From
the Department of Clinical Science, Intervention and Technology
the Division of Medical Imaging and Technology
Karolinska Institutet, Stockholm, Sweden

NEURORADIOLOGICAL ASPECTS OF MULTIPLE SCLEROSIS:
FROM EARLY SIGNS TO LATE DISEASE STAGES

Tobias Granberg

Karolinska Institutet

Stockholm 2015

Published by Karolinska Institutet.
Printed by E-print AB 2015

ISBN 978-91-7549-996-3

Department of Clinical Science, Intervention and Technology
Division of Medical Imaging and Technology

Neuroradiological aspects of Multiple Sclerosis: from early signs to late disease stages

THESIS FOR DOCTORAL DEGREE (Ph.D.)

which will be publicly defended in Lecture hall 9Q,
Alfred Nobels allé 8, Karolinska Institutet, Huddinge

Friday September 4th 2015

By
Tobias Granberg

Principal Supervisor:
Associate professor Maria Kristoffersen-Wiberg
Karolinska Institutet
Department of Clinical Science,
Intervention and Technology
Division of Medical Imaging and Technology

Co-supervisor(s):
Professor Sten Fredrikson
Karolinska Institutet
Department of Clinical Neuroscience
Division of Neurology

Professor Peter Aspelin
Karolinska Institutet
Department of Clinical Science,
Intervention and Technology
Division of Medical Imaging and Technology

Doctor Juha Martola
Karolinska Institutet
Department of Clinical Science,
Intervention and Technology
Division of Medical Imaging and Technology

Opponent:
Associate professor Anders Svenningsson
Umeå University
Department of Pharmacology
and Clinical Neuroscience
Division of Clinical Neuroscience

Examination Board:
Professor Sven Ekholm
University of Gothenburg
The Institute of Clinical Sciences
Department of Radiology

Professor Pia Maly Sundgren
Lund University
Department of Diagnostic Radiology
Clinical Sciences Lund

Associate professor Magnus Andersson
Karolinska Institutet
Department of Clinical Neuroscience
Division of Neurology

Stockholm 2015

To my dear wife Alexandra and

my parents Jan-Olov and Ritha,

as well as my sisters, Erika, Lovisa and Stina.

"*After climbing a great hill, one only finds that there are many more hills to climb*"

— Nelson Mandela (1918-2013)

ABSTRACT

Background: Multiple sclerosis (MS) is a chronic inflammatory and degenerative disease of the central nervous system and a leading cause of neurological disability in young adults. Magnetic resonance imaging (MRI) has improved the diagnostic process in MS, but has also led to incidental MS-like findings. The growing therapeutic arsenal and the variable clinical expression of MS makes MRI important for evaluating treatment response and advanced volumetric measurements are common endpoints in MS treatment trials. More feasible MRI measurements are, however, needed in order to implement quantitative MRI biomarkers in clinical practice, where they may aid in individualizing treatment and care for MS patients.

Purpose: The aim of this thesis is to describe neuroradiological aspects of MS, from its earliest signs to late stages, by describing the frequency and significance of incidental MRI findings suggestive of MS, and by studying corpus callosum atrophy as a biomarker for cognitive and physical disability in MS over a wide range of disease duration.

Study I, a systematic review, showed that incidental brain MRI findings suggestive of MS without typical MS symptoms, and with no better explanation of the findings, are of clinical importance. This entity is preferably called radiologically isolated syndrome (RIS) and persons with RIS often have subclinical cognitive impairment and radiological measurements similar to those seen in MS. RIS progresses radiologically in a majority of cases and about one third of the patients are diagnosed with MS over a mean follow-up time of five years.

Study II, a retrospective cohort study, showed that RIS is an uncommon finding. In a yearly sample of the brain MRI examinations of 2105 patients at Karolinska University Hospital, only one case of RIS was found (0.05%). The patient later developed clinically active MS.

Study III compared the performance and feasibility of the two leading radiological methods for assessing corpus callosum atrophy, corpus callosum area (CCA) and corpus callosum index in a cross-sectional evaluation of the participants in Study IV. Both measurements could be obtained in less than a minute with excellent repeatability. CCA had the strongest correlations with cognitive and physical disability, and was most accurate in differentiating patients from controls and relapse-remitting MS from progressive forms of MS.

Study IV was a 17-year longitudinal cohort study of 37 MS patients that were evaluated clinically, neuropsychologically and radiologically, and a matched healthy control group. The disease durations spanned over five decades, reflecting a panorama of early to late stages of the disease. The corpus callosal atrophy rate decreased with increasing disease duration. The normalized corpus callosum area was correlated with cognitive ($r = 0.79$, $p < 0.001$) and physical ($r = -0.55$, $p < 0.001$) disability, outperforming commonly used volumetric methods.

Conclusions: RIS is a rare but clinically important condition that in many cases constitutes preclinical MS. CCA is a feasible measurement of corpus callosum atrophy for MS research and clinical practice, and outperforms classical volumetric measurements as a biomarker for cognitive and physical disability in MS.

SAMMANFATTNING

Bakgrund: Multipel skleros (MS) är en kronisk inflammatorisk och degenerativ sjukdom som drabbar hjärna och ryggmärg. Skadornas utbredning varierar, vilket leder till att symtomen kan skilja sig påtagligt åt mellan individer. Undersökning med magnetkamera (MR) kan påvisa tecken till MS och bidrar till förbättrad MS-diagnostik, men också till bifynd som radiologiskt liknar MS hos personer som undersöks av andra skäl. De senaste två decennierna har behandlingsmöjligheterna vid MS förbättrats, vilket ökat betydelsen av MR för att utvärdera terapieffekten. Avancerade MR-volymmått av hjärnan har därför kommit att bli viktiga utfallsmått i läkemedelsstudier. MR-mått måste dock vara praktiska för att kunna tillämpas i kliniskt arbete, där de kan bidra till individanpassad behandling vid MS.

Syfte: Den här avhandlingen syftar till att beskriva neuroradiologiska aspekter av MS, från sjukdomens tidigaste tecken till dess sena stadier, genom att beskriva förekomsten och betydelsen av oväntade MR-fynd som liknar MS, och genom att studera atrofi av hjärnbalken (corpus callosum) som markör för fysisk och kognitiv funktionsnedsättning vid MS.

Studie I, en systematisk översiktsartikel, visade att MR-bifynd som liknar MS hos personer som inte har typiska MS-symtom är kliniskt betydelsefulla. Personer med detta tillstånd (lämpligen kallat radiologiskt isolerat syndrom, RIS) uppfyller inte kriterierna för MS, men har ofta kognitiva funktionsnedsättningar och MR-mätvärden som liknar de som ses vid MS. Hos de flesta ses en progress av MR-fynden och en tredjedel utvecklar MS inom fem år.

Studie II, en retrospektiv kohortstudie, visade att RIS är ett ovanligt tillstånd. Vid en genomgång av samtliga MR-undersökningar som utfördes under ett års tid vid Karolinska Universitetssjukhuset i Huddinge återfanns endast 1 fall av RIS bland 2105 personer (0,05%).

Studie III jämförde två radiologiska metoder för mätning av atrofi i corpus callosum vid MS. De två metoderna, corpus callosum area (CCA) och corpus callosum index (CCI), tillämpades i patientgruppen som beskrivs i Studie IV. Båda metoderna kunde mätas inom en minut med utmärkt reproducerbarhet. CCA var starkast korrelerat till kognitiv och fysisk funktionsnedsättning vid MS. CCA var även mest tillförlitligt i att skilja MS-patienter från friska kontrollpersoner och i att skilja MS med progressivt och skovvist förlopp.

I **Studie IV** följdes 37 MS-patienter neurologiskt, neuropsykologiskt och radiologiskt under 17 år. Patienternas sjukdomsdurationer omfattade fem decennier, avspeglande tidiga till sena faser av MS. Hjärnbalksatrofin avtog med tiden och normaliserat CCA var starkt kopplat till kognitiv funktionsnedsättning och måttlig korrelerat till fysisk funktionsnedsättning. Dessa korrelationer var starkare än motsvarande samband för volymmått av hjärnvävnaderna.

Slutsatser: RIS är ett ovanligt men kliniskt betydelsefullt tillstånd som i många fall utgör en preklinisk fas av MS. CCA är ett praktiskt mått för att bedöma hjärnbalksatrofi, vilket presterar bättre än CCI och volymmått av hjärnan som markör för kognitiv och fysisk funktionsnedsättning vid MS. CCA kan således vara ett lämpligt mått för MS-forskning och kliniskt arbete.

LIST OF SCIENTIFIC PAPERS

This thesis is based on the following four papers, which will be referred to in the text by their roman numerals.

I. **Radiologically isolated syndrome – incidental magnetic resonance imaging findings suggestive of multiple sclerosis, a systematic review.**
Granberg T, Martola J, Kristoffersen-Wiberg M, Aspelin P and Fredrikson S.
Multiple Sclerosis Journal. 2013 Mar;19(3):271–80.

II. **Radiologically isolated syndrome: an uncommon finding at a university clinic in a high-prevalence region for multiple sclerosis.**
Granberg T, Martola J, Aspelin P, Kristoffersen-Wiberg M and Fredrikson S.
BMJ Open. 2013 Nov;3(11):e003531.

III. **MRI-defined corpus callosal atrophy in multiple sclerosis: a comparison of volumetric measurements, corpus callosum area and index.**
Granberg T, Bergendal G, Shams S, Aspelin P, Kristoffersen-Wiberg M, Fredrikson S, Martola, J.
Journal of Neuroimaging. E-published ahead of print 2015 Mar 19.
DOI: 10.1111/jon.12237.

IV. **Corpus callosum atrophy is strongly associated with cognitive impairment in multiple sclerosis: results of a 17-year longitudinal study.**
Granberg T, Martola J, Bergendal G, Shams S, Damangir S, Aspelin P, Fredrikson S and Kristoffersen-Wiberg M.
Multiple Sclerosis Journal. E-published ahead of print 2014 Dec 4.
DOI: 10.1177/1352458514560928.

CONTENTS

LIST OF ABBREVIATIONS

2D	Two-dimensional
3D	Three-dimensional
ASL	Arterial spin labeling
AUC	Area under the curve
BICAMS	Brief international cognitive assessment for multiple sclerosis
BOLD	Blood-oxygen-level dependent
BPF	Brain parenchymal fraction
BV	Brain volume
CCA	Corpus callosum area
CCI	Corpus callosum index
CIS	Clinically isolated syndrome
CNS	Central nervous system
CSF	Cerebrospinal fluid
CT	Computed tomography
DIR	Double inversion recovery
DIS	Dissemination in space
DIT	Dissemination in time
DMT	Disease modifying therapy
DTI	Diffusion tensor imaging
EDSS	Expanded disability status scale
FLAIR	Fluid attenuated inversion recovery
fMRI	Functional magnetic resonance imaging
GMF	Grey matter fraction
GMV	Grey matter volume
ICC	Intra-class correlation
IgG	Immunoglobulin G
IQR	Interquartile range
LV	Lesion volume
MPRAGE	Magnetization-prepared rapid acquisition gradient echo
MRI	Magnetic resonance imaging

MS	Multiple sclerosis
MSFC	Multiple sclerosis functional composite
nCCA	Normalized corpus callosum area
nLV	Normalized lesion volume
OR	Odds ratio
PACS	Picture archiving communicating system
PASAT	Paced auditory serial addition test
PET	Positron emission tomography
PPMS	Primary progressive multiple sclerosis
PRISMA	Preferred reporting items for systematic reviews and meta-analyses
PSIR	Phase-sensitive inversion recovery
RIS	Radiologically isolated syndrome
RRMS	Relapsing–remitting multiple sclerosis
SD	Standard deviation
SDMT	Symbol digit modalities test
SPMS	Secondary progressive multiple sclerosis
T	Tesla
WMF	White matter fraction
WMV	White matter volume

1 INTRODUCTION

1.1 MULTIPLE SCLEROSIS

1.1.1 Overview and historical background

Multiple sclerosis (MS) is a common chronic immune-mediated disease that affects the central nervous system (CNS), leading to neurological dysfunction.[1] The name originates from the fact that the disease causes damage at *multiple* locations in the brain and spinal cord where the inflammatory lesions leave *sclerotic* scars.[2] In Swedish, MS can be translated as "många ärrhärdar" or more loosely as "Många Skadeställen".[3]

MS mainly affects young otherwise healthy persons with a mean age at MS onset of 29 years. The disease often leads to both physical and cognitive disability, but the disease course is hard to predict as some patients will have a benign course, while others will have a relentless disease progression. In either case, MS has significant effects on both an individual and a community level, where the disease is estimated to cause costs of 9 billion euros per year in the European Union alone.[2]

The first comprehensive descriptions of the disease stems from the late 18th century, and early pathological descriptions came in the first half of the 19th century.[4,5] The disease is considered to have been characterized as a separate disease entity by the French physician Jean-Martin Charcot in 1868.[6] In the near one and a half century that has passed since Charcot's description of the disease, there has been ever increasing research interest in MS, not least after the introduction of effective disease-modifying therapies (DMT) and the subsequent increasing therapeutic arsenal. These research efforts have dramatically improved our knowledge and understanding of the disease. A summary of the current knowledge base with focus on neuroradiological aspects is presented below.

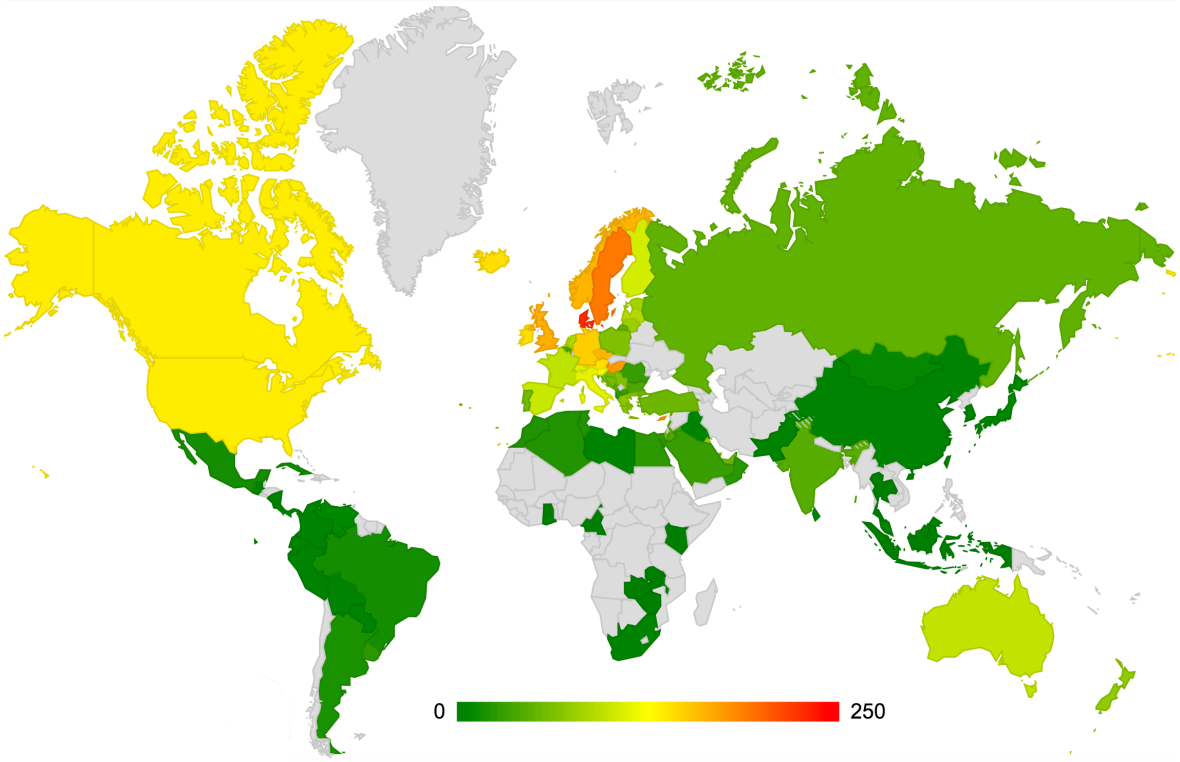
1.1.2 Epidemiology

Globally, around 2.5 million people have MS, but there are large variations in prevalence and incidence of MS across different regions, as illustrated in Figure 1.[7] MS is a relatively common disease in Scandinavia, and Sweden has one of the highest reported frequencies of MS with an incidence of 10.2 per 100,000 person-years and a prevalence of 189 per 100,000 inhabitants.[8,9] There is also a prominent sex difference in MS, where women are more than twice as likely to be affected by MS. In Sweden, the current female to male ratio is 2.5.[8] The prevalence of MS is increasing, which is mainly attributed to increases in life expectancy.[10]

The skewed geographical distribution of MS has been attributed to a combination of genetic and environmental factors and has been the focus of numerous epidemiological studies trying to better understand MS pathophysiology. It has for example been shown that close relatives of MS patients have a higher risk of developing MS than the general population. Monozygotic twins of MS patients have a lifetime risk of developing MS around 30%, while first degree relatives have a risk of around 2-5% and half-siblings around 1%,[11] which can be

compared to the general population risk of around 0.2% in Sweden.[8] Genetic studies have so far uncovered more than 100 risk gene variants for MS, but many of these variants are common in the general population and each variant only carries a modestly increased risk for MS. Interestingly, all of the identified genes are associated with immunity.[12]

Figure 1. *National prevalence of MS per 100,000 inhabitants. Data based on a WHO survey in 2004, updated by the MS International Federation in 2013.*[13]



The importance of environmental factors is highlighted by the fact that the risk of developing MS changes with migration. The MS risk becomes intermediate of that attributed to the region of origin and the new region, with a greater adaption to the new region's risk when moving before adolescence.[14] A phenomenon that has gained much interest is that the prevalence of the disease follows a North and South gradient from the equator, with increasing prevalence closer to the poles.[10,15] This has also been shown in Sweden, where the prevalence of MS increases with 1.0–1.5% per degree of north latitude.[8] Solar ultraviolet radiation, and secondarily vitamin D_3-levels that are dependent on skin exposure to sunlight as well as dietary intake of vitamin D, have therefore been proposed as protective factors.[16]

An umbrella review of environmental risk factors for MS recently found that among 44 studied possible risk factors (including comorbidities, infections, trauma, vaccinations and toxic substances), only three risk factors were supported by strong epidemiological evidence: immunoglobulin G (IgG) seropositivity for Epstein-Barr virus (EBV) nuclear antigen (random effects odds ratio, OR, 4.5), infectious mononucleosis (OR 2.2) and smoking (OR 1.5).[17] Although the association of MS and EBV exposure is significant, it is important to remember that a large majority of the healthy adult population are seropositive for EBV.[18]

1.1.3 Etiology and pathophysiology

The cause of MS remains unknown, but there is a growing body of knowledge regarding the pathophysiology of MS, which is likely to assist in identifying its genesis, although this discovery may continue to elude us for a long time ahead.

A majority of the nerve fibers, axons, in the brain are insulated by oligodendrocytes with a substance called myelin that consists of lipids (42%), water (40%) and proteins (18%).[19] In MS, the myelin is damaged, impeding nerve conduction and triggering neurological symptoms.[2] The histo-pathological hallmarks of MS are demyelination and perivenous inflammation, illustrated in Figure 2, with axonal loss, gliosis and neuronal degeneration.[20] The demyelination is caused by both immunological and neurodegenerative processes, but it is still debated which of these two components that are the primary and secondary driving force of the disease.[21]

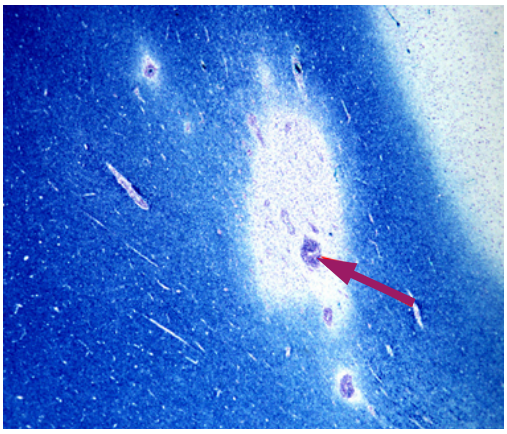


Figure 2. *Microscopic image with Luxol fast blue staining of a MS lesion showing perivenous inflammation (arrow) and demyelination (whiter area).[22] Image courtesy of professor Stephen DeArmond.*

MS is considered to be driven by self-reactive mononuclear cells (monocytes, B- and T-cells) which migrate across the blood brain barrier into the CNS.[2] Although T-cells have mainly been in focus, the importance of B-cells is highlighted by intrathecal IgG production, the clinical response to anti-CD20 therapies and the relationship of meningeal follicles with cortical lesions.[23,24] Intriguingly, there is large inter-individual heterogeneity in the inflammatory response seen in MS lesions microscopically with four different histopathological patterns.[20] It is, however, unclear what implications these findings may have for diagnosis, subtyping and treatment of MS.

