White matter changes in patients with cognitive impairment

- clinical and pathophysiological aspects

Michael Jonsson, MD

UNIVERSITY OF GOTHENBURG

Institute of Neuroscience and Physiology

Department of Psychiatry and Neurochemistry

The Sahlgrenska Academy

University of Gothenburg 2012

ISBN 978-91-628-8447-5

Institute of Neuroscience and Physiology

Department of Psychiatry and Neurochemistry

University of Gothenburg

Printed by Ineko AB

Gothenburg 2012

To my family

"Il faut cultiver son jardin"

Voltaire, *Candide* (1759)

ABSTRACT

Cerebral white matter changes (WMC), detected with computed tomography (CT) or magnetic resonance imaging (MRI), represent a common condition in elderly people. However, the prognostic, symptomatological and biochemical constituents of WMC are only partially known. The aim of the present study was to evaluate WMC in relation to clinical manifestations in patients with mild cognitive impairment (MCI) and dementia and, by means of cerebrospinal fluid (CSF) analyses, to study different structural biomarkers possibly reflecting the pathophysiological process of WMC in non-disabled patients.

In study I, significant associations were found between WMC and age, sex, hypertension, ischemic heart disease and TIA/minor stroke. Furthermore there were significant associations between WMC and apathy, mental slowness, disinhibition, gait disturbance and focal neurological symptoms, but not with depressed mood.

In study II, CSF was analyzed for biomarkers known to be related to Alzheimer's disease [AD; the 1-40 and 1-42 fragments of amyloid-β, α- and β-cleaved soluble amyloid precursor proteins (sAPPα, sAPPβ), total tau (T-tau), hyperphosphorylated tau (P-tau)] and vascular dementia [VaD; neurofilament protein light subunit (NFL), sulfatide, and CSF/S-albumin ratio]. NFL and sulfatide but not the AD biomarkers were related to WMC.

In study III, low CSF levels of the myelin lipid sulfatide but not biomarker deviations associated with axonal degeneration (NFL), or AD were found to be related to progressive WMC.

In study IV it was found that WMC were associated with ventricular atrophy which in turn was associated with neuropsychological dysfunction. Furthermore, tissue inhibitor of metalloproteinase 1 (TIMP-1), NFL and sAPPβ were related to both ventricular atrophy as well as WMC. Matrix metalloproteinase 9 (MMP-9) was the only marker representing WMC progression.

The results indicate that WMC in patients with cognitive impairment are independently related to a dysexecutive-related behavioural symptom profile, vascular disorders and a non-AD biochemical profile associated with vessel-wall pathology and demyelination. The findings have may have implications for definition and nosological knowledge of AD and vascular cognitive disorder.

POPULÄRVETENSKAPLIG SAMMANFATTNING

Demens är vanligt hos äldre och och definieras som ett tillstånd av tilltagande nedsättning av kognitiva funktioner så till den grad att det utgör en betydande nedsättning av tidigare social och yrkesmässig funktionsnivå, utöver vad som kan förväntas av det normala åldradet. Demens innebär ett avsevärt lidande för den drabbade individen såväl som för anhöriga. I och med att befolkningen blir allt äldre drabbas allt fler. De vanligaste orsakerna till demens anses vara Alzheimers sjukdom, vaskulär (blodkärlsrelaterad) demens och blandtillstånd mellan de två (blanddemens). Tillsammans utgör dessa ca 80 % av alla fall. Vaskulär demens, också kallad småkärlssjuka, har på senare år blivit alltmer uppmärksammad som möjlig faktor bakom såväl intellektuell som psykisk och fysisk funktionsnedsättning hos äldre. En utmärkande förändring i hjärnan vid vaskulär demens är vitsubstansförändringar, som man kan se hos levande människor vid datortomografi eller magnetkameraundersökning av hjärnan.

I det första delarbetet studerade vi huruvida förekomsten av vitsubstansförändringar är relaterad till andra kärlriskfaktorer och kliniska neuropsykiatriska symptom oberoende av specifik demensdiagnos. Vi valde ut 176 patienter med de vanligaste demenssjukdomarna Alzheimers sjukdom, vaskulär demens och blanddemens. I materialet ingick även en mindre grupp av patienter med så kallad lindrig kognitiv störning som vi vet är ett tillstånd med förhöjd risk att utveckla demens. Samtliga patienter hade genomgått datortomografi eller magnetkameraundersökning av hjärnan. Resultaten visade att vitsubstansförändringar i sig, oberoende av demensdiagnos, är relaterade till en specifik så kallad dysexekutiv neuropsykiatrisk profil där symptom som apati, mental förlångsamning och nedsatt motorik dominerar. Denna symptomprofil var relativt enkel att påvisa med ett diagnosinstrument som kallas STEP. Dessutom kunde vi bekräfta att vitsubstansförändringar är relaterade till ålder och annan kärlsjukdom.

I arbete 2-4 studerade vi huruvida neurokemiska markörer i cerebrospinalvätska (CSV) kunde öka vår förståelse om varför vitstubstansförändringar uppkommer och vilken betydelse de har. I den vita substansen dominerar långa nervutskott (axoner). Dessa ligger normalt inbäddade i fettrika myelinskidor vilket ger en snabb spridning av nerimpulser i axonerna.

I det andra delarbetet mätte vi nivåer av olika markörer som brukar relateras till Alzheimers sjukdom och vaskulär demens. Resultaten visade att vitsubstansförändringar var relaterade till markörer för såväl myelinnedbrytning som för sönderfall av axoner. Samtidigt såg vi att graden av vitsubstansförändringar inte var korrelerade med markörer för Alzheimers sjukdom.

I det tredje delarbetet studerade vi huruvida de olika strukturella biomarkörerna i CSV vid utgångsbesöket var korrelerade till ökade vitsubstansförändringar mätta vid ett återbesök tre år senare. På det sättet försökte vi spåra de mekanismer som kan ligga bakom den aktiva sjukdomsprocessen. Resultaten visade att markören för nedbrytning av myelin var den enda som förutspådde tilltagande vitsubstansförändringar. Detta resultat talar för att nedbrytningen av myelin föregår nervcellssönderfallet.

I det fjärde delarbetet studerade vi ett utökat och känsligare batteri av CSV-markörer i relation till hjärnavbildningsmarkörer för subkortikal sjukdom i form av atrofi/förtvining av hjärnsubstans men även i relation till en funktionell hjärnavbildningsteknik, som med hjälp av vattenmolekylers rörelser utefter vitsubstansskikt kan ge en förfinad bild av vitsubstansförändringar. Vi undersökte även markörernas relation till prestation på olika neuropsykologiska tester. Resultaten talade åter för att markörer för myelinomsättning var de enda som förutspådde progress av vitsubstansförändringar, men att resultat på neuropsykologiska tester var relaterade till

radiologiska tecken på förtvinig av hjärnsubstans och dysexekutiva neuropsykologiska symptom, såväl på tvärsnittsnivå som vad gäller försämring över 3 år.

Sammanfattningsvis talar studierna för att vitsubstansförändringar i hjärnan är relaterade till en specifik neuropsykiatrisk symptomprofil och vaskulära faktorer. Studierna på CSV talar för att den primära neuropatologiska mekanismen är en demyelinisering som sekundärt leder till en axonal degeneration. Denna process leder i sin tur till en hjärnatrofi som syns vid magnetkameraundersökning. Den leder också till ett antal neuropsykologiska symptom som särskilt återfinns vid vaskulär demens. Vaskulär sjukdom är sannolikt det som sätter igång och driver sjukdomsprocessen.

Den vaskulära sjukdomen skall förstås behandlas med primärpreventiva (kost och motion) och sekundärpreventiva (läkemedel och andra interventioner mot hypertoni, diabetes och hyperlipidemi) metoder. Det är i dagsläget dock okänt om dessa interventioner verkligen förebygger uppkomsten av vitsubstansförändringar och vaskulär demens. Här behövs fler studier. Vidare skulle det vara intressant att undersöka om läkemedelskandidater som stimulerar återbildning av myelin i den vita substansen skulle kunna användas i behandlingen av vitsubstansförändringar och med dem associerade symptom.

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ORIGINAL PAPERS (I-IV)

LIST OF ORIGINAL PAPERS

This thesis is based on the following papers, referred to in the text by their Roman numerals:

I. Jonsson M, Edman Å, Lind K, Rolstad S, Sjögren M, Wallin A. Apathy is a prominent neuropsychiatric feature of radiological white-matter changes in patients with dementia. (*International Journal of Geriatric Psychiatry 2010 Jun;25(6):588-95).*

II. Jonsson M, Zetterberg H, van Straaten E, Lind K, Syversen S, Edman Å, Blennow K, Rosengren L, Pantoni L, Inzitari D, Wallin A. Cerebrospinal fluid biomarkers of white matter lesions – cross-sectional results from the LADIS study *(European Journal of Neurology 2010, 17: 377-382).*

III. Jonsson M, Zetterberg H, Rolstad S, Edman Å, Gouw A, Bjerke M, Lind K, Blennow K, Pantoni L, Inzitari D, Wallin A. Low cerebrospinal fluid sulfatide predicts progression of white matter lesions – the LADIS study. *(Submitted).*

IV. Bjerke M, Jonsson M, Nordlund A, Gouw A, Blennow K, Zetterberg H, Pantoni L, Inzitari D, Schmidt R, and Wallin A. Cerebrovascular biomarker profile is related to white matter hyperintensities and ventricular atrophy in a LADIS substudy. *(Manuscript).*

