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MRI IN MULTIPLE SCLEROSIS

EARLY DETECTION, CORTICAL LESIONS AND GADOLINIUM RETENTION

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MRI IN MULTIPLE SCLEROSIS - EARLY DETECTION, CORTICAL LESIONS AND GADOLINIUM RETENTION
THESIS FOR DOCTORAL DEGREE (Ph.D.)

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This thesis is dedicated to my amazing wife Caroline Forslin and my two wonderful daughters

ABSTRACT

Background: Multiple sclerosis (MS) is a common neuroinflammatory and -degenerative disease. Magnetic Resonance Imaging (MRI) facilitates the diagnosis of MS, providing means to initiate early treatment and improving the long-term outcome. MRI can unveil MS in a pre-/subclinical phase as incidental findings in persons without typical MS symptoms, a condition called Radiologically Isolated Syndrome (RIS). New MRI techniques like synthetic MRI and phase-sensitive inversion recovery (PSIR) can further improve the visualization of MS pathology. Gadolinium-based contrast agents (GBCAs) are used in MS to evaluate disease activity but lead to retention of small amounts of gadolinium in the brain. These new developments highlight the need to constantly evaluate current and new MRI methods in an evidence-based manner to make informed decisions on how to optimize MS diagnostics.

Study I investigated the incidence of RIS from a population-based perspective, in a Swedish region with high MS-incidence. All 2272 brain MRI scans performed in the county during one year were assessed by a senior radiologist and neuroradiologist for RIS. Only two cases of RIS was found, constituting merely 0.8 cases per 100,000 person-years, which is low compared to the MS-incidence in Sweden of 10.2 cases per 100,000 person-years.

Study II compared the ability of conventional and synthetic PSIR to detect leukocortical lesions in 21 MS patients and studied the clinical relevance of the findings. The study showed that conventional and synthetic PSIR have comparable sensitivity for leukocortical MS lesions with excellent inter-rater agreement. Furthermore, the detected leukocortical lesion volumes were associated with lower cognitive scores, highlighting the clinical relevance.

Study III retrospectively investigated gadolinium retention with conventional T1-weighted brain MRI. The dentate nucleus and globus pallidus were assessed in an 18-year follow-up study of 23 patients and 23 cross-sectional controls. Possible associations with cognitive deficits were also explored. The study showed that a higher number of GBCA administrations was associated with higher T1-signal intensity index in both the dentate nucleus and globus pallidus. After correcting for several factors related to MS disease severity, an association remained between higher signal intensity and lower verbal fluency performance.

Study IV used simultaneous T1- and T2-relaxometry (synthetic MRI) to quantitatively assess the relationship between GBCA administrations and MRI relaxation rates (R1 and R2) in a prospective cohort of 85 MS patients along with 21 healthy controls without GBCA exposure. A higher number of administrations of linear, but not macrocyclic, GBCAs was associated with a dose-dependent increase in R1 and R2 in the studied structures (dentate nucleus, globus pallidus, caudate nucleus and thalamus). Furthermore, higher relaxation rates were associated with lower cognitive performance, but not increased physical disability or fatigue.

Conclusions: RIS is a relatively rare phenomenon in a region with high MS-incidence. The RIS-incidence may, however, increase with improved MRI technologies and availability. Synthetic MRI provides PSIR that allows detection of leukocortical lesions with a similar sensitivity to conventional PSIR, without additional scan time. Linear GBCAs are associated with brain gadolinium retention that causes both T_1 and T_2 effects on MRI. These MRI signal changes are associated with lower cognitive performance, but that does not necessarily imply causality since the correlations may be confounded by MS pathology.

SAMMANFATTNING

Bakgrund: Multipel skleros (MS) är en vanlig neuroinflammatorisk och -degenerativ sjukdom. Undersökning med magnetkamera (MR) underlättar diagnostisering av MS, vilket bidrar till att tidig behandling kan sättas in och därmed förbättra den långsiktiga prognosen för MS-patienter. MR av hjärnan kan upptäcka MS i en pre-/subklinisk fas som bifynd hos personer utan typiska MS-symtom - ett tillstånd som kallas radiologiskt isolerat syndrom (RIS). Nya MR-tekniker som syntetisk MR och ”phase-sensitive inversion recovery” (PSIR) kan ytterligare förbättra visualiseringen av MS-plack. Gadoliniumbaserade MR-kontrastmedel används för att utvärdera sjukdomsaktivitet vid MS, men leder också till ansamling i hjärnan av små mängder gadolinium, som är en tungmetall. Befintliga och nya MR-teknikers för- och nackdelar behöver kontinuerligt och systematiskt utvärderas för att optimera MS-diagnostiken på ett evidensbaserat vis.

Studie I, som utfördes i Västmanland, undersökte förekomsten av RIS ur ett befolkningsbaserat perspektiv. Alla 2272 MR-undersökningar av hjärnan som utfördes i länet under ett år bedömdes avseende kriterier för RIS av en senior radiolog, neuroradiolog och neurolog. Endast två fall av RIS hittades, vilket utgör 0,8 fall per 100 000 personår, vilket är lågt jämfört med MS-incidensen i Sverige, som är 10,2 fall per 100 000 personår.

Studie II jämförde de två MR-sekvenserna konventionell och syntetiskt PSIR avseende deras förmåga att detektera MS-plack, som engagerar både vitsubstans och hjärnbarken, samt den kliniska relevansen av dessa fynd. Studien, som inkluderade 21 MS-patienter, visade att konventionell och syntetisk PSIR har jämförbar känslighet för dessa MS-plack med utmärkt överensstämmelse mellan bedömarna. Vidare kopplas volymen av dessa MS-plack till lägre kognitiv förmåga, vilket poängterar den kliniska relevansen.

Studie III undersökte möjlig ansamling av gadolinium i hjärnan med konventionell T1-viktad MR. Intensiteten i två hjärnstrukturer (nucleus dentatus och globus pallidus) bedömdes hos 23 MS-patienter, som följts under 18 år, och 23 friska personer. Eventuella kopplingar till kognitiv nedsättning undersöktes också. Fler antal kontrastmedelsadministrationer var kopplat till högre T1-signalintensitetsindex i bägge de undersökta hjärnstrukturerna. Efter korrektion för flera MS-relaterade sjukdomsfaktorer kvarstod en statistisk koppling mellan högre signalintensitet och lägre verbal ordflödesförmåga.

Studie IV använde samtidig T1- och T2-relaxometri (syntetisk MR) för att kvantitativt bedöma förhållandet mellan gadoliniumbaserade kontrastmedelsadministrationer och MR-relaxationstider (R1 och R2) i en prospektiv tvärsnittsstudie av 85 MS-patienter tillsammans med 21 friska kontroller, som inte exponerats för MR-kontrastmedel. Ett högre antal administreringar av linjära, men inte makrocykliska, kontrastmedel kopplades till en dosberoende ökning av R1 och R2 i de studerade strukturerna (nucleus dentatus, globus pallidus, nucleus caudatus och thalamus). Vidare kopplades högre relaxationstider i några strukturer i sin tur till lägre kognitiv förmåga, men inte till ökad fysisk funktionsnedsättning eller utmattning.

Slutsatser: RIS är ett relativt sällsynt fenomen i en region med hög MS-incidens. RIS-incidensen kan emellertid komma att öka med förbättrad MR-teknik och tillgänglighet. Syntetisk MR skapar en syntetisk PSIR som möjliggör detektion av MS-plack som passerar gränsen mellan vitsubstans och hjärnbarken. Syntetisk PSIR har liknande känslighet som konventionell PSIR, utan ytterligare undersökningstid i MR-kameran. Linjära MR-kontrastmedel är förknippade med gadoliniumretention i hjärnan som orsakar både T1- och T2-effekter på MR; Dessa MR-signalförändringar är statistiskt associerade med lägre kognitiv funktionsförmåga, men det betyder inte nödvändigtvis att det föreligger ett orsakssamband eftersom kopplingen delvis kan förklaras av MS-sjukdomen.

LIST OF SCIENTIFIC PAPERS

I. **Incidence of Radiologically Isolated Syndrome: A Population-Based Study.**
Forslin Y, Granberg T, Jumah AA, Shams S, Aspelin P, Kristoffersen-Wiberg M, Martola J, Fredrikson S.
American Journal of Neuroradiology. 2016 Jun;37(6):1017-22.

II. **Retention of Gadolinium-Based Contrast Agents in Multiple Sclerosis: Retrospective Analysis of an 18-Year Longitudinal Study.**
Forslin Y, Shams S, Hashim F, Aspelin P, Bergendal G, Martola J, Fredrikson S, Kristoffersen-Wiberg M, Granberg T.
American Journal of Neuroradiology. 2017 Jul;38(7):1311-1316.

III. **Detection of Leukocortical Lesions in Multiple Sclerosis and their association with Physical and Cognitive Impairment: A comparison of Conventional and Synthetic Phase-Sensitive Inversion Recovery MRI**
Forslin Y, Bergendal Å, Hashim F, Martola J, Shams S, Kristoffersen-Wiberg M, Fredrikson S, Granberg T.
American Journal of Neuroradiology. 2018 Nov;39(11):1995-2000.

IV. **Gadolinium Retention in the Brain: an MRI Relaxometry Study of Linear and Macrocyclic Gadolinium-Based Contrast Agents in Multiple Sclerosis**
Forslin Y, Martola J, Bergendal Å, Fredrikson S, Kristoffersen-Wiberg M, Granberg T.
Submitted to a scientific journal

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LIST OF ABBREVIATIONS

2D	Two-dimensional
3D	Three-dimensional
ANOVA	Analysis of variance
CIS	Clinically isolated syndrome
CNS	Central nervous system
CSF	Cerebrospinal fluid
DIR	Double inversion recovery
DIS	Dissemination in space
DIT	Dissemination in time
DMT	Disease-modifying therapy
DN	Dentate nucleus
DWI	Diffusion-weighted imaging
EDSS	Expanded disability status scale
EMA	European medicines agency
FLAIR	Fluid attenuated inversion recovery
GBCA	Gadolinium-based contrast agents
GP	Globus pallidus
LCL	Leukocortical lesions
MAGNIMS	Magnetic resonance imaging in multiple sclerosis
MPRAGE	Magnetization-prepared rapid acquisition gradient echo
MRI	Magnetic resonance imaging
MS	Multiple sclerosis
NSF	Nephrogenic systemic fibrosis
PSIR	Phase sensitive inversion recovery
RRMS	Relapsing-remitting multiple sclerosis
SDMT	Symbol digit modalities test
SII	Signal intensity index
SP	Secondary progressive (multiple sclerosis)
T	Tesla
T1WI	T1-weighted imaging
T2WI	T2-weighted imaging
WM	White matter

1 INTRODUCTION

1.1 MULTIPLE SCLEROSIS

1.1.1 Background and diagnosis

Multiple sclerosis (MS) is a neuroinflammatory and neurodegenerative disease that causes permanent damage to the central nervous system (CNS). In Greek, *sclerosis* means hardening and MS therefore refers to the multiple sites in the brain and spinal cord with hardened scar tissue. The inflammatory and degenerative processes in MS lead to a loss of myelin, which acts as an insulator of axons. The demyelination causes a disruption of the transmission of action potentials and can lead to permanent axonal loss, which gives the clinical symptoms in MS patients.[1] MS is a leading cause of neurological deficits in the young population in the Western world.[1] In Sweden, the disease is most commonly diagnosed at the age of 30 years and MS occurs more than twice as often in women than in men. The Swedish MS prevalence is around 189 per 100,000 and the incidence 6.0 to 10.2 per 100,000 person-years, which are among the highest rates globally.[2–4]

The basis for diagnosing MS currently relies on the McDonald criteria, which require evidence of lesions in two or more locations in the CNS - 'dissemination in space' (DIS), which occurred at two different occasions - 'dissemination in time' (DIT). Definitions of DIS and DIT have changed over the years. The MS diagnosis was until recently made on the basis of the McDonald criteria from 2010,[5] which was a revision of the two earlier versions from 2005 and 2001,[6,7] in which magnetic resonance imaging (MRI) has taken an increasingly larger role with each new revision. From the 2010 revision of the McDonald criteria, the radiological evaluation made it possible to demonstrate both DIS and DIT with a single MRI scan if at least one detected lesion showed activity - i.e. enhancement with a gadolinium-based contrast agent (GBCA), in combination with non-enhancing lesions. The diagnostic criteria for MS were, however, updated once again in 2017.[8] A major revision from a radiological perspective is the inclusion of MS lesions affecting the cortex. Obtaining a reasonable *in vivo* detection rate of cortical MS pathology is, however, challenging since it requires 7 Tesla (T) MRI or dedicated sequences such as phase-sensitive inversion recovery (PSIR) or double inversion recovery (DIR).[9,10] The previous 2010 McDonald classification of DIS and DIT, which have been used in the studies of this thesis, are compared to the updated 2017 version in Table 1. A large difference in the latest revision from a clinical perspective is that the presence of oligoclonal bands in the cerebrospinal fluid (CSF) in patients with only one clinical episode is enough to fulfill DIT and thereby the criteria for MS diagnosis if MRI can demonstrate DIS. Furthermore, Table 2 shows a comparison between three latest radiological DIS classifications from 2005, 2010 and the present classification from 2017,[8,11,12] and Figure 1A illustrates an MS patient fulfilling all these classifications for DIS. It is, however, very important to note that the radiological

classification for DIS should not be applied blindly to all types of patients performing brain MRI scans.