1.1.4 Diagnosis

There is no pathognomonic feature of MS, nor is there an absolute diagnostic test for MS. The diagnosis therefore relies on diagnostic criteria demonstrating that the CNS is affected by the disease at two different locations, called dissemination in space (DIS), at two different points in time, called dissemination in time (DIT).[25]

The MS diagnostic criteria are constantly being revised according to new research findings in order to facilitate and increase the accuracy of MS diagnostics. In 1965, Schumacher et al. introduced the first modern criteria, which were solely based on clinical findings.[26] In 1983, Poser et al. incorporated paraclinical methods such as cerebrospinal fluid (CSF) analysis, evoked potentials and neuroimaging. Brain and spinal MRI lesions were integrated as a key diagnostic feature in the MS diagnostic criteria in 2001 by McDonald et al.,[27,28] and the McDonald criteria have since been revised in 2005 and in 2010.[25,29]

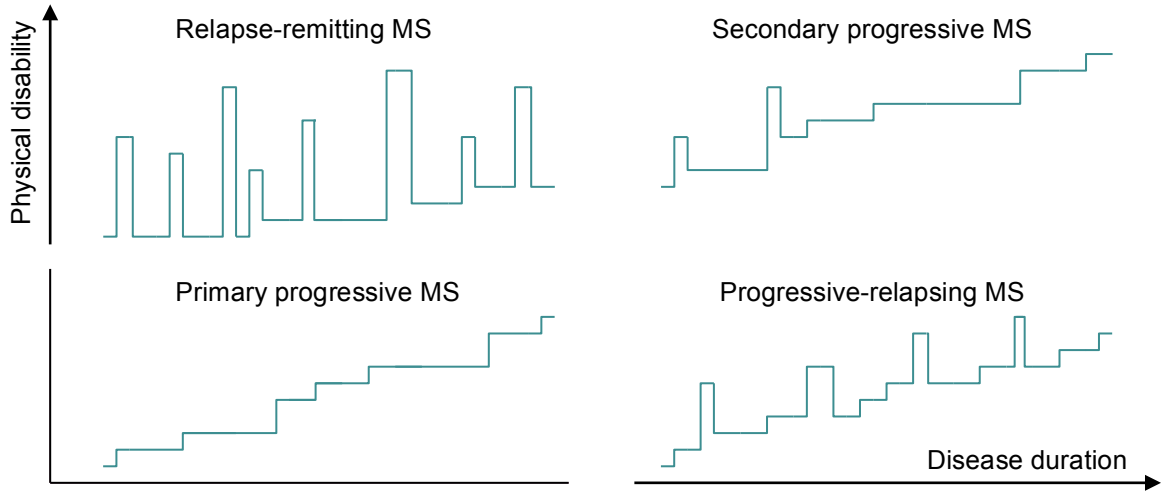
Clinically isolated syndrome (CIS) is a distinct entity where there is only clinical evidence of one symptomatic episode suggestive of MS and yet no evidence of DIT. CIS has been important as a pre-diagnostic stage of MS, but the prevalence of CIS is expected to decrease with the use of the latest McDonald criteria as a single MRI scan can now demonstrate both DIS and DIT.[25,30] The constantly adapting MS criteria mean that longitudinal MS research is studying a moving target, complicating comparisons with older studies.[31]

1.1.5 Clinical manifestations and subtypes

The clinical expression of MS is variable as any part of the CNS can be affected.[1] Frequent symptoms include sensory disturbances (numbness, tingling, pain, itching, vertigo), visual problems, affected motor abilities (walking difficulties, muscle spasms, tremor) and autonomic functions (sexual difficulties, bladder and bowel dysfunctions), as well as more diffuse symptoms (fatigue, depression, cognitive impairment).[1]

MS is classically divided in four subtypes with different disease courses,[32] illustrated in Figure 3. The most common form (85%) is relapse-remitting MS (RRMS), where there are acute episodes of worsening with full or partial recovery, interspersed with a period of remission until the next relapse. A relapse usually lasts less than a few months and the mean number of relapses is 0.4 per year.[2] Typically, relapses eventually become less frequent with accumulating disability, and about two third of RRMS patients will go on to a secondary progressive phase (SPMS) with a steady decline in neurological functions after 15-20 years.[33]

Figure 3. *Illustration of the disease course of the four classical MS subtypes. The drastic changes in physical disability represent relapses.*



In 15% of MS patients there is a progressive decline from onset called primary progressive MS (PPMS), or progressive-relapsing MS (PRMS) if there are superimposed relapses.[32] PPMS patients have a more even female to male ratio and are on average 10 years older at onset than RRMS patients. PPMS typically has a faster disease progression than RRMS, why patients with PPMS and SPMS will be about the same age when they reach important disability milestones.[21] The diagnostics of PPMS is more complicated than for RRMS as it is a less common presentation and does not provide relapses to prove DIT. Adding to the complexity is the higher age of the patients, leading to more comorbidities and a higher

incidence of other neurodegenerative diseases. The criteria for PPMS require one year of progressive worsening without remission and supportive evidence by two of the following: ≥1 brain lesions (periventricular, juxtacortical or infratentorial), ≥2 spinal cord lesions or abnormal CSF findings (oligoclonal bands, elevated IgG index).[25]

While the subtypes are helpful in giving a broad understanding of disease progression, this stratification does not fully reflect the individual clinical expression of MS. In 2014, a complementary categorization was introduced where the combination of clinical relapses and imaging findings are used to describe the disease as active/non-active and as having progression/no progression. This update has eliminated the need for the rarely used PRMS subtype, as it is now described as PPMS with disease activity.[30]

Similarly to the varying symptomatology, the disease activity and progression is highly individual, making it hard to predict the clinical outcome.[2] Clinical predictors associated with a poor prognosis are incomplete recovery after the first episode, a short remission until the first relapse and bladder/bowel disturbances at onset.[34]

1.1.6 Treatment

The treatment of MS has been revolutionized in the last two decades. The first effective disease-modifying therapies (DMTs) were introduced in the mid-1990s and since then the number of available treatments has increased substantially with many new DMT classes.[35] Early treatment has been supported by the fact that axonal damage is closely related to inflammation and occurs early in MS, and that the radiological and clinical progression of CIS can be delayed by DMTs.[36] It has also been shown that early treatment decelerates the longterm progression of RRMS,[37] and reduces the mortality rate 20 years later with 46%.[38]

Treatment options in RRMS have recently been further improved by the introduction of the first oral therapies.[39] Autologous hematopoietic stem cells transplantation has shown remarkable results in the treatment of aggressive MS.[40,41] There is also hope for finding effective therapy in progressive MS as treatment with statins, which are believed to have immunomodulatory effects, has shown promising results in reducing brain atrophy.[42] This expanded therapeutic arsenal does, however, complicate the treatment choice in individual patients as the treatment efficacies and side effects differ. Neuroradiological biomarkers can therefore play an important role in aiding neurologists to tailor treatment for their patients.[43]

1.1.7 Clinical measurements of disability

MS is the leading non-traumatic cause of neurological disability in young adults in Europe and the United states, which affects the patients' health-related quality of life.[1,2] In the natural progression of MS, patients typically need a cane when walking after two decades and a wheelchair after three decades of disease duration.[2]

The physical disability in MS is overt and there are numerous methods to quantify it. The consistently most used rating scale is the expanded disability status scale (EDSS) that was introduced by Kurtzke et al. in 1983.[44] It is a 10-grade scale of physical disability, illustrated

in Figure 4. Despite its wide use in clinical trials and MS research, the scale does have several limitations. The scoring is based on subjective neurological assessment, with poor reproducibility, and the scale is non-linear, which causes statistical limitations.[45]

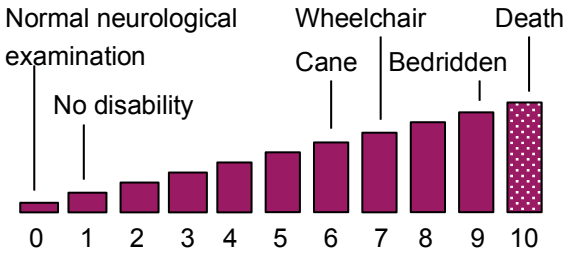


Figure 4. *The expanded disability status scale.*

The major argument against EDSS is that it does not reflect non-physical MS disability. There has hence been a lot of effort put into finding suitable replacement scales. The most commonly used alternative is the multiple sclerosis functional composite (MSFC), which is a multi-dimensional scale reflecting ambulation (25 m timed walk), hand function (9 hole peg test) as well as attention and information processing speed (paced auditory serial addition test, PASAT). Although MSFC solves many of the issues of EDSS, it has not yet come to replace EDSS, probably due to its limited practicability with 30 minutes of testing time.[45]

Cognitive impairment is common in MS (43-70%) and is present even in the earliest stages of the disease. Although the cognitive deficits can be subtle, they are disabling and affect the patients' health-related quality of life. The most consistently reported cognitive deficits include memory and visual learning disturbances, affected sustained and divided attention, information processing speed and abstract reasoning. Meanwhile, general intelligence and language functions remain relatively intact.[46]

The two most recognized neuropsychological test batteries are the *brief repeatable battery of neuropsychological tests* and the *minimal assessment of cognitive function in MS*. These take up to 1.5 hour to administer,[47] which is why recommendations have been stipulated for brief cognitive monitoring in order to make it more practical. The primary recommended test is the symbol digit modalities test (SDMT), measuring information processing speed.[48] In SDMT, the participant deciphers symbols into numbers with the help of a numerical key during 90 seconds. The test has good reproducibility[49] and is sensitive to cognitive decline as it reflects a neuroanatomically widely dispersed frontoparietal network.[50,51] We have chosen to focus on SDMT in our studies, which is in line with the aforementioned recommendations. In order to more globally detail cognitive functioning, we have also administered three complimentary tests for which the main concepts presented below:

- FAS, a phonemic verbal fluency test, reflecting an anatomically well defined frontotemporal function,[52] as the participant is asked to name as many words as possible during one minute starting with each of the letters F, A and S.[53]
- Rey-Osterrieth complex figure test - copy, assessing visuospatial constructional ability located in the parietal lobes and executive functions located prefrontally.[54]
- Rey auditory verbal learning test, evaluating verbal learning and memory associated with the medial temporal lobe. The participant is presented with 15 words and asked to repeat them, which is iterated five times (encoding). After 30 minutes the participant tries to recall as many words as possible (free retrieval), which involves additional prefrontal regions.[55]

1.2 MAGNETIC RESONANCE IMAGING IN MULTIPLE SCLEROSIS

MRI it the most important imaging method in MS due to its excellent tissue contrast, which exceeds the capabilities of computed tomography (CT), not least in the posterior fossa that is a common location for MS pathology. MRI's superiority for MS lesion detection in comparison with CT was reported as early as in the first published application of MRI in MS in 1981,[56] as shown in Figure 5. Overall, MRI can visualize positive findings in more than 95% of MS patients.[57] The key concepts of MRI in the diagnostic investigation, treatment surveillance, clinical trials and for increasing our pathophysiological understanding of MS are presented below.

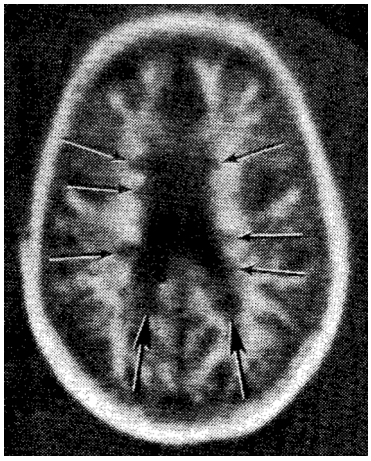


Figure 5. *The first published brain MRI in MS visualizing multiple lesions (arrows) in an 18-year-old female with MS.*[56]

1.2.1 Background and basic MRI physics

MRI is an advanced non-invasive imaging technique that is the gold standard imaging modality in many neurological disorders. The underlying concepts have been awarded several Nobel prizes.[58] One of the major advantages with MRI is that, unlike CT, it is not based on ionizing radiation. The imaging is instead constructed by using the interaction between atomic nuclei in the imaged subject and radio waves under the influence of strong magnetic fields. Clinical MRI typically operates at magnetic field strengths of 1.5 or 3.0 Tesla (T), equivalent of up to 60,000 times the strength of Earth's magnetic field.[59]

Some atomic nuclei have an inherent magnetic property called spin and basically act as "mini-magnets". The most abundant atomic nuclei with a spin in the body are the hydrogen atoms, also called protons. The protons align parallel or antiparallel with the main magnetic field in the MRI scanner and spin around their own axis. A few more protons align parallel with the main field (10 per million per 1.5 T), causing a small magnetic vector that can be imaged under the right circumstances. By applying magnetic field gradients and radio waves with the right frequency (resonating with the protons' spin) the direction of the protons can be altered. The radio waves and gradient fields are collectively called pulse sequences and are used to "tip" the magnetic vector so that it can be measured with metallic coils acting as antennas. Different MRI contrast weightings are obtained by manipulating the net magnetic vector and measuring the effects, resulting in excellent soft tissue contrast and high sensitivity for pathological changes.[59]

1.2.2 Conventional MRI sequences

Standard MRI sequences in MS include PD-, T1- and T2-weighted images along with fluid-attenuated inversion recovery (FLAIR) and contrast-enhanced T1-weighted images. An example of these tissue contrasts is illustrated in Figure 6. The improved signal-to-noise ratio that 3 T provides compared to 1.5 T can generally be used to improve the lesion contrast, spatial resolution and/or reduce the acquisition time.

Proton density (PD) weighted images simply reflect the amount of signal that is obtained from the tissues, as the signal is proportional to the number of protons.[59] In clinical practice, this weighting is suitable for detecting lesions in areas that can be prone to image artifacts on other sequences, i.e. mainly to detect or confirm infratentorial and spinal lesions.

T2-weighted images are sensitive to water content, for instance the CSF. MS lesions are typically seen as hyperintensities on T2-weighted images due to increased water content in edema or loss of normal tissue that is replaced by CSF. The findings are, however, not specific for MS. Some of these lesions will also eventually disappear.[57] By adding an inversion pulse to reduce the signal from free water such as CSF, a T2-**FLAIR** is obtained. This increases the sensitivity for MS lesions and FLAIR images are therefore important in clinical practice.[60] FLAIR images are, however, prone to artifacts, why lesions generally have to be confirmed on other sequences (i.e. PD-, T1- or T2-weighted images).[57] Three-dimensional (3D) acquisition of the FLAIR images may increase lesion sensitivity additionally.[61,62]

T1-weighted images are often used to confirm the location of MS lesions, but only 10-30% of T2-hyperintense lesions are also seen on traditional T1 sequences. The contrast in T1-weighted images is to a large extent dependent on the lipid content of the tissues, and the image intensity is reduced as the fatty myelin is damaged in MS, displayed as low signal (hypointensities) in the T1-weighted images. MS lesions with low T1-signal compared to the normal-appearing white matter are referred to as "black holes" and are more strongly correlated to axonal loss and physical disability than MS lesions only seen on T2-weighted images.[60,63] 3D T1-weighted sequences such as magnetization-prepared rapid acquisition with gradient echo (MPRAGE) add the beneficial possibilities of multi-planar reconstructions and volumetric analysis of the brain.[64] The proportion of lesions that are seen on T1-weighted images increase with MPRAGE sequences and the field strength.[65]

Contrast-enhancement after intravenous administration of Gadolinium-based contrast media is most commonly imaged on T1-weighted images where the paramagnetic Gadolinium shortens T1-relaxation times, and increases the signal intensity.[59] The blood brain barrier may be disrupted if there is active inflammation in the CNS, leading to increased permeability for cells, macromolecules

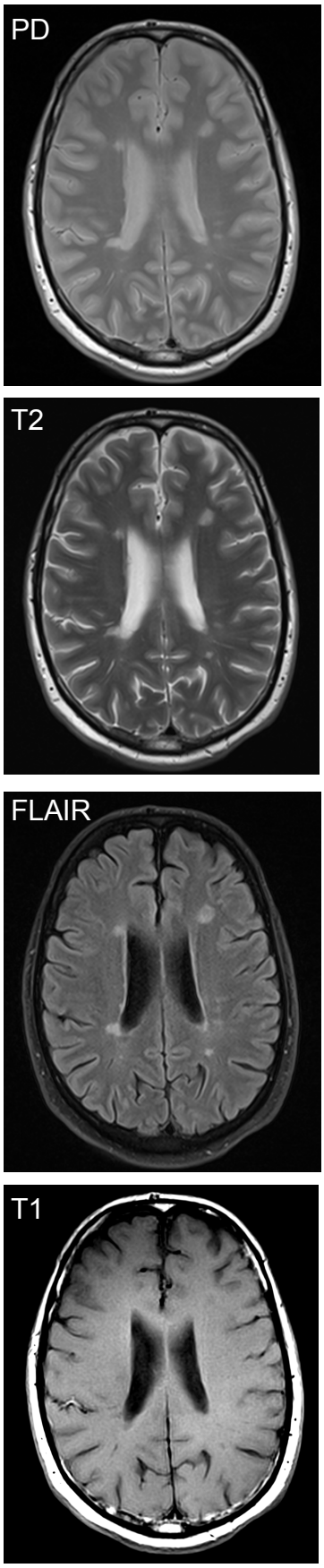


Figure 6. *Axial non-contrast MRI in a 49-year-old male MS patient. PD, T2, FLAIR and T1 indicates image type/weighting.*

and contrast media. Drastic changes in signal intensity between native and contrast-enhanced images are thus strongly indicative of active inflammation.[60]

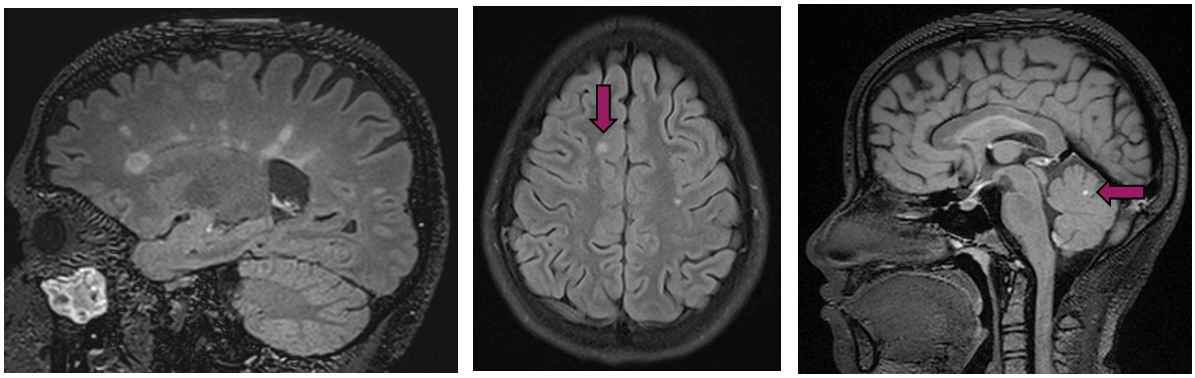
Most MS lesions initially go through a phase with contrast-enhancement that typically resolves within 2-6 weeks.[66,67] The sensitivity for contrast-enhancing lesions is highly dependent on the dose and the timing of imaging. Although higher contrast media doses result in more detected contrast-enhancing lesions, standard doses are generally recommended in order to reduce the risk for side effects such as hypersensitivity reactions and nephrogenic systemic sclerosis.[57,67] Dynamic brain MRI scans have reported that newly formed MS-lesions usually show a centrifugal (outwards) filling of contrast media, while subacute lesions more typically show an early ring enhancement with centripetal (inwards) filling.[68] Reactivated chronic lesions typically display ring enhancement.[69]

1.2.3 Lesion morphology and topography

MS lesions are typically ovoid or rounded as they are centered along a venule and have certain predilection sites, illustrated in Figure 7 and 8. MS lesions are typically:

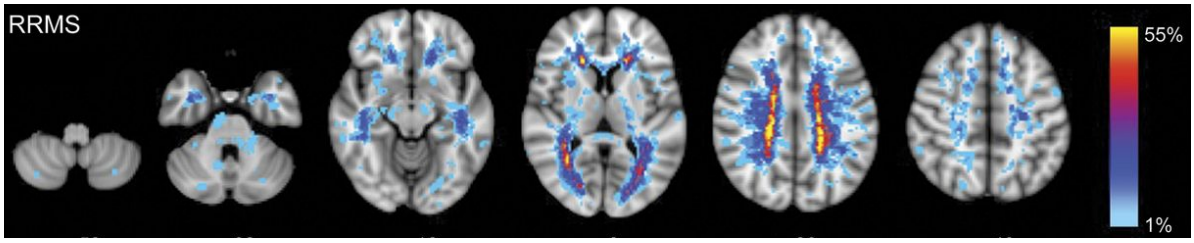
- ***Periventricular:*** These lesions are centered around venous vessels radiating perpendicularly from the ventricles and the corpus callosum into the centrum semiovale. They can therefore have a finger-like appearance, a radiological sign called Dawson's fingers.[57] The surface of the corpus callosum adjacent to the lateral ventricles is affected by lesions in 55-95% of all MS patients.[70]
- ***Juxtacortical:*** MS lesions commonly affect the short communicating fibers, called U-fibers, that project tangentially alongside the cortex inbetween associated cortical areas and thus compose the white matter adjacent to the cortex.[43]
- ***Infratentorial:*** Lesions below the cerebellar tentorium are common in MS and lesions in the brainstem and cerebellum are helpful in increasing the specificity of MS suspected white matter abnormalities.[71,72] T2-weighted images have classically been considered to be more sensitive for infratentorial lesions than FLAIR images, but this may not be true for 3D FLAIR acquisitions. Detection of infratentorial lesions may also be affected by pulsation and flow artifacts.[73]

Figure 7. *Periventricular MS lesions in a "Dawson's fingers" pattern (left), a juxtacortical lesion (middle, arrow) and a contrast-enhancing infratentorial lesion (right, arrow).*



- ***Spinal:*** The spinal cord is also affected in MS and spinal lesions are mainly located in the cervical medulla.[74] Spinal lesions are often symptomatic and tend to correlate relatively well with EDSS (due to EDSS' focus on physical mobility), but there are also asymptomatic spinal lesions.[75,76] These can be used as diagnostic clues as asymptomatic spinal lesions are uncommon in other diseases and may strengthen the radiological suspicion of MS in patients with equivocal brain white matter anomalies. However, spinal imaging is complicated by the small size and mobility of the spinal cord in combination with flow, pulsation and susceptibility artifacts.[77]
- ***Optical nerves***: About half of all MS patients experience at least one optical neuritis, which is also a frequent presenting symptom. Dedicated fat-suppressed coronal images of the optical nerves and chiasm should be obtained if there are visual symptoms.[57,78]

Figure 8. *MS lesion distribution in 50 RRMS patients projected on the Montreal Neurological Institute (MNI) standard space template. The colour scale represents the probability (range 1-55%) of finding MS lesions at the different sites.*[79]



1.2.4 The diagnostic role of MRI in MS

The radiological classification of suspected MS lesions have been revised along with the clinical diagnostic criteria. Paty et al. defined the earliest radiological classification in 1988,[80] followed by Barkhof et al. in 1997, which was later refined by Tintoré et al. in 2000.[81] The current MRI classification, defined by Swanton et al.,[82] simplified the requirements for DIS while increasing the overall accuracy of the diagnostics, exemplified in Table 1.

