cerebrospinal fluid, Aβ=amyloid beta, t-tau=total tau, p-tau=phosphorylated tau. *The routine analyses in Nijmegen and the LeARN analyses were dichotomized according to the Amsterdam cut-offs (see methods). **The routine analyses in Nijmegen were dichotomized according to the Nijmegen cut-offs and the LeARN analyses according to the Amsterdam cut-offs (see methods).

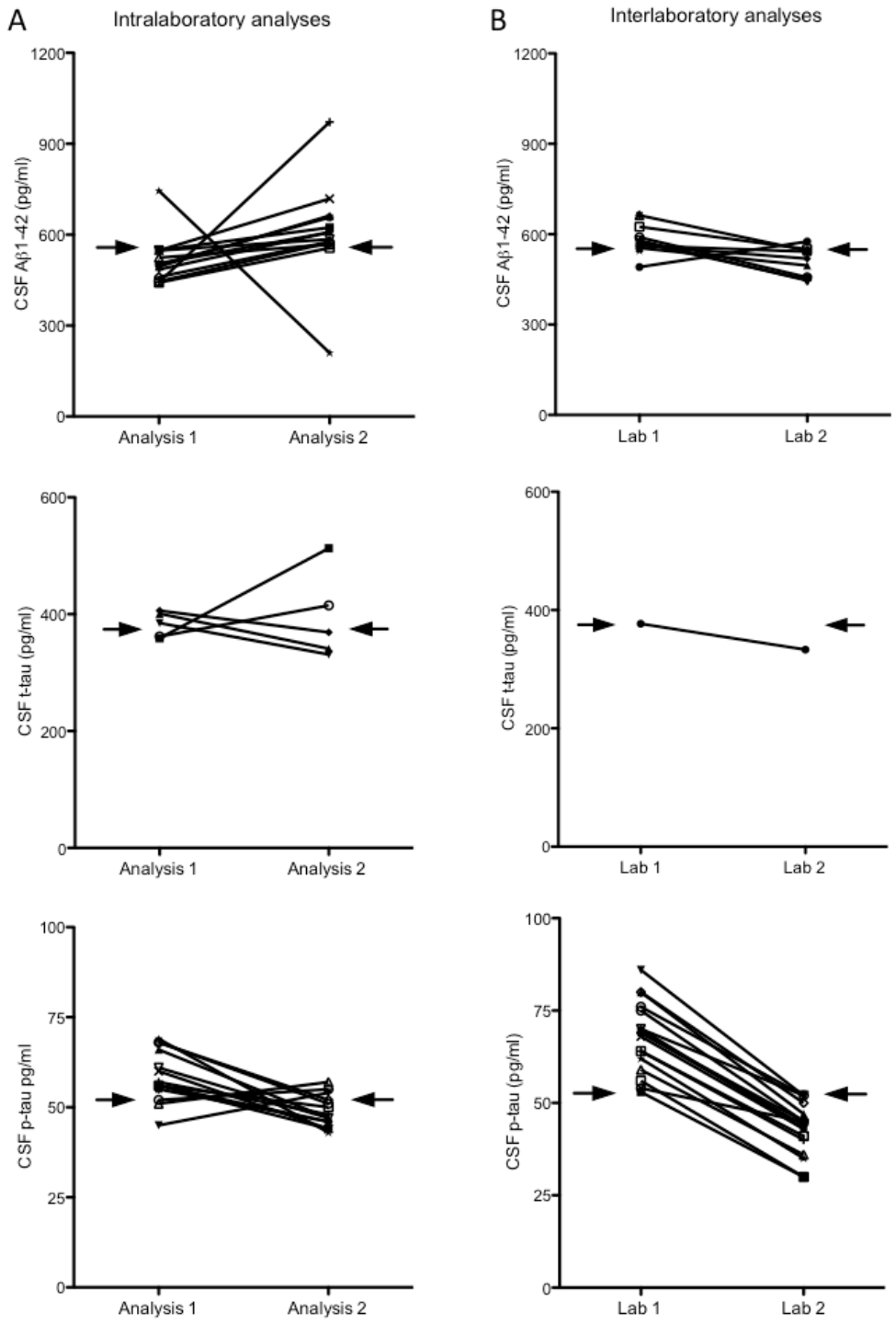


Figure 4. Change in CSF marker classification according to cut-offs

Results are CSF levels only for subjects in which reanalysis let to a different biomarker classification using the cut-offs from Amsterdam to define abnormal CSF values. On the left (A) are changes in biomarker levels for intralaboratory reanalysis and on the right (B) for interlaboratory reanalysis. The arrow represents the applied Amsterdam CSF cut-off. Analysis 1 is routine practice and analysis 2 is performed as part of the LeARN study. CSF=cerebrospinal fluid, Aβ=amyloid beta, t-tau=total tau, p-tau=phosphorylated tau.

DISCUSSION

We observed clear variability in CSF AD biomarker levels between repeated analyses in the same laboratory as well as between two different laboratories. Our study is the first to show that this variability frequently led to a change in CSF-based AD diagnosis when predefined cut-offs for abnormal CSF values were applied.

CSF intralaboratory variability (based on CV) was largest for Aβ1-42, consistent with previous studies.[5-7] Change in AD-like scores after repeated analyses in the same laboratory was, however, highest for CSF p-tau (29%) followed by Aβ1-42 (26%) and t-tau (10%). This large change for p-tau is likely due to a smaller range of values of p-tau and values being closer to the cut-off compared to other markers, such that a small change in observed concentration more easily leads to a different classification.

CSF interlaboratory variability (based on CV) was largest for p-tau, unlike findings from previous studies, which found that interlaboratory variability was largest for Aβ1-42.[5-7] Change in AD-like scores after repeated analyses in different laboratories was also higher for CSF p-tau (22%) than for Aβ1-42 (12%) and t-tau (1%) when the same cut-offs were used for each site. A rather unexpected finding was that the interlaboratory variability of Aβ1-42 was smaller than the intralaboratory variability.

A major finding was that CSF analytical variability frequently led to a change in diagnostic CSF-based AD classification. Importantly, also a diagnosis of a CSF AD profile based on Aβ1-42 and t-tau or p-tau changed in 12-16% of the cases. As most subjects whose AD classification changed after reanalysis had values around the cut-off points, it could be helpful to use a range around a cut-off point rather than a fixed cut-off point.

The intra- and interlaboratory variability in CSF results can result from differences in preanalytical and analytical procedures, and lot-to-lot variation of analytical kits.[9,15] The intralaboratory variability is likely due to lot-to-lot variation as other procedures remained essentially the same. Additional support for this comes from the observation that internal control values showed a striking lot-to-lot variation of up to 20%. The change in internal control values was of the same order of magnitude and same direction of change as the change in CSF scores from the patient samples. Another possible explanation for the intralaboratory variability is variability in freezing conditions and storage time. However, previous studies showed that these factors have a minor impact on variability of CSF values.[16,17]

Interlaboratory variability may also be caused by lot-to-lot variation. Indeed, lots used in each lab showed only minor overlap (data not shown). Differences in analytical procedures may also have contributed to the interlaboratory variability. However, both laboratories used similar protocols, were both trained in a hands-on workshop,[9] and were similarly experienced.

Another source of variability between laboratories, which may influence CSF-based AD classification, is the difference in cut-offs used. We, therefore, tested interlaboratory variability for CSF-based AD classification both with the same CSF cut-offs as well as lab-specific cut-offs. While lab-specific cut-offs for Aβ1-42 and t-tau did not differ much, there

was a large difference for p-tau (85 pg/ml in Nijmegen vs. 52 pg/ml in Amsterdam). This was also reflected in the interlaboratory variability in CSF-based AD classification. Using the same cut-off, abnormal p-tau was more common when samples were analyzed in Nijmegen than in Amsterdam. Change in classification was mostly due to subjects with scores around the cut-off (Figure 4B). Using the lab-specific cut-off, however, abnormal p-tau was more common when samples were analyzed in Amsterdam than in Nijmegen. Here change in classification was mainly due to differences in cut-offs. This clearly indicates that lab-specific cut-offs may also influence comparability between laboratories.

Our study has several limitations. The cut-offs that were used may have influenced our findings on change in CSF-based AD diagnosis. However, as no universal CSF cut-offs are available, we applied routinely used validated cut-offs. One of the major strengths of this study was the large number of CSF samples used to study interlaboratory variability, as most previous studies were based only on a few samples.[5-7] In addition, our study design allows generalization to other CSF centers that analyze CSF AD biomarkers using ELISA, as it reflects CSF procedures in general clinical practice.

Together, our findings suggest that variability in CSF analyses is common between and within laboratories, in particular for Aβ1-42 and p-tau. A substantial part of this variability seems to be explained by lot-to-lot variation of analytical kits. The variability has a large impact on CSF-based AD diagnosis or treatment decisions in clinical settings, suggesting that we should be careful when interpreting CSF findings and always interpret them within a clinical context[17] and with reference to internal standards. For the moment this rightly restricts the indication for CSF biomarker testing in diagnostic guidelines as complimentary and non-obligatory.[19,20] The recent consensus on standardization of preanalytical aspects of CSF analyses,[15,17] as well as the ongoing worldwide quality control study[6] and standardization projects (www.neurodegenerationresearch.eu) will help to move towards a standardized and harmonized implementation of CSF markers in clinical routine.

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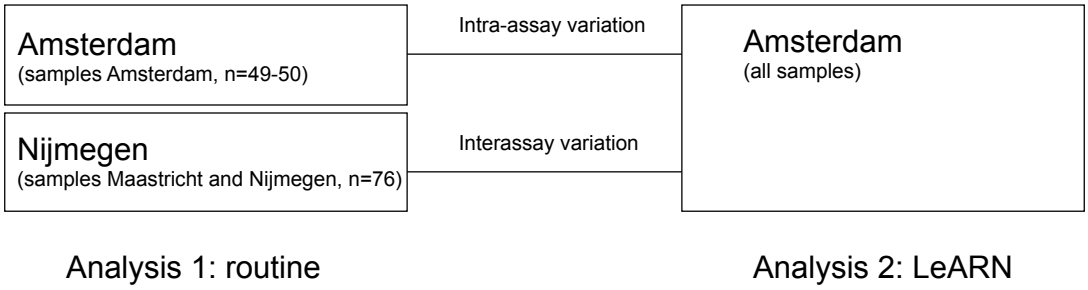
SUPPLEMENTAL DATA

Supplemental Table 1. Baseline patient demographics

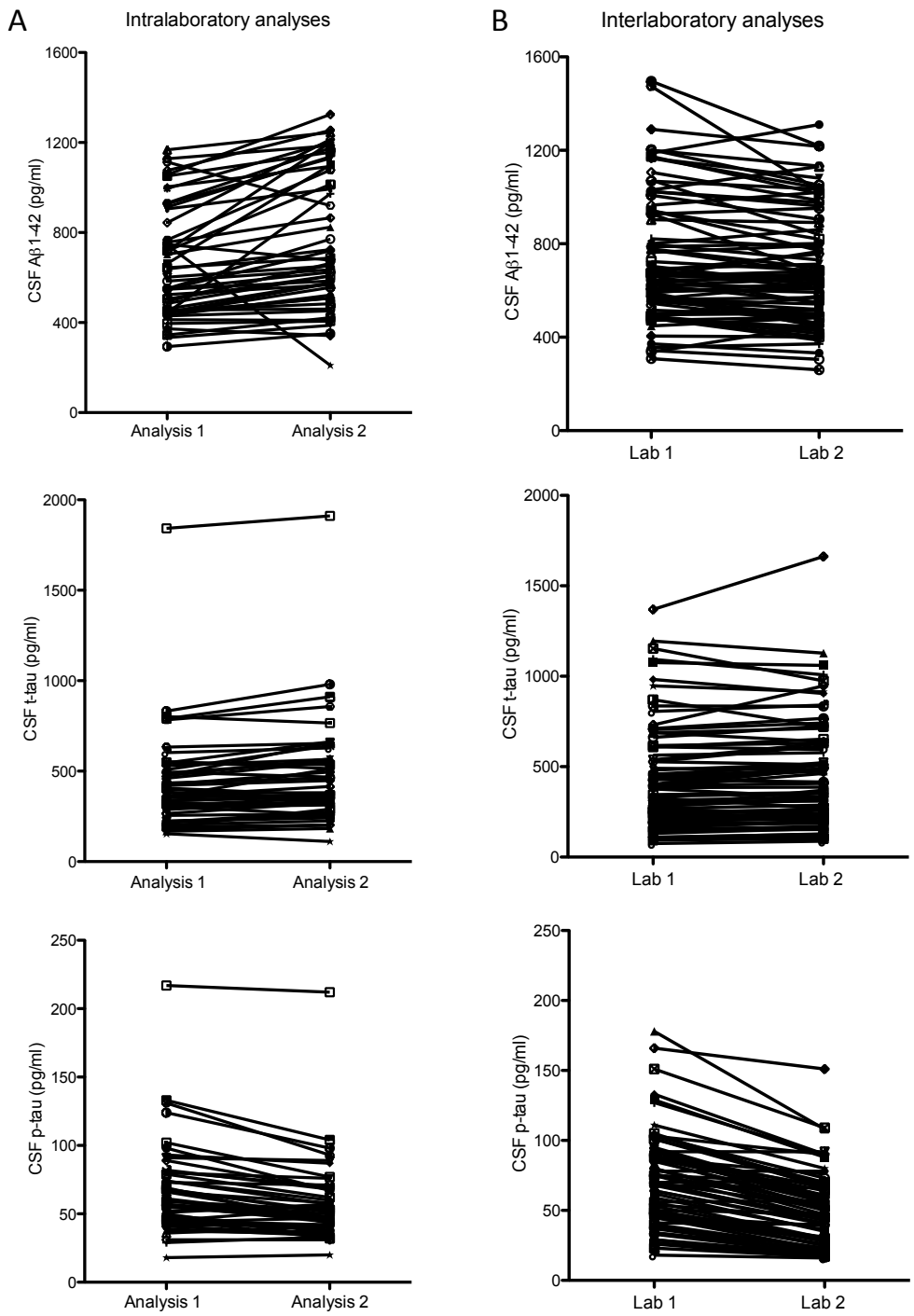
	Total sample (n=126)	Amsterdam cohort (n=50)	Maastricht & Nijmegen cohort (n=76)
Age	67.0 (9.2)	65.0 (7.7)	68.3 (9.8)
Female, n	36 (29%)	12 (24%)	24 (32%)
MMSE	26.1 (2.9)	25.2 (3.0)	26.8 (2.7)
CDR	0.6 (0.3)	0.7 (0.4)	0.5 (0.3)
Clinical diagnosis, n			
SCI	41 (33%)	9 (18%)	32 (42%)
MCI	33 (26%)	8 (16%)	25 (33%)
AD-type dementia	32 (25%)	17 (34%)	15 (20%)
Other dementia	20 (16%)	16 (32%)	4 (5%)

Results are mean (SD) or number (%), presented for the total sample and separately for the sample for intralaboratory analyses (Amsterdam cohort) and the sample for interlaboratory analyses (Maastricht & Nijmegen cohort). MMSE=Mini-Mental State Examination, CDR=Clinical dementia rating scale, SCI=subjective cognitive impairment, MCI=mild cognitive impairment, AD=Alzheimer's disease.



Supplemental Figure 1. CSF analyses by laboratory

Laboratories that performed CSF analyses for clinical routine are presented on the left (analysis 1); the laboratory that performed CSF analyses for the LeARN study is presented on the right (analysis 2).



Supplemental Figure 2. Change in CSF levels after reanalysis

Results on the left (A) are changes in CSF biomarker levels for intralaboratory reanalysis and on the right (B) for interlaboratory reanalysis. Analysis 1 is routine practice and analysis 2 is performed as part of the LeARN study. CSF=cerebrospinal fluid, Aβ=amyloid beta, t-tau=total tau, p-tau=phosphorylated tau.

PART II

DIAGNOSTIC CRITERIA FOR PREDEMENTIA ALZHEIMER'S DISEASE

CHAPTER 7

COMPARISON OF INTERNATIONAL WORKING GROUP CRITERIA AND NATIONAL INSTITUTE ON AGING - ALZHEIMER'S ASSOCIATION CRITERIA FOR ALZHEIMER'S DISEASE

Alzheimer's & Dementia 2012;8:560-563

Pieter Jelle Visser, Stephanie Vos, Ineke van Rossum, Philip Scheltens

ABSTRACT

Two sets of research criteria for Alzheimer's disease (AD) are now available: those published by an International Working Group (IWG) in 2007 and the recommendations published by the National Institute of Aging and the Alzheimer Association (NIA-AA) in 2011. They provide both guidelines for the diagnosis of asymptomatic and symptomatic AD. The co-existence of two sets of criteria for the same disorder raises the question as to which set of criteria should be preferred. A comparison of the criteria revealed differences in approach, terminology and use of cognitive markers and biomarkers. Most persons who meet the IWG criteria will also meet the NIA-AA criteria and vice versa. However, the NIA-AA criteria allow for a subclassification of persons based on biomarker results within each diagnostic category. Further research is needed to validate the criteria. Modifications are likely to be made before the criteria can be used in daily practice.

INTRODUCTION

Two sets of research criteria for Alzheimer's disease (AD) are now available: those published by an International Working Group (IWG) in 2007 and those published by the National Institute of Aging and the Alzheimer Association (NIA-AA) in this Journal in 2011 [1-4]. The co-existence of two sets of criteria for the same disorder raises the question as to which set of criteria should be used. In this commentary, we will compare the two sets of criteria, highlight their strengths and limitations, and discuss future perspectives.

APPROACH AND TERMINOLOGY

AD can be subdivided in three stages based on the presence of AD biomarkers and cognitive impairments: a prepathology stage (biomarkers normal, no cognitive impairments) an asymptomatic stage (biomarkers abnormal, no cognitive impairments), and a symptomatic stage (biomarkers abnormal, cognitive impairments). The symptomatic stage can be further subdivided in a subjective cognitive impairments stage (AD-SCI), a mild cognitive impairment stage (AD-MCI), and a dementia stage (AD-dementia). Both the IWG criteria and NIA-AA recommendations deal with the asymptomatic and symptomatic stages of AD but the approaches and terminology differ (figure).

IWG criteria

The IWG proposes two sets of criteria. Both sets of criteria require assessment of AD biomarkers. The first set of criteria refers to subjects with asymptomatic AD and is referred to as 'preclinical AD'. In the 2010 revision, 'preclinical AD' is subdivided in 'asymptomatic at risk for AD' and 'presymptomatic AD' [5]. This latter category applies to asymptomatic subjects who are carrier of autosomal dominant AD mutations. The second set of criteria is for subjects with symptomatic AD and is referred to as 'AD'. Subjects who meet these criteria can be subclassified as 'prodromal AD' or 'predementia AD' (the AD-MCI stage in the figure) or 'AD dementia' (the AD-dementia stage in the figure). Persons with AD-SCI cannot yet be classified by the criteria. The authors indicate that further research is needed before it can be decided whether subjects with AD-SCI should be considered as having AD or as being asymptomatic at risk for AD [5].

NIA-AA recommendations

The NIA-AA proposes 3 sets of criteria in case biomarkers are available: one for the asymptomatic stage called 'preclinical AD', one for the AD-MCI stage called 'MCI due to AD', and one for the AD-dementia stage called 'dementia due to AD'. In case no biomarkers can be used for the diagnosis, subjects should be classified according to clinical criteria. Persons with dementia can be diagnosed as probable or possible AD and persons with MCI as MCI.

<table>
<tr><td rowspan="2">AD stages</td><td rowspan="2">Prepathology AD</td><td rowspan="2">Asymptomatic AD</td><td colspan="3">Symptomatic AD</td></tr>
<tr><td>AD-SCI stage</td><td>AD-MCI stage</td><td>AD-dementia stage</td></tr>
<tr><td rowspan="2">IWG</td><td rowspan="2"></td><td rowspan="2">Preclinical AD
Asymptomatic at risk for AD</td><td rowspan="2">-</td><td colspan="2">AD</td></tr>
<tr><td>Prodromal AD</td><td>AD dementia</td></tr>
<tr><td>NIA-AA</td><td></td><td>Preclinical AD
Stages 1-2</td><td>Stage 3</td><td>MCI due to AD</td><td>Dementia due to AD</td></tr>
</table>

Figure. Terminology of AD stages
AD=Alzheimer's disease; SCI=Subjective cognitive impairment; MCI=Mild cognitive impairment; IWG=International Working Group; NIA-AA= National Institute of Aging-Alzheimer's Association.

COGNITIVE CRITERIA

The main difference between the cognitive criteria in the two sets is in how they deal with non-memory impairments.

IWG criteria

The AD criteria require impairment on an episodic memory test that preferably has encoding specificity. Dementia is not necessary, although a subdivision in prodromal AD and AD dementia can be made as described above. In the 2010 update, persons with non-memory impairments can meet the AD criteria as well but only if they have a well-defined syndrome such as primary progressive non-fluent aphasia, logopenic aphasia, frontal variant of AD, or posterior cortical atrophy. These subjects are referred to as atypical AD [5].

NIA-AA recommendations

In the NIA-AA criteria memory impairment is not necessary for the diagnosis. The criteria for MCI due to AD require MCI, either amnestic or non-amnestic. These MCI criteria are similar to those published in 2004 [6-7]. The criteria for dementia due to AD require impairments in multiple cognitive domains and interference in activities of daily living. For the diagnosis of dementia, memory impairment is not necessary either.

BIOMARKER CRITERIA

Both sets of criteria rely essentially on the same biomarkers for AD pathology, i.e. amyloid beta 1-42 in cerebrospinal fluid (CSF), amyloid deposits as measured by PET scanning, tau in CSF, hippocampal atrophy, or hypoperfusion or hypometabolism as measured by PET or SPECT imaging. They differ in the way they how they combine these biomarkers for the diagnosis.

IWG criteria

In the original criteria biomarkers were subdivided according to modality (CSF, PET, SPECT, or MRI). In the 2010 revision biomarkers were classified as pathophysiological markers (markers for amyloid or tau) and topographical markers (other biomarkers) [5]. An abnormal score for any AD biomarker (pathophysiological or topographical) is sufficient to fulfill the biomarker criterion for AD (table 2). In subjects with preclinical AD, pathophysiological markers should be abnormal (table 1).

Table 1. IWG and NIA-AA criteria for asymptomatic AD

	IWG	NIA-AA Stage 1	Stage 2	Stage 3
Cognitive criteria	No impairment	No impairment	No impairment	Subtle cognitive decline
Biomarker criteria	Amyloid or tau abnormal	Amyloid abnormal	Amyloid and injury marker abnormal	Amyloid and injury marker abnormal

IWG=International Working Group; NIA-AA= National Institute of Aging-Alzheimer's Association.

Table 2. IWG and NIA-AA criteria for AD-MCI and AD-dementia

	IWG	NIA-AA		
Cognitive criteria				
- AD-MCI	Memory impairment	MCI		
- AD-dementia	Memory impairment	Dementia		
Biomarker criteria			Amyloid marker	Injury marker
- AD-MCI	Any amyloid or injury marker	High likelihood	+	+
- AD-dementia				
		Intermediate likelihood	+	?
			?	+
		Uninformative	+	-
			-	+

IWG=International Working Group; NIA-AA= National Institute of Aging-Alzheimer's Association; AD=Alzheimer's disease; MCI=Mild cognitive impairment. + = Abnormal; - = Normal; ? = Not tested.

NIA-AA recommendations

The NIA-AA criteria make a distinction between amyloid markers and neuronal injury markers (other biomarkers). Persons with preclinical AD are classified in three stages based on biomarkers and cognitive markers (table 1). Persons with abnormal amyloid markers only are in stage 1, subjects with both abnormal amyloid and injury markers are in stage 2. Persons with both abnormal amyloid and injury markers and subtle cognitive impairments, including SCI, are in stage 3.

