Neurodegenerative Biomarkers in Healthy Elderly - with special reference to the preclinical pattern of biological and cognitive markers for Alzheimer's disease

Stomrud, Erik

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Erik Stomrud, MD

Clinical Memory Research Unit
Department of Clinical Sciences, Malmö
Faculty of Medicine

LUND UNIVERSITY

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Supervisors
Elisabet Londos, Associate professor
Lennart Minthon, Associate professor
Oskar Hansson, Associate professor
Lund University

External Faculty Examiner
Maria Eriksdotter-Jönhagen,
Associate professor
Karolinska Institutet
Stockholm, Sweden

Chair at Thesis Defence
Bengt Jeppsson, Professor
Lund University

Examination Board
Henrik Anckarsäter, Professor
Göteborg University
Martin Ingelsson, Associate professor
Uppsala University
Åsa Westrin, Associate professor
Lund University

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Neurodegenerative biomarkers in healthy elderly - with special reference to the preclinical pattern of biological and cognitive markers for Alzheimer's disease

Abstract

Background: Alzheimer's disease (AD) is a neurodegenerative disorder characterized by tau and amyloid brain pathology. With the gradual degeneration of neurons, cognitive symptoms will arise and the affected individual will eventually develop AD dementia. The neuropathologic hallmarks of AD have been observed also in cognitively healthy individuals, which has led to the assumption that the disorder has a long preclinical phase. Several biological markers for detecting and predicting AD have been validated over the years, where CSF biomarkers are one of the most recent and accurate markers. The generally perceived notion today is that these biological markers will be altered also in the preclinical phase. An additional aspect is that a review made in this thesis of the control samples in CSF articles, suggests that controls have been selected without efforts to minimize the misclassification of preclinical AD individuals. Therefore the aim of this study was to investigate biological and cognitive markers for AD in a group of cognitively healthy elderly individuals who were used as clinical control subjects in research studies.

Setting: The study sample consisted of 62 cognitively healthy elderly individuals from a clinical control group. They were followed for 4.5 years at three occasions and underwent assessments of EEG activity and regional cerebral blood flow (rCBF) as well as repeated assessments of cognitive function and CSF biomarker levels. The CSF biomarkers were Aβ42, total tau (t-tau) and hyperphosphorylated tau (p-tau). The cognitive testing included among others the MMSE, the ADAS-cog, cognitive speed (AQT), and subjective memory impairment.

Results: In the sample there were individuals with clinically pathological assessments on each separate biological marker. CSF Aβ42 levels predicted development of subjective memory impairment affecting quality of life at the 3 years follow-up and correlated with delayed word recall and cognitive speed at the 4.5 years follow-up. Additionally, the individuals with decreasing CSF Aβ42 levels during the follow-up performed cognitively worse than those with stable levels at the 4.5 years follow-up. CSF tau levels on the other hand correlated with an increase of the low-frequent theta activity on EEG and showed covariance with rCBF in the right medial frontal lobe and the left fronto-parieto-temporal area. In each case the correlations were stronger for p-tau levels compared to t-tau levels. Increase in theta activity was also correlated with slower cognitive speed.

Discussion: In this group of cognitively healthy elderly individuals there were individuals with deteriorated cognitive and biological markers associated with AD. These markers further correlated to one another in specific patterns, where it was the known AD-associated changes of the markers (i.e. low CSF Aβ42, high CSF t-tau and p-tau, decreased EEG rhythm, and decreased rCBF) that were primarily related. The findings could imply that the biomarkers might indicate early neurodegenerative changes of the brain and that these changes could be detectable before extensive cognitive impairment. The findings could also suggest that preclinical AD might be present in this "healthy" study sample. Hence, pathological processes prevailing in AD might bridge the clinically created arbitrary division of normal and non-normal aging of the brain.

Key words: Alzheimer's disease, dementia, MCI, preclinical Alzheimer's disease, biomarkers, cerebrospinal fluid, amyloid beta protein, tau protein, phosphorylated tau protein, EEG, SPECT, cerebral blood flow, cognition, memory, early diagnosis, control groups.

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Lund University, Sweden

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"*The important thing in science is not so much to obtain new facts as to discover new ways of thinking about them*"

Sir William Bragg

ABSTRACT ENGLISH

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– with special reference to the preclinical pattern of biological and cognitive markers for Alzheimer's disease

Erik Stomrud, MD

Background: Alzheimer's disease (AD) is a neurodegenerative disorder characterized by tau and amyloid brain pathology. With the gradual degeneration of neurons, cognitive symptoms will arise and the affected individual will eventually develop AD dementia. The neuropathologic hallmarks of AD have been observed also in cognitively healthy individuals, which has led to the assumption that the disorder has a long preclinical phase. Several biological markers for detecting and predicting AD have been validated over the years, where CSF biomarkers are one of the most recent and accurate markers. The generally perceived notion today is that these biological markers will be altered also in the preclinical phase. An additional aspect is that a review made in this thesis of the control samples in CSF articles, suggests that controls have been selected without efforts to minimize the misclassification of preclinical AD individuals. Therefore the aim of this study was to investigate biological and cognitive markers for AD in a group of cognitively healthy elderly individuals who were used as clinical control subjects in research studies.

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with rCBF in the right medial frontal lobe and the left fronto-parieto-temporal area. In each case the correlations were stronger for p-tau levels compared to t-tau levels. Increase in theta activity was also correlated with slower cognitive speed.

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SAMMANFATTNING SVENSKA

Neurodegenerativa biomarkörer hos friska äldre

– med fokus på tidiga förändringar av biologiska och kognitiva markörer vid Alzheimers sjukdom.

Erik Stomrud

Alzheimers sjukdom är den vanligaste demenssjukdomen och cirka 14 miljoner personer världen över lever med Alzheimers demens. En person som insjuknat i en demenssjukdom får problem med minnet och/eller andra intellektuella funktioner som leder till svårigheter att klara av vardagen. Vid Alzheimers sjukdom beror detta på att de två proteinerna amyloid och tau omsätts felaktigt och inlagras i hjärnan i form av senila plack och tangles, vilka kan ses vid mikroskopisk undersökning av hjärnvävnaden. Denna felaktiga omsättning leder till att nervcellerna i hjärnan fungerar sämre och att de till slut bryts ned med förlust av hjärnvävnad som följd. Mycket talar dessutom för att dessa processer vid Alzheimers sjukdom startar flera decennier innan den drabbade personen får problem med minnet.

Det finns idag flera undersökningar och tester för att påvisa dessa sjukdomsprocesser. En av de markörer som har visat sig vara bäst på att urskilja personer med Alzheimers demens från friska individer är analys av ryggvätska. Här mäts mängden av en speciell typ av proteinet amyloid (Aβ42) samt totala mängden av proteinet tau (t-tau) och mängden hyperfosforylerat tau (p-tau). Utöver detta kan vid Alzheimers sjukdom förändringar ses även av den elektriska aktiviteten i hjärnan (EEG-undersökning) och av blodflödet i olika delar av hjärnan (SPECT-undersökning). Eftersom sjukdomsprocesserna vid Alzheimers sjukdom tros föregå försämrat minne, har det spekulerats i att även dessa undersökningar skulle kunna påvisa sjukdomstecken innan minnet påverkas.

I avhandlingen redovisas en sammanställning av urvalsprocessen för de friska kontroller som har använts inom forskning om ryggvätskemarkörer. Sammanställningen visar att individerna väljs enbart utifrån minnestester och att i en tredjedel av fallen har individerna haft subjektiva problem med minnet. Eftersom sjukdomsprocesserna troligen börjar långt innan minnesproblemen visar sig finns det därmed en stor risk att individer med Alzheimers sjukdom i dessa studier bedömts som friska äldre, vilket kan påverka resultaten i studierna. Därför valde jag i denna avhandling att i en grupp intellektuellt friska äldre individer studera biologiska och intellektuella markörer som i tidigare studier visat samband med Alzheimers sjukdom.

I studien följdes 62 stycken intellektuellt friska äldre individer med olika tester under 4,5 år. De genomgick upprepade minnestester, ryggvätskeprov vid två tillfällen, en EEG-undersökning och en SPECT-undersökning. Flera intellektuella funktioner testades, däribland närminne, intellektuell snabbhet och subjektiva minnesproblem. Ryggvätskeprovet innebar att ryggvätska tappades ut från ländryggen och att nivåerna av proteinerna Aβ42, t-tau och p-tau mättes.

I studien sågs att det för varje specifikt test av de biologiska markörerna fanns individer med värden som i en klinisk vardag skulle ha bedömts som avvikande. Vidare sågs i gruppen att de undersökta markörerna var relaterade till varandra i specifika mönster. Lägre nivåer av proteinet Aβ42 i ryggvätskan kunde förutsäga sannolikheten att utveckla subjektiva minnesproblem efter 3 år men hade också ett samband med sämre resultat på minnestester avseende närminne och intellektuell snabbhet vid uppföljningen efter 4,5 år. Höga nivåer av t-tau och p-tau hade däremot ett samband med förlångsammad elektrisk aktivitet framför allt i bakre delen av hjärnan men även med sänkt blodflöde i den främre högra hjärnhalvan och ökat blodflöde i den bakre vänstra hjärnhalvan. Förlångsammad elektrisk aktivitet var också relaterat till sänkt intellektuell snabbhet.

Studien visade således samband mellan de undersökta markörerna i en grupp intellektuella friska äldre individer. Vid en närmare genomgång kunde detta samband ses just mellan de förändringar som i tidigare studier visat samband med Alzheimers sjukdom. Resultaten skulle kunna tala för att sjukdomsprocesserna vid Alzheimers sjukdom även finns hos friska äldre samt att de i studien undersökta markörerna möjligen skulle kunna påvisa dessa processer. För att säkert kunna fastställa detta behöver emellertid resultaten upprepas i större studier med längre uppföljningstid. Sammanfattningsvis talar resultaten i denna studie för att sjukdomsprocesserna vid Alzheimers sjukdom överskrider den tidigare symtombaserade, kliniska uppdelningen i vad som är ett friskt respektive ett avvikande åldrande av hjärnan.

LIST OF ORIGINAL PUBLICATIONS

I

Stomrud E, Hansson O, Blennow K, Minthon L, Londos E.
Cerebrospinal Fluid Biomarkers Predict Decline in Subjective Cognitive Function over 3 Years in Healthy Elderly.
Dementia and Geriatric Cognitive Disorders 2007; 24: 118–124

II

Stomrud E, Hansson O, Minthon L, Blennow K, Rosén I, Londos E.
Slowing of EEG Correlates with CSF Biomarkers and Reduced Cognitive Speed in Elderly with Normal Cognition over 4 Years.
Neurobiology of Aging xxx (2008) xxx–xxx;
doi:10.1016/j.neurobiolaging.2008.03.025

III

Stomrud E, Hansson O, Zetterberg H, Blennow K, Minthon L, Londos E.
Longitudinal CSF Biomarkers Correlate with Cognitive Decline in Healthy Elderly.
Archives of Neurology 2009; accepted

IV

Stomrud E, Forsberg A, Hägerström D, Ryding E, Blennow K, Zetterberg H, Minthon L, Hansson O, Londos E
CSF Biomarkers Correlate with Regional Cerebral Blood Flow on SPECT in Healthy Elderly.
Submitted for publication

CONTENTS

ABBREVIATIONS AND DEFINITIONS

ABBREVIATIONS

Aβ	Amyloid beta
Aβ42	Amyloid beta 1-42
AD	Alzheimer's disease
ADAS-cog	Alzheimer's Disease Assessment Scale - cognitive subscale
ADL	Activities of daily living
APOE	Gene coding for apolipoprotein E
APOE-ε4 allele	Allele coding for isoform apolipoprotein E4
APP	Amyloid precursor protein
AQT	A Quick Test on cognitive speed
CDR	Clinical dementia rating
CDT	Clock drawing test
CERAD	The Consortium to Establish a Registry for Alzheimer's Disease
CSF	Cerebrospinal fluid
CT	Computed tomography
DLB	Dementia with Lewy bodies
DSM-IV	Diagnostic and Statistical Manual of Mental Disorders, 4th edition
EC	Entorhinal cortex
FTD	Frontotemporal dementia
HMPAO	Hexamethylpropylene amine oxime
ICD-10	International Classification of Diseases, 10th revision, by the World Health Organisation
ICF	International Classification of Functioning, Disability and Health, by the World Health Organisation
MCI	Mild cognitive impairment
MemQoL	Quality of life assessment subscale memory

MMSE	Mini Mental State Examination
MRI	Magnetic resonance imaging
MTL	Medial temporal lobe
NFT	Neurofibrillary tangles
NIA-Reagan	National Institute on Aging and the Reagan Institute
NINCDS-ADRDA	National Institute of Neurological and Communicative Disorders and Stroke and the Alzheimer's Disease and Related Disorders Association
PET	Positron emission tomography
Pre-MCI	Stage preceding mild cognitive impairment
P-tau	Hyperphosphorylated tau protein
rCBF	Regional cerebral blood flow
SBU	The Swedish Council on Technology Assessment in Health Care
SPECT	Single photon emission computed tomography
SPM	Statistical Parametric Mapping, software program by The Wellcome Trust Centre for Neuroimaging, London, UK
T-tau	Total tau protein
^{99m}Tc	Metastable nuclear isomer of technetium-99
qEEG	Quantitative electroencephalography
WHO	The World Health Association

DEFINITIONS

Alzheimer's disease	Presence of neuropathology changes associated with post-mortem definition of the disease.
AD dementia	Fulfilment of criteria for dementia with concurrent explanatory presence of AD neuropathology changes or fulfilment of clinical criteria of AD
Prodromal AD	Presence of substantial AD neuropathology changes and cognitive symptoms however not enough to yield a dementia diagnosis
Preclinical AD	From the first AD neuropathology changes up to the first appearance of measurable cognitive symptoms

THESIS AT A GLANCE

	Hypothesis	Setting	Results	Conclusion
I–IV	In a group of cognitively healthy elderly individuals there will exist individuals with deteriorated biologic markers for Alzheimer's disease, and these will be associated with proxy markers for future dementia, such as worse cognitive performance.	Repeated cognitive function, repeated CSF biomarkers, EEG activity, and regional cerebral blood flow were assessed in 62 cognitively healthy elderly individuals over a period of 4.5 years. *Study design: prospective, longitudinal observation study*	CSF Aβ42 levels correlated with cognitive performance whereas CSF tau levels correlated with EEG activity and rCBF change. The combination of CSF Aβ42 and p-tau levels predicted cognitive decline and correlated with EEG activity and cognitive speed.	In a group of cognitively healthy elderly individuals there existed individuals with deteriorated levels of AD-associated biological markers and these could in part be associated with worse cognitive performance. *Hypothesis: true & falce*
I	AD-associated changes in the CSF biomarkers Aβ42, t-tau and p-tau correlate with and precede cognitive decline in a group of cognitively healthy elderly individuals.	Baseline CSF biomarker level analysis in 54 cognitively healthy elderly with cognitive follow-up after 3 years. *Study design: longitudinal*	Baseline CSF Aβ42 levels and the combination of high CSF p-tau and low CSF Aβ42 levels could predict development of subjective memory impairment affecting quality of life after 3 years with reasonable accuracy.	CSF biomarkers are related to future cognitive impairment before its onset and imply that AD-related biological signs might be detectable already in preclinical disease stages. *Hypothesis: true*

	Hypothesis	Setting	Results	Conclusion
II	AD-associated changes in the CSF biomarkers Aβ42, t-tau and p-tau are related to a concurrent change in the brain electric activity in a group of cognitively healthy elderly individuals.	Assessment of CSF biomarkers, quantitative EEG activity and cognitive performance in 33 cognitively healthy elderly individuals. *Study design: cross-sectional*	Increased CSF tau levels and tau/Aβ42 ratio correlated with increased relative theta activity furthermost in the right posterior part of the brain. These changes in CSF biomarkers and theta activity was further associated with slowing of cognitive speed.	Neuropathologic processes represented by CSF biomarkers correlate with cerebral function visualized by EEG rhythm and cognitive speed in cognitively unimpaired individuals. The biomarkers in this study might indicate early abnormal degenerative changes in the brain. *Hypothesis: true*
III	Individuals with a progressive deterioration towards an AD pattern in the CSF biomarkers Aβ42, t-tau and p-tau levels will perform cognitively worse than those with stable levels.	Change in CSF biomarker levels over 4.5 years in relation to cognitive performance at 4.5 years follow-up in 37 cognitively healthy elderly individuals. *Study design: longitudinal*	Both low CSF Aβ42 levels and substantial longitudinal decrease in CSF Aβ42 levels were associated with worse delayed episodic memory and cognitive speed. Substantial longitudinal increase in CSF p-tau levels was additionally associated with worse cognitive speed.	In these cognitively unimpaired individuals a concurrent decrease in CSF Aβ42 levels and cognitive performance might be observed, which would implicate that CSF biomarker levels might reflect very early neurodegenerative processes in the brain. *Hypothesis: true & falce*
IV	AD-associated changes in the CSF biomarkers Aβ42, t-tau and p-tau are related to a concurrent change in regional cerebral blood flow (rCBF) in a group of cognitively healthy elderly individuals.	Assessment of CSF biomarkers, rCBF, and cognitive performance in 32 cognitively healthy elderly individuals *Study design: cross-sectional*	Increased CSF p-tau and t-tau levels were associated with decreased rCBF in the right medial frontal lobe and increased rCBF in the left fronto-parieto-temporal area.	In cognitively unimpaired individuals AD-related biochemical processes represented by CSF biomarkers correlates with AD-related cerebral function visualized by rCBF. Hence, preclinical degenerative changes in the brain might be present as indicated by these biomarkers. *Hypothesis: true & falce*

INTRODUCTION 1

BACKGROUND 1|1

Imagine a train out of control rushing towards you down a railway track. This is how I have chosen to visualize development of Alzheimer's disease (AD) throughout my doctoral-studies. Everything starts at the point when the stationary train is set in motion. In this metaphor this event represents the onset of the first pathologic changes in the brain. At first no one notices that the train is moving with increasing speed. Not until it reaches the place where you are standing do you become aware of it. The time that has elapsed up until this moment represents the preclinical stage of the disease where the pathology can be extensive but cognitive symptoms limited. Later, as the symptoms progress, they ultimately reach a threshold where they become evident. To continue the train journey, you try to board the train but the speed is very high and you have to run along beside it for quite a while. Finally, you manage to throw yourself on board only to realize that the end of the track is in view and a crash will soon be unavoidable. You look for the emergency brake, but find that the train is not equipped with one. The only thing left to do is to jump off the train and stand on the side to see it crash. Returning to AD, health care providers try to investigate and diagnose the disease while the pathology continues to progress in the individual. Once we "boarded" the disease and diagnosed it we discover that we can neither cure it nor slow it down. The only thing we can do is to give proper care and symptomatic treatment as we see the individual deteriorate until the time of a premature death.

In order to change the series of events for the rushing train several actions could be taken. The first would be to make it impossible for the train to be set in motion to begin with. The second would be to install an emergency brake and the third would be to notice the rushing train earlier, especially if there was an emergency brake on board. Again, translated to AD the two first actions would mean the development of a preventive and disease-modifying or –arresting treatment. This is currently ongoing with several potential substances being tested. The third possible action would be to diagnose the disease earlier, already in the preclinical stages, before the point where extensive irreversible brain damage has occurred. Only then preventive treatment will be of value. Hence, increased knowledge of preclinical AD will be imperative if the new treatment strategies prove to be effective. I hope that this study will contribute to the acquirement of new knowledge and a better understanding of this preclinical stage in AD development.

RESEARCH APPROACH 1|2

PRE-EXISTING UNDERSTANDING 1|2|1

A central point of departure has been the opinion that the symptomatic, clinical definition of dementia and AD dementia in particular[1, 2] is not enough to understand the disorders as they present in the everyday contact with patients. This sense of inadequacy has further increased as disease-modifying or -arresting treatments are under development that will alter the underlying neuropathologic changes in the brain. Consequently, focus needs to shift from treating AD solely as a symptomatic condition towards treating it as a biological condition. This is further reinforced with the revised NINCDS-ADRDA research criteria where markers of biological changes have strengthened their position as proxy markers for the post-mortem histologic definition of the disease that is suggested as the gold standard.[3] In this biologically focused perspective, it is presumed that a biological sign that differs by more than two standard deviations from a validated, arbitrary reference value is most likely an indication of pathology and disease. It is this pre-existing understanding from which the scientific questions, hypotheses, aims and study design in this thesis have been formulated and hence determine the conclusions this study can provide.

ISSUES THE STUDY CAN ANSWER 1|2|2

There are methodological restrictions to the scientific questions that can be addressed in this study. The study is designed in such a way that it may indicate associations between the investigated variables, which could then be discussed in the light of neurodegenerative disease. However, the study is relatively underpowered, which could lead to type-II bias. Hence, absence of associations between the investigated variables in the study does not conclude that the variables are not related. Therefore, the study is limited in its ability to extract conclusions from negative findings and this will therefore be avoided in the thesis. If the restrictions are interpreted for this specific study it would suggest that: associations between markers for AD could indicate shared underlying pathology. However, claims whether the inter-individual spread of these markers is due to natural variation or disease cannot be made. Similarly, the outcome measurements available in the study prohibit attempts to estimate diagnostic validity of the markers for detecting incipient AD.

The sample in this study consists of cognitively healthy elderly individuals. Thus, the variance in cognitive performance and the incidence of longitudinal cognitive decline is limited. In order to overcome the limitation in available outcome measurements, only markers for AD have been investigated, which have been extensively validated for the manifest disease. If both experimental biomarkers and outcome measurements with little variance would have been used, then the ability to find true associations would be unacceptably low and attempts to draw conclusions would be practically impossible. Furthermore, the study has not been designed in a way that enables it to make statements regarding other dementia disorders. This could be a confounding factor since other causes of dementia might influence the cognitive performance variables.

One of the reasons behind the limited sample size, leading to absence in power, has been that invasive investigations have been performed without clinical indication. However, in dementia research these investigations are seldom performed on cognitively healthy controls, which will be shown in the CSF article analysis below. An even more unique element in dementia research is invasive investigations performed repeatedly on the same healthy individual. Nevertheless, one of the strengths of the current study is the sample size, albeit the power limitations. The fact that invasive and brain imaging radiating investigations have been performed on the same individuals additionally strengthens the novelty of this study.

Instead of providing the "true" preclinical pattern of the investigated markers for AD, the purpose of this study has been to explore the preclinical pattern of these AD-associated biological markers in a group of cognitively healthy elderly individuals. The attempts to explore this essentially unknown area of research must be regarded as initial steps that will need to be reproduced with larger and better powered studies. However, the current study could highlight those preclinical relationships that merit further investigation.

CONTRIBUTION OF THE AUTHOR 1|2|3

As a physician I am inclined to adopt an interest in exploring the biological perspective of AD. Additionally, I have a special interest in the clinical situation where the patient meets the professionals in the health care setting, which also contribute to the direction of this study. Therefore, the scientific questions of this study originate from the clinical questions that arise in these meetings. Hence, this study does not explore the actual techniques for investigating AD markers or the mechanisms behind the changes of the markers. Instead, the

point of departure is the desire to achieve a deeper understanding for how to interpret these markers preclinically and how to balance between them if they are in conflict. This is the same considerations that are made in the patient – physician relationship in order to identify the cause of the patient's problem.

THEORETICAL POSITIONS 1|3

The scientific questions in this study can never be treated as isolated phenomenons, separate from the rest of the AD research field. In fact there are adjacent scientific issues within which the study acts, that the researcher and the readers should be made aware of in order to appreciate the relevance of the study and its findings.

PATHOLOGY VERSUS MANIFESTATION 1|3|1

The first issue is whether to treat AD as a disorder of biological changes or of cognitive manifestation. This question is part of the more general discussion of symptoms versus aetiology. The first aspect of this issue is the WHO classification of functioning, disability and health (IFC),[4] in which a distinction can be made between body structure and function, impairment and disability (Figure 1). Body structure and function is, in this case, a biological term of physiological or anatomical properties of the body. Impairments refer to a deviation from a generally accepted standard in the individual capacity. It is not the same as the underlying pathology but instead a manifestation of this pathology. Hence, limping could be the effect of both congenital damage and of an adult-life injury. Finally, disability could be defined as the individual's interaction with the surrounding world. Impairment hereby does not have to lead to a disability since both the individual's behaviour as well as adaptation of the surrounding can compensate for the impairment. In the case of AD neuropathologic features and reduced cerebral blood flow are examples of body structure and function, whereas decline in cognitive performance is an example of impairment and finally, problems with ADL is an example of disability (Figure 1). This discussion is the core issue in the discussion of whether dementia is a disorder defined by the cognitive symptoms (impairments) or neuropathologic changes (body structure and function). Even though the relationships between the positions are not always conclusive, they are by definition not in opposition to one another. Instead they have different importance in different situations, which can be very evident in clinical practice. Therefore, the need to differentiate between symptoms and body changes, manifestations and pathology, clinical description and

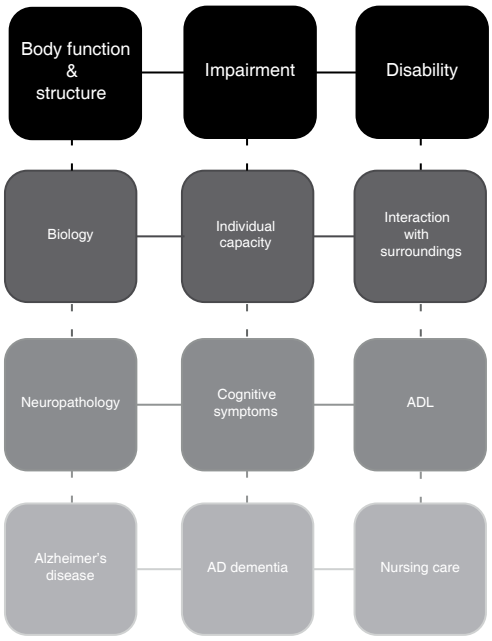

Figure 1. The International Classification of Functioning, Disability and Health (ICF) by WHO (top line) and how it can be interpreted for disease in general (second line), dementia disorders (third line), and Alzheimer's disease in particular (fourth line).

aetiology, in dementia diagnostics has been stressed by many for a long time.[3, 5-11] The direct impact this has on the current study is that I refer to the biological body changes as Alzheimer's disease (AD) and to the clinical descriptive, manifestation of pathology as AD dementia. In summary, the terms dementia and AD imply more than solely if an individual has a disease or not. The linguistic and declarative reality is quite simply more complex and it is in this reality that the current study tries to navigate.

SEQUENCE OF CHANGE 1|3|2

The second issue, which is derived from the prior one, is the importance of determining in which order changes in markers occur. In general, changes in body structure and function (pathology) can be assumed to precede impairments (manifestation of pathology). In AD it has further been suggested that change in functions precede change in structure.[12] Hence, the neuron will malfunction before it deteriorates and contributes to the progressive cell loss. With a logical step backward it would be reasonable to assume that the intracellular pathological process in its turn precedes malfunction of the neuron. Consequently, a logi-

cally derived rationale can be proposed for the probable internal order in which different investigations and biological markers for AD could be suspected to be sensitive in reflecting the disease.

NORMALITY 1|3|3

The third and final issue concerns the concept of normality. An exhaustive discussion covering the entire spectra of normality will not be possible in this thesis, since it spreads over several fields of sciences. However, in the light of the current study a number of aspects should be emphasized. Firstly, the word "normal" has different meanings depending on the circumstance in which it is used. For instance, in a biological setting, normal would be used to represent normal distribution, i.e. the Gaussian distribution, which is the probability distribution of a variable that tends to cluster around an average. In clinical practice, in the health care setting, normal would instead represent the values of a variable that lies within an arbitrary reference range (often set to cover 95 % of expected values in a representative population). In a philosophical setting it could be speculated that normal represents a preset or unspoken reference state, which is regarded as the desirable state and which the majority of individuals strive to attain. In the Dorland's Illustrated Medical Dictionary the word normal is defined as "*agreeing with the regular and established type*" and in the Swedish Academy Glossary it is defined in part as "*an example that constitutes guiding principle or pattern*".[13, 14]

This discussion leads to the second aspect, which is relevant in medical research. Controls are used as "types" and "patterns" to which others are compared and they are often referred to as "normal" individuals. Is, however, the value of a variable in a control individual by definition normal? In dementia research I would most often have to say no. The reason is that different scientific questions have different requirements for the controls. Sometimes the controls need to represent a general public and sometimes they need to be *free of disease*. Since selection of controls in dementia research is based on cognitive manifestations instead of a probable pre-existing, underlying pathology, the controls can seldom fulfil the requirement *free of disease* when needed. Thus, it is reasonable to speculate that deviant values in control individuals could be regarded as pathologic as well as non-pathologic. Hence, this issue influences research on biological markers for AD by shifting the way of thinking from the statement "it is normal since it occurs in the controls!" to the question "is it normal merely because it occurs in controls?"

STUDY DESIGN 1|4

The design of this study is based on a quantitative research setting. It is a prospective observational study and the collected longitudinal data allows both cross-sectional and longitudinal analyses, which will be specified in each paper.

The development of the current study and its aims can be traced back to the need for a control group at the memory clinic at Malmö University Hospital that could be used in research studies with different patient samples. As time went by and more research started to include biological markers, concerns arose in the research directory group that the controls were possibly not *free from disease* though they were cognitively healthy. The division of study samples based solely on cognition appeared very problematic due to the suspected long preclinical phase of AD. It was therefore decided that a clinical follow-up of the individuals was necessary, and I was recruited to do this follow-up. The first preliminary results from this follow-up indicated that perhaps these healthy controls were not free from disease, which was later published as the first paper of this thesis. These findings led to the arrangement of an additional, more extensive follow-up, which also included investigations of biological markers. Hence, longitudinal data was extended to include more than only cognitive performance.