Table 1. *Comparison of the two latest revisions of the radiological classifications for MS lesions.*

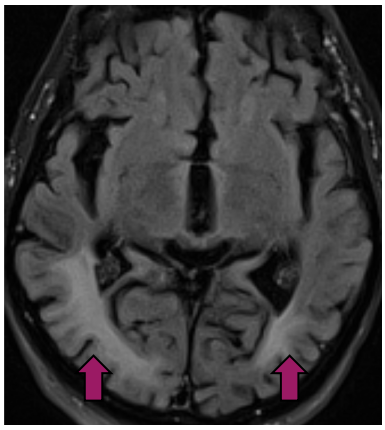
MS criteria	**McDonald 2005**[29]	**McDonald 2010**[25]
Radiological classification	**Barkhof-Tintoré**[81]	**Swanton**[71]
Demonstration of DIS	At least 3 out of: ≥3 periventricular lesions ≥1 juxtacortical lesion ≥1 infratentoriell or spinal lesion ≥1 contrast-enhancing or ≥9 lesions	At least 2 out of: ≥1 periventricular lesion ≥1 juxtacortical lesion ≥1 infratentoriell lesion ≥1 spinal lesion
Demonstration of DIT	- New lesion(s) ≥1 month after the initial clinical event - Contrast-enhancing lesion(s) ≥3 months after the initial clinical event	- New/contrast-enhancing lesion(s) on follow-up *and/or* - Concomitant asymptomatic contrast-enhancing lesion(s)
Sensitivity, specificity[82]	60%, 88%	72%, 87%

There are regional differences in MS that also have to come into consideration from a radiological perspective. For instance, Asian MS patients are typically older, have a lower incidence of oligoclonal bands and more commonly present with an optico-spinal form of the

disease. This means that the McDonald criteria have to be modified when applied to Asian patients and that the imaging protocols should also focus on optical nerve and spinal imaging in these patients.[57]

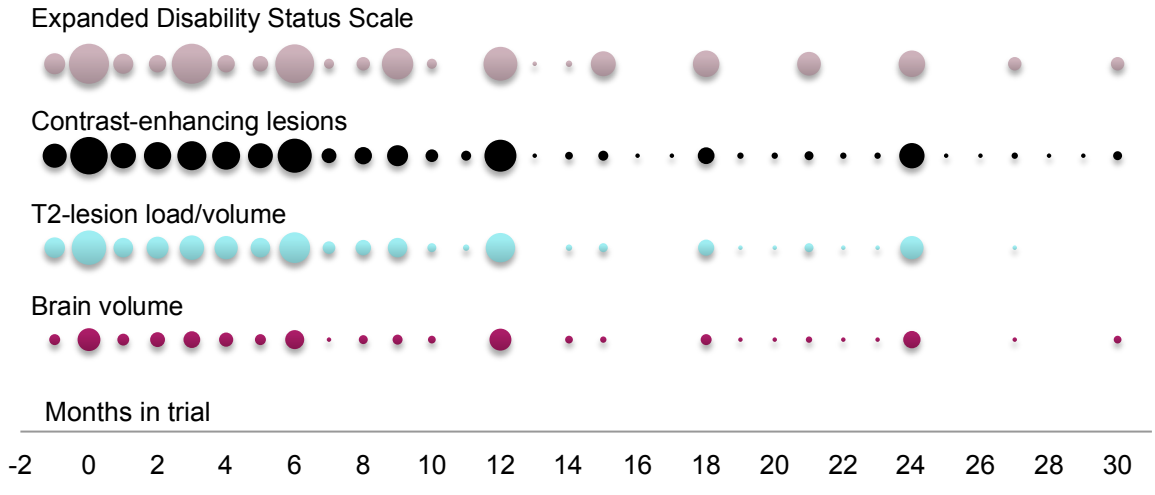
Surveillance: MRI is used in clinical practice to monitor treatment response.[57] MRI activity is defined as new, enlarging or contrast-enhancing lesions.[30] Lack of activity indicates suppression of the inflammation and supports continuation of the current treatment, while activity may indicate a need for more frequent follow-ups or a change of therapy.[57] MRI is also used to detect progressive multifocal leukoencephalopathy (PML), a rare but serious side effect of the drug natalizumab where there is an infection or re-activation of JC virus in oligodendrocytes, a virus that many healthy persons carry in a dormant state. PML has a heterogenous MRI appearance but is often seen as large diffuse white matter lesions (see Figure 9) with restricted diffusion and may have contrast-enhancement and/or cyst-like appearance.[83]

Figure 9. *FLAIR of a MS patient with PML showing diffuse white matter changes (arrows). Image courtesy of Juha Martola.*



Treatment trials: MRI's excellent ability to demonstrate disease activity is used to study the efficacy of MS drugs. MRI based measurements are used as the primary outcome in phase II studies and are important secondary endpoints in phase III trials. An illustration of the use of radiological outcome measures in MS trials is presented in Figure 10. T2 lesion load/volume and contrast-enhancing lesions are the two most commonly used measures. There are strong correlations between the effect of treatment on MRI activity and clinical relapses, supporting the rationale of using imaging surrogate markers.[84] The trend towards lower relapse rates in MS patients and a decreasing number of untreated patients make clinical outcome measures harder to reach and highlights the importance of imaging biomarkers as outcome measures.[67]

Figure 10. *Frequency of MRI-based endpoints in phase I-IV clinical trials from 1993-2014 (88 studies). The sizes of the circles represent the number of studies using the respective measurements at a certain trial time point. The largest circles (EDSS at month 0 and 3) represent 80 trials.*[85]



Pathophysiological insights: MRI has in many cases overtaken autopsies and biopsies as the most important instruments to study the pathological processes in MS due to practical reasons, its non-invasive nature, reproducibility and repeatability.[60] Non-conventional MRI techniques (see section 1.2.8) have also added to MRI's multi-dimensionality and provided several new quantitative measures of focal and diffuse MS pathology.[86]

Red flags: It is important to remember that white matter changes are frequent in healthy individuals and increase with age. They are also common in other diseases than MS. Mimics of MS are numerous and other causes for white matter abnormalities are, amongst others: normal aging, small-vessel disease, vasculitis, acute disseminated encephalomyelitis (ADEM), PML, encephalitis, neuroborreliosis (Lyme disease), sarcoidosis, toxic substances, leukodystrophies, Susac's syndrome, cerebral autosomal-dominant arteriopathy with subcortical infarcts and leukoencephalopathy (CADASIL), alcohol overconsumption and vitamin B12 deficiency.[66]

Brain white matter changes should therefore be interpreted thoughtfully if the findings are atypical for MS or if there is a better alternative explanation for the patients' presenting symptoms. Findings that should prompt caution in interpreting findings as MS include: [66]

- No brain lesions (i.e. only spinal and/or optical nerve lesions)
- Extensive, symmetric or diffuse brain white matter changes
- Sparing of the U-fibers and the corpus callosum
- Contrast-enhancement of a majority of lesions
- Extensive spinal lesions
- Mass effect
- Cerebrovascular lesions
- A lack of dynamics (i.e. enlargement, shrinkage or disappearance of existing lesions or formation of new lesions) on follow-up imaging

One of the most challenging tasks in clinical practice is differentiating whether white matter abnormalities are more likely to be MS lesions or ischemic-degenerative lesions. Main differences are summarized in Table 2.

Table 2. *Diagnostic clues for characterizing white matter changes.*

MS lesions	Ischemic-degenerative lesions
• Younger patients (15-40 years) • Often female • Periventricular lesions radiating from corpus callosum • Corpus callosal lesions and atrophy • Juxtacortical lesions • Infratentorial lesions often affect the middle cerebellar peduncles • Contrast-enhancing lesions • Dynamic lesions (see above)	• Older patients (>40 years) • Male predominance • Lesions in watershed areas • Lack of lesions in MS predilection areas • Sparing of the U-fibers and corpus callosum • No contrast-enhancing lesions

1.2.5 Radiologically isolated syndrome

Non-specific white matter anomalies on brain MRI are common incidental findings that increase in frequency with age. By definition, these unspecific changes are of unclear or no clinical significance.[87] Sometimes, however, there are incidental white matter anomalies with a radiological pattern similar to those seen in MS, in persons without typical MS symptoms.[88]

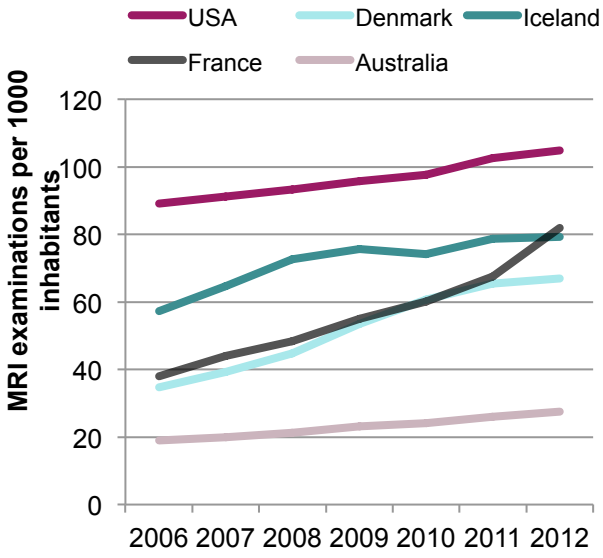
The terminology for such incidental radiological findings has been diverse. In 2009, two alternative definitions were proposed: radiologically isolated syndrome (RIS),[89] and radiologically uncovered asymptomatic possible inflammatory-demyelinating disease (RAPIDD).[90] Although none of the terms are perfect, RIS has become the convention.[88] The RIS criteria as defined by Okuda et al. are presented in Table 3. In the past years RIS has become a hot topic in neurology mainly due to disagreements on the clinical management of these findings.[91,92]

Table 3. *The Okuda criteria for RIS.*[89]

A	Incidental white matter abnormalities in the CNS meeting the following MRI criteria: 1. Ovoid, well-circumscribed and homogeneous foci with or without involvement of the corpus callosum 2. T2 hyperintensities measuring >3mm and fulfilling Barkhof criteria (≥3 out of 4) for dissemination in space[27] 3. CNS white matter anomalies not consistent with a vascular pattern
B	No historical accounts of remitting clinical symptoms consistent with neurologic dysfunction
C	The MRI anomalies do not account for clinically apparent impairments in social, occupational, or generalized areas of functioning
D	The MRI anomalies are not due to the direct physiologic effects of substances (recreational drug abuse, toxic exposure) or a medical condition
E	Exclusion of individuals with MRI phenotypes suggestive of leukoaraiosis or extensive white matter pathology lacking involvement of the corpus callosum
F	The CNS MRI anomalies are not better accounted for by another disease process

In contrast to MS, there are few epidemiological studies of RIS due to its recent definition. As elaborated in Study I, incidental MS findings have been described in large autopsy studies in Europe and North America with a frequency of 0.08-0.2% in unselected materials,[93–95] and 0.3% in patients with psychiatric disorders.[96] The radiological equivalent of these findings, RIS, is likely to increase with the increasing use of MRI, seen in Figure 11.[97]

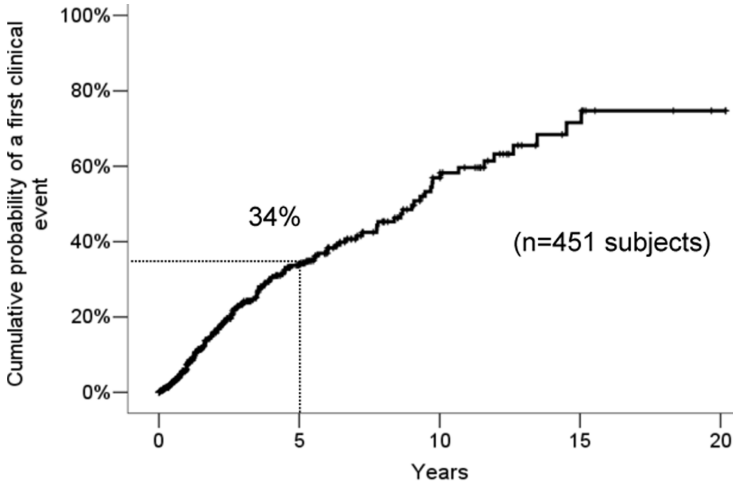
Figure 11. *MRI examinations per year per 1000 inhabitants as reported by the Organisation for Economic Co-operation and Development.*[97]



The hospital-based frequency of RIS in Pakistan, a low prevalence region for MS,[98] has been reported to be as high as 0.7% in the ages of 15-40 years.[99] In relatives of MS patients, the RIS frequency has been reported to be 2.9%.[29]

As found in Study I,[88] and later confirmed by a multi-center study by the Radiologically Isolated Syndrome Consortium,[100] about two thirds of persons with RIS will show a radiological progression with new, enlarging or contrast-enhancing lesions during follow-ups of five years. Meanwhile, one third will develop clinical symptoms and thereby convert to CIS or MS in the same time span, as seen in Figure 12. As such, RIS can be viewed as potential preclinical or subclinical stage of MS and is therefore an important area for future research to better understand MS pathophysiology.[88,100]

Figure 12. *Kaplan-Meier chart visualizing the risk of clinical progression in RIS.*[100]



1.2.6 Traditional radiological biomarkers

The historically most commonly used MRI measurement is the T2 lesion count/load/burden, which has a modest correlation with the clinical expression of MS, particularly in late disease stages with high EDSS values.[57,101,102] This discrepancy, called the clinico-radiological paradox,[74] is likely attributed to a number of reasons:

- Lesions in non-eloquent areas. Only every 5-10th MS lesion is symptomatic.[78,103]
- Diffuse white matter changes are difficult to demarcate visually and changes in the normal-appearing white matter can only be quantified with non-conventional imaging methods.[104]
- The low sensitivity for cortical MS lesions on standard MRI sequences.[78,105]
- Lack of optical tract and spinal imaging in some MRI protocols for MS.[57]
- The dual role of the immune system, involved in both de- and remyelination, meaning that inflammatory changes can be both destructive and reperative.[60]
- Limitations of EDSS, the classical clinical outcome measure, as mentioned in section 1.1.7.[45]
- The plasticity of the brain, where some individuals are better able to compensate for losses in neuronal function.[74,106]

Despite the aforementioned paradox, MRI does have a prognostic value in MS. Typical MS lesions carries a ten-year risk of converting from CIS to MS of around 80%, while the risk is only 20% in CIS patients without typical MS lesions.[101,107] Longitudinal studies have shown that mainly T1 hypointense lesions are predictive of cognitive decline.[47] Meanwhile, contrast-enhancing lesions have low prognostic value in terms of predicting relapses and disability.[108] Overall, long-term longitudinal MRI studies are scarce, why there is a need for further studies to identify predictive radiological biomarkers in MS.[47]

1.2.7 Corpus callosum as an imaging biomarker

The corpus callosum is an anatomical structure that connects the two cerebral hemispheres. It is the largest cerebral commissure and mainly consists of myelinated axons that provide inter-hemispheric interaction.[109] The corpus callosum is significantly affected in MS, both through focal lesions and through Wallerian degeneration caused by distant damage to fibers projecting through it.[110] Corpus callosal morphology is therefore a logical choice for a MS imaging biomarker. As corpus callosum is easily visualized with MRI, there has been early interest in it for radiological MS research.[111–113]

Technical developments in image processing and volumetry have shifted research focus to more advanced imaging techniques such as volumetry and non-conventional MR measures.[86] Studies have, however, shown that corpus callosal atrophy can actually be more strongly correlated with information processing speed than MS lesion volume.[113–115] Corpus callosum morphology can distinguish MS patients from controls and differentiate subtypes of MS.[116–118] Furthermore, corpus callosum atrophy has been reported to be correlated with physical disability in 5 and 9 year long perspectives, and to predict conversion from CIS to MS.[119–122]

There are manual, semi-automated and automatic methods for corpus callosum atrophy quantification, where manual methods or operator-supervised methods are considered to be the gold standard.[123] The most commonly used manual methods are the corpus callosum area (CCA) and the corpus callosum index (CCI).[124] These two measurements are further discussed in Methods, section 3.5.

1.2.8 Volumetry

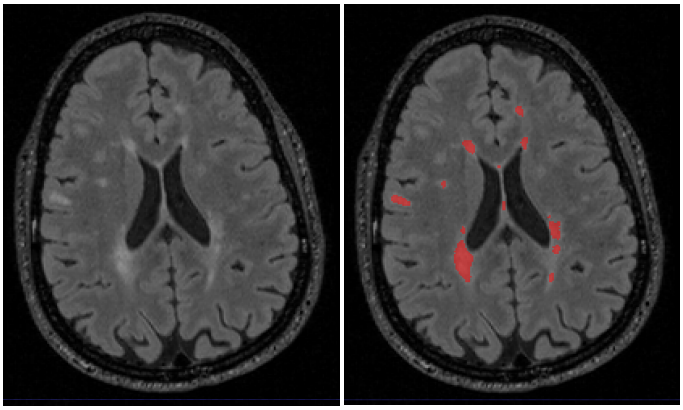
The annual brain atrophy rate is around 0.1-0.3% in normal aging and substantially higher, 0.6-1.0%, in MS regardless of the disease subtype.[60,78] Brain atrophy is seen in all stages of MS and is more strongly correlated to physical disability than T2 lesion load. It also correlates with cognitive performance and health-related quality of life.[74] Brain atrophy is therefore the most commonly used measure of neurodegeneration in treatment trials. Whole-brain atrophy measures are unspecific and reflect many different aspects of the accumulating pathological changes in MS.[78] Contributors to the tissue loss are neuronal damage with Wallerian degeneration and axonal loss with subsequent gliosis.[60] It is important to remember, however, that changes in cerebral volume can also be due to physiological factors (hydration status), treatment with anti-inflammatory drugs (pseudo-atrophy with reductions of edema) and technical reasons (scanner and software differences).[78]

Segmentations of grey matter and white matter are now commonly used in research and increase the specificity of the atrophy. Grey matter atrophy is closely associated with neuronal loss, reductions of synaptic density and loss of cortical connectivity, why it is strongly correlated to cognitive impairment.[60] Segmentations of grey and white matter are most accurately performed on 3D T1-weighted sequences with near-isotropic resolution with a voxel size of around 1 mm^3. A caveat is that white matter MS lesions may have a signal

intensity mimicking grey matter, which may bias the results. MS lesion segmentation and filling (replacing the lesions with white matter intensity) is therefore recommended.[125]

Lesion segmentation, as exemplified in Figure 13, is complex and there are numerous different approaches to estimate the MS lesion volume (LV). Multi-channel approaches, using image registration of multiple sequence types including FLAIR are recommended to ensure accurate lesion delineation due to the heterogeneity of signal intensities of MS lesions.[125] Manual lesion segmentation remains the gold standard.[67]

Figure 13. *Axial FLAIR (left) and lesion segmentation (red, right) in a 28-year-old female with 12 years disease duration of RRMS. Lesion volume was 13 milliliters.*



An increasing use of 3D MRI sequences and an expanding variety of volumetric software reflect the growing interest in volumetric brain measurements in neuroscience research. Although the post-processing procedures have been facilitated by improved graphical user interfaces and reductions processing times, these quantitative biomarkers have yet to become implemented in the clinical workflow.[86,104] Main reasons include lack of resources or time for image processing, reproducibility issues and difficulties in interpretation of the data on an individual basis.

1.2.9 Non-conventional and emerging imaging techniques

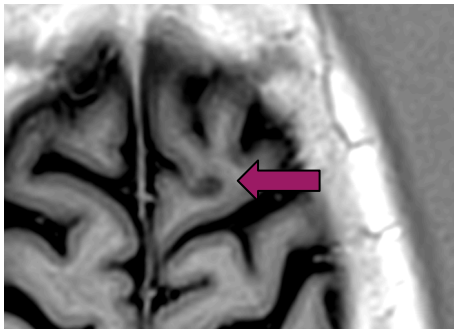
Many metabolic and molecular imaging methods are used exclusively in a research setting due to their complexity, technical limitations and costs. These methods are important in expanding our understanding of MS and can help us identify important disease mechanisms and novel MS treatment targets. The two first examples below, however, are new MRI sequences and alternative methods for reading the scans that are emerging and may come into clinical use in the near future.[63]

Image registration methods can aid the radiological readings by better utilizing our commonly acquired MRI data. On example is subtraction MRI where follow-up scans are overlaid on baseline scans to increase sensitivity for lesional change and atrophy.[126] Another application is FLAIR* where FLAIR sequences are registered to susceptibility weighted images, which increases the specificity of white matter abnormalities by identifying central venules in MS lesions.[127]

Sequences for cortical lesion detection have given a renaissance to cortical MS pathology previously known from autopsy studies. These lesions are important as they increase the specificity of the diagnostic MRI criteria for MS.[128] They are also independent predictors of two-year grey matter atrophy and worsening of physical disability.[129] The two main new sequences are double inversion recovery (DIR), where a second inversion pulse for fat

suppression is applied to a T2-FLAIR, and phase-sensitive inversion recovery (PSIR) where phase information is used in the reconstruction of a T1-weighted inversion recovery, as seen in Figure 14.[105]

Figure 14. *Axial PSIR in a 49-year-old male MS patient visualizing a cortical lesion (arrow).*



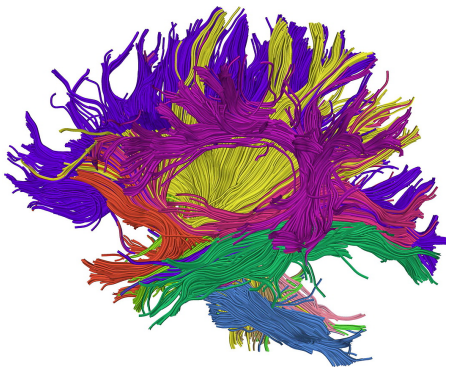
Arterial spin labeling (ASL) is an MRI technique that quantifies cerebral perfusion. Protons of the inflowing arterial blood are magnetically labeled and then interact with protons in the imaging slice, thereby changing the MRI signal proportionally to the perfusion. Acute MS lesions typically have increased perfusion, while reductions in perfusion are seen in chronic lesions, subcortical grey matter and normal-appearing white matter.[60]

Functional MRI (fMRI) is sensitive to changes in the cerebral blood flow that indirectly reflect cortical activation in task-based paradigms or in a "resting" state. fMRI can be based on ASL or blood-oxygen-level dependent (BOLD) contrast imaging where the difference in magnetic properties of saturated and unsaturated hemoglobin is utilized. fMRI has consistently shown abnormal activation of accessory cortical areas compared to controls. These abnormal activation patterns change with the disease course and are thought to reflect adaptive mechanisms that limit the functional effects of structural damage.[43]

Magnetic transfer ratio is an indirect measure of tissue integrity where a saturation pulse is applied with a frequency differing from the expected spectrum of free protons, called an off-resonance pulse. This pulse mainly affects protons bound to macromolecules that typically relax faster than we can obtain a signal. There is, however, interactions between bound and free protons and some of this magnetization will be transferred to the free protons that we can image.[130] The magnetic transfer ratio is strongly correlated to demyelination and axonal damage and quantifies tissue damage in normal-appearing white matter and before lesions become visible. The measurement is strongly correlated to mobility and cognitive function, but is only viable for comparisons on a group level and has limited reproducibility.[43,78]

Diffusion tensor imaging (DTI) is used to study the integrity and major direction of white matter tracts by magnetically labeling protons and studying their diffusivity.[60] DTI allows for tractography, illustrated in Figure 15,[131] where the integrity of white matter tracts can be studied together with structural connectivity. DTI is a sensitive measure of axonal integrity with pronounced changes in focal lesions, intermediate changes in dirty-appearing white matter and more subtle changes in normal-appearing white matter.[43] Tractography has shown that tract abnormalities are only partly explained by MS lesion location, highlighting the role of diffuse MS pathology.[47]

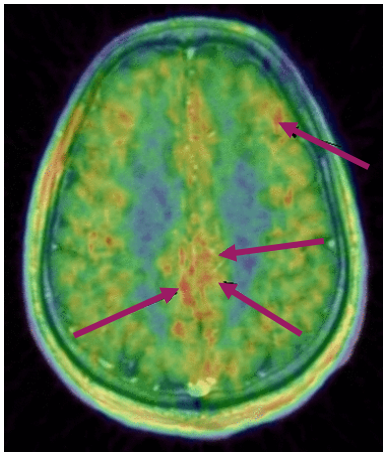
Figure 15. *Whole-brain DTI based tractography.*[131]



Magnetic resonance spectroscopy shows early metabolic changes in MS and can reveal diffuse tissue damage in MS that is not visible on conventional MRI. Reduced N-acetylaspartate is a sensitive marker for neuronal degeneration/dysfunction and predicts brain atrophy.[132] Demyelination leads to increased levels of choline, representing cell-membrane phospholipid turnover, and lipids such as myo-inositol.[78] In active inflammation the levels of lactate and glutamate increase, suggesting a role for glutamate excitotoxicity.[132] There are also increased concentrations of sodium in MS as a sign of neurodegeneration.[63]

Positron emission tomography (PET) is a method where radioactive isotopes are coupled to a molecule of biochemical importance and administered intravenously, making it possible to visualize the distribution of the radioligand down to pico-nano molar levels. PET reveals altered glucose metabolism and cerebral blood flow in MS and changes in neurotransmitter levels. It can also show de- and remyelination and has unveiled widespread microglial activation, independent from relapses, in white and grey matter as depicted in Figure 16.[133]

Figure 16. *^{11}C-PK11195 PET showing cortical microglial activation (arrows) in MS.*[133]



Ultra-high field strength (7 T) is becoming increasingly available for research. The first human 7 T MRI scanner in Sweden was installed in Lund as a national research resource, inaugurated in May 2015. The high field strength offers increased signal-to-noise ratio that can be used to improve temporal or spatial resolution. There are also increased susceptibility effects that are advantageous for certain applications, but that also provide challenges in terms of inhomogeneities.[134]

Myelin water imaging is a novel technique based on relaxometry where there is a separation of the MRI signal based on its relaxation times. The underlying theory is that protons bound in the myelin sheaths have faster relaxation times than those in extracellular and intracellular fluids and CSF.[135] The proportion of the fast relaxing myelin-bound water has been validated as an *in-vivo* surrogate measurement of myelin.[136] Myelin water imaging is a promising candidate for a specific MRI-derived biomarker in MS as it reflects clinical variability in MS and changes with disease progression,[137] but there are still technical limitations and its full clinical potential remains to be studied.[135]

2 AIMS OF THIS THESIS

The overall purpose of this thesis was to describe neuroradiological aspects of multiple sclerosis, from early signs to late stages, with an emphasis on MRI findings, aiming to facilitate diagnosis and individualization of treatment and care for patients with RIS and MS.

