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Search for reliable diagnostic markers for Alzheimer's Disease.

Akademisk avhandling

som för avläggande av medicine doktorsexamen
vid Karolinska Institutet offentligen försvaras
Fredagen den 19 Maj 2000 kl 09.00
I Hörsalen, Plan 4, Novum, Huddinge sjukhus

av

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Leg. Läkare

Stockholm 2000

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"By George!" cried the inspector.
"How ever did you see that?"
"Because I looked for it."

Sir Arthur Conan Doyle, md (1859-1930) in:
The Adventure of the Dancing Men.

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List of Original Publications

The thesis is based on the following papers, which will be referred to in the text by their roman numerals:

I Andreasen N, Vanmechelen E, van de Voorde A, Davidsson P, Hesse C, Tarvonen S, Räihä I, Sourander L, Winblad B, Blennow K. Cerebrospinal fluid tau protein as a biochemical marker for Alzheimer's disease: a community-based follow-up study.
J Neurol Neurosurg Psychiat 1998;64:298-305.

II Andreasen N, Hesse C, Davidsson P, Minthon L, Wallin A, Winblad B, Vanderstichele H, Vanmechelen E, Blennow K. Cerebrospinal fluid β-amyloid$_{(1-42)}$ in Alzheimer Disease: Differences Between Early- and Late-Onset Alzheimer Disease and Stability During the Course of Disease.
Arch Neurol 1999;56(6):673-80.

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

IV Andreasen N, Minthon L, Davidsson P, Vanderstichele H, Vanmechelen E, Winblad B, Blennow K. Evaluation of CSF-tau and CSF-Aβ42 as diagnostic markers for Alzheimer's disease in clinical practice. Submitted.

V Andreasen N, Minthon L, Vanmechelen E, Vanderstichele H, Davidsson P, Winblad B, Blennow K. Cerebrospinal fluid tau and Abeta42 as predictors of development of Alzheimer's disease in patients with mild cognitive impairment. Neurosci Lett 1999 Sep 24;273(1):5-8

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Abstract

The diagnosis of Alzheimer's disease (AD) is rather difficult not at least in the earlier stages of the disease. The diagnostic accuracy has been reported to be around 65-90%. These figures however, come from academic centers with a special interest in the disease and are based on patients followed for several years. One can speculate that the accuracy is lower at the non-specialist level. This thesis deals with the problem of finding reliable biomarkers in the cerebrospinal fluid (CSF) with acceptable sensitivity and specificity which can be utilized as diagnostic tools not only for the specialist.

The defining lesions of AD are the neurofibrillary tangles (NFT) and senile plaques (SP), composed of hyperphosphorylated tau and b-amyloid (Aβ) respectively. Therefore, we focused on total CSF-tau and Aβ42 as markers for AD, both individually and in combination with each other.

Paper I demonstrates a high sensitivity of CSF-tau for the diagnosis of AD but a lower specificity against other dementias. Specificity for more limited cerebral lesions such as ALS and Parkinsons disease, depression and normal aging is good. Moreover, we demonstrate that the stability between baseline CSF-tau and follow-up is good.

Paper II demonstrates high sensitivity for CSF-Aβ42 as a diagnostic marker for AD and a low intra-individual variation. Low CSF-Aβ42 levels are found even early in the course of the disease.

In *paper III* we again focus on CSF-tau but this time in a large prospective sample of consecutive AD patients from a community based sample. CSF-tau was analysed in routine clinical neurochemistry. We confirm the high sensitivity and specificity for depression and normal aging demonstrated in paper I. An increase in CSF-tau is seen very early in the course of the disease.

In *paper IV* we combine CSF-tau and CSF-Aβ42, looking at all patients referred to the clinic during one year. Both assays were analysed in routine clinical neurochemistry. Again we can demonstrate high sensitivity and specificity. We suggest the use of the combination of the two biomarkers in common clinical practice as an inexpensive and reliable tool in the diagnosis of AD.

In *paper V* we proceed with the early diagnosis. As we previously found high CSF-tau and low CSF-Aβ42 very early in the clinical course of AD we wanted to examine the potential of the two markers in MCI and indeed we found them useful even here. Since the sample was relatively small, more studies are required to confirm our findings.

Our conclusion is that the combination of the biomarkers tau and Aβ42 in the CSF have high sensitivity in the diagnosis of AD even in the very early course of the disease. The specificity for psychiatric disorders (e.g. depression) and some neurological disorders (e.g. Parkinson and ALS) is also good, but lower specificity is found for other dementias. Thus, we also stress that these biomarkers cannot be used alone, but should be part of the full clinical work-up.

Abbreviations

Aβ	β-amyloid
Aβ40, Aβ42	the 40 and 42 amino acid forms of β-amyloid
AD	Alzheimer's disease
ADL	Activities of daily living
ANOVA	Analysis of variance
ApoE	Apolipoprotein E
APP	Amyloid precusor protein
BBB	Blood Brain Barrier
CERAD	Consortium to Establish a Register for AD
CRP	C-reactive protein
CT	Computed Tomography
CJD	Creutzfeldt-Jacob's Disease
CSF	Cerebrospinal fluid
CV	Coefficient of variance
EAD	Early onset Alzheimer's disease
EEG	Electroencephalography
ELISA	Enzyme-Linked Immuno-Sorbent Assay
FAD	Familiar Alzheimer's Disease
FTD	Frontotemporal dementia
LAD	Late onset Alzheimer's disease
LA	Leucoaraiosis
LP	Lumbar puncture
MCI	Mild cognitive impairment
MIX	Mixed dementia
MMSE	Mini-Mental State Examination
MRI	Magnetic resonance imaging
NSE	Neuron specific enolase
NFT	Neurofibrillary tangles
PDP	The Piteå Dementia Project
PS-1	Presenilin 1
PS-2	Presenilin 2
PHF	Paired helical filaments
PHF-tau	Hyperphosphorylated tau
PLPH	Post-lumbar puncture headache
PSP	Progressive supranuclear palsy
TSH	Thyroid stimulating hormone
S	Serum
SP	Senile plaques
VAD	Vascular dementia
WML	White matter lesions

Introduction

During the 1970s and 1980s dementia and those it affected became an increasing problem for the Swedish healthcare system as well as for other developed countries with an aging population. As the Head of the Department of Rehabilitation in Piteå, Nothern Sweden I had two problems to face. First, our nursing homes and other forms of sheltered-living were full of patients who had gone through either a deficient diagnostic evaluation or none at all. This caused severe management problems for caregivers. Second, the patients´quality of life was clearly impaired. It became necessary to establish a department, which could primarily work with the clinical assessment and accurate diagnosis of patients with cognitive impairment.

By doing so we faced the next problem which was the somewhat weak clinical criteria used for the diagnosis of sporadic Alzheimer's disease (AD). It became more and more urgent to find good, reliable and inexpensive diagnostic markers for AD. Consequently I started "The Piteå Dementia Project" in 1986.

This work deals with the problem of finding reliable diagnostic markers for AD. Because the cerebrospinal fluid (CSF) may be considered the window to the brain I have focused my research on finding such biomarkers there.

History

The word "dementia," meaning "out of sense," is deviated from Latin: (de = no and mens = mind). Dementia is a clinical diagnosis, which is comprised of an assembly of symptoms and can be defined as: a global decline in memory and intellectual, personal and emotional capacity without impairment of consciousness (Lishman, 1987), yet sufficient enough to interfere with social and/or occupational performance (ICD-10, 1987; NIH, 1988; APA, 1994). As early as 1896 Kraepelin stressed the connection between dementia and aging (Kraepelin, 1896).

The most common form of dementia is AD. The German psychiatrist Alois Alzheimer was the first to describe this disease in 1907 with the case study of a 51 year old woman who presented with paranoid delusions. At the first visit he found memory impairment, as well as reading and writing difficulties. Later in the course of the illness she developed hallucinations, dysphasia, dysgnosia, dyspraxia and spatial disorientation. Post mortem he found gross cortical atrophy and on a microscopic level, nerve cell loss, senile plaques and neurofibrillary tangles (Alzheimer, 1907). In his textbook from the beginning of last century Kraepelin introduced the name "Alzheimer's disease" (AD) for the presenile dementia which appeared before the age of 65 (Kraepelin 1910).

Until 1950 arteriosclerosis was generally considered to be the major cause of dementia in the elderly. In 1951 Fisher showed that senile dementia was unrelated to cerebral arteriosclerosis (Fischer, 1951). This led to renewed interest in the findings of Alzheimer (Blessed et al, 1968). Today we realize that the case identified by Alzheimer was merely the tip of the iceberg and that dementia is one of the major health problems of old age. Moreover, it has an impact on the family caretaker for many years and is a big problem for the health care system.

Epidemiology

Early epidemiological studies were often based on indirect data collection through case records or informants (for review se Dohrenwend, 1990). With the introduction of DSM-III in 1980 a more systematic diagnostic system developed which gave renewed interest in epidemiological studies of dementia, especially AD.

During the recent years incidence and prevalence data have been reported in a large number of studies from several continents. The incidence and prevalence of AD appear to be fairly constant geographically and stable over time, at least in Europe (Hoffman et al, 1991). In their review, Jorm et al found that the prevalence of moderate to severe dementia doubled every 5.1 years from 1% in the age-span of 65-69 years to 39% at 90-95 years (Jorm et al, 1987). Ritchie and colleagues have confirmed these numbers (Ritchie et al, 1992). Recently Fratiglioni has reviewed studies publicized during the last 10 years (Fratiglioni et al, 1999) finding that both incidence and prevalence increase with age even in the very old. She also found little geographical variation although it is still unclear if the reported higher frequency of VAD in the Asian population is due to differential distribution of genetic and/or environmental factors, or due to methodological differences.

We have less detailed information about the geographical variation of other dementia forms, such as vascular dementia (VAD) and frontotemporal dementia (FTD). In addition, most studies on the incidence and prevalence of dementia are based on population surveys, which include all degrees of severity. This type of design is necessary in order to study the geographical variation. Moreover, the majority of the studies are performed near universities, i.e., in urban areas.

Dementia incidence and prevalence estimated from studies are summarized in table 1.

Table 1.
Published estimates of incidence and prevalence rates of dementia in various studies.

Authors	Year	Place	Age	Study type	Annual Incid. rate,%	Prev. rate,%	AD Propor- tion,%
Nielsen	1981	Rural Denmark	60+	Population survey	1.2	-	-
Shaji	1996	Rural India	60+	Population survey	-	3.4	41
Rocca	1990	Rural Italy	65+	Population survey	-	8.4	39
Mölsa	1982	Finland	65+	Population survey	0.4	2.0	50
Folstein	1991	Baltimore, US	65+	Community survey	-	4.5	44
Sulkava	1985	Finland	65+	Population survey	-	6.7	50
Li	1991	Beijing, China	65+	Population survey	0.3	1.1-1.8	40
Copeland	1992	Liverpool, UK	65+	Population survey, (moderate-severe cases)	0.92	2.4-3.8	68
Saunders	1993	Liverpool, UK	65+	Population survey	-	4.7	-
Morgan	1993	Nottingh, UK	65+	Population survey	0.9	-	-
Bickel	1994	Mannh, GFR	65+	Population survey	1.5	-	58
Boothby	1994	London, UK	65+	Population survey	2.0	-	48
Yoshitake	1995	Hisayama, Jap	65+	Population survey	2.0	-	38
Prencipe	1996	Rural Italy	65+	Population survey	-	8.0	64
Letenneur	1994	Bordeaux, Fr	65+	Population survey	1.6	-	70
Bachman	1993	Framingh, US	65+	Population survey	6.5	-	58
Jagger	1992	Melt Mowb,UK	75+	Population survey (mod-severe cases)	-	5.2	-
Engedal	1988	Oslo, Norway	75+	Population survey mild cases	-	6.7	-
				mod-severe cases	-	3.8	-
Fratiglioni	1991-1997	Stockh, Sweden	75+	Population survey	1.5	11.9	74
Hofman	1991	23 centr, Europe	75+	Population surveys	-	13.5	-
Juva	1993	Helsinki,Finland	75+	Population survey (moderate dementia)	-	11.8	-
Paykel	1994	Cambridge,UK	75+	Population survey	4.3	-	-
Hagnell	1992	Rural,Sweden	80+	Population survey	Lifetime AD-risk26-32%		
Magnusson	1989	Iceland	82+	Population survey	1.0	10	-
Gussekloo	1995	Leyden, NL.	85+	Population survey	6.9	-	-
Fichter	1996	Munich, GFR	85+	Population survey	11.6	-	-
Aevarsson	1996	Gothenburg, S	85-88	Population survey	9.0	-	36
Newens	1993	Newcastle,UK	45-64	In-patient records	0.007	0.03	64
Park	1994	Rural,South Kor	65+	Population survey + Clinical assessment	-	10.8	60

From a community health point of view however, the milder forms of dementia are of less interest than those which call for some form of medical care, institutionalization or other community action, for example when planning the volume of the various forms of sheltered living in a certain area.

The estimated prevalence range was 2.4 – 10.8 and for the Piteå study 2.2 (Andreasen et al, 1999). The estimate was near the lower range of those of other studies, or roughly one half of the average estimate in these studies. This is most likely a reflection of the fact that more severe cases were included in this study.

Genetics

As early as the 1940s and 1950s Sjögren and coworkers showed that AD was inherited as an autosomal dominant disease in some families (Sjögren et al, 1952). Further they demonstrated that first generation had a three to four times higher risk of developing AD. They described two kinds of hereditary: one with an early onset and autosomal dominant heredity and one with late onset and a more complex heredity. Even in late onset AD there is a substantial hereditary component with an increased risk for a first degree relative (Fratiglioni et al, 1993).

Trisomy 21
It is known that patients with Down's syndrome, with a trisomy 21, develop an early Alzheimer encephalopathy (Olson and Shaw, 1969). It was therefore interesting when St. George-Hyslop demonstrated a genetic linkage to chromosome 21 in some of the families with early onset AD (St. George-Hyslop et al, 1987). Later, in 1990, he and his group showed that AD is a genetically heterogeneous disorder by demonstrating that not all families with hereditary AD showed linkage to chromosome 21 (St. George-Hyslop et al, 1990).

Chromosome 21
Today we recognize five different mutations on the amyloid precusor protein (APP) gene on chromosome 21. Although we only know of some 20 families with these mutations, they are of great importance in explaining a pathway towards AD. In 1991 three different mutations were discovered in codon 717 in families with early onset Alzheimer´s Disease (EAD): V717I (Goate et al, 1991), V717F (Chartier-Harlin et al, 1991) and V717G (Murrell et al, 1991). "The Swedish mutation," which is a double mutation at position 670/671 was found a year later (Mullein et al, 1992; Lannfelt et el, 1994). The same year "The Dutch mutation" was identified. This was a mutation in codon 692 (Hendriks et al, 1992). The latest mutation identified on chromosome 21 is I716V (Beckman et al, 1997), and this mutation leads to EAD in the mid fifties.

Chromosome 14
The finding of mutations on chromosome 21 stimulated the search for additional mutations on other chromosomes. In 1993 Schellenberg could demonstrate linkage to the long arm of chromosome 14 (Schellenberg et al, 1993). Two years later five mutations in a novel gene from this region (S182) were identified in several cases of early onset familiar AD (FAD) (Sherrington et al, 1995). S-182 has later been renamed presenilin 1 or PS-1. The predicted

protein has 467 amino acids and multiple transmembrane domains. This locus has been shown, through linkage studies, to be the major cause of autosomal dominant early onset FAD. Today more than 50 AD-causing mutations have been demonstrated in the PS-1 gene (Cruts and Van Broeckhoven, 1998 a review).

Chromosome 1
Shortly after the detection of the PS-1 gene a second family member, presenilin 2 (PS-2) was isolated on chromosome 1 (Levy-Lahad et al, 1995), in a region already identified as the location for the gene causing FAD in families of Volga German ancestors (Rogaev et al, 1995; Bird et al, 1988). One Italian family has also been shown to possess the PS-1 gene mutation (Sorbi et al, 1997).

Chromosome 19 (ApoE)
ApoE is a protein involved in cholesterol transport, and it has been suggested to be involved in the recycling of lipids and in the turnover of myelin and neuronal membranes (Poirier, 1994). It has been shown to been found in astrocytes and increase after injury (Poirier et al, 1991) and is synthesized by astrocytes (Ignatius et al, 1986).

Previous studies (for review see Poirier, 1994) have suggested that after cell damage, released lipids are taken up by astrocytes and therein linked to ApoE. At the time of regeneration, ApoE-lipid complexes are secreted and reused in myelin and neuronal membrane synthesis. This process involves ApoE specific receptors (Handelmann et al, 1992).

The gene for ApoE is located on chromosome 19 (Olaisen et al, 1982) and it has three common alleles, designated ε2, ε3 and ε4. In the normal population the frequencies of ApoE phenotypes are ApoE2 7-8%, E3 77-78% and E4 14-16% (Roses, 1996). The frequency of ApoE4 is increased to about 40% among AD patients (Strittmatter and Roses, 1995). A dose dependent risk for developing AD is observed with one or two copies of the ε4 allele (Corder et al, 1993; Roses, 1995 review). The presence of the ε4 allele appears to decrease the age of onset of AD by about 3-4 years for one ε4 allele and 8-10 years for two ε4 alleles (Saunders et al, 1993b) and at the same time increase the burden of Aβ deposited in the brain (Strittmatter, 1995; Näslund et al, 1995).
The observation of AD cases with no ApoE-ε4 alleles and of healthy aged individuals with two ApoE-ε4 alleles indicates that ApoE-ε4 is neither necessary nor sufficient to cause AD (Lannfelt et al, 1994). On the other hand if dementia already exists, the possession of a ε4 allele strongly suggests that it is an AD (Saunders et al, 1996; Kakulas et al, 1996; Smith and Jobst, 1996; Welsh-Bohmer et al, 1997; Mayeux et al, 1998). About 60-70% of all

patients with AD have an e4 allele while it is seen in only 15-20% of the general population (Strittmatter, 1995).

