Predictors of cognitive decline in memory clinic patients

Christin Andersson
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“What the caterpillar calls the end of the world, the rest of the world calls butterfly”

Richard Bach
ABSTRACT

**Background:** The major challenge in memory clinics is to predict development as well as non-development of dementia among the heterogeneous group of patients with cognitive complaints. Since the advent of pharmacological treatment possibilities for patients with Alzheimer’s disease (AD), much research has been focused on predictors for dementia since initiation of treatment in the preclinical phase may prevent development of dementia. At present, there are no tests specific enough to alone predict cognitive progression or stability. There are also no biochemical markers available to monitor the neurodegenerative disease process in AD. Thus there is a need for valid and clinically easy-to-use methods to differentiate patients who are at high risk of cognitive decline from those who will not progress. **Objectives:** The overall aim of this thesis was to examine which clinical methods that can be used – and in what combinations – to differentiate between patients at high and low risk for cognitive decline and dementia. **Material & Methods:** The thesis includes four retrospective studies that were performed among patients admitted to the Memory Clinic at Karolinska University Hospital in Huddinge. All patients were non-demented at baseline. They were clinically followed up during approximately 3 years because suspicion of a progressive cognitive disorder could not be ruled out at baseline. **Study I** investigated the efficacy of a number of neuropsychological tests for prediction of subsequent cognitive decline and conversion to dementia. **Study II** investigated the relationship between episodic memory function, APOE ε4 allele status and levels of cerebrospinal fluid (CSF) biomarkers: total-tau (T-tau), hyperphosphorylated tau (P-tau) and the 42 amino acid form of beta amyloid (Aβ42). **Study III** investigated the relationship between longitudinal changes in CSF biomarkers and cognitive function. **Study IV** investigated what combination of clinical methods at baseline that was most predictive of cognitive decline and conversion to dementia. **Results:** Rey Auditory Verbal Learning Test (RAVLT) was the most efficient neuropsychological test for prediction of memory impairment and dementia. Cutoff levels in baseline RAVLT were defined and could identify patients at high and low risk for subsequent cognitive decline. Among moderately memory impaired patients, low CSF Aβ42 values differentiated those who did decline from those who did not. The relationship between episodic memory and CSF biomarkers was affected by the APOE ε4 allele, where APOE ε4 carriers who declined cognitively had pathological CSF 1-tau, P-tau and Aβ42. Severely memory impaired patients showed significantly increasing P-tau levels during cognitive decline and progression to AD while patients with normal or moderately impaired memory showed unchanging P-tau levels and remained cognitively stable. CSF T-tau and Aβ42 did not change during follow-up.

**Conclusion:** RAVLT is a useful test for identifying patients at high and low risk for cognitive decline. Adding CSF Aβ42 values increase the ability to differentiate those who will decline from those who will not, especially among moderately memory impaired patients. The APOE ε4 allele may affect the CSF biomarker levels among patients with memory impairment. Increasing P-tau levels during cognitive decline suggest that P-tau may be useful as a longitudinal marker of the early neurodegenerative process in AD.
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<th>Description</th>
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<tbody>
<tr>
<td>AD</td>
<td>Alzheimer’s Disease</td>
</tr>
<tr>
<td>Aβ42</td>
<td>The 42 amino acid form of β-amyloid</td>
</tr>
<tr>
<td>aMCI</td>
<td>amnestic MCI</td>
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<tr>
<td>ANOVA</td>
<td>Analysis of Variance</td>
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<tr>
<td>APOE</td>
<td>Apolipoprotein E (gene)</td>
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<tr>
<td>ApoE</td>
<td>Apolipoprotein E (protein)</td>
</tr>
<tr>
<td>APP</td>
<td>Amyloid Precursor Protein</td>
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<tr>
<td>CART</td>
<td>Classification and Regression Tree Analysis</td>
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<tr>
<td>CI</td>
<td>Confidence Interval</td>
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<tr>
<td>CSF</td>
<td>Cerebrospinal fluid</td>
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<td>CT</td>
<td>Computed Tomography</td>
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<td>DLB</td>
<td>Dementia with Lewy Bodies</td>
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<td>FTD</td>
<td>Frontotemporal Dementia</td>
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<tr>
<td>LP</td>
<td>Lumbar Puncture</td>
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<tr>
<td>LSD</td>
<td>Least Significant Difference</td>
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<tr>
<td>MCI</td>
<td>Mild Cognitive Impairment</td>
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<tr>
<td>MIM</td>
<td>Moderate Impairment in Memory</td>
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<td>MMSE</td>
<td>Mini Mental State Examination</td>
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<tr>
<td>MRI</td>
<td>Magnetic Resonance Imaging</td>
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<td>MTA</td>
<td>Medial Temporal lobe Atrophy</td>
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<tr>
<td>naMCI</td>
<td>non-amnestic MCI</td>
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<tr>
<td>NIM</td>
<td>No Impairment in Memory</td>
</tr>
<tr>
<td>NINCDS-ADRDA</td>
<td>National Institute of Neurological and Communicative Disorders and Stroke and the Alzheimer’s Disease and Related Disorders Association</td>
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<tr>
<td>NFT</td>
<td>Neurofibrillary Tangles</td>
</tr>
<tr>
<td>NUD</td>
<td>Non Ultra Descriptum (etiology uncertain)</td>
</tr>
<tr>
<td>RAVLT</td>
<td>Rey Auditory Verbal Learning Test</td>
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<tr>
<td>SCI</td>
<td>Subjective Cognitive Impairment</td>
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<tr>
<td>SIM</td>
<td>Severe Impairment in Memory</td>
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<tr>
<td>SP</td>
<td>Senile Plaques</td>
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<tr>
<td>SPECT</td>
<td>Single Photon Emission Computed Tomography</td>
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<tr>
<td>T-tau</td>
<td>Total tau</td>
</tr>
<tr>
<td>PET</td>
<td>Positron Emission Tomography</td>
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<tr>
<td>P-tau</td>
<td>Hyperphosphorylated tau</td>
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<tr>
<td>VaD</td>
<td>Vascular Dementia</td>
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<tr>
<td>VCI</td>
<td>Vascular Cognitive Impairment</td>
</tr>
<tr>
<td>WAIS-R</td>
<td>Wechsler Adult Intelligence Scale – Revised</td>
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1 INTRODUCTION
1.1 COGNITIVE DISORDERS AND DEMENTIA

1.1.1 Mild cognitive impairment

The concept of mild cognitive impairment (MCI) has been used in the literature for the last decades to cover the continuum, often described as a transitional state, between normal cognitive function and dementia. In 1999, Ronald Petersen and coworkers at the Mayo Clinic defined MCI predominantly as an isolated impairment in memory [Petersen et al. 1999]. Before that, several terms were used to describe the lower extremity of the normal cognitive range and these terms have been presented in a review by Ritchie & Touchon (2000): benign senescent forgetfulness, age-associated memory impairment (AAMI), late-life forgetfulness, age-associated cognitive decline (AACD), ageing-related cognitive decline, mild cognitive decline, mild neurocognitive decline and cognitive impairment no dementia (CIND) [Ritchie & Touchon 2000]. The concept of MCI is sometimes but not always used in a restricted sense, referring only to cognitive changes preceding Alzheimer’s disease (AD). This is however not the most common use of the term and it does not in itself exclude changes of other etiologies, e.g. vascular disease or even reversible conditions such as depression. A partly overlapping term that is sometimes used in the literature is vascular cognitive impairment (VCI), introduced by Vladimir Hachinski (1993) as a descriptive term for cognitive impairment of different degrees caused by vascular disease or brain damage [Hachinski & Bowler 1993]. Thus, the concept of VCI is restricted to cognitive changes due to vascular disease. In this thesis, the concept of MCI is used to refer to cognitive impairments regardless of etiology.

From many studies it has been concluded that patients with MCI are at an increased risk for developing dementia when compared to individuals with normal cognitive functioning [Petersen et al. 1999, DeCarli 2003]. However, the conversion rates presented in the literature vary considerably, ranging from 4% in a community sample, to 31% in a clinical setting [Bruscoli & Lovestone 2004]. In a study from the Karolinska University Hospital Memory Clinic, the majority of patients with MCI remained stable or improved when reassessed on a regular basis, while approximately one third progressed to dementia during three years [Wahlund et al. 2003]. Even when follow-up times are longer, up to 10 years, it has been suggested that most MCI patients remain stable, although the risk of conversion to dementia is strongly influenced by the age of the patients [Visser et al. 2006]. Patients with MCI thus constitute a very heterogeneous group. There is a lack of a precise definition of the concept leading to variable results in studies where alternate criteria for the construct have been used [Petersen 2004]. Attempts have been made to formulate criteria for different subtypes of MCI, integrating clinical and epidemiological perspectives on the concept of MCI [Winblad et al. 2004; Petersen 2004]. The proposed subtypes are based on the heterogeneity of the clinical presentation of MCI and are described as: (i) amnestic MCI (aMCI) single domain, (ii) aMCI multiple domain, (iii) non-amnestic MCI (naMCI) single domain and (iv) naMCI multiple domain [Petersen 2004, Winblad et al. 2004]. Estimates of the prevalence of MCI vary according to the definition used. It has been suggested that the prevalence of MCI is approximately 19% among subjects younger than 75 years of age and about 29% among subjects older than 85 years of age.
[Lopez et al. 2003]. Of all the patients with MCI, it is estimated that about 30% have aMCI. Of these, approximately 12% progress to AD each year, and after 6 years, up to 80% have progressed to AD [Petersen 2004]. Vascular causes of MCI are less well studied although it is suggested that vascular MCI may be more common and possibly treatable [DeCarli 2003].

Controversy exists whether the proposed subtypes of MCI have different etiologies and have an influence on subsequent type of dementia diagnosis, as has been suggested [Petersen 2004; Winblad et al. 2004]. Some studies suggest that AD is typically preceded by aMCI [DeCarli 2003] and that patients with aMCI typically progress to other types of dementia. Others however, have failed to show that subtypes of MCI would be useful in defining early stages of various types of dementia [Fischer et al. 2007]. Controversy also exists whether patients with MCI are at an increased risk of death when compared to subjects with normal cognitive functioning. It has been shown that aMCI is associated with increased mortality when compared to reference subjects, and that the mortality is higher in multiple domain aMCI than in single domain aMCI [Hunderfund et al. 2006]. However, it has also been reported that patients with naMCI – both single and multiple domain – are at an increased risk of death when compared to aMCI patients [Yaffe et al. 2006], while others have not been able to find an increased mortality risk among MCI patients when compared to non-impaired patients [Guehne et al. 2006].

Despite the proposed MCI consensus guidelines [Winblad et al. 2004], attempts to implement the criteria show that the precise definitions of the MCI subtypes are still a matter of interpretation. Kramer et al. (2006) point out that the methods to determine cognitive functioning vary across studies and their results suggest that aMCI is often more diffuse than what is outlined in the criteria [Kramer et al. 2006]. Furthermore, MCI criteria applied in drug trials have only shown low to moderate accuracy for predementia AD, especially among younger patients [Visser et al. 2005]. Others stress that there are many more studies on aMCI than naMCI and suggest that aMCI should receive serious consideration for inclusion in DSM-V, while naMCI should remain a research entity for further investigation [Petersen & O’Brien 2006].

In neuropathological studies on patients with MCI, neurofibrillary tangles (NFT) have been found in the hippocampus and other medial temporal regions and senile plaques (SP) accumulate in the hippocampus and neocortex and become more prevalent during progression to AD [Small et al. 2006]. The clinical impairment in MCI has been reported to correlate best with the neurofibrillary changes while the amyloid burden has been found less discriminative [Petersen et al. 2006]. In aMCI patients, the neuropathological features have been reported to be intermediate between the neurofibrillary changes of aging and the pathologic features of very early AD [Petersen et al. 2006]. NFTs in the ventromedial temporal lobe have been reported as a possible substrate for the memory decline in MCI [Markesbery et al. 2006].

Recently, non-invasive scanning techniques to detect patterns of amyloid deposition and NFTs in the brain have been developed. For example, an amyloid-imaging position-emission tomography (PET) tracer termed Pittsburgh Compound-B (PIB) have been developed, and by assessment of the PIB retention, quantitative information on
amyloid deposits in living subjects can be provided [Klunk et al. 2004]. It has been shown that PIB retention in patients with MCI is intermediate between healthy controls and patients with AD, and furthermore that PIB retention correlate with CSF levels of beta amyloid$_{42}$ (Aβ42), total tau (T-tau) and verbal episodic memory respectively [Forsberg et al, 2007, in press]. Another amyloid ligand tracer (FDDNP) has also been shown to differentiate patients with MCI from patients with AD and from subjects with no cognitive impairment, and this method is potentially useful to determine regional patterns of amyloid plaques and tau NFT in the living brain [Small et al. 2006].

1.1.2 Alzheimer’s disease

In Sweden, approximately 140 000 people suffer from some kind of dementia disorder [SBU report]. Worldwide, it is estimated that approximately 28 million people suffer from dementia [Wimo et al. 2006] and the costs are high [Jönsson et al. 2006]. The number of people affected is expected to double every 20 years, with 4.6 million new cases around the world every year; i.e. one new case every 7 seconds [Ferri et al. 2005]. The prevalence of dementia at the age of 65 is approximately 1%, and at 90 years of age the prevalence is over 50% [SBU report]. The most prominent risk factor for dementia is thus increasing age, and women over 85 years are affected at a higher rate than men at the same age [SBU report].

