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RELIABILITY OF VISUAL ASSESSMENT OF MEDIAL TEMPORAL LOBE ATROPHY

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Yesterday is past,
tomorrow is future,
today is a gift –
that's why it´s called PRESENT

Master Oogway

ABSTRACT

Background: Medial temporal lobe atrophy (MTA) has been found to be an early sign of Alzheimer's disease (AD). Visual assessment of MTA (vaMTA) is a rapid, cost-efficient and clinically adaptable visual interpretation method for rating MTA, based on coronal magnetic resonance imaging (MRI) scans. The method was developed by Scheltens et al in the 1990s.

Purpose: The aim of this thesis was to investigate the reliability of vaMTA using the Scheltens rating scale: on a long-term basis, compared with volumetric calculation, compared with multivariate analyses and, finally, tested in a clinical situation

In **Study I**, MRI scans of 100 patients were visually assessed six times over a 1-year period. Two radiologists, with different backgrounds, performed the assessments independently of each other. The results showed a high degree of reproducibility when performed by an experienced investigator. The reproducibility drops when assessment is rarely performed.
Study II was a comparison between vaMTA and measurement of hippocampal volume in 544 non-demented elderly individuals from the SwedishNational Study of Ageing and Care in Kungsholmen (SNAC-K). A significant correlation was found between the two methods. Cut-off values for MTA scores in normal ageing were also suggested.
In **Study III** the reliability of Scheltens' visual assessment rating scale for assessing MTA was compared with that of a multivariate MRI classification method, orthogonal projections to latent structures (OPLS), and manually measured hippocampal volumes to distinguish between subjects with AD and healthy elderly controls (CTL). A comparison between the different techniques was also performed in predicting future developments from mild cognitive impairment (MCI) to AD. The prediction accuracies in distinguishing between AD patients and CTL were high for all three modalities. All three methods were also highly accurate in identifying subjects who converted from MCI to AD at 1-year follow-up.
Finally, in **Study IV,** vaMTA scores were used in a validation study of the proposed new "Dubois criteria" in Alzheimer's disease, in which MTA is one of four important biomarkers. A retrospective study of 150 patients was carried out to compare the traditional diagnostic criteria for dementia with the new criteria suggested by Dubois et al. The results showed a lack of accuracy for the new AD criteria, as they were valid for only 55% of the clinically diagnosed patients with full-blown AD in this study.

Conclusion: Visual assessment of MTA using the MTA scale is reliable when performed on a daily basis. Medial temporal lobe atrophy scores have a significant correlation to hippocampal volume measurements, can predict conversion from MCI to AD with similar accuracy as can volumetric calculations and multivariate analysis, and can be used as supportive biomarker in the work-up of AD.

LIST OF PUBLICATIONS

I. **Overtime reliability of MTA rating in a clinical setting**
Cavallin L, Løken K, Engedal K, Øksengård AR, Wahlund LO, Bronge L, Axelsson R
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II. **Comparison between visual assessment of MTA and hippocampal volumes in an elderly non-demented population**
Cavallin L, Bronge L, Zhang Y, Øksengård AR, Wahlund LO, Fratiglioni L, Axelsson R
Submitted to Acta Radiologica, (first revision submitted 12 02 11)

III. **Sensitivity and specificity of medial temporal lobe visual ratings and multivariate regional MRI classification in Alzhemer's disease**
Westman E/Cavallin L, Muehlboeck JS, Zhang Y, Mecocci P, Vellas B, Tsolaki M, Kloszewska I, Soininen H, Spenger C, Lovestone S, Simmons A, Wahlund LO for the AddNeuroMed consortium
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IV. **Lack of accuracy for the proposed 'Dubois criteria' in Alzheimer's disease: a validation study from the Swedish brain power initiative**
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Contents

LIST OF ABBREVIATIONS

AC	Anterior commisure
AD	Alzheimer's disease
ADNI	Alzheimer' Disease Neuroimaging Initiative
APOE4	Apolipoprotein E4 genotype
APP	Amyloid precursor protein
Aβ42	Beta amyloid 1-42
BALI	Brain Atrophy and Lesion Index
BBB	Blood-brain barrier
BRASS	Brain Registration Analysis and Software suite
CBF	Cerebral blood flow
CDR	Clinical Dementia Rating
CSF	Cerebral spinal fluid
CT	Computed tomography
CTL	Control subject
DLB	Dementia of Lewy body type
DSM-IV	Diagnostic and Statistical Manual of Mental Disorders, 4th edition
Dx	Right side
EEG	Electrencephalogram
ELISA	Enzyme-linked immuno-sorbent assay
FDG	Fluoro-deoxy-glucose
FFE	Fast Field echo
FLD	Frontal lobe dementia
FOV	Field of view
FSE	Fast spin echo
FTD	Fronto temporal dementia
GCS	Global cortical atrophy
GDS	Geriatric Depression Scale
GEE	Generalized estimating equation
ICAD	International Conference in Alzheimer's Disease
ICC	Inter-class correlation coefficients
ICD-10	International Statistical Classification of Diseases and Related Health Problems, 10th revision
ICV	Intracranial volume
IgG	Immunoglobulin G
κ	Kappa
KL	Kirsti Løken
LBD	Lewy body dementia
LC	Lena Cavallin
MANOVA	Multivariate analysis of variance
MCI	Mild Cognitive Impairment
MCI-c	Mild Cognitive Impairment-converters
MCI-s	Mild Cognitive Impairment - stable

MMSE	Mini Mental State Examination
MPRAGE	Magnetization prepared rapid acquisition gradient echo
MR	Magnetic resonance
MRI	Magnetic resonance imaging
MTA	Medial temporal lobe atrophy
MTL	Medial temporal lobe
NFT	Neurofibrillary tangle
NIA	National Institute of Ageing
NINCDS-ADRDA	National Institute of Neurological and Communicative Disorder and Stroke and the Alzheimer's Disease and Related Disorders Association
OPLS	Ortogonal projections tolatent structures
PA	Posterior atrophy
PC	Posterior commisure
PET	Positron emission tomography
PIB	Pittsburgh compound B
PLS	Partial least squares
P-tau	Phosphorylated tau protein
RAVLT	Rey Auditory Verbal Learning Test
ROI	Region of interest
SCI	Subjective cognitive impairment
SD	Semantic dementia
SE	Spin echo
Sin	Left side
SMILE	Stockholm Medical Image Laboratory and Education
SNAC-K	Swedish National Study of Ageing and Care in Kungsholmen
SPECT	Single photon Emission Computed Tomography
T	Tesla
TE	Time of echo
tfl	Time of flight
TR	Time of repetition
3D	Three-dimensional
2D	Two-dimensional
T-tau	Total tau
UNS	Unspecified
VaD	Vascular dementia
vaMTA	Visual assessment of medial temporal lobe atrophy
wκ	Weighted kappa
WML	White matter lesion
WMC	White matter changes

1 INTRODUCTION

1.1 BACKGROUND

In 1907 Alois Alzheimer was the first to describe both the clinical and pathological findings of presenile dementia. As a psychiatrist and a brain researcher specializing in neuro histo pathology he received the patient records and brain of a 56-year-old woman named Auguste D. He had met the woman years before, as a psychiatrist, and had followed her disease progression from a distance. Her symptoms were initially described as progressive cognitive impairment, focal symptoms, hallucinations, delusions and psychosocial incompetence. At autopsy she was found to have moderate hydrocephalus, cerebral atrophy, and arteriosclerosis of the small cerebral vessels. Alzheimer described the histopathological findings in the cerebral cortex as "peculiar changes in the neurons, with fibrils with characteristic thickness and peculiar impregnability". He also found and described "numerous small miliary foci in the superior layer of the cerebral cortex and these foci seemed to be the storage of a peculiar material". Alzheimer wrote a three-page report on this case, titled, "Über eine eigenartige Erkrankung der Hirnrinde", describing the disease that was later named after him (1).

A retrospective study of this first medical record of Alzheimer's dementia, by Maurer et al in 1997 (2), confirms the patient's diagnosis according to the International Statistical Classification of Diseases and Related Health Problems, 10th revision (ICD-10), criteria for dementia. The miliary foci were later named "senile or neurotic plaques" and the intracellular fibrils were called "neurofibrillary tangles". It was only in the 1980s that the plaques were found to be made of beta-amyloid (Aβ) protein and neurofibrillary tangles (NFTs) of hyperphosphorylated tau protein (P-tau).

Since 1907, much research has been done regarding dementia disorders, and the research is on-going. In the last 100 years new methods to investigate brain degenerative disorders have been given much credit. Research has included areas such as pathology, molecular biology, genetics, laboratory science, radiology and clinical science.

1.2 DEMENTIA

Dementia, derived from the Latin term *de mens*, meaning "without mind", is an acquired clinical syndrome of long duration and progressiveness. Dementia is forgetfulness (3), but it is also impairment of thinking, reasoning, communication, orientation and practical abilities. To learn new things or to maintain learned skills becomes difficult. Personality changes resulting in aggressiveness, emotional bluntness, lack of insight, judgment and empathy, and disinhibition are other symptoms of dementia.

"Dementia" is a general term and does not need to be linked to a specific aetiology. Alzheimer's dementia is only one form of dementia. Other forms of dementia are fronto-temporal dementia (FTD), Lewy-body dementia (LBD), vascular dementia

(VaD) and mixed dementias. The symptoms and findings vary between these diseases. Criteria for dementia are given in the ICD-10 and Diagnostic and Statistical Manual of Mental Disorders, 4th edition (DSM-IV) (3, 4).

The definitive diagnosis is, despite the increasing use of numerous new biomarkers, still a pathological diagnosis. Only at autopsy and using the microscope is the diagnosis finally confirmed or rejected.

1.3 MILD COGNITIVE IMPAIRMENT

Mild cognitive impairment (MCI) is a heterogeneous condition and is said to be a transition zone between normal ageing and dementia (5). Mild cognitive impairment can convert to dementia and the conversion rate is approximately 12-15%/year. The majority of MCI-patients remain stable and about 12% regain normal cognition (5,6).Mild cognitive impairment might convert to Alzheimer´s disease, VaD, FTD, DLB or mixed dementias (5).

1.4 ALZHEIMER'S DISEASE

Alzheimer's disease (AD) precedes the most common form of dementia, Alzheimer's dementia, and is considered a major threat to public health in the 21st century, world-wide. Its incidence increases dramatically with age, doubling in frequency every 5 years after the age of 60, afflicting 1% of all 60–64-year-olds and 30–40% of those aged 85 years and older all over the world (6, 7).

Alzheimer's disease is recognized as a group of diseases, a syndrome, that varies according to factors related to age, etiology and heredity. It is a neurodegenerative disorder divided into a familial and a sporadic type. The sporadic type of AD is a heterogeneous group with multifactorial pathogenesis. Two well-known risk factors for sporadic AD are old age and an ε4 allele of apolipoprotein E (ApoE) on chromosome 19. The sporadic type of AD represents more than 99% of all cases. Inherited familial forms of AD with gene mutations on chromosomes 1, 14 or/and 21 represent less than 1 % of all AD cases.

Disease in individuals younger than ≤ 65 years of age is referred to as early-onset or presenile AD while AD in individuals≥65 is referred to as late -onset or senile AD. The inherited form of familial AD has the highest representation in early-onset AD. The early symptoms of AD are failure in short term memory (episodic memory impairment), learning problems and difficulties in processing new information. As the disease progresses there is increased speech impairment and a general decline in cognitive functions are affected.

The research criteria for AD were outlined in 1984 by the National Institute of Neurological and Communicative Disorders and Stroke and the Alzheimer's Disease and Related Disorders Association (NINCDS-ADRDA). The NINCDS-ADRDA criteria outline the symptoms of the disease and describe the diagnosis of possible, probable, and definite AD (8). In possible AD, criteria of dementia must be fulfilled but the dementia can be caused by other diseases as well as AD. Probable AD is also based on dementia criteria with progressive deterioration of memory and other cognitive

functions over time. The diagnosis of definite AD requires histo-pathological confirmation at autopsy (8).The clinical diagnostic criteria for AD, used in hospitals, are ICD-10 and DSM-IV.

Across the world procedures vary for investigating AD. At Minnesmottagningen, Karolinska University Hospital in Huddinge, Sweden the diagnostic work-up is performed according to a clinical frame-work including a team of geriatricians, neuropsychologists, nurses, occupational therapists and speech therapists headed by a senior consultant. The diagnosis is given by a senior consultant in consensus with the team-members involved (5). In the clinical frame-work episodic memory is tested with Rey Auditory Verbal Learning Test (RAVLT). Two other examples from the test-battery are the Mini Mental State Examination (MMSE) and clocktest. Venous blood punctures are performed for ordinary and extended check-ups and for APOE ε-genotyping. Lumbar puncture is performed for liquor analysis albumin, immuno-globulin G (IgG) and the biomarkers betaamyloid 1- 42 (Aβ-42), total –tau (T-tau) and phosphorylated tau (P-tau). An electroencephalogram (EEG) is used to measure and record of electrical brain activity. Brain imaging is performed with computed tomography (CT) and/or magnetic resonance imaging (MRI), to exclude intracranial bleeding, infarctions, tumour and to verify or out-rule brain atrophy. Singlephoton emission computed tomography (SPECT) and positron emission tomography (PET) can be used for assessment of brain perfusion and cerebral glucose metabolism.

1.4.1 Biomarkers

The understanding of underlying biological factors of AD has advanced during recent years. Biomarkers of different kinds have been recognized and included in the assessment. Brain imaging investigations are performed with CT, MRI SPECT and PET.

In cerebrospinal fluid (CSF) biomarkers such as Aβ42, T-tau and P-tau can be detected, analysed and quantified. Up till now three genetic mutations for AD have been found visualized on chromosomes 1, 14 and 21,and can be used as biomarkers of the disease, especially in early-onset AD.

1.4.1.1 Brain imaging

The first CT scanner in Sweden was installed at Karolinska Hospital, Stockholm, in 1975. Brain imaging with CT has been in public use since around 1980, initially just to confirm or rule out intracranial bleeding. The early clinical use of CT in AD investigations was primarily to exclude bleeding, tumours and hydrocephalus.

In 1980 one of the first CT studies of normal ageing was performed by Jacoby et al (9). In 1982 Soininen et al compared CT findings in demented and non-demented patients and found that ventricular dilatation in demented correlated significantly to cognitive loss (10).

Today CT is still in use and multidetector-row CT has been shown to be a suitable imaging tool to investigate brain atrophy, as an alternative to MRI (11). Two Nobel prizes have been won for work in MRI, in 1952 and in 2003. Since 1984 and the installation of the first MRI machine in Sweden, at the psychiatric clinic at St Görans

Hospital in Stockholm, tremendous developments have taken place in the MRI field. The role of MRI in investigating demented patients and especially patients with AD was soon recognized (12, 13, 14), with focus on the medial temporal lobe (MTL), hippocampus and entorhinal cortex.

Nuclear medicine imaging assesses cerebral perfusion or blood flow with a conventional gamma camera using SPECT and gamma-emitting isotopes and evaluates impaired glucose metabolism in the brain by means of a PET camera and 18F-fluoro-deoxy-glucose (FDG) (15, 16, 17). These methods have been used for almost 30 years to evaluate deterioration in the AD brain (18, 19) and FDG is the most evaluated PET tracer in AD research.

To increase the specificity of nuclear medicine, imaging tracers reflecting specific processes in the AD brain such as amyloid deposition are of interest. The first human study with amyloid tracer Pittsburgh compound-B (PIB) was carried out at the Uppsala, Sweden, PET centre in collaboration with the Pittsburgh group and was published in2004 (20). Pittsburgh compound-B crosses the blood-brain barrier (BBB) and binds with high affinity to aggregates of Aβ. Retention of PIB is significantly higher in the frontal and temporo-parietal association cortices as well as in striatum in AD patients compared with healthy controls (20). Binding of PIB to Aβ is a very early sign in AD and when scanning in patients with mild cognitive impairment (MCI), increasing PIB retention is seen mainly in those patients who later will convert to AD (21).

1.4.1.2 Cerebrospinal fluid

Cerebrospinal fluid is in direct contact with the extracellular space in the brain. This means that biochemical changes in the brain are reflected in the liquor. Damage to the BBB results in high inflammatory cell content and increases albumin concentration. High IgG presence in CSF indicates BBB damage and possibly intracerebral production of IgG, as seen in neuroinflammatory brain diseases such as multiple sclerosis. There are many biomarkers related to AD in the CSF. In clinical practice, Aβ42, T-tau and P-tau are used the most.

1.4.1.2.1 Aβ1-42

Amyloid beta peptide-42 forms the insoluble main component of the neuritic plaque with a length of 42 amino acids after cleavage from amyloid precursor protein (APP). Alois Alzheimer saw the plaques in his microscope already in 1907 but not until 1984 was the protein purified (22). A decrease in Aβ42 content of about 50% is seen in CSF in AD patients compared with non-demented controls of the same age. In an autopsy study (23) an inverse correlation between Aβ42 levels in CSF and the number of plaques was demonstrated.

1.4.1.2.2 Tau-protein

The NFT is one of the main neuropathological findings in AD. It is composed of an abnormal fibrillar material accumulated in the cytoplasm in large neurons. The main component in the NFT is tau protein.

Tau protein is a microtubule-associated protein normally located in the neuronal axons. Its biological functions are to stabilize the microtubule network and regulate axonal transport (24). Tau is usually found in six different isoforms (25). All forms can be found in the NFT. In AD the tau protein undergoes hyperphosphorylation, which affects the normal microtubule binding and stabilization. The NFT is accumulated intracellulary in AD, leading to neuronal function loss and cell death. In CSF it is possible to analyse the amount of both T-tau and P-tau.