The specific objectives of each study were:

Study I To identify relevant articles regarding incidental MRI findings suggestive of MS, summarize the nomenclature and current knowledge of such findings and to give recommendations for future studies.

Study II To estimate the annual frequency of RIS at a university clinic in a region with a high incidence and prevalence of MS.

Study III To compare the feasibility and performance of corpus callosum area and corpus callosum index as radiological biomarkers for cognitive and physical disability in MS. A secondary aim was to compare corpus callosum area and corpus callosum index to volumetric brain measurements in these regards.

Study IV To study the progression of corpus callosal atrophy in MS and assess the longitudinal use of normalized corpus callosum area as a biomarker for cognitive and physical disability in MS.

3 MATERIALS AND METHODS

3.1 ETHICAL CONSIDERATIONS

Study I, consisting of a systematic-review, did not require ethical approval as it did not involve any participants, experimental procedures or management of sensitive personal data.

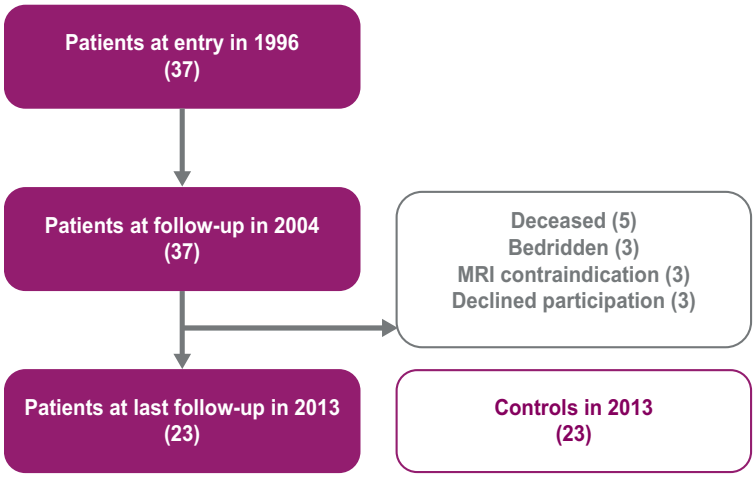
The Regional Ethical Review Board in Stockholm approved Study II (registration number 2011/1085-31/3) as well as Study III and IV (registration numbers 04-906/4 and 2012/858-31/2). Written informed consent was obtained from all participants.

3.2 PROCEDURES AND PARTICIPANTS

Study I. This systematic review was performed according to the preferred reporting items for systematic reviews and meta-analyses (PRISMA) statement on the 2nd of March 2012.[138] The search strings, inclusion criteria and exclusion criteria were predefined, as detailed in the Supplementary appendix for Study I.[88] The databases used were Embase, PubMed, Scopus and Web of Knowledge. Two raters (Tobias Granberg and Juha Martola) independently evaluated all abstracts and any discrepancies in inclusion/exclusion were decided by a third rater (Maria Kristoffersen-Wiberg). All articles included were read to full extent and their references were scrutinized to identify any additional studies of interest.

Study II. In this retrospective study conducted in 2012, all brain MRI examinations performed at Huddinge sjukhus in 2001 were anonymously screened for white matter anomalies fulfilling the Okuda RIS criteria.[89] The sample year was chosen due to the availability of digital storage of radiological and clinical data, and in order to investigate the 10-year prognosis of identified RIS cases. Persons of interest in the study, where more clinical information was needed, were de-anonymized in order to obtain written informed consent for inclusion in the study and for reviewing their clinical patient charts.

Figure 17. *Patient participation in the longitudinal study.*



Study III and IV. In this longitudinal cohort study, 37 MS patients were recruited from the outpatient clinic at the Department of Neurology, Huddinge sjukhus and followed with MRI, neurological assessment and neuro-psychological testing from 1996 with follow-ups in 2004 and 2013. An age- and gender-matched healthy control group was recruited at the last follow-up. A flow chart of the patient participation in the study is shown in Figure 17 and the demography of the participants is detailed in Table 4.

Table 4. *Demography of the participants in Study III and IV.*

	All patients 1996	Remaining patients* 1996	Remaining patients* 2004	Remaining patients* 2013	Controls 2013
N	37	23	23	23	23
Sex, N, females/males	26/11	18/5	18/5	18/5	18/5
Age, years	42 (10)	39 (8.1)	48 (8.1)	57 (8.0)**	57 (7.2)**
MS subtype, N, RR/SP/PP	23/11/3	18/5/0	13/10/0	3/20/0	-
Disease duration, years	11 (8.5)	10 (6.9)	19 (6.8)	27 (6.9)	-
EDSS, median (range)	4.5 (0.0-8.0)	3.5 (0.0-6.5)	5.0 (1.0-7.5)	6.0 (1.5-8.0)	-
DMT	65%	17%	52%	22%	-

**The 23 patients followed to 2013. **p = 0.95. Mean values if not otherwise specified. Standard deviations are reported in parenthesis. DMT = Disease Modifying Therapy, RR = Relapse-Remitting MS, SP = Secondary Progressive MS, PP = Primary Progressive MS.*

3.3 CLINICAL EVALUATIONS

Study II. The clinical information in the referrals and, when needed, the clinical patient charts were evaluated by a medical doctor (Tobias Granberg) with the support of an experienced MS neurologist (Sten Fredrikson) according to the Okuda criteria as specified in Table 3.[89] Special regards were taken to criteria B-F, i.e. that the white matter anomalies were not better explained by another disease process or substance and that there were no remitting neurological symptoms or impairments in daily activities. All possible RIS cases had been examined by a neurologist as part of the clinical work up. Final decisions on inclusion or exclusion based on Okuda criteria B-F were made by consensus of the two raters.

Table 3 (reiterated). *The Okuda criteria for RIS.*[89]

A	Incidental white matter anomalies in the CNS meeting the following MRI criteria: 1. Ovoid, well-circumscribed and homogeneous foci with or without involvement of the corpus callosum 2. T2 hyperintensities measuring >3mm and fulfilling Barkhof criteria (≥3 out of 4) for dissemination in space.[27] 3. CNS white matter anomalies not consistent with a vascular pattern
B	No historical accounts of remitting clinical symptoms consistent with neurologic dysfunction
C	The MRI anomalies do not account for clinically apparent impairments in social, occupational, or generalized areas of functioning
D	The MRI anomalies are not due to the direct physiologic effects of substances (recreational drug abuse, toxic exposure) or a medical condition
E	Exclusion of individuals with MRI phenotypes suggestive of leukoaraiosis or extensive white matter pathology lacking involvement of the corpus callosum
F	The CNS MRI anomalies are not better accounted for by another disease process

Study III and IV. Physical disability was scored according to EDSS by an experienced MS neurologist (Sten Fredrikson) at all time points (1996, 2004 and 2013). An experienced neuropsychologist (Gösta Bergendal) administered the neuropsychological testing at all three time points in conjunction with the MRI examinations. The main focus of the neuropsychological testing was SDMT.

In 2013, a more comprehensive neuropsychological test battery was administered with additional testing including a verbal fluency test (FAS), Rey-Osterrieth complex figure test – copy and Rey auditory verbal learning test with encoding (0 min) and delayed recall (30 min). The test battery was designed to reflect conceptually different cognitive functions with different neuroanatomical correlates, please see section 1.1.7 for further details on these tests. All raw test scores were converted to *z*-scores of normative data based on age, gender and educational level. Test results were defined as abnormal if the result deviated more than two standard deviations (SD) from the mean of the norm.[139]

3.4 MAGNETIC RESONANCE IMAGING

Study II. All brain MRI examinations were acquired at Huddinge hospital using two 1.5 T scanners, Siemens Vision and Symphony (Siemens Healthcare, Erlangen, Germany) with MRI protocols dedicated to the clinical queries in the referrals. Incidental white matter anomalies were in many cases further characterized with a dedicated MS protocol, especially in young patients where the MS incidence rate is high. The MS protocol was standardized in accordance with the "Stockholm prospective assessment of MS" study (STOP-MS) and the acquisition parameters are presented in Table 5.

Table 5. *MRI parameters of the standardized MS protocol in Study II.*

Sequence	Plane	Number of slices	Slice thickness (mm)	Repetition time (ms)	Echo time (ms)	Inversion time (ms)	Flip angle (°)
T1 MPRAGE	Axial	128	1.5	13.5	7	300	15
PD/T2 TSE	Axial	54	3.0	4761	22/90	-	180
T2 TSE	Sagittal	19	4.0	3500	96	-	180
FLAIR^{Gd}	Axial	27	5.0	9000	110	2500	180
T1 SE^{Gd}	Axial	27	5.0	570	14	-	90

^{Gd}Acquired after intravenous administration of gadolinium-based contrast media. FLAIR = fluid attenuated inversion recovery, MPRAGE = three-dimensional magnetization prepared rapid acquisition gradient echo, PD = proton density, (T)SE = (turbo) spin echo.

Study III and IV. Imaging was performed at Karolinska University Hospital on 1.5 T MRI scanners: General Electrics Signa (General Electric Healthcare, Milwaukee, USA) in 1996, Siemens Vision in 2004 and Siemens Avanto in 2013 (Siemens Healthcare, Erlangen, Germany). Care was taken to optimize comparability of the measurements over time by harmonizing the acquisition parameters, which are presented in detail in Table 6.

Table 6. *MRI parameters in Study III and IV.*

	Sagittal FSE/TSE T2			MPRAGE		FLAIR	
Time point	1996	2004	2013	2004	2013	2004	2013
Number of slices	11	19	19	160	160	19	126
Slice thickness (mm)	5.0	4.0	4.0	1.4	1.4	5.0	1.4
Gap between slices (mm)	0.0	0.4	0.4	-	-	1.5	0.0
In-plane resolution (mm)	1.0x1.0	1.0x1.0	1.0x1.0	1.0x1.0	1.0x1.0	1.0x1.0	1.0x1.0
Repetition time (ms)	4000	3500	4290	1350	1910	9000	5000
Echo time (ms)	76	96	103	7	3.08	110	411
Inversion time (ms)	-	-	-	3000	1100	2500	1800
Flip angle (°)	90	180	150	15	15	180	120
Number of averages	1	2	2	1	1	2	1

FLAIR = fluid attenuated inversion recovery, FSE/TSE = fast/turbo spin echo, MPRAGE = three-dimensional magnetization prepared rapid acquisition gradient echo.

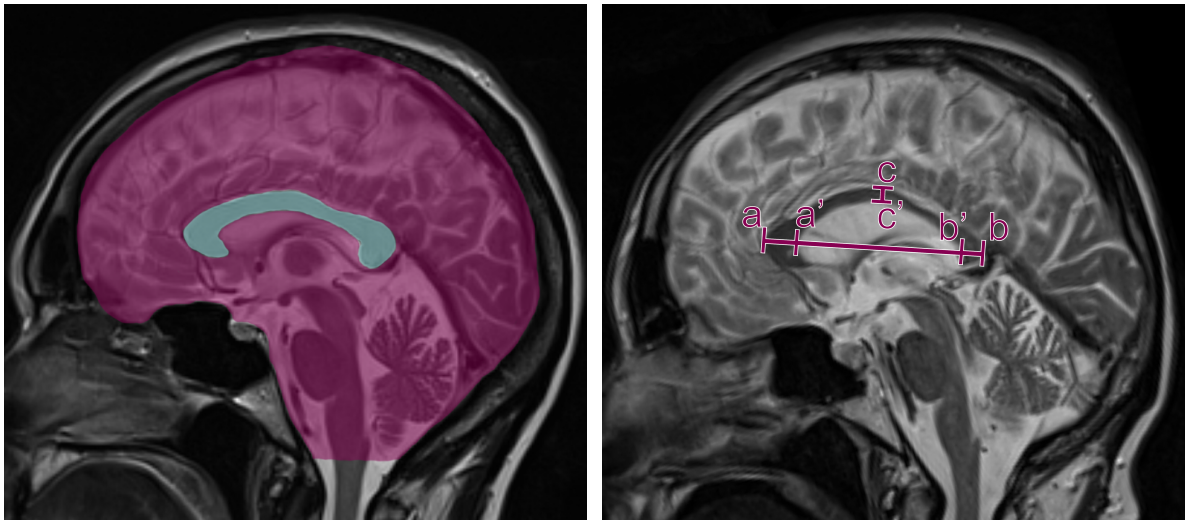
3.5 RADIOLOGICAL EVALUATIONS

Study II. All brain MRI examinations were first anonymized and systematically screened by a medical doctor (Tobias Granberg) with three years experience of neuroradiological research who had received training in assessing white matter changes by two neuroradiologists (Maria Kristoffersen-Wiberg and Juha Martola). The white matter anomalies were assessed according to the Barkhof classification,[27] as stipulated by Okuda et al.[89] Juxtacortical lesions were defined as involving the U-fibers, i.e. "touching the cortex". In order to preserve a high sensitivity for possible RIS, the same rater also screened the clinical radiological readings in order to further identify any white matter anomalies suggestive of MS. All findings in the clinical radiological readings were further summarized in order to report on the disease panorama of the clinic. All plausible RIS cases according to the Okuda criteria,[89] were re-assessed by a neuroradiologist with long experience in MS (Juha Martola) according to the same classification, blinded to the clinical information and the clinical radiological readings.

Study III and IV. All radiological two-dimensional (2D) measurements of corpus callosum were performed on mid-sagittal MRI slices oriented by the inter-hemispheric fissure, the great cerebral vein (vein of Galen), and the cerebral aqueduct (aqueduct of Sylvius). The measurements were performed on standard radiological workstations using integrated measuring tools in the Picture Archiving Communicating System (PACS; IDS7, Sectra, Sweden). The measurements used in study III and IV were performed by a neuroradiologist (Juha Martola). Intra-rater agreement was studied at a second rating session 6 months later and inter-rater agreement was analyzed by comparing the ratings of the whole sample with those of a resident in radiology (Tobias Granberg) and an MD/PhD student (Sara Shams). All measurements were performed in a randomized order, blinded to the clinical data, previous ratings and each other's ratings.

The corpus callosum area was obtained by manual tracing of its outer contour. For the longitudinal evaluations in Study IV, the measurement was normalized (nCCA) to the intracranial surface area in the same slice.[119] The corpus callosum index (CCI) was measured as defined by Figueira et al.[117] Both of these measurements are illustrated in Figure 18.

Figure 18. *Corpus callosum measurements on mid-sagittal T2-weighted MRI. To the left is a 51-year-old healthy female control and to the right a 52-year-old female MS patient. The corpus callosum area (left, turquoise) is normalized by dividing it by the intracranial surface (plum).*[124] *Corpus callosum index (right, plum) is calculated by summing the anteroposterior length of the genu (aa'), the splenium (bb') and the craniocaudal height of the body of corpus callosum (cc'), divided its length (ab) according to the equation: (aa'+bb'+cc')/ab.*[117]



The above-mentioned manual radiological measurements were performed on T2-weighted images. However, in recent literature T1-weighted images are more frequently used for corpus callosum measurements, why the methods were also applied to sagittal reconstructions of the MPRAGE sequences. This complimentary comparison was quantified through an intra-rater agreement analysis of a resident in radiology (Tobias Granberg) across sequences.

3.6 VOLUMETRY

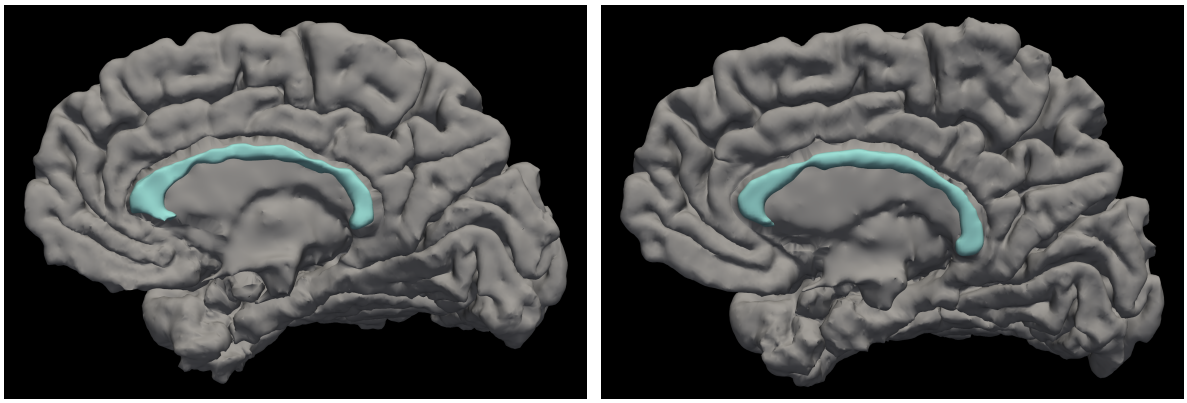
In Study III and IV, the MPRAGE and FLAIR sequences from 2004 and 2013 were used for volumetry. All volumetric processing was quality controlled by a resident in radiology (Tobias Granberg) and image quality, with emphasis on motion artifacts and ghosting, was assessed prior to performing segmentations to ensure adequate image quality.

The longitudinal stream of Freesurfer 5.3.0 (Harvard University, Boston, USA) was used to obtain brain tissue segmentations. Manual interventions to ensure accurate segmentations included removing misclassified meningeal tissue, adding control points to adjust intensity normalization failures and filling in white matter topological errors.

MS lesion segmentations were performed using Lesion Segmentation Toolbox 1.2.3 (Technische Universität München, Munich, Germany) for Statistical Parametric Mapping 8 (University College London, United Kingdom) using a multi-channel approach with both MPRAGE and FLAIR sequences with a kappa value "0.3", lesion belief map "GM", resulting in the MS lesion volume (LV). All reported processing times are based on running above-mentioned software on a MacBook Pro (3 GHz Intel Core i7 processor, 8 GB DDR3 1600 MHZ RAM) with OS X 10.8.5.

The volumes of interest were the brain volume (BV), grey matter volume (GMV), white matter volume (WMV) and corpus callosum volume (CCV). The five sub-segmentations of corpus callosum provided by Freesurfer were summed to obtain the CCV, illustrated in Figure 19. The estimated total intracranial volume was used as a measurement of the intracranial volume (ICV). For the longitudinal evaluation in Study IV, all brain tissue measurements were normalized to the ICV, resulting in the brain parenchymal fraction (BPF), grey matter fraction (GMF), white matter fraction (WMF) and normalized lesion volume (nLV).

Figure 19. *Corpus callosum volume in a male MS patient with SPMS in 2004 (left: 2.2 milliliters) and 2013 (right: 1.7 milliliters). In 2004 the patient was 38 years old with 15 years disease duration. EDSS scores were 7.5 at both time points. SDMT scores were -0.8 SD and -1.7 SD.*



3.7 STATISTICAL ANALYSIS

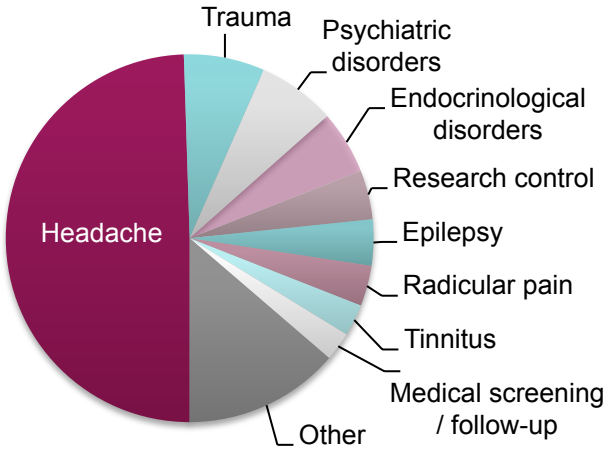
SPSS 22.0 (IBM, USA, 2013) was used to perform statistical analyses in Study III and IV. Normality of data was evaluated using the Shapiro-Wilk normality test. Group comparisons of parametric data were performed using independent or paired t-test, while non-parametric independent data were analyzed with the Mann-Whitney U test. Correlation analyses in parametric data were evaluated with Pearson correlation coefficient and non-parametric data (such as EDSS and LV) were analyzed using Spearman's rho. In accordance with statistical convention, correlation coefficients (*r*) of 0.2-0.4 were considered weak, 0.4-0.6 moderate, 0.6-0.8 strong and 0.8-1.0 very strong.[140] Intra- and inter-agreement analyses for continuous measurements (such as corpus callosum area and index) were assessed using intraclass correlation coefficient (ICC) with a two-way mixed effects model for absolute agreement on single measures. In accordance with statistical convention, ICCs of < 040 were considered poor, 0.40-0.75 fair to good and >0.75 excellent.[140] Classification accuracy in Study III was studied via the area under the curve (AUC) in receiver operating characteristic (ROC) curves with a nonparametric assumption of distribution. Statistical significance was pre-defined as an α-level of 0.05. Due to multiple comparisons in Study III and IV, Bonferroni corrections were applied. The corrected α-level was 0.006 for Study III and 0.007 for Study IV.