The role of the ApoE in the pathogenesis of AD is somewhat unclear. Antibodies to ApoE have been found to stain SP (Namba et al, 1991) and AD patients with two ApoE4 alleles were found to have more and denser SP (Schmeckel et al, 1993; Rebeck et al, 1993).

In vitro studies have shown that Aβ forms complexes with the E3 isoform, but not with the E4 isoform (Zhou et al, 1996; Yang et al, 1997). The binding of Aβ to ApoE ε3 prevents the aggregation of Ab in SP which are an intricate part of AD pathology (Wisniewski et al, 1994; Castano et al, 1995; Pillot et al, 1997).

ApoE has also been found in NFT in brain tissue (Richey et al, 1995). Tau binds to ApoE3 but not to ApoE4 (Strittmatter et al, 1994). The binding of tau to ApoE3 is in the microtubule-binding repeat region which is the same region that appears to promote self-assembly of tau into NFT (Goedert, 1993). This data suggest that ApoE3 protect from phosphorylation while ApoE4 increases the phosphorylation of tau (Strittmatter and Roses, 1995).

Health Economics

As the prevalence of AD increases with increasing age (Jorm et al, 1987, Ritchie et al, 1992), the cost of dementia care has grown rapidly during the last few decades. The proportion of the oldest old (+85) has increased considerably (Statistical Yearbook of Sweden 1998, 1999), and is expected to rise in the coming decades. The number of demented people will therefore also increase if no cure emerges. This implies that even the costs for care of the demented will escalate substantially (Wimo et al, 1997a; for a review se Wimo et al, 1997b).

Bearing this in mind an economic assessment of dementia care and treatment should be a priority task for any health-care system. The gross cost of illness due to dementia in Sweden, with a population of approximately 9 million, was estimated to be SEK 30.7 billion, corresponding to 3,8 billion dollars in 1991 (Wimo et al, 1997a). The estimate for Denmark is DDK 77,000 (about $10,000) per person and year (Kronborg Andersen et al, 1999), in the US it is $1.75 trillion in 1991 (Ernst and Hay, 1994), and for Canada $3,9 billion (Ostbye and Crosse, 1994). In 1996 the cost per year in the US was $ 18,408 for a single patient with mild AD, $30,096 for a patient with moderate AD and $36,132 for a patient with severe AD (Leon et al, 1998).

Although these costs cannot be directly translated from one country to another because of differences in the health care systems, there is a similar trend that allows the data to be used more generally and not exclusively on the study population (Winblad et al, 1997).

Pathology

Macroscopic findings.

The macroscopic changes of the AD brain consist of diffuse, symmetrical brain atrophy of all the layers of the cortex, with shrinkage and widening of sulci, mainly in the frontal and temporal lobe. Subcortical structures are less affected (Brun and Gustafson, 1976; Tomlinson and Corsellis, 1984; Turkheimer et al, 1984; Terry, 1985; Perry, 1986; Braak and Braak, 1994). Global atrophy of the cortex is more pronounced in EAD whereas in late onset Alzheimer´s Disease (LAD) (Salat et al, 1999), the atrophy is most pronounced in the temporal lobe (Duyckaertes et al, 1985). Brain weight is frequently reduced in AD (Double et al, 1996). As brain weight normally depends on the patients´ sex, age and constitution, the degree of atrophy should be measured in regards to these factors. In uncomplicated cases of AD, the large cerebral vessels may show minimal arteriosclerotic changes consistent with aging.

Microscopic findings.

The histological features of AD are complex and varied. The main findings in the AD brain are neurofibrillary tangles (NFT), senile plaques (SP) and degeneration of neurons and synapses (Alzheimer, 1907; Coleman and Flood, 1987; Terry et al, 1994; Blennow et al, 1996a; Goedert, 1997). These are followed by astrocytic and microglia response, vascular and white matter changes, granulovascular degeneration and Hirano bodies (Mann et al, 1988; Hirano, 1994 a review; Brun, 1995). These changes may also be seen to a lesser extent in the normal aging brain. The definitive histopathological diagnosis of AD is based on the Consortium to Establish a Registry for Alzheimer's Disease (CERAD) criteria (Mirra et al, 1991), and includes atrophy of the brain, increase in the defoliation of the dendritic tree, neuronal loss, NFT and neuritic plaques. The hippocampal formation seems to be the earliest region in which a loss of neurons can be shown in AD. The pattern of neurodegeneration results in a functional isolation of the hippocampus from the cortical association areas (Hyman et al, 1984; Ball et al, 1985; Simic, 1997)
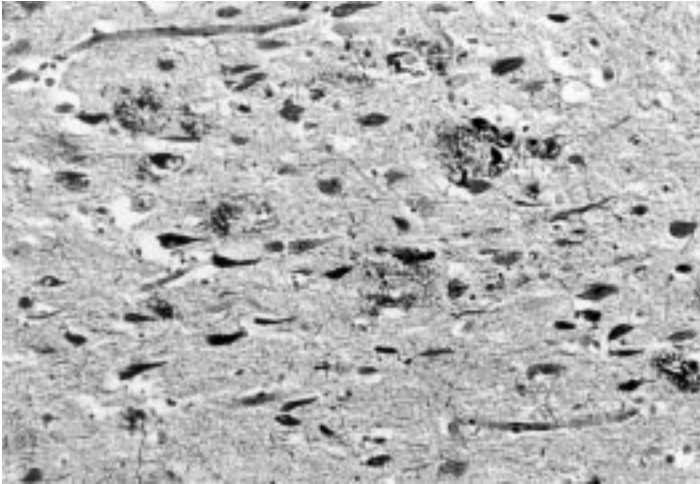
Picture by Nenad Bogdanovic.

Neurofibrillary Tangles.
NFTs are one of the hallmarks of AD. They are however not specific for AD, but may be found in a variety of degenerative brain disorders such as Dementia pugilistica, Progressive supranuclear palsy (PSP), Down's syndrome, Postencephalitic Parkinsonism and others (Dickson, 1997 a review). NFT are composed of abnormal fibrillary material that has accumulated in the cytoplasm of the large neurons. Similar material is found in the dendrites and axons (Goedert, 1998).

NFTs contain paired helical filaments (PHF), which appear to be formed primarily from abnormal aggregations of hyperphosphorylated tau-protein (Binder et al, 1985; Kosik, 1990). Six major isoforms of tau are present in the human brain. The tau gene is located on chromosome 17q21 (Sato-Harada et al, 1996) and contains eleven exons (Andreadis et al, 1992).

Tau-protein is a normal brain phosphoprotein, which binds to microtubules in the neuronal axons, thereby promoting the assembly and stability (Kowall and Kosik, 1987; Goedert, 1993). When hyperphosphorylated, as in AD, tau protein looses its ability to act as the "glue" that binds the microtubules together thereby causing instability in the axon and diminishing its transport ability (Ferreira et al, 1989; Iqbal and Grundke-Iqbal, 1997). The hyperphosphorylation of tau has been postulated to play an important role in the pathophysiology of AD (Trojanowski et al, 1990). Tau-protein is normally secreted to the CSF, but at present it is not possible to differentiate between normal tau and hyperphosphorylated tau (PHF-tau) with commercial methods of analysis. The level of total tau in the CSF is assumed to reflect neuronal, preferably axonal degeneration (Blennow et al, 1995) but it may also be related to the presence of NFTs in the brain (Tapiola et al, 1997).

Senile plaques

Senile plaques (SP) consists of a central amorphous, acellular core material called β-amyloid (Aβ), surrounded by a variety of neuronal and glial processes. The main protein in the core structure is Aβ42 (Glenner and Wong, 1984; Masters et al, 1985) which is a proteolytic product from the APP (Kang et al, 1987) and is stained by Congo staining. The APP gene is located on chromosome 21 (St. George-Hyslop et al, 1987). The amyloid deposits are found in different regions of the normal aging brain as well as in the initial stage of AD but then preferentially in the associative areas.

Aβ is metabolized along two pathways (fig 1). In the first pathway the cleavage of the APP molecule leads to a shorter non-amyloidogenic protein ending at Val-39 (Aβ39) or Val-40 (Aβ40). In the second pathway the cleavage is close to the Aβ fragment yielding a longer and potentially amyloidogenic fragment containing the intact Aβ sequence (Aβ42) (Selkoe, 1991, a review). The different forms of amyloid deposits contain different C-terminal truncated forms of Aβ, with Aβ42 predominating in diffuse plaques (Iwatsubo et al, 1994; Tamaoka et al, 1994), in SP cores (Miller et al, 1993; Roher et al, 1993) and in cortical homogenate (Gravina et al, 1995). Shorter forms predominate in both vascular amyloid (Prelli et al, 1988; Miller et al, 1993) and in CSF (Vigo-Pelfrey et al, 1993).

Apolipoprotein E

ApoE is situated on the surface of lipoprotein particles. It is engaged in carrying lipids (phospholipids and cholesterol) between cells (Mahley, 1988). Next to the liver the brain is the most important site of ApoE production (Elshourbagy et al, 1985). It is synthesized and secreted by the astrocytes but not the neurons (Diedrich et al, 1991). ApoE plays an important role in the coordinated storage and redistribution of phospholipids and cholesterol within the area of nerve damage (Poirier, 1994, Mahley and Huang, 1999 a review). Three different alleles (ε2, ε3 and ε4) exist at a single gene locus on chromosome 19 coding for 3 isoform of ApoE: ApoE2, ApoE3 and ApoE4. The different isoforms are found in; ApoE2 = 7-8%, ApoE3 = 77-78%, and ApoE4 = 14-16% of the general population (Farrer e t al, 1997).

Fig 1.

APP and Aβ metabolism

Secretase sites
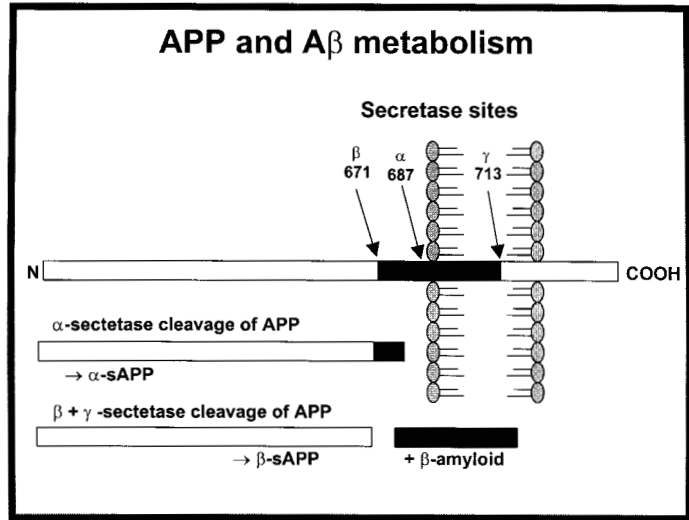
*Drawing by
Kaj Blennow*

ApoE has been identified as a susceptibility gene for LAD (Corder et al, 1993). Inheritance of an ε4 allele is associated with a dose-related higher risk and younger age of onset for AD. Having one ε4 increases the risk by about three times and a double ε4 by eight. On the other hand the ε2 allele reduce the possibility of developing AD and increases the age of onset (Corder et al, 1994).

The pathogenic mechanism of ApoE in AD is not fully understood. In vitro studies have shown that ApoE3 binds to the microtubule-associated protein tau with high avidity, while ApoE4 does not bind tau. This suggests that ApoE3, but not ApoE4, by binding to tau, slows the degree of tau phosphorylation and self-assembly into paired helical filaments (Strittmatter et al, 1994). Moreover the ε4 allele increases the amount of SP (Schmechel et al, 1993; Bales et al, 1997). It has also been suggested that intraneuronal ApoE, by interaction with tau protein, may influence the neuronal pathology in AD already early in the course of the disease (Han et al, 1994).

Cholinergic neurons are significantly reduced in AD cases with the ε4 allele (Arendt et al, 1997) which leads to a more pronounced acetylcholinergic deficit (Soininen et al, 1995). This may explain why treatment with acetylcholinesterase inhibitors is more successful in individuals without the ε4 allele (Farlow et al, 1998). Increased cholinergic neuron vulnerability and reduced plasticity in ε4 carriers suggests a less efficient handling of phospholipids and cholesterol in the CNS cells.

Diagnostics

AD exhibits etiological, clinical and pathological heterogeneity and besides rare cases produced by mutations on chromosome 1,14, and 21, the vast proportion of cases are sporadic. Characteristic pathological findings include neuronal loss, NFT and SP, but the severity of each of these changes varies considerably between individual patients. Up until now there is no consensus for biochemical markers for AD that would allow presymptomatic detection or definitive premorbid diagnosis. Therefore, an accurate diagnosis is more difficult, especially in the early phase of AD. In view of existing and upcoming pharmacological therapy, this is when accuracy is most crucial.

The diagnosis is an elusive goal despite the fact that the disease was described almost one hundred years ago. Up until recently there has been a lack of interest to improve methods to diagnose the disease. A common attitude among both professionals and the general public has been that the establishment of a correct diagnosis is inconsequential. The patients are over retirement age and there is no cure to be offered.

What was not taken into account was that even without a pharmacological approach to the disease a correct diagnosis would lead to a better understanding of the needs of the patients and thereby better care and quality of life. One can wonder why heart failure commands to greater efforts and resources from the medical profession than brain failure. After all the heart is nothing but a pump, - apart from in poetry!

The diagnosis of AD today is based on three different criteria-based diagnostic systems: The International classification of Diseases, 10th revision (ICD-10) (WHO, 1992), the Diagnostic and Statistical Manual of Mental Disorders, 4th edition (DSM-IV) (APA, 1994), and the National Institute of Neurological and Communicative Disorders and Stroke – Alzheimer's Disease and Related Disorders Association NINCDS-ADRDA Work Group criteria (McKhann et al, 1984).

ICD-10
The ICD-10 criteria focus on deterioration in both thinking and memory sufficient enough to impair activity of daily living (ADL). The decline in memory affects registration, storage and retrieval of new information. The guidelines require dementia, insidious onset, slow deterioration and the absence of other diseases, which can induce dementia.

Table 2 DSM-IV criteria. (Reproduced from APA 1987)

A   Development of multiple cognitive deficits manifested by both
     Memory impairment (impaired ability to learn new information or to recall previously learned information)
     One (or more) of the following cognitive disturbances:
       Aphasia
       Apraxia (impaired ability to carry out motor activities despite intact motor function)
       Agnosia (failure to recognize or identify objects despite intact sensory function)
       Disturbance in executive functioning (i.e., planning, organizing, sequencing, abstracting)

B.   The cognitive deficits in criteria A1 and A2 each cause significant impairment in social or occupational functioning and represent a significant decline from a previous level of functioning.

C.   The course is characterized by gradual onset and continuing cognitive decline.

D.   The cognitive deficits in criteria A1 and A2 are not due to any of the following:
       1. Other CNS conditions that cause progressive deficits in memory and cognition
       2. Systemic conditions that are known to cause dementia (e.g., hypothyroidism, vitamin B_{12} or folic acid deficiency, niacin deficiency, hypercalcemia, neurosyphilis, HIV infection)
       3. Substance-induced conditions

E.   The deficits do not occur exclusively during the course of a delirium

F.   The disturbance is not better accounted for by another Axis I Disorder (e.g. Major Depressive Disorder, Schizophrenia).

DSM-IV

DSM-IV is based exclusively on clinical findings. It defines dementia as a syndrome with multiple cognitive deficits including memory impairment and at least one of the following cognitive disturbances: aphasia, apraxia, agnosia or disturbances of executive functioning. These criteria are intended to serve only as guidelines for diagnosis and their application is dependent on clinical judgement (Erkinjuntti et al, 1997). Subtypes are EAD (onset at < 65 years of age) and LAD (onset at >65 years of age), se table 2.

NINCDS-ADRDA

The NINCDS-ADRDA criteria define definite, probable and possible AD. The differences between those three reflect available clinical information. Criteria for *definite AD* requires histopathological evidence of AD plus clinical criteria for probable AD. *Probable AD* requires deficits in two or more areas of cognition; progressive worsening of memory and other cognitive functions, no disturbances of consciousness, onset between the ages 40 to 90 and absence of systemic brain disorders that could explain the memory or cognitive decline. The diagnosis is supported by progressive deterioration of specific functions such as aphasia, apraxia, agnosia and impaired ADL. *Possible AD* is diagnosed when the patient has dementia with a clinical course that resembles AD, but also has a second brain disorder or systemic illness that is

sufficient to produce dementia but is not considered to be the cause of the dementia and the patient has a progressive deficit in the absence of any other cause. See table 3.

For an accurate diagnosis of AD it is vital to rule out other disease that can mimic dementia. Hence the clinical work-up must include a detailed medical history of concurrent somatic and psychiatric diseases, heredity, social and professional status and a description of the course of the actual symptoms. A thorough neurological examination is required as well as an evaluation of the cognitive status. The latter should be done with help from at least the Mini-Mental State examination (MMSE) (Folstein et al, 1975) but more sophisticated rating scales such as: the ADAS-cog scale (Rosen et al, 1984), the Blessed-Roth Dementia scale (Blessed et al, 1968), the Clinical Rating Test (CDR) (Morris, 1993) or the Gottfries-Bråne-Steen (GBS) (Gottfries et al, 1982) can be used.

Table 3. NINCDS-ADRDA criteria (Reproduced from McKhann 1984)

1 The criteria for the clinical diagnosis of "probable AD" include:
- Dementia established by clinical examination and documented by the mental status testing and confirmed by neuropsychological tests
- Deficits in two or more areas of cognition
- Progressive worsening of memory and other cognitive functions
- No disturbance of consciousness
- Onset between ages 40 and 90, most often after age 65
- Absence of systemic disorder or other brain diseases that in and of themselves could account for the progressive deficits in memory and cognition.