ABBREVIATIONS

Aβ, β-amyloid

AD, Alzheimer´s disease

ADDTC, Alzheimer´s Disease Diagnostic and Treatment Centers

APP, amyloid precursor protein

ARWMC, age-related white matter changes

BBB, blood-brain barrier

CADASIL, cerebral autosomal dominant arteriopathy with subcortical infarcts and leukoencephalathy

CSF, cerebrospinal fluid

CT, computed tomography

CVD, cerebrovascular disease

DLB, dementia with Lewy bodies

DSM-III-R, diagnostic and statistical manual of mental disorders, 3rd edition, revised

DSM-IV, diagnostic and statistical manual of mental disorders, 4th edition

ELIZA, enzyme-linked immunosorbent assay

FTD, frontotemporal dementia

ICD-10, international classification of diseases, 10th edition

MCI, mild cognitive impairment

MBP, myelin basic protein

MID, multi infarct dementia

MMP, matrix metalloproteinase

MMSE, mini-mental state examination

MRI, magnetic resonance imaging

NFL, neurofilament protein light subunit

NINCDS-ADRDA, National Institute of Neurological and Communicative Disorders and Stroke - Alzheimer´s Disease and Related Disorders Association

NINDS-AIREN, National Institute of Neurological Disorders and Stroke - Association Internationale pour la Recherché et l'Enseignement en Neurosciences

P-tau, hyperphosphorylated tau

PDD, Parkinson´s disease with dementia

PSD, post stroke dementia

RIND, reversible ischaemic neurologic deficit

sAPPα, soluble N-terminal APP, cleaved at the α-site

sAPPβ, soluble N-terminal APP, cleaved at the β-site

STEP, stepwise comparative status analysis

SID, strategic infarct dementia

SVD, subcortical vascular dementia

T-tau, total tau

TIA, transitoric ischaemic attack

TIMP, tissue inhibitor of metalloproteinase

VaD, vascular dementia

WMC, white matter changes

WMH, white matter hyperintensities

WML, white matter lesions

INTRODUCTION

Cerebral white matter changes (WMC) represent a common and often progressive condition in elderly people and contribute to disability [1]. However, the pathophysiology and clinical significance of WMC are still under debate.

In the literature, several synonymous expressions and related acronyms for white matter changes, (with or without the plural s), occur:

Leukoaraiosis (Greek. leuko-white, araiosis-rarefaction [2])

White matter changes – WMCs

Age related white matter changes – ARWMCs

White matter lesions – WMLs

White matter hyperintensities – WMHs/WMHIs

BASIC NOSOLOGY

Dementia

Dementia is common in the elderly and is defined as a decline in several cognitive domains (memory, language, praxis, gnosis, executive function) that is severe enough to constitute a significant decline in professional or social functioning [3]. Although dementia is a heterogeneous concept which encompasses conditions with different and often mixed aetiologies resulting in diverse clinical presentations, the cognitive, behavioural and functional decline generally results in an impaired quality of life for the afflicted patient and close relatives. There are about 140,000 patients with dementia in Sweden and the related annual cost to society has been estimated at SEK 40 billion. The prevalence increases with age and is about 1% at the age of 65, about 20% at the age of 80 and about 50% at the age of 90. Thus, with the globally anticipated increase in life expectancy, dementia is also

a growing challenge to the health care system. The most prevalent dementia type diagnoses are Alzheimer's disease (AD), vascular dementia (VaD), dementia with Lewy body bodies (DLB), Parkinson's disease with dementia (PDD), and Frontotemporal dementia (FTD).

Mild cognitive impairment

Mild cognitive impairment (MCI) is a condition defined by a history of cognitive impairment that is detectable with cognitive testing, but with no or only a minimal decline in social functioning or the activities of daily living and thus, by definition, does not meet the criteria for dementia. As a result of a collaborative international working group on MCI, specific recommendations for general MCI criteria were proposed in 2004 and include the following: (i) the person is neither normal nor demented; (ii) there is evidence of cognitive deterioration shown by either an objectively measured decline over time and/or a subjective report of decline by self and/or informant in conjunction with objective cognitive deficits; and (iii) the activities of daily living are preserved, and complex instrumental functions are either intact or minimally impaired [4]. MCI can have different aetiologies and may be a sign of prodromal dementia as it constitutes an elevated risk of progress to dementia within a few years, but many MCI patients are stable and some even recover at follow-up after a few years [5].

Alzheimer's disease

Alzheimer's disease (AD) is considered to be the most prevalent cause of dementia, constituting about 60% of all cases [6]. The clinical course is characterised by an insidious onset and smooth progression of impaired memory, visuospatial abilities and language, eventually leading to a general functional decline [3,7,8]. The condition is named after the German psychiatrist and neuropathologist Alois Alzheimer (1864-1915) who in 1906 published a case report on a 56 year old

woman with progressive memory loss, disorientation and hallucinations, supplemented with post mortem neuropathological findings of the senile plaques and neurofibrillary tangles that have subsequently become the hallmarks of AD [9].

The most commonly used clinical criteria for the diagnosis of AD in research studies are still those proposed in 1984 by NINCDS-ADRDA (National Institute of Neurological and Communicative Disorders and Stroke and the Alzheimer's Disease and Related Disorders Association) [10], originally intended to differentiate AD from VaD. The basic clinical criteria for the diagnosis of probable AD are: dementia established by clinical examination and documented by brief clinical tests such as the Mini-Mental Test [11] or Blessed Dementia Scale [12], and confirmed by neuropsychological tests; deficits in two or more areas of cognition; progressive worsening of memory and other cognitive functions; no disturbance of consciousness; onset between the ages of 40 and 90, most often after 65; and the absence of systemic disorders or other brain diseases that in and of themselves could account for the progressive deficits in memory and cognition.

In 2007, Dubois and colleagues presented new research criteria for pre-dementia AD [13]. These postulate that AD is one neuropathological process with a spectrum of clinical presentations from pre-symptomatic to full-blown dementia and that biomarkers for AD pathology are important adjuncts to the clinical phenotyping of the patient to make an accurate pre-dementia diagnosis of AD. Dubois and colleagues recently expanded on this reasoning in a new lexicon in which they reconceptualised the diagnosis around both a specific pattern of cognitive changes (amnestic memory impairment) and structural/biochemical evidence of AD pathology [14]. Along similar lines, the Alzheimer's Association and the National Institute of Aging have worked on new diagnostic criteria and guidelines for AD that were published in 2011. These guidelines postulate three clinically relevant stages of the disease with a continuum between and within each stage. The first stage is a preclinical phase, which might last a decade or more. During this phase there is evidence of abnormal

biomarker patterns, such as low cerebrospinal fluid (CSF) Aβ42 levels and/or increased amyloid tracer retention on positron emission tomography (PET), but without signs of cognitive impairment [15]. This phase is at present considered clinically irrelevant and is proposed only as a research framework for longitudinal studies to better understand disease progression or early intervention with disease-modifying therapy. The second stage is designated MCI due to AD ("prodromal AD" according to the Dubois lexicon [14]) [16]. During this stage, biomarkers positively identify the underlying cause of the cognitive impairment. The third stage is Alzheimer's dementia [8]. The criteria for this stage resemble the old McKhann criteria [10], but they are more specific.

Vascular dementia

Vascular dementia (VaD) is considered to be the second most prevalent form of dementia which constitutes about 20% of all cases. Vascular dementia is a heterogeneous concept and includes subtypes such as post-stroke dementia (PSD), strategic infarct dementia (SID) and subcortical vascular dementia (SVD) on the basis of diverse vascular pathogenesis [17]. Several pathological vascular mechanisms (e.g. thromboembolism, vessel wall damage, cerebrovascular insufficiency, hyperviscosity, haemorrhage) can lead to cognitive impairment [17,18] and thus there are also several types of VaD, ischaemic as well as haemorrhagic, [e.g. PSD, SID, Binswanger's disease, lacunar dementia (état criblé) [19], CADASIL, ischaemic-hypoperfusive VaD, haemorrhagic VaD]. PSD has been regarded as typical for large vessel (thromboembolic) and SVD for small vessel (hypoperfusive) VaD.

Comparative studies show a lack of agreement among the criteria [3,7,20,21], commonly used for VaD over the last two decades. The NINDS-AIREN (National Institute of Neurological Disorders and Stroke and Association Internationale pour la Recherché et l'Enseignement en Neurosciences) criteria were proposed in 1993 [21]. Still the most widely used criteria for VaD in clinical trials, they stipulate that the clinical diagnosis of probable VaD must fulfil all of the following main criteria:

1. *Dementia:* defined as impairment of memory and two or more cognitive domains severe enough to interfere with ADL.

   Exclusion criteria: disturbance of consciousness, delirium, psychosis, severe aphasia, or major sensorimotor impairment precluding neuropsychological testing. Also excluded are systemic disorders or other brain diseases (such as AD) that in and of themselves could account for deficits in memory and cognition.

2. *Cerebrovascular disease,* defined by the presence of focal signs consistent with stroke at neurological examination (with or without a history of stroke), and evidence of relevant cerebrovascular disease (CVD) on computed tomography (CT) or magnetic resonance imaging (MRI) including *multiple large vessel infarcts or a single strategically placed infarct* (angular gyrus, thalamus, basal forebrain, or posterior cerebral artery or anterior cerebral artery territories), *as well as multiple basal ganglia and white matter lacunes* or periventricular *white matter lesions,* or combinations thereof.

3. *A relationship between the above two disorders:* a) onset of dementia within three months of a recognised stroke; b) abrupt deterioration in cognitive functions; or c) fluctuating, stepwise progression of cognitive deficits.