Persons with MCI or dementia due to AD are classified based on amyloid and neuronal injury markers (table 2). If both amyloid and neuronal injury markers are abnormal, the likelihood for AD is high. If only amyloid or neuronal injury markers have been measured and the test is abnormal, the likelihood is intermediate. If the amyloid marker is abnormal and the neuronal injury marker normal or vice versa, the biomarkers are considered uninformative.

DISCUSSION

Strengths and limitations

A strength of the IWG criteria for AD is that that they cover the clinical spectrum from MCI to dementia. This circumvents the arbitrary and sometimes difficult distinction between MCI and dementia. Limitations are the strong emphasis on memory impairment as non-demented subjects with AD may present with non-memory impairments [8-9]. Moreover, the term prodromal AD for subjects with AD-MCI may be confusing. The authors use this term to indicate that these subjects are prodromal to the dementia stage. However, 'prodromal' could be easily understood as prodromal to AD itself, although these subjects meet the IWG AD criteria.

A strength of the NIA-AA recommendations is the distinction between amyloid and injury markers in the criteria for symptomatic AD. Also the broad definition of cognitive impairment including non-amnestic MCI is useful. A limitation is the use of three sets of criteria for a single disease. This entails that the diagnosis change with disease progression, which may cause confusion to clinicians and patients. Another limitation is that the ranking of biomarkers in persons with MCI or dementia may be difficult to apply. Moreover, a substantial number of subjects may have 'uninformative' biomarker test results. Unpublished data from our own cohorts showed that 60% of the subjects with MCI had conflicting scores for CSF amyloid beta 1-42, CSF tau, and hippocampal atrophy.

A limitation of both sets of criteria is that in persons with MCI and dementia the amyloid and injury markers are given the same diagnostic accuracy for AD. The amyloid cascade hypothesis, however, states that amyloid pathology marks the start of AD and that neuronal injury is a secondary event [10]. Amyloid markers will therefore have a higher sensitivity for AD than injury markers while injury markers will have a higher specificity [11].

Classification of subjects according to the criteria

Most persons that meet the IWG criteria will also meet the NIA-AA criteria and vice versa. Subjects with non-amnestic MCI who meet the NIA-AA MCI due to AD criteria but not the IWG criteria may form the only exception. An important difference between the NIA-AA criteria and the IWG criteria is that the NIA-AA criteria make a subclassification based on biomarker results within each diagnostic group. For example, a person with MCI who meets the IWG criteria will meet the MCI due to AD NIA-AA criteria as well and will then be subclassified to have a high or intermediate likelihood for AD or to have uninformative biomarker results.

Use of the criteria

The current use of the criteria will be for research, as emphasized by both the IWG and the NIA-AA working group. Which set of criteria should be preferred will depend on the purpose of the study. For example, recent AD-MCI trials with amyloid modifying treatments selected subjects with amyloid markers [12-13]. These persons would meet the IWG AD

criteria or the NIA-AA MCI due to AD criteria with intermediate likelihood for AD. In case a study requires including subjects with an increased risk for cognitive decline, a combination of amyloid and injury markers could be used, such as the NIA-AA MCI or dementia due to AD criteria with a high likelihood for AD [14-18].

Future perspectives

The new criteria are useful attempts to translate the results from AD biomarker studies into guidelines for diagnosis. Further research is needed to validate the criteria. It seems useful to reconsider the ranking of biomarkers in the criteria such that amyloid markers are used for the diagnosis and injury markers for the staging of the disease and for prognosis [14-18]. In the meantime, clinicians can apply biomarkers for diagnostic purposes outside the framework of diagnostic criteria. In persons with dementia biomarkers may for example help to differentiate between AD and frontotemporal lobar dementia [19]. In persons with MCI, AD biomarkers may be tested if the patient asks to know what is the likely cause of the cognitive impairments [20]. Patients should not be tested for AD biomarkers with the only goal to classify them according to the new criteria.

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CHAPTER 8

PRECLINICAL ALZHEIMER'S DISEASE AND ITS OUTCOME: A LONGITUDINAL COHORT STUDY

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Stephanie J.B. Vos, Chengjie Xiong, Pieter Jelle Visser, Mateusz S. Jasielec, Jason Hassenstab, Elizabeth A. Grant, Nigel J. Cairns, John C. Morris, David M. Holtzman, Anne M. Fagan

ABSTRACT

Background

New research criteria for preclinical Alzheimer's disease have been proposed, which include stages for cognitively normal individuals with abnormal amyloid markers (stage 1), abnormal amyloid and neuronal injury markers (stage 2), or abnormal amyloid and neuronal injury markers and subtle cognitive changes (stage 3). We aimed to investigate the prevalence and long-term outcome of preclinical Alzheimer's disease according to these criteria.

Methods

Participants were cognitively normal (clinical dementia rating [CDR]=0) community-dwelling volunteers aged at least 65 years who were enrolled between 1998 and 2011 at the Washington University School of Medicine (MO, USA). CSF amyloid-β1–42 and tau concentrations and a memory composite score were used to classify participants as normal (both markers normal), preclinical Alzheimer's disease stage 1–3, or suspected non-Alzheimer pathophysiology (SNAP, abnormal injury marker without abnormal amyloid marker). The primary outcome was the proportion of participants in each preclinical AD stage. Secondary outcomes included progression to CDR at least 0.5, symptomatic Alzheimer's disease (score of at least 0.5 for memory and at least one other domain and cognitive impairments deemed to be due to Alzheimer's disease) and mortality. We undertook survival analyses using subdistribution and standard Cox hazards models and linear mixed models.

Findings

Of 311 participants, 129 (41%) were classed as normal, 47 (15%) as stage 1, 36 (12%) as stage 2, 13 (4%) as stage 3, and 72 (23%) as SNAP, and 14 (5%) remained unclassified. The 5-year progression rate to CDR at least 0.5, symptomatic Alzheimer's disease was 2% for participants classed as normal, 11% for stage 1, 26% for stage 2, 56% for stage 3, and 5% for SNAP. Compared with individuals classed as normal, participants with preclinical Alzheimer's disease had an increased risk of death after adjusting for covariates (hazard ratio 6.2, 95% CI 1.1–35.0; p=0.040).

Interpretation

Preclinical Alzheimer's disease is common in cognitively normal elderly people and is associated with future cognitive decline and mortality. Thus, preclinical Alzheimer's disease could be an important target for therapeutic intervention.

INTRODUCTION

Alzheimer's disease (AD) starts with a preclinical phase in which AD neuropathological abnormalities begin to accumulate but cognitive ability is normal.[1-3] Now that biomarkers for AD have become available, identification of preclinical AD in vivo in cognitively normal individuals is possible.[4] Information regarding the occurrence and outcome of preclinical AD is crucial for the understanding of AD pathophysiology and the design of secondary prevention trials.

Research criteria for preclinical AD have been proposed by the Preclinical Working Group of the National Institute on Aging (NIA) and Alzheimer's Association (AA).[5] The NIA-AA criteria for preclinical AD propose ordered stages for cognitively normal individuals with abnormal amyloid markers (stage 1), abnormal amyloid and neuronal injury markers (stage 2), and abnormal amyloid and neuronal injury markers and subtle cognitive changes (stage 3).[5] In a 2012 study in which structural and amyloid imaging markers were used to categorise individuals according to these stages,[6] the rate of short-term (1 year) progression to mild cognitive impairment (MCI) or dementia increased with advancing preclinical AD stage.

The aim of this study was to identify the prevalence and long-term outcome of preclinical AD according to these criteria in a cohort of cognitively normal individuals. We used CSF markers to define NIA-AA preclinical AD stages and assessed the long-term cognitive and mortality outcomes of participants in each stage. We also tested whether the proportion and cognitive outcome of preclinical AD were affected by age or APOE genotype.

METHODS

Participants

Participants were cognitively normal community-dwelling volunteers enrolled between June, 1998, and September, 2011, in longitudinal studies of memory and ageing at the Knight Alzheimer's Disease Research Center (KADRC) of the Washington University School of Medicine (St Louis, MO, USA). Details of recruitment and assessment methods for these participants have been published.[7] Participants in the KADRC cohort were living independently in the community at study entry and underwent annual clinical assessment unless prevented by death, illness, refusal, or relocation from St Louis. Participants were selected from the larger KADRC cohort based on the following criteria: completion of baseline cognitive and CSF assessment; baseline clinical dementia rating (CDR) score of 0; at least 65 years of age at the time of lumbar puncture; at least one annual clinical follow-up assessment; and good general health. The Human Research Protection Office at Washington University School of Medicine approved the KADRC studies, including the Healthy Aging and Senile Dementia study (P01AG003991), the Alzheimer's Disease Research Center study (P50AG05681), and the Antecedent Biomarkers for AD: the Adult Children Study (P01-AG026276). Written informed consent was obtained from all participants at enrolment.

Procedures

Participants underwent annual cognitive assessment, which included CDR and CDR sum of boxes (CDR-SB),[8] mini-mental state examination (MMSE), and a psychometric test battery.[9] The CDR is a global dementia staging system that assesses the presence or absence of dementia and, when present, its severity. The global CDR stages are 0, indicating cognitive normality, and 0.5, 1, 2, and 3, indicating very mild impairment or very mild dementia, and mild, moderate, and severe dementia, respectively.[10] The CDR-SB is a more quantitative representation of cognitive impairment than the global CDR and is derived directly from individual ratings in six cognitive and functional domains, or boxes (memory, orientation, judgment and problem solving, community affairs, home and hobbies, and personal care). The CDR-SB is the total score of all the separate boxes (range 0–18, with 0 as the best score). Participants with a CDR score of 0 typically have scores of 0 on all box scores; however, a global CDR score of 0 can also be assigned in the presence of one box score of 0.5 in a non-memory domain. Baseline CDR score and diagnosis were assigned by trained clinicians and were based on the cognitive assessment closest to the time of the lumbar puncture.

We used CSF markers to define NIA-AA preclinical AD stages. CSF amyloid-β_{1-42} ($A\beta_{1-42}$) was used as a marker of amyloid and CSF tau was used as a marker of neuronal injury. CSF samples (20–25 mL) were collected once at study entry at 0800 h after overnight fasting. Lumbar punctures (lumbar vertebrae L4/L5) were done by trained neurologists using a 22-gauge atraumatic Sprotte spinal needle (Pajunk Medical Systems, Norcross, GA, USA). Samples were gently inverted to avoid possible gradient effects, briefly centrifuged at low speed, and aliquoted (0.5 mL) into polypropylene tubes before being frozen at −84°C. Samples were analysed for total tau (t-tau), phosphorylated tau_{181} (p-tau_{181}), and $A\beta_{1-42}$ by ELISA (INNOTEST; Innogenetics, Ghent, Belgium).

CSF markers were dichotomised (normal or abnormal) by defining a cutoff that could best differentiate participants in our cohort who had CDR 0 at baseline from those in an independent cohort who had CDR 0.5, symptomatic AD (for demographics of this cohort, see appendix), on the basis of the Youden index (sensitivity+specificity−1). The resultant optimum cutoffs for abnormal were less than 459 pg/mL for $A\beta_{1-42}$, greater than 339 pg/mL for t-tau, and greater than 67 pg/mL for p-tau_{181}.

The NIA-AA criteria for preclinical AD do not define the subtle cognitive changes needed for classification as stage 3. Since episodic memory is usually the earliest cognitive domain to be affected in AD,[11,12] we used an episodic memory composite score as the measure of cognition to define stage 3. The composite score was based on factor analyses[9] and consisted of the sum of the three free recall trials from the Buschke free and cued selective reminding test,[13] total scores from the easy and hard trials of associate learning from the Wechsler memory scale-revised,[14] and the total number of correctly recalled units from the Wechsler memory scale-revised logical memory immediate recall test. Raw scores from each test were converted to Z scores using a normative reference sample of participants from the KADRC who were cognitively normal at enrolment and who did not progress to a dementia diagnosis during follow-up (ie, remained CDR 0).[15] The normative

reference sample, as described in detail by Johnson and colleagues,[15] was composed of participants who were enrolled in ongoing studies at the KADRC between Oct 1, 1979, and Dec 31, 2006. 82 (26%) of the 311 participants in the present study contributed cognitive scores (almost exclusively from annual visits before CSF collection) to the normative reference sample. The mean of the three tests was used to create a composite episodic memory score and was converted again into a Z score within the population. The cutoff at the lowest tenth percentile of the distribution in our sample (less than −1.25 SD) was applied to signify memory impairment.

At baseline, participants were classified as normal if both episodic memory and CSF markers met our criteria for normal, in stage 1 if only $A\beta_{1-42}$ was abnormal, in stage 2 if $A\beta_{1-42}$ and either t-tau or p-tau_{181} were abnormal, and in stage 3 if additionally the participant's cognitive ability was below the memory test threshold (panel 1). Participants were classified in the suspected non-Alzheimer pathophysiology (SNAP) group if they had abnormal t-tau or p-tau_{181} in the presence of normal $A\beta_{1-42}$, regardless of episodic memory ability (panel 1).[16] Participants who did not fit within one of the groups were included in the unclassified group and, in view of the uncertainty of their classification, were excluded from the main analyses.

Panel 1. Glossary of preclinical AD stages and symptomatic AD

Normal group	CDR 0[1], amyloid (-), neural injury (-), subtle cognitive decline (-)
Preclinical AD stage 1	CDR 0, amyloid (+)[2], neural injury (-), subtle cognitive decline (-)
Preclinical AD stage 2	CDR 0, amyloid (+), neural injury (+)[3], subtle cognitive decline (-)
Preclinical AD stage 3	CDR 0, amyloid (+), neural injury (+), subtle cognitive decline (+)[4]
SNAP group[5]	CDR 0, amyloid (-), neural injury (+), subtle cognitive decline (-/+)
Unclassified group	CDR 0, amyloid (-/+), neural injury (-), subtle cognitive decline (+)
Symptomatic AD	CDR>0[6], memory and at least one other domain received a score of ≥0.5 and the clinician felt the cognitive impairments to be due to AD (probable AD), no reference to biomarkers

[1]CDR 0, Clinical Dementia Rating=0, no dementia, [2]Amyloid, CSF Ab42 (+) <459pg/mL, [3]Neural injury, CSF tau (+) >339pg/mL or ptau181 (+) >67pg/mL, [4]Subtle cognitive decline, episodic memory composite score (+) in the lowest 10th percentile, [5]SNAP, Suspected Non-Alzheimer Pathophysiology, [6]CDR>0, CDR 0.5= very mild dementia, CDR 1=mild dementia, CDR 2=moderate dementia, CDR 3=severe dementia.

The primary outcome measure was the proportion of participants in each preclinical AD stage, as defined by CSF markers and scores on episodic memory tests. Secondary outcome measures were cognitive decline on the CDR-SB and MMSE, progression to CDR at least 0.5, symptomatic AD at the latest available follow-up before dropout, and mortality.

In our KADRC research cohort, the clinical diagnosis of AD in individuals with a CDR score of 0.5 or greater is based on criteria from the National Institute of Neurological and Communicative Disorders and Stroke and the Alzheimer's Disease and Related Disorders Association,[17] in accordance with standard protocols.[7] In individuals with a CDR score of 0.5, AD was diagnosed if a score of at least 0.5 was given for memory and at least one other

domain and the clinician deemed the cognitive impairments to be due to AD (probable AD; referred to as CDR 0.5, symptomatic AD). CDR 0.5, symptomatic AD differs from MCI as defined in the criteria of MCI due to AD or prodromal AD,[18,19] although it is similar (appendix). As part of the assessment to define whether a participant was cognitively impaired with or without symptomatic AD, a thorough, informant-based and participant-based interview was completed. Cognitive examination, consisting of assessment of recent and long-term memory, executive function, reasoning, language, and visuospatial function, was also undertaken by trained clinicians. The CDR rating and diagnosis are based on both present and historical cognitive ability. In contrast to MCI, an absolute cutoff score on a specific cognitive test is not used to define the presence of cognitive impairment. Moreover, classification as CDR 0.5, symptomatic AD requires clinical change in two cognitive domains, whereas subjective complaints and impairment in any cognitive domain is sufficient to meet the criteria for MCI (appendix).

In a subset of participants for whom autopsy samples were available, neuropathological examination was undertaken using established protocols.[20] AD neuropathological changes were rated using the NIA-AA guidelines.[21] We also tested whether the prevalence and long-term outcome of preclinical AD were affected by age or APOE genotype. TaqMan assays (Applied Biosystems, Foster City, CA, USA) for both ABI#C_3084793_20 and ABI#C_904973_10 were used for APOE genotyping, as described previously.[22]

Statistical analysis

Baseline differences between the stages were analysed using ANOVA for continuous variables and Fisher's exact tests and logistic regression models for categorical variables. Missing data from cognitive tests at follow-up were modelled with mixed models. We first undertook an omnibus test for joint significance of the stage variables and proceeded with subgroup analyses only if this overall test was statistically significant. We did competing-risks survival analyses using Fine and Gray's[23] subdistribution hazards model (subdistribution hazards ratio [SHR]) to investigate the predictive accuracy of the preclinical AD stages for progression to CDR at least 0.5, symptomatic AD during the available follow-up period, with normal individuals as a reference group, uncorrected and corrected for baseline age, sex, education, and APOE genotype. Unlike standard Cox hazards models that usually treat mortality as censoring, these models regard mortality as a competing event that can impede progression to symptomatic AD. Standard Cox proportional hazards models (hazard ratio [HR]) were used to assess the predictive capacity of preclinical AD stages for mortality during the follow-up period, in both unadjusted and adjusted analyses. The relation between the stages and rate of change in CDR-SB and MMSE over time were assessed with general linear mixed models including linear time effects,[24] adjusted for baseline age, sex, education, and APOE genotype. Analyses included baseline score and all available follow-up scores. The final models were specified with a random intercept and slope, because these models provided the best measures on Akaike's

information criterion[25] for analysis of the corresponding clinical and cognitive measures compared with models with other covariance structures. Specific values for each covariate were used to create adjusted plots for the survival analyses and mixed models. Predicted curves or slopes for each of the AD stage groups were plotted within each combination of APOE ε4 and sex with age (72.6 years) and education (15.5 years) fixed at the sample means. These means represent the total group of participants, excluding the unclassified group because this group was not included in these analyses. Subdistribution hazards models were implemented using the STCRREG command in STATA 12 (Stata, College Station, TX, USA). All other statistical analyses were done with SPSS version 19.0 (Chicago, IL, USA), with significance set at $p<0.05$.

RESULTS

Table 1 lists demographics and baseline characteristics. 129 of 311 (41%) participants were classified in the normal group, 47 (15%) as stage 1, 36 (12%) as stage 2, 13 (4%) as stage 3, 72 (23%) as being in the SNAP group, and 14 (5%) remained unclassified. The appendix shows the distribution of participants across the stages. MMSE and memory scores were lower in stage 3 than in the other groups (table 1). Preclinical AD (stage 1–3) was more prevalent in individuals older than 72 years (median age of sample) than in those aged 72 years or younger (37% vs 26%; $p=0.044$) and was more prevalent in APOE ε4 carriers than in non-carriers (47% vs 23%; $p<0.0001$; appendix). The mean interval between lumbar puncture and the closest cognitive assessment was 2.6 months (SD 2.1).

110 (35%) participants were available at 5 years of follow-up and 14 (5%) were available at 10 years. 39 patients (13%) were lost to follow-up and 20 (6%) died during follow-up. After a median follow-up of 3.9 years (range 1–15), progression to CDR at least 0.5, symptomatic AD had occurred in two (2%) participants in the group classed as normal, six (13%) in stage 1, nine (25%) in stage 2, seven (54%) in stage 3, four (6%) in the SNAP group, and four (29%) in the unclassified group (table 2). Of the 32 participants who progressed, 22 (69%) were diagnosed with CDR 0.5, symptomatic AD at their last follow-up, six (19%) had CDR 1, symptomatic AD, and four (13%) had CDR 2, symptomatic AD.

Table 1. Demographics and baseline characteristics

	All N=311	Normal group N=129	Stage 1 N=47	Stage 2 N=36	Stage 3 N=13	SNAP group N=72	Unclassified N=14
Age	72.9 (6.0)	70.6 (4.6)^{1,2,3,S,U}	73.8 (6.9)^{N,3,U}	74.4 (5.8)^{N,3,U}	78.1 (4.9)^{N,1,2,S}	73.6 (5.8)^{N,3,U}	78.3 (6.7)^{N,1,2,S}
Female, n	171 (55%)	73 (57%)	23 (49%)	23 (64%)^{3}	4 (31%)^{2}	43 (60%)	5 (36%)
Education, y	15.4 (2.9)	15.2 (2.6)^{1}	16.9 (3.2)^{N,2,3,S,U}	15.3 (2.7)^{1}	14.3 (2.7)^{1}	15.3 (3.1)^{1}	14.4 (2.9)^{1}
Race, n*							
Caucasian	287 (92%)	118 (91%)	38 (81%)	36 (100%)	13 (100%)	70 (97%)	12 (86%)
African American	22 (7%)	10 (7%)	9 (19%)	-	-	1 (1%)	2 (14%)
Indian	1 (0.5%)	1 (1%)	-	-	-	-	-
AIAN	1 (0.5%)	-	-	-	-	1 (1%)	-
APOE-e4+, n	106 (34%)	32 (25%)^{1,2,3}	22 (47%)^{N}	18 (50%)^{N}	9 (69%)^{N,S,U}	22 (31%)^{3}	3 (21%)^{3}
MMSE**	28.9 (1.3)	29.2 (1.0)^{1,3,U}	28.6 (1.4)^{N,3}	28.9 (1.3)^{3}	27.5 (2.2)^{N,1,2,S}	29.1 (1.1)^{3,U}	28.3 (1.6)^{N,S}
CDR-SB	0.03 (0.1)	0.03 (0.1)	0.04 (0.1)	0.03 (0.1)	0.08 (0.2)	0.02 (0.1)	0.04 (0.1)
Episodic memory, z-score	0.0 (1.0)	0.20 (0.8)^{3,U}	0.23 (0.9)^{3,U}	0.01 (0.9)^{3,U}	-2.05 (0.4)^{N,1,2,S}	0.14 (1.0)^{3,U}	-1.45 (0.2)^{N,1,2,S}
Aβ1-42, pg/mL	610 (259)	712 (207)^{1,2,3,S}	355 (75)^{N,S,U}	350 (71)^{N,S,U}	321 (63)^{N,S,U}	770 (237)^{N,1,2,3,U}	641 (288)^{1,2,3,S}
T-tau, pg/mL	328 (188)	222 (59)^{2,3,S}	210 (66)^{2,3,S}	552 (243)^{N,1,S,U}	592 (150)^{N,1,S,U}	460 (153)^{N,1,2,3,U}	202 (63)^{2,3,S}
P-tau181, pg/mL	61 (30)	43 (10)^{2,3,S}	43 (12)^{2,3,S}	93 (36)^{N,1,U}	95 (20)^{N,1,U}	85 (25)^{N,1,U}	36 (9.8)^{2,3,S}
Follow-up, y (range)	3.9 (1-15)	4.2 (1-13)	4.0 (1-11)	3.2 (1-13)	2.0 (1-6)	3.0 (1-15)	4.2 (1-8)
Progression to CDR≥0.5 symptomatic AD, n	32 (10%)	2 (2%)^{1,2,3,U}	6 (13%)^{N,3}	9 (25%)^{N,S}	7 (54%)^{N,1,S}	4 (6%)^{2,3,U}	4 (29%)^{N,S}
Mortality, n	20 (6%)	2 (2%)^{1,3}	5 (11%)^{N}	3 (8%)	4 (31%)^{N,S}	5 (7%)^{3}	1 (7%)

Results are mean (SD), median (range), or number (%). Preclinical AD stages are defined by CSF markers based on optimal Youden cut-offs: Abnormal CSF Aβ1-42 <459 pg/mL, t-tau >339 pg/mL, p-tau >67 pg/mL. Episodic memory is a composite score of the Associate Learning Test, Logical Memory Test, and Selective Reminding Test, with a cut-off at the lowest 10th percentile: -1.25 SD. AD=Alzheimer's disease, SNAP=Suspected Non-Alzheimer Pathophysiology, AIAN=American Indians and Alaska Natives, *APOE*=apolipoprotein E, MMSE=Mini Mental State Examination (range 0-30, with 30 as the best score), CDR=Clinical Dementia Rating Scale (range 0-3, with 0 as the best score), CDR-SB=Clinical Dementia Rating scale Sum of Boxes (range 0-18, with 0 as the best score), Aβ=beta amyloid, t-tau=total tau, p-tau=phosphorylated tau. *Fisher's exact test p-value=0.007. **The MMSE score was missing for 1 participant in stage 1 at baseline. ^{N}p<0.05 compared to the normal group, ^{1}p<0.05 compared to stage 1, ^{2}p<0.05 compared to stage 2, ^{3}p<0.05 compared to stage 3, ^{S}p<0.05 compared to the SNAP group, ^{U}p<0.05 compared to the unclassified group. See Appendix for exact p-values.

