From DEPARTMENT OF NEUROBIOLOGY, CARE SCIENCES AND SOCIETY (NVS), H1
Karolinska Institutet, Stockholm, Sweden

COGNITION IN MULTIPLE SCLEROSIS WITH SPECIAL EMPHASIS ON MRI FINDINGS AND CEREBROSTEROL

Gösta Bergendal

Karolinska Institutet

Stockholm 2014

Published by Karolinska Institutet.

ISBN 978-91-7549-591-0

Printed by
REPROPRINT AB
Stockholm 2014
www.reproprint.se
Gårdsvägen 4, 169 70 Solna

Karolinska Institutet

Institutionen för neurobiologi, vårdvetenskap och samhälle (NVS)

Cognition in Multiple sclerosis with special emphasis on MRI findings and cerebrosterol

AKADEMISK AVHANDLING
som för avläggande av medicine doktorsexamen vid Karolinska Institutet offentligen försvaras i Föreläsningssal Månen (9Q), Alfred Nobels Allé 8, 9tr; Huddinge sjukhus

Torsdagen den 5/6-14, kl 14:00

av
Gösta Bergendal
Leg. psykolog

Huvudhandledare:
Docent Ove Almkvist,
Karolinska Institutet
Institutionen för neurobiologi, vårdvetenskap och samhälle (NVS), H1

Bihandledare:
Professor Sten Fredrikson
Karolinska Institutet
Institutionen för Klinisk neurovetenskap
Sektionen för neurologi

Docent Maria Kristoffersen-Wiberg
Karolinska Institutet
Institutionen för Klinisk vetenskap, intervention och teknologi
Sektionen för medicinsk avbildning och teknik

Professor Agneta Herlitz
Karolinska Institutet
Institutionen för klinisk neurovetenskap

Fakultetsopponent:
Docent Anders Svenningsson
Umeå Universitet
Institutionen för klinisk neurovetenskap

Betygsnämnd:
Professor Kristian Borg
Karolinska Institutet
Institutionen för kliniska neurovetenskaper

Docent Aniko Bartfai
KIDS/Karolinska Institutet
Institutionen för kliniska vetenskaper

Docent Gullvi Flensner
Högskolan Väst
Institutionen för omvårdnad hälsa och kultur

Stockholm 2014

ABSTRACT

Multiple sclerosis (MS) is a progressive inflammatory and degenerative disease of the central nervous system (CNS). This thesis focuses on cognition in MS, with special emphasis on long-term magnetic resonance imaging (MRI) findings and cerebrosterol plasma levels.

In study I, the effects of MS on a variety of cognitive aspects were evaluated longitudinally over an eight-year follow-up period in 31 patients who had been diagnosed as having relapsing-remitting MS, secondary progressive MS (SP-MS) or primary progressive MS. A selective pattern of decline was found at baseline in the whole group, with marked decline in information-processing speed (IPS). These deficits in IPS at baseline predicted further cognitive decline over the follow-up period. A differential pattern of cognitive decline over time was noticed in the subgroups, with the most pronounced decline in the SP-MS group; in these patients, the deterioration in visual IPS was clearly more marked than that in auditory IPS. A high disability score (on the expanded disability status scale; EDSS) during follow-up was associated with cognitive decline. These findings indicate that tests measuring IPS are especially strong predictors of cognitive decline over longer periods in patients with MS.

In Study II, 25 patients with MS and 25 matched control participants were tested with a picture-naming test (Boston Naming Test; BNT) and a letter-word fluency test (using the letters FAS). In the BNT, the MS patients used less distinct descriptions and substitutions and had significantly more off-target substitutions than the control group. The MS patients were significantly less effective in using strategies for retrieval in the word fluency FAS test than the control participants. These results suggest that language function becomes impaired in MS, with semantically nonspecific naming responses and less effective use of strategies for retrieval in word fluency.

In Study III, 22 MS patients were given tasks investigating IPS, covering the following aspects: cognitive (symbol digit modalities test; SDMT), sensory (visual and auditory reaction time tests), motor (finger-tapping speed test) and auditory interhemispheric transfer (verbal dichotic listening test; VDL). These parameters were related to the area of the corpus callosum in the brain (CCA), measured with MRI at baseline and at follow-up nine years later. The relative brain volume (RBV) and the T2 lesion load were taken into account. The results showed that the CCA, but not the RBV or the T2 lesion load, was associated with the SDMT score, and that the higher the annual rate of change in the CCA, the poorer the performance in the left ear VDL, with a subsequently more pronounced advantage in the right ear VDL. These results indicate that corpus callosum is related to a clearly cognitive component, rather than a sensory-motor component.

Study IV analyzed the relationships between cognitively demanding information processing (measured with the SDMT), clinical status (EDSS), plasma cerebrosterol

24OHC levels, and MRI-normalized measurement of RBV, grey and white matter volumes, and ventricular cerebrospinal fluid volume in a cross-sectional sample of 21 MS patients. The results showed that slow IPS in SDMT was related to neurodegeneration, particularly loss of grey matter volume, and high cerebrosterol plasma concentrations, reflecting membrane turnover in the CNS. Poor EDSS was associated with high plasma cerebrosterol levels, which is hypothetically a biomarker of MS progression.

Conclusions: Deterioration in IPS seems to be a central aspect of cognitive decline in MS. Slow IPS occurs already at an early phase in the disease and predicts long term cognitive decline. The MS patients have less effective strategies for lexical substitution and retrieval than healthy persons. The poor lexical processing in the MS patients could putatively be due to slow IPS, especially in the markedly speed demanding word fluency test. CCA in contrast to RBV and the T2 lesion load, appears to be exclusively related to a cognitively demanding IPS task but not to sensory-motor speed tasks, which suggests that CC is especially important for cognitive speed processes. Slow IPS seems to be primarily associated with low grey matter volume and high plasma concentration level of cerebrosterol while disability appears to be related to high plasma level of cerebrosterol. Since there were no interaction between cerebrosterol and the neurodegenerative predictors, it is putative that the cytotoxic properties of the cerebrosterol independently could cause neuronal cell death and thereby affect IPS independently of neurodegeneration.

LIST OF PUBLICATIONS

I. Bergendal G, Fredrikson S, Almkvist O. Selective decline in information processing in subgroups of Multiple Sclerosis: An 8-year longitudinal study. Eur Neurol 2007;57:193-202.

II. Tallberg IM, Bergendal G. Strategies of lexical substitution and retrieval in multiple sclerosis.
Aphasiology 2009;23:1184-1195.

.

III. Bergendal G, Martola J, Stawiarz L, Kristoffersen-Wiberg M, Fredrikson S, Almkvist O. Callosal atrophy in multiple sclerosis is related to cognitive speed. Acta Neurol Scand 2013;127:281-289.

IV. Bergendal G, Stawiarz L, Fredrikson S, Karrenbauer VD, Almkvist O. Information processing speed in Multiple Sclerosis is related to grey matter volume and plasma levels of cerebrosterol.
Manuscript

CONTENTS

LIST OF ABBREVIATIONS

ANCOVA	Analysis of covariance
ANOVA	Analysis of variance
ARC	Annual rate of change
ART	Auditory reaction time
BBB	Blood brain barrier
BDNF	Brain-derived neurotrophic factor
BNT	Boston Naming Test
CCA	Corpus callosum area
CIS	Clinically isolated syndrome
CNS	Central nervous system
COWAT	Controlled Oral Word Association Test
CSF	Cerebrospinal fluid
CT	Computed tomography
DTI	Diffusion tensor imaging
EBV	Epstein-Barr virus
EDSS	Expanded disability status scale
EF	Executive functioning
fMRI	Functional magnetic resonance imaging
FSIQ	Full-scale intelligence quotient
FT	Finger-tapping test
HLA	Human leukocyte antigen
HQOL	Health related quality of life
IL	Interleukin
IPS	Information processing speed
MRI	Magnetic resonance imaging
MS	Multiple sclerosis
MSSS	Multiple sclerosis severity score
MTI	Magnetization transfer imaging
NGF	Nerve growth factor
PASAT	Paced auditory serial attention test
PP-MS	Primary progressive multiple sclerosis
PR-MS	Progressive relapsing multiple sclerosis
QoL	Quality of life
RAVLT	Rey auditory verbal learning test

RBV	Relative brain volume (normalized)
RO Copy	Rey-Osterrieth complex figure copy
RR-MS	Relapsing remitting multiple sclerosis
SDMT	Symbol digit modalities test
SLDT	Swedish lexical decision test
SP-MS	Secondary progressive multiple sclerosis
TNF	Tissue necrosis factor
VDL	Verbal dichotic listening
VRT	Visual reaction time
WAIS-R	Wechsler adult intelligence scale
WM	Working memory

1 THESIS SUMMARY – MAIN SECTION

1. GENERAL BACKGROUND

1.1 Multiple sclerosis: Introduction

Multiple sclerosis (MS) is a chronic, autoimmune, inflammatory, neurodegenerative, demyelinating neurological disease of the central nervous system (CNS). It has unknown etiology and a duration of many years. From a historical perspective, there are early descriptions of neurological symptoms and the subsequent development of persisting dysfunction which appear to describe MS; for example, in the diary written by the English aristocrat Augustus D'Este during the period 1830-40 (Fredrikson and Kam-Hansen 1989; Landtblom et al. 2010). In 1860 Jean-Martin Charcot identified and named the disease "sclerose en plaques disseminées". He noted the association between loss of the fatty insulation layer composed of myelin around the nerve fibers and the patterns of neurological impairment, and he suggested diagnostic criteria (Charcot´s triad) based on the occurrence of dysarthria, ataxia and tremor (Charcot 1877). Later research using improved diagnostic techniques such as magnetic resonance imaging (MRI) has confirmed Charcot's clinical findings and added to our understanding of MS.

1.2 Epidemiology

MS primarily strikes young and middle-aged adults aged 20-40 years (Weinshenker et al. 1989). The average age of disease onset is around 30 years (Sibley 1990; Weinshenker et al. 1991). In rare cases, the onset of MS can occur early in childhood. MS is two to three times as common in women as in men (Goodin 2014). The prevalence of MS varies with the geographic position, with the highest frequency in temperate climate zones at southern or northern latitudes, such as in Western Europe. Scotland and the Outer Hebrides, in particular, have a high prevalence, which can be up to 300 cases per 100000 people. England and Wales have rates varying from 74 to 112 per 100000 (Rejdak 2010). Sweden also belongs in these areas of high prevalence (Andersen 2012). Interestingly, some ethnic groups living in high prevalence areas appear to be resistant to MS, for example some of the First Nations people of western Canada (Rejdak 2010).

1.3 Etiology

The etiology of MS seems to be complex and multifactorial. It appears that a genetic factor contributes to the etiology. First-degree relatives of a person with MS are at higher risk of contracting the disease than the general population. Monozygotic twins

are reported to have a higher risk of contracting MS than dizygotic twins (Dyment et al. 2004; Baranzini et al. 2010). Individuals who live with MS patients but are not genetically related to them are at no higher risk than the general population. It has been found that the human leukocyte antigen (HLA) system can bind body-specific proteins in some human tissue types, thereby contributing to the autoimmune activation of T-cells in MS (Fagius et al. 2007). The HLA region on chromosome 6 is associated with MS and the presence of HLA-DR 2 in people living in northern Europe is connected with the disease (Dyment 2004). There are indications that the CYP27B1 gene is also associated with MS, by encoding the vitamin D-activating 1-alpha hydroxylase enzyme (Ramagopalan 2011).

Environmental factors are also part of the etiology of MS. It has been observed that people who migrate before puberty from areas at high risk of MS to areas at low risk are less likely to contract the disease (Kurtzke 1993). Such observations have given rise to a viral or infective hypothesis. However, animal studies have failed to isolate the causative organism, or to transfer the disease to other animals. The focus has been on the Epstein-Barr virus (EBV) as a possible viral trigger of MS. EBV is a common cause of mononucleosis which has a similar geographic distribution to MS. In a case-control study, Hernan et al. (2001) showed that patients who had previously developed mononucleosis had an increased prevalence of MS. Ascherio et al. (2001) demonstrated in a prospective study that individuals with symptomatic EBV infection or with stronger antibody responses to EBV were more likely to develop MS than those without. Despite these findings, no causal relationship has been established between MS and EBV. Human herpesvirus 6 and *Chlamydophyla pneumoniae* have also been suggested as possible causative agents of MS (Swanborg et al. 2003). Sunlight exposure and vitamin D levels are environmental factors that appear to be important etiological factors in MS, because of the relationship between geographical differences in sunlight exposure and the association between latitude and the prevalence of MS around the world, and the effects of sunlight on serum vitamin D levels in association with the immunomodulatory effect of vitamin D on the homeostasis of T-cells (Correale et al. 2009). Epidemiological research has also identified a history of smoking as an environmental risk factor for MS (Handel and Ramagopalan 2011).

It has been hypothesized that environmental factors could interact with the genes via epigenetic mechanisms to regulate gene expression, thus modulating the disease presentation (Burell et al. 2011, Huynh et al. 2013).

1.4 Pathophysiology and biochemistry of MS

It has been hypothesized that the pathogenesis of MS is related to exposure of the patient to an environmental agent during childhood which triggers epigenetic mechanisms, thereby inducing potentially auto-reactive T-cells. These potentially auto-reactive T-cells are activated after a period of latency by a systemic trigger (such as infection), are attached to the internal wall of the blood vessel by the adhesion

molecules on their surface, and then pass through the blood-brain barrier (BBB) from the blood into the brain tissue, producing proinflammatory cytokines and initiating a local cell-mediated immune reaction.

Myelin and neuronal antigens are then released and the antigens cross from the brain tissue across the BBB to be presented to T-cells as foreign, thereby inducing activated auto-reactive T-cells which proliferate; the receptors on these fit the proteins from the myelin sheath. These activated proliferated T-cells go through the BBB and come in contact with protein fragments of myelin presented by the receptors on HLA molecules, whereby they are further activated to produce cytokines which attract more activated T-cells and activate microglia, astrocytes and endothelial cells, giving rise to more proinflammatory cytokines such as interleukin (IL)-1 and tissue necrosis factor-α. These cytokines upregulate the adhesion molecules in the endothelium at the BBBand, together with chemo-attractant cytokines (chemochines), they reinforce the recruitment of T-cells. In this context, B-cells produce auto-antibodies which bind to the myelin and contribute to its destruction. These auto-antibodies also facilitate the destruction by macrophages of the myelin sheath (Fagius et al. 2007; Rejdak et al. 2010). The macrophages secrete an enzyme (protease) which breaks down the myelin (Smith 2001). The auto-antibodies and other cytotoxic substances such as reactive oxygen, perforin and granzyme boost the effects of the pro-inflammatory cytokines (Lord et al. 2003; Morales et al 2006) and cause the death of myelin-producing oligodendrocytes and demyelination, resulting in conduction block.

At the same time, suppressor T-cells are producing immunomodulatory cytokines such as IL-4 and transforming growth factor-β, and neuroprotective neurotrophic factors such as nerve growth factor (NGF) and brain-derived neurotrophic factor (BDNF), which mediate cell survival and proliferation in the CNS (Kalinowska-Lyszczarz and Losy 2012), thereby reducing the inflammation and helping the oligodendrocytes to survive and proliferate. The plasticity resulting from these mechanisms allows some remyelination and functional recovery to take place. Persistent demyelination does, however, cause axonal loss (Rejdak et al. 2010).

The neuron-specific enzyme Cyp46A1(24-s hydroxylase) oxidizes cholesterol from the destroyed brain tissue into the water-soluble metabolite cerebrosterol (24-hydroxycholesterol; 24OHC), which is a steroid acid that freely passes the BBB, and the degraded cholesterol is thus removed from the CNS (Lütjohann et al. 2003). Leoni and Caccia (2011) reported that levels of plasma cerebrosterol were correlated with brain atrophy in relapsing-remitting MS (RR-MS) and primary progressive MS (PP-MS) patients. Karrenbauer et al. (2006) demonstrated a negative correlation between the cerebrosterol-cholesterol ratio in plasma and the volume of T2-weighted lesions in RR-MS and PP-MS patients, and a positive correlation between the cerebrosterol-cholesterol ratio and the volume of T1-weighted hypointense lesions in RR-MS patients. Increased levels of plasma cerebrosterol were found in MS patients with inflammatory active gadolinium-enhancing T2 lesions but not in MS patients without this type of lesion (Leoni et al. 2004). According to Leoni and Caccia (2013),

focal inflammatory activity could be associated with increased turnover of cholesterol in the brain.

Grey matter lesions are different from white matter lesions in that they have fewer inflammatory characteristics such as infiltration by macrophages and lymphocytes, which suggests a lack of an adaptive immune response (Rejdak 2010). The process of cerebral atrophy in MS is not fully understood, but it is assumed that neuro-inflammation triggers the cerebral degeneration (Amato 2004;Fagius et al. 2007).

1.5 Diagnosis of MS

The diagnosis of MS is clinical. In 1982, the Poser Committee (Poser et al. 1983) set up criteria aiming to ensure that trials and research protocols would only include patients with true MS. The McDonald criteria, which were introduced in 2001, revised in 2005, and revised again in 2010, included evidence from new MRI techniques and simplified the diagnosis of PP-MS, making the role of spinal cord lesions clearer; these criteria are now the main diagnostic standard for MS (Polman et al. 2011).