Thus, in order to meet the NINDS-AIREN criteria for VaD, memory is the cardinal cognitive symptom and two other cognitive deficits based on neuropsychological examination are required. Furthermore, the cognitive disorder must develop within three months of a stroke. VaD is ruled out if brain imaging fails to reveal vascular lesions. Recommendations for which vascular lesions are to be included, as well as their degree of severity, are specified. These criteria were questioned at an early stage for being too strict in terms of the temporal association with stroke, and on the basis of

the limited existing knowledge of the cognitive symptoms actually related to CVD [22,23]. They are therefore often used in a modified form in studies of patients with VaD. Furthermore, they might be applied to large vessel VaDs such as PSD and SID, but not to the small vessel SVD.

While PSD and SID typically début within a few months of a clinical stroke, SVD – often with considerable diffuse WMC – typically has a more insidious onset and continuous progression, much akin to that of AD. As a disease entity, SVD is the most homogenous and probably the most common form of VaD. Criteria for SVD have been formulated by several research groups [24-26]. Those produced by Erkinjuntti et al. have become the preferred criteria (Table 1).

CADASIL (Cerebral Autosomal Dominant Arteriopathy with Subcortical Infarcts and Leukoencephalopathy) is caused by a mutation of the Notch3 gene of chromosome 19 and was the first described form of VaD that could be coupled to a genetic deviation [27-29]. Migraine with aura can appear as a début symptom already during adolescence. Patients with CADASIL often suffer from recurring transitoric ischaemic attacs (TIA) or minor strokes as early as in the 4^{th} or 5^{th} decade of life. The typical radiological finding is that of subcortical lacunar infarcts and diffuse WMC that tend to progress with age. The typical clinical course is that of a stepwise or smoothly progressive cognitive decline with a frontosubcortical syndrome with a dysexecutive cognitive profile and a gait disturbance. Depressive symptoms are also common. CADASIL has come to be regarded as a model disease for SVD as it afflicts younger subjects where co-existing Alzheimer pathology is less probable to contribute to the clinical picture.

Table 1. The criteria for the clinical diagnosis of subcortical vascular dementia (Erkinjuntti et al., J Neural Transm Suppl 2000) include all of the following:

A. Cognitive syndrome including both

I) Dysexekutive syndrome: impairment in goal formulation, initiation, planning, organizing, sequencing, executing, set-shifting and maintenance, abstracting.

II) Memory deficit (may be mild): impaired recall, relative intact recognition, less severe forgetting, benefit from cues.

And which indicate a deterioration from a previous higher level of functioning, and are interfering with complex (executive) occupational and social activities not due to physical effects of cerebrovascular disease alone.

B. Cerebrovascular disease including both

I) Evidence of relevant cerebrovascular disease by brain imaging

II) Presence/history of neurologic signs as evidence for cerebrovascular disease (hemiparesis, lower facial weakness, Babinski sign, sensory deficit, dysarthria, gait disorder, extrapyramidal signs consistent with subcortical brain lesions).

Clinical features supporting the diagnosis of subcortical vascular dementia include the following:

- Episodes of mild upper motor neuron involvement such as drift, reflex asymmetry, incordination.
- Early presence of gait disturbances (small-step gait or marche a petits-pas or magnetic, apraxic-ataxic or Parkinsonian gait).
- History of insteadiness and frequent, unprovoked falls
- Early urinary frequency, urgency, and other urinary symptoms not explained by urologic disease.
- Dysarthria, dysphagia, extrapyramidal signs (hypokinesia, rigidity).
- Behavioral and psychological symptoms such as depression, personality change, emotional incontinence, psychomotor retardation.

Features that make the diagnosis of subcortical vascular dementia uncertain or unlikely include:

- Early onset of memory deficit and progressive worsening of memory and other cognitive functions such as language (transcortical sensory aphasia), motor skills (apraxia), and perception (agnosia), in the absence of corresponding focal lesions on brain imaging.
- Absence of relevant cerebrovascular disease lesions on brain imaging.

Most prevalent dementia subtypes AD – VaD and mixed dementia

Thus, SVD and AD more typically pass a stadium of MCI in their clinical course of functional decline from normal age-related function to a manifest dementia syndrome. This circumstance can be challenging in clinical differential diagnostics. When the pathologies coexist, as is often the case, and both are judged to contribute to the cognitive and functional decline, the term mixed dementia is often used [30-36]. However, there is no established consensus on how to evaluate the relative contributions of the two pathologies. Vascular risk factors have also been associated with AD and the majority of AD patients also have radiologically detectable WMC. Is there a specific clinical neuropsychiatric symptom profile typically related to WMC regardless of the clinical dementia type diagnosis? Are there neurochemical markers in CSF reflecting the two pathologies? A clinical examination based on a structured interview with the patient and a close relative, in combination with a more thorough neuropsychological assessment, brain imaging (MRI) and CSF analysis, increases the diagnostic accuracy, particularly in early stages of the disease.

WHITE MATTER CHANGES

Brain imaging

Diffuse WMC of varying degree are common brain imaging findings in elderly people, especially in subjects with vascular risk factors. On CT, they typically emerge as patchy or diffuse areas of hypodensity of the periventricular or deep white matter. On MRI, they instead emerge as hyperintense areas of punctuate, confluent or diffuse nature on T2-weighted sequences [37], reflecting areas with a relatively higher water and lower fat content. Severity degree of WMC can be rated with different semi-quantative visual scales [37-40] (Figure 1) or be quantified with manual or automated volumetric methods.

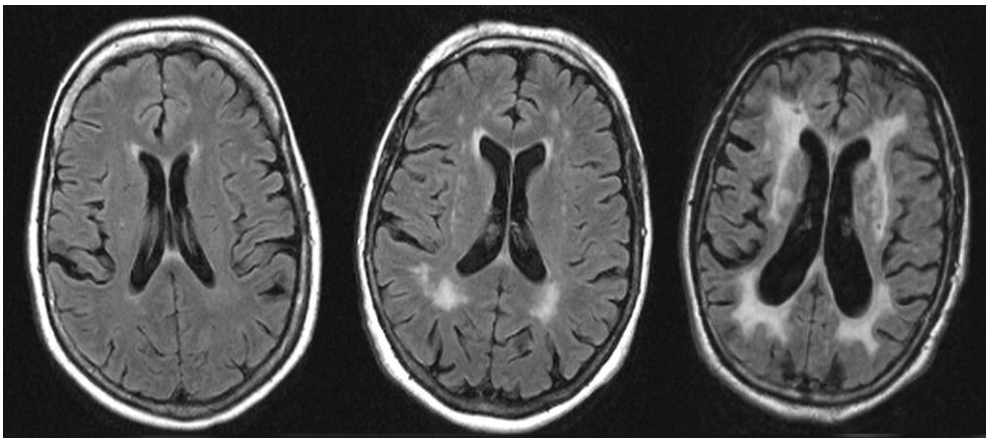


Figure 1: White matter changes of mild, moderate and severe degree according to the Fazekas scale

Lacunar infarcts are up to 15 mm wide and appear as spheric hyperintense foci on T2-weighted MRI sequences. They are typically localized to the basal ganglia, internal capsule, thalamus, and pons. They are often seen in parallel to, or even within, the more diffuse WMC and are also regarded as a sign of small vessel disease [41,42].

Diffusion weighted imaging (DWI) and diffusion tensor imaging (DTI) are techniques based on MRI scans. They focus on the mobility of tissue water within cellular and extracellular compartments. In healthy white matter the tissue water molecules move along the axis of the neural fibres as their mobility is restricted by intact membrane structures. Diffusion in other directions is an early sign of impaired tissue integrity. The calculated average diffusion coefficient (ADC) can be used to quantify the degree of tissue destruction within the lesions as well as the incipient morphological destruction in the surrounding tissue still normal appearing on conventional MRI [43].

Vascular risk factors

Although prevalence figures vary considerably in different studies, WMC appear to be more frequent in individuals with vascular risk factors and in patients with dementia, VaD in particular. Besides age,

the vascular factors most consistently associated with WMC are hypertension and other manifestations of arteriosclerotic disease such as stroke and ischaemic heart disease. However, it has also been suggested that other vascular factors such as hypotension, cardiac arrhythmias, diabetes mellitus and hypercholesterolemia are associated with the development of WMC [25,44-47].

Pathology/Pathophysiology

WMC represent a periventricular and subcortical leukoencephalopathy, manifested as demyelination, axonal loss and lacunar infarcts. While the diffuse WMC are characterized by varying degree of incomplete ischemic damage, the lacunes are regarded as the ultimate consequences of complete infarctions due to arteriolar occlusion. This neuropathological picture is caused by several microvascular changes due to arteriosclerosis, hyalinosis and fibrinoid necrosis of vessels with or without lumen occlusion. [48-50]. Thus, different pathological processes with various etiologies can cause small vessel disease in the brain. Arteriolosclerosis is typically associated with diffuse WMC, while lipohyalinosis and atherosclerosis are typically associated with lacunar infarcts and, sometimes, intracerebral haemorrhages. Cerebral amyloid angiopathy is associated with both diffuse WMC and small cortical infarcts as well as lobar haemorrhages [51,52]. Arteriolosclerosis is also associated with increased vessel wall permeability and with plasma protein extravasation/organisation which may give rise to WMC. Thus, the vascular brain injuries are related to specific vessel disorders. However, arteriolar dysfunction, irrespective of the vessel disease, remodels the extracellular matrix, further compromising the arteriolar function.