1.4.1.2.3 Total tau

All isoforms of tau, irrespective of phosphorylation, are detected and analysed in T-tau. Total tau reflects the amount of neuronal degeneration. In AD there is more than 100% increase in T-tau in CSF, compared with controls (26).

1.4.1.2.4 Phosphorylated tau

The amount of P-tau in CSF correlates with the NFT burden in the brain. Phosphorylated tau is increased more than 100% in AD compared with controls.

In a clinical situation all three CSF markers are tested, even if the combination of Aβ42 and T-tau is said to have the strongest evidence in the clinical diagnosis of AD (27).Sometimes the T-tau/P-tau ratio is used to discriminate other dementias from AD (28).Varying laboratory routines, however, make test results difficult to compare between centres, making general cut-off levels difficult to establish (27).

1.4.1.3 Genetics

Genetic predisposition is a well-known risk factor for AD. Families with early age (≤65 years) at onset of AD were recognized as early as the 1930s (29). Many of those families had a clear autosomal dominant inheritance with almost 100% penetrance. The association between Down's syndrome and AD has been known since 1929. In a prospective study by Lai and Williams (30) the prevalence of AD in Down's syndrome was 55–75% above 50 years of age.

Most AD cases are late onset. There is no real genetic biomarker in this group. Two well-known heavy risk factors for late-onset AD are old age and APOE ε4 (31).

1.4.1.3.1 Chromosome 19

Apolipoprotein E has been found to be involved in cholesterol metabolism. The encoding apolipoprotein E gene is located on chromosome 19. It consists of three allele variants, ε2, ε3 and ε4, with varying frequency across the world. In the Western world ε2 occurs in 10%, ε3 in 75% and ε4 in 15% of the population (32).

Two APOE ε4 alleles have been shown to increase the risk of developing AD. It has also been reported that presence of two ε4 alleles may lead to early onset of AD (31). The APOE gene is a risk factor and not a causative factor for AD. Irrespective of this, APOE gene testing is frequently performed in the work-up of AD.

1.4.1.3.2 Chromosome 21

The APP gene is located on chromosome 21. In 1991 the missing link between AD, Aβ and the APP gene was identified as a mutation in the APP gene (33). Since then many different mutations have been found in the APP gene, which have been reported to account for an estimated 16% of all early-onset AD cases (34). The mutations are autosomal dominant and result in malfunction of APP and an excessive Aβ production or increased Aβ42/40 ratio (35).

1.4.1.3.3 Chromosome 1 and 14

Four years after the discovery of the APP gene mutation, genes on chromosome 14 (36) and chromosome 1 (37) were linked to familial AD. They were called presenilin 1 (on chromosome 14) and presenilin 2 (on chromosome 1). These two genes have a similar genetic structure and encode two highly homologous proteins. Over 170 pathogenic mutations of presenilin 1 have been found, and 14 of presenilin 2. These mutations commonly result in an increase in the Aβ42/40 ratio (35). The mutations in presenilin 1 represent about 80% of all early-onset familial AD cases.

The mutations on the three chromosomes 1, 14 and 21 are all involved in the increase of production, deposition and aggregation of Aβ42 in the brain

1.5 STRUCTURAL CHANGES IN THE AGEING BRAIN

A human infant is born with an immature brain. Myelination begins during the fifth foetal month and proceeds rapidly up to an age of 2 years. The myelination process slows markedly after 2 years of age but continues in fibres of associative brain areas up to the third and fourth decade of life (38). Many intervention studies currently target adults 60 years of age and older(39), but studies in individuals 18-60 years of age have however demonstrated decline of memory as early as in the twenties (40)This means that memory decline begins short after reaching maturity and maybe even before myelination is completed.

In the normal ageing brain it is possible to detect degenerative changes by CT and MRI. Whole brain volume can be calculated using MRI volumetry. By conducting repeated investigations over a time period, it is possible to see progression by using image subtraction or calculating atrophy progression with volumetry (39, 41). Age-related ventricular volume expansion, loss of cortical thickness and hippocampal volume losses are also possible to subtract or calculate, as is done in longitudinal studies (39). The atrophy rate/year can be calculated. In older ages there is an acceleration of the atrophy rate and the border between normal ageing and pathological ageing may be more difficult to determine.

1.5.1 Medial temporal lobe

The MTL consists of amygdala, hippocampus, parahippocampal gyrus entorhinal cortex, choroid fissure, temporal horn of the lateral ventricle, and the collateral sulcus. In AD and other forms of dementias MTA is in focus and has been for a long period of time.

Staging of AD neuropathology was performed by Braak and Braak in the 1990s (42). According to these authors, neuropathological AD changes start in the entorhinal cortex. This is also the region were the myelination process ended. The degeneration spreads in reverse direction to the myelination process, namely from the transentorhinal cortex to entorhinal cortex, to the amygdala and from here to the hippocampus (42, 43, 44).Primary sensory and motor areas are spared until late in the disease process. Senile plaques or neuritic or amyloid plaques are formed by extracellular Aβ42 and the NFTs in neurons are formed by P-tau protein.The disease progression is closely linked to progression of neurofibrillary formations throughout the brain (42).

At autopsy, when both plaques and tangles are seen in the entorhinal cortex and hippocampus, the diagnosis is confirmed as AD. Neuronal loss is a prominent factor caused by tangle overload intracellularly in the temporal neocortex and hippocampus. The death of neurons leads to increasing volume loss in the MTL, detectable by CT and MRI, and a hypoperfusion in the region, detectable by SPECT and PET.

Medial temporal lobe atrophy is suggested to be a diagnostic marker for AD (45).One of the most important and most studied structures in the MTL is the hippocampus formation.

1.5.2 Hippocampus

The development of the hippocampus has been explained as a progressive infolding of the foetal gyrus dentatus, cornu ammonis, subiculum and parahippocampal gyrus around the progressively smaller hippocampus sulcus (46). By the 21st week of gestation the hippocampus and surrounding structures appear similar as in the adult brain (47). The fully inverted hippocampus has an oval configuration in coronal MR images. Incomplete hippocampal inversion is found in 19% of the population and in these cases the hippocampus retains a round or pyramidal shape on the affected side. Unilateral hippocampus malrotations are seen in 13% of individuals and only on the left side. Bilateral, incomplete inversion is seen in 6% (47) of the population. The hippocampus on the right side is normally somewhat larger than on the left side and females on average have a larger hippocampus, normalized to intracranial volume (ICV), than males (39).

The hippocampus together with the cingulate cortex, olfactory cortex and amygdala is part of the limbic system. The hippocampus plays an important role in the consolidation of information from short-term memory to long-term memory and also in spatial navigation.

Damages to the hippocampus may affect it unilaterally or bilaterally and may be caused by infarction, tumour, encephalitis or MTL epilepsy, among other causes. Patients with unilateral hippocampal damage leaving the structure intact on the contralateral side, can retain nearly normal memory function (48). Bilateral damage to the hippocampus results in profound difficulties in forming new memories and also affects memory formed before the damage. The most famous patient without hippocampus function was Henry Gustav Molaison. Both his hippocampus formations were surgically removed in an attempt to relieve his epileptic seizures (49). After surgery he was

unable to form new episodic memories. Also he could not remember what happened shortly before the operation, but was able to retain some memories from his childhood (50).In early AD, episodic memory impairment is one of the first symptoms and it is also the core symptom in new research criteria for AD (51).

1.5.2.1 Hippocampus in normal ageing

For many years it was an accepted fact that neurons were irreplaceable. Neurons did not regenerate. They lived and they died. In 1998 Eriksson et al published a paper on neurogenesis in the human brain (52). They showed that new neurons are formed in the hippocampus in the adult human brain. This new nerve cell formation is found in the subgranular zone in dentate gyrus in the hippocampus. Later, neurogenesis was shown to also take place in the subventricular zone and the olfactory bulb (53) and in a more pronounced way than in the hippocampus (53).There is a normal volume loss in the hippocampus with age. After about 70 years of age there is a significant difference in the linear atrophy slope progression. This age was therefore suggested to be the starting point of an acceleration of atrophy in the hippocampus (39).

1.5.2.2 Hippocampus in Alzheimer's disease

Alzheimer's disease starts in the entorhinal cortex and from there expands to the hippocampus.The neuron loss in the hippocampus in AD is located to the subiculum and dentate granular layer (54). Note that new nerve cells are formed just beneath, in the hippocampal subgranular layer in the dentate gyrus (52).

Hippocampal atrophy is a common feature in advanced AD. This atrophy is easily detected by both CT and MRI (11, 14, 45). Autopsy studies have reported 36–60% reduction in hippocampal volume in late AD compared with normal ageing (55). Volumetric measurements of the hippocampus and visual assessment of MTA atrophy based on MRI sequences are used in the work-up and diagnosis of MCI and AD (45). Subjects with MCI have a higher risk to progress to AD (56-59) but a substantial proportion remains stable or revert back to normal.

In early-onset AD, atrophy may first be noticed in the precuneus region while the hippocampus may stay unaffected even late in the disease process (60). Hippocampus atrophy is, furthermore, not specific to AD (61) and can be present also in other cognitive disorders. In frontal lobe dementia (FLD), for example, hippocampus atrophy and frontotemporal atrophy may be strongly marked. Hippocampus atrophy can also be pronounced in vascular dementia (VaD), dementia of Lewy body type (DLB) and semantic dementia and in mixed forms (SD) (62, 63) but it can also be a normal finding in the very old (39).

1.5.3 Evaluating brain atrophy

1.5.3.1 Autopsy

Senium means old age. Already in the 7th century B.C., Pythagoras divided life into five distinct stages at the ages of 1-7, 8-21, 22-49, 50-63 and 64-81 years. The two oldest groups were described as "a period of decline and decay of human body and regression of mental capacities" (64, 65).

Roger Bacon in the 13th century was probably the first to refer to the brain as a source of memory and thoughts. He thought the ventricular system in the brain consisted of three ventricles, the anterior ventricle for the imagination, the middle one for thought and judgment and the posterior ventricle for memory (64, 66).

Dissection of the human body has in different ways been performed for thousands of years, at the beginning for mummifying purposes only, but later on with an interest in learning human anatomy and pathology. In the 17th century when dissections of the human body for anatomical study became less taboo there was an increasing trend towards researching underlying organic changes in the brain as the source of mental disorders (66). In 1864 Wilks (64, 67) described decrease in brain weight as a sign of atrophy. He also described brain atrophy, saying that "instead of sulci meeting, they are widely separated and their intervals are filled with serum … and the full depth of the sulci can be seen" (64, 67).

It may have been Galileo Galilei who invented the microscope in 1609, but it was only after improvements in microscopy, in the late 19th century, and with new histochemical techniques, that we could properly look at parts of the nervous system for the first time. In 1898 Redlich described two cases of senile dementia with miliary sclerosis (64, 68), and in 1907 using the new Bielschowsky stain, Alzheimer described the findings in the first recorded early-onset AD case (1, 2). Today's techniques of performing a brain autopsy and staining are improved since 1907, but are built on the same principles as Alzheimer's technique.

Macroscopically the AD brain at autopsy shows widening of the ventricles and sulci, cortical thinning and MTA. Brain weight loss is more pronounced in younger than in older AD patients (69).

A problem today is that the overall autopsy rate is decreasing and continues to decline in the Western countries (70). For this reason, brain banks for brain donations and to ensure research possibilities are being formed all over the world. The Brain Bank of Karolinska Institute was started in 1972 thanks to the pioneering work of Prof. Bengt Winblad. The bank now can provide brain tissue for research on neurological diseases, for experimental purposes and for education (71).

1.5.3.2 Visual assessment

Visual assessment of brain changes, on MRI or CT images, is what neuroradiologists perform every day, often resulting in different assessments for different radiologists depending on skill and experience. In an attempt to standardize the visual assessments in clinical studies, among others the Fazekas group and Wahlund team have created grading scales for white matter changes (WMCs). Scheltens' group in Amsterdam have devised grading scales for global cortical atrophy (GCA), visual assessment of MTA (vaMTA) and, recently, a grading scale for visual assessment of posterior atrophy (PA). A new multi-functional rating scale, the Brain Atrophy and Lesion Index (BALI); was presented by Chen et al in 2010 (72).

1.5.3.2.1 Global cortical atrophy

The GCA scale gives the mean score for cortical atrophy throughout the entire cerebrum and is based on a study by Pasquier et al (73) in 1996. The visual assessment of GCA is based on consideration of the width of the sulci and the volume of the gyri in all lobes of the cerebrum and can also be used for the cerebellum. It is a 4-point scale, where 0 = no cortical atrophy; 1 = mild atrophy, and opening of the sulci; 2 = moderate atrophy, with volume loss of the gyri; and 3 = severe atrophy, also known as end-stage, "knife-blade" atrophy. In the initial study, dilatation of the ventricles was also staged (73). The cortical atrophy can be general, focal and/or asymmetric and it can be a pathological sign or a normal finding in the very old.

With repeated MRI investigations and documentation of increasing cortical atrophy and ventricular enlargement, GCA staging is a valuable tool for diagnosis of AD (74, 75). Assessment of focal cortical atrophy may identify patients with FLD, SD, primary progressive aphasia and posterior cortical atrophy (74).

1.5.3.2.2 Posterior atrophy

Alzheimer's disease is typically associated with MTA, but MTA can also be present in other forms of dementia such as FTD, VaD, DLB and can be seen in normal ageing. In early-onset AD or in individuals with prominent visual problems due to AD, atrophy can be seen in the posterior cingulate gyrus, precuneus and parietal lobes. Sometimes this atrophy is seen without any sign of MTA (60, 76, 77).

Visual assessment of posterior cortical atrophy, on MRI, has been described in a newly published paper by Koedam et al (77). A visual rating scale for PA has been developed, consisting of an assessment in three dimensions, sagittal, coronal and axial. It is a 4-point scale, where 0 = no atrophy; 1 = mild widening of the sulci without evident volume loss of the gyri; 2 = substantial widening of the sulci and volume loss of the gyri; 3 = severe end-stage atrophy. The anatomical structures that are evaluated in three dimensions are the posterior cingulate sulcus, parieto-occipital sulcus, precuneus and parietal lobe.

This study showed that visual assessment of PA was able to discriminate individuals with AD from other dementias and controls, while vaMTA failed to discriminate AD from other dementias. This suggests that a combination of vaMTA and visual assessment of PA may increase the sensitivity for AD (77).

1.5.3.2.3 Medial temporal lobe atrophy

Visual assessment of MTA was developed in the early 1990s by Philip Scheltens' team in Amsterdam. The first publication was a validation of visually assessed atrophy using linear measurements of the MTL (78). The assessment was revised in 1995 (79) and apart from some small adjustments the method for visual assessment has remained unchanged since then. There are other methods for assessment of MTA, but most of them are further developments of Scheltens' original method (59, 80).

The vaMTA is based on a 5-point grading scale. The assessment is performed on a coronal MRI image which can be angulated perpendicular to the anterior-posterior commissure (AC-PC) line or perpendicular to the long axis of the hippocampus, which is a coronal line along the brainstem axis (59, 79, 81). Structures evaluated are the choroid fissure, the temporal horn of the lateral ventricle and the hippocampus formation including the hippocampus proper, subiculum, parahippocampal gyrus and dentate gyrus.

The MTA scores range from 0 = no atrophy; to 1 = slightly increased width of the choroid fissure; 2 = increasing width of the choroid fissure, slightly increased width of the temporal horn and slightly decreasing height of hippocampus; 3 = wide open choroid fissure, increasing width of the temporal horn and progressive decrease in hippocampal height; and 4 = end-stage atrophy with wide open choroid fissure and temporal horns and a minimal hippocampus (79).

With the new, multi-detector CT technique it is possible to achieve good-quality CT images in any desired plane for vaMTA. A comparison between 64-detector row CT and 1.5 Tesla (T) MRI performed by Wattjes et al (11) showed good agreement between CT and MRI. The correlation between hippocampal volume and MTA has been shown to be statistically significant, but the correlation coefficient is low (81). Perhaps MTA is more likely to reflect whole brain atrophy compared with just hippocampal atrophy. In a work by Knoops et al this has been suggested (82).

1.5.3.3 Linear measurements

Even before the CT and MRI era, linear measurements were performed on plain films. An indirect measurement of atrophy was the Evans ratio. This involves dividing, using a pneumoencephalogram, the maximal anterior width of the lateral ventricles by the maximal intracranial width in an anterior-posterior plain film view (83).

Linear measurements have also been used in CT to evaluate brain atrophy (84, 85). The measurements were used to calculate ratios between structures in order to compare controls with diseased individuals. The dilatation of the lateral and third ventricles was thought to be an effect of central atrophy. In longitudinal studies increasing distances or decreasing ratios were noted for AD patients compared with controls (86).

In a recent study by Zhang et al (87) linear measurements on CT images were investigated in a population of AD patients and healthy elderly. Only two ratios of significance were found, the temporal horn ratio and the suprasellar cistern ratio. An increase in the temporal horn ratio reflects the enlargement of ventricles caused by MTA. Also, the suprasellar cistern ratio increases with increasing MTA (87).Linear measurements in MRI investigations of atrophy are not often seen, but a few studies have reported significant results when comparing linear measurements in individuals with AD or MCI, and controls (78, 88).

1.5.3.4 Volumetry

In volumetry, a structure or a cavity can be outlined and calculated, discovering even very small differences in or between individuals in longitudinal studies (89). Volumetry

is mostly used in MRI, but before the MRI era, CT volumetry was used, especially in calculating ventricular volumes (86).