The most common form of dementia is AD, accounting for approximately 60-70% of all dementia cases [Fratiglioni et al. 2000]. The disease was named in 1910 by the German psychiatrist Emil Kraepelin (1856-1926) after his colleague Alois Alzheimer (1864-1915) who published a case report in 1907 on a patient named Auguste D [Alzheimer 1907; Maurer et al. 1997]. Auguste D was admitted to the Frankfurt hospital in Germany in 1901 at the age of 51 because of progressive cognitive impairment, including rapidly increasing memory impairments, focal symptoms, hallucinations, delusions and psychosocial incompetence [Maurer et al. 1997]. Despite the long history of the study of dementia, the struggle to define the disease and to address issues related to differential diagnostics is a relatively recent phenomenon [Khachaturian 2005]. The clinical criteria for AD were outlined in 1984 by a work group established by the National Institute of Neurological and Communicative Disorders and Stroke (NINCDS) and the Alzheimer’s disease and Related Disorders Association (ADRA) [McKhan et al. 1984]. Before that, the DSM-III criteria for the diagnosis of dementia were used and the field of clinical research was considered impeded by the lack of consensus on diagnostic criteria and lack of standardized assessment instruments [Khachaturian 2005]. This situation is in much comparable to the current debate concerning the concept of MCI.

The NINCDS-ADRDA criteria outline three levels of certainty for the diagnosis of AD: (i) probable, (ii) possible and (iii) definite. The criteria for the clinical diagnosis of probable AD require that dementia is established by clinical examinations, and the diagnosis can be made with confidence if there is an insidious onset and a progressive worsening of memory and other cognitive functions in the absence of other systemic or brain diseases that could account for the cognitive deficits [McKhan et al. 1984]. The diagnosis of probable AD is supported by progressive deterioration in specific
cognitive functions such as language, motor skills and perception [McKhann et al. 1984]. Patients with AD in early stages typically present with memory impairment joined next by semantic and other linguistic impairments and emerging difficulties with demanding visuospatial processing [Lambon Ralph et al. 2003]. A diagnosis of definite AD requires histopathologic confirmation at autopsy [McKhann et al. 1984].

Auguste D died at the age of 56, and in Alois Alzheimer’s publication from 1907, he described plaques, neurofibrillary tangles (NFT) and arteriosclerotic changes after autopsy of the brain [Maurer et al. 1997]. These features, extracellular amyloid deposits and intraneuronal neurofibrillary changes, are today recognized as typical of AD [Small & Cappai 2006]. The main protein constituent of plaques is beta-amyloid (Aβ), a peptide of 40–42 amino acids that is formed after cleavage of the amyloid precursor protein (APP) [Bhennow 2005]. Neuropathological studies have shown that amyloid accumulation in early stages exhibit considerable interindividual variation. In contrast, NFT exhibit a characteristic distribution pattern in the brain, permitting the differentiation of six different stages of AD [Braak & Braak 1991]. Among subjects with severe neurofibrillary changes high densities of amyloid deposits are consistently found, while those rich in amyloid are not always affected by neurofibrillary changes [Braak & Braak 1991]. In the AD disease process, the amyloid cascade hypothesis proposes accumulation of Aβ caused by disturbances in APP metabolism occurring as one of the earliest molecular event, whereas tau pathology and NFT formation is thought to be a later event. Thus, the accumulation of Aβ is described as the primary influence driving AD pathogenesis, and the formation of NFT containing tau protein is proposed to result from an imbalance between Aβ production and Aβ clearance [Hardy & Selkoe 2002].

There is today no cure for AD, but during the last ten years, symptomatic treatment using acetyl cholinesterase inhibitors is available. Clinical trials have shown stabilizing effects on cognitive functions for 1 year [Winblad et al. 2001] up to 3 years [Wallin et al. 2007].

1.1.3 MCI and AD – continuum or different entities

The view of MCI as a transitional phase between one state where cognition is normal, and another where dementia is present, has been asserted by some and criticized by others. The view of MCI and AD as distinct entities have been criticized as misleading because it is inconsistent with the disease process in AD, characterized by progressive synaptic and neuronal dysfunction resulting in an insidious onset of symptoms that subsequently progress to clinically manifest dementia [Morris et al. 2006]. MCI patients have also been found to often show neuropathologic features of AD at autopsy; suggesting that MCI, at least aMCI, is in fact early AD [Morris et al. 2006; Marksberg et al. 2006]. Accordingly, MCI and AD are mainly differentiated by degree of impairment rather than structural differences. Others however, have shown that the neuropathologic changes found at autopsy in patients with aMCI are intermediate between those found in normal aging and in early AD [Petersen RC et al. 2006], thus suggesting that MCI should be considered as an entity distinct from normal aging and from clinically probable AD [Petersen & O’Brien 2006]. The view of cognitive
impairment as a continuum without a certain threshold that demarcates demented from non-demented has also been discussed in relation to the concept of VCI, and the term comprises both non-demented and demented subjects with cognitive deficits of vascular origin [Hachinski & Bowler 1993].

The different views of MCI as a transitional phase or as an early phase of dementia are of crucial importance to how the research questions in the field of dementia are formulated: is the purpose to identify patients with a disease or is the purpose to predict who will later fall ill among healthy or asymptomatic subjects? For example, some argues that MCI only seems to increase the risk for dementia, not because it predisposes for AD but because 20% of those with MCI already have AD [Ganguli et al. 2006]. Identifying risk factors for a future disease must be one thing and identifying individuals who presently suffer from a specific disease must be another. Nonetheless, it is a tricky question. When a person deteriorates cognitively and functionally and is said to convert from MCI to AD, did he or she suffer from AD already at the MCI phase, or did he or she fall ill just when the symptoms became obvious? There are no clear answers to these questions.

There are studies that have shown cognitive deficits, such as low mental and linguistic ability, in subjects who develop dementia in late life several decades before the onset of dementia [Snowdon et al. 1996; Whalley et al. 2000]. The pathogenic process of AD has also been proposed to start decades before dementia is present [Price & Morris 1999]. It is thus not clear whether the cognitive deficits found early in life among patients who later develop dementia are the effects of pathological processes in the brain, or whether lower childhood and adolescent abilities are related to other factors, such as lower socioeconomic status and poorer health. Higher mental ability may allow access to better health information and better lifestyle choices [Whalley et al. 2000].

1.1.4 Differential dementia diagnostics

It has been debated in the literature whether Auguste D, Alois Alzheimer’s patient, really suffered from the disease that was named after her doctor, or whether she had a different type of dementia [O’Brien 1996]. In 1995, the hospital file of Auguste D that had been missing since 1909 was found [Maurer et al. 1997]. The file contained Alzheimer’s detailed descriptions of his patient’s symptoms. The neuropathological findings at autopsy were also reported, including descriptions of plaques, neurofibrillary tangles and arteriosclerotic changes [Maurer et al. 1997]. It has been pointed out that they were not quite the same as the textbook symptoms of AD and that her clinical symptoms were more complex than what is now called AD [O’Brien 1996]. Others reject the assertion that Auguste D did not suffer from the disease she has come to symbolize as ill-founded, and state that the arteriosclerotic changes that were mentioned by Alois Alzheimer were not considerable [Graeber et al. 1998]. However, the proposition that Auguste D did not suffer from AD leads to a thought-provoking question: if the first AD case was not AD, what is AD?

In recent years, studies have shown that the neuropathological findings in AD and vascular dementia (VaD) show a considerable overlap. With age, VaD patients have
shown increasing accumulation of Aβ in the brain when compared to elderly without cerebrovascular disease, suggesting that VaD patients acquire Alzheimer-like pathology in old age [Lewis et al. 2006]. It has also been found that vascular factors are associated with AD changes in the brain [Korf et al. 2005], and that practically all risk factors for AD have a vascular component that decreases cerebral perfusion [de la Torre 2002]. Thus, findings support the notion that AD and VaD may not reflect mutually exclusive diagnoses but share both vascular and neurodegenerative pathological features [Lewis et al. 2006]. Naturally, the overlap between AD and VaD at the microscopic level has its clinical counterpart: differential clinical diagnostics between AD and VaD in late phase dementia are difficult. In preclinical phases, some differential clinical features have been proposed, such as different cognitive profiles, structural imaging differences and possibly different patterns of biomarker levels in CSF [Mega 2002]. However, despite these differential features, it has been shown that the correspondence between clinical and pathologic diagnoses of VaD is poor, and this may in part be due to flaws in the neuropathological gold standard and to comorbidity of the patients and co-occurrence of VaD and AD changes [Knopman 2006].

As an illustration of the fuzziness surrounding the concept of MCI and also the indistinct boundary between the preclinical MCI phase and the clinical phase of dementia, this section closes with a famous poem by John Godfrey Saxe:

**Blind men and the Elephant**
(by John Godfrey Saxe)

It was six men of Indostan
To learning much inclined,
Who went to see the Elephant,
(Though all of them were blind),
That each by observation
Might satisfy his mind
The first approached the Elephant,
And happening to fall
Against his broad and sturdy side,
At once he began to bawl:
“God bless me! but the Elephant
Is very like a wall!”
The Second, feeling of the tusk,
Cried, “Ho! What have we here
So very round and smooth and sharp?
To me ‘tis mighty clear
This wonder of an Elephant
Is very like a spear!”
The Third approached the animal,
And happening to take
The squirming trunk within his hands,
Thus boldly up and spake:
“‘I see,“ quoth he, “the Elephant
Is very like a snake!”
The Fourth reached out an eager hand,
And felt about the knee.
“ ’What most this wondrous beast is like
Is mighty plain,” quoth he;
“ ’Tis clear enough the Elephant
Is very like a tree!”
The Fifth who chanced to touch the ear,
Said: “E’en the blindest man
Can tell what this resembles most;
Deny the fact who can
This marvel of an Elephant
Is very like a fan!”
The Sixth no sooner had begun
About the beast to grope,
Than, seizing on the swinging tail
That fell within his scope,
“I see,” quoth he, “the Elephant
Is very like a rope!”
And so these men of Indostan
Disputed loud and long,
Each in his own opinion
Exceeding stiff and strong,
Though each was partly in the right,
And all were in the wrong!

**Moral:**
So off in theologic wars,
The disputants, I ween,
Rail on in utter ignorance
Of what each other mean,
And prate about an Elephant
Not one of them has seen!
1.2 PROPOSED PREDICTORS AND DIAGNOSTIC TOOLS

1.2.1 Subjective cognitive impairment

Subjective cognitive complaints are commonly reported by older adults and studies of the prevalence of memory complaints in community-based samples vary greatly, from 22% to 56% [DeCarli 2003]. The prevalence of such complaints has been shown to increase with age [Reid & MacLullich 2006; DeCarli 2003]. It has been suggested that subjective cognitive complaints may be an early sign of incipient dementia and in recent years people who experience memory or other cognitive problems have been encouraged to seek medical attention at specialist memory clinics. The validity, or clinical significance, of subjective complaints is however debated [Jorm et al. 2001]. Controversy exists whether subjective complaints are related to actual cognitive performance and/or are predictive of future cognitive decline and dementia [Reid & MacLullich 2006; Kliegel et al. 2005]. Different results have been reported from studies using clinical samples and population-based samples, probably caused by selection bias.

In memory clinics, the amount of demented patients with reduced insight into their cognitive deficits are probably larger than in the general population, and the prevalence of patients with cognitive complaints, despite normal cognitive functioning, is also probably proportionally higher since subjects with normal cognition without subjective cognitive complaints are unlikely to attend memory clinics. Consequently, clinically based studies have found no strong associations between subjective complaints and cognitive performance [Kliegel et al. 2005], and cognitive complaints have been reported to correlate to depressive symptoms rather than to actual cognitive performance [Kliegel et al. 2005; Roth et al. 2005; Suhr 2003]. Moreover, several studies have shown that health complaint ratings in general, independent of objective health, is associated with neuroticism [Kliegel et al. 2005]. It has also been found that memory complaints in older people who do not show actual cognitive impairment or decline may reflect psycho-affective or health problems, and that adequate intervention in these problems may improve quality of life in these persons [Comijs et al. 2002]. These relationships may be explained by a tendency among depressed subjects to perceive and recall more cognitive failures and other negative things about themselves [Kliegel et al. 2005]. However, others have found that subjective memory complaints are not just secondary to symptoms like depression, but in part reflect realistic self-observations of cognitive decline [Schmand et al. 1997] and it has been suggested that cognitive complaints in the absence of cognitive deficits in neuropsychological tests may reflect a “pre-MCI” stage of a neurodegenerative disease process.

Memory complaints have been found to predict the neuropathologic diagnosis of AD among initially non-demented subjects older than 74 years [Jorm et al. 2004]. Furthermore, patients with subjective complaints have shown similar patterns of grey matter loss in the medial temporal lobe, frontaltemporal and other neocortical regions as MCI patients when compared to healthy controls [Saykin et al. 2006]. However, others in turn have shown no significant correlation between self-reported memory complaints and cognitive performance or later development of dementia, although in contrast, informant-reported memory loss has been shown to predict future diagnosis of AD [Cart et al. 2000]. Subjective cognitive complaints, preferentially corroborated by an
informant, are included in several definitions and proposed diagnostic criteria of MCI, mainly to reduce the rate of false positive diagnoses [Kliegel et al. 2005].