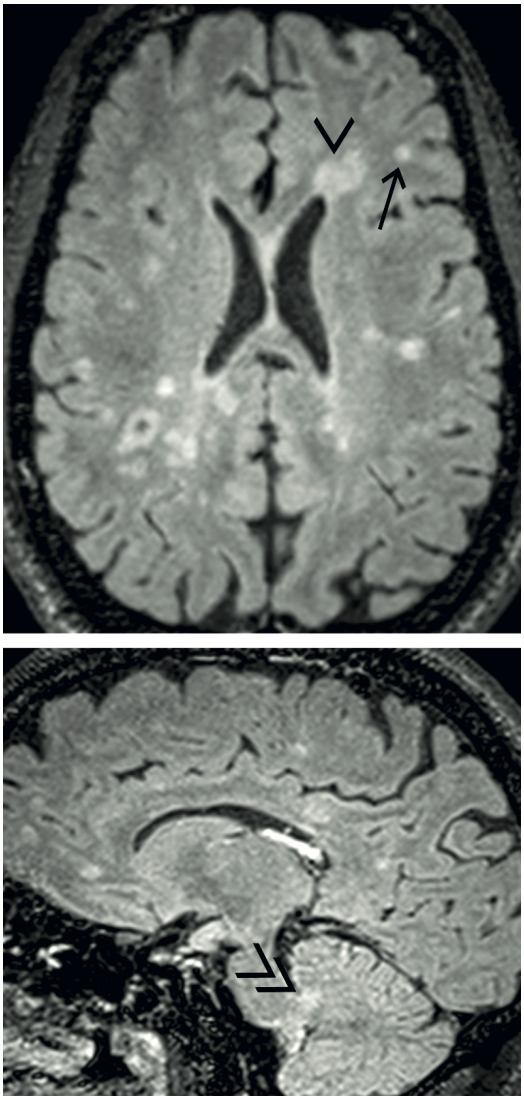


Figure 1A. *A T2-weighted FLAIR image, from Karolinska University Hospital, of a 25-year-old MS patient with periventricular (open arrow), juxtacortical (closed arrow) and infratentorial (double open arrows) WM anomalies typical for MS.*

It is essential to exclude any differential diagnosis when assessing a patient with suspicion of MS, since other diseases can also lead to brain MRI findings that formally fulfill the radiological classification for DIS. The list of differential diagnoses of MS is extensive and an expert panel has tried to systematize the investigation. They came up with 79 clinical, laboratory and radiological "red flags" that are suggestive of an alternative diagnosis than MS and warrants further investigation.[13] Some common differential diagnoses with white matter (WM) changes that can mimic MS on MRI are vascular diseases (Figure 1B), migraine or other neuroinflammatory diseases such as neuromyelitis optica.

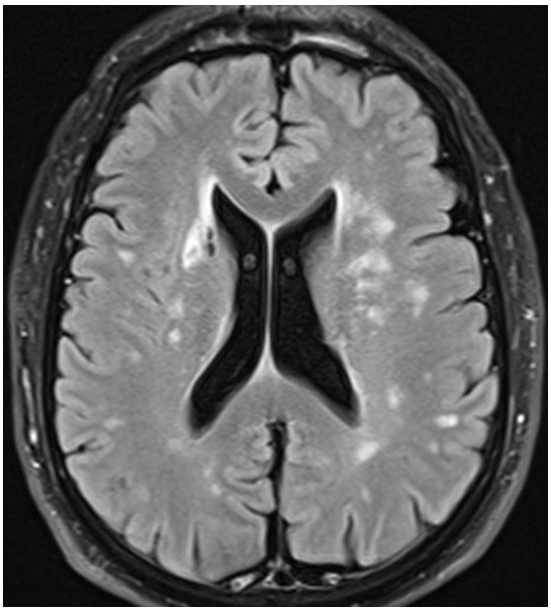


Figure 1B. *A T2-weighted FLAIR image, from Karolinska University Hospital, of a patient with periventricular and subcortical WM anomalies consistent with age-related vascular disease.*

Table 1. *Comparison of the 2010 and 2017 revisions of the McDonald criteria.*[5,8]

Clinical attacks	2010 Lesions with objective clinical evidence	2017 Lesions with objective clinical evidence	2010 Additional criteria to make the diagnosis	2017 Additional criteria to make the diagnosis
≥ 2	≥ 2 or 1 lesion(s) with **reasonable** historical evidence of a previous attack	≥ 2 lesions	None. Clinical evidence alone is enough and differential diagnosis should have been considered.	**None, although MRI is always recommended if available.**
≥ 2	1 lesion	1 lesion	DIS; OR await further clinical attack implicating a different site in the CNS	**None if the clinical attack corresponding to a lesion is clear-cut.** If it is not a clear-cut DIT, it can also be fulfilled by an additional attack or another lesion on MRI
1	≥ 2 lesions	≥ 2 lesions	DIT; OR await a second clinical attack	DIT; OR await a second clinical attack OR **presence of oligoclonal bands in CSF**
1	1 lesion	1 lesion	DIS; OR await further clinical attack suggesting a different location in the CNS AND DIT; OR await a second clinical attack	DIS; OR await further clinical attack suggesting a different location in the CNS AND DIT; OR await a second clinical attack OR **presence of oligoclonal bands in CSF**
0			1 year of disease progression AND at least 2 of: DIS in brain MRI according to Swanton classification; DIS in the spinal cord based on ≥ 2 T2 lesions or positive CSF	

DIS=Dissemination In Space; CNS=Central Nervous System; CSF=Cerebrospinal fluid; DIT=Dissemination In Time; MRI=Magnetic Resonance Imaging.
Changes between the 2010 and 2017 revision in ***bold****.*

Table 2. *Comparison of the three latest versions of the radiological DIS classification*

DIS McDonald 2005[11]	DIS McDonald 2010[12]	DIS McDonald 2017[8]
Lesions in at least 3 out of: • ≥3 periventricular • ≥1 juxtacortical • ≥1 infratentorial or spinal • ≥1 contrast-enhancing *or* ≥ 9 lesions	**Lesions in at least 2 out of:** • ≥1 periventricular • ≥1 juxtacortical • ≥1 infratentorial • ≥1 spinal cord	**Lesions in at least 2 out of:** • ≥1 Periventricular • ≥1 Juxtacortical/**cortical** • ≥1 Infratentorial • ≥1 Spinal
Sensitivity 60% Specificity 88%	Sensitivity 72% Specificity 87%	Sensitivity and specificity similar as DIS 2010[14,15]

DIS=Dissemination In Space

The time from symptom onset to MS diagnosis has been decreasing over the last decades because of developments in the diagnostic criteria and improvements in diagnostic techniques and routines.[16] Early treatment initiation has been shown to be of vital importance to improve long-term outcome.[17] The goal of each revised version of the diagnostic criteria has been to establish an MS diagnosis early with high sensitivity and specificity to thereby allow treatment initiation as early as possible.[8] The sensitivity and specificity for the 2017 revised DIS classification is not yet fully investigated. However, initial investigations have shown that the new McDonald criteria (mostly due to positive CSF sample) enables earlier diagnosis of MS after a first clinical event,[18] with higher sensitivity,[14,15] but perhaps with somewhat reduced specificity – at least according to a recent study.[19]

1.1.2 Physical and cognitive evaluation

MS is clinically characterized by the attacks and/or progression of physical disability. The global motor disability in MS is most commonly measured by using the Expanded Disability Status Scale (EDSS).[20] EDSS is an ordinal scale ranging from 0 to 10, where 0 represents normal neurological functioning without findings in the neurological examination, while a score of 10 means death due to MS. The scores represent neurological impairment and increasing disability with certain landmarks, e.g. at EDSS 6 assistance is required to walk and at EDSS 7 the patient uses a wheel-chair. Furthermore, a majority of the MS patients also report fatigue that negatively affect their health-related quality of life.[21] Fatigue is often evaluated using the Fatigue Severity Scale, a self-reported questionnaire with a 7-point grading scale and 9 questions, which has been shown to have great reliability and consistency.[21,22]

While the physical manifestations of the disease may be obvious, up to 70 % of MS patients also have cognitive deficiencies that may be less apparent.[23] It has also been suggested that MS in some cases can present with predominantly cognitive dysfunction.[24,25] The awareness of cognitive impairment in MS has increased over the years and there are different neuropsychological tests and test batteries that are used for detecting cognitive impairment in MS. These test batteries are designed to evaluate different domains that are affected by MS. The most common test battery for evaluating cognitive function in MS, the Brief Repeatable Battery, is a fairly comprehensive set of tests that includes assessments of memory, language, attention, information processing speed and visuospatial function.[26,27]

More recently, a test battery called Brief International Cognitive Assessment for MS was recommended as a standard for evaluating cognition in MS. One study concluded that this newer test battery is more suitable than the Brief Repeatable Battery to use in everyday practice and research, especially when resources and time are scarce.[28] However, in clinical practice, it may sometimes only be feasible to perform one single test and in those instances Symbol Digit Modalities Test (SDMT) is recommended since it is relatively sensitive to cognitive impairment in MS and only takes 90 seconds to perform.[29] As SDMT is commonly used in clinical follow-ups of MS patients in our hospital, it was natural to include this test as measurement of cognitive function. The tests that were used in this thesis were:

- **SDMT**, reflecting the information processing speed. The test participant has to decipher symbols (representing the digits 0-9) and then decode a sequence of symbols into digits as fast as possible. SDMT reflects primarily frontoparietal functions and has been shown to have good repeatability.[29,30]
- **Verbal Fluency Test**, examining the word generation capacity – i.e. the performance to generate as many words as possible beginning with specific letters (in our case F, A and S, although other letter combinations exist).[31] Decline in verbal fluency performance has predominantly been related to damage in the left frontal lobe.[32,33]
- **Rey Auditory Verbal Learning Test** (RAVLT), assessing verbal memory function by recalling 15 words in five repetitions and then at a sixth delayed recall after 30 min. The test has been related to function in primarily the medial temporal lobes and temporal poles as well as hippocampal volumes for the delayed recall.[31,34]
- **Rey-Osterrieth complex figure test – copy** (ROCFT), assessing the visuospatial organization and visual memory capacity by presenting a geometrically complex figure that is then re-drawn by hand from memory.[31] ROCFT performance has been related to right hemisphere and prefrontal lobe functions.[35,36]

1.1.3 MS subtypes

In 1996, the National MS Society in the United States presented a description of four MS subtypes: relapsing-remitting MS (RRMS), secondary progressive MS, primary progressive MS and progressive-relapsing.[37] These MS subtypes became internationally acknowledged and have been used in research and clinics to categorize the clinical phenotype of MS. The term 'relapse' refers to a period of clinical worsening of symptoms brought on by intermittent episodes of inflammation in the CNS. The progressive degenerative aspect of MS is still under debate, whether it is a secondary effect of inflammation or a separate disease process. As further understanding was made regarding the underlying pathology as well as the clinical course of MS, a revision of the subtyping was made in 2014,[38,39] and implemented in the 2017 revision of the diagnostic McDonald criteria.[8] However, radiologically isolated syndrome (RIS), that in many cases can be considered to be the earliest detectable form representative of MS pathology, was not included as a subtype of MS. In the revision, progressive-relapsing MS was removed as a subtype.

The revision further proposed that the different forms of MS should be specified as either active or non-active, based on the presence of clinical relapses or MRI activity (new/enlarging or contrast-enhancing lesions). The description of patients with progressive MS (Primary or Secondary progressive MS) should also include if any “progression” is present, i.e. gradual worsening of clinical symptoms. The potential advantages and caveats

of the new MS classification remain to be further studied, but it can hopefully help clinicians to better individualize MS therapies and facilitate research.