According to the Poser criteria, MS can be definite or probable. Within these, it can be clinically definite/probable or laboratory-supported definite/probable.

The criteria for clinically definite MS are: 1. at least two attacks and clinical evidence of two separate lesions; or 2. at least two attacks and clinical evidence of one lesion plus paraclinical evidence [including computed tomography (CT), MRI, evoked potential, hyperthermia challenge, and urodynamic studies] of another separate lesion. The two attacks must be located in different parts of the CNS and each of them must last for at least 24 hours and be separated by a period of at least one month. Occasionally, symptoms which are reliable and adequate for locating an MS lesion can be accepted instead of clinical evidence.

Criteria for laboratory-supported definite MS include: 1. at least two attacks and either clinical or paraclinical evidence of one lesion plus a positive cerebrospinal fluid (CSF; i.e. elevated IgG index or oligoclonal bands); 2. one attack and clinical evidence of two separate lesions plus a positive CSF; or 3. one attack and clinical evidence of one lesion plus paraclinical evidence of another separate lesion and a positive CSF. The two attacks must appear in different parts of the CNS and each must last at least 24 hours and be separated by at least one month. One of the attacks must involve a different part of the CNS from that demonstrated by the clinical or paraclinical evidence.

Criteria for clinically probable MS include: 1. at least two attacks and clinical evidence of one lesion; 2. one attack and clinical evidence of two separate lesions; or 3. one attack plus clinical evidence of one lesion and paraclinical evidence of another separate lesion. The two attacks must involve separate parts of the CNS.

Criteria for laboratory-supported probable MS are at least two attacks plus a positive CSF. The two attacks must be associated with different parts of the CNS and be separated by at least one month, and each attack must have a duration of at least 24 hours.

The revised McDonald criteria (2010) for a diagnosis of MS are:

1. Two or more attacks (or relapses) and two or more objective clinical lesions. No additional information is needed.

2. Two or more attacks and one objective clinical lesion plus dissemination of the disease in space, as demonstrated by MRI, or a positive CSF plus two or more MRI lesions consistent with MS, or a further clinical attack involving a different site.

3. One attack and two or more objective clinical lesions plus dissemination of the disease in time, as demonstrated by MRI or a second clinical attack.

4. One attack and one objective clinical lesion (monosymptomatic presentation) plus dissemination of the disease in space, as demonstrated by MRI, or a positive CSF plus two or more MRI lesions consistent with MS, as well as dissemination in time, as demonstrated by MRI or a second clinical attack.

5. Insidious neurological progression suggestive of MS. One year of disease progression must be retrospectively or prospectively observed and there must be at least two of the following: a positive brain MRI [i.e. nine T2 lesions or four or more T2 lesions with positive visual evoked potential results]; a positive spinal cord MRI (i.e. two focal T2 lesions); a positive CSF.

1.6 Clinical presentation

The clinical presentation and course of MS is highly variable and individual (Lublin and Reingold 1996; O´Connor 2002). In 85% of MS patients, the first indication of MS onset involves an episode of neurological disturbance (called the clinically isolated syndrome; CIS) (Miller et al. 2012). The CIS suggests the possibility of MS and usually manifests as a lesion in the optic nerve (optic neuritis), spinal cord or cerebellum. MS can also present as cognitive changes, seizures or encephalopathy. Fifty to seventy percent of adults who have a CIS have multiple asymptomatic white matter lesions in the brain (Miller et al. 2012).

According to consensus reached late last century, there are four main types of MS (Lublin and Reingold 1996).These are a. RR-MS, which is characterized by clearly defined disease attacks with sequelae and full or partial recovery with periods between disease attacks characterized by a lack of disease progression; b. PP-MS, which is characterized by disease progression from the onset with occasional plateaus and temporary minor improvements; c. secondary progressive MS (SP-MS), which occurs after an initial relapsing-remitting course and is characterized by progression

with or without occasional relapses, minor remissions and plateaus; and d. progressive relapsing MS (PR-MS), which is characterized by a progressive disease course from the onset, with clear acute relapses, with or without full recovery, and periods between relapses characterized by continuing disease progression. PR-MS is rare. It has, in fact, been questioned whether PP-MS and PR-MS are truly different, because their natural histories are so similar (Kremenchutzky 1999).

The typical pattern of clinical progression in MS is that an initial phase of RR-MS, in which recovery from each relapse is incomplete so that the symptoms accumulate, develops into a progressive disease course (SP-MS). In a long-term geographically based study, Weinshenker et al. (1989) demonstrated that approximately 50% of patients with RR-MS switch to SP-MS during the first 10 years after disease onset and around 90% of RR-MS patients develop SP-MS within 25 years.

The term benign MS is used to describe a subtype of RR-MS which is characterized by minor progression of disability [according to the expanded disability status scale (EDSS) (Kurtzke 1983)] 10 years after the disease onset (McAlpine 1961). Malignant MS is characterized by rapid disease progression, with development of significant disability or death within a few months of the onset of the disease (Lublin and Reingold 1996).

Long-term studies have provided evidence that patients reach the stage at which walking is impaired within a median of 10 years, the stage at which there is need of unilateral support while walking within a median of 15-20 years, and the stage at which they can walk only a few steps within a median of 30 years (Weinshenker and Ebers 1987).

Life expectancy in patients with MS varies with different studies. An analysis of registry data from Vancouver, British Columbia, and London, Ontario, Canada, demonstrated that for most age groups, life expectancy is shortened by six to seven years (Sadovnick 1992). In a study of patients attending the MS clinics in Vancouver between 1972 and 1988, 47.1% died from complications of MS and 28.6% of the remaining deaths were due to suicide (Sadovnick et al. 1991).

It is generally accepted that the following clinical and demographic factors can negatively affect the prognosis of MS: older age at onset (>40 years); motor, cerebellar, sphincter or multiregional symptoms; frequent relapses during the first years of the disease; a short interval between the first two attacks; incomplete remissions; fast progress of disability; and a short time from the onset of the disease to the start of the progressive phase (O´Connor 2002).

1.7 Clinical measures of disease progression

1.7.1 Clinical evaluation of disability

The commonly used clinical measures of MS disability are the EDSS (Kurtzke 1983) and the multiple sclerosis severity score (MSSS) (Roxburgh et al. 2005).

The EDSS is based on eight functional systems: pyramidal, cerebellar, brainstem, sensory, bowel and bladder, visual, cerebral and "other". Each of these functional systems is rated during neurological examination on a 0-to-5 or 0-to-6 scale, except for the "other" functional system, which is scored zero or one. The functional system ratings give a total score out of 10, in increments of 0.5. This total score is named the EDSS score. An EDSS score of 0 signifies normal neurological status. An EDSS score of 10 signifies death. The EDSS rates the level of disability, focusing especially on motor function measured as the ability to walk. Thus, the EDSS puts much emphasis on the pyramidal tract and brain stem function but pays relatively little attention to mental processes such as mood and cognition. Although there is a high prevalence of mood disorders in patients with MS, this affective dysfunction is not recognized by the EDSS, which can erroneously give the impression that the MS patient is in good overall emotional condition (Feinstein 1997). With respect to cognitive dysfunction, the cerebral functional system rates cognitive impairment on a scale of 2 (mild), 3 (moderate), 4 (marked) or 5 (demented). Thus, it is possible for a demented MS patient to have a total EDSS score of 5.0, signifying an only moderate degree of disability, which would of course be misleading. Another weakness of the EDSS scale is that it is ordinal and not linear and is thus not sensitive to changes in clinical status over time. Furthermore, EDSS has a limited ability to discriminate between individuals in terms of the extent of their disability (Hobart et al. 2000).

The MSSS was developed from a database of 9892 MS patients by applying an arithmetic method to correct each patient's EDSS score for disease duration; the extent of each patient´s disability was compared with the distribution of disability scores in patients with the same disease duration to create a reference table (Roxburgh et al. 2005). According to Roxburgh et al., the MSSS has the advantage of detecting different rates of disease progression more efficiently than the EDSS. The MSSS can be used for comparing groups of patients, but disease fluctuations prevent its use for predicting future disability for individuals.

1.7.2 Radiological measures in MS

MRI-based measures are important for the clinical diagnosis and management of MS and for gaining an understanding of the disease mechanisms (Filippi and Rocca 2007). The conventional MRI measures include measurement of brain atrophy and lesions; hypointense T1 lesions and hyperintense gadolinium-enhancing T2 lesions indicate active inflammation. The extent of the T2 lesions in the CNS is of clinical importance early in the MS course and has a prognostic value in predicting transformation from a CIS to RR-MS (Barkhof et al. 1997)). T2 lesions are more common in the white matter than in the grey matter on conventional MRI and are

frequently located in periventricular white matter, the inner surface of the corpus callosum, the juxtacortical grey-white junction, the infratentorial brain regions, and the spinal cord (Neema et al. 2007). Kutzelnigg et al. (2005) have demonstrated that white matter lesions are predominantly focal and periventricular to start with, changing with time to being more subtle and diffuse; these changes are accompanied by a substantial increase in the extent of demyelination in the grey matter. The white matter T2 lesions can disappear to a variable extent, which indicates potential for repair (Meier et al. 2007). Despite the importance of conventional MRI for understanding the pathophysiology of MS on a macroscopic level, these lesion measures are not specific enough to detect any underlying microscopic pathology which might affect the white and grey matter, and are weak in comparison with the EDSS status (Neema et al. 2007). In a study in which brain slices from chronic MS patients were analyzed histopathologically with both conventional and quantitative MRI, Seewann et al. (2011) found that cortical lesions which are visible with conventional MRI are associated with a higher total lesion load, and concluded that when cortical lesions are detected with MRI, they represent only a small part of the pathology, meaning that the essential underlying pathology is missed. Thus, the macroscopic lesions visible with MRI may actually extend further in normal-appearing brain tissue.

T1-hypointensive lesions (so-called black holes), primarily seen in the supratentorial cerebral regions, usually begin as gadolinium-enhanced active inflammatory lesions. Sailer et al. (2001) demonstrated in a prospective study that there was a correlation between changes in the T1 lesion volume and progressive cerebral atrophy, whereas no such association was seen between the T2 lesion volume and atrophy; they concluded that there may be a direct link between T1 hypointensive abnormalities and cerebral atrophy.

The mechanisms causing atrophy are not fully understood. It has been assumed that brain atrophy is due to cortical inflammatory pathology, demyelination, axonal loss, and neuronal apoptosis (Peterson et al. 2001). Studies have suggested that axonal loss could be independent of the mechanisms causing demyelination, that it is caused by an abnormal glial-axonal interaction, and that it can occur in the absence of inflammation (Bitsch et al. 2000; Garbern et al. 2002). These findings have given support to the assumption that although cortical atrophy can be associated with white matter inflammation, an independent process causing atrophy could also be present (Amato 2004). Atrophy in the CNS affects the infratentorial areas as well as the supratentorial areas such as the cortical (Portaccio et al. 2006) and subcortical regions (Lin et al. 2008). Radiological measures of cerebral atrophy include assessment of the volume of grey matter, the volume of ventricular CSF, and the RBV normalized brain volume i.e. brain parenchymal fraction. Grey matter atrophy, which is recognized as an marker of disease progression in MS (Fisher et al. 2008), precedes white matter atrophy because the rate of atrophy is higher in the grey matter than in the white matter in the early stages of MS (Dalton et al. 2004). Grey matter atrophy can occur as early as in the CIS and in the initial stages of RR-MS (Dalton et al. 2004), and is reported to be significantly correlated with disability (Fisher et al. 2008). Fisher et al.

(2008) demonstrated that the rate of grey matter atrophy accelerates at the point where RR-MS switches to SP-MS. The new ultra-high-field MRI technology, which uses magnets of 3.0 tesla or more, offers greater sensitivity in detecting lesions and the opportunity of further exposing the underlying cortical pathology. In comparison with conventional MRI with lower field strengths, high-field MRI can detect more T2 and T1 lesions in the early stages of the disease with better precision (Neema et al. 2007). Other new MRI techniques include magnetization transfer imaging (MTI) and diffusion tensor imaging (DTI). MTI was developed for detecting disease activity and monitoring disease progression in MS. It can detect a decrease in the exchange of protons, which appears in pathological brain tissue, as a decreased magnetization transfer ratio, which is assumed to primarily mirror demyelination and axonal loss. DTI detects alterations in the direction, randomness and velocity of water movement in the tissue, mapping the diffusion of water three-dimensionally, calculating the magnitude, degree and orientation of diffusion anisotropy, and thus measuring demyelination and loss of axonal integrity (Neema et al. 2007).

1.7.3 Cognitive impairment in MS

The prevalence of cognitive impairment in MS is reported to be between 40 and 70% (Chiaravalloti and DeLuca 2008). Cognitive dysfunction is often seen early in the disease course (Nocentini et al. 2006) and is present in all MS subtypes (Portaccio et al. 2009). It has been reported in the CIS (Khalil et al. 2011). Kujala et al. (1997) demonstrated that incipient cognitive decline in MS tends to worsen over time, but that normal cognitive functioning can remain intact. Cognitive impairment increases with long disease durations and the development of a progressive disease course (Achiron et al. 2005), and is generally more severe in SP-MS than in RR-MS (Beatty et al. 1989). Studies comparing PP-MS and SP-MS patients have generally found greater cognitive dysfunction in the SP-MS group (Chiaravalloti and DeLuca 2008). The cognitive domains most frequently impaired in MS are information processing speed (IPS) and memory (Rao et al. 1991). Impairment is also often seen in executive functioning (EF) (Lazeron et al. 2004). Crystallized knowledge and language comprehension are, in contrast, relatively resistant to MS (Bobholz and Rao 2003).

IPS is associated with the ability to keep information in the working memory (WM) and the speed with which it is simultaneously manipulated and modified. Thus deficits in WM and IPS affect each other. Studies in large populations of MS patients have shown that the frequency of deficits in IPS is higher than that in WM, especially in SP-MS patients (DeLuca et al. 2004). The performance of MS patients in tests investigating IPS has been reported to decline more rapidly than that in tests measuring other cognitive domains (Denney et al. 2008). The symbol digit modalities test (SDMT; oral form) and the paced auditory serial addition test (PASAT) are commonly used for measuring IPS (Lezak 1995). An expert committé (BICAMS) of members representing the main cultural groups has recommended the SDMT as the test of IPS. The reason for this recommendation was that it is congenial for both patient and assessor, has good psychometric properties and clinical validity expressed

by an association with employment status and is also satisfactory validated against conventional brain MRI parameters as well as fMRI (Langdon et al. 2012). In a 3-year longitudinal study, Amato et al. (2010) found that poor performance in the SDMT at baseline predicted further cognitive decline with time.

Attention is associated with both IPS and WM. MS patients have an impaired ability in complex attention tasks involving sustained attention and divided attention, with the focus on several tasks simultaneously (McCarthy 2005).

Long-term memory is reported to be impaired in 40-65% of MS patients (Rao et al. 1993). Recent research has demonstrated that a poor performance in long-term memory tasks in MS patients is due to difficulties in the initial encoding of information and not primarily to dysfunctional retrieval of information (Thornton et al. 2002). MS patients need to put more effort into repetitions to reach learning criteria but, when they have done that, their ability to recall and to recognize the initial information is as good as in healthy controls (DeLuca et al. 1998). The Rey auditory verbal learning test (RAVLT) is widely used for assessing memory (Lezak 1995). When evaluating memory function in MS patients, it is important to keep in mind that their performance in memory tests could also be affected by deficits in EF and IPS.

The EFs are associated with the frontal lobes of the brain; they include complex goal-directed behavior (planning), initiation and inhibition of behavior, shifting of behavior (flexibility), updating (WM), and anticipating outcomes by simulation (Baddeley 1986; Filley 2000). Executive dysfunction does occur in MS patients, but less frequently than deficits in IPS and memory (Bobholz and Rao 2003). In a community-based sample, Drew et al. (2008) found that 17% of MS patients had deficits in EFs, including fluency, set-shifting (moving attentional focus) and inhibition. Tests for fluency are common among tests measuring EFs; for example, the controlled oral word-association test (COWAT), which measures phonemic (word) fluency, and the category test, which measures semantic fluency (Lezak 1995; Spreen and Strauss 1998). In a quantitative review of 35 studies, Henry and Beatty reported that increased neurological disability or a progressive MS course were associated with deficits in tests of phonemic and semantic fluency.