1.2.2 Episodic memory – the time machine in the brain

Episodic memory, a concept that was introduced by Endel Tulving, has been called the basis of mental time travel because it allows humans to travel backwards in time and to re-experience past events [Tulving 2002]. It has been suggested that the essence of episodic memory lies in the conjunction of three concepts: self, autonoetic awareness (i.e. awareness that mental representations or memories are different from direct observations of the world) and subjectively sensed time [Tulving 2002]. In most studies, episodic memory involves the retrieval of verbal or non-verbal materials, usually in the form of word lists, stories, pictures or faces, which were presented in an earlier study episode [Small et al. 2004].

Many studies have shown that memory impairment is a strong predictor for conversion to dementia among MCI patients [Almkvist 1998; Petersen 1999; Petersen 2001; Arnáz & Almkvist 2003], and that verbal memory tests are helpful markers of subsequent dementia [Estévez-González et al. 2003; Frank & Byrne 2000]. However, it has been suggested that memory impairment is neither sufficient nor necessary for a diagnosis of preclinical AD since some patients do not present with delayed recall impairments prior to AD, and it has been stressed that criteria for preclinical AD should not focus exclusively on memory dysfunction [Visser et al. 2002]. However, there is no consensus of what constitutes an impaired level of performance that fulfills the MCI criteria applied. In a clinical setting, fulfillment of MCI criteria is ultimately determined through clinical judgment using information from various diagnostic tools including neuropsychological tests [Winblad et al. 2004].

1.2.2.1 Neuropsychological assessment

The concepts of episodic memory and other cognitive domains are theoretical constructs rather than physical entities that are directly measurable. These types of constructs can only be validated in relation to other constructs, of which some may be directly observable and others not [Spector 1992]. The majority of constructs concerning memory or other cognitive functions have been developed through studies and observations of patients with different types of neurological damage and disease. Amnesia and other types of memory dysfunction have different etiologies and neurobiological correlates, and the specific aspects of memory that are affected in different types of brain damage or disease vary a lot [Butters & Delis 1995].

Episodic memory is often assessed by different kinds of word lists, for example Rey Auditory Verbal Learning Test (RAVLT) [Schmidt 1996]. This test was originally constructed by a Swiss psychologist named Edouard Claparède during 1910’s [Boake 2000]. The original version was called ‘Test de mémoire des mots’. RAVLT consists of 15 unrelated words that are presented verbally over five consecutive learning trials, with immediate recall assessed following each presentation [Butters & Delis 1995]. The Huddinge version of RAVLT is administered with five learning trials (i.e. total
sum), and a sixth recall trial administered after a 30 minutes delay (i.e. delayed recall).

The reliability of RAVLT has been thoroughly examined over the years. It has been shown that alternate forms of auditory-verbal learning test lists are comparable in degree of difficulty [Uchiyama et al. 1995], and also in other important parameters, e.g. number of words generated during each learning trial, and number of confabulations generated by the patient [van den Burg & Kingma 1999]. Édouard Claparède was careful in his statements about the validity of the test and questioned whether the test measured memory function in general or only memory for words. Correlation studies were performed, showing an association between the number of words recalled and the results in a corresponding non-verbal test where only visual stimuli were presented [Boake 2000]. Furthermore, factor analysis studies using healthy subjects have identified two main factors in RAVLT: acquisition and retention, and both total sum (i.e. learning trials I-V) and delayed recall in RAVLT were associated with the retention factor [Vakil & Blachstein 1993]. In healthy brains however, different aspects of memory are highly correlated. In AD patients by contrast, it has been shown that there is no significant correlation between the total sum and delayed recall tasks, suggesting that these different parameters of the test assess different aspects of memory [Larrabee 2003]. Thus, it is emphasized that the composition of the study group may affect the correlations between underlying factors or constructs [Delis et al. 2003]. Furthermore, the test-retest reliability was also evaluated, and norms for children, adolescents and adults were published already in the 1920’s. The norms have been updated in recent years [Schmidt 1996].

In overt dementia, other cognitive domains than memory are also affected, e.g. language, visuospatial function, executive function and psychomotor speed. Again, these are theoretical constructs and thus not directly measurable. Various neuropsychological tests have been developed to measure function in these domains respectively. For example, visuospatial function is usually assessed by drawing, construction or figure classification tasks and psychomotor speed is appraised by tempo related tasks.

### 1.2.3 Apolipoprotein E

The apolipoprotein (APOE) gene exists in three common isoforms encoded by distinct alleles: ε2, ε3 and ε4. One allele is inherited from the father and one from the mother and there are therefore six possible combinations of the APOE genotype from these three alleles. ApoE protein plays a central role in the metabolism and transport of cholesterol in the body [Strittmatter et al. 1993]. The ε4 allele of the APOE gene has been identified as a major genetic risk factor for the development of AD and subjects with two ε4 alleles are at the highest risk of dementia [Corder et al. 1993]. Protein interactions between ApoE and tau or Aβ are proposed as mechanisms that could explain this genetic effect [Galasko et al. 1998] However, the APOE ε4 allele is also proposed as a risk factor for other types of dementia, for example Lewy body dementia (DLB) [Hardy et al. 1994], VaD [Frisoni et al. 1994], Pick’s disease [Klman et al. 2000] and semantic dementia [Andersen et al. 2000]. ApoE protein is increased following injury and is also increased in several chronic neurodegenerative disorders.
ApoE is bound to SP and NFT in the brains of AD patients and also to amyloid plaques in other dementing disorders [Strittmatter et al. 1993].

In the Swedish population, a higher frequency of the e4 allele has been observed when compared to other Caucasian populations (Table 1) [Eggertsen et al. 1993]. Similar frequencies have been reported from other Nordic countries. Furthermore, there is a north-to-south decreasing gradient of the e4 allele in Europe, and this gradient coincides with the decreasing prevalence of coronary artery disease from north to south, but no such gradient for the prevalence of AD has been found [Lucotte et al. 1997; Kumar et al. 2002].

| Table 1. APOE genotypes in the Swedish population, (n=407) |
|-----------------|----------|
| e2e2            | 1 %      |
| e2e3            | 10 %     |
| e2e4            | 3 %      |
| e3e3            | 59 %     |
| e3e4            | 16 %     |
| e4e4            | 11 %     |

Eggertsen et al. 1993

Although the APOE e4 genotype is a risk factor for developing AD, the e4 carrier status does not in itself predict cognitive decline or conversion to dementia; thus the clinical utility of the APOE genotype is limited [Devanand et al. 2005]. For example, it is estimated that even patients homozygous for the e4 allele have a greater than 50% chance of escaping the disease and the APOE genotype contributes no more to the prediction of AD than knowledge of the family history of dementia [Liddell et al. 2001].

1.2.4 Biological markers in cerebrospinal fluid

Cerebrospinal fluid (CSF) is a colorless fluid that surrounds the brain and spine. Biochemical changes in the brain are thought to be reflected in CSF. Thus, CSF is an obvious source of biomarkers for brain disorders [Blennow 2005]. CSF is produced primarily in the choroid plexus of the cerebral ventricles at a rate of 0.3 ml/min and with a total volume between 80-150 ml; CSF is replaced approximately three times per day [Raedler & Wiedemann 2006]. The CSF production has been reported to decrease with increasing age, and this, together with the ventricular dilatation that occurs with aging presumably influence measured concentrations of lumbar CSF constituents [May et al. 1990]. CSF contains sugars, lipids, electrolytes and proteins with most constituents present in similar or lower concentrations than in blood, because the blood-CSF-barrier restricts the entry of proteins into CSF [Raedler & Wiedemann 2006].

At present, there are three CSF biomarkers for AD that have been extensively evaluated
in the literature: total-tau (T-tau), hyperphosphorylated tau (P-tau) and the 42 amino acid form of beta-amyloid (Aβ42). These CSF biomarkers may reflect the pathogenic processes of the disorder. Patients with AD typically show decreased CSF levels of Aβ42 and increased levels of T-tau and P-tau when compared to healthy controls [Andreasen et al. 2001; Blennow 2004; Sunderland et al. 2003]. The decreased levels of Aβ42 in CSF may reflect the accumulation of Aβ in the brain [Andreasen & Blennow 2005] since depositions of senile plaques in the brain have been found to correlate with low levels of Aβ42 in CSF [Strozyk et al. 2003]. However, low levels of Aβ42 are also found in other disorders, such as DLB [Andreasen et al. 2001], amyotrophic lateral sclerosis (ALS) [Sjögren et al. 2002], multiple system atrophy (MSA) [Holmberg et al. 2003] and Creutzfeldt-Jacob disease (CJD) [Otto et al. 2000]. Thus the specificity of Aβ42 for AD is low.

Tau is a normal protein in the brain that promotes the assembly and stability of neuronal axons by binding to microtubules [Goedert 1993]. Increased levels of CSF T-tau most likely reflect neuronal and axonal damage [Blennow 2005]. In its hyperphosphorylated state, tau loses the ability to assemble and stabilize microtubules, causing axonal instability, which impairs the transport ability [Andreasen et al. 2001]. Hyperphosphorylated tau also promotes tau aggregation [Blennow & Vanmechelen 2003]. The increased CSF levels of P-tau found in patients with AD most likely reflect the phosphorylation state of tau and the subsequent NFT formation [Blennow 2005]. T-tau have in studies shown high sensitivity for differentiation of AD from normal aging and depression but lower specificity against other dementia disorders, such as VaD, FTD and other neurological disorders [Andreasen et al. 2001; Blennow 2004; Blennow & Vanmechelen 2005]. It has been suggested that elevated CSF P-tau levels is a more specific marker for AD than T-tau and Aβ42 [Blennow & Vanmechelen 2005], although there is recent evidence that P-tau may be increased also in other neurological disorders, such as multiple sclerosis, characterized by axonal damage [Bartosik-Psujek & Stemmlasiak 2006]. It has been proposed that there are two types of biomarkers for AD: state markers and stage markers. A state marker reflects the intensity or activity of a specific disease process and a stage marker reflects how far the degenerative disease process has proceeded [Blennow 2005].

1.2.5 Medial temporal lobe atrophy

Atrophy of brain tissue describes a loss of neurons and the connections between them, and this can be visualized using brain imaging techniques like magnetic resonance imaging (MRI), computed tomography (CT) or positron emission tomography (PET). Brain imaging is also used to exclude expansivc pathological processes in the brain, e.g. tumors, subdural hematomas or hydrocephalus. In research, much interest has been focused on whether atrophy of the medial temporal lobe can serve as an early diagnostic marker for AD [Wahlund et al. 2005] since the entorhinal cortex and hippocampus are related to the earliest pathological changes in AD [Braak & Braak 1991]. It has been found that some patients with MCI show severe medial temporal lobe atrophy (MTA) although the absence of MTA does not exclude the development of dementia [Visser et al. 1999]. Likewise, it has been shown that visual assessment of MTA on brain MRI is a powerful predictor of conversion to dementia in relatively
young MCI patients, although not all patients with MTA at baseline developed dementia during a follow-up of approximately 3 years [Karl et al. 2004]. It has also been suggested that gray matter loss in the medial temporal lobe among healthy elderly subjects with subjective cognitive complaints but normal memory performance may reflect a pre-MCI stage [Saykin et al. 2006]. Furthermore, combined measurements of MTA and other diagnostic tools have also been proposed. For example it has been shown that a combination of MTA and pathological CSF biomarkers predicts dementia development among MCI patients [Bouwman et al. 2006].

Measurements of the volumes of the entorhinal, transentrichal, perirhinal cortices and the hippocampus and amygdala have yielded mixed results in AD patients [Teipel et al. 2006]. It has been suggested that the entorhinal volume is more useful than hippocampal volume to differentiate patients who convert to dementia from those who do not; although there is evidence that both these regions degenerate before the onset of overt dementia [Dickerson et al. 2001]. Others however, have found no difference or inferior accuracy for entorhinal cortex [Teipel et al. 2006]. To summarize, it has been found that atrophy of the hippocampus can differentiate patients with AD from healthy subjects, but there is a lack of evidence because of insufficient quality of studies concerning the usefulness of MTA as a diagnostic marker in a more general setting [Wahlund et al. 2005].

1.2.6 Depression

Depression is a common symptom in dementia [Kennedy & Salesmati 2001; Visser et al. 2000] although it may also cause cognitive symptoms among non-demented elderly [Biringer et al. 2005]. Thus, there is controversy whether depression should be regarded as a possible risk factor for subsequent dementia, or as a confounding factor in dementia research. However, since studies show that preclinical AD is often accompanied by depression, it has been recommended that research that aims at investigating preclinical AD should not exclude patients on the basis of depression [Visser et al. 2000]. It has also been suggested that depression is a risk factor for subsequent MCI among cognitively normal elderly subjects, especially among subjects carrying the APOE ε4 allele [Geda et al. 2006]. The cognitive function of male subjects seems to be more vulnerable to depressive symptoms than the cognitive function of female subjects [Biringer et al. 2003; Geda et al. 2006]. Furthermore, there is evidence that chronic psychological distress is a risk factor for development of AD and that this relationship probably reflects neurobiologic mechanisms other than the pathologic hallmarks of AD, i.e. plaques and tangles in the brain [W. Lopez et al. 2003].