1.1.4 Radiologically isolated syndrome

The availability and use of MRI have increased in all OECD countries over the last decades.[40] Concurrently, improved MRI techniques provide higher image quality, higher spatial resolution and/or shorter scanning time. The higher quality and use of MRI increase the diagnostic accuracy, but also increase the frequency of incidental MRI findings.[41]

MRI scans may unveil incidental WM anomalies with MS-like appearance among persons without previous suspicion of MS. The diagnosis of RIS was introduced by Okuda and colleges the diagnostic RIS criteria were defined (Table 3).[42] The most common clinical indication for the initial MRI imaging leading to RIS diagnosis is headache.[41] This has highlighted MRI as a method to possibly detect MS disease activity before it presents with clinical symptoms. RIS is clinically important since one-third of these patients will develop clinical symptoms consistent with MS within 5 years.[41,43]

Okuda's RIS criteria from 2009 was used in Study I of this thesis, but to better stratify RIS cases that are more likely to be subclinical MS, the MRI in MS group (MAGNIMS) recently published new assessment guidelines including consensus recommendations regarding the management of RIS.[44] Early treatment is very important in MS to limit the accumulated neurological damage.[17] However, it is unclear how RIS, which apparently in some cases is a manifestation of MS pathology in a pre-/subclinical form, should be monitored and if any MS treatment should be given. The MAGNIMS group concluded that there is not yet enough evidence to motivate initiation of disease-modifying therapies (DMTs) for RIS. Studies have identified risk factors for conversion of RIS to MS, such as a presence of spinal or infratentorial lesions.[43,45] Those persons should at least benefit from regular clinical and radiological follow-ups.[44] All DMTs have side effects and risks, which may outweigh the beneficial effects of an early treatment regime in RIS that eventually do not convert to MS. Therefore a multicenter treatment trial, to better explore the benefits of a treatment approach for RIS, is ongoing.[46]

Table 3. *Okuda's criteria for RIS that have been used in this thesis.*

Criterion	Requirement
	Incidental WM anomalies in the CNS meeting the following MRI criteria:
A	**A1.** Ovoid, well-circumscribed, and homogeneous foci with or without involvement of the corpus callosum
	A2. T2 hyperintensities measuring >3mm and fulfilling Barkhof criteria.[11] (≥3 out of 4) for dissemination in space
	A3. CNS WM 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 WM pathology lacking involvement of the corpus callosum
F	The CNS MRI anomalies are not better accounted for by another disease process

1.1.5 RIS epidemiology

There are only a few studies on the incidence of RIS.[41] These previous studies have been hospital-based studies from Sweden (Huddinge) and Pakistan. Interestingly, the higher incidence region for MS (Sweden) had a low frequency of RIS-cases (0.05%),[47] while the low-incidence region for MS (Pakistan) showed a higher RIS frequency (0.7%).[48] One study in relatives to patients with MS showed that up to 3% fulfilled RIS criteria, based on the 2005 DIS classification, and 10% based on the 2010 DIS classification.[49]

1.1.6 MS therapy

MRI findings play a central role in personalizing MS treatment by making it possible to evaluate treatment efficacy in individual patients. The basis of MS treatment is DMTs. Since the first approval for treating RRMS with interferon β in 1993, many different DMTs have been developed. The DMTs aim to modulate immune functions thereby reducing the inflammatory activity, which in clinical trials has been shown to reduce the annual relapse rate, the number of new T2-lesions and the number of contrast-enhancing lesions.[50] There are several DMTs to choose from when individualizing the treatment in regards to each patient's risks and disease severity. Interferon β (Avonex, Betaferon, Plegridy, Rebif) and glatiramer acetate (Copaxone), which are administered as subcutaneous injections, have previously been considered to be first-line treatment. More recently oral alternatives as first line drugs have been registered, e.g. teriflunomide (Aubagio) and dimethyl fumarate (Tecfidera).[51]

If the patients respond poorly to the first-line treatment (by clinical and radiological assessments), or if a more aggressive disease course is expected, the treatment can be escalated/initiated with alternatives such as natalizumab (Tysabri) or fingolimod (Gilenya) or the newly registered kladribin (Mavenclad),[52] which have higher expected efficacy but also more potential severe adverse events such as opportunistic infections. In Sweden off-label use of rituximab (MabThera) has proven to be a highly effective treatment with a favorable risk-benefit ratio with large potential cost savings compared to DMTs with indications for MS.[53] A similar newly registered alternative to rituximab is ocrelizumab (Ocrevus). Additionally, if the inflammatory activity is high or very high (frequent clinical attacks or many contrast-enhancing lesions on MRI) alternatives such as alemtuzumab (Lemtrada) or Autologous Hematopoietic Stem Cell Transplantation (AHSCT) may be considered.[50,54]

1.2 MAGNETIC RESONANCE IMAGING

1.2.1 MRI usage

The clinical availability and use of Magnetic Resonance Imaging (MRI) for clinical diagnostic purposes has more than doubled in the last two decades.[40] It is becoming one of the most important paraclinical tools for diagnostic investigations. In contrast to X-ray based techniques, such as computer tomography (CT), and nuclear medicine investigations that uses radioactive isotopes, MRI does not generate any potentially harmful ionizing radiation. Furthermore, MRI produces images with a much higher soft tissue contrast that increases the detection and characterization of specific findings, which in turn improves the diagnostic accuracy.[55] One of the diseases where MRI has taken a primary role in both the diagnostics and clinical follow-up routine is MS.[8]

MRI is usually a safe diagnostic tool, but there are, however, some safety considerations: Ferromagnetic objects that come to close to the strong magnetic fields can become dangerous projectiles and medical devices or implants can dysfunction. Energy from radiofrequency waves can cause heating of tissue as well as external material that potentially can harm the patient. High noise during scanning requires hearing protection.[56]

1.2.2 MRI physics

In short, clinical MRI techniques utilize very strong magnetic fields (in clinical settings usually 1.5 or 3 T) in combination with radio waves from transmitter coils that interact, resonate, with the inherent physical properties of the proton nuclei (most prevalent in water molecules) in the tissues. This creates echoes of radio waves from the protons that are measured by receiver coils (a sort of antenna) and used to create images of the body. A more foundational explanation of MRI physics and image reconstruction is beyond the scope of this thesis, but is reviewed in many text books, or articles on the subject.[57]

1.3 MULTIPLE SCLEROSIS AND MRI

1.3.1 Current MRI protocols for MS diagnostics in Sweden

MRI applications, especially in MS, have had an increasing importance since the introduction of MRI images (such as illustrated in Figure 2)[58] that clearly showed its superiority to Computer Tomography in detecting specific pathologies.

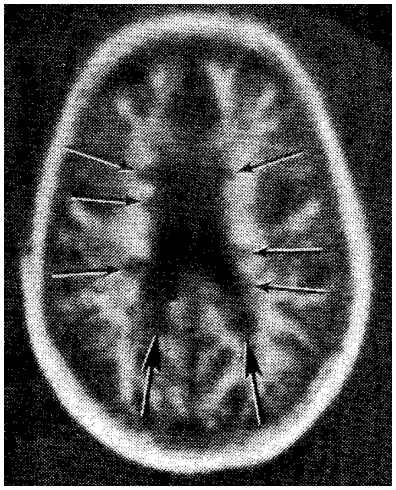


Figure 2. *An 18-year old female MS patient from the first published brain MRI of MS. The arrows show multiple MS lesions.*[58]

To improve the MS diagnostics nationally and to standardize the use of MRI in MS investigation and follow-ups in Sweden, neurologists and neuroradiologists wrote a national consensus agreement 2016 of the use of MRI in MS. The document included recommendations on MRI protocols, indications and frequency of use, as well as recommended the use of GBCA.[59] The currently recommended MRI protocols are illustrated in Figure 3. A revision of the national guidelines of MRI in MS is expected in 2019 will likely reduce the usage of GBCAs during routine MS follow-ups.

Figure 3. *Swedish national MRI protocols for investigation and follow-up of MS.*[59]

MRI investigation

1. 3D T1 (non-enhanced)
2. SWI
3. Gadolinum-contrast agent
4. Axial T2
5. 3D T2-FLAIR
6. 3D T1 (post contrast)

MRI follow-up

1. Gadolinium-contrast agent
2. Axial T2
3. 3D T2-FLAIR
4. 3D T1 (post contrast)

1.3.2 Conventional MRI sequences

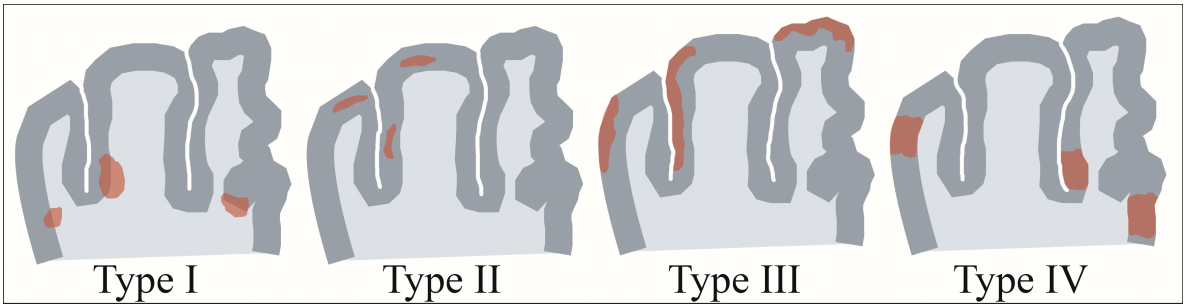
For standard assessment of the MS lesions on brain MRI, T1-weighted imaging (T1WI), T2-weighted imaging (T2WI) and fluid attenuating inversion recovery (FLAIR) images are currently primarily used. If possible, 3D sequences should ideally be used to achieve higher spatial resolution to reduce partial volume effects and thereby improve the detection rate of small lesions.[60] T1WI with administration of GBCA is used to assess whether an MS lesion is currently inflammatorily active, what is referred to as a contrast-enhancing lesion. In contrast-enhancing lesions, there is a bright T1WI signal within the lesion due to leakage of the GBCA across the blood-brain-barrier. Detection of contrast-enhancing lesions or new/enlarging MS lesions on follow-up MRI is considered “disease activity” and especially valuable information for the clinician when deciding on the choice of therapy.

1.3.3 Detection of cortical lesions with MRI

Histopathological studies of MS have shown widespread involvement not only of the WM but also the grey matter in MS.[61,62] Almost all MS patients have some degree of MS pathology in the cortex, even in the early stages of the disease, which causes a decline in cognitive abilities.[63] It is clinically valuable to include assessment of cortical lesions, as they are a predictor of cognitive impairment and would increase diagnostic accuracy if included in the radiological readings.[64,65]

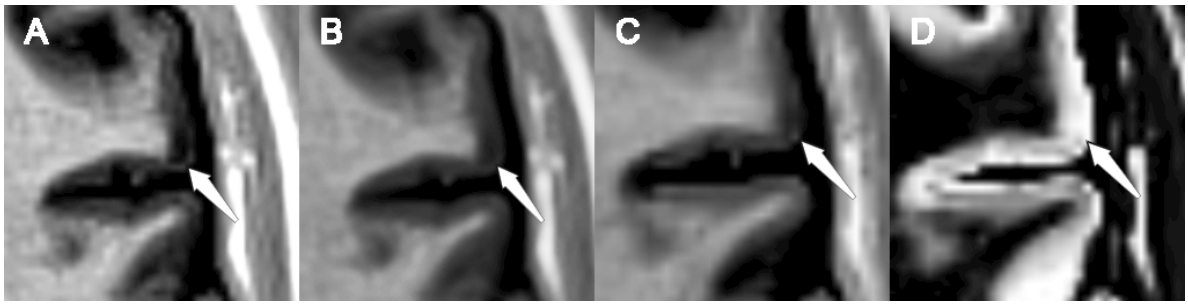
The grey matter MS pathology is, however, hard to detect *in vivo* since the myelin content in the cortex is lower than in the WM, which reduces the contrast between demyelinated lesions and surrounding brain parenchyma.[64] Figure 4 illustrates one common classification for different types of cortical lesions,[62] of which leukocortical lesions (LCL, Type I) are the most easily detectible on MRI.[66,67]

Figure 4. *Classification of cortical lesions according to Bø L et al.*[62]:
Type I: Leukocortical
Type II: Intracortical
Type III: Subpial lesion
Type IV: The whole cortex



The detection of subpial lesions (type III) are especially difficult due to partial volume effects between the cortex and CSF.[68] The inclusion of cortical lesions in the 2017 McDonald criteria therefore poses opportunities in improving MS diagnostics but also challenges since conventional MRI sequences have limited sensitivity to
lesions in the cortex. Newer MRI sequences like DIR and PSIR can detect 1.5-5 times more cortical lesions than conventional sequences.[69,70] It has been suggested to add PSIR or DIR into clinical MRI protocols, if such sequences are available.[71] There are, however, technical limitations with these techniques (e.g. low signal-to-noise ratio, flow artifacts, non-uniformity of the magnetic field in the cortex) and the inter-rater agreement for cortical lesions remains moderate.[71] Therefore, the cortical lesion criterion for DIS was integrated with the more conventional juxtacortical lesions in the 2017 McDonald criteria.[8,60] Figure 5A shows how an intracortical lesion can be more or less easy to detect on different MRI sequences and Figure 5B shows a leukocortical lesion visualized with DIR. Another way to improve the detection of cortical lesions is to increase the MRI field strength. T2* imaging at 7 T MRI has recently been shown (with histopathological verification) to more than double the detection rate of cortical lesions compared to a 3 T scanner, and can also detect diffuse cortical MS pathology beyond focal lesions.[72,73]

Figure 5A. *A cortical MS lesion in a 42-year-old male with RRMS and 15-year disease duration illustrated with the following MRI sequences: MPRAGE (A), PSIR (B), Synthetic MRI PSIR (C) and Synthetic MRI DIR (D). The MRI scan was performed at Karolinska University Hospital in Huddinge*