2 The diagnosis of "probable AD" is supported by:
- Progressive deterioration of specific cognitive functions such as aphasia, apraxia and agnosia
- Impaired activities of daily living and altered patterns of behavior
- Family history of similar disorder, particularly if confirmed neuropathology
- Laboratory results of: normal LP as evaluated by standard techniques
- Normal pattern or nonspecific changes in EEG, such as increase slow wave activity
- Evidence of cerebral atrophy on CT with progression documented by serial observation

3 Other clinical features consistent with the diagnosis of "probable AD":
- After exclusion of causes of dementia other than AD, include
- Plateaus in the course of progression of the illness
- Associated symptoms of depression, insomnia, incontinence, delusions, illusions, hallucinations, catastrophic verbal, emotional, or physical outbursts, sexual disorders, and weight loss
- Other neurologic abnormalities in some patients, especially with more advanced disease and including motor signs such as increased muscle tone, myoclonus, or gait disorder
- Seizures in advanced disease
- CT normal for age

4 Features that make the diagnosis "probable AD" uncertain or unlikely include:
- Sudden apoplectic onset
- Focal neurological findings such as hemiparesis, sensory loss, visual field deficits, and incoordination early in the course of the illness
- Seizures and gait disturbances at the onset or very early in the course of the illness

5 Clinical diagnosis of "possible AD":
- May be made on the basis of the dementia syndrome, in the absence of other neurologic, psychiatric, or systemic disorder sufficient to cause dementia, and in the presence of variations in the onset, in the presentation, or in the clinical course
- May be made in the presence of a second systemic or brain disorder sufficient to produce dementia, which is not considered to be the cause of the dementia
  Should be used in research studies when a single, gradual progressive severe cognitive deficit is identified in the absence of other identifiable cause

6 Criteria for diagnosis of "definite AD".
- The clinical criteria for probable AD and histopathologic evidence obtained from a biopsy or autopsy

NINDS-AIREN

Criteria for the diagnosis of vascular dementia (VAD) is defined according to the National Institute of Neurological Disorders and Stroke - Association Internationale pour la Recherche et l'Enseignement en Neurosciences (NINDS-AIREN) criteria (Roman et al, 1993).

Laboratory tests

Blood and Urine tests

The laboratory evaluation that is routinely performed includes: sedimentation rate, C-reactive protein (CRP), complete blood count, serum electrolytes (including calcium and albumin), glucose, creatinine, liver function tests, thyroid stimulating hormone (TSH), serum vitamin B_{12} and urine analysis. Optional tests are: serum folate, methylmalonic acid, serum ethanl, blood serology for Lues, Borrelia, HIV or other viruses, urine collection for heavy metals or toxicology (Marcusson et al, 1995).

Lumbar puncture

Quincke introduced the procedure of lumbar puncture in 1891. It has a low incidence of complications (Blennow et al, 1993a) and does not require hospitalization. Though it is seldom performed by a general practitioner (GP) it is certainly possible. The most common complication is the post-lumbar puncture headache (PLPH), which is characterized by a frontal, occipital or diffuse headache, with onset typically within a few hours after LP. The symptoms are considered to be caused by a persistent leak of CSF through the needle hole in the arachnoid and dural membranes into the epidural space (Gass et al, 1971; Lieberman et al, 1971; Gorelick and Biller, 1986; Blennow et al, 1993a; Gielen, 1989 a review). The incidence of PLPH has however been reduced to less than 2% due to the use of fine-gauged needles and by allowing the patient to rest in the supine position for one hour after the procedure (Blennow et al, 1993a).

As mentioned before the CSF can be considered to be the window to the brain and therefore it is most likely that the central processes in the brain in AD will be reflected here. Analysis provides information about possible damage to the blood brain barrier (BBB) as well as information about inflammatory or infectious processes in the brain. Increased white cellcount in CSF and intrathecal immunoglobulin production, evaluated using the IgG and IgM index and/or CSF specific oligoclonal bands, is found in chronic cerebral infections (such as neurolues, HIV and Borrelia), and inflammatory disorders (such as multiple sclerosis and systemic lupus erythematosus). As these disorders may mimic AD, they have to be excluded, which is most conveniently done through an LP. Further, it gives information about the different markers for AD such as CSF-tau and CSF-Aβ42, which are described in detail later in this thesis.

Brain imaging

Brain imaging is an important part of the standard investigation.
CT-scan
Computed tomography (CT) is the most often used method of x-ray investigation. It can be used to rule out conditions other than dementia. Approximately 5% of the patients evaluated for cognitive decline are found to have a treatable condition such as a subdural hematoma, hydrocephalus, cyst or brain tumor (Blennow, 1990). CT can also be used to estimate the degree of atrophy especially of the gyri, sulci and ventricles (Wippold et al, 1991). The use of an ordinary CT is somewhat troublesome as there is a substantial overlap of the atrophy seen in AD with that which can be expected in normal aging (George et al, 1983; Creasey et al, 1986). Data from the OPTIMA project of Oxford support that in AD the memory structures of the medial temporal lobe are affected early and severely in AD as seen on CT with temporal lobe orientation (Jobst et al, 1992). Furthermore the rate of progression of the atrophy was estimated to be ten times greater than that seen in normal aging (Smith, 1996).

The magnetic resonance imaging (MRI)
MRI has provided new opportunities to evaluate atrophy in AD. The T_1 weighted images are very useful in defining the anatomical structures surrounding the hippocampal fissure, while the T_2 image increase the sensitivity for detection of underlying abnormalities, especially ischemic lesions. Volumetric studies of the medial temporal lobe structures, in particular the hippocampus and amygdala show significant and consistent atrophy in AD patients (Rusinek et al, 1991; Julin et al 1997). The procedure is however rather time consuming and in a recent paper a visual assessment is proposed which only requires 1/10 of the time required today (Wahlund et al, 1999). MRI is an expensive method and is seen more as a complement to CT in clinical routine.

Electroencephalography (EEG)
EEG has been used in the diagnosis of brain diseases since the 1920s (Berger, 1929). In 1958 a standardized method for human EEG was implemented (Jasper, 1958). The EEG-activity has frequencies between 0.5 and 30 Hz and amplitudes of about 100 mV. The normal activity in an adult human at rest and awake is 8-12 Hz and most pronounced over the occipital lobe (Elmståhl et al, 1994). The typical EEG findings in AD are a decrease in alpha-frequency and increased slow-wave activity in delta and theta frequency (Johanneson et al, 1979; Katzman, 1981; McKhann et al, 1984; Rosen et al, 1993; Minthon et al, 1993). Diffuse slowing of the EEG pattern is seen in almost all dementias (Cummings, 1983; Petit, 1999). In AD there is often a distinct change early in the disease, while the EEG tends to be normal in mild cognitive impairment (MCI) (Jelic et al, 1996; Claus et al, 1999). However,
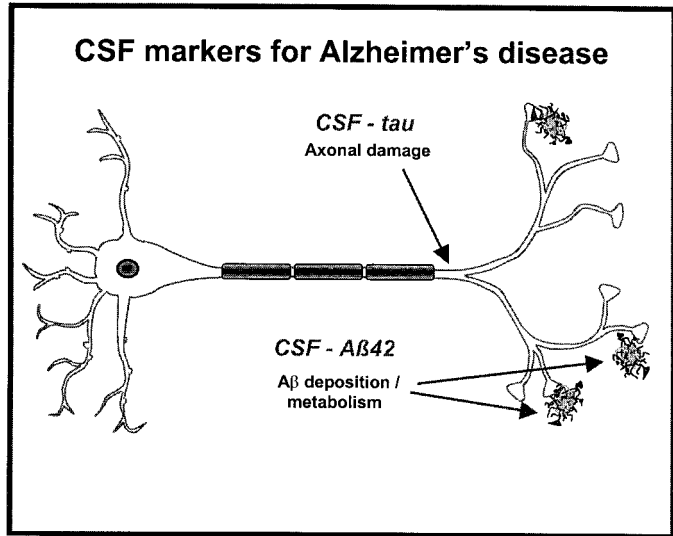
frequency analysis by means of quantitative EEG has shown in these subjects tendency to increased EEG slowing as assessed by means of alpha/theta ratio (Jelic et al, 1998). In the later stages of the disease the general slowing of the alpha-activity will be more pronounced and epileptiformic potentials can appear (Erkinjuntti et al, 1988; Rosen et al, 1993; Rosen, 1997).

The cost of a full clinical work up (January 2000) at Piteå River Valley Hospital is listed below (table 4). The doctors´ and nurses´ fee and the cost of the clinic is not estimated.

Table 4. The current cost of a clinical work-up at Piteå River Valley Hospital

Blood parameters
ESR, C-reactive protein
Complete blood count
Serum folate and serum B_{12} Blood glucose
Serum potassium, sodium, chloride and creatinine
Serum albumin and calcium (three times)
Alkaline phosphatases (ALP)
Aspartate aminotransferase (ASAT)
Alanine aminotransferase (ALAT)
g-glutamyl transferase
Thyroid-stimulating hormone, free thyroxin, triiodothyronine
Morning urine (protein, glucose, acetoacetate, pH, nitrate, Hb, LPC)   **449 SEK**

CSF parameters
Erythrocyte, Leucocyte, Chloride, Albumin, Glucose   **190 SEK**
Isoelectric focusing   **648 SEK**
Tau, Aβ42   **561 SEK**

Genetics
ApoE genotype   **310 SEK**

Brain imaging
CT-scan   **1400 SEK**
EEG   **1450 SEK**

Neuropsychological testing   **3000 SEK**

Total   **8008 SEK**

Fig 2.

CSF markers for Alzheimer's disease

CSF - tau
Axonal damage

CSF - Aß42
Aβ deposition /
metabolism

*Drawing by
Kaj Blennow*

Biochemical markers.

Three major potential uses for biomarkers are obvious. First, they might be important in the diagnosis of AD especially in the early course of the disease and in differentiating cognitive impairment in AD from that in normal aging. Second, such markers might help in selecting those patients sensitive to treatment and in differentiating between various therapeutic compounds. Third, they might be also useful for monitoring the treatment as they reflect changes in the defining processes of AD. One might even expect that these changes would be detectable much earlier than with traditional clinical trials where the outcome measures are more vague and subjective. Thus the study-time in such trials may be shortened with lower costs and the diagnosis will be more exact thereby making it possible for new compounds to become available to the AD patient earlier.

As mentioned before, the central microscopic changes in AD are an increased number of NFT, SP and degeneration of neurons and synapses, compared to that of non-demented individuals of the same age. Finding biochemical markers that reflect these changes would be ideal. Diagnosis would become more reliable, more cost efficient and even the non-specialist could make a more accurate diagnosis and thereby treat the disease at an earlier stage.

Traditionally one has looked for changes in the blood that may reflect these processes. Unfortunately this has been unsuccessful. ApoE genotyping has instead been suggested as a marker for AD. ApoE, a protein on the surface of

lipoproteins, is produced by astrocytes. It participates in the transportation of cholesterol and other lipids and is involved in the response to tissue injury (Poirier, 1994) where it provides the injured nerve cells with cholesterol and phospholipids for maintenance and repair of membranes (Mahley, 1988). Furthermore ApoE is involved in the growth of neurites, dendrite remodelling and synaptogenesis (Poirier, 1994).

Three different alleles of ApoE code for three different proteins, ApoE2, ApoE3 and ApoE4. In a large neuropathologic study of 2188 individuals from 26 AD centers, Mayeux et al (1998) showed that the sensitivity for e4 allele for AD was 65% and the specificity was 68% while the sensitivity and specificity for the clinical diagnosis was 93% and 55% respectively. With the combination of the e4 allele and the clinical diagnosis the sensitivity was 61% and the specificity 84%. Thus the determination of the ApoE genotype has most clinical value in patients that already fulfill the criteria of clinical AD.

Since the intercellular space of the brain is in direct contact with the CSF, biochemical changes in the brain may be reflected by CSF analyses. Therefore it seems reasonable to search in the CSF for one or more markers for AD. As it is believed that the disease has a preclinical course of 20-30 years (Davies et al, 1988; Price and Morris, 1999) one might expect to find CSF changes early in the course of the disease, perhaps even in the preclinical stages. The CSF biochemical markers should reflect those central pathogenic processes in AD: formation of plaques and tangles, and loss of neurons and synapses (Blennow et al, 1996a).

Quincke introduced LP in 1891. This procedure, however, has been some-what controversial for a long time because of the fear of complications, particularly the post-lumbar puncture headache (PLPH). While this is a common complication among younger individuals it has been shown to occur in less than 2% among elderly patients (Blennow et al, 1993a).

There are some methodological problems dealing with CSF-samples that should be taken into consideration. The sampling of CSF, the processing of the sample and the transportation and storage can influence the outcome of many of the analyses.

First it is important if the analyte will pass the blood-brain barrier (BBB). This is seen with many proteins with higher levels in serum than in the CSF (Tibblin et al, 1977). If so the CSF level will reflect the perifery rather than the CNS. This may also be of importance in the sampling since even a minor puncture hemorrhage will result in a falsly increased CSF level e.g. for IgM (Blennow et al, 1996b). A gradient within the brain and along the spinal cord is seen for many proteins (Blennow et al, 1993b).

It is important that the samples are treated correctly, and that the correct equipment is used. Many hydrophobic proteins will adhere to test tubes for example, and Aβ42 levels will decrease to 65% in polystyrene or glass tubes

It is also important to know if the sample can be frozen, and if so, can this be done before or after transport? What is the optimal temperature for freezing? Is it suitable with a long storage times and will repeated freeze-thaw cycles lead to proteolytic breakdown or aggregation of the protein of interest (Vanderstichele et al, 1998).

Tau protein
Tau-protein is a normal human brain phosphoprotein mainly synthesized in neurons (Kosik, 1989). This protein binds to microtubuli in the axons of the nerve cells thereby promoting mikrotubuli assembly and stability (Goedert, 1993; for review se Goedert 1996). The microtuble-bound tau is part of the cytoskeleton, which is important for the plasticity and regeneration of the neurons (Delacourte and Buee, 1997; for review se Brandt, 1996). Tau normally exists in 6 isoforms in the human brain (Goedert et al, 1989). As the degree of phosphorylation of tau increases the binding to the microtubule is inhibited (Drechsel et al, 1992). In AD abnormal hyperphosphorylated tau is found in paired helical filaments (PHF) which are the main components of the NFT (Grundke-Iqbal et al, 1986; Goedert, 1993; for review se Johnson and Hartigan, 1998), neuropil threads and senile plaque neurites (Terry, 1979; Grundke-Iqbal et al, 1986).

Aside from binding to microtubules tau has other functions. Interaction with phospholipase C-γ (Hwang et al, 1996) suggests a role in signal transduction, and it has been shown that tau has a role in regulating motor-protein mediated transport of organelles (Sato-Harada et al, 1996). Very high CSF-tau levels are found in rapedly progressive degenerative diseases such as Creutzfeldt-Jacob disease (Arai et al, 1995; Otto et al, 1997) and in acute destructive disorders such as stroke (Arai et al, 1995; Hessse et al, submitted). This suggests that the level of CSF-tau reflects the degree of neuronal/axonal degeneration. Studies have shown that the tau gene is located on chromosome 17 (Abel et al, 1993).

Since hyperphosphorylation of tau is a vital hallmark of AD it would be of interest to study PHF-tau in the CSF. Up until now the attempts to develop a commercial laboratory method for routine analyses of PHF-tau in normal clinical practice has not been successful (Wang et al, 1991; Vandermeeren et al, 1993). Blennow et al (1995) has shown, however, that total CSF-tau correlates well with PHF-tau. Tau in CSF has been shown to increase in AD in at least 30 studies (Wang et al, 1991; Vandermeeren, 1993 et al; Arai et al, 1995; Blennow et al, 1995; Hock et al, 1995; Jensen et al, 1995; Mori et al, 1995; Motter et al, 1995; Munroe et al, 1995; Nitsch et al, 1995; Tato et al,

1995; Vigo-Pelfrey et al, 1995; Rösler et al, 1996; Galasko et al, 1997; Galasko et al, 1998b). Some studies have even shown an increase of CSF-tau in VAD (Blennow et al, 1995; Skoog et al, 1995; Wallin et al, 1999) while others have found normal levels (Mori et al, 1995; Arai et al, 1998a). Even in patients suffering from head trauma there is an increase in the CSF-tau (Zemlan et al, 1999) as well as in FTD (Blennow et al, 1995; Arai et al, 1997; Green et al, 1999; Sjögren et al, 2000), however, Mecocci et al (1998) have found normal levels in FTD.

The highest levels of CSF-tau have been found in acute stroke (Arai et al, 1995; Hesse et al, submitted) but even in Creutzfeldt Jakob´s disease (CJD) levels of up to one hundred times that of normal individuals have been found (Arai et al, 1995, Otto et al, 1997). In contrast many other chronic neurologic diseases such as Parkinson´s disease (Blennow et al, 1995), progressive supranuclear palsy (Urakami et al, 1999), cerebellar degeneration (Mitani et al, 1998), HIV (Ellis et al, 1998) as well as psychiatric disorders such as depression (Blennow et al, 1995) have normal or very mildly elevated CSF-tau levels.

Another important issue is the stability of CSF-tau. Is there a variation during a 24-hour period or from day to day or year to year? It has been shown that there is reasonable stability during the disease (Sunderland et al, 1999). The stability of CSF-tau might indicate that it could be used as an outcome measure in the assessment when monitoring treatment with neuroprotective compounds.