1.2.7 Vascular risk factors

In recent years, neuropathological studies have shown that vascular and neurodegenerative changes in the brain coexist and overlap much more than previously proposed. Consequently, AD and VaD may share both vascular and neurodegenerative pathological features [Lewis et al. 2006].
Studies have shown that blood pressure among middle-aged subjects is often high in those who later develop dementia [Skoog & Gustafson 2006]. A recent epidemiological study found that high midlife cholesterol levels, obesity and high blood pressure increase the risk for AD and dementia in an additive manner [Kivipelto & Solomon 2006]. However, blood pressure starts to decrease years before onset of AD and continues to decrease during the disease process [Skoog & Gustafson 2006]. Thus, among the oldest old, it has been shown that low blood pressure rather than high is associated with cognitive deficits according to the Mini Mental State Examination (MMSE) [Folstein et al. 1975; Hestad et al. 2005]. Likewise, it has also been shown that cholesterol levels that were high in midlife were significantly lower in patients five years prior to a clinical diagnosis of AD [Kivipelto & Solomon 2006]. Other vascular factors that have been proposed to increase the risk for memory deficits and dementia are diabetes [Nash & Fillit 2006] and high homocysteine levels [Nurk et al. 2005]. Thus there is evidence that risk factors affecting arteriosclerosis increase the risk for dementia.

1.3 THE MEMORY CLINIC CHALLENGE

Since the advent of pharmacological treatment possibilities for patients with AD, research has been focused on MCI and predictors of dementia because initiation of treatment in the preclinical phase might be of benefit to the patients [Winblad et al. 2004]. Patients are referred to multidisciplinary outpatient clinics world-wide, and the Karolinska University Hospital memory clinic is one of them. In these clinics, patients are usually younger, have milder cognitive symptoms and a wider range of diagnoses and etiologies of the cognitive impairment – including reversible conditions like depression, anxiety, post-traumatic syndromes, whiplash sequelae, normal pressure hydrocephalus, obstructive sleep apnea and hyper-/ hypothyroidism – than patients in more traditional geriatric psychiatry clinics [Hejl et al. 2003; Hejl et al. 2002; Vraamark Elberling et al. 2002; Luce et al. 2001; Hogh et al. 1999; Kopelman et al. 1996]. A larger proportion of younger memory clinic patients also show normal cognitive function in neuropsychological investigations as compared to elderly patients [Vraamark Elberling et al. 2002].

The major challenge in memory clinics is two-fold: (i) to identify patients who are at high risk for developing dementia and (ii) to identify patients who are not at short-term risk of dementia. In other words, the challenge is to predict development as well as non-development of dementia [Busse et al. 2003]. Even though there is no remedial treatment for dementia, studies have shown that delays in treatment may have detrimental effect on patient’s long-term well-being [Winblad et al. 2006]. The need for an early diagnosis is also important for facilitating the situation both for the patients and their spouses, by communication and information about the diagnosis, assessment and treatment of concomitant depression, referral to Alzheimer patient organizations and sensitivity to the carer’s needs [Brækhus et al. 1998]. Moreover, it is equally important to identify patients who will remain stable, considering both the socio- economical cost of recurrent dementia investigations in principally healthy individuals, and the psychologically strenuous situation for the patients whose concerns about their cognitive functioning might be further increased by annual reexaminations to monitor signs of deterioration.
In a short term perspective, the challenge must be to identify patients who are in a preclinical phase of dementia; i.e. to differentiate patients whose subtle symptoms are caused by a dementing disease process from those whose symptoms are caused by other, possibly reversible conditions. The challenge to identify patients in a long term perspective; i.e. to predict which healthy individuals who will later develop a disease must be a challenge for future research.

1.3.1 Karolinska University Hospital Memory Clinic

In 1999, four-hundred-and-two (402) outpatients went through an extensive dementia investigation at the out-patient clinic. The distribution of clinical diagnoses during this year is shown in Figure 1. MCI was the largest diagnostic group followed by patients with AD, VaD and FTD (27%). In 2005, four-hundred-and-thirty-five (435) of the outpatients at the Karolinska University Hospital Memory Clinic were referred to extensive dementia investigation (188 males and 247 females). The mean age of the patients was 63.0±10.5 years and the mean MMSE-score was 27.0±3.0. The majority of patients were diagnosed as non-demented (38% subjective cognitive impairment, SCI; and 38% MCI; Figure 2), and 19% were diagnosed with AD, VaD or FTD (Figure 2).

The increasing numbers of patients with SCI and the decreasing number of AD, VaD and FTD patients in 2005 as compared to 1999, illustrates that patients seek medical help for memory problems at an increasingly earlier stage and shows the importance to find clinically useful predictors to differentiate patients who will decline cognitively and progress to dementia from those who will not.
2 AIMS

The overall aim of the thesis was to examine which clinical methods that can be used – and in what combinations – to differentiate between patients at high and low risk for cognitive decline and dementia.

The specific aims of the studies were:
I: to investigate whether application of cutoff levels in a verbal episodic memory test (Rey Auditory Verbal Learning Test, RAVLT) is a useful method for identifying patients who are at high and low risk for cognitive decline and subsequent dementia.

II: to investigate the relationships between episodic memory function, APOE genotype, CSF biomarkers and longitudinal cognitive decline.

III: to investigate longitudinal measurements of CSF biomarkers (T-tau, P-tau and Aβ42) and conversion to dementia in patients who were defined by their episodic memory performance according to RAVLT at inclusion and who were clinically followed up during a mean time period of 3 years.

IV: to investigate what combination of baseline CSF biomarker levels, APOE ε4 allele status and episodic memory functioning (according to RAVLT test results) that predicts progression to AD during a 3 year follow-up among initially non-demented memory clinic patients.
3 MATERIAL AND METHODS

3.1 SUBJECTS

3.1.1 General characteristics

The study samples in papers I-IV of the thesis were selected retrospectively among patients who were initially admitted to the Karolinska Huddinge Memory Clinic because of subjective and/or objective memory complaints, and who visited the clinic for at least one neuropsychological follow-up investigation during the years 2002–2004 (study I-III) and 2002-2005 (study IV). All patients eligible for the studies were thus examined with a comprehensive neuropsychological test battery at two separate occasions, at baseline and at follow-up, approximately 3 years apart. The patients were referred to follow-up investigations since suspicions of a progressive cognitive disorder could not be ruled out at baseline. Importantly, the suspicion of possible progression could have been raised from any of the investigations conducted as part of the clinical routine at baseline (see section 3.2.1 for further details on the clinical routine).

No exclusion of patients was made based on patient characteristics, e.g. comorbidity and/or psychiatric illnesses such as depression, since we wanted to find predictors for cognitive decline in a naturally heterogeneous sample in a memory clinic. It has been recommended that research that aims to investigate preclinical AD should not exclude subjects with depression since preclinical AD is often accompanied by depression [Visser et al. 2000]. Likewise, there are other conditions that may cause cognitive symptoms, for example diabetes and hypertension [Nash & Fillit 2006], and we did not wish to exclude these patients since they may be of increased risk for dementia. Exclusion of patients in the separate studies was only made depending on missing data in any of the clinical investigations relevant for the specific study (see below for further details). Demographic data for the study samples in study I-IV is summarized in Table 2.

<table>
<thead>
<tr>
<th></th>
<th>2003*</th>
<th>2005</th>
<th>Study I</th>
<th>Study II</th>
<th>Study III</th>
<th>Study IV</th>
</tr>
</thead>
<tbody>
<tr>
<td>n</td>
<td>293</td>
<td>435</td>
<td>224</td>
<td>124</td>
<td>39</td>
<td>153</td>
</tr>
<tr>
<td>Female sex</td>
<td>55%</td>
<td>57%</td>
<td>58%</td>
<td>54%</td>
<td>48%</td>
<td>61%</td>
</tr>
<tr>
<td>Age</td>
<td>62.3 (9.7)</td>
<td>63.0 (10.5)</td>
<td>60.7 (8.5)</td>
<td>61.2 (9.3)</td>
<td>61.3 (7.6)</td>
<td>61.1 (8.2)</td>
</tr>
<tr>
<td>MMSE</td>
<td>27.0 (3.1)</td>
<td>27.0 (3.0)</td>
<td>28.2 (1.7)</td>
<td>28.1 (2.0)</td>
<td>28.3 (1.8)</td>
<td>28.4 (1.7)</td>
</tr>
<tr>
<td>Education, yrs</td>
<td>11.8 (4.0)</td>
<td>missing data</td>
<td>13.1 (4.3)</td>
<td>13.1 (4.3)</td>
<td>13.5 (4.2)</td>
<td>12.5 (4.1)</td>
</tr>
<tr>
<td>APOE</td>
<td>0</td>
<td>56%</td>
<td>58%</td>
<td>48%</td>
<td>36%</td>
<td>52%</td>
</tr>
<tr>
<td>ε4</td>
<td>1</td>
<td>37%</td>
<td>35%</td>
<td>45%</td>
<td>56%</td>
<td>41%</td>
</tr>
<tr>
<td>alleles</td>
<td>2</td>
<td>7%</td>
<td>7%</td>
<td>7%</td>
<td>8%</td>
<td>7%</td>
</tr>
</tbody>
</table>

* Solomon A, personal communication
3.1.2 Study I

3.1.2.1 Step I

Thirty-three (33) patients were selected retrospectively among subjects who were diagnosed with MCI at baseline and who visited the memory clinic for a follow-up investigation approximately 14 months later (range: 6-30 months). The mean age at baseline was 60.6 years (SD = 6.0), and the mean MMSE-score at baseline was 28.2 (SD = 1.6). MCI was diagnosed according to modified Petersen criteria, described by Wahlund et al. [2003]: (1) memory complaints; (2) intact activities of daily living, (3) objective signs of decline in relation to the assumed premorbid functioning level or >1.5 SD below age-matched controls in any cognitive domain, and (4) not fulfilling criteria for dementia.

3.1.2.2 Step II

The step II sample consisted of 224 patients (58% females) who were clinically monitored with cognitive testing during approximately 3 years (range: 0.5–10.7 years). Patients from the Step I were excluded from the Step II sample. The mean age at baseline was 60.7 years (SD = 8.5), and the mean MMSE score was 28.2 (SD = 1.7). Patients who had not completed the Rey Auditory Verbal Learning Test (RAVLT) at baseline were excluded. Initiation of treatment with cholinesterase inhibitors during the follow-up interval was allowed.

3.1.3 Study II

The study sample included 124 memory clinic patients (54% females). Patients were excluded on the basis of: i) missing RAVLT, ii) lumbar puncture (LP) examination had not been performed, iii) when the LP was performed at the last visit to the clinic and there was no neuropsychological testing available after the LP, and iv) when all three CSF biomarkers (T-tau, P-tau and Aβ42) had not been analyzed. Since, the LP examination was not always performed at the first visit to the clinic, the mean time between the LP and the neuropsychological follow-up investigation was 25.5 months (range: 0.3–8.8 years), i.e. somewhat shorter than the mean follow-up time in the other studies. The mean age at baseline was 61.2 years (SD=9.3), and the mean MMSE score was 28.1 (SD=2.0). None of the patients were treated with cholinesterase inhibitors before the LP.

3.1.4 Study III

The study sample comprised of 39 patients (48% females) who were clinically diagnosed as non-demented at baseline and who were clinically followed up with both neuropsychological testing and lumbar punctures (LPs) at two separate occasions. The mean time between baseline and the follow-up investigation was approximately 3 years (range: 0.6–6.8 years). The mean time between the LP and neuropsychological examination was 1.2 months at baseline and 1.5 months at follow-up. The mean age of the patients at baseline was 61.3 (SD=7.6), and the mean MMSE score was 28.3
(SD=1.8). Initiation of treatment with cholinesterase inhibitors between baseline and follow-up was allowed. All diagnoses were established independently of CSF biomarker levels, APOE genotype and the SIM, MIM and NIM categorization from the RAVLT results. All patients were diagnosed as non-demented at baseline; 14 patients had no cognitive impairment and 25 patients had MCI. At follow-up, 15 patients had no cognitive impairment, 10 patients had MCI, and 14 patients had progressed to dementia (of these, 10 had AD, 2 FTD, 1 had DLB and 1 had dementia non ultra descriptum [NUD]).

3.1.5 Study IV

The study sample comprised 153 patients (61% females) who were followed up at the clinic during a mean time period of approximately 3 years (range: 0.4–11.0 years). Inclusion criteria were: i) LP with CSF analyses of T-tau, P-tau and Aβ42 performed within 1 year from the first neuropsychological examination and ii) analysis of APOE genotype. The mean age at baseline was 61.1 years (SD=8.2), and the mean MMSE score was 28.4 (SD=1.7). At follow-up, 27 patients had progressed to AD, 76 had MCI and 50 patients had normal cognition. Patients who converted to other types of dementia than AD were excluded. AD was diagnosed according to DSM-IV criteria [American Psychiatric Association 1994], and MCI according to the Winblad et al. criteria [Winblad et al. 2004].

3.2 PROCEDURES – STUDY I-IV

3.2.1 Clinical procedure – overview

The dementia investigations at the Karolinska University Hospital memory clinic typically included: medical history, patient and informant interview, neurological examination, mini mental state examination (MMSE), blood tests including APOE genotyping, neuropsychological assessment, speech pathology examination, functional assessment, brain imaging (magnetic resonance imaging, MRI or computed tomography, CT and sometimes single positron emission computed tomography, SPECT), electroencephalography (EEG) and lumbar puncture (LP) with analyses of T-tau, P-tau and Aβ42 in CSF.

3.2.2 Diagnostic procedure

The clinical diagnoses were established at multi-professional conferences where a team of neuropsychologists, nurses, occupational therapist, physicians, speech pathologist and social worker participated. MCI was defined using modified Petersen criteria described by Wahlund et al. [2003] (study I), or by Winblad et al. criteria [2004] (study III-IV). Dementia and AD were defined according to the DSM-IV diagnostic criteria (study I, II, IV) [American Psychiatric Association 1994] or following the recommendations of the National Institute of Neurological and Communicative Disorders and Stroke (NINCDS) and the Alzheimer’s Disease and Related Disorders...
Association (ADRSA) work group (III) [McKhann et al. 1984]. FTD was defined according to clinical consensus diagnostic criteria for FTD [Neary et al. 1998]. DLB was defined using the consensus guidelines for the clinical diagnosis of DLB [McKeith et al. 1996].