Table 2. Prediction of preclinical AD stages for CDR≥0.5 symptomatic AD and mortality

	Progression to CDR≥0.5 symptomatic AD					Mortality risk			
	5-year progression rate	Uncorrected SHR (95% CI)	Difference compared to other groups	Corrected SHR (95% CI)	Difference compared to other groups	Uncorrected HR (95% CI)	Difference compared to other groups	Corrected HR (95% CI)	Difference compared to other groups
Normal group	2%	Reference	1: p=0.0160 2: p=0.0002 3: p<0.0001 S: p=0.1986	Reference	1: p=0.0791 2: p=0.0004 3: p=0.0001 S: p=0.2670	Reference	1: p=0.0363 2: p=0.0449 3: p=0.0001 S: p=0.0968	Reference	1: p=0.1897 2: p=0.0781 3: p=0.0010 S: p=0.0710
Stage 1	11%	7.0 (1.4-34.1)	2: p=0.0606 3: p=0.0004 S: p=0.1977	4.6 (0.8-25.6)	2: p=0.0536 3:p=0.0021 S: p=0.3646	5.9 (1.1-31.0)	2: p=0.9233 3: p=0.0149 S: p=0.5807	3.7 (0.5-26.4)	2: p=0.5903 3: p=0.0128 S: p=0.6365
Stage 2	26%	18.1 (3.9-83.1)	1:p=0.0606 3: p=0.0656 S: p=0.0047	14.3 (3.3-61.9)	1:p=0.0536 3:p=0.1587 S: p=0.0053	6.3 (1.0-38.3)	1: p=0.9233 3: p=0.0364 S: p=0.5673	6.0 (0.8-44.2)	1: p=0.5903 3: p=0.0403 S: p=0.8560
Stage 3	56%	49.2 (10.1-240.4)	1: p=0.0004 2: p=0.0656 S: p<0.0001	33.8 (6.1-186.7)	1: p=0.0021 2: p=0.1587 S: p=0.0004	31.5 (5.6-176.0)	1: p=0.0149 2: p=0.0364 S: p=0.0048	31.5 (4.1-244.6)	1: p=0.0128 2: p=0.0403 S: p=0.0195
SNAP group	5%	3.1 (0.6-17.6)	1: p=0.1977 2: p=0.0047 3: p<0.0001	2.5 (0.5-12.6)	1: p=0.3646 2: p=0.0053 3: p=0.0004	4.1 (0.8-21.5)	1: p=0.5807 2: p=0.5673 3: p=0.0048	5.2 (0.9-30.9)	1: p=0.6365 2: p=0.8560 3: p=0.0195

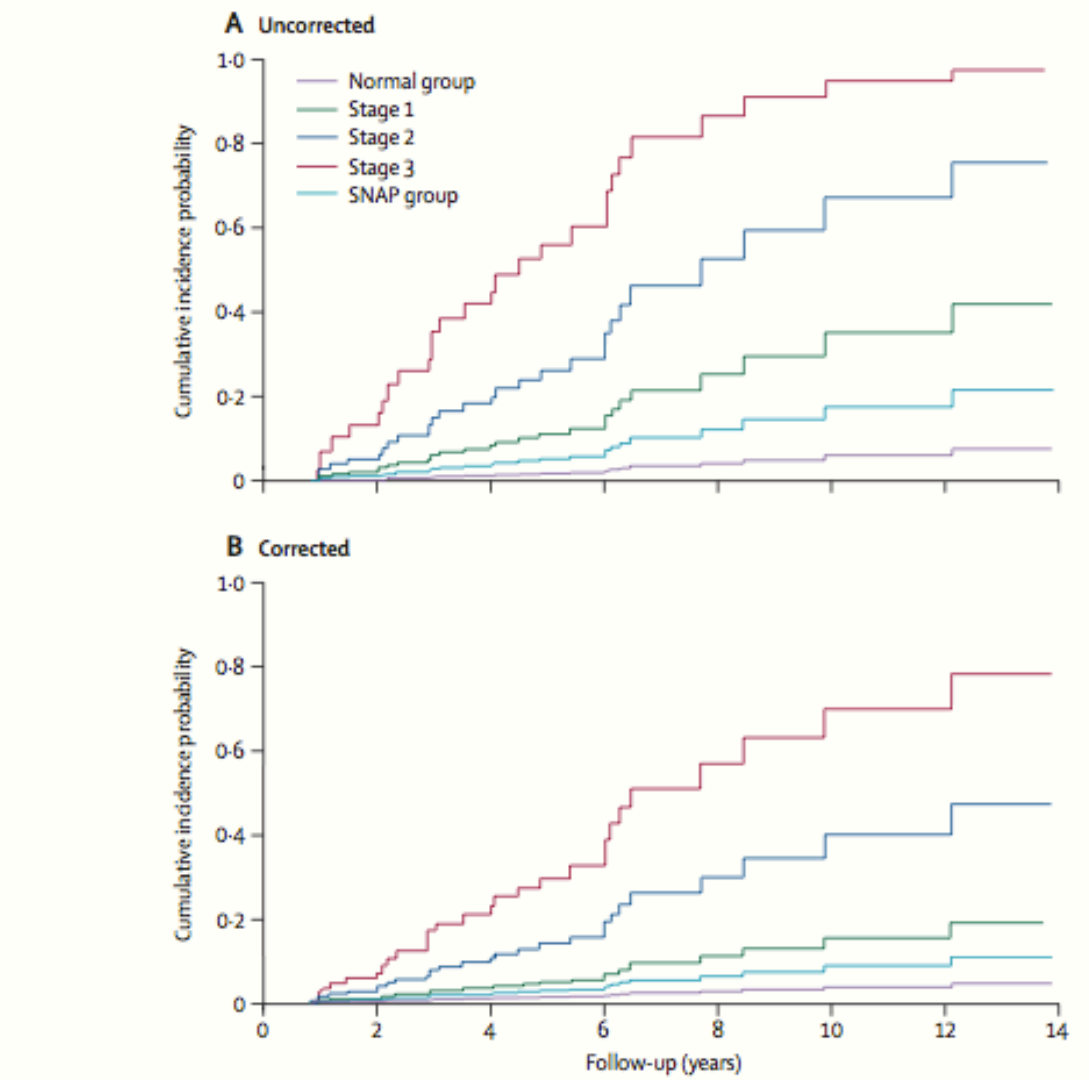
Results are 5-year progression rate (cumulative incidence rate) to CDR≥0.5 symptomatic AD and associated subhazard ratio (SHR, 95% CI) for progression to CDR≥0.5 symptomatic AD calculated using a Fine & Gray subdistribution hazards model, and hazard ratio (HR, 95% CI) for mortality calculated using Cox regression analyses. Analyses are shown as both uncorrected and corrected for baseline age, gender, education, and *APOE* genotype. AD=Alzheimer's disease, CDR=Clinical Dementia Rating scale (range 0-3, with 0 as the best score), SNAP=Suspected Non-Alzheimer Pathophysiology, SHR=Subhazard Ratio, HR=Hazard Ratio. N=normal group, 1=stage 1, 2=stage 2, 3=stage 3, S=SNAP group.

Survival analyses showed that, taking into account mortality, participants in each preclinical AD stage had a higher risk of progression to CDR at least 0.5, symptomatic AD than did participants classed as normal (stage 1 SHR 7.0, 95% CI 1.4–34.1, p=0.016; stage 2 SHR 18.1, 3.9–83.1, p=0.0002; stage 3 SHR 49.2, 10.1–240.4, p<0.0001; table 2, figure). Preclinical AD stages also differed from each other, with more severe stages associated with higher risk of progression to symptomatic AD, although the difference between stages 2 and 3 was not significant (p=0.066). The progression rate of participants in the SNAP group did not differ from that of individuals classed as normal (p=0.20). Only plots for APOE ε4 negative women are shown because this is the most populous group of the four combinations from the two dichotomous factors, APOE ε4 and sex. However, effects of preclinical AD stages (either on risk of converting to a higher CDR or on rate of change in MMSE) remained the same regardless of the combinations of APOE ε4 and sex. After correction for covariates, results remained essentially the same, except that progression in stage 1 was no longer different from that of individuals classed as normal (SHR 4.6, 95% CI 0.8–25.6, p=0.079), which was mainly driven by the correction for age (data not shown). The estimated 5-year progression (cumulative incidence) rate to CDR at least 0.5, symptomatic AD was 2% for participants classed as normal, 11% for those in stage 1, 26% for those in stage 2, 56% for those in stage 3, and 5% for those in the SNAP group. The risk of progression was not different between older (>72 years) and younger (≤72 years) individuals with preclinical AD (SHR 2.0, 95% CI 0.7–5.5; p=0.19) or between APOE ε4 carriers and non-carriers with preclinical AD (SHR 1.1, 0.5–2.6, p=0.76; appendix).

20 (6%) participants died during follow-up (table 1). Compared with individuals classed as normal, participants with preclinical AD (stage 1–3) had an increased risk of death after adjusting for covariates (HR 6.2, 95% CI 1.1–35.0; p=0.040), which increased with advancing stage (table 2). There was weak evidence of a difference in risk of death in the SNAP group compared with the normal group (HR after adjustment 5.2, 95% CI 0.9–30.9; p=0.071). Of the nine participants with preclinical AD who underwent autopsy, eight received a neuropathological diagnosis of AD with intermediate-to-high neuropathological AD change and one received a diagnosis of AD with low neuropathological AD change (table 3). Three of four participants with SNAP who underwent autopsy had low level neuropathological AD change according to NIA-AA criteria; thus this pathological change is unlikely to explain the cognitive impairments. Of these three individuals with SNAP, two were in NIA-AA Aβ stage 1 and one was in NIA-AA Aβ stage 2 but this individual had a low neurofibrillary tangle score and vascular comorbidity.[21] All participants with SNAP had a neuritic plaque score of 0. Other coexisting pathological abnormalities in these individuals were minor and were deemed unlikely to have contributed substantially to the cognitive status. The time to death after baseline lumbar puncture ranged from 2 to 11 years for all participants; therefore, AD pathology might have accumulated by the time of autopsy.

The annual rate of increase in CDR-SB was higher in each preclinical stage than in the normal group (stage 1 p=0.029, stage 2 p<0.0001, stage 3 p=0.0009), in stages 2 and 3 than in the SNAP group (stage 2 p=0.0021 and stage 3 p=0.0048), and in stage 3 compared with

stage 1 (p=0.037; table 4; appendix). The annual rate of decrease in the MMSE was higher in participants in stages 2 and 3 than in participants in the normal group (stage 2 p=0.0021, stage 3 p=0.023) and in those in the SNAP group (stage 2 p=0.0047, stage 3 p=0.028; table 4; appendix). The appendix shows individual cognitive trajectories on the CDR-SB and MMSE.



Number of participants

Normal	129	112	73	29	10	4	1	0
Stage 1	47	36	28	16	4	3	0	0
Stage 2	36	31	15	9	5	1	1	0
Stage 3	13	10	5	2	0	0	0	0
SNAP	72	61	28	15	11	6	6	2

Figure. Progression to CDR at least 0.5, symptomatic AD by preclinical AD stage

Cumulative incidence probability of CDR at least 0·5, symptomatic AD for each preclinical AD stage (A) uncorrected for covariates and (B) corrected for age, sex, education, and APOE genotype. AD=Alzheimer's disease. CDR=clinical dementia rating scale (range 0–3, with 0 as the best score). SNAP=suspected non-Alzheimer pathophysiology.

Table 3. Clinical, biomarker and neuropathologic features of participants who died (n=20)

Case #	Stage	Age at baseline and sex	Edu-cation (years)	MMSE baseline /last	CSF Aβ1-42 (pg/mL)	CSF T-tau (pg/mL)	Cause of death	Time to death (y from baseline)	Last in-person clinical diagnosis (y from baseline)	Last in-person CDR	Clinical diagnosis at death[1]	CDR at death[1]	Primary/ secondary/ other NP diagnosis	NIA-AA*
1	Normal	74 F	12	28 / 27	465	250	Severe burns	14	Uncertain dementia (11)	0.5	-	-	No autopsy	-
2	Normal	70 M	12	29 / 29	812	261	Severe burns	2	No dementia (1)	0	-	-	Pending	-
3	Stage 1	78 F	12	30 / 27	433	333	Myocardial infarction	11	Symptomatic AD (10)	0.5	Primary Vascular dementia	1	AD	Intermediate
4	Stage 1	81 F	18	30 / 22	264	246	Inanition	9	Symptomatic AD (6)	2	Symptomatic AD	3	AD / TDP-MTL	High
5	Stage 1	78 F	18	28 / 26	315	251	Inanition	9	No dementia (5)	0	Symptomatic AD	3	AD / HS	Intermediate
6	Stage 1	90 F	20	30 / 27	272	182	Congestive heart failure	5	No dementia (3)	0	No dementia	0	AD / SVD	Low
7	Stage 1	91 F	18	28 / 27	383	242	Inanition	4	Uncertain dementia (4)	0.5	Uncertain dementia	0.5	AD / SVD	Intermediate
8	Stage 2	80 F	16	29 / 28	457	543	Bronchopneumonia	6	Uncertain dementia (3)	0.5	Symptomatic AD	0.5	AD / SVD / AGD and SDH	Intermediate
9	Stage 2	85 M	8	29 / 28	411	582	Unknown	4	No dementia (3)	0	-	-	No autopsy	-
10	Stage 2	82 F	12	30 / 29	267	490	Unknown	3	No dementia (2)	0	-	-	No autopsy	-
11	Stage 3	88 M	12	28 / 27	303	575	Congestive heart failure	2	Symptomatic AD (2)	0.5	Symptomatic AD	0.5	AD	Intermediate
12	Stage 3	79 F	16	28 / 27	283	393	Multiple organ failure	4	No dementia (2)	0	No dementia	0	AD / DLB	High
13	Stage 3	71 F	12	29 / 24	318	548	Ovarian cancer	5	Symptomatic AD (4)	0.5	Symptomatic AD	1	AD / SVD	High
14	Stage 3	78 M	16	28 / 28	410	463	Motor vehicle accident	1	No dementia (1)	0	-	-	No autopsy	-
15	SNAP	90 M	19	26 / 25	682	520	Respiratory failure	2	No dementia (1)	0	Symptomatic AD	0.5	AD / AGD	Low
16	SNAP	90 F	12	29 / 30	786	739	Unknown	3	No dementia (3)	0	No dementia	0	AD / SVD / astrocytoma	Low
17	SNAP	86 F	18	28 / 28	738	495	Atherosclerotic heart disease	5	Symptomatic AD (4), with CVD	1	Symptomatic AD	1	SVD / NFT-MTL	Not
18	SNAP	75 F	14	28 / 30	1109	624	Metastatic carcinoma	4	Uncertain dementia (3)	0.5	Uncertain dementia	0.5	AD	Low
19	SNAP	84 M	12	29 / 27	486	364	Respiratory failure	3	No dementia (2)	1	-	-	No autopsy	-
20	Unclassi-fied	76 F	12	29 / 25	670	156	Mitral valve stenosis	9	Symptomatic AD (8)	1	Symptomatic AD	2	AD / SVD / LB	Low

Aβ=beta amyloid, AD=Alzheimer's disease, AGD=argyrophilic grain disease, CDR=Clinical Dementia Rating scale, CSF=cerebrospinal fluid, CVD= cardiovascular disease contributing to cognitive impairment, DLB=dementia with Lewy bodies, HS=hippocampal sclerosis, LB=Lewy bodies unspecified, NFT-MTL=neurofibrillary tangles in medial temporal lobe, NP=neuropathology, p-tau=phosphorylated tau, SDH=subdural hematoma, SNAP=Suspected Non-Alzheimer Pathophysiology, SVD= small vessel disease with infarcts/microinfarcts, TDP-MTL=TDP-43-proteinopathy in medial temporal lobe, t-tau=total tau, y=years. *NIA-AA criteria: AD neuropathologic change: not, low, intermediate, or high. Only intermediate or high neuropathologic change is considered sufficient to account for dementia. [1]clinical diagnosis and CDR assigned after participant death, based on review of historical clinical records and interview with informant as to cognitive abilities of the participant just prior to their death.

Table 4. Annual rate of change in CDR-SB and MMSE according to preclinical AD stage

Stages	Slope CDR-SB	P-value slope	Difference compared to other groups	Slope MMSE	P-value slope	Difference compared other groups
Normal group	0·03 (0·03)	P=0·2784	1: p=0·0289 2: p<0·0001 3: p=0·0009 S: p=0·3870	-0·01 (0·03)	P=0·6955	1: p=0·2324 2: p=0·0021 3: p=0·0233 S: p=0·9857
Stage 1	0·15 (0·04)	P=0·0014	2: p=0·0638 3: p=0·0373 S: p=0·2096	-0·09 (0·05)	P=0·0966	2: p=0·0655 3: p=0·1019 S: p=0·2925
Stage 2	0·28 (0·05)	P<0·0001	1: p=0·0638 3: p=0·3848 S: p=0·0021	-0·24 (0·06)	P=0·0003	1: p=0·0655 3: p=0·6227 S: p=0·0047
Stage 3	0·37 (0·10)	P=0·0002	1: p=0·0373 2: p=0·3848 S: p=0·0048	-0·31 (0·12)	P=0·0145	1: p=0·1019 2: p=0·6227 S: p=0·0279
SNAP group	0·07 (0·04)	P=0·0685	1: p=0·2096 2: p=0·0021 3: p=0·0048	-0·01 (0·04)	P=0·7578	1: p=0·2925 2: p=0·0047 3: p=0·0279

Data are slopes (SE) corrected for age, gender, education, and *APOE* genotype and comparison to other groups. AD=Alzheimer's disease, CDR-SB=Clinical Dementia Rating scale Sum of Boxes (range 0-18, with 0 as the best score), MMSE=Mini Mental State Examination (range 0-30, with 30 as the best score), SNAP=Suspected Non-Alzheimer Pathophysiology. N=normal group, 1=stage 1, 2=stage 2, 3=stage 3, S=SNAP group.

DISCUSSION

In this study, we show that preclinical AD can be defined by CSF markers, is common in individuals aged at least 65 years, and is associated with an increased risk of cognitive decline, progression to CDR at least 0.5, symptomatic AD, and mortality (panel 2).

31% of participants in our cohort had preclinical AD (stages 1–3), which is consistent with findings from clinicopathological studies[1-3] and the population-based Mayo Clinic Study of Aging (MCSA), which used imaging measures (appendix).[16] The validity of our biomarker-based diagnosis of preclinical AD was further supported by the finding that eight of nine participants with preclinical AD who underwent autopsy had intermediate-to-high AD neuropathological changes. The distribution across the preclinical AD stages was also similar to that reported in the MCSA.[16]

Individuals with preclinical AD progressed faster to CDR at least 0.5, symptomatic AD than did those in the normal and SNAP groups. Progression rates differed between the preclinical AD stages; thus, stages 1, 2, and 3 represent different and progressive disease severities. Findings from the MCSA also showed an increased rate of cognitive decline with advancing stage, although only 1 year of follow-up data were reported.[6]

Mortality risk was higher in participants with preclinical AD than in those in the normal group and also increased with advancing stage. To our knowledge, no other studies have examined mortality risk in preclinical AD, but our findings are consistent with clinical studies in individuals with incident or very mild AD dementia.[26,27] There is no clear explanation for the increased mortality risk. Risk factors for AD might also be associated with other life-threatening diseases. Alternatively, AD-related cognitive impairments might increase mortality risk because they may hamper diagnosis and management of other diseases or increase the risk of accidents.[28,29] Increased mortality risk might also have resulted from AD pathological abnormalities, which might compromise the physiological response to other illnesses.[30] However, further research is needed to understand the relation between preclinical AD and mortality.

The proportion of participants with preclinical AD was higher in older individuals and in APOE ε4 carriers than in younger individuals and non-carriers, which is in line with findings from previous studies.[31] However, neither age (<72 years vs ≥72 years) nor APOE genotype predicted rate of decline, although these subanalyses had limited statistical power owing to the small sample sizes. Although APOE ε4 is often a good predictor of cognitive decline in unselected populations, the absence of its prognostic utility in individuals with AD pathological abnormalities is consistent with findings from previous studies.[32]

23% of participants in this study had SNAP, in line with findings from the MCSA.[16] Cognitive decline in the SNAP group was similar to that in normal individuals, although there was weak evidence of increased mortality. There were no to low AD pathological changes on autopsy, suggesting that these individuals might have other diseases.

The selection of cutoffs is crucial for categorisation of NIA-AA stages.[5] We used the Youden index to define the CSF cutoffs. These values were lower than those previously used in a similar cohort ($A\beta_{1-42}$ <500 pg/mL, t-tau >440 pg/mL, and p-tau_{181} >78 pg/mL).[33] Use of the previous cutoffs would lead to a slightly higher proportion of preclinical AD (40%), but the progression to symptomatic AD remained the same (appendix). Our cognitive cutoff at the tenth percentile was in line with that used in the MCSA.[6,16]

Also, the choice of cognitive tests might affect the NIA-AA staging and outcome. We defined subtle cognitive changes as low scores on a memory composite test. If subtle cognitive change was defined as a low score in any cognitive domain (episodic memory, semantic memory, working memory, or visuospatial score, as described by Johnson and colleagues[15]), the number of individuals in stage 3 and the unclassified group would increase. However, progression rates to CDR at least 0.5, symptomatic AD in these groups would be lower (appendix). Although we used a composite score of three memory tests on the basis of factor analyses, the use of a specific memory test could have led to different results.

Participants in stage 3 differed from those with MCI or early dementia in that they had a CDR score of 0 and therefore no change in cognitive function and no interference in activities of daily living. Still, some of these participants might have met psychometric criteria for MCI. Findings from a study in autosomal dominant AD mutation carriers[34]

showed that individuals with preclinical AD might have cognitive impairments without disturbance in functional abilities, and thus appear clinically normal.

14 participants remained unclassified and their outcome has not been investigated previously. They had an increased risk of progression to symptomatic AD but not of mortality compared with the normal group (appendix). Although amyloid pathology might be present in these individuals, future studies are needed to clarify their characteristics and outcome.

Our results are consistent with those recently reported in the MCSA,[6, 16] although there were important differences in study design. The MCSA used imaging markers for staging individuals and cognitive tests to define clinical diagnosis rather than the CDR. Furthermore, the biomarker cutoffs were defined as those yielding 90% sensitivity for diagnosing AD dementia from a separate AD cohort and a global cognitive test score was used to define subtle cognitive change. The similarity in findings between the studies suggests that CSF and imaging markers might be equally effective for identification of individuals with preclinical AD and prediction of clinical outcome. However, this suggestion does not imply that CSF and imaging makers are equivalent. Head-to-head comparison might yield a different conclusion.