In summary, the main vascular pathology related to diffuse WMC is arteriolosclerosis of the small vessels perforating the white matter [41,53]. The vessel wall thickening leads to a reduced cerebral blood flow and an impaired vascular autoregulation [41,51]. An increased vessel wall permeability with leakage of plasma proteins to the perivascular brain parenchyma result in a blood-brain barrier

(BBB) dysfunction, and decreases drainage of extracellular fluid [53]. Inflammatory reactions are also involved [54]. It is believed that a chronic ischemic-hypoxic state leads to the loss of oligodendrocytes, demyelination and axonal degeneration, the hallmarks of established WMC, [41,53]. However, the pathophysiological mechanisms leading from vascular pathology to brain tissue damage are not fully understood [41]. One way to study this issue would be to analyse cerebrospinal CSF biomarkers in relation to WMC.

Biochemical markers

CSF is a clear liquid that is continuously produced in the plexus choroideus and ependymal cells in the walls of the lateral and third ventricles of the brain. It flows via the fourth ventricle into the subarachnoidal space surrounding the brain and spinal cord and is eventually recycled to the blood stream through the arachnoid villi in the sinus sagittalis superior. The total volume of CSF is about 140 ml and with a recycling rate of about six to eight hours. CSF functions as a shock absorber that protects the brain against mechanical trauma. It maintains homeostasis and also serves as a medium for nutrients, neurotransmitters and hormones and for the removal of metabolic waste products from the neural tissue. CSF is separated from the blood by the BBB but is in continuum with the extracellular fluid of the brain. Samples of CSF are collected by means of a lumbar puncture with a thin needle between the 3rd and 4th or the 4th and 5th lumbar vertebrae in the lower back.

The analysis of CSF for neurochemical markers has become a routine procedure and a cornerstone of contemporary dementia investigations together with brain imaging and neuropsychological testing [55]. As CSF is in direct contact with the extracellular compartment of the brain, biochemical changes in the brain are reflected in the CSF. There are several studies supporting the value of CSF analyses of T-tau (Total-tau), P-tau (hyperphosphorylated tau), and β-amyloid (reflecting neuronal

loss, neurofibrillary tangles and senile plaques, respectively) in establishing the clinical AD diagnosis, and in predicting future transition from MCI to overt AD [56,57].

The association between radiologically detected WMC and biomarker levels in CSF has been examined in cross-sectional studies on patients with SVD. An elevated CSF/serum albumin ratio has been demonstrated in patients with SVD and WMC as a sign of an impaired integrity of the BBB [58-60]. Sulfatide, a lipid enriched in myelin, which is elevated in CSF in patients with WMC, has been regarded as a marker of demyelination [61-64]. Neurofilament protein light subunit (NFL), a cytoskeletal protein in large myelinated axons, has been found to be significantly increased in patients with SVD [65] and in relation to WMC in other clinical forms of dementia [66]. These three biomarkers are not affected in AD patients without WMC. In a longitudinal study of MCI, a combined biomarker pattern of NFL, T-tau, P-tau and β-amyloid at baseline was able to identify quite well those MCI patients who subsequently deteriorated to overt SVD, in contrast to healthy controls, stable MCI patients, and those patients who deteriorated to AD or mixed dementia [67]. Other possible markers, such as myelin basic protein (MBP), matrix metalloproteinases MMPs and the tissue inhibitors of metalloproteinases (TIMPs) have been related to the pathological process of WMC [68].

Neuropsychiatric and neuropsychological manifestations

A frontosubcortical syndrome with gait disturbance and a dysexecutive symptom profile (impairment of goal formulation, planning, organising, sequencing, executing, set-shifting and maintenance, abstracting) has been related to VaD, including WMC [25,26,69,70].

Since the latter half of the 1990s, research has tended to focus more on the earlier MCI stages of vascular cognitive impairment (VCI) [70-78]. As it was also shown that VCI often constitutes a

progressive condition with a high risk of transition to dementia within a few years [79], interest in this concept has grown considerably. The significance of subcortical vascular disease, rather than multiple cortical infarcts, as the pathogenetic denominator of VaD has then also been increasingly appreciated.

Research in recent years has added further to our knowledge of the impact of WMC on cognitive functions in subjects with no or only mild functional impairment (MCI) and the prognostic significance of these WMC in terms of the functional outcome. This can be illustrated with a recently published review of results so far achieved from the LADIS study (Leukoaraiosis And DISability – see Material and Methods below) [1]. In summary, severe WMC turned out to be associated with worse performance in tests of global cognitive and executive functions, speed and motor control, attention, naming and visuoconstructional praxis [80] with an increased risk of further cognitive impairment and dementia [81], and were a strong and independent predictor for the overall transition to functional disability or death within three years [82]. The degree and location, mainly deep frontal, of WMC were also associated with an impaired motor performance, gait and balance [83,84] as well as depressive symptoms [85,86] and predicted further depressivity [87,88]. The progression of diffuse WMC and the number of incident lacunes at follow-up were associated with a deterioration in executive functions, and speed and motor control [89].

In conclusion, these results further strengthen the notion of WMC as an often progressive condition that contributes to disability, and that the associated neuropsychiatric and neuropsychological manifestations already in early stages of cognitive decline can be described as a progressive dysexecutive-related or frontosubcortical syndrome.

OVERALL AIM

The overall aim of this thesis has been to evaluate WMC in relation to clinical neuropsychiatric symptoms and vascular risk factors in patients with cognitive impairment and, by means of CSF analyses, to study different structural biomarkers possibly reflecting the pathophysiological process of WMC.

SPECIFIC AIMS

To study the possible association between WMC, vascular factors and a broad spectrum of neuropsychiatric symptoms in cognitively impaired patients, particularly whether WMC are independently associated with a dysexecutive-related behavioural symptom profile and vascular disorders, regardless of specific clinical dementia diagnoses.

To study how the degree of WMC rated on MRI scans of the brain in non-disabled elderly individuals relates to CSF levels of structural biomarkers associated with AD (amyloid-β 1-40, amyloid-β 1-42, α- and β-cleaved soluble amyloid precursor proteins, T-tau, P-tau) and VaD (NFL, sulfatide, and CSF/S-albumin ratio).

To study the mechanisms leading from vascular pathology to brain tissue damage in WMC by analysing CSF biomarkers associated with AD and SVD in relation to progression of WMC as rated on MRI.

To study the relationship between CSF biomarker levels and changes in normal appearing brain tissue (NABT) as rated with diffusion weighted imaging (DWI), WMC and atrophy (MRI) as well as their association to neuropsychological assessments.

MATERIAL AND METHODS

The prospective revised dementia study

Paper I is based on data from the longitudinal P-rev study which was performed at the Institute of Clinical Neuroscience, Section of Psychiatry and Neurochemistry, Sahlgrenska University Hospital, Sweden 1991-1997. The overall aim of the P-rev study was, by means of a thorough mapping of symptomatology, clinical course and biochemistry, to increase the diagnostic accuracy of the most prevalent forms of dementia, AD and VaD, but more primarily to study the occurrence of diagnostic subgroups not necessarily captured within traditional diagnostic criteria or, more specifically, to study the possible heterogeneity in AD (early onset AD and late onset AD, respectively) and VaD (MID and SVD, respectively). Patients with findings of an isolated acquired cognitive/emotional dysfunction or a manifest dementia syndrome (DSM-III-R) were enrolled. The assessment comprised a standardized clinical dementia investigation including physical, neurological, psychiatric and cognitive examinations, laboratory tests, brain imaging (CT or MRI) and a careful review of the medical history prior to diagnosis. [60,65].

The LADIS study

Papers II-IV are based on studies conducted as single-centre substudies of the Gothenburg cohort of the LADIS (Leukoaraiosis And DISability) project. The LADIS project is a European multi-centre study of the prospective role of WMC as an independent determinant of the transition from a healthy functional status to disability in elderly individuals. Further aims have been to establish whether WMC are an independent predictor of: i) death from any cause or specific causes, ii) cardiovascular/cerebrovascular events, iii) dementia, iv) depression, and to study the progression of WMC on MRI images in relation to functional, clinical and quality of life outcomes [90]. Patients enrolled in LADIS had different degrees of WMC but, at inclusion, no or only mild functional

impairment (e.g. MCI). The patients were clinically assessed with a standardized battery of somatic, neurologic, psychiatric, cognitive, functional and quality of life measures and followed up annually for three years, after which a second MRI was performed.

The P-rev and LADIS studies were both approved by the University of Gothenburg's Ethics Committee. All patients, or their closest relative, gave their informed consent for participation in the studies, both of which were conducted in accordance with the Helsinki Declaration.

Patients

In paper I, 176 consecutive patients (90 women and 86 men, age 70.1 ± 6.4 years) with AD [10], VaD [21,23], mixed dementia [WHO7] and MCI [4,91] were recruited from subjects participating in the P-rev study. Cross-sectional baseline data were collected. Degree of dementia was rated as mild in 90 patients, moderate in 72 patients och severe in three patients [92]. Eleven patients were diagnosed as MCI. The patients were referred to the clinic from their general practitioners, other specialists, or through self-referral and were inpatients on a ward specialized in dementia investigations.

In paper II-IV, 53 non-disabled individuals (25 women and 28 men, age 74 ± 4.8 years) with WMC were recruited between March 2002 and January 2003 from the Gothenburg cohort (N=63) of the LADIS study. The basis for enrollment to these CSF substudies was the availability for a lumbar puncture. Cross-sectional baseline data were used in study II-IV with added three year follow-up data on subgroups of participants in paper III-IV. Most of the LADIS subjects were recruited among outpatients at the neuropsychiatric clinic at Sahlgrenska University Hospital, Mölndal, Sweden, and fulfilled the criteria for MCI [4,91] but also included were some elderly individuals recruited among cognitively healthy controls in the Gothenburg MCI study [93] or from other clinics and primary health care centers.