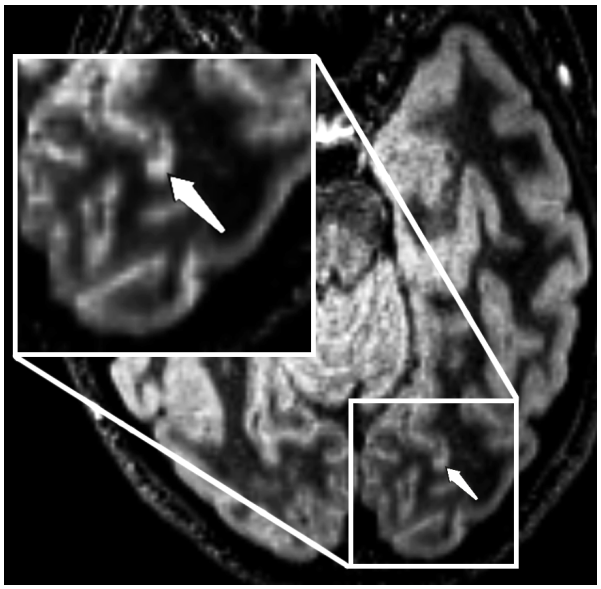


Figure 5B. *DIR sequence illustrating a leukocortical lesion (extending from the white matter into the cortex) in a 45-year-old woman with 3 years' disease duration of RRMS.*

1.3.4 Synthetic MRI

Synthetic MRI is an MRI technique that is able to quantify absolute MRI parameters (R1, R2, proton density with correction for field inhomogeneities), which can then be used to synthesize MRI weighted images with different virtual echo, repetition and inversion times.[74,75] This turbo-spin-echo saturation-recovery sequence uses four acquisitions with alternating slice acquisition order, which effectively results in four different saturation delays; This, in combination with two echo times, results in 16 different images for each voxel that is used for the fitting of the T1- and T2-relaxation curves. Synthetic MRI can thereby provide images with multiple weighted images, such as T1WI, T2WI, T1- and T2-weighted FLAIR as well as DIR and PSIR from a single acquisition. The technique is currently, however, based on a 2D acquisition with a need for gaps between slices to avoid interactions between slices. This being said, the quantitative aspect of the technique makes it possible to accurately compare the MRI measurements longitudinally and between patients.[75] Figure 5B exemplifies the detection of a cortical lesion with conventional and synthetic MRI.

1.3.5 Detection of central venules with MRI

Since early histopathological descriptions of MS, it has been known that MS lesions occur around small venous vessels, called a central venule.[76] T2*-weighted (as illustrated in Figure 6)[77] or susceptibility weighted imaging (SWI) have been shown to be able to detect these central veins *in vivo*. It has therefore been suggested that adding the detection of central veins into the MS diagnostic procedure would improve the radiological specificity for suspected MS cases, and may add prognostic information.[78] Studies have shown that both 3 and 7 T MRI can visualize central veins in many MS lesions.[79] In a study comparing the two field strengths, 7 T was able to demonstrate around twice as many lesions with central veins compared to 3 T. The authors concluded that 7 T in this way is helpful to discriminate between microangiopathic and MS WM lesions.[80] Apart from the potential to be useful in the radiological evaluation of MS, T2*-weighted sequences are also sensitive to blood residuals and can therefore be helpful when excluding differential diagnoses, during investigation of patients with suspected MS.[77]

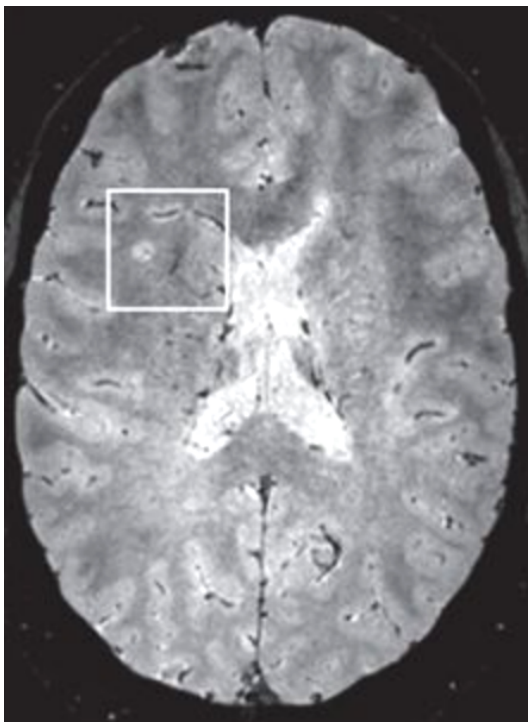


Figure 6. *A T2*-weighted image showing a central vein in a subcortical MS-lesion.*[77]

1.4 MRI CONTRAST AGENTS

1.4.1 MRI contrast agents

The development of MRI contrast agents was initially motivated by how iodine-based contrast agents had showed added value for Computed Tomography for certain clinical applications, such as differentiating tumors and peritumoral edema.[81,82] The subsequent development of contrast agents also for MRI has since been shown to have many diagnostic advantages to improve image quality and give additional information on functional tissue characterization.[83] Contrast-enhanced MRI has been shown to be especially important to detect focal lesions, such as tumors, abscesses and metastasis; characterize lesions (e.g. benign vs. malignant tumors); give functional information and for use in MR angiography.[84] MRI contrast agents can have paramagnetic or superparamagnetic properties. Typically, specific metal ions are used in the contrast agents, which affect the T1- and T2-relaxation in the adjacent tissue. This strengthens the image contrast between the voxels containing the contrast agent and surrounding tissue with no or lower concentration of the contrast agent. The absolute majority of contrast agents are paramagnetic, which mainly shorten the T1-, but to some degree also shorten the T2-relaxation. The paramagnetic metal used in contemporary clinical settings is gadolinium in different types of GBCAs.[85] There are also other examples of MRI contrast agents that have been used clinically. Manganese is such a paramagnetic metal with similar relaxation properties as gadolinium but it is currently not available in any

commercial products.[85] Contrast agents containing iron ions are instead superparamagnetic, which affects mainly the T2 relaxation, leading to signal loss on T2-weighted images. Iron-containing agents are presently not commercially available either.[85]

1.4.2 Gadolinium-based contrast agents

Gadolinium is a metal belonging to the lanthanides. GBCAs are used for a wide spectrum of indications to improve diagnostics. During the early years of GBCAs, it was considered safe in all patients,[86] but after safety concerns regarding nephrogenic systemic fibrosis (NSF), see 1.4.3, it was considered safe only in patients with normal renal function.[87] Possible short-term reactions include allergic reactions (e.g. hives, sneezing, anaphylactoid reactions), physiologic reactions (such as nausea) or complaints related to the injection site (pain or coolness). The total amount of short-term adverse events has been reported to be < 2.4%, although serious short-term adverse events are as uncommon as 0.01-0.03%, with no statistically significant difference in the risk between the different GBCAs[88] Most types of GBCAs are excreted through renal filtration and have a half-life of around 60-90 minutes, but a few GBCAs have partial excretion through the liver. Table 4 lists the characteristics of the GBCAs that have been commercially available as well as the types that recently were withdrawn from the market in Europe.[89]

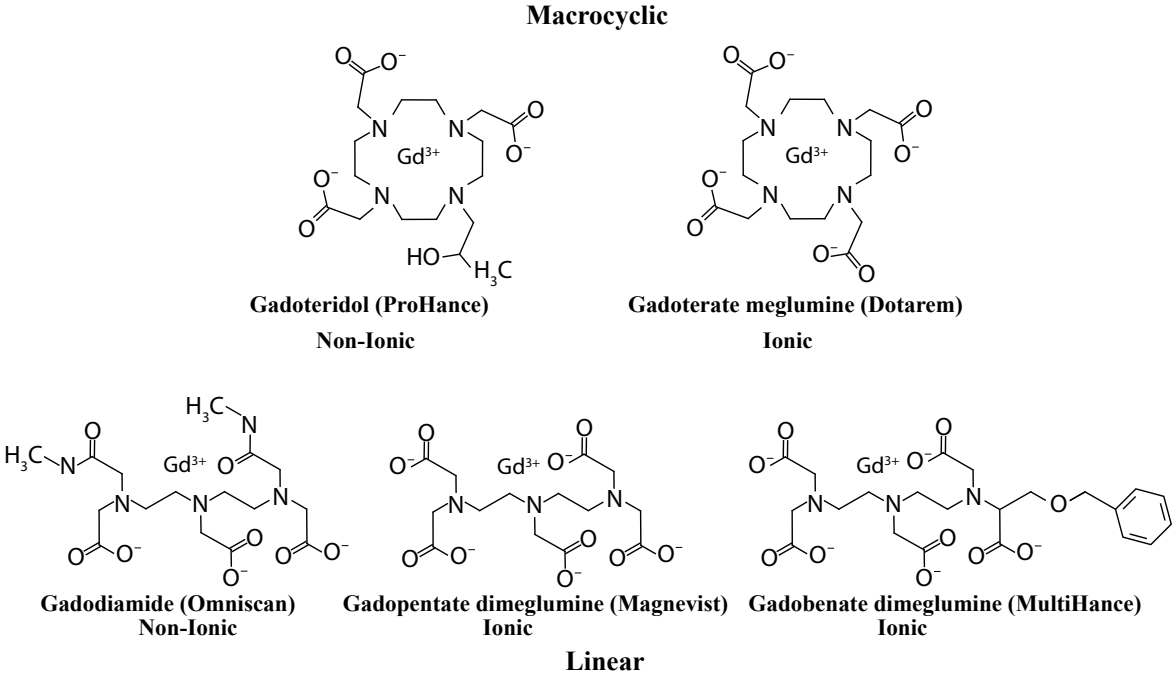
Unchelated gadolinium (Gd^{3+}) is a toxic substance that may cause liver necrosis, hematological effects, splenic degeneration and blockage of calcium channels.[85,90] Gd^{3+} is therefore chelated when used in GBCAs to prevent toxic effects. The chelate binds to the gadolinium and stabilizes it.[85] The chemical stability varies depending on the chelate. Those with lower stability have a higher degree of *in vivo* dissociation of Gd^{3+}, which is insoluble in its free form or soluble when bound to peptides, proteins or other macromolecules. Dissociation of Gd^{3+} *in vivo* almost immediately leads to binding to other molecules, such as citrate and phosphate.[91] The different GBCAs are categorized into linear (open-chain chelates with lower stability) and macrocyclic types (with higher stability).[92] They can further be categorized by their ionic potentials or how strong their relaxation capabilities are. It has been shown *in vitro* that the non-ionic linear GBCAs are the least chemically stable and ionic macrocyclic GBCAs have the highest stability.[93] A visualization of the molecular structures of the GBCAs studied in this thesis is presented in Figure 7.

Table 4. *Characteristics of clinically used GBCAs.*[94]

Gadolinium Based Contrast Agents	Product Name	Chelate Class	Ionicity	Hepato-biliary excretion	EMA decision for market approvals
Gadodiamide	Omniscan	**Linear**	Non-ionic	No	Suspend
Gadoversetamide	OptiMARK	**Linear**	Non-ionic	No	Suspend
Gadopentetate dimeglumine	Magnevist	**Linear**	Ionic	No	Suspend (except for intra-articular administration)
Gadobenate dimeglumine	MultiHance	**Linear**	Ionic	Yes, 1-4%	Restrict to liver scans
Gadoxetate disodium	Primovist, Eovist	**Linear**	Ionic	Yes, 42-51%	Restrict to liver scans
Gadofosveset trisodium	Ablavar, Vasovist	**Linear**	Ionic	Yes, 5%	Already discontinued
Gadoterate meglumine	Dotarem, Magnescope	**Macrocyclic**	Ionic	No	Continued
Gadobutrol	Gadavist, Gadovist	**Macrocyclic**	Non-ionic	No	Continued
Gadoteridol	ProHance	**Macrocyclic**	Non-ionic	No	Continued

EMA = European Medicines Agency.

Figure 7. *Examples of a few of the molecular structures of GBCAs from the different classes.*



1.4.3 Nephrogenic systemic fibrosis

Since its introduction 1987, over 450 million doses of GBCAs have been administered with relatively few safety concerns,[88,95,96] except for some rare adverse events.[97,98] However, in 2006, an association was made between nephrogenic systemic fibrosis (NSF) and GBCA administrations.[87] NSF is characterized by fibrosis of the skin and internal organs. Most cases of NSF have been observed with the linear non-ionic contrast agent gadodiamide (Omniscan), which is the least chemically stable GBCA.[93] It is therefore believed that it is the dissociated form, Gd^{3+}, which activates the immune response causing the excessive collagen production leading to NSF. The majority of NSF cases have been observed in patients with renal failure. Most of the GBCAs are excreted through renal filtration. Patients with impaired renal function therefore retain chelated Gd^{3+} in the body for a longer period of time. This increases the risk of Gd^{3+} to dissociate and thereby leads to higher parenchymal exposure.[99] Since the discovery of NSF, patients with risk factors for renal dysfunction undergo an evaluation of their renal function prior to any MRI scan with GBCAs. This has led to a marked drop in the number of NSF cases.[88] Furthermore, many hospitals have also been inclined to mainly use the chemically more stable macrocyclic GBCAs.