The major strengths of this study are the large sample size of well-characterised participants and the long follow-up period of up to 15 years (mean 4 years). However, our study has several limitations. Because participants agreed to take part in a longitudinal study, including multiple neuroimaging procedures and serial lumbar punctures, they are unlikely to be representative of the general population; nor were they selected at random from the population. However, our sample is similar to other research samples of cognitively normal older adults and people with early symptomatic AD.[35] Also, the number of participants who progressed to symptomatic AD in each stage was small and results should therefore be interpreted carefully. Furthermore, AD clinical diagnosis at follow-up was neuropathologically validated in only a small subset of participants. Thus, some participants might have been misclassified, although the rate of confirmation of AD diagnosis post mortem at the KADRC is high (93%).[7] Similar to participants in the MCSA, participants in our study were mainly white and highly educated, and findings might not apply to individuals with other backgrounds. Although we included cognitively normal individuals (CDR 0), 18 of them had a CDR-SB score of 0.5 (one score of 0.5 in a non-memory domain) and could be considered suspicious because these people might not be truly unimpaired. However, analyses without these participants revealed similar results (appendix).

Although we regard this study as preliminary and hypothesis generating, our findings have several important implications. First, these findings show that preclinical AD is common and can be diagnosed by CSF markers, as shown by neuropathological validation in eight of nine participants who underwent autopsy. The strong association between preclinical AD and future cognitive decline and mortality makes preclinical AD an important target for therapeutic intervention. Second, they show that the proposed NIA-AA staging of preclinical AD represents different disease stages in view of differences in rate of

progression. Third, the findings from this study have implications for the design of secondary prevention trials. Screening of individuals for biomarker assessment according to their age and APOE genotype might be useful, and trials could stratify individuals by preclinical AD stage. The rate of cognitive decline was low compared with that in individuals with MCI[36] or dementia.[37] Thus, trials of preclinical AD need large sample sizes or a longer follow-up to identify effects on cognitive outcome measures. Furthermore, mortality should be considered as an endpoint in trials. Fourth, both occurrence and outcome of preclinical AD are dependent on tests and CSF cutoffs used, which highlights the need for standardisation.

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SUPPLEMENTAL DATA

Supplemental Table 1. Baseline demographics of the independent CDR 0·5 symptomatic AD sample (N=74)

Age	74·5 (5·4)
Female, n	36 (49%)
Education, y	14·5 (3·1)
Race, n	
Caucasian	71 (96%)
African American	2 (3%)
Native Hawaiian and Pacific Islander	1 (1%)
APOE-ε4+, n	51 (69%)
MMSE	25·9 (3·0)
CDR-SB	2·5 (1·0)
Episodic memory, z-score	-1·6 (1·0)
Aβ1-42, pg/mL	424 (219)
T-tau, pg/mL	607 (293)
P-tau181, pg/mL	97 (48)

Results are mean (SD) or number (%). Episodic memory is a composite score of the Associate Learning Test, Logical Memory Test, and Selective Reminding Test. AD=Alzheimer's Disease, *APOE*=Apolipoprotein E, MMSE=Mini Mental State Examination (range 0-30, with 30 as the best score), CDR-SB=Clinical Dementia Rating scale Sum of Boxes (range 0-16, with 0 as the best score), Aβ=beta amyloid, p-tau=phosphorylated tau, t-tau=total tau.

Supplementary Table 2. Overview of concepts for mild cognitive impairments

Construct	Definition			
	Psychometric test impairment	Functional impairment	Clinical dementia diagnosis according to DSM IV or ICD-10	Biomarker status
CDR 0·5 symptomatic AD[1]	Defined cut-offs not utilized	Very mild to mild change in daily functioning in memory and at least 1 non-memory domain	Some	Not needed
Amnestic MCI[2]	Yes, in memory domain	Subjective report of cognitive decline	No	Not needed
MCI due to AD[3]	Yes, in any cognitive domain	Cognitive concern reflecting a change in cognition	No	Abnormal
Prodromal AD[4]	Yes, in memory domain	Complaints of memory decline	No	Abnormal

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Supplemental Table 3. Exact p-values for the pairwise comparisons of Table 1.

	Normal vs. stage 1	Normal vs. stage 2	Normal vs. stage 3	Normal vs. SNAP	Normal vs. unclas-sified	Stage 1 vs. stage 2	Stage 1 vs. stage 3	Stage 1 vs. SNAP	Stage 1 vs. unclas-sified	Stage 2 vs. stage 3	Stage 2 vs. SNAP	Stage 2 vs. unclas-sified	Stage 3 vs. SNAP	Stage 3 vs. unclas-sified	SNAP vs. unclas-sified
Age	0·0007	0·0003	<0·0001	0·0003	<0·0001	0·6531	0·0146	0·8184	0·0084	0·0393	0·4849	0·0254	0·0074	0·9222	0·0039
Female	0·3678	0·4333	0·0859	0·6665	0·1451	0·1763	0·2501	0·2482	0·3864	0·0465	0·6756	0·0778	0·0627	0·7855	0·1060
Education	0·0004	0·7729	0·2942	0·7115	0·3502	0·0127	0·0037	0·0033	0·0044	0·2665	1·000	0·3138	0·2329	0·9123	0·2775
APOE-e4	0·0059	0·0045	0·0025	0·3787	0·7804	0·7731	0·1601	0·0744	0·1008	0·2380	0·0507	0·0758	0·0125	0·0173	0·4943
MMSE	0·0101	0·2195	<0·0001	0·5203	0·0101	0·3397	0·0024	0·0646	0·3532	0·0003	0·5019	0·1159	<0·0001	0·0791	0·0308
CDR-SB	0·3347	0·8380	0·1167	0·8884	0·7059	0·5697	0·3502	0·3239	0·8482	0·1961	0·7719	0·8300	0·1134	0·3621	0·6642
Episodic memory	0·8437	0·2302	<0·0001	0·6128	<0·0001	0·2409	<0·0001	0·5646	<0·0001	<0·0001	0·4567	<0·0001	<0·0001	0·0671	<0·0001
Aβ1-42	<0·0001	<0·0001	<0·0001	0·0396	0·1847	0·8954	0·5672	<0·0001	<0·0001	0·6425	<0·0001	<0·0001	<0·0001	<0·0001	0·0209
T-tau	0·5470	<0·0001	<0·0001	<0·0001	0·5634	<0·0001	<0·0001	<0·0001	0·8438	0·3126	0·0004	<0·0001	0·0005	<0·0001	<0·0001
P-tau181	0·9111	<0·0001	<0·0001	<0·0001	0·1929	<0·0001	<0·0001	<0·0001	0·2537	0·7014	0·0638	<0·0001	0·0955	<0·0001	<0·0001
Follow-up	0·6396	0·7969	0·1251	0·9818	0·8928	0·5625	0·0934	0·6575	0·8906	0·2185	0·8249	0·7838	0·1415	0·2084	0·8876
Progression to CDR≥0·5	0·0077	0·0002	<0·0001	0·1338	0·0005	0·1575	0·0033	0·1770	0·1716	0·0641	0·0069	0·7962	0·0001	0·1875	0·0145
Mortality	0·0180	0·0604	0·0003	0·0673	0·2077	0·7250	0·0850	0·4808	0·7018	0·0623	0·7953	0·8893	0·0187	0·1436	0·9787

Results are p-values of pairwise comparisons of Table 1. Aβ= amyloid-beta, *APOE*=Apolipoprotein E, CDR-SB=Clinical Dementia Rating Scale Sum of Boxes, MMSE=Mini-Mental State Examination, p-tau=phosphorylated tau, SNAP=Suspected Non-Alzheimer Pathophysiology, t-tau= total tau.

Supplemental Table 4. Distribution of preclinical AD stages

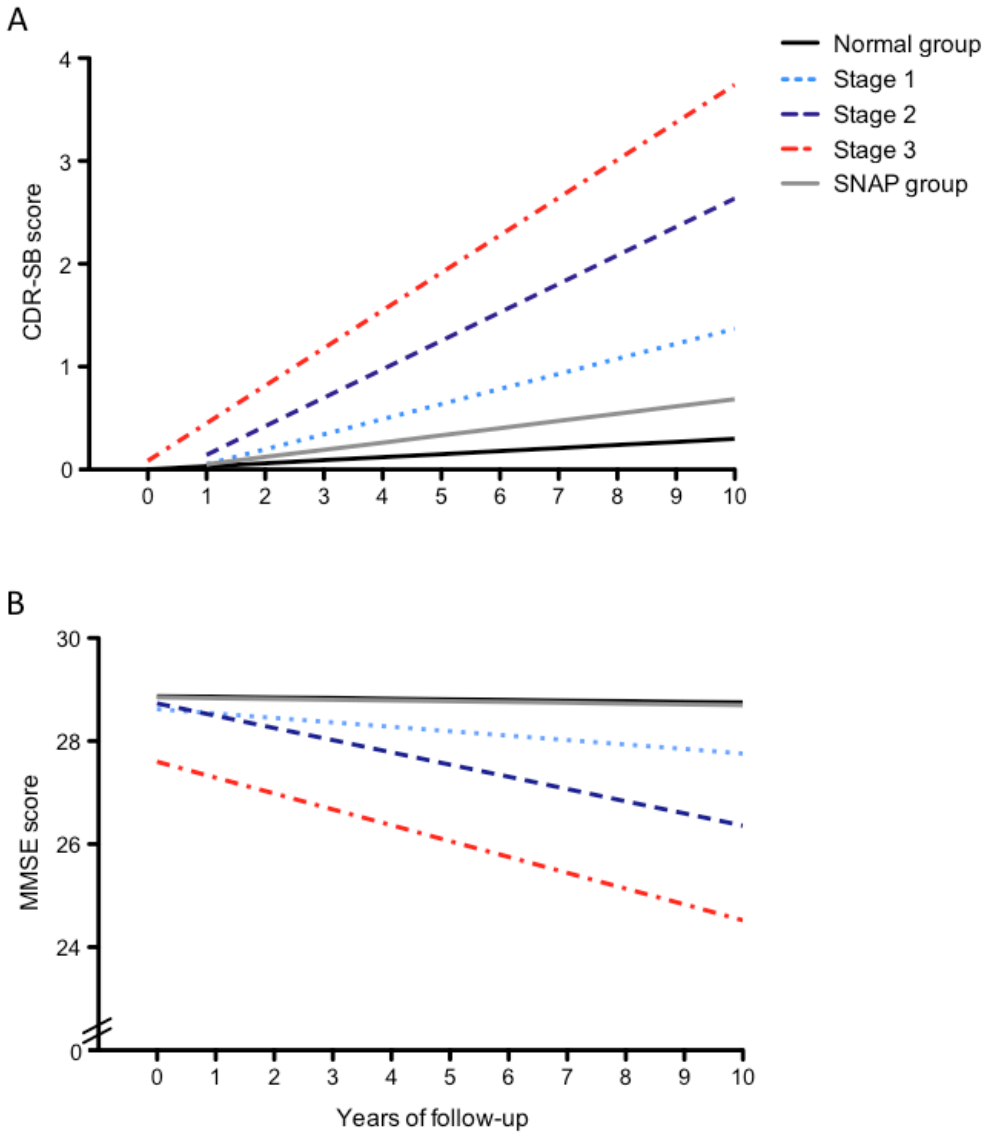
Stage	CSF Aβ1-42 <459 pg/mL	CSF t-tau >339 pg/mL	CSF p-tau181 >67 pg/mL	Cognition <-1·25 SD	N (%)	Overall N (%)
Normal group	-	-	-	-	129 (41·5)	129 (41·5)
Stage 1	+	-	-	-	47 (15)	47 (15)
Stage 2	+	+	-	-	8 (3)	36 (12)
	+	-	+	-	5 (2)	
	+	+	+	-	23 (7)	
Stage 3	+	+	-	+	1 (0·5)	13 (4)
	+	+	+	+	12 (3·5)	
SNAP group	-	+	-	-	14 (4·5)	72 (23)
	-	+	-	+	2 (0·5)	
	-	-	+	-	9 (3)	
	-	+	+	-	45 (14·5)	
	-	+	+	+	2 (0·5)	
Unclassified	-	-	-	+	11 (3·5)	14 (4·5)
	+	-	-	+	3 (1)	

Results are number (%) of participants identified in the different stages based on CSF markers with optimal Youden cut-offs: Abnormal CSF Aβ1-42 <459 pg/mL, t-tau >339 pg/mL, p-tau181 >67 pg/mL. Cognition was an episodic memory composite score of the Associate Learning Test, Logical Memory Test, and Selective Reminding Test, with a cut-off at the lowest 10th percentile: -1·25 SD. Aβ=beta amyloid, AD=Alzheimer's Disease, p-tau=phosphorylated tau, SNAP=Suspected Non-Alzheimer Pathophysiology, t-tau=total tau.

Supplemental Table 5. Preclinical AD and its outcome according to age and *APOE* genotype

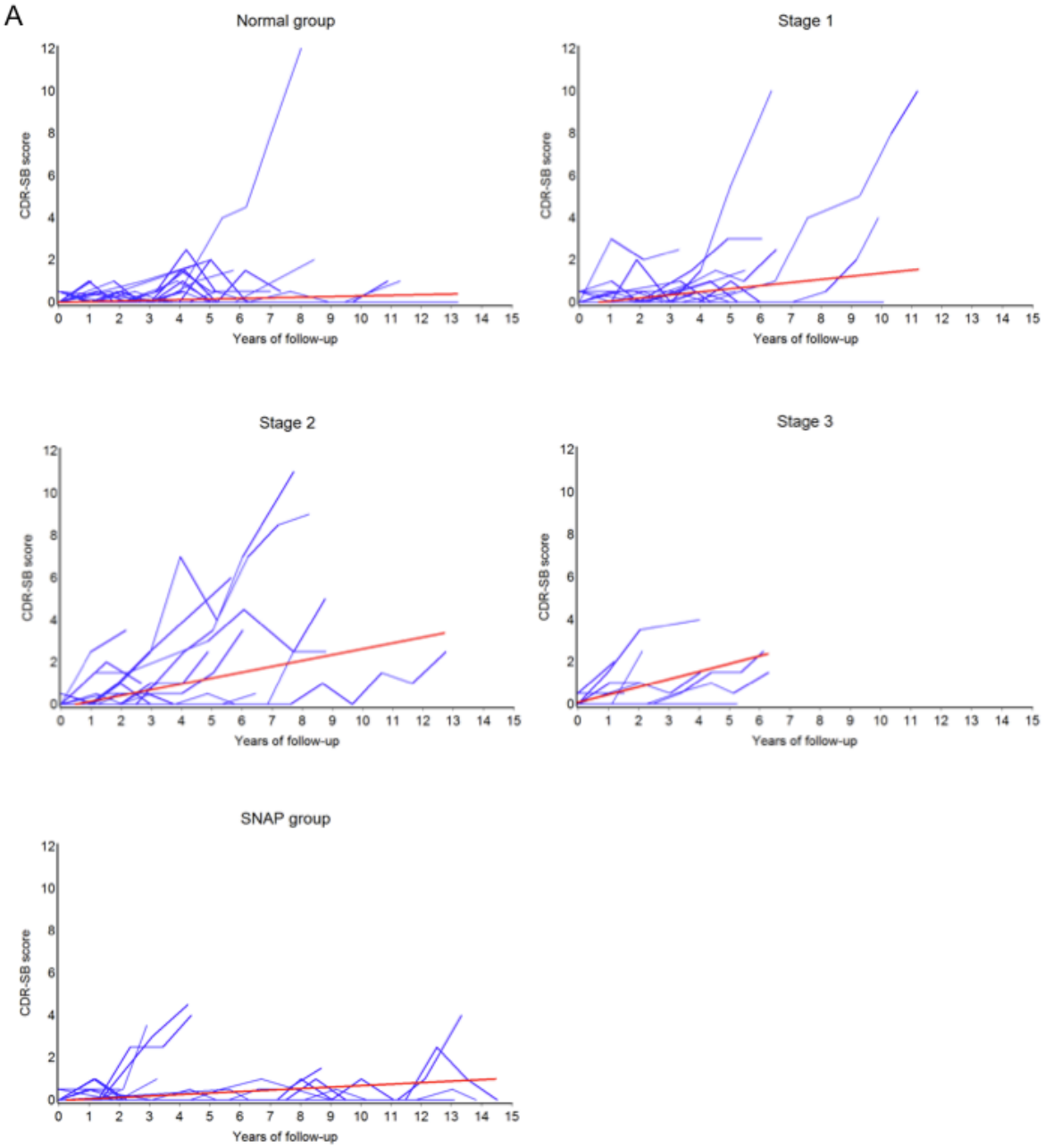
A	Baseline prevalence		Progression to CDR≥0·5 symptomatic AD	
	Age ≤72 (n=169)	Age >72 (n=142)	Age ≤72 (n=6)	Age >72 (n=25)
No preclinical AD	125 (74%)	90 (64·5%)*	1 (1%)	9 (10%)
Normal group	90 (53·5%)	39 (27·5%)	0 (0%)	2 (5%)
SNAP group	31 (18·5%)	41 (29%)	1 (3%)	3 (7%)
Unclassified	4 (2%)	10 (7%)	0 (0%)	4 (40%)
Preclinical AD	44 (26%)	52 (36·5%)	5 (11%)	17 (33%)
Stage 1	25 (15%)	22 (15·5%)	1 (4%)	5 (23%)
Stage 2	17 (10%)	19 (13%)	2 (12%)	7 (37%)
Stage 3	2 (1%)	11 (8%)	2 (100%)	5 (45·5%)
B	**Baseline prevalence**		**Progression to CDR≥0·5 symptomatic AD**	
	APOE-ε4- (n=205)	*APOE*-ε4+ (n=106)	*APOE*-ε4- (n=18)	*APOE*-ε4+ (n=13)
No preclinical AD	158 (77%)	57 (53%)***	7 (4%)	3 (5%)
Normal group	97 (47%)	32 (30%)	2 (2%)	0 (0%)
SNAP group	50 (24·5%)	22 (21%)	2 (4%)	2 (9%)
Unclassified	11 (5·5%)	3 (2%)	3 (27%)	1 (33%)
Preclinical AD	47 (23%)	49 (47%)	11 (23%)	11 (22%)
Stage 1	25 (12%)	22 (21%)	2 (8%)	4 (18%)
Stage 2	18 (9%)	18 (17%)	6 (33%)	3 (17%)
Stage 3	4 (2%)	9 (9%)	3 (75%)	4 (44%)

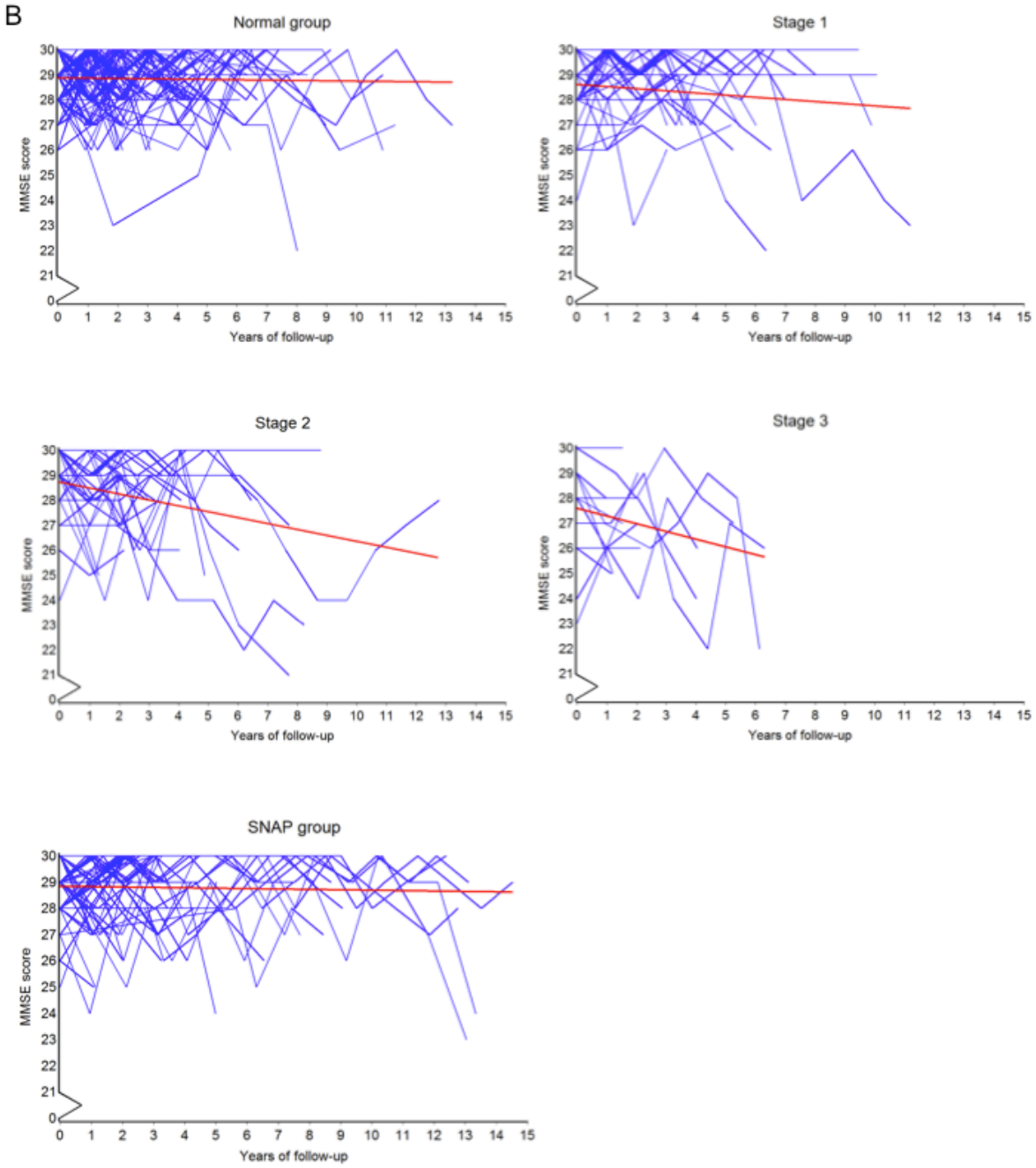
Data are baseline number (%) of participants with and without preclinical AD, and number (%) of these participants that progressed to CDR≥0·5 symptomatic AD by dichotomous age (A) and *APOE* genotype (B) grouping. AD=Alzheimer's Disease, *APOE*=Apolipoprotein E, CDR=Clinical Dementia Rating scale, SNAP=Suspected Non-Alzheimer Pathophysiology. *P<0·05 compared to age ≤72, ***p<0·001 compared to *APOE*-e4-, based on Chi-squared tests for 2 by 2 tables.



Supplemental Figure 1. Annual rate of change in CDR-SB and MMSE by preclinical AD stage

Graphs show the estimated annual rate of change in CDR-SB (A) and MMSE (B), based on slopes according to each preclinical AD stage, corrected for age, gender, education, and *APOE* genotype. The black line represents participants in the normal group; light blue, stage 1; dark blue, stage 2; red, stage 3; and grey, SNAP. CDR-SB=Clinical Dementia Rating scale Sum of Boxes (range 0-18, with 0 as the best score), MMSE=Mini Mental State Examination (range 0-30, with 30 as the best score).


A
Normal group
Stage 1
Stage 2
Stage 3
SNAP group
CDR-SB score
Years of follow-up



Supplemental Figure 2. Individual cognitive trajectories for CDR-SB and MMSE by preclinical AD stage

Graphs show individual cognitive trajectories (blue) and the overall estimated annual rate of change (red) for CDR-SB (A) and MMSE (B) by preclinical AD stage. CDR-SB=Clinical Dementia Rating scale Sum of Boxes (range 0-18, with 0 as the best score), MMSE=Mini Mental State Examination (range 0-30, with 30 as the best score), SNAP= Suspected Non-Alzheimer Pathophysiology.