Paper II included all the 53 enrolled LADIS subjects with baseline data on MRI and CSF biomarkers available.

Paper III included those 37 subjects (15 women and 22 men, age at baseline 73.6 ± 4.6 years) from paper II who were eligible for a follow-up MRI after three years. Regarding basic demographic data, the missing 16 subjects from paper II were significantly older, had fewer years of education and lower MMSE scores at baseline but the gender distribution and WMC load at baseline were similar as in the group with completed follow-up MRI.

Paper IV included the 46 subjects (22 women and 24 men; age 74 ± 5 years) from paper II, where baseline data on WMC and atrophy (MRI), DWI evaluation metrics (44 subjects) and results from CSF analyses and neuropsychological assessments were available. WMC progression results (RPS) on 37 subjects (paper III) were also added. Furthermore, a demographically comparable subpopulation (34 subjects) was included based on availability of data from MRI and neuropsychological baseline and follow-up assessments.

Methods

Evaluation of vascular factors (paper I)

The presence of vascular factors was identified through information from the patient and a close relative, medical records, and clinical findings. Vascular factors considered were diabetes mellitus, hypotension, hypertension, ischaemic heart disease, cardiac arrhythmia, TIA/RIND, major stroke, and other atherosclerotic conditions (e.g. stenosis of the carotid arteries, claudicatio intermittens). The presence of hypertension was considered if previously diagnosed, with antihypertensive treatment being present or if the blood pressure at examination, recorded as the mean value of three

standardised readings, was obviously hypertensive (>180/>100 mm Hg). The frequences of the different vascular risk factors are shown in table 2 together with results.

Evaluation of neuropsychiatric symptoms (paper I)

Neuropsychiatric (cognitive, neurological and psychiatric) signs and symptoms were assessed by means of the Stepwise comparative status analysis (STEP) [94], a comprehensive observational instrument consisting of 35 items relating to various symptoms associated with dementia (listed in table 3 together with results). Each symptom has been defined in order to be assessed independently, such as apathy, which enables the examiner to distinguish it from confounding symptoms as mental slowness or depressed mood. The assessment relies mainly on what the examiner observes. However, as some symptoms are better observed over time, a description from a close relative of the patient and from the ward staff is also considered. Variables first evaluated are symptoms related to consciousness, sensorium and emotional ability (modified items from the Comprehensive Psychopathological Rating Scale [95]). Thereafter, memory function, and intellectual and central sensorimotor functions are assessed using a brief cognitive battery (e.g. orientation, memory, visuo-spatial function, abstracting) and finally the basic neurological status. For the present purpose only the absence or presence of symptoms was taken into account. Cognitive function was also evaluated through the Mini-Mental State Examination [11].

Evaluation of neuropsychological symptoms (paper IV)

The neuropsychological assessment battery comprised tests of speed and attention, episodic memory and executive functions. Trailmaking A and B [96], Symbol Digit Modalities Test and digit cancellation were used for the speed and attention domain. For episodic memory the California Verbal Learning Test (CVLT) was used [97]. For executive functions, Stroop Colour Word Test [98], verbal fluency, a maze task, Digit Span [99] backwards and subtraction scores from Trailmaking (B time − A time) were used. Each test score was z-transformed in order to construct composite z-

scores within each domain. The composite z-score expresses the general level of performance within that domain. For the episodic memory composite z-score, the sum of the z-scores of learning trials 1-5, delayed recall and recognition on CVLT were used. The z scores of neuropsychological tests fr which higher score represented poorer performance were inverted (-z) for calculation of composite scores.

Radiological evaluations (papers I-IV)

In all studies (I-IV) the scans were performed at Sahlgrenska University Hospital, Gothenburg, Sweden. The radiological evaluations were performed by experienced raters, blinded to clinical details.

In paper I, CT scans (76 patients) were performed without contrast enhancement and with 10 mm contiguous slices through the cerebrum. MRI scans (100 patients) were performed on a 1.0 T magnet. Conventional spin-echo sequences were used, including proton density-weighted and T2-weighted images (TR/TE 2,250/20-80 msec), and T1-weighted axial scans (TR/TE 500/15 msec). In these patients, the brain was examined with 6 mm contiguous slices in the transversal and sagittal planes. The presence and degree of WMC were evaluated with a semi-quantitative scale [39,40,66,100]. For the present purpose, the patients were divided into groups based on presence or absence of WMC.

In papers II-IV*,* MRI scans were performed according to a standardised protocol (LADIS) in which a 1.5-T scanner was used, and the series included axial T2-weighted images, axial fluid-attenuated inversion recovery (FLAIR) images, and coronal or sagittal 3D T1 sequence, as previously described [101]. The WMC were rated at the Image Analysis Center of the VU Medical Center in Amsterdam, the Netherlands. The Fazekas scale was used for staging WMC as mild (single lesions < 10mm, areas of "grouped" lesions < 20mm in any diameter), moderate (single lesions 10-20 mm, areas of

"grouped" lesions > 20 mm in any diameter, no more than "connecting bridges" between individual lesions) or severe (single lesions or confluent areas of hyperintensity ≥ 20 mm in any diameter) [38]. The basis for rating was always the most severe abnormality, even when seen only on one slice. Furthermore, volumetric quantification of WMC was performed on the same sequences, including the infratentorial region [101].

In papers III-IV, the progression of WMC was assessed visually on FLAIR images, comparing baseline and three year follow-up scans for each patient side-by-side, using a modified version of the Rotterdam progression scale (RPS) [102,103]. The absence or presence of progression (0 and 1, respectively) was scored in three periventricular regions (frontal caps, occipital caps, bands), four subcortical regions (frontal, parietal, occipital, temporal), basal ganglia, and the infratentorial region [46,102], thus resulting in a total score ranging from 0 (no progression) to 9 (widespread progression).

In paper IV, DWI was performed on the 1.5-T whole-body system with a pulsed gradient spin-echo sequence with echo planar imaging readout with 2 *b* factors (b=0 sec/mm^2 and b=900-1000 sec/mm^2). The diffusion gradients were applied along the three principal directions and the voxel size was 1.95X1.95X1.95 mm. The DWI metrics included the average apparent diffusion coefficient (ADC), or mean diffusivity, of both WMC and normal–appearing brain tissue (NABT) of white matter and grey matter. Furthermore, relative peak height (rPH) and peak position (PP) of NABT was assessed by histogram analysis. Further details are found elsewhere [104]. DWI metrics were analysed and ventricular and sulcal atrophy was assessed at the Department of Neurology at the Medical University of Graz. A template-based atrophy rating scale previously assessed in other studies [101,105] ranging from one (no atrophy) to eight (severe atrophy) was employed. All MRI measurements assessed in this study were baseline values except for the progression of white matter changes.

Biochemical analyses (paper II-IV)

Patients included in study II-IV, were subjected to lumbar puncture through the L3/L4 or L4/L5 interspace, at baseline. The CSF was collected in polypropylene tubes and centrifuged at 2000 x g, 4°C. The supernatant was gently mixed to avoid gradient effects and the CSF was further aliquoted and stored at –80°C pending analyses. A serum sample was also drawn. The biomarkers known to be related to AD, i.e. Aβ1-42, T-tau and P-tau181, were analysed with a commercially available multiplex beadbased assay from Innogenetics utilizing the Luminex® xMAP® technology (INNO-BIA AlzBio3) as previously described [106]. The α-cleaved soluble APP (sAPP-α) and β-cleaved soluble APP (sAPP-β) levels were determined with a multiplex electrochemiluminescent enzyme-linked immunosorbent (ELISA) assay from Meso Scale Discovery (MSD®) according to manufacturer's advice (MULTI-SPOT® sAPPα/sAPPβ). This assay employs the 6E10 antibody to capture sAPP-α and a neoepitope-specific antibody to capture sAPP-β. Both isoforms are detected by SULFO-TAG–labeled anti-APP antibody p2-1. Furthermore, the CSF level of Aβ40 as well as the MMP (MMP-1, -2, -3, -9 and -10) and TIMP-1 levels were determined using MSD® assays (MULTI-ARRAY Human Aβ40 Ultra-Sensitive, MULTI-SPOT® Human MMP 3-Plex Ultra-Sensitive, MULTI-SPOT® Human MMP 2-Plex Ultra-Sensitive, and MULTI-ARRAY® Human TIMP-1, respectively). The kit that captures the Aβ40 fragment uses the 6E10 antibody in combination with an end-specific SULFO-TAG–labeled anti-Aβ40 antibody (Meso Scale Discovery), i.e. detecting Aβx-40. Further, the sulfatide concentration was determined by thin layer chromatography while NFL was determined by ELISA [107,108]. In paper II-III, the detection limit of the NFL ELISA was 250 ng/L, while the NFL ELISA (UmanDiagnostics NF-light ®) utilized in paper IV was more sensitive. The analysis of MBP was performed with an ELISA (ACTIVE® MBP) purchased from Diagnostic Systems Laboratories. Albumin levels in serum and CSF were measured by immunonephelometry on an Immage immunochemistry system (Beckman Coulter Inc, Fullerton,

Ca). The CSF/serum albumin ratio was calculated as previously described [109]. All biomarkers were analysed at baseline.

Statistical methods

Group comparisons were performed using analysis of variance, Kruskal-Wallis test or Mann-Whitney U test. Dichotomous comparisons were conducted with x2-test. Correlation analyses were performed using the Spearman rank correlation. Stepwise linear or logistic regressions were performed for predictions of dependant variables.