1.4.4 Gadolinium retention

It had already in the early 1990s been shown in rodent models (with normal renal function) that gadolinium can be retained in many body parts, such as the skin, bone and liver after administrations of GBCAs.[100,101] It was later shown that the gadolinium retention in those tissues were up to 30 times greater with non-ionic linear GBCAs than macrocyclic GBCAs.[102] In 2014, Kanda and colleagues published results showing that the number of linear GBCA administrations was associated with increased T1-signal in the DN and GP in patients with normal renal function.[103] In this pivotal study, a Signal Intensity Index (SII) was calculated to quantify the image brightness by dividing the measurement of the investigated brain structure with measurements of another reference region within the same image acquisition according to the following equation:

$$\text{SII} = \frac{\text{Signal intensity measured in region of interest (ROI)}}{\text{Signal intensity measured in a reference region}}$$

This SII method made retrospective analyses of brain MRI scans possible, although with the assumption that retention of gadolinium would not be significant in the reference region. Numerous MRI studies with similar methodology have later shown that administrations of linear GBCAs are associated with higher T1-weighted SII also in other brain structures.[104–109] Furthermore, histopathological studies have since validated that the increased SII corresponds to gadolinium retention.[106,107,110,111] A study using a quantitative approach (T1-relaxometry) also corroborated these findings by showing that a higher number of linear GBCA administrations was associated with higher T1-relaxation rates in the DN.[109] A similar relaxometry-based method did not show any association for the macrocyclic GBCA gadobutrol,[112,113] although a third (more recent) study did show a higher T1-relaxation rate in the GP in the gadobutrol-group compared to controls.[114] It is not fully established which forms of gadolinium (dissociated or bound in the original chelate) cause the higher T1 signal intensity in these brain regions. The fact that it accumulates in a higher degree for linear GBCAs suggests that it gets retained to a larger extent in a de-chelated form.[91]

It is unclear if gadolinium retention in the brain causes any clinically important or even detectable deficits. Studies that investigate potential negative neurological or cognitive effects and symptoms of gadolinium retention are thus needed, especially for patient groups that are repeatedly exposed to GBCAs, such as MS patients. A large study from Ontario, Canada, of 99,739 patients who had received GBCAs for a non-neurological indication, did not show any significantly higher risk to develop Parkinson's disease than among 146,818 patients who had performed non-GBCA-enhanced MRI. However, the analysis had a limitation in that merely 2.4% of the patients receiving GBCA had gotten more than three GBCA administrations.[100] Another recent study in MS patients did not show any association to worsening of EDSS scores.[115] The studies in this thesis are among the first to scientifically try to investigate the relationship of GBCA administrations and cognitive impairment. Preliminary results from a study at the Mayo clinic did not show GBCA-exposure to be a significant risk factor for cognitive performance.[116]

1.4.5 Gadolinium-based contrast agents in MS

GBCA-enhancing lesions provides temporal information of the MS disease activity, as contrast-enhancing lesions have most likely arisen within a few weeks before the MRI scan.[117] It may also increase the sensitivity to detect new MS lesions during follow-up scans.[118] It has been considered important to include GBCAs both in diagnostic investigations and follow-up MRI scans in order to have the best possible sensitivity to disease activity, which facilitates selection of appropriate DMTs and change of treatment regime when necessary. However, the increased efficacy of MS treatments has led to a lower frequency of contrast-enhancing lesions on follow-up MRI scans.[119] As MS patients in the early disease stages are recommended to be followed-up with MRI at least every 6 months (first and second follow-up) and then every 12 months, it is important to moderate the use of GBCAs to avoid unnecessary exposure.[59] When the disease treatment has stabilized (i.e. no detectable disease progression), follow-up scans without GBCAs should be considered.[59]

2 AIMS

This thesis aimed to explore the clinical value of conventional and new MRI applications in MS and how they could be used to evaluate different features of MS in pre-/subclinical and more advanced disease stages respectively. The thesis further aimed to explore brain gadolinium retention in MS and its potential negative effects. The aims of the studies were:

Study I
To investigate the population-based incidence of RIS in an area with high prevalence of MS and how it was affected by different versions of the DIS classification.

Study II
To compare the ability to detect leukocortical lesions in MS between conventional and synthetic versions of PSIR and volumetrically investigate the associations with cognitive and physical dysfunction.

Study III
To investigate the longitudinal relationship of administrations of GBCAs with increased SII in the DN and GP, as well as the association of SII with neuropsychological performance in MS.

Study IV
To use MR-relaxometry to quantify the R1 and R2 values in different brain regions and explore their associations to exposure of linear and macrocyclic GBCA in MS. A secondary aim was to assess possible associations between relaxometry values and cognitive dysfunction.

3 MATERIAL AND METHODS

3.1 ETHICAL CONSIDERATIONS

All studies were approved by the Regional ethics review board in Stockholm. Registration numbers:

- **Study I:** 2014/385-31/1
- **Study II:** 2013/1635-31/2
- **Study III:** 21/95, 04-906/4, 2012/858-31/2, 2016/1468-31
- **Study IV:** 2013/1635-31/2, 2016/1468-31

3.2 PATIENTS, PARTICIPANTS AND RADIOLOGICAL ASSESSMENT

All radiological assessments and measurements in the studies were performed blinded to all MRI scanning details as well as all clinical and patient data.

Study I. All performed brain MRI scans (in the year 2013) from the only two MRI centers in Västmanland County, were included in the study. In the first step, all MRI scans were anonymized and assessed by an experienced radiologist (Ayad Antwan). All MRI scans without any WM anomalies as well as patients with known MS were excluded. In a second step, a neuroradiologist (Juha Martola) evaluated the radiological parts of the RIS criteria (Okuda's RIS criteria A and E, presented in more detail in Table 3). For comparison of the importance of different DIS classifications, both the 2005 and 2010 versions were evaluated. Written informed consent, was obtained to study patient history and neurological examinations in the patient charts, were retrieved from the patients that fulfilled the classification for DIS. Exclusions due to other likely causes for WM anomalies (Okuda's RIS criteria B-D and F) were made in consensus between a radiology resident (Yngve Forslin) and a professor of neurology (Sten Fredrikson) with specialization in MS.

Study II. In total, 21 MS patients were prospectively recruited from the Neurology Department at Karolinska University Hospital in Huddinge, to perform conventional and synthetic PSIR in addition to the normal follow-up protocol for MS. Patients with contraindications for MRI investigation, other neurological diseases or previous trauma to the head, were excluded. The demography of the patients is illustrated in Table 5. The conventional and synthetic PSIR images of the 21 MS patients were anonymized and randomly divided into two collections. Each collection contained images of only one set of either conventional or synthetic PSIR from all 21 patients. Two neuroradiologists (Farouk Hashim and Juha Martola) then individually assessed these collections separately during two rating sessions. The two neuroradiologists together then performed a third consensus assessment, which was considered "gold standard". All assessments were performed with a delay of 12 weeks to avoid any bias caused by potential recollection from previous evaluations.

Study III. Over 18 years, 23 MS patients were prospectively followed-up with at least 3 MRI scans in 1996, 2004 and 2013. During the last follow-up in 2013, 23 age- and gender-matched healthy controls were also recruited for comparison. The patients had received different numbers of the linear GBCAs gadodiamide (Omniscan) and gadopentetate dimeglumine (Magnevist). Only 6 patients had received one administration of the macrocyclic agent gadoterate meglumine (Dotarem), as further detailed in Table 5. After anonymizing and randomizing the order of MRIs, a neuroradiologist (Farouk Hashim) performed manual measurements on the T1-weighted images using 2D regions of interest in the clinical Picture Archiving and Communication System (Sectra IDS7/dx, v. 15.1, Sectra Imaging IT Solutions AB, Linköping, Sweden). For anatomical guidance, and to avoid MS lesions, simultaneous assessment was performed of the corresponding T2-weighted images. To quantify the T1-signal intensity, a SII was calculated with the middle cerebellar peduncle and thalamus as reference regions for the DN and GP respectively.

Study IV. In the period from January to June 2015, 88 consecutive patients with MS who performed brain MRI at Karolinska University Hospital in Huddinge were recruited for additional imaging with Synthetic MRI. After exclusion of 3 patients due to imaging artifacts (1) and previous brain trauma (2), the study included 85 MS patients who had previously received GBCAs. Additionally, 21 healthy controls were included in the study. The patients were stratified into three groups based on the type of previously administered GBCAs with a macrocyclic group consisting of 15 patients who had only received the macrocyclic agent gadoterate meglumine (Dotarem) and a linear group of 11 patients who only had received the linear gadodiamide (Omniscan) and/or gadopentetate dimeglumine (Magnevist). The remaining 59 patients had received between 1-4 administrations of gadoterate meglumine (Dotarem) and 1-9 administration of gadodiamide and/or gadopentetate dimeglumine. This last group was therefore entitled the mixed group. The median times since the last GBCA administration was 7 months for the macrocyclic group, 52 months for the linear group and 12 months for the mixed group. See Table 5 for more details. In all study participants, the longitudinal (R1) and transversal (R2) relaxation values from Synthetic MRI were extracted from manual segmentations, by a resident in radiology (Yngve Forslin), of the DN, GP, CN and thalamus. These deep grey-matter structures were chosen as they were easy to delineate and had been shown to be predilection sites of gadolinium retention.[103–105,120,121] To assess the repeatability of the technique, scan-rescan acquisitions with repositioning of Synthetic MRI was performed in 14 MS patients and 17 healthy controls.

3.3 DEMOGRAPHY OF THE STUDIES

3.3.1 Study I

Västmanland County had a population of 259,000 inhabitants in the study year 2013.[122] Since the healthcare in Sweden is divided into counties, the known population in combination with a complete coverage of all MRIs in the region made it possible to calculate a population-based incidence of RIS. In total, 1,907 individuals (1,091 females, 816 males) in the ages 0-91 years underwent a total of 2,272 brain MRI scans during 2013 (range 1-6 scans per individual) in Västmanland, Sweden. This equaled to 877 brain MRI scans per 100,000 person-years. The mean age at the MRI scans was 47 years and Figure 8 illustrates age distribution in the study.

Figure 8. *The age distribution by gender of the participants in Study I.*

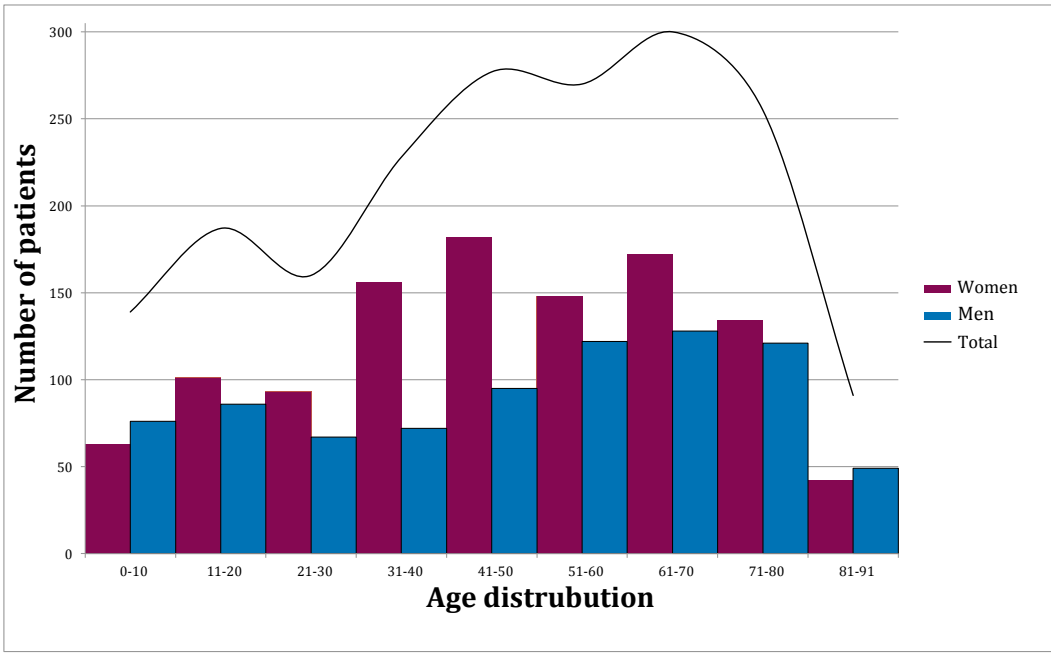


Table 5. *Demography of the study population in Study II, III and IV and available data on T1- and T2-related measurements and GBCA-exposure.*