Supplemental Text 1. Cohort information

The overall rate of progression from CDR 0 to CDR 0.5 or greater in our sample was 10% (32/311) over an average of 4 years of follow-up. This percent is comparable to the Mayo Clinic Study of Aging (MCSA), a population based longitudinal program in which many participants have multiple imaging procedures and some have lumbar puncture. The overall rate of progression from cognitive normality to MCI in the MCSA sample was 20% (296/1450) with a median follow-up of 3·4 years, but the MCI construct in the MCSA program was unstable as 34% of MCI individuals reverted to cognitive normality at subsequent follow-up.[1] Removing the MCSA individuals who later reverted to normal leaves 196 of the 1450 (13·5%) cognitively normal persons who developed MCI/symptomatic AD, very similar to our 10%.

Reference

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Supplemental Table 6. Preclinical AD and its outcome using different classification approaches

A) Use of different CSF cutoffs; Aβ1-42 <500, t-tau <440, p-tau181 <78 pg/mL

	Normal group	Stage 1	Stage 2	Stage 3	SNAP group	Unclassified group
Proportion stages, n	131 (42%)	85 (27%)	28 (9%)	13 (4%)	37 (12%)	17 (6%)
Progression to CDR≥0·5 symptomatic AD, n	2 (1.5%)	9 (11%)	7 (25%)	7 (54%)	2 (5%)	5 (29%)
Mortality, n	1 (1%)	7 (8%)	3 (11%)	4 (31%)	4 (11%)	1 (6%)

B) Stage 3 defined as a score in the lowest 10th percentile of any cognitive domain

	Normal group	Stage 1	Stage 2	Stage 3	SNAP group	Unclassified group
Proportion stages, n	104 (33%)	37 (12%)	28 (9%)	21 (7%)	71 (23%)	50 (16%)
Progression to CDR≥0·5 symptomatic AD, n	2 (2%)	6 (16%)	8 (29%)	8 (38%)	4 (6%)	4 (8%)
Mortality, n	0 (0%)	5 (14%)	1 (4%)	6 (29%)	4 (6%)	4 (8%)

C) Participants with a baseline CDR-SB of 0·5 excluded (n=293)

	Normal group	Stage 1	Stage 2	Stage 3	SNAP group	Unclassified group
Proportion stages, n	123 (42%)	43 (15%)	34 (12%)	11 (4%)	69 (24%)	13 (4%)
Progression to CDR≥0·5 symptomatic AD, n	2 (2%)	6 (14%)	9 (27%)	6 (55%)	4 (6%)	3 (23%)
Mortality, n	2 (2%)	5 (12%)	2 (6%)	4 (36%)	4 (6%)	0 (0%)

Results are number (%) for (A) previously applied CSF cut-offs of our center, (B) a different definition of stage 3 based on the episodic memory, semantic memory, working memory, and visuospatial composite score (described in Johnson et al 2009), and (C) only participants with CDR-SB=0 at baseline. AD=Alzheimer's disease, CDR-SB=Clinical Dementia Rating Scale Sum of Boxes, CSF=cerebrospinal fluid, SNAP= Suspected Non-Alzheimer Pathophysiology.

Supplemental Table 7. Prediction of the unclassified group for CDR≥0·5 symptomatic AD, mortality, and annual cognitive decline

A) Prediction of the unclassified group for CDR≥0·5 symptomatic AD and mortality

	Progression to CDR≥0·5 symptomatic AD					Mortality risk			
	5-year progression rate	Uncorrected SHR (95% CI)	Difference compared to normal group	Corrected SHR (95% CI)	Difference compared to normal group	Uncorrected HR (95% CI)	Difference compared to normal group	Corrected HR (95% CI)	Difference compared to normal group
Normal group	2%	Reference		Reference		Reference		Reference	
Unclassified group	34%	24·2 (4·7-125·5)	P=0·0002	16·4 (2·8-95·8)	P=0·0019	5·3 (0·5-59·9)	P=0·1749	2·8 (0·2-36·9)	P=0·4305

Results are 5-year progression rate (cumulative incidence rate) to CDR≥0·5 symptomatic AD and associated subhazard ratio (SHR, 95% CI) for progression to CDR≥0·5 symptomatic AD calculated using a Fine & Gray subdistribution hazards model, and hazard ratio (HR, 95% CI) for mortality calculated using Cox regression analyses.. Analyses are shown as both uncorrected and corrected for baseline age, gender, education, and *APOE* genotype. AD=Alzheimer's disease, CDR=Clinical Dementia Rating scale, SNAP=Suspected Non-Alzheimer Pathophysiology, SHR=Subhazard Ratio, HR=Hazard Ratio.

B) Annual rate of change in CDR-SB and MMSE for the unclassified group

Stages	Slope CDR-SB	P-value slope	Difference compared to normal group	Slope MMSE	P-value slope	Difference compared to normal group
Normal group	0·03 (0·03)	P=0·2661	Reference	-0·01 (0·03)	P=0·6673	Reference
Unclassified group	0·33 (0·08)	P=0·0001	P=0·0007	-0·24 (0·10)	P=0·0127	P=0·0258

Data are slopes (SE) corrected for age, gender, education, and APOE genotype and comparison to other groups. AD=Alzheimer's disease, CDR-SB=Clinical Dementia Rating scale Sum of Boxes (range 0-18, with 0 as the best score), MMSE=Mini Mental State Examination (range 0-30, with 30 as the best score), SNAP=Suspected Non-Alzheimer Pathophysiology.

CHAPTER 9

PREVALENCE AND PROGNOSIS OF PRODROMAL ALZHEIMER'S DISEASE:

A COMPARISON OF THE INTERNATIONAL WORKING GROUP CRITERIA AND NATIONAL INSTITUTE OF AGING-ALZHEIMER'S ASSOCIATION CRITERIA

Stephanie J.B. Vos, Frans Verhey, Andy Simmons, Michaela Litchmore Dunbar, Giovanni Frisoni, Cristina Muscio, Hilkka Soininen, Sanna-Kaisa Herukka, Erik Salmon, Christine Bastin, Sebastiaan Engelborghs, Stefan Van der Mussele, Isabel Santana, Raquel Lemos, Alexandre Mendonça, Dina Silva, Flavio Nobili, Silvia Morbelli, Robert Perneczky, Mira Didic, Bart van Berckel, Lars-Olof Wahlund, Harald Hampel, Magda Tsolaki, Lennart Minthon, Philip Scheltens, Pieter Jelle Visser and the Alzheimer's Disease Neuroimaging Initiative

ABSTRACT

Objective

Our aim was to compare the International Working Group (IWG) criteria and the National Institute of Aging-Alzheimer Association (NIA-AA) criteria for prodromal AD.

Methods

1243 subjects were recruited from the EADC network and ADNI. Inclusion criteria were baseline MCI diagnosis and data of at least one AD biomarker (CSF Aβ, CSF tau, FDG-PET or MRI) and one clinical follow-up available. 494 (40%) subjects had both an amyloid and neural injury marker.

Results

According to the IWG criteria, 670 (54%) subjects were classified as having prodromal AD. They had at least one abnormal biomarker and impairment in episodic memory. 353 (53%) of these subjects progressed to AD-type dementia compared to 128 (22%) subjects in the normal group. According to the NIA-AA criteria, 74 (15%) were classified in the low-AD-likelihood group (no amyloid deposition or neural injury), 250 (51%) in the high-AD-likelihood group (amyloid deposition and neural injury), 29 (6%) in the conflicting biomarker group with amyloid deposition without neural injury, and 141 (29%) in the Suspected Non-Alzheimer Pathology group (SNAP; neural injury without amyloid deposition). At follow-up, 10 (14%) subjects in the low-AD-likelihood group, 155 (62%) in the high-AD-likelihood group, 7 (24%) in the group with amyloid deposition and neural injury, and 42 (30%) in the SNAP group progressed to AD-type dementia.

Conclusions

Our findings support the use of biomarkers in subjects with MCI according to the IWG and NIA-AA criteria and highlight the importance of differentiating between amyloid and neural injury markers to give an optimal prognosis.

INTRODUCTION

Mild cognitive impairment (MCI) usually represents the stage between normal cognitive functioning and dementia and often Alzheimer's disease (AD)-type dementia.[1] However, not all subjects with MCI have AD pathology.[2, 3] Increasing knowledge gained on the underlying pathophysiology of AD in the last years has led to the development of two sets of research criteria for prodromal AD: those by the International Working Group (IWG)[4, 5] and those by the National Institute of Aging and the Alzheimer Association (NIA-AA).[6]

These new research criteria allow detection of AD before the dementia stage and assume that AD pathophysiological changes in the MCI stage increase the confidence that these subjects will progress to AD-type dementia. The IWG criteria require memory impairment and at least one abnormal AD biomarker for prodromal AD, whereas the NIA-AA criteria did not specify cognitive impairment and related the number of positive biomarkers to the likelihood that the diagnosis of MCI is due to AD. Recent studies showed that these criteria have a fair to good predictive ability for the progression to AD-type dementia.[7-9] However, it is unknown how these sets of criteria differ in identification of prodromal AD and in prediction of AD-type dementia.

The aim of the present study was to compare the prevalence and prognostic value of these two sets of diagnostic criteria in subjects with MCI by means of a large multicenter sample and well-established AD biomarkers.

METHODS

Subjects

Subjects were recruited from several independent multicenter studies: the DESCRIPA study,[10] AddNeuroMed study,[11] the European Alzheimer's Disease Consortium (EADC) PET project,[12] and the American Alzheimer's Disease Neuroimaging Initiative (ADNI) study[13] (downloaded in February 2012); and from 7 cohorts of the EADC (Amsterdam, Antwerp, Brescia, Coimbra, Kuopio, Liege, Lisbon). All studies are described more in detail elsewhere.

Inclusion criteria of the present study were baseline diagnosis of MCI, availability of at least one of the following biomarker data at baseline: beta amyloid (Aβ)1-42 in cerebrospinal fluid (CSF), tau in CSF, qualitative or quantitative measures of medial temporal lobe atrophy (MTA) or hippocampal volume (HCV) on magnetic resonance imaging (MRI), or cerebral glucose metabolism on brain 18F-fluorodeoxyglucose positron emission tomography (18F FDG PET), and at least one clinical follow-up assessment. Exclusion criteria were diagnosis of dementia at baseline or any other somatic, psychiatric or neurological disorder that might have caused the cognitive complaints or impairments.

Clinical and cognitive assessment

Clinical and cognitive assessments were performed according to the routine protocol at each site, including a clinical interview, scoring on the MMSE, neuropsychological assessment and biomarker assessment.

Baseline diagnosis of MCI was made according to the criteria of Petersen et al.[1] Raw scores on neuropsychological tests were converted to z scores or equivalent scores at each center. Cognitive impairment was defined as z score <-1.5 SD or equivalent score on at least one cognitive test. Subjects with a z score <-1.5 SD on a memory test were classified as having amnestic MCI. Supplementary Table 1 describes the neuropsychological tests that were used at each center. Decline in cognition was calculated as the difference between the MMSE score at baseline and the score at last follow-up. Primary outcome measures were prevalence of prodromal AD and progression to AD-type dementia according to the DSM-IV[14] and NINCDS-ADRDA[15] criteria. Secondary outcome measure was cognitive decline on the MMSE. The study was approved by the Medical Ethics Committee of each center and all the recruited subjects provided written informed consent.

Biomarker assessment

Biomarker assessment was performed according to the routine protocol at each site. We used routine biomarker cut-offs of each center to define abnormal biomarkers. All biomarker cut-offs are listed in Supplemental Table 2.

Classification according to the IWG and NIA-AA criteria

For the IWG criteria, subjects were classified in 2 groups. Subjects were classified in the prodromal AD group if they had memory impairment and at least one abnormal biomarker. They were classified in the normal group if all biomarkers were normal or if they had no memory impairment.

For the NIA-AA criteria, we differentiated between amyloid (e.g. CSF Aβ1-42) and neuronal injury markers (e.g. CSF tau, FDG-PET, MTA or HCV) for classification. Of the subjects who had an amyloid and neuronal injury marker, subjects were classified in the low-AD-likelihood group if both amyloid and neuronal injury markers were normal, in the high-AD-likelihood group if both amyloid and at least one neuronal injury marker were abnormal, in the conflicting biomarker Isolated Amyloid Pathology (IAP) group if the amyloid marker was abnormal and injury marker normal, and in the conflicting biomarker Suspected Non-Alzheimer Pathophysiology (SNAP) group if the injury marker was abnormal and amyloid marker normal.[9, 16] Of the subjects who had only one biomarker available, subjects were classified in the intermediate-AD-likelihood group if the marker that was tested was abnormal. Biomarker info was considered uninformative/inconclusive if only one of them was tested and normal.

Statistical analyses

Statistical analyses were done with SPSS version 19.0 (Chicago, IL, USA) with significance set at $p<0.05$. Baseline differences between the biomarker subgroups were analyzed using ANOVA for continuous variables and χ^2 tests or logistic regression models for categorical variables. Standard Cox proportional hazards models were used to test the predictive ability of the IWG and NIA-AA criteria for AD-type dementia. Analyses were corrected for age,

gender, and center. The relation between biomarker groups of the IWG and NIA-AA criteria and change on the MMSE was assessed by slope analyses with general linear mixed models. The analyses included the baseline score and last follow-up score (up to 9 years after baseline). The model was specified with a random intercept and slope and with center as a random effect because this model provided the best -2 log likelihood compared with models with simpler covariance structures. Analyses were corrected for age and gender.

RESULTS

Sample demographics

We included 1243 subjects. 494 subjects had both an amyloid and neuronal injury marker (CSF Aβ1-42 and tau/MRI) and 749 subjects had only a neuronal injury marker (FDG-PET n=192; MRI n=557). Supplemental Table 3 shows the number of subjects for each biomarker by center and Supplemental Table 4 presents baseline sample characteristics for the total sample and the separate biomarker samples.

IWG criteria

Of the 1243 subjects, 670 (54%) were classified as having prodromal AD according to the IWG criteria. Differences in baseline demographics and outcome between the prodromal AD group and the normal group are presented in Table 1. Subjects with prodromal AD were more likely to progress to AD-type dementia than subjects in the normal group with normal biomarkers (HR=3.7, p<0.001; Figure 1, Table 2). Subjects with prodromal AD also showed a larger decline on the MMSE than subjects in the normal group (p<0.001; Figure 2, Table 3). Of the subjects in the normal group with abnormal biomarkers (n=163), 52 (32%) progressed to AD-type dementia compared to 75 (18%) of the subjects in the normal group with normal biomarkers (n=410; HR=1.9, p=0.001).

NIA-AA criteria

Of the subjects with both an amyloid and neuronal injury marker available (n=494), 74 (15%) were classified in the low-AD-likelihood group according to the NIA-AA criteria, 250 (51%) in the high-AD-likelihood group, 29 (6%) in the conflicting IAP group, and 141 (29%) in the conflicting SNAP group. Of the subjects with only a neuronal injury marker available (n=749), 413 (55%) were classified in the intermediate-AD-likelihood group, and 336 (45%) in the inconclusive group. Baseline demographics and outcome of the biomarker groups are presented in Table 1. The high-AD-likelihood, intermediate-AD-likelihood, and SNAP group had a higher risk of progression to AD-type dementia compared to the low-AD-likelihood group (high HR=7.8, p<0.001; intermediate HR=6.4, p<0.001; SNAP HR=2.5, p=0.02; Figure 1, Table 2). The IAP group tended to have a higher risk of progression to AD-type dementia than the low-AD-likelihood group (HR=2.3, p=0.10). Decline on the MMSE showed a similar pattern for the biomarker groups (Figure 2, Table 3).

Table 1. Demographics and clinical outcome by biomarker subgroups

	IWG criteria		NIA-AA criteria					
	Normal group Normal markers or no aMCI N=573	Prodromal AD aMCI and at least 1 marker + N=670	Low AD likelihood Amyloid - Injury - N=74	High AD likelihood Amyloid + Injury + N=250	Conflicting A IAP Amyloid + Injury - N=29	Conflicting B SNAP Amyloid - Injury + N=141	Intermediate AD likelihood Amyloid ? Injury + N=413	Uninformative /inconclusive Amyloid ? Injury - N=336
Age, y	71.3 (7.8)^{P}	72.5 (7.4)	69.0 (8.9)^{H,I,U}	72.5 (7.0)^{L,A,U}	68.2 (8.4)^{H,I}	71.0 (8.1)^{I}	73.5 (7.0)^{L,A,B,U}	70.9 (7.7)^{L,H,I}
Female, n	320 (56%)^{P}	324 (48%)	41 (55%)	116 (46%)^{U}	11 (38%)^{U}	66 (47%)^{U}	207 (50%)^{U}	203 (60%)^{H,A,B,I}
Education, y	10.3 (5.0)^{P}	11.7 (4.6)	10.7 (3.8)^{H}	12.4 (4.7)^{L,B,I,U}	12.0 (4.5)	11.3 (4.5)^{H,U}	10.8 (4.6)^{H}	10.3 (5.2)^{H,B}
Follow-up, y	2.5 (1.5)^{P}	2.3 (1.4)	2.9 (1.8)^{H,I}	2.3 (1.1)^{L,B}	2.7 (1.7)^{I}	2.8 (1.7)^{H,I}	2.1 (1.3)^{L,A,B,U}	2.5 (1.5)^{I}
APOE-e4	161 (38%)^{P}	279 (57%)	16 (26%)^{H,I}	158 (67%)^{L,A,B,I,U}	12 (43%)^{H}	40 (32%)^{H,I}	133 (55%)^{L,H,B,U}	81 (38%)^{H,I}
MMSE baseline	27.2 (2.4)^{P}	26.4 (2.3)	26.4 (3.0)^{A,U}	26.2 (2.4)^{A,I,U}	27.7 (1.6)^{L,H,B,I}	26.6 (2.5)^{A,U}	26.6 (2.2)^{H,A,U}	27.3 (2.3) ^{L,H,B,I}
MMSE last follow-up	25.9 (4.0)^{P}	23.1 (4.6)	25.7 (4.2)^{H,I}	22.4 (4.9)^{L,A,B,I,U}	26.6 (3.4)^{H,I}	24.9 (4.9)^{H,U}	24.0 (4.0)^{L,H,A,U}	26.0 (4.0)^{H,B,I}
Progression to AD-type dementia	128 (22%)^{P}	353 (53%)	10 (14%)^{H,B,I}	155 (62%)^{L,A,B,I,U}	7 (24%)^{I,H}	42 (30%)^{L,H,I,U}	201 (49%)^{L,H,A,B,U}	65 (19%)^{H,B,I}
Progression to other dementia	41 (7%)	17 (3%)	9 (12%)^{H,I,U}	2 (1%)^{L,B,I,U}	1 (3%)	11 (8%)^{H}	18 (4%)^{L,H}	16 (5%)^{L,H}

Results are mean (SD) for continuous variables or frequency (%). For APOE: n=. MMSE=Mini-Mental State Examination (range 0-30). IWG=International Working Group, NIA-AA=National Institute of Aging and Alzheimer's Association, IAP=isolated amyloid pathology, SNAP=suspected non-Alzheimer pathophysiology, *APOE*=apolipoprotein E, AD=Alzheimer's Disease. $^{L}p<0.05$ compared to low AD likelihood, $^{H}p<0.05$ compared to high AD likelihood, $^{A}p<0.05$ compared to conflicting A group IAP, $^{B}p<0.05$ compared to conflicting B group SNAP, $^{I}p< 0.05$ compared to intermediate AD likelihood, $^{U}p<0.05$ compared to uninformative/inconclusive.

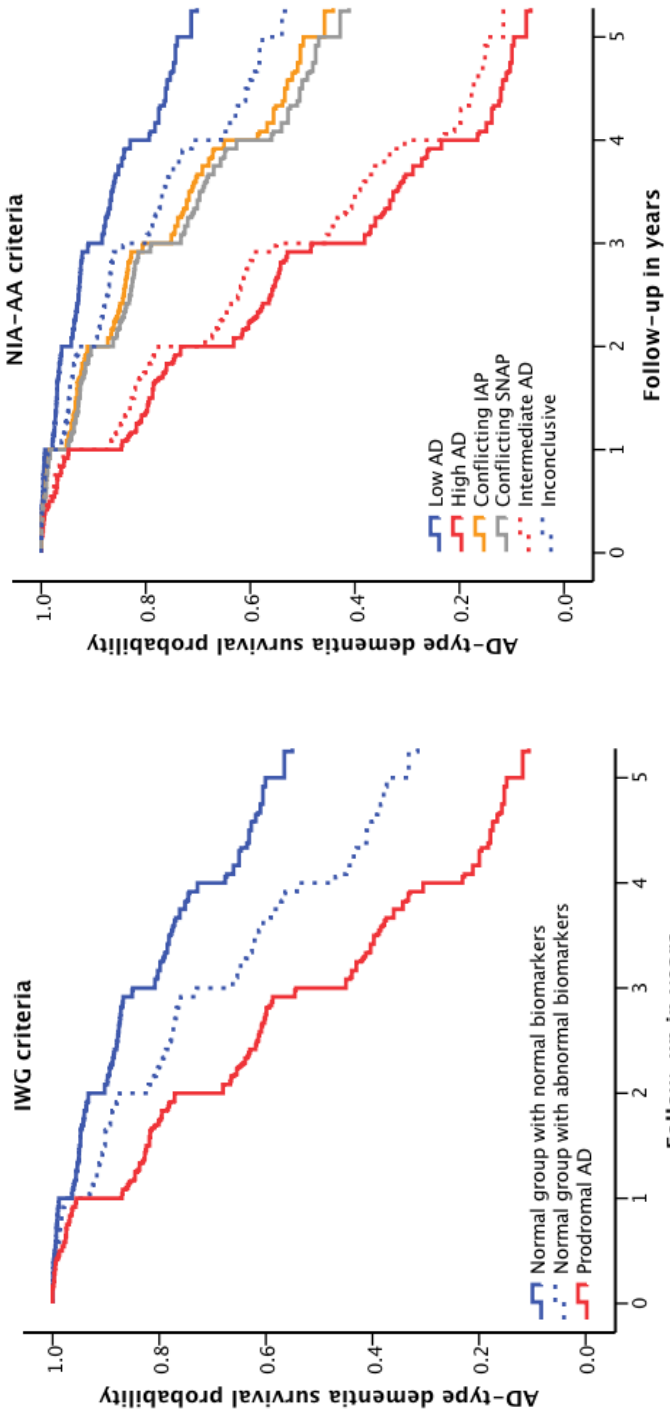

Figure 1. AD-type dementia survival probability by the IWG and NIA-AA criteria

The graphs represent the AD-type dementia survival probability according to the IWG criteria (on the left) and the NIA-AA criteria (on the right). IWG: The normal group represents subjects with no memory impairment and normal biomarkers. The normal group with abnormal biomarkers represents those with no memory impairment and at least one abnormal biomarker. The prodromal AD group represents subjects with memory impairment and at least one abnormal biomarker. NIA-AA: The low AD likelihood group represents subjects without amyloid pathology and neuronal injury, the high AD likelihood group represents subjects with both amyloid and neuronal injury, the IAP group is a conflicting biomarker group with amyloid pathology without neuronal injury, the SNAP group is a conflicting biomarker group with neuronal injury without amyloid pathology, the intermediate AD group represents subjects with neuronal injury without information on amyloid pathology, the inconclusive group represents subjects without neuronal injury without information on amyloid pathology. AD=Alzheimer's disease, IWG=International Working Group, NIA-AA=National Institute of Aging and Alzheimer's Association, IAP=isolated Alzheimer pathology, SNAP=suspected non-Alzheimer pathophysiology.