RESULTS

Vascular factors and neuropsychiatric symptoms (paper I)

In paper I, a majority of the included patients (59%) had detectable WMC of varying degree as assessed with CT or MRI. According to bivariate analysis, the patients with WMC were significantly older than those without WMC ($P < 0.001$) and more often men ($P = 0.038$). No associations were seen regarding degree of dementia and presence or absence of WMC.

Table 2. Vascular factors related to absence or presence of white matter changes.

	White matter changes			
Vascular factors	**Total group (n = 176)**	**Absence of WMCs (n = 73)**	**Presence of WMCs (n = 103)**	**Adjusted for MRI/CT (p-value*)**
Hypertension	47 (27%)	13 (18%)	34 (33%)	**0.017**
Hypotension	18 (10%)	9 (12%)	9 (9%)	0.841
Diabetes mellitus	12 (7%)	2 (3%)	10 (10%)	0.085
TIA/RIND	25 (14%)	6 (8%)	19 (18%)	**0.034**
Major stroke	6 (3%)		6 (6%)	0.977
Ischemic heart disease	41 (23%)	12 (16%)	29 (28%)	**0.029**
Cardiac arrhythmia	27 (15%)	9 (12%)	18 (18%)	0.173
Other atherosclerotic vascular diseases	11 (6%)	5 (7%)	6 (6%)	0.805
Number of vascular factors	1.1 ± 1.3 (0 - 5)	0.8 ± 1.0 (0 - 3)	1.3 ± 1.4 (0 - 5)	**0.002**
BP supine systolic	144 ± 20 (100 - 210)	138 ± 18 (100 - 195)	148 ± 20 (110 - 210)	**0.000**
BP supine diastolic	80 ± 9 (60 - 115)	78 ± 7 (60 - 100)	81 ± 10 (60 - 115)	**0.003**
BP erected systolic	131 ± 21 (90 - 215)	125 ± 17 (90 - 175)	136 ± 22 (90 - 215)	**0.001**
BP erected diastolic	76 ± 9 (50 - 100)	74 ± 8 (50 - 95)	77 ± 10 (55 - 100)	**0.032**

BP = blood pressure

Values are given in percentages or as mean values ± SD and min-max

*Logistic regression. White matter changes as dependent and vascular factors as independent in the model, adjusted for MRI/CT

The total number of vascular factors present was significantly related to the presence of WMC and individual associated vascular factors were hypertension, history of TIA/RIND and ischaemic heart disease. Levels of examined blood pressure were consistently correlated to the presence of WMC. (Table 2)

Among neuropsychiatric symptoms in the STEP protocol, there were significant associations between WMC and apathy, mental slowness, disinhibition, gait disturbance, and focal neurological symptoms. However, there were no significant associations between WMC and depressed mood or the parietotemporal symptoms (e.g. impaired memory, orientation, abstract thinking and visuospatial abilities) usually associated with AD. (Table 3)

Table 3. Neuropsychiatric primary variables related to absence or presence of white matter changes.

White matter changes				
Neuropsychiatric symptoms	**Total group (n = 176)**	**Absence of WMCs (n = 73)**	**Presence of WMCs (n = 103)**	**Adjusted for MRI/CT (p-value *)**
Reduced wakefulness	4 (2%)	0 (0%)	4 (4%)	0.977
Concentration difficulties	73 (42%)	27 (37%)	46 (45%)	0.073
Hallucinatory behaviour	10 (6%)	5 (7%)	5 (5%)	0.655
Paranoid symptoms	18 (10%)	5 (7%)	13 (13%)	0.116
Restless movements	36 (20%)	10 (14%)	26 (25%)	0.066
Depressed mood	28 (16%)	13 (18%)	15 (15%)	0.660
Elevated mood	8 (4%)	3 (4%)	5 (5%)	0.869
Apathy	127 (72%)	42 (58%)	85 (82%)	**<0.001**
Disinhibition	49 (28%)	12 (16%)	37 (36%)	**0.005**
Vulnerability to stress	114 (66%)	48 (66%)	66 (65%)	0.209
Perseveration	94 (53%)	35 (48%)	59 (57%)	0.441
Mental slowness	108 (62%)	36 (49%)	72 (71%)	**0.002**
Memory disturbance	169 (96%)	68 (93%)	101 (98%)	0.861
Disorientation	120 (68%)	54 (74%)	66 (64%)	0.731
Reduced capacity for abstract thinking	150 (87%)	57 (79%)	66 (64%)	0.140
Visuospatial disturbances	124 (71%)	53 (73%)	71 (70%)	0.621
Poverty of language	65 (38%)	26 (37%)	39 (39%)	0.588
Sensory aphasia	43 (25%)	17 (23%)	26 (26%)	0.755
Visual agnosia	7 (4%)	5 (7%)	2 (2%)	0.111
Apraxia	33 (19%)	12 (16%)	21 (20%)	0.428
Dysarthria	12 (7%)	5 (7%)	7 (7%)	0.452
Dysphagia	0 (0%)	0 (0%)	0 (0%)	
Positive masseter reflex	26 (15%)	9 (12%)	17 (17%)	0.253
Tremor	18 (10%)	4 (6%)	14 (14%)	0.113
Rigidity	25 (14%)	9 (12%)	16 (16%)	0.682
Paratonia	31 (18%)	10 (14%)	21 (20%)	0.254
Hypokinesia	30 (17%)	10 (14%)	20 (19%)	0.759
Marche à petits pas	24 (14%)	3 (4%)	21 (21%)	**0.024**
Increased reflexes	54 (31%)	18 (25%)	36 (35%)	0.393
Babinski´s phenomenon	11 (6%)	1 (1%)	10 (10%)	0.082
Ataxia	14 (8%)	5 (7%)	9 (9%)	0.363
Body agnosia	59 (34%)	25 (34%)	34 (33%)	0.316
Myoclonus	1 (1%)	0 (0%)	1 (1%)	0.991
Focal neurologic symptoms	40 (23%)	11 (15%)	29 (28%)	**0.028**
Other emotional, cognitive or neurologic symptoms	5 (3%)	3 (4%)	2 (2%)	0.374

Frequencies concern existence of the Neuropsychiatric primary variable (regardless of severity)
*Logistic regression. White matter changes as dependent and neuropsychiatric symptoms as independent in the model, adjusted for MRI/CT

The subsequent stepwise multiple logistic regression analysis revealed that the neuropsychiatric symptoms apathy ($P < 0.001$) and mental slowness ($P = 0.024$), together with age ($p < 0.001$), were the most consistent of clinical features predicting the presence of WMC. As expected, MRI as a radiological method also predicted the detection of WMC ($p < 0.001$). (Table 4)

Table 4. Multivariable Logistic model for absence or presence of WMCs.

Variable	ß (SE)	Odds Ratio (95% CI)	p-value
Intercept	-9.987 (2.286)		
Apathy	0.932 (0.255)	2.5 (1.5-4.2)	<0.001
Mental slowness	0.509 (0.225)	1.7 (1.1-2.6)	0.024
MRI/CT	1.612 (0.400)	5.0 (2.3-11.0)	<.0001
Age (years)	0.116 (0.030)	1.1 (1.1-1.2)	<0.001

White matter changes as dependent variable, $p < 0.05$.

Biochemical markers (paper II-IV)

In paper II, fifteen of the 53 enrolled LADIS subjects had mild, 23 had moderate and 15 had severe degree of WMC according to the Fazekas scale. The corresponding WMC volumetric measures of hyperintense signal are displayed in table 5 together with biochemical characteristics. There were no significant differences regarding age and sex between the groups. The CSF-NFL levels differed between the three groups ($P < 0.001$) with the highest levels in the group with severe WMC. CSF sulfatide concentration was slightly elevated in the moderate and severe groups compared to the mild group but the association was less strong ($P = 0.036$) and there was a substantial overlap between groups. T-tau, P-tau and the different amyloid markers did not differ significantly between the groups, nor did the CSF/S-albumin ratio. The group of patients with mild WMC tended to have higher CSF levels of sAPP-α and sAPP-β, as well as Aβ40 and Aβ42, as compared to the groups with moderate and severe degrees of WMC but these relations were non-signifikant. (Table 5)