Volumetry using MRI can be performed on all regions that have well-defined borders. For example, in the diagnosis of AD hippocampus, amygdala and entorhinal cortex, whole-brain volume or cortical thickness can be outlined and calculated. All these regions are of interest in the work-up of AD, but volumetry is time-consuming and so far has been used only in research.

Manual outlining of the hippocampus, amygdala and entorhinal cortex is often performed on a three-dimensional (3D) T1-weighted MRI sequence (39). The structures may be outlined in the coronal projection (39) or in a sagittal projection (90) but all three image planes, sagittal, coronal and axial orientations, are used to control the delineations.

The programs used for manual volumetric calculation differ, as do the techniques to outline the structures. Besides the manual outlining, semi-automatic and automatic methods can be used. The variety of software programs available for semi-automatic and automatic segmentation techniques (90, 91) is large.

Manual outlining is highly dependent on the investigator. A manual segmentation of hippocampus volume, performed by a skilled investigator, is better than other techniques such as vaMTA or automatic calculation of hippocampal volume (91) in detecting atrophy.

An important advantage in using automatic segmentation rather than manual assessment is the consistency and reproducibility of the segmentations, which almost completely eliminates investigator bias (90).

1.6 NEW PROPOSED CRITERIA FOR ALZHEIMER'S DISEASE

The clinical criteria for AD were outlined in 1984 by the NINCDS-ADRDA (8). These criteria are based on a two-step work-up. Firstly the diagnosis of dementia has to be made. Secondly the aetiology of the dementia disorder is established based on distinct observations of symptoms and clinical signs.

Since 1984 the general use of CT, MRI, PET, biomarkers in CSF, and genetics has made it possible to diagnose AD much earlier in the disease process than was previously possible and in some cases even before any symptoms have appeared. This has raised the need for new, updated criteria for AD diagnosis in order to bring the time point of the diagnosis forward to precede the development of the dementia syndrome where the diseased person is no longer in charge of themselves.

The first step in this direction came in 2007 with a publication by Dubois et al (51). In this paper new research criteria for AD were based on a core criterion and supportive biomarkers. Early and significant episodic memory impairment is the core criterion. Supportive features to the core criterion are presence of MTA, abnormal CSF markers,

specific patterns on functional neuroimages with PET, and proved AD autosomal dominant mutation within the immediate family. According to these research criteria, the core criterion and one of the supportive factors are enough for diagnosing "probable AD" (51).

In 2009 the National Institute on Aging (NIA) and the Alzheimer's Association sponsored a series of round table meetings whose purpose was to establish a process for revising diagnostic and research criteria for the continuum of AD (Fig. 1).

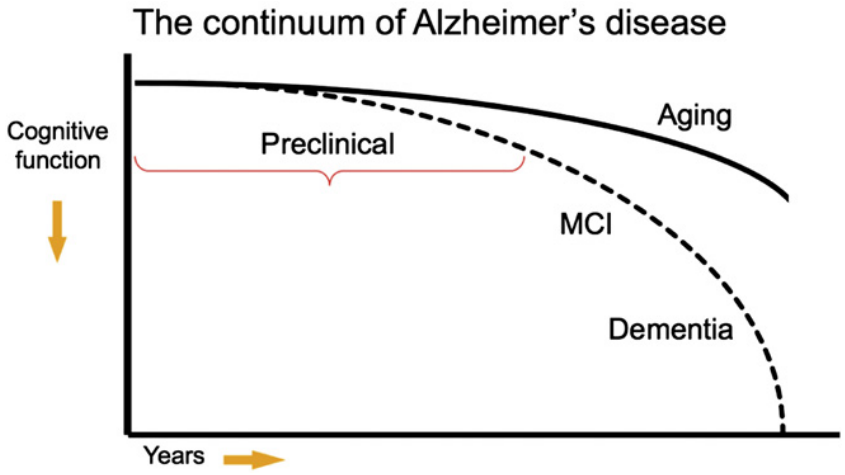


Fig. 1. Model of the clinical trajectory of Alzheimer's disease (AD). The stage of preclinical AD precedes mild cognitive impairment (MCI) and encompasses the spectrum of presymptomatic autosomal dominant mutation carriers, asymptomatic biomarker-positive older individuals at risk for progression to MCI due to AD and AD dementia, as well as biomarker-positive individuals who have demonstrated subtle decline from their own baseline that exceeds that expected in typical aging, but would not yet meet criteria for MCI. Note that this diagram represents a hypothetical model for the pathological-clinical continuum of AD but does not imply that all individuals with biomarker evidence of AD-pathophysiological process will progress to the clinical phases of the illness. Figure and figure text from Sperling R et al 2011 (92).

The recommendation from these advisory meetings was to form three separate workgroups, each with an assigned task to formulate diagnostic criteria for the asymptomatic preclinical phase of AD, the symptomatic preclinical phase of AD and the dementia phase of AD, respectively. A fourth separate group was later tasked with revising neuropathological criteria for AD (93). The recommendations from the initial three workgroups were presented in a symposium at the 2010 International Conference in Alzheimer's Disease (ICAD) meeting in Honolulu, Hawaii. The documents were revised and all three documents were submitted simultaneously in 2011. The recommendation from the neuropathology workgroup was supposed to be in late 2011 (93).

A general problem for the members in the workgroups has been to define specific cut-off points for normal versus abnormal values for neuroimaging and CSF biomarkers. Regarding CSF biomarkers, work needs to be done with regard to uniform assessment,

standardization and use of the laboratory techniques to evaluate the biomarkers Aβ, T-tau and P-tau (93).

In 2010 Jack et al published a hypothetical model of dynamic biomarkers of Alzheimer's pathological cascade (94). This cascade shows the dynamics of the biomarkers' relation during stages of cognitively normal individuals in the preclinical stage, through MCI to dementia (Fig. 2). In his hypothetical model of the disease cascade there may be abnormal accumulation of Aβ, detectable with PET-PIB first in the frontal lobes, 10-20 years before symptoms become noticeable. Brain Aβ accumulation is necessary but not sufficient to produce clinical symptoms (92). Cognitive symptoms are directly related to neurodegeneration and increasing T-tau and P-tau and these may be pathological in the late preclinical stage or early MCI. Magnetic resonance imaging or volumetry of MTL structures may turn pathologic inthe MCI stage parallel to the cognitive reduction debut, but only in the dementia stage does clinical function drop (94). None of the biomarkers is static; they change over time and follow a non-linear time course which is hypothesized to be sigmoidal in shape.

This diagram makes it easier to understand the disease development in AD, but note that this a hypothetical model (Fig. 2).

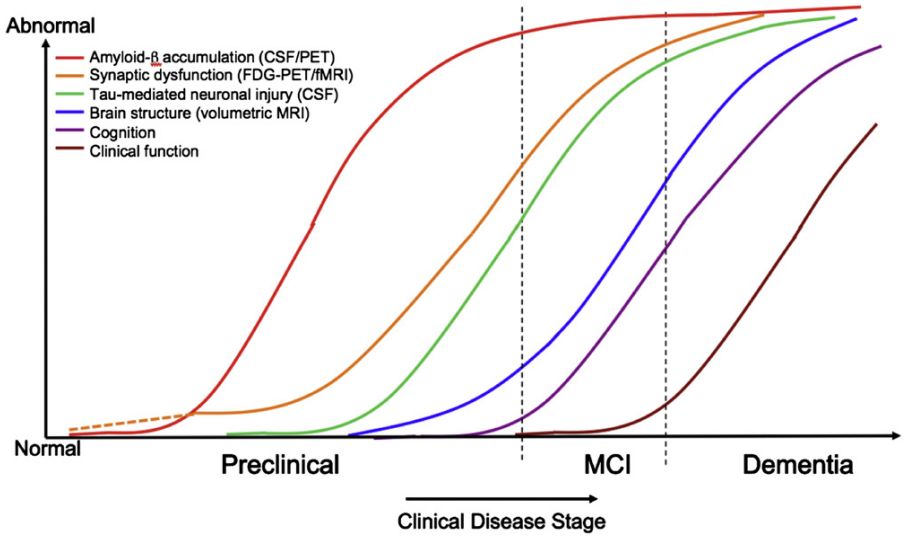


Fig. 2. Hypothetical model of dynamic biomarkers of the AD expanded to explicate the preclinical phase: Ab as identified by cerebrospinal fluid Ab42 assay or PET amyloid imaging. Synaptic dysfunction evidenced by fluorodeoxyglucose (F18) positron emission tomography (FDG-PET) or functional magnetic resonance imaging (fMRI), with a dashed line to indicate that synaptic dysfunction may be detectable in carriers of the ε4 allele of the apolipoprotein E gene before detectable Ab deposition. Neuronal injury is evidenced by cerebrospinal fluid tau or phospho-tau, brain structure is evidenced by structural magnetic resonance imaging. Biomarkers change from normal to maximally abnormal (y-axis) as a function of disease stage (x-axis). The temporal trajectory of two keyindicators used to stage the disease clinically, cognitive and behavioral measures, and clinical function are also illustrated.
Figure and figure text are slightly modified by Sperling R et al (92) but orginates from Jack CR et al, (93).

The results of the three workgroups were formulated into diagnostic criteria for three stages of AD: the asymptomatic preclinical phase of AD, the symptomatic preclinical phase of AD, and the dementia phase of AD. The first stage, the asymptomatic preclinical phase of AD, is subdivided into three phases, according to the preclinical workgroup (94).These three phases in the first stage are for preclinical research use only and are built on absence or presence of Aβ, markers of neuronal injury and evidence of subtle cognitive change.

The second stage, the symptomatic predementia phase of AD, has also been named "mild cognitive impairment due to AD" by Albert et al (95). For a patient to be referred to as second stage AD, the core criteria for MCI, listed below, must be met first.

The core criteria for the diagnosis of MCI are:
1. Concern regarding a change in cognition
2. Impairment in one or more cognitive domains
3. Preservation of independence in functional abilities
4. Not demented

In the third stage, the dementia or "dementia due to AD" stage (96), the core criteria for dementia have to be fulfilled first.

The core criteria for dementia are:

1. Interference with the ability to function at work or at usual activities
2. Decline from previous levels of functioning and performing
3. Condition not caused by delirium or major psychiatric disorder
4. Cognitive impairment
5. Cognitive or behavioural impairment in at least two of following domains:
    a. impaired ability to acquire and remember information
    b. impaired reasoning and handling of complex tasks
    c. impaired visuospatial functions
    d. impaired language functions
    e. changes in personality

In this stage there are seven different phases more or less confirming the diagnosis of "dementia due to AD", according to McKhann et al (96). As with the second stage, additive features are absence or presence of biomarker of AD type, Aβ, and neuronal injury, together with fulfilled core criteria of AD.

2 AIMS OF THE THESIS

The overall purpose of this thesis was to test the reliability of vaMTA in different settings. This was done in four studies with specific aims as follows:

StudyI To investigate intra- and inter- rater agreement of visual assessment of MTA over a period of one year.

Study II To evaluate visual assessment of MTA compared with hippocampus volume in an elderly non-demented population. A second aim was to create cut-off normal MTA score values for different age groups for use in a clinical evaluation.

Study III The first aim was to compare the performance of the multivariate technique, orthogonal projections to latent structures (OPLS), with visual assessment of MTAand hippocampal volume to differentiate between AD cases and controls. The second aim was to assess how well the three different approaches predicted conversion from MCI, at baseline, to AD at one year follow-up.

Study IV To validate the proposed new research criteria for AD, from 2007, in a naturalistic sample of patients with memory disturbances. The second aim was to assess the concordance between the proposed new research criteria and the clinical evaluation according to the traditional disease classification systems, DSM-IV and ICD-10, in diagnosing AD.

3 MATERIALS AND METHODS

Three of the studies, studies I, II and IV, were approved by the ethical committee at Karolinska Institutet. The Norwegian part of study I was approved in a letter of notification from Professor Knut Engedal at Hukommelsesklinikken, Ulleval University Hospital, Oslo, Norway. Study III was approved by the ethical review boards in each of the participating countries (the United Kingdom (UK), Finland, France, Greece, Italy and Poland) verified by a letter of notification from Professor Simon Lovestone at the MRC Centre for Neurodegeneration Research, King's College London, UK.

3.1 PATIENTS

Study I: In this retrospective study, 100 out-clinic patients with memory problems were included, 50 from Minnesmottagningen, Karolinska University Hospital, Huddinge, Stockholm, Sweden, and 50 from Hukommelsesklinikken, Oslo University Hospital, Ulleval, Oslo, Norway. All patients underwent an MRI protocol for geriatric patients, according to local clinical standards. A clinical report was written before including patients in the study.

The Swedish population were examined during 2001–2004 and the Norwegian population between 2006 and 2008. Patients in the Swedish group were younger (44–69, mean 59, years) compared with those in the Norwegian group (52–87, mean 78, years). The inclusion criterion was a good-quality MRI with a coronal T1 sequence covering the hippocampus head and neck and at least parts of the hippocampal body. Patients were selected to represent all grades of MTA.

Study II: In this comparative study, 555 subjects, a sub-sample of an epidemiological study of 3,363 healthy, non-demented elderly individuals (≥60 years of age), were included in the Swedish National Study of Ageing and Care in Kungsholmen (SNAC-K). This is a longitudinal, multidisciplinary study on ageing and health, which began in 2001. The SNAC-K was created to detect the influence of lifetime genetic, environmental and biological factors on medical, psychological and social health in late adulthood. The SNAC-K samples were stratified for age and year of assessment. Ten age cohorts were chosen, with different assessment intervals: 6-year intervals in the younger cohorts (60–78 years old), and 3-year intervals in the older cohorts (aged ≥ 81). The MRI examinations were performed during 2001–2003. Subjects with severe cerebral diseases that directly affect brain atrophy were excluded.

On average, the selected 555 subjects were slightly younger, more educated, and cognitively advantaged compared with the remaining sample. After a quality assessment of the baseline MRI images, 544 subjects (60–97 years old), 318 females and 226 males, were finally included in the MRI study of volumetric measurements while all 555 (320 females and 235 males) were included in the visual assessment study. When comparing the two studies the same 544 subjects were used. Because of the limited sample size in the age groups ≥87 years, we categorized all participants aged 87 and older as one group. In total, we therefore investigated seven age groups: 60, 66, 72, 78, 81, 84 and ≥87 year olds.

Study III: All patients in this study were enrolled in the AddNeuroMed project, part of Innovative Medicines in Europe (InnoMed), a European Union (EU) programme designed to make drug discovery more efficient. Data were collected from six different sites across Europe: the University of Kuopio, Finland; the University of Perugia, Italy; Aristotle University of Thessaloniki, Greece; King's College London, in the UK; the University of Lodz, Poland; and the University of Toulouse, France. Magnetic resonance imaging images from *257 subjects were included in this study, 75 AD patients, 101 MCI patients and 81 healthy controls.

All AD and MCI subjects were recruited from local memory clinics of the six participating sites while the control subjects were recruited from non-blood-related members of the patients' families, caregivers' relatives or from social centres for the elderly. The inclusion criteria for AD were: NINCDS-ADRDA and DSM-IV criteria for probable AD, Mini Mental State Examination (MMSE) score range between 12 and 28, Clinical Dementia Rating (CDR) score≥0.5, and ≥65 years old. The inclusion criteria for MCI were: MMSE score range between 24 and 30, Geriatric Depression Scale (GDS) score≤5, CDR = 0.5, and age ≥65 years. The distinction between MCI and controls was in CDR scores. Controls rated 0 and MCI patients scored 0.5. Exclusion criteria were significant neurological or psychiatric illness other than AD, VaD or large infarcts. Patients with significant unstable systematic illness or organ failure were likewise excluded.

*****_A misestimation in the published paper III stated 252 subjects_

Study IV: From a total of 600 patient files with consecutive new referrals to Minnesmottagningen, Karolinska University Hospital, Huddinge, during the years 2001–2006, 150 patients had performed additional work-up according to the new criteria set by Dubois et al (51). These 150 subjects were included in this retrospective study. The mean MMSE score was 26 (range 18–30), mean age 59 (range 41–78) years, and mean CDR 0.5. The inclusion criteria in the study were an objective history of episodic memory dysfunction, CSF analyses, SPECT and/or vaMTA. None of the patients had undergone all the procedures available in the work-up of dementia. According to the cerebral MRI and/or CT scan, patients with intracerebral tumours, normal pressure hydrocephalus and cortical strokes were excluded. As our sample were retrospectively drawn from the routine clinical records and did not represent a preselected research cohort, they were a true naturalistic sample where the clinical presentation of each patient set the agenda for adding diagnostic procedures

3.2 METHODS

3.2.1 Magnetic resonance imaging

Study I: Magnetic resonance imaging was performed at the Department of Radiology at Karolinska University Hospital, Huddinge, Sweden, using one of their three 1.5 T MRI scanners: Siemens Vision, Symfoni or Avanto (all from Siemens, Erlangen, Germany). A T1-weighted 3D magnetization-prepared, rapid-gradient echo sequence (MPRAGE), time of repetition (TR) 11.4 ms, time echo (TE) 4.4 ms and flip angle 10, was used to grade MTA. The coronal plane was selected perpendicular to a line

between the AC and PC in the midsagittal plane. This sequence yields 72 continuous coronal slices with a slice thickness of 2.5 mm, covering the whole brain. A good separation between grey and white matter was obtained.