The current study is an attempt to widen the knowledge of the preclinical pattern of biological markers, to explore possible mutual associations between the biological markers and to investigate possible relationships with cognitive impairment. However, this biological perspective of the disease will not be contradictory to the descriptive definition of the disease based on cognitive manifestations. As mentioned above, it is instead a separate, co-existing aspect of the disease. Consequently, the aim of this study to increase the knowledge in one of these aspects does by definition not restrict the knowledge or validity of the other.

THE CONTRIBUTION TO EXISTING RESEARCH 1|5

In the research of biological markers for AD the investigations of controls have been limited to creating reference values or as representing the "normal". This has provided a certain preclinical knowledge, which is reviewed in the following chapter, but more research is needed. At the start of this thesis only one article about CSF biomarkers with focus on cognitively healthy elderly individuals had previously been published. Research on biological markers for AD needs to un-

dergo the same development as research on AD neuropsychology and neuropathology where published longitudinal research on cognitively healthy individuals can be found. At the same time it is pertinent to ask the question: How has the preclinical knowledge, that already exists, been adopted by the AD research community? This question gives rise to two new questions: "What is the rationale for control selection?" and "What kinds of controls have been used in the previous research of biological markers if represented by the CSF biomarkers?" In the following two sections these questions will be investigated.

CONTROL SELECTION 1|5|1

Selection of controls is a difficult process if the case-control samples are to be comparable. Different selection principles have been proposed but it is often impossible to satisfy them all and conflicts between them inevitably arise. Wacholder et al. have proposed four principles for control selection: 1) study base, individuals should be enrolled from the same study base to reduce selection bias; 2) deconfounding, known confounders should be controlled for and possible confounders should have as little variability as possible to avoid confounding bias; 3) comparable accuracy, errors in measurements or obtaining information should be equal between groups to reduce information bias; 4) efficiency, constraining the other principles by limiting the time and resources available.[15] The principles however do not demand equality, merely comparability.[15] The validity of study outcomes in fact depends on the careful consideration in control selection.[16] Chui H has further suggested that study validity is decided by: 1) appropriate comparison groups, at least one of which is free of the targeted disease; 2) a clear description of the spectrum of patients and controls; 3) an independent and blind comparison of the test result with an appropriate reference ("gold") standard.[17]

In the text above there are two phrases that become relevant for this study. Firstly … *known confounders should be controlled for* … and secondly … *at least one of which (groups) is free of the targeted disease*. A problem that arises in dementia research is that normative studies and control groups in the elderly traditionally do not exclude those who have preclinical dementia.[18] It is well known that the sensitivity of cognitive tests is low for detecting future dementia and that the biomarkers studied often change earlier than clinical cognitive symptoms do when neuropathology is present.[3, 19, 20] Hence, a situation may arise in which preclinical cases are misclassified and in the end may lead to overestimation of normative age-related changes and delay in detecting pathological impairments.[18]

In summary, a careful control selection is crucial in the process to determine the normative data, validity and clinical applicability of a diagnostic biomarker. Misclassification of individuals with the targeted pathology should be avoided. The threshold for controls should basically be disease or not (pathology) instead of diagnosis or not (manifestation), since clinical diagnosis emerges far too late in AD.[21] The question that arises then, is how has this been applied to real life research settings up until now?

ANALYSIS OF CSF ARTICLES 1|5|2

To investigate how carefully the selection of controls in CSF studies has been made over the last years, a systematic evaluation was performed of the controls used in published articles on CSF biomarkers in AD. The detailed method for and the flow chart of the article selection are described in Box 1 and Figure 2.

Box 1. Method for the CSF article selection

Inclusion criteria

1) PubMED search on June 3rd, 2006
2) Combination of MESH-keywords
   a. Alzheimer Disease
   b. Biological Marker / cerebrospinal fluid
3) Published from 2003 to June 3rd, 2006
4) Original study using a normal material
5) English

Exclusion criteria

1) Non-AD dementia
2) Technique assessment independent of specific neuropathologic disease

Evaluated variables

1) Impact factor of publishing journal
2) Study design
3) Control selection process
4) Control examination

A PubMED search was conducted according to the set criteria and limits above. The identified articles in the set time period were reviewed and those articles that fulfilled the inclusions criteria and did not fulfil any exclusion criteria were selected. The remaining articles underwent an in-depth review where the information that was presented for the controls was abstracted. The impact factor of the publishing journal was determined by the Journal Citation Reports® in 2006, with one exception (see matrix in the supplements). An overview of the article selection process is presented in Figure 2.

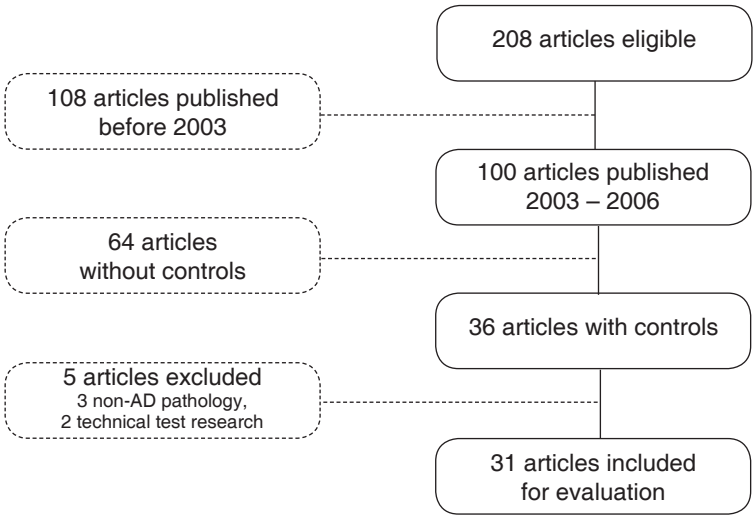

Figure 2. Flowchart of the article selection in the systematic evaluation of the controls used in CSF research on Alzheimer's disease.

Between 1985 and June 2006, 208 articles on CSF biomarkers and AD were published. With the exception of 1998 a substantial increase in the annual number of published articles was evident during the beginning of this decade (Figure 3). Of the 31 evaluated articles almost one third (10 articles) have used control material consisting of individuals with subjective memory impairment who do not fulfil the dementia criteria (Figure 4). Moreover, over two thirds (21 articles) have used other patient groups, such as patients with neurological complaints/disorders, psychiatric disorders, spinal anaesthesia before surgical intervention or subjective cognitive impairment. Whether these individuals are truly cognitively unaffected, i.e. healthy controls, is evidently uncertain. Only 3 articles have used either population based randomization or volunteering as control selection. Furthermore, 6 articles do not provide information on whether or not cognitive assessment was made on the controls. Examination for medical conditions that could affect cognition has been stated in 20 articles and examination for psychiatric conditions in 16 articles. Thus, the number of articles examining for these possible confounding factors does not exceed the number that has used possibly unreliable samples. As a reader I can only assume that the controls in the remaining articles had been examined at selection. In alignment with the findings of this review, previous systematic reviews on diagnostic tools have reported sufficient diagnostic evaluation of the control group in only one third of available studies.[22]

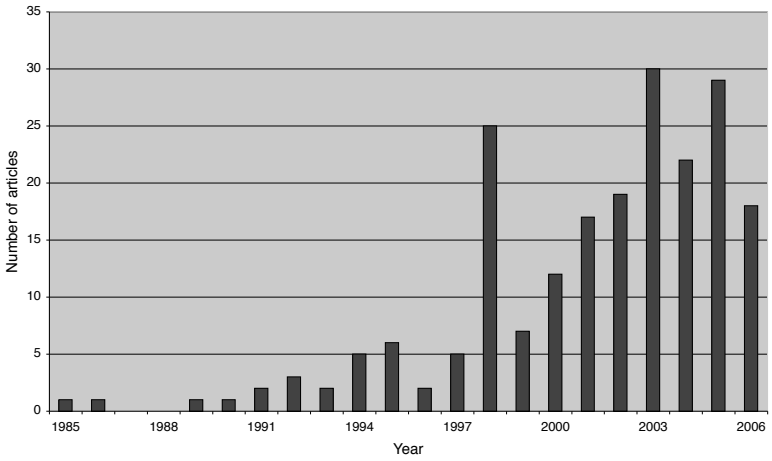

Figure 3. The number of published articles on CSF in AD before 2006.

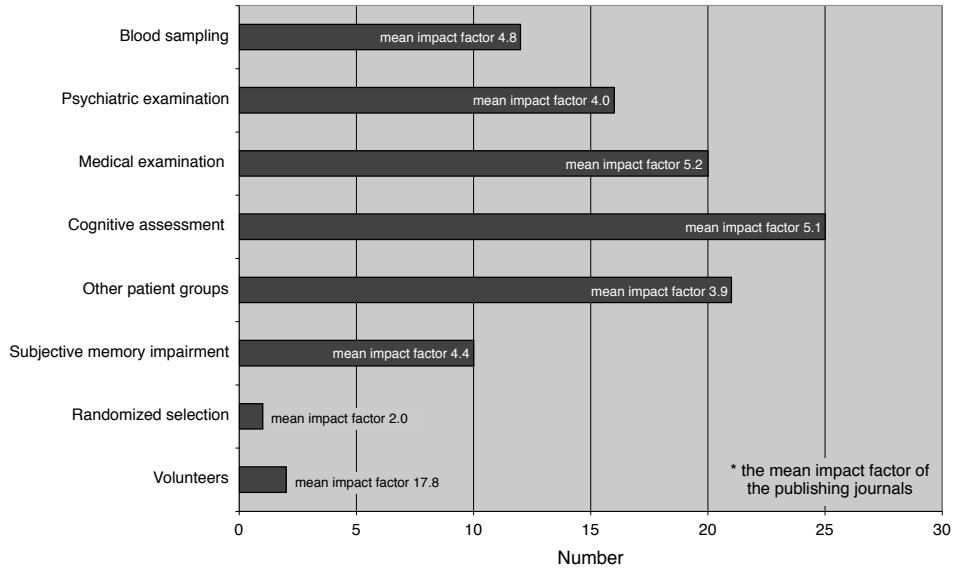

Figure 4. The number of CSF articles that described their control selection divided into different important points of information. The mean impact factor of the publishing journals was calculated for each point of information.

Moreover, one could hypothesize that high impact journals would set higher requirements for the control selection. However, the impact factor of the journal is not related to whether the controls include individuals with subjective cognitive impairment or other conditions. The only exception is the two very high impact factor journals of 12 and higher, which contain the only articles that used healthy volunteers. For the complete evaluation of the CSF articles please go to the matrix in the supplements (Table 4).

In summary, the selection of controls in the evaluated articles could increase the susceptibility of misclassification of incipient AD and thus could be in conflict with the deconfounding principle.[15] Several post-mortem studies have also shown that the neuropathological burden is greater in individuals with cognitive impairment than those cognitively unaffected.[23] Consequently, the frequent use of individuals with subjective memory impairment as "healthy" controls is questionable. An additional interesting aspect is that the controls had been investigated for physical disorders in 7 of the 10 articles that used cognitively impaired individuals as controls, which are conditions that would less likely affect CSF results compared to subjective cognitive impairment of unknown origin. Hence, the knowledge of avoiding confounding factors is there, but the correlation between subjective memory impairment and possible future dementia disorder is apparently overlooked. Fortunately, it appears as if an increasing number of studies after 2006 have been published with cognitively non-impaired individuals as controls. This could perhaps be due to a decrease in the reluctance of performing lumbar puncture without clinical indication, but this is merely speculation.

LOOKING FORWARD 1|5|3

The answer to the general question of how the current knowledge has been adopted in previous research can be summarized as questionable. If the focus is instead turned to the future, a few circumstances emerge that require closer attention in future studies of biological markers for AD. The first of these circumstances is the need to identify the requirements of the control selection in the specific study. The second is to gather the knowledge that currently exists for the investigated marker's preclinical pattern. The third and last is to adopt the outcomes of the former questions to the specific study. With this thesis I wish to contribute to increasing the knowledge in the second of these circumstances. In the introduction, I have therefore tried to show that there is both a need and a possibility of accomplishing this. In the case of the subsequent need to adopt the outcomes in actual study design, this is the future responsibility and duty of each individual researcher.

STRUCTURE OF THE THESIS 1|6

In the introduction, the intention is to give the background to why the scientific questions and design of the study are valid and important to make. The chosen disposition and topics of the introduction are intended as an effort to present the scientific considerations that occur in medical science but not always declared. In the following chapter I have chosen to quite extensively review the preclinical pattern of neuropathology, cognitive manifestations and relevant biological markers for this study. This is followed by the aim, design and results of the study and its separate papers. Finally the entire study is discussed and commented with the intention to take this discussion to a higher level than is possible for the articles on their own.

CURRENT KNOWLEDGE 2

DEMENTIA DISORDERS 2|1

The diagnosis of dementia is a clinical diagnosis determined by the fulfilment of criteria defined by either DSM IV or ICD-10.[1, 2] It is an arbitrarily set threshold based on the degree of decline in social and occupational activities due to impairments in different cognitive functions (Box 2). The term dementia says nothing about the underlying biological events and hence the condition can be caused by several different disorders. Some of the most common forms of dementia are: AD, vascular dementia, dementia with Lewy bodies (DLB) and frontotemporal lobe dementia (FTD).[11, 20] These diagnoses are all defined by post-mortem neuropathologic changes. In clinical practice, however, there is a need for a diagnosis when the patient is still alive. Therefore, clinical classification criteria have become available to determine the type of possible dementia disorder.[1, 2, 24-28] The diagnosis of AD, however, is made primarily through excluding other dementia disorders (Box 3).[20, 24]

The focus of the last decade has shifted more and more towards the underlying neuropathologies of dementia disorders and the biomarkers reflecting them. In alignment with this shift, the revised NINCDS-ADRDA research criteria for diagnosis of AD have set the cognitive requirements lower than needed for a dementia diagnosis. This makes it possible for an individual to be regarded as having AD without having dementia.[3] The underlying disease (AD diagnosis) is hereby separated from the symptoms and their effect on ADL functions (dementia

Box 2. Dementia short facts.

DEMENTIA[1, 2, 342]

Criteria

A. Memory disturbance.

B. Disturbances in other cognitive functions (DSM IV: aphasia, apraxia, agnosia, or executive function) (ICD-10: thinking, judgement, language, orientation, comprehension, calculation, or learning capacity).

C. Progressive disturbances with decline from previous higher levels of function. (ICD-10 = requires at least 6 months)

D. Impairments in social functioning and ADL functions.

E. Deficits also in the absence of delirium.

Prevalence

From 1 % (60–64 years of age) to 24–33 % (85 years of age or older) in developed countries. Worldwide 24.3 million people in year 2001 and 81.1 millions in year 2040.

Box 3. Alzheimer's disease short facts.

ALZHEIMER'S DISEASE [20, 124, 343–345]

Neuropathology:
Amyloid plaques, neurofibrillary tangles, synapse degeneration and neuron cell loss.

Risk factors:
High age. Genetic (APOE-ε4 allele and first line relative with AD). Somatic (middle-age hypertension). Intellectual (Low educational level and reduced mental activity in late life).

Cognitive symptoms at diagnosis:
Progressive episodic-memory impairment, aphasia, apraxia, and agnosia.

Prevalence:
From 0.6 % (60–64 years of age) to 14–20 % (85 years of age or older).
Worldwide 14 million people in year 2001 and 49 millions in year 2040. (AD dementia approximately 60 % of dementia cases)

Treatment – symptomatic:
Cholinesterase inhibitors (rivastigmine, donepezil and galantamine) and NMDA-receptor antagonist (memantine)

Treatment – disease-modifying:
None clinically available. Future possible agents: secretase modulators, Aβ immunotherapy, Aβ fibrillisation inhibitors, and anti-tau drugs.

Prognosis:
Life expectancy shortened and is in mean 3.5 years from diagnosis.

diagnosis). This is possible due to the fact that AD is a neurodegenerative disease in which the onset of neuropathologic changes precedes symptoms by decades.[29-32] A coherent terminology of AD development has been suggested in the revised NINCDS-ADRDA research criteria[3], in which AD dementia refers to the phase in which symptoms is severe enough to meet both dementia and AD diagnostic criteria. Prodromal AD refers to the phase in which symptoms of AD are present though not severe enough to yield a dementia diagnosis. The prodromal AD phase includes the mild cognitive impairment (MCI) condition. Preclinical AD refers to the long asymptomatic phase from the first neuropathologic changes to the first appearance of measurable symptoms (Figure 5).

The MCI classification is, as with the diagnosis of dementia, a clinical, criteria-based definition (Box 4).[33-37] It is an arbitrarily set threshold primarily dependant on the relative absence of decline in social and occupational activities despite presence of impairments in different cognitive functions. MCI can have the same underlying diseases as the dementia condition and a majority of the individuals with MCI progress to a dementia diagnosis before their death. There are however individuals who remain cognitively stable over time or even regain their earlier cognitive function.[35, 37, 38] The DSM IV has a similar definition called mild neurocognitive disorder, but it is stricter and requires measurable deficits in at least two cognitive functions.[1] It has further been suggested that a pre-MCI

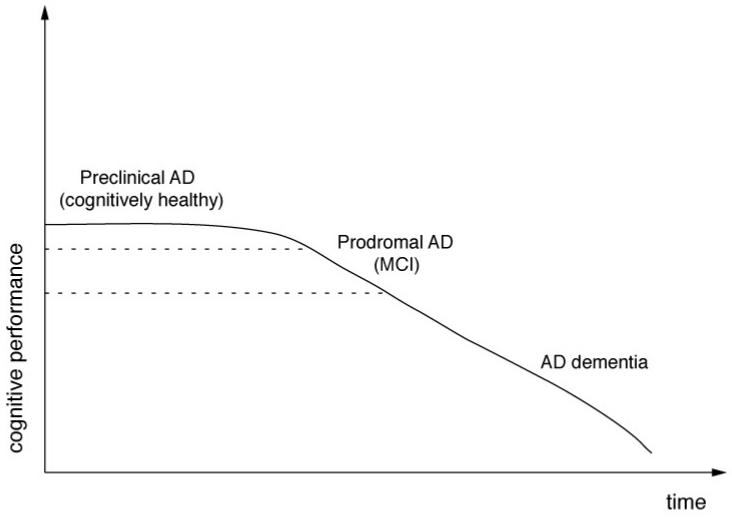

Figure 5. The natural development of a neurodegenerative disorder such as Alzheimer's disease with its preceding stages.

Box 4. Mild cognitive impairment short facts.

MCI [33, 35-37]

Criteria:

A. Cognitive complaint (subjective or through informant)
B. Objective cognitive impairment
C. Preserved general cognitive function
D. Largely intact ADL functions
E. Not clinically demented.

Prevalence:

Between 3 % and 19 % in an older population. Yearly incidence rate estimated to 8 58/1000 individuals.

Prognosis:

Progression-rate to dementia is 5–15 % per year and up to 80 % have progressed after 6 years.

phase exists, with non-measurable subjective memory impairment up to 15 years prior to developing MCI. Individuals with this subjective memory impairment have been observed to have a five-fold increase in the risk of developing MCI compared to individuals without subjective complaints.[39, 40] However, this definition is theoretical by nature since it would be impossible to apply it to clinical practice due to a large inter-individual overlap and heterogeneous underlying pathogenesis.

In summary, dementia disorders are classified according to their symptoms and effect on an individual's ADL functions. The classification ranges from asymptomatic via MCI to dementia. Meanwhile, the individual can concurrently be classified according to the underlying neuropathology (AD, DLB, FTD, etc.) irrespective of the symptom classification (Figure 6).

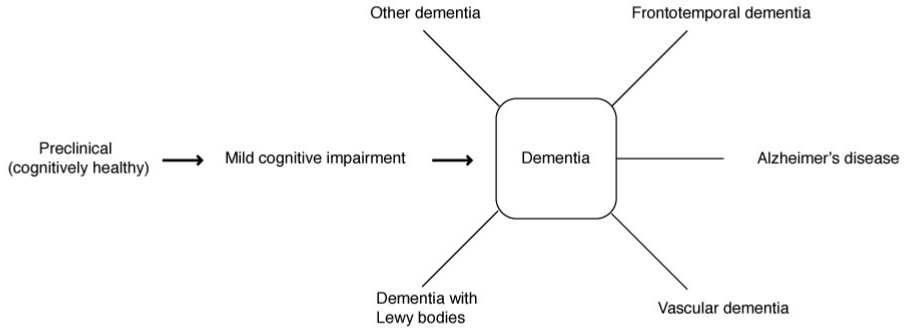

Figure 6. The development of dementia and different dementia subtypes.

AD AND ITS PRECEDING STAGES 2|2

NEUROPATHOLOGY 2|2|1

GENERAL (CLASSIFICATION CRITERIA)

Amyloid plaques (senile and neuritic) and neurofibrillary tangles (NFT) are hallmarks of the neuropathologic diagnosis of AD.[20, 24, 41-43] The extracellular plaques are composed of amyloid β depositions aggregated with different cerebral cells, whereas the intracellular NFT are composed of hyperphosphorylated tau protein aggregated in the cytoplasm (Figure 7).[20, 43-46] In AD, these histologic features are further accompanied by synapse degeneration, neuron loss and disturbance of acetylcholine transmitter activity.[10, 20, 41, 42, 47] Several neuropathologic classification criteria have been proposed based on these changes but with different requirements for type, amount and localisation of the changes. Some of the most common criteria are the Khachaturian consensus criteria,[41] the Braak neurofibrillary staging system,[42] the NIA-Reagan criteria[48] and the CERAD[49].

Neither the generating force behind AD pathogenesis nor the precise sequence of neuropathogenic events are fully understood but some central hypotheses have been suggested. One of these is the amyloid cascade hypothesis, which argues that the accumulation of amyloid β in the brain is the trigger mechanism.[45] Increased production and oligomerization of amyloid β_{1-42} occur from an alternative cleavage pathway of the precursor protein (APP) and the subsequent ac-

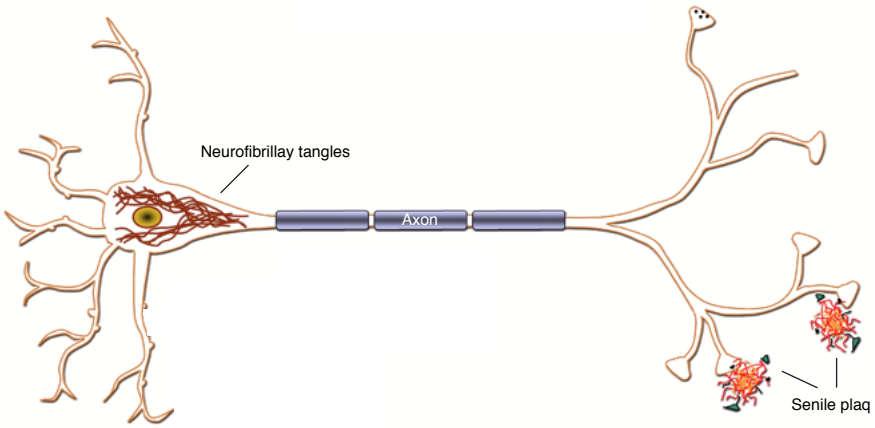

Figure 7. A neuron with AD pathology, i.e. intracellular neurofibrillary tangles (NFT) in the cytoplasm and extracellular deposits of senile plaques close to the synapses.
Modification of drawings by Professor K. Blennow. With permission.

cumulation into plaques leads to synapse and neuron damage. This in turn alters the phosphorylation of tau protein thus causing neuronal cell dysfunction and cell death. In support of this theory there are reports of neuronal toxicity of human Aβ oligomers[50] as well as of an abundance of soluble Aβ42 and subsequent plaques in individuals with Down's syndrome or hereditary AD due to an overexpression of APP. Hence, these individuals have a greatly increased risk of developing AD despite a later ensuing tau pathology.[51-55] Others argue, however, that tau abnormalities are the triggering mechanism.[44] It has been shown that pathologic hyperphosphorylation of tau protein leads to soluble tau proteins in the cells, which aggregate into neuropil threads and non-degradable NFT in the cytoplasm. The neuron can survive for many years with these deposits but becomes increasingly dysfunctional and will eventually deteriorate leading to cell loss. Beside these two hypotheses several other theories have been launched, suggesting that inflammatory processes, oxidative stress, or mitochondrial dysfunction are triggering mechanisms.[20]

In the dispute between the tau hypothesis and the amyloid cascade hypothesis, both pathologies are argued to occur exclusively from one another.[43] However, there is evidence from both sides of situations where the other hypothesis cannot fully explain post-mortem findings. As an alternative, Price and Morris therefore have suggested a combination of the two pathologies where both are crucial for the development of AD, as opposed to one being superior to the other.[31] They propose that NFT alone slowly increase ubiquitously with age,[29,

[56, 57] whereas plaques do not accumulate with age.[57, 58] Only when plaques are present, the NFT increase in an accelerated AD pattern. If the neurons manage to survive during these pathologic processes the individual remains clinically asymptomatic but because of the loss of neurons, due to progressive cell death, cognitive impairment emerges.[59-64]

In summary, it is the general notion that the neuropathologic features in clinically diagnosed AD dementia include NFT, senile and neuritic plaques, synapse destruction, neuron cell loss and regional brain tissue loss.

AD

In early AD, NFT accumulate in the hippocampus, entorhinal cortex (EC) and subiculum whereas plaques are first observed in the EC and neocortex.[20, 29, 43, 44, 57, 65-69] It is, moreover, in these areas that the subsequent synapse dysfunction, neuron loss and brain atrophy is first observed.[3, 59-64, 70] In general, it appears as if the areas of the human brain that have evolved last, are the ones to be first affected by AD neuropathology.[44] Later, at the time of the clinical AD dementia diagnosis, the neuron loss is substantial compared to non-demented individuals and the location reflects the cognitive impairments present.[20, 61-64] By now the entire temporal lobes, and especially their medial parts, are affected most intensely, followed by progression to the parietal lobes and eventually the frontal lobes.[44, 71, 72]

It should be added that in individuals with clinical AD dementia the presence of multiple pathologies is frequent (20-50 %).[73, 74] Multiple brain pathologies are suggested to increase the risk for an individual to develop dementia with an odds ratio of 20.7. These individuals also perform worse on cognitive tests and require only a mean of 1.9 NFT to develop dementia compared to a mean of 15.7 in individuals without multiple pathologies.[73, 75] AD pathology and cerebrovascular lesions is the most commonly observed combination.[73, 74]

In summary, neuron loss and multiple pathologies appears to be the primary events directly related to the cognitive impairment seen in AD dementia. These findings could hereby support the theories of vulnerability and reserve capacities or in other words why only some individuals develop clinical AD dementia in the presence of a certain amount of NFT and plaques.[75-77]

MCI

Although MCI is a heterogeneous condition, it is associated with an increased risk of developing AD dementia. This is also evident in the neuropathologic features of MCI individuals (CDR 0.5), where a substantial amount of NFT and plaques are often observed. [29, 60, 68, 78-82] The frequency of AD neuropathology in these individuals is reported to lie between 80-100 %, [78, 82] with the highest frequencies in groups with amnestic MCI. One study has reported intermediate numbers of neuritic plaques in neocortical areas and NFT in the medial temporal lobe (MTL) in MCI individuals compared to mild AD dementia and controls.[81] Moreover, memory function was most closely related to the number of NFT in the MTL, which is in alignment with other studies.[66, 81]

Neuron loss in MTL and especially in layer II of the EC is substantial in MCI individuals. The degree of this neuron loss is intermediate to that seen in AD dementia and cognitive healthy individuals or equivalent to that seen in mild AD dementia.[61, 64] The degree of layer atrophy and neuron loss is further related to the level of cognitive impairment.[64] Multiple pathologies are another histologic feature of AD dementia that can be seen on intermediate levels in MCI individuals. It has also been suggested that amnestic MCI, as compared to non-amnestic MCI, more closely resembles mild AD dementia and has a higher frequency of multiple pathologies.[73]

In summary, the histological features of AD precede dementia diagnosis and are almost fully developed before or concurrent with the first objective cognitive impairment.

PRECLINICAL

Neuropathologic investigations of older individuals without any measurable cognitive impairment (CDR 0) almost exclusively report the presence of NFT and plaques in the brain in an appreciable number of individuals.[23, 29, 30, 56-58, 60, 65-67, 73, 79, 80, 82-87] The frequency of AD neuropathology varies between different studies, which could be explained by different classification criteria being used but also by difficulties of standardizing a definition of a non-demented individual. In general frequencies of 10–40 % are reported, [23, 30, 56, 57, 80, 84, 86] however frequencies as high as 90 % have been reported as well as frequencies below 10 % according to the most strict criteria.[82, 84]

The first areas affected by these changes in non-demented individuals are primarily regions related to AD neuropathology, thus the MTL for the NFT and the temporal neocortex for the plaques.[29, 43, 57, 65-68] In contrast to MCI and AD

dementia there is no substantial neuron loss in individuals without cognitive impairment regardless of whether or not there is an abundance of NFT or plaques.[59-64] In addition, the occurrence of multiple pathologies is much more scarce (19 %) compared to cognitively impaired individuals (56%) although single pathologies are common (48 %).[73] Furthermore, some studies have reported correlations between the presence of AD neuropathology and poorer episodic memory, [23, 30, 56, 57, 66, 80] although other studies have not been able to reproduce these findings.[84, 85] This lack of coherence in the findings might be due to the same causes as discussed above for neuropathology frequencies, i.e. different classification criteria and the inexact definition of a non-demented individual.