Senile plaques
The second defining lesions of AD are the SP with a central core consisting of Aβ, surrounded by astrocytic processes, microglia, abnormal and degenerating nerve processes (Tomlinson and Corsellis, 1984). The SP are made up primarily of aggregated forms of Aβ (Glenner and Wong, 1984; Masters et al, 1985), which are proteolytic products from APP (Kang et al, 1987). Aβ is secreted into the extracellular space (Haass et al, 1992), which is continuous with CSF. Several isoform of Aβ exist, most of them being produced during normal cell metabolism (Haass et al, 1992). Forms of Aβ are found in the brain tissue of AD with heterogeneity at the N- and C-terminals.

The primary constituent of the SP and diffuse plaques is the Aβ isoform with 42 amino acids, Aβ42 (Miller et al, 1993; Roher et al, 1993; Iwatsubo et al, 1994 and1998; Prior et al, 1996). The shorter form Aβ40 has been found to predominate in vascular amyloid (Prelli et al, 1988) and in the CSF (Kuo et al, 1990; Vigo-Pelfrey et al, 1993). Because Aβ42 is more hydrophobic than shorter variants of Aβ (Jarret et al, 1993; Harigaya et al, 1995), it is possible that this form is more prone to aggregate in SP. Thus, the reduction of CSF-Aβ42 level in patients with AD may be secondary to an aggregation in the

amyloid deposits and SP, decreasing the amount of Aβ42 that can be secreted to the extracellular space, and thereby lowering the levels remaining in the CSF (Kuo et al, 1990). Another possible explanation is that reduction is secondary to amyloid mis-metabolism (Motter et al, 1995; Tamaoka et al, 1997).

Several studies using assays specific for Aβ42 have found decreased CSF-levels in AD patients (Motter et al, 1995; Ida et al, 1996; Tamaoka et al, 1997; Vanderstichele et al, 1998; Galasko et al, 1998b; Hulstaert et al, 1999). Longitudinal data show the same stability for CSF-Aβ42 as is shown for CSF-tau (Kanai et al, 1998). Although it is controversial whether, and how, Aβ may cause AD, this molecule is at the very least a critical marker for the disease process.

After acute ischemic stroke, there is a marked increase in CSF-tau within 1-2 days, peaking after 2-3 weeks, and returning to normal after 3-4 months. The level of CSF-Aβ42, however, remains unchanged (Hesse et al, submitted). This data supports the hypothesis that the level of CSF-tau reflects neuronal damage and degeneration, whereas the level of CSF-Aβ42 is not simply a product of neurodegeneration.

As CSF-Aβ42 and CSF-tau reflect two of the defining lesions in AD it is close at hand to combine the two analyses in the diagnosis of AD and thereby increase the sensitivity and specificity. This has been shown in some resent studies (Kanai et al, 1998; Galasko et al, 1998b; Hulstaert et al, 1999)

Table 5. Accuracy of the clinical diagnosis of AD

No Pat	Sensitivity	Specificity	Study	Country	Year
40	0,95	0,79	Lopez et al	USA	1999
56	0,93	0,23	Varma et al	UK	1999
134	0,83	-	Lim et al	USA	1999
61	0,58-0,83	-	Nagy et al	UK	1999
1833	0,96	-	Mayeux et al	USA	1998
201	0,88	-	Mirra et al	USA	1997
234	0,77	-	Pearl et al	USA	1997
72	0,95	0,81	Larson et al	USA	1996
53	0,96	0,86	Kosunen et al	Finland	1996
93	0,90	-	Klatka et al	USA	1996
100	0,90	-	Rasmusson et al	USA	1996
106	0,87	-	Gearing et al	USA	1995
196	0,86	-	Victoroff et al	USA	1995
76	0,71	0,95	Coen et al	Ireland	1994
170	0,90	-	Galasko et al	USA	1994
60	0,81	0,73	Blacker et al	USA	1994
94	0,98	0,69	Kazee et al	USA	1993
30	0,84	-	Harell et al	USA	1993
650	0,78	-	Mendez et al	USA	1992
142	0,84	-	Mirra et al	USA	1991
25	0,68	-	Risse et al	USA	1990
43	0,92	0,65	Kokull et al	USA	1990
675	0,77	-	Jellinger et al	Austria	1990
54	0,80	-	Boller et al	USA	1989
150	0,87	-	Joachim et al	USA	1988
57	0,64-0,86	-	Tierney et al	Canada	1988

Aims of the Thesis

The aim of this thesis is to investigate the qualification of CSF-tau and CSF-Aβ42 as biomarkers for AD in common clinical practice.

Study I

To study the sensitivity and specificity of CSF-tau as a biomarker of AD. Further to evaluate the stability of CSF-tau over time and evaluate if CSF-tau differs between patients with or without the ApoE ε4 allele.

Study II

To study the potential of CSF-Aβ42 as a biomarker of AD. To evaluate the sensitivity and specificity of CSF-Aβ42, the intra-individual biological variation of CSF-Aβ42 levels in patients with AD, and to examine the possible effects of differential binding between Aβ42 and ApoE isoforms on CSF-Aβ42 levels.

Study III

To further study the sensitivity, specificity and stability of CSF-tau in a large longitudinal community based sample, and to evaluate if CSF-tau is influenced by different co-variates such as gender, age, duration or severity of the disease or possession of the ApoE ε4 allele.

Study IV

To evaluate the utility of the combination of CSF-tau and CSF-Aβ42 as diagnostic markers in clinical practice.

Study V

To investigate the utility of CSF-tau and CSF-Aβ42 as predictors of development of AD in patients with MCI.

Materials and Methods

Patient sample

All studies were part of "The Piteå Dementia Project" (PDP), a project conducted through the Department of Rehabilitation at The Piteå River Valley Hospital in Norrbottens Län, Sweden. The project was started in January 1986 and is still in progress. This is a longitudinal study of clinically diagnosed cognitively impaired patients in a geographically well defined area in which a systematic case-finding procedure has been used (Andreasen et al, 1999).

The County Health Authority of Norrbotten County runs the Piteå River Valley Hospital. It serves the population of the Piteå River Valley, which incorporates four communities with a total population, in November 1993, of $\approx$ 62 000 persons. The city of Piteå is the largest city in the region. All four communities in the area have a well-established primary health care service covering the entire population.

Since 1990 national law requires the municipalities in Sweden to provide suitable housing, when needed, for those citizens that have some form of handicap. This includes dementia. The housing is subsided by the community if the citizen is unable to live in his or her normal dwelling despite maximum home-care. The four communities of Piteå River Valley have a common policy which states that the mental status of all persons with dementia, who are in need of community-provided housing must be assessed at the Neurogeriatric Section of the Department of Rehabilitation at Piteå River Valley Hospital. This implies that all persons with a cognitive disturbance severe enough to require medical attention will be seen at the Piteå River Valley Hospital.

During the period from 1990 to 1995 we assessed 619 patients. We found that the mean annual incidence of clinically relevant dementia was 295 per 100.000 persons at risk (Andreasen et al, 1999). The mean prevalence rate of dementia was 755 per 100.000 persons (Andreasen et al, 1999). This means that approximately 300 persons per 100.000 population over the age of 40 were in need of medical attention or social service interaction due to dementia disorders (Andreasen et al, 1999).

Examination of the patients

The patient-evaluations were performed in a standardized way, including the following items:

1.Clinical examination
The clinical assessment included a medical history obtained from the patient and from relatives or caregivers. A medical examination, which include a somatic, neuropsychiatric and neurological status was performed on all patients (Andreasen et al, 1999).

2. Neuropsychological assessment
Neuropsychological assessment was performed by a speech pathologist, with special postgraduate training in neuropsychological test methods. The tests used were Luria's neuropsychological test and Raven's matrices, Yerk's test, Koh's cubes, Trail making test, and Frostig's test (Christensen, 1984; Lezak, 1983,1995). The following items were measured: expressive and impressive language ability, spatial ability, ability of abstract and logic thinking, executive ability, and short-term memory.

3. Activity of daily living
Activity of daily living (ADL) was assessed according to the Sunnaas method (Vardenberg et al, 1991). According to this method, twelve items, such as eating, urinary or faecal continence and the ability to move indoors, were assessed on a scale ranging from "cannot" (=0) to "can do without problems" (=3).

In addition, the patients were observed during the evaluation period (one week) by the staff. These observations were noted on a special chart, which was used in the final diagnostic assessment.

4. Blood and urine samples
Blood was drawn from an antecubital vein for determination of hemoglobin (Hb), erythrocyte particle count (EPC) and volume fraction (EVF), mean erythrocyte cell volume (MCV), mean erythrocyte cell hemoglobin content (MCH) and concentration (MCHC), thrombocyte particle count (TPK), leukocyte particle count (LPK), leukocyte differential count, proportion of deviating leukocyte cell forms, erythrocyte sedimentation rate (ESR), acute C-reactive protein (CRP), serum folate, serum B_{12}, bilirubin, alkaline phosphatase (ALP), gamma-GT, AST, ALT, LD, alpha-amylase, blood glucose, serum potassium, sodium, chloride, calcium, albumin, creatinine, thyroid stimulating hormone (TSH), free thyroxin (FT_4) and triiodothyronine (FT_3), and ApoE genotyping.

Morning urine was collected for determination of protein and glucose, acetoacetate, pH, nitrate, hemoglobin and leukocyte count.

5. Lumbar puncture

LP was performed at baseline and at follow-up on all patients who consented to this investigation. The procedure was done between 11 and 12 a.m. Access to the CSF was made through the L3/L4 or L4/L5 interspace. Two 12 ml samples were collected in polypropylene tubes. Immediate analyses including a cell count, and after centrifugation, glucose and total protein, were performed at the local hospital. CSF was sent by ordinary mail to the Clinical Neurochemistry Laboratory at Sahlgren's University Hospital (a distance of 1.400 km).

At arrival (the day after the LP), isoelectric focusing and quantitative determinations of albumin, IgG and IgM in CSF and serum (S) were performed, and the albumin ratio (CSF/S), and IgG index and IgM index were calculated for evaluation of possible blood-brain barrier damage or intrathecal immunoglobulin production (Blennow et al, 1993c). The remaining CSF was stored at -80^0C for later analyses.

In study I, II and V the determinations were performed on frozen CSF in one session. In study III and IV the tests were done continuously as part of the clinical routine at the Department of Clinical Neuroscience, Unit of Neurochemistry, University of Göteborg, Sahlgren's University Hospital, Mölndal, Sweden.

6. Electroencephalography and CT-scan

EEG was performed on all subjects, and the α-activity frequency and possible focal abnormalities were recorded. Computerized tomography (CT) of the brain was performed by an experienced radiologist. Cortical atrophy, ventricular enlargement, white matter lesions (WML) and focal lesions (infarcts and lacunes) were recorded.

Patients included

The number of patients included in the studies is shown in table 6 and the basic clinical data in table 7.

Table 6. Number of patients included in study I – V					
Study	Duration of study	No. of patients included	No. of patients studied	No. of controls included	Inclusion criteria
1	6	377	75	18	All patients with 2 LP
2	2	131	53	21	All AD patients with 2 LP
3	5	909	407	65	All AD patients
4	1	265	246	100	All patients
5	3	56	16	15	All MCI → AD patients

Study I
In study 1 all 75 patients were drawn from the PDP. During the study period of 6 years 377 patient were examined and in 75 cases a follow-up investigation with an LP was performed. An additional group consisted of 37 patients with VAD from the Department of Geriatrics, University of Turku, Finland. In this group an follow-up investigation was performed after approximately three years, including a new CT scan to evaluate progression of leucoaraiosis (LA). In these cases only one LP was performed at baseline. The 9 ALS patients were examined at the Department of Neurology, Boden Hospital, Sweden. One control group consisted of 18 patients referred to the Department of Neurology, Boden Hospital, Sweden with only mild neurological symptoms (e.g. Cephalgia or Vertigo) but with no cognitive symptoms. These patients´ medical records were followed for approximately 10 years and not one developed symptoms of dementia. Another control group consisted of 18 individuals from Mölndal Hospital, Sweden, and included patients admitted for minor surgery (prostate hyperplasia or knee arthrosis) performed under spinal anesthesia. The controls were examined before the operation and had no history or signs of cognitive decline, or other psychiatric or neurological disorders.

Table 7. Basic clinical data on patients in study I – V

Study	No. of patients	Diagnosis	Gender (M/F)	Age	Duration	MMSE
1	43	AD	14/29	75	45	20
	11	MIX	5/6	79	33	21
	21	VAD	13/8	79	39	23
	37	VAD (SF)	21/16	74	-	-
	9	ALS (Boden)	5/4	72	-	-
2	53	AD	16/37	71.4	-	22.8
	17	EAD		64.1	-	23.6
	36	LAD		74.9	-	22.4
3	407	AD	168/239			
	274	Prob AD	92/182	74.8	42.9	22
	133	Poss AD	76/57	76.5	33.3	22.1
4	246	Total				
	105	Prob AD	39/66	75.9	39.6	22.8
	58	Poss AD	34/24	77.4	40.8	21.6
	23	VAD	14/9	76.5	38.4	22.3
	20	MCI	8/12	70.9	31.2	27.8
	9	LBD	5/4	74.6	25.2	20.9
5	16	MCI	8/8	71	-	≥28

Abbreviations: SF = Finland, Prob = probable, poss = possible. Age in years, duration in months.

Study II
This study consisted of 53 patient with AD from the PDP and the Department of Clinical Neuroscience, Neuropsychiatric Clinic, Malmö University Hospital, Sweden (with the same diagnostic criteria). The 21 healthy controls came from Mölndal Hospital, as described above.

Study III
The patients included in this study came from the PDP, were diagnosed with AD and had undergone an LP within the last 5-years. Out of 909 referred individuals, 484 (53,2%) had AD. Of those, 407 consented to an LP giving an acceptance rate of 84,1%. Out of these, 274 patients had probable AD and 133 possible AD. The healthy control group of 65 individuals from Mölndal Hospital, as described above.

Study IV

In this study, all consecutive patients admitted for investigation of cognitive symptoms during the course of one year (265 individuals from the PDP) were included. Of these, 246 patients had an LP performed, giving an acceptance rate of 92,8%. A large multi-center study (Hulstaert et al, 1999) was used as a control group. This study included CSF samples from 100 healthy volunteers or patients with disorders not associated with pathologic conditions of the brain.

Study V

This was the study of individuals admitted for evaluation of MCI and who later (after 1-2 years) developed AD with clinical dementia. We studied 16 patients. We included patients with MCI at baseline investigation, who at follow-up, showed a drop in MMSE score of two points or more, and who also showed one or more additional symptoms of dementia. We did not include patients that had not progressed to dementia at the follow-up examination. The rationale for this was that only a proportion of patients with MCI converts to dementia during each year of follow up (Petersen et al, 1997). This means that a very extensive follow-up period (>5 years) is needed to be certain that MCI patients will not develop dementia. Inclusion of these patients would thus have given inconclusive results with the follow-up period used in the present study. For the control group a large multi-center study (Hulstaert et al, 1999) was used, as described above.

Diagnostic criteria

In the final diagnostic assessment, information from all tests in the PDP study was used. Diagnoses were coded according to the Swedish version of the International Classification of Diseases, 9th edition in study I and 10th edition in the other studies.

In patients with dementia or psychiatric diseases the conditions were first diagnosed according to the DSM-lll-R criteria for study I and DSM-IV for study II-V.

AD

Probable and possible AD was defined according to the National Institute of Neurological Disorders and Communicative Disorders - Alzheimer's Disease and Related Disorders Association (NINCDS-ADRDA) criteria (McKhann et al, 1984).

MCI

Mild cognitive impairment (MCI) was defined as a decline in memory greater than would be expected for the patient's age (Petersen et al, 1997), but with

no other symptom than memory impairment reported or identified. An inclusion criterion was also a MMSE score of 27 or above. All MCI patients with symptoms or signs of other dementia disorders, such as vascular dementia and alcoholic dementia were excluded.

VAD
Probable and possible VAD and mixed AD/VAD was defined according to the National Institute of Neurological Disorders and Stroke - Association Internationale pour la Recherche et l'Enseignement en Neurosciences (NINDS-AIREN) criteria (Roman et al, 1993).

FTD
Probable or possible FTD was defined according to the consensus statement criteria (Brun et al, 1994). The frontal lobe syndrome was defined as a syndrome with predominance of personality change and the following emotional-motivational deficits: (1) emotional bluntness (indifference, aspontaneity, apathy, lack of initiative); (2) impaired control and modulation of emotions (disinhibition, tearfulness, aggressiveness, impulsiveness, inadequate smiling, indolent euphoria); and (3) lack of judgement and insight.

Controls
The control groups consisted of patients admitted for minor surgery. They were individuals without histories, symptoms or signs of psychiatric or neurological disease, malignant disease or systemic disorders (e.g. collagenosis, rheumatoid arthritis or infectious disease). None of the controls were receiving pharmacological therapy for neurological or psychiatric disorders. Their cognitive status was examined using the MMSE (Folstein et al, 1975). Individuals with scores below 28 were not included. All controls were examined at Mölndal Hospital, Sweden.

All diagnostic elements of the clinical work-up were considered and contributed to the final diagnosis and all diagnoses were set by the author (N.A.). In study I the diagnosis were reviewed by one of the other authors (K.B.).

Neurochemical Methods

The Swedish Board accredits the laboratory used in the studies in this thesis for Accreditation and Conformity Assessment (SWEDAC) in co-operation with the European co-operation for Accreditation (EA).
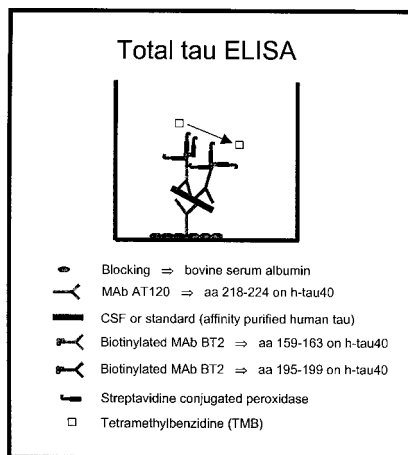
BBB-barrier function

Quantitative determination of albumin in serum (S) and CSF was performed using the Behring Nephelometer Analyzer (Behringwerke AG, Marburg, Germany). The CSF/S albumin ratio (Tibblin et al, 1977) was calculated as: [CSF-albumin (mg/l)/S-albumin (g/l)], and was used to measure the blood-brain barrier (BBB) function.