The diagnoses referred to in studies I, II and IV are the clinical diagnoses established at the multi-professional team conferences described above. In study III, the diagnoses were established independently of RAVLT test results (i.e. the SIM, MIM and NIM categorization), CSF biomarker levels and APOE genotype by an experienced specialist in geriatrics.

3.2.3 Neuropsychological assessment

A neuropsychological test battery, assessing function in several cognitive domains, was administered at baseline and at follow-up. Table 3 shows the tests that were included in the neuropsychological test battery and a schematic overview of the cognitive domains that each test is associated with. In study II-IV, combined measurements of function in each cognitive domain were used; i.e. language, visuospatial function, memory, psychomotor speed and executive function. Please note that the overview presented in Table 3 is arbitrary and that other classifications are possible. Performance within each cognitive domain can always fractionate further, and beneath the processing of each cognitive task lays a complex system of processing modules which together produce the current performance [Funnell 2004].

Neuropsychological test results were standardized by z-transformations using a control group of subjects consisting of patients’ relatives, members of the Swedish Pensioner Society of the Huddinge community, and non-mutation carriers from Alzheimer’s disease families [Amäiz & Almkvist 2003].

3.2.4 Mini Mental State Examination

The mini-mental state examination (MMSE) is a brief test instrument for assessment of cognitive performance [Folstein et al. 1975]. The MMSE is widely used as a measure of global cognitive function when screening for dementia. It is also used to monitor disease progression and response to treatment, both in clinical settings and as a research tool. The specificity for individual clinical syndromes that cause cognitive problems is limited although it is sensitive to detect dementia symptoms [Crum et al. 1993]. The test requires 5-10 minutes to administer and consists of eleven questions covering orientation, memory, attention, ability to name, follow verbal and written commands, write a sentence and copy a complex polygon similar to a Bender-Gestalt Figure [Bender 1938] and the maximum score is 30 [Folstein et al. 1975]. A population-based study on cognitive performance as measured by the MMSE has shown that the results vary by age and education. This may partly be explained by an increasing prevalence of dementia with age and also an increased prevalence of many diseases and developmental disorders among low educational and lower socioeconomic class individuals [Crum et al. 1993].
Table 3. Neuropsychological tests and an overview of cognitive domains.

<table>
<thead>
<tr>
<th>Test</th>
<th>Primary cognitive domain</th>
<th>Additional cognitive domains</th>
<th>Reference</th>
</tr>
</thead>
<tbody>
<tr>
<td>Information (WAIS-R)</td>
<td>language skill</td>
<td>general knowledge/semantic memory</td>
<td>Bartfai et al. 1994</td>
</tr>
<tr>
<td>Similarities (WAIS-R)</td>
<td>language skill</td>
<td>abstraction ability/logical reasoning</td>
<td>Bartfai et al. 1994</td>
</tr>
<tr>
<td>Block Design (WAIS-R)</td>
<td>visuospatial function</td>
<td>executive function, psychomotor speed</td>
<td>Bartfai et al. 1994</td>
</tr>
<tr>
<td>Clock Drawing test</td>
<td>visuospatial function</td>
<td>executive function, semantic memory</td>
<td>Lezak 1995</td>
</tr>
<tr>
<td>Rey Osterrieth Complex Figure</td>
<td>visuospatial function</td>
<td>executive function, attention, planning</td>
<td>Lezak 1995</td>
</tr>
<tr>
<td>Figure Classification</td>
<td>visuospatial function</td>
<td>executive function, attention, logical reasoning</td>
<td>Andersson et al. 1978</td>
</tr>
<tr>
<td>Digit Symbol (WAIS-R)</td>
<td>psychomotor speed</td>
<td>executive function, attention</td>
<td>Bartfai et al. 1994</td>
</tr>
<tr>
<td>TMT A (time)</td>
<td>psychomotor speed</td>
<td>executive function, attention</td>
<td>Reitan &amp; Wolfson 1993</td>
</tr>
<tr>
<td>TMT B (correct connections)</td>
<td>executive function</td>
<td>mental shifting, attention, psychomotor speed</td>
<td>Reitan &amp; Wolfson 1993</td>
</tr>
<tr>
<td>Verbal fluency (FAS)</td>
<td>executive function</td>
<td>attention, self-monitoring, semantic memory</td>
<td>Fernaeus &amp; Almquist 1998</td>
</tr>
<tr>
<td>Digit Span (WAIS-R)</td>
<td>short-term memory</td>
<td>working memory, executive function, attention</td>
<td>Bartfai et al. 1994</td>
</tr>
<tr>
<td>RAVLT</td>
<td>verbal episodic memory</td>
<td>learning, delayed recall, executive function</td>
<td>Schmidt 1996</td>
</tr>
<tr>
<td>WMS-R logical memory</td>
<td>verbal episodic memory</td>
<td>logical memory, executive function, attention</td>
<td>Wechsler 1987</td>
</tr>
<tr>
<td>Free &amp; Cued recall, 12 word list</td>
<td>verbal episodic memory</td>
<td>executive function, attention, memory, recognition,</td>
<td>Bäckman &amp; Forsell 1994</td>
</tr>
</tbody>
</table>

3.2.5 APOE genotyping

Patient DNA samples for APOE genotype analyses were extracted from peripheral white blood cells using standard methods [Hixson JE et al. 1990] (study II-IV). APOE genotypes were determined by a microsequencing method on microtitre plates (Affigene ApoE; Sangtec Medical, Bromma, Sweden).

3.2.6 Lumbar puncture and CSF analyses

Lumbar puncture (LP) was performed in the L3/L4 or L4/L5 interspace of the spinal cord, with the patient sitting [Blennow 2005; Andreasen & Blennow 2005]. CSF was obtained by the LP and the samples were aliquoted in polypropylene tubes and stored at
−80 °C until analysis. CSF T-tau was determined using a sandwich ELISA constructed to measure total tau, both normal tau and hyperphosphorylated tau [Blennow et al. 1995]. CSF P-tau was determined using a sandwich ELISA, constructed to specifically measure tau phosphorylated at Thr181 [Vanmechelen et al. 2000]. CSF Aβ42 was analyzed using a sandwich ELISA, constructed to specifically measure β-amyloid 1-42 [Andreasen et al. 1999]. Frozen samples were sent on dry ice to the Clinical Neurochemistry Laboratory in Gothenburg, Sweden, where all the analyses were performed. Two internal CSF pools were run on each ELISA plate to assure reproducibility.

All CSF samples used in the studies were collected as part of the clinical routine. For the purpose of this thesis project, T-tau and Aβ-2 levels were reanalyzed in some of the samples; T-tau levels had previously been analyzed in all the samples that were used in study II (n=124) and Aβ42 levels had previously been analyzed in 51 of the 124 samples in study II. For the purpose of this project, T-tau levels were reanalyzed in 65% of the samples (n=80/124) and Aβ42 levels in 43% of the samples (n=22/51). The clinical analyses were performed using the same methods at the same laboratory as described above. The levels obtained from the clinically analyzed CSF samples correlated significantly with the levels obtained in the reanalyzed samples (T-tau r=.85, p<.00001; Aβ42 r=.74, p<.0001). When frozen CSF samples for reanalysis were unavailable, CSF level data from the clinical assessment routine was used in the statistical analyses.

3.3 DATA ANALYSIS

All statistical analyses were performed in Statistica data analysis software system, version 7.0 (StatSoft Inc, 2004).

3.3.1 Study I

3.3.1.1 Step I

The patients were divided into three clusters based on the standardized results (z-scores) in verbal and visual memory tests (RAVLT, RCF memory and free and cued recall) from the follow-up investigation, using cluster analysis [Anderberg 1973]. Thus, three clusters with different memory functioning levels at follow-up were created. Analyses of variance (ANOVAs) with Least Significant Difference (LSD) post hoc tests were used to compare the clusters regarding demographic variables (age, gender, education) and MMSE scores at baseline, and to confirm that all clusters differed significantly in the follow-up memory test scores. Second, the baseline neuropsychological tests were simultaneously investigated as to their predictive value for memory function at follow-up by Classification and Regression Tree Analysis (CART) [Crichton et al. 1997], using the three clusters described above as outcome. The CART analysis finds the optimal combination of variables (i.e. among the baseline neuropsychological test results) to correctly classify patients into the outcome variable specified (i.e. memory clusters). The CART analysis also generates cutoff levels in the
tests with best predictivity for the outcome measure used. The CART models are fit
with the use of nonparametric statistical methods suitable for small data sets [Ligthart et
al. 2007].

3.3.1.2 Step II

The RAVLT cutoff values from the CART analysis in step I (Figure 3, page 32) were
used to assign the patients to three memory groups from their standardized baseline
results in this test: severe impairment in memory (SIM), moderate impairment in
memory (MIM) and no impairment in memory (NIM). One-way ANOVAs were used
to compare demographic variables (gender, age and educational level), baseline MMSE
scores and baseline neuropsychological test results of the SIM, MIM and NIM groups.
The primary outcome measure in step II was cognitive decline during the follow-up
interval and was determined in each neuropsychological test by multiple linear
regression analyses (controlling for effects of age, gender, education, pharmacological
treatment and follow-up time). The mean z-score result in each cognitive domain at
follow-up was also calculated. The proportions of omitted tests at baseline, and the test
attrition rates between baseline and follow-up in SIM, MIM and NIM, were compared
by difference tests. To circumvent a possible attrition bias, each patient’s cognitive
status was also judged individually from the neuropsychological profile at follow-up.
Positive predictive values were calculated for NIM, MIM and SIM, respectively,
showing the proportion in each group with severe cognitive deficits at follow-up,
defined as impairments of more than 1.5 SD in memory, and in at least two non-
memory tests from more than one non-memory cognitive domain. The incidence of
dementia at follow-up (according to diagnoses established at team consensus
conferences) was also investigated by calculating positive predictive values.

3.3.2 Study II

First, patients were divided into 3 memory groups, according to cut-off scores in the
RAVLT that were statistically derived in Study I: (i) severely impaired memory (SIM),
(ii) moderately impaired memory (MIM) and no impairment in memory (NIM).
Second, patients in SIM, MIM and NIM respectively were dichotomized on the basis of
no or at least 1 APOE ε4 allele, thus creating 6 subgroups.

Correlations were analyzed by Spearman rank order correlations and p was set at <0.01.
Group comparisons were made using one-way ANOVA, followed by LSD post hoc
analyses for pairwise comparisons. Differences between proportions were compared
using two-sided t tests. Multiple linear regression analyses were used to compare CSF
biomarker levels across groups and to investigate longitudinal cognitive decline,
controlling for effects of gender, age, education, follow-up time and baseline
performance in each cognitive domain. The mean z-score performance in each
cognitive domain at follow-up was also calculated.
3.3.3 Study III

The patients were divided into three groups according to their episodic memory test results at baseline, using the cut-off scores in RAVLT described above (section 3.3.1).

Correlation coefficients were calculated using non-parametric Spearman rank order correlation analyses. Demographic data and neuropsychological test results were analyzed using one-way ANOVA followed by Bonferroni post hoc analyses for group comparisons. Longitudinal changes within SIM, MIM and NIM were analyzed using repeated measures ANOVA followed by Bonferroni post hoc analyses for group comparisons. Level of statistical significance was set at p<.05. Data are expressed as means and 95% confidence intervals (CI) unless otherwise specified.

3.3.4 Study IV

Classification and regression tree analysis (CART) was used to investigate what combination of baseline variables that best predicted the diagnosis at follow-up, and to estimate cutoff levels in the variables with the highest predictivity [Crichton et al. 1997; Ligthart et al. 2007] Thus, clinical diagnosis at follow-up was used as categorical outcome measure in the analysis and the predictor variables were: sex, education, CSF T-tau, P-tau and Aβ42, APOE ε4 alleles, RAVLT learning (total sum) and RAVLT retention (delayed recall). The CART analysis simultaneously finds out the optimal combination of predictor variables to classify patients into the outcome categories (i.e. clinical diagnosis at follow-up).

Furthermore, the cutoff levels generated by the CART-analysis in the most predictive variables were applied to baseline data. Group comparisons were made using one-way ANOVA followed by Bonferroni post hoc analysis. Cognitive decline during follow-up was investigated using t-tests for dependent samples. Level of statistical significance was set at p<.05.

3.4 ETHICAL APPROVALS

Study I-IV were approved by the ethical committee at Karolinska University Hospital in Huddinge (No. 01/03, studies I-IV; No. 134/03, studies II-IV).
4 RESULTS AND DISCUSSION

4.1 STUDY I

4.1.1 Step I

Among all the neuropsychological tests performed at baseline, the CART analysis identified a combination of delayed recall and total sum from the RAVLT as the best predictors for optimal classification of patients into the correct outcome cluster. The cutoff scores in baseline RAVLT that were generated by the CART analysis are shown in Figure 3. The other memory tests that were administered at baseline (RCF memory, Free & Cued recall, 12 word list) did not discriminate equally well between all three outcome clusters, and there were no significant differences between the clusters in non-memory cognitive tests at baseline.