In general, the consensus is shifting more and more towards regarding non-demented individuals with abundant AD-associated neuropathology as pre-clinical AD.[23, 29, 56, 57, 68, 80, 85] However, it still has to be emphasized that a majority of non-demented older individuals only have a limited amount of AD neuropathology if any at all.[84] It appears to be the quantity and distribution rather than the quality of the changes that determines whether neuron loss and subsequent cognitive impairment is present.[60, 68, 79] Meanwhile, if the histological changes represent a preclinical AD state, the individuals would not be healthy. Then the generally perceived notion that AD neuropathology is abundant in cognitively healthy elderly control subjects would be false and instead indicate presence of disease.[56, 68]

In conclusion, all histologic features associated with AD can in fact be observed in brains of cognitively healthy older individuals. Neuropathologic findings are however of little use in the clinical every-day life since we cannot perform autopsies for neuropathologic investigation when the individual is still alive. Hence proxy markers are needed to identify the neuropathologic changes and the possible preclinical AD individuals.

COGNITIVE FEATURES 2|2|2

AD

Cognition in AD dementia can be regarded in several ways. It is the most important symptom of the disease and disturbances in cognition are required for diagnosis.[3] It is in fact what defines the clinical disease.[1, 2] Meanwhile, it is also an important marker of disease severity and is increasingly being used as a risk and prediction marker for future development of AD dementia for example in MCI.

At the time of dementia diagnosis, episodic memory and foremost delayed recall are the cognitive functions most closely related to AD.[3, 20, 88-91] In the revised NINCDS-ADRDA criteria it has even been suggested that solitary epi-

sodic memory impairment is sufficient for a diagnosis of AD.[3] Furthermore, large community cohort studies have reported that episodic memory, semantic memory, visuospatial functions, executive function and perceptual speed are the most precise in differentiating very mild AD dementia from healthy controls.[92-95] In general the classical initial cognitive impairment triad in AD dementia beside episodic memory deficits is apraxia (praxis), agnosia (visuospatial function) and aphasia (language).[3, 20, 88] With the progression of the disease these cognitive functions become more severely reduced and are followed by impairments in other cognitive functions. In the later stages of the disease frontal lobe cognitive impairments (i.e. personality change, fluctuating mood with aggression, apathy, etc) can be seen. In the end, almost all cognitive functions are severely impaired and the individual loses much of the ability to communicate with the surrounding world.[88]

In addition, non-cognitive disturbances such as depression are also common in AD at the time of clinical diagnosis.[88] In general, the sequence of impaired cognitive functions follows the locations of the neuropathologic changes of the disease.[44, 96] It is important to emphasize that episodic memory deficits are not pathognomonic for AD dementia and when the deficits are present other causes such as depression, somatic disease, non-AD dementia, etc have to be considered.[3]

MCI

Deficits in cognitive functions are the definition of the clinical condition MCI.[35, 38, 97, 98] Due to its heterogeneous pathogenesis, the combination of impaired cognitive function will differ between MCI individuals. Episodic memory is often thought to be impaired in the individuals who progress to AD dementia and has the strongest predictive value for this conversion.[19, 35, 90, 96, 99-102] Other cognitive impairments seen in individuals with MCI that progress to AD dementia include executive function, perceptual speed and new learning.[35, 90, 94, 96, 101, 103] It has further been reported that MCI with impairments in multiple cognitive functions have a significantly increased likelihood of converting to dementia.[89, 94, 103-105] However, differences in the prognosis between different impaired cognitive functions in MCI have not been observed and AD dementia and vascular dementia are similar in the extent of their cognitive deficits in the MCI stage.[19, 35, 82, 90] There is also a considerable overlap in cognitive performance in MCI individuals converting to AD dementia and those who remain stable.[94] Hence, the pattern of cognitive impairment is not sufficient to separately predict conversion from MCI to AD dementia.

PRECLINICAL

The term preclinical in the discussion of cognitive impairment is rather contradictory since the period by definition precedes the first cognitive impairments. It is therefore inevitable that the information in this section overlaps with that in the MCI section above. However, variance in cognitive performance between individuals does not necessarily mean that someone has to be impaired.

Many large longitudinal epidemiological community cohort studies of non-demented elderly have investigated the preclinical cognitive traits of incipient AD. The longest follow-up period exceeds 20 years in one of the studies.[106] In all of these studies, impairment in episodic memory is a characteristic cognitive feature of AD dementia and one of the first and most severely affected in the development of the disease.[89-91, 94, 99, 100, 102, 103, 106-113] In addition, other memory functions are also reported to be affected in these early stages, [112-117] as well as other cognitive functions such as cognitive speed, executive functions, abstract reasoning, new learning and attention.[89, 94, 103, 104, 106, 113, 115-117] The length of time from the first observed impairment to the dementia diagnosis differs between studies, depending on the length of follow-up and the cognitive function tested for. In general, onset of cognitive impairment six to nine years before AD dementia diagnosis is reported.[90, 100, 106-108, 110-112, 114, 115, 118, 119]

Overall, it appears to be delayed memory that is the main and best validated predictive cognitive feature of future AD dementia.[89-91, 99, 100, 106, 108, 110-112] However, despite the stringency in the findings of these large community sample studies there are a couple of aspects that need to be taken into consideration. As mentioned previously, these cognitive deficits are not pathognomonic for AD. The normal aging process probably causes deficits in the same cognitive functions as preclinical AD, i.e. episodic memory, perceptual speed and executive functions.[19, 94] Nevertheless, this age association impairment could be due to the presence of preclinical AD in the control samples, leading to overestimation of age affect that has been discussed in the introduction.[18] It has been argued that when it comes to cognition, preclinical AD should be treated as both quantitatively and qualitatively different from age-associated cognitive decline.[91] Finally, it was reported in one study that one third of individuals who develop AD dementia do not present any cognitive deficits 3 years prior to diagnosis and only 38 % had both subjective memory complaints and domain-specific cognitive impairments.[107] Hence, some individuals tend not to progress from normal aging to clinical AD dementia through a long period with cognitive impairment.

In summary, impairments in cognitive performance are crucial for and define AD dementia but do not alone have sufficient ability to act as a proxy marker for future AD dementia.

DIAGNOSTIC BIOMARKERS 2|2|3

In AD, neuropathologic changes have previously been regarded as the diagnostic gold standard and clinical criteria as the major proxy marker for probable AD.[3, 24] However, biomarkers within molecular science and brain imaging have in the last decades increasingly positioned themselves within the research field. The general definition of a biomarker is that it should detect a fundamental and preferably unique feature of neuropathology of the specific disorder.[120, 121] Its qualities should be confirmed by at least two independent, well powered studies with specified control subjects.[120] Furthermore, a biomarker can have several purposes, for example as a diagnostic marker, as a marker for monitoring a disease or preceding conditions and as a prognostic or predictive marker for future disease development.[122] Fox and Growdon have further suggested a division of biomarkers into marker of trait, state or rate. A trait marker predicts the likelihood or susceptibility of developing a certain disease, a state marker is a diagnostic marker and a rate marker detects progression of the pathophysiology.[121] Sometimes the synonym "stage" is used instead of "rate".[123]

In an attempt to define the requirements of a valid biomarker a working group at NIA-Reagan have proposed that: "*The ideal biomarker for AD ... should have a sensitivity of 80 % for detecting AD and a specificity of 80 % for distinguishing other dementias; it should be reliable, reproducible, non-invasive, simple to perform, and inexpensive.*"[120] However, the requirements of non-invasive, simple to perform and inexpensive will inevitably be relative. First, these requirements cannot be mandatory if superior and compelling diagnostic data is presented for the biomarker.[122] Second, no clear definition can be made of the requirements and what is expensive and complicated for one individual might be regarded as inexpensive and simple by another.

According to recent large systemic reviews/technical assessments from NINCDS-ADRDA and SBU in Sweden, the biomarkers that are currently best validated for early diagnosis of AD are CSF biomarkers, structural imaging with MRI and functional imaging with PET (Table 5 in the supplements).[3, 124] This was also indicated in the older American Academy of Neurology practice parameters from 2001[125] and is supported by several other published reviews.[20, 35, 113, 126, 127]

In the CSF it is amyloid $\beta_{1\text{-}42}$, total tau-protein, hyperphosphorylated tau protein and combinations of the three that have been best validated with sensitivities of 85–94 % and specificities of 83–100 %.[3, 123, 128] In MRI it is age-corrected MTL atrophy that best distinguish and predict AD dementia with sensitivities and specificities greater than 85 %.[3, 20, 127, 129] In assessments of atrophy, CT is inferior to MRI, however due to cost and access in clinical practice CT is useful to structurally identify treatable differential causes of cognitive impairment.[124, 127] The PET investigation can measure blood flow, glucose metabolism and protein aggregates of amyloid and tau, and the pooled evidence for discriminative and predictive sensitivities and specificities have been reported to be greater than 80%.[3, 127, 130] SPECT, in similarity with PET, measures blood flow but has generally produced sensitivities and specificities between 70–80 %, which is below the recommended requirements of a diagnostic marker as described above.[3, 131, 132] Hence, SPECT is inferior to PET in accuracy although currently often less expensive and more accessible in clinical practice.[127] The EEG activity alters with the development of AD, however the diagnostic accuracy is substantially inferior to the other biomarkers and the clinical utility is concluded to be insufficient to suggest EEG as a biomarker for initial evaluation.[124, 133] APOE-ε4 allele is already one of the biological trait markers for AD and increases the susceptibility of AD development. However, it has no determining position in the diagnostic evaluation of AD dementia.[20, 124] Additional biomarkers that could be of value in the future are plasma/serum/blood markers, urine markers and functional MRI.[122, 126, 127] The combination of different biomarkers has furthermore been reported to add to the diagnostic accuracy.[129, 131, 134-137]

As mentioned above all these biomarkers reflect different processes and hence will be in the transition from healthy to pathologic levels at different times of the disease development. Almost exclusively, these transitions should occur in preclinical stages since they reflect neuropathologic processes leading to decline in cognitive function. It can be noted that a few studies report that glucose metabolism in the EC in PET, the hippocampal atrophy rate, theta activity on qEEG and CSF biomarkers have predicted development of MCI in healthy elderly.[134, 138-142] As discussed in the introduction, it can be speculated that trait markers occur first (APOE-ε4 allele), followed by state markers reflecting pathogenesis (CSF biomarkers) and later state markers reflecting cerebral and neuronal function (rCBF PET and EEG activity). Finally, state markers reflecting structure would change (MTL atrophy on MRI). The current level of evidence in preclinical AD for the biomarkers investigated in this thesis will be summarized separately in the "Biomarkers" section below.

COGNITIVE TESTS 2|3

MMSE 2|3|1

The MMSE is one of the most used cognitive screening tests in the world. Attention, verbal recall, expressive language, visual construction, orientation and calculations are the main cognitive functions tested.[143] The normative values have been derived from several large community-based population studies and are suggested to lie between 24 – 28 points.[144-147] In comparison, Folstein et al. suggested a normative value of 27 in the original MMSE study.[143] The reliability of the test is reported high (0.83–0.89) and the sensitivity and specificity are suggested to lie between 69–91 % and 87–99 % respectively.[148] However, in a systematic review by SBU in 2006, these numbers could not be calculated since no study with the required quality standard had been published.[124] The MMSE is known to be of little value in early stages of cognitive decline and 25 % in a population-based elder control sample score 29 or 30 points out of 30 points.[145] Confounding factors are reported to be age (3 points decline over 20 years), educational level (1 point lower with low educational level) and possibly gender.[145, 146, 148] However in a large population study, the MMSE score correlated with age but did not change over time in the same individual. This could suggest that the tested cognitive functions are not affected by increasing age but instead by underlying preclinical neuropathology that increases in a population with increasing age.[149]

ADAS-cog 2|3|2

The ADAS-cog is similar to other cognitive screening tests such as the MMSE but with the exception that some cognitive functions are tested somewhat more thoroughly. The cognitive functions tested are, among others, attention, verbal recall, expressive language, visual construction and orientation. The more cognitively impaired the higher results on the test, which spans from 0 to a maximum of 70 points (without delayed recall) or 85 points (with delayed recall).[150] Normative values are estimated to around 5 points (without delayed recall).[151-154] Reliability of the ADAS-cog has been reported to be high and the sensitivity and specificity are 90–100 % between healthy controls and AD dementia.[150, 153, 155] Small confounding effects have been reported by age (primarily on delayed recall), educational level and gender.[151]

AQT 2|3|3

AQT is a test where 40 figures are to be named according to their colour, form and combination colour and form as quickly as possible (figure 14). It is a test of cognitive speed and is dependent on visual recognition and verbal fluency, naming and automaticity.[156] The naming of the dual colour-form task is the most cognitively challenging. CBF measurements during the test session have reported activation of parietal lobes and deactivation of frontal and fronto-temporal lobes compared to resting levels. This is a similar pattern to those seen in tests of working memory with visual input.[156] Normative values are set to < 60 sec in the naming of both colour and form, whereas a result > 70 sec is suggested to be pathologic. Results between 60 – 70 sec are said to be possibly pathologic.[157] The sensitivity and specificity have been reported to be 97 % in one study, compared to a sensitivity of 100 % and a specificity of 84 % for the MMSE in the same sample.[158] The reliability of AQT was estimated to 0.88–0.96 with the same objections as for the sensitivity and specificity.[157] Increasing age has been the only observed confounding factor and is associated with a slower performance.[156, 157]

CLOCK DRAWING TEST 2|3|4

In the clock drawing test (CDT) the test subject is instructed to draw a clock and put the hands to show 10 minutes past 11 (Figure 8). This can be qualitatively as well as quantitatively evaluated and a large number of assessment scales have been proposed.[159] Cognitive functions involved in the test are visuospatial function, constructional praxis, comprehension and semantic input with the re-

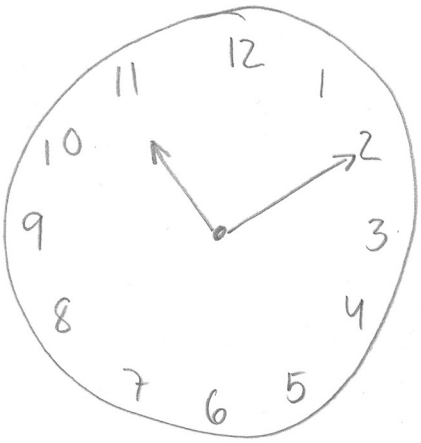

Figure 8. Correctly drawn hours and hands on the clock drawing test.

quirement of abstract thinking.[159] Good reliability measures have been reported however not specified and is not applicable between the different assessment scales.[160-162] The normative value is estimated to 8.5 points in the 10 point maximum score assessment scale used in the current study.[160] Sensitivity and specificity are suggested to be around 85 % in systematic reviews and thus valuable for cognitive screening.[124, 159] However, others have reported much poorer numbers, and with great differences between the assessment scales.[161] In spite of this, several studies have suggested that the CDT is more sensitive for detecting early cognitive impairments compared to the MMSE.[159, 163] The CDT, however, has not been able to predict conversion to AD dementia in MCI samples, which limits its use.[164, 165] Educational level has been reported to be a confounder but the test has the advantage of being useful even for illiterates.[159, 161] Both age and gender are suggested confounders.[161]

CUBE COPYING 2|3|5

Cube copying is included in other cognitive screening tests such as the ADAS-cog. The test subject is instructed to make a copy of a transparent, three-dimensional figure in the shape of a cube (Figure 9).[150, 166] It can be both qualitatively and quantitatively evaluated, and Maeshima et al. have suggested that the numbers of correct vertexes and plane-drawing lines should be calculated.[167] Cube copying tests several cognitive functions including visuospatial function and constructional praxis. The three-dimensional aspect of the test is proposed to make it more sensitive to early changes in cognition than other copying tests.[168] Healthy elderly perform 18 – 20 points out of a maximum of 20 points. The test has been shown to discriminate healthy controls from AD dementia and to

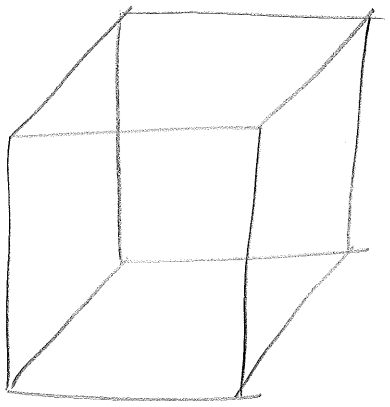

Figure 9. A correctly drawn cube on the cube copying test.

a certain extent predict conversion to AD dementia in individuals with MCI.[165, 166] Low educational level has been observed to be a confounder, whereas the reported confounding effect of age and gender is not as clear.[169, 170]

BIOMARKERS 2|4

CSF 2|4|1

At present, the best validated biomarkers for AD in the CSF are Aβ42, t-tau and p-tau.[128, 129, 136, 171, 172] Aβ42 constitutes a crucial component in the core of senile and neuritic plaques that are seen in AD. It is a proteolytic product from the trans-membrane APP in the neuron cell wall. The APP is encoded on chromosome 21 and can be metabolized in two pathways where one leads to the extracellular release of Aβ42 by cleavage of the β-and γ-secretase (Figure 10).[171, 173] Tau is instead a major component in the NFT that are seen in AD. It is an intracellular protein found in the neuronal axons and contributes to the neuron's stability and axonal transportation. If hyperphosphorylation of the tau occurs it loses these abilities and aggregates into intracellular paired helical filaments, which make up the NFT in the cytoplasm of the neuron. With neuron degeneration, tau is released into the CSF. T-tau reflects all tau released whereas p-tau reflects the hyperphosphorylated tau released. Hence, p-tau is suggested to reflect whether a pathologic phosphorylation state was present in the degenerated neurons (Figure 11).[171, 173, 174]

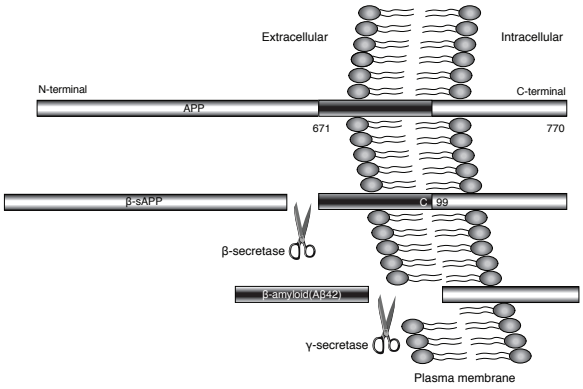

Figure 10. Metabolism of the amyloid precursor protein (APP) with amyloid β1–42 generation.
Modification of drawings by Professor K. Blennow. With permission.

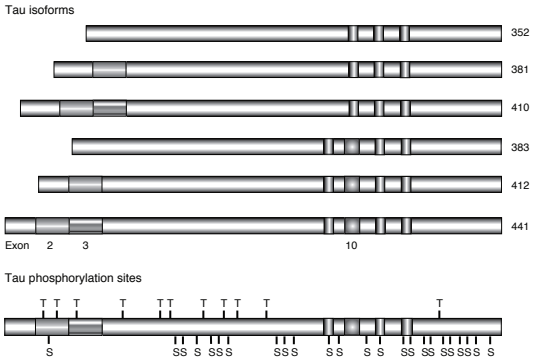

Figure 11. Tau protein isoforms and below possible physphorylation sites of tau, either the amino acids threonine (T) or serine (S).
Modification of drawings by Professor K. Blennow. With permission.

DIAGNOSTIC ABILITY

In AD a decrease in CSF Aβ42 levels and an increase in CSF t-tau and p-tau levels can be found.[3, 123, 128, 136, 171, 172, 175] The magnitude of these changes has been assessed to a 50 % decrease in CSF Aβ42 and a 300 % increase in CSF t-tau.[136, 172] Numerous studies have reported these changes and estimated the sensitivities and specificities to well over 80 % in the discrimination between AD dementia and healthy controls. Progression in individuals with MCI has further been predicted with equally good accuracy.[123, 128, 176-179] The diagnostic accuracy has been additionally improved by using combinations or ratios of the three.[136, 171, 178-181] There have been reports suggesting that the main predictive ability for conversion in MCI is in the CSF t-tau and p-tau levels, [182] whereas the ability to differentiate AD from other causes of cognitive impairment has instead been attributed to the CSF Aβ42 and p-tau levels.[123, 171] In addition, AD-associated changes in CSF biomarkers (low CSF Aβ42 and high CSF t- and p-tau) have by some also been associated with a more advanced cognitive decline in early stages of cognitive impairment.[183]

The separate changes in the CSF biomarkers are not specific to AD.[171, 173] A decrease in CSF Aβ42 levels is also seen in conditions such as DLB, [184] amyotrophic lateral sclerosis[185] and multiple system atrophy.[186] An increase in CSF t-tau levels is seen in conditions with massive neuron loss such as Creutzfeldt-Jakob disease (CJD) and transiently after acute stroke.[187, 188] Mild increases of CSF t-tau levels can also be found in FTD.[189] In contrast, CSF p-tau is not especially increased in CJD or after stroke and hence is suggested to be a more specific biomarker for AD.[171, 173] In some studies old age has been associated with a gradual increase of tau in the CSF[173, 190-193] but this has been negated in other studies.[194-198] In con-

trast, most studies have been consistent in showing no correlation between age and CSF Aβ42 levels.[193, 194, 198]

PRECLINICAL

In contrast to other biomarkers, the predictive value of CSF biomarkers has been investigated already in preclinical stages. Four such studies have, to our knowledge, been published until the middle of 2009 (not including the manuscripts of this doctoral thesis). The first study reported that CSF Aβ42 levels were lower in non-demented, healthy older individuals who developed dementia within 3 years.[139] The second reported that high CSF t-tau:Aβ42 and p-tau:Aβ42 ratios predicted conversion from CDR 0 (cognitively healthy) to CDR greater than 0 (MCI or dementia) in an community-based sample of elderly over 5 years.[138] The third reported that cognitively healthy controls with high CSF tau:Aβ42 ratio had a significantly increased risk of converting to MCI within 4 years.[140] The fourth and last study reported that low CSF Aβ42 levels were the sole predictor for cognitive decline tested with MMSE or development of dementia over an 8 years period in a community-based older cohort.[141] In summary, these studies suggest that AD-associated changes in CSF biomarkers could possibly be seen in preclinical stages and could be related to poorer cognitive performance. In addition to these studies, decreased CSF Aβ42 levels have been correlated to decreased whole brain volume in healthy controls.[199] An interesting aspect in that study was that in mildly demented individuals the correlation was instead observed with increased CSF t-tau and p-tau levels. Nevertheless, other studies have denied such correlations between CSF biomarkers and whole-brain volume in samples with manifest AD dementia.[200]

LONGITUDINAL STABILITY

Longitudinal changes in CSF biomarker levels have also been previously investigated. With few exceptions, [191, 198, 201] CSF t-tau and p-tau levels have been reported to remain stable regardless if investigated in AD dementia, MCI or healthy controls.[182, 192, 194-196, 202-208] CSF Aβ42 levels on the other hand have both been reported stable, [191, 194, 198, 205, 207-210] as well as declining over time.[203, 204] An explanation to the difference in results is that the studies with stable levels have had an insufficient length of follow-up.[204] Another possible explanation could be the time point in the AD development at which the investigation was performed, since one study reported longitudinal decrease in CSF Aβ42 levels only in prodromal AD.[191]

CSF VERSUS OTHER BIOMARKERS

Correlations with other biomarkers for AD are not uniform. One explanation to this could be the disease stage at which the markers have been investigated. For example, the CSF biomarkers have been correlated with neuropathologic features of AD, [211-214] but in one study of manifest AD dementia no such correlation was seen.[200] The CSF biomarkers can also be claimed to correlate with cognitive performance since they differentiate groups of healthy elderly, MCI and dementia from one another. However, the CSF biomarkers and cognitive performance do seldom correlate within each group,[174, 196, 197, 215] with some exceptions.[176, 191] In most studies APOE-ε4 allele carriers have lower CSF Aβ42 levels[140, 179, 216-220] with some exceptions, [190, 200, 210] whereas studies report inconclusive results for CSF t-tau levels.[140, 174, 179, 192, 200, 209, 212, 218-222] Finally, hippocampal volume loss measured by MRI has in some studies been associated with AD-associated CSF changes.[134, 205, 223]

CAUSES AND UNDERLYING MECHANISMS

The underlying mechanism behind why APP is increasingly cleaved in the Aβ42 pathway and why tau becomes hyperphosphorylated is not fully known. Similarly, the mechanisms behind the AD-associated decrease in measurable CSF Aβ42 levels are not either completely known. An additional disturbing fact is that AD development in Down's syndrome and familial AD, due to APP gene mutations, is characterized by an increase in APP and hence Aβ42 production in young ages.[53] However, at the time of dementia diagnosis even individuals with Down's syndrome will display a decrease in Aβ42.[54] Nevertheless, some of the proposed causes behind the decrease in CSF Aβ42 levels have been the actual aggregation into plaques, decreased Aβ42 production with the neuron loss, increased clearance from the CSF and deficit drainage of CSF. In contrast, the increase in CSF tau levels is thought to depend on the degeneration of neurons.[173]

In summary, changes in CSF biomarker levels are as yet among the best for the discrimination and prediction of AD dementia. In addition, they also appear promising in the preclinical stages, even though the findings need to be reproduced.

EEG 2|4|2

EEG is the recording of the brain's spontaneous electric activity due to the synchronous firing of large numbers of neurons with similar orientation. The EEG recording is assessed by the rhythmic activity, which can be divided into and quantified (qEEG) within different frequency bands. The most common are the delta (0-4 Hz), theta (4-7 Hz), alpha (8-12) and beta (< 30Hz) frequencies (Figure 16).

DIAGNOSTIC ABILITY

The characteristic qEEG changes in AD are a diffuse, low-frequent activity (low mean frequency) due to a decrease in high frequencies (alpha and beta) and an increase in low frequencies (delta and theta). In addition, a decrease can be seen in fast-wave coherence (the synchronicity of EEG rhythms between two locations).[224-232] The degree of these changes correlates with the disease stage.[224, 225, 227]

A proposed sequence of the AD-associated changes in EEG activity has been extrapolated from data on MCI samples. First, an increase in relative theta activity is seen, followed by a decrease in relative beta activity, a decrease in alpha activity and at last an increase in delta activity.[227, 230, 233] The increase in relative theta activity has also been reported to differentiate early stages of AD dementia from healthy controls and to have intermediate characteristics in MCI.[142, 224, 227, 230, 233-240] Already in individuals with subjective memory impairment, the increase in relative theta activity has been reported to predict cognitive decline.[142] Concurrent changes in other frequency bands have been reported but the affected frequencies differ between the studies.[224, 230, 235, 236, 239, 241] In contrast, some studies have instead reported solitary changes restricted to relative alpha frequency to be associated to AD.[224, 228, 235] All these AD-associated changes in EEG activity are further supported by studies other than longitudinal case-control studies. For example, administration of anti-cholinergic substances (scopalamine) to older individuals has been found to enhance the AD-associated changes in EEG activity, whereas administration of pro-cholinergic substances (acetylcholinesterase inhibitor) reduces the same.[242-246]

EEG RHYTHM VERSUS OTHER BIOMARKERS

The low-frequency EEG activity that is characteristic of an AD brain has further been correlated to other AD-associated changes such as hippocampal atrophy

on MRI, [247] increased CSF tau levels, [248] decreased rCBF, [249-252] regional hypometabolism, [253] autopsy-confirmed neuron cell loss, [254] presence of the APOE-ε4 allele, [255-257] decrease in ADL-functions[226, 258] and reduced memory performance.[227, 228, 236, 259] Moreover, the low-frequency EEG activity has been suggested to correlate strongest with AD when located in the posterior, temporo-parietal areas.[227, 228, 249, 260] Finally, it should be stated that there are some contradictory studies that report no alterations in EEG rhythm in MCI or mild AD dementia compared to healthy controls.[261, 262] Furthermore, slowing of EEG rhythm and increase in relative theta activity has been associated with normal aging as well as other encephalopathies such as encephalitis, acute stroke and head trauma.[256, 260]

CAUSES AND UNDERLYING MECHANISMS

The mechanisms behind slowing of the EEG rhythm and increase in the relative theta activity in AD are not fully elucidated. One suggested mechanism is cholinergic dysfunction.[230, 243, 256, 263-266] The enhancement and reversibility of AD-associated changes in EEG activity in individuals when subjected to anti-cholinergic and pro-cholinergic substances, supports this theory.[242-246] Alterations in hippocampal theta activity by cholinergic transmissions have further been observed in animal models.[267-269] Another suggested mechanism is cortical hypoperfusion, since a relationship between theta activity and decreased rCBF has been observed.[249-252] Both mechanisms are suggested to involve hippocampal neurons, which would make the hippocampus a potentionally important area of theta activity regulation.[247, 268]

Overall, individuals with AD dementia have significantly altered EEG rhythm compared to healthy individuals. There are reports that these changes are present and measurable already when the first cognitive symptoms appear. The diagnostic and predictive accuracy has, however, not been sufficient to make qEEG an initial investigational marker in clinical practice.[133] The strength of EEG lies instead in the fact that it is a non-invasive, non-radiating and relatively inexpensive investigation with high-density spatial mapping and good replicable ability.[239] It could therefore be of use as a second line investigational tool.[133]

SPECT 2|4|3

DIAGNOSTIC ABILITY

Regional CBF can be measured with SPECT by obtaining images with a gamma camera after injection of a radionuclide. In AD, the change in resting rCBF occurs in certain, mainly bilateral patterns of reduction. These reductions are initially seen in the EC, posterior cingulate, precuneus and hippocampus.[270-272] From the start of cognitive symptoms up to moderate AD dementia a reduction is seen in the medial temporal lobes and later in parietal and posterior temporal lobes. In the more severe stages of the disease the frontal lobes become affected.[132, 270, 273-279] And though the frontal lobe reductions are suggested to occur late there have been observations of local frontal engagement concurrent with the early posterior reductions.[274, 276-278, 280-282]

REGIONAL CBF VERSUS OTHER BIOMARKERS

These suggested early posterior changes in rCBF best predicted conversion to AD dementia in MCI individuals[270, 271, 274, 277, 281, 283-287] as well as the speed of cognitive decline.[285] Moreover, the structural distribution of neuropathology according to Braak and the impairment in cognitive functions in AD dementia have been temporally correlated to the sequence of change in rCBF.[44, 271, 273, 288] However, discordance in the location of the hypoperfusion, histologic neuropathology and atrophy can be found in the earliest stages of the disease.[288-290] Increase in CSF tau levels have both been suggested and rejected to correlate to posterior hypoperfusion in MCI and manifest AD dementia.[272, 278, 285] In contrast to histologic and structural features of AD, the hypoperfusion does not appear to progress with the deterioration of the disease.[273] Finally, the early changes in rCBF have been observed to be reversible if pro-cholinerg substances are administered.[132, 276] Nevertheless, compared to functional imaging with other modalities, rCBF measured by SPECT is inferior in sensitivity and specificity and has even had problems reaching a diagnostic accuracy of 80 %.[3, 120, 131, 132, 241] According to one study 20 % of individuals with AD dementia are indistinguishable to healthy controls when hippocampal blood flow is measured with SPECT.[279]

CAUSES AND UNDERLYING MECHANISMS

The mechanisms behind the hypoperfusion in AD are not fully understood, especially in the early stages. One proposed mechanism is a deafferentation of the areas with hypoperfusion due to remote neuronal loss in other areas.[132, 273,

[276, 279, 283, 290, 291] This could explain why hypoperfusion is first seen in the posterior cingulate gyrus, whereas neuron loss and structural changes are first seen in the hippocampus and the EC.[44, 288, 289] The non-progressiveness and reversibility of the hypoperfusion in AD could on the other hand suggest that the underlying mechanisms precede the progressive neuron loss.[273] Other suggested underlying mechanisms are active oxidative stress in the posterior association cortex and a cholinergic deficit.[292, 293] Hence, it is still uncertain whether hypoperfusion is the cause or the effect of the neuronal dysfunction associated with AD.