4 RESULTS

4.1 STUDY I

In total, 79 unique relevant publications were identified in the systematic literature search, out of which 60 articles were peer-reviewed articles. Incidental MRI findings suggestive of MS had a divergent terminology. In total, 12 patient cohorts and 6 case reports were identified from Brazil, France, Italy, Spain, Turkey and USA with 394 combined reported cases. Mean ages in the cohorts ranged from 30-42 years and the overall age range was 16-70 years. The female to male ratio of the reported cases was 2.0:1 (excluding a study focusing on the effects of pregnancy on RIS progression where there were naturally only female participants).

Figure 20. *Indications for the initial MRI unveiling RIS, N=394.*



As illustrated in Figure 20, the most common indication for the brain MRI unveiling the MS-like radiological findings was headache, composing nearly half of all reported cases. Two cohort studies provided neuropsychological test data, showing that thorough neuropsychological testing reveals subclinical cognitive impairments in persons with RIS. Persons with RIS showed similar but less pronounced deficits compared with MS patients in terms of information processing speed, verbal fluency, short term memory, cross-tapping and go/no-go test performance.[141,142] Furthermore, it was shown that RIS patients have lesion distributions, lesion volumes and brain volumes,[75,142–144] as well as spectroscopy findings similar to those found in MS.[143]

Due to differences in the terminology and methodology used in the studies, the ability to perform a meta-analysis was limited. However, of all reported RIS cases roughly two thirds progressed radiologically, i.e. had new, enlarging or contrast-enhancing lesions on follow-up MRI examinations, during five-year follow-up. Meanwhile, about one third of the patients developed symptoms consistent with demyelinating disease and were thus diagnosed with MS (or CIS). Cervical spine lesions were identified as the most important predictor of clinical progression with other predictors being a high lesion load (especially in combination with abnormal CSF findings), contrast-enhancing lesions, cervical spine lesions, infratentorial lesions, younger age and pathological visual evoked potentials.

We found that management of RIS was controversial with three alternative proposed approaches: wait (no intervention, instructing the patient to seek healthcare if symptoms develop), follow (regular clinical and radiological follow-ups with decreasing frequency) and treat (prescribing off-label DMT). In studies where treatment was reported, 10% of the patients received MS medications.

4.2 STUDY II

In total 2105 individuals were examined with a brain MRI during the sample year. Ages ranged from 0 to 90 years, with a median age of 48 years. There were 1202 females (57%) and 903 males (43%) in the cohort. The results of Study II are illustrated in the flow chart in Figure 21 and the panorama of radiological findings in the cohort is summarized in Table 7. For more details on the step-by-step procedures and clinical details of the excluded patients, please see the original article. In the yearly sample, only one case of RIS was identified, equaling a frequency of 0.05% in the whole cohort. In the age range 15-40 years (661 individuals), where MS is more common, the RIS frequency was 0.15%.

The patient with RIS was a 43-year-old female who was healthy except for migraines. She had no previous symptoms suggestive of MS and no heredity for neurological diseases. She was referred for a brain MRI due to frequent migraines at work (1-3/week). The initial brain MRI demonstrated 15 T2-hyperintense lesions: 12 periventricular, 2 juxtacortical and 1 contrast-enhancing, fulfilling the Barkhof classification for DIS.[27] The MRI findings are illustrated in Figure 22. Thorough neurological examination provided normal findings. A lumbar puncture revealed oligoclonal bands and a raised IgG index. She was planned for a follow-up half a year later, but after three months she presented with dysesthesia and ataxia in the upper extremities. MRI showed three new brain lesions and a cervical lesion. She was diagnosed with MS and started on interferon β therapy. Over the coming years she continued to have Lhermitte's sign but a benign course with only one distinct relapse in 10 years with lower extremity weakness. To date, she remains active and works full time.

Figure 21 *(left). Flow-chart illustrating the findings in Study II (N).* ***Figure 22*** *(right). Axial T1-weighted MRI illustrating a juxtacortical lesion (top) and the contrast-enhancing lesion (bottom).*

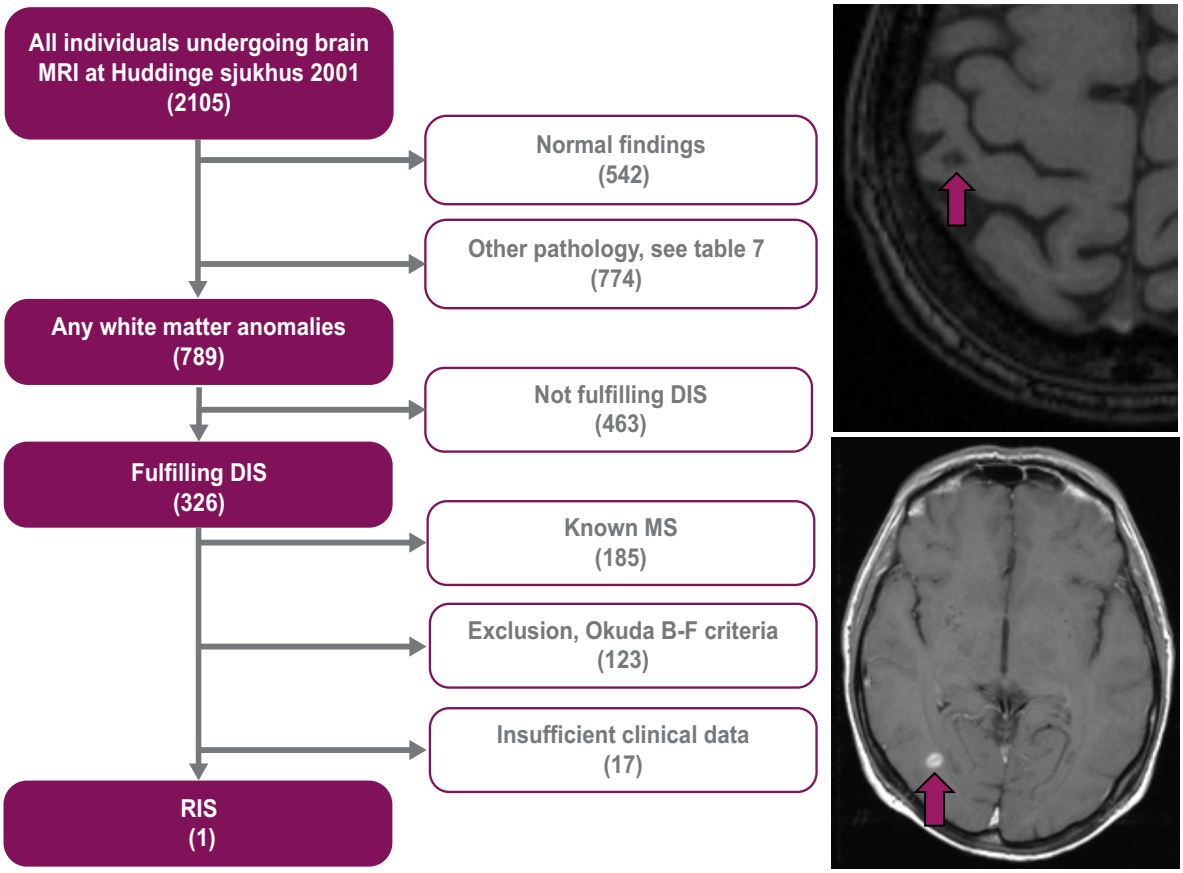


Table 7. *Radiological findings in the yearly sample of brain MRIs at Huddinge hospital 2001. Individual patients may be represented in multiple categories*

Radiological findings	Number of individual patients
Within normal limits	**542**
Cerebrovascular disorders	**326**
- Infarctions	222
- Vascular malformations, aneurysms, dissections and occlusions	73
- Intracranial bleedings and contusions	28
- Other	3
White matter and neurodegenerative disorders	**1143**
- Atrophy	285
- Basal ganglia disorders	12
- Hydrocephalus	20
- Marked perivascular spaces	37
- Possible inflammatory white matter changes	356
- Unspecific or degenerative white matter changes	433
Infectious, inflammatory and metabolic disorders	**88**
- Intracranial infections	32
- Optical neuritis	37
- Metabolic disorders	4
- Vasculitis	4
- Other	11
Neoplasms	**179**
- Meningiomas	44
- Intra-axial malignancies	39
- Pituitary adenomas	31
- Vestibular schwannomas	19
- Unspecified or other types of neoplasms	46
Cysts and malformations	**74**
- Parenchymal, arachnoid and pineal cysts	42
- Malformations or dysplasias	16
- Pituitary gland cysts or disorders	16
Sinonasal and orbital disorders	**191**
- Sinusitis, mastoiditis, mucosal thickening	187
- Other	4

4.3 STUDY III

Reliability and feasibility: The inter-rater ICC for all three raters was 93% for CCA and 94% for CCI. The intra-rater ICC for the neuroradiologist was 97% for CCA and 96% for CCI. When comparing measurements obtained on T1- and T2-weighted images the intra-rater ICCs were 93% for CCA and 90% for CCI. The mean time to obtain CCA measurements was 43 seconds per subject and time point, compared with 18 seconds for CCI. Volumetric measurements took longer to acquire and edit, with a mean processing time of 14 hours and an average editing time of 33 minutes per subject and time point.

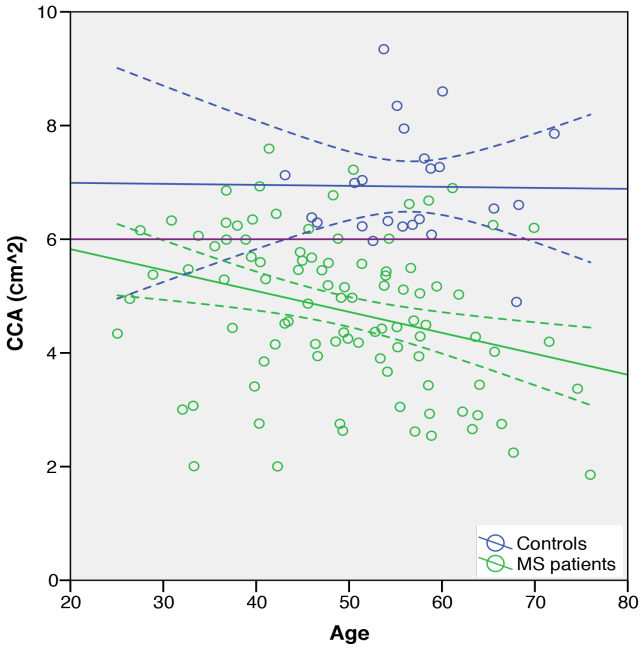
Cognitive and physical disability: CCA was the measurement with the strongest correlation with both SDMT and EDSS. All corpus callosum-based measurements (CCA, CCI and CCV) had stronger correlations with SDMT than conventional volumetric measurements. Generally, all radiological measurements had less strong correlations with EDSS, with no more than moderate correlations. Further details are found in Table 8.

Table 8. *Radiological measurements cross-sectional correlation with EDSS and SDMT.*

	Correlation to SDMT (*r, p*)	Correlation to EDSS (*r, p*)
CCA	0.82, < 0.001*	-0.56, < 0.001*
CCI	0.73, < 0.001*	-0.45, 0.001*
CCV	0.72, < 0.001*	-0.55, < 0.001*
BV	0.50, 0.001*	-0.45, 0.001*
GMV	0.67, < 0.001*	-0.50, < 0.001*
WMV	-0.16, 0.30	0.002, 0.99
LV	-0.69, < 0.001*	0.52, < 0.001*

*Correlation coefficients and p-values adjusted for age, sex and disease duration. *Correlations that remained significant after correction for multiple testing. BV = brain volume, CCA = corpus callosum area, CCI = corpus callosum index, corpus callosum volume, GMV = grey matter volume, LV = lesion volume, WMV = white matter volume.*

Figure 23. *Change of corpus callosum area with age. Linear regression lines with 95%-confidence intervals. The purple line illustrates the suggested 6 cm^2 cut-off.*



Classifying performance: Overall, the corpus callosum-based measurements were more accurate than the other radiological measurements in differentiating MS patients from controls. The overall accuracy (AUC) was 91% for CCA and 90% for CCI for all time points. The accuracy based on the two later time points (where volumetric data was available) is presented in Table 9. In the current population, a cut-off CCA of 6 cm^2 separated patients from controls with a sensitivity of 79% and a specificity of 91%, as illustrated in Figure 23.

The greatest accuracy in differing patients with a relapse-remitting subtype from progressive subtypes (PPMS and SPMS) was seen for LV, CCA and CCV, illustrated in ROC curves in

Figure 24. These results were corroborated in a leave-one-out cross-validation analysis, which can also be seen in Table 9.

Figure 24. *Accuracy in differentiation of MS patients from controls (left) and MS patients with progressive from relapse-remitting clinical courses (right).*

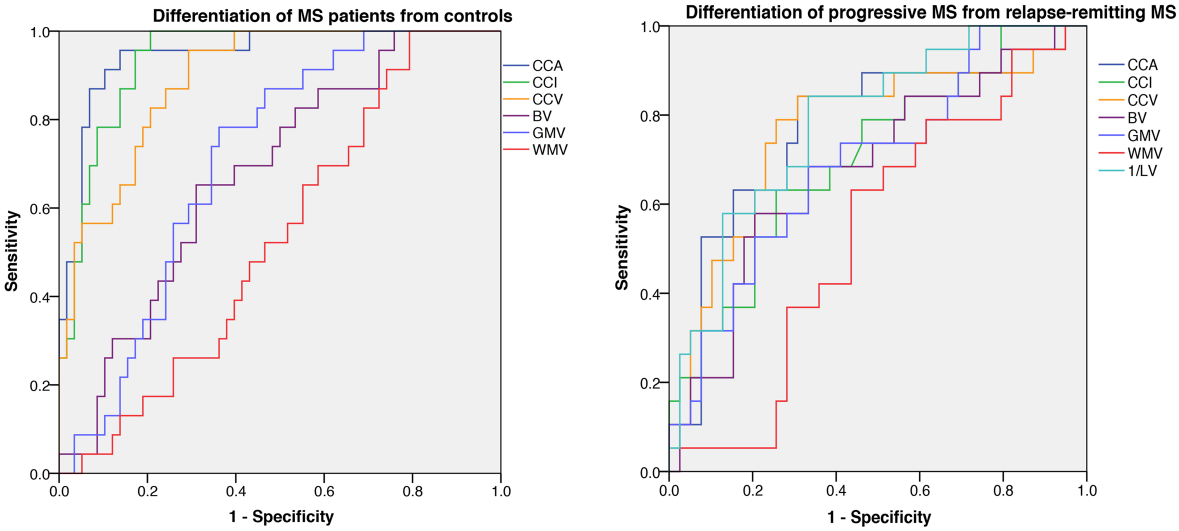


Table 9. *Performance of the radiological measurements in differentiation tasks.*

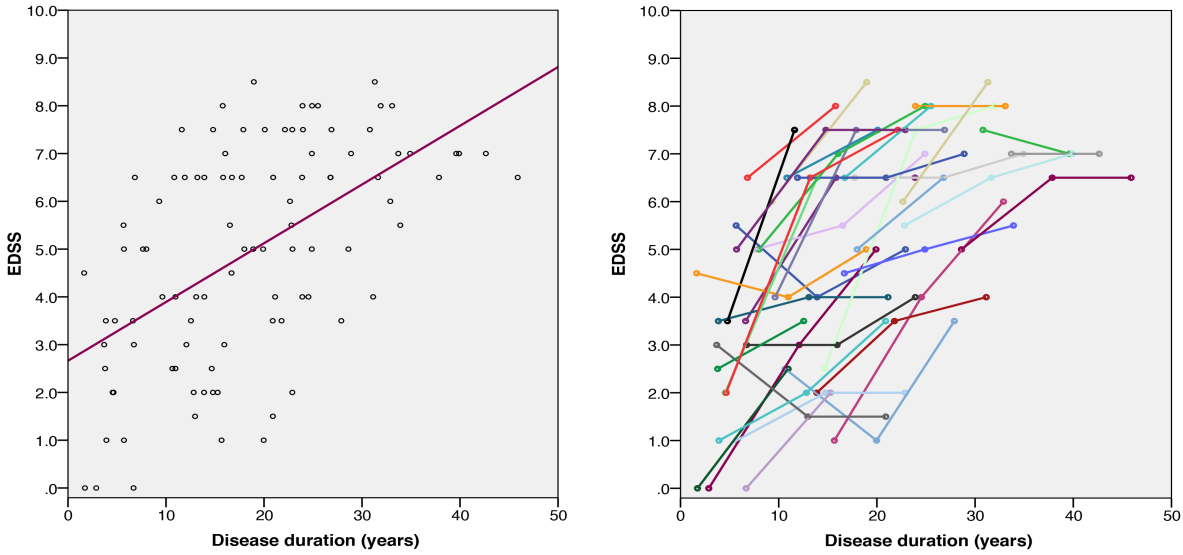
	Differentiating MS patients from controls		**Differentiating RRMS from progressive subtypes of MS**	
	Accuracy	Validation accuracy**	Accuracy	Validation accuracy**
Corpus callosum area	95%	86 %	77%	72%
Corpus callosum index	94%	84%	71%	62%
Corpus callosum volume	89%	78%	77%	71%
Brain volume	68%	63%	68%	62%
Grey matter volume	71%	63%	70%	64%
White matter volume	53%	49%	53%	47%
Lesion volume	-	-	78%	63%

*RRMS = Relapse-Remitting MS. **Leave-one-out cross-validation analysis.*

4.4 STUDY IV

Physical disability: Overall, there was a great span of disease duration (1.6-46 years) and EDSS scores (0.0-8.0) throughout the study. The physical disability increased moderately with the disease duration after adjustments for age and sex ($r = 0.41$, $p < 0.001$). With longer disease durations, the EDSS scores tended to plateau as visualized in Figure 25.

Figure 25. *Physical disability over disease duration for the 37 MS patients at all time points. Scatter plot with linear regression line (left) and individual EDSS scores connected by colored lines (right).*



Cognitive disability: All but four patients performed below the norms in at least one of the five tests in 2013. One patient chose not to participate in the testing, why 18 out of 22 (82%) were cognitively impaired. There were no difference in clinical or radiological characteristics between the MS patients with normal and subnormal cognitive performance, please see the original article for details.[145] There were large individual differences in SDMT performance and SDMT had a weak negative correlation with disease duration ($r = -0.37$, $p < 0.001$).

Corpus callosum measurements: Intra- and inter-rater ICCs for nCCA were excellent (both 96%, $p < 0.001$). As specified in Table 10, nCCA differed significantly between patients and controls. In the control group, the nCCA was not correlated with age ($r = -0.035$, $p = 0.88$).

Table 10. *Comparison of the radiological measurements of MS patients and controls.*

	MS patients	Controls	*p* value
N	23	23	
Normalized corpus callosum area	2.85 (0.79)	4.56 (0.59)	< 0.001*
Brain parenchymal fraction	67.4 (4.0)	70.0 (2.2)	0.007*
Grey matter fraction	35.3 (4.4)	37.8 (1.7)	0.016
White matter fraction	27.5 (4.1)	29.0 (2.0)	0.130

Mean values in % with standard deviations in parenthesis.

**Correlations that remained significant after correction for multiple testing.*

The nCCA tended to decrease with increasing disease duration, corrected for age and sex (r = -0.18, p = 0.087). The mean decrease in CCA was 6.6 mm^2 (1.2%) per year in the 23 patients followed to 2013, with a higher atrophy rate between the two first time points (8.6 mm^2, 1.6%) than the last two (4.4 mm^2, 0.9%). The decrease in corpus callosal atrophy is seen in Figure 26. An individual example of corpus callosal atrophy is seen in Figure 27.