Table 2. AD-type dementia survival probability by the IWG and NIA-AA criteria

	Hazard Ratio (95% CI)	P-value
IWG		
Normal group with normal biomarkers	Reference	
Normal group with abnormal biomarker(s)	1.9 (1.3-2.8)	0.001
Prodromal AD	3.7 (2.9-4.9)	<0.001
NIA-AA		
Low AD likelihood	Reference	
High AD likelihood	7.8 (3.7-16.3)	<0.001
Conflicting IAP	2.3 (0.8-6.3)	0.10
Conflicting SNAP	2.5 (1.2-5.2)	0.02
Intermediate AD likelihood	6.4 (3.0-13.5)	<0.001
Uninformative/inconclusive	1.8 (0.8-4.0)	0.13

Results are Hazard Ratios (95% CI) for progression to AD-type dementia calculated using Cox regression analyses and corrected for age, gender, and center for all the biomarker groups. IWG=International Working Group, NIA-AA=National Institute of Aging and Alzheimer's Association, IAP=isolated amyloid pathology, SNAP=suspected non-Alzheimer pathophysiology, AD=Alzheimer's Disease.

NIA-AA subanalyses

The NIA-AA criteria do not differentiate between the several neuronal injury markers. We tested whether the use of different injury markers resulted in different findings. When MRI and CSF tau were used separately in combination with CSF Aβ1-42, results were generally the same (Supplemental Table 5). However, analyses based on CSF tau showed higher progression rates for the SNAP group and lower progression rates for the high-AD-likelihood group compared to the analyses based on MRI (SNAP: 36 vs. 28%, high: 64 vs. 71%).

In the subgroup of subjects who had an amyloid marker (CSF Aβ1-42) and two injury markers (CSF tau and MRI; n=408), we found that an abnormal amyloid marker in combination with two abnormal injury markers was better predictive for progression to AD-type dementia than an abnormal amyloid marker in combination with only one abnormal injury marker (74% vs. 41-44%; Supplemental Table 6). Subjects with conflicting injury markers in combination with amyloid deposition had similar progression rates (41 vs. 44%; Supplemental Table 6).

The conflicting IAP and SNAP groups unexpectedly showed similar progression rates to AD-type dementia. We compared CSF Aβ1-42 levels subjects with SNAP who progressed to AD-type dementia with those who did not progress. For ELISA analyses (n=116), we found that SNAP progressors had lower CSF Aβ1-42 levels compared to non-progressors (607 [95% CI 574-641] vs. 847 [95% CI 791-904] pg/mL, p<0.001). For xMAP analyses (ADNI, n=35), we did not find a statistically significant difference between the progressors and non-progressors although this could be a power issue (231 [95% CI 202-260] vs. 248 [95% CI 237-259] pg/mL, p=0.22).

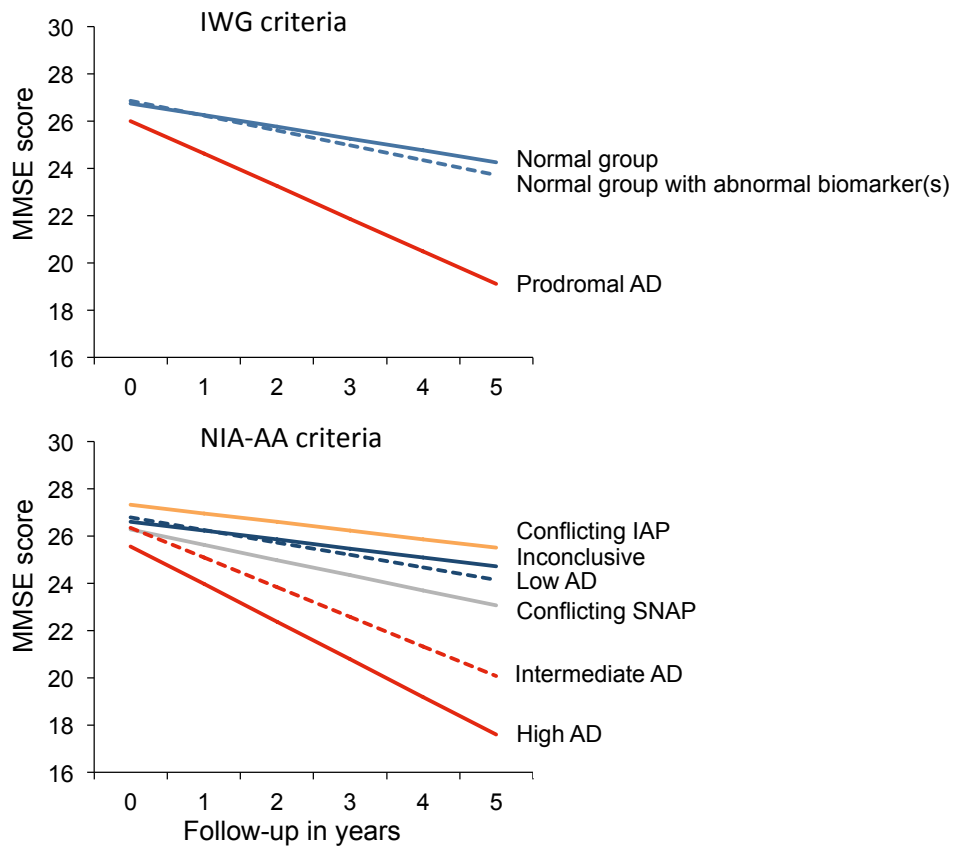

Figure 2. Annual MMSE change for each biomarker group by the IWG and NIA-AA criteria
IWG: The normal group represents subjects with no memory impairment and normal biomarkers. The normal group with abnormal biomarkers represents those with no memory impairment and at least one abnormal biomarker. The prodromal AD group represents subjects with memory impairment and at least one abnormal biomarker NIA-AA: The low AD likelihood group represents subjects without amyloid pathology and neuronal injury, the high AD likelihood group represents subjects with both amyloid and neuronal injury, the IAP group is a conflicting biomarker group with amyloid pathology without neuronal injury, the SNAP group is a conflicting biomarker group with neuronal injury without amyloid pathology, the intermediate AD group represents subjects with neuronal injury without information on amyloid pathology, the inconclusive group represents subjects without neuronal injury without information on amyloid pathology. AD=Alzheimer's disease, IWG=International Working Group, NIA-AA=National Institute of Aging and Alzheimer's Association, IAP=isolated amyloid pathology, SNAP=suspected non-Alzheimer pathophysiology.

Table 3. Annual MMSE change for each biomarker group by the IWG and NIA-AA criteria

	Slope MMSE	P-value slope
IWG criteria		
Normal group with normal biomarkers	-0.50 (0.10)	P<0.001
Normal group with abnormal biomarkers	-0.63 (0.16)	P<0.001
Prodromal AD	-1.38 (0.08)**	P<0.001
NIA-AA criteria		
Low AD	-0.38 (0.23)	P=0.10
High AD	-1.59 (0.13)**	P<0.001
Conflicting IAP	-0.36 (0.39)	P=0.35
Conflicting SNAP	-0.64 (0.17)	P<0.001
Intermediate AD	-1.25 (0.12)*	P<0.001
Inconclusive	-0.53 (0.11)	P<0.001

*P<0.05 compared to the normal group/low AD, **p<0.001 compared to the normal group with normal biomarkers/low AD. AD=Alzheimer's disease, IAP=isolated amyloid pathology, SNAP=suspected non-Alzheimer pathophysiology.

DISCUSSION

This study is the first large-scale study to compare the recently published IWG and NIA-AA criteria for MCI. Our findings showed that subject classifications based on the IWG and NIA-AA criteria were good predictors of likelihood of future cognitive decline and progression to AD-type dementia in subjects with MCI.

The IWG criteria required one abnormal biomarker and episodic memory impairment for classification of prodromal AD. We found that these subjects were 3 times more likely to develop AD-type dementia compared to those classified as being normal. One of the drawbacks of the IWG classification is that the normal group also includes subjects with abnormal biomarkers with non-memory impairment, as they may progress to AD-type dementia.[17] We found that the use of any impairment instead of memory impairment to define prodromal AD led to an increase in prevalence of prodromal AD of more than 10 %. However, progression rates remained similar (data not shown).

The NIA-AA criteria differentiated between amyloid markers and neuronal injury markers and related the number of abnormal biomarkers to the likelihood of that MCI is due to AD. Our results showed that this is beneficial for diagnosis and prognosis since subjects with both abnormal amyloid and neuronal injury markers were almost eight times more likely to develop AD-type dementia compared to those with both normal amyloid and neuronal injury markers. Subjects with one of the two biomarkers abnormal were two to three times more likely to develop AD-type dementia than subjects with both normal biomarkers. Some subjects only had an injury marker available (intermediate or inconclusive group). In that case, the outcome can be very heterogeneous because some have AD, i.e. amyloid pathology, and others have not. In our study, the intermediate group

showed a progression rate to AD-type dementia that was similar to that of the conflicting biomarker groups together.

The similar progression rates to AD-type dementia of the conflicting amyloid and neuronal injury groups were particularly surprising, as subjects with SNAP (abnormal injury marker and normal amyloid marker) are expected to have lower progression rates than those with an abnormal amyloid marker and normal injury marker.[18] Interestingly, subjects with SNAP who progressed to AD-type dementia had CSF Aβ1-42 levels closer to the cut-off value. It could be that these subjects have more comorbidities so that less amyloid pathology is needed to progress to AD-type dementia.[19] Alternatively, SNAP could be an atypical form of AD with less pronounced amyloid pathology. It is also possible that the CSF Aβ1-42 cut-offs used at each center were not optimal to identify all subjects with prodromal AD.

The NIA-AA criteria do not differentiate between the several neuronal injury markers. We showed that subjects with abnormal MRI in combination with an abnormal amyloid marker had higher progression rates to AD-type dementia than subjects with abnormal CSF tau in combination with abnormal amyloid marker. This is in line with the hypothetical model of AD biomarkers,[20, 21] which states that subjects with abnormal MRI are further in the disease process than those with abnormal CSF tau. Moreover, in a subgroup of subjects who had two injury markers available, we found that abnormality of both CSF tau and MRI in combination with an abnormal amyloid marker led to higher progression rates compared to only one abnormal injury marker in combination with an abnormal amyloid marker, suggesting that the former group is even further in the disease process. When we compared the predictive ability of conflicting injury markers, we found that abnormal CSF tau and normal MRI in combination with an abnormal amyloid marker had a similar predictive ability for AD-type dementia as normal tau and abnormal MRI in combination with abnormal amyloid marker. It may be that subjects with abnormal MRI had CSF tau levels just below the cut-off. Alternatively, tau and MRI abnormality could be parallel rather than serial pathways in the disease process.

In order to apply the IWG or NIA-AA criteria, the use of cut-offs for biomarkers and cognition is required. We chose to define cognitive impairment as 1.5 SD below the mean or equivalent score and used routine biomarker cut-offs of each center to define abnormal biomarkers. However, the use of different cut-off could have led to different results, which is a well-known drawback of using dichotomous data and also highlights the need for standardization of cut-offs.

This study had several limitations. No autopsy data were available, which might have led to diagnostic AD misclassifications. Furthermore, different centers used different cognitive tests and biomarker assessment protocols. This might have introduced heterogeneity between centers but findings may better reflect current clinical practice. Moreover, we corrected for center in statistical analyses and used Z-scores for cognitive tests defined at each center. The major strength of our study is the large sample size of well-characterized subjects.

Our results have several implications. First, they show that prodromal AD can be identified based on biomarkers and the IWG and NIA-AA criteria. As these two sets of criteria showed good predictive ability for future cognitive decline they may be useful for selection of subjects for therapeutic trials. Second, knowledge about both amyloid pathology and neuronal injury is preferred to increase the certainty whether someone will develop AD-type dementia or not. Although a longer follow-up is needed to see whether all subjects with only amyloid pathology will become demented. Moreover, the use of multiple neuronal injury markers in combination with an amyloid marker can help to identify the subjects that will soon progress to AD-type dementia. Third, our findings suggest that a more lenient CSF Aβ1-42 cut-off may be needed to identify subjects with SNAP who have prodromal AD.

Together, our findings support the use of biomarker subgroups of the IWG and NIA-AA criteria for MCI, given its good predictive ability for future cognitive decline. Our results suggest that knowledge about both amyloid pathology and neuronal injury is preferred to give an optimal prognosis. More research on conflicting biomarkers and on longitudinal biomarker data is needed for final validation of the use of biomarkers in clinical practice.

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SUPPLEMENTAL DATA

Supplemental Table 1. Neuropsychological tests by center and by cognitive domain

Center	Neuropsychological tests			
	Memory	Language	Executive function	Visuoconstruction
AddNeuroMed (multicenter)	CERAD word list	CERAD verbal fluency animals 1 minute	-	CERAD visuoconstruction
ADNI (multicenter)	RAVLT	Verbal fluency animals 1 minute	TMT part A and B	-
Antwerp	RBANS word list, WMS verbal paired associations, logical memory, RBANS story recall, HDS	RBANS verbal fluency animals 1 minute	TMT part A and B	RBANS figure copy, cube drawing, luria meander
Brescia	RAVLT, story Brescia	Verbal fluency animals 1 minute, phonemic fluency 1 minute	TMT part A and B	Rey copy figure
Coimbra	ADAS-Cog word list, free & cued recall	Fluency animals 1minute	TMT part A and B	ADAS-Cog
Descripa (multicenter)	Rey auditory verbal learning test and CERAD word list were primary tests	Verbal fluency animals 1 minute	TMT part A and B	Rey figure copy test and CERAD figures were primary tests
EADC PET (multicenter)	Rey auditory verbal learning test and CERAD word list were primary tests	Verbal fluency animals 1 minute	TMT part A and B	Rey figure copy test and CERAD figures were primary tests
Kuopio	CERAD word list	-	-	CERAD visuoconstruction
Liege	RI-48 word list	Verbal fluency animals 2 minutes	Stroop	-
Lisbon	CVLT	Verbal fluency supermarket items 1 minute	TMT part A and B	Cube copy
VUmc Amsterdam	Rey auditory verbal learning test	Verbal fluency animals 1 minute	TMT part A and B	Rey figure copy test

If multiple tests were performed for a cognitive domain, tests were ranked by preference; if scores of the first test were not available, we used scores of the second tests etc. CERAD=The Consortium to Establish a Registry for Alzheimer's Disease, RAVLT=Rey auditory Verbal Learning Test, TMT=Trail Making Test, RBANS=Repeatable Battery for the Assessment of Neuropsychological Status, WMS=Wechsler Memory Scale, HDS= Hierarchic Dementia Scale, ADAS-Cog=Alzheimer's Disease Assessment Scale-cognitive subscale, CVLT= Californian Verbal Learning Test. Descripa tests per center are described elsewhere.

Supplemental Table 2. Biomarker cut-offs per center

	CSF Aß1-42*	CSF tau*	CSF p-tau*	FDG-PET	HCV	MTA
AddNeuroMed	-	-	-	-	Z-score <-0.0003 (calculated in healthy controls)	> 2
ADNI	<192[a]	>399[a]	>70[a]	-	< 5.389[b], LEAP method	-
Antwerp	Z-score <-1.5 SD	Z-score <-1.5 SD	Z-score <-1.5 SD	-	-	-
Brescia	<500[c]	>450/>500[c]	-	-	< 5th percentile of volume distribution of controls[d]	> 2
Coimbra	Lab reference values	Lab reference values	Lab reference values	-	-	> 2
Descripa	<551[e]	>375[e]	>52[e]	-	< 5.389[b], LEAP method	> 2
EADC PET	-	-	-	AD profile	-	-
Kuopio	<452	>399	>70	-	Right: men<14.9, women<15.3[f] Left: men<14.3, woman<14.1[f]	-
Liege	-	-	-	AD profile	-	-
Lisbon	<445	>300	>61	-	Z-score <-1.5 SD	-
Amsterdam VUmc	<551[e]	>375[e]	>52[e]	-	< 5.389[b], LEAP method	> 2

*CSF analyses were performed using commercially available sandwich ELISAs for all centers except for the ADNI study, which used the multiplex xMAP Luminex platform. PET=Positron Emission Tomography, CSF=cerebrospinal fluid, Aß=amyloid-beta, FDG= fludeoxyglucose, HCV=hippocampal volume, MTA=medial temporal lobe atrophy. [a]Shaw LM, Vanderstichele H, Knapik-Czajka M, et al. Cerebrospinal fluid biomarker signature in Alzheimer's disease neuroimaging initiative subjects. Ann Neurol 2009;65:403–413.[b]Vos SJ, van Rossum IA, Verhey F, et al. Prediction of Alzheimer disease in subjects with amnestic and nonamnestic MCI. Neurology 2013;80:1124-1132; [c]Sjogren M, Vanderstichele H, Ågren H, et al. Tau and Abeta42 in cerebrospinal fluid from healthy adults 21–93 years of age: establishment of reference values. Clin Chem 2001;47:1776–1781; [d]Galuzzi S, Geroldi C, Ghidoni R, et al. The new Alzheimer's criteria in a naturalistic series of patients with mild cognitive impairment. J neurol 2010; 257, 2004-14; [e]Mulder C, Verwey NA, van der Flier WM, et al. Amyloid-ß(1-42), total tau, and phosphorylated tau as cerebrospinal fluid biomarkers for the diagnosis of Alzheimer disease. Clin Chem 2010;56:248 –253. [f]Herukka SK, Pennanen C, Soininen H, Pirttila T. CSF Abeta42, tau and phosphorylated tau correlate with medial temporal lobe atrophy. J Alzheimers Dis 2008;14:51-7.

Supplemental Table 3. Number of subjects by center and by biomarker

Center	Total N	Amyloid marker	Injury marker			
		CSF Aß1-42	CSF tau	FDG-PET	HCV	MTA
AddNeuroMed	99	-	-	-	98	85
ADNI	300	155	155	-	300	-
Antwerp	41	41	41	-	-	-
Brescia	94	66	66	-	90	70
Coimbra	37	17	17	-	-	36
Descripa	270	104	104	-	214	235
EADC PET	143	-	-	143	-	-
Kuopio	50	19	19	-	48	-
Liege	49	-	-	49	-	-
Lisbon	36	2	2	-	36	-
Amsterdam VUmc	126	90	90	-	98	112

CSF=cerebrospinal fluid, Aß=amyloid-beta, FDG= fludeoxyglucose, PET=Positron Emission Tomography, HCV=hippocampal volume, MTA=medial temporal lobe atrophy, EADC=European Alzheimer's Disease Consortium.

Supplemental Table 4. Sample demographics by biomarker groups

	Total sample N=1243	1 marker FDG-PET N=192	1 marker MRI N=557	1 marker FDG-PET or MRI N=749	2 markers CSF Aß and tau N=86	2/3 markers CSF Aß and tau or MRI N=494	3 biomarkers CSF Aß and tau and MRI N=408
Age, y	71.9 (7.6)	71.8 (8.0)	72.6 (7.2)	72.3 (7.4)	72.7 (7.5)	71.3 (7.8)	71.0 (7.9)
Female, n	644 (52%)	93 (48%)	317 (57%)	410 (55%)	46 (54%)	234 (47%)	188 (46%)
Education, y	11.1 (4.8)	10.4 (4.4)	10.6 (5.0)	10.6 (4.9)	10.8 (3.4)	11.8 (4.5)	12.0 (4.7)
aMCI, n	956 (77%)	191 (99%)	395 (71%)	586 (78%)	68 (79%)	370 (75%)	302 (74%)
Follow-up, y	2.4 (1.4)	2.6 (1.7)	2.2 (1.3)	2.3 (1.4)	3.0 (2.1)	2.6 (1.5)	2.5 (1.3)
APOE-e4	440 (48%)	36 (47%)	178 (47%)	214 (47%)	32 (45%)	226 (50%)	194 (51%)
MMSE baseline	26.7 (2.4)	26.6 (2.1)	27.1 (2.3)	26.9 (2.3)	25.8 (2.8)	26.4 (2.5)	26.5 (2.4)
MMSE last follow-up	24.4 (4.6)	23.8 (3.9)	25.3 (4.1)	24.9 (4.1)	23.2 (5.3)	23.8 (5.0)	23.9 (4.9)
Progression to AD-type dementia	481 (39%)	100 (52%)	166 (30%)	266 (36%)	38 (44%)	214 (43%)	176 (43%)
Progression to other dementia	58 (5%)	16 (8%)	18 (3%)	34 (5%)	5 (6%)	23 (5%)	18 (4%)

Results are mean (SD) or number (%) for sample demographics of subjects with only a neuronal injury marker and subjects with amyloid and neuronal injury marker(s). PET=Positron Emission Tomography, CSF=cerebrospinal fluid, Aß=amyloid-beta, FDG= fludeoxyglucose, MRI=magnetic resonance imaging, aMCI=amnestic mild cognitive impairment, APOE=Apolipoprotein, MMSE=Mini-Mental State Examination, AD=Alzheimer's disease.

CHAPTER 10

GENERAL DISCUSSION

SUMMARY

NEDERLANDSE SAMENVATTING

AUTHOR AFFILIATIONS

PUBLICATIONS

AUTHOR INFORMATION

ACKNOWLEDGEMENTS

GENERAL DISCUSSION

The Alzheimer's disease field is experiencing an exciting evolution with biomarkers at the forefront. For a long time, AD has been considered a clinicopathological disease and the clinical diagnosis has not been taken into account the long preclinical and prodromal stages of AD. Thanks to advances in the AD biomarker field, our understanding of the early course of AD has substantially improved over the past years. AD is now regarded as a clinicobiological disease that can be identified in vivo prior to the onset of dementia.

The aim of this thesis was to investigate how we should use biomarkers for early diagnosis and prognosis of Alzheimer's disease. For this purpose, in part I of this thesis, we examined the role of AD biomarkers in subjects with MCI. In part II of this thesis, we studied the prevalence and prognosis of preclinical and prodromal AD, according to the recently proposed IWG and NIA-AA criteria for AD. We will now discuss our findings and the implications for research, AD trials and clinical practice. We will conclude with recommendations for future research.

Diagnostic and prognostic value of AD biomarkers in subjects with MCI

AD markers for prediction of AD-type dementia in subjects with MCI

Several AD markers are available but it is still unclear which is the optimal biomarker for predicting progression from MCI to AD-type dementia. Our studies indicated that the CSF Aß1-42/tau ratio was a better predictor than CSF Aß1-42, CSF tau, and HCV. In chapter 2, we compared the predictive ability of CSF Aß1-42, t-tau, p-tau, and a combined measure of Aß1-42 and tau for AD-type dementia in subjects with MCI by means of a meta-analysis. We found that all single markers had a similar predictive value with an odds ratio around 7, whereas the predictive value of the combination of CSF Aß1-42 and tau was more than twice as high (odds ratio 18). This is in line with previous studies.[1, 2] We also compared the predictive ability of CSF Aß1-42/tau ratio with that of HCV on MRI for AD-type dementia after 2 years and found that CSF and MRI markers were both good predictors, independent of cognition and APOE genotype (chapter 3). However, the CSF Aß1-42/tau ratio had a higher sensitivity and an overall predictive value that was more than twice as high than that of HCV. Several previous studies also found that CSF markers were a better predictor for progression to AD-type dementia than MRI markers [3-6] while other studies found that MRI markers were the best predictor.[7-9] In chapter 4, we showed that CSF Aß1-42, CSF tau, and HCV all had a similar predictive accuracy for AD-type dementia and the CSF Aß1-42/tau ratio was again the best predictor. The better performance of the CSF Aß1-42/tau ratio compared to other CSF markers and HCV is probably because information on an early AD process (amyloid pathology) will help to identify those with AD and additional information on a more downstream process (tau pathology) will help to give an appropriate prognosis.[10, 11]

In Chapter 4, we compared the predictive ability of CSF and MRI markers and *APOE*-genotype for AD-type dementia in subjects with aMCI and naMCI because AD pathology may

present differently in both MCI subgroups and therefore AD biomarkers may have a different predictive accuracy for AD-type dementia. While progression rates to AD-type dementia are known to be higher in aMCI,[12, 13] we demonstrated that AD markers were equally predictive in both MCI subgroups. However, sensitivity of CSF and MRI markers was higher and specificity lower in subjects with aMCI. This was mainly because subjects with aMCI had more abnormal biomarker scores than subjects with naMCI. AD biomarkers may therefore not be as sensitive for early diagnosis of AD in subjects with naMCI compared to aMCI when the same cut-offs are applied in each subgroup. This is particularly interesting in light of the proposed NIA-AA criteria for MCI due to AD,[14] as they consider both aMCI and naMCI as possible prodromal stages of AD-type dementia.