In summary, AD-associated changes in rCBF can be observed and when used in combination with other markers, such as MTL atrophy or CSF biomarkers, the diagnostic and predictive accuracy can be valuably improved.[132, 272, 285, 294, 295] Changes in rCBF can also possibly contribute to the differentiation of AD from other causes of cognitive impairment.[132, 276, 294-297]

AIM, OBJECTIVES AND HYPOTHESES 3

The neuropathologic changes in AD development precede the first measurable cognitive symptoms by decades. In spite of this, healthy clinical control samples are often selected solely due to their unaffected cognitive performance. In addition, these selection processes have not been fully controlled for possible cognitive impairment as described in the introduction. Hence, these "healthy" control samples could inevitably include individuals with AD neuropathology. Several of the recent biological markers for AD could be useful already in early, preclinical stages. Studies supporting these theories, however, are scarce. Therefore, the aim of this doctoral thesis was:

– To investigate biological and cognitive markers for AD in a group of cognitively healthy elderly individuals who have been used as clinical control subjects in research studies.

CSF biomarkers, qEEG recordings, rCBF measurements and several cognitive screening tests were the validated markers for AD available for collection in this study. The incidence of conversion to dementia in this study is expected to be low, due to the sample size and the length of follow-up, thus excluding conversion as an outcome measurement. Instead, measurable objective cognitive decline was used as an outcome measurement. In light of these prerequisites the aim of this thesis was specified in three main objectives:

1. To study the distribution of the biological and cognitive marker levels in the group. (Paper I – IV)
2. To study the cross-sectional and longitudinal relationships between the biological markers and cognitive performance on an individual level. (Paper I and III)
3. To study the relationship between the different biological markers on an individual level. (Paper II and IV)

From the current level of evidence for preclinical AD and the preclinical pattern of markers for AD, hypotheses were set for the thesis as well as for each paper.

Main thesis hypothesis:

– In a group of cognitively healthy elderly individuals there will be individuals with deteriorated biologic markers for AD such as CSF biomarkers, rCBF and electric activity and these proposed deteriorations in biological markers will be associated with proxy markers for future dementia, such as declining cognitive performance.

Individual paper hypotheses:

1. AD-associated changes in the CSF biomarkers Aβ42, t-tau and p-tau correlate with and precede cognitive decline in a group of cognitively healthy elderly individuals. (Paper I)
2. AD-associated changes in the CSF biomarkers Aβ42, t-tau and p-tau are related to a concurrent change in the brain electric activity in a group of cognitively healthy elderly individuals. (Paper II)
3. Individuals with a progressive deterioration towards an AD pattern in the CSF biomarkers Aβ42, t-tau and p-tau levels will perform cognitively worse than those with stable levels. (Paper III)
4. AD-associated changes in the CSF biomarkers Aβ42, t-tau and p-tau are related to a concurrent change in rCBF in a group of cognitively healthy elderly individuals. (Paper IV)

METHODS AND MATERIAL 4

STUDY DESIGN 4|1

The papers of this doctoral thesis are derived from a longitudinal study of clinical healthy controls at the Clinic of Neuropsychiatry at Malmö University Hospital, Malmö, Sweden. Recruitment was done through advertisements posted at locations frequented by the elderly, as well as through information directed towards non-blood relatives to patients visiting the clinic. At the initial evaluation the participants underwent a thorough examination, including medical history, physical and psychiatric examinations, and cognitive testing. In addition, they underwent a CT scan according to routine clinical practice, in order to exclude structural brain damages. Inclusion criteria at baseline were: intact ADL functions, no complaints of memory loss and cognitive test results within expected normal range. Exclusion criteria at baseline were: active physical or mental disease that could affect cognitive status, including advanced pathology on the CT scan, fulfilment of criteria for AD[24] or other dementia types and fulfilment of criteria for MCI.[34, 35] When indicated, additional inclusion and exclusion criteria were added and specified in the paper concerned.

After inclusion the participants underwent a number of physical and cognitive investigations with two clinical follow-up visits over a period of 4.5 years. The physical investigations in the study were all well documented and reliable methods used to distinguish AD patients from healthy controls. They included repeated CSF analysis, APOE genotyping, EEG and SPECT-CT. The temporal order of investigations is specified in Figure 12. It should be added that the baseline CSF was collected within 6 months after inclusion. Moreover, the EEG recording was performed approximately 6 months after the cognitive assessment at the 3-year follow-up and less than one year before the cognitive assessment and CSF collection at the 4.5-year follow-up.

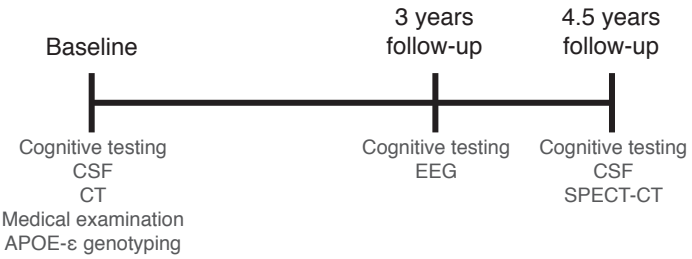

Figure 12. The investigations and assessments performed at baseline and each follow-up.

The incidence of AD dementia in a group of cognitively healthy individuals followed for 4.5 years is too low to allow these diagnoses to serve as outcome measurements in this study.[298] Neuropathologic post-mortem examinations are similarly not feasible with the current follow-up period. Instead, subjective or objective cognitive decline without the need to fulfil any diagnosis criteria has been set as a clinical outcome in the longitudinal analyses. The first paper was written prior to the 4.5-year follow-up which limited the available cognitive outcome measurements in this paper.

The initial baseline evaluations were performed by a number of experienced physicians at the Clinic of Neuropsychiatry at the Malmö University Hospital, Malmö, Sweden. The locations and administrators of the other investigations are specified in each section below.

STUDY POPULATION 4|2

The participants in this study were initially recruited to constitute a healthy elderly control sample for studies of clinical dementia disorder. However, as specified in the introduction, the interest to separately study these presumed healthy individuals is increasing. Due to the original purpose of the study population it was important to cognitively optimize the group in order to minimize the presence of neurodegenerative pathology. The flowchart of the participants in the study is presented in Figure 13. One individual summoned to the initial evalu-

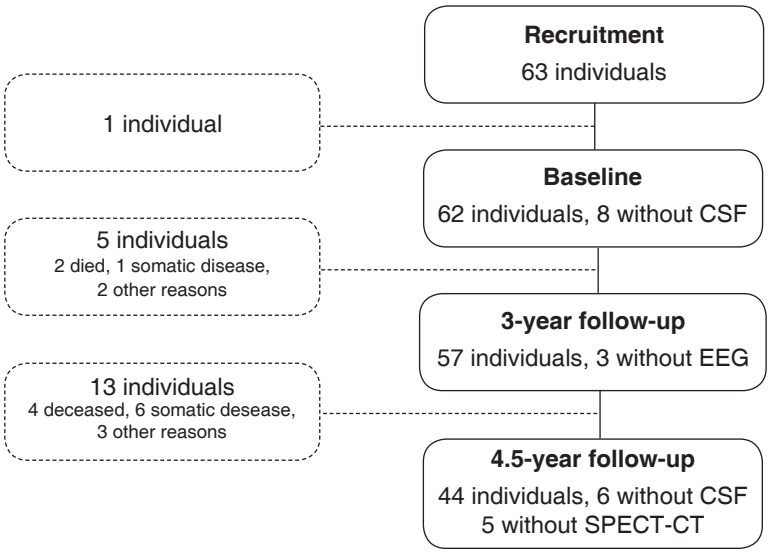

Figure 13. Participant flow chart for the entire study period including reasons for drop-out.

ation did not meet the inclusion criteria of unaffected cognitive function. Of the 62 participants at baseline 57 remained at the 3-year follow-up and 44 at the 4.5-year follow-up. The number of participants that successfully underwent each investigation is also stated in Table 1. Since different participants failed to complete different investigations the specific number of participants in each study might differ from that of the follow-ups in general.

The baseline characteristics are presented in Table 2. The mean age and sex distribution is similar to that seen in many AD studies. The frequency of APOE-ε4 allele was similar to that reported in a previous control sample and lower compared to the AD sample in the same study.[299] A pathologic albumin ratio could

Table 1. The investigations that were performed at each assessment occasion including the number of participants that completed each of these investigations.

Investigations (n)	0 – 6 months (baseline)	3 – 3.5 years (first follow-up)	4.5 years (second follow-up)
Cognitive assessments	● (62)	● (57)	● (44)
Biologic markers			
CSF collection	● (54)		● (38)
EEG		● (54)	
SPECT-CT			● (39)
CT	● (62)		
APOE genotyping	● (62)		
Medical examination	● (62)		

Table 2. Baseline characteristics of the participants including demographics, medical history, and cognitive assessments.

Participant characteristics (baseline)	Number	Percent
Sex (F/M)	41 / 21	
Age (mean ±SD)	72.7 ± 8.0	
APOE-ε4 (homozygote)	18 (1)	29 % (2 %)
Pathologic albuminratio	5	9.6 %
Hypertension	9	15 %
Diabetes	1	1.7 %
Thyroid-disease history	7	11.7 %
Depression history	2	3.2 %
Antidepressant therapy	3	5 %
Smoking, active	4	6 %
Smoking, previous	28	45 %
MMSE score (mean ±SD)	29.2 ± 0.9	

be a sign of cerebrovascular disease[293] and is in the study seen in a minority if the participants. Of the somatic disease history, all disease frequencies are below the numbers reported in adult population-based studies and systematic reviews.[300-303] The very high mean MMSE score and low SD value reflect the effort to cognitively optimize the study population and it should be noted that the 25th to 75th percentile lies between 29 points and the maximum 30 points of the test. This can be compared to only 25 % in previous large population-based samples.[145] Unfortunately, educational level and ethnicity have not been registered for the participants. It can be presumed, however, that they have at least 7 years education in the publicly financed mandatory elementary school system, which existed in Sweden during the first 6 decades of the 20th century. Furthermore, there is no indication that any of the participants grew up in a country that did not have a similar mandatory school system.

The participants who dropped out of the study before the 4.5-year follow-up performed almost 1.5 points lower on the delayed word recall task on the ADAS-cog ($p < 0.001$) compared to the remaining participants. However, no difference in the crude MMSE was observed. Since declining episodic memory could be a sign of neurodegenerative disease, this could lead to fewer preclinical cases in the remaining group. If so, a possible presence of preclinical AD in the study sample would be harder to detect. No differences in age, sex, APOE genotype, or baseline CSF biomarker levels were observed between the groups. At the 4.5-year follow-up, no remaining participants had developed AD dementia. There were 3 participants whose cognitive performance bordered on an MCI diagnosis. They were re-evaluated one year later, however, without any of them progressing to a definite MCI. It was not possible to evaluate for the presence of possible MCI, AD dementia or other dementia in the dropouts.

COGNITIVE ASSESSMENTS 4|3

The participants performed all cognitive tests consecutively during one visit on each assessment occasion. The cognitive tests used were the MMSE[143], the ADAS-cog[150], AQT[156], CDT[160] and cube copying[166]. Additionally, the participants were assessed for presence of subjective memory impairment affecting quality of life (memQoL) as well as their living situation at the 3-year follow-up. Unfortunately, all cognitive tests were not performed at each assessment occasion (Table 3). At the 4.5-year follow-up the cognitive testing was done in conjunction to the subsequent CSF collection. At the other occasions, the cognitive testing was performed separate from the other investigations. All cognitive assessments

Table 3. The cognitive tests that were performed at each assessment occasion.

Cognitive tests	0 – 6 months (baseline)	3 – 3.5 years (first follow-up)	4.5 years (second follow-up)
MMSE	●	●	●
ADAS-cog	●		●
AQT		●	●
CDT		●	●
Cube-copying	● †	●	●
MemQoL*		●	●
RUD-Lite ®**		●	

† Taken from the ADAS-cog. * Subjective memory impairment affecting quality of life.
** Resource utilization.

were administered at the Clinic of Neuropsychiatry at the Malmö University Hospital, Malmö, Sweden by several experienced nurses at the baseline visit and by the author at the 3- and 4.5-year follow-up visits.

The MMSE and the ADAS-cog were performed according to clinical routine for each test. The 85 point version of the ADAS-cog was used, which includes the valuable delayed word recall task. Low total score on the MMSE and high total score on the ADAS-cog reflect impaired cognitive functions. In AQT the participant is asked to name 40 figures as quickly as possible according to their colour, form and colour-form, respectively (Figure 14). The test administrator times each of the three tasks and notes any mistakes in naming. In the CDT the participant is asked to place the hours of the clock on a preset circle and the hands pointing at 10 minutes past 11 (Figure 8). To quantify the test performance we used the Ten-Point Clock Test rating scale, where points are given for placing the hours (1, 2, 4, 5, 7, 8, 10, 11) in the right sector (8 points) and the hands on ten minutes past eleven (2 points).[160] In cube copying the participant is asked to make a copy of a transparent cube below the original (Figure 9). The assessment of the drawings were based on a modification of a scoring system

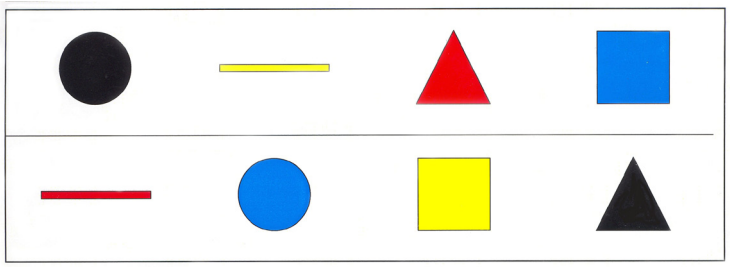

Figure 14. AQT, subtest colour-form. Short version used for giving instructions to the test subject

by Maeshima et al. where points are given for 8 correct vertexes and 12 correct parallel lines.[167] A vertex was defined as three lines connecting maximum 3 cm apart and the parallel limit was set to 10°. In the papers of this thesis the two separate measures have been combined into one numeric variable (20 points).

In quality of life assessment the Quality-of-Life Alzheimer's Disease instrument (QoL AD) was used, in which thirteen domains (physical health, energy, mood, living situation, memory, family, marriage, friends, self, ability to do chores, ability to do things for fun, money and life as a whole) are rated on a 4-point scale by the participant.[304] Assessment levels are poor, not so good, good and excellent, and the first two levels were regarded as considerable decline. The memory item was used to assess the reduction of quality of life by developed subjective memory impairment (memQoL). By adding the disturbance factor of quality of life to subjective memory impairment, the use of the variable as a proxy marker for cognitive impairment is strengthened. Finally, the participants' current living situation was categorised according to a shortened version of Resource Utilization in Dementia (RUD-Lite®) specifying the type of housing.[305] The 4 options available were regular apartment/house, service flats for senior citizens, old people's home, or nursing home.

CSF 4|4

The same procedures were performed in the first and second CSF collection and analysis (Figure 15). Lumbar puncture was performed in the sitting position. The CSF samples were obtained in the L3/L4 or L4/L5 interspaces. After disposal of the first 1 ml of CSF, the following 10 ml were collected in plastic (polypropylene) tubes to avoid absorbance of amyloid β by the tube wall. All CSF samples were mixed gently to avoid possible gradient effects. No CSF sample contained more than 500 erythrocytes/μl. The CSF samples were centrifuged at 2.000 g at 4 °C for 10 min to eliminate cells and other insoluble material, and were then immediately frozen and stored at –80 °C pending biochemical analyses, without being thawed or refrozen. The CSF samples were analysed for Aβ42, t-tau and p-tau (tau phosphorylated at threonine 181) with xMAP Luminex technology using the INNO-BIA AlzBio3 kit (Innogenetics, Ghent, Belgium) and the same batch of reagents.[306] The results are presented in ng/l. In the longitudinal CSF study (paper III) random samples of stored baseline CSF were analysed together with the follow-up CSF in order to secure concordant assay values between the two analysis occasions. Serum samples were collected in conjunction to the first CSF collection and baseline CSF/serum albumin ratio was calculated for evaluation of the blood-brain barrier function.

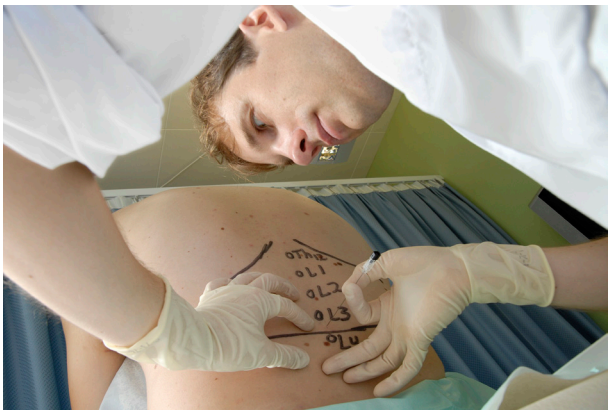

Figure 15 A. Lumbar puncture for CSF collection. The L3-L4 interspace is palpated with the test subject in sitting position. *Photo by Medicinsk Informationsteknik*

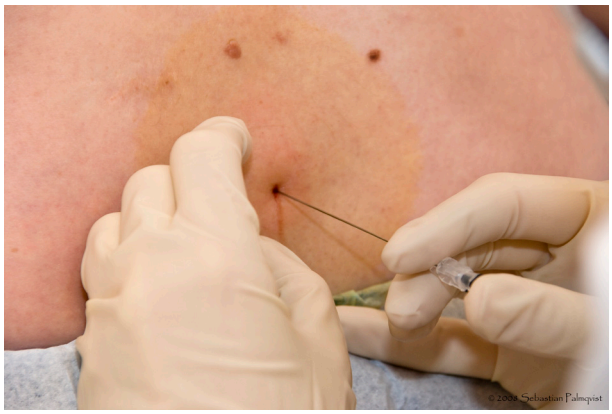

Figure 15 B Lumbar puncture for CSF collection. After local anaesthetic infiltration in the skin, a needle is inserted in the L3-L4 interspace and into the spinal canal. *Photo by S. Palmqvist. With permission*

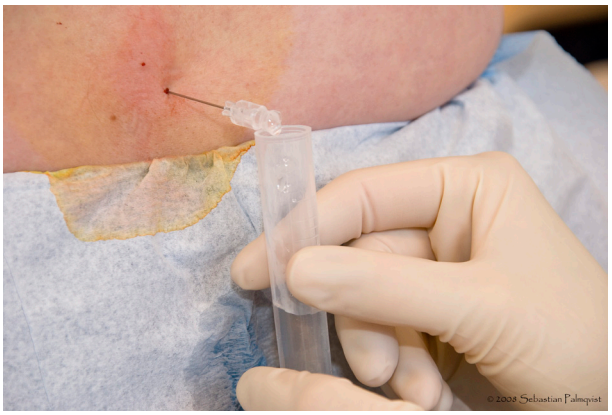

Figure 15C Lumbar puncture for CSF collection. Cerebrospinal fluid is tapped into specific test tubes before the needle is withdrawn. *Photo by S. Palmqvist With permission*

The lumbar punctures were performed at the Clinic of Neuropsychiatry at Malmö University Hospital, Malmö, Sweden by experienced physicians at baseline and the author at follow-up. The CSF samples were analyzed at the Unit of Neurochemistry at Sahlgrenska University Hospital, Göteborg, Sweden. The CSF/serum albumin ratio was determined at the Department of Clinical Chemistry at Lund University Hospital, Lund, Sweden. CSF cell counts were performed at the Department of Clinical Chemistry at Malmö University Hospital, Malmö, Sweden.

BLOOD TESTS 4|5

Baseline venous blood samples were collected from an arm vein by experienced nurses and separated into several test tubes due to the different requirements of each specific blood analyses. The samples were handled according to clinical practice at the Malmö University Hospital. The blood was analysed for blood cells, electrolytes, creatinine, liver-bile duct damage, blood lipids, vitamin B deficit, blood glucose, thyroid function, brain natriuretic peptide (BNP), prothrombin time (PT-INR), C-reactive protein (CRP), sexual hormones and APOE genotype. The blood samples were collected at the Clinic of Neuropsychiatry at Malmö University Hospital, Malmö, Sweden and the analyses were performed at the Department of Clinical Chemistry at the same hospital.

EEG 4|6

EEG was recorded for 20 minutes from 19 electrodes with Nervus (Viasys Healthcare Inc, Madison WI) equipment, according to the 10–20 system and a sampling frequency of 256 Hz, high pass filter 0.16 Hz and low pass filter at 500 Hz (Figure 16). In order to certify that the analysis was performed on an EEG recorded with the patient fully awake, 10 sec epochs of artefact free EEG were selected in the eyes-closed situation within 5–20 sec after interaction with the patient either by verbal communication, or following eye closure on command. Post-interaction epochs were further used in order to optimize and standardize the influence of fluctuation in arousal, which predominantly causes variability in the theta activity. Several of these 10 s epochs were then analysed and one epoch was selected based on its alpha stability for further calculations.

Quantification of the EEG data reconstructed in CA mode, 2 sec epochs (Hamming filter), was performed by using commercially available software (Nervus Reader 3.4, Viasys Healthcare Inc Madison, WI). Peak frequency of

posterior dominant activity, log absolute power and relative power of delta (0.5-4 Hz), theta (4–8 Hz), alpha (8–13 Hz), beta (13–32 Hz) were calculated by FFT analysis for each quadrant of the skull: left anterior (F3,F7,T3, C3), right anterior (F4, F8, T4,C4), left posterior (P3,T5,O1), right posterior (P4,T6,02). Average values for all four quadrants are presented as well as values for each quadrant (Figure 16).

The EEG recordings were performed at the Department of Neurophysiology at Malmö University Hospital, Malmö, Sweden. The subtraction, transformation and calculation of the EEG data were performed by an experienced physician and neurophysiologist, Professor Emeritus I. Rosén (co-author paper II).

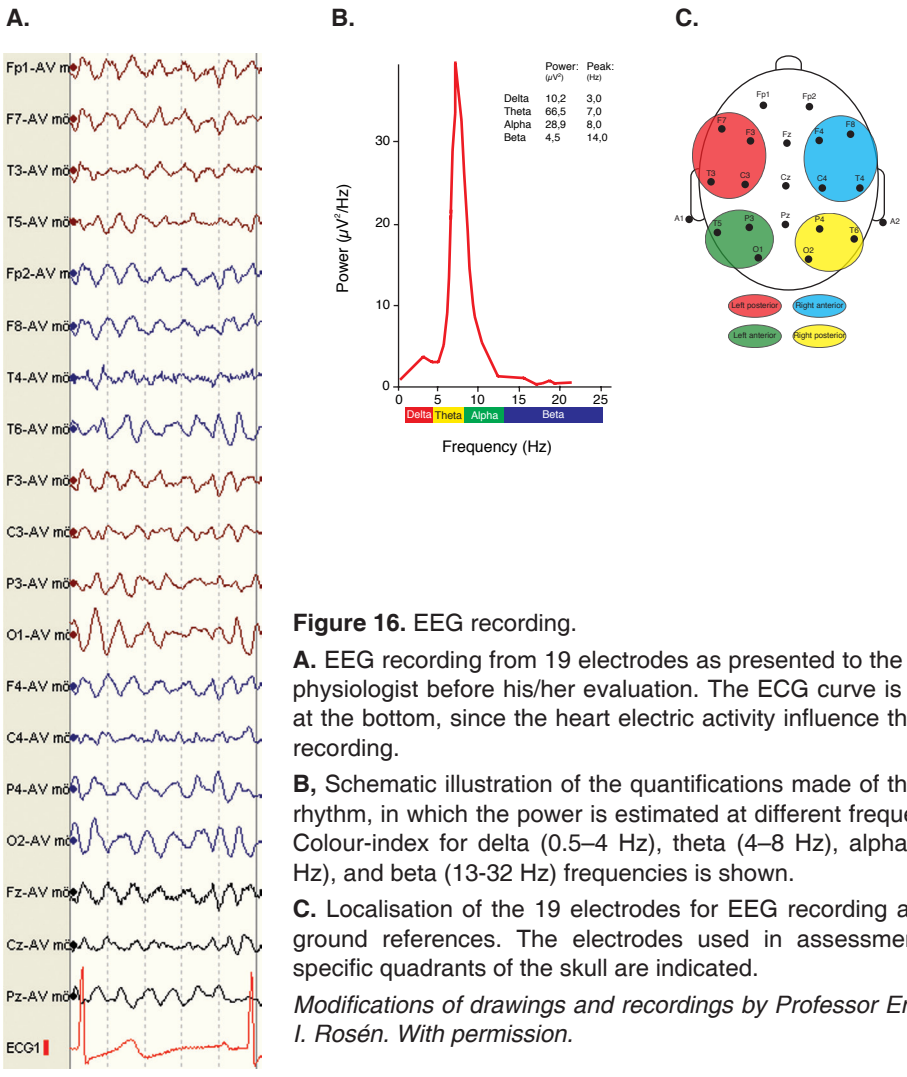

Figure 16. EEG recording.

A. EEG recording from 19 electrodes as presented to the neurophysiologist before his/her evaluation. The ECG curve is shown at the bottom, since the heart electric activity influence the EEG recording.

B, Schematic illustration of the quantifications made of the EEG rhythm, in which the power is estimated at different frequencies. Colour-index for delta (0.5–4 Hz), theta (4–8 Hz), alpha (8–13 Hz), and beta (13-32 Hz) frequencies is shown.

C. Localisation of the 19 electrodes for EEG recording and the ground references. The electrodes used in assessments for specific quadrants of the skull are indicated.

Modifications of drawings and recordings by Professor Emeritus I. Rosén. With permission.

SPECT-CT 4|7

^{99m}Tc-exametazime and SPECT-CT (Siemens Symbia® T2, Siemens Medical Solutions) were used to measure the rCBF (Figure 17). The participants were given an intravenous injection of 900 MBq ^{99m}Tc-exametazime (Ceretec®, GE Health Care) in a well-lit setting apart from ambient noise, resting in a supine position, awake with eyes open. Image acquisition began 30 minutes later with a total acquisition time of about 40 min. The gamma camera was equipped with low energy high-resolution collimators, rotating in a non-circular orbit of 360 degrees and recording 128 projections per detector head. The recorded images were reconstructed into a 128 × 128 × 128 voxel matrix using 3D-OSEM (Flash 3D, Siemens Medical Solution) with DEW scatter correction and attenuation correction based on the sequentially performed low dose CT measurements. The spatial resolution of the images was about 10 mm FWHM (full-width half maximum) at the centre of rotation.

Statistical parametric mapping (SPM5) (The Wellcome Trust Centre for Neuroimaging, London, UK) together with MATLAB 7.1 (The Mathworks) were used for voxel-based analyses. The SPECT images were normalized into a standard stereotactic space.[307, 308] The images were then smoothed by means of an isotropic Gaussian filter (16-mm FWHM). In the statistical analysis voxels were normalized to the global level of CBF. Analysis of covariance between

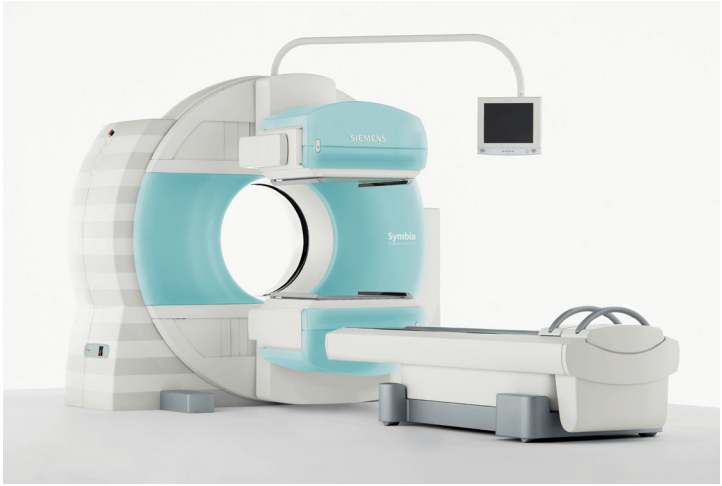

Figure 17. The SPECT-CT scan. The circular component of the machine in the back is the CT scan. The upper and lower cubical components in front of the circular component are the dual head gamma camera. The test subject lies on the bed in the front during scanning.
With permission from Siemens Medical Solution, Siemens AG

the SPECT measurements versus CSF biomarker levels and results on cognitive tests was performed by entering a regressor of respective covariate. Voxels were considered significant at a threshold of $p < 0.001$, uncorrected. Clusters were considered significant at a threshold of $p < 0.05$, corrected for multiple comparisons.