CSF-tau

CSF-tau was determined using a sandwich ELISA (Innotest hTAU-Ag, Innogenetics, Gent, Belgium), constructed to measure total tau (both normal tau and PHF-tau), as described previously in detail (Vandermeeren et al, 1993; Blennow et al, 1995) (Fig 3).

In study I and V, all analyses were performed at one occasion. In study III and IV the analyses were done as routine clinical neurochemical analyses, which were run approximately every second week (study III) or every week (study IV). All samples were run as duplicates. For use as internal controls, two CSF pools were made, one low-tau pool (CSF samples from patients with psychiatric and minor neurological disorders) and one high-tau pool (CSF samples from patients with AD), and were stored in aliquots at -80°C. These control samples were run on each ELISA plate.

Fig 3. Schematic drawing of the ELISA for CSF-tau determination.

Total tau ELISA

Blocking ⇒ bovine serum albumin
MAb AT120 ⇒ aa 218-224 on h-tau40
CSF or standard (affinity purified human tau)
Biotinylated MAb BT2 ⇒ aa 159-163 on h-tau40
Biotinylated MAb BT2 ⇒ aa 195-199 on h-tau40
Streptavidine conjugated peroxidase
Tetramethylbenzidine (TMB)

Drawing by Kaj Blennow

CSF-Aβ42

CSF-Aβ42 was determined using a sandwich ELISA [INNOTEST Aβ42 Innogenetics, Gent, Belgium] designed specifically to measure Aβ42, as described previously (Vanderstichele et al, 1998). The monoclonal antibody 21F12, which is highly specific for the C-terminus of the Aβ peptide, was used as capturing antibody, while the biotinylated monoclonal antibody 3D6, specific to the N-terminus, was used as a detector. Characteristics of the antibodies were described previously (Citron et al. 1997, Johnson-Wood et al. 1997). No cross-reactivity was observed with Aβ40 or shorter peptides, and Aβ43 was only detectable with a 40-fold lower affinity (Vanderstichele et al, 1998). High-performance liquid chromatography (HPLC) purified Aβ42 (Bachem), was used as standard. Intra-assay variability is less than 5% and inter-assay variability less than 10%. Sensitivity for the ELISA for CSF-samples was 50 pg/ml (5 SD above the absorbance level in the blank). The assay was linear in the range of 200-1500 pg/ml. All analyses were run on the same batch of antibodies and ELISA plates (Fig 4).

In study II and V, all analyses were performed at one occasion. In study IV the analyses were done as routine clinical neurochemical analyses, which were run approximately every week. All samples were run as duplicates. For use as internal controls, two CSF pools were made, one normal-Aβ42 pool (CSF samples from patients with psychiatric and minor neurological disorders) and one low-Aβ42 pool (CSF samples from patients with AD), and were stored in aliquots at -80ºC. These control samples were run on each ELISA plate.

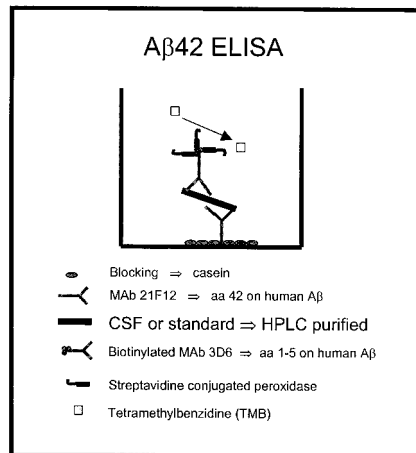**Fig 4. Schematic drawing of the ELISA for CSF-Aβ42 determination.**

Aβ42 ELISA

Blocking ⇒ casein
MAb 21F12 ⇒ aa 42 on human Aβ
CSF or standard ⇒ HPLC purified
Biotinylated MAb 3D6 ⇒ aa 1-5 on human Aβ
Streptavidine conjugated peroxidase
Tetramethylbenzidine (TMB)

Drawing by Kaj Blennow

Test tubes

To determine whether test tubes of different materials affect the measured levels of CSF-tau or CSF-Aβ42, lumbar puncture was performed on 7 patients with AD, and CSF was collected in polyetene tubes. Thereafter, fresh CSF was pipetted into three different types of tubes: polypropylene, polystyrene, and glass, incubated at 37°C for 3h, and finally assayed for CSF-tau and CSF-Aβ42. CSF-NSE, a glycolytic enzyme located in the neural cytoplasm (Pickel et al, 1976), was also determined as a reference protein, using a sandwich ELISA (Vanmechelen et al, 1997).

ApoE

Determination of the ApoE isoforms in study I, II and III was performed using isoelectric focusing (IEF) and Western blotting, with minor modifications (Kane and Gowland, 1986). In study IV the determination of ApoE alleles was performed by PCR followed by mini-sequencing as described in detail by Blennow et al (2000).

Statistical Methods

Study I

In all diagnostic groups, CSF-tau followed the normal distribution curve, but with a positive skewness and the variance was also higher in groups with higher means. Therefore, all data was log transformed before statistical comparisons between groups, which was performed using factor analysis of variance (ANOVA), with post hoc analyses (Tukey's HSD test for unequal N) for comparisons between two groups. The cut-off level (mean +2SD) for CSF-tau in the healthy control group was also determined after log transformation. The Spearman's correlations coefficient was used for correlations.

Study II

For group comparisons of clinical and biochemical variables, analysis of variance (ANOVA) with post-hoc comparisons (Tukey) was used. The Spearman coefficient was used for correlations. The reference limit was estimated as the 0.95 fractile of the control values using a rank-based method (IFCC, 1987).

Study III

Comparisons between groups were performed using factor analysis of variance (ANOVA), with post hoc analyses (Tukey's HSD test for unequal N) for comparisons between two groups. The Pearson's correlations coefficient was used for correlations.

The data were also investigated by principal component analysis (PCA) as implemented in the SIMCA-S software (version 6.0, Umetri AB, Sweden). Multivariate correlations between CSF-tau levels and clinical (sex, age, duration of disease, MMSE score and rate of progression) and neurochemical (CSF/serum albumin ratio, ApoE genotype) descriptors were examined using partial least squares (PLS) with cross validation (CV) as validation tool.

The relationship between sensitivity and specificity for both AD vs. controls and AD vs. dysthymia was described using Receiver Operating Characteristics (ROC) curve.

Study IV
Comparisons between groups were performed using factor analysis of variance (ANOVA), with post hoc analyses (Tukey's HSD test for unequal N) for comparisons between two groups. The Pearson's correlation coefficient was used for correlations. The sensitivity (i.e., the proportion of AD cases with high tau and low $A\beta42$ levels) and specificity (i.e. the proportion of other patients with normal tau and $A\beta42$ levels) were calculated using the cut-off line from the multi-center study (Hulstaert, 1999). The positive and negative predictive values for the combination of tau and Ab42 were calculated compared with the non-demented and depression groups.

Study V
For group comparisons of clinical and biochemical variables, the Mann-Whitney U-test was used, while the Pearson coefficient was used for correlations.

Ethics

The Ethics Committees of the Universities of Göteborg, Malmö and Umeà, Sweden approved all studies. All patients (or a relative) and controls gave their informed consent to participate in the studies. The studies were conducted in accordance with the provisions to the Helsinki Declaration.

Results

1. Comparison between AD, controls, other dementias and brain disorders.

1a. CSF-tau.

In paper I we found that at baseline investigation, CSF-tau was increased in AD as compared with ALS (p<0.002), neurological controls (p<0.0001), and healthy controls (p<0.0001), while it did not significantly differ between the AD, VAD or MIX groups. There was also a significant increase in CSF-tau in VAD compared with neurological controls (p<0.005) and healthy controls (p<0.0001), and in MIX compared with neurological controls (p<0.005) and healthy controls (p<0.0001).

Table 8. Comparison between AD, controls, other dementias and brain disorders.

Diagnosis	Paper I	Paper Ia	Paper III	Paper IV	Paper V
MCI				517±190	663±574
AD	796±382		690±341	759±417	
MIX	756±296		661±447	699±275	
LBD				238± 97	
VAD	708±382	234±173		461±280	
ALS	386±135				
Depression			231±101	400±115	
Neurol controls	356±193				
Controls	190± 57	190± 57	227±101	264±102	286±141

Paper Ia = Samples from Turku, Finland
The CSF-tau values are given in pg/ml ± SD

CSF-tau was also increased in the VAD samples from Turku, Finland as compared with healthy controls (p<0.01). However, when the VAD group was divided into patients with (n= 10) and without (n= 27) progressive leucoaraiosis (LA) at the one-year follow-up, an increase in CSF-tau was only found in VAD without progressive LA (p<0.005), but not in VAD with progressive LA compared with healthy controls.

In paper III, CSF-tau was increased at baseline investigation in the probable (p<0.0001) and possible AD groups (p<0.0001) as compared with the depression and the control group, while no significant difference was found between the depression and the control groups.

In paper IV we found a significant increase in CSF-tau in AD as compared with MCI (p=0.04), LBD (p<0.0001), VAD (p=0.001), and non-demented (p<0.0001). An increase in CSF-tau was also found in MIX compared with LBD (p=0.002), non-demented (p<0.0001), and VAD (p=0.042). No significant differences were found between the other diagnostic groups.

In paper V baseline CSF-tau was significantly increased in MCI patients as compared with the controls (p= 0.005).

1b CSF-Aβ42

In paper II CSF-Aβ42 levels were significantly decreased in the AD group as compared with the control group (p<0.001). The reference limit was 1130 pg/mL. When comparing the AD subgroups, CSF-Aβ42 was significantly lower in the EAD group as compared with the LAD group (p<0.001).

Table 9. Comparison between CSF Aβ42 and type of dementia

Diagnosis	Paper II	Paper IV	Paper V
MCI		640$\pm$260	507$\pm$221
AD	709$\pm$304	523$\pm$108	
EAD	422$\pm$170		
LAD	845$\pm$255		
MIX		572$\pm$225	
LBD		568$\pm$183	
VAD		704$\pm$321	
Depression		901$\pm$109	
Controls	1678$\pm$ 436	897$\pm$ 242	773$\pm$233

The CSF-Aβ42 values are given in pg/ml $\pm$ SD

In paper IV there was a marked decrease in CSF-Aβ42 in AD as compared with VAD (p=0.006), depression (p=0.003), and non-demented (p<0.0001). A decrease in CSF-Aβ42 was also found in MIX compared with depression (p=0.022) and non-demented (p<0.0001), in MCI compared with non-demented (p=0.006), and in LBD compared with non-demented (p=0.004). No significant differences were found between the other diagnostic groups.

In paper V, CSF-Aβ42 at baseline was significantly decreased in MCI patients as compared with the control group (p= 0.003).

2. Relation between age and CSF-biomarkers.

1a. CSF-tau.
In paper I there was no significant correlation between age and CSF-tau, neither in the AD, VAD, MIX, neurological controls, nor healthy control groups.

Diagnosis	Paper I		Paper III		Paper IV		Paper V	
MCI							-0.335	0.222
AD	-0.03	NS	0.02	NS	-0.10	0.324		
MIX	-0.01	NS	0.14	NS				
VAD	-0.01	NS						
Depression			0.74	<0.0001				
Neurol control	0.41	NS						
Health control	0.13	NS	0.24	NS			0.068	0.810

Table 10. Relation between age and CSF-tau.

Values are given as r-values.

In paper III there was no significant correlation between age and CSF-tau in the AD, possible AD or control groups while a significant correlation was found in the depression group.

In paper IV, there was no significant correlation between age and CSF-tau within the AD group

In paper V there was no significant correlation between age and CSF-tau, neither in the MCI nor in the control group.

1b. CSF-Aβ42
In paper II there was a significant positive correlation between age and CSF-Aβ42 level in the AD group, whereas no such correlation was found in the control group.

Within the AD group in paper IV, there was no significant correlation between age and CSF-Aβ42.

Table 11. Relation between age and CSF-Aβ42.						
Diagnosis	Paper II		Paper IV		Paper V	
AD	0.46	<0.001	0.0003	0.976	0.0506	0.839
Health control	-0.15				-0.186	0.506

Values are given as r-values.

There was no significant correlation between age and CSF-Aβ42, neither in the MCI nor in the control groups in paper V.

3. Stability of CSF-biomarkers between baseline and follow-up.

1a CSF-tau
In paper I CSF-tau did not significantly differ between baseline and follow-up investigations, neither in the AD group, the VAD group nor the MIX group. Within the AD group, there was a significant correlation between baseline and follow-up CSF-tau levels (r= 0.77; p<0.0001). Similar correlations were found within the VAD (r= 0.91; p<0.001) and MIX (r= 0.72; p<0.02) groups.

Table 12. Stability of CSF-tau between baseline and follow-up.						
Diagnosis	Paper I		Paper III		Paper V	
	Baseline	Follow-up	Baseline	Follow-up	Baseline	Follow-up
MCI					663±574	697±404
AD	796±382	791±411	697±318	710±337		
MIX	756±296	697±287	526±285	549±287		
VAD	708±382	695±378				
Depression			231±110	224±108		

CSF-tau values are given in pg/ml ± SD

In paper III CSF-tau did not significantly differ between baseline and follow-up investigations, neither in the probable AD, possible AD, nor dysthymia groups. Within the probable AD group, there was a significant correlation between baseline and follow-up CSF-tau levels (r= 0.64; p<0.0001). Within

the possible AD group, the correlation was r= 0.75 (p<0.0001). The patients with depression also showed a significant correlation between baseline and follow-up CSF-tau levels (r=0.96; p<0.0001).

In paper V, similar levels were found at baseline and follow-up for CSF-tau. There was also a highly significant correlation between baseline and first follow-up for CSF-tau (r= 0.795: p= 0.0001).

1b CSF- Aβ42
In paper II we studied whether CSF-Aβ42 levels change during the course of the disease. In the AD group, CSF-Aβ42 levels did not significantly differ between baseline and the first follow-up investigation at approximately 10 months, nor did the CSF-Aβ42 levels in the subgroups, EAD and LAD differ significantly between baseline and the first follow-up investigation.

| Table 13. Stability of CSF- Aβ42 between baseline and follow-up.				
Diagnosis	Paper II		Paper V	
	Baseline	Follow-up	Baseline	follow-up
MCI			663±574	697±404
AD	709±304	701±309		
EAD	422±170	406±145		
LAD	845±255	840±265		

The CSF-Aβ42 values are given in pg/ml ± SD

There was also a highly significant correlation between baseline and first follow-up CSF-Aβ42 levels (r= 0.90; p<0.0 01), and the coefficient of variation between baseline and follow-up levels was low (10.8%). The corresponding correlations were r= 0.80 (p<0.001) in the EAD subgroup and r= 0.81 (p<0.001) in the LAD subgroup.

In 10 patients with AD, 3 longitudinal CSF samples were collected (at baseline, and at 10- and 20-month follow-up visits). Also in these patients, the CSF-Aβ42 level showed no significant change, and most patients showed stable levels over time.

Fig 5. CSF-Aβ42 at baseline and follow-up 10 and 20 months later in 10 patients
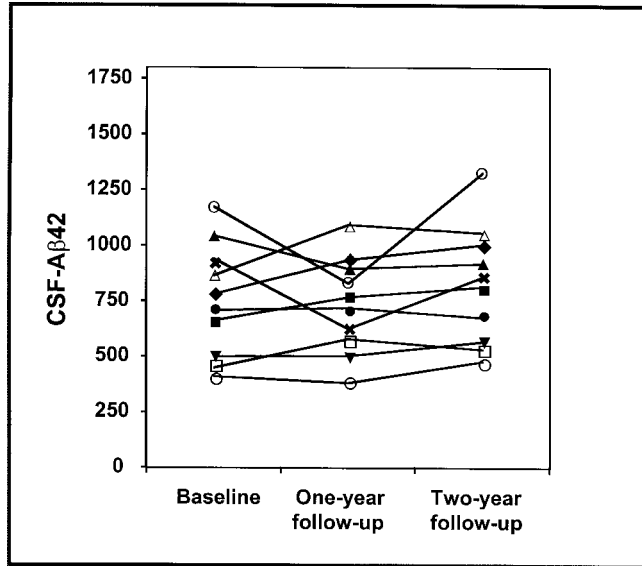
In paper V, similar levels were found at baseline and follow-up for CSF-Aβ42. There was also a highly significant correlation between baseline and first follow-up for CSF-Aβ42 (r= 0.942; p= 0.0001).

4. Relation to duration of disease.

1a CSF-tau
In paper I we did not find any significant correlation between duration of dementia and CSF-tau neither in the AD group, nor in the VAD or MIX group.

There was also no significant correlation between duration of dementia and CSF-tau either in the AD group, or in the possible AD groups in paper III and IV.

Table 14. Relation between CSF-tau and duration of disease.

Diagnosis	Paper I		Paper III		Paper IV	
AD	0.22	NS	-0.04	NS	0.01	NS
MIX	0.07	NS	-0.25	NS	-0.20	NS
VAD	-0.19	NS			-0.09	NS

Values are given as r-values.

In paper IV we did not find any significant correlation between duration of dementia and CSF-tau neither in the AD group, nor in the VAD or MIX group.

1b CSF- Aβ42
There was no significant correlation in paper II between duration of dementia and level of CSF-Aβ42 in the total AD group. Neither was there a significant correlation between duration of dementia and level of CSF-Aβ42 in the EAD nor in the LAD subgroups.

Table 15. Relation between CSF-Ab42 and duration of disease.