Figure 26. *Normalized corpus callosum area over time for the 37 MS patients at all time points. Scatter plot with a linear regression line (left) and individual corpus callosum measurements connected by colored lines in the graph (right).*

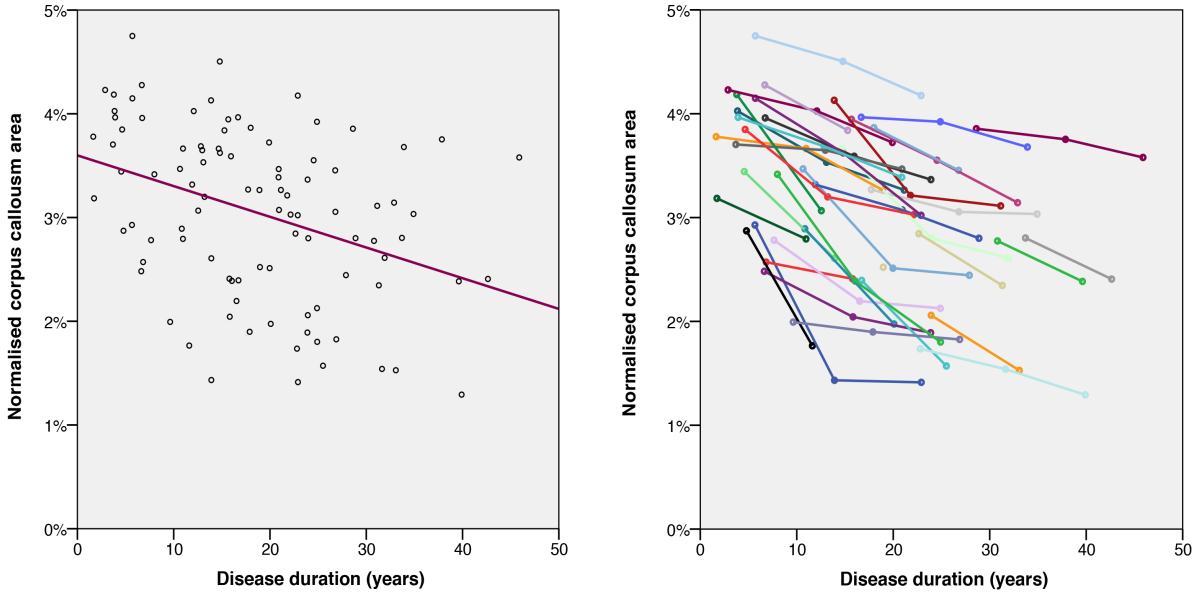
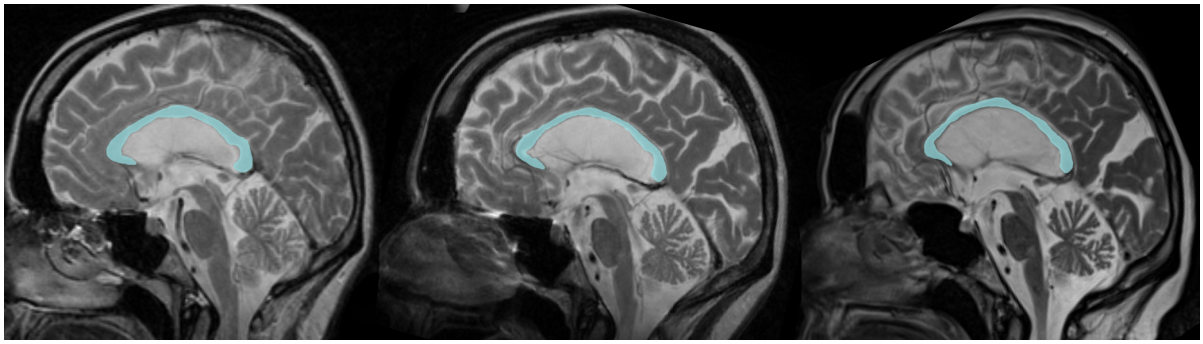


Figure 27. *The corpus callosum area in 1996 (left, 606 mm^2), 2004 (middle, 452 mm^2) and 2013 (right 418 mm^2) in a female MS patient. At the first time point she was 34 years old, had a disease duration of 19 years with RRMS and an EDSS of 2.5. At the later two time pints she had converted to SPMS and had EDSS scores of 7.5 and 8.0. SDMT scores were -1.2, -2.0 and -2.3 SD respectively.*



The nCCA was strongly correlated with the information processing speed as measured by SDMT (r = 0.79, p < 0.001) and moderately correlated with the EDSS (r = -0.55, p < 0.001) after adjusting for age, sex and disease duration. As shown in Table 11, these correlations remained significant after correcting for multiple comparisons. The longitudinal correlations are featured in Table 12, where only nCCA in 2004 showed a strong correlation with the SDMT in 2013.

Table 11. *Radiological measurements cross-sectional correlation with SDMT and EDSS.*

	Correlation to SDMT (*r, p*)	**Correlation to EDSS (*r, p*)**
Normalized corpus callosum area	0.79, < 0.001*	-0.55, < 0.001*
Brain parenchymal fraction	0.16, 0.31	-0.32, 0.017
Grey matter fraction	0.62, < 0.001*	-0.45, 0.001*
White matter fraction	-0.34, 0.028	0.10, 0.48
Normalized lesion volume	-0.72, < 0.001*	-0.49, < 0.001*

Table 12. *Radiological measurements at previous time points and their longitudinal correlation with SDMT and EDSS in 2013.*

		Correlation to SDMT (*r, p*)	**Correlation to EDSS (*r, p*)**
Normalized corpus callosum area	1996	0.58, 0.015	-0.46, 0.021
	2004	0.76, < 0.001*	-0.48, 0.016
Brain parenchymal fraction	2004	0.30, 0.24	-0.33, 0.20
Grey matter fraction	2004	0.62, 0.008	-0.53, 0.028
White matter fraction	2004	-0.020, 0.94	-0.054, 0.84
Normalized lesion volume	2004	-0.64, 0.006*	0.27, 0.30

*All normalized values are reported in %. Correlation coefficients and p-values adjusted for age, sex and disease duration. *Correlations that remained significant after correction for multiple testing.*

In order to study the predictive correlation of the measurements, the correlations with the end measurements were adjusted for the SDMT and EDSS at each time point. This revealed no independently significant predictive correlations of any of the radiological measurements with the end SDMT/EDSS results, as detailed in Supplementary Table 2 of the original article.[145]

Volumetry: BPF was the only volumetric measurement that differed significantly between MS patients and controls, as described in Table 10. The volumetric measurements overall showed stronger association with SDMT than EDSS. As detailed in Table 11, GMF and nLV were strongly correlated with SDMT and moderately correlated with EDSS after adjusting for age, sex and disease duration. From a longitudinal perspective, only nLV in 2004 showed a significant correlation with the SDMT in 2013. The results of all volumetric measurements at all time points are reported in Table 12.

5 DISCUSSION

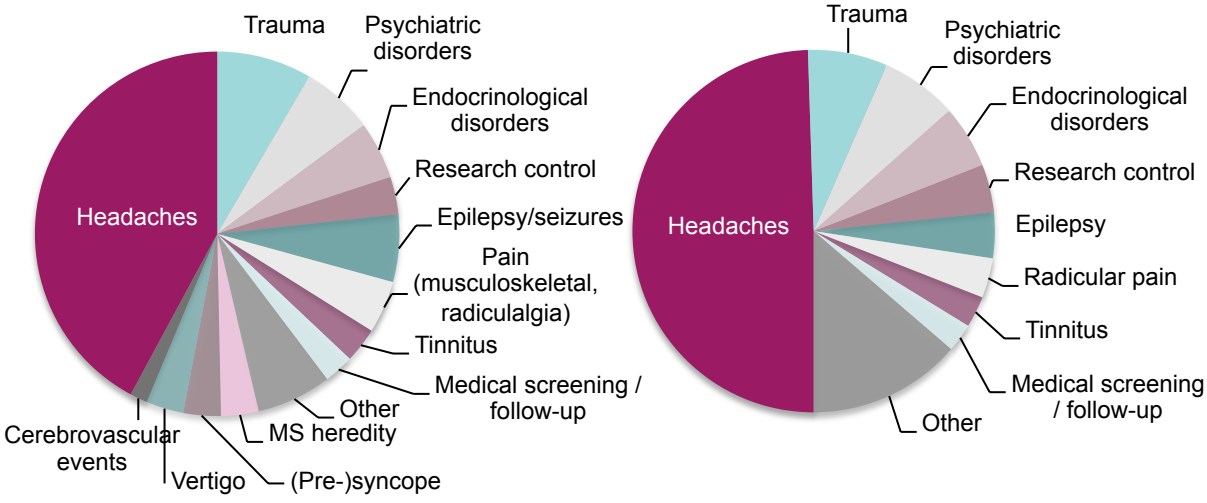
Study I: This systematic review was the first study to methodically summarize the knowledgebase regarding incidental MRI findings suggestive of MS. The results of the study have been important as they converged the terminology and the definition of this entity towards radiologically isolated syndrome, which is now the standard nomenclature.

The most important finding in Study I was that one third of the persons with RIS develop typical MS symptoms, and thereby convert to MS, within five years. This finding, in combination with the consistent reports of neuropsychological and neuroradiological similarities between MS and RIS, clearly suggests that RIS in many cases is a preclinical stage of MS. These results have recently been corroborated in a large retrospective multicenter study, which also confirmed that asymptomatic cervical lesions are the most important predictor of clinical progression.[100] Since the publication of Study I, the concept of RIS as subclinical/preclinical MS has been further underpinned by studies showing that:

- Metabolic changes indicative of axonal degeneration are detected by magnetic resonance spectroscopy in persons with RIS, similar to what is expected in MS.[146]
- Thalamic atrophy is more pronounced than reductions in WMV and GMV in RIS, which is in line with previous studies of CIS, pediatric MS and early RRMS.[147]
- White matter integrity measured with DTI is reduced in RIS compared to healthy controls, but to a lesser degree than in RRMS. However, functional connectivity measured with resting-state fMRI is not affected in RIS, while there are changes in RRMS. This suggests that the CNS damage is not as pronounced in RIS as in MS and/or that that RIS patients have a functional reserve.[148]

The above-mentioned multi-center study of RIS also reported similar indications for the MRI unveiling RIS, as displayed in Figure 28, highlighting headaches as the major indication.[100]

Figure 28. *Comparison of the MRI indications unveiling RIS in Study I (right, compiled data N=394),*[88] *and in the multi-center study by Okuda et al. (left, N=451).*[100]



The high frequency of headaches in RIS cases is noteworthy, but it remains unclear whether there is a causative relationship between the white matter anomalies and the headaches. Headaches are common in the general population and in MS, but headache prevalence estimates vary greatly, making it uncertain if headaches are more frequent in MS than in healthy individuals. Further complicating the issue is that headache is a known side effect of DMTs such as interferons,[149] and that persons with migraine have a higher prevalence of white matter abnormalities.[150] Epilepsy is another MRI indication in RIS of special interest since seizures have been described as an unusual presenting symptom of MS and that the prevalence of epilepsy is about three times higher in MS patients than in the general population.[151] However, neither headaches nor epilepsy are considered typical MS symptoms and it is therefore hard to ascertain whether these correlations should be interpreted as an atypical onset of MS if the affected individuals later convert to MS.

The subclinical cognitive deficits seen in RIS make it hard to distinguish the RIS entity from "cognitive MS", which is a rare sort of MS where cognitive impairment or behavioral changes are the primary and predominant manifestation of the disease.[152,153] However, by definition, persons with RIS do not fulfill the MS criteria and should not have cognitive deficits that affect their daily activities. It remains to be studied if persons with RIS that have subclinical cognitive deficits are more likely to develop cognitive MS.

Another point of interest is the fact that 15% of the reported cases in the multi-center study had a family history of MS,[100] since first degree relatives of MS patients have a 15-25 times higher risk of developing MS than the general population.[11] The only study to date reporting on the prevalence of RIS in MS relatives showed that 2 out of 68 (2.9%) participants fulfilled the RIS criteria.[154] However, MS heredity does not seem to be an independent predictor of conversion from RIS to MS,[100] and prospective studies of relatives to MS patients are needed to determine the natural prevalence and significance of RIS in relatives of MS patients.

The most surprising finding of Study I was that every tenth patient with RIS is treated with off-label immunosuppressive MS medications. As early treatment is beneficial in MS, it stands to reason that the same may be true for RIS that eventually evolves into MS. A caveat for this extrapolation is that it remains unclear if the clinical course of MS with a RIS onset differs from classical onset MS, which could possibly affect the risk-benefit analysis for treatment. Prospective follow-up of RIS is the recommended strategy by the MS Phenotype Group until treatment trials have been performed.[30]

Strengths of the study were the systematic approach, following the PRISMA guidelines, and the use of multiple raters who individually analyzed the results of the literature searches. A limitation of the study was the semi-quantitative nature of the results as differences in terminology, methodology an possible overlaps of the described RIS cohorts hindered a precise meta-analysis to be conducted.

Study II: The main finding in this observational study was that the frequency of RIS was much lower than expected. RIS findings constituted just 0.05% of a yearly sample of brain MRI examinations at a tertiary hospital in the Stockholm region, which has a high incidence and prevalence of MS. Interestingly, the only case of RIS showed a fast conversion to MS, but then a relatively benign disease course.

The incidence of RIS is expected to increase with MRI usage and the MS incidence. The low frequency of RIS findings is therefore somewhat counterintuitive when compared with the only previous epidemiological study of RIS, which was conducted in Pakistan, a region with low MS incidence and prevalence. In the same age group (15-40 years), we found a lower RIS frequency of 0.15% compared to 0.7% in Pakistan. One explanation for this discrepancy could be the fact that the awareness of MS and MS symptoms is higher in the general population and among doctors in high-incidence regions for MS. Unexpected MS findings would thus be less frequent. Differences in study methodology and MRI availability may also have contributed to the differences in results.

When interpreting the results of Study II, there are two major limitations that have to be taken into account. One is the fact that the initial screening of the radiological data was not conducted by a radiologist, but instead by a trained rater (Tobias Granberg), who at the time of analysis was a medical student. Although all patients of interest were re-evaluated by a neuroradiologist, it can be argued that the use of a second rater or a rater with longer clinical experience could possibly have increased the number of identified patients of interest. Another limitation is the fact that the sample was taken from a university hospital in a region with high MS incidence and prevalence, which may not be generalizable to other regions or non-tertiary hospitals.

In an effort to increase our understanding of the epidemiology of RIS, we decided to account for these limitations in a second study that we conducted in 2014, which is currently under review. A similar approach was used but the radiological screening was performed by a radiologist in a population-based sample containing all brain MRI's performed in Västmanland county, Sweden, in 2013. The RIS incidence rate in Västmanland was found to be similar to our findings in Study II, suggesting that above-mentioned limitations may not have influenced the results substantially.

Study III and IV: There are two major implications from the results of these two studies. One is that the results encourage us to refocus research and clinical interest in corpus callosum morphology because of its special strategic importance and its close association with physical and cognitive disability in MS. The second is that corpus callosum atrophy is easily quantifiable with excellent reproducibility with 2D measurements that can be obtained on conventional sequences within less than a minute by trained raters.

Study III constituted the first comparative study of the two most commonly used 2D measurements of corpus callosum – corpus callosum area (CCA) and corpus callosum index (CCI). Both proved to have excellent repeatability and reproducibility across raters with

varying neuroradiological experience and across sequence types. The acquisition time for CCI was faster than for CCA (18 versus 43 seconds), while the necessary manual edits of the automatic volumetry were far more time-consuming. All corpus callosum measurements (CCA, CCI and CCV) were more closely associated with cognitive and physical disability than commonly used volumetric measurements (BV, WMV, GMV and LV), which is in line with previous studies.[115,121,155] CCA consistently proved to be the measurement with the strongest relationship with the clinical parameters, both SDMT (r = 0.82, p < 0.001) and EDSS (r = -0.56, p < 0.001), after adjusting for age, sex and disease duration. CCA also had the highest accuracy in differentiating MS patients from controls (95%) and RRMS from progressive forms of MS (77%).

Study IV showed that the corpus callosal atrophy rate decreased with increasing disease duration and that the nCCA was strongly correlated with SDMT and EDSS even during nearly two decades of follow-up. The plateauing corpus callosal atrophy and physical disability indicate that the underlying pathology in highly myelinated areas is more aggressive in the early phases of the disease, encouraging early treatment. This interpretation is supported by histopathological studies showing that the most extensive axonal damage occurs in the early phases of MS.[156]

Interestingly, there was no correlation between nCCA and age in the healthy control group while the classic brain volumetric measurements tended to decrease with age. The relative resistance to age-dependent change is in line with previous studies.[157–159] This suggests that corpus callosum morphology may be a sensitive marker for MS pathology, especially at higher ages/later disease stages.

Discouragingly, none of the radiological measurements had an independent predictive value in terms of predicting the clinical outcome. The results did, however, show interesting trends indicating that predictive values may be seen in larger cohorts.

The slightly better performance of CCA compared to CCV is somewhat surprising as 3D measurements are generally preferable in most brain quantifications. This may not be true for corpus callosum as volume-based corpus callosum measurements struggle with the issue of defining the mediolateral anatomical borders of corpus callosum. This is due to the fact that corpus callosum consists of white matter tracts projecting between the two hemispheres and is thus in continuity with the white matter in both cerebral hemispheres. This issue is avoided in cross-sectional 2D measurements and may be the reason that CCA performs better as a biomarker in our studies. The fact that CCA is a measurement of the whole cross-sectional area of corpus callosum while CCI mainly reflect atrophy in the three measuring points in the genu, body, and splenium may explain the difference in performance between CCA and CCI.

The major strengths of the studies are the longitudinal perspective, where the cohort has been studied over 17 years, and the fact that the same experienced neurologist and neuropsychologist have performed the clinical assessments throughout the study. Another strong point is the use of a matched healthy control group for comparison. The results also

have a good generalizability as the studied cohort reflects different subtypes of MS, a wide range of disability levels and disease durations spanning over five decades.

The studies provide data on the longterm clinical and radiological progression in MS, which is a rarity due to the limited availability of MRI two decades ago. The scarce MRI availability is also the cause of the studies' relatively small sample size, which is a major limitation of the studies. The loss of participants to the last time point is unfortunately a natural effect of the long follow-up as most of the patients that we were unable follow to 2013 were deceased or too disabled to participate. Despite the few participants, the results were highly significant and remained so after correcting for known confounders and multiple comparisons. The classification performance in Study III also remained stable in cross-validation testing.

Another limitation is that three different scanners were used due to the natural need for hardware upgrades at the radiological department. To compensate for this, efforts were made to harmonize imaging parameters over time and all imaging was performed on 1.5 T scanners. Freesurfer measurements furthermore have good reproducibility across different scanners and even across field strengths, especially when using the longitudinal processing stream, as we did.[160] Normalizations for head size were also used when analyzing the data longitudinally in Study IV, as this is an effective way of reducing inter-scanner variability.[125]

Possible confounders in neuropsychological testing are depression and fatigue, and a major limitation of study III and IV is that no tests were performed to diagnose these conditions. This was a conscious choice since the neuropathological test battery and the MRI protocol at the last time point took 2.5 hours and adding further testing was deemed too impractical and uncomfortable for the participants. It also remains unclear how findings of depression and/or fatigue should have been handled since their impact on neuropsychological testing is unclear, with conflicting results in the literature.[46] It cannot arbitrarily be managed statistically and excluding patients with depression would both decrease the power of the study and limit the generalizability of the results as the lifetime risk of depression in MS is high.[161]

Lastly, the effects of medication with MS drugs on the clinical and radiological assessments are hard to appreciate as the frequency and type of MS treatment naturally varied throughout the study.

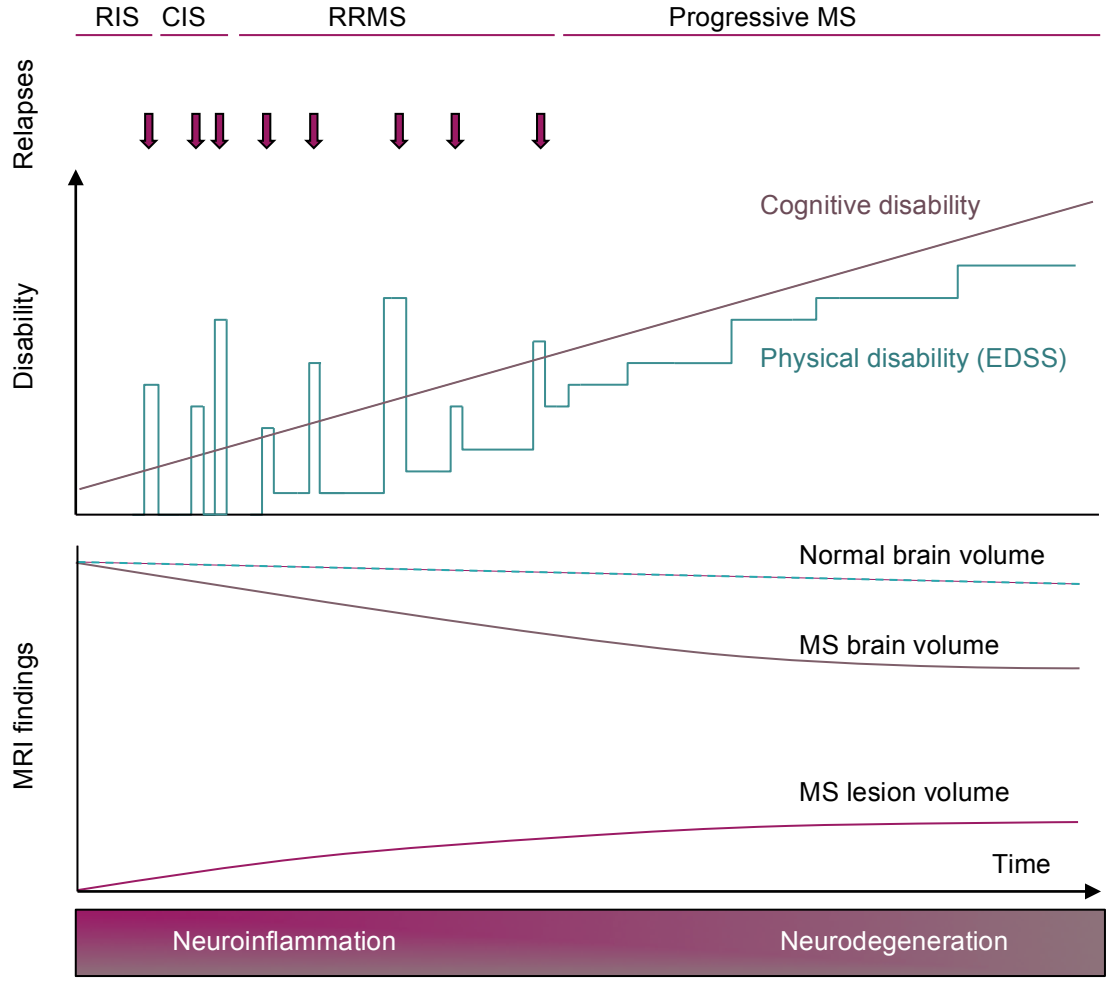
General discussion: The findings of this thesis support the idea that RIS can be the earliest sign of MS, a glimpse of the disease before clinical conversion to CIS or MS. Figure 29 is an illustration that conceptualizes the integration of RIS in the MS paradigm.

Patients will enter the trajectory at different phases in the illustration. Some will enter with RIS if they have happened to have an MRI (due to other indications or atypical symptoms) before MS onset. Others will present as CIS or RRMS with typical MS-symptoms and relapses, with the majority of cases eventually reaching a progressive disease stage.