The ^{99m}Tc-HMPAO SPECT-CT scans were performed at the Department of Neurophysiology at Lund University Hospital, Lund, Sweden. Image analysis with SPM was performed at the Division of Psychiatry, Department of Clinical Neuroscience, Karolinska Institutet, Stockholm, Sweden by PhD A. Forsberg (co-author paper IV).

STATISTICS 4|8

Statistical analysis was performed using the SPSS statistics, which in 2009 was renamed PASW statistics (SPSS Inc., Chicago, IL, USA). The versions 12.0.1 (paper I), 14.0.1 (paper II), 15.0.1 (paper III) and 17.0.1 (paper IV) for Windows were used.

Non-parametric tests and median values were used when the variables were not normally distributed. Spearman rank correlation coefficient was used to test the degree of linear correlation between continuous variables (cognitive assessments, CSF biomarkers level, EEG rhythm and age). Dichotomized data was analyzed with Mann-Whitney U test (gender, memQoL, longitudinal CSF biomarkers change, outliers and dropouts). Kruskal-Wallis test was used to test nominal data with more than two labels (APOE genotype). Prediction of cognitive decline (paper I) was estimated and described with the sensitivity and specificity numbers when using cut-off-levels that optimized the Youden index[309] as well as with the odds ratio using a logistic regression model. Level of significance was set to $p < 0.05$, where not stated otherwise.

ETHICS 4|9

Each performed investigation in this study was approved by the regional ethics committee of Lund University, Lund, Sweden. (LU 383-98, LU 184-01, LU 450/2006) The participants gave their written consent to participate at each assessment occasion. None of the participants had obligations towards or were dependant upon any of the clinics or co-workers involved.

On each test occasion the participants received financial reimbursement according to the investigations involved. Transportation expenses to and from

the test clinics were always compensated. At baseline the participants received a sum of 1000 SKR. The same amount was received at the 4.5-year follow-up. At the 3-year follow-up, which only included cognitive testing and the non-invasive EEG recording, the participants received gift certificates for a value of 250 SKR. The CSF samples were stored in a registered biobank (reg. nr. 68). The SPECT investigation involved exposure to radiation and a specific approval by the Isotope Committee at Lund University Hospital was acquired (IK 623).

MAIN RESULTS 5

The full version of each paper is placed in the appendix of the thesis. The main features of each paper are summarized below.

PAPER I 5|1

HYPOTHESIS

AD-associated changes in the CSF biomarkers Aβ42, t-tau and p-tau correlate with and precede cognitive decline in a group of cognitively healthy elderly individuals.

SETTING

CSF analysis and cognitive assessment were performed at baseline with an additional cognitive follow-up three years later. Fifty-seven cognitively healthy elderly individuals underwent repeated assessments and CSF was successfully collected in 54 of these individuals. The CSF was analyzed for Aβ42, t-tau and p-tau 181. The cognitive assessment included the MMSE, AQT, subjective cognitive impairment and current living situation.

RESULTS

No participant developed dementia or MCI during the 3 years. However subjective memory impairment affecting quality of life (memQoL) was reported in 25 % of the participants at follow-up. Participants with pathologic memQoL were slower on follow-up AQT colour-form compared to those without subjective memory impairment (Mann Whitney-U test, $p < 0.01$) and moreover had lower baseline CSF Aβ42 levels (Mann Whitney-U test, $p < 0.05$). CSF Aβ42 predicted pathologic memQoL with an area under the curve of 0.722 using a ROC-curve (Mann Whitney-U test, $p < 0.05$). Pathologic memQoL was best predicted by the combination of CSF Aβ42 and CSF p-tau with a sensitivity of 71.4 % and specificity of 75.7 % if using cut-off levels CSF Aβ42 ≤ 670 ng/l and CSF P-tau ≥ 55 ng/l (Youden index 0.471). Moreover, the same cut-off levels had a maximum odds ratio of 9.5 ($p = 0.004$) for future pathologic memQoL. Low baseline CSF Aβ42 levels further correlated with a higher prevalence of living in service flats for senior citizens as well as lower total MMSE scores at follow-up. However, the MMSE score range was narrow with a 1 point difference between the 25th and 75th percentile and included the maximum score. CSF t-tau and p-tau levels individually did not correlate with any cognitive measurements.

COMMENTS

In this group of cognitively healthy elderly individuals there were participants with CSF biomarker levels that in cognitively impaired individuals would be regarded as pathologic. Additionally, the CSF biomarkers were not normally distributed which would be expected of biological markers in strictly healthy individuals. The predictive ability for cognitive impairment of low CSF Aβ42 levels separately as well as in combination with high CSF P-tau levels are in alignment with findings in other current and recently published studies on healthy elderly.[138-141] These changes in CSF biomarkers have also been associated with AD dementia and conversion to AD dementia in MCI individuals.[171, 178, 180] In this paper memQoL was used as a proxy marker for cognitive impairment since no participant developed MCI or dementia and the association with cognitive speed results supported this usage. In light of the subjective nature and heterogeneity of memQoL it is impressive that CSF biomarker levels were able to indicate future cognitive decline in a sample such as this. In conclusion, CSF biomarker levels in this paper might indicate neurodegeneration and hence AD-related biological signs could possibly be detectable already in healthy elderly individuals.

CONCLUSION

AD-associated changes in CSF biomarkers could precede cognitive decline in a group of cognitively healthy elderly individuals.

PAPER II 5|2

HYPOTHESIS

AD-associated changes in the CSF biomarkers Aβ42, t-tau and p-tau are related to a concurrent change in the brain electric activity in a group of cognitively healthy elderly individuals.

SETTING

EEG measurement and CSF collection were performed on 33 elderly individuals with repeated normal cognitive test scores over 4.5 years, including total MMSE scores of 27 or higher. The CSF collection was performed at the 4.5-year follow-up and the EEG at most one year earlier. Cognitive assessments were performed in conjunction with both investigations and consisted of the MMSE, the ADAS-cog (only at the 4.5-year follow-up), cube copying, clock drawing test and AQT. The CSF was analyzed for Aβ42, t-tau and p-tau 181. The EEG recordings underwent quantification with calculation of mean peak frequency and

relative power of the delta (0.5–4 Hz), theta (4–8 Hz), alpha (8–13 Hz) and beta (13–32 Hz) frequencies. The frequency power was calculated for each quadrant of the skull as well as average value for the entire skull.

RESULTS

Increased CSF p-tau and t-tau levels correlated with increased relative theta activity ($r_s = 0.556$; $p < 0.001$) whereas no correlation was seen for CSF Aβ42. However, combined as a ratio, CSF p-tau/Aβ42 and t-tau/Aβ42 correlated stronger with relative theta activity ($r_s = 0.622$; $p < 0.001$). Consistently, p-tau had stronger correlations compared to t-tau. The correlations remained for each quadrant of the skull, with the strongest in the right posterior ($r_s = 0.643$; $p < 10^{-4}$) quadrant followed by the left posterior ($r_s = 0.591$; $p < 0.001$), the right anterior ($r_s = 0.575$; $p < 0.001$) and the left anterior quadrant ($rs = 0.510$; $p < 0.01$). Both increased relative theta activity and high p-tau/Aβ42 or t-tau/Aβ42 ratios correlated with slower results on AQT ($r_s > 0.461$; $p < 0.01$). Also, with cognitive speed the strongest correlation for relative theta activity was seen in the posterior quadrants ($r_s > 0.503$; $p < 0.01$). No correlation was observed with the other frequency bands or cognitive tests. In the theta frequency statistical outliers were observed that in a clinical setting would be regarded as pathological EEG measurements. None of these participants differed in cognitive performance and the correlations remained even if they were excluded.

COMMENTS

In this paper cerebral function visualized by EEG rhythm and cognitive speed correlated with neuropathologic processes represented by CSF biomarkers. The correlations observed furthermore included changes previously associated with AD (i.e. increased CSF tau, theta activity and cognitive speed). The findings are in alignment with the only previous study of CSF and EEG in healthy elderly, which reported a correlation between CSF t-tau and slowing of EEG rhythm.[248] Furthermore, several previous studies have suggested theta activity to change early in AD development[224, 227, 230, 235, 237, 238] and especially in the posterior regions.[227, 249] The posterior localisation of the correlations is also in alignment with the classical AD neuropathology distribution. The primary location for the first tau pathology in AD is the medial temporal lobe (MTL)[44] whereas Aβ42-associated pathology is first seen in dorso-frontal regions.[129, 171] This could partly explain the difference in the correlation patterns of CSF p-tau and t-tau versus that of CSF Aβ42, since relative theta power has been associated with MTL atrophy. Moreover, neuronal cell degeneration is often suggested as the origin of

increased relative theta activity[230, 243, 249, 250] and the stronger correlation of p-tau in the current paper would then suggest degeneration of neurons with a pathologic hyperphosphorylation state. In conclusion CSF biomarkers and EEG theta activity might indicate early abnormal degenerative changes in the brain already in cognitively healthy elderly.

CONCLUSION

AD-associated changes in CSF biomarkers could be associated with a specific slowing of the EEG activity in a group of cognitively healthy elderly individuals.

PAPER III 5|3

HYPOTHESIS

Individuals with a progressive deterioration towards an AD pattern in the CSF biomarkers Aβ42, t-tau and p-tau levels will perform cognitively worse than those with stable levels.

SETTING

In this longitudinal study CSF collection and cognitive assessment was performed at baseline and four years later. At baseline, 54 participants had a successful CSF collection and 37 of these had an additional second successful CSF collection at follow-up and are therefore included in the paper. Baseline CSF collection was performed within 6 months after the cognitive assessment. The CSF was analyzed for Aβ42, t-tau and p-tau 181. Longitudinal assessments included mean difference in CSF biomarker levels as well as dichotomization in regard to the magnitude of a possible change in CSF biomarker levels. Dichotomization was done for 10 %, 15 % and 20 % change in an AD-associated pathologic direction for each of the CSF biomarkers. The cognitive assessments included the MMSE and the ADAS-cog with the addition of AQT and clock drawing at follow-up.

RESULTS

CSF biomarkers and cognitive performance did not correlate at baseline, whereas low CSF Aβ42 levels correlated with lower delayed word recall score (r_s = -0.437, $p < 0.01$) and AQT score (r_s > -0.385, $p < 0.05$) at follow-up. Baseline CSF Aβ42 levels could not predict cognitive performance at follow-up, which could suggest occurrence of individual changes in CSF Aβ42 levels during the follow-up period. The mean longitudinal change in Aβ42 levels were however not associ-

ated with cognitive performance. Instead, the participants who decreased more than 15 % in CSF Aβ42 levels performed worse on ADAS-cog delayed word recall at follow-up ($z = –2.18$, $p < 0.05$) and those who decreased more than 20 % performed worse on AQT at follow-up ($z = -2.35$, $p < 0.05$). CSF p-tau and t-tau levels did not correlate with cognitive performance at follow-up, however those participants that increased more than 20 % in CSF p-tau levels performed worse on AQT at follow-up ($z = –2.13$, $p < 0.05$). No additional effect was seen if combinations of CSF Aβ42, p-tau and t-tau were used. The 17 dropouts had slightly higher baseline ADAS-cog delayed word recall score (i.e. worse) than the remaining participants but otherwise did not differ. Of possible confounders age showed small associations with some cognitive test performances and presence of APOE-ε4 allele was associated with higher follow-up CSF T-tau levels and a greater longitudinal decrease in CSF Aβ42 levels.

COMMENTS

In this paper CSF Aβ42 levels correlated with cognitive performance and individuals with a relative extensive longitudinal decrease in CSF Aβ42 levels had poorer cognitive results compared to those with stable levels. This is in alignment with the findings in the three other papers of this thesis, namely that CSF Aβ42 has been primarily associated with cognitive performance whereas CSF p-tau has primarily been associated with other cerebral functions such as electric activity and blood flow. However, in contrast to some of the other studies, no additional effect was observed if the AD-associated combination of CSF Aβ42 and p-tau was used, even though longitudinal increase in CSF p-tau levels correlated by itself with cognitive speed. In this paper it was delayed episodic memory and cognitive speed, cognitive functions known to be affected early in AD, that correlated with CSF biomarkers. Thus, a possible concurrent decrease in CSF Aβ42 levels and cognitive performance could have taken place during the follow-up period. Such a transition in markers for AD has even been proposed to occur preclinically since longitudinal stability of CSF biomarker levels has not correlated to the extent of cognitive decline in manifest disease.[195, 196, 204] In summary, this paper suggests that CSF biomarkers previously associated with AD appear to correlate with decline in cognitive functions known to be affected early in AD development in a group of cognitively healthy elderly individuals. Hence, the results could implicate that CSF biomarkers might be able to detect the very early neurodegenerative processes of AD.

CONCLUSION

A progressive deterioration in CSF Aβ42 levels could be related to decline in cognitive performance in a group of cognitively healthy elderly individuals.

PAPER IV 5|4

HYPOTHESIS

AD-associated changes in the CSF biomarkers Aβ42, t-tau and p-tau are related to a concurrent change in rCBF in a group of cognitively healthy elderly individuals.

SETTING

In this cross-sectional study, 32 cognitively healthy elderly individuals underwent CSF collection, SPECT imaging and cognitive assessment. The CSF was analyzed for Aβ42, t-tau and p-tau 181. The SPECT images were analysed with SPM to investigate covariance between rCBF versus CSF biomarkers and cognitive performance. The cognitive assessments included the MMSE, the ADAS-cog and AQT.

RESULTS

High CSF p-tau and t-tau levels correlated with decreased rCBF in the right superior posterior medial frontal lobe ($p < 0.001$) and increased rCBF in the left fronto-parieto-temporal border zone area ($p < 0.05$). The covariance between rCBF versus CSF p-tau had a higher degree of significance at cluster level compared to that versus CSF t-tau. CSF Aβ42 was not associated with rCBF. Neither CSF p-tau and t-tau levels nor rCBF in the current right frontal and left posterior locations were associated with cognitive performance.

COMMENTS

In this paper CSF p-tau and t-tau levels were associated with rCBF. This association between CSF tau and rCBF has previously been both reported and denied.[272, 278, 285] The negative covariance between CSF p-tau and t-tau levels versus rCBF was found in the right medial frontal lobe at the location of the supplementary motor cortex (SMC). Previous rCBF studies on AD and MCI individuals have reported a similar frontal rCBF decrease, however most often as secondary findings concurrent with decreases in more posterior areas of the brain.[274, 278, 280, 282, 288] In addition, the SMC is highly involved in executive functions[310, 311], which is one of the earliest cognitive functions to be affected in AD development.[89, 95]

Meanwhile, the positive covariance in the paper was located in the left fronto-parieto-temporal area. Increased rCBF has often been reported in AD and MCI individuals[274, 277, 312, 313] and this increase has been hypothesized to be a possible initial compensatory mechanism in situations of neuronal stress, for example incipient AD.[312, 313] Hence, in the current paper, biological markers related to AD (i.e. CSF biomarkers and rCBF) appear to already correlate in cognitively healthy elderly individuals. These findings could hereby suggest that these biomarkers might indicate preclinical neurodegenerative changes in the brain.

CONCLUSION

AD-associated changes in CSF p-tau and t-tau levels could be related to specific right medial frontal and left fronto-parieto-temporal changes in rCBF in a group of cognitively healthy elderly individuals.

COMMENTS 6

In the previous chapter, comments were made for each specific paper. However, there are more general aspects and circumstances, which influence the entire study. In the "Thesis at a glance" section a structured summary of the thesis is presented. Here below certain issues will be discussed more closely. First, it should be recognized that the explorative nature of this study limits one from drawing extensive conclusions. The findings in this study are only representative for this sample and can not be generalized to explain definite patterns in population-based samples. Instead the conclusions in this study are valuable in a wider perspective because they can be used to generate hypotheses for future research in other study samples.

THE OBJECTIVES 6|1

1st OBJECTIVE

The first objective of this thesis was to study the distribution of markers. The variance in cognitive performance was, for obvious reasons, limited due to the cognition-based selection process. However, the biological markers in this study did not show the same narrow distribution. Instead the values of the biological markers ranged over a relatively large interval. Furthermore, it could be noticed that the CSF biomarkers did not have a normal distribution, which could be expected from a biological marker in a control sample without extensive disease pathology. In fact the distribution of CSF biomarker levels in this group of cognitively healthy individuals resembles the distribution in groups with AD individuals.[314] The characteristic of this specific distribution is the occurrence of a prolonged tail of individuals towards the direction of levels associated with AD (Figure 18).

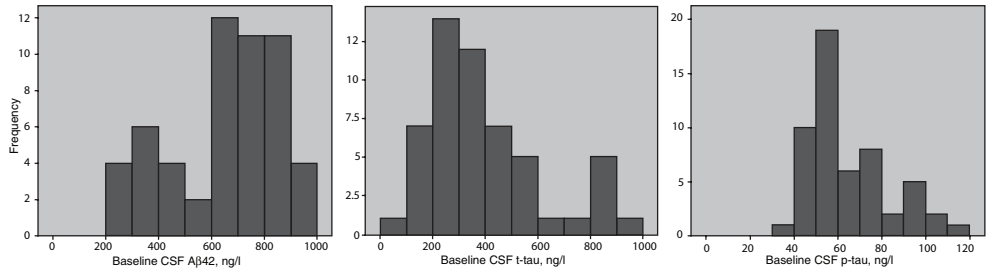

Figure 18. Distribution of CSF Aβ42, t-tau and p-tau levels in the study sample of cognitively healthy elderly individuals. A prolonged tale of the distribution can be seen in the directions that are associated with AD dementia.

Finally, within the current study sample statistical outliers (> 1.5 SD of the median value) were observed in the papers for some biomarkers, further suggesting a relatively wide range of values. If the individuals in the sample were assessed for each separate biomarker with AD dementia clinical reference standards from the participating neurophysiology and neurochemistry departments, several clinically pathologic biomarker levels were observed. Four clinically pathologic EEG recordings were qualitatively assessed by the neurophysiologist. Ten participants had pathologic rCBF when qualitatively assessed by the SPECT-supervising neurophysiologist and around the same number had participants CSF biomarker levels outside the cut-offs used at the memory-clinic.

2nd OBJECTIVE

The second objective was to study the relationship between biological markers and cognitive performance. The study suggests that in the sample there is a longitudinal relationship between these variables, which gives rise to a cross-sectional relationship during the follow-up. As has been previously stated in the thesis, the crude cognitive tests used created difficulties in detecting true differences. Despite this there were cognitive functions that correlated with the biological markers.

The first interesting aspect is that it was cognitive functions associated with early AD development, i.e. delayed episodic memory and cognitive speed, which correlated with the biomarkers. Secondly, it is not surprising that the MMSE scores did not provide any relevant correlations with the biological markers in this study. Thirdly, in the first paper, impairments in a subjective measurement of cognitive function at the 3-year follow-up were related to CSF biomarker levels. Later at the 4.5-year follow-up, it was instead impairments in objective measurements of cognitive functions that correlated with CSF biomarker levels. Hence, this shift could strengthen the belief that a true association between CSF biomarker levels and cognitive decline exists in this group of cognitively healthy elderly. Finally, it should be held in mind that the variance in cognitive performance could have other underlying causes than neurodegenerative disease and in the current study these possible confounding factors could not be excluded or completely adjusted for.

3rd OBJECTIVE

The third objective was to study the relationship between the different biological markers. The study suggests that in this sample the different biomarkers have an association to one another and as for the cognitive variables it is changes in the biomarkers previously related to AD dementia that correlated with one another.

The first comment that could be made concerns the difference in correlation patterns of the CSF biomarkers seen in this study. The CSF tau levels appear to be more closely related to the other biological markers whereas the CSF Aβ42 levels appear more closely related to the cognitive performance. Despite the limitations in the ability to draw conclusions the findings are in alignment with findings in other studies. It is primarily CSF Aβ42 levels that have correlated to cognitive performance in samples of healthy elderly.[139, 141] In AD dementia and MCI it is instead CSF tau levels that have correlated to the cognitive performance.[43] The few studies that have investigated the relationship between rCBF or EEG activity to CSF biomarkers have reported correlations only to CSF tau.[248, 272, 285] A difference in the correlation pattern between the CSF biomarkers is further supported by the findings of Fagan et al. where whole-brain atrophy correlated to CSF Aβ42 levels in the healthy controls but to CSF tau levels in individuals with very mild dementia.[199] Hence, the difference in correlation patterns of the CSF biomarkers between the papers of this study does not make the relationships less likely to reflect true associations.

The second comment of this third objective concerns the difference in spatial location of the correlations. CSF tau levels correlated primarily with right frontal and left posterior rCBF but right posterior EEG activity. This difference in locations could be explained by that the changes in the biological markers with spatial assessments are possibly caused by remote effects that originate from the regions of the first neuropathologic changes.[132, 276, 289] In addition, the localisations of each correlation were never in opposition to what had been previously reported for that specific biomarker.

The third comment regards the fact that several previous studies have not been able to observe the current correlations between biological markers and cognitive function within manifest AD dementia samples. There can be several reasons for this but one of them can depend on the kind of marker that is studied. A state marker that reflects disease or no disease will, at one point in the disease development, change to reach a plateau-level where it stabilizes. Hence, from this moment the state marker will not correlate to a rate marker that reflects the progression of the disease and therefore continuously change.

THE STUDY SAMPLE 6|2

At the baseline of this study the aim was to recruit a cognitively optimized control group, i.e. "supernormal" individuals. The result of this is that conversion to MCI or dementia would be less likely compared to that in a population-based

or hospital-based control sample. As calculated in the discussion of paper I, the theoretical likelihood of someone developing dementia in this study is low. The fate of the dropouts is further unknown and conversion to MCI or dementia in that group has not been possible to assess. Nevertheless, at baseline the drop-outs had significantly worse cognitive test scores on the MMSE (1 point) and the ADAS-cog (1 point) compared to the remaining participants at the 4.5-year follow-up. In summary, it could be reasonable that conversion to MCI and AD dementia was not available as outcome measurements in this study.

However, three participants at the 4.5-year follow-up were on the borderline to fulfil the MCI criteria due to a deviant score on a single cognitive test. These individuals were re-assessed after an additional one year but did not deteriorate further and instead some improved their deviant score. In light of this, these few individuals were not treated as a separate group in the longitudinal analyses but they were excluded from the cross-sectional follow-up analyses as specified in the papers concerned. On a general note it should be mentioned that the sample could never be as cognitively optimized at follow-up as it was aimed to be at baseline.

THE AIM AND THE FUTURE 6|3

The aim of this study was to investigate biological and cognitive markers for AD in a group of cognitively healthy elderly individuals. All papers of this study could suggest that biological markers and foremost CSF biomarkers might indicate neuropathology in this sample. In most of the cases the findings of this study can be linked with a possible connection to AD and primarily its early stages. Thus, the study could suggest that biological markers and foremost CSF biomarkers might indicate early neurodegenerative changes of the brain in this sample.

The key word in the previous sentence is "*could*", even though this potential ability of the biological markers is more probable now than it was before this study was performed. Hence, the study confirms that biological markers are an interesting area of future preclinical AD research. It reinforces the importance of planning and performing new preclinical studies, which should be larger and better powered than the current one. It also suggests that it could be relevant to continue the cognitive follow-up of the participants in the current study. Finally, it supports the need of thorough control selections in the future, if the sample is to be treated as "free from disease". To conclude, the pathological processes prevailing in AD might bridge the clinically created arbitrary division of normal and non-normal aging of the brain.

CONCLUSIONS 7

Specific hypotheses were in advance proposed for each paper as described in the "Aims, objectives and hypotheses" of the thesis. Based on these hypotheses, the study concludes that:

- In a group of cognitively healthy elderly individuals there existed individuals with deteriorated levels in AD-associated biological markers.
- These deteriorations in biological marker levels could in part be associated with worse cognitive performance.
- Biological markers previously associated with AD might indicate early neurodegenerative changes in the brain also in cognitively unimpaired individuals.

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REFERENCES 10

1. American Psychiatric Association. *Diagnostic and Statistical Manual of Mental Disorders. Fourth Edition (DSM-IV)*. Washington D.C.: American Psychiatric Association; 1994.

2. World Health Organisation. *The ICD-10. International Classification of Mental and Behavioural Disorders: Clinical Descriptions and Diagnostic Guidelines.* Geneva, Switzerland: World Health Organisation; 1992.

3. Dubois B, Feldman HH, Jacova C, Dekosky ST, Barberger-Gateau P, Cummings J, Delacourte A, Galasko D, Gauthier S, Jicha G, et al. Research criteria for the diagnosis of Alzheimer's disease: revising the NINCDS-ADRDA criteria. *Lancet Neurol.* 2007; **6** (8): 734–746.

4. World Health Organisation. *The ICF. International Classification of Functioning, Disability and Health.* Geneva, Switzerland: World Health Organisation; 2001.

5. Essen-Möller E, Wohlfahrt S. VI, 4. Suggestions for the amendment of the official swedish classification of mental disorders. *Acta Psychiatrica Scandinavica.* 1947; **22** (S47): 551–555.

6. Roth M. Classification and aetiology in mental disorders of old age: some recent developments. In: Kay DWK, Walk A, eds. *Recent developments in Psychogeriatrics – A Symposium*: Br J Psychiatry; 1971:1–18.

7. Katzman R. Alzheimer's disease. *N Engl J Med.* 1986; **314** (15): 964–973.

8. Mathuranath PS, Xuereb JH, Bak T, Hodges JR. Corticobasal ganglionic degeneration and/or frontotemporal dementia? A report of two overlap cases and review of literature. *J Neurol Neurosurg Psychiatry.* 2000; **68** (3): 304–312.

9. Burns A, Iliffe S. Alzheimer's disease. *BMJ.* 2009; **338**: b158.

10. Ritchie K, Lovestone S. The dementias. *Lancet.* 2002; **360** (9347): 1759–1766.

11. Burns A, Iliffe S. Dementia. *BMJ.* 2009; **338**: b75.

12. Small SA. Alzheimer disease, in living color. *Nat Neurosci.* 2005; **8** (4): 404–405.

13. Dorland's Illustrated Medical Dictionary. 25th ed. Philadelphia: W. B. Saunders; 1974.

14. The Swedish Academy. The Swedish Academy glossary. 13th ed. Stockholm: The Swedish Academy; 2006: 619.

15. Wacholder S, McLaughlin JK, Silverman DT, Mandel JS. Selection of controls in case-control studies. I. Principles. *Am J Epidemiol.* 1992; **135** (9): 1019–1028.

16. Dorn HF. Some applications of biometry in the collection and evaluation of medical data. *J Chronic Dis.* 1955; **1** (6): 638–664.

17. Chui H. Diagnosis: Introduction. In: Qizilbash N, Schneider LS, Chui H, et al., eds. *Evidence-based Dementia Practice*. First ed. Oxford: Blackwell Science Ltd; 2002: 81–85.

18. Sliwinski M, Lipton RB, Buschke H, Stewart W. The effects of preclinical dementia on estimates of normal cognitive functioning in aging. *J Gerontol B Psychol Sci Soc Sci.* 1996; **51** (4): P217–225.

19. Backman L, Jones S, Berger AK, Laukka EJ, Small BJ. Multiple cognitive deficits during the transition to Alzheimer's disease. *J Intern Med.* 2004; **256** (3): 195–204.

20. Blennow K, de Leon MJ, Zetterberg H. Alzheimer's disease. *Lancet.* 2006; **368** (9533): 387–403.

21. Lasky T, Stolley PD. Selection of cases and controls. *Epidemiol Rev.* 1994; **16** (1): 6–17.

22. Chui H. Diagnosis: Reaching a Diagnosis of Dementia. In: Qizilbash N, Schneider LS, Chui H, et al., eds. *Evidence-based Dementia Practice*. First ed. Oxford: Blackwell Science Ltd; 2002:93.

23. Bennett DA, Schneider JA, Arvanitakis Z, Kelly JF, Aggarwal NT, Shah RC, Wilson RS. Neuropathology of older persons without cognitive impairment from two community-based studies. *Neurology.* 2006; **66** (12): 1837–1844.

24. McKhann G, Drachman D, Folstein M, Katzman R, Price D, Stadlan EM. Clinical diagnosis of Alzheimer's disease: report of the NINCDS-ADRDA Work Group under the auspices of Department of Health and Human Services Task Force on Alzheimer's Disease. *Neurology.* 1984; **34** (7): 939–944.

25. McKeith IG, Dickson DW, Lowe J, Emre M, O'Brien JT, Feldman H, Cummings J, Duda JE, Lippa C, Perry EK, et al. Diagnosis and management of dementia with Lewy bodies: third report of the DLB Consortium. *Neurology.* 2005; **65** (12): 1863–1872.

26. Chui HC, Victoroff JI, Margolin D, Jagust W, Shankle R, Katzman R. Criteria for the diagnosis of ischemic vascular dementia proposed by the State of California Alzheimer's Disease Diagnostic and Treatment Centers. *Neurology.* 1992; **42** (3 Pt 1): 473–480.