1.7.4 Cognition in MS in relation to MRI characteristics

MRI results appear to be correlated with cognitive functioning in MS patients, and can be used to assist in the assessment of cognitive function (Filippi et al. 2010). It has been demonstrated that MS patients with greater lesion loads are more cognitively impaired than those with less of a lesion burden (Swirsky-Sacchetti et al. 1992). Cortical lesions and atrophy predict cognitive dysfunction independently of each other, leading to the assumption that they represent two different processes (Calabrese et al. 2009). However, in cross-sectional and longitudinal studies, brain atrophy appears to have a stronger correlation with cognitive performance than T2 and T1

lesion volumes, which have only a modest correlation with functioning in cognitive tests (Rovaris et al. 2006). Summers et al. (2008) showed that brain atrophy early in the clinical course predicts cognitive impairment several years later in RR-MS patients. The width of the third ventricle is reported to be especially strongly associated with cognitive dysfunction (Benedict 2006). Atrophy in the white matter has been shown to affect cognitive functions that are dependent on the rapid transfer of information, such as IPS and WM (Sanfilipo et al. 2006). Loss of volume in the white matter structure corpus callosum due to atrophy is reported to reduce attention and IPS measured by the PASAT (Lin et al. 2008). Yaldizli et al. reported that atrophy of the posterior segment of corpus callosum in MS patients, correlated with decline in IPS and verbal fluency. Calabrese et al. (2010) found widespread cortical thinning in MS patients with mild cognitive impairment, indicating that cognitive dysfunction could be associated with an underlying global pathology. Grey matter atrophy is related to impairment of verbal memory, verbal fluency and attention (Amato et al. 2004; Benedict et al. 2006). It has been reported that atrophy in the thalamus in MS patients is a strong predictor of cognitive dysfunction. Hippocampal atrophy in MS patients is associated with dysfunction in memory encoding and retrieval (Sicotte et al. 2008). Tekok-Kilic (2007) showed that left frontal atrophy in MS patients is related to deficits in verbal memory performance while right frontal atrophy is associated with dysfunction in visual memory and WM. Studies concerning the relationship between functional anisotropy measured by DTI and cognitive performance in MS patients have given contradictory results (Chiaravalloti and DeLuca 2008).

1.7.5 Cognition in MS and functional MRI (fMRI)

Studies of MS and cognition using functional imaging techniques have mainly focused on WM, attention and EFs (Chiaravalloti and DeLuca 2008). In many fMRI studies, cortical activation associated with rising blood-oxygenation levels has been found in similar regions in MS patients and controls, but the magnitude of activation was higher in the MS group. This has been explained as the result of recruitment of other cortical networks as a compensatory mechanism (Filippi et al. 2004). This increase in activity has also been shown in a study of MS patients with normal performance levels in cognitive tests and in accordance with the hypothesis of compensatory recruitment of cortical networks, the cognitive performance level declines when the increased activation is no longer sufficient to compensate for the decreased cortical neuronal integrity (Langdon 2011). Studies of cortical activation in relation to working memory tasks using fMRI, have found that the same cortical regions are activated in MS patients and healthy persons but the magnitude of activation is higher in MS patients than in normal controls (Wishart et al. 2004). Penner et al. (2003) reported that MS patients with mild cognitive impairment had stronger activation than controls in the right dorsolateral prefrontal cortex, right superior temporal gyrus, right lateral cerebellum, left angular gyrus and bilateral inferior parietal cortex, during attention tasks. Furthermore, they found that severely

cognitively impaired MS patients also had higher activation in the left inferior parietal lobe than the control group. In an fMRI study focusing on a task requiring planning, which is an aspect of EF, Lazeron et al. (2004) found similar levels of activation in the bilateral frontal and parietal lobes in MS patients and healthy normal controls.

1.7.6 Cognition in MS in relation to neurochemistry

The relationship between brain neurochemistry and cognitive dysfunction in MS is relatively unexplored in comparison with the association between cognitive deficits and morphological brain MRI characteristics. However, recent findings have provided evidence suggesting an association between the lack of neuroprotective NGF and cognition in MS. Thus, it has been reported that poor performance in RR-MS patients in a spontaneous word list generation task and a memory recall task was related to low concentrations of the neurotrophin beta-NGF in plasma (Kalinowska-Lyszczarz et al. 2012). Patanella et al. (2010) demonstrated a correlation between low levels of BDNF and poor performances in a speeded divided-attention test and a visual scanning test.

It is known from previous studies that levels of cerebrosterol are positively correlated with brain atrophy (Leoni and Caccia 2011) and the presence of T2-weighted lesions (Karrenbauer et al. 2006) in RR-MS and PP-MS, which evokes the question of whether they are also associated with cognitive dysfunction in MS. In an epidemiological study, Hughes et al. (2012) demonstrated that healthy people with high cerebrosterol levels and high 24OHC/27OHC ratios were at greater risk of developing cognitive impairment over 8 years' follow-up. Leoni & Caccia (2013 and 2014) reported a correlation between cognitive tests (one was SDMT measuring IPS) and decreased cerebrosterol levels in patients with Huntington´s disease. However up to now the relationship between cognitive dysfunction and level of plasma cerebrosterol has not been studied in MS patients and therefor deserves attention.

1.7.7 Cognition in MS in relation to genetics

Relatively few studies have investigated the relationship between genetics and cognitive functioning in MS. However, the few available studies indicate a possible link between the apolipoprotein E (APOE) epsilon-4 allele and cognitive functioning in MS patients (Ghaffar and Feinstein 2009). Koutsis et al. (2007) and Shi et al. (2008) reported an association between carriage of this allele and impairment of verbal memory. Aspects of genetic protection against cognitive deficits have also been reported; Weinstock-Guttman et al. (2011) found that MS patients with a variant of brain-derived neurotrophic factor (BDNF) gene were more protected against visual memory deficits than MS patients who did not have this gene variant.

1.7.8 Cognition in MS in relation to demographic variables

Male MS patients appear to show cognitive deficits to a greater extent than women (Beatty and Aupperle 2002; Schoonheim et al. 2014). MS does not interact with age, and does not affect normal cognitive deterioration with age (Bodling et al. 2009). Thus, MS patients go through the same process of cognitive aging as healthy adults. Higher education levels have been shown to protect against cognitive decline in MS. against declines in IPS (Benedict et al. 2010;Sumowski et al. 2009).

1.7.9 Cognition in MS in relation to disease variables

Studies of the relationship between disability (measured by the EDSS) and cognitive decline have given contradictory results (Heaton et al. 1985; Rao et al. 1991). In these studies, disease duration can be a confounding factor, since it is associated with greater physical disability and the development of a progressive disease course, which is associated in turn with more pronounced cognitive decline. Chiaravalloti and DeLuca (2008) conclude in their systematic review that cognitive impairment is only mildly associated with physical disability.

1.7.10 Cognition in MS and fatigue

Fatigue is reported to affect up to 87% of patients with MS (Krupp et al. 1988). The findings concerning effects of self-reported fatigue on cognitive functioning have yielded ambiguous results. Some studies have not found any significant relationship between self-reported fatigue and objective performance on cognitive tests (Bailey et al. 2007). However, declines in sustained task performance have been reported in MS patients compared with healthy controls (Schwid et al. 2003).

1.7.11 Cognition in MS and depression

The prevalence of depression in MS patients is reported to be between 27% and 54% (Joffe et al. 1987;Minden et al. 1987). Depression affects a variety of cognitive functions, including WM and IPS (Arnett et al. 1999).

1.7.12 Cognitive functioning and pharmacological treatment

There are limited and inconsistent data concerning the effects of disease-modifying medications on cognitive functioning in MS. Interferon beta-1a is reported to have a beneficial effect on IPS and memory. Fisher et al. (2000) and Pliskin et al. (1996) showed that interferon beta-1b also has a mild positive effect on performance in a visual memory task. However, there is no evidence for any beneficial effects

associated with glatiramer acetate, natalizumab, or fingolimod on cognitive function in MS patients (Amato et al. 2013).

1.7.13 Cognition in relation to cognitive training and rehabilitation

There are relatively few studies on the treatment of cognitive deficits using training and rehabilitation. The rehabilitation programs which have been developed are intended to improve attentional deficits, communication skills and memory impairment (Chiaravalloti and DeLuca 2008). In a study of MS patients with moderate-to-severe memory impairment, Basso et al. (2006) found that a self-generated memory technique in which the target word was encoded by actively generating it from a cue (first letter) was a more effective way of learning than traditional didactic encoding. Similarly, a spaced learning technique with 5-minute breaks between each learning trial resulted in better acquisition of everyday functional tasks than a traditional learning model without such intervals in a study of MS patients and healthy controls (Goverover et al. 2008). There are methodological limitations in many of the studies focusing on cognitive training and rehabilitation; for example, the use of a qualitative rather than a quantitative research design, reliance on case studies, lack of control groups, and a follow-up period that is too short (Amato et al. 2013; Chiaravalloti and DeLuca 2008). According to a Cochrane review by Otajärvi-Rosti and Hämäläinen (2014) the interventions and outcome measures in studies concerning neuropsychological rehabilitation in MS are heterogenous, which makes it difficult to compare these reports. When evaluating all twenty studies together Otajärvi-Rosti and Hämäläinen found low-level evidence for that neuropsychological rehabilitation improves cognition in MS. Nevertheless when they analysed the studies one by one, 18 of these studies appeared to have a positive effect on cognition. Thus cognitive training improved memory span and working memory. When cognitive training was combined with other neuropsychological rehabilitation methods such as learning compensatory strategies, attention, immediate verbal memory and delayed memory were improved.

1.7.14 Cognition in relation to daily living and quality of life (QoL)

MS patients with cognitive deficits experience difficulties performing everyday activities. Goverover et al. (2006) reported that performance on tests measuring IPS was associated with the level of daily life activities that demanded quick responses. Furthermore, there was a correlation between poor QoL and cognitive dysfunction (Cutajar et al. 2000). Cutajar et al. reported that poor performance in frontal-lobe-related executive tasks, measured using the Luria frontal lobe syndrome test and the Rivermead behavioural memory test, was associated with worsening QoL. In a longitudinal study, Fujii et al. (2004) reported that WM (measured using the digit span task) predicted QoL (measured using the brief QoL inventory (BQOLI). Flensner et al. (2013) reported that perceived problems with memory, attention and

concentration, measured by the Perceived Deficit Questionnaire (PDQ) and fatigue measured by the Fatigue Severity Scale (FSS) were contributing factors in the health-related quality of life (HQOL) domain of bodily pain and vitality. However, other studies have not found any significant correlation between cognition in MS and QoL. A weak point in most studies demonstrating such a relationship seems to be that they have not taken clinical variables such as disease duration and disability (EDSS) into account or corrected for these factors. In a study of 120 MS patients and 44 healthy controls that controlled for EDSS score, fatigue, cognition, mood disorder, personality and presence of behavior disorders, Benedict et al. (2005) found no significant relationship between cognitive function and health-related QoL (HQOL). However Benedict et al. found that vocational status was predicted by cognitive function as well as disease duration and conscientiousness. Similarly, in a study of 359 MS patients, Van Schependom et al. (2014) noted an absence of significant relationship between performance in a task measuring WM and IPS (PASAT) and HQOL, when EDSS score, disease duration and onset type were into account. However, they did find a significant relationship between walking distance (EDSS) and manual control (measured by the 9-hole peg test) and HQOL. A fundamental problem in these studies is that self-reporting of HQOL by the MS patients could be biased because of their cognitive impairment.

2 AIMS OF THE THESIS

The overall aim of the thesis was to explore and describe various aspects of cognition in MS, particularly in relation to long-term MRI findings and plasma concentrations of cerebrosterol.

The specific aims of the studies were:

I: to investigate how different aspects of cognitive function change over an 8-year period in a cohort of MS patients and in subgroups within this cohort, and to relate these changes to demographic (age, gender, and education) and clinical (age at onset, disease duration, and disability measured by EDSS) characteristics at baseline and follow-up.

II: to investigate lexical substitution and retrieval in MS patients in comparison with a matched control group of healthy individuals.

III: to investigate the associations between cognitive, sensory and motor IPSs as well as verbal dichotic listening (VDL), and the area of the corpus callosum in MS patients at baseline and at follow-up approximately 9 years later.

IV: to investigate the relationship between IPS (measured by SDMT) and cholesterol degradation (expressed as plasma levels of cerebrosterol), taking into account normalized MRI estimates of RBV, volumes of grey and white matter, and ventricular CSF volume, and disability (EDSS) in a cross-sectional sample of MS patients.

3 MATERIALS AND METHODS

3.1 Subjects

Study I, an eight-year longitudinal study, included 31evaluable subjects from a cohort of 32 MS patients (21 females) seen consecutively at the outpatient ward of the Department of Neurology, Karolinska University Hospital Huddinge, Sweden, during June to December 1995. The patients had clinically definite MS according to the Poser criteria (Poser et al. 1983). At follow-up about 8 years after the baseline measurements, in 2003, 1 male individual in the sample died. The patients were classified according to the Lublin classification as RR-MS (n=10), SP-MS (n=17), and PP-MS (n=4) at baseline in 1995. At follow-up in 2003, a switch from RR-MS to SP-MS had occurred in 5 patients. At baseline, the mean age of all included subjects was 42.9 years (SD=10.1y), the mean duration of disease was 12.1 years (SD=9.1y), and the mean EDSS score was 4.1 (SD=2.3). The EDSS score at follow-up was 5.5 (SD=2.2).

There were more women than men in the evaluable patient group (21/10), which approximately mirrors the distribution of the genders in the total MS patient population. The evaluable patients had a mean disease duration of about one decade at baseline. There were no significant differences in gender distribution or disease duration between subgroups at baseline, according to one-way ANOVA testing. Patients in the SP-MS and PP-MS groups were on average middle-aged on admission. The RR-MS patients were significantly younger and less well educated than the SP-MS and PP-MS patients, while the latter groups had similar mean ages and levels of education. Physical disability (EDSS) was relatively severe in the SP-MS and PP-MS patients and did not differ between these groups, whereas it was relatively mild in patients with RR-MS (Table 1).

Table 1. Demographic and clinical characteristics (Age at disease onset, Duration of disease and Disability according to EDSS) in a cohort diagnosed 1995 with Multiple Sclerosis.

	Subgroups			
	Relapsing Remitting	Secondary Progressive	Primary Progressive	All subjects*
N (females/males)	10(4/6)	17(14/3)	4(3/1)	31 (21/10)
Age, y	36.5±8.9	45.8±10.0	46.3±7.2	42.9±10.1
Age at disease onset, y	28.0±8.9	31.6±8.1	33.8±7.0	30.7±8.2
Education, y	9.9±1.5	12.7±2.5	12.7±0.6	11.9±2.4
Duration, y	8.5±8.3	14.2±8.8	12.6±12.0	12.1±9.1
EDSS	2.2±1.9	4.6±2.0	5.8±2.1	4.1±2.3

*1 deceased male patient excluded

Study II, which was a cross-sectional study carried out during 2004-2005, included 25 evaluable patients who had participated in study I, and 25 healthy controls. The remaining six patients from study I were not evaluable for study II: four did not want to participate, one had moved abroad, and one was prevented from participating for other reasons. The patient group included 16 women and 9 men (RR-MS: n=7, SP-MS: n=15, PP-MS: n=3) with a mean age of 44.4 years (SD=10.1y) and the control group included 17 women and 8 men with a mean age of 41 years (SD=16.0y). The mean duration of disease in the MS group was 20 years (SD=9.4y) and the mean EDSS score was 5.8 (SD=2.1). The mean duration of formal education was 11.8 years (SD=2.4y) in the MS group and 13.0 years (SD=2.8y) in the control group. The 25 healthy controls were not significantly different in mean age, mean duration of formal education, and estimated full-scale intelligence quotient (FSIQ) from the patients with MS.

Study III was carried out in 22 MS patients (16 females and 6 males) who had all participated in study I. The patients were neuropsychologically tested and investigated by MRI at baseline in 1995 and at follow-up about nine years later, in 2004-2005. This study was run partly in parallel with studies II and IV, and all of the evaluable patients also took part in studies I and II. The remaining nine patients of the original 31 from study I did not participate in study III because: four patients did not want to participate, one had moved abroad, one was unable to undertake MRI because

of an intracranial surgical clip, and three PP-MS patients were excluded because of their small number. At baseline there were eight RR-MS patients and 14 SP-MS patients. Three patients had changed from RR-MS to SP-MS at follow-up. The mean age at baseline was 42.7 years (SD=10.9y), the mean duration of education was 11.7 years (SD=2.6y), the mean age at disease onset was 29.4 years (SD=8.3y), and the mean disease duration was 13.3 years (SD=9.1y). The EDSS disability score was 4.0 (SD=2.0) at baseline and 5.5 (SD=2.2) at follow-up.

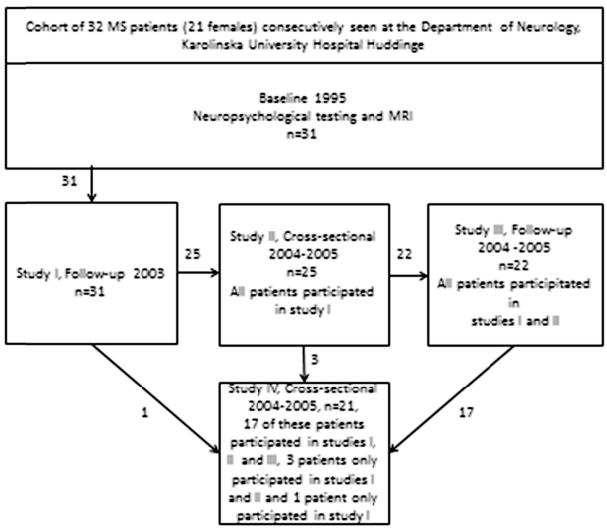
Study IV was a cross-sectional study carried out during 2004-2005, partly in parallel with studies II and III. All the patients (14 females and 7 males) in study IV were part of the original cohort from study I. Among the individuals in study IV, 1 had only participated in study I, 3 had participated in study I and II and 17 in study I, II and III. The MS subgroups consisted of 7 RR-MS patients, 11 SP-MS patients, and 3 PP-MS patients. The mean age at examination was 48.3 years (SD=9.5y), the mean duration of education was 11.6 years (SD=2.2y), the mean age at disease onset was 29.9 years (SD=8.3y), the mean disease duration was 18.5 years (SD=9.0y), and the mean EDSS score was 5.5 (SD=2.4).