At the Curato Røntgeninstitutt in Oslo, Norway, two different MRI protocols were used, depending on the year of investigation. A Siemens Avanto 1.5 T MRI machine was used for all patients. For 13 patients investigated in 2006, the protocol included a coronal T1-weighted two-dimensional (2D) sequence with TR 450 ms, TE 9.4 ms and two acquisitions. The coronal sequence was angled perpendicular to the AC–PC line. The slice thickness was 4 mm, with a 0.4 mm gap between images. This sequence yielded 20 continuous slices. During the period 2007–2008 a coronal T1-weighted 3D time of flight (tfl) was used, with TR 1900 ms and TE 3.08. Slice thickness varied between 0.9 and 1 mm. The number of continuous slices varied between 41 and 110. The coronal orientation was perpendicular to the AC–PC line. This sequence was used for the remaining 37 patients from this centre. In both Norwegian protocols the head, neck and parts/whole body of hippocampus and MTL were covered and a good separation between grey and white matter was obtained.

No corrections were made post-MRI to the images, regarding angulation in the coronal plane or sidewise asymmetry in the images. This means that the sequences included in this study were identical to the native sequences.

Study II: The MRI scan was performed on a 1.5T scanner (Philips Intera, The Netherlands). In the SNAC-K project, 3D fast field echo (FFE) T1 MRI sequences were used. The 3D FFE T1 images (TR = 15 ms, TE = 7 ms, flip angle = 5°, number of slices = 128, thickness = 1.5 mm, field of view (FOV) = 240, matrix = 256*256) were used both for volumetric measurements of the hippocampus and ICV, and for vaMTA. The transversal images were reoriented to coronal sections perpendicular to the AC–PC line, according to the Talairach atlas (97) suitable for both volumetric and visual assessments.

In a subgroup of 21 subjects, reorientation to coronal sections was performed both perpendicularly to the AC–PC line and parallel to the brainstem, in order to investigate whether there was any significant statistical difference when rating images with different angulation.

Study III: Data acquisition for the AddNeuroMed study was designed to be compatible with the Alzheimer Disease Neuroimaging Initiative (ADNI) (98). The imaging protocol for both studies included a high resolution sagittal 3D T1-weighted MPRAGE volume (voxel size1.1x1.1x1.2 mm^3). The MPRAGE volume was acquired using a custom pulse sequence specifically designed for the ADNI study to ensure compatibility across scanners (98). Full brain and skull coverage was required and detailed quality control was carried out on all MR images according to the AddNeuroMed quality control procedure (99).

Study IV: A standardized protocol for geriatric purposes was used, performed on a 1.5 T Siemens Vision (Symfoni and Avanto) scanner (Siemens, Erlangen, Germany). Visual assessments were based on a T1-weighted 3D MPRAGE sequence, TR = 11.4 ms, TE = 4.4 ms and flip angle = 10. The coronal plane was chosen, perpendicular to a line between the AC and PC in the midsagittal plane. This sequence yields 72 continuous coronal slices, with a slice thickness of 2.5 mm, covering the whole brain. A good separation between grey and white matter was obtained.

3.2.1.1 Visual assessment of the medial temporal lobe

Studies I-IV: The MTL was bilaterally visually assessed regarding atrophy on coronal MRI images. The MTA scale was invented by Philip Scheltens et al(published in 1992 (78) for the first time) for use on plain films. Later the technique was modified (79) for use with digital images. Visual assessment of MTA was performed on one specific coronal MRI slice, behind the amygdala and mamillary bodies, in the position of the cerebral peduncles. It was based on a visual estimation of the MTL volume. The visual assessment included the hippocampus proper, dentate gyrus, subiculum, parahippocampal gyrus, entorhinal cortexand surrounding CSF spaces such as the temporal horn and choroid fissure (Table 1). The right and left side were rated separately. Scores ranged from 0 to 4 (Fig. 3), with 0 = no atrophy; 1 = slightly increased width of the choroid fissure; 2 = increasing width of the choroid fissure, slightly increased width of the temporal horn and slightly decreasing hippocampal height; 3 = wide open choroid fissure, increasing width of the temporal horn and progressive decrease of hippocampal height; and 4 = end-stage atrophy with wide open choroid fissure and temporal horns and a small hippocampal volume (79).

Table 1. Visual assessment of medial temporal lobe atrophy (vaMTA), using the scale by Philip Scheltens et al (78, 79) Copyright © 1992, *British Medical Journals* (with permission).

MTASCORE	WIDTH OF CHOROID FISSURE	WIDTH OF TEMPORAL HORN	HEIGHT OF HIPPOCAMPUS
0	N	N	N
1	↑	N	N
2	↑↑	↑	↓
3	↑↑↑	↑↑	↓↓
4	↑↑↑	↑↑↑	↓↓↓

N normal, ↑increasing, ↓decreasing. Atrophy increases with an increased score-number.

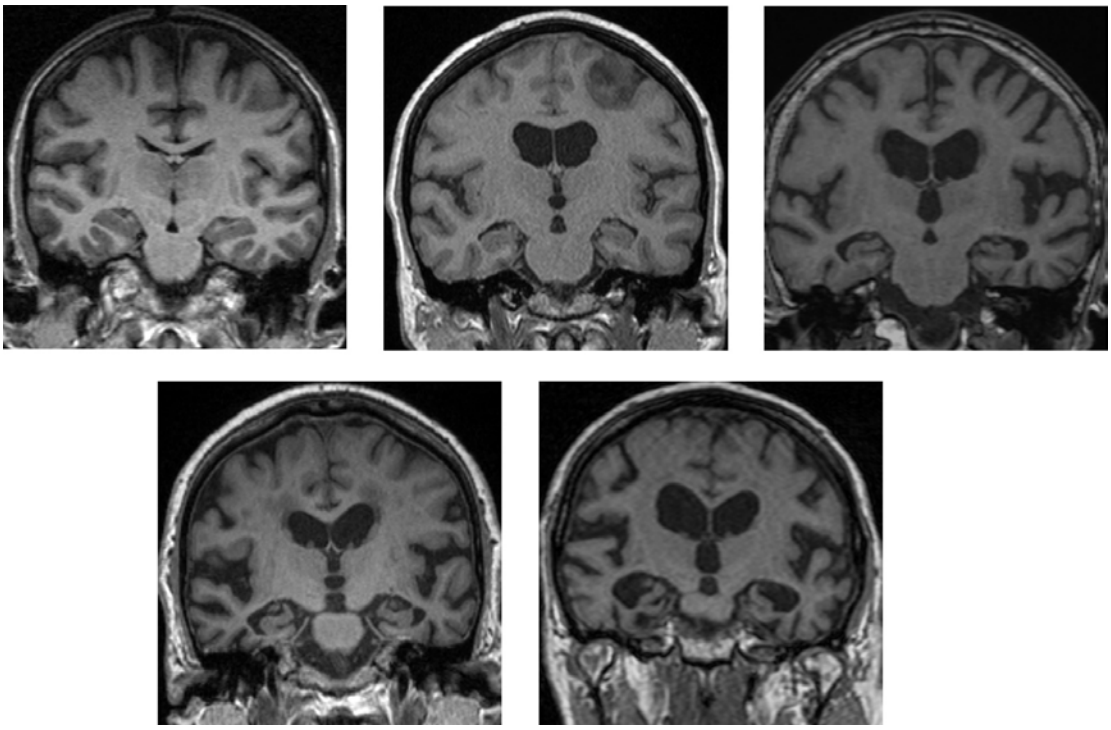


Fig. 3. Visual assessment of medial temporal lobe atrophy (vaMTA). The images show, from left to right, MTA scores 0–4.

3.2.1.2 Volumetric measurements

Studies II and III: Magnetic resonance images were transferred to a HERMES workstation (Nuclear Diagnostics, Stockholm, Sweden). The transversal 3D T1 FFE images were reoriented to coronal sections perpendicular to the AC–PC line, according to the Talairach atlas (97). STEREOLOGY (100), volumetric software in HERMES, was used to obtain ICV. This is a semi-automatic method for estimating large brain structures which it is time-consuming to delineate. Intracranial volume was used to adjust the hippocampal volume to different head sizes. With a region of interest (ROI) tool in HERMES Multi Modality, hippocampal formation was manually delineated and the hippocampal volume was calculated. The volumetric method and volumetric work in these two studies has been described in detail in a previous publication by Zhang et al from 2010 (39).

3.2.1.3 Regional volume segmentation

Study III: In this study Free-Surfer was used, a pipeline developed by Fischl and Dale (101) which produces measurements of regional cortical thickness and volumetric measures (Fig. 4). Cortical reconstruction and volumetric segmentation includes removal of non-brain tissue using a hybrid watershed/surface deformation procedure (102), automated Talairach transformation, segmentation of the subcortical white matter and deep grey matter volumetric structures (including hippocampus, amygdala, caudate, putamen, ventricles) (102-104), intensity normalization (105), tessellation of the grey matter white matter boundary, automated topology correction (106, 107), and surface deformation following intensity gradients to optimally place the grey/white and grey/CSF borders at the location where the greatest shift in intensity defines the transition to the other tissue class (101, 108, 109). Once

thecortical models are complete, registration to a spherical atlas takes place which utilizes individual cortical folding patterns to match cortical geometry across subjects (110). This is followed by parcellation of the cerebral cortex into units based on gyral and sulcal structure (111, 112). The left and right side of volumes and thicknesses were averaged. The regional cortical thickness measures were originated from 34 areas and the regional volumes were measured from 23 areas. All volumetric measures from each subject were normalized to the subject's ICV. Thickness measures were not normalized.

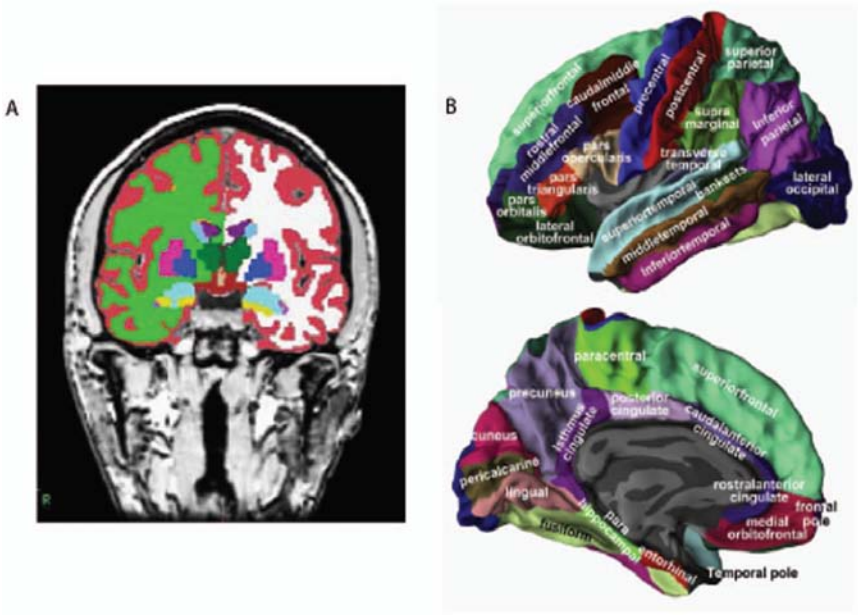


Fig. 4a and b. Representations of ROIs included as candidate input variables in the multivariate OPLS model. (A) Regional volumes. (B) Regional cortical thickness measures. DOI:10.1371/journal.pone.0022506.g001 PLoS ONE.

3.2.2 Single-Photon-Emission-Computed-Tomography

Study IV: The cerebral metabolism reflected by cerebral blood flow (CBF) was examined by SPECT using a visual assessment combined with a semi-quantitative method, with the Brain Registration Analysis and Software suite (BRASS) (113). After injection of 1000 MBq Tc-99m-HMPAO in quiet surroundings with the patient's eyes closed, SPECT perfusion was performed according to standard clinical procedure. Acquisition was started 30 minutes after injection. Data were collected in 60 projections evenly spread through 360° with a triple-headed rotating gamma camera (Picker IRIX, Cleveland, OH, USA) and a total acquisition time of 22 minutes. Tomographic slices were reconstructed using an iterative algorithm (Hosem, Nuclear Diagnostics AB, Stockholm, Sweden) with Chang attenuation correction (attenuation coefficient: 0.12/cm). Data were formatted as a 3D dataset with 128 x 128 x 64 voxel (64 transversal sections) and a voxel size of 1.75 x 1.75 x 3.5 mm^3. The reconstructed datasets were post-filtered with a Butterworth filter (cut-off 1.0/cm). The resolution in a tomographic slice was measured at 10.2 mm full width at half maximum.

3.2.3 Cerebrospinal fluid analysis

Study IV: Cerebrospinal fluid was obtained by standard procedure lumbar puncture in all patients agreeing to undergo this procedure. All samples were stored at –80°C until analysis. Cerebrospinal T-tau, Aβ 42 and P-tau were determined using a sandwich enzyme-linked immunosorbent assay (ELISA) constructed to measure these proteins (114). Two internal CSF pools were run on each ELISA to assure its reproducibility (115). The reference values (Aβ42 <427 ng/l, T-tau >445 ng/l or P-tau >74 ng/l) published by Wallin et al (114) were used as cut-off values.

3.2.4 Clinical assessments

Study IV: Since the core feature in the suggested new criteria by Dubois et al (51) is persistent reduction in episodic memory function, we included only patients who had been examined based on the RAVLT (116). This is a list of 15 words presented to the patient five times, reflecting episodic memory capacity (immediate and delayed recall). The cut-off for the pathological delayed recall test was set at <6 (out of the total of 15 words), according to previous findings (117). Global cognitive function was assessed using the MMSE (118), performed according to standard procedure by the routine clinical physicians seeing the patients at the first consultation at the memory clinic. Memory function including the RAVLT was assessed by a trained neuropsychologist. A grading of the cognitive impairment using CDR (119) was performed by a senior consultant when the patients were discussed in the diagnostic consensus meeting. The diagnosis was made according to the consensus criteria for MCI by Winblad et al. (120) , the ICD-10/DSM-IV criteria for dementia (3, 4), the NINCDS-ADRDA for AD (8), the NINCDS-AIREN for VaD (121) and the Manchester criteria for FTD (115). For the diagnosis of subjective cognitive impairment (SCI), we used the ICD-10 classification of "Z03.3 = observation for possible neuro-organic disorder" (3) when the patient had subjective memory problems that could not be objectively confirmed. When the dementia diagnosis was unclear, the ICD-10 classification for unspecified dementia (dementia UNS) was used (3).

3.2.5 Statistical analysis

Study I: Intra- and inter-rater reliability was performed with kappa and weighted kappa statistics (122, 123). The extent of the agreement between the repeated assessments from the radiologists concerning each patient is the proportion between the number of pairs for which there was agreement, and the total numbers of possible pairs of assignments. These values were calculated and stored in a data file. In order to compare the two raters and the two sites with respect to a patient's "agreement score" a generalized estimating equations (GEE) model was performed using the GENMOD procedure in SAS(SAS Institute Inc., Cary, NC, USA) (124). The GEE strategy is a useful approach for repeated measurement analysis of ordered categorical outcomes. A p-value < 0.05 was considered statistically significant.

Study II: Multivariate analysis of variance (MANOVA) was used to compare the differences in brain structure volumes between women and men, adjusted for age and ICV. Dependent *t*-tests were performed to identify the difference in hippocampal volume between the right and left side (122).

Spearman rank order correlation coefficient (r_s) was used to measure the association between hippocampal volume and vaMTA, MTA score. Analyses were performed using Statistica version 9.0 (StatSoft®, Inc., Tulsa, OK, USA). In comparisons between gender, age, and sides regarding MTA score, we used a GEE model and performed analyses using the GENMOD procedure in SAS, version 9.1 (SAS Institute Inc., Cary, NC, USA). The GEE strategy is a useful approach for repeated measurements analysis of ordered categorical outcomes. The model was set up for the within-group factor side (Sin, Dx) and the between-groups factors gender (F, M) and age (seven categories). Intra-rater reliability was analysed using kappa and weighted kappa for ordered categorical data, and intraclass correlation coefficient (ICC) for continuous data (volumetry).

Study III: Magnetic resonance images were analysed using OPLS (125-127), a supervised multivariate data analysis method included in the software package SIMCA (Umetrics AB, Umeå, Sweden). A very similar method, Partial Least Squares (PLS) to latent structures, has previously been used in several studies to analyse MRI data (128, 129). The two methods OPLS and PLS give the same predictive accuracy, but the advantage of OPLS is that the model created to compare groups is rotated, which means that the information related to class separation is found in the first component of the model, the predictive component. The other orthogonal components in the model, if any, relate to variation in the data not connected to class separation. Focusing the information related to class separation on the first component makes data interpretation easier (127).

Pre-processing was performed using mean centring and unit variance scaling. Mean centring improves the interpretability of the data, by subtracting the variable average from the data. By doing so the dataset is repositioned around the origin. Large variance variables are more likely to be expressed in modelling than low variance variables. Consequently, unit variance scaling was selected to scale the data appropriately. This scaling method calculates the standard deviation of each variable. The inverse standard deviation is used as a scaling weight for each MRI measure. Altogether 57 variables were used for OPLS analysis. No feature selection was performed, meaning all measured variables were included in the analysis. A model containing age was also created to test if there were any significant differences between the diagnostic groups in relation to this variable.

Sensitivity and specificity were calculated from the cross-validated prediction values of the OPLS models and for the visual assessment. Finally, the positive and negative likelihood ratios (LR+ = sensitivity/100-specificity) and LR- = (100-sensitivity/-specificity) were calculated. A positive likelihood ratio between 5 and 10 or a negative likelihood ratio between 0.1 and 0.2 increases the diagnostic value in a moderate way, while a value >10 or <0.1 significantly increases the diagnostic value of the test (130).

Finally the AD versus control (CTL) models were used as training sets to investigate how well they could predict conversion from MCI to AD after 1-year follow-up and how they compare to the visual assessment. To easily compare the performance of the three methods we also calculated the sensitivity (MCI converters predicted as AD) at a fixed specificity (MCI stable predicted as CTL). We set the specificity for all three methods for this comparison to that of the visual assessment since this cannot be changed, and recalculated the sensitivity and specificity for the other two methods.