Combination of AD markers for prediction of AD-type dementia in subjects with MCI

Instead of investigating single markers for AD, it may be more beneficial to combine several markers for optimal diagnosis and prognosis of AD. While the CSF Aß1-42/tau ratio was the best predictor in our MCI studies, the combination with HCV increased predictive accuracy for AD-type dementia (chapter 3), as also shown in other studies.[3, 4, 7-9] This is likely because both markers reflect different underlying AD pathology and become abnormal at different stages of the disease. Our test sequence analyses suggested that it is most efficient to perform first a CSF assessment for prediction of AD-type dementia after 2 years. Volumetric assessment of hippocampal volume may be considered in subjects who have a normal CSF Aß1-42/tau, mainly because we found a small group of subjects with a normal CSF ratio and abnormal HCV that progressed to AD-type dementia (n=5).

In chapter 5, we reported that abnormal CSF t-tau, CSF p-tau, and HCV are good markers of faster future cognitive decline and progression to AD-type dementia in subjects with MCI and CSF amyloid pathology, i.e. prodromal AD. This is in line with a previous study[15] and highlights again the importance of combining neuronal injury markers with amyloid markers for better prognosis. We did not find large differences in predictive ability between injury markers but results in chapter 5 and 9 showed that subjects with two abnormal injury markers and amyloid pathology were more likely to progress to AD-type dementia compared to those with only one abnormal injury marker and amyloid pathology. Overall, because of the early position of amyloid markers in the AD pathological cascade, they may have a better diagnostic value, whereas neuronal injury markers are later markers and may therefore have a better prognostic value.[16-19]

New diagnostic criteria for preclinical and prodromal Alzheimer's disease

The introduction of the concepts preclinical and prodromal AD in the revised criteria for AD[14, 20-22] is interesting both from a diagnostic and therapeutic perspective, as we can identify AD now before the dementia stage, i.e. at the predementia stages. The classification of subjects based on both cognitive and biomarker status helps to increase the certainty of whether a subject has underlying AD pathology or not and, if so, determines what disease stage the subject is at and aids to make a prognosis.

NIA-AA criteria for preclinical AD

In chapter 8, we showed that the use of CSF markers and episodic memory according to the NIA-AA criteria for preclinical AD enables identification of subjects with preclinical AD. We found that 31% of the cognitively healthy participants met criteria for preclinical AD, with 15% of the subjects in stage 1, 12% in stage 2, and 4% in stage 3. These findings together with our autopsy data provide support for the accumulation of AD pathology in cognitively healthy elderly already.[23-25] Furthermore, the preclinical AD stages strongly predicted cognitive decline, progression to CDR 0.5 that clinicians believed was due to AD, and also mortality. Subjects in a more advanced preclinical AD stage were more likely to progress to CDR 0.5 symptomatic AD, as also shown in a previous study.[26]

Even though these criteria seem promising for identifying preclinical AD, they have some limitations. Not all subjects with AD fit within the proposed classification groups.[27] 5% of the subjects remained unclassified based on the NIA-AA criteria (chapter 8). The majority of these subjects had subtle cognitive changes in the presence of normal biomarkers whereas a few had also amyloid pathology. As this group was likely to progress to AD-type dementia (29%), the underlying pathology of these subjects needs further investigation. Furthermore, the criteria did not specify subtle cognitive decline in detail to define preclinical AD stage 3, which leads to possible variability. Using any cognitive domain versus episodic memory to define stage 3 led to different results in chapter 8. The number of subjects in stage 3 and the unclassified group was larger if any cognitive domain was used but progression rates were lower than if episodic memory was used. Another problem is the need to use cut-offs to define biomarkers as normal vs. abnormal, as the use of different cut-offs may lead to different findings. Results in chapter 8 showed that a more lenient cut-off will lead to more subjects identified with preclinical AD, however progression rates remained essentially the same.

IWG and NIA-AA criteria for prodromal AD

In chapter 9, we showed that prodromal AD was common and that these subjects showed much higher progression rates to AD-type dementia compared to those without prodromal AD. According to the IWG criteria, 54% of the subjects had prodromal AD and these subjects showed more cognitive decline than those with normal biomarkers. According to the NIA-AA criteria, 51% of the subjects were in the high-AD-likelihood group (both amyloid pathology and neuronal injury), 15% in the low-AD-likelihood group (normal markers), 6% in the IAP conflicting group (amyloid pathology without neuronal injury), and 29% were in the SNAP conflicting group (neuronal injury without amyloid injury). As expected, those with the highest likelihood showed the highest progression rates for AD-type dementia (62%) and those with the lowest likelihood the lowest progression rates (14%). Subjects in the IAP and SNAP groups showed intermediate and similar progression rates (24-30%). This latter finding was unexpected given their underlying pathology.

In their present form, the NIA-AA criteria may be favored over the IWG criteria for a few reasons. The NIA-AA criteria suppose that an increased number of abnormal

biomarkers is related to an increased likelihood that MCI is due to AD,[14] which was indeed the case in our study. In contrast, the IWG criteria require only one abnormal biomarker (any) for prodromal AD and only an abnormal amyloid marker for asymptomatic subjects at risk for AD,[20, 21] which makes identification of subjects who are most likely to progress soon to AD-type dementia more difficult. For instance, we cannot tell whether subjects with amyloid pathology without information on neuronal injury are at an early or later process of the disease. Also, subjects with neuronal injury without information on amyloid pathology may have AD but may also have other diseases. Another important difference is the definition of cognition. The IWG criteria require episodic memory impairment while the NIA-AA criteria do not specify cognitive impairment. Our results in chapter 4 show that also subjects with naMCI can progress to AD-type dementia, although the progression rates are lower. Again, the use of biomarker cut-offs is also a limitation for these both sets of criteria.

Standardization of AD biomarkers

AD biomarkers are increasingly being used in clinical practice but the absence of standardized analyses for biomarkers constitutes a major deficiency and may influence the diagnostic AD classification of subjects. Findings in chapter 6 show that there is still considerable intra-and interlaboratory variability of CSF analyses, consistent with previous reports.[28-32] A substantial part of the observed variability may be explained by lot-to-lot variation of analytical kits but future studies on lot-to-lot comparison will need to confirm this suggestion. Moreover, we found that the variability frequently influences AD diagnosis when predefined cut-offs were used to classify CSF biomarkers as normal or abnormal. Importantly, also a diagnosis of a CSF AD profile based on Aβ1-42 and t-tau or p-tau changed in 12-16% of the cases. While most of the subjects in our study with a change in AD classification after reanalysis had CSF biomarker values around the cut-off points, the variability may have a large impact on CSF-based AD diagnosis or treatment decisions in clinical settings, implying that we should be careful when interpreting CSF findings and always interpret them within a clinical context. Another point of concern is the use of cut-offs to define the continuous biomarker scores as normal or abnormal. An agreement on universal cut-offs is needed,[33] because the use of different cut-offs can lead to different diagnoses in patients (chapter 6, 8). Other studies have shown that also imaging markers still suffer from poor standardization and reproducibility.[34] Ongoing[30, 35] and future worldwide standardization projects will help to move towards a standardized and harmonized implementation of AD biomarkers in clinical routine.

Methodological considerations

Strengths

The use of large sample sizes increased the power of our studies and allowed us to perform multivariate analyses. The longitudinal study designs with up to 2 (chapter 3), 5 (chapter 4, 5), 9 (chapter 9), and 15 years (chapter 8) of follow-up gave us the opportunity to investigate the short-term as well as long-term prognosis of subjects. By means of a meta-

analysis we could get insight into the predictive ability of CSF biomarkers by pooling data from previous studies and taking into account the sample size of all studies (chapter 2).

Limitations

Our studies had several limitations. Because subjects agreed to take part in longitudinal studies, including neuroimaging procedures and lumbar punctures, they are unlikely to be representative of the general population. Subjects were also relatively young elderly and findings may therefore not apply to the oldest old. The DESCRIPA study (chapter 3-5) and EADC study (chapter 9) were multi-center memory clinic-based studies, which allowed generalization to other memory clinics but not to the general population. In contrast, data from the community-dwelling-volunteer-based study of Washington University in St. Louis (chapter 8) allowed generalization to a broader population although it may be restricted to Caucasians and highly educated people. Furthermore, the DESCRIPA and EADC study were based on clinical routine rather than standardized protocols. This might have introduced heterogeneity between centers but findings may better reflect current clinical practice. Moreover, we corrected for center in statistical analyses and used Z-scores or equivalent scores for cognitive tests defined at each center. In the DESCRIPA study, biomarker analyses were also performed at one center.

Other limitations were the loss to follow-up and missing data, because of which valuable information could have been missing. Although follow-up was relatively long, a longer follow-up would have helped to investigate whether all subjects with abnormal biomarkers eventually will develop AD-type dementia. Moreover, we used clinical diagnoses as outcome measure instead of autopsy diagnoses (except for a small subgroup in chapter 8), which may have led to misclassification of subjects and which could have hampered the validation of biomarkers. Furthermore, multiple testing could be a problem in some studies but we mostly provided p-values to correct for multiple testing. In addition, estimation of temporal dynamics in biomarkers changes was based on cross-sectional biomarker studies but longitudinal biomarker data are needed to examine this thoroughly.

IMPLICATIONS OF AD BIOMARKERS

Amyloid cascade hypothesis

Findings in this thesis may have implications for the amyloid cascade hypothesis,[36, 37] which has dominated research for the past two decades. This hypothesis states that AD pathophysiology starts with amyloid deposition already 10 to 15 years before the first clinical symptoms appear, which initiates multiple downstream events leading to neuronal injury and eventually to clinical symptoms. In line with this, Jack and colleagues recently proposed a hypothetical AD biomarker model, which suggests that abnormal CSF Aß1-42 can be detected first, followed by amyloid PET, CSF tau, and MRI and FDG PET. [38, 39] Our findings are partially in favour of the amyloid cascade and the hypothetical biomarker model. We indeed found that subjects with both amyloid deposition and neuronal injury

showed more cognitive decline than those with only amyloid pathology, likely because they are further in the disease process. Furthermore, the higher sensitivity of the CSF Aß1-42/tau ratio for progression to AD-type dementia than that of HCV may indicate that CSF markers become abnormal at an earlier stage than MRI biomarkers. However, we also recognized some inconsistencies with regard to the model. We did not find a difference in overall predictive accuracy between the single CSF markers and HCV. Several subjects had hippocampal atrophy but normal CSF tau, which may suggests that pathways are not the same for all subjects. Also, subjects with normal CSF Aß1-42 and abnormal CSF tau or HCV had an increased risk for AD-type dementia, but as discussed below this CSF Aß1-42 classification may partly be a cut-off issue. Therefore, while the cascade is a useful model, careful interpretation of amyloid and neuronal injury markers is likely needed for diagnosing and staging AD.

Isolated neuronal injury

Some of the subjects in our study did not fit within the amyloid cascade because they had neuronal injury without amyloid pathology. At present, they are therefore suggested to have suspected non-Alzheimer pathophysiology (SNAP).[27] Both in cognitively healthy controls and in subjects with MCI, the SNAP group represented a large group of subjects (23-29%). Interestingly, cognitively healthy controls with SNAP did not progress to AD-type dementia or showed substantial cognitive decline at follow-up (chapter 8), consistent with a previous study.[26] They were less likely to be APOE-ε4 carriers than subjects with preclinical AD but they do not appear to differ regarding cerebrovascular imaging lesions or vascular risk factors.[26] Because of low progression rates to AD-type dementia, they may have other neurodegenerative diseases. Our autopsy data showed that they had no to low AD pathology change at autopsy and other pathologies included argyrophilic grain disease, neurofibrillary tangles in medial temporal lobe, or small vessel disease with infarcts/microinfarcts. These pathologies were however not sufficient to account for cognitive impairments. It could also be that tau pathology reflects normal cognitive aging in these subjects.

We and others[40] found that subjects with MCI and SNAP showed progression rates to AD-type dementia similar to those with isolated amyloid pathology but they tended to be less likely *APOE*-ε4 carriers than those with prodromal AD with amyloid pathology (chapter 9). Intriguingly, subjects with MCI and SNAP who progressed to AD-type dementia had intermediate CSF Aß1-42 levels (just above the predefined cut-off), and they may therefore reflect a subgroup of subjects for whom less amyloid pathology is needed to develop AD-type dementia (chapter 9). This could also explain our findings in chapter 3, where MRI analyses could only add to the predictive accuracy for AD-type dementia in a small group of subjects with MCI with a normal CSF Aß1-42/tau ratio. Remarkably, most of these subjects that progressed to AD-type dementia also appeared to have rather intermediate CSF Aß1-42 levels in the presence of abnormal tau. The intermediate CSF Aß1-42 levels in subjects with MCI and SNAP and relatively high progression rates to AD-type dementia (30%) after a mean follow-up of 3 years suggest that they may have atypical AD. This could be explained

by the variety in CSF Aß1-42 levels in cognitively healthy elderly or by the variability in type of aggregated Aß.[41] It may also result from lower cognitive reserve[42] or from pathological heterogeneity because of multicomorbitidy.[17, 43] Overall, the high prevalence of SNAP in healthy controls as well as in subjects with MCI and the high progression rate to AD-type dementia in this latter group highlight the importance to further explore the comorbid diseases, underlying pathology, clinical characteristics and outcome of these subjects.

Clinical trials

The identification of preclinical and prodromal AD is particularly interesting for AD trials, as development of therapeutic strategies is now aimed at truly modifying the underlying disease pathogenic processes rather than simply ameliorating dementia symptoms. Inclusion of subjects with preclinical and prodromal AD will ensure appropriate therapeutic targeting and will lead to higher progression rates to AD-type dementia and therefore smaller sample sizes (chapter 2). Age and *APOE* genotype may be useful for prescreening of individuals for biomarker assessment,[44] given the higher prevalence of AD pathology in *APOE*-ε4 carriers[45] or older subjects,[46] as also shown in chapter 8. Furthermore, trials may stratify individuals based on their predementia AD stage[47] and number of abnormal biomarkers since their prevalence and prognosis differ (chapter 8, 9). We showed that subjects at a more advanced stage will progress faster to AD-type dementia and therefore a smaller sample size is needed for trials. However, a larger number will be required for screening given the lower prevalence with advancing stage (chapter 3, 8, 9). Also, neuronal injury markers could be used as outcome in AD trials[48, 49, 50] since they are closely related to cognition and may be more reliable measures of AD pathophysiological changes than cognitive outcomes. Amyloid markers could be used as outcome to provide efficacy of the intervention.[51, 52] Moreover, our study in preclinical AD suggests that mortality should be considered as an outcome in trials, as subjects with preclinical AD had an increased risk to die at follow-up, with a higher risk at more advanced stages (chapter 8).

Clinical practice

Biomarker assessment is currently complementary and recommended in case of substantial clinical uncertainty after other diagnostic tests.[53, 54] However, as biomarker research progresses, also biomarker assessment in early AD stages becomes more common in clinical practice, albeit still controversial. An important advantage of diagnosis at the prodromal AD stage is that it helps the patient and family to understand and accept the underlying cause of their cognitive problems, which reduces uncertainty and in this way may increase quality of life. Patients will also be more likely to get earlier appropriate care and they can anticipate the future. However, there are also several disadvantages. The main drawback is that there is currently no effective treatment.[55] An early diagnosis my also provoke stress and catastrophic reactions. Furthermore, no clear prognosis can be given to patients with prodromal AD, as the time to dementia is uncertain and not all subjects may become demented.[56] Both compensatory mechanisms and cognitive reserve may influence the

progression to dementia.[42, 57] Moreover, the prevalence of other brain pathologies could have impact on the relationship between AD pathology and clinical symptoms,[58 59, 60] which is especially common at an older age.[61]

A diagnosis of AD at the preclinical stage is currently restricted to research settings. As these subjects have no cognitive problems and there is no effective treatment available, an early diagnosis raises lots of ethical questions because it may do more harm than good to these subjects. Once preventive treatments become available, biomarkers will start playing a more prominent role in preclinical AD. A plasma biomarker for AD would be ideal then but so far attempts to develop an accurate plasma AD marker have been unsuccessful (chapter 2).[62]

Our data suggest that we should differentiate between upstream and more downstream markers of AD to give the best diagnosis and prognosis (chapter 3-5,8,9). For amyloid diagnosis, amyloid PET imaging or CSF Aß1-42 can be used. When we compare both markers, amyloid imaging may be less invasive and there may be less variation across analyses, while CSF analyses are less expensive and subjects are not exposed to radiation. Importantly, some studies have shown that amyloid pathology can be detected in CSF before it is seen on amyloid PET scans[63, 64] but more research is needed to confirm this. It should also be noted that PET amyloid markers primarily measure insoluble fibrillar forms of Aß, while reduced Aß in CSF may also reflect aggregation of other Aß species such as oligomers. Besides, clinicians should bear in mind that amyloid pathology is not specific for AD, as it may also be seen in subjects with Lewy body dementia or vascular dementia.[65, 66] Results should therefore always be carefully interpreted in clinical context. Furthermore, an injury marker should be used to give a prognosis. The advantage of using CSF markers is that they give info on both amyloid pathology (Aβ) and neuronal injury (tau), and we showed that CSF tau is a good prognostic marker (chapter 5). The advantage of MRI is that it is often already routinely done to exclude other diseases and additional volumetric assessment of the hippocampus may be useful to predict outcome as shown in chapter 3 and 5. Our test sequence analyses suggest that CSF assessment should be performed first for short-term prediction of AD-type dementia and that HCV assessment may be considered in subjects who have a normal CSF Aß1-42 /tau ratio.

At present, the application of the new criteria for prodromal AD in clinical practice comes with several problems. It is for instance unclear what we should tell patients with conflicting biomarker results. We do not know yet whether all subjects with a SNAP biomarker profile (neuronal injury without amyloid pathology) have AD and will progress to AD-type dementia or not. This is also true for patients with isolated amyloid pathology, since maybe not all of them will progress to AD-type dementia over time. Moreover, we found that several subjects have values around the biomarker cut-off, which may influence their biomarker classification and diagnosis. The CSF Aß1-42/tau ratio may be a good alternative because it may catch more people with AD that just fall below the Aß1-42 or tau cut-offs when these markers are used separately. Before implementation of biomarkers in routine clinical practice, standardized and harmonized procedures for biomarker

assessment need to be developed (chapter 6) and the encountered difficulties with the revised AD criteria must be resolved (chapter 8 & 9).

The shift from diagnosing AD at the dementia stage towards diagnosing AD at the predementia stage might raise the question what AD actually is, i.e. the underlying pathophysiological disease process or the clinical syndrome of progressive cognitive decline. Until recently, the clinical diagnosis of probable AD-type dementia was the best alternative for definite AD diagnosis at autopsy. Now, biomarkers allow us to provide an earlier AD diagnosis independent of clinical expression of disease severity. However, several individuals with amyloid pathology will never develop any AD symptoms or AD-type dementia.[56] Given the lack of effective treatment and individual risk prediction for AD-type dementia, one may wonder whether we should label someone with an AD diagnosis before they are demented, i.e. in need of care. For clinicians it is important to keep in mind that patients do not suffer from the pathology but from the cognitive impairments and that a diagnosis at the preclinical stage is therefore currently unwarranted in clinical settings. In contrast, a diagnosis of AD at the prodromal stage can help to explain the cause of their cognitive problems, even though several uncertainties about the future may persist. Nonetheless, biomarker use can lead to an incorrect classification of patients as being at high risk for AD-type dementia, i.e. false positives, which may have an adverse effect on the patient's well being. At present, the main advantage is that we can rule out AD in several patients with MCI that have normal biomarkers, which will provide relief in these patients. Overall, for each patient with MCI with uncertain etiology, the potentials and limitations of biomarker use should always be carefully discussed before application.[67]

FUTURE DIRECTIONS

The last decade, large improvements have been made in the AD field thanks to biomarker developments but several questions remain unanswered. It is not known yet which set of biomarkers can best diagnose AD and predict AD-type dementia over time and what would be the optimal test sequence. More studies on head-to-head comparison of markers in longitudinal clinical studies with an extensive follow-up and preferably also post-mortem examination will be needed. Also more longitudinal biomarker studies are required to get better insight into the temporal dynamics of AD biomarkers because currently most studies are based on cross-sectional biomarker analyses. Furthermore, the time to AD-type dementia differs between subjects and not all subjects with AD pathology develop AD-type dementia. This needs further exploration and may for instance relate to mixed pathologies and cognitive reserve. Also the factors that predispose someone to develop AD pathology should be studied more in detail, as vascular factors and lifestyle factors likely play a role in midlife already. In addition, isolated tau pathology needs more investigation, especially in light of our SNAP findings. All of this will help to better understand preclinical and prodromal AD, which may lead to better treatment opportunities in the future.

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SUMMARY

The advances in AD biomarker research over the last years have improved our understanding of the early course of AD and allow us now to identify subjects with AD before they are demented, which opens new opportunities for AD treatment and prevention. At present, it is still unclear how biomarkers or biomarker combinations should be used for diagnosis and prognosis of AD for clinical and research purposes. In this thesis, we studied the role of biomarkers for diagnosis and prognosis of Alzheimer's disease in cognitively normal subjects, subjects with amnestic MCI (aMCI), and subjects with non-amnestic MCI (naMCI). In part I, we described the diagnostic and prognostic ability of single and combined AD biomarkers in subjects with MCI. In part II, we focused on the recently proposed diagnostic criteria for preclinical and prodromal AD. These criteria highlight the use of biomarkers to increase the certainty of whether a subject has underlying AD pathology or not.

In **Chapter 2**, we provided a review and meta-analysis of the use of CSF and plasma AD biomarkers for the prediction of AD-type dementia in subjects with MCI. We showed that the combination of Aβ1-42 and tau in CSF had a better predictive accuracy for AD-type dementia than single CSF Aβ1-42, t-tau or p-tau markers. Plasma levels of Aβ1-40 tended to predict progression to AD-type dementia, while plasma levels of Aβ1-42, the ratio Aβ1-42/Aβ1-40 and homocysteine did not predict outcome. Furthermore, in a fictive trial design with drugs that aim to prevent the progression from MCI to AD-type dementia, use of the combination of CSF Aβ1-42 and tau markers in the selection of subjects could substantially reduce the sample size and trial costs.

In **Chapter 3**, we compared the predictive ability of the CSF Aβ1-42/tau ratio and HCV on MRI for AD-type dementia in subjects with MCI of the European multi-center memory-clinic-based DESCRIPA study and determined the optimal test sequence of these biomarkers. While both markers were good predictors for progression to AD-type dementia and cognitive decline in subjects with MCI, we showed that the CSF Aβ1-42/tau ratio was the best predictor for progression. Test sequence analyses demonstrated that HCV could increase the predictive accuracy of the CSF Aβ1-42/tau ratio. This increase in predictive accuracy for AD-type dementia and cognitive decline was because HCV predicted AD-type dementia and cognitive decline in subjects with a normal CSF Aβ1-42/tau ratio.