The demographic and clinical characteristics of patients in studies I-IV are summarized in table 2, and a graphic overview of participation in the studies is presented in Fig 1.

Table 2. Demographic and clinical characteristics for the study samples in study I-IV

Characteristic	Study I baseline 1995	Study II cross-sectional 2004-2005	Study III baseline 2004-2005	Study IV cross-sectional 2003-2004
N (females/males)	31 (21/10	25(16/9)	21 (16/6)	21(14/7)
Age, y	42.9±10.1	44.4±10.1	42.7±10.9	48.3±9.5
Age at onset, y	30.7±8.2	24.4±	29.4±8.3	29.9±8.3
Education, y	11.9±2.4	11.9±2.4	11.7±2.6	11.6±2.2
Duration, y	12.1±9.1	20.0±9.4	13.3±9.1	18.5±9.0
EDSS	4.1±2.3	5.8±2.1	4.0±2.0	5.5±2.4
EDSS at follow-up	5.5±2.2		5.5±2.2	

Figure 1. Overview of participants in studies I, II, III and IV.



3.2 Procedures – Studies I-IV

3.2.1 Clinical neurological examination

A clinical neurological examination and diagnosis was carried out at baseline in 1995 and at follow-up in 2003 in study I, by the neurologist Sten Fredrikson. In study II, this neurological examination was carried out in 2004-2005, in study III it was carried out at baseline in 1995 and at follow-up in 2004-2005, and in study IV it was carried out in 2004-2005, all by the same neurologist. In all the studies, the examination included classification of the MS type and the clinical course, and assessment of disability in terms of the EDSS (Kurtzke 1983). The neurologist was kept blinded for the neuropsychological test results.

3.2.2. Neuropsychological assessment of cognitive functions

In studies I, III and IV, all the neuropsychological testing was done by the same neuropsychologist Gösta Bergendal, and in study II the testing was performed by Gösta Bergendal and the speech therapist Ing-Mari Tallberg. The neuropsychologist was kept blinded to the patients' clinical diagnosis and neurological status in studies I-IV and the speech therapist was kept blinded similarly in study II.

In study I, neuropsychological assessment of cognitive function, using a battery of 15 evidence-based neuropsychological tests chosen to cover different aspects of information processing, memory, language function, various executive functions, spatial function, and motor function, was carried out at baseline in 1995 and at follow-up in 2003. The following neuropsychological tests were used:

1. The Swedish version of the vocabulary test (half-scale) from the revised Wechsler adult intelligence scale (WAIS-R), to measure verbal ability (Wechsler 1981; Bartfai et al. 1994).

2. The similarities test from the WAIS-R, to measure verbal abstraction (Wechsler 1981; Bartfai et al. 1994).

3. The COWAT, to measure word fluency (using the letters FAS) (Lezak 1995).

4. The Rey-Osterieth complex figure (RO) copy test, to assess visuospatial ability by copying a complex figure (Lezak 1995).

5. The picture arrangement test, to assess visual-based comprehension, from the WAIS-R (Wechsler 1981; Bartfai et al. 1994).

6. The digit span test from the WAIS-R, to measure verbal short-term memory and WM (Wechsler 1981; Bartfai et al. 1994).

7. The Corsi block-tapping span test, to measure visuo-spatial short-term memory (Lezak 1995).

8. The RAVLT, to assess verbal episodic memory according to total learning and retention (Lezak 1995).

9. The Rey-Osterrieth retention test, to measure visuo-spatial episodic memory (Lezak 1995).

10. The SDMT test (oral form), to measure IPS (Lezak 1995).

11. The visual reaction time (VRT) and auditory reaction time (ART) from the automated psychological test system (Levander and Elithorn 1987).

12. The finger-tapping (FT) test, to assess motor speed in the right and left hands (Lezak 1995).

The administration of this test battery was completed in about 90 min.

In study II, the pre-morbid FSIQs for the MS patients and controls were estimated using the Swedish lexical decision test (SLDT) (Almkvist et al. 2007). Current global cognitive functioning was assessed for each patient using a summary test (equivalent to the FSIQ) based on the following tests: vocabulary (WAIS-R), similarities (WAIS-R), digit span (WAIS-R), block design (WAIS-R) and SDMT score transformed to the corresponding digit symbol score in the WAIS-R test. The FSIQ was calculated

using the following linear addition process to give the estimated current FSIQ, from the WAIS-R manual (Bartfai et al 1994): 2 x the weighted scores on the verbal tests plus 1.5 x the weighted scores on the performance tests. It was not necessary to assess the controls for anything other than FSIQ (using the SLDT), since premorbid and current intelligence are highly correlated in healthy persons.

The lexical ability of the MS and control groups was tested using the following tests:

1. The Boston Naming test (BNT) a confrontation picture-naming test (Kaplan et al. 1983). The following measures were calculated in the BNT: the number of correct responses and omitted responses, the semantic specificity, and the number of off-target associative responses (visual, semantic, contextual and phonemic).

2. The COWAT (FAS) for measuring word fluency (Lezak 1995). This included analysis of the cluster size and the number of switches between clusters, according to a scoring procedure developed by Troyer et al. (1997). In this context, clusters were defined as groups of successively produced words beginning with the same two first letters, words that differed by one single vowel sound, rhymes, and homonyms.

In study III, IPS (cognitive, sensory and motor) was measured at baseline in 1995 and at follow-up in 2004-2005 using the following tests:

1. The SDMT (oral form) (Lezak 1995) for measuring IPS with a cognitive component.

2.The VRT and ART tests from the automated psychological test system for measuring sensory speed (Levander and Elithorn 1987).

3. The FT test for measuring motor speed in the right and left hands (Lezak 1995).

At follow-up, auditory inter-hemispheric transfer was assessed using the Verbal Dichotic Listening (VDL) (Hugdahl 1988).

In study IV, IPS was assessed using the SDMT according to the standard oral procedure (Lezak 1995).

3.2.3 Magnetic resonance imaging and analysis

The MRI imaging in studies III and IV was performed at the Karolinska University Hospital.

In study III, the MRI examinations were performed at baseline in 1995 by Leszek Stawiarz and again at follow-up in 2004-2005 by Leszek Stawiarz, Juha Martola and Maria Kristoffersen Wiberg, using a 1.5T scanner and a standard head coil. At baseline, a fast spin echo technique was used for image acquisition. Sagittal T2-

weighted continuous 5-mm imaging sections were obtained. A turbo spin echo technique was used at follow-up and sagittal T2-weighted 4 mm imaging sections were obtained. The T2 lesion load at baseline and follow-up was defined as the total number of T2-weighted lesions. A two dimensional analysis of corpus callosum area CCA was carried out using the Sectra PACS system (Linköping, Sweden). In this procedure, the CCA was measured three times and the mean of these measurements was calculated. The volumes of the lateral ventricles, the third ventricle and the brain parenchymal tissue expressed as the RBV (brain volume divided with intracranial volume) were evaluated on the original T1-weighted images, using a volumetric method and the semi-automated tool in the Hermes MultiModality - region growing program. The procedures for MR imaging, evaluation of CCA, and volumetric measurement are described in Martola et al. (2007).

In study IV, the MRI examinations were performed in 2004-2005; the results were evaluated by Leszek Stawiarz, using a 1.5T scanner (Magnetom, Siemens, Erlangen, Germany) with a standard head coil. The MRI protocol was an extended clinical protocol including T1-weighted pulse sequences (MPRAGE) with 1.5 mm axial slices, TR=13.5, TE=7, TI=300 ms, field of view=25 cm, and matrix=256x256. Images from this sequence were also used for the volumetric analysis. The FSL software package v. 4.1 (FMRIB Analysis Group, Center for Functional Magnetic Resonance Imaging of the Brain, Oxford) (Smith et al. 2004) was used to evaluate the volumes of brain tissue. The fully automated SIENAX (Structural Image Evaluation, Using Normalization of Atrophy) method was used to estimate normalized brain volumes from a single image. The brain volume, ventricular space volume, and volumes of grey matter and white matter were obtained and normalized for skull size (normalization to MNI152 standardized stereotaxic brain template) (Jenkinson et al. 2001). The brain volume was measured as the RBV proportion in relation to intracranial volume, while the others were measured in cm^3.

3.2.4 Biochemical analysis

In study IV, Virginija Danylaite Karrenbauer was responsible for the blood sampling logistics in 2004-2005. Biochemical analysis of cerebrosterol concentrations in plasma was carried out at the Department of Clinical Biochemistry at Karolinska University Hospital, Huddinge. After taking the blood sample, the plasma was separated by centrifugation and frozen for storage. Isotope-dilution mass spectrometry was then carried out according to the procedure described by Dzeletovic et al. (1995) to determine the concentrations of cerebrosterol in the plasma.

3.2.5 Statistical analyses

The statistical software packages used were the IBM SPSS version 14, USA, in studies I and II, and the IBM SPSS Statistics version 19, USA, in studies III and IV.

3.2.5.1 Study I

The demographic and cognitive characteristics of the total sample of patients with MS and the subgroups (RR-MS, SP-MS and PP-MS) were described using means ± standard deviation (SD). Z-scores were used to compare the results of the various neuropsychological tests. These test results were computed on the basis of reference values from test manuals and handbooks. Two-way analysis of variance (ANOVA) was used for the subgroups between factors and the times of assessment, at baseline in 1995 and at follow-up in 2003, and within factors for each neuropsychological test. Two-way analysis of covariance (2 subgroups x 2 times; ANCOVA) was used for each neuropsychological measure in order to see whether there was cognitive decline over time, for differences between subgroups, and to analyze the influence of age, gender, education and EDSS scores. At this initial subgroup analysis, the PP-MS group was excluded because of its small size. The subgroups were also compared by multivariate analysis of variance. The influence of single demographic and clinical disease variables was analyzed by means of two-way ANOVA on each cognitive variable, using a median split of the background variables (age, gender, education, age at onset, disease duration, and EDSS score at baseline and follow-up). A Bonferroni correction was done for the associations found between various neuropsychological measures and clinical variables in order to control the familywise error rate, i.e. the probability of false significant results (type I errors), which can occur when making multiple comparisons.

3.2.5.2 Study II

Means and standard deviations were calculated for the various test results. One-way ANOVA was used to compare the MS group with the healthy control group with respect to demographic variables (age and education) and language variables.

Pearson's correlation coefficients were used to calculate the correlations between lexical variables and between demographics and lexical variables. A Bonferroni correction was applied to these correlation analyses.

3.2.5.3 Study III

Separate stepwise regression analyses were performed for each neuropsychological test at baseline and follow-up as well as for the annual rate of change (ARC) in CCA in each neuropsychological test, to evaluate the effect of CCA on information processing. Demographics (age at baseline, gender, years of education), clinical characteristics (onset of symptoms, duration of disease and severity of disease as assessed by EDSS), as well as MRI measures (RBV and T2 lesion load), were included as possible predictors in order to adjust for possible confounding factors. Because of the small sample size, it was not appropriate to test for interactions among the explanatory variables.

3.2.5.4 Study IV

When presenting data (demographics, SDMT scores, brain measurements, EDSS scores, and cerebrosterol levels), descriptive statistics (means±SD) were used. The relationships between SDMT, EDSS, demographics (age, gender and education), volumetric MRI, and cerebrosterol results were calculated using Pearson correlation coefficients. Two types of stepwise multiple regression analyses were performed using a backward elimination procedure. The dependent variable in one of the stepwise regression analyses was the SDMT raw score and in the other the EDSS score. In both stepwise regression analyses, MRI normalized measures of brain volume, grey and white matter and ventricular CSF volumes, cerebrosterol levels and demographics were added as possible predictors of SDMT and EDSS.

3.3 Ethical considerations

Ethical approval for study I was obtained from the Local Research Committee at Huddinge Hospital (21/95); the Research Ethics Committee KI, South (Dnr 55/03); and the regional Ethics Committee in Stockholm (04-906/4). An ethical application for study II was added to the ethical application for study I and was approved (Dnr 55/03). The Regional Ethics Committee, Division 4, gave ethical approval for study III (Dnr 04-906/4). Ethical approval for study IV was received from the Research Ethics Committee KI, South (Dnr 322/02), and the Regional Ethics Committee, KI (Dnr 02-548).

The participants were clearly informed about the procedures and aims of the study before participation. The information given to the participants was in neutral written form which allowed easy refusal to participate without having to explain reasons. All data concerning participants were treated in a safe, anonymous manner.

4 RESULTS

4.1 Study I

At baseline, the whole MS patient group already had poor results (cut-off at −1.5 SD) on the ISP tests symbol digit, VRT and ART (table 3). At this stage, when the average disease duration was about 12 years, there were no significant differences in mean test performance results between the subgroups (RR-MS, SP-MS and PP-MS) except for in the Corsi span test. The disease duration at baseline was associated with impaired performance in the RO copy test, the symbol digit test, the VRT test, and the FT test for the dominant and non-dominant hands.

The greatest decline over the eight years' follow-up in the total MS group was seen in the VRT results, followed by the RO Copy, the FT right hand and the Corsi span test results (table 3). The cognitive deficits in the total group at baseline and follow-up were most pronounced in the three ISP tests (symbol digit, VRT and ART) while

there was no significant decline in either the verbal ability test or the learning and memory tests during the follow-up period.

There was a differential pattern of cognitive decline over time in the subgroups of the MS patients. Thus, the SP-MS group had a marked decline in VRT, while the scores for this test did not decline significantly in the RR-MS and PP-MS groups MS patients with optic neuritis did not perform any worse than those without optic neuritis in the VRT test. In contrast, none of the MS subgroups showed any significant decline over time in the ART test Nor was there any significant decline over time in any of the subgroups nor any group differences in the vocabulary test and the other non-speeded tests.

The results revealed that old age had a negative influence on most of the neuropsychological test results. In contrast, there were no obvious interactions between gender and a decline in cognition. There was also no such interaction for education overall, except for a significant effect on vocabulary. There was no interaction between a decline in cognitive functioning and gender, age at onset of disease, or disease duration. The results suggested an absence of any evident relationship between disability and cognitive functioning at baseline. In contrast, a high EDSS score (>6) at follow-up had a significantly negative effect on performance in tests measuring motor speed, visual IPS, verbal learning and retention.

Initial impairment predicted further cognitive decline. Tests measuring IPS were especially strongly connected with decline over time.

In summary, the results for the total MS group showed an initial selective pattern with a marked decline in the IPS tasks already at baseline and a pronounced decline over time, especially in visual IPS, while there was no significant time-related deterioration in auditory IPS. Initial marked cognitive deficits, especially in IPS tests, predicted further cognitive decline. In the subgroups, there was a differential pattern over time, with the most pronounced cognitive deterioration occurring in the SP-MS group, which showed a marked deterioration in visual IPS compared to auditory IPS. High EDSS scores at follow-up were related to cognitive decline.

4.2 Study II

The control group had a significantly higher number of correct responses, including target responses, subordinated responses and synonyms, in the BNT than the MS patients and had significantly fewer omitted responses (table 4).

The MS patients' responses in the BNT were less specific and contained more inexact and more superordinated descriptions as substitutions for correct target responses (table 4). The MS group also made significantly more semantically off-target associations than the controls in the BNT.

On the word fluency COWAT (FAS), the MS patients generated significantly fewer correct words and switched significantly fewer times between clusters than the controls. However, there were no differences concerning the cluster size (table 5).

For all participants, there was a significant positive correlation (Pearson correlation coefficient) between the BNT variables (number of correct responses) and the current FSIQ, and a negative correlation between the number of nonspecific responses and the FSIQ. Furthermore, there was a significant positive correlation between the COWAT variables (number of correct words, cluster size and cluster switches) and the current FSIQ. However, there was no significant correlation between the BNT and the COWAT with relation to demographic variables (age and education).

There was a correlation between substitutive responses and retrieval strategies. Thus, a low number of correct responses on the BNT was significantly correlated with a high occurrence of nonspecific responses and off-target associations. There was also a significant correlation between the number of correct responses on the BNT and the COWAT results. A significant correlation was seen between correct responses on the BNT and frequent switches between clusters on the COWAT. However, no correlation was found between correct responses on the BNT and a high cluster size on the COWAT. A high total number of correct words on the COWAT correlated with a low occurrence of nonspecific responses and with a low frequency of off-target associations on the BNT, and frequent switches on the COWAT was correlated with a low proportion of nonspecific responses on the BNT.