Diagnosis	Paper I		Paper IV	
AD	-0.16	NS	-0.03	NS
EAD	0.35	NS		
LAD	0.08	NS		
MIX			0.05	NS
VAD			-0.37	NS

Values are given as r-values.

In paper IV there was no significant correlation between duration of dementia and CSF-Aβ42, either in AD, MIX or VAD groups.

5. Relation to severity of dementia.

1a CSF-tau
There was no significant correlation between severity of dementia, estimated using MMSE, and CSF-tau, neither in the AD group, nor in the VAD or the MIX group in paper I.

Diagnosis	Paper I		Paper III		Paper IV	
Table 16. Relation between CSF-tau and severity of dementia.						
AD	-0.15	NS	-0.11	NS	-0.11	NS
MIX	-0.26	NS	-0.04	NS	0.04	NS
VAD	0.17	NS			-0.28	NS

Values are given as r-values.

There was no significant correlation between severity of dementia and CSF-tau in paper III, neither in the probable nor possible AD groups.

In paper IV we did not find any significant correlation between severity of dementia and CSF-tau neither in the AD group, nor in the VAD or MIX group.

1b CSF- Aβ42
In paper II there was no significant correlation between severity of dementia, estimated using the MMSE and CSF-Aβ42 level in the AD group, nor was there any correlation between MMSE score and CSF-Aβ42 level in the EAD nor LAD subgroups.

Table 17. Relation between CSF-Ab42 and severity of dementia.

Diagnosis	Paper II		Paper IV	
AD	-0,02	NS	0.25	NS
EAD	-0.14	NS		
LAD	0.13	NS		
MIX			0.17	NS
VAD			0.48	p= 0.019

Values are given as r-values.

In paper IV we did not find any significant correlation between severity of dementia and CSF-Aβ42 neither in the AD nor the MIX groups, while a slight positive correlation was found in the VAD group.

6. Relation to progression of disease.

1a CSF-tau
Progression of dementia in AD was measured as (30–MMSE score divided by duration of disease) and CSF-tau, and also as change in MMSE score between baseline and follow-up divided by time between the investigations.

Table 18. Relation between CSF-tau and progression of disease.

Measure	Diagnosis	Paper I		Paper III	
Progression between onset and baseline	Probable AD	-0.04	NS	0.01	NS
	Possible AD			-0.31	NS
Progression between baseline and follow-up	Probable AD	0.03	NS	-0.06	NS
	Possible AD			-0.21	NS

Values are given as r-values.

In paper I, there was no significant correlation between rate of progression and CSF-tau using any of these measures.

Neither in paper III was there any significant correlations between progression of dementia and CSF-tau, neither in probable nor possible AD patients.

1b CSF- Aβ42

In paper II, progression of dementia in AD was measured as (30–MMSE score divided by duration of disease).

Table 19. Relation between CSF-Aβ42 and progression of disease.

Diagnosis	Paper II	
AD	0.08	NS
EAD	-0.24	NS
LAD	-0.15	NS

Values are given as r-values.

We found no significant correlation between progression of dementia and CSF-Aβ42, either in the total AD group, or in the EAD or LAD subgroups.

7. Relation to the ApoE ε4 allele

1a CSF-tau

In paper I we compared CSF-tau levels in patients with and without the ApoE4 allele. No significant differences were found, neither within the AD group (n=13 without ApoE4 vs. n=22 with ApoE4), nor the VAD group (n=8 without ApoE4 vs. n=13 with ApoE4). Only two MIX patients did not possess an ApoE4 allele, making statistical analyses impossible. Neither were there any significant differences in change in CSF-tau levels between baseline and one-year follow-up, in the AD group, or the VAD group.

In paper IV, there was no significant difference between patients with the ApoE4 allele, regarding CSF-tau levels, either in the AD, MIX, VAD or LBD groups.
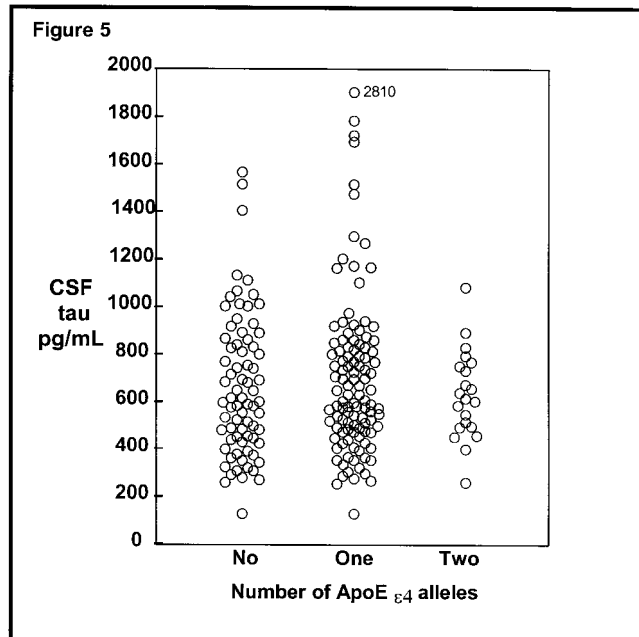
Table 20. Relation between CSF-tau and the ApoE ε4 allele.

| Diagnosis | Paper I | | Paper IV | |
	With ApoE4	without ApoE4	with ApoE4	without ApoE4
AD	738+323	875+415	730+319	892+590
MIX	765+505	672+301	680+234	716+310
VAD			338+257	486+284
LBD			188+70	262+105

The CSF-tau values are given in pg/ml + SD

In paper III there was no significant difference in CSF-tau between AD patients without (n=75), with one (n=110) or with two (n=21) ApoE ε4 alleles (Figure 6).

Figure 6.

Figure 5

55

1b CSF- Aβ42

In paper II we compared CSF-Aβ42 levels between individuals with and without the ApoE ε4 allele. No significant differences were found, in the AD group (n=16 without ε4 and n=36 with ε4), or in the control group (n=13 without ε4 vs. n=8 with ε4).

Table 21. Relation between CSF-Aβ42 and the ApoE ε4 allele.

| Diagnosis | Paper II | | Paper IV | |
	With ApoE4	without ApoE4	with ApoE4	without ApoE4
AD	731±393	690±259	622±228	482±137
MIX			649±251	484±153
LBD			624±203	456± 53
VAD			762±203	322± 62
Control	1702±339	1641±587		

The CSF-Aβ42 values are given in pg/ml ± SD

The impact of the ApoE ε4 allele on the CSF level of Aβ42 was also studied in paper IV. In this paper we found a significant difference in CSF-Aβ42 between patients with and without the ApoE ε4 allele, within the AD ($p<0.0001$), MIX ($p=0.004$), VAD ($p<0.0001$) groups. No significant difference was found in the LBD group ($p=0.215$), possibly due to the low number of patients (n= 6 vs. n=3).

8. Confounding factors

In paper II we examined whether test tubes of different materials had an influence on the analytical results. We found that Aβ42 levels showed a 33% reduction in glass tubes and a 36% reduction in polystyrene tubes, commonly used in laboratories, but did not change in polypropylene tubes (Fig. 7).
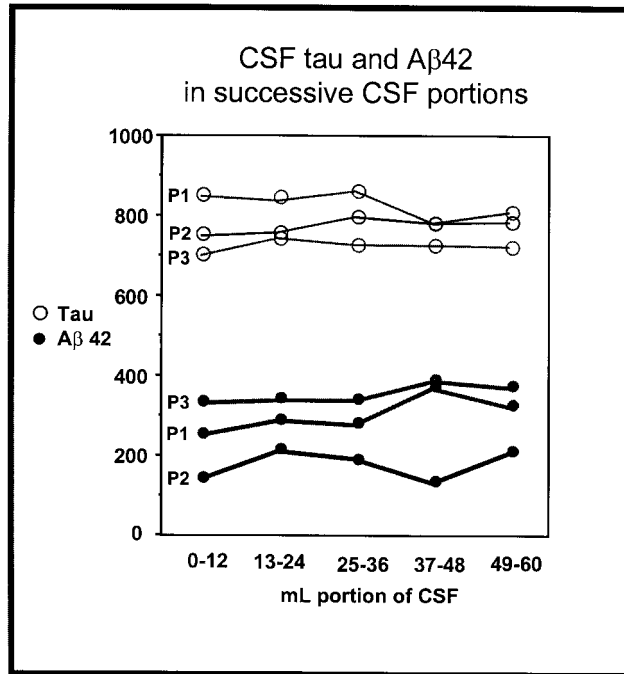
Fig 7. Influence of test tubes on CSF biomarkers
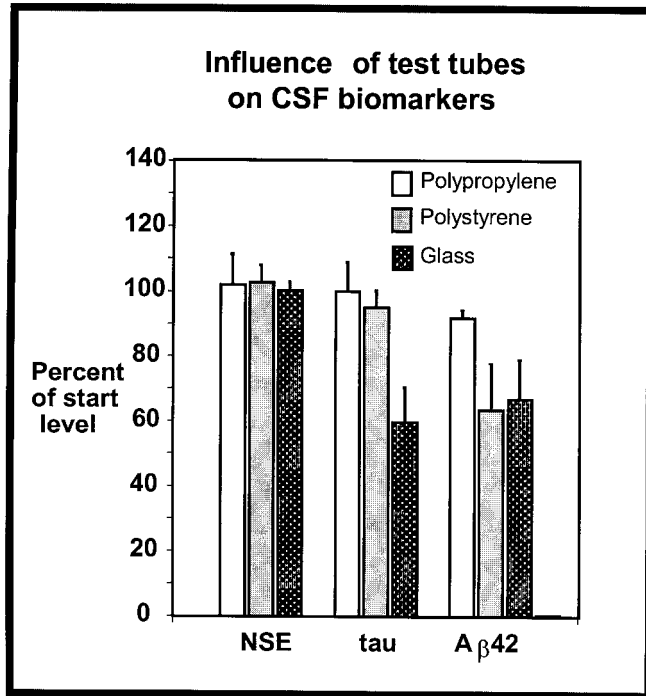
We also examined if there was any gradient in the CSF as is known for both proteins such as albumin (Blennow et al, 1993b) and monoamines (Blennow et al, 1993c). However, no gradient was found either for CSF-tau or CSF-Aβ42 as shown in fig 8.

Fig 8. CSF-tau and Aβ42 in 6 successive CSF portions of 3 patients.


9. Analytical stability

CSF-tau was run as a routine clinical neurochemical analysis in paper III, and both CSF-tau and CSF-Aβ42 in paper IV. Analyses were run approximately every second week (paper III) or every week (paper IV). All samples were run in duplicate. For use as internal controls, two CSF pools were made, one normal pool and one AD pool, which were run on each ELISA plate.

The coefficient of variance (CV) for these controls was 13.8% for the low control and 6.3% for the high control. In paper IV the CV for the internal control samples was 18.9% for CSF-tau and 10.7% for CSF-Aβ42 for the normal control and 10.1% for CSF-tau and 11.0% for CSF-Aβ42 for the AD control.

In paper IV we also studied the analytical variation for the CSF-Aβ42 assay and the stability of CSF-Aβ42 by re-analyzing stored CSF samples on one ELISA plate. In 41 CSF samples, the correlation between CSF-Aβ42 run in clinical routine at different times during one year, and the same samples re-run at one occasion was r= 0.964, p<0.0001 (Fig. 9).

Fig 9. Evaluation of analytical stability of CSF- Aβ42
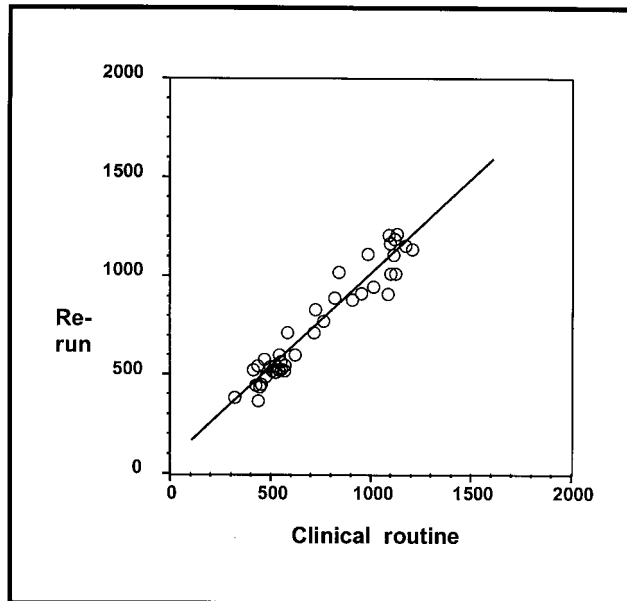
10. Sensitivity and Specificity

The individual valuables for each of the five studies are described in table 22.

In paper IV the sensitivity for the combination of CSF-tau and CSF-Aβ42 in patients possessing the ApoE ε4 allele increased from 95.2% to 73/74 (98.5%) for AD, from 90.2% to 27/27 (100%) for MIX and from 75% to 7/8 (87.5%) for MCI. In VAD, all three ApoE ε4 positive patients had pathological values for CSF-tau and CSF-Aβ42.

Table 22. Sensitivity and specificity in study I-V

	Biomarker	Sensitivity	Specificity
AD			
I	tau	95%	-
II	Aβ42	92%	-
III	tau	93%	86%
IV	tau + Aβ42	94%	-
MCI			
V	tau + Aβ42	75%	-
MIX			
I	tau	100%	-
IV	tau + Aβ42	89.7%	-
VAD			
I	tau	-	14%
IV	tau + Aβ42		48%
Control			
I	tau	-	94%
II	Aβ42	-	
III	tau	-	
IV	tau + Aβ42	-	88.9%
V	tau + Aβ42	-	80%

Discussion

The last twenty years has represented a tremendous challenge for those involved in the research of AD. Our knowledge has increased immensely, yet we have only taken a few steps on the path towards fully understanding the disease. A cure for AD is unfortunately still a mirage. On our way towards the final goal a reliable diagnostic technique is crucial, especially since early diagnosis is so important in view of the fact that first disease-arresting therapeutic principles will probably be emerging soon.

This thesis will deal with one of the efforts in this pursuit: biochemical markers in the CSF for AD. The focus has been to search for biochemical markers, which mirror the defining lesions in the disease and to determine to what extent we can rely on these markers in the diagnosis.

CSF-markers in AD and other types of dementia

We found a pronounced increased CSF-tau in AD patients, in agreement with numerous studies, published before and after our studies (Vandermeeren et al, 1993; Arai et al, 1995; Blennow et al, 1995; Jensen et al, 1995; Motter et al, 1995; Vigo-Pelfrey et al, 1995; Tato et al, 1995; Mori et al, 1995; Skoog et al, 1995; Nitsch et al, 1995; Munroe et al, 1995; Hock et al, 1995; Riemenschneider et al, 1996; Isoe et al, 1996; Blomberg et al, 1996; Lannfelt et al, 1996; Galasko et al, 1997; Shoji et al, 1998; Kanai et al, 1998; Nishimura et al, 1998; Mecocci et al, 1998; Kurz et al, 1998; Sunderland et al, 1999; Hulstaert et al, 1999; for review se Kaye, 1996; Trojanowski, 1996; Arai et al, 1998b; Galasko et al, 1998b). Thus, the elevation of CSF-tau in AD is consistently and firmly established.

We also showed increased CSF-tau in AD patients in clinical routine. This finding is a step towards the inclusion of CSF markers such as tau in the everyday clinical work-up of patients with cognitive impairment.

Increased CSF-tau was also found in MCI patients who later developed AD. Thus, this biomarker may be of value in the very early phase of AD.

The specificity is not optimal, however, since increased levels were also found in VAD. On the other hand, when studying the Finnish VAD group, in which follow-up examinations including MRI have been performed, an increase in CSF-tau was found only in the group without progressive LA. The neuropathological basis of LA has been suggested to be a demyelination of

the white matter surrounding the cerebral ventricles, probably caused by a form of arteriolosclerosis in the long penetrating vessels supplying the white matter. Other changes, such as amyloid angiopathy may also contribute (for review see Wallin and Blennow, 1991). A possible theory is that VAD patients with progressive LA may define a more "pure" form of VAD, where besides progressive LA, even have an increasing number of brain infarcts (Tarvonen-Schröder et al, 1995). In this group we found normal CSF-tau levels. In contrast, in the VAD group without progressive LA, higher CSF-tau levels were found. One possible explanation for this is that VAD patients may, in addition to cerebrovascular pathology, have concomitant AD pathology, which is impossible to exclude clinically. Indeed, several neuropathological studies have found that a high proportion (40-80%) of clinically diagnosed VAD patients have notable concomitant AD pathology (Jellinger, 1996; Kosunen et al, 1996).

Total CSF-tau is elevated in AD, but also in other degenerative disorders of the brain (Klatka et al, 1996; Mayeux et al, 1998). The mechanism(s) leading to the increase in CSF-tau in AD is still unclear. After acute ischemic stroke, there is a marked transient increase in CSF-tau and also a correlation with the size of the infarct (Hesse et al, submitted). These findings suggest that the level of tau in CSF reflects the degree of neuronal damage or degeneration.

Further, in Creutzfeldt-Jacob's disease (CJD), a disorder with very intense neuronal degeneration, a severe increase in CSF-tau is found (Otto et al, 1997), while in more localized cerebral degeneration such as Parkinson's disease, CSF-tau is normal or only slightly increased (Arai et al, 1995; Blennow et al, 1995; Consensus report, 1998). Also in psychiatric disorders, such as depression, CSF-tau is found to be normal (Blennow et al, 1995). These findings further support that CSF-tau reflects the degree of neuronal/axonal damage and degeneration. However, alternative explanations for the increase in CSF-tau in AD include a relation to the formation of neocortical neurofibrillary tangles (Zapotoczky, 1998), or possibly an increased expression of tau as an attempt to regenerate degenerating neuronal axons.