In **Chapter 4**, the predictive accuracy of CSF Aβ1-42, CSF tau, CSF Aβ1-42/tau, HCV on MRI, and APOE genotype for AD-type dementia was compared in subjects with aMCI and naMCI of the DESCRIPA study. We found that the overall predictive accuracy for AD-type dementia was similar for subjects with aMCI and naMCI, although sensitivity of CSF and MRI markers was higher and specificity lower in subjects with aMCI. Biomarkers also predicted decline on the MMSE in both MCI groups and decline in memory and overall cognition in subjects with naMCI. While there are differences in the relationship of biomarkers to outcome in aMCI and naMCI, our findings suggest that the symptomatic manifestations of

the AD pathophysiological process is broader than the amnestic presentation at the MCI stage and also includes non-amnestic MCI.

In **Chapter 5**, we assessed the progression rate to AD-type dementia and identified prognostic markers in subjects with MCI and evidence of abnormal amyloid-ß in CSF from the DESCRIPA study. In these subjects with prodromal AD/MCI due to AD, we found that 57% of the subjects progressed to AD-type dementia after a mean follow up of 2.2 years. High CSF levels of t-tau and p-tau and hippocampal atrophy predicted progression to AD-type dementia and decline in MMSE score. Our results implicate that markers of AD-related neuronal injury could help to identify those subjects with MCI due to AD that will more rapidly progress to dementia.

In **Chapter 6**, we investigated the influence of CSF intralaboratory and interlaboratory variability on diagnostic CSF-based AD classification in a memory-clinic population of the Dutch multi-center LeARN study. We observed clear variability in CSF AD biomarker levels between repeated analyses in the same laboratory as well as between two different laboratories, in particular for Aβ1-42 and p-tau. A substantial part of this variability could be explained by lot-to-lot variation of analytical kits. Our findings showed that the variability frequently led to a change in CSF-based AD diagnosis when predefined cut-offs for abnormal CSF values were applied, which may have a large impact on treatment decisions in clinical settings. Together, our results indicate that we should be careful when interpreting CSF findings and always interpret them within a clinical context.

In **Chapter 7**, we compared the theoretical framework of the International Working Group (IWG) criteria and National Institute on Aging–Alzheimer's Association (NIA-AA) criteria for Alzheimer's disease. The criteria revealed differences in approach, terminology and use of cognitive markers and biomarkers. Most persons who meet the IWG criteria will also meet the NIA-AA criteria and vice versa. However, the NIA-AA criteria allow for a subclassification of persons based on biomarker results within each diagnostic category. Validation and likely also modification of these criteria are needed before they can be applied in clinical practice.

In **Chapter 8**, we described the prevalence and prognosis of subjects with preclinical AD according to CSF biomarkers, episodic memory and the proposed NIA-AA criteria. Subjects were recruited from longitudinal studies of memory and aging in cognitively normal community-dwelling volunteers at the Knight Alzheimer's Disease Research Center (ADRC) of the Washington University School of Medicine in St. Louis (USA). We showed that preclinical AD can be defined by CSF markers, is common in individuals aged at least 65 years, and is associated with an increased risk of cognitive decline, progression to CDR at least 0.5 symptomatic AD, and mortality. Progression rates differed between the preclinical AD stages; thus, stages 1, 2, and 3 likely represent different and progressive disease severities. Hence, preclinical AD could be an important target for therapeutic intervention.

In **Chapter 9**, the prevalence and prognosis of prodromal AD was examined based on well-established biomarkers and the proposed IWG and NIA-AA criteria. Subjects were recruited from multiple centers of the European Alzheimer's Disease Consortium (EADC)

and the Alzheimer's Disease Neuroimaging Initiative (ADNI). Our findings showed that subject classifications based on the IWG and NIA-AA criteria were both good predictors of likelihood of future cognitive decline and progression to AD-type dementia in subjects with MCI. However, our results indicated that knowledge about both amyloid pathology and neuronal injury is preferred to give an optimal prognosis. Moreover, the use of multiple neuronal injury markers in combination with an amyloid marker can help to identify the subjects that will soon progress to AD-type dementia. Also, a more lenient CSF Aβ1-42 cut-off may be needed to identify subjects with SNAP who have prodromal AD.

In **Chapter 10,** we provided a general discussion of our findings, its implications for research, AD trials and clinical practice, and recommendations for future research. Furthermore, this last chapter includes a summary of the findings, list of author affiliations, list of publications, information about the author, and acknowledgements.

SAMENVATTING

De vooruitgang de afgelopen jaren in biomarker onderzoek bij de ziekte van Alzheimer (AD) heeft ons begrip van de vroege fase van AD verbeterd en staat ons toe om nu mensen te identificeren met AD vooraleer ze dement zijn. Dit zorgt voor nieuwe mogelijkheden voor behandeling en preventie van AD. Op dit moment is het nog steeds onduidelijk hoe biomarkers of biomarker combinaties gebruikt moeten worden voor de diagnose en prognose van AD, zowel voor klinische als wetenschappelijke doeleinden. In dit proefschrift hebben we de rol van biomarkers voor de diagnose en prognose van AD bestudeerd bij cognitief normale mensen, mensen met milde amnestische stoornissen en mensen met milde non-amnestische stoornissen. In deel I hebben we de diagnostische en prognostische waarde van aparte of gecombineerde biomarkers voor AD bij mensen met milde cognitive stoornissen (MCI) beschreven. In deel II hebben we ons gericht op de recent voorgestelde diagnostische criteria voor preklinische en prodromale AD. Deze criteria benadrukken het gebruik van biomarkers om meer zekerheid te krijgen of er al dan niet sprake is van onderliggende AD pathologie.

In **Hoofdstuk 2** hebben we een overzicht en meta-analyse gepresenteerd van het gebruik van AD biomarkers in hersenvocht (CSF) en plasma. We toonden aan dat de combinatie van Aβ1-42 en tau in CSF een betere voorspeller was voor AD-type dementie dan Aβ1-42, t-tau of p-tau apart. Aβ1-40 in plasma toonde een tendens om progressie naar AD-type dementie te voorspellen, terwijl plasma waardes van Aβ1-42, de ratio Aβ1-42/Aβ1-40, en homocysteïne de uitkomst niet voorspelden. In een fictief trial design met medicatie om progressie van MCI naar AD-type dementia te voorkomen vonden we dat het gebruik van de combinatie van CSF Aβ1-42 en tau markers voor de selectie van mensen het aantal mensen nodig voor de trial evenals de kosten voor de trial aanzienelijk kon verminderen.

In **Hoofdstuk 3** hebben we de voorspellende waarde van de CSF Aβ1-42/tau ratio en hippocampus volume (HCV) vergeleken voor progressie naar AD-type dementie bij mensen met MCI binnen de Europese DESCRIPA studie waaraan meerdere Europese centra (geheugenpoliklinieken) deelnamen. Daarnaast hebben we de optimale testvolgorde van deze biomarkers bepaald. Ondanks dat beide markers goede voorspellers waren voor progressie naar AD-type dementie en cognitieve achteruitgang vonden we dat de CSF Aβ1-42/tau ratio de beste voorspeller was voor progressie. Analyses voor testvolgorde toonden aan dat HCV de voorspellende waarde van de CSF Aβ1-42/tau ratio kon doen toenemen. Deze toename in voorspellende accuraatheid voor AD-type dementie en cognitive achteruitgang was er doordat HCV progressie voorspelde bij mensen met een normale CSF Aβ1-42/tau ratio.

In **Hoofdstuk 4** werd de voorspellende accuraatheid van CSF Aβ1-42, CSF tau, CSF Aβ1-42/tau, HCV, en APOE genotype voor AD-type dementie vergeleken bij mensen met amnestische MCI (aMCI) en non-amnestische MCI (naMCI). We vonden dat de algemene voorspellende waarde voor AD-type dementie vergelijkend was bij mensen met aMCI en naMCI, hoewel de sensitiviteit van CSF en MRI markers hoger was en de specificiteit lager

was bij mensen met aMCI. Biomarkers voorspelden ook achteruitgang op de MMSE in beide MCI groepen en achteruitgang in geheugen en algemene cognitie bij mensen met naMCI. Ondanks dat er verschillen waren in de relatie tussen biomarkers en uitkomst bij aMCI en naMCI tonen onze resultaten aan dat de symptomatische manifestaties van het AD pathofysiologisch proces breeder zijn dan de amnestische presentatie in de MCI fase en ook non-amnestische MCI bevat.

In **Hoofdstuk 5** onderzochten we het aantal mensen dat AD-type dementie ontwikkelde en identificeerden we prognostische markers bij mensen met MCI en abnormaal amyloid-ß in CSF van de DESCRIPA study. Bij deze mensen met prodromale AD/MCI als gevolg van AD vonden we dat 57% AD-type dementie kreeg na een gemiddelde follow-up van 2.2 jaar. Hoge CSF waardes van t-tau en p-tau en hippocampus atrofie voorspelden progressie naar AD-type dementie en achteruitgang op de MMSE. Onze resultaten impliceren dat markers voor AD-gerelateerde neuronale schade zouden kunnen helpen om mensen met MCI als gevolg van AD te identificeren wat sneller AD-type dementie zullen krijgen.

In **Hoofdstuk 6** onderzochten we de invloed van CSF variabiliteit tussen en binnen laboratoria op de diagnostische classificatie van AD gebaseerd op CSF data in een geheugenpolipopulatie van de Nederlandse LeARN studie waaraan meerdere centra deelnamen. We observeerden duidelijke variabiliteit in CSF AD biomarker waardes tussen herhaalde metingen in hetzelfde laboratorium evenals tussen twee verschillende laboratoria, in het bijzonder voor Aβ1-42 en p-tau. Een substantieel gedeelte van deze variabiliteit zou kunnen verklaard worden door variatie tussen lots van analytische kits. Onze bevindingen toonden aan dat de variabiliteit vaak leidde tot verandering van AD diagnose wanneer vooraf gedefineerde afkappunten gebruikt werden om abnormale CSF waardes te bepalen, hetgeen een grote impact kan hebben op behandelbeslissingen in klinische settings. Algeheel tonen onze resultaten aan dat we voorzichtig moeten zijn bij het interpreteren we CSF waardes en dat we CSF waardes altijd in een klinische context moeten interpreteren.

In **Hoofdstuk 7** vergeleken we het theoretische kader van de International Working Group (IWG) criteria en de National Institute on Aging–Alzheimer's Association (NIA-AA) criteria. Deze criteria tonen verschillen in aanpak, terminologie en gebruik van cognitieve markers en biomarkers. De meeste mensen wat aan de IWG criteria voldoen zullen ook aan de NIA-AA criteria voldoen en andersom. Hoe dan ook, de NIA-AA criteria staan subclassificatie van mensen toe gebaseerd op biomarker resultaten binnen elke diagnostische categorie. Validatie en wellicht ook aanpassingen van deze criteria zijn nodig alvorens deze toegepast kunnen worden in de klinische praktijk.

In **Hoofdstuk 8** beschreven we de prevalentie en prognose van mensen met preklinische AD gebaseerd op CSF biomarkers, episodisch geheugen, en de voorgestelde NIA-AA criteria. Deelnemers werden gerecruteerd van longitudinale studies naar geheugen en veroudering bij cognitief normale vrijwilligers uit de samenleving aan het Knight Alzheimer's Disease Research Center (ADRC) van Washington University School of Medicine

in St. Louis (VS). We toonden aan dat preklinische AD gemeten kan worden met CSF markers, veel voorkomt bij mensen van 65 jaar en ouder, en geassocieerd is met een verhoogd risico op cognitieve achteruitgang, progressie naar een CDR van 0.5 of groter met symptomatische AD, en mortaliteit. Progressie verschilde tussen de preklinische AD fases; dus fase 1, 2, en 3 representeren wellicht een verschillende en progressieve ziekte-ernst. Dit geeft aan dat preklinische AD een belangrijk doelwit kan zijn voor therapeutische interventie.

In **Hoofdstuk 9** onderzochten we de prevalentie en prognose van prodromale AD door het gebruik van erkende biomarkers en de voorgestelde IWG en NIA-AA criteria. Mensen werden geworven van verschillende centra van het European Alzheimer's Disease Consortium (EADC) en Alzheimer's Disease Neuroimaging Initiative (ADNI). Onze bevindingen toonden aan dat classificaties van individuen gebaseerd op de IWG en NIA-AA criteria goede voorspellers waren voor de kans op cognitieve achteruitgang of progressie naar AD-type dementie in de toekomst. Desalniettemin toonden onze resultaten aan dat kennis over zowel amyloïde pathologie als neuronale schade de voorkeur heeft om een optimale prognose te geven. Ook kan het gebruik van meerdere neuronale schade markers in combinatie met een amyloïde marker helpen om de mensen te identificeren wat sneller AD-type dementie zullen ontwikkelen. Daarnaast kan een liberaler CSF Aβ1-42 afkappunt nodig zijn om de mensen met enkel neuronale schade en prodromale AD (SNAP) te identificeren .

Hoofdstuk 10 bevat een algemene discussie van onze bevindingen, de implicaties hiervan voor onderzoek en kliniek en aanbevelingen voor verder onderzoek. Dit laastse hoofdstuk bevat tevens een samenvatting van de resultaten, overzicht van auteursaffiliaties, lijst van publicaties, informatie over de auteur en dankwoord.

AUTHOR AFFILIATIONS

Pauline Aalten, PhD
Department of Psychiatry and Neuropsychology, Maastricht University, School for Mental Health and Neuroscience, Alzheimer Center Limburg, Maastricht, the Netherlands

Frederik Barkhof, MD, PhD
Department of Radiology, VU University Medical Center, Amsterdam, the Netherlands

Christine Bastin, PhD
Cyclotron Research Centre, University of Liège, Belgium

Bart N.M. van Berckel, MD, PhD
Department of Nuclear Medicine and PET Research, VU University Medical Center, Amsterdam, The Netherlands

Kaj Blennow, MD, PhD
Clinical Neurochemistry Laboratory, Göteborg University, Sahlgrenska University Hospital, Mölndal, Sweden

Leah Burns, MPH
Global Health Economics & Outcomes Research, Bristol-Myers Squibb Company, Wallingford, Connecticut, USA

Nigel J. Cairns, PhD
Department of Neurology, Department of Pathology and Immunology, The Knight Alzheimer's Disease Research Center, Washington University School of Medicine, St. Louis, Missouri, USA

Mira Didic, MD PhD
Service de Neurologie and Neuropsychologie, Pôle de neurosciences cliniques, AP-HM Timone, INSERM U751, Université de la Méditerranée, Marseille, France

Sebastiaan Engelborghs, MD, PhD
Reference Center for Biological Markers of Dementia (BIODEM), Laboratory of Neurochemistry and Behavior, Institute Born-Bunge, University of Antwerp, Antwerp, Belgium; and Department of Neurology and Memory Clinic, Hospital Network Antwerp, Middelheim and Hoge Beuken, Antwerp, Belgium

Anne M. Fagan, PhD
Department of Neurology, Washington University School of Medicine, The Knight Alzheimer's Disease Research Center, St. Louis, Missouri, USA.

Giovanni B. Frisoni, MD
IRCCS San Giovanni di Dio Fatebenefratelli, Laboratory of Epidemiology and Neuroimaging and Translational Research Unit, Brescia, Italy

Lutz Froelich, MD, PhD
Department of Geriatric Psychiatry, Zentralinstitut für Seelische Gesundheit, University of Heidelberg, Mannheim, Germany

Wiesje van der Flier, PhD
Alzheimer center & Department of Neurology, Neuroscience Campus Amsterdam, VU University Medical Center, Amsterdam, The Netherlands

Elizabeth A. Grant, PhD
Division of Biostatistics, The Knight Alzheimer's Disease Research Center, Washington University School of Medicine, St. Louis, Missouri, USA

Harald Hampel, MD, PhD
Department of Psychiatry, Psychosomatic Medicine and Psychotherapy, Goethe-University, Frankfurt, Germany

Ron Handels, MSc
Department of Psychiatry and Neuropsychology, Maastricht University, School for Mental Health and Neuroscience, Alzheimer Center Limburg, Maastricht, the Netherlands

Jason Hassenstab, PhD
Department of Psychology, The Knight Alzheimer's Disease Research Center, Washington University School of Medicine, St. Louis, Missouri, USA

Sanna-Kaisa Herukka, MD, PhD
Department of Neurology, School of Medicine, University of Eastern Finland and Kuopio University Hospital, Kuopio, Finland

David M. Holtzman, MD, PhD
Department of Neurology, The Knight Alzheimer's Disease Research Center, Hope Center for Neurological Disorders, Washington University School of Medicine, St. Louis, Missouri, USA

Mateusz S. Jasielec, MS
Division of Biostatistics, Washington University School of Medicine, St. Louis, Missouri, USA

Dirk L. Knol, PhD
Department of Epidemiology and Biostatistics, VU University Medical Center, Amsterdam, the Netherlands

Raquel Lemos, PhD
Faculty of Psychology and Educational Sciences, University of Coimbra, Coimbra, Portugal

Gilbert L'Italien, PhD
Global Health Economics & Outcomes Research, Bristol-Myers Squibb Company, Wallingford, Connecticut, USA

Michaela Litchmore-Dunbar, BSc
Department of Education Support, King's College London, Institute of Psychiatry, London, UK

Alexandre de Mendonça, MD
Neurological Clinical Research Unit, Instituto de Medicina Molecular, Lisboa, Portugal

Lennart Minthon, MD, PhD
Clinical Memory Research Unit, Department of Clinical Sciences Malmö, Lund University, Malmö, Sweden

Silvia Morbelli, MD, PhD
Nuclear Medicine, Department of Internal Medicine, University of Genoa, Genoa, Italy

John C. Morris, MD, PhD
Department of Neurology, Department of Pathology and Immunology, The Knight Alzheimer's Disease Research Center, Washington University School of Medicine, St. Louis, Missouri, USA

Cristina Muscio, PhD
IRCCS San Giovanni di Dio Fatebenefratelli, Laboratory of Epidemiology, Neuroimaging and telemedicine, Brescia, Italy

Flavio Nobili, MD
Clinical Neurophysiology Service, Department of Neurosciences, Ophthalmology and Genetics, University of Genoa, Genoa, Italy

Marcel G.M. Olde Rikkert, MD, PhD
Department of Geriatric Medicine, Radboud University Nijmegen Medical Centre, Nijmegen, The Netherlands; and Donders Institute for Brain, Cognition and Behaviour, Radboud University Nijmegen and Radboud Alzheimer Centre, Nijmegen, The Netherlands

Robert Perneczky, MD, PhD
Department of Psychiatry and Psychotherapy, Technische Universität München, Munich, Germany

Inez Ramakers, PhD
Department of Psychiatry and Neuropsychology, Maastricht University, School for Mental Health and Neuroscience, Alzheimer Center Limburg, Maastricht, the Netherlands

Ineke A. van Rossum, MD, PhD
Alzheimer center & Department of Neurology, Neuroscience Campus Amsterdam, VU University Medical Center, Amsterdam, The Netherlands

Daniel Rueckert, PhD
Department of Computing, Imperial College London, London, UK

Erik Salmon, MD, PhD
Memory Clinic, Department of Neurology, CHU Liège, Belgium; and Cyclotron Research Centre, Liège, Belgium

Isabel Santana, MD, PhD
Department of Neurology, Coimbra University Hospital, Coimbra, Portugal

Philip Scheltens, MD, PhD
Alzheimer center & Department of Neurology, Neuroscience Campus Amsterdam, VU University Medical Center, Amsterdam, The Netherlands

Dina Silva, PhD
Dementia Clinics, Faculty of Medicine, University of Lisbon, Lisbon, Portugal; and Instituto de Farmacologia e Neurociências, Edifício Egas Moniz, Faculdade de Medicina de Lisboa, Lisboa, Portugal

Andy Simmons, PhD
Department of Clinical Neuroscience, Centre for Neuroimaging Science, King's College London, Institute of Psychiatry, London, UK

Hilkka Soininen, MD, PhD
Department of Neurology, University and University Hospital of Kuopio, Kuopio, Finland

Charlotte E. Teunissen, PhD
Department of Clinical Chemistry, VU University Medical Center, Amsterdam, the Netherlands

Magda Tsolaki, MD, PhD
Aristotle University of Thessaloniki, Memory and Dementia Center, 3rd Department of Neurology, "G Papanicolaou" General Hospital, Thessaloniki, Greece

Stefan Van der Mussele, MSc
Reference Center for Biological Markers of Dementia (BIODEM), Laboratory of Neurochemistry and Behavior, Institute Born-Bunge, University of Antwerp, Antwerp, Belgium; and Department of Nursing and Midwifery Sciences, Faculty of Medicine and Health Sciences, University of Antwerp, Antwerp, Belgium

Marcel M. Verbeek, PhD
Department of Neurology and Department of Laboratory Medicine, Radboud University Nijmegen Medical Centre, Nijmegen, The Netherlands; and Donders Institute for Brain, Cognition and Behaviour, Radboud University Nijmegen and Radboud Alzheimer Centre, Nijmegen, The Netherlands

Frans R.J. Verhey, MD, PhD
Department of Psychiatry and Neuropsychology, Maastricht University, School for Mental Health and Neuroscience, Alzheimer Center Limburg, Maastricht, the Netherlands

Pieter Jelle Visser, MD, PhD
Department of Psychiatry and Neuropsychology, Maastricht University, School for Mental Health and Neuroscience, Alzheimer Center Limburg, Maastricht, the Netherlands; and Alzheimer center & Department of Neurology, Neuroscience Campus Amsterdam, VU University Medical Center, Amsterdam, The Netherlands

Lars-Olof Wahlund, MD, PhD
Department of NVS, Section of Clinical Geriatrics, Karolinska Institutet, Karolinska University Hospital, Huddinge, Sweden

Robin Wolz, PhD
Department of Computing, Imperial College London, London, UK

Chengjie Xiong, PhD
Division of Biostatistics, The Knight Alzheimer's Disease Research Center, Washington University School of Medicine, St. Louis, Missouri, USA

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AUTHOR INFORMATION

Stephanie Vos is born in Tongeren on July 30 1986 and grew up in Riemst (Belgium). After graduating from high school at the Heilig Graf-instituut Bilzen in 2004, she started to study Psychology at Maastricht University. During her training she got selected to follow an Honors program in Psychology. In 2007, Stephanie started the Research Master Neuropsychology, for which she performed a research and clinical internship at the Department of Psychiatry and Neuropsychology of the Academical Hospital of Maastricht. In 2009, she graduated cum laude and started working as a PhD candidate at the Department of Psychiatry and Neuropsychology under supervision of Prof. dr. Frans Verhey and Dr. Pieter Jelle Visser. As part of her PhD project, she worked for 3 months at Washington University in St. Louis to collaborate with Prof. dr. Anne Fagan and colleagues. Presently, Stephanie Vos works as a postdoctoral researcher at the Department of Psychiatry and Neuropsychology.

Stephanie Vos is geboren in Tongeren op 30 juli 1986 en groeide op in Riemst (België). In 2004 rondde ze haar middelbare school af aan het Heilig Graf-instituut te Bilzen, waarna ze aan haar opleiding Psychologie begon aan de Universiteit Maastricht. Tijdens deze opleiding werd ze geselecteerd om een Honors programma in Psychologie te volgen. In 2007 begon ze aan de onderzoeksmaster Neuropsychologie en in kader hiervan deed ze een stage op de afdeling Psychiatrie en Neuropsychologie van het Academisch ziekenhuis Maastricht. In 2009 studeerde ze cum laude af en startte ze haar werk als promovendus op de afdeling Psychiatrie en Neuropsychologie van de Universiteit Maastricht onder leiding van Prof. dr. Frans Verhey en Dr. Pieter Jelle Visser. Als onderdeel van haar promotieproject heeft ze 3 maanden gewerkt aan Washington University in St. Louis om samen te werken met Prof. dr. Anne Fagan en collega's. Stephanie Vos werkt momenteel als postdoc onderzoeker op de afdeling Psychiatrie en Neuropsychologie.

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