In summary, the MS group had fewer correct responses and more omitted responses on the BNT than the control group. Furthermore, the MS patients gave less-specific descriptions as substitutions for the target word. Also, the MS patients had fewer words on the COWAT and fewer switches between clusters than the controls. Good performances in the BNT and COWAT were correlated with a high current FSIQ in both the MS and control groups. There was also a correlation between the performances on the COWAT and the BNT.

4.3 Study III

The RR-MS and SP-MS subgroups did not differ regarding demographic characteristics (age, gender, and education) but the SP-MS group had a significantly higher EDSS score at baseline and follow-up. The SP-MS group also had a significantly smaller CCA at baseline and at follow-up. However, the rate of corpus callosum atrophy did not differ between the groups. The RBV was significantly smaller in the SP-MS group than in the other groups at baseline, but not at follow-up. There were no differences between groups regarding the ARC in the RBV, the T2 lesion load at baseline or at follow-up, or the ARC in the T2 lesion load.

A significant difference between the groups in favor of the RR-MS group appeared in the SDMT performance at both baseline and follow-up, and in the VRT and FT (dominant and non-dominant hands) tests at follow-up.

In the whole group at baseline (1995), a small CCA was associated with a poor performance on the SDMT and a long disease duration and high T2 lesion load were associated with a poor performance on the FT test for both hands (fig. 3).

At follow-up (2004-2005), a small CCA was also related to poor performance on the SDMT (fig. 4). Furthermore, an association between a high EDSS score and a poor FT speed for both hands was seen at follow-up. At the same time-point, a small RBV was associated with a good performance in the right ear in the VDL test (VDLr), while a small CCA was associated with a poor left ear performance on the VDL test (VDLl).

There was no significant association between performance in the SDMT and the ARC for any other variable. However, the VRT was significantly associated with the ARC in the CCA. A significant association between the ART and the ARC in the RBV and disease duration was also seen.

VDLr performance was significantly positively associated with the ARC in the RBV. However there was no significant association between VDLr performance and the ARC in the CCA or the ARC in the T2 lesion load. On the other hand, the VDLl performance was significantly negatively associated with the ARC in the CCA. The higher the ARC in CCA, the lower percentage of correct responses were registered on the left ear and the higher percentage of correct responses on the right ear.

In summary, the main result was that the CCA, in contrast to the RBV and the T2 lesion load, was positively correlated with performance in the SDMT, both at baseline and at follow-up.

4.4 Study IV

The MS patients in study 4 had moderate physical disability according to the EDSS score and a clearly lower mean performance on the SDMT than healthy adults (Lezak 1995). The mean grey matter volume was slightly smaller than normal values in the whole sample (Lüders et al. 2002). Furthermore, the mean levels of plasma cerebrosterol in the sample were higher than in healthy people (Dzeletovic et al. 1995).).

A poor performance in the SDMT was significantly correlated with a small grey matter volume, a small brain volume, a large ventricular volume and high levels of cerebrosterol. However, there were no significant correlations between the SDMT and either white matter volume or duration of education. A strong correlation was seen between a high EDSS score and high levels of plasma cerebrosterol. In contrast, there were only weak correlations between the EDSS scores and a small brain volume or large ventricular volume (table 6).

Age was significantly negatively correlated with brain volume and significantly positively correlated with ventricular volume. The duration of education was

significantly positively correlated with the volume of grey matter and significantly negatively correlated with the volume of white matter. A significant correlation was seen between high levels of plasma cerebrosterol and a large ventricular volume. However, there was no significant correlation between high levels of plasma cerebrosterol and the MRI volumetric measures.

A stepwise multiple regression analysis was done, using backward elimination of possible predictors, MRI volumetric measures, and concentrations of cerebrosterol, in order to find predictors related to SDMT performance. This analysis showed that brain volume, grey matter volume and plasma levels of cerebrosterol were significantly and independently related to performance in the SDMT. When age, gender and education were added to the MRI measures and the plasma levels of cerebrosterol as possible predictors of SDMT, the prediction gained no further power. There were no obvious differences between the groups (RR-MS, SP-MS and PP-MS) concerning the relationships between possible predictors and the SDMT performance.

In order to investigate whether each predictor (grey matter volume, brain volume and cerebrosterol level) had a true independent effect on SDMT performance or whether these predictors interacted, a number of stepwise analyses were carried out. Interaction variables were added and all possible pairwise combinations of predictors were analyzed with respect to their shared variance. For all three combinations of predictors (grey matter volume and cerebrosterol, grey matter volume and brain volume, and brain volume and cerebrosterol), the shared variance for the predictors was relatively small.

A stepwise regression analysis was also performed with EDSS as the dependent variable and the MRI volumetric measures (brain volume, the volumes of grey and white matter and the ventricles) and plasma levels of cerebrosterol as independent variables. This analysis showed that high EDSS scores were significantly related to high plasma levels of cerebrosterol. Adding age at onset of disease as a predictor clearly improved this relationship.

In summary, a poor performance on the SDMT was associated with a low volume of grey matter and high levels of cerebrosterol, while a high EDSS score was associated with high levels of cerebrosterol.

5 DISCUSSION

5.1 The focus of this thesis

This thesis investigates cognitive functioning in patients with MS, with a special focus on long-term MRI findings and plasma cerebrosterol levels.

5.2 Study I

In study I, the first finding was that even at baseline, there was selective decline in visual IPS tasks, with impaired VRT but not ART, in the whole MS group. Previous

studies confirm that IPS is the most frequently impaired cognitive domain early in the MS course (Nocentini et al. 2006). Impaired IPS can even occur in the CIS (Khalil et al. 2001). However, the selective pattern, with a more marked decline in visual IPS than in auditory IPS, is new. Since patients with optic neuritis in study I, did not perform worse in VRT, than those without optic neuritis, it could be assumed that impaired VRT does not depend on optic nerve dysfunction. It is possible that the more pronounced decline in visual IPS is partly because the optic tract has a larger extension in space than the auditory neural network and therefore could be more vulnerable to MS lesions. The anterior loop of the optic radiation (Meyer´s loop) travels around the inferior horn of the lateral ventricles and at the middle aspect of the temporal horn, the optic radiation is located at the roof and lateral wall of the lateral ventricles (Eberling and Reulen 1988). MS lesions indicating demyelination frequently have a bilateral periventricular distribution, especially in the tract of the lateral angles of the lateral ventricles (Allen 1991) and therefore the anterior loop of the optic radiation is putatively exposed for those lesions, which could contribute to the decline in information processing.

In contrast to this prominent impairment in IPS, performance in the verbal tests was not affected. Putatively, the functions measured by different tests may have different thresholds with regard to the amount of cerebral dysfunction required before visible impairment appears. Possibly compensatory brain mechanisms are backing up the diverse cognitive functions in different degree, thereby making them variously resistant to brain pathology. The differences could also be the result of MS affecting different brain regions at different times during the disease course, and the tests measuring functions in different locations in the CNS. It is possible that the type of MS course is important for the time at which and the degree to which specific regions in the CNS are affected, and the time at which cerebral dysfunction is measurable by a specific test.

The second finding in study I was that incipient cognitive decline, especially in IPS, predicts further cognitive decline over time in the MS patients, which agrees with previous findings (Amato et al. 2010). This could be due to underlying progressive neurodegenerative brain pathology. There is evidence of early grey matter atrophy in the CIS and the initial stages of RR-MS (Dalton et al. 2004). Morgen et al. (2006) found cortical atrophy in early RR-MS which correlated with impairments in IPS and EFs measured by the PASAT. In contrast, cognitive functions that were well preserved at baseline tended to be better preserved at follow-up eight years later. The finding in study I that deterioration over time is especially marked in IPS is supported by other reports (Denney et al. 2008). The markedly pronounced decline in information processing could putatively due to that it is an unspecific and global function, being affected by brain pathology independently of it´s location.

The third finding in study I was the differential pattern of cognitive decline over time in the subgroups, with the most marked decline in the SP-MS group, particularly in relation to the RR-MS group and most apparent in visual information processing

speed as compared to auditory information processing, after correction for group differences concerning age, education and EDSS score. The more marked cognitive decline in the SP-MS group than the RR-MS group and PP-MS group is consistent with earlier reports. Beatty et al. (1989) found that SP-MS patients were more severely impaired than RR-MS patients, and Denney et al. (2005) reported that an SP-MS group had greater cognitive deterioration than a PP-MS group. It has been demonstrated that grey matter atrophy accelerates when RR-MS switches to SP-MS (Fisher et al. 2008), which could have contributed to the more severe cognitive decline in the SP-MS group. Possibly, the remissions in the RR-MS group slow down the disease progress thereby sparing it from cognitive decline to a higher degree than the SP-MS and PP-MS group. The small sample size in the subgroups calls for caution in the interpretation of the results, however.

The fourth main finding was the association between a high EDSS score (>6) and selective cognitive decline, primarily in IPS, at follow-up but not at baseline, for the whole MS group, is consistent with some reports of a relationship between cognitive functioning and EDSS (Lynch et al. 2005). However studies of the relationship between cognitive status and EDSS have yielded disparate results and some have not found any significant relationship of that sort (Amato et al. 2010). In contrast to an interaction in study I, between a high degree of disability at follow-up and high age in relation to cognitive decline, neither gender, age at onset nor disease duration had any effect on cognition. It is also notable that education had no effect on cognitive performance except vocabulary. It is possible that the association occurred because many of the MS patients had switched to an SP-MS course during follow-up, possibly with an associated higher degree of neurodegeneration and more pronounced physical disability; the older age of the participants could also have been a factor.

In conclusion, the notation in study I of an early selective cognitive decline in MS, primarily in visual information processing in comparison to auditory information processing is a new finding. The observation that early cognitive deterioration in MS, mainly in information processing speed, predicts further cognitive decline and has no association initially with physical disability, suggests an underlying sub-clinical disease factor, putatively neurodegeneration, running side by side with visible cognitive decline. The absence of confounding influence by gender, education, age at onset and disease duration in speeded information task performance strengthens the impression of a possible underlying independent pathological factor(s). In contrast, aging and high degree of disability at follow-up effects cognition, particularly information processing speed negatively. Putatively the differential pattern in study I, with the SP-MS group seeming to have a more pronounced cognitive impairment than the other groups, most obvious in visual reaction time compared with auditory reaction time, could to some part due to a that SP-MS patients have a more developed neuropathology. However these group differences must be interpreted with special caution with respect to the small sample size.

5.3 Study II

Study II differed from studies I, III and IV in that it investigated language function, and not primarily IPS. This study showed greater deficits in the MS patients than in the control group in the use of strategies for substituting target words and strategies for letter-word fluency retrieval, as measured by the BNT and the COWAT (FAS), which is consistent with previous findings. Thus the finding in study II that the MS patients had a higher frequency of errors in the Boston Naming Test is in accordance with a similar finding by Laatu et al. (2001) and the smaller number of words as well as more frequent use of dysexecutive strategies in FAS fluency also seen in study II, is consistent with the findings by Tröster et al. (1998). Furthermore in study II, the poor performance in Boston Naming Test and FAS fluency was corresponding to a general drop in the MS patients current FSIQ.

A limitation of study II was that it did not investigate language function in relation to brain MRI measures. While few studies have looked into the relationship between language function and neuroradiological findings, Amato (2004) has demonstrated an association between poor performance in verbal fluency and grey matter atrophy. PET and fMRI studies have shown that word generation tasks as FAS fluency are associated with activation of left frontal regions Cabeza and Nyberg (2000). On the other hand lexical-semantic retrieval of name associated with a picture in the Boston Naming Test has been reported to activate the left middle temporal gyrus and underlying white matter (Baldo et al. 2013). Putatively neurodegeneration in MS could affect these fronto-temporal areas of importance for word-generation and lexical-semantic retrieval directly or indirectly by giving a slow information processing. Earlier studies on verbal learning have proposed that information search processes do not work effectively in MS patients because of slowed mental activity (Faglioni et al. 2000). However the reason for the impaired lexical processing in MS patients showed in study II is unclear.

In contrast to the verbal deficits noted in study II, aspects of verbal ability appeared well preserved in study I. There were no deficits in the verbal ability tests involving vocabulary, similarities and word fluency at baseline nor any decline at follow-up in study I. Studies investigating language in MS patients have generally shown that language function is relatively well preserved, although subtle comprehension deficits have been reported (Langdon 2011). The paradoxical contrast between the well preserved word fluency in study I and the poor performance in word fluency in study II is possibly the result of the control group in study II, and the more refined analysis of word fluency, including the strategies used, which may have helped to detect subtle lexical deficits. It may also be possible that the cut-off level in the reference data in the test manual used in study I was too low to accurately calculate the z-scores in the word fluency test.

It may also be possible that the poorer performance in word fluency in MS patients compared with controls might have been partly affected by the controls having had 1.2 years more formal education, despite all efforts to match the groups according to their demographic characteristics. This may have positively affected the word fluency

test results in the control group, and may also have given this group an advantage in the BNT, thereby making the finding of lexical deficits in the MS group less representative of total the MS population. However, the difference in mean duration of education between the MS patients and the control group was not significant, and it is reasonable to assume that the findings in study II reflect real, although subtle, language disturbances in the MS patients, which could be expected to influence their social self-esteem and QoL in a negative way.

In conclusion, an interesting angle is the notation that the MS group had poorer lexical strategies for substitution and retrieval than healthy control persons and that there was a correlation between the quality of substitutive responses and the adequacy of retrieval strategies. It can be assumed that the poor lexical processing in the MS group in study II is associated with slow IPS, especially in the word fluency test since time pressure is most pronounced in this task; however, this hypothesis needs to be tested in future research.

5.4 Study III

This study focused on the relationship between atrophy in the corpus callosum and performance in a cognitively demanding speed task (SDMT), a sensory speed task (VRT and ART) and a motor speed task (FT) over nine years, as well as measuring auditory inter-hemispheric transfer at follow-up (VDL). The corpus callosum is a white matter commissure which connects the hemispheres, allowing inter-hemispheric connections between subcortical and cortical neurons.

In this study, the main finding was that, in contrast to the RBV and the T2 lesion load, the CCA was associated exclusively with performance in the SDMT. The smaller the CCA was at baseline, the poorer was the performance in the SDMT at baseline; and the smaller the CCA was at follow-up, the poorer was the performance in the SDMT at follow-up. The RBV and the T2 lesion load were associated with performance in sensory-motor tests but not with SDMT performance. Adding MRI measures (RBV and T2 lesion load), demographic characteristics (age, gender, and education), and clinical characteristics (age at onset, disease duration, severity of disease according to EDSS, and MS course), did not alter the strength of the relationship between the corpus callosum and performance in the SDMT. This suggests a relationship between atrophy in the corpus callosum and a decline in cognitive IPS early in the initial stages of MS when clinical disability is not yet marked. The finding confirms the assumption of an underlying sub-clinical neurodegenerative factor running side by side with clinically visible cognitive deterioration that was made in study I.

The relationship between the CCA and IPS in cognitive tasks has not been studied to any great extent. However, a cross-sectional study by Yaldizli et al. (2014) has demonstrated that atrophy of the posterior corpus callosum segment is associated with poor performance in the SDMT. In a cross-sectional MRI study, Lin et al. also found

an association between a small CCA and deficits in attention and IPS as measured by the PASAT. The finding in study III of an association between atrophy of white matter in the corpus callosum and cognitive IPS does not align with the report by Batista et al. (2012), who found performance in the SDMT to be associated with the grey matter volume but not with the white matter volume. It is possible that IPS depends more on the corpus callosum than on other white matter regions because of the area's importance in inter-hemispheric connectivity. This assumption is in agreement with the finding in a diffusion tensor imaging study by Roosendaal et al. (2009) that impaired IPS as measured by the letter digit substitution test (LDST), was associated with abnormal fractional anisotropy in the corpus callosum. Putatively, the corpus callosum and the neocortex contribute in different ways to cognitively demanding information processing, in the sense that the white matter fibers in the corpus callosum may be responsible for inter-hemispheric connectivity and speed, whereas the neocortex may serve as an associative component on a higher functional level.

In study III, there was no relationship between a high T2 lesion load and a poor SDMT performance. However, T2 lesion load at baseline was associated with impairment in FT speed. The absence of a relationship between the T2 lesion load and the SDMT performance fits the results of several cross-sectional and longitudinal studies, in which atrophy was frequently found to have a stronger association with cognitive performance than with the overall T2 lesion load (Rovaris et al. 2006). Zivadinov et al. (2001), for example, reported that cognitive deterioration relied significantly more on the development of brain atrophy than on the T2 lesion load in a longitudinal study of brain atrophy and cognitive disturbance in the early phase of RR-MS.