![Diagram](image)

Figure 3. Cutoff levels in RAVLT results that were generated from the CART analysis. These cutoff levels (described as raw test scores and standardized z-scores) were used to define the baseline memory groups in Step II.

Furthermore, we investigated the relationships between the three memory groups (SIM, MIM and NIM) and available medical data at follow-up (Table 4, unpublished data). In this small sample, the majority of patients in SIM (83%) had developed dementia at follow-up, 2.2 years after baseline examination, as compared to 40% in MIM and no patient in NIM.
Table 4 APOE genotype status, hippocampal atrophy and clinical diagnosis at follow-up in SIM, MIM and NIM respectively, in the small study sample from Study 1, Step I.

<table>
<thead>
<tr>
<th>APOE genotype</th>
<th>SIM</th>
<th>MIM</th>
<th>NIM</th>
<th>Row n</th>
</tr>
</thead>
<tbody>
<tr>
<td>e4 carrier</td>
<td>92% (11/12)</td>
<td>13% (2/15)</td>
<td>33% (2/6)</td>
<td>n=33</td>
</tr>
<tr>
<td>MRI: hippocampal atrophy (yes/no)</td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Baseline</td>
<td>0% (0/10)</td>
<td>0% (0/15)</td>
<td>0% (0/5)</td>
<td>n=30</td>
</tr>
<tr>
<td>3.5 years</td>
<td>75% (6/8)</td>
<td>13% (1/8)</td>
<td>0% (0/2)</td>
<td>n=18</td>
</tr>
<tr>
<td>Clinical diagnosis at follow-up (2.2 yrs)</td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Dementia</td>
<td>83% (10/12)</td>
<td>43% (6/15)</td>
<td>0% (0/6)</td>
<td>n=16/33</td>
</tr>
<tr>
<td>MCI</td>
<td>17% (2/12)</td>
<td>47% (7/15)</td>
<td>83% (5/6)</td>
<td>n=14/33</td>
</tr>
<tr>
<td>SCI</td>
<td>0% (0/12)</td>
<td>13% (2/15)</td>
<td>13% (1/6)</td>
<td>n=3/33</td>
</tr>
</tbody>
</table>

4.1.2 Step II

When the cutoff scores that were derived from the statistical analysis in Step I were applied to a different larger sample (n=224), we found at baseline that 58 patients had severe impairment (SIM), 84 had moderate impairment (MIM) and 82 had no impairment in memory (NIM).

When baseline and follow-up neuropsychological data were compared, the NIM group, whose performance was normal at baseline, remained cognitively normal at follow-up. Multiple regression analyses, adjusting for effects of age, sex, education and follow-up time, using NIM as an unchanging reference group, showed that SIM deteriorated significantly in at least one test of language, visuospatial function, executive function and memory, while MIM showed no significant decline in any of those domains (Figure 4).

Figure 4. Cognitive change between baseline and follow-up in SIM, MIM and NIM.
4.2 STUDY II

Episodic memory function, according to RAVLT scores, was weakly but significantly correlated with CSF marker levels only among ε4+ subjects (T-tau: \( r = -0.41, p = .001; \) P-tau: \( r = -0.36, p = .005; \) Aβ42: \( r = 0.47, p = .0001 \) [Figure 5a]), but not among ε4− subjects (T-tau: \( r = -0.02, p = .87; \) P-tau: \( r = -0.02, p = .90; \) Aβ42: \( r = 0.09, p = .52 \) [Figure 5b]).

![Correlation between CSF Aβ42 and verbal episodic memory among APOE ε4 positive subjects](image)

![Correlation between CSF Aβ42 and verbal episodic memory among APOE ε4 negative subjects](image)

Figure 5a-b. Correlations between verbal episodic memory according to RAVLT z-scores and CSF Aβ42 levels among APOE ε4 positive subjects (a) and APOE ε4 negative subjects (B).

The patients were divided into 3 memory groups, SIM, MIM and NIM, and were dichotomized on the basis of no or at least one APOE ε4 allele, thus creating six subgroups. When comparing these 6 subgroups, SIM ε4+ and MIM ε4+ groups showed significantly lower Aβ42 levels than the other groups. T-tau was increased in SIM ε4+
when compared to all the other groups, except from SIM e4-. P-tau was significantly increased in SIM e4+ when compared to all the other groups, including the SIM e4- group (Figure 6a-c). However, both SIM e4+ and SIM e4- declined cognitively during follow-up. Using multiple regression analyses adjusting for the effects of gender, age, education, and follow-up time using NIM as controls, T-tau was significantly higher in SIM e4+ when compared to all other groups, including SIM e4-.

Figure 6 a-c. Standardized T-tau (a), P-tau (b) and Aβ42 (c) levels in CSF by z-transformation using reference values from healthy controls, published by Hansson et al. (2006). Data are means (■) 95% Confidence Intervals (Δ) and raw data (Δ).
4.3 STUDY III

The main result of study III was that initially non-demented patients with severely impaired episodic memory (SIM) at baseline, who declined cognitively over three years and progressed to dementia at a high rate, showed significantly increasing CSF P-tau levels during follow-up (Figure 7a). Most of the patients in MIM and NIM remained cognitively stable and did not convert to dementia during the follow-up period. Both these groups showed unchanging P-tau-levels (Figure 7a). CSF T-tau levels were high and Aβ42 levels were low among SIM patients, both at baseline and at follow-up, and these CSF biomarkers did not change significantly in any of the three memory groups during follow-up (Figure 7b-c). To summarize, longitudinally increasing P-tau levels were found in those patients that converted to dementia while the non-converters showed stable levels.

Figure 7a-c. CSF P-tau (a), T-tau (b) and Aβ42 (c) during three years in NIM, MIM and SIM respectively.
4.4 STUDY IV

In study I-III, cognitive decline was used as the primary outcome measure. In study IV, we used the clinical diagnosis at follow-up as the primary outcome, to investigate what baseline variables that were the best predictors of progression to AD among initially non-demented patients. The investigated baseline variables were: sex, education, levels of T-tau, P-tau and Aβ42 in CSF, APOE ε4 genotype, RAVLT learning (total sum) and RAVLT retention (delayed recall).

CSF Aβ42 and RAVLT results at baseline were identified as the best predictors of conversion to dementia and also the best predictors of normal cognition (NC) at follow-up. Using CART-analysis, we found that the majority of patients who progressed to AD during the 3 years follow-up interval could be identified at baseline from a combination of low Aβ42 levels in CSF and low RAVLT delayed recall results (group A, Figure 8). Most patients with low Aβ42 levels, but better preserved RAVLT delayed recall at baseline remained non-demented at follow-up (78%), although some (22%) were diagnosed with AD at follow-up (group B, Figure 8). All patients with a combination of CSF Aβ42 levels above the cutoff level generated by the CART analysis and no impairment in RAVLT learning at baseline remained non-demented at follow-up (group D, Figure 8). Most of the patients with Aβ42 levels above the cutoff level and slightly impaired RAVLT learning results (93%) remained non-demented at follow-up (group C, Figure 8).

Figure 8. shows the result from the CART-analysis. Among the baseline variables, the analysis identified CSF Aβ42, RAVLT delayed recall and RAVLT learning (total sum) as the best predictors for clinical diagnosis at follow-up. The tree graph also shows the cutoff levels that were generated by the CART analysis.

The four groups (A-D) showed different CSF biomarker levels. Both T-tau and P-tau levels were significantly higher in group A as compared to the other groups (p<.05). Furthermore, group A-D showed different cognitive profiles during 3 years according
to longitudinal neuropsychological testing. Group A deteriorated significantly in visuospatial tests, attention/executive function, working memory and MMSE-scores (Figure 9b, d-e, h). Group A showed the same decline pattern in all other cognitive domains except from psychomotor speed, although these changes did not reach statistical significance (Figure 9a, c, f-g). Group B deteriorated significantly in tests of psychomotor speed, attention/executive function, working memory and MMSE-scores during follow-up (Figure 9c-d, e, h). Group C and D showed no significant decline in any of the cognitive domains during follow-up (Figure 9a-h).

Figure 9a-h. Longitudinal cognitive decline in group A-D.
4.5 SUMMARY

To summarize, we have shown that the degree of memory impairment, as measured by the verbal episodic memory test Rey Auditory Verbal Learning Test (RAVLT), at the first referral to a memory clinic is highly decisive of the cognitive prognosis during a mean follow-up period of approximately 3 years. Our data suggests that the RAVLT cutoff scores that were provided in Study I, is a clinically useful method to identify patients at high risk for cognitive decline and dementia (SIM), and also to identify those who are not at short-term risk (NIM). The RAVLT cutoff scores also identified a group of patients where the majority remained cognitively stable, while some patients (16-20%) declined cognitively and were diagnosed with dementia at follow-up (MIM). The ability to differentiate between patients at high and low risk for progression to dementia may be increased by LP examination and analyses of Aβ42 levels in CSF, especially among the patients with moderate memory impairment at baseline (MIM).

Furthermore, we investigated the relationships between verbal episodic memory, levels of CSF biomarkers (T-tau, P-tau and Aβ42), APOE genotype and longitudinal cognitive decline in a large group of patients. Our results showed that the APOE e4 allele is associated with pathological levels of CSF biomarkers in SIM patients, and that episodic memory correlated with CSF biomarker levels only among APOE e4 positive patients. Despite the differences in CSF biomarker levels between e4 positive and e4 negative patients, no differences in cognitive function were found between these two groups with severe memory impairment. Neither were there any differences between these two groups in longitudinal cognitive decline or in number of patients who were diagnosed with dementia at follow-up. Data thus showed that the APOE e4 allele status affected the relationship between episodic memory and levels of T-tau, P-tau and Aβ42 in CSF. It remains to be determined whether the APOE genotype affects the expression of biomarker levels in CSF, or whether the APOE genotype and the different biomarker patterns reflect different types of underlying disease processes. Longitudinally increasing P-tau levels in CSF in severely memory impaired patients, the majority of whom converted to dementia, were found.

4.6 THE ORIGIN OF THE THESIS PROJECT

The common denominator of all the patients that participated in this retrospective, clinically-based thesis project is that they were all referred to follow-up investigations at the Karolinska University Hospital in Huddinge because suspicion of a progressive cognitive disorder could not be ruled out after the clinical investigations at the baseline visit to the clinic.
The origin of this thesis project was the clinical impression and experience that a large amount of the patients who had been judged to be at increased risk for dementia, and who were clinically monitored for cognitive progression, showed no signs of cognitive decline despite long follow-up periods of several years. On the other hand, initially non-demented patients – often classified as affected by MCI at baseline – who subsequently developed dementia had to wait until their symptoms progressed further and clinical signs of cognitive or functional deterioration were obvious in order to receive pharmacological treatment with acetyl cholinesterase inhibitors. The main purpose of this project was to find methods to improve the identification of patients at high risk for cognitive decline and dementia and to discriminate those from patients who were not at short-term risk for cognitive progression.

The results from our studies clearly corroborated our clinical experience, that a large amount of patients remained cognitively stable and non-demented during the mean follow-up period of three years. Importantly, the results also clearly revealed evidence that differences existed already at baseline between the patients who progressed to dementia and those who did not, and that these differences may be used clinically to improve diagnostics of early dementia, and also to improve the ability to identify cognitive normality among patients with subjective complaints.

4.7 THE SIM, MIM AND NIM CATEGORIZATION & COGNITIVE DECLINE

Although MCI is a common term in dementia research it is without a doubt a rather volatile concept. Despite attempts to reach consensus, there are no precise definitions of the concept, not even for aMCI [Kramer et al. 2006]; i.e. the MCI subtype that has attracted most attention in studies on predictors for dementia [Petersen & O’Brien 2006]. The most common approach in longitudinal dementia research is often the use of various definitions of MCI as an inclusion criterion, and clinical or research diagnoses at follow-up as the main outcome measure. This approach is doubly problematic; at baseline as well as at follow-up. At baseline because the concept of MCI is diffuse, and at follow-up because a definite diagnosis of dementia can only be established post-mortem [McKhann et al. 1984].

In this thesis project we have applied a different approach: and defined our patients at baseline using a statistically derived categorization scheme, based on episodic memory function; SIM, MIM and NIM. Furthermore, we have used longitudinal cognitive decline as the main outcome measure, and conversion to dementia as secondary outcome in all studies, except in study IV. Critics may allege that introduction of additional concepts, such as SIM, MIM and NIM, only adds to the fuzziness surrounding the concept of MCI. However, it is important to remember that our SIM, MIM and NIM categorization of patients is to be thought of as a memory impairment categorization scheme that does not compete, nor replace, the concept of MCI or other proposed diagnostic classification systems.

Our SIM, MIM and NIM categorization is based on cutoff scores derived from the
statistical analyses in study 1, step I. Petersen et al. (2006) point out that classifications based on a particular cutoff score on a specific test, inevitably result in an inherent instability in the outcome. For example, if a patient scores just below the cutoff level at one occasion and just above the cutoff at another occasion, that person would be described as reverting from MCI to normal [Petersen & O’Brien 2006], although the difference between the test results is insignificant. That is of course a correct statement, and possible bias caused by a probable gray zone around the cutoff score may arise. However, when MCI is based on clinical judgment and the label is not clearly defined, there will also always be cases where the MCI classification is at least equally uncertain; at one occasion a patient may be judged as MCI, and at another time – or by a different physician – the person may be judged as e.g. normal or demented.