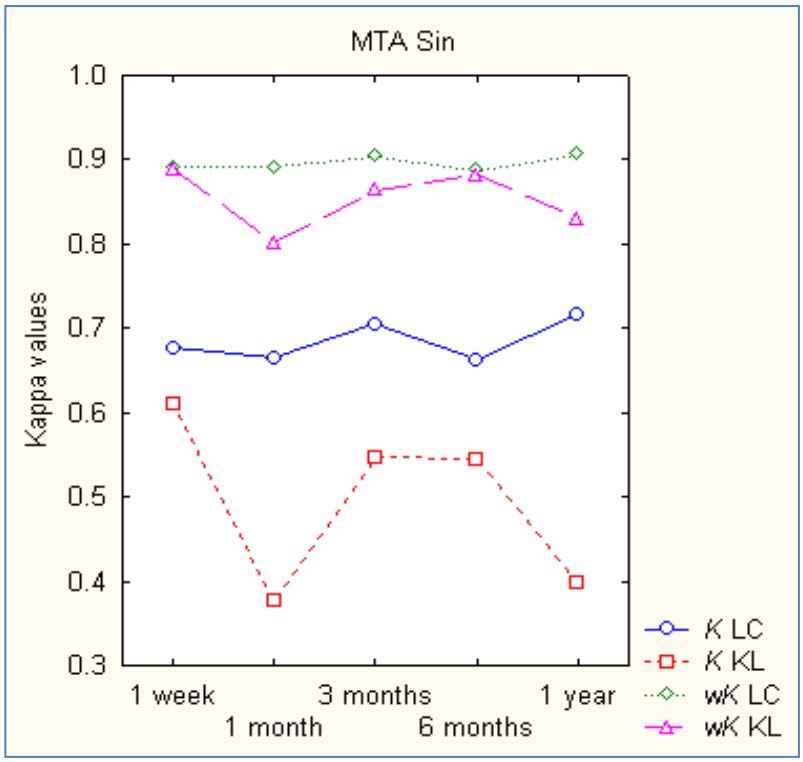
Study IV: Spearman rank order correlation coefficient was used to measure the association between the diagnosis of MCI and the biomarkers. The agreement between the diagnosis of MCI and the MRI, SPECT and RAVLT was analysed with κ- and weighted κ statistics. For the κ analyses, the variables were divided into three categories in the following way: diagnoses (0 + 1 = normal + SCI; 2 = MCI; and 3 = AD), MRI (0 + 1 = no atrophy; 2 = slight atrophy; and 3 + 4 = extensive atrophy), SPECT (0 = no pathology; 1 = slight pathology; and 2 = extensive pathology) and RAVLT delayed recall (3 + 4 = sum score 15-8, 2 = sum score 7–4 and 1 = sum scores 3–0). Judgments of correlation coefficients and κ values were made according to Landis and Koch (122) classifying the estimates as follows < 0.20 = poor, 0.21–0.40 = fair, 0.41–0.60 = moderate, 0.61–0.80 = good and 0.81–1.00 = very good. The sign test was used to analyze the difference between sides.

4 RESULTS

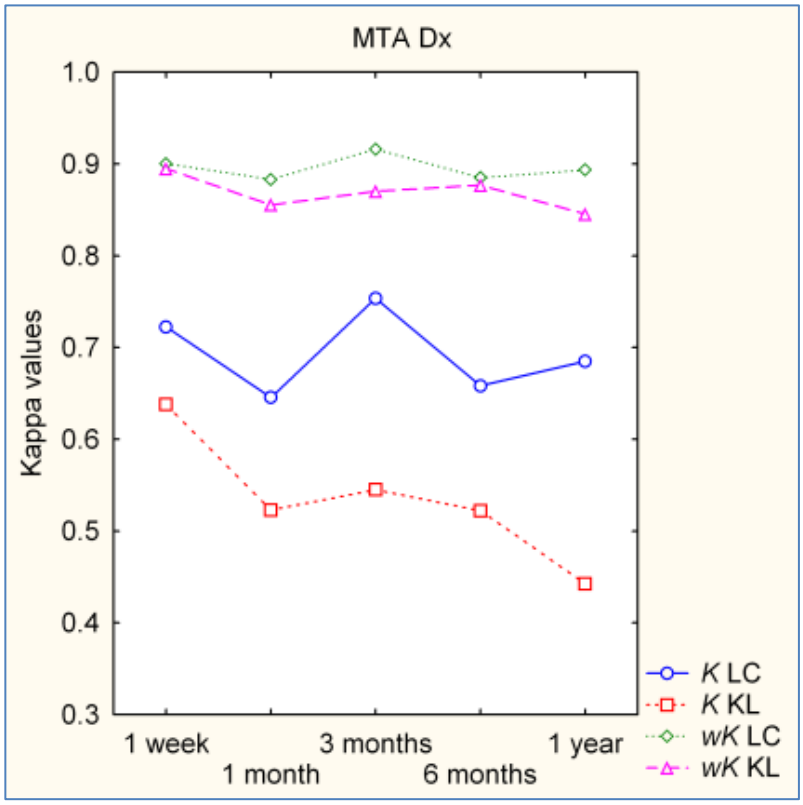
Study I: Consensus ratings at time zero were compared with ratings at 1 week, 1 month, 3 months, 6 months and 1 year and revealed variation between sides for both investigators. For the neuroradiologist, simple kappa ranged between 0.65 and 0.75 on the right side and between 0.66 and 0.72 on the left side. For the general radiologist, a decline was seen on the right side from 0.64 to 0.44, and on the left side from 0.61 to 0.38 at the 3-month and 0.40 at the 1-year rating. Intra-rater weighted kappa showed almost perfect agreement (wκ 0.81–1.00) for both radiologists, with few variations over the year and little variation between the right and left side. The weighted kappa values varied between 0.88 and 0.92 on both sides for LC and between 0.86 and 0.89 on the right side and between 0.80 and 0.89 on the left side for KL (Fig. 5a and b).

Inter-rater reliability was fair to moderate. The agreement between the two investigators decreased during the year and dropped from κ 0.48 to κ 0.32 on the right side and from κ 0.48 to κ 0.28 on the left side. Weighted kappa values for inter-rater agreement showed little variation over the year, ranging from 0.79 to 0.84 on the right side and from 0.73 to 0.84 on the left side, with a dip at 1 month (κ 0.73), which corresponded to the simple kappa at 1 month (κ 0.28), on the left side (Fig. 6a and b).

There was no statistically significant difference in MTA rating between the Swedish and Norwegian examinations ($p=0.35$), and there was no statistically significant interaction between country and investigator ($p=0.57$). There was no significant difference between the three T1-weighted sequences used for evaluating the MTL: 3D MP-RAGE, 3D tfl or coronal 2D T1. There was no difference in kappa when comparing thin (0.9–2.5 mm) with thick images (4 mm). A statistically significant difference in MTA rating was found between the two radiologists ($p<0.001$).

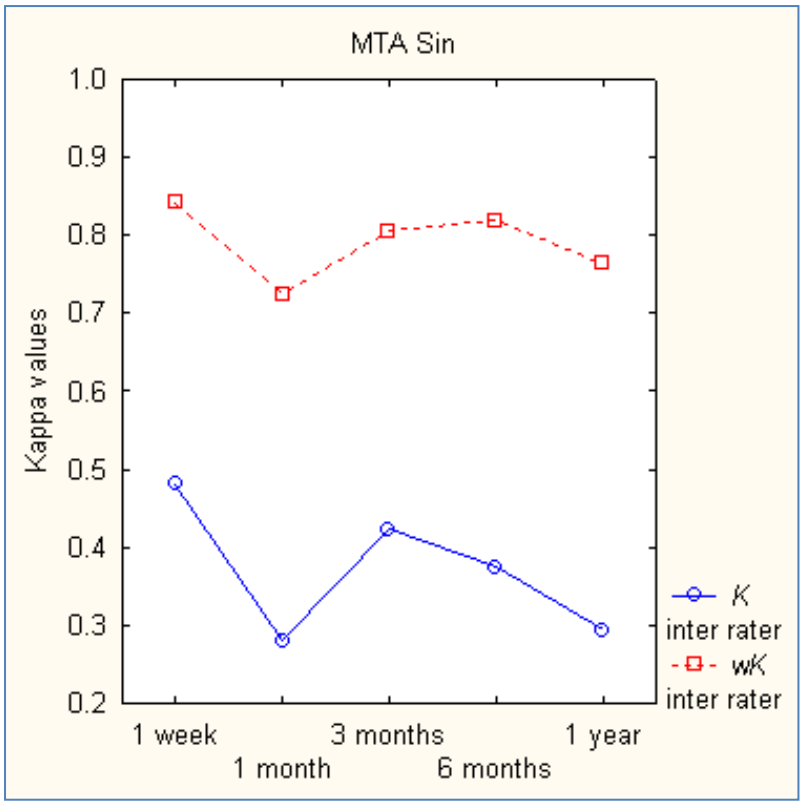


a)

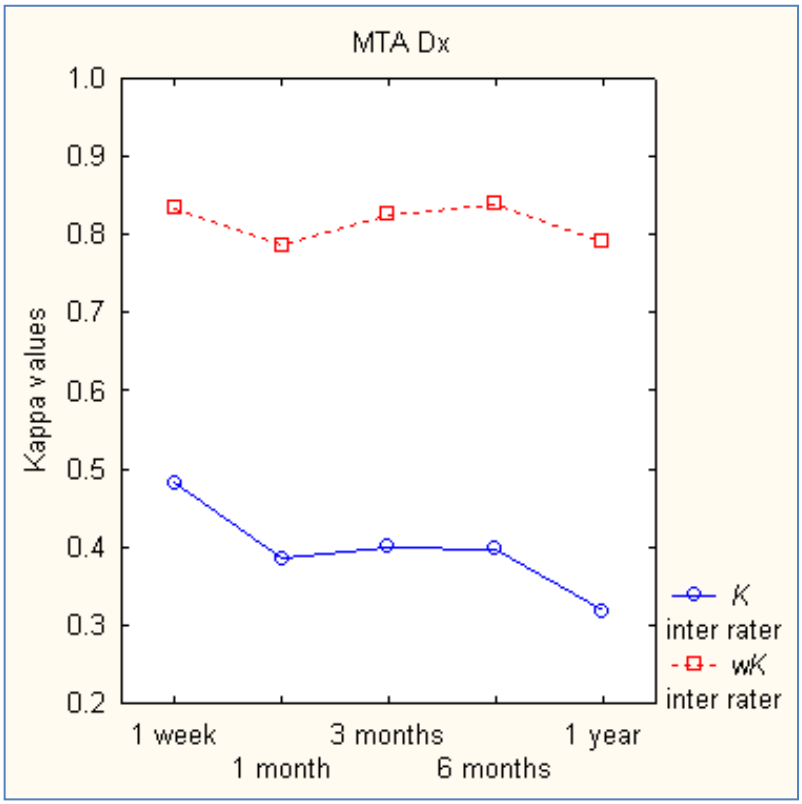


b)

Fig. 5a and b. Agreement between consensus ratings at time zero and individual ratings over a period of 1 year, shown as kappa (*K* LC, *K* KL) and weighted kappa (*wK* LC, *wK* KL) for both investigators. Sin = left; Dx = right.



a)



b)

Fig. 6a and b. Left and right side inter-rater kappa (κ) and weighted kappa (wκ) values for the two investigators LC and KL. All the five ratings during the year from time zero are shown. Sin = left; Dx = right.

Study II: Sixty-five per cent or 362/555 of our study population of non-demented elderly were 60–72 years old and only 6% or 36/555 were ≥ 87 years of age. When we evaluated MTA score v. age we found a statistically significant difference between age cohorts ($p<0.001$). Pair-wise comparisons between cohorts revealed a significant difference between all groups, except between the two oldest age groups. The gender difference when using MTA score was statistically significant ($p<0.001$), showing a higher MTA score (i.e. more atrophy) for men than for women, but no statistically significant difference ($p=0.25$) in MTA score was found between the right and the left MTL.

Among the non-demented elderly individuals included, there was a spread of MTA scores across the different age cohorts (Table 2). In the two youngest age groups (60 and 66 years old) there was a predominance of MTA scores 0 and 1. Scores 0–2 were most prevalent in the age groups 72, 78 and 81 years, while in the two oldest age groups, MTA scores of 0–3 were found and even ratings up to 4 could be seen in the oldest age group.

Table 2. Frequency (%) of medial temporal lobe atrophy (MTA) scores in the different age cohorts, based on 555 individuals and 1,110 medial temporal lobes.

AGE COHORTS	MTA 0	MTA 1	MTA 2	MTA 3	MTA 4
60 yrs	46	51	3	0	0
66 yrs	36	57	7	0	0
72 yrs	23	58	18	1	0
78 yrs	17	56	24	3	0
81 yrs	4	59	31	6	0
84 yrs	9	36	46	9	0
≥87 yrs	1	49	36	11	3

A comparison of hippocampal volume/ICV with MTA score (Fig. 7) revealed that there was a wide range of volumes for each MTA score and a great variation of MTA scores with each volume. As seen in the Figure, there is a great overlap of actual volumes between different MTA scores.

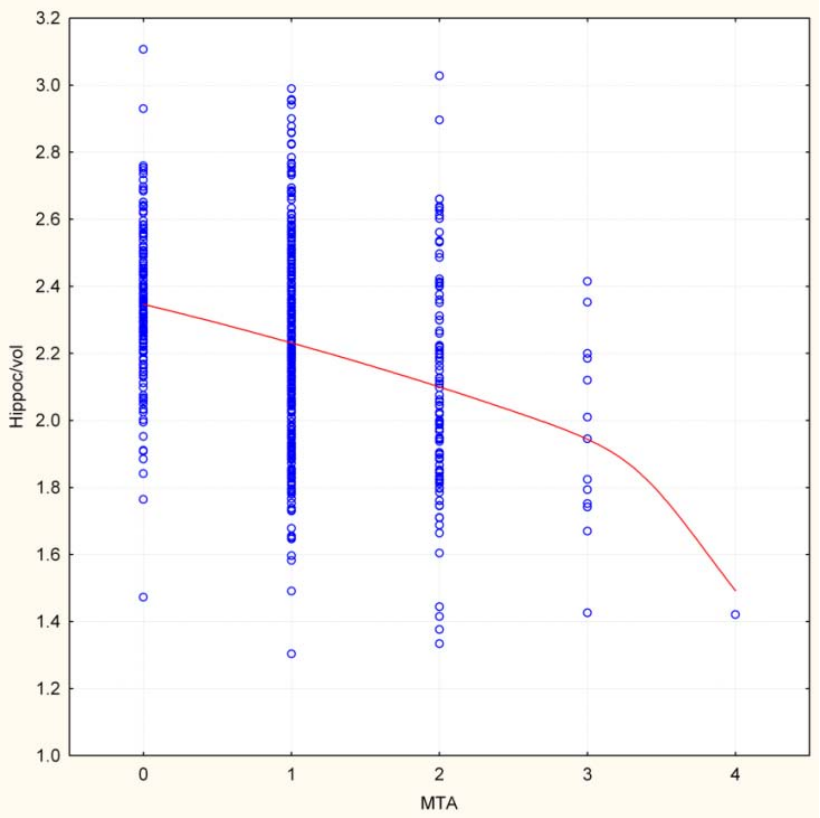
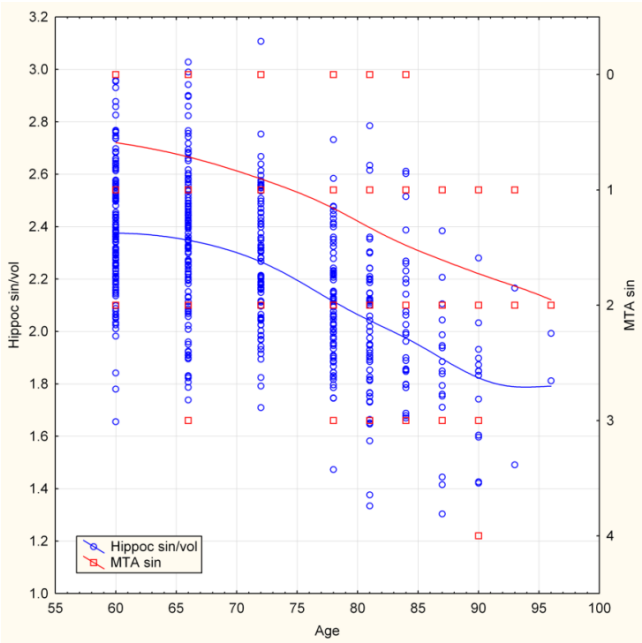
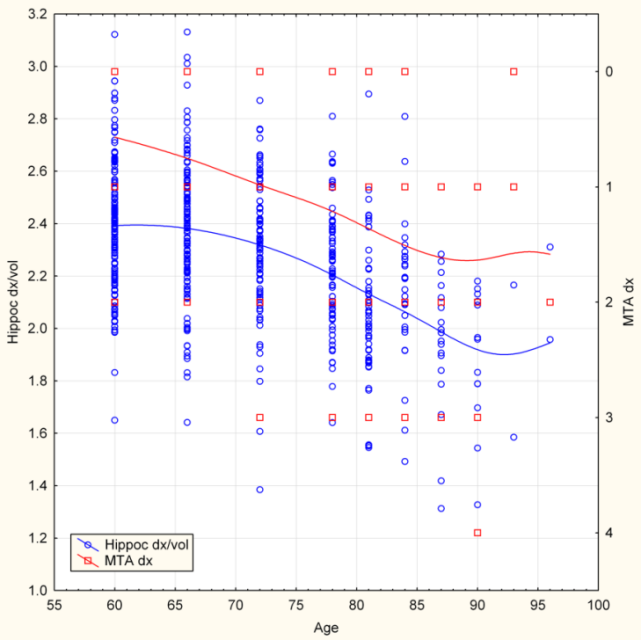


Fig. 7. Scatter plot of normalized hippocampal volume versus medial temporal lobe atrophy (MTA). The red line is the association between hippocampal volume and MTA.