In the second paper we found a decrease of CSF-Aβ42 in AD, confirming earlier findings (Motter et al, 1995; Nitch et al, 1995; Pirttila et al, 1996; Ida et al, 1996; Tamaoka et al, 1997; Vanderstichele et al, 1998; Shoji et al, 1998; Galasko et al, 1998a+b). In paper IV we reconfirmed our previous findings that also in routine clinical use CSF-Aβ42 was reduced in AD.

We found in paper V that even in MCI which then proceeds to AD there is a decreased CSF-Aβ42 which is in agreement with the findings of others (Galasko et al, 1998b). This finding further supports the use of CSF biomarkers also in the early phase of the disease.

The mechanism(s) leading to a reduction in the CSF-Aβ42 level in patients with AD is still unclear. One possible explanation is that the reduction is secondary to the progressive degeneration of neurons. After acute ischemic stroke however, there is a marked increase in CSF-tau within 1-2 days, which reaches a peak after 2-3 weeks, and returns to normal values after 3-4 months, whereas the level of CSF-Aβ42 remains unchanged (Hesse et al, submitted). These data support the hypothesis that the level of CSF-tau reflects neuronal damage and degeneration, whereas the level of CSF-Aβ42 does not seem to simply reflect neurodegeneration.

Aβ is secreted to the extracellular space, which is continuous with the CSF. In both AD and control brains, Aβ exists in a water-soluble form (Kuo et al, 1998), whereas in AD, a portion of Ab aggregates and is incorporated into the highly insoluble fibrils in the plaques. These amyloid deposits consist primarily of Aβ42 (Miller et al, 1993; Roher et al, 1993; Tamaoka et al, 1994). Because Aβ42 is more hydrophobic than the shorter variants of Aβ (Jarret et al, 1993), it is possible that this form is more prone to aggregate in SP. Thus, alternatively, the reduction of CSF-Aβ42 level in AD patients may be secondary to the aggregation in the Aβ deposits and SP, leading to a decrease in the amount of Aβ42 that can be secreted to the extracellular space. Thereby resulting in lower levels remaining in the CSF, as suggested previously (Kuo et al, 1990). However, alternative explanations include reduced production or secretion of Aβ in the AD brain

Relation between CSF-biomarkers and aging

Neither in papers I, III or IV could we detect any correlation of CSF-tau with age. Since there were minor age-differences between the diagnostic groups, we performed multiple ANOVAs, which showed a significant effect by diagnosis but not by age for CSF-tau. Thus the differences found were probably not caused by age differences.

Since there were minor age-differences between the diagnostic groups also in paper IV, we performed multiple ANOVAs, which showed a significant effect by diagnosis but not by age for CSF-Aβ42. Also in paper V, there was no significant correlation between CSF-Aβ42 and age. These findings are in agreement with other studies (Motter et al, 1995; Nitch et al, 1995; Shoji et al, 1998, Galasko et al, 1998b).

In paper II however, we found a significant positive correlation between CSF-Aβ42 and age within the AD group, meaning that the CSF-Aβ42 levels were significantly lower in the EAD than in the LAD group. There is no obvious

explanation for this discrepancy between papers II and IV. However, one may speculate that it may be related to the selection of patients, since the AD patients in paper II were selected, while in paper IV, all consecutive patients during one year were included.

Stability of CSF-markers between baseline and follow-up

In study I, III and V, CSF-tau levels were stable between baseline and follow-up. We also studied whether CSF-Aβ42 levels change over time during the disease process. In study II, the CSF-Aβ42 levels were stable between baseline and follow-up investigations. Further, in study V, we found high correlations for CSF-Aβ42 between baseline and follow-up in patients with AD, and the percentage of patients with abnormal values were identical at baseline and follow-up.

These findings show that CSF-tau and CSF-Aβ42 levels are stable for each individual patient, i.e. that the total biological and methodological variations for the biomarkers are low.

A useful diagnostic marker for AD must give reproducible results over time, in order to be able to monitor biochemical changes due to the effect of potential new therapeutic strategies. Our results therefore suggest that it might be possible to detect the effect of a drug that decelerates the neuronal/ axonal degeneration or alters tau gene expression level in AD by monitoring CSF-tau. Similarly, it might be possible to detect the effect of a drug that affects the expression and/or deposition of Aβ by monitoring CSF-Aβ42.

Monitoring the CSF levels of these biomarkers may be a more sensitive tool than clinical rating scales, which besides being subjective also may be less sensitive, since a measurable change in cognition may require an extended period of evaluation while the biochemical effect may be detected much faster.

Relation between CSF-biomarkers and duration and severity of AD

We found it of interest to study if CSF-tau changes with time during the disease process. In none of the studies were we able to find any correlation between CSF-tau and duration of dementia. Most previous studies have not either found any significant correlation between CSF-tau and duration of dementia (Blennow et al, 1995; Nitsch et al, 1995; Golombowski et al, 1997; Galasko et al, 1998b), though some (Tato et al, 1995; Blomberg et al, 1996) have found a positive correlation.

Similarly, we found no correlation between CSF-Aβ42 levels and duration of dementia in the studies, which is also in agreement with other studies (Kanai et al, 1998).

In paper I, III and IV we tested if CSF-tau varies with the severity of dementia. We were not able to find any correlation. In most previous studies, no significant correlation between CSF-tau and severity of disease/MMSE scores has been found (Arai et al, 1995; Blennow et al, 1995; Motter et al, 1995; Vigo-Pelfrey et al, 1995; Nitch et al, 1995). We found high CSF-tau levels in patients with a very short clinical duration (6-12 months) and high MMSE scores (>23). Tato and co-workers, however, (1995) found a decrease in CSF-tau with higher MMSE scores. Neuropathological studies have found that the abundance of PHF-tau related pathology correlates with the severity of dementia (Braak and Braak, 1991; Arriagada et al, 1992). If the elevation of CSF-tau in AD is linked to formation of PHF-tau and NFT, increasing levels would be expected during the progression of the disease. Instead, our findings further support that CSF-tau levels reflect the degree of neuronal degeneration and that the activity of the degenerative process does not change during the different phases of clinical dementia.

In paper II and IV we investigated whether CSF-Aβ42 level varies with severity of dementia. We did not find any correlation. Two previous studies could not either show any significant correlation between CSF-Aβ42 level and severity of disease or MMSE scores (Motter et al, 1995; Ida et al, 1996). Again, the MMSE is a relatively insensitive instrument for reflecting the course of the disease, and the 1-year follow-up is a relatively short time period.

These findings support that high CSF-tau and low CSF-Aβ42 are present in the earlier stages of the disease. Because relatively few patients with MMSE scores below 15 were included in the studies, we cannot exclude the possibility that CSF-tau or CSF-Aβ42 levels may change in the very late course of the disease.

The findings that high CSF-tau and low CSF-Aβ42 levels are present during the earlier stages of the disease make these biomarkers useful as diagnostic markers early in the disease process when diagnosis is most difficult.

The next step was to investigate if high CSF-tau and low CSF-Aβ42 levels would be found even before the onset of clinical dementia. Consequently it was of interest to also study patients with MCI, and indeed, we found a high correlations for both CSF-tau and CSF-Aβ42 between baseline and follow-up, in agreement with our previous studies. Furthermore, the percentage of patients with abnormal values was high, and identical at baseline and follow-up. Thus, high CSF-tau and low CSF-Aβ42 levels are present in the earlier stages of the disease and probably already before the onset of clinical dementia. These findings further support that these biomarkers may also be useful as a diagnostic marker early in the disease process when diagnosis is most difficult. This may be of considerable importance when selecting patients with early memory disturbances for treatment and for participation in clinical drug trials.

Relation between CSF-biomarkers and rate of progression

As we believe that CSF-tau may reflect the degree of neuronal/axonal damage and degeneration we wanted to test if CSF-tau varies with the rate of progression of the disease, i.e. if higher CSF-tau values are found in patients with a more aggressive disease. Further, although most AD patients have high CSF-tau levels, the variability between different patients is high, ranging from about 200 pg/mL to very high levels of about 2000 pg/mL. Such variation may be related to warying degree of disease aggressiveness.

We therefore studied the rate of progression measured as change in MMSE between onset and baseline or between baseline and follow-up (which may give a more accurate measure) per time unit. We were not able to find any correlation between CSF-tau and rate of progression of dementia.

We also investigated whether CSF-Aβ42 levels vary with the rate of progression of the disease. In the EAD group, there was a tendency towards a correlation between CSF-Aβ42 levels and rate of progression of dementia, where lower levels of CSF-Aβ42 correlated with faster progression. We did not however, find any correlation between CSF-Aβ42 levels and rate of progression of dementia, but again, the MMSE is a relatively insensitive instrument for reflecting the course of the disease, and the 1-year follow-up is a relatively short time period.

Relation between CSF-biomarkers and the ApoE ε4 allele.

A higher frequency of the ApoE4 allele is found in AD than in the general population (Corder et al, 1993; Poirier et al, 1993; Saunders et al, 1993b). Some lines of evidence suggest a link between ApoE and tau protein. *First*, ApoE immunoreactivity is found in neurofibrillary tangles (Corder et al, 1993; Namba et al, 1991; Wisniewski and Frangione, 1992), which are composed of a hyperphosphorylated form of tau (Grundke-Iqbal et al, 1986; Ihara et al, 1986). *Second*, in vitro studies have shown that ApoE3 binds to the microtubule-associated protein tau with high avidity, while ApoE4 does not bind tau (Strittmatter et al, 1993). ApoE3 is able to bind to each of the four microtubule-binding repeats of tau, while ApoE4 only binds to repeat III, and not to repeats I, II, or IV (Huang et al, 1995). The binding between full-length tau and ApoE3 is abolished by phosphorylation of tau at serine$_{262}$, the only residue known to be phosphorylated on PHF-tau that lies in the microtubule-binding repeat region of tau (Huang et al, 1995). Using in situ binding techniques, ApoE has however, been found to bind to NFT in dephosphorylated tissue, probably by phosphorylation-dependent binding to tau (Richey et al, 1995), but the binding to NFT did not differ between brains with different ApoE alleles (Richey et al, 1995). ApoE is found to be decreased in CSF in AD patients (Hesse et al, 2000).

In study I, we showed that CSF-tau levels do not differ between AD or VAD patients with or without the ApoE4 allele. Neither in study III were we able to find any significant correlation between CSF-tau and between AD patients that do, or do not, possess the ApoE ee4 allele. To further evaluate such potential influences, we also performed a PLS analysis, which is a more powerful tool for finding correlations in data-sets where uni-variate correlations do not exist, but covariance between descriptors might be present, i.e. the clinical and neurochemical estimates in the present study. The PLS however, showed no predictive power for CSF-tau for the different clinical and neurochemical parameters examined.

These findings are in agreement with previous CSF-studies (Motter et al, 1995; Skoog et al, 1995; Nitch et al, 1995), and suggest that differential binding affinity between ApoE isoforms and tau does not influence the CSF-level of tau.

We studied whether CSF-Aβ42 level varies between patients that do, or do not, possess the ApoE ε4 allele. Several lines of evidence suggest a link between ApoE and Aβ. *First*, ApoE immunoreactivity is found in SP (Namba et al, 1991; Wisniewski and Frangione, 1992). *Second*, results of in vitro studies show that ApoE binds to Aβ. Results regarding different binding affinity between Aβ and ApoE isoforms are controversial, however, and seem mainly to depend on the experimental procedure (Strittmatter et al, 1993;

LaDu et al, 1994; Richey et al, 1995). CSF-Aβ42 levels did not differ, however, between patients with AD, with or without the ApoE4 allele, neither in study II nor study IV. These results are also in agreement with those from a previous CSF study (Motter et al, 1995), and suggest that possible differential binding affinity between the different ApoE isoforms and Aβ does not affect the CSF-level of Aβ.

Confounding factors

Population

When studying AD it is of great importance that the diagnosis is as accurate as possible. Therefore one must use as many parameters as feasible in this effort. Academic centers report accuracy rates for the clinical diagnosis of AD between 65-90% (Tierney et al, 1988; Galasko et al, 1994; Klatka et al, 1997; Bowler et al, 1998), although some studies have reported even lower figures (Mayeux et al, 1998). Moreover in academic centers one might expect that the cases presented are often more complicated and troublesome for the GP.

Furthermore in academic centers the patients are often evaluated by several different doctors, often doctors in training, which makes the assessments more heterogeneous. In these studies we use a community-based sample where all patients in the area are seen at the same center and by the same doctor. This may diminish the variation, and make the setting more uniform (Andreasen et al, 1999).

At the same time it is very important that the controls are "clean" from AD. In study I we had two control groups. One with healthy controls consisting of subjects without histories, symptoms or signs of psychiatric or neurological disease, malignant disease, or systemic disorders (e.g. rheumatoid arthritis, infectious disease). No individual had symptoms of cognitive impairment, and in individuals over 60 years of age, the cognitive status was examined using the Mini-Mental State Examination (Folstein et al, 1975). Individuals with scores below 28 were not included. The other group consisted of "neurological" controls with mild neurological symptoms such as headache and vertigo, where the medical examination did not reveal any organic brain disorder. None of the patients had cognitive impairment, and all patients were followed for 10 years through medical records and none developed dementia.

We found in paper I that CSF-tau was almost twice as high in the neurological controls as in the healthy controls. This may indicate that in spite of our efforts some incipient AD patients or other degenerative disorders

were harbored in the neurological control group, leading to a dilution of the results.

In paper II-V we studied healthy elderly individuals as controls, whereas some previous studies used patients with neurological disorders without cognitive impairment (Tamaoka et al, 1997).

Gradient in the CSF
It is known that monoamines for example have a gradient through the spinal tract (Blennow et al, 1993b). We examined if there was any gradient in the CSF of tau or Aβ42. CSF was sampled in six serial portions on three patients, but no gradients were found either for CSF-tau or CSF- Aβ42. These findings also suggest that the volume of CSF sampled is not critical for tau and Aβ42 determinations.

No correlation exist between BBB defects and CSF-tau and CSF-Aβ concentrations, suggesting that both CSF-tau and CSF-Aβ42 are produced within the central nervous system (Vanderstichele et al, 1998)

To eliminate time differences acting as a confounder we performed the LPs at the same time of the day (between eleven a.m. and twelve p.m.).

Transportation and handling of CSF samples
Repeated freezing and thawing of the CSF-samples do not influence CSF-tau and CSF-Aβ42 levels (Vanderstichele et al, 1998). CSF samples were sent at room temperature over a substantial distance (≈1000 miles), and were frozen after arrival to the lab until analysis. These findings suggest that the handling of CSF samples for routine analyses is not a critical confounding factor.

Influence of test tubes
Incubation experiments in test tubes of different materials showed that the level of neuron-specific enolase NSE, a cytosolic hydrophilic enzyme, did not significantly differ when incubated in different test tubes. In contrast, CSF-Aβ42 levels showed a 33% reduction in glass tubes, a 36% reduction in polystyrene tubes, commonly used in laboratories, and no change in polypropylene tubes. Therefore, we collected all CSF samples in polypropylene test tubes, to which Aβ does not adhere.

Taking into account all these confounding factors, the high sensitivity suggests that CSF-tau and CSF-Aβ42 level may be useful as biochemical markers for AD, especially when differentiating between AD and normal aging.

Performance of the ELISAs
The stability of the ELISAs, as determined by running both high and low control samples on each plate, was also acceptable, and in the range expected for immunoassays. The coefficient of variance (CV) for the internal control samples was low for CSF-tau, CSF-Aβ42 and for the normal controls. We also studied the analytical variation for the CSF-Aβ42 assay and the stability of CSF-Aβ42 by re-analyzing stored CSF samples previously analyzed in clinical routine on one ELISA plate. In these CSF samples, the correlation between CSF-Aβ42 run in clinical routines at different times during one year, and the same samples re-run at one occasion was high. These findings suggest that the ELISAs are robust.

Sensitivity and Specificity

The main objective of this thesis is to find reliable biomarkers for AD. As discussed earlier we have concentrated on biomarkers in the CSF. The two main defining lesions in AD are NFT and SP. The cardinal constituent of the NFT is PHF-tau and in SP the Aβ42, and therefore we have studied tau and Aβ42 respectively in the CSF. The central issue is the sensitivity and specificity of the two markers, individually and in combination with each other.

In the first paper we studied tau in patients with AD, MIX VAD, ALS versus two control groups: controls from a neurologic clinic and healthy controls. We found a high sensitivity in AD and MIX which is well over that which is recommended by the Consensus Group on Molecular and Biochemical markers of AD by the Ronald and Nancy Reagan Research Institute and the National Institute of Aging (Consensus report, 1998). On the other hand we found a low specificity against VAD, ALS and neurological controls. The specificity against healthy controls was clearly acceptable.

As discussed before we believe that CSF-tau reflect the neuronal degeneration. It is not surprising therefore, that we also found elevated CSF-tau values in VAD patients. In the first place, many reports have shown a concomitant AD pathology when the diagnosis was controlled postmortem (Tierney, 1988; Galasko et al, 1994; Klatka et al, 1997). In the second place, the cell death in VAD alone leads to elevated CSF-tau values (Hesse et al, submitted). On the other hand we should therefore expect to find normal CSF-tau values in ALS as this is a more localized degeneration (Arai et al, 1995; Blennow et al, 1995).

In paper III we wanted to examine the sensitivity and specificity of CSF-tau as a diagnostic marker in clinical practice for AD in a large sample. We found a

sensitivity of 93% and thus were able to confirm the high sensitivity seen in the first paper. We even studied which cutoff level would produce the best sensitivity and specificity. With ROC curve analyses we found that a cut-off level of 302 pg/mL resulted in a sensitivity of 93%, a specificity of 86% for AD versus elderly controls, while the corresponding values were 74% and 96% for AD versus dysthymia. Academic centers report accuracy rates for the clinical diagnosis of AD between 65-90% (Tierney et al, 1988; Galasko et al, 1994; Klatka et al, 1997), although some studies have reported even lower figures (Mayeux et al, 1998).