27. Roman GC, Tatemichi TK, Erkinjuntti T, Cummings JL, Masdeu JC, Garcia JH, Amaducci L, Orgogozo JM, Brun A, Hofman A, et al. Vascular dementia: diagnostic criteria for research studies. Report of the NINDS-AIREN International Workshop. *Neurology.* 1993; **43** (2): 250–260.

28. The Lund and Manchester Groups. Clinical and neuropathological criteria for frontotemporal dementia. *J Neurol Neurosurg Psychiatry.* 1994; **57** (4): 416–418.

29. Price JL, Morris JC. Tangles and plaques in nondemented aging and "preclinical" Alzheimer's disease. *Ann Neurol.* 1999; **45** (3): 358–368.

30. Hulette CM, Welsh-Bohmer KA, Murray MG, Saunders AM, Mash DC, McIntyre LM. Neuropathological and neuropsychological changes in "normal" aging: evidence for preclinical Alzheimer disease in cognitively normal individuals. *J Neuropathol Exp Neurol.* 1998; **57** (12): 1168–1174.

31. Price JL, Morris JC. So what if tangles precede plaques? *Neurobiol Aging.* 2004; **25** (6): 721–723; discussion 743–726.

32. Davies L, Wolska B, Hilbich C, Multhaup G, Martins R, Simms G, Beyreuther K, Masters CL. A4 amyloid protein deposition and the diagnosis of Alzheimer's disease: prevalence in aged brains determined by immunocytochemistry compared with conventional neuropathologic techniques. *Neurology.* 1988; **38** (11): 1688–1693.

33. Petersen RC. Mild cognitive impairment as a diagnostic entity. *J Intern Med.* 2004; **256** (3): 183–194.

34. Petersen RC, Doody R, Kurz A, Mohs RC, Morris JC, Rabins PV, Ritchie K, Rossor M, Thal L, Winblad B. Current concepts in mild cognitive impairment. *Arch Neurol.* 2001; **58** (12): 1985–1992.

35. Gauthier S, Reisberg B, Zaudig M, Petersen RC, Ritchie K, Broich K, Belleville S, Brodaty H, Bennett D, Chertkow H, et al. Mild cognitive impairment. *Lancet.* 2006; **367** (9518): 1262–1270.

36. Ritchie K, Touchon J. Mild cognitive impairment: conceptual basis and current nosological status. *Lancet.* 2000; **355** (9199): 225–228.

37. DeCarli C. Mild cognitive impairment: prevalence, prognosis, aetiology, and treatment. *Lancet Neurol.* 2003; **2** (1): 15–21.

38. Winblad B, Palmer K, Kivipelto M, Jelic V, Fratiglioni L, Wahlund LO, Nordberg A, Backman L, Albert M, Almkvist O, et al. Mild cognitive impairment – beyond controversies, towards a consensus: report of the International Working Group on Mild Cognitive Impairment. *J Intern Med.* 2004; **256** (3): 240–246.

39. Reisberg B, Prichep L, Mosconi L, John ER, Glodzik-Sobanska L, Boksay I, Monteiro I, Torossian C, Vedvyas A, Ashraf N, et al. The pre-mild cognitive impairment, subjective cognitive impairment stage of Alzheimer's disease. *Alzheimers Dement.* 2008; **4** (1 Suppl 1): S98–S108.

40. Reisberg B, Ferris SH, de Leon M, Torossian C, Kadiyala S, Zhu W. Subjective cognitive impairment: the pre-mild cognitive impairment stage of brain degeneration: longitudinal outcome after a mean of 7 years follow-up. *Neuropsychopharmacology.* 2005; **30** (Suppl 1): S81.

41. Khachaturian ZS. Diagnosis of Alzheimer's disease. *Arch Neurol.* 1985; **42** (11): 1097–1105.

42. Braak H, Braak E. Neuropathological stageing of Alzheimer-related changes. *Acta Neuropathol.* 1991; **82** (4): 239–259.

43. Goedert M, Spillantini MG. A century of Alzheimer's disease. *Science.* 2006; **314** (5800): 777–781.

44. Braak E, Griffing K, Arai K, Bohl J, Bratzke H, Braak H. Neuropathology of Alzheimer's disease: what is new since A. Alzheimer? *Eur Arch Psychiatry Clin Neurosci.* 1999; **249 Suppl 3**: 14–22.

45. Hardy J, Selkoe DJ. The amyloid hypothesis of Alzheimer's disease: progress and problems on the road to therapeutics. *Science.* 2002; **297** (5580): 353–356.

46. Masters CL, Simms G, Weinman NA, Multhaup G, McDonald BL, Beyreuther K. Amyloid plaque core protein in Alzheimer disease and Down syndrome. *Proc Natl Acad Sci U S A.* 1985; **82** (12): 4245–4249.

47. Terry RD, Masliah E, Salmon DP, Butters N, DeTeresa R, Hill R, Hansen LA, Katzman R. Physical basis of cognitive alterations in Alzheimer's disease: synapse loss is the major correlate of cognitive impairment. *Ann Neurol.* 1991; **30** (4): 572–580.

48. The National Institute on Aging and Reagan Institute Working Group on Diagnostic Criteria for the Neuropathological Assessment of Alzheimer's Disease. Consensus recommendations for the postmortem diagnosis of Alzheimer's disease. *Neurobiol Aging.* 1997; **18** (4 Suppl): S1–2.

49. Mirra SS, Heyman A, McKeel D, Sumi SM, Crain BJ, Brownlee LM, Vogel FS, Hughes JP, van Belle G, Berg L. The Consortium to Establish a Registry for Alzheimer's Disease (CERAD). Part II. Standardization of the neuropathologic assessment of Alzheimer's disease. *Neurology.* 1991; **41** (4): 479–486.

50. Lesne S, Koh MT, Kotilinek L, Kayed R, Glabe CG, Yang A, Gallagher M, Ashe KH. A specific amyloid-beta protein assembly in the brain impairs memory. *Nature.* 2006; **440** (7082): 352–357.

51. Teller JK, Russo C, DeBusk LM, Angelini G, Zaccheo D, Dagna-Bricarelli F, Scartezzini P, Bertolini S, Mann DM, Tabaton M, et al. Presence of soluble amyloid beta-peptide precedes amyloid plaque formation in Down's syndrome. *Nat Med.* 1996; **2** (1): 93–95.

52. Wisniewski KE, Wisniewski HM, Wen GY. Occurrence of neuropathological changes and dementia of Alzheimer's disease in Down's syndrome. *Ann Neurol.* 1985; **17** (3): 278–282.

53. Englund H, Anneren G, Gustafsson J, Wester U, Wiltfang J, Lannfelt L, Blennow K, Hoglund K. Increase in beta-amyloid levels in cerebrospinal fluid of children with Down syndrome. *Dement Geriatr Cogn Disord.* 2007; **24** (5): 369–374.

54. Tapiola T, Soininen H, Pirttila T. CSF tau and Abeta42 levels in patients with Down's syndrome. *Neurology.* 2001; **56** (7): 979–980.

55. Iwatsubo T, Mann DM, Odaka A, Suzuki N, Ihara Y. Amyloid beta protein (A beta) deposition: A beta 42 (43) precedes A beta 40 in Down syndrome. *Ann Neurol.* 1995; **37** (3): 294–299.

56. Price JL, McKeel DW, Jr., Buckles VD, Roe CM, Xiong C, Grundman M, Hansen LA, Petersen RC, Parisi JE, Dickson DW, et al. Neuropathology of nondemented aging: presumptive evidence for preclinical Alzheimer disease. *Neurobiol Aging.* 2009; **30** (7): 1026–1036.

57. Schmitt FA, Davis DG, Wekstein DR, Smith CD, Ashford JW, Markesbery WR. "Preclinical" AD revisited: neuropathology of cognitively normal older adults. *Neurology.* 2000; **55** (3): 370–376.

58. Mackenzie IR. Senile plaques do not progressively accumulate with normal aging. *Acta Neuropathol.* 1994; **87** (5): 520–525.

59. Price JL, Ko AI, Wade MJ, Tsou SK, McKeel DW, Morris JC. Neuron number in the entorhinal cortex and CA1 in preclinical Alzheimer disease. *Arch Neurol.* 2001; **58** (9): 1395–1402.

60. Katzman R, Terry R, DeTeresa R, Brown T, Davies P, Fuld P, Renbing X, Peck A. Clinical, pathological, and neurochemical changes in dementia: a subgroup with preserved mental status and numerous neocortical plaques. *Ann Neurol.* 1988; **23** (2): 138–144.

61. Gomez-Isla T, Price JL, McKeel DW, Jr., Morris JC, Growdon JH, Hyman BT. Profound loss of layer II entorhinal cortex neurons occurs in very mild Alzheimer's disease. *J Neurosci.* 1996; **16** (14): 4491–4500.

62. West MJ, Coleman PD, Flood DG, Troncoso JC. Differences in the pattern of hippocampal neuronal loss in normal ageing and Alzheimer's disease. *Lancet.* 1994; **344** (8925): 769–772.

63. West MJ, Kawas CH, Stewart WF, Rudow GL, Troncoso JC. Hippocampal neurons in pre-clinical Alzheimer's disease. *Neurobiol Aging.* 2004; **25** (9): 1205–1212.

64. Kordower JH, Chu Y, Stebbins GT, DeKosky ST, Cochran EJ, Bennett D, Mufson EJ. Loss and atrophy of layer II entorhinal cortex neurons in elderly people with mild cognitive impairment. *Ann Neurol.* 2001; **49** (2): 202–213.

65. Giannakopoulos P, Hof PR, Mottier S, Michel JP, Bouras C. Neuropathological changes in the cerebral cortex of 1258 cases from a geriatric hospital: retrospective clinicopathological evaluation of a 10-year autopsy population. *Acta Neuropathol.* 1994; **87** (5): 456–468.

66. Guillozet AL, Weintraub S, Mash DC, Mesulam MM. Neurofibrillary tangles, amyloid, and memory in aging and mild cognitive impairment. *Arch Neurol.* 2003; **60** (5): 729–736.

67. Tomlinson BE, Blessed G, Roth M. Observations on the brains of non-demented old people. *J Neurol Sci.* 1968; **7** (2): 331–356.

68. Morris JC, Price AL. Pathologic correlates of nondemented aging, mild cognitive impairment, and early-stage Alzheimer's disease. *J Mol Neurosci.* 2001; **17** (2): 101–118.

69. Thal DR, Rub U, Schultz C, Sassin I, Ghebremedhin E, Del Tredici K, Braak E, Braak H. Sequence of Abeta-protein deposition in the human medial temporal lobe. *J Neuropathol Exp Neurol.* 2000; **59** (8): 733–748.

70. Price JL. Aging, preclinical Alzheimer disease, and early detection. *Alzheimer Dis Assoc Disord.* 2003; **17 Suppl 2**: S60–62.

71. Coleman PD, Flood DG. Neuron numbers and dendritic extent in normal aging and Alzheimer's disease. *Neurobiol Aging.* 1987; **8** (6): 521–545.

72. Alzheimers sjukdom. In: Marcusson J, Blennow K, Skoog I, Wallin A, eds. *Alzheimers sjukdom och andra kognitiva sjukdomar*. 2nd ed. Stockholm: Liber AB; 2003:41–43.

73. Schneider JA, Arvanitakis Z, Bang W, Bennett DA. Mixed brain pathologies account for most dementia cases in community-dwelling older persons. *Neurology.* 2007; **69** (24): 2197–2204.

74. Jellinger KA, Attems J. Neuropathological evaluation of mixed dementia. *J Neurol Sci.* 2007; **257** (1–2): 80–87.

75. Snowdon DA, Greiner LH, Mortimer JA, Riley KP, Greiner PA, Markesbery WR. Brain infarction and the clinical expression of Alzheimer disease. The Nun Study. *JAMA.* 1997; **277** (10): 813–817.

76. Stern Y. Cognitive reserve. *Neuropsychologia.* 2009; **47** (10): 2015–2028.

77. Mayeux R. Epidemiology of neurodegeneration. *Annu Rev Neurosci.* 2003; **26**: 81–104.

78. Morris JC, Storandt M, Miller JP, McKeel DW, Price JL, Rubin EH, Berg L. Mild cognitive impairment represents early-stage Alzheimer disease. *Arch Neurol.* 2001; **58** (3): 397–405.

79. Crystal H, Dickson D, Fuld P, Masur D, Scott R, Mehler M, Masdeu J, Kawas C, Aronson M, Wolfson L. Clinico-pathologic studies in dementia: nondemented subjects with pathologically confirmed Alzheimer's disease. *Neurology.* 1988; **38** (11): 1682–1687.

80. Galvin JE, Powlishta KK, Wilkins K, McKeel DW, Jr., Xiong C, Grant E, Storandt M, Morris JC. Predictors of preclinical Alzheimer disease and dementia: a clinicopathologic study. *Arch Neurol.* 2005; **62** (5): 758–765.

81. Markesbery WR, Schmitt FA, Kryscio RJ, Davis DG, Smith CD, Wekstein DR. Neuropathologic substrate of mild cognitive impairment. *Arch Neurol.* 2006; **63** (1): 38–46.

82. Storandt M, Grant EA, Miller JP, Morris JC. Longitudinal course and neuropathologic outcomes in original vs revised MCI and in pre-MCI. *Neurology.* 2006; **67** (3): 467–473.

83. Price JL, Davis PB, Morris JC, White DL. The distribution of tangles, plaques and related immunohistochemical markers in healthy aging and Alzheimer's disease. *Neurobiol Aging.* 1991; **12** (4): 295–312.

84. Knopman DS, Parisi JE, Salviati A, Floriach-Robert M, Boeve BF, Ivnik RJ, Smith GE, Dickson DW, Johnson KA, Petersen LE, et al. Neuropathology of cognitively normal elderly. *J Neuropathol Exp Neurol.* 2003; **62** (11): 1087–1095.

85. Goldman WP, Price JL, Storandt M, Grant EA, McKeel DW, Jr., Rubin EH, Morris JC. Absence of cognitive impairment or decline in preclinical Alzheimer's disease. *Neurology.* 2001; **56** (3): 361–367.

86. Neuropathology Group of the Medical Research Council Cognitive Function and Ageing Study (MRC CFAS). Pathological correlates of late-onset dementia in a multicentre, community-based population in England and Wales. . *Lancet.* 2001; **357** (9251): 169–175.

87. Hansen LA, DeTeresa R, Davies P, Terry RD. Neocortical morphometry, lesion counts, and choline acetyltransferase levels in the age spectrum of Alzheimer's disease. *Neurology.* 1988; **38** (1): 48–54.

88. Forstl H, Kurz A. Clinical features of Alzheimer's disease. *Eur Arch Psychiatry Clin Neurosci.* 1999; **249** (6): 288–290.

89. Backman L, Jones S, Berger AK, Laukka EJ, Small BJ. Cognitive impairment in preclinical Alzheimer's disease: a meta-analysis. *Neuropsychology.* 2005; **19** (4): 520–531.

90. Nestor PJ, Scheltens P, Hodges JR. Advances in the early detection of Alzheimer's disease. *Nat Med.* 2004; **10 Suppl**: S34–41.

91. Salmon DP, Bondi MW. Neuropsychological assessment of dementia. *Annu Rev Psychol.* 2009; **60**: 257–282.

92. Almkvist O, Backman L. Detection and staging of early clinical dementia. *Acta Neurol Scand.* 1993; **88** (1): 10–15.

93. Almkvist O, Winblad B. Early diagnosis of Alzheimer dementia based on clinical and biological factors. *Eur Arch Psychiatry Clin Neurosci.* 1999; **249 Suppl 3**: 3–9.

94. Backman L, Wahlin A, Small BJ, Herlitz A, Winblad B, Fratiglioni L. Cognitive Functioning in Aging and Dementia: The Kungsholmen Project. *Aging, Neuropsychology, and Cognition.* 2004; **11** (2): 212–244.

95. Perry RJ, Hodges JR. Attention and executive deficits in Alzheimer's disease. A critical review. *Brain.* 1999; **122 (Pt 3)**: 383–404.

96. Perry RJ, Hodges JR. Fate of patients with questionable (very mild) Alzheimer's disease: longitudinal profiles of individual subjects' decline. *Dement Geriatr Cogn Disord.* 2000; **11** (6): 342–349.

97. Petersen RC, Smith GE, Waring SC, Ivnik RJ, Tangalos EG, Kokmen E. Mild cognitive impairment: clinical characterization and outcome. *Arch Neurol.* 1999; **56** (3): 303–308.

98. Petersen RC, Stevens JC, Ganguli M, Tangalos EG, Cummings JL, DeKosky ST. Practice parameter: early detection of dementia: mild cognitive impairment (an evidence-based review). Report of the Quality Standards Subcommittee of the American Academy of Neurology. *Neurology.* 2001; **56** (9): 1133–1142.

99. Tierney MC, Szalai JP, Snow WG, Fisher RH, Nores A, Nadon G, Dunn E, St George-Hyslop PH. Prediction of probable Alzheimer's disease in memory-impaired patients: A prospective longitudinal study. *Neurology.* 1996; **46** (3): 661–665.

100. Estevez-Gonzalez A, Kulisevsky J, Boltes A, Otermin P, Garcia-Sanchez C. Rey verbal learning test is a useful tool for differential diagnosis in the preclinical phase of Alzheimer's disease: comparison with mild cognitive impairment and normal aging. *Int J Geriatr Psychiatry.* 2003; **18** (11): 1021–1028.

101. Albert MS, Moss MB, Tanzi R, Jones K. Preclinical prediction of AD using neuropsychological tests. *J Int Neuropsychol Soc.* 2001; **7** (5): 631–639.

102. Backman L, Small BJ, Fratiglioni L. Stability of the preclinical episodic memory deficit in Alzheimer's disease. *Brain.* 2001; **124** (Pt 1): 96–102.

103. Arnaiz E, Almkvist O. Neuropsychological features of mild cognitive impairment and preclinical Alzheimer's disease. *Acta Neurol Scand Suppl.* 2003; **179**: 34–41.

104. Arnaiz E, Almkvist O, Ivnik RJ, Tangalos EG, Wahlund LO, Winblad B, Petersen RC. Mild cognitive impairment: a cross-national comparison. *J Neurol Neurosurg Psychiatry.* 2004; **75** (9): 1275–1280.

105. Sacuiu S, Sjogren M, Johansson B, Gustafson D, Skoog I. Prodromal cognitive signs of dementia in 85-year-olds using four sources of information. *Neurology.* 2005; **65** (12): 1894–1900.

106. Elias MF, Beiser A, Wolf PA, Au R, White RF, D'Agostino RB. The preclinical phase of alzheimer disease: A 22-year prospective study of the Framingham Cohort. *Arch Neurol.* 2000; **57** (6): 808–813.

107. Palmer K, Backman L, Winblad B, Fratiglioni L. Early symptoms and signs of cognitive deficits might not always be detectable in persons who develop Alzheimer's disease. *Int Psychogeriatr.* 2008; **20** (2): 252–258.

108. Small BJ, Fratiglioni L, Viitanen M, Winblad B, Backman L. The course of cognitive impairment in preclinical Alzheimer disease: three- and 6-year follow-up of a population-based sample. *Arch Neurol.* 2000; **57** (6): 839–844.

109. Tierney MC, Szalai JP, Dunn E, Geslani D, McDowell I. Prediction of probable Alzheimer disease in patients with symptoms suggestive of memory impairment. Value of the Mini-Mental State Examination. *Arch Fam Med.* 2000; **9** (6): 527–532.

110. Masur DM, Sliwinski M, Lipton RB, Blau AD, Crystal HA. Neuropsychological prediction of dementia and the absence of dementia in healthy elderly persons. *Neurology.* 1994; **44** (8): 1427–1432.

111. Welsh K, Butters N, Hughes J, Mohs R, Heyman A. Detection of abnormal memory decline in mild cases of Alzheimer's disease using CERAD neuropsychological measures. *Arch Neurol.* 1991; **48** (3): 278–281.

112. Tierney MC, Yao C, Kiss A, McDowell I. Neuropsychological tests accurately predict incident Alzheimer disease after 5 and 10 years. *Neurology.* 2005; **64** (11): 1853–1859.

113. Twamley EW, Ropacki SA, Bondi MW. Neuropsychological and neuroimaging changes in preclinical Alzheimer's disease. *J Int Neuropsychol Soc.* 2006; **12** (5): 707–735.

114. Linn RT, Wolf PA, Bachman DL, Knoefel JE, Cobb JL, Belanger AJ, Kaplan EF, D'Agostino RB. The 'preclinical phase' of probable Alzheimer's disease. A 13-year prospective study of the Framingham cohort. *Arch Neurol.* 1995; **52** (5): 485–490.

115. Jacobs DM, Sano M, Dooneief G, Marder K, Bell KL, Stern Y. Neuropsychological detection and characterization of preclinical Alzheimer's disease. *Neurology.* 1995; **45** (5): 957–962.

116. Blacker D, Lee H, Muzikansky A, Martin EC, Tanzi R, McArdle JJ, Moss M, Albert M. Neuropsychological measures in normal individuals that predict subsequent cognitive decline. *Arch Neurol.* 2007; **64** (6): 862–871.

117. Chen P, Ratcliff G, Belle SH, Cauley JA, DeKosky ST, Ganguli M. Patterns of cognitive decline in presymptomatic Alzheimer disease: a prospective community study. *Arch Gen Psychiatry.* 2001; **58** (9): 853–858.

118. Amieva H, Jacqmin-Gadda H, Orgogozo JM, Le Carret N, Helmer C, Letenneur L, Barberger-Gateau P, Fabrigoule C, Dartigues JF. The 9 year cognitive decline before dementia of the Alzheimer type: a prospective population-based study. *Brain.* 2005; **128** (Pt 5): 1093–1101.

119. Fabrigoule C, Rouch I, Taberly A, Letenneur L, Commenges D, Mazaux JM, Orgogozo JM, Dartigues JF. Cognitive process in preclinical phase of dementia. *Brain.* 1998; **121 (Pt 1)**: 135–141.

120. The Ronald and Nancy Reagan Research Institute of the Alzheimer's Association and the National Institute on Aging. Consensus report of the Working Group on: "Molecular and Biochemical Markers of Alzheimer's Disease". *Neurobiol Aging.* 1998; **19** (2): 109–116.

121. Fox N, Growdon JH. Biomarkers and Surrogates. *NeuroRx : the journal of the American Society for Experimental NeuroTherapeutics.* 2004; **1** (2): 181.

122. Sunderland T, Hampel H, Takeda M, Putnam KT, Cohen RM. Biomarkers in the diagnosis of Alzheimer's disease: are we ready? *J Geriatr Psychiatry Neurol.* 2006; **19** (3): 172–179.

123. Blennow K, Hampel H. CSF markers for incipient Alzheimer's disease. *Lancet Neurol.* 2003; **2** (10): 605–613.

124. SBU. *Dementia diseases.* Vol 172. First ed. Stockholm: SBU, The Swedish Council on Technology Assessment in Health Care; 2006.

125. Knopman DS, DeKosky ST, Cummings JL, Chui H, Corey-Bloom J, Relkin N, Small GW, Miller B, Stevens JC. Practice parameter: diagnosis of dementia (an evidence-based review). Report of the Quality Standards Subcommittee of the American Academy of Neurology. *Neurology.* 2001; **56** (9): 1143–1153.

126. Frank RA, Galasko D, Hampel H, Hardy J, de Leon MJ, Mehta PD, Rogers J, Siemers E, Trojanowski JQ. Biological markers for therapeutic trials in Alzheimer's disease. Proceedings of the biological markers working group; NIA initiative on neuroimaging in Alzheimer's disease. *Neurobiol Aging.* 2003; **24** (4): 521–536.

127. Small GW, Bookheimer SY, Thompson PM, Cole GM, Huang SC, Kepe V, Barrio JR. Current and future uses of neuroimaging for cognitively impaired patients. *Lancet Neurol.* 2008; **7** (2): 161–172.

128. Hampel H, Teipel SJ, Fuchsberger T, Andreasen N, Wiltfang J, Otto M, Shen Y, Dodel R, Du Y, Farlow M, et al. Value of CSF beta-amyloid1-42 and tau as predictors of Alzheimer's disease in patients with mild cognitive impairment. *Mol Psychiatry.* 2004; **9** (7): 705–710.

129. de Leon MJ, Mosconi L, Blennow K, DeSanti S, Zinkowski R, Mehta PD, Pratico D, Tsui W, Saint Louis LA, Sobanska L, et al. Imaging and CSF studies in the preclinical diagnosis of Alzheimer's disease. *Ann. N. Y. Acad. Sci.* 2007; **1097**: 114–145.

130. Herholz K, Salmon E, Perani D, Baron JC, Holthoff V, Frolich L, Schonknecht P, Ito K, Mielke R, Kalbe E, et al. Discrimination between Alzheimer dementia and controls by automated analysis of multicenter FDG PET. *Neuroimage.* 2002; **17** (1): 302–316.

131. Chong MS, Sahadevan S. Preclinical Alzheimer's disease: diagnosis and prediction of progression. *Lancet Neurol.* 2005; **4** (9): 576–579.

132. Devous MD, Sr. Functional brain imaging in the dementias: role in early detection, differential diagnosis, and longitudinal studies. *Eur J Nucl Med Mol Imaging.* 2002; **29** (12): 1685–1696.

133. Jelic V, Kowalski J. Evidence-based evaluation of diagnostic accuracy of resting EEG in dementia and mild cognitive impairment. *Clin EEG Neurosci.* 2009; **40** (2): 129–142.

134. de Leon MJ, DeSanti S, Zinkowski R, Mehta PD, Pratico D, Segal S, Clark C, Kerkman D, DeBernardis J, Li J, et al. MRI and CSF studies in the early diagnosis of Alzheimer's disease. *J Intern Med.* 2004; **256** (3): 205–223.

135. Bouwman FH, Schoonenboom SN, van der Flier WM, van Elk EJ, Kok A, Barkhof F, Blankenstein MA, Scheltens P. CSF biomarkers and medial temporal lobe atrophy predict dementia in mild cognitive impairment. *Neurobiol Aging.* 2007; **28** (7): 1070–1074.

136. Hampel H, Burger K, Teipel SJ, Bokde AL, Zetterberg H, Blennow K. Core candidate neurochemical and imaging biomarkers of Alzheimer's disease. *Alzheimers Dement.* 2008; **4** (1): 38–48.

137. Brys M, Glodzik L, Mosconi L, Switalski R, De Santi S, Pirraglia E, Rich K, Kim BC, Mehta P, Zinkowski R, et al. Magnetic resonance imaging improves cerebrospinal fluid biomarkers in the early detection of Alzheimer's disease. *J Alzheimers Dis.* 2009; **16** (2): 351–362.

138. Fagan AM, Roe CM, Xiong C, Mintun MA, Morris JC, Holtzman DM. Cerebrospinal fluid tau/beta-amyloid (42) ratio as a prediction of cognitive decline in nondemented older adults. *Arch. Neurol.* 2007; **64** (3): 343–349.

139. Skoog I, Davidsson P, Aevarsson O, Vanderstichele H, Vanmechelen E, Blennow K. Cerebrospinal fluid beta-amyloid 42 is reduced before the onset of sporadic dementia: a population-based study in 85-year-olds. *Dement. Geriatr. Cogn. Disord.* 2003; **15** (3): 169–176.

140. Li G, Sokal I, Quinn JF, Leverenz JB, Brodey M, Schellenberg GD, Kaye JA, Raskind MA, Zhang J, Peskind ER, et al. CSF tau/Abeta42 ratio for increased risk of mild cognitive impairment: a follow-up study. *Neurology.* 2007; **69** (7): 631–639.

141. Gustafson DR, Skoog I, Rosengren L, Zetterberg H, Blennow K. Cerebrospinal fluid beta-amyloid 1-42 concentration may predict cognitive decline in older women. *J Neurol Neurosurg Psychiatry.* 2007; **78** (5): 461–464.

142. Prichep LS, John ER, Ferris SH, Rausch L, Fang Z, Cancro R, Torossian C, Reisberg B. Prediction of longitudinal cognitive decline in normal elderly with subjective complaints using electrophysiological imaging. *Neurobiol Aging.* 2006; **27** (3): 471–481.

143. Folstein MF, Folstein SE, McHugh PR. "Mini-mental state". A practical method for grading the cognitive state of patients for the clinician. *J Psychiatr Res.* 1975; **12** (3): 189–198.

144. Huppert FA, Cabelli ST, Matthews FE. Brief cognitive assessment in a UK population sample – distributional properties and the relationship between the MMSE and an extended mental state examination. *BMC Geriatr.* 2005; **5**: 7.

145. Tombaugh TN, McIntyre NJ. The mini-mental state examination: a comprehensive review. *J Am Geriatr Soc.* 1992; **40** (9): 922–935.

146. Crum RM, Anthony JC, Bassett SS, Folstein MF. Population-based norms for the Mini-Mental State Examination by age and educational level. *JAMA.* 1993; **269** (18): 2386–2391.

147. Iverson GL. Interpretation of Mini-Mental State Examination scores in community-dwelling elderly and geriatric neuropsychiatry patients. *Int J Geriatr Psychiatry.* 1998; **13** (10): 661–666.

148. Cullen B, O'Neill B, Evans JJ, Coen RF, Lawlor BA. A review of screening tests for cognitive impairment. *J Neurol Neurosurg Psychiatry.* 2007; **78** (8): 790–799.

149. Jacqmin-Gadda H, Fabrigoule C, Commenges D, Dartigues JF. A 5-year longitudinal study of the Mini-Mental State Examination in normal aging. *Am J Epidemiol.* 1997; **145** (6): 498–506.