	Study II	**Study III**		**Study IV**			
	MS patients (N=21)	**Healthy controls (N=23)**	**MS patients with GBCA-administrations (N=23)**	**Healthy Controls (N=21)**	**Mixed MS group (N=59)**	**Linear MS group (N=11)**	**Macrocyclic MS group (N=15)**
Females/Males, N	14/7	18/5	18/5	11/10	18/41	8/3	11/4
Age at the MRI scan, years	45 ± 12	56 ± 7	59 ± 6	36 ± 14	44 ± 10	51 ± 11	37 ± 14
Disease duration, years	15 ± 10	-	19 ± 10	-	13 ± 8	20 ± 9	5 ± 6
MS subtype, RR/SP/PP (for Study II at baseline in 1995-96)	13/7/1	-	18/5/0 (baseline) 16/7/0 (2004) 2/21/0 (2013)	-	39/17/3	5/6/0	14/1/0
Disease modifying therapy, N (%)	14 (67%)	-	-	-	44 (75%)	5 (46%)	13 (87%)
Number of GBCA administrations, median (range)	-	0	2 (0-12)	-	⇓	⇓	⇓
Number of Dotarem administrations, median (interquartile range)	-	-	See in text 3.2	0	2 (2)	0	3 (1)
Number of Magnevist and Omniscan administrations, median (interquartile range)	-	-	3 (4)	0	4 (3)	3 (5)	0
Number of months since last GBCA-administration, median (range)	-	-	-	-	13 (3-172)	52 (24–172)	7 (3-14)

	Study II	Study III		Study IV			
SII (Study III) or R1 (Study IV), Dentate nucleus, mean (±Standard Deviation), middle cerebellar peduncle as reference (Study III)	-	1.01 ± 0.03*	1.03 ± 0.04*	1.23±0.05	1.29±0.05	1.28 ± 0.05	1.27 ± 0.04
SII or R1, Globus Pallidus, thalamus as reference	-	1.10 ± 0.04**	1.07 ± 0.06**	1.24±0.06	1.29±0.06	1.31 ± 0.05	1.28 ± 0.05
R1, Thalamus	-	-	-	1.12±0.04	1.15 ± 0.05	1.14 ± 0.05	1.14 ± 0.04
R1, Caudate Nucleus	-	-	-	0.91±0.03	0.95 ± 0.04	0.96 ± 0.03	0.94 ± 0.02
R2, Dentate Nucleus	-	-	-	15.9±0.96	16.6 ± 1.21	16.7 ± 1.96	16.0 ± 1.45
R2, Globus Pallidus	-	-	-	19.6 ± 1.24	20.9 ± 1.47	20.5 ± 1.74	21.3 ± 2.78
R2, Thalamus	-	-	-	14.6 ± 0.47	14.6 ± 0.50	14.5 ± 0.54	14.4 ± 0.38
R2, Caudate Nucleus	-	-	-	13.9 ± 0.50	14.8 ± 0.83	14.4 ± 0.62	14.2 ± 0.76
Expanded disability status scale, score, median (interquartile range), *N*	2.0 (2.0)	-	4.0 (2.9)	-	2.0 (2.0) *N=54*	2.5 (4.0) *N=11*	2.0 (1.25) *N=13*
Single Digit Modality Test, *z*-scores, median (interquartile range), *N*	-0.48 (1.46)	-	-1.03 (1.99)	-	-1.04 (1.42) *N=36*	-0.19 (1.66) *N=5*	-1.30 (1.48) *N=12*
Verbal Fluency Test, *z*-scores, *N*	-0.37 ±1.37	-	-0.66 ± 1.16	-	-0.23 ± 1.14 *N=17*	0.09 ± 1.69 *N=4*	-1.20 ± 1.88 *N=6*
Fatigue Severity Scale, mean score, *N*	4.53 ± 1.79	-	-	-	4.26 ± 1.86 *N=14*	4.20 ± 2.12 *N=2*	4.33 ±1.21 *N=3*

Values reported as mean ± standard deviations, unless otherwise specified. Relaxation values in ms^{-1}. RR=relapsing-remitting, SP=secondary progressive, PP=primary progressive. SII = Signal Intensity Index. SII-difference between controls and MS patients in Study III: unpaired t-test
**P = 0.001, **P < 0.001.*

3.4 CLINICAL EVALUATIONS

In Study I, all patients fulfilling DIS classification were assessed retrospectively by assessing their clinical patient charts with regards to RIS exclusion criteria. Those who fulfilled the RIS criteria at their MRI scan 2013 had thereafter been followed-up clinically by a neurologist to investigate MS diagnosis, including thorough clinical history, neurological examination and CSF sampling. In Study II, III and IV, EDSS was used as a measurement of global motor dysfunction together with SDMT for information processing speed and a verbal fluency test. In Study III, the neuropsychological evaluations were performed at baseline, after 9 and 18 years and additionally included RAVLT (verbal learning with encoding and 30 min delayed recall) and ROCFT (organization and visuospatial construction ability). In Study II and III the cognitive testing was performed in conjunction with the MRI scans, by the same experienced neuropsychologist (Åsa Bergendal), while in Study IV the cognitive scores were retrospectively collected from the clinical charts. In Study IV only scores within 6 months prior or after the MRI scan were included. Based on the patients age and sex, the cognitive test scores were transformed into normalized to z-scores based on normative data in the literature.

3.5 IMAGE ACQUISITION

Study I. All three scanners that existed in Västmanland County were covered by the study: two 1.5 T MRI scanners (Siemens Avanto and Symphony; Siemens Healthcare, Erlangen, Germany) and one 3 T MRI scanner (Philips Ingenia; Philips Medical Systems, Best, The Netherlands). The MRI brain protocols were individualized and differed depending on the indication for the scan, but they all included at least T1WI and T2WI with a slice thickness of ≤5 mm. The majority of the MRI protocols also included a T2-weighted FLAIR sequence and 42% of the scans were performed with GBCA-administration. The protocols used for the following indications were the only that did not include T2-weighted FLAIR sequence: arterial angiography, control post-intracranial hemorrhage or after aneurysm coiling, meningioma follow-up, investigating vestibular schwannoma or tumors near the pons. The details of the standard brain MRI protocol that were most often used in each of the three MRI scanners are presented in Table 6.

Table 6. *A general overview of the standard brain MRI protocols used in the three different MRI scanners in Västmanland county.*

MRI scanner	Philips Ingenia	Siemens Symphony	Siemens Avanto
Field strength	3 T	1.5 T	1.5 T
Standard brain MRI protocol	*Axial T1WI, sagittal T1WI, axial T2WI, coronal T2WI, axial FLAIR, axial DWI*	*Axial T1WI, sagittal T1WI, axial T2WI, coronal T2WI, axial FLAIR, axial DWI*	*Sagittal T1WI, axial T2WI, coronal FLAIR, axial SWI, axial DWI*
Slice thickness	*≤5 mm*	*≤5 mm*	*≤5 mm*
Number (and percentage) of scans with/without contrast media	*790 (35%)/924 (41%)*		*169 (7.4%)/389 (17%)*

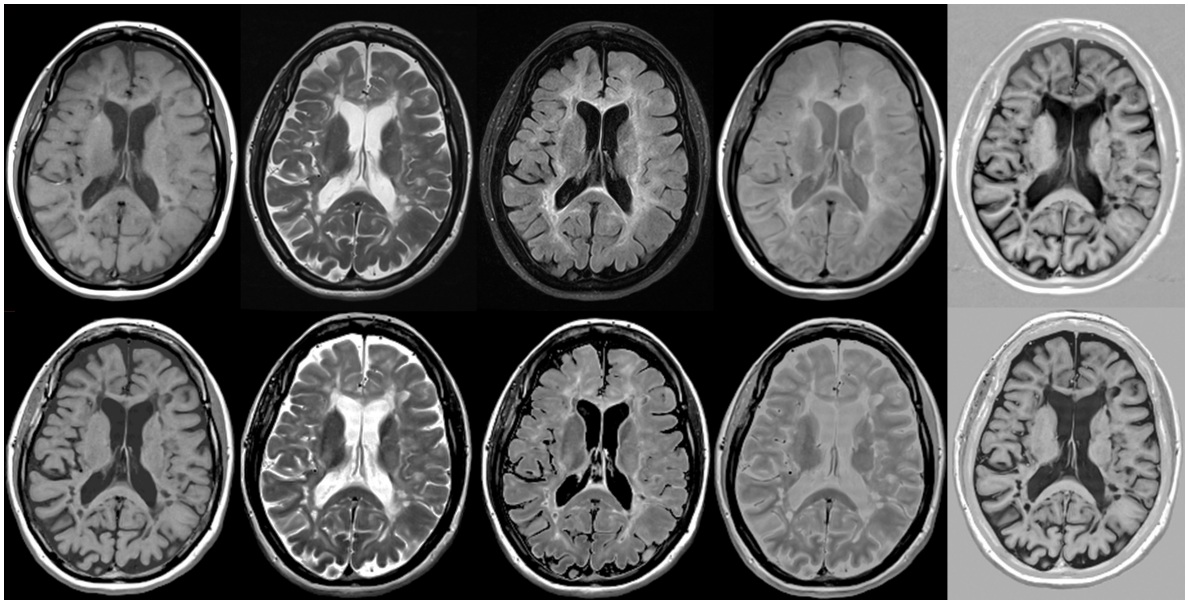
Study II and IV. A Siemens Trio 3 T MRI scanner (Siemens Healthcare, Erlangen Germany) was used for all participants in both studies. In addition to the normal clinical MRI protocol for MS,[59] Synthetic MRI sequence was added to the protocol. The longitudinal and transversal relaxation rates were automatically extracted by a least square fit model that was applied to the data in the SyMRI software (v.11.0 Beta for Mac, Synthetic MR, Linköping, Sweden). Image acquisition parameters for Study II and IV are detailed in Table 7. In **Study II**, a conventional PSIR sequence was also added to the protocol. The synthetic PSIR in **Study II** was extracted by using a reconstruction of the longitudinal magnitude data of the T1 inversion parameters in the quantitative MRI sequence.

Table 7. *Image acquisition parameters used in Study II and IV.*

	Study II		**Study IV**	**Study II and IV**	
Sequence type	**Synthetic PSIR**	**Conventional PSIR**	**Synthetic MRI**	**MPRAGE**	**FLAIR**
Acquisition type	2D axial	2D axial	2D axial	3D sagittal	3D sagittal
Matrix size	256 x 204	256 x 204	256 x 204	256 x 256	256 x 256
In-plane resolution, mm	0.9 x 0.9	0.9 x 0.9	0.9 x 0.9	1.0 x 1.0	1.0 x 1.0
Slices, N	34	34	30	176	160
Slice thickness, mm	3.0	3.0	4.0	1.0	1.0
Slice gap, mm	1.5	1.5	1.2	-	-
Flip angle, °	120	120	120	9.0	120, T2-variable
Repetition time, ms	4820* [6000]	6000	4260*	2300	6000
Echo time, ms	22/100* [10]	10	22/100*	2.98	388
Inversion time(s), ms	150/580/ 2000/4130* [500]	500	150/580/ 2000/4130*	900	2100
Acquisition time, min	7:47	3:32	6:50	5:15	7:02

**Synthetic MRI acqusisition parameters. Virtual settings for synthetic PSIR in brackets. FLAIR=fluid-attenuated inversion recovery, MPRAGE=magnetization-prepared rapid acquisition gradient echo, PSIR=phase-sensitive inversion recovery.*

Figure 9. *A comparison between the respective T1WI, T2WI, T2-weighted FLAIR images, proton density-weighted images, and PSIR images from conventional (top row) and synthetic (bottom row) MRI. The images are from a 50-year old female MS patient who was included in Study II.*



Study III. Brain MRI scans were performed at baseline, after 9 years and 18 years. The majority of patients also had MRI scans with GBCA administrations between these examinations. A non-enhanced T1-weighted spin echo sequence was acquired at baseline and at the 18-year follow-up with 5 mm slice thickness. At up to four examinations over the last nine years (minimum at 9- and 18-year follow up) an MPRAGE sequence with 1x1x1.5 mm resolution was included in the MRI protocol. The MRI acquisition parameters are described in Table 8.