By this alternate approach, using the SIM, MIM and NIM categorization scheme, we identified a group of patients who was at high risk of cognitive decline and dementia (SIM). Interestingly, the neuropsychological baseline profile of SIM showed a high resemblance with the concept of aMCI. Furthermore, the number of patients in SIM who converted to dementia during 3 years in our studies (Table 5), correspond well to the conversion rates reported for aMCI patients after 6 years of follow-up [Petersen 2004]. It may be argued that SIM has an advantage over aMCI since it is more clearly defined from cutoff scores in an easily administered neuropsychological test.

The baseline profile of the MIM group was reminiscent of what has been described as multiple domain naMCI [Petersen et al. 2001]. The prognosis of this group was more ambiguous than that of SIM; most patients remained non-demented during follow-up while approximately one fifth progressed to dementia. This further underlines the resemblance between MIM and naMCI, since naMCI does not convert to dementia at an equally high rate as aMCI [Fischer et al. 2007].

The subjective complaints in the NIM group were not associated with cognitive performance, or with longitudinal cognitive decline, since this group presented with normal cognitive function both at baseline and at follow-up. Our data are thus consistent with findings reporting a lack of association between SCI and objective performance in memory clinic samples [Kliegel et al. 2005].

Thus, the results from our studies, using the SIM, MIM and NIM categorization, corroborate findings that aMCI is highly predictive of subsequent dementia [Petersen et al. 1999; Petersen et al. 2001; Petersen 2004]. Consequently, we agree with the proposition that aMCI should receive serious consideration for inclusion in DSM-V, while naMCI should remain a research entity for further investigation [Petersen & O’Brien 2006]. In summary, the studies show that the SIM, MIM and NIM categorization scheme is a clinically useful tool to predict cognitive decline and conversion to dementia, as well as cognitive stability, during a mean time period of approximately three years, among middle-aged memory clinic patients.

Table 5 shows an overview of the characteristics of SIM, MIM and NIM based on the results from the studies in this thesis.
Table 5. Overview of the characteristics of SIM, MIM and NIM

<table>
<thead>
<tr>
<th>Characteristics</th>
<th>SIM</th>
<th>MIM</th>
<th>NIM</th>
<th>Study</th>
</tr>
</thead>
<tbody>
<tr>
<td>Cognitive decline during ≈3 years</td>
<td>yes</td>
<td>no</td>
<td>no</td>
<td>I, II, III</td>
</tr>
<tr>
<td>Severe cognitive deficits at follow-up (≥60%)</td>
<td>yes</td>
<td>no</td>
<td>no</td>
<td>I</td>
</tr>
<tr>
<td>Diagnosis of dementia at follow-up (≥60%)</td>
<td>yes</td>
<td>no</td>
<td>no</td>
<td>I, II, III</td>
</tr>
<tr>
<td>CSF T-tau (&gt;400 pg/ml)</td>
<td>yes</td>
<td>no</td>
<td>no</td>
<td>II, III</td>
</tr>
<tr>
<td>CSF P-tau (&gt;60 pg/ml)</td>
<td>yes</td>
<td>no</td>
<td>no</td>
<td>II, III</td>
</tr>
<tr>
<td>CSF Aβ42 (&lt;555 pg/ml)</td>
<td>yes</td>
<td>no</td>
<td>no</td>
<td>II, III</td>
</tr>
<tr>
<td>Increasing CSF P-tau levels during ≈3 years</td>
<td>yes</td>
<td>no</td>
<td>no</td>
<td>III</td>
</tr>
</tbody>
</table>

APOE ε4 allele (%)  
- SIM: 67%  
- MIM: 37%  
- NIM: 56%  
- Study II

Comparable concepts  
- SIM: aMCI  
- MIM: naMCI  
- NIM: SCI  
- Study I, II, IV

1 Reference value used at the clinical chemistry laboratory at the Karolinska University Hospital  
2 Except at baseline in study III  
3 Cutoff level derived from the statistical analysis in study IV  
4 Except among ε4 negative patients in study II  
5 APOE ε4 positive MIM patients showed significantly lower Aβ42 levels than NIM in study II

4.8 MEMORY IMPAIRMENT

We found that RAVLT, a verbal episodic memory test that includes both free and delayed recall tasks, was most discriminative of patients at high and low risk for cognitive decline. Furthermore, RAVLT was superior to the other memory tests that were performed at baseline (Logical Memory, free and cued recall, WMS-R [Wechsler 1987]; free and cued recall, 12 word list [Bäckman & Fosell 1994]; Rey-Osterrieth Complex Figure, immediate recall [Lezak 1995]). These other tests did not include repeated learning trials and a delayed recall task. Instead, they consisted of one single learning trial and immediate recall and cued recall tasks. Therefore, these other tests might not measure the same aspects of memory. Recall based on multiple presentations of material has been shown to be different from recall based on singular presentations, and it has been found that recall based on multiple presentations provide the most functionally relevant assessments [Johnstone et al. 2000]. It has also been shown that subjects in preclinical phases of AD are able to utilize cognitive reserve capacity to improve functioning [Bäckman & Small 1998], thus suggesting that tests that offer less opportunity for cognitive support are more suitable as diagnostic tools in early phases of dementia than tests that include e.g. cued recall or recognition tasks. Thus, other memory tests that assess memory and learning in similar ways as the RAVLT (e.g. other versions of auditory verbal learning tests) may be equally predictive for
progression to dementia.

One of the questions raised by our studies is whether the patients, who presented with severely impaired memory and fairly normal results in non-memory cognitive domains at baseline (SIM), suffer from the same or a different disease process as the patients in the MIM group, who presented with only slight or moderate memory and/or other cognitive impairments. It has been proposed that patients with subcortical VaD present with less pronounced memory impairments and more symptoms of frontal dysfunction, such as retrieval deficits and perseverations than AD patients [Looi & Sachdev 1999; Tierney et al. 2001; Traykov et al. 2002; Traykov et al. 2005]. Although highly speculative, our data may suggest that SIM represents a group of patients with symptoms described as typical of AD and that MIM rather represents patients with a different symptomatology and underlying disease process.

4.9 PATHOPHYSIOLOGICAL CONSIDERATIONS

In the AD disease process, the amyloid cascade hypothesis proposes accumulation of Aβ as one of the earliest molecular events, whereas tau pathology is thought to be a later event [Hardy & Selkoe 2002]. The initial memory loss in AD may be caused by synaptic failure. This in turn may be a consequence of the accumulation of soluble Aβ oligomers, possibly interfering with critical neuronal activities including long term potentiation, which may play an important role in learning and consolidation of long-term memory [Oddo et al. 2006; Shors & Matzel 1997]. The patients in study III that converted to dementia showed low CSF Aβ42 levels already at baseline, accompanied by severely impaired memory and subsequently increasing P-tau levels during cognitive progression. These findings are consistent with the amyloid cascade hypothesis; suggesting that accumulation of Aβ is an early event [Hardy & Selkoe 2002], and that CSF P-tau levels may reflect subsequent formation of NFT [Blinnow & Vanmechelen 2003]. Also in line with this hypothesis, a recent study reported that low CSF levels of Aβ42 predict later cognitive decline and dementia among initially non-demented women who were followed up during 8 years [Gustafson et al. 2007].

Very few studies have investigated longitudinal changes in CSF T-tau, P-tau and Aβ42, both in healthy controls and in patients with progressive neurodegenerative disorders. The study samples are small and results are inconsistent. Furthermore, CSF P-tau is the least studied of these three CSF biomarkers. To our knowledge, there is only one other study that has shown increasing P-tau levels (phosphorylated at threonine 231; P-tau231) in association with longitudinal hippocampal volume loss in MCI patients [de Leon et al. 2006]; thus this finding is consistent with our results that showed increasing CSF P-tau231 levels during progression from MCI to AD. However, de Leon et al. (2006) showed decreasing CSF Aβ42 levels in their patients while our study showed unchanging Aβ42 levels during follow-up. Decreasing CSF Aβ42 levels during progression in AD patients were also reported by Tapiola et al. (2000), while other studies found no significant CSF Aβ42 changes at follow-up [Andreasen et al. 1999; Kanai et al. 1998]. CSF T-tau levels have in some studies been reported to increase among AD patients during follow-up [Blomberg et al. 1996; Kanai et al. 1998; Kanai
et al. 1999), while others have shown relatively stable T-tau levels over extended periods of time [Andreasen et al. 1998; Sunderland et al. 1999; Tapiola et al. 2000].

As shown in Table 5, SIM patients showed increased T-tau and P-tau levels when compared to NIM and MIM, and both SIM and MIM patients who were positive for the APOE ε4 allele had significantly lower Aβ42 levels than the other groups. Above (in section 4.8) we speculated from a memory perspective that the different cognitive profiles between patients in SIM and MIM may not merely reflect different phases of disease processes, but rather different underlying disease processes in these groups. The differences in CSF biomarker patterns, together with the cognitive differences between these two groups, speak in favor of this interpretation. The SIM group showed increased T-tau and P-tau levels and low levels of Aβ42, while the MIM group showed decreased Aβ42 levels, but normal tau levels. The pattern found in SIM has been suggested to be typical of AD [Andreasen et al. 2001; Sunderland et al. 2003; Blennow 2004], and the CSF pattern found in MIM, have been reported in patients with VaD and DLB [Andreasen et al. 2001; Blennow & Vanmechelen 2003].

There was also a difference in APOE ε4 allele genotype prevalence between the SIM and MIM groups (Table 5). There were more ε4 positive patients in SIM than in MIM in study II. In study III, the number of APOE ε4 positive patients was increased in all memory groups, probably caused by the selection of patients who had been longitudinally followed up with LP, which resulted in a bias towards an increased number of patients who carried the APOE ε4 allele. In study II, we showed that CSF biomarker levels differed between ε4 positive and ε4 negative patients in SIM; SIM ε4+ patients showed increased levels of T-tau and P-tau and decreased levels of Aβ42 levels when compared to SIM ε4- patients. Moreover, APOE ε4+ patients in MIM also showed low levels of Aβ42, but normal levels of T-tau and P-tau. It is not clear whether the APOE genotype affects the expression of biomarker levels in CSF or whether the different biomarker patterns reflect different types of underlying disease processes in these groups. Other studies have also reported relationships between CSF Aβ42 levels and the APOE ε4 allele [Andreasen et al. 2001; Galasko et al. 1998; Ganzet et al. 2003; Tapiola et al. 2000; Riemenschneider et al. 2000], not only among demented patients, but also among healthy controls [Prince et al. 2004; Sunderland et al. 2004]. These results support the suggestion that the presence of the APOE ε4 allele may drive the Aβ accumulation in the brain [Lewis et al. 2006].

In line with the findings presented above – that the cognitive profile of SIM resemble aMCI which has been shown to be associated with AD, and these SIM patients show a CSF biomarker pattern reported to be typical of AD – together with the well-known finding that the APOE ε4 allele increases the risk for AD, it is tempting to interpret the pathological CSF biomarker levels among SIM ε4+ subjects and the normal levels among SIM ε4- patients as reflecting different types of disease processes in these two groups, rather than an APOE ε4 allele effect on CSF biomarkers within the same underlying disease. Also in line with the argumentation above, the findings in study II, that MIM ε4+ patients showed decreased Aβ42 levels and normal tau levels, may support our hypothesis that MIM patients maybe suffer from a different neurodegenerative process than the patients in SIM, since the CSF biomarker pattern found in MIM has been reported in patients with VaD and DLB [Andreasen et al. 2001;
Blenow & Vanmechelen 2003]. Thus the findings from study I-III may suggest – in different ways – that SIM patients develop AD at a high rate and that MIM patients who progress are more likely to develop other types of dementia.

Study IV showed that group A and B showed low CSF levels of Aβ42 at baseline while their baseline RAVLT delayed recall results differed; group A showed impaired delayed recall results while group B had fairly normal results at baseline. Furthermore, the cognitive profile of group A corresponded best to SIM and the cognitive profile of group B to MIM. Again, this difference may possibly suggest that the differences between patients in group A and B reflect different underlying disease processes.

On the other hand one could argue that the memory function in group B was not yet impaired at baseline, but at follow-up group B deteriorated slightly in delayed recall. A recent study has also suggested that amyloid deposition in the brain reaches a plateau in the early clinical stages of AD, and precedes cognitive decline [Engler et al. 2006]. Again, this could be in line with the suggestion that deposition of β-amyloid in the brain and reduction of Aβ42 in CSF is an early pathogenic event in AD [Hardy & Selkoe 2002]. Thus, as an alternative to the interpretation presented earlier in this section, our findings may reflect that patients in group A and B are in different stages of the same neurodegenerative disease process (i.e. AD), rather than affected by different underlying diseases. However, if patients in group B were in an earlier stage of disease (i.e. AD) than group A, then one would expect that group B at follow-up should resemble group A at baseline. This was not the case.

4.10 METHODOLOGICAL CONSIDERATIONS

4.10.1 Outcome measures

Many studies use clinical diagnoses as the main outcome measurement in studies on predictors for dementia. The validity of studies on predictors for a certain outcome target, e.g. dementia diseases, is always highly dependent on the reliability of the outcome measurement used [Zetterberg et al. 2003]. A wider range of outcome measures can be recommended to provide a more comprehensive account of the long-term effects in longitudinal studies of dementia [Winblad et al. 2006]. In study I, cognitive status (defined as severe cognitive deficits and clinical diagnoses of dementia) was used as an additional outcome measure to the primary outcome of cognitive decline. Although these outcome measures partially overlap, it was clear from the results, that the group level analyses of cognitive decline masked the cognitive deterioration of some individuals in the MIM group, which was revealed by the additional analyses of cognitive status at follow-up. The additional analyses also showed that that the proportion of patients in SIM, showing severe cognitive deficits at follow-up (84%), was higher than the proportion that was diagnosed as demented (64%). This discrepancy may reflect that in some patients, although showing severe cognitive deficits, their ability to perform activities of daily living was not impaired enough to fulfill the dementia criteria.