Hippocampal volumes normalized to ICV and the inverted MTA score (Fig. 8a and b) showed almost parallel curves across the different age groups. The correlation between hippocampal volume/ICV and MTA score was highly significant (r_s=-0.26, p <0.001, on the right side, and r_s=-0.32, p <0.001, on the left side) but the correlation coefficient was low. The volume of the hippocampus decreased with age, while the MTA score increased. Inverting MTA scores on the right vertical axis made the curves easier to read and understand.



a)



b)

Fig. 8a and b. Left hippocampus/MTA (4a) and right hippocampus/MTA (4b). Scatter plots showing normalized hippocampal volume in cm³ (blue) along left Y-axis v.age on X-axis and inverted medial temporal lobe atrophy score (red) along right Y-axis v. age. The blue line is the association between hippocampal volume and age. The red line is the association between MTA and age.

Intra-rater reliability of vaMTA was κ 0.76 on the right side and κ 0.77 on the left side in 100 subjects, with weighted kappa (wκ) 0.88 and 0.89, respectively. The ICCs of the volumetric measurements were 0.93, according to Zhang et al (39).

When comparing MTA scores from the two different coronal angulations of the transverse T1-weighted FFE sequence in 21 subjects by reorienting the images, no statistically significant difference in MTA score between the two angulations was observed (p=0.69 on the right side and p=1.0 on the left side). Kappa was calculated to be κ 0.60 on the right side and κ 0.65 on the left side. Weighted kappa was 0.84 on the right side and 0.85 on the left side.

Study III: Two models were created using total hippocampal volume, automated regional volume and cortical thickness measurements to compare AD cases with controls. The first model, using the total manual hippocampal volume, accounted for 100% of the variance of the original data (R2(X)) and its cross-validated predictability, Q2(Y) = 0.61. The second model, using regional MRI measures, resulted in one predictive component with R2(X) = 60% and cross-validated predictability Q2(Y) = 0.45.

The separation between patients with AD and controls and the predictive power of the models Q2(Y) can be seen in Figure 9a using automated regional volume and cortical thickness measurements as input. As can be seen, there is a distinct separation between subjects with AD and controls. This model resulted in a prediction accuracy of 82.7%. Figure 9b illustrates the variables of importance for distinction between the two groups. The pattern of atrophy, including hippocampus, amygdala and entorhinal cortex among other temporal lobe regions, together with volume measurements of CSF, is similar to previous analyses by the AddNeuroMed cohort using regional MRI measurements and an OPLS model (131).

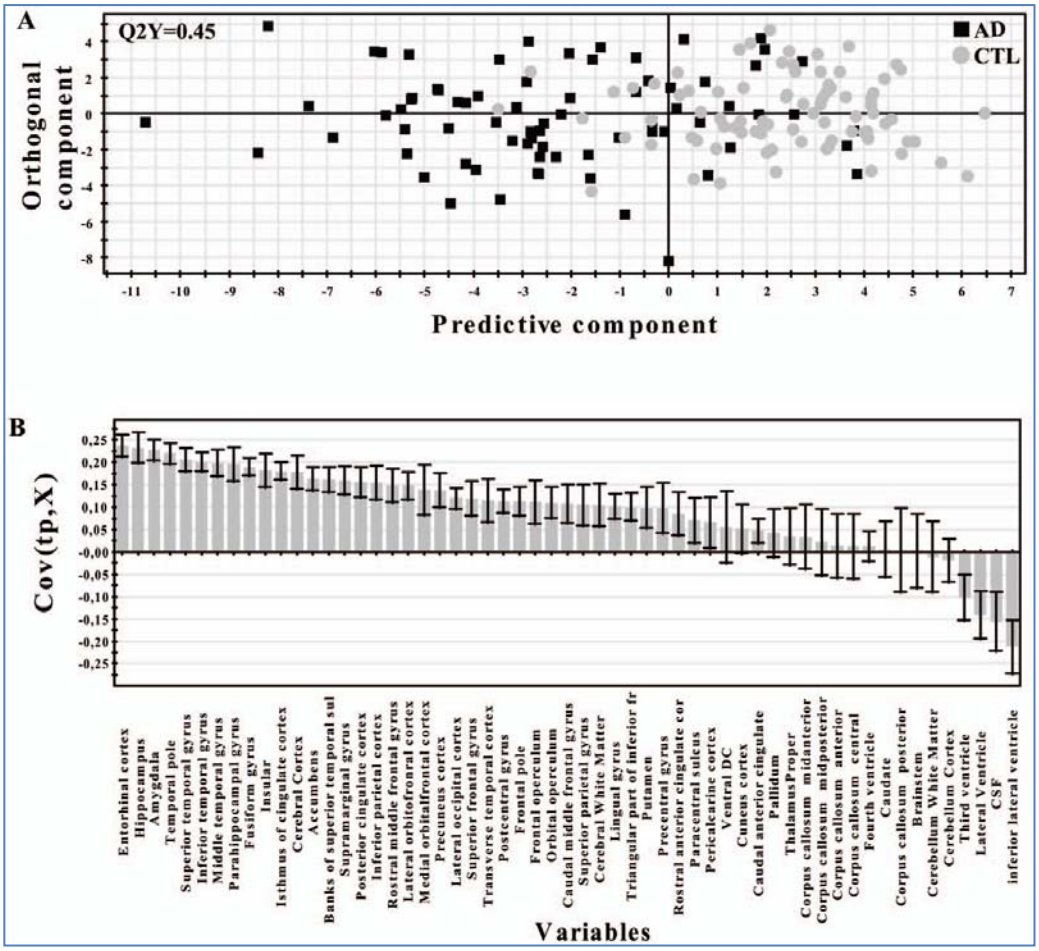


Fig. 9a and b. OPLS cross validated score plots and MRI measures of importance for the separation between AD and CTL. (A) The scatter plot visualises group separation and the predictability of the AD vs. CTL model. Each black square represents an AD subject and each gray circle a control subject. Control subjects to the left of zero and AD subjects to the right of zero are falsely predicted. Q2 (Y).0.05 (statistically significant model). (B) Measures above zero have a larger value in controls compared to AD and measures below zero have a lower value in controls compared to AD. A measure with a high covariance is more likely to have an impact on group separation than a measure with a low covariance. Measures with jack knifed confidence intervals that include zero have low reliability. PLoS ONE DOI:10.1371/journal.pone.0022506.g003

Visual assessment using the Scheltens scale resulted in a prediction accuracy of 80.8% and total hippocampal volume yielded a prediction accuracy of 89.1%. In summary, the best predictive result was obtained from manual hippocampal measures closely followed by the automated image pipeline with OPLS and lastly the visual rating assessment. In the investigation of how the three different approaches would predict conversion from MCI at baseline to AD at 1 year clinical follow-up, all MCI subjects were classified as either AD or control using OPLS models (AD v. CTL). The visual assessment for the MCI subjects were performed in the same way, using the same cut offs as described previously, resulting in an assessment of abnormal or normal brain changes with respect to age. The results demonstrated that 68% of the

MCI-c subjects were classified as more AD-like and 68% of the MCI-s subjects were classified as more control-like at base line using visual rating.

Using automated regional MRI measures as input to the OPLS model, 74% of the MCI-c subjects who converted to AD at 1-year follow-up were predicted to be more AD-like and 70% of the MCI-s subjects were predicted to be more control-like at baseline. For the total hippocampal volume, 79% of the MCI-c subjects who converted to AD at 1-year follow-up were predicted as more AD-like and 54% of the MCI-s subjects were predicted to be more control-like. When setting the specificity (MCI-s predicted as CTL) to a fixed value (the specificity of the visual assessment) to make the comparison of the methods easier, the results were slightly altered.

To conclude, the best results were obtained from the OPLS model with automated regional MRI measures as input, with 79% of MCI-c subjects converted to AD at 1-year follow-up predicted to be more AD-like compared with 68% for the manual hippocampal volumes and 68% for Scheltens visual assessment.

Study IV: The distribution of the clinical diagnosis made by the two independent geriatricians according to the traditional classification systems was: SCI (n = 18), MCI (n = 78), AD (n = 23), VaD (n = 15), FLD (n = 3), unspecified (UNS) dementia (n = 10) and normal (n = 3) (Fig.10).

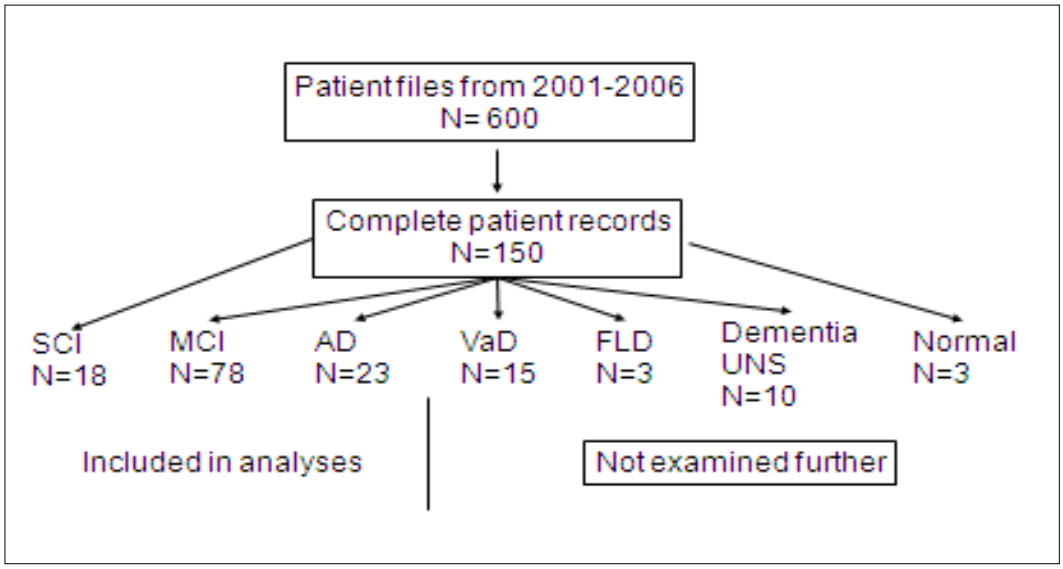


Fig.10. Flow chart of patients whose files were entered into the dataset for statistical analysis. AD = Alzheimer's disease; FLD = frontal lobe dementia; MCI = mild cognitive impairment; SCI = subjective cognitive impairment; UNS = unspecified; VaD = vascular dementia.

The concordance between the routine clinical staff who used the results of the biomarkers (MRI, SPECT scans and the CSF analyses) in the clinical work-up and the study physicians blinded to the biomarkers in question was 90% for the AD diagnosis and 66% for all other diagnoses combined.

Our main focus was to investigate the applicability of the Dubois criteria (51) to identify possible prodromal AD cases in our clinically diagnosed SCI and MCI patients.

A second aim was to validate the Dubois criteria (51) in patients with clinically diagnosed AD, according to the traditional diagnostic criteria. For the statistical analyses, we therefore excluded the patients with all other clinical dementia diagnoses (VaD, FLD and dementia UNS, n = 28) and the three patients who were cognitively normal. Thus, 119 patients with SCI, MCI or AD were initially studied. Of these, only 110 patients had performed the RAVLT. The majority had had a rating of the MTA and CBF done and in about 50% of these patients, analyses of dementia biomarkers in CSF were performed (Table 3).

Table 3. The frequency of the test performance for patients (n = 150) included in the study.

TESTS	RAVLT	MTA	CBF	CSF T-TAU	CSFP-TAU	CSF AB42
No of patients	110	122	117	63	46	54

None of the included patients performed all six tests. Aβ42 = amyloid protein beta 1-42; CBF = cerebral blood flow rated using single-photon emission computer tomography (SPECT); CSF = cerebrospinal fluid; MTA = medial temporal lobe atrophy; p-tau = phosphorylated tau protein; t-tau = total tau protein; RAVLT = Rey Auditory Verbal Learning Test.

The normal range and the cut-off values of biomarkers are shown in Table 4.

Table 4. Normal range of biomarkers and the cut-off values used in this study.

BIOMARKERS	RANGE	PATHOLOGICAL CUT-OFF VALUE
RAVLT words	0-15	<6
MTA score	0-4	>2
CBF score	0-2	>1
CSF Aβ ng/l	>427	<427
CSF t-tau ng/l	>445	>445
CSF p-tau ng/l	<74	>74

Of the 18 patients with the clinical diagnosis of SCI, only three met the core criteria set by Dubois. Two of the SCI patients met one additional Dubois criterion, having pathological CSF. Thus, compared with the traditional criteria, the Dubois criteria could identify two prodromal AD patients among the SCI cases. Similarly, the Dubois criteria identified five prodromal AD patients among the 78 MCI patients, four of whom had all three additional pathological features and one who had pathological CBF.

Among the total number of clinically diagnosed SCI and MCI patients (n = 96), 26% (n = 25) met the core criteria of pathological episodic memory levels tested with RAVLT but had no additional AD features, according to the Dubois criteria. Therefore, ≥1 additional feature identified 7.3% (7/96) "true" prodromal Alzheimer cases in the group of 96 clinically non-demented patients.

We also investigated whether the suggested criteria by Dubois et al. (51) could identify AD cases in a sample of 23 patients with a clinical diagnosis of AD, according to the standard (ICD-10 or DSM-IV) criteria; 95.6% (22/23) patients had a pathological episodic memory, while 54.5% (12/22) of them had ≥1 additional AD feature. Thus, 54.5% of the patients with an AD diagnosis, according to the traditional diagnostic criteria, also met the criteria of Dubois et al (51).

The κ analyses showed a poor to moderate concordance (wκ = 0.18–0.52) between the added procedures and biomarkers (RAVLT, MRI, SPECT and CSF) and the clinical diagnoses (SCI, MCI and AD). However, delayed recall, objectively assessed by RAVLT, was the most sensitive biomarker to detect AD cases (wκ = 0.52). At a group level, there were no significant correlations between any of the suggested procedures or biomarkers and the diagnosis of MCI.

5 DISCUSSION

Study I: In parts, the results of this study are as expected. The hypothesis was that both intra- and inter-rater kappa values would diverge both with time between investigators and from the consensus rating. The results for the general radiologist (KL) supported this hypothesis, with decreasing kappa values over the year related to baseline. For the neuroradiologist (LC), the kappa decreased to a level of about 0.7 and fluctuated around this value over the year, showing a fairly stable evaluation from time point to time point.

The difference in experience between the two radiologists may be one explanation for this result. Even though the two investigators had been trained together, they did not work together and differed greatly in experience. Both had performed vaMTA for about 2 years prior to this study. However, while the neuroradiologist performed vaMTA many times daily, the general radiologist did a few times a week. It is also possible that the neuroradiologist had a higher impact on the consensus rating, caused by her greater experience. This may be another reason why the general radiologist's reliability values declined over time.

An educational platform in MTA rating, on a department or country basis, may be one way of achieving a more reliable assessment. It can be created through a training programme including 50–100 cases, iterative training and personal feedback in close relation to the assessments. A similar programme, evaluating cardiac CT, is already being successfully run at the Department of Radiology of Karolinska University Hospital in Stockholm (132).

Our results showed that there were no statistically significant differences when rating either of the three T1-weighted sequences, nor different image thickness or angulation of images in the coronal plane, used in this study. This means that a reliable assessment of MTA is probably not that sensitive to minor changes in technique, concerning MRI machines, sequences, coronal angulation and, to some extent, even image thickness.

Study II: The comparison between hippocampal volumes and vaMTA using MTA scores showed a highly significant correlation. The correlation coefficient using Spearman rank correlation was, however, low (-0.26 Dx, -0.32 Sin), but corresponds to that reported in other studies (81). The relatively low, but significant, correlations indicate that only a part of the variation in MTA score is explained by the variation in total hippocampal volume. There can be a number of possible explanations for this. One explanation is that the two methods describe different things. In calculation of the volume of the hippocampus, the head, body and tail are outlined manually in every MRI scan of the 8 cm long structure. Surroundings are not included in the measurement. In vaMTA, only one single coronal image was evaluated. In this method, surrounding structures are included in the evaluation, including the temporal horn of the lateral ventricle, and the choroid fissure, parahippocampal gyri and collateral and parahippocampal sulci (81). This may support the idea that vaMTA assesses whole-brain atrophy rather than atrophy of the hippocampus (82). A second explanation is that when measuring the volume of the hippocampus, atrophy in one part may affect the

total volume to a greater or lesser degree, depending on where the changes are located in the hippocampus. The vaMTA just tells us what the MTL looks like in one image slice, but the primarily affected part may lie somewhere else along the hippocampus.

One possible explanation for the absence of significant side differences with vaMTA is that the visual assessment technique may not be sensitive enough to discriminate subtle differences. Another possible explanation is that the grading scale has too few rating steps in vaMTA.

The normal values of MTA in this study can be of great use in daily clinical practice. To know the range of normality in different age groups will be a helpful diagnostic tool for geriatricians and neurologists. It is worth mentioning, however, that at the individual level the MTA score may be outside the normal range but still be normal.