Table 8. *MRI scanners and sequence parameters in Study III.*

Time point	Baseline	9-year follow-up	18-year follow-up	
MRI scanner	GE Signa	Siemens Vision	Siemens Avanto	
Field strength, Tesla	1.5	1.5	1.5	
Sequence type	TSE	MPRAGE	TSE	MPRAGE
Field of view, mm	250	240	220	260
Acquisition matrix	192x256	256x256	224x256	256x256
Slice thickness, mm	5	1.5	5	1.5
Repetition time, ms	640	1350	553	1910
Echo time, ms	13	7	9.1	3
Inversion time, ms	-	300	-	1100
Flip angle, °	90	15	90	15
Number of averages	2	1	1	1

TSE=Turbo Spin Echo, MPRAGE=magnetization-prepared rapid acquisition gradient echo

3.6 VOLUMETRICS

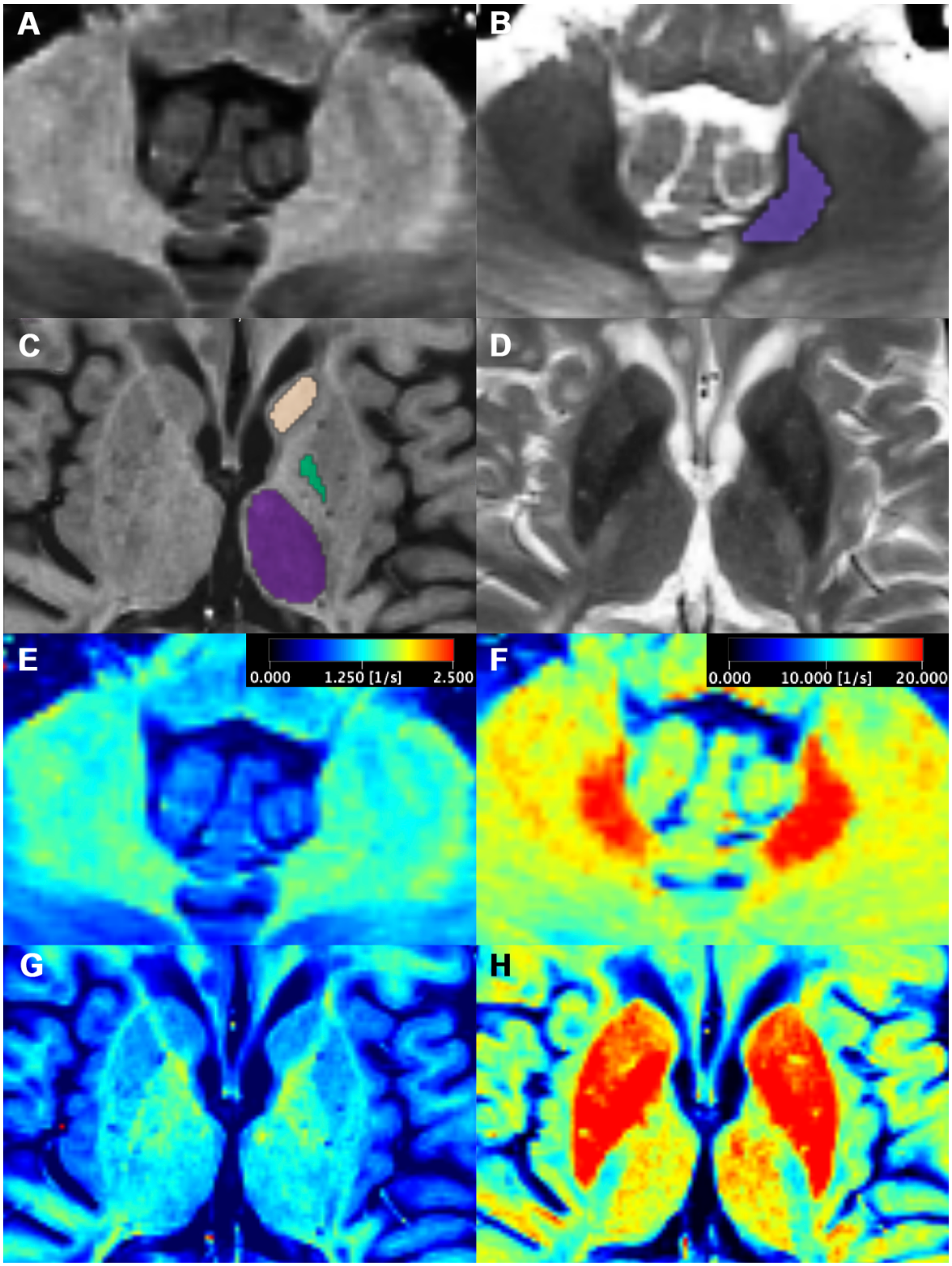
WM segmentations in Study II, III and IV, WM lesions were segmented using Lesion Segmentation Toolbox v. 2.0.12 (Technische Universität München, Munich, Germany) for Statistical Parametric Mapping v. 12 (http://www.fil.ion.ucl.ac.uk/spm/software/spm12; University College London, London, UK) based on T2-weighted FLAIR images.[123,124] The probability maps of the WM lesions were binarized using FMRIB Software Library v. 5.0.9 (Oxford University, Oxford, United Kingdom),[125] with a 0.1 binarization threshold and manually corrected using ITK-SNAP v. 3.4.0 (University of Pennsylvania, Philadelphia, USA).[126] In Study II, the corrections were made first by a resident in radiology (Yngve Forslin) and finally reviewed by a neuroradiologist (Farouk Hashim). In Study III and IV, the corrections to the WM lesion masks were made by another resident in radiology (Tobias Granberg).

LCL segmentations in Study II were performed manually and separately on both the conventional and synthetic PSIR images in ITK-SNAP by a neuroradiologist (Farouk Hashim) based on the consensus agreement.

Brain parenchymal volume segmentations in Study III was retrieved with the longitudinal stream of Freesurfer v. 5.3.0 (Harvard University, Boston, USA)[127] and normalized to the estimated total intracranial volume to obtain the Brain Parenchymal Fraction.

Region of interest segmentations (the DN, GP, CN and thalamus) in Study IV were performed by a resident in radiology (Yngve Forslin) using ITK-SNAP on synthetic T1WI and T2WI, as illustrated in Figure 10. In order to reduce partial volume effects, the edges of the structures were avoided. By using FMRIB Software Library tools version 6.0 (http://fsl.fmrib.ox.ac.uk/fsl),[128] each structures' relaxation values were retrieved from the Synthetic MRI R1- and R2-maps.

Figure 10. *Segmentations from Study IV in an MS patient who have had received 6 previous linear GBCA-administrations.* ***A*** *and* ***C*** *are T1-weighted images that show hyperintensities in the DN and the segmentations of the GP, CN and thalamus.* ***E*** *and* ***G*** *illustrates the corresponding R1-images.* ***B*** *and* ***D*** *illustrates T2-weighted images and the segmentation of the DN.* ***F*** *and* ***H*** *represents the corresponding R2-images in. The color scale for the represent the relaxation values 0–2.5 s^{-1} for R1 and 0-20 s^{-1} for R2. (Segmentations are only shown on the side of the left brain hemisphere).*



3.7 STATISTICS

Software and statistical significance level
SPSS 22.0 was used in Study I and II and SPSS 24.0 was use in Study III and IV. The statistical significance level was pre-defined as a $P<0.05$. Following Benjamini and Hochbergs version for adjustment of the false discovery rate,[129] this level equaled 0.030 in Study II, 0.029 in Study III and 0.023 in Study IV.

Normality
Shapiro-Wilks test was used to evaluate normal distribution. When applicable, normal distribution was achieved with logarithmic transformation.

Group comparisons
Unpaired t-test was used to compare SII in MS patients and healthy controls in Study III. For non-normal distributed data, Wilcoxon signed-rank test was used for group comparisons in Study II. In Study IV, one-way Analysis of variance (ANOVA) with Hochberg post-hoc test was used to compare the relaxation rates in the different subgroups and healthy controls.

In Study III, the MS patients were divided into groups depending on the number of received GBCA-administrations (0, 1-4 and 5-12) to be used in the repeated measures ANOVA to explore longitudinal changes in the SII during the 18-year follow-up.
In Study IV, repeatability was assessed by the mean delta of the relaxation times of the two scans.

In Study II, the Intraclass correlation coefficient was used to assess inter-rater agreement.

Regression analyses
Multiple linear regression was used in Study II to explore associations between LCL (independent variable) and cognitive test scores (dependent variables). In Study III and IV, multiple regression analyses were similarly used to explore associations between the number of GBCA and SII and relaxation values, respectively. In Study III SII and in Study IV R1 and R2 were respectively used as independent variables to assess associations with global motor function and neuropsychological test scores.
In study IV - as the macrocyclic and linear group merely had 6 and 4 patients respectively who had performed verbal fluency test and 3 and 2 patients who had performed fatigue severity scale test, the regression analyses for relaxation rates, verbal fluency performance and fatigue were only made within the mixed subgroup. Regression analyses for the information processing speed scores were made for both the mixed (N=36) and macrocyclic group (N=12) and EDSS for all groups.

4 RESULTS

4.1 STUDY I

4.1.1 RIS incidence

Out of the 1,907 patients, 1,297 had no WM changes and 121 were patients with known MS. As illustrated in Figure 11, 447 patients were excluded due to Okuda's RIS criteria A and E, leaving 42 patients fulfilling the 2010 DIS classification. Out of these, only 20 patients also fulfilled the stricter 2005 DIS classification. The most common indication for MRI among the patients with DIS was headache. The clinical causes for exclusions due to comorbidities that can lead to WM anomalies, such as dementia and cardiovascular events, are detailed in Table 9. After exclusions, two patients were found to fulfill the concurrent criteria for RIS. They both fulfilled the 2005 and 2010 classification for DIS. This equaled to a cumulative incidence of 0.1% per year among all performed brain MRIs in the county. From a population-based standpoint the incidence was 0.8 cases per 100,000 citizens and year. During the screening process of the 2,272 MRI examinations, the findings from the clinical radiological readings were tabulated by a resident in radiology (Yngve Forslin) and is summarized in Table 10.

Figure 11. *Flow-chart of the screening process in Study*

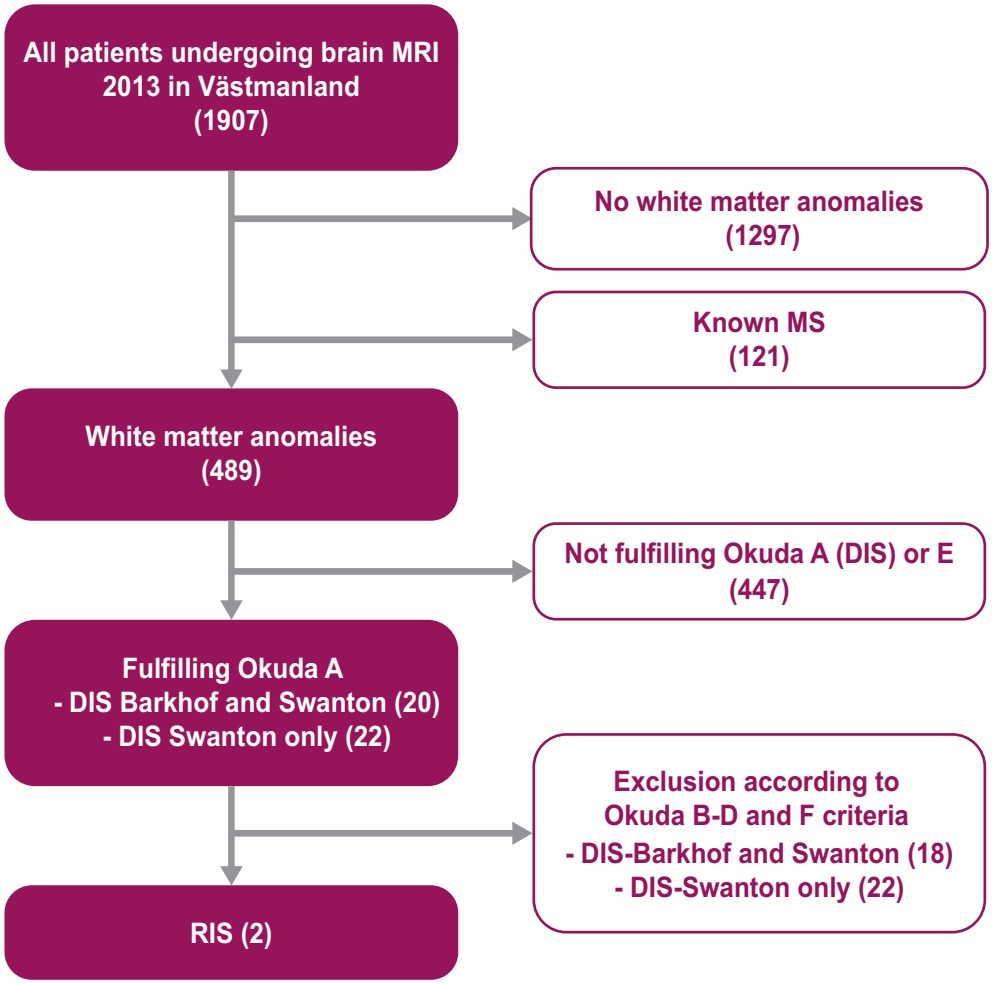


Table 9. *Clinical parts of the RIS criteria B-D and F, as described by Okuda and colleagues[3] and reasons for exclusions (right column).*