Further, some elderly controls, although asymptomatic, may harbor presymptomatic AD lesions in their brains. Thus, sensitivity and specificity rates above these levels should not be expected for diagnostic markers for AD when evaluated in clinically diagnosed patients without post mortem examination.

In our second paper we concentrated on CSF-Aβ42. We found a marked decrease in CSF-Aβ42 in AD patients, most pronounced in EAD patients but also in LAD patients as compared with healthy, age-matched controls.

Using the lower 0.95 fractile of the control values as a reference limit, the sensitivity of the assay for diagnosis of AD was high (92%). Thus, we were able to confirm previous findings of reduced CSF levels of Ab42 in patients with AD (Motter et al, 1995; Ida et al, 1996; Tamaoka et al, 1997; Vanderstichele et al, 1998).

The high sensitivity of CSF-Aβ42 in study II compared with previous studies has many possible explanations. First of all, we studied healthy elderly individuals as controls, whereas some previous studies used patients with neurological disorders without cognitive impairment (Tamaoka et al, 1997). Alternate explanations include differences in selection and characteristics of patients. In our study we used a community-based sample as described earlier (Andreasen et al, 1999) whereas most other samples are drawn from a highly selected group of patients visiting academic centers.

Although the inter-individual variation in CSF-Aβ42 level was low within each diagnostic group, two patients with AD in study II had deviating CSF-Aβ42 levels. The patient with LAD (CSF-Aβ42 level, = 1569 pg/mL) had typical histories and clinical findings of AD. Review of the medical records of the patient with EAD with a high CSF-Aβ42 level (1876 pg/mL) revealed, however, that this patient had a history of repeated head trauma, when practicing his profession as a construction worker and as a "head-specialist" elite soccer player. At the age of 45, he experienced concentration difficulties, and reduced short-term memory, and his wife also reported changes in his personality such as aggressiveness and suspiciousness. He was given a diagno-

sis of AD, but has not shown any deterioration during 18 months of follow-up. Thus, this patient has an atypical AD, suggestive of a variant of dementia pugilistica.

The high sensitivity but low specificity of both CSF-tau and CSF-Aβ42 incited us to study the combination of CSF-tau and CSF-Aβ42. In paper IV we studied all patients seen for cognitive disturbance at our department during one year. In this study we used the cut-off line from the multi-center study (Hulstaert et al, 1999).

By using this we found that the sensitivity for the two CSF markers to identify AD pathology in AD and MIX patients was high (95% for AD and 90% for MIX). Higher sensitivity figures than the ones obtained in the present study may not be expected for diagnostic markers for AD when evaluated in clinically diagnosed patients. Studies with neuropathologically confirmed cases and controls are needed to with certainty determine the sensitivity and specificity of CSF-tau as a diagnostic marker for AD.

In our studies we found that either CSF-tau was increased and/or CSF-Aβ42 decreased in AD patients even very early in the course of the disease, when the MMSE scores were high. As the diagnosis is most elusive in the early phase of the disease we were interested in the transition between incipient AD and very mild AD, knowing that individuals with MCI appear to have an increased risk of developing AD at a rate of 10% to 15% per year (Grundman, 1996). We therefore studied patients with MCI (Petersen et al, 1997; Almkvist and Winblad, 1999) which at follow-up had proceeded to AD in study V.

Using the same cut-off value as in the previous study (Hulstaert et al, 1999), 80% of the controls had normal levels of both CSF-tau and CSF-Aβ42, while 20% of the controls had abnormal values. In contrast, of the MCI patients, 88% had high CSF-tau and low CSF-Aβ42, while 12% had normal levels of both markers. Thus, the sensitivity for high CSF-tau and/or low CSF-Aβ42, for the prediction of AD in MCI patients was 88%, while the specificity was 80%.

Thus in both studies the sensitivity and specificity values of CSF-tau and CSF-Aβ42 fulfill the criteria set by the Consensus Group on Molecular and Biochemical markers of AD by the Ronald and Nancy Reagan Research Institute and the National Institute of Aging (Consensus report, 1998), that is to say that a clinically useful diagnostic marker must be able to detect AD early in the course of the disease having a sensitivity and a specificity exceeding 80%.

Criticism of material and methods used

All the patients that are included in this thesis are drawn from "the Piteå Dementia Project". In this project all patients with cognitive disturbances severe enough to be noticed by the patient, his relatives or the health care system are referred to the Department of Rehabilitation for clinical assessment and work up. Since the authorities require a clinical work-up at this department, it means that all or nearly all prevalent cases with some degree of memory impairment most probably were detected (Andreasen et al, 1999).

As the study has continued for more than 10 years and dementias are progressive diseases, we believe that we have only missed those early dementia patients who died from other causes before their dementia became severe enough to warrant admission for work-up. Moreover, this age segment of the population is very stable, with a very low migration, which means that very few patients moved into or away from the area. Compared to case finding by screening, our case-finding procedure was probably more efficient, since cases that were missed at one point in time were highly likely to show up later during the sampling period.

In addition, all cases were subjected to a uniform work-up performed by one doctor and a well-trained staff. The data collection therefore is fairly complete, uniform and free from bias of such a magnitude that it would affect the results.

The incidence and prevalence of dementia obtained in this area (Andreasen et al, 1999) is comparable with other epidemiological studies taking into consideration that this is a community-based study. Thus one can presume that the patients are representative and there is little selection bias.

The controls are selected among healthy elderly volunteers and from patients admitted for minor surgery. The clinical investigation, the MMSE test and the medical history did not unveil any dementia. We did not perform any follow-up either clinically or through medical case records on this group. As it is known, AD pathology starts at least 20-30 years before clinical onset (Davies et al, 1988; Price and Morris, 1999) we might therefore expect a certain number of incipient AD patients among the controls. These incipient cases will therefore inevitably have an effect on the sensitivity of the studies.

The diagnosis of AD is based on clinical exclusion criteria (McKhann et al, 1984). According to these criteria diagnosis is only definite at necropsy. The studies in this thesis are all performed on patients seen at the Piteå River Valley Hospital. The nearest department of pathology is situated 60 miles

away, which gives us no opportunity to perform a postmortem on our patients. Moreover most of our patients died in community-provided housing and the attitude from the staff and from relatives of the patients were rather negative towards a postmortem. Therefore there may be some misdiagnosed AD patients.

Moreover it is known that many VAD patients harbor a concomitant AD pathology, which is impossible to exclude clinically (Jellinger, 1998; Kosunen et al, 1996). Therefore the prevalence of VAD in the studies is likely to be somewhat high, leading to an underestimation of the specificity of the biochemical markers.

The study is longitudinal and most patients are seen every six months for some time, especially if the diagnosis is somewhat uncertain. This implies that the risk of misdiagnosis will be minimized. Only one investigator with vast experience in diagnosing dementia diseases was involved in Piteå. On the other hand this solitude might bias his judgement. Therefore we decided to reevaluate all the diagnoses in study I by one other author (K.B.) and found good concordance between the two investigators.

The estimation of the duration of the disease is very uncertain. At the first visit the patient or his relative often describe the duration as short, - less than one year. When the investigator suggests that the duration may have been longer it may be extended by another year. At the follow-up the relatives often spontaneously disclose that after discussing the matter with the family and seeing the course of the disease that there have been signs of cognitive disturbances for five to ten years or even more. This implies that the uncertainty of the duration is substantial and moreover that it is of importance that the investigator realizes this when interviewing the relatives for medical history. There is a tendency towards a growing awareness of the disease in the population which makes the relatives more observant.

The MMSE score (Folstein et al, 1975) is used for the estimation of the degree of dementia and the progression of the disease. This is a rather blunt instrument with a typical S-shaped curve with a minor decrease in the beginning and in the advanced stages of the disease with a more significant decline in the middle period of the disease (Butler et al, 1996). Furthermore, some patients, often those with a higher education, may have AD even with a high MMSE score, and may have a slower deterioration (Christensen, 1997; Gauthier et al, 1997). A more sensitive test, for example the ADAS-cog (Rosen ET al, 1984), would have been a better choice but had to be excluded due to the workload it entails. Thus, we cannot exclude the possibility that the CSF-level of tau and Aβ42 reflects the intensity of the degenerative process in AD.

Lumbar puncture is an invasive method. Furthermore it is considered unpleasant to the patient and entails certain risks. In the hands of an experienced investigator the discomfort for the patient during the procedure is minimal and the risk of a post-lumbar puncture headache is less than 2% (Blennow et al, 1993a). The risk of introducing an infection is insignificant, given that aseptic procedure is undertaken. At our center we have performed more than 3000 LPs with no complication of infections. With standard procedures for tapping, handling, transporting and storing the samples, including freezing and thawing we have shown that the procedure is robust.

The sensitivity of CSF-tau and CSF-Aβ42 is acceptable as biomarkers for AD and well over what is demanded in the consensus report (1998). We have shown that total CSF-tau and CSF-Aβ42 can be used as markers even in the very early stage of AD as well as in MCI patients. Further we speculate that CSF-tau and CSF-Aβ42 can be used in monitoring new drugs in order to detect protective qualities earlier. As we have stressed the total CSF-tau must be judged as a marker of axonal degeneration, which is certainly seen in AD. A test assessing the PHF-tau would more likely reflect the central processes in AD and thereby more specifically measure the changes in the central processes of AD. One might even expect that the specificity especially against VAD might increase with such a marker. Further studies are needed to evaluate this, and studies in our group are in progress.

Very few studies are done with biomarkers on MCI patients. Our study is very small with only 16 patients, and more and larger studies are required to evaluate this issue.

The demand for biomarkers that can detect AD preclinically will increase as new medical compounds are developed that can slow down or stop the central pathological processes of the disease. As the pathological processes start 20-30 years before clinical symptoms appear it is essential to make an inventory of existing CSF banks with material from individuals who have later (10-20 years) developed AD. Such studies are in progress in our group.

Summary

The need for good, reliable markers for AD is pressing. Earlier, when we had no treatment available, we could afford to wait until a follow-up investigation clearly indicated AD before the diagnosis was definitely established. Moreover, most patients presented late in the course of the disease when the clinical picture was more clear-cut. At our center the mean MMSE score was about 19 in the starting years of the PDP. During the last ten years the awakening interest and knowledge from the public and patients have encouraged the patients to seek help earlier and earlier in the course of the disease. The mean MMSE-score of the patients today is approximately 25. In view of existing (acetylcholine esterase inhibitors) and emerging (e.g. neuroprotective) therapeutic compounds, there is an urgent need for an exact and early diagnosis.

As the population of our country grows older the prevalence of AD will increase (Jorm et al, 1987). It will be an impossible task for the specialized centers to evaluate every patient with cognitive impairment. Therefore a substantial amount of patients must be evaluated at Primary care centers or centers with no special interest in dementia diseases. This present us with a huge challenge to develop inexpensive and reliable markers for AD which are easy to use and as non-time consuming as possible for the non-specialist physician.

In summary, CSF-tau and CSF-Aβ42 have a high sensitivity and specificity for AD also in a large community-based series of consecutive AD cases, where the analyses were run continuously in a clinical neurochemical laboratory. The increase in CSF-tau in AD probably reflects the neuronal/axonal degeneration, which is one of the fundamental features of AD neuropathology, while decreased CSF-Aβ42 may reflect the deposition of Aβ in the brain which is the other fundamental feature of AD. Increased CSF-tau and decreased CSF-Aβ42 is found very early in the disease processes in AD, and is stable during the course of the disease. There are no confounding factors such as concentration gradients (Vanmechelen et al, 1998), and samples can be sent either at room temperature or frozen, since this does not influence the results. Although LP may be regarded as a moderately invasive test, it is easy to perform, and entails only a minimal risk for complications (Blennow et al, 1993a). Last, ELISA assays are inexpensive to perform. Therefore, we suggest that CSF-tau and CSF-Aβ42 are included in the clinical work-up of patients with cognitive impairment. The clinical utility is high in patients with early symptoms of dementia, especially when differentiating between incipient AD and age-associated memory impairment and dysthymia or depressive pseudodementia, which are the most problematic differential diagnoses, especially in the early stages of the disease (Zapotoczky, 1998).

CSF diagnostic markers however, should not be used alone (Blennow and Vanmechelen, 1998). Instead, the accuracy of the clinical diagnosis of AD may increase if the diagnosis is based on the summarized information gained from the clinical examination, brain-imaging techniques (SPEC, CT/MT scans), CSF diagnostic markers, and other biochemical diagnostic tools, e.g. ApoE genotyping (Blennow and Vanmechelen, 1998). Similarly, the clinical diagnosis of myocardial infarction is based on the combination of clinical symptomatology, electrocardiogram, and biochemical markers (e.g. creatine kinase). Studies with neuropathologically confirmed cases are needed to, with certainty, determine the sensitivity and specificity of CSF-tau and CSF-Aβ42 as diagnostic marker for AD.

Conclusion

In paper I our findings confirm the high sensitivity of CSF-tau for the diagnosis of AD, but high CSF-tau was also found in VAD, resulting in a lower specificity. High CSF-tau however, is preferentially found in VAD patients without progressive LA, which may constitute a group with concomitant AD pathology. High CSF-tau may be present during the whole course of the disease in AD. Further, we show that CSF-tau levels do not differ between AD or VAD patients with and without the ApoE4 allele. These findings are in agreement with previous CSF-studies (Motter et al, 1995; Skoog et al, 1995; Nitch et al, 1995), and suggest that differential binding affinity between ApoE isoform and tau does not influence the CSF-level of tau.

In paper II we confirm previous findings that sensitivity of CSF-Aβ42 is high as a diagnostic marker for AD. The intra-individual biological variation in CSF-Aβ42 level is low. Low CSF-Aβ42 levels are also found in the earlier stages of dementia in patients with AD. These findings suggest that CSF-Aβ42 analyses may be of value in the clinical diagnosis of AD, especially in the early course of the disease, where this is particularly difficult.

In paper III we found that CSF-tau has a sensitivity and specificity for AD exceeding 85% also in a large community based series of consecutive AD cases (n=407), where the analyses were run continuously in a clinical neurochemical laboratory. The increase in CSF-tau is found very early in the disease process in AD, is stable over time, and has a low inter-individual variation at repeated sampling.

In paper IV we found the combination of CSF-tau and CSF-Aβ42 has a high sensitivity and predictive value for AD also in a large community-based series of consecutive cases, where the analyses were run continuously in a clinical neurochemical laboratory.

In the last study we found results suggesting that high CSF-tau and low CSF-Aβ42 be found already in the pre-dementia phase of AD. The sensitivity and specificity for these biochemical markers are additive.

Our findings suggest that they may have a role in the diagnostic work-up of MCI patients, to help to identify those that will develop AD. This will be especially important when drugs with potential effect on the progression of AD will reach the clinical phase.

Our findings suggest that the biomarkers CSF-tau and CSF-Aβ42 may be included in the clinical work-up of patients with cognitive impairment, especially to differentiate early AD from normal aging and psychiatric disorders such as depressive pseudodementia. We further stress that these biomarkers shall not be used exclusively but in conjunction with traditional methods, such as history, clinical findings, blood samples, EEG, SPECT, CT scan, ADL assessment and neuropsychological tests.

Acknowledgements

I wish to express my warm and sincere thanks to all the patients and healthy volunteers who participated in this study, and to the staff and my colleagues at the Department of Rehabilitation and staff on Neurochemical laboratory of Mölndal for their generous support.

I especially wish to express my sincere thanks to:

Kaj Blennow. My very dear friend. With you as guiding star, the pavement on my scientific path has been much less troublesome than I feared. For your generous and continuous support throughout these studies. Your knowledge has been a never ever drying well where I knew I always could draw the wisdom I was without. Your hospitality is outstanding, and your home has been as a safe house on my scientific journey.

Kurt Svärdsudd. My very good friend and brother in law. You taught me the fundamental rules of science. Long before I understood that this thesis could be the result of my work you had realized this. You gently coached me and guided me so that I became able to organize my otherwise rather chaotic way of handling the everyday clinic.

Bengt Winblad. For your constructive and greatly appreciated criticism and your support and for sharing your wide knowledge and enthusiasm in science. Without your support and believing in my project, this thesis would never have been delivered. I also want to express my appreciation of our friendship both on a personal and a scientific level.

Christina Sjödin. My research nurse and supporter who have helped me from the beginning of the study in 1986. You always believed in the project and somehow in between all your other duties managed to find the time for research. I am very grateful that you have put up with me and my temper especially in the numerous stress situations that have occurred.

Lennart Minthon. For your enthusiasm and good discussions with constructive criticism. I especially appreciate your engagement in the final work with this thesis.

Lennart Wennberg. Head nurse of ward department 2A. With your safe and confident manner you secured the ward when my project engulfed me.

My co-authors. E Vanmechelen, A Van de Voorde, P Davidsson, C Hesse, S Tarvonen, I Räihä, L Sourander, A Wallin, H Vanderstichele, J Gottfries, A Clarberg. You generously supported me with your best ideas.

Olle Sunnergaard. For your support both as a colleague at the hospital and with fruitful scientific conversations.

Tommy Sundell. For warmhearted helps and support through the last years. When my project was questioned, both within or outside the profession, you always stood by my side and defended me

Jan-Erik Andersson. Who "inhered" my project and me and supported me so that I under the last half year had the opportunity to complete this thesis.
Sture Berglund. For kindly assisting me to carry out my vision of the beautiful sky in the morning in Norrbotten and letting me use your painting production "Dawn" to embellish the cover of this thesis.
Roberta Boson. For linguistic corrections of this thesis.
My family. My sons Christian and Jan. My wife, Britt, for your love, patience and support. For never question my time on the job or the endless hours at the computer. Not least for unbelievable ground service during the finishing of this thesis.

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