Study III: Automated computerized MRI methods to aid in the diagnosis of AD will only be implemented in clinical practice if they are carefully investigated and validated. In this study the overall accuracy was higher for the OPLS technique (83%) compared with the visual rating scale (81%) in distinguishing AD cases from controls. Although manual measurement of total hippocampal volume still yielded the best prediction accuracy (89%), the time-consuming nature of manual measurements makes them impractical in a clinical setting.

At 1-year follow-up 19 of the 101 subjects with MCI converted from MCI to AD while 82 remained stable. It is, however, difficult to confidently state that one method is better than the other due to the small numbers of subjects who converted to AD. The three methods predicted 15/19, 14/19 and 13/19 of converters, respectively, to be more AD like, which is a small absolute difference that has a larger impact on the percentage accuracy. Large studies with longer follow-up times are warranted to better investigate this issue.

Although neuropathologically confirmed diagnosis is preferred when evaluating these types of automated models, it is very difficult to obtain large neuropathologically confirmed datasets in practice.

Neurodegeneration in AD is estimated to start 20–30 years before the clinical diagnosis is given (133) and if a diagnosis is to be made at the prodromal stage of the disease it may be necessary to combine several different markers of the disease. Previously we have shown that combining regional MRI measures with magnetic resonance spectroscopy results improves classification results, doubling the positive likelihood ratio (134).

Study IV: In our SCI and MCI sample, the Dubois criteria (51) identified only a few patients with pre-AD, showing that the traditional classification criteria, at least in our sample, are still useful in this heterogeneous patient material. Our sample is a naturalistic patient sample from a memory clinic but is highly selective with regard to age. This clinic is tasked with investigating all patients with memory disturbances <65 years of age in the whole Stockholm region. Consequently, our clinical sample is younger than in other memory clinic settings and the proportion of patients with SCI

and MCI as compared with patients with dementia is high. The pre-test probability for being diagnosed as having AD is therefore lower compared with other clinical settings with older patient populations.

The actual pre-AD cases in our sample may be truly low as the use of the Dubois criteria suggests here, although the conversion rate of MCI to AD in our clinic after 3 years' follow-up (8) is in concordance with international findings.

The rates of conversion from MCI to AD may vary according to age, neuropsychological profile and the definition of MCI (135, 136). Interestingly, the Dubois criteria identified only twelve cases of AD in our sample of 23 patients diagnosed as having a dementia syndrome, due to AD, according to the traditional classification systems.This may be due to the fact that the traditional criteria takes the evolving symptomatology and clinical signs into account, while the new research criteria merely focus on episodic memory disturbance and various pathological biomarkers. Another reason may be that AD is not just one disease but a heterogeneous group of diseases.

One problem with the proposed Dubois criteria (51) is that there are no established cut-off levels for dementia biomarkers in CSF. This makes identification of possible pre-AD cases in clinical samples vary according to which cut-offs are used. When using MTA as a biomarker there is biological variability with age, which has to be taken into account for inclusion and for outcome measures in clinical trials (137).Our study shows that MTA was normal or only questionably pathological in 82.6% of the clinically diagnosed AD patients. A possible interpretation is that our sample was younger, with a low degree of global cognitive deficits, and that it may be more relevant to measure atrophy in more anterior areas than suggested by the Scheltens method (78).Magnetic resonance image scanning and CSF sampling will possibly be difficult to apply as part of regular routine in dementia screening in Europe due to local regulations and attitudes (138).

All these findings urge us also to focus on the development of other possible biomarkers that are less time-consuming and financially demanding to use in all clinical settings.

6 CONCLUSIONS AND FUTURE ASPECTS

Visual assessment of MTA has proved to be a fast and reliable method. In this thesis it has been shown that the method is reliable over time when performed by a trained neuroradiologist. When the method is compared with manual volumetry and automatic measurement, MTA rating is not the best method for differentiating between AD cases and controls, but the differences between methods are very small. Visual assessment of MTA is useful even for predicting conversion from MCI to AD and in this respect it is nearly as good as volumetry or automatic multivariate analysis.

When testing the Dubois research criteria from 2007 in a clinical setting our result are not in line with results from another retrospective study which showed almost 100% agreement between the new research criteria and neuropathology .In our study all patients were re-diagnosed and the re-diagnostic work-up was done to avoid all circularity bias. In late 2011 the debate was still ongoing whether these new research criteria are possible to implement in clinical practice. There is a need for new up-dated clinical criteria for AD, but they have to be carefully tested in prospective studies especially in the preclinical and asymptomatic stages of the disease, before being generally accepted.

Visual assessment of MTA is not a very well-known or well-used method in Sweden. In neuroradiology departments it may be heard of (though seldom used), but rarely in rural hospitals. At geriatric and neurology departments, however, the method is better known, at least in university hospitals.

From a clinical perspective, vaMTA can add important information for the diagnostic work-up. The method is fast and can be performed together with the regular neuro-radiology report. It is possible to use the method on both MRI and CT images, with reliable results. It is also possible to compare measures from CT and MRI examinations with each other and get comparable results. In our department we use both CT and MRI for evaluating atrophy progression and have found it clinically valuable.Taking all this into account, it is possible just to use good-quality CT in the investigation of dementia especially in the very old. The scanning time is a few seconds and there are only minor limitations according to patient co-operation. In the near future we can foresee an increasing demand for vaMTA from our colleagues in geriatric and neurology departments and as radiologists we have to meet this request with increased knowledge about the method.

It is, however, very important to point out that MTA does not give a diagnosis, it just visualizes atrophy, and provides no information about the mechanisms behind it.

Even though both manual volumetric measurement of the hippocampus and automatic multivariate analysis are slightly better than visual assessment in detecting atrophy, they are much more time-consuming. Rating MTA takes a few minutes, while volumetry, manually outlined, takes about 40 minutes for two hippocampi. With today's technique, automatic segmentation of the whole brain including both volumes and cortical thickness takes around 13 computer hours for between one and eight

patients, but then the whole brain is measured and not just the hippocampus. When trying to implement automatic segmentation or volumetric measurement in a clinical setting today the time-consuming evaluation will be a problem, when great numbers of brains need to be investigated daily.

In the future, however, automatic multivariate analysis may be the method of choice combined with visual assessment of atrophy of all brain regions. It may be possible to use this in the same way as in evaluating SPECT with BRASS. First the radiologist performs visual assessment. This is then compared with an automatic method for confirmation or for correction.

The BRASS evaluation takes about 5 minutes to perform but much human brainpower and computer power has to be used to reduce the evaluating time for multivariate analysis to be comparable to BRASS and possible to use in clinical routine assessment. Other automatic and semi-automatic methods and techniques are being developed that can be used to evaluate different brain volumes and cortical structures and a great deal of research in this field is currently underway.

Further new areas for using visual rating scales will be developed, as well. New areas for visual rating scales are under development. Recently a rating scale for visual assessment of posterior atrophy was published by Koendam et al. Other areas of special interests for visual assessment may be the amygdala and entorhinal cortex. A technique for visual assessment of atrophy in basal ganglia will also be highly desirable.

New AD- and dementia-biomarkers are under development and right know focus is primarily in finding biomarkers in the blood. To calculate gene expression in blood test may be a further way to identify patients with high likelihood of having AD or other dementias.

The use of PET-PIB and other new PET markers will definitely increase in the future in early assessment of AD. At the moment, PET-PIB is, however, only available for research purposes. It is still too expensive for clinical use and also there are too few PET cameras today to meet the increasing demand for this method in Sweden.

In the meantime, until such problems are solved, vaMTA and visual assessment of other brain regions will remain the method of choice in clinical radiology when investigating dementias.

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REFERENCES

1. Alzheimer A (1907) Über eine eigenartige Erkrankung der Hirnrinde.Allgemeine Zeitschrift für Psychiatrie und Psychisch-gerichtliche Medizin 64:146-148.

2. Maurer K, Volk S, Gerbaldo H (1997) August D and Alzhemer's disease. Lancet 349:1546-1549.

3. WHO: The ICD-10 classification of mental and behavioural disorder. Clinical descriptions and diagnostic guidelines. Geneva: WHO 1992.

4. Diagnostic and Statistical Manual of Mental Disorder, 4th edition. DSM-IV, 1994.

5. Thompson PM, Hayashi KM, Dutton RA et al (2007) Tracking Alzheimer's disease. Ann N Y Acad Sci 1097:183–214.

6. Jorm AF, Korten AE, Henderson AS (1987) The prevalence of dementia: a quantitative integration of the literature. Acta Psychiatr Scand 76(5):465–479.

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

8. Wahlund LO, Philstrand E, Jonhagen ME (2003) Mild cognitive impairment: experience from a memory clinic. Acta Neurol Scand Suppl. 179:21-24.

9. Jacoby RJ, Levy R, Dawaon JM (1980) Computed tomography in the elderly. I. The normal population. Br J Psychiatry 136:249-255.

10. Soininen H, Puranen M, Riekkinen PJ (1982) Computed tomography findings in senile dementia and normal aging. J Neurol Neurosurg Psychiatry 45(1):50-54.

11. Wattjes MP, Henneman WJ, van der Flier WM, de Vries O, Träber F, Geurts JJ, Scheltens P, Vrenken H, Barkhof F (2009) Diagnostic imaging of patients in a memory clinic: comparison of MR imaging and 64-detector row CT. Radiology 253 (1):174–183.

12. Seab JP et al (1988) Quantitative NMR measurements of hippocampal atrophy in Alzheimer's disease. Magn Reson Med 8 (2):200-208.

13. Kesslak JP, Nalcioglu O, Cotman CW (1991) Quantification of magnetic resonance scans for hippocampal and parahippocampal atrophy in Alzheimer's disease. Neurology 41 (1):51-54.

14. Jack CR, et al (1992) MR-based hippocampal volumetry in the diagnosis of Alzhemer's disease. Neurology 42(1):183-188.

15. Kaneko K, et al (2004) Posterior cingulated hypoperfusion in Alzheimer's disease, senile dementia of Alzheimer type, and other dementias evaluated by three-dimentional stereotactic surface projections using Tc-99m HMPAO SPECT. Clinical nuclear medicine 29:362-366.

16. Cavallin L, Danielsson R, Öksengard AR, Wahlund LO, Juhlin P, Frank A, Engman EL, Svensson L, Kristofferssen Wiberg M (2006) Can Dynamic Susceptability Contrast Magnetic Resonance Imaging replace Single Photon Emission Tomography in the diagnosis of patients with Alheimer´s disease? A pilot study. Acta Radiol 47:977-985.

17. Cavallin L, Axelsson R, Wahlund LO, Öksengard AR, Svensson L, Juhlin P, Kristoffersen Wiberg M, Frank A (2008) Voxel-based correlation between co-registered SPECT and DSC MRI in subjects with suspected Alzheimer's disease. Acta Radiol 49:1154-1161.

18. de Leon MJ et al (1983) Positron emission tomographc studies of aging and Alzhemer's disease. AJNR Am J Neuroradiol 4:568-571

19. Foster NL, et al (1983) Alzheimer's disease: focal cortical changes shown by positron emission tomography. Neurology 33:961-965

20. Klunk WE, et al (2004) Imaging brain amyloid in Alzhemer's disease with Pittsburg Compound-B. Ann Neurol 55:306-319

21. Forsberg A, et al (2008) PET imaging of amyloid deposition in patients with mild cognitive impairment. Neurobiol Aging 29:1456-1465

22. Glenner GG, Wong CW (1984) Alzheimer's disease: initial report of the purification and characterization of a noval cerebrovascular amyloid protein. Biochem Biophys Res Commun 120:885-890

23. Strozyk D et al (2003) CSF Abeta 42 levels correlate with amyloid-neuropathology in a population-based autopsy study. Neurology 60:652-556.

24. Gotz J, Ittner LM, Kins S (2006) Do axonal defects in tau and amyloid precursor protein transgenic animals, model axonopathy in Alzheimer's disease? J Neurochem 98:993-1006.

25. Buee L, Bussiere T, Buee-Scherrer V, Delacourte A, Hof PR (2000) Tau protein isoforms, phosphorylation and role in neurodegenerative disorders. Brain Res Rev 33:95-130.

26. Blennow K, Wallin A, Ågren H, Spenger C, Siegfried J, Vanmechelen E (1995) Tau protein in cerebrospinal fluid: a biochemical diagnostic marker for axonal degeneration in Alzheimer's disease? Mol Chem Neuropathology 26:231-245.

27. Hampel A, Mitchell A, Blennow K, Frank A et al (2004) Core biological marker candidates of Alzheimers disease – perspectives for diagnosis prediction of outcome and reflection of biological activity. J Neuroal Transm 111:247-272.

28. Riemenschneider M, Wagenpfeil S, Vanderstichele H, Otto M, Wiltfang J, Kretzschmar H, Vanmechelen E, Forstl H, Kruz A (2003) Phospho-tau/total-tau ratio in cerebrospinal fluid discriminates Creutzfeldt-Jakob disease from other dementias. Mol Psychiatry 8:343-347.

29. Lowenberg K, Waggoner R (1934) Familial organic psychosis (Alzheimer's type). Arch Neurol Psychiatr 31:737-754.

30. Lai F, Williams RS (1989) A prospective study of Alzheimer's disease in Down syndrome. Arch Neurol 46:849-853.

31. Ashford JW (2004) APOE genotype effects on Alzheimer's disease onset and epidemiology. J Mol Neurosci 23:157-165.

32. St George-Hyslop PH (2000) Genetic factors in the genesis of Alzheimer's disease. Ann N Y Acad Sci 924:1-7.

33. Goate A et al (1991) Segregation of a missense mutation in the amyloid precursor protein gen with familial Alzheimer's disease. Nature 349:704-706.

34. Raux G et al (2005) Molecular diagnosis of autosomal dominant early onset Alzheimer's disease: an update. J Med Genet 42:793-795.

35. Brouwers N, Sleegers K, Van Broeckhoven C (2008) Molecular genetics of Alzheimer's disease: an update. Annals of medicine 40:562-583.

36. Sherrington R et al (1995) Cloning of a gene bearing missense mutations in early onset familial Alzheimer's disease. Nature 375:754-760.

37. Levy-Lahad E et al (1995) Candidate gene for the chromosome 1 familial Alzheimer's disease locus. Science 269:973-977.

38. Yakovlev PI, Lecours AR (1967) The myelogenetic cycles for regional maturation of the brain. In: Minkowski A, ed Regional development of the brain in early life. Oxford; Blackwell 3-70.

39. Zhang Y, Qiu C, Lindberg O, Bronge L, Aspelin P, Bäckman L, Fratiglioni L, Wahlund LO (2010) Acceleration of hippocampal atrophy in a non-demented elderly population: the SNAC-K study. Int Psychogeriatr 22:14–25.

40. Salthouse, TA (2009). When does age-related cognitive decline begin? Neurobiology of Aging, 30:507–514.

41. Sluimer JD, van der Flier WM, Karas GB, Fox NC, Scheltens P, Barkof F, Vrenken H (2008) Whole-brain atrophy rate and cognitive decline: longitudinal MR study of memory clinic patients. 248:590-598.

42. Braak H, Braak E (1991) Neuropathological staging of Alzheimer-related changes. Acta Neuropathol 82:239-259.

43. Braak H, Braak E (1996) Development of Alzheimer related neurofibrillary changes in the neocortex inversely recapitulate cortical myelination genisis. Acta Neuropathol 92:197-201.

44. Braak H, Braak E (1996) Evolution of neuropathology of Alzheimer's disease. Acta Neurol Scand Suppl 165:3-12.

45. Wahlund LO, Julin P, Johansson SE, Scheltens P (2000) Visual rating and volumetry of the medial temporal lobe on magnetic resonance imaging in dementia: a comparative study. J Neurol Neurosurg Psychiatry 69(5):630–635.

46. Kier EL, Kim JH, Fulbright RK, Bronen RA (1997) Embryology of the human fetal hippocampus: MR imagning, anatomy and histology. AJNR Am J Neuroradiol 18:525-532.

47. Bajic D, Wang C, Kumlien E, Mattson P, Lundberg S, Eeg-Olofsson O, Raininko R (2008) Eur Radiol 18:138-142.

48. Di Gennaro G, Grammaldo LG, Quarato PP, Esposito V, Mascia A, Sparano A, Meldolesi GN, Picardi A (2006) Severe amnesia following bilateral medial temporal lobe damage on two distinct occasions. Neurol Sci 27:129-133.

49. Scoville WB, Milner B (1957) Loss of recent memory after bilateral hippocampal lesions. J Neurol Neurosurg Psych 20:11-21.

50. Squire LR (2009) The legacy of patient H.M for neuroscience. Neuron 61:6-9.

51. Dubois B, Feldman HH, Jacova C, Dekosky ST, Barberger-Gateau P, Cummings J, Delacourte A, Galasko D, Gauthier S, Jicha G, Meguro K, O'Brien J, Pasquier F, Robert P, Rossor M, Salloway S, Stern Y, Visser PJ, Scheltens P (2007) Research criteria for the diagnosis of Alzheimer's disease: revising the NINCDS-ADRDA criteria. Lancet Neurol 6:734–746.

52. Eriksson PS. Perfilieva E, Bjork-Eriksson T, Alborn AM, Norborg C, Peterson DA Gage FH (1998) Neurogenisis in the adult human hippocampus. Nat Med 4:1313-1317.

53. Curtis MA, Kam M, Nannmark U, Andersson MF, Axell MZ, Wikkelso C, Holtas S (2007) Human neuroblasts migrate to the olfactory bulb via a lateral ventricular extension. Science 315:1243-1249.

54. Šimić G, Kostović I, Winblad B, Bogdanović N (1997) Volume and number of neurons of the human hippocampal formation in normal aging and in Alzheimer's disease. J Comp Neurol 379:482-494

55. Bobinski M, Weigel J, Wisniewski HM et al (1995) Atrophy of hippocampal formation subdivisions correlates with stage and duration of Alzheimer's disease. Dementia 6:205-210.