RIS criteria	Requirement	Number of excluded patients	Examples
B	No historical accounts of remitting clinical symptoms consistent with neurologic dysfunction	13	Optical neuritis (4), dysesthesia (4), hemiparesis (3), walking difficulties and positive Babinski's sign (1), Lhermitte's sign (1)
C	The MRI anomalies do not account for clinically apparent impairments in social, occupational, or generalized areas of functioning	3	Dementia (3)
D	The MRI anomalies are not due to the direct physiologic effects of substances (recreational drug abuse, toxic exposure) or a medical condition	2	Long-term alcohol abuse (2)
E	Exclusion of individuals with MRI phenotypes suggestive of leukoaraiosis or extensive WM pathology lacking involvement of the corpus callosum	*	
F	The CNS MRI anomalies are not better accounted for by another disease process	22	Stroke (5), myocardial infarction (4), malignant tumor with chemotherapy (4), diabetes mellitus type 2 with long-term complications and hypertension (3), transient ischemic attacks (3), aortic stenosis (2), carotid artery stenosis (1),

***** *The radiological RIS criteria E and A3 (Table 3) were assessed at the same time.*

MRI findings	N*
Normal findings	**654**
Neurodegenerative disorders	**718**
Degenerative or unspecific white matter changes	489
Marked perivascular spaces	50
Other degenerative findings	33
Cerebrovascular disorders	**444**
Infarctions	257
Intracranial bleedings	154
Arterial stenosis, dissections	13
Aneurysms	11
Venous thrombosis	4
Vasculitis	3
Progressive supranuclear palsy	2
Wernicke-encephalopathy	1
Other cerebrovascular disorders	2
Intracranial neoplasms	**247**
Meningiomas	104
Gliomas	59
Suspected neoplasm or neoplasm of unknown origin	38
Metastasis	30
Intracranial lipomas	6
Lymphomas	4
Schwannomas	3
Other neoplasms	3
Neuroinflammatory disorders	**160**
MS findings in known MS patients	121
Other and suspected demyelinating disorders	39
Sinonasal disorder	**158**
Sinusitis, mastoiditis, polyps	156
Other ear/nose pathology	2
Intracranial cysts	**94**
Arachnoid cysts	30
Pineal body cysts	28
Neuroglial cyst	7
Fissure choroid cyst	7
Colloid cyst	4
Epi-/dermoid cyst	3
Unspecified cysts	15
Malformations	**84**
Cavernous venous malformation	20
Developmental venous anomaly	20
Arnold-Chiari malformations	11
Mega cisterna magna	7
Agenesis of the corpus callosum	5
Dandy-Walker malformations	2
Other malformations	9
Hydrocephalus	**50**
Normal pressure hydrocephalus	40
Secondary hydrocephalus	10
Infectious disorders	**16**
Encephalitis	5
Meningitis	5
Other infectious disorders	6

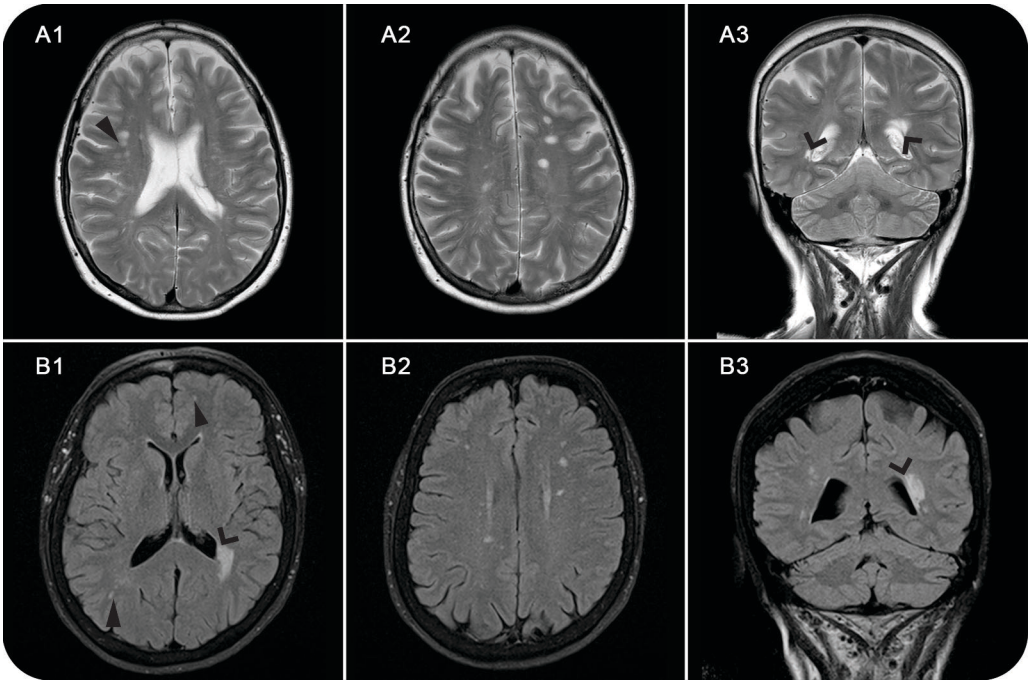
Table 10. *An overview of the radiological findings in the 1907 patients examined during the year 2013 in Västmanland County, Sweden.*

**Each finding has only been counted once per patient, although different types of findings in the same patient have been counted separately.*

4.1.2 The RIS patients

Both RIS patients had 3 periventricular, 1 juxtacortical and more than 9 WM anomalies, thus fulfilling both the 2005 and 2010 classifications for RIS. The first person with RIS was a previously healthy 61-year-old female, who performed a brain MRI scan due to headache and unspecific vertigo. She had no contrast-enhancing lesions. CSF-analysis showed oligoclonal IgG-bands, but a normal IgG-index. No neurological symptoms or findings were presented at the clinical assessment and she had no recollection of any previous MS-associated symptoms. The second patient with RIS was a 66-years-old female that had performed the MRI scan after two episodes of suspected epileptic seizures. The clinical investigation (including electroencephalogram) had normal findings and no diagnosis of epilepsy was made. CSF-analyses were negative. The neurological examination was normal and there was no history of prior neurological events. The persons with RIS did not fulfill the concurrent McDonald 2010 criteria for MS and did not receive any therapy. They were followed-up and after 1 year – still with no symptoms that indicated MS. Images of the RIS-patients are illustrated in Figure 12.

***Figure 12**. The first (A1-A3) and second (B1-B3) person who fulfilled the RIS criteria. Periventricular lesions are shown with open arrows, and juxtacortical lesions with closed arrows.*

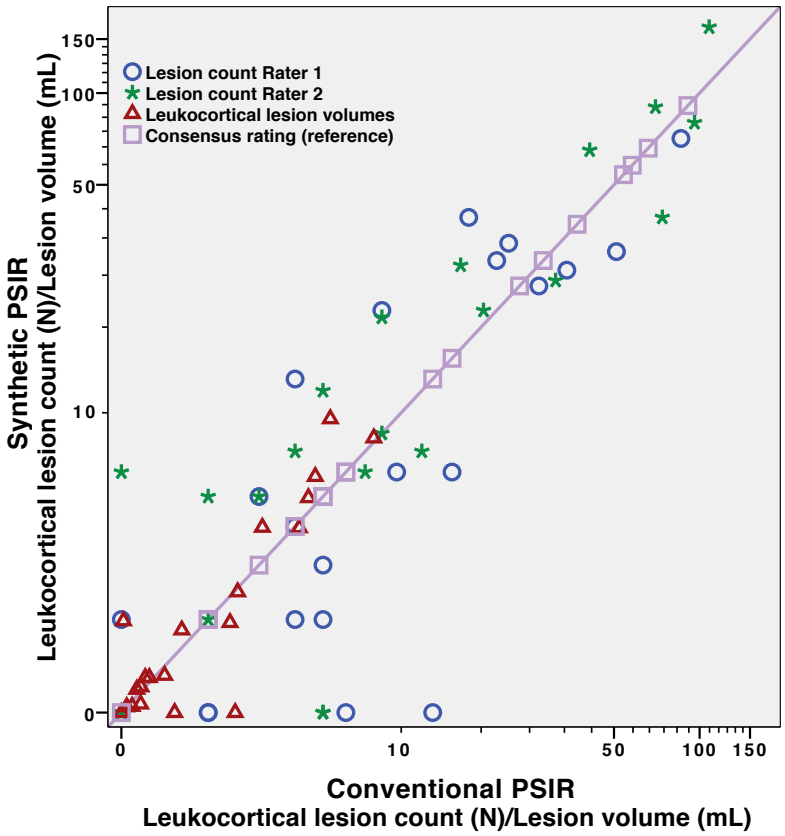


4.2 STUDY II

4.2.1 Radiological comparison of conventional and synthetic PSIR

The Intraclass Correlation Coefficient showed an excellent agreement between the raters for the LCL assessment (conventional PSIR: 0.79, P<0.001; synthetic PSIR: 0.87, P<0.001). Similar agreements were shown between each rater and the consensus agreement ratings (conventional PSIR: 0.91 and 0.97; synthetic PSIR: 0.92 and 0.94, P<0.001 for each rater respectively). The number of detected LCL on the two MRI sequences did not differ significantly from each other (P=0.47 and P=0.08 for the raters respectively). Figure 13 illustrates the relationship between the two raters' individual and consensus ratings. Apparently, there seems to be a larger dispersion between the neuroradiologists' ratings in patients with fewer lesions and less relative difference for patients with higher lesion counts. There was, however, a significant but relatively minor difference between one rater's LCL count on conventional PSIR and the consensus rating (*P*=0.008). Wilcoxon Sign Rank test further showed no difference between the manual LCL volume segmentations between conventional and synthetic PSIR (*P*=0.17). The comparisons between the counts and volumes are shown in Table 11. Figure 14 illustrates two LCL and how they were segmented on conventional and synthetic PSIR.

Figure 13. *Illustration of the lesion volumes (red triangles) and the relation between each rater's lesion counts (blue circles and green stars) on conventional and synthetic PSIR, as well as their relation to the consensus rating (purple squares and line).*



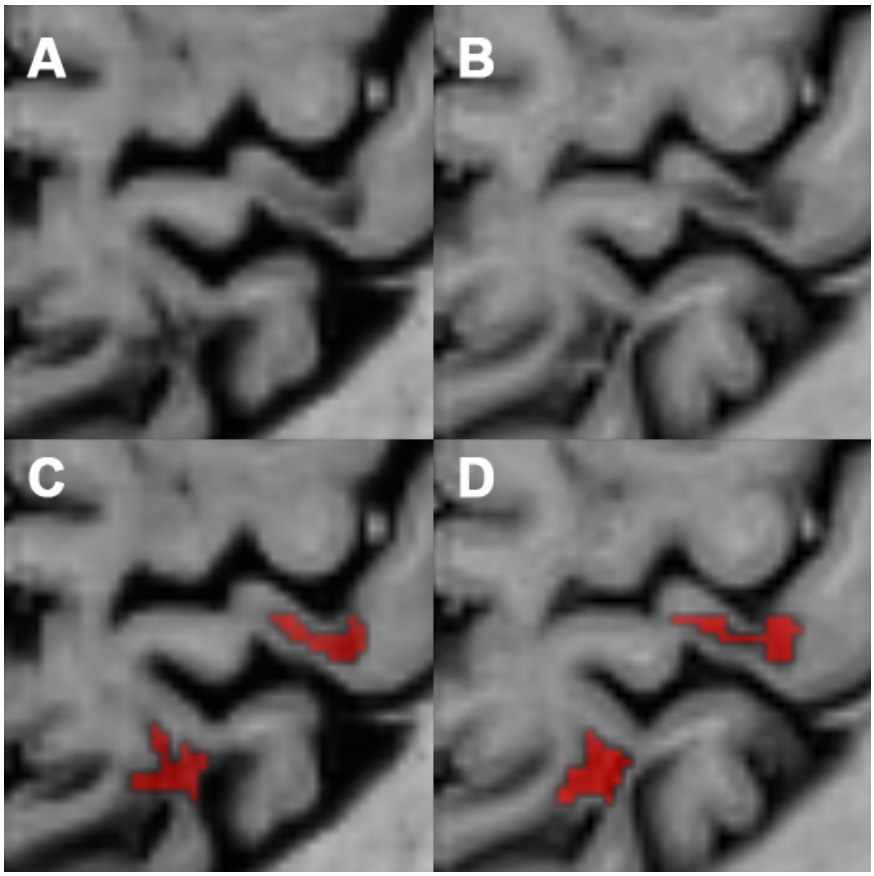


Figure 14. *Comparison of two leukocortical lesions and their segmentations on conventional (**B** and **D**) and synthetic PSIR (**A** and **C**).*

Table 11. *Comparisons of the radiological assessments of leukocortical lesions on conventional and synthetic PSIR.*

	Conventional PSIR	Synthetic PSIR	P-value conventional vs. synthetic PSIR*	Consensus rating	P-value conventional/synthetic PSIR vs. consensus rating*
Leukocortical lesion count, Rater 1, N	7±17	5±26	0.47	5±30	0.14/0.06
Leukocortical lesion count, Rater 2, N	7±34	7±29	0.08	5±30	0.008/0.96
Leukocortical lesion volume, mL	0.53±2.46	0.32±2.89	0.17		

*The values are presented as median ± interquartile range. *P-value by Wilcoxon sign rank test.*

4.2.2 Associations to cognitive and motor function