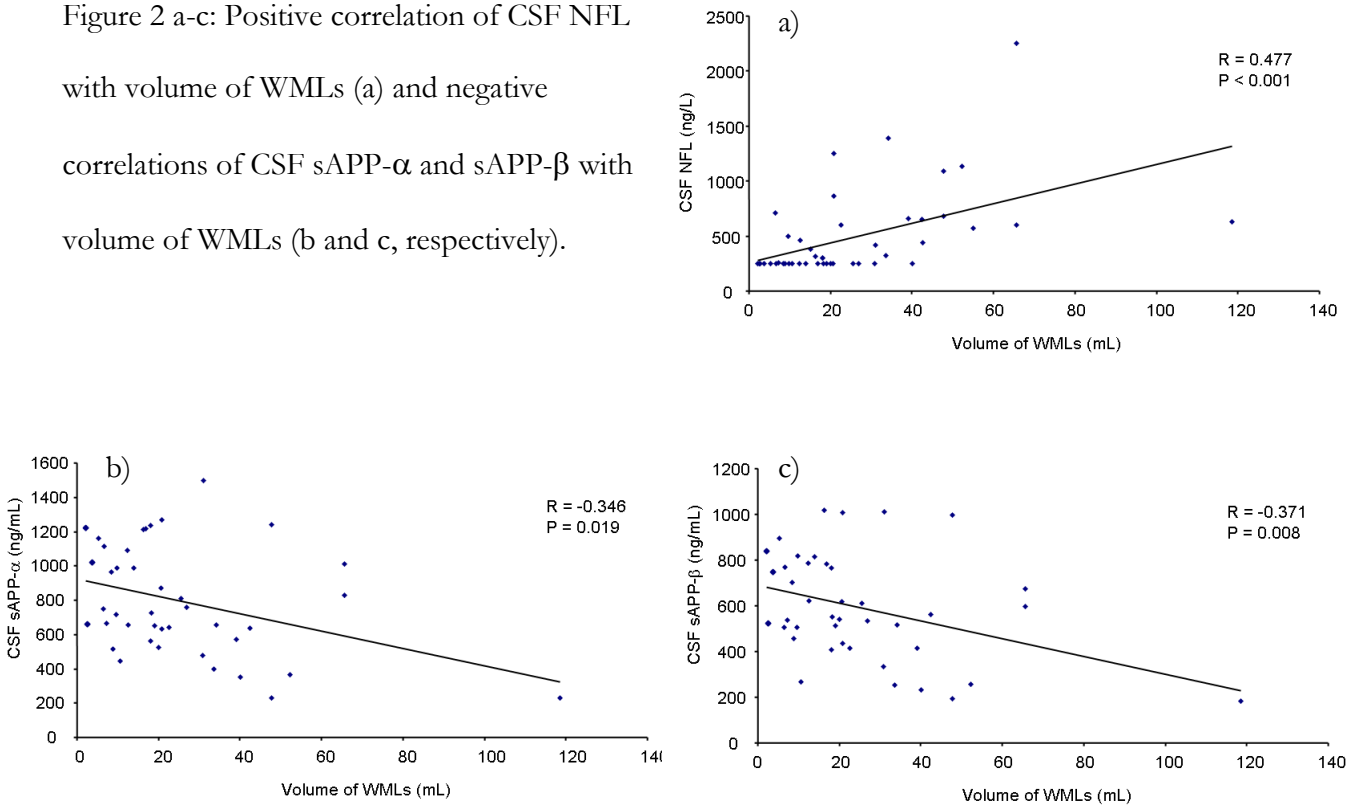
Table 5. Biochemical characteristics according to degree of white matter lesions[a]

	Mild WMLs (n=15)	Moderate WMLs (n=23)	Severe WMLs (n=15)	*P* value
Volume of WMLs (mL)	6.4 (2.2-12)	20 (8.9-43)	48 (18-120)	<0.001
NFL (ng/L)	250 (250-750)	300 (250-1250)	630 (250-2200)	<0.001
T-tau (ng/L)	450 (160-920)	370 (110-1500)	470 (90-1200)	NS
P-tau$_{181}$ (ng/L)	57 (34-77)	56 (28-100)	58 (25-140)	NS
Aβ40 (ng/L)	3500 (1400-6100)	2900 (1300-7800)	3000 (800-7400)	NS
Aβ42 (ng/L)	730 (280-990)	550 (250-920)	540 (330-860)	NS
sAPP-α (ng/mL)	960 (440-1200)	650 (350-1300)	630 (230-1500)	NS
sAPP-β (ng/mL)	710 (270-890)	550 (230-1020)	480 (180-1010)	NS
Sulfatide (nM)	250 (200-400)	250 (150-400)	300 (200-450)	0.036
CSF/S-albumin	6.4 (3.9-13)	6.4 (4.0-11)	6.6 (3.3-11)	NS

[a]Data are presented as median (range).

Volumetric WMC measures also correlated positively with NFL levels (r = 0.477, P < 0.001) and negatively with the levels of sAPP-α and sAPP-β (r = -0.346 and r = -0.371, respectively, P < 0.02). (Figure 2 a-c)

Figure 2 a-c: Positive correlation of CSF NFL with volume of WMLs (a) and negative correlations of CSF sAPP-α and sAPP-β with volume of WMLs (b and c, respectively).



Paper III included the 37 LADIS subjects that were eligible for a follow-up MRI after three years. Progression of WMC, as assessed with the RPS score, ranged from 0 to 8 points within the group (median RPS = 2). Subjects with more pronounced progression (RPS >2; n = 15) had a lower mean CSF sulfatide concentration (230 ± 41 nmol/L) at baseline as compared to subjects with no or minimal progression [(RPS 0-2; n = 22) (285 ± 69 nmol/L)] according to univariate analyses (*P* = .009). Sulfatide was the only biomarker that predicted the RPS score according to the regression analysis, explaining 18.9 % of the total variance.

In paper IV, it was shown that there was no direct relationship between the WMC volume and neuropsychological dysfunction. However, increased WMC volume was correlated to ventricular atrophy which in turn was associated with neuropsychological dysfunction. Furthermore, the increase in ventricular dilatation and WMC volume were both related to increased levels of sAPPβ, NFL and TIMP-1, biomarkers previously associated with cerebrovascular disease. Progression of WMC, assessed through the RPS score, was correlated to increased baseline levels of MMP-9 indicating a potential role of this biomarker in white matter damage. Furthermore, the NABT metrics were related to the outcome in executive functions and speed and motor control at baseline, while the biomarkers P-tau and MMP-10 were associated with memory function and speed and motor control. All three of them were intercorrelated at baseline. The associations of NABT and P-tau with neuropsychological functions were, however, lost at follow-up possibly due to a drop in patients with significantly worse memory functions at baseline.

DISCUSSION

The symptom profile with apathy and mental slowness found to be related to WMC in **paper I** is usually referred to as a frontosubcortical neuropsychiatric symptom profile as it often appears in patients with tissue lesions related to the vascular system in the deep frontosubcortial brain regions, i.e. in those regions that are affected by WMC [26,50,110,111]

In the present study apathy was defined in the wide sense, i.e. a syndrome comprising loss of motivation and initiative, lack of interest, and emotional reduction as has been previously described [112-114]. Loss of motivation is also a characteristic feature of depression, and depressive symptoms have been found to be related to WMC [115]. However, given that depressed mood, the fundamental feature of depression, was not related to WMC in the present study it appears difficult to believe that the white matter-related apathy findings were equivalents of depression. Instead, mental slowness, a cognitive processing disturbance, was found to be independently related to WMC. This result supports the idea that cognitive impairment is a feature of WMC. Although apathy is an increasingly recognized concomitant of a wide range of central nervous system disorders, there is as yet little consensus regarding methods for detecting apathy, or for distinguishing it from depression or cognitive dysfunction, or for assessing its severity [116]. The method used in the present paper is a simplified low-tech method for the identification apathy and related symptoms. It may also be useful in conditions other than WMC.

Although, the present study deals with the most common dementia disorders, i.e. AD, VaD and mixed AD/VaD, which together account for at least 80% of all dementia cases [60] it was considered appropriate not to differentiate the patient material with regard to clinical diagnoses, for the present purposes. The reason for this was that WMC are thought to reflect similar pathology [117] irrespective of the dementia type diagnosis, and it has been suggested that WMC constitute an overlapping factor between AD and VaD [49,118,119]. As a matter of fact, in a recent large

European multi-centre study of dementia, apathy was found to be an independent neuropsychiatric syndrome across all common dementia subtypes [120,121]. Although data on WMC were lacking in that study, one reasonable possibility is that WMC were the common denominator behind the apathy syndrome.

WMC were found to be associated with a history of TIA/RIND and also with vascular factors of a non-stroke type (blood pressure, ischaemic heart disease). These findings support the hypothesis that WMC are a manifestation of a vascular disorder. This is in line with previous studies on the relationship between WMC and vascular factors. However, the absence of independent relationships between WMC and vascular factors is puzzling and at variance with the results of other studies. One explanation could be that the relationships are not captured behind the multivariate outcomes. Another explanation might be the use of dichotomous variables which are less likely to detect associations than variables with several points of measurement. A third, more speculative, explanation would be that the vascular factors measured in the study mainly reflect macrovascular lesions rather than the microvascular lesions that have been suggested to be the main lesions behind WMC.

The result in paper II that CSF NFL is related to WMC in non-disabled elderly is in agreement with previous findings in demented patients [66]. NFL is mainly found in large myelinated axons that extend subcortically [122], and thus CSF NFL increases preferentially in disorders with white-matter damage such as multiple sclerosis [123] and SVD, [65,66,124,125], and after traumatic brain injury [126]. Large myelinated axons are also abundant in the pyramidal tract, and CSF NFL increases in patients after spine trauma [127], and in amyotrophic lateral sclerosis [128]. T-tau, on the other hand, is highly expressed in cortical axons [129], and increases in CSF preferentially in disorders with cortical damage or degeneration, such as AD [130] and Creutzfeldt-Jakob disease [131]. The lack of association of WMC with the AD biomarkers speaks against a primary pathogenic link between

white matter disease and AD. In contrast, negative correlations between volumes of WMC and CSF levels of soluble APP suggest that small vessel disease in the brain may result in reduced expression and/or processing of APP, which in turn may result in less deposition of amyloid in the brain. Notably, the group of patients with mild WMC tended to have higher CSF levels of, not only sAPP-α and sAPP-β, but also Aβ40 and Aβ42, than the groups with a moderate or severe degree of WMC, which also suggests a general downregulation of APP metabolite production in response to white matter disease. Similar results have recently been seen in multiple sclerosis [57]. Sulfatide, a marker of damage to myelin [61], was slightly elevated in patients with a moderate or severe degree of WMC, but the association was weak and the overlap between groups considerable.

The main finding in paper III was that of a significant, however inverse, association between the CSF concentration of the demyelination marker sulfatide and WMC progression. By contrast, neither impaired BBB-function (CSF/serum albumin ratio) and axonal degeneration (NFL), nor the AD biomarkers (T-tau, P-tau, Aβ42) [132] were associated with the progression of WMC. Together, these findings indicate that WMC pathogenesis is a process different from the neurodegenerative process seen in AD.