SPMS and PPMS has been grouped together with the rationale that they have similar ages at onset of progression.[21] This grouping is controversial as the subtypes differ substantially from

a clinical point of view. However, histopathologically and radiologically the PPMS do not seem to differ considerably from the other subtypes, although RRMS patients tend to have larger lesions.[162,163] It is therefore argued that a primary-progressive disease course without disease activity could possibly reflect a later stage of the disease where there has been an occult inflammatory phase, maybe due to the clinico-radiological paradox (see section 1.2.6) or other individual differences. Whether PPMS patients have a subclinical inflammatory phase is debated, but there is data suggesting that many PPMS patients do have contrast-enhancing lesions, especially in the early phases (<5 years disease duration) of their disease.[164] Interestingly, about 10% of persons with RIS go on to develop PPMS and thereby lack a classical inflammatory phase of the disease.[100]

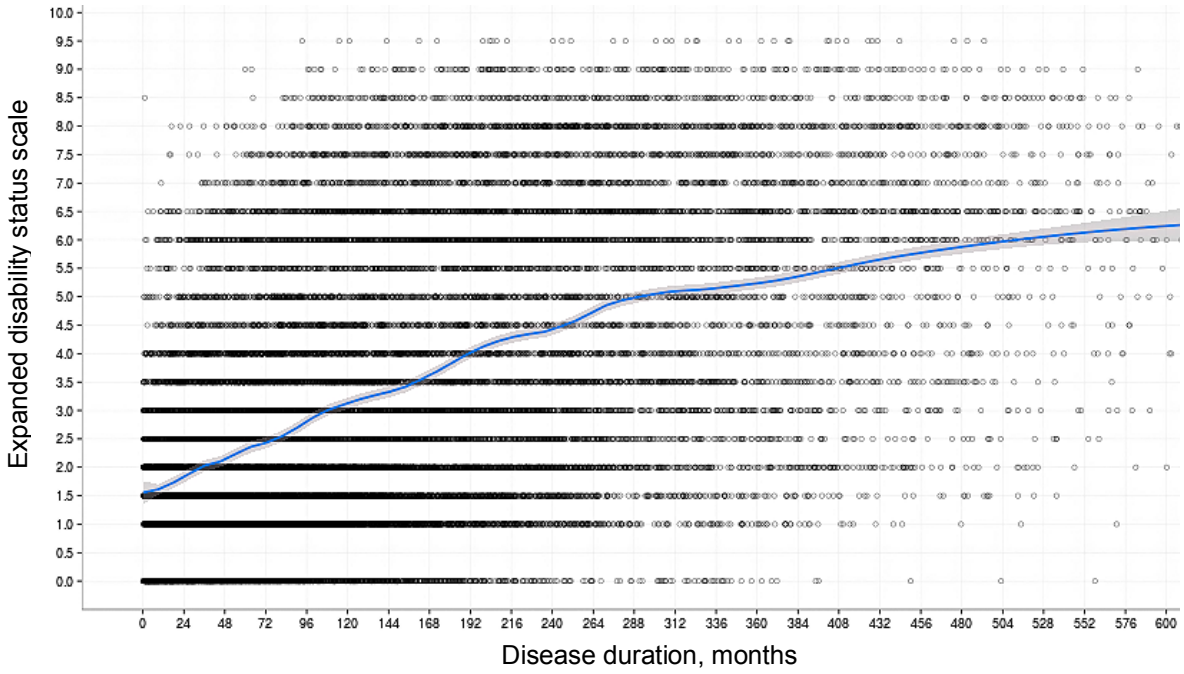
Figure 29. *An illustration of how RIS may be interpreted as part of the MS paradigm.*



In the illustration, there is a plateau of EDSS as seen in Study IV. This is consistent with data from the Swedish MS registry (see Figure 30). MS lesions have been reported to increase by about 5-10% per year,[101] and the reported annual brain atrophy rates are around 0.6-1.0%.[60,78] Based on our last two time points the lesion volume only increased by 2.2% per year and the brain volume reduction was a mere 0.2% per year. This suggest that the accumulated damage has a biological upper limit as the disease reaches a state where there is less healthy tissue left to loose, which is why the MRI findings in the figure above eventually plateau. This concept is supported by previous studies that suggest that the threshold for the plateauing effect is

around the time that patients reach EDSS 4,[102] after which the rate of progression of physical disability is quite similar for patients with relapse-remitting and progressive onset.[165]

Figure 30. *An illustration of all registered EDSS scores of MS patients in Stockholm in the Swedish MS registry with a locally weighted scatterplot smoothing regression line. Image courtesy of Leszek Stawiarz at the Swedish Neuro Registries.*



In order for the RIS concept to gain further traction it is important that the diagnostic criteria are intuitive. An aspect that is rarely discussed in terms of RIS is the complexity of the Okuda criteria. These issues are discussed in detail below.

- A corner-stone issue is that the findings in the current definition have to be incidental. This requirement makes it impossible to prospectively study RIS in general populations or in relatives of MS patients as the findings are then not truly incidental.
- The phrasing of "remitting clinical symptoms consistent with neurologic dysfunction" is imprecise with a lack of clarity in terms of what indications for MRI are to be accepted within the RIS entity.
- The impact on the activities of daily life (criterion C) is hard to evaluate. It also seems likely that even subclinical cognitive impairment in RIS may discreetly affect everyday life activities.
- Clinical findings suggestive of MS in neurological examinations are not mentioned. It remains an open question whether it is reasonable to classify a symptomless person with clear neurological findings suggestive of MS as having RIS.
- The concept of "no better explanation" is diffuse and met in several different criteria: A3, D, E and F.
- The DIS classification (A2) is not in line with modern MS diagnostic criteria. This aspect is of special importance as the choice of DIS classification may decrease or increase the incidence and prevalence of RIS as well as the proportion of persons that will eventually convert to MS.

- The concept and significance of MRI evidence of DIT is not mentioned. This is of importance as contrast-enhancing lesions and dynamics with new lesions are more suggestive of MS than ischemic-degenerative white matter abnormalities.
- Formulations could be more consise. An example is the redundancy in A1: "with or without involvement of the corpus callosum".

A new simplified RIS definition is therefore hereby proposed:

Table 13. *Proposition for new RIS criteria.*

A	MRI findings fulfilling the current diagnostic MRI criteria for DIS.*
B	No symptoms or neurological findings typical for MS.**
C	The findings should not be more likely or better explained by another disease process, comorbidities or substances***

**Currently the 2010 McDonald criteria.*[25]

***Symptoms should be interpreted with the consultation of an experienced MS neurologist. Only symptoms that do not render a CIS or MS diagnosis, following a thorough physical neurological examination, are accepted.*

****The concept of "no better explanation" has been thoroughly discussed by Charil et al.*[66]

6 CONCLUSIONS

In summary, the first two studies of this thesis showed that unexpected brain MRI findings suggestive of MS are relatively uncommon in a region with a high prevalence and incidence of MS. These incidental radiological findings, which are preferably called radiologically isolated syndrome, are despite of this of clinical importance since persons with RIS are at high risk of developing MS. RIS is thus in many cases a preclinical stage of MS. RIS is therefore conceptually important to study since it may give an insight into the earliest stages of MS and why some individuals have no or atypical initial MS-symptoms.

The second two studies of this thesis describe the progression of corpus callosal atrophy over 17 years in a cohort of MS patients with disease durations spanning over nearly five decades, thereby including both early and late stages of the diseases. Novel findings include that the corpus callosum atrophy rate decreases with the disease duration and that CCA remains the preferred method for studying corpus callosal atrophy, even though new methods such as CCI and CCV have been introduced. The studies add to the growing body of evidence that corpus callosum atrophy is a more sensitive MRI biomarker than classical volumetric measurements. This suggests that CCA may be a suitable quantitative paraclinical biomarker for cognitive and physical disability in MS research and possibly in clinical practice since it is a fast method with excellent reproducibility.

7 FUTURE ASPECTS

Although RIS has become a hot topic in the MS field, there is still work that needs to be done before RIS can be widely accepted as a preclinical and/or subclinical stage of MS and integrated into the MS classification tree. The definition of RIS has to be simplified and harmonized with the current diagnostic criteria for MS. The concept of "no better explanation" also has to be concretized to preserve a high specificity of RIS in terms of the risk for conversion to MS.

Large prospective studies will have to show whether persons with RIS are more likely to have a similar or more benign clinical course of MS and the rationale for treating RIS with MS drugs. Hopefully, we will soon be able to tell if we can prevent or delay the conversion of RIS to MS as the first treatment trial for RIS is already planned.[166] The relative sparsity of RIS encourages collaborative research efforts. The findings of this thesis and other RIS studies can aid us in constructing guidelines for management of RIS, preferably on a national or international level, in order to standardize the care for persons with RIS.

The new therapeutic options in MS have created a need for prompt diagnosis and good predictors of the disease course to tailor the treatment for optimal results. Radiological practice has to implement new quantitative methods to respond to this need. There are many emerging imaging techniques (see 1.2.9) that may prove to be specific and robust enough for clinical use. Until then, measurements of corpus callosum atrophy could be a favorable approach for improving the diagnostics and surveillance in MS.

CCA is a viable option due to its excellent reproducibility and that it can be performed by trained raters. The possibility to apply CCA to previously acquired sagittal 2D data also provides an opportunity to make use of previously acquired data in a way that is not possible with volumetry. The findings regarding CCA are therefore likely to be welcomed by research groups that are in need of quantitative MRI biomarkers but do not have the resources for volumetric analyses.

Despite the practicality of CCA it may still prove challenging to implement it in a clinical setting as the method requires a short introduction and would be additional work for the rater in the clinical workflow. Cut-off values for different degrees of corpus callosal atrophy would also be necessary for interpretation on an individual level. The suggested 6 cm^2 CCA value will have to be validated in other cohorts. An attractive option for clinical use that we are currently exploring is the development of a visual rating scale for corpus callosal atrophy where the visual interpretation could be complemented with CCA measurements in uncertain cases.

8 ACKNOWLEDGEMENTS

I wish to thank everyone who contributed to this thesis. In particular, I would like to thank:

My main supervisor, Docent **Maria Kristoffersen-Wiberg**, for her kind support and positive reinforcement ever since my first research project in 2009. You have given me the opportunity to grow freely as a researcher with kind encouragement. I appreciate that you have always given me your full endorsement to also explore interesting aspects and side-projects outside the scope of this thesis.

Professor **Sten Fredrikson**, my supervisor, for planting the idea of focusing on multiple sclerosis in my research by his inspiring lectures in neurology when I was a medical student in 2010. Like Baloo, I believe that you have tried to "teach me everything you know" about MS. I hope that I managed to absorb as much as possible of your profound knowledge of MS.

Professor **Peter Aspelin**, my supervisor, for his full support, positive outlook and strategic advice throughout my doctoral process. You have made these studies possible and have been a keystone in the foundation of my scientific learning. I believe that the Aspelin legacy will continue to be of great importance in radiology.

Doctor **Juha Martola**, my supervisor, for creating an important milestone in the longitudinal cohort study in 2004 for his doctoral thesis. Your enthusiastic engagement in my doctoral project via e-mails and flying across the Baltic Sea from Helsinki countless times for meetings and ratings have been fundamental for this thesis.

My co-author **Doctor Gösta Bergendal**, who have taught me a great deal on the neuropsychological aspects of MS and have been an excellent partner for long discussions on cognitive aspects of MS. I hope you are taking good care of D*uck*tor Bergendal and look forward to our continuing collaboration.

My co-author and fellow PhD student **Sara Shams**, who with amazing energy have expanded and advanced the research project on cerebral microbleeds that was once my entry point to radiological research. I am glad to have you in our research group with your high work ethic and optimistic perspectives.

My clinical supervisor, **Farouk Hashim**, who encouraged me as a medical student to pursue a career in radiology. You have shown me countless interesting cases and thereby inspired me follow you onto this career path. I hope that I in return will be able to enthuse you to explore the scientific aspects of it and that we will collaborate in many future projects.

My mentor, Docent **Christina Sjöstrand**, who has been a great partner to share and discuss my doctoral experience with during the early phases of my studies.

Our administrator **Helena Forssell**, who has been a great help in guiding me through the jungle of forms, rules and traditions that are a part of the doctoral process.

MRI physicists **Martin Uppman** and **Love Engström-Nordin** for helping me design and test image acquisition protocols and for teaching me the principles of MRI and how to use the MRI scanners.

MRI nurses **Maria Andersson**, **Eliisa Asp**, **Hassan Ataei-Chegeni**, **Kerstin Eriksson**, **Marie Persson Edsborg**, **Hannele Siirilä**, **Soroush Sohrabian**, **Pia Sonfors**, **Karin Telin** and **Handan Özalp** for their interest in my studies and for teaching me the practicalities of using the MRI scanners.

My fellow PhD student and clinical colleague **Yngve Forslin**, who has joined our group with great eagerness to carry on and expanding our work on neuroradiological aspects of MS.

My collaborators **Johan Berglund**, **Carmen Cananau**, **Lena Cavallin**, **Virginija Danylaité Karrenbauer**, **Daniel Ferreira**, **Katharina Fink**, **Henrik Sjöström**, **Leszek Stawiarz**, **Lars-Olof Wahlund** and **Eric Westman**, with whom I have been involved in interesting research projects outside of this thesis.

My clinical colleagues **Babak Flahat**, **Ole Kvorning**, **Susanne Müller**, **Ella Popovici**, **Marie Sund**, the **Neuro group** and my **fellow medical students, interns and residents** for inspiration and encouragement during the different stages of my PhD studies.

Our residency director **Marie-Louise Wretling** for encouraging me to continue on the radiology pathway ever since my medical studies and for endorsing a mixture of clinical work, research and teaching during the residency.

My friend **Karin Johnsson** for valuable comments on this thesis.

I would also like to give special acknowledgement to **my family and friends** for your love and for supporting me in pursuing this scientific exploration. Without your support and encouragement, this would not have been achievable.

Last, but not least, I would like to take this opportunity to thank all the study **participants** and the **patients' families** for their time and effort, which made this research possible.

This research has made use of the resources of **SMILE** medical imaging laboratory at Karolinska University Hospital and **neuGRID**, a grid computational framework financed by the European Commission's Seventh Framework Programme. The studies have been funded by **Karolinska Institutet** and **Stockholms Läns Landsting** through an ALF grant.

9 REFERENCES

1. Compston A, Coles A. Multiple sclerosis. *Lancet*. 2002;359(9313):1221–1231.

2. Scolding N. *Multiple sclerosis*. 1st ed. Oxford University Press; 2012.

3. Fagius J, Röhl A. *Multipel skleros*. Karolinska Institutet University Press; 2007.

4. 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(2):237–243.

5. Landtblom A-M, Fazio P, Fredrikson S, et al. The first case history of multiple sclerosis: Augustus d'Esté (1794-1848). *Neurol. Sci.* 2010;31(1):29–33.

6. Kumar DR, Aslinia F, Yale SH, et al. Jean-Martin Charcot: The Father of Neurology. *Clin. Med. Res.* 2011;9(1):46–49.

7. Dua T. *Multiple sclerosis resources in the world.* World Health Organization; 2008.

8. Ahlgren C, Odén A, Lycke J. High nationwide prevalence of multiple sclerosis in Sweden. *Mult. Scler.* 2011;17(8):901–908.

9. Ahlgren C, Odén A, Lycke J. High Nationwide Incidence of Multiple Sclerosis in Sweden. *PLoS ONE*. 2014;9(9).

10. Koch-Henriksen N, Sørensen PS. The changing demographic pattern of multiple sclerosis epidemiology. *Lancet Neurol.* 2010;9(5):520–532.

11. Dyment DA, Ebers GC, Dessa Sadovnick A. Genetics of multiple sclerosis. *Lancet Neurol.* 2004;3(2):104–110.

12. International Multiple Sclerosis Genetics Consortium (IMSGC), Beecham AH, Patsopoulos NA, et al. Analysis of immune-related loci identifies 48 new susceptibility variants for multiple sclerosis. *Nat. Genet.* 2013;45(11):1353–1360.

13. Multiple Sclerosis International Federation. *Atlas of Multiple Sclerosis.* Available at: http://www.msif.org/about-us/advocacy/atlas/. Accessed April 8, 2015.

14. Marrie RA. Environmental risk factors in multiple sclerosis aetiology. *Lancet Neurol.* 2004;3(12):709–718.

15. Simpson S, Blizzard L, Otahal P, et al. Latitude is significantly associated with the prevalence of multiple sclerosis: a meta-analysis. *J. Neurol. Neurosurg. Psychiatry*. 2011;82(10):1132-41.

16. Ascherio A, Munger KL. Environmental risk factors for multiple sclerosis. Part II: Noninfectious factors. *Ann. Neurol.* 2007;61(6):504–513.

17. Belbasis L, Bellou V, Evangelou E, et al. Environmental risk factors and multiple sclerosis: an umbrella review of systematic reviews and meta-analyses. *Lancet Neurol.* 2015;14(3):263–273.

18. Evans AS, Kaslow RA. *Viral Infections of Humans: Epidemiology and Control.* Springer Science & Business Media; 1997.

19. Siegel GJ. *Basic Neurochemistry*. 6th ed. Lippincott-Raven; 1999.

20. Lucchinetti C, Brück W, Parisi J, et al. Heterogeneity of multiple sclerosis lesions: implications for the pathogenesis of demyelination. *Ann. Neurol.* 2000;47(6):707–717.

21. Trapp BD, Nave K-A. Multiple sclerosis: an immune or neurodegenerative disorder? *Annu. Rev. Neurosci.* 2008;31:247–269.

22. Stephen DeArmond. *Multiple Sclerosis Microscopic Pathology.* Available at: http://missinglink.ucsf.edu/lm/ids_104_Demyelination/Didactic/MSMicroPath.htm. Accessed April 29, 2015.

23. Magliozzi R, Howell O, Vora A, et al. Meningeal B-cell follicles in secondary progressive multiple sclerosis associate with early onset of disease and severe cortical pathology. *Brain*. 2007;130(4):1089–1104.

24. Hauser SL, Waubant E, Arnold DL, et al. B-Cell Depletion with Rituximab in Relapsing–Remitting Multiple Sclerosis. *N. Engl. J. Med.* 2008;358(7):676–688.

25. Polman CH, Reingold SC, Banwell B, et al. Diagnostic criteria for multiple sclerosis: 2010 Revisions to the McDonald criteria. *Ann. Neurol.* 2011;69(2):292–302.

26. Schumacher GA, Beebe G, Kibler RF, et al. Problems of Experimental Trials of Therapy in Multiple Sclerosis: Report by the Panel on the Evaluation of Experimental Trials of Therapy in Multiple Sclerosis. *Ann. N. Y. Acad. Sci.* 1965;122(1):552–568.

27. Barkhof F, Filippi M, Miller DH, et al. Comparison of MRI criteria at first presentation to predict conversion to clinically definite multiple sclerosis. *Brain*. 1997;120(11):2059–2069.

28. McDonald WI, Compston A, Edan G, et al. Recommended diagnostic criteria for multiple sclerosis: guidelines from the International Panel on the diagnosis of multiple sclerosis. *Ann. Neurol.* 2001;50(1):121–127.

29. Polman CH, Reingold SC, Edan G, et al. Diagnostic criteria for multiple sclerosis: 2005 revisions to the "McDonald Criteria." *Ann. Neurol.* 2005;58(6):840–846.

30. Lublin FD, Reingold SC, Cohen JA, et al. Defining the clinical course of multiple sclerosis: the 2013 revisions. *Neurology*. 2014;83(3):278–286.

31. Sormani MP, Tintorè M, Rovaris M, et al. Will Rogers phenomenon in multiple sclerosis. *Ann. Neurol.* 2008;64(4):428–433.

32. 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. *Neurology*. 1996;46(4):907–911.

33. Rovaris M, Confavreux C, Furlan R, et al. Secondary progressive multiple sclerosis: current knowledge and future challenges. *Lancet Neurol.* 2006;5(4):343–354.

34. Langer-Gould A, Popat RA, Huang SM, et al. Clinical and demographic predictors of long-term disability in patients with relapsing-remitting multiple sclerosis: a systematic review. *Arch. Neurol.* 2006;63(12):1686–1691.

35. Oh J, Calabresi PA. Emerging injectable therapies for multiple sclerosis. *Lancet Neurol.* 2013;12(11):1115–1126.

36. Lublin F. History of modern multiple sclerosis therapy. *J. Neurol.* 2005;252(3):iii3–iii9.

37. Trojano M, Pellegrini F, Fuiani A, et al. New natural history of interferon-beta-treated relapsing multiple sclerosis. *Ann. Neurol.* 2007;61(4):300–306.

38. Goodin DS, Reder AT, Ebers GC, et al. Survival in MS: a randomized cohort study 21 years after the start of the pivotal IFNβ-1b trial. *Neurology*. 2012;78(17):1315–1322.

39. Martin R. Multiple sclerosis: Closing in on an oral treatment. *Nature*. 2010;464(7287):360–362.

40. Burman J, Iacobaeus E, Svenningsson A, et al. Autologous haematopoietic stem cell transplantation for aggressive multiple sclerosis: the Swedish experience. *J. Neurol. Neurosurg. Psychiatry*. 2014;85(10):1116–1121.

41. Mancardi GL, Sormani MP, Gualandi F, et al. Autologous hematopoietic stem cell transplantation in multiple sclerosis A phase II trial. *Neurology*. 2015;84(10):981–988.

42. Chataway J, Schuerer N, Alsanousi A, et al. Effect of high-dose simvastatin on brain atrophy and disability in secondary progressive multiple sclerosis (MS-STAT): a randomised, placebo-controlled, phase 2 trial. *Lancet*. 2014.

43. Filippi M, Rocca MA. MR Imaging of Multiple Sclerosis. *Radiology*. 2011;259(3):659–681.

44. Kurtzke JF. Rating neurologic impairment in multiple sclerosis An expanded disability status scale (EDSS). *Neurology*. 1983;33(11):1444.

45. Cohen JA, Reingold SC, Polman CH, et al. Disability outcome measures in multiple sclerosis clinical trials: current status and future prospects. *Lancet Neurol.* 2012;11(5):467–476.

46. Chiaravalloti ND, DeLuca J. Cognitive impairment in multiple sclerosis. *Lancet Neurol.* 2008;7(12):1139–1151.

47. Rocca MA, Amato MP, De Stefano N, et al. Clinical and imaging assessment of cognitive dysfunction in multiple sclerosis. *Lancet Neurol.* 2015;14(3):302–317.

48. Langdon DW, Amato MP, Boringa J, et al. Recommendations for a Brief International Cognitive Assessment for Multiple Sclerosis (BICAMS). *Mult. Scler.* 2012;18(6):891–898.

49. Benedict RHB, Amato MP, Boringa J, et al. Brief International Cognitive Assessment for MS (BICAMS): international standards for validation. *BMC Neurol.* 2012;12:55.

50. Forn C, Belloch V, Bustamante JC, et al. A symbol digit modalities test version suitable for functional MRI studies. *Neurosci. Lett.* 2009;456(1):11–14.

51. Forn C, Belenguer A, Belloch V, et al. Anatomical and functional differences between the Paced Auditory Serial Addition Test and the Symbol Digit Modalities Test. *J. Clin. Exp. Neuropsychol.* 2011;33(1):42–50.

52. Birn RM, Kenworthy L, Case L, et al. Neural systems supporting lexical search guided by letter and semantic category cues: A self-paced overt response fMRI study of verbal fluency. *NeuroImage*. 2010;49(1):1099–1107.

53. Barry D, Bates ME, Labouvie E. FAS and CFL Forms of Verbal Fluency Differ in Difficulty: A Meta-analytic Study. *Appl. Neuropsychol.* 2008;15(2):97–106.

54. Shin M-S, Park S-Y, Park S-R, et al. Clinical and empirical applications of the Rey–Osterrieth Complex Figure Test. *Nat. Protoc.* 2006;1(2):892–899.