The stronger influence of atrophy than of lesion load on cognitive performance raises the question of whether neurodegeneration (as expressed by atrophy) and neuroinflammation (shown as gadolinium-enhanced T2 lesions) represent processes that are at least partly independent of each other. This view corresponds to findings in the prospective 18-month follow-up study by Sailer et al. (2001), in which no correlation was seen between T2 lesion volume and atrophy over the follow-up period. Earlier experimental studies have also supported the assumption that atrophy can be independent of mechanisms causing demyelination. For example, it has been reported that axonal loss could be due to an abnormal glial-axonal interaction and could occur with only low-grade inflammation (Bitsh et al. 2000) or could even appear in the total absence of demyelination and inflammation (Garbern et al. 2002). It is assumed that there is an interplay between inflammation, neurodegeneration and the dynamic vulnerability of intact axons (Compston and Coles 2008).

A differential relationship between the VDL (differing between right and left ear performance) and the ARC in the CCA and RBV was seen in the MS patients in study III. The higher ARC in the CCA in all MS patients at follow-up was related to poorer performance in VDLl, with a more marked advantage in VDLr, in the whole MS group. A relationship between a higher ARC in RBV and more correct responses

with VDLr was also found. Earlier research has also shown an association between callosal atrophy and impaired VDLl in RR-MS patients (Pelletier et al. 2001). Putatively, the association between impaired VDLl and callosal atrophy occurs because left-ear listening, unlike right-ear listening, is highly dependent on the auditory callosal pathway and the left ear is therefore more vulnerable to the disconnection of this pathway by atrophy than the right ear. It seems reasonable that right-ear listening might appear to be improved in association with callosal atrophy because of the resulting elimination of sensory interference from the left ear. It seems paradoxical that the CCA in study III at follow-up was more strongly associated with the SDMT performance than with VDL, since the latter is dependent on inter-hemispheric transfer. Putatively, this was because auditory inter-hemispheric transfer is backed up by ipsilateral pathways and VDL is therefore less dependent on callosal functionality than the SDMT.

A comparison of the subgroups revealed a differential pattern. The SP-MS group performed significantly poorer in the cognitive speed task (SDMT) than the RR-MS group, both at baseline and at follow-up. Furthermore, the SP-MS patients performed less well than the RR-MS group in the sensory-motor speed-demanding VRT and FT tasks at follow-up. This differential pattern of decline in cognitive speed over time in the subgroups, with an inferior performance in the SP-MS group in comparison with the RR-MS group, is in accordance with previous findings (Comi et al. 1995; De Luca et al. 2004). Demographic characteristics did not confound this difference between the subgroups. However, the EDSS score was higher in the SP-MS group and could therefore have influenced the results in the motor speeded FT task, especially at follow-up when it was most marked. The finding that the SP-MS and RR-MS patients achieved low results in all tests in relation to reference data and that they did not differ with respect to RBV at follow-up or ARC in the CCA suggests that both groups were impaired in IPS and had brain atrophy. Since no significant differences were seen between the RR-MS and SP-MS groups in the ARC in the CCA, it is possible that the smaller CCA in the SP-MS group in this study occurred because this latter group tended to have a more advanced disease and a longer disease duration than the RR-MS group.

In conclusion, the main finding that CCA in contrast to RBV and T2 lesion load, was exclusively associated with the cognitive speed-demanding task and not with the sensory-motor speed tasks, makes it putative that CCA has a particular importance for cognitive speed. The small sample size brings an amount of uncertainty into the results. A shortcoming in the present study is the lack of differentiation between different regions of the CCA. However, the finding that the CCA is mainly associated with cognitive speed rather than sensory-motor speed represents a new feature which could be further enlightened in future research using a larger sample size.

5.5 Study IV

Study IV widens the focus in study III of the relationship between cognition in MS patients and MRI findings by investigating the levels of cerebrosterol, which has been proposed as a marker for brain cholesterol turnover in the CNS (Bretillon et al. 2000; Björkhem 2006). Brain cholesterol is involved in cell membrane structure and function, regulating the membrane proteins (Leoni & Caccia (2011). Cerebrosterol has a variety of functions in the CNS. It has an important role in the transport of cholesterol between astrocytes and neurons (Leoni & Caccia 2013), is a structural component of cellular membranes, and is involved in the formation and regulation of synapses and growth of dendrites, and exocytosis (i.e. dumping the content of synaptic vesicles in the synaptic cleft) (Leoni & Caccia 2014). Furthermore, low concentrations of cerebrosterol protect neuronal cells against oxidative stress (Noguchi et al. 2014). Leoni (2004) reported that gadolinium-enhanced T2 lesions indicating focal inflammatory activity were associated with increased turnover of cholesterol in the brain.

The main finding in study IV was that a slow IPS in MS patients was associated with small grey matter volumes and high levels of plasma cerebrosterol, and that a poor clinical status (EDSS) was associated with high levels of cerebrosterol. The findings of this study agree with those of previous studies that have investigated the relationships between cognition and plasma cerebrosterol levels. Hughes et al. (2012) found that participants with high plasma levels of cerebrosterol and high 24OHC/27OHC ratios were at greater risk of developing cerebrovascular disease and impairment longitudinally over 8 years. A correlation between cognitive tests (among them the SDMT) and decreased plasma levels of cerebrosterol in patients with Huntington´s disease was reported by Leoni & Caccia (2013 and 2014). However, our study is the first to investigate the relationships between cognition and plasma cerebrosterol levels and between the EDSS score and plasma cerebrosterol levels in MS patients.

The MRI normalized measure of brain volume, volume of grey matter and plasma levels of cerebrosterol (24OHC) were found to be independent predictors of SDMT-performance. With respect to the absence in our stepwise regression analysis of an interaction between 24OHC and the neurodegenerative predictors of SDMT in the MS patients, it seems probable that neuronal cell death implying axonal loss could be induced by the well-known cytotoxicity of 24OHC, thereby affecting SDMT performance independently of neurodegeneration. Thus previous studies have shown that cerebrosterol 24OHC has potent cytotoxic properties (Kölsch et al. 1999) and induces a programmed neuronal cell death by apoptosis or necroptosis i.e. a form of more chaotic cell death expressed as necrosis, depending of the enzyme serine/threonine kinase 1 (Yamanaka et al. 2011; Noguschi et al. 2014).

It is notable that the mean level of plasma cerebrosterol tended to be higher in the MS patient group in study IV than in the healthy reference group (Dzeletovic et al. 1995). Two patients out of the whole group (n=21) had very high values (>2SD). The other

patients had plasma cerebrosterol levels within the range for healthy people. The somewhat higher than normal mean plasma cerebrosterol levels in the patients in study IV might be partly dependent on the fact that the MS patients were older (mean age 48.3±9.5 years) than the healthy volunteers in the reference group (15 males, mean age 36.7 years; and 16 females, mean age 38.4 years). Bretillon et al. (2000) have shown that plasma cerebrosterol concentrations increase with age. However, including age in addition to cerebrosterol did not give further power to the prediction of performance in the SDMT.

Among the neurodegenerative predictors in the model with the SDMT result as a dependent variable, grey matter volume appeared to be most strongly related to SDMT performance. In contrast, white matter volume did not have enough predictive strength to be included in the stepwise regression model. Furthermore, there was no significant correlation (Pearson) between SDMT performance and white matter volume. This result indicates that grey matter is particularly important for IPS. Some previous studies confirm the relationship between SDMT performance and loss of grey matter volume (Nocentini et al. 2012; Batista et al. 2012). Riccitelli et al. (2011) reported that performance in an IPS-demanding task (PASAT 3) was associated with distinct patterns of regionally distributed grey matter loss in the fronto-temporal lobes, the left hypothalamus and the thalami. In contrast, other studies have shown an association between the SDMT results and white matter atrophy and no association between SDMT performance and grey matter atrophy (Sanfilipo et al. 2006). It is conceivable that the stronger association between a small grey matter volume and IPS seen in study IV could have occurred because most of the MS patients in Sanfilipo's study had RR-MS with subsequently less grey matter atrophy than the patients in study IV most of whom were diagnosed with SP-MS.

In a model with functional status on EDSS as the dependent variable and brain volume, grey matter, white matter and ventricular volumes as well as plasma levels of 24OHC as independent variables, high plasma concentration of 24OHC significantly predicted EDSS. Putatively the cytotoxicity of 24OHC also affects neurons which the EDSS functions depend on, inducing apoptosis or necroptosis. This could possibly contribute to an impaired clinical ability. In contrast none of the brain volumetric measures provided any power to the prediction of ESSS in the stepwise regression model. However EDSS showed significant pairwise correlations (Pearson) with brain volume as well as ventricular space. Age at onset added strength to the prediction of EDSS. This is in accordance with the finding of increasing disability in old age for both sexes (Bove et al. 2013).

The absence of a correlation between the EDSS score and the MRI volumetric measures contrasts with the relationship between the SDMT performance and the MRI measures, primarily grey matter volume. An absence of correlation between grey matter volumes and the EDSS performance was also reported in a study of RR-MS patients by Chard et al. (2002). In another study of RR-MS patients, Morgen et al. (2006) found a correlation between grey matter volume and performance in the

PASAT (processing speed and working memory) but no correlation between grey matter volume and the EDSS score.

The difference between the EDSS and the SDMT concerning the presence of a relationship with grey matter volume may be due to the latter involving more cognitively demanding tasks and therefore depending more exclusively on activity in higher associative cortical areas. Forn et al. (2011) showed in an fMRI study of healthy participants that IPS measured using the SDMT mainly activates a wide frontoparietal network in the cortex. Disability measured using the EDSS, on the other hand, can be affected by pathology in many different locations in the CNS in addition to the cortex, as well as in non-CNS locations (Chard & Miller 2009). Thus, the EDSS quantifies disability in eight functional systems (pyramidal, cerebellar, brainstem, sensory, bowel and bladder, visual, cerebral, and "other"). Putatively, these functions in the EDSS have different thresholds with respect to how far the grey matter pathology has developed before an impairment is obvious, and neuroplasticity could possibly make these resistant to atrophy to varying degrees. Thus, the variety of functions in the EDSS possibly lessens its association with grey matter volume and makes it less specific in comparison with the SDMT.

In conclusion, the results of study IV suggest that information processing speed in MS is affected by neurodegeneration, primarily loss of grey matter volume and levels of plasma cerebrosterol a marker of membrane turnover and that these predictors are independent. A correlation between the EDSS performance and plasma cerebrosterol levels indicates that the latter could be a putative biomarker for MS disease progression. However, the small number of MS patients may make the results uncertain. In future research, it would be desirable to validate these results by using a larger sample size, preferably with a follow-up design. A future study could also be enriched by including the aspect of health related quality of life, and vocational status in relation to information processing speed, MRI findings and cerebrosterol.

5.6 Methodological limitations and strengths

5.7 Sample selection and representativeness

The total number of MS patients in the original cohort in 1995 was relatively small (n=32) but only one patient had dropped out from study I at follow-up eight years later. The patients in the original cohort were consecutively recruited, which favored the representativeness of the samples. However, in studies II-IV, a number of patients from the original cohort were missing and the small group size, especially in study IV, probably diminished the representativeness of the samples. Also, a small sample size implies less power for the test and subsequently an enhanced risk of a type II error in interpretation of the results, i.e. that the null hypothesis will not be rejected when it is false. In studies I and III, the long follow-up period could, to some extent, have compensated for the small sample size.

It is also important to take into account the distribution of the subgroups, in the context of representativeness. The typical distribution of MS patients is said to be RR-MS 45-60%, SP-MS 30-45% and PP-MS 10-15%. In the original sample in 1995, from which the MS patients in studies I-IV emanated, the distribution of subgroups was RR-MS 32%, SP-MS 55% and PP-MS 13%. This distribution deviates from the typical subgroup distribution, with more SP-MS patients. The high number of SP-MS patients in our cohort may have occurred because patients had switched from RR-MS to SP-MS during the time from disease onset to 1995. In the following years, switches to SP-MS continued to occur in the MS patients from the cohort, reinforcing the higher proportion of SP-MS patients. Because SP-MS patients are reported to have poorer results in cognitive tasks, there is an obvious risk that the dominance of SP-MS patients in study II resulted in the MS group performing worse (in comparison with the control group) than a more representative group would have. These elements of uncertainty concerning the representativeness of the MS sample necessitate caution in the interpretation of the results.

Another methodological limitation was the lack of a control group in studies I, III and IV. Instead of comparing with a control group, population-based normative data from manuals and handbooks were used in these studies. It is obvious that this is not optimal. Because the population-based normative data may have been of varying quality, there may have been deficits in the standardization of the test data or the normative data may have been too old. However, in the studies undertaken in this thesis, normative data from authorized well-reputed manuals and handbooks were used. Despite the advantage of having a control group, it would probably have been difficult to engage controls for the long follow-up periods in studies I and III.

When comparing the results of studies I-IV, it is important to keep in mind that the MS patient samples were from the same original cohort and were thus not independent of each other. A possible consequence of this is that the results of these studies could have tendencies in common which might have influenced the comparison of results between studies.

Another weak methodological point in the studies was the insufficient control for effects of MS medication.

Training effects which can appear with retesting using the same task, should also be taken into account. These are particularly relevant for those patients who participated in all the studies and performed the same test tasks several times. However, the long intervals between test and retest, and the use of parallel test versions in the studies, probably counterbalanced the influence of training effects to a certain extent.

Although the test instruments used in studies I-IV have well documented reliability (i.e. consistency of the measurements) and validity (i.e. accuracy in the sense that the test measures what it is supposed to measure), there is a certain risk that fatigue may have contributed to some degree to inconsistencies in the test measures in the MS patients.

5.7 Concluding remarks

The main conclusion to be drawn from studies I-IV is that slow IPS appears to be a primary aspect of cognitive decline in MS patients. In these studies, deterioration in IPS began early in the MS course and predicted long-term cognitive decline. IPS could be assumed to be important for the functionality of other domains such as language. Cognitive speed in MS was influenced by the neuroradiological characteristics as well as membrane turnover of brain cerebrosterol, which interacted with the clinico-demographic variables to various degrees. The disease course in MS, including the development of cognitive decline, has commonly been viewed as primarily driven by neuroinflammation. However, these studies indicate that neurodegeneration and the neurotoxic cholesterol oxidation product cerebrosterol also independently contribute to cognitive impairment and clinical disability in the disease.

The early cognitive decline, which was seen in the MS patients in study I, seems to be selective by occurring in visual IPS rather than auditory IPS and this is a new finding. Study II contributes to our understanding of the language pathology in MS, in that strategies for lexical substitution and retrieval were poorer in patients with MS than in healthy people and that there was a correlation between the relevance of substitutive responses in a naming task and the efficiency of retrieval strategies in a letter fluency task. In study III, the finding that the callosal area, in contrast to the RBV and the T2 lesion load, was exclusively associated already initially with outcomes of a cognitive speed-demanding task and not with those of sensory-motor speed tasks is also a new finding. Similarly, the findings in study IV, that IPS was influenced by grey matter volume and plasma cerebrosterol levels and that disability measured by the EDSS correlated with plasma cerebrosterol levels are new.

With respect to the small sample, caution must be used in interpreting these results. It would be fruitful to view the results as preliminary and useful for generating hypotheses, with the need for validation in future studies with improved designs and larger population samples. Hopefully, these fragments of information can be integrated into the cumulated knowledge from earlier research and contribute to improved diagnostics as well as a better understanding of cognition in MS.

2 ACKNOWLEDGEMENTS

I wish to thank all the persons who have made the thesis possible. It is difficult to make this list complete.

My main supervisor and friend associate professor Ove Almkvist, whom I first became acquainted with 1995 at Huddinge Hospital, is an inspiring and encouraging mentor. He has guided me through the research projects with a good sense of patience, humour and brightness.

Professor Sten Fredrikson, my first assisting supervisor, whom I also know from 1995, has taken a great responsibility for my research and is an important person. He serves as a good model as researcher and teacher. In his calm and mildly humoristic manner, he has given me good advice and confidence, not least when I have experienced shortcomings.

My second assisting supervisor, associate professor Maria Kristoffersen-Wiberg has taken an important part in the MRI related parts of the thesis, and shown kind interest in my research.

My third assisting supervisor Agneta Herlitz, encouraged me to apply for the doctoral education at Karolinska Institutet. She has kept a sharp eye on me, as a good fay.

Professor Peter Aspelin is a dynamic and inspiring person, with an generous engagement in his co-workers research. It is nice to take part in discussions with him. He has kindly sponsored the consultance of a statistician for analyzing data from study III.

Warmest thanks to associate professor Ing-Mari Tallberg, my co-author in study II. She invited me generously to take part in research concerning language pathology in MS patients. I appreciate the friendship with her.

Great thanks to my co-author PhD, MD Virginija Karrenbauer, who is a charming person with much enthusiasm as well as knowledge within the field of cerebrosterol and neurology. She kindly invited me to the cerebrosterol-related research.

Also many thanks to my co-author physicist, MSc Leszek Stawiarz who is a nice and unselfish person, who often gives advice to me and other researchers in their MRI related projects.

Thanks to my co-author in study III, PhD, MD Juha Martola. Juha is inspiring by his solid knowledge within neuroradiology and individualistic as well as fearless attitude towards challenges irrespective of if they concern research or wilderness expeditions.

I wish to thank Professor Ingemar Björkhem “Mr Cerebrosterol”. He is a pionjeer within the neurochemical field, and without his achievements, I and my co-authors would never have had the faintest idea about cerebrosterol.