The present study is a sub-study within the frame of the LADIS project which dictates the careful inclusion of patients with WMC of small vessel disease origin only. Although the study sample was not large, there was a fair distribution of subjects over varying degrees of WMC at baseline. Previous studies on sulfatide in CSF in relation to WMC in humans have found significantly elevated CSF sulfatide levels in patients with VaD [61], SVD[64]., and in normal pressure hydrocephalus and subcortical arteriosclerotic encephalopathy [42]. In these studies the authors have viewed the elevated levels of CSF sulfatide in relation to WMC of vascular origin as a reflection of demyelination.

Remyelination is the natural regenerative response to demyelination. A number of molecules (e.g. growth factors, cytokines) are released by microglia and astrocytes in response to demyelination and regulate the proliferation and differentiation of multipotent progenitor cells to mature oligodendrocytes, capable of producing new myelin sheaths that enwrap the demyelinated axons [133]. It is not until the final stages of differentiation that oligodendrocytes express sulfatide and myelin proteins forming mature myelin membranes. Sulfatide then appears to act back as a negative regulator for further oligodendrocyte differentiation [134]. Successful remyelination may result in functional recovery after transient demyelination [133,135]. However, these mechanisms sometimes can not fully compensate for the demyelination. This has been related to an age factor [136], but also to the possibility that intensity or chronicity of demyelination eventually outpaces the remyelinating capacity [137], as may be the case in multiple sclerosis (MS).

CSF biomarkers in MS have recently been reviewed where tentative markers for demyelination (e.g. myelin basic protein and antibodies to other myelin proteins) and remyelination (e.g. neuronal cell adhesion molecule, and growth factors as ciliary neurotrophic factor, brain-derived neurotrophic factor, nerve growth factor and neurotrophin 3) were evaluated. In brief, there is a demand for biomarkers for remyelination to further explore the heterogeneity in MS and to guide the development of novel repair-promoting strategies [138]. Sulfatide was not mentioned among these biomarker candidates. However, Ilyas and colleagues have detected antibodies to sulfatide in CSF from MS patients and hypothesised that these antibodies could act detrimentally on the remyelination process by inhibiting the differentiation of progenitor oligodendrocyte cells [139].

In our study, a lower level of sulfatide in baseline CSF predicted the progression of WMC. This finding could be seen as a sign of an age-related decline in the regenerative capacity as mentioned above, a shortage of myelin due to immature oligodendrocytes, but also as a reflection of an ongoing

remyelination attempt that is eventually outpaced by the chronic ischemic/hypoxic-induced demyelination process.

Previous post mortem studies on humans with concomitant AD pathology and WMC have revealed a significant reduction of the number of oligodendrocytes in relation to the severity of WMC, whereas no correlations were seen between oligodendrocytes and age or degree of cortical AD pathology [140,141]. In a more recent study, myelin loss in WMC was quantified in post mortem brains of patients with VaD, AD, (mixed AD/VaD however excluded), dementia of Lewy body type and healthy controls. Reduced myelin density was found in all three dementia groups compared to the controls, but was most severe in VaD. In the AD group, myelin loss was coupled with an increased number of oligodendrocytes, while in the VaD group myelin loss was instead associated with a reduction of size of oligodendrocytes. The authors concluded that these differences suggest diverse mechanisms behind the demyelination in VaD (hypoxic-ischaemic damage to oligodendrocytes) and AD (secondary to axonal degeneration) and, further, that mild to moderate ischemia might stimulate remyelination, whilst severe ischemia may lead to unsuccessful remyelination due to an impaired recruitment of oligodendrocyte precursor cells [142].

In our study, the CSF level of NFL did not predict progression of WMC in spite of the fact that NFL was the most significant marker of WMC severity at baseline, whereas the association with sulfatide was only slight, (paper II) [143]. The absence of positive NFL findings suggests that axonal degeneration does not reflect the ongoing disease process per se but rather a consequence of an insufficient remyelination process as suggested by the relationship between sulfatide and the progression of WMC.

Besides limited sample size, the study drawbacks (Papers II and III) are the somewhat insensitive neurochemical method used for determining CSF NFL with a lower limit of detection of 250 ng/l,

and the thin layer chromatography method used for determining CSF sulfatide which provides rough values as multiples of 25 nmol/l. Another restriction is the limited sensitivity of the T2-weighted MRI for quantification of the true extent of tissue destruction in white matter disease. More sensitive radiological methods such as diffusion weighted/tensor MRI could possibly display more obvious correlations with neurochemical markers [144,145]. In paper IV, a more sensitive assay for the analysis of NFL was applied as well as a more sensitive radiological method in the form of DWI.

In paper IV, we added atrophy measures (MRI) and DWI as a more sensitive radiological method. We also used a more sensitive assay for the analysis of NFL and additional CSF biomarkers in the form of MMP (MMP-1, -2, -3, -9 and -10) and TIMP-1. Furthermore, we studied the relations of the radiological and biochemical markers with neuropsychological test results. Ventricular atrophy was directly associated with executive function and speed and motor control at baseline and follow-up and further related to the volume of WMC. Both these imaging markers correlated to the biochemical markers reflecting CVD (sAPPβ, NFL and TIMP-1), thus giving the subcortical vascular disease a biomarker fingerprint. Increased levels of MMP-9 have also previously been related to subcortical vascular disease and was in the present study the only marker representing WMC progression. A possible link between increased levels of MMP-9 and WMC might be a hypoxic induced expression of the enzyme and a concomitant hypoxia induced degeneration of oligodendrocytes. Whether there is a cause and effect relationship remains to be investigated. Changes in the NABT metrics seem to mirror more of a cortical profile as reflected by the correlation to sulcal atrophy as well as to P-tau and MMP-10 both of which has been related to AD [68].

CONCLUSION

In general we have found evidence of a specific symptom pattern and a neurochemical fingerprint of white matter involvement that are different from those usually found in Alzheimer's disease. Our findings suggest that white matter involvement is caused by a vessel-wall related disease process that involves myelin, axonal and tissue remodelling events that are different from those of early Alzheimer's disease. These findings should be taken into account for instance in treatment trials.

ACKNOWLEDGEMENTS

I would like to express my sincere gratitude to all who have made this work possible:

Anders Wallin, my supervisor, for your scientific guidance and encouraging discussions and for sharing your expertise in this interesting field of medical science. You often incited the project a step further at moments of despair.

Henrik Zetterberg, my co-supervisor, for your scientific guidance and for sharing your expertise in neurochemistry with a constant and contagious enthusiasm and for your many initiatives for new research projects.

Maria Bjerke, co-author, for sharing your expertise in neurochemistry and, furthermore, for your devoted help and guidance with the practical procedures in the final stage of the thesis work.

Åke Edman, for co-authoring and for taking part in the clinical LADIS work. For many a long session with reading and analysing scientific articles and for your guidance in how to write them.

Sindre Rolstad, for co-authoring and for your help with statistics and article submitting procedures and for taking part in the clinical LADIS work.

Karin Lind, co-author and co-worker in the LADIS project. My closest colleague and fellow research student, for many fruitful discussions on clinical and research matters and on life as a whole.

Kaj Blennow, for sharing your expertise in neurochemistry and research and for your many initiatives for new research projects.

Steinar Syversen, co-author, for sharing your expertise in geriatric medicine and cardiology and for your valuable contributions to the LADIS study.

Ewa Styrud, **Eva Bringman** and **Christina Holmberg**, research nurses, for your dedicated work in several clinical trials and for adding high spirits to the research group.

Kerstin Gustavsson and **Ingela Isblad**, for your splendid work as research nurses in the LADIS project as well as several years of clinical co-work.

Calle Eckerström and **Erik Olsson**, for sharing your brain imaging expertise and for contributing with images included in this thesis.

Mattias Göthlin, for your excellent database work and your constant readiness to assist.

Arto Nordlund, for sharing your expertise in the field of neuropsychology and for taking part in the LADIS work.

Jacob Stålhammar, for your enthusiastic help with illustrations.

Niklas Mattsson and **Erik Portelius**, for sharing your expertise in the field of neurochemistry and for fertile ideas for future projects.

Sonja Klingén, head of the neuropsychiatric clinic. You have with great generosity and benevolence made this project possible.

Magnus Sjögren, my co-supervisor early on, for many interesting discussions and for supervising with great enthusiasm during the early phase of the project.

Ulla Ohlson, research administrator at the institution, for much assistance with procedural paper work.

To all **LADIS study participants**.

The research group at Mölndal memory clinic for providing a fruitful climate for scientific work.

All other **members of staff** at the memory clinic in Mölndal for making it a great place to work.

All **members of staff** at the neurochemistry laboratory in Mölndal, especially laboratory technicians **Åsa Källén**, **Monica Christiansson** and **Sara Hullberg** for your technical assistance with the LADIS CSF samples.

Agnes Jonsson-Blomberg, for your valuable software technical assistance early on in the LADIS project.

My wife **Margareta** and my daughters, **Agnes** and **Freja**, for listening and trying to understand.

This work was made possible by grants from the Handlanden Hjalmar Svenssons Forskningsfond, Pfannenstills Forskningsstiftelse, The Alzheimer Research Fund, Axel Linders Stiftelse, The Foundation Gamla Tjänarinnor, Stiftelsen Demensfonden, Sahlgrenska University Hospital, The Swedish Research Council, Swedish Brain Power and Stiftelsen Psykiatriska Forskningsfonden.

The LADIS study was funded by the European Union with grants under the V European Framework Programme (Quality of life and management of living resources 1998-2002), contract No QLRT – 2000 – 00446 as a concerted action.

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