55. Litvan I, Grafman J, Vendrell P, et al. Multiple memory deficits in patients with multiple sclerosis: Exploring the working memory system. *Arch. Neurol.* 1988;45(6):607–610.

56. Young IR, Hall AS, Pallis CA, et al. Nuclear magnetic resonance imaging of the brain in multiple sclerosis. *Lancet*. 1981;2(8255):1063–1066.

57. Lövblad K-O, Anzalone N, Dörfler A, et al. MR Imaging in Multiple Sclerosis: Review and Recommendations for Current Practice. *Am. J. Neuroradiol.* 2010;31(6):983–989.

58. Nobel Media. The Nobel Prize in Physiology or Medicine for 2003. Available at: http://www.nobelprize.org/nobel_prizes/medicine/laureates/2003/press.html. Accessed April 1, 2015.

59. McRobbie DW, Moore EA, Graves MJ, et al. *MRI from Picture to Proton*. 2nd ed. Cambridge University Press; 2007.

60. Filippi M, Rocca MA, Barkhof F, et al. Association between pathological and MRI findings in multiple sclerosis. *Lancet Neurol.* 2012;11(4):349–360.

61. Bink A, Schmitt M, Gaa J, et al. Detection of lesions in multiple sclerosis by 2D FLAIR and single-slab 3D FLAIR sequences at 3.0 T: initial results. *Eur. Radiol.* 2006;16(5):1104–1110.

62. Polak P, Magnano C, Zivadinov R, et al. 3D FLAIRED: 3D fluid attenuated inversion recovery for enhanced detection of lesions in multiple sclerosis. *Magn. Reson. Med.* 2012;68(3):874–881.

63. Ciccarelli O, Barkhof F, Bodini B, et al. Pathogenesis of multiple sclerosis: insights from molecular and metabolic imaging. *Lancet Neurol.* 2014;13(8):807–822.

64. Brant-Zawadzki M, Gillan GD, Nitz WR. MP RAGE: a three-dimensional, T1-weighted, gradient-echo sequence--initial experience in the brain. *Radiology*. 1992;182(3):769–775.

65. Sinnecker T, Mittelstaedt P, Dörr J, et al. Multiple sclerosis lesions and irreversible brain tissue damage: A comparative ultrahigh-field strength magnetic resonance imaging study. *Arch. Neurol.* 2012;69(6):739–745.

66. Charil A, Yousry TA, Rovaris M, et al. MRI and the diagnosis of multiple sclerosis: expanding the concept of "no better explanation". *Lancet Neurol.* 2006;5(10):841–852.

67. Barkhof F, Simon JH, Fazekas F, et al. MRI monitoring of immunomodulation in relapse-onset multiple sclerosis trials. *Nat. Rev. Neurol.* 2012;8(1):13–21.

68. Gaitán MI, Shea CD, Evangelou IE, et al. Evolution of the blood–brain barrier in newly forming multiple sclerosis lesions. *Ann. Neurol.* 2011;70(1):22–29.

69. Ge Y, Grossman RI, Udupa JK, et al. Brain Atrophy in Relapsing-Remitting Multiple Sclerosis and Secondary Progressive Multiple Sclerosis: Longitudinal Quantitative Analysis. *Radiology*. 2000;214(3):665–670.

70. Garg N, Reddel SW, Miller DH, et al. The corpus callosum in the diagnosis of multiple sclerosis and other CNS demyelinating and inflammatory diseases. *J. Neurol. Neurosurg. Psychiatry*. E-pub ahead of Apr 9 2015. DOI: 10.1136/jnnp-2014-309649.

71. Swanton JK, Fernando K, Dalton CM, et al. Modification of MRI criteria for multiple sclerosis in patients with clinically isolated syndromes. *J. Neurol. Neurosurg. Psychiatry*. 2006;77(7):830–833.

72. Montalban X, Tintoré M, Swanton J, et al. MRI criteria for MS in patients with clinically isolated syndromes. *Neurology*. 2010;74(5):427–434.

73. Moraal B, Roosendaal SD, Pouwels PJW, et al. Multi-contrast, isotropic, single-slab 3D MR imaging in multiple sclerosis. *Eur. Radiol.* 2008;18(10):2311–2320.

74. Barkhof F. The clinico-radiological paradox in multiple sclerosis revisited. *Curr. Opin. Neurol.* 2002;15(3):239–245.

75. Okuda DT, Mowry EM, Cree BAC, et al. Asymptomatic spinal cord lesions predict disease progression in radiologically isolated syndrome. *Neurology*. 2011;76(8):686–692.

76. Kearney H, Miller DH, Ciccarelli O. Spinal cord MRI in multiple sclerosis—diagnostic, prognostic and clinical value. *Nat. Rev. Neurol.* 2015;11(6):327–338.

77. Lycklama G, Thompson A, Filippi M, et al. Spinal-cord MRI in multiple sclerosis. *Lancet Neurol.* 2003;2(9):555–562.

78. Ge Y. Multiple Sclerosis: The Role of MR Imaging. *Am. J. Neuroradiol.* 2006;27(6):1165–1176.

79. Matthews L, Marasco R, Jenkinson M, et al. Distinction of seropositive NMO spectrum disorder and MS brain lesion distribution. *Neurology.* 2013;80(14):1330-7.

80. Paty DW, Oger JJ, Kastrukoff LF, et al. MRI in the diagnosis of MS: a prospective study with comparison of clinical evaluation, evoked potentials, oligoclonal banding, and CT. *Neurology*. 1988;38(2):180–185.

81. Tintoré M, Rovira A, Martínez MJ, et al. Isolated demyelinating syndromes: comparison of different MR imaging criteria to predict conversion to clinically definite multiple sclerosis. *Am. J. Neuroradiol.* 2000;21(4):702–706.

82. Swanton JK, Rovira A, Tintore M, et al. MRI criteria for multiple sclerosis in patients presenting with clinically isolated syndromes: a multicentre retrospective study. *Lancet Neurol.* 2007;6(8):677–686.

83. Chataway J, Miller DH. Natalizumab Therapy for Multiple Sclerosis. *Neurotherapeutics*. 2013;10(1):19–28.

84. Sormani MP, Bonzano L, Roccatagliata L, et al. Magnetic resonance imaging as a potential surrogate for relapses in multiple sclerosis: a meta-analytic approach. *Ann. Neurol.* 2009;65(3):268–275.

85. Multiple Sclerosis Discovery Forum. The Design of MRI-Related Clinical Trials in MS. Available at: http://www.msdiscovery.org/clinical-trial-design. Accessed March 22, 2015.

86. Bakshi R, Thompson AJ, Rocca MA, et al. MRI in multiple sclerosis: current status and future prospects. *Lancet Neurol.* 2008;7(7):615–625.

87. Morris Z, Whiteley WN, Longstreth WT Jr, et al. Incidental findings on brain magnetic resonance imaging: systematic review and meta-analysis. *BMJ*. 2009;339:b3016.

88. Granberg T, Martola J, Kristoffersen-Wiberg M, et al. Radiologically isolated syndrome – incidental magnetic resonance imaging findings suggestive of multiple sclerosis, a systematic review. *Mult. Scler.* 2013;19(3):271–280.

89. Okuda DT, Mowry EM, Beheshtian A, et al. Incidental MRI anomalies suggestive of multiple sclerosis: the radiologically isolated syndrome. *Neurology*. 2009;72(9):800–805.

90. Siva A, Saip S, Altintas A, et al. Multiple sclerosis risk in radiologically uncovered asymptomatic possible inflammatory-demyelinating disease. *Mult. Scler.* 2009;15(8):918–927.

91. Brassat D, Lebrun-Frenay C, On behalf of the "Club Francophone de la SEP." Treat patients with radiologically isolated syndrome when the MRI brain scan shows dissemination in time: Yes. *Mult. Scler.* 2012;18(11):1531–1532.

92. Bourdette D, Yadav V. Treat patients with radiologically isolated syndrome when the MRI brain scan shows dissemination in time: No. *Mult. Scler.* 2012;18(11):1529–1530.

93. Georgi W. Multiple sclerosis. Anatomopathological findings of multiple sclerosis in diseases not clinically diagnosed. *Schweiz. Med. Wochenschr.* 1961;91:605–607.

94. Gilbert JJ, Sadler M. Unsuspected multiple sclerosis. *Arch. Neurol.* 1983;40(9):533–536.

95. Engell T. A clinical patho-anatomical study of clinically silent multiple sclerosis. *Acta Neurol. Scand.* 1989;79(5):428–430.

96. Johannsen LG, Stenager E, Jensen K. Clinically unexpected multiple sclerosis in patients with mental disorders. A series of 7301 psychiatric autopsies. *Acta Neurol. Belg.* 1996;96(1):62–65.

97. OECD Health Data 2012. Available at: http://www.oecd.org/els/health-systems/oecdhealthdata2012-frequentlyrequesteddata.htm. Accessed April 27, 2013.

98. Wasay M, Khatri IA, Khealani B, et al. MS in Asian countries. *Int. MS J.* 2006;13(2):59–65.

99. Wasay M, Rizvi F, Azeemuddin M, et al. Incidental MRI lesions suggestive of multiple sclerosis in asymptomatic patients in Karachi, Pakistan. *J. Neurol. Neurosurg. Psychiatry*. 2011;82(1):83–85.

100. Okuda DT, Siva A, Kantarci O, et al. Radiologically Isolated Syndrome: 5-Year Risk for an Initial Clinical Event. *PLoS ONE*. 2014;9(3):e90509.

101. Fisniku LK, Brex PA, Altmann DR, et al. Disability and T2 MRI lesions: a 20-year follow-up of patients with relapse onset of multiple sclerosis. *Brain.* 2008;131(3):808–817.

102. Li DKB, Held U, Petkau J, et al. MRI T2 lesion burden in multiple sclerosis: a plateauing relationship with clinical disability. *Neurology*. 2006;66(9):1384–1389.

103. Filippi M, Rocca MA, De Stefano N, et al. Magnetic resonance techniques in multiple sclerosis: the present and the future. *Arch. Neurol.* 2011;68(12):1514–1520.

104. Neema M, Stankiewicz J, Arora A, et al. MRI in multiple sclerosis: what's inside the toolbox? *Neurother. J.* 2007;4(4):602–617.

105. Calabrese M, Filippi M, Gallo P. Cortical lesions in multiple sclerosis. *Nat. Rev. Neurol.* 2010;6(8):438–444.

106. Tomassini V, Matthews PM, Thompson AJ, et al. Neuroplasticity and functional recovery in multiple sclerosis. *Nat. Rev. Neurol.* 2012;8(11):635–646.

107. Brex PA, Ciccarelli O, O'Riordan JI, et al. A Longitudinal Study of Abnormalities on MRI and Disability from Multiple Sclerosis. *N. Engl. J. Med.* 2002;346(3):158–164.

108. Kappos L, Moeri D, Radue EW, et al. Predictive value of gadolinium-enhanced magnetic resonance imaging for relapse rate and changes in disability or impairment in multiple sclerosis: a meta-analysis. Gadolinium MRI Meta-analysis Group. *Lancet*. 1999;353(9157):964–969.

109. Mooshagian E. Anatomy of the Corpus Callosum Reveals Its Function. *J. Neurosci.* 2008;28(7):1535–1536.

110. Evangelou N, Konz D, Esiri MM, et al. Regional axonal loss in the corpus callosum correlates with cerebral white matter lesion volume and distribution in multiple sclerosis. *Brain*. 2000;123(9):1845–1849.

111. Simon JH, Holtås SL, Schiffer RB, et al. Corpus callosum and subcallosal-periventricular lesions in multiple sclerosis: detection with MR. *Radiology*. 1986;160(2):363–367.

112. Simon JH, Schiffer RB, Rudick RA, et al. Quantitative determination of MS-induced corpus callosum atrophy in vivo using MR imaging. *Am. J. Neuroradiol.* 1987;8(4):599–604.

113. Barkhof FJ, Elton M, Lindeboom J, et al. Functional correlates of callosal atrophy in relapsing-remitting multiple sclerosis patients. A preliminary MRI study. *J. Neurol.* 1998;245(3):153–158.

114. Sánchez MP, Nieto A, Barroso J, et al. Brain atrophy as a marker of cognitive impairment in mildly disabling relapsing-remitting multiple sclerosis. *Eur. J. Neurol.* 2008;15(10):1091–1099.

115. Bergendal G, Martola J, Stawiarz L, et al. Callosal atrophy in multiple sclerosis is related to cognitive speed. *Acta Neurol. Scand.* 2013;127(4):281–289.

116. Yaldizli Ö, Glassl S, Sturm D, et al. Fatigue and progression of corpus callosum atrophy in multiple sclerosis. *J. Neurol.* 2011;258(12):2199–2205.

117. Figueira FFA, Santos VS dos, Figueira GMA, et al. Corpus callosum index: a practical method for long-term follow-up in multiple sclerosis. *Arq. Neuropsiquiatr.* 2007;65(4A):931–935.

118. Sampat MP, Berger AM, Healy BC, et al. Regional White Matter Atrophy–Based Classification of Multiple Sclerosis in Cross-Sectional and Longitudinal Data. *Am. J. Neuroradiol.* 2009;30(9):1731.

119. 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(4):375–380.

120. Yaldizli Ö, Atefy R, Gass A, et al. Corpus callosum index and long-term disability in multiple sclerosis patients. *J. Neurol.* 2010;257(8):1256–1264.

121. Vaneckova M, Kalincik T, Krasensky J, et al. Corpus Callosum Atrophy - A Simple Predictor of Multiple Sclerosis Progression: A Longitudinal 9-Year Study. *Eur. Neurol.* 2012;68(1):23–27.

122. Kalincik T, Vaneckova M, Tyblova M, et al. Volumetric MRI markers and predictors of disease activity in early multiple sclerosis: a longitudinal cohort study. *PloS One*. 2012;7(11):e50101.

123. Dagdeviren ZA, Oguz K, Cinsdikici MG. Three techniques for automatic extraction of corpus callosum in structural midsagittal brain MR images: Valley Matching, Evolutionary Corpus Callosum Detection and Hybrid method. *Eng. Appl. Artif. Intell.* 2014;31:101–115.

124. Granberg T, Bergendal G, Shams S, et al. MRI-Defined Corpus Callosal Atrophy in Multiple Sclerosis: A Comparison of Volumetric Measurements, Corpus Callosum Area and Index. *J. Neuroimaging*. E-pub ahead of print Mar 19 2015. DOI: 10.1111/jon.12237.

125. Vrenken H, Jenkinson M, Horsfield MA, et al. Recommendations to improve imaging and analysis of brain lesion load and atrophy in longitudinal studies of multiple sclerosis. *J. Neurol.* 2013;260(10):2458–2471.

126. Liguori M, Meier DS, Hildenbrand P, et al. One year activity on subtraction MRI predicts subsequent 4 year activity and progression in multiple sclerosis. *J. Neurol. Neurosurg. Psychiatry*. 2011;82(10):1125–1131.

127. Sati P, George IC, Shea CD, et al. FLAIR*: A Combined MR Contrast Technique for Visualizing White Matter Lesions and Parenchymal Veins. *Radiology*. 2012;265(3):926–932.

128. Filippi M, Rocca MA, Calabrese M, et al. Intracortical lesions: relevance for new MRI diagnostic criteria for multiple sclerosis. *Neurology*. 2010;75(22):1988–1994.

129. Calabrese M, Rocca MA, Atzori M, et al. Cortical lesions in primary progressive multiple sclerosis A 2-year longitudinal MR study. *Neurology*. 2009;72(15):1330–1336.

130. Henkelman RM, Stanisz GJ, Graham SJ. Magnetization transfer in MRI: a review. *NMR Biomed.* 2001;14(2):57–64.

131. O'Donnell LJ, Westin C-F. An Introduction to Diffusion Tensor Image Analysis. *Neurosurg. Clin. N. Am.* 2011;22(2):185–196.

132. De Stefano N, Filippi M. MR Spectroscopy in Multiple Sclerosis. *J. Neuroimaging*. 2007;17:31S–35S.

133. Kiferle L, Politis M, Muraro PA, et al. Positron emission tomography imaging in multiple sclerosis–current status and future applications. *Eur. J. Neurol.* 2011;18(2):226–231.

134. Metcalf M, Xu D, Okuda DT, et al. High-Resolution Phased-Array MRI of the Human Brain at 7 Tesla: Initial Experience in Multiple Sclerosis Patients. *J. Neuroimaging*. 2010;20(2):141–147.

135. Alonso-Ortiz E, Levesque IR, Pike GB. MRI-based myelin water imaging: A technical review. *Magn. Reson. Med.* 2014.

136. Laule C, Kozlowski P, Leung E, et al. Myelin water imaging of multiple sclerosis at 7 T: correlations with histopathology. *NeuroImage*. 2008;40(4):1575–1580.

137. Kolind S, Matthews L, Johansen-Berg H, et al. Myelin water imaging reflects clinical variability in multiple sclerosis. *NeuroImage*. 2012;60(1):263–270.

138. Moher D, Liberati A, Tetzlaff J, Altman DG, PRISMA Group. Preferred reporting items for systematic reviews and meta-analyses: the PRISMA statement. *BMJ*. 2009;339:b2535.

139. Lezak MD ed. *Neuropsychological assessment*. 5th ed. Oxford University Press; 2012.

140. Salkind NJ. *Encyclopedia of Measurement and Statistics 3-Volume Set*. 1st ed. SAGE Publications; 2006.

141. Lebrun C, Blanc F, Brassat D, et al. Cognitive function in radiologically isolated syndrome. *Mult. Scler.* 2010;16(8):919–925.

142. Amato MP, Hakiki B, Goretti B, et al. Association of MRI metrics and cognitive impairment in radiologically isolated syndromes. *Neurology*. 2012;78(5):309–314.

143. De Stefano N, Stromillo ML, Rossi F, et al. Improving the characterization of radiologically isolated syndrome suggestive of multiple sclerosis. *PloS One*. 2011;6(4):e19452.

144. Giorgio A, Stromillo ML, Rossi F, et al. Cortical lesions in radiologically isolated syndrome. *Neurology*. 2011;77(21):1896–1899.

145. Granberg T, Martola J, Bergendal G, et al. Corpus callosum atrophy is strongly associated with cognitive impairment in multiple sclerosis: Results of a 17-year longitudinal study. *Mult. Scler.* E-pub ahead of print Dec 5 2014. DOI: 10.1177/1352458514560928.

146. Stromillo ML, Giorgio A, Rossi F, et al. Brain metabolic changes suggestive of axonal damage in radiologically isolated syndrome. *Neurology*. 2013;80(23):2090–2094.

147. Azevedo CJ, Overton E, Khadka S, et al. Early CNS neurodegeneration in radiologically isolated syndrome. *Neurol. Neuroimmunol. Neuroinflammation*. 2015;2(3):e102.

148. Giorgio A, Stromillo ML, Leucio AD, et al. Appraisal of Brain Connectivity in Radiologically Isolated Syndrome by Modeling Imaging Measures. *J. Neurosci.* 2015;35(2):550–558.

149. Applebee A. The clinical overlap of multiple sclerosis and headache. *Headache*. 2012;52(S2):111–116.

150. Swartz RH, Kern RZ. Migraine is associated with magnetic resonance imaging white matter abnormalities: A meta-analysis. *Arch. Neurol.* 2004;61(9):1366–1368.

151. Koch M, Uyttenboogaart M, Polman S, et al. Seizures in multiple sclerosis. *Epilepsia*. 2008;49(6):948–953.

152. Zarei M, Chandran S, Compston A, et al. Cognitive presentation of multiple sclerosis: evidence for a cortical variant. *J. Neurol. Neurosurg. Psychiatry*. 2003;74(7):872–877.

153. Assouad R, Louapre C, Tourbah A, et al. Clinical and MRI characterization of MS patients with a pure and severe cognitive onset. *Clin. Neurol. Neurosurg.* 2014;126:55–63.

154. Gabelic T, Ramasamy DP, Weinstock-Guttman B, et al. Prevalence of Radiologically Isolated Syndrome and White Matter Signal Abnormalities in Healthy Relatives of Multiple Sclerosis Patients. *Am. J. Neuroradiol*. 2014;35(1):106-12.

155. Klawiter EC, Ceccarelli A, Arora A, et al. Corpus Callosum Atrophy Correlates with Gray Matter Atrophy in Patients with Multiple Sclerosis. *J. Neuroimaging*. 2015;25(1):62–67.

156. Kuhlmann T, Lingfeld G, Bitsch A, et al. Acute axonal damage in multiple sclerosis is most extensive in early disease stages and decreases over time. *Brain.* 2002;125(10):2202–2212.

157. Sullivan EV, Rosenbloom MJ, Desmond JE, et al. Sex differences in corpus callosum size: relationship to age and intracranial size. *Neurobiol. Aging*. 2001;22(4):603–611.

158. Mitchell TN, Free SL, Merschhemke M, et al. Reliable callosal measurement: population normative data confirm sex-related differences. *Am. J. Neuroradiol.* 2003;24(3):410–418.

159. Jäncke L, Mérillat S, Liem F, et al. Brain size, sex, and the aging brain: Brain Size, Sex, and Aging. *Hum. Brain Mapp.* 2015;36(1):150-69.

160. Reuter M, Schmansky NJ, Rosas HD, et al. Within-subject template estimation for unbiased longitudinal image analysis. *Neuroimage*. 2012;61(4):1402–1418.

161. Chwastiak L, Ehde DM, Gibbons LE, et al. Depressive Symptoms and Severity of Illness in Multiple Sclerosis: Epidemiologic Study of a Large Community Sample. *Am. J. Psychiatry*. 2002;159(11):1862–1868.

162. Antel J, Antel S, Caramanos Z, et al. Primary progressive multiple sclerosis: part of the MS disease spectrum or separate disease entity? *Acta Neuropathol.* 2012;123(5):627–638.

163. Kuchling J, Ramien C, Bozin I, et al. Identical lesion morphology in primary progressive and relapsing-remitting MS -an ultrahigh field MRI study. *Mult. Scler.* 2014;20(14):1866–1871.

164. Ingle GT, Thompson AJ, Miller DH. Magnetic resonance imaging in primary progressive multiple sclerosis. *J. Rehabil. Res. Dev.* 2002;39(2):261–271.

165. Confavreux C, Vukusic S, Moreau T, et al. Relapses and Progression of Disability in Multiple Sclerosis. *N. Engl. J. Med.* 2000;343(20):1430–1438.

166. Okuda D, Frenay CL, Siva A, et al. Multi-center, randomized, double-blinded assessment of dimethyl fumarate in extending the time to a first attack in radiologically isolated syndrome (ARISE Trial). *Neurology*. 2015;84(S14):P7.207.