Study I and III has in part received financial support from the Psychology Clinic, Karolinska University Hospital, Huddinge.

I am also grateful for the assistance with blood sampling, from the study nurses Ewa Waldebäck, Marita Ingemarsson and Anna Aronsson at MS center, Department of Neurology, Karolinska University Hospital, Huddinge whom have performed blood sampling.

Great thanks to Antona Wagstaff, PrePublication Services, Oakura, New Zealand, who has given excellent assistance with the language check-up.

The statistician Jakob Bergström, KI, was consulted and I want to give him my thanks.

The inspiring discussions with the neurologists at Neurological Department, Karolinska University, Huddinge have added fuel to my interest in MS.

Professor Jan Hillert, at Neurological Department, has shown a kind interest in study III. His MS seminars have been stimulating and useful for me.

Thanks also to PhD, nurse Kristina Gottberg and PhD, Specialist physiotherapist in neurology Ulrika Einarsson for useful discussions.

My collegues at the Psychology Clinic are also giving a good support by their friendship and positive attitude.

Last but not least I want to thank Li, my lovely wife. Without her, there had probably never been any thesis.

3 REFERENCES

Achiron A, Polliack M, Rao SM, Barak Y, Lavie M, Appelboim et al. Cognitive patterns and progression in multiple sclerosis: construction and validation of percentile curves. J Neurol Neurosurg Psychiatry 2005;76:744-749.

Allen IV. Pathology of multiple sclerosis. In: McAlphine´s multiple sclerosis. Eds. Mathews WB, Compston A, Allen IV, Martyn CN. Churchill Livingstone: New York 1991;Ch 12:341-78.

Almkvist O, Adveen M, Henning L, Tallberg IM. Estimation of premorbid cognitive function based on word knowledge: The Swedish Lexical Decision Test (SLDT). SJoP 2007;48:271-79.

Amato MP, Bartolozzi ML, Zipoli V, Portaccio E, Mortilla M, Guidi L. et al. Neocortical volume decrease in relapsing-remitting MS patients with mild cognitive impairment. Neurology 2004;63:89-93.

Amato MP, Langdon D, Montalban X, Benedict RHB, DeLuca J, Krupp LB, Thompson AJ, Comi G. Treatment of cognitive impairment in multiple sclerosis: position paper. J Neurol 2013;260:1452-68.

Amato MP, Portaccio E, Goretti B, Zipoli V, Iudice A, Pina Della D. et al. Relevance of cognitive deterioration in early relapsing-remitting MS: a 3-year follow-up study. Mult Scler 2010;16:1474-82.

Andersen O. From the Gothenburg cohort to the Swedish MS registry. Acta Neurol Scand 2012;126:13-19.

Arnett PA, Higgonson CI, Voss WD, Bender WI, Wurst JM, Tippin JM. Depression in multiple sclerosis: relationship to working memory capacity. Neuropsychology 1999;13:546-56.

Arnett PA, Higgonson CI, Voss WD, Wright B, Bender WI, Wurst JM, Tippin JM. Depressed mood in multiple sclerosis: relationship to capacity-demanding memory and attentional functioning. Neuropsychology 1999;13:434-46.

Ascherio A, Munger KL, Lennette ET, Spiegelman D, Hernán MA, Olek MJ. et al. Epstein-Barr virus antibodies and risk of multiple sclerosis: a prospective study. JAMA 2001;286:3083-8.

Baddeley A. 1986 Working memory. Oxford University Press, London.

Bailey A, Channon S, Beaumont JG. The relationship between subjective fatigue and cognitive fatigue in advanced multiple sclerosis. Mult Scler 2007;13:73-80.

Baldo JV, Arévalo A, Patterson JP, Dronkers NF. Grey and white matter correlates of picture naming: Evidence from a voxel-based lesion analysis of the Boston Naming Test. Cortex 2013;49:658-667.

Baranzini SE, Mudge J, van Velkinburgh JC, Khankhanian P, Khrebtukova I, Miller NA. et al. Genome, epigenome and RNA sequences of monozygotic twins discordant for multiple sclerosis. Nature 2010;464:1351-56.

Barkhof F, Filippi M, Miller DH, Scheltens P, Campi A, Polman CH. et al. Comparison of MRI criteria at first presentation to predict conversion to clinically definite multiple sclerosis. Brain 1997;120:2059-2069.

Bartfai A, Nyman H, Stegman B: Wechsler Adult Intelligence Scale Revised: WAIS-R Manual. Stockholm, Psykologiförlaget, 1994.

Basso MR, Lowery N, Courtney G, Combs D, Johnson J. Self-generated learning in people with multiple sclerosis. JINS 2006;12:640-48.

Batista S, Zivadinov R, Hoogs M, Bergsland N, Heininen-Brown M, Dwyer MG. et al. Basal ganglia, thalamus and neocortical atrophy predicting slowed cognitive processing in multiple sclerosis. J Neurol 2012;259:139-46.

Beatty WW, Aupperle RL. Sex differences in cognitive impairment in multiple sclerosis. Clin Neuropsychol 2002;16:472-80.

Beatty WW, Goodkin DE, Monson N, Beatty PA. Cognitive disturbances in patients with relapsing remitting multiple sclerosis. Arch Neurol 1989;46:1113-19.

Benedict RHB, Bruce JM, Dwyer MG, Abdelrahman N, Hussein S, Weinstock-Guttman B. et al. Neocortical atrophy, third ventricular width, and cognitive dysfunction in multiple sclerosis. Arch Neurol 2006;63:1301-06.

Benedict RHB, Morrow SA, Weinstock Guttman BW, Cookfair D, Schretlen DJ. Cognitive reserve moderates decline in information processing speed in multiple sclerosis patients. Journal of the International Neuropsychological Society 2010;16:829-35.

Benedict RHB, Wahlig E, Bakshi R, Fishman I, Munschauer F, Zivadinov R, Weinstock-Guttman B. Predicting quality of life in multiple sclerosis: accounting for physical disability, fatigue, cognition, mood disorder, personality, and behavior change. J Neurol Sci 2005;231:29-34.

Bitsch A, Schuchardt J, Bunkowski S, Kuhlmann T, Bruck W. Acute axonal injury in multiple sclerosis. Correlation with demyelination and inflammation. Brain 2000;123:1174-83.

Björkhem I. Crossing the barrier, oxysterols as cholesterol transporters and metabolic modulators in the brain. J Intern Med 2006;260:493-508.

Bobholz JA, Rao SM. Cognitive dysfunction in multiple sclerosis: a review of recent developments. Curr Opin Neurol 2003;16:283-8.

Bobholz JA, Rao SM. Cognitive Dysfunction in multiple sclerosis: a review of recent developments. Curr Opin Neurol 2003;16:283-88.

Bodling AM, Denney DR, Lynch SG. Cognitive aging in Patients with multiple sclerosis: A cross-sectional analysis of speeded processing. Arch Clin Neuropsych 2009;24:761-67.

Bove R, Musallam A, Healy BC, Houtchens M, Glanz BI, Khoury S. et al. No sex-specific difference in disease trajectory in multiple sclerosis patients before and after age 50. BMC Neurology 2013;13:73.

Bretillon L, Lütjohann D, Stahle L, Widhe T, Bindl L, Eggertsen G. et al. Plasma levels of 24S-hydroxycholesterol reflect the balance between cerebral production and hepatic metabolism and are inversely related to body surface. J. Lipid Res 2000;41:840-45.

Burell AM, Handel AE, Ramagopalan SV, Ebers GC, Morahan JM. Epigenetic mechanisms in multiple sclerosis and the major histocompatibility complex (MHC). Discov Med 2011;11:187-96.

Cabeza R, Nyberg L. Imaging cognition II: An empirical review of 275 PET and fMRI studies. J Cognitive Neurosci 2000;12:1-47.

Calabrese M, Agosta F, Rinaldi F, Mattisi I, Grossi P, Favaretto A. et al. Cortical lesions and atrophy associated with cognitive impairment in relapsing-remitting multiple sclerosis. Arch Neurol 2009;66:1144-50.

Calabrese M, Rinaldi F, Mattisi I, Grossi P, Favaretto A, Atzori M. et al. Widespread cortical thinning characterizes patients with MS with mild cognitive impairment. Neurology 2010;74:321-28.

Charcot JM. Lectures on the Diseases of the Nervous System. 1877.

Chard D, Miller D. Grey matter pathology in clinically early multiple sclerosis: Evidence from magnetic resonance imaging. J Neurol Sci 2009;282:5-11.

Chard DT, Griffin CM, Parker GJM, Kapoor R, Thompson AJ, Miller DH. Brain atrophy in clinically early relapsing-remitting multiple sclerosis. Brain 2002;125:327-37.

Chiaravalloti ND, DeLuca J. Cognitive impairment in multiple sclerosis. Lancet Neurol 2008;7:1139-51.

Comi G, Filippi M, Martinelli V, Campi A, Rodegher M, Alberoni M, Sirabian G, Canal N. Brain MRI correlates of cognitive impairment in primary and secondary progressive multiple sclerosis. J Neurol Sci 1995;132:222-7.

Compston A, Coles A. Multiple sclerosis. Lancet 2008;372:1502-17.

Correale J, Ysrraelit MC, Gaitán MI. Immunomodulatory effects of vitamin D in multiple sclerosis. Brain 2009;132:1146-60.

Cutajar R, Ferriani E, Scandellari C, Sabattini L, Trocino C, Marchello LP. et al. Cognitive function and quality of life in multiple sclerosis patients. J Neurovirol 2000;6:S186-90.

Dalton CM, Chard DT, Davies GR, Miszkiel KA, Altmann DR, Fernando K. et al. Early development of multiple sclerosis is associated with progressive grey matter atrophy in patients presenting with clinically isolated syndromes. Brain 2004;127:1101-07.

DeLuca J, Chelune GJ, Tulsky DS, Lengenfelder J, Chiaravalloti ND. Is speed of processing or working memory the primary information processing deficit in multiple sclerosis? J Clin Exp Neuropsychol 2004;26:550-62.

DeLuca J, Chelune GJ, Tulsky DS, Lengenfelder J, Chiaravalloti ND. Is speed of processing or working memory the primary information processing deficit in Multiple sclerosis? J Clin Exp Neuropsychol 2004;26:550-62.

DeLuca J, Gaudino EA, Diamond BJ, Christdoulou C, Engel RA. Acquisition and storage deficits in multiple sclerosis. J Clin Exp Neuropsychol 1998;20:376-90.

Denney DR, Lynch SG, Parmenter BA. A 3-year longitudinal study of cognitive impairment in patients with primary progressive multiple sclerosis: speed matters. J Neurol Sci 2008;267:129-36.

Denney DR, Sworowski LA, Lynch SG. Cognitive impairment in three subtypes of multiple sclerosis. Arch Clin Neuropsychol 2005;20:967-81.

Drew M, Tippett LJ, Starkey NJ, Isler RB. Executive dysfunction and cognitive impairment in a large community-based sample with Multiple Sclerosis from New Zealand: a descriptive study. Arch Clin Neuropsychol 2008;23:1-19.

Dyment DA, Ebers GC, Sadovnick AD. Genetics of multiple sclerosis. Lancet Neurol 2004;3:104-10.

Dzeletovic S, Breuer O. Determination of cholesterol oxidation products in human plasma by isotope dilution-mass spectrometry. Anal Biochem 1995;225:73-80.

Eberling U, Reulen HJ. Neurosurgical topography of the optic radiation in the temporal lobe. Acta Neurochir (Wien) 1988;92:29-36.

Fagius J, Andersen O, Hillert J, Olsson T, Sandberg M. Multipel skleros. Karolinska Institutet University Press 2007.

Faglioni P, Bertolani L, Botti C, Merelli E. Verbal learning strategies in patients with multiple sclerosis. Cortex 2000;36:243-63.

Feinstein A. Multiple sclerosis, depression and suicide. Clinicians should pay more attention to psychopathology. BMJ 1997;315:691-2.

Filippi M, Rocca MA, Benedict RH, DeLuca J, Geurts JJ, Rombouts SA, Ron M, Comi G. The contribution of MRI in assessing cognitive impairment in multiple sclerosis. Neurology 2010;75:2121-28.

Filippi M, Rocca MA. Conventional MRI in Multiple Sclerosis. J Neuroimaging 2007;17:3S-9S.

Filippi M, Rocca MA. Cortical reorganization in patients with MS. J Neurol Neurosurg Psychiatry 2004;75:1087-89.

Filley CM. Clinical neurology and executive dysfunction. Seminars in Speech and Language 2000;21:95-108.

Fischer JS, Priore RL, Jacobs LD, Cookfair DL, Rudick RA, Herndon RM. Neuropsychological effects of Interferon beta-1a in relapsing multiple sclerosis. Ann Neurol 2000;48:6:885-92.

Fisher E, Lee JC, Nakamura K, Rudick RA. Gray matter atrophy in multiple sclerosis: a longitudinal study. Ann Neurol 2008;64:255-65.

Flensner G, Landtblom AM, Söderhamn O, EK AC. Work capacity and health-related quality of life among individuals with multiple sclerosis reduced by fatigue: a cross-sectional study. BMC Public Health 2013;13:224.

Forn C, Belenguer A, Belloch V, Sanjuan A, Parcet MA, Avila C. Anatomical and functional differences between the Paced Auditory Serial Addition Test and the Symbol Digit Modalities Test. J Clin Exp Neuropsychol 2011;33:42-50.

Fredrikson S, Kam-Hansen S. The 150-year anniversary of multiple sclerosis: does its early history give an etiological clue? Perspect Biol Med 1989;32:237-43.

Fujii DE, Wylie AM, Nathan JH. Neurocognition and long-term prediction of quality of life in outpatients with severe and persistent mental illness. Schizophr Res 2004;69:67-73.

Garbern JY, Yool DA, Moore GJ, Wilds IB, Faulk MW, Klugmann M. Patients lacking the major CNS myelin protein, proteolipid protein 1, develop length-dependent axonal degeneration in the absence of demyelination and inflammation. Brain 2002;125:551-61.

Ghaffar O, Feinstein A. APOE ε4 and cognitive dysfunction in multiple sclerosis: A review. J Neuropsychiatry Clin Neurosci 2010;22:155-65.

Goodin D. The epidemiology of multiple sclerosis: insights to disease pathogenesis. In Handbook of clinical neurology. In: Handbook of clinical neurology. Ed. Goodin D. Elsevier 2014.

Goverover Y, Genova H, Hillary FG, De Luca J. The relationship between neuropsychological measures and the Timed Instrumental Activities of Daily Living task in multiple sclerosis. Mult Scler 2006;12:1-9.

Goverover Y, Hillary FG, Chiaravalloti N, Arango-Lasprilla JC, DeLuca J. A functional application of the spacing effect to improve learning and memory in persons with multiple sclerosis. J Clin Exper Neuropsychol 2009;31:513-22.

Handel AE, Ramagopalan SV. Smoking and Multiple Sclerosis: A Matter of Global Importance. Neuroepidemiology 2011;37:243-44.

Heaton RK, Nelson LM, Thompson DS, Burk JS, Franklin GM. Neuropsychological findings in relapsing-remitting and chronic-progressive multiple sclerosis. J Consult Clin Psychol 1985;53:103-110.

Henry JD and Beatty WW. Verbal fluency deficits in multiple sclerosis. Neuropsychologia 2006;44:1166-74.

Hernan MA, Zhang SM, Lipworth L, Multiple sclerosis and age at infection with common viruses. Epidemiology 2001;12:301-6.

Hobart J, Freeman J, Thompson A. Kurtzke scales revisited: the application of psychometric methods to clinical intuition. Brain 2000;123:1027-40.

Hugdahl K. (ed). Handbook of dichotic listening: theory, methods and research. Chichester: John Wiley & Sons Ltd., 1988.

Hughes TM, Kuller LH, Lopez OL, Becker JT, Evans RW, Sutton-Tyrell K, Rosano C. Markers of cholesterol metabolism in the brain show stronger associations with cerebrovascular disease than Alzheimer´s disease. J Alzheimers dis 2012;30:53-61.

Huynh JL, Casaccia P. Epigenetic mechanisms in multiple sclerosis: implications for pathogenesis and treatment. Lancet Neurol 2013;12:195-206.

Jenkinson M, Smith SM. A global optimization method for robust affine registration of brain images. Med Image Anal 2001;5:143-56.

Joffe RT, Lippert GP, Gray TA, Sawa G, Horvath Z. Mood disorder and multiple sclerosis. Arch Neurol 1987;44:376-8.

Kalinowska-Lyszczarz A, Losy J. The role of Neurotrophins in Multiple Sclerosis-Pathological and Clinical Implications. Int J Mol Sci 2012;13:13713-13725.

Kalinowska-Lyszczarz A, Pawlak MA, Michalak S, Losy J. Cognitive deficit is related to immune-cell beta-NGF in multiple sclerosis patients. J Neurol Sci 2012;321:43-48.

Karrenbauer VD, Leoni V, Tuan Lim E, Giovannoni G, Ingle GT, Sastre-Garriga JS. et al. Plasma cerebrosterol and magnetic resonance imaging measures in multiple sclerosis. Clin Neurol Neurosurg 2006;108:456-60.