150. Rosen WG, Mohs RC, Davis KL. A new rating scale for Alzheimer's disease. *Am J Psychiatry.* 1984; **141** (11): 1356–1364.

151. Graham DP, Cully JA, Snow AL, Massman P, Doody R. The Alzheimer's Disease Assessment Scale-Cognitive subscale: normative data for older adult controls. *Alzheimer Dis Assoc Disord.* 2004; **18** (4): 236–240.

152. Zec RF, Landreth ES, Vicari SK, Feldman E, Belman J, Andrise A, Robbs R, Kumar V, Becker R. Alzheimer disease assessment scale: useful for both early detection and staging of dementia of the Alzheimer type. *Alzheimer Dis Assoc Disord.* 1992; **6** (2): 89–102.

153. Liu HC, Teng EL, Chuang YY, Lin KN, Fuh JL, Wang PN. The Alzheimer's Disease Assessment Scale: findings from a low-education population. *Dement Geriatr Cogn Disord.* 2002; **13** (1): 21–26.

154. Grundman M, Petersen RC, Ferris SH, Thomas RG, Aisen PS, Bennett DA, Foster NL, Jack CR, Jr., Galasko DR, Doody R, et al. Mild cognitive impairment can be distinguished from Alzheimer disease and normal aging for clinical trials. *Arch Neurol.* 2004; **61** (1): 59–66.

155. Weyer G, Erzigkeit H, Kanowski S, Ihl R, Hadler D. Alzheimer's Disease Assessment Scale: reliability and validity in a multicenter clinical trial. *Int Psychogeriatr.* 1997; **9** (2): 123–138.

156. Wiig EH, Nielsen NP, Minthon L, McPeek D, Said K, Warkentin S. Parietal lobe activation in rapid, automatized naming by adults. *Percept Mot Skills.* 2002; **94** (3 Pt 2): 1230–1244.

157. Jacobson JM, Nielsen NP, Minthon L, Warkentin S, Wiig EH. Multiple rapid automatic naming measures of cognition: normal performance and effects of aging. *Percept Mot Skills.* 2004; **98** (3 Pt 1): 739–753.

158. Nielsen NP, Wiig EH, Warkentin S, Minthon L. Clinical utility of color-form naming in Alzheimer's disease: preliminary evidence. *Percept Mot Skills.* 2004; **99** (3 Pt 2): 1201–1204.

159. Shulman KI. Clock-drawing: is it the ideal cognitive screening test? *Int J Geriatr Psychiatry.* 2000; **15** (6): 548–561.

160. Manos PJ, Wu R. The ten point clock test: a quick screen and grading method for cognitive impairment in medical and surgical patients. *Int J Psychiatry Med.* 1994; **24** (3): 229–244.

161. Seigerschmidt E, Mosch E, Siemen M, Forstl H, Bickel H. The clock drawing test and questionable dementia: reliability and validity. *Int J Geriatr Psychiatry.* 2002; **17** (11): 1048–1054.

162. Philpot M. The clock-drawing test: a critique. *Int Psychogeriatr.* 2004; **16** (3): 251–256.

163. Moretti R, Torre P, Antonello RM, Cazzato G, Bava A. Ten-Point Clock Test: a correlation analysis with other neuropsychological tests in dementia. *Int J Geriatr Psychiatry.* 2002; **17** (4): 347–353.

164. Griffith HR, Netson KL, Harrell LE, Zamrini EY, Brockington JC, Marson DC. Amnestic mild cognitive impairment: diagnostic outcomes and clinical prediction over a two-year time period. *J Int Neuropsychol Soc.* 2006; **12** (2): 166–175.

165. Buchhave P, Stomrud E, Warkentin S, Blennow K, Minthon L, Hansson O. Cube copying test in combination with rCBF or CSF A beta 42 predicts development of Alzheimer's disease. *Dement Geriatr Cogn Disord.* 2008; **25** (6): 544–552.

166. Maeshima S, Osawa A, Maeshima E, Shimamoto Y, Sekiguchi E, Kakishita K, Ozaki F, Moriwaki H. Usefulness of a cube-copying test in outpatients with dementia. *Brain Inj.* 2004; **18** (9): 889–898.

167. Maeshima S, Itakura T, Nakagawa M, Nakai K, Komai N. Visuospatial impairment and activities of daily living in patients with Parkinson's disease: a quantitative assessment of the cube-copying task. *Am J Phys Med Rehabil.* 1997; **76** (5): 383–388.

168. Ericsson K, Forssell LG, Holmen K, Viitanen M, Winblad B. Copying and handwriting ability in the screening of cognitive dysfunction in old age. *Arch Gerontol Geriatr.* 1996; **22** (2): 103–121.

169. Shimada Y, Meguro K, Kasai M, Shimada M, Ishii H, Yamaguchi S, Yamadori A. Necker cube copying ability in normal elderly and Alzheimer's disease. A community-based study: The Tajiri project. *Psychogeriatrics.* 2006; **6** (1): 4–9.

170. Paganini-Hill A, Clark LJ. Preliminary assessment of cognitive function in older adults by clock drawing, box copying and narrative writing. *Dement Geriatr Cogn Disord.* 2007; **23** (2): 74–81.

171. Blennow K. CSF biomarkers for mild cognitive impairment. *J. Intern. Med.* 2004; **256** (3): 224–234.

172. Blennow K. Cerebrospinal fluid protein biomarkers for Alzheimer's disease. *NeuroRx.* 2004; **1** (2): 213–225.

173. Andreasen N, Blennow K. CSF biomarkers for mild cognitive impairment and early Alzheimer's disease. *Clin Neurol Neurosurg.* 2005; **107** (3): 165–173.

174. Itoh N, Arai H, Urakami K, Ishiguro K, Ohno H, Hampel H, Buerger K, Wiltfang J, Otto M, Kretzschmar H, et al. Large-scale, multicenter study of cerebrospinal fluid tau protein phosphorylated at serine 199 for the antemortem diagnosis of Alzheimer's disease. *Ann. Neurol.* 2001; **50** (2): 150–156.

175. Sunderland T, Linker G, Mirza N, Putnam KT, Friedman DL, Kimmel LH, Bergeson J, Manetti GJ, Zimmermann M, Tang B, et al. Decreased beta-amyloid1-42 and increased tau levels in cerebrospinal fluid of patients with Alzheimer disease. *JAMA.* 2003; **289** (16): 2094–2103.

176. Visser PJ, Verhey F, Knol DL, Scheltens P, Wahlund LO, Freund-Levi Y, Tsolaki M, Minthon L, Wallin AK, Hampel H, et al. Prevalence and prognostic value of CSF markers of Alzheimer's disease pathology in patients with subjective cognitive impairment or mild cognitive impairment in the DESCRIPA study: a prospective cohort study. *Lancet Neurol.* 2009; **8** (7): 619–627.

177. Diniz BS, Pinto Junior JA, Forlenza OV. Do CSF total tau, phosphorylated tau, and beta-amyloid 42 help to predict progression of mild cognitive impairment to Alzheimer's disease? A systematic review and meta-analysis of the literature. *World J Biol Psychiatry.* 2008; **9** (3): 172–182.

178. Hansson O, Zetterberg H, Buchhave P, Londos E, Blennow K, Minthon L. Association between CSF biomarkers and incipient Alzheimer's disease in patients with mild cognitive impairment: a follow-up study. *Lancet Neurol.* 2006; **5** (3): 228–234.

179. Shaw LM, Vanderstichele H, Knapik-Czajka M, Clark CM, Aisen PS, Petersen RC, Blennow K, Soares H, Simon A, Lewczuk P, et al. Cerebrospinal fluid biomarker signature in Alzheimer's disease neuroimaging initiative subjects. *Ann Neurol.* 2009; **65** (4): 403–413.

180. Mattsson N, Zetterberg H, Hansson O, Andreasen N, Parnetti L, Jonsson M, Herukka SK, van der Flier WM, Blankenstein MA, Ewers M, et al. CSF biomarkers and incipient Alzheimer disease in patients with mild cognitive impairment. *JAMA.* 2009; **302** (4): 385–393.

181. Herukka SK, Hallikainen M, Soininen H, Pirttila T. CSF Abeta42 and tau or phosphorylated tau and prediction of progressive mild cognitive impairment. *Neurology.* 2005; **64** (7): 1294–1297.

182. Brys M, Pirraglia E, Rich K, Rolstad S, Mosconi L, Switalski R, Glodzik-Sobanska L, De Santi S, Zinkowski R, Mehta P, et al. Prediction and longitudinal study of CSF biomarkers in mild cognitive impairment. *Neurobiol Aging.* 2009; **30** (5): 682–690.

183. Snider BJ, Fagan AM, Roe C, Shah AR, Grant EA, Xiong C, Morris JC, Holtzman DM. Cerebrospinal fluid biomarkers and rate of cognitive decline in very mild dementia of the Alzheimer type. *Arch Neurol.* 2009; **66** (5): 638–645.

184. Andreasen N, Minthon L, Davidsson P, Vanmechelen E, Vanderstichele H, Winblad B, Blennow K. Evaluation of CSF-tau and CSF-Abeta42 as diagnostic markers for Alzheimer disease in clinical practice. *Arch Neurol.* 2001; **58** (3): 373–379.

185. Sjogren M, Davidsson P, Wallin A, Granerus AK, Grundstrom E, Askmark H, Vanmechelen E, Blennow K. Decreased CSF-beta-amyloid 42 in Alzheimer's disease and amyotrophic lateral sclerosis may reflect mismetabolism of beta-amyloid induced by disparate mechanisms. *Dement Geriatr Cogn Disord.* 2002; **13** (2): 112–118.

186. Holmberg B, Johnels B, Blennow K, Rosengren L. Cerebrospinal fluid Abeta42 is reduced in multiple system atrophy but normal in Parkinson's disease and progressive supranuclear palsy. *Mov Disord.* 2003; **18** (2): 186–190.

187. Otto M, Wiltfang J, Tumani H, Zerr I, Lantsch M, Kornhuber J, Weber T, Kretzschmar HA, Poser S. Elevated levels of tau-protein in cerebrospinal fluid of patients with Creutzfeldt-Jakob disease. *Neurosci Lett.* 1997; **225** (3): 210–212.

188. Hesse C, Rosengren L, Andreasen N, Davidsson P, Vanderstichele H, Vanmechelen E, Blennow K. Transient increase in total tau but not phospho-tau in human cerebrospinal fluid after acute stroke. *Neurosci. Lett.* 2001; **297** (3): 187–190.

189. Green AJ, Harvey RJ, Thompson EJ, Rossor MN. Increased tau in the cerebrospinal fluid of patients with frontotemporal dementia and Alzheimer's disease. *Neurosci Lett.* 1999; **259** (2): 133–135.

190. Glodzik-Sobanska L, Pirraglia E, Brys M, de Santi S, Mosconi L, Rich KE, Switalski R, Saint Louis L, Sadowski MJ, Martiniuk F, et al. The effects of normal aging and ApoE genotype on the levels of CSF biomarkers for Alzheimer's disease. *Neurobiol Aging.* 2009; **30** (5): 672–681.

191. Kanai M, Matsubara E, Isoe K, Urakami K, Nakashima K, Arai H, Sasaki H, Abe K, Iwatsubo T, Kosaka T, et al. Longitudinal study of cerebrospinal fluid levels of tau, A beta1–40, and A beta1–42(43) in Alzheimer's disease: a study in Japan. *Ann Neurol.* 1998; **44** (1): 17–26.

192. Andreasen N, Minthon L, Clarberg A, Davidsson P, Gottfries J, Vanmechelen E, Vanderstichele H, Winblad B, Blennow K. Sensitivity, specificity, and stability of CSF-tau in AD in a community-based patient sample. *Neurology.* 1999; **53** (7): 1488–1494.

193. Sjogren M, Davidsson P, Tullberg M, Minthon L, Wallin A, Wikkelso C, Granerus AK, Vanderstichele H, Vanmechelen E, Blennow K. Both total and phosphorylated tau are increased in Alzheimer's disease. *J. Neurol. Neurosurg. Psychiatry.* 2001; **70** (5): 624–630.

194. Andreasen N, Minthon L, Vanmechelen E, Vanderstichele H, Davidsson P, Winblad B, Blennow K. Cerebrospinal fluid tau and Abeta42 as predictors of development of Alzheimer's disease in patients with mild cognitive impairment. *Neurosci. Lett.* 1999; **273** (1): 5–8.

195. Andreasen N, Vanmechelen E, Van de Voorde A, Davidsson P, Hesse C, Tarvonen S, Raiha I, Sourander L, Winblad B, Blennow K. Cerebrospinal fluid tau protein as a biochemical marker for Alzheimer's disease: a community based follow up study. *J. Neurol. Neurosurg. Psychiatry.* 1998; **64** (3): 298–305.

196. Sunderland T, Wolozin B, Galasko D, Levy J, Dukoff R, Bahro M, Lasser R, Motter R, Lehtimaki T, Seubert P. Longitudinal stability of CSF tau levels in Alzheimer patients. *Biol. Psychiatry.* 1999; **46** (6): 750–755.

197. Blomberg M, Jensen M, Basun H, Lannfelt L, Wahlund LO. Increasing cerebrospinal fluid tau levels in a subgroup of Alzheimer patients with apolipoprotein E allele epsilon 4 during 14 months follow–up. *Neurosci. Lett.* 1996; **214** (2–3): 163–166.

198. Mollenhauer B, Bibl M, Trenkwalder C, Stiens G, Cepek L, Steinacker P, Ciesielczyk B, Neubert K, Wiltfang J, Kretzschmar HA, et al. Follow-up investigations in cerebrospinal fluid of patients with dementia with Lewy bodies and Alzheimer's disease. *J. Neural. Transm.* 2005; **112** (7): 933–948.

199. Fagan AM, Head D, Shah AR, Marcus D, Mintun M, Morris JC, Holtzman DM. Decreased cerebrospinal fluid Abeta(42) correlates with brain atrophy in cognitively normal elderly. *Ann Neurol.* 2009; **65** (2): 176–183.

200. Engelborghs S, Sleegers K, Cras P, Brouwers N, Serneels S, De Leenheir E, Martin JJ, Vanmechelen E, Van Broeckhoven C, De Deyn PP. No association of CSF biomarkers with APOEepsilon4, plaque and tangle burden in definite Alzheimer's disease. *Brain.* 2007; **130** (Pt 9): 2320–2326.

201. Bouwman FH, van der Flier WM, Schoonenboom NS, van Elk EJ, Kok A, Rijmen F, Blankenstein MA, Scheltens P. Longitudinal changes of CSF biomarkers in memory clinic patients. *Neurology.* 2007; **69** (10): 1006–1011.

202. Blennow K, Zetterberg H, Minthon L, Lannfelt L, Strid S, Annas P, Basun H, Andreasen N. Longitudinal stability of CSF biomarkers in Alzheimer's disease. *Neurosci. Lett.* 2007; **419** (1): 18–22.

203. Huey ED, Mirza N, Putnam KT, Soares H, Csako G, Levy JA, Copenhaver B, Cohen RM, Sunderland T. Stability of CSF beta-amyloid(1–42) and tau levels by APOE genotype in Alzheimer patients. *Dement. Geriatr. Cogn. Disord.* 2006; **22** (1): 48–53.

204. Tapiola T, Pirttila T, Mikkonen M, Mehta PD, Alafuzoff I, Koivisto K, Soininen H. Three-year follow-up of cerebrospinal fluid tau, beta-amyloid 42 and 40 concentrations in Alzheimer's disease. *Neurosci. Lett.* 2000; **280** (2): 119–122.

205. de Leon MJ, DeSanti S, Zinkowski R, Mehta PD, Pratico D, Segal S, Rusinek H, Li J, Tsui W, Saint Louis LA, et al. Longitudinal CSF and MRI biomarkers improve the diagnosis of mild cognitive impairment. *Neurobiol Aging.* 2006; **27** (3): 394–401.

206. de Leon MJ, Segal S, Tarshish CY, DeSanti S, Zinkowski R, Mehta PD, Convit A, Caraos C, Rusinek H, Tsui W, et al. Longitudinal cerebrospinal fluid tau load increases in mild cognitive impairment. *Neurosci. Lett.* 2002; **333** (3): 183–186.

207. Andersson C, Blennow K, Almkvist O, Andreasen N, Engfeldt P, Johansson SE, Lindau M, Eriksdotter-Jonhagen M. Increasing CSF phospho-tau levels during cognitive decline and progression to dementia. *Neurobiol Aging.* 2008; **29** (10): 1466–1473.

208. Zetterberg H, Pedersen M, Lind K, Svensson M, Rolstad S, Eckerstrom C, Syversen S, Mattsson UB, Ysander C, Mattsson N, et al. Intra-individual stability

of CSF biomarkers for Alzheimer's disease over two years. *J. Alzheimers Dis.* 2007; **12** (3): 255–260.

209. Kanai M, Shizuka M, Urakami K, Matsubara E, Harigaya Y, Okamoto K, Shoji M. Apolipoprotein E4 accelerates dementia and increases cerebrospinal fluid tau levels in Alzheimer's disease. *Neurosci. Lett.* 1999; **267** (1): 65–68.

210. Andreasen N, Hesse C, Davidsson P, Minthon L, Wallin A, Winblad B, Vanderstichele H, Vanmechelen E, Blennow K. Cerebrospinal fluid beta-amyloid (1–42) in Alzheimer disease: differences between early- and late-onset Alzheimer disease and stability during the course of disease. *Arch. Neurol.* 1999; **56** (6): 673–680.

211. Tapiola T, Alafuzoff I, Herukka SK, Parkkinen L, Hartikainen P, Soininen H, Pirttila T. Cerebrospinal fluid {beta}-amyloid 42 and tau proteins as biomarkers of Alzheimer-type pathologic changes in the brain. *Arch Neurol.* 2009; **66** (3): 382–389.

212. Tapiola T, Overmyer M, Lehtovirta M, Helisalmi S, Ramberg J, Alafuzoff I, Riekkinen P, Sr., Soininen H. The level of cerebrospinal fluid tau correlates with neurofibrillary tangles in Alzheimer's disease. *Neuroreport.* 1997; **8** (18): 3961–3963.

213. Buerger K, Ewers M, Pirttila T, Zinkowski R, Alafuzoff I, Teipel SJ, DeBernardis J, Kerkman D, McCulloch C, Soininen H, et al. CSF phosphorylated tau protein correlates with neocortical neurofibrillary pathology in Alzheimer's disease. *Brain.* 2006; **129** (Pt 11): 3035–3041.

214. Strozyk D, Blennow K, White LR, Launer LJ. CSF Abeta 42 levels correlate with amyloid-neuropathology in a population-based autopsy study. *Neurology.* 2003; **60** (4): 652–656.

215. Hulstaert F, Blennow K, Ivanoiu A, Schoonderwaldt HC, Riemenschneider M, De Deyn PP, Bancher C, Cras P, Wiltfang J, Mehta PD, et al. Improved discrimination of AD patients using beta-amyloid (1–42) and tau levels in CSF. *Neurology.* 1999; **52** (8): 1555–1562.

216. Galasko D, Chang L, Motter R, Clark CM, Kaye J, Knopman D, Thomas R, Kholodenko D, Schenk D, Lieberburg I, et al. High cerebrospinal fluid tau and low amyloid beta42 levels in the clinical diagnosis of Alzheimer disease and relation to apolipoprotein E genotype. *Arch Neurol.* 1998; **55** (7): 937–945.

217. Sunderland T, Mirza N, Putnam KT, Linker G, Bhupali D, Durham R, Soares H, Kimmel L, Friedman D, Bergeson J, et al. Cerebrospinal fluid beta-amyloid1-42 and tau in control subjects at risk for Alzheimer's disease: the effect of APOE epsilon4 allele. *Biol. Psychiatry.* 2004; **56** (9): 670–676.

218. Tapiola T, Pirttila T, Mehta PD, Alafuzofff I, Lehtovirta M, Soininen H. Relationship between apoE genotype and CSF beta-amyloid (1–42) and tau in patients with probable and definite Alzheimer's disease. *Neurobiol. Aging.* 2000; **21** (5): 735–740.

219. Prince JA, Zetterberg H, Andreasen N, Marcusson J, Blennow K. APOE epsilon4 allele is associated with reduced cerebrospinal fluid levels of Abeta42. *Neurology.* 2004; **62** (11): 2116–2118.

220. Andersson C, Blennow K, Johansson SE, Almkvist O, Engfeldt P, Lindau M, Eriksdotter-Jonhagen M. Differential CSF biomarker levels in APOE-epsilon4-positive and -negative patients with memory impairment. *Dement Geriatr Cogn Disord.* 2007; **23** (2): 87–95.

221. Golombowski S, Muller-Spahn F, Romig H, Mendla K, Hock C. Dependence of cerebrospinal fluid Tau protein levels on apolipoprotein E4 allele frequency in patients with Alzheimer's disease. *Neurosci Lett.* 1997; **225** (3): 213–215.

222. Lasser RA, Dukoff R, Levy J, Levin R, Lehtimaki T, Seubert P, Sunderland T. Apolipoprotein E epsilon 4 allele in association with global cognitive performance and CSF markers in Alzheimer's disease. *Int J Geriatr Psychiatry.* 1998; **13** (11): 767–774.

223. Leow AD, Yanovsky I, Parikshak N, Hua X, Lee S, Toga AW, Jack CR, Jr., Bernstein MA, Britson PJ, Gunter JL, et al. Alzheimer's disease neuroimaging initiative: a one-year follow up study using tensor-based morphometry correlating degenerative rates, biomarkers and cognition. *Neuroimage.* 2009; **45** (3): 645–655.

224. Coben LA, Danziger W, Storandt M. A longitudinal EEG study of mild senile dementia of Alzheimer type: changes at 1 year and at 2.5 years. *Electroencephalogr Clin Neurophysiol.* 1985; **61** (2): 101–112.

225. Schreiter-Gasser U, Gasser T, Ziegler P. Quantitative EEG analysis in early onset Alzheimer's disease: correlations with severity, clinical characteristics, visual EEG and CCT. *Electroencephalogr Clin Neurophysiol.* 1994; **90** (4): 267–272.

226. Nobili F, Copello F, Vitali P, Prastaro T, Carozzo S, Perego G, Rodriguez G. Timing of disease progression by quantitative EEG in Alzheimer' s patients. *J Clin Neurophysiol.* 1999; **16** (6): 566–573.

227. Prichep LS, John ER, Ferris SH, Reisberg B, Almas M, Alper K, Cancro R. Quantitative EEG correlates of cognitive deterioration in the elderly. *Neurobiol Aging.* 1994; **15** (1): 85–90.

228. Babiloni C, Binetti G, Cassetta E, Dal Forno G, Del Percio C, Ferreri F, Ferri R, Frisoni G, Hirata K, Lanuzza B, et al. Sources of cortical rhythms change as a function of cognitive impairment in pathological aging: a multicenter study. *Clin Neurophysiol.* 2006; **117** (2): 252–268.

229. Kai T, Asai Y, Sakuma K, Koeda T, Nakashima K. Quantitative electroencephalogram analysis in dementia with Lewy bodies and Alzheimer's disease. *J Neurol Sci.* 2005; **237** (1–2): 89–95.

230. Jeong J. EEG dynamics in patients with Alzheimer's disease. *Clin Neurophysiol.* 2004; **115** (7): 1490–1505.

231. Besthorn C, Forstl H, Geiger-Kabisch C, Sattel H, Gasser T, Schreiter-Gasser U. EEG coherence in Alzheimer disease. *Electroencephalogr Clin Neurophysiol.* 1994; **90** (3): 242–245.

232. Wada Y, Nanbu Y, Koshino Y, Yamaguchi N, Hashimoto T. Reduced interhemispheric EEG coherence in Alzheimer disease: analysis during rest and photic stimulation. *Alzheimer Dis Assoc Disord.* 1998; **12** (3): 175–181.

233. Penttila M, Partanen JV, Soininen H, Riekkinen PJ. Quantitative analysis of occipital EEG in different stages of Alzheimer's disease. *Electroencephalogr Clin Neurophysiol.* 1985; **60** (1): 1–6.

234. Grunwald M, Busse F, Hensel A, Kruggel F, Riedel-Heller S, Wolf H, Arendt T, Gertz HJ. Correlation between cortical theta activity and hippocampal volumes in health, mild cognitive impairment, and mild dementia. *J Clin Neurophysiol.* 2001; **18** (2): 178–184.

235. Jelic V, Johansson SE, Almkvist O, Shigeta M, Julin P, Nordberg A, Winblad B, Wahlund LO. Quantitative electroencephalography in mild cognitive impairment: longitudinal changes and possible prediction of Alzheimer's disease. *Neurobiol Aging.* 2000; **21** (4): 533–540.

236. Jelic V, Shigeta M, Julin P, Almkvist O, Winblad B, Wahlund LO. Quantitative electroencephalography power and coherence in Alzheimer's disease and mild cognitive impairment. *Dementia.* 1996; **7** (6): 314–323.

237. Rossini PM, Del Percio C, Pasqualetti P, Cassetta E, Binetti G, Dal Forno G, Ferreri F, Frisoni G, Chiovenda P, Miniussi C, et al. Conversion from mild cognitive impairment to Alzheimer's disease is predicted by sources and coherence of brain electroencephalography rhythms. *Neuroscience.* 2006; **143** (3): 793–803.

238. Lehmann C, Koenig T, Jelic V, Prichep L, John RE, Wahlund LO, Dodge Y, Dierks T. Application and comparison of classification algorithms for recognition of Alzheimer's disease in electrical brain activity (EEG). *J Neurosci Methods.* 2007; **161** (2): 342–350.

239. Jackson CE, Snyder PJ. Electroencephalography and event-related potentials as biomarkers of mild cognitive impairment and mild Alzheimer's disease. *Alzheimers Dement.* 2008; **4** (1 Suppl 1): S137–143.

240. Huang C, Wahlund L, Dierks T, Julin P, Winblad B, Jelic V. Discrimination of Alzheimer's disease and mild cognitive impairment by equivalent EEG sources: a cross-sectional and longitudinal study. *Clin Neurophysiol.* 2000; **111** (11): 1961–1967.

241. Wolf H, Jelic V, Gertz HJ, Nordberg A, Julin P, Wahlund LO. A critical discussion of the role of neuroimaging in mild cognitive impairment. *Acta Neurol Scand Suppl.* 2003; **179**: 52–76.

242. Ebert U, Kirch W. Scopolamine model of dementia: electroencephalogram findings and cognitive performance. *Eur J Clin Invest.* 1998; **28** (11): 944–949.

243. Osipova D, Ahveninen J, Kaakkola S, Jaaskelainen IP, Huttunen J, Pekkonen E. Effects of scopolamine on MEG spectral power and coherence in elderly subjects. *Clin Neurophysiol.* 2003; **114** (10): 1902–1907.

244. Adler G, Brassen S. Short-term rivastigmine treatment reduces EEG slow-wave power in Alzheimer patients. *Neuropsychobiology.* 2001; **43** (4): 273–276.

245. Brassen S, Adler G. Short-term effects of acetylcholinesterase inhibitor treatment on EEG and memory performance in Alzheimer patients: an open, controlled trial. *Pharmacopsychiatry.* 2003; **36** (6): 304–308.

246. Kogan EA, Korczyn AD, Virchovsky RG, Klimovizky S, Treves TA, Neufeld MY. EEG changes during long-term treatment with donepezil in Alzheimer's disease patients. *J Neural Transm.* 2001; **108** (10): 1167–1173.

247. Grunwald M, Hensel A, Wolf H, Weiss T, Gertz HJ. Does the hippocampal atrophy correlate with the cortical theta power in elderly subjects with a range of cognitive impairment? *J Clin Neurophysiol.* 2007; **24** (1): 22–26.

248. Jelic V, Blomberg M, Dierks T, Basun H, Shigeta M, Julin P, Jensen M, Lannfelt L, Winblad B, Wahlund LO. EEG slowing and cerebrospinal fluid tau levels in patients with cognitive decline. *Neuroreport.* 1998; **9** (1): 157–160.

249. Mattia D, Babiloni F, Romigi A, Cincotti F, Bianchi L, Sperli F, Placidi F, Bozzao A, Giacomini P, Floris R, et al. Quantitative EEG and dynamic susceptibility contrast MRI in Alzheimer's disease: a correlative study. *Clin Neurophysiol.* 2003; **114** (7): 1210–1216.

250. Rodriguez G, Nobili F, Rocca G, De Carli F, Gianelli MV, Rosadini G. Quantitative electroencephalography and regional cerebral blood flow: discriminant analysis between Alzheimer's patients and healthy controls. *Dement Geriatr Cogn Disord.* 1998; **9** (5): 274–283.

251. Kwa VI, Weinstein HC, Posthumus Meyjes EF, van Royen EA, Bour LJ, Verhoeff PN, Ongerboer de Visser BW. Spectral analysis of the EEG and 99m-Tc-HMPAO SPECT-scan in Alzheimer's disease. *Biol Psychiatry.* 1993; **33** (2): 100–107.

252. Passero S, Rocchi R, Vatti G, Burgalassi L, Battistini N. Quantitative EEG mapping, regional cerebral blood flow, and neuropsychological function in Alzheimer's disease. *Dementia.* 1995; **6** (3): 148–156.

253. Buchan RJ, Nagata K, Yokoyama E, Langman P, Yuya H, Hirata Y, Hatazawa J, Kanno I. Regional correlations between the EEG and oxygen metabolism in dementia of Alzheimer's type. *Electroencephalogr Clin Neurophysiol.* 1997; **103** (3): 409–417.

254. Rae-Grant A, Blume W, Lau C, Hachinski VC, Fisman M, Merskey H. The electroencephalogram in Alzheimer-type dementia. A sequential study correlating the electroencephalogram with psychometric and quantitative pathologic data. *Arch Neurol.* 1987; **44** (1): 50–54.