In study III, research diagnoses were established independently of the CSF biomarker
levels, APOE genotype and the SIM, MIM and NIM categorization. In study IV, where clinical diagnoses at follow-up were used as outcome measure in the CART-analysis, it would have been a clear advantage if research diagnoses had been established independently of the predictor variables, to avoid possible bias from circular reasoning. However, the purpose of the study was to find the best predictors of cognitive decline as well as cognitive stability – defined by the clinical diagnosis at follow-up – and to establish cutoff levels in the most predictive variables. In the second part of the study we defined our patients from the cutoff levels generated from the CART analysis and examined the longitudinal cognitive decline in these CART-generated groups (A-D). Possible bias from circular reasoning might therefore be minimized since the relevance of the CART-generated groups was further investigated by longitudinal cognitive function according to neuropsychological test results. Thus, the validity of the cutoff levels was not solely dependent on the clinical diagnosis at follow-up. Furthermore, the cutoff levels that were established for the RAVLT in study IV was similar to the cutoff levels that were established in study I, which were validated using longitudinal cognitive decline as the primary outcome measure.

The longitudinal approach is a clear strength of this thesis project. Each individual serves as his or her own control in the studies, which is advantageous when individual differences and changes over time are as marked as in the dementia research area [Gray & Della Sala 2004]. The mean follow-up time for the patients in the studies was approximately 3 years. However, there was a considerable variation between subjects in the time between baseline and the last neuropsychological follow-up. This is a common problem in clinical studies, where the needs of the patients rather than the needs of the researches naturally dictate the conditions. The advantages of a larger and naturalistic study sample have to be balanced against the benefit of a fixed follow-up interval. We chose the larger study sample and have tried to minimize the effect of these inter-individual differences in the statistical analyses by controlling for the effects of follow-up time.

4.10.2 Reliability

According to classical test theory, all test results are to some degree affected by measurement error, as well as by other factors [Turner et al. 2001]. In neuropsychological testing, such other factors are for example lack of motivation or temporary attentional deficits. Evidently, when assessing human behavior of any kind there are numerous sources of possible variations leading to measurement errors. Therefore, heavy demands are put on examination of the reliability of neuropsychological tests [Turner et al. 2001]. However, measurement errors do not only affect neuropsychological tests, and awareness of these problems is also called for when it comes to non-behavioral assessments, like e.g. analyses of protein levels in CSF. It has been shown that the outcome of CSF analyses may be influenced by confounding factors, such as a tendency for proteins to stick to the walls of test tubes made of polystyrene [Lewczuk et al. 2006]. Thus, only tubes made out of polypropylene should be used in studies of CSF proteins Blennow & Hampel 2003]. It has also been shown that Aβ42 and tau concentrations are stable in CSF samples that are immediately frozen and stored for longer periods at −80°C, while Aβ42
concentrations decrease by approximately 20% during the first two days when stored at 4, 18 and 37°C [Schoonenboom et al. 2005]. Furthermore, it has been found that the inter-laboratory variation, i.e. variation in CSF levels obtained at different laboratories, is in the range of 20-30%; and thus, the CSF levels reported from different research groups may differ significantly [Lewczuk et al. 2006].

4.10.3 Sample selection and representativity

Study I-IV were performed among fairly young memory clinic patients with subjective cognitive symptoms and fairly normal or slightly impaired general cognition according to the MMSE. The possibility to generalize the results to an older patient population with more overt cognitive symptoms is thus uncertain.

Paper I-IV were retrospective studies. The study samples were selective in the sense that only patients, who were clinically followed up after baseline investigations, since a progressive cognitive disorder was neither completely confirmed nor rejected, were included. Thus, there could be a risk of narrowing the generalizability of the studies if both healthy individuals and patients with clear symptoms suggestive of subsequent dementia were excluded. However, the results in our studies showed that our samples were cognitively heterogeneous since they consisted of patients with cognitive functions ranging from normal to impaired performance. In study II, approximately 30% of the sample showed normal cognitive performance (NIM) and remained normal throughout the follow-up period. Another third of the sample showed severe memory impairment and declined cognitively over time (SIM), and the remaining sample had slight impairments in memory and the majority of these patients did not decline at the follow-up (MIM). These proportions were representative of the diagnostic outcome of all patients investigated at the Karolinska Huddinge Memory Clinic during 1 year [Wahlund et al. 2003], and the mean age of these patients was also similar to the mean patient age in our study.

The prevalence of APOE ε4 carriers was higher among the patients in all our studies than has been reported from the general population [Eggersten et al. 1993]. This is probably caused by a tendency among people to seek help for subtle memory problems if they have had a relative suffering from dementia. A clear overrepresentation of ε4 allele positive patients was consistently found in our study samples, especially in SIM and NIM patients (Table 5). In SIM, the overrepresentation is probably reflecting the association between the APOE ε4 allele and dementia, and the high proportion of patients developing dementia during follow-up in this group. In NIM, the overrepresentation of the APOE ε4 allele is more likely one of the reasons why these patients were referred to follow-up investigations in the first place, despite their normal memory functions both at baseline and at follow-up. Furthermore, it has been found that the APOE ε4 allele frequency is higher in clinic-based AD samples than in community-based AD samples, probably because clinic-based samples contain younger patients with more severe disease who have higher APOE ε4 allele load [Tsuang et al. 1996]. Statistically, the high prevalence of APOE ε4 carriers in NIM suggests that this group is at an increased risk for AD or types of dementia [Corder et al. 1993; Hardy et al. 1994; Frisoni et al. 1994 Kalman et al. 2000; Andersen et al. 2000]. However, it has
been reported that APOE genotype reliably predicted AD only when memory test performance was included in the predictive model [Tierney et al. 1996]. Consequently, it has been suggested that APOE genotyping make an additional contribution to predict AD only in cognitively more impaired and homogeneous groups, but not in heterogeneous samples with less cognitive impairment [Lee et al. 2006]. Thus, it remains to be determined whether the e4 positive patients with normal memory function, from the heterogeneous memory clinic sample in our studies, are at an increased long-term risk of dementia or not.

In our studies as well as in many others, memory impairment was found to be a strong predictor for conversion to dementia. Furthermore, we showed that the degree of memory impairment was highly decisive of the cognitive prognosis during a mean time period of three years. However, there have been some contradicting findings concerning the predictive value of episodic memory. In some studies, no additive contribution of memory tests for prediction of dementia has been found over e.g. other psychometric measures [Storandt et al. 2002] or CSF biomarkers [de Leon et al. 2006]. This may in part be explained by selection of too homogenous samples. Since episodic memory is affected in the early course of the disease, individuals may reach floor on these tests at an early stage, and even more so as severity increases [Storandt et al. 2002]. Thus, in mild AD, language and visuospatial function – commonly affected in later phases than episodic memory – may predict further progression of clinically evident AD better than episodic memory tests, because language and visuospatial tests have no floor effect in mild AD [Lee et al. 2006]. Consequently, it is very important to study early predictors for dementia in appropriately heterogeneous samples, as we have attempted to do in the studies of this thesis.

4.10.4 Dementia diagnostics

In the studies of this thesis project we have not primarily focused on differential diagnostics of dementia, although we have discussed different possible underlying disease processes in relation to different clinical profiles. In general, the term dementia refers to a cognitive state and is a non-specific term that encompasses different disease processes. The term dementia in the studies of this thesis refers to unspecified types of dementia diseases, such as AD, VaD, DLB, FTD etc. The most important reason for this use of the concept is that our main approach was to use cognitive decline and not dementia diagnoses as the main outcome measure in the studies. A second reason is that differential diagnostics is difficult. Thus, it is easier to determine whether a patient fulfills diagnostic criteria for dementia or not, than to determine the specific etiology of the cognitive status.

The prevalence of VaD among patients investigated at the Karolinska Memory Clinic in 1999 and 2005 was 2-4% (Figure 1 and 2). These numbers differ substantially from what has been reported in the literature, where VaD consistently has been found to account for 15-20% of all dementia cases [Fratigioni et al. 2000]. Thus, it is not clear whether this means that VaD is underdiagnosed at our memory clinic, or whether AD is that much more common than VaD in younger patients who seek help at an early stage? An underrepresentation of patients with VaD has also been reported from other
dementia and memory clinics [Knopman 2006]. Both VaD and AD increase with
advancing age and no age-related variations have been found in the incidence of AD
and VaD that may explain why AD is more common than VaD in dementia and
memory clinics [Knopman 2006]. However, stroke survivors and patients with vascular
symptoms probably seek help at other specialist units and this may be an explanation
why VaD is less prevalent in our sample than in the general population.
5 CONCLUSIONS AND FUTURE PERSPECTIVES

In this thesis we have examined a number of methods that are used at the memory clinic at Karolinska University Hospital in Huddinge, in the clinical examination and diagnostics of patients who seek help for subjective cognitive problems. The methods examined in study I-IV are neuropsychological tests, LP with CSF analyses of T-tau, P-tau and Aβ42 and APOE genotyping, controlling for effects of demographic data such as age, education and follow-up time. To conclude:

- The cutoff levels in baseline RAVLT results that are provided in study I is a useful clinical method to identify patients who are at high and low risk for cognitive decline and dementia (SIM and NIM respectively).

- From cutoff levels in baseline RAVLT results, a group of patients was also identified whose cognitive prognosis was more uncertain (MIM); some patients (16-20%) progressed to dementia while the majority remained cognitively stable. Among these patients, the ability to predict cognitive decline and conversion to dementia was improved by adding LP examination and analyses of Aβ42 levels in CSF. Thus, our data suggests that neuropsychological memory assessment and LP with CSF analyses are complementing, useful methods in memory clinic diagnostic work; both to predict conversion to dementia but also to improve the ability to identify cognitive normality among patients with subjective complaints.

- A longitudinal increase in CSF P-tau levels was found among patients who decline cognitively and progress to AD. This increase suggests that CSF P-tau may be suitable as a longitudinal marker of the neurodegenerative disease process during progression from MCI to AD.

- A pathological CSF biomarker pattern that has previously been described as typical of AD – with increased T-tau and P-tau levels and decreased Aβ42 levels – was clearly associated with cognitive decline and progression to dementia, only among APOE ε4 positive patients with severe memory impairment and not among severely memory impaired patients without the APOE ε4 allele. It remains to be determined whether the APOE genotype affects the expression of biomarkers in CSF, or whether the different biomarker patterns reflect different types of disease processes in patients with progressive cognitive dysfunction.

Important questions are raised by this thesis project. In studies on predictors for dementia, the validity of the results is always dependent on the outcome measurement used. One of the concerns during this thesis project has been the lack of a gold standard for clinical establishment of AD and other types of dementia disorders, and one of the struggles has been to choose an outcome measure reliable enough to find valid predictors for dementia. Until there is a gold standard available for clinical differential diagnostics in dementia disorders, further studies using different types of outcome measures are needed. This is crucial in order to increase the understanding of the
variety of the clinical presentations in different dementia diseases, since there is still a lot to wish for concerning the correlation between clinical and pathologic diagnoses of dementia disorders.

RAVLT was found to be the most efficient neuropsychological test for identifying patients at high and low risk for cognitive decline and conversion to dementia. The cutoff levels provided in study I may be used clinically to determine the risk level for patients seeking help for subjective cognitive complaints and concerns about their memory. As always however, one has to be careful before applying group level data to clinical settings and to judgments at the individual level. At present, it is recommended that these cutoff levels are used with reason. At the general practitioner level, the cutoff levels may be used as guidance when determining which patients should be referred to specialist memory clinics, or as a complement to the general practitioner’s other methods to establish a diagnosis of dementia. At the specialist memory clinic level, the cutoff levels may be used as a complement to other currently used screening methods, like e.g. the crude MMSE test, especially to help identification of cognitive normality among subjectively impaired subjects. When the RAVLT cutoff scores indicate moderately impaired function (MIM), it is recommended that additional methods are used in order to find patients at risk for cognitive decline and conversion to dementia; preferentially LP with CSF analysis of Aβ42.

In order to establish the individual level clinical usefulness of the cutoff scores found for both RAVLT and Aβ42 in our studies, prospective studies on patients with subjective memory complaints in primary care as well as in memory clinics are needed. Moreover, using RAVLT as an assessment tool in prospective population based studies on initially cognitive normal persons with several years of follow-up would be important to study if RAVLT can be used as predictor for cognitive decline in the general population.

Can CSF P-tau be used as a marker for staging of the neurodegenerative disease process? Is Aβ42 more a marker for the initiation of the neurodegenerative process? Prospective studies on the longitudinal relationships between CSF biomarker changes and cognitive decline in a large number of patients with subjective memory complaints may answer these questions. Longitudinal studies on cognitive and biomarker changes in CSF should also be related to brain imaging information obtained by MRI and PET investigations.

The association between CSF biomarker levels and APOE genotype is intriguing. It would be interesting to focus on the APOE ε4 negative patients who cognitively declined and had normal CSF dementia biomarkers. In what way do they differ from the APOE ε4 positive patients who cognitively declined but had pathological biomarkers?

The above suggested prospective studies would increase the understanding of the different phases of the neurodegenerative disease process in preclinical and clinical dementia, improve our understanding of the underlying pathophysiological mechanisms and further improve early diagnostics.
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