Khalil M, Enzinger C, Langkammer C. Cognitive impairment in relation to MRI metrics in patients with clinically isolated syndrome. Mult scler 2011;17:173-80.

Kölsch H, Lütjohann D, Tulke A, Björkhem I, Rao ML. The neurotoxic effect of 24-hydroxycholesterol on SH-SY5Y human neuroblastoma cells. Brain Res 1999;818:171-75.

Koutsis G, Panas M, Giogkaraki E, Potagas C, Karadima G, Sfagos C, Vassilopoulos D. APOE epsilon4 is associated with impaired verbal learning in patients with MS. Neurology 2007;68:546-49.

Kremenchutzky M, Cottrell D, Rice G, The natural history of multiple sclerosis: a geographically based study. 7. Progressive-relapsing and relapsing-progressive multiple sclerosis: a re-evaluation. Brain 1999;122:1941-50.

Krupp LB, Alvarez LA, LaRocca NG, Scheinberg LC. Fatigue in multiple sclerosis. Arch Neurol 1988;45:435-7.

Kujala P, Portin P, Ruutiainen J. The progress of cognitive decline in multiple sclerosis A controlled 3-year follow-up. Brain 1997;120:289-97.

Kurtzke JF. Epidemiologic evidence for multiple sclerosis as an infection. Clin Microbiol Rev 1993;6:382-427.

Kurtzke JF. Rating neurologic impairment in multiple sclerosis: an expanded disability scale. Neurology 1983;33:1444-52.

Kutzelnigg A, Lucchinetti CF, Stadelmann C. et al. Cortical demyelination and diffuse white matter injury in multiple sclerosis. Brain 2005;128:2705-12.

Landtblom AM, Fazio P, Fredrikson S, Granieri E. The first case history of multiple sclerosis: Augustus d'Esté (1794-1848). Neurol Sci 2010;31:29-33.Laatu S, Revonsuo A, Hämäläinen P, Ojanen V, Ruutiainen J. Visual object recognition in multiple sclerosis. J Neurol Sci 2001;185:77-88.

Langdon DW. Cognition in multiple sclerosis. Curr Opin Neurol 2011;24:244-249.

Langdon DW, Amato MP, Boringa J, Brochet B, Foley F, Fredrikson S, Hämäläinen P, Hartung HP, Krupp L, Penner IK, Reder AT, Benedict RHB. Recommendations for a brief international cognitive assessment for multiple sclerosis (BICAMS). Mult Scler J 2012;18:891-98.

Lazeron RH, Rombouts SA, Scheltens P, Polman CH, Barkhof F. An fMRI study of planning-related brain activity in patients with moderately advanced multiple sclerosis. Mult Scler 2004;10:549-55.

Lazeron RH, Rombouts SA, Scheltens P, Polman CH, Barkhof F. An fMRI study of planning-related brain activity in patients with moderately advanced multiple sclerosis. Mult Scler 2004;10:549-55.

Leoni V, Caccia C. 24S-hydroxycholesterol in plasma: A marker of cholesterol turnover in neurodegenerative diseases. Biochimie 2013;95:595-612.

Leoni V, Caccia C. Oxysterols as biomarkers in neurodegenerative diseases. Chem Phys Lipids 2011;164:515-24.

Leoni V, Caccia C. Study of cholesterol metabolism in Huntington´s disease. Biochem Biophys Res Commun 2014, In press.

Leoni V, Mastermann T, Mousavi FS, Wretlind B, Wahlund LO, Diczfalusy U. et al. Diagnostic use of cerebral and extracerebral oxysterols. Clin Chem Lab Med 2004;42:186-91.

Levander S, Elithorn A: Department of Psychiatry and Behavioural Medicine, University of Trondheim, Norway, 1987.

Lezak M. Neuropsychological Assessment. New York, Oxford University Press, 1995.

Lin X, Tench CR, Morgan PS, Constantinescu CS. Use of combined conventional and quantitative MRI to quantify pathology related to cognitive impairment in multiple sclerosis. J Neurol Neurosurg Psychiatry 2008;79:437-41.

Lord SJ, Rajotte RV, Korbutt GS, Bleackley RC. Granzyme B: a natural born killer. Immunol Rev 2003;193:31-8.

Lublin FD, Reingold SC. Defining the clinical course of multiple sclerosis: results of an international survey. National Multiple Sclerosis Society (USA) Advisory Committee on Clinical Trials of New Agents in Multiple Sclerosis. Neurology1996;46:907-11.

Lüders E, Steinmetz H, Jäncke L. Brain size and grey matter volume in the healthy human brain. Neuroreport 2002;13:2371-74.

Lütjohann D, von Bergmann K. 24S-Hydroxycholesterol: a Marker of Brain Cholesterol Metabolism. Pharmacopsychiatry 2003;36:102-06.

Lynch SG, Parmenter BA, Denney DR. The association between cognitive impairment and physical disability in multiple sclerosis. Mult Scler 2005;11:469-76.

Martola J, Stawiarz L, Fredrikson S. et al. Progression of non-age-related callosal brain atrophy in multiple sclerosis: a 9-year longitudinal MRI study representing four decades of disease development. J Neurol Neurosurg Psychiatry 2007;78:375-80.

McAlpine D. The benign form of multiple sclerosis. A study based on 241 cases seen within 3 years of onset and followed up until the 10th year or more of the disease. Brain 1961;84:186-203.

McCarthy M, Beaumont JG, Thompson R, Peacock S. Modality-specific aspects of sustained and divided attentional performance in multiple sclerosis. Arch Clin Neuropsychol 2005;20:705-18.

Meier DS, Weiner HL, Guttman CRG. Time-Series Modeling of Multiple Sclerosis Disease Activity: A Promising Window on Disease Progression and Repair Potential? Neurotherapeutics 2007;4:485-98.

Miller DH, Declan TC, Ciccarelli O. Clinically isolated syndromes. Lancet Neurol 2012;11:157-69.

Minden SL, Orav J, Reich P. Depression in multiple sclerosis. Gen Hosp Psychiatry 1987;9:426-34.

Morales Y, Parisi JE and Lucchinetti CF. The Pathology of Multiple Sclerosis: Evidence for Heterogeneity. In: Advances in Neurology. Vol 98, Multiple sclerosis and demyelinating diseases. Ed. Mark S. Freedman . Lippincott Williams & Wilkins 2006.

Morgen K, Sammer G, Courtney SM, Wolters T, Melchior H, Blecker CR. et al. Evidence for a direct association between cortical atrophy and cognitive impairment in relapsing-remitting MS. NeuroImage 2006;30:891-98.

Neema M, Stankiewicz J, Arora A, Guss ZD, Bakshi R. MRI in Multiple Sclerosis: What´s Inside the Toolbox? Neurotherapeutics 2007;4:602-17.

Nocentini U, Bozzali M, Spanò B, Cercignani M, Serra L, Basile B. et al. Exploration of the relationships between regional grey matter atrophy and cognition in multiple sclerosis. Brain Imaging Behav 2012, Epub ahead of print.

Nocentini U, Pasqualetti P, Bonavita S, Bucafusca M, De Caro MF, Farina D. et al. Cognitive dysfunction in patients with relapsing-remitting multiple sclerosis. Mult Scler 2006;12:77-87.

Noguchi N, Saito Y,Urano Y. Diverse functions of 24(S)-hydroxycholesterol in the brain. Biochem Biophys Res Commun 2014 (in press)

O´Connor P; Canadian Multiple Sclerosis Working Group. Key issues in the diagnosis and treatment of multiple sclerosis. An overview. Neurology 2002;59:1-33.

Patanella AK, Zinno M, Quaranta D, Nociti V, Frisullo G, Gainotti G. et al. Correlations between peripheral blood mononuclear cell production of BDNF, TNF-alpha, IL-6, IL-10 and cognitive performances in multiple sclerosis patients. J Neurosci Res 2010;88:1106-12.

Pelletier J, Suchet L, Witjas T, Habib M, Guttman CR, Salamon G. et al. A longitudinal study of callosal atrophy and interhemispheric dysfunction in relapsing-remitting multiple sclerosis. Arch Neurol 2001;58:105-11.

Penner IK, Rausch M, Kappos L, Opwis K, Radu EW. Analysis of impairment related functional architecture in MS patients during performance of different attention tasks. J Neurol 2003;250:461-72.

Peterson JW, Bö L, Mörk S, Chang A, Trapp BD. Transected neurites, apoptotic neurons, and reduced inflammation in cortical multiple sclerosis lesions. Ann Neurol 2001;50:389-400.

Pliskin NH, Hamer DP, Goldstein DS, Towle VL, Reder AT, Noronha A. Improved delayed visual reproduction test performance in multiple sclerosis patients receiving interferon beta-1b. Neurology 1996;47:1463-68.

Polman CH, Reingold SC, Banwell B, et al. Diagnostic Criteria for Multiple sclerosis: 2010 Revisions to the McDonald Criteria. Ann Neurol 2011;69:292-302.

Portaccio E, Amato MP, Bartolozzi ML, et al. Neocortical volume decrease in relapsing-remitting multiple sclerosis with mild cognitive impairment. J Neurol Sci 2006;245:195-99.

Portaccio E, Stromillo ML, Goretti B. et al. Neuropsychological and MRI measures predict short-term evolution in benign multiple sclerosis. Neurology 2009;73:498-503.

Poser CM, Paty DW, Scheinberg L. et al. New diagnostic criteria for multiple sclerosis: guidelines for research protocols. Ann Neurol 1983;13:227-31.

Ramagopalan SV, Dyment DA, Zameel Cader M. et al. Rare Variants in the CYP27B1 Gene Are Associated with Multiple Sclerosis. Ann Neurol 2011;70:881-886.

Rao S, Leo G, Bernardin L, Unverzagt F. Cognitive dysfunction in multiple sclerosis: frequency, patterns and predictions. Neurology 1991;41:685-91.

Rao SM, Grafman J, DiGuilio D. et al. Memory dysfunction in multiple sclerosis: its relation to working memory, semantic encoding and implicit learning. Neuropsychology 1993;7:364-74.

Rejdak K, Jackson S, Giovannoni G. Multiple sclerosis: a practical overview for clinicians. British Medical Bulletin 2010;95:79-104.

Riccitelli G, Rocca MA, Pagani E, Rodegher ME, Rossi P, Falini A. et al. Cognitive impairment in multiple sclerosis is associated to different patterns of grey matter atrophy according to clinical phenotype. Hum Brain Mapp; 2011:1535-43.

Roosendaal SD, Geurts JJG, Vrenken H, Hulst HE, Cover KS, Castelijns JA. et al. Regional DTI differences in multiple sclerosis patients. Neuroimage 2009;44:1397-1403.

Rosti-Otajärvi EM and Hämäläinen PI. Neuropsychological rehabilitation for multiple sclerosis (Review). The Cochrane collaboration. John Wiley & Sons, Ltd. Pub. in The Cochrane Library 2014;Issue 2.

Rovaris M, Comi G, Filippi M. MRI markers of destructive pathology in multiple sclerosis-related cognitive dysfunction. J Neurol Sci 2006;245:11-116.

Roxburgh RHSR, Seaman SR, Masterman T. et al. Multiple sclerosis severity score: Using disability and disease duration to rate disease severity. Neurology 2005;64:1144-51.

Sadovnick AD, Ebers GC, Wilson RW, Paty DW. Life expectancy in patients attending multiple sclerosis clinics. Neurology 1992;42:991-94.

Sadovnick AD, Eisen K, Ebers GC, Paty DW. Cause of death in patients attending multiple sclerosis clinics. Neurology 1991;41:1193-6.

Sailer M, Losseff NA, Wang L, Gawne-Cain L, Thompson AJ, Miller D. T1 lesion load and cerebral atrophy as a marker for clinical progression in patients with multiple sclerosis. A prospective 18 months follow-up study. Eur J Neurol 2001;8:37-42.

Sanfilipo MP, Benedict RHB, Weinstock-Guttman B, Bakshi R. Gray and white matter brain atrophy and neuropsychological impairment in multiple sclerosis. Neurology 2006;66:685-92.

Sibley WA. Diagnosis and course of multiple sclerosis. In: Neurobehavioral aspects of multiple sclerosis. Ed. Rao SM. Oxford University Press 1990.

Schoonheim MM, Vigeveno RM, Rueda Lopes FC, Pouwels PJW, Polman CH, Barkhof F. et al. Sex-specific extent and severity of white matter damage in multiple sclerosis: Implications for cognitive decline. Hum Brain Mapp 2014;35:2348-58.

Schwid SR, Tyler CM, Schneid EA. et al. Cognitive fatigue during a test requiring sustained attention: a pilot study. Mult Scler 2003;9:503-08.

Seewann A, Vrenken H, Kooi E-J, van der Valk P, Knol DL, Polman CH. et al. Imaging the tip of the iceberg: visualization of cortical lesions in multiple sclerosis.

Shi J, Zhao CB, Vollmer TL, Tyry TM, Kuniyoshi SM. APOE epsilon 4 allele is associated with cognitive impairment in patients with multiple sclerosis. Neurology 2008;70:185-90.

Sicotte NL, Kern KC, Giesser BS. et al. Regional hippocampal atrophy in multiple sclerosis. Brain 2008;131:1134-1141.

Smith M. The Role of proteolytic enzymes in autoimmune demyelinating diseases: An update. In: Role of Proteases in the Pathophysiology of Neurodegenerative Diseases. Eds. Lajtha A, Banik NL. Kluwer Academic/Plenum Publishers, New York 2001.

Spreen O, Strauss E. 1998 A compendium of neuropsychological tests. 2nd edn. Oxford University Press, Oxford.

Summers MM, Fisniku LK, Anderson VM, Miller DH, Cipolotti L, Ron M. Cognitive impairment in relapsing-remitting multiple sclerosis can be predicted by imaging performed several years earlier. Mult Scler 2008;14:197-204.

Sumowski JF, Chiaravalloti N, Wylie G, DeLuca J. Cognitive reserve moderates the negative effect of brain atrophy on cognitive efficiency in multiple sclerosis. J Int Neuropsychol Soc 2009;15:606-12.

Swanborg RH, Whittum-Hudson JA, Hudson AP. Infectious agents and multiple sclerosis – are Chlamydia pneumoniae and human herpes virus 6 involved? J Neuroimmunol 2003;136:1-8.

Swirsky-Sacchetti T, Mitchell DR, Seward J. Neuropsychological and structural brain lesions in multiple sclerosis: a regional analysis. Neurology 1992;42:1291-95.

Tekok-Kilic A, Benedict RH, Weinstock-Guttman B. et al. Independent contributions of cortical gray matter atrophy and ventricle enlargement for predicting neuropsychological impairment in multiple sclerosis. Neuroimage 2007;36:1294-3000.

Thornton AE, Raz N, Tucke KA. Memory in multiple sclerosis: contextual encoding deficits. J Int Neuropsychol Soc 2002;8:395-409.

Tröster A, Fields J, Testa J, Paul R, Blanco C, Hames K. et al. Cortical and subcortical influences on clustering and swiching in the performance of verbal fluency tasks. Neuropsychologia 1998;36:295-304.

Troyer A, Moscovitch M, Winocour G. Clustering and switching as two components of verbal fluency: Evidence from younger and older healthy adults. Neuropsychology 1997;11;138-46.

Van Schependom J, D´hooghe MB, De Schepper M, Cleynhens K, D´hooghe M, Haelewyck MC. et al. Relative contribution of cognitive and physical disability components to quality of life in MS. J Neurol Sci 2014;336:116-121.

Wechsler D: Wechsler Adult Intelligence Scale – Revised: Manual. San Antonio/Tx, Psychological Corporation, 1981.

Weinshenker BG, Bass B, Rice GPA, et al. The natural history of multiple sclerosis: a geographically based study. I. Clinical course and disability. Brain 1989;112:133-146.

Weinshenker BG, Ebers GC. The natural history of multiple sclerosis. Can J Neurol Sci 1987;14:255-61.

Weinshenker BG, Rice GPA, Noseworthy JH, et al. The natural history of multiple sclerosis: A geographically based study. 3 Multivariate analysis of predictive factors and models of outcome. Brain 1991; 114:1045-1056.

Wishart HA, Saykin AJ, McDonald BC. et al. Brain activation patterns associated with working memory in relapsing-remitting MS. Neurology 2004;62:234-38.

Yaldizli Ö, Penner IK, Frontzek K, Naegelin Y, Amann M, Papadopoulou A. et al. The relationship between total and regional corpus callosum atrophy, cognitive impairment and fatigue in multiple sclerosis patients. Mult Scler J 2014;20:356-64.

Yamanaka K, Saito Y, Yamamori T, Urano Y, Noguchi N. 24(S)-hydroxycholesterol induces neuronal cell death through necroptosis, a form of programmed necrosis. J Biol Chem 2011;286:2466-73.

Zivadinov R, Sepcic J, Nasuelli D, De Masi R, Monti Bragadin L, Tommasi MA. et al. A longitudinal study of brain atrophy and cognitive disturbances in the early phase of relapsing-remitting multiple sclerosis. J Neurol Neurosurg Psychiatry 2001;70:773-80.