255. Ponomareva NV, Korovaitseva GI, Rogaev EI. EEG alterations in non-demented individuals related to apolipoprotein E genotype and to risk of Alzheimer disease. *Neurobiol Aging.* 2008; **29** (6): 819–827.

256. Rossini PM, Rossi S, Babiloni C, Polich J. Clinical neurophysiology of aging brain: From normal aging to neurodegeneration. *Prog Neurobiol.* 2007; **83** (6): 375–400.

257. Babiloni C, Benussi L, Binetti G, Cassetta E, Dal Forno G, Del Percio C, Ferreri F, Ferri R, Frisoni G, Ghidoni R, et al. Apolipoprotein E and alpha brain rhythms in mild cognitive impairment: a multicentric electroencephalogram study. *Ann Neurol.* 2006; **59** (2): 323–334.

258. Onishi J, Suzuki Y, Yoshiko K, Hibino S, Iguchi A. Predictive model for assessing cognitive impairment by quantitative electroencephalography. *Cogn Behav Neurol.* 2005; **18** (3): 179–184.

259. van der Hiele K, Vein AA, van der Welle A, van der Grond J, Westendorp RG, Bollen EL, van Buchem MA, van Dijk JG, Middelkoop HA. EEG and MRI correlates of mild cognitive impairment and Alzheimer's disease. *Neurobiol Aging.* 2007; **28** (9): 1322–1329.

260. Elmstahl S, Rosen I, Gullberg B. Quantitative EEG in elderly patients with Alzheimer's disease and healthy controls. *Dementia.* 1994; **5** (2): 119–124.

261. Gawel M, Zalewska E, Szmidt-Salkowska E, Kowalski J. Does EEG (visual and quantitative) reflect mental impairment in subcortical vascular dementia? *J Neurol Sci.* 2007; **257** (1–2): 11–16.

262. Kwak YT. Quantitative EEG findings in different stages of Alzheimer's disease. *J Clin Neurophysiol.* 2006; **23** (5): 456–461.

263. Holschneider DP, Waite JJ, Leuchter AF, Walton NY, Scremin OU. Changes in electrocortical power and coherence in response to the selective cholinergic immunotoxin 192 IgG-saporin. *Exp Brain Res.* 1999; **126** (2): 270–280.

264. Soininen H, Reinikainen KJ, Partanen J, Helkala EL, Paljarvi L, Riekkinen PJ. Slowing of electroencephalogram and choline acetyltransferase activity in post mortem frontal cortex in definite Alzheimer's disease. *Neuroscience.* 1992; **49** (3): 529–535.

265. Riekkinen P, Jr., Sirvio J, Riekkinen P. Relationship between the cortical choline acetyltransferase content and EEG delta-power. *Neurosci Res.* 1990; **8** (1): 12–20.

266. Steriade M. Acetylcholine systems and rhythmic activities during the waking – sleep cycle. *Prog Brain Res.* 2004; **145**: 179–196.

267. Chapman CA, Lacaille JC. Cholinergic induction of theta-frequency oscillations in hippocampal inhibitory interneurons and pacing of pyramidal cell firing. *J Neurosci.* 1999; **19** (19): 8637–8645.

268. Lopes da Silva F. Neural mechanisms underlying brain waves: from neural membranes to networks. *Electroencephalogr Clin Neurophysiol.* 1991; **79** (2): 81–93.

269. Siok CJ, Rogers JA, Kocsis B, Hajos M. Activation of alpha7 acetylcholine receptors augments stimulation-induced hippocampal theta oscillation. *Eur J Neurosci.* 2006; **23** (2): 570–574.

270. Johnson KA, Jones K, Holman BL, Becker JA, Spiers PA, Satlin A, Albert MS. Preclinical prediction of Alzheimer's disease using SPECT. *Neurology.* 1998; **50** (6): 1563–1571.

271. Kogure D, Matsuda H, Ohnishi T, Asada T, Uno M, Kunihiro T, Nakano S, Takasaki M. Longitudinal evaluation of early Alzheimer's disease using brain perfusion SPECT. *J Nucl Med.* 2000; **41** (7): 1155–1162.

272. Okamura N, Arai H, Maruyama M, Higuchi M, Matsui T, Tanji H, Seki T, Hirai H, Chiba H, Itoh M, et al. Combined Analysis of CSF Tau Levels and [(123) I]Iodoamphetamine SPECT in Mild Cognitive Impairment: Implications for a Novel Predictor of Alzheimer's Disease. *Am J Psychiatry.* 2002; **159** (3): 474–476.

273. Bradley KM, O'Sullivan VT, Soper ND, Nagy Z, King EM, Smith AD, Shepstone BJ. Cerebral perfusion SPET correlated with Braak pathological stage in Alzheimer's disease. *Brain.* 2002; **125** (Pt 8): 1772–1781.

274. Johnson KA, Moran EK, Becker JA, Blacker D, Fischman AJ, Albert MS. Single photon emission computed tomography perfusion differences in mild cognitive impairment. *J Neurol Neurosurg Psychiatry.* 2007; **78** (3): 240–247.

275. Burns A, Philpot MP, Costa DC, Ell PJ, Levy R. The investigation of Alzheimer's disease with single photon emission tomography. *J Neurol Neurosurg Psychiatry.* 1989; **52** (2): 248–253.

276. Matsuda H. Role of neuroimaging in Alzheimer's disease, with emphasis on brain perfusion SPECT. *J Nucl Med.* 2007; **48** (8): 1289–1300.

277. Huang C, Eidelberg D, Habeck C, Moeller J, Svensson L, Tarabula T, Julin P. Imaging markers of mild cognitive impairment: multivariate analysis of CBF SPECT. *Neurobiol Aging.* 2007; **28** (7): 1062–1069.

278. Tsolaki M, Sakka V, Gerasimou G, Dimacopoulos N, Chatzizisi O, Fountoulakis KN, Kyriazis G, Papanastasiou J, Kazis A. Correlation of rCBF (SPECT), CSF tau, and cognitive function in patients with dementia of the Alzheimer's type, other types of dementia, and control subjects. *Am J Alzheimers Dis Other Demen.* 2001; **16** (1): 21–31.

279. Zakzanis KK, Graham SJ, Campbell Z. A meta-analysis of structural and functional brain imaging in dementia of the Alzheimer's type: a neuroimaging profile. *Neuropsychol Rev.* 2003; **13** (1): 1–18.

280. Staffen W, Schonauer U, Zauner H, Spindler I, Mair A, Iglseder B, Bernroider G, Ladurner G. Brain perfusion SPECT in patients with mild cognitive impairment and Alzheimer's disease: comparison of a semiquantitative and a visual evaluation. *J Neural Transm.* 2006; **113** (2): 195–203.

281. Huang C, Wahlund LO, Almkvist O, Elehu D, Svensson L, Jonsson T, Winblad B, Julin P. Voxel- and VOI-based analysis of SPECT CBF in relation to clinical and psychological heterogeneity of mild cognitive impairment. *Neuroimage.* 2003; **19** (3): 1137–1144.

282. Caffarra P, Ghetti C, Concari L, Venneri A. Differential patterns of hypoperfusion in subtypes of mild cognitive impairment. *Open Neuroimag J.* 2008; **2**: 20–28.

283. Hirao K, Ohnishi T, Hirata Y, Yamashita F, Mori T, Moriguchi Y, Matsuda H, Nemoto K, Imabayashi E, Yamada M, et al. The prediction of rapid conversion to Alzheimer's disease in mild cognitive impairment using regional cerebral blood flow SPECT. *Neuroimage.* 2005; **28** (4): 1014–1021.

284. Borroni B, Anchisi D, Paghera B, Vicini B, Kerrouche N, Garibotto V, Terzi A, Vignolo LA, Di Luca M, Giubbini R, et al. Combined 99mTc-ECD SPECT and neuropsychological studies in MCI for the assessment of conversion to AD. *Neurobiol Aging.* 2006; **27** (1): 24–31.

285. Hansson O, Buchhave P, Zetterberg H, Blennow K, Minthon L, Warkentin S. Combined rCBF and CSF biomarkers predict progression from mild cognitive impairment to Alzheimer's disease. *Neurobiol Aging.* 2009; **30** (2): 165–173.

286. Habert MO, Horn JF, Sarazin M, Lotterie JA, Puel M, Onen F, Zanca M, Portet F, Touchon J, Verny M, et al. Brain perfusion SPECT with an automated quantitative tool can identify prodromal Alzheimer's disease among patients with mild cognitive impairment. *Neurobiol Aging* (2009), 10.1016/j.neurobiolaging.2009.01.013

287. Nobili F, Frisoni GB, Portet F, Verhey F, Rodriguez G, Caroli A, Touchon J, Calvini P, Morbelli S, De Carli F, et al. Brain SPECT in subtypes of mild cognitive impairment. Findings from the DESCRIPA multicenter study. *J Neurol.* 2008; **255** (9): 1344–1353.

288. Matsuda H, Kitayama N, Ohnishi T, Asada T, Nakano S, Sakamoto S, Imabayashi E, Katoh A. Longitudinal evaluation of both morphologic and functional changes in the same individuals with Alzheimer's disease. *J Nucl Med.* 2002; **43** (3): 304–311.

289. Caroli A, Testa C, Geroldi C, Nobili F, Barnden LR, Guerra UP, Bonetti M, Frisoni GB. Cerebral perfusion correlates of conversion to Alzheimer's disease in amnestic mild cognitive impairment. *J Neurol.* 2007; **254** (12): 1698–1707.

290. Minoshima S, Cross DJ, Foster NL, Henry TR, Kuhl DE. Discordance between traditional pathologic and energy metabolic changes in very early Alzheimer's disease. Pathophysiological implications. *Ann N Y Acad Sci.* 1999; **893**: 350–352.

291. Meguro K, Blaizot X, Kondoh Y, Le Mestric C, Baron JC, Chavoix C. Neocortical and hippocampal glucose hypometabolism following neurotoxic lesions of the entorhinal and perirhinal cortices in the non-human primate as shown by PET. Implications for Alzheimer's disease. *Brain.* 1999; **122 (Pt 8)**: 1519–1531.

292. Nobili F, Brugnolo A, Calvini P, Copello F, De Leo C, Girtler N, Morbelli S, Piccardo A, Vitali P, Rodriguez G. Resting SPECT-neuropsychology correlation in very mild Alzheimer's disease. *Clin Neurophysiol.* 2005; **116** (2): 364–375.

293. Moorhouse P, Rockwood K. Vascular cognitive impairment: current concepts and clinical developments. *Lancet Neurol.* 2008; **7** (3): 246–255.

294. Dougall NJ, Bruggink S, Ebmeier KP. Systematic review of the diagnostic accuracy of 99mTc-HMPAO-SPECT in dementia. *Am J Geriatr Psychiatry.* 2004; **12** (6): 554–570.

295. Jagust W, Thisted R, Devous MD, Sr., Van Heertum R, Mayberg H, Jobst K, Smith AD, Borys N. SPECT perfusion imaging in the diagnosis of Alzheimer's disease: a clinical-pathologic study. *Neurology.* 2001; **56** (7): 950–956.

296. Talbot PR, Lloyd JJ, Snowden JS, Neary D, Testa HJ. A clinical role for 99mTc-HMPAO SPECT in the investigation of dementia? *J Neurol Neurosurg Psychiatry.* 1998; **64** (3): 306–313.

297. Hanyu H, Shimizu S, Hirao K, Kanetaka H, Sakurai H, Iwamoto T, Koizumi K, Abe K. Differentiation of dementia with Lewy bodies from Alzheimer's disease using Mini-Mental State Examination and brain perfusion SPECT. *J Neurol Sci.* 2006; **250** (1–2): 97–102.

298. Fratiglioni L, Launer LJ, Andersen K, Breteler MM, Copeland JR, Dartigues JF, Lobo A, Martinez-Lage J, Soininen H, Hofman A. Incidence of dementia and major subtypes in Europe: A collaborative study of population-based cohorts. Neurologic Diseases in the Elderly Research Group. *Neurology.* 2000; **54** (11 Suppl 5): S10–15.

299. Poirier J, Davignon J, Bouthillier D, Kogan S, Bertrand P, Gauthier S. Apolipoprotein E polymorphism and Alzheimer's disease. *Lancet.* 1993; **342** (8873): 697–699.

300. SBU. *Treatment of depressive disorders.* Vol 166/1+2+3. First ed. Stockholm: SBU, The Swedish Council on Technology Assessment in Health Care; 2004.

301. SBU. *Intensive glucose lowering treatment in diabetes*. Stockholm: SBU, The Swedish Council on Technology Assessment in Health Care; 2009.

302. SBU. *Moderately Elevated Blood Pressure.* Vol 170/1+2. First ed. Stockholm: SBU, The Swedish Council on Technology Assessment in Health Care; 2004.

303. Golden SH, Robinson KA, Saldanha I, Anton B, Ladenson PW. Clinical review: Prevalence and incidence of endocrine and metabolic disorders in the United States: a comprehensive review. *J Clin Endocrinol Metab.* 2009; **94** (6): 1853–1878.

304. Logsdon RG, Gibbons LE, McCurry SM, Teri L. Assessing quality of life in older adults with cognitive impairment. *Psychosom Med.* 2002; **64** (3): 510–519.

305. Wimo A, Winblad B. Resource Utilization in Dementia: "RUD Lite". *Brain Aging.* 2003; **3** (1): 48–59.

306. Olsson A, Vanderstichele H, Andreasen N, De Meyer G, Wallin A, Holmberg B, Rosengren L, Vanmechelen E, Blennow K. Simultaneous measurement of beta-amyloid (1–42), total tau, and phosphorylated tau (Thr181) in cerebrospinal fluid by the xMAP technology. *Clin. Chem.* 2005; **51** (2): 336–345.

307. Friston KJ, Ashburner J, Frith CD, Poline JB, Heather JD, Frackowiak RSJ. Spatial registration and normalization of images. *Human Brain Mapping.* 1995; **3** (3): 165–189.

308. Talairach J, Tournoux P. *Co-planar stereotaxic atlas of the human brain : an approach to cerebral imaging.* New York: Thieme Medical Publishers; 1988.

309. Youden WJ. Index for rating diagnostic tests. *Cancer.* 1950; **3** (1): 32–35.

310. Nachev P. Cognition and medial frontal cortex in health and disease. *Curr Opin Neurol.* 2006; **19** (6): 586–592.

311. Rushworth MF. Intention, choice, and the medial frontal cortex. *Ann N Y Acad Sci.* 2008; **1124**: 181–207.

312. Sojkova J, Beason-Held L, Zhou Y, An Y, Kraut MA, Ye W, Ferrucci L, Mathis CA, Klunk WE, Wong DF, et al. Longitudinal cerebral blood flow and amyloid deposition: an emerging pattern? *J Nucl Med.* 2008; **49** (9): 1465–1471.

313. Dai W, Lopez OL, Carmichael OT, Becker JT, Kuller LH, Gach HM. Mild cognitive impairment and alzheimer disease: patterns of altered cerebral blood flow at MR imaging. *Radiology.* 2009; **250** (3): 856–866.

314. Samgard K, Zetterberg H, Blennow K, Hansson O, Minthon L, Londos E. Cerebrospinal fluid total tau as a marker of Alzheimer's disease intensity. *Int J Geriatr Psychiatry.* 2009; **DOI:10.1002/gps.2353**.

315. Rota E, Bellone G, Rocca P, Bergamasco B, Emanuelli G, Ferrero P. Increased intrathecal TGF-beta1, but not IL-12, IFN-gamma and IL-10 levels in Alzheimer's disease patients. *Neurol Sci.* 2006; **27** (1): 33–39.

316. Bibl M, Mollenhauer B, Esselmann H, Lewczuk P, Klafki HW, Sparbier K, Smirnov A, Cepek L, Trenkwalder C, Ruther E, et al. CSF amyloid-beta-peptides in Alzheimer's disease, dementia with Lewy bodies and Parkinson's disease dementia. *Brain.* 2006; **129** (Pt 5): 1177–1187.

317. Biroccio A, Del Boccio P, Panella M, Bernardini S, Di Ilio C, Gambi D, Stanzione P, Sacchetta P, Bernardi G, Martorana A, et al. Differential post-translational modifications of transthyretin in Alzheimer's disease: a study of the cerebral spinal fluid. *Proteomics.* 2006; **6** (7): 2305–2313.

318. Castano EM, Roher AE, Esh CL, Kokjohn TA, Beach T. Comparative proteomics of cerebrospinal fluid in neuropathologically-confirmed Alzheimer's disease and non-demented elderly subjects. *Neurol Res.* 2006; **28** (2): 155–163.

319. Mielke MM, Zandi PP, Blennow K, Gustafson D, Sjogren M, Rosengren L, Skoog I. Low serum potassium in mid life associated with decreased cerebrospinal fluid Abeta42 in late life. *Alzheimer Dis Assoc Disord.* 2006; **20** (1): 30–36.

320. Galimberti D, Schoonenboom N, Scheltens P, Fenoglio C, Venturelli E, Pijnenburg YA, Bresolin N, Scarpini E. Intrathecal chemokine levels in Alzheimer disease and frontotemporal lobar degeneration. *Neurology.* 2006; **66** (1): 146–147.

321. Strekalova H, Buhmann C, Kleene R, Eggers C, Saffell J, Hemperly J, Weiller C, Muller-Thomsen T, Schachner M. Elevated levels of neural recognition molecule L1 in the cerebrospinal fluid of patients with Alzheimer disease and other dementia syndromes. *Neurobiol Aging.* 2006; **27** (1): 1–9.

322. Blasko I, Lederer W, Oberbauer H, Walch T, Kemmler G, Hinterhuber H, Marksteiner J, Humpel C. Measurement of thirteen biological markers in CSF of patients with Alzheimer's disease and other dementias. *Dement Geriatr Cogn Disord.* 2006; **21** (1): 9–15.

323. Kapaki E, Liappas I, Paraskevas GP, Theotoka I, Rabavilas A. The diagnostic value of tau protein, beta-amyloid (1–42) and their ratio for the discrimination of alcohol-related cognitive disorders from Alzheimer's disease in the early stages. *Int J Geriatr Psychiatry.* 2005; **20** (8): 722–729.

324. Schoonenboom NS, Mulder C, Van Kamp GJ, Mehta SP, Scheltens P, Blankenstein MA, Mehta PD. Amyloid beta 38, 40, and 42 species in cerebrospinal fluid: more of the same? *Ann Neurol.* 2005; **58** (1): 139–142.

325. Maruyama M, Higuchi M, Takaki Y, Matsuba Y, Tanji H, Nemoto M, Tomita N, Matsui T, Iwata N, Mizukami H, et al. Cerebrospinal fluid neprilysin is reduced in prodromal Alzheimer's disease. *Ann Neurol.* 2005; **57** (6): 832–842.

326. Zhang J, Goodlett DR, Quinn JF, Peskind E, Kaye JA, Zhou Y, Pan C, Yi E, Eng J, Wang Q, et al. Quantitative proteomics of cerebrospinal fluid from patients with Alzheimer disease. *J Alzheimers Dis.* 2005; **7** (2): 125–133; discussion 173–180.

327. Georganopoulou DG, Chang L, Nam JM, Thaxton CS, Mufson EJ, Klein WL, Mirkin CA. Nanoparticle-based detection in cerebral spinal fluid of a soluble pathogenic biomarker for Alzheimer's disease. *Proc Natl Acad Sci U S A.* 2005; **102** (7): 2273–2276.

328. Ahmed N, Ahmed U, Thornalley PJ, Hager K, Fleischer G, Munch G. Protein glycation, oxidation and nitration adduct residues and free adducts of cerebrospinal fluid in Alzheimer's disease and link to cognitive impairment. *J Neurochem.* 2005; **92** (2): 255–263.

329. Walter A, Korth U, Hilgert M, Hartmann J, Weichel O, Fassbender K, Schmitt A, Klein J. Glycerophosphocholine is elevated in cerebrospinal fluid of Alzheimer patients. *Neurobiol Aging.* 2004; **25** (10): 1299–1303.

330. Schoonenboom NS, Pijnenburg YA, Mulder C, Rosso SM, Van Elk EJ, Van Kamp GJ, Van Swieten JC, Scheltens P. Amyloid beta (1–42) and phosphorylated tau in CSF as markers for early-onset Alzheimer disease. *Neurology.* 2004; **62** (9): 1580–1584.

331. Lewczuk P, Esselmann H, Bibl M, Beck G, Maler JM, Otto M, Kornhuber J, Wiltfang J. Tau protein phosphorylated at threonine 181 in CSF as a neurochemical biomarker in Alzheimer's disease: original data and review of the literature. *J Mol Neurosci.* 2004; **23** (1–2): 115–122.

332. Ryberg H, Soderling AS, Davidsson P, Blennow K, Caidahl K, Persson LI. Cerebrospinal fluid levels of free 3-nitrotyrosine are not elevated in the majority of patients with amyotrophic lateral sclerosis or Alzheimer's disease. *Neurochem Int.* 2004; **45** (1): 57–62.

333. Clark CM, Xie S, Chittams J, Ewbank D, Peskind E, Galasko D, Morris JC, McKeel DW, Jr., Farlow M, Weitlauf SL, et al. Cerebrospinal fluid tau and beta-amyloid: how well do these biomarkers reflect autopsy-confirmed dementia diagnoses? *Arch Neurol.* 2003; **60** (12): 1696–1702.

334. Puchades M, Hansson SF, Nilsson CL, Andreasen N, Blennow K, Davidsson P. Proteomic studies of potential cerebrospinal fluid protein markers for Alzheimer's disease. *Brain Res Mol Brain Res.* 2003; **118** (1–2): 140–146.

335. Ganzer S, Arlt S, Schoder V, Buhmann C, Mandelkow EM, Finckh U, Beisiegel U, Naber D, Muller-Thomsen T. CSF-tau, CSF-Abeta1-42, ApoE-genotype and clinical parameters in the diagnosis of Alzheimer's disease: combination of CSF-tau and MMSE yields highest sensitivity and specificity. *J Neural Transm.* 2003; **110** (10): 1149–1160.

336. Saez-Valero J, Fodero LR, Sjogren M, Andreasen N, Amici S, Gallai V, Vanderstichele H, Vanmechelen E, Parnetti L, Blennow K, et al. Glycosylation of acetylcholinesterase and butyrylcholinesterase changes as a function of the duration of Alzheimer's disease. *J Neurosci Res.* 2003; **72** (4): 520–526.

337. Bagli M, Papassotiropoulos A, Hampel H, Becker K, Jessen F, Burger K, Ptok U, Rao ML, Moller HJ, Maier W, et al. Polymorphisms of the gene encoding the inflammatory cytokine interleukin-6 determine the magnitude of the increase in soluble interleukin-6 receptor levels in Alzheimer's disease. Results of a pilot study. *Eur Arch Psychiatry Clin Neurosci.* 2003; **253** (1): 44–48.

338. Schonknecht P, Pantel J, Hunt A, Volkmann M, Buerger K, Hampel H, Schroder J. Levels of total tau and tau protein phosphorylated at threonine 181 in patients with incipient and manifest Alzheimer's disease. *Neurosci Lett.* 2003; **339** (2): 172–174.

339. Kapaki E, Paraskevas GP, Zalonis I, Zournas C. CSF tau protein and beta-amyloid (1–42) in Alzheimer's disease diagnosis: discrimination from normal ageing and other dementias in the Greek population. *Eur J Neurol.* 2003; **10** (2): 119–128.

340. Andreasen N, Vanmechelen E, Vanderstichele H, Davidsson P, Blennow K. Cerebrospinal fluid levels of total-tau, phospho-tau and A beta 42 predicts development of Alzheimer's disease in patients with mild cognitive impairment. *Acta Neurol Scand Suppl.* 2003; **179**: 47–51.

341. Buerger K, Zinkowski R, Teipel SJ, Arai H, DeBernardis J, Kerkman D, McCulloch C, Padberg F, Faltraco F, Goernitz A, et al. Differentiation of geriatric major depression from Alzheimer's disease with CSF tau protein phosphorylated at threonine 231. *Am J Psychiatry.* 2003; **160** (2): 376–379.

342. Ferri CP, Prince M, Brayne C, Brodaty H, Fratiglioni L, Ganguli M, Hall K, Hasegawa K, Hendrie H, Huang Y, et al. Global prevalence of dementia: a Delphi consensus study. *Lancet.* 2005; **366** (9503): 2112–2117.

343. Birks J. Cholinesterase inhibitors for Alzheimer's disease. *Cochrane Database Syst Rev.* 2006; (1): CD005593.

344. Barclay LL, Zemcov A, Blass JP, Sansone J. Survival in Alzheimer's disease and vascular dementias. *Neurology.* 1985; **35** (6): 834–840.

345. Williams MM, Xiong C, Morris JC, Galvin JE. Survival and mortality differences between dementia with Lewy bodies vs Alzheimer disease. *Neurology.* 2006; **67** (11): 1935–1941.

SUPPLEMENTS 10

Supplement 1

CSF articles analysis matrix

Supplement 2

Evidence for diagnostic biomarkers

Supplement 1: CSF articles analysis matrix

Article	Journal	Impact factor	Study design	Number of controls	Volunteers	Randomized selection	Subjective memory impairment	Other patient group	Specification	Cognitive assessment	Physical examination	Psychiatric examination	Blood sampling
Rota et al. 2006 [315]	Neurol Sci	0.8	P - C	25				●	Neurologic complaints	●			
Bibl et al. 2006 [316]	Brain	7.3	P - C	23			●	●	Neurologic complaints	●	●	●	
Biroccio et al. 2006 [317]	Proteomics	6.1	P - C	27				●	Neurologic complaints		●		
Castaño et al. 2006 [318]	Neurol Res	1.6	R - C	43						●			
Mielke et al. 2006 [319]	Alzheimer Dis Assoc Disord	2.0	R - C	86		●					●	●	●
Hansson et al. 2006 [178]	Lancet Neurol	12.2	P - L	39	●					●	●	●	
Galimberti et al. 2006 [320]	Neurology	4.9	P - L	40				●	Neurologic complaints		●		
Strekalova et al. 2006 [321]	Neurobiol Aging	5.3	P - C	46				●	Exclude subarachnoidal hemorrhage				
Blasko et al. 2006 [322]	Dement Geriatr Cogn Disord	2.6	P - C	27				●	Surgical intervention	●			
de Leon et al. 2006 [205]	Neurobiol Aging	5.3	P - L	9			●	●	Subjective memory impairment	●	●	●	●
Kapaki et al. 2005 [323]	Int J Geriatr Psychiatry	2.2	P - C	50				●	Surgical intervention	●	●	●	●
Schoonenboom et al. 2005 [324]	Ann Neurol	7.6	P - C	26			●	●	Subjective memory impairment	●			
Maruyama et al. 2005 [325]	Ann Neurol	7.6	P - L	27			●	●	Subjective memory impairment	●			
Zhang et al. 2005 [326]	J Alzheimers Dis	5.1*	P - C	31						●	●	●	●
Georganopoulou et al. 2005 [327]	Proc Natl Acad Sci U S A	10.2	P - L	15						●			
Ahmed et al. 2005 [328]	J Neurochem	4.6	P - C	18				●	Neurologic complaints		●		
Walter et al. 2004 [329]	Neurobiol Aging	5.3	P - C	30				●	Surgical intervention				
de Leon et al. 2004 [134]	J Intern Med	4.0	P - L	2						●			
Schoonenboom et al. 2004 [330]	Neurology	4.9	P - L	21			●	●	Subjective memory impairment	●			
Lewczuk et al. 2004 [331]	J Mol Neurosci	2.6	P - C	9				●	Neurological / psychiatric complaints	●	●	●	●
Ryberg et al. 2004 [332]	Neurochem Int	3.0	P - C	19						●	●	●	
Clark et al. 2003 [333]	Arch Neurol	4.9	P - L	69			●	●	Subjective memory impairment	●	●	●	●
Puchades et al. 2003 [334]	Brain Res Mol Brain Res	1.6	P - C	7			●	●	Psychiatric disorder	●	●	●	●
Ganzer et al. 2003 [335]	J Neural Transm	2.5	P - C	68			●	●	Dementia disorder	●	●	●	●
Sunderland et al. 2003 [175]	JAMA	23.3	P - C**	72	●					●	●		●
Sáez-Valero et al. 2003 [336]	J Neurosci Res	3.2	P - C	106						●	●	●	●
Bagli et al. 2003 [337]	Eur Arch Psychiatry Clin Neurosci	2.3	P - C	25			●	●	Dementia disorder	●	●	●	●
Schönknecht et al. 2003 [338]	Neurosci Lett	1.9	P - C	16				●	Surgical intervention	●		●	
Kapaki et al. 2003 [339]	Eur J Neurol	2.2	P - L	46				●	Surgical intervention	●	●		●
Andreasen et al. 2003 [340]	Acta Neurol Scand Suppl	0.3	P - L	32			●	●	Subjective memory impairment	●	●	●	
Buerger et al. 2003 [341]	Am J Psychiatry	8.3	P - C	21						●	●	●	
Total					2	1	10	21		25	20	16	12
Mean impact factor (without impact > 12)		4.9 (4.0)			17.8	2.0	4.4	3.9		5.1	5.2	4.0	4.8

P = prospective, R = retrospective, C = cross-sectional, L = longitudinal. * as of 2009-06-23. ** manuscript including a separate meta-analysis part

Supplement 2: Evidence for diagnostic biomarkers

	NINCDS-ADRDA [3] 2007	SBU [124] 2006
CT	n/a	●●●
MR	●●●	●●●
PET	●●●	●●
SPECT	●●	●●
CSF	●●●	●●●
qEEG	n/a	●
Gene mutation testing	●●●	n/a
APOE-ε4 allele	n/a	●

● Limited diagnostic value

●● Moderate diagnostic value

●●● High diagnostic value

n/a not applicable