56. Petersen RC, et al (2009) Mild cognitive impairment: ten years later. Arch neurol 66:1447-1455.

57. Ritchie K, Artero S, Touchon J (2001) Classification criteria for mild cognitive impairment: a population-based validation study. Neurology 56 (1):37-42.

58. Mitchell J et al, (2009) Outcome in subgroups of mild cognitive impairment (MCI) is highly predictable using a simple algorithm. J Neurol 256:1500-1509.

59. Urs R, Potter E, Barker W, et al (2009)Visual rating system for assessing magnetic resonance images: a tool in the diagnosis of mild cognitive impairment and Alzheimer's disease. J Comput Assist Tomogr 33:73–78.

60. Karas G, Scheltens P, Rombouts S, van Schijndel R, Klein M, Jones B, van der Flier W, Vrenken H, Barkhof F (2007) Precuneus atrophy in early-onset Alzheimer's disease: a morphometric structural MRI study. Neuroradiol 49:967–976.

61. Scheltens P (2001) Structural neuroimaging of Alzheimer's disease and other dementias. Aging Clin Exp Res 13:203-209.

62. Laakso MP, Partanen K, Riekkinen P, Lehtovirta M, Helkala EL, Hallikainen M, Hanninen T, Vainio P, Soininen H (1996) Hippocampal volumes in Alzheimer's disease, Parkinson's disease with and without dementia, and in vascular dementia: an MRI study. Neurology 46:678–681.

63. Galton CJ, Patterson K, Graham K, Lambon-Ralph MA, Williams G, Antoun N, Sahakian BJ, Hodges JR (2001) Differing patterns of temporal atrophy in Alzheimer's disease and semantic dementia. Neurology 57:216–225.

64. Berchtold NC, Cotman CW (1998) Evolution in conceptualization of dementia and Alzheimer's disease: greec-roman period to 1960s. Neurobiol of Aging19:173-189.

65. Halpert BP (1983) Development of the term "senility" as a medical diagnosis. Minn Med 66:421-424.

66. Torack R (1979) Adult dementia:history, biopsy, pathology. Neurosurg 4:434-442.

67. Wilks S (1864) Clinical notes on atrophy of the brain. J Ment Sci 10:10-19.

68. Redlich E (1898) Über miliare sklerose der Hirndrinde bei senile atrophie. J Psychiat Neurol 17:208-216.

69. Mann DMA, Yates PO, Marcyniuk B (1985) Some morphometric observations on the cerebral cortex and hippocampus in presenile Alzheimer's disease, senile dementia of Alzheimer type and Down syndrome in middle age. J Neurol Sci 69:139-159.

70. Sinard JH (2001) Factors affecting autopsy rates, autopsy request rates and autopsy findings at a large academic medical center. Exp Mol Pathol 70:333-343.

71. Hompage of Department of Neurobiology, Care Sciences and Society, Karolinska Institutet Huddinge, Stockholm, Sweden.

72. Chen W, Song X, Zhang Y, Darvesh S, Zhang N (2010) An MRI-based semiquantitative indes for the evaluation of brain atrophy and lesions in Alzheimer's disease, mild cognitive impairment and normal aging. Dement Geriatr Cogn Disord 30:121-130.

73. Pasquier F, Leys D, Weerts JG, Mounier-Vehier F, Barkhof F, Scheltens P (1996) Inter- and intraobserver reproductibility of cerebral atrophy assessment on MRI scans with hemispheric infarcts. Eur Neurol 36:268-272.

74. Scheltens P, Pasquier F, Weerts J, Barkhof F, Leys D (1997) Qualitative assessment of cerebral atrophy on MRI: inter and intra-observer reproductibility in dementia and normal aging. Eur Neurol 37:95-99.

75. DeCarli C Haxby JV, Gillette JA, Teichberg D, Rapoport SI Schapiro MB (1992) Longitudinal changes in lateral ventricular volume in patients with dementia of the Alzheimer type. Neurology 42:2029-2036.

76. Lehmann M, Koedam E, Barnes J, Barlett J, Ryan N, Pijnenburg Y, Barkhof F, Wattjes M, Scheltens P, Fox N (20011) Posterior cerebral atrophy in the absence of medial temporal lobe atrophy in pathologically-confirmed Alzheimer's disease. Neurobiology of Aging DOI: 10.1016/j.neurobiolaging.2011.4.003.

77. Koedam E, Lehmann M, van der Flier W, Scheltens P, Pijnenburg Y, Fox N, Barkhof F, Wattjes M (2011) Visual assessment of posterior atrophy development of a MRI rating scale. Eur Radiol Open access DOI 10.1007/s00330-011-2205-4.

78. Scheltens P, Leys D, Barkhof F, Huglo D, Weinstein HC, Vermersch P, Kuiper M, Steinling M, Wolters E, Valk J (1992) Atrophy of medial temporal lobe on MRI in "probable" Alzheimer's disease and normal ageing: diagnostic value and neuropsychological correlations. J Neurol Neurosurg Psychiatr 55:967-972.

79. Scheltens P, Launer LJ, Barkhof F, et al (1995). Visual assessment of medial temporal lobe atrophy on magnetic resonance imaging: interobserver reliability. J Neurol 242:557–560.

80. Varon D, Loewenstein D, Potter E, Greig M, Agron J et al (2011) Minimal atrophy of the entorinal cortex and hippocampus: progression of cognitive impairment. Dement Geriatr Cogn Disord 31:276-283.

81. Wahlund LO, Julin P, Lindqvist J, et al. Visual assessment of medial temporal lobe atrophy in demented and healthy control subjects: correlation with volumetry. Psychiatry Res 1999;90:193–199.

82. Knoops AJ, van der Graaf Y, Appelman AP, Gerritsen L, Mali WP, Geerlings MI (2009) Visual rating of the hippocampus in non-demented elders: does it measure hippocampal atrophy or other indices of brain atrophy? The SMART-MR study. Hippocampus 19:1115–1122.

83. Toma A, Holl E, Kitchen N, Watkins L (2011) Evans index revisited: the need for an alternative in normal pressure hydrocephalus. Neurosurg 68:939-944.

84. Sabattini L (1982) Evaluation and measurement of the normal ventricular and subarachnoid spaces by CT. Neuroradiology 23:1-5.

85. Synek V, Reuben JR (1976) The ventricular-brain ratio using planimetric measurement of EMI scans. Br J Radiol 49:233-237.

86. De Leon MJ, George AE, Reisberg B et al (1989) Alzheimer's disease: longitudinal CT studies of ventricular change. AJR Am J Roentgenol 152:1257-1262.

87. Zhang Y, Londos E, Minthon L, Wattmo C, Lui H, Aspelin P, Wahlund LO (2008) Usefulness of computered tomography linear measurement in diagnosing Alzheimer's disease. Acta Radiol 49:91-97.

88. Saka E, Dogan EA, Topcuoglo MA, Senol U, Balkan S (2007) Linear measures of temporal lobe atrophy on brain magnetic resonance imaging (MRI) but not visual rating of white matter changes can help discrimination of mild cognitive impairment (MCI) and Alzheimer's disease (AD) Arch Gerontol Ger 44:141-151.

89. Conz L, Morita ME, Coan AC, Kobayashi E, Yasuda CL, Pereira AR, Lopes-Cendes F (2011) Longitudinal MRI volumetric evaluation in patients with familiar mesial temporal lobe epilepsy. Front Neurol 14:1-5.

90. Doring T, Kubo T, Cruz C, Juruena M, Fainberg J, Dominigues R, Gasparetto E (2011) Evaluation of hippocampal volume based on MRI imaging in patients with bipolar affective disorder applying manual and automatic segmentation techniques. J Magn Res Imag 33:565-572.

91. Kloppel S, Stonnington CM, Barnes J Chen F, Chu C, et al. (2008) Accuracy of dementia diagnosis: a direct comparison between radiologists and a computerized method. Brain 131:2969-2974.

92. Sperling R, Aisen P, Beckett L, Benett L, Craft S, Fagan A et al (2011) Toward defining the preclinical stages of Alzheimer's disease: recommendations from the National Institute of Aging-Alzheimer's Association workgroups on diagnostic guidelines for Alzheimer's disease. Alz Dement 7:280-292.

93. Jack C, Albert M, Knopman D, McKhann G, Sperling R, CarrilloM, Thies B, Phelps C (2011) Introduction to the recommendations from the National Institute on Aging-Alzhemer's association workgroups on diagnostic guidelines for Alzheimer's disease. Alzheimer's & Dementia 7:257-262, DOI 10.1016/j.jalz.2011.03.004.

94. Jack C, Knopman D, Jagust W, Shaw L, Aisen P, Weiner M, Petersen R, Trojanowski J (2010) Hypothetical model of dynamic biomarker of the Alzheimer's pathological cascade. Lancet Neurol 9:119-128.

95. Albert M, DeKosky S, Dickson D, Dubois B, Feldman H, Fox N, Gamst A et al (2011) The diagnosis of mild cognitive impairment due to Alzheimer's

disease: recommendations from the National Institute of Aging-Alzheimer's Association workgroups on diagnostic guidelines for Alzheimer's disease. Alz Dement 7:270-279.

96. McKhann G, Knopman D, Chertkow H, Hyman B, Jack C, Kawas C, Klunk W et al (2011) The diagnosis of dementia due to Alzheimer's disease: recommendations from the National Institute of Aging-Alzheimer's Association workgroups on diagnostic guidelines for Alzheimer's disease. Alz Dement 7:263-269.

97. Talairach J, Tournux (1993) Referentially orientated cerebral MRI anatomy: an atlas of stereotaxic anatomic correlations for gray and white matter. Thieme Medical Publishers, New York.

98. Jack C, Bernstein M, Fox N, Thompson P, Alexander G, et al (2008) The Alzheimer's Disease Neuroimaging Initiative (ADNI) MRI methods. J Magn Reson Imaging 27:685-691.

99. Simmons A, Westman E, Muehlboeck S, Mecocci P, Vellas B et al (2009) MRI measures of Alzheimer's disease and the AddNeuroMed study. Ann N.Y. Acad Sci 1180:47-55.

100. Sheline YI, Black KJ, Lin DY et al (1996) Stereological MRI volumetry of the frontal lobe. Psychiatry Res 67:203–214

101. Fischl B, Dale AM (2000) Measuring the thickness of the human cerebral cortex from magnetic resonance images. Proc Natl Acad Sci USA 97:11050-11055.

102. Segonne F, Dale AM, Busa E, Glessner M, Salat D, et al (2004) A hybrid approach to the skull stripping problem in MRI. Neuroimage 22:1060-1075

103. Fischl B, Salat DH, Busa E, Albert M, Dieterich M, et al (2002).Whole brain segmentation: automated labelling of neuroanatomical structures in the human brain. Neuron 33:341-355.

104. Fischl B, Salat DH, van der Kouwe AJ, Makris N, Segonne F, et al (2004) Sequence-independent segmentation of magnetic resonance images. Neuroimage 23:69-84.

105. Sled JG, Zijdenbos AP, Evans AC (1998) A nonparametric method for automatic correction of intensity non uniformity in MRI data. IEEE Trans Med Imaging 17:87-97.

106. Fischl B, Liu A, Dale AM (2001) Automatied manifold surgery: constructing geometrically accurate ant topological correct models of the human cerebral cortex. IEEE Trans Med Imaging 20:70-80.

107. Segonne F, Pacheco J, Fischl B (2007) Geometrically accurate topology-correction of cortical surface using nonseparating loops. IEEE Trans Med Imaging 26:518-529.

108. Dale AM, Fischl B, Serano MI (1999) Cortical surface-based analysis. I. Segmentation and surface reconstruction. Neuroimage 9:179-194.

109. Dale AM, Sereno MI (1993) Improved localization of cortical activity by combining EEG and EMG and MEG with MRI cortical surface reconstruction: a linear approach. J Cogn Neurosci 5:162-176.

110. Fischl B, Serano MI, Tootell RB, Dale AM (1999) High-resolution intersubject averaging and a coordinate system for the cortical surface, Hum Brain Mapp 8:272-284.

111. Desikan RS, Segonne F, Fischel B, Quinne BT, Dickerson BC, et al (2006) An automated labelling system for subdividing the human cerebral cortex on MRI scans into gyral based regions of interest. Neuro Image 31:968-980.

112. Fischl B, van der Kouwe A, Destrieux C, Halgren E, Segonn F, et al (2004) Automatically parcellation the human cerebral cortex. Cereb Cortex 14:11-22.

113. Radau PE, Slomka PJ, Svensson L, Wahlund LO (2000) Automated segmentation and registration technique for HMPAO-SPECT imaging of Alzheimer's patients. Proc SPIE Image Processing 3979:372-384.

114. Wallin AK, Blennow K, Andreassen N, Minthon L (2006) CSF-biomarkers for Alzheimer's disease: levels of β-amyloid, tau and phosphorylated-tau relate to clinical syndroms and survival. Dement Geriatr Cogn Disord 21:131-138.

115. Brun A, Englund E, Gustafson L, Passant U, Mann DMA, Neary D, et al (1994)The Lund and Manchester Groups: Clinical and neuropathological criteria for frontotemporal dementia. J Neurol Neurosurg Psychiatry 57:416-418.

116. Schmidt M (1996) Rey auditory verbal learning test – A handbook. Los Angeles, Western Psychological Services.

117. Andersson C, Lindau M, Almquist O, Engfeldt P, Johansson SE, Jonhagen ME (2006) Identifying patients at high and low risk of cognitive decline using Rey Auditory Verbal Learning Test among middle aged memory clinic outpatients. Dement Geriatr Cogn Disord 21:251-259.

118. Folstein MF, Folstein SE, McHugh PR (1975) Minimental-state: a practical method for grading the cognitive state of patients for the clinician. J Psychiatr Res 12:189-198.

119. Morris JC (1993) Clinical dementia rating. Neurology 43:2412–2414.

120. Winblad B, Palmer K, Kivipelto M, Jelic V et al (2004) Mild cognitive impairment – beyond controversies, towards a concensus: report of the International Working Group on Mild Cognitive Impairment. J Intern Med 256:240-246.

121. Roman GC, Tatemichi TK, Erkunjuntti T, Cummings JL, Masedeu JC, Garcia JH, et al (1993)Vascular dementia: diagnostic criteria for research studies. Report of the NINCDS-AIREN International Workshop. Neurology 43:250–260.

122. Landis JR, Koch GG (1977) The measurement of observer agreement of categorical data. Biometrics 33:159–174.

123. Cohen J. (1968) Weighted kappa: nominal scale agreement with provision for scaled disagreement or partial credit. Psychol Bull;70:213–220.

124. Stokes M, Davis C, Koch GG. Categorical Data Analysis Using the SAS System, Second Edition, Cary, NC: SAS Institute Inc 2000.

125. Westman E, Simmons A, Zhang Y, Muehlboeck JS, Tunnard C, et al (2011) Multivariate analysis of MRI data for Alzheimer's disease, mild cognitive impairment and healthy controls. Neuroimage 54:1178-1187.

126. Trygg J (2002) Orthogonal projections to latent structures (O-PLS). J Chemom 16:119-128.

127. Wiklund S, Johansson E, Sjostrom L, Mellerowicz EJ, Edlund U, et al (2008) Visualization of GC/TOF-MS-based metabolomics data for identification of biochemically interesting compounds using OPLS class models. Anal Chem 80:115-122.

128. Levine B, Kovacevic N, Nica EI, Cheung G, Gao F, et al (2008) The Toronto traumatic brain injury study: Injury severity and quantified MRI. Neurol 70:771-778.

129. McIntosh AR, Lobaugh NJ (2004) Partial least squares analysis of neuroimaging data: applications and advances. Neuroimage 23:250-263.

130. Qizilbash N, Schneider L, Chui H, Tariot P, Brodaty H, et al (2002) Evidenced-based Dementia Practice. Oxford, UK: Blackwell Publishing pp 20–23.

131. Westman E, Simmons A, Muehlboeck S, Mecocci P, Vellas B, et al. (2011) AddNeuroMed and ADNI: similar patterns of Alzheimer's atrophy andautomated MRI classification accuracy in Europe and North America NeuroImage58:818-828.

132. Ripsweden J, Mir-Akbari, Brolin EB, et al (2009) Is training essential for interpreting cardiac computed tomography? Acta Radiologica 2;194-200.

133. Blennow K, de Leon MJ, Zetterberg H (2006) Alzheimer's disease. Lancet 368:387-403.

134. Westman E, Wahlund LO, Foy C, Poppe M, Cooper A, et al (2010) Combining MRI and MRS to distinguish between Alzheimer's disease and healthy controls. J Alzheimer's Dis 22:171-181.

135. Visser PJ, Kester A, Jolles J, Verhey F (2006) Ten-year risk of dementia subjects with mild cognitive impairment. Neurol 67:1201-1207.

136. Hodges JR, Erzincliglu S, Patterson K (2006) Evolution of cognitive deficits and conversion to dementia in patients with mild cognitive impairment: a very-long-term follow-up study. Dement Geriatr Cogn Disord 21:380-391.

137. Galluzzi S, Talassi E, Belussi M, et al (2008) Multi-center comparison of medial temporal atrophy in patients with Alzheimer's disease – data from the ICTUS study. Dement Geriatr Cogn Disord 26:314–322.

138. Visser PJ, Verhey FRJ, Boada M, Bullock R, De Deyn PP, Frisoni GB, Froelich L, et al (2008) DESCRIPA study group: Development of screening guidelines and clinical criteria of predementia Alzheimer's disease. Neuroepidem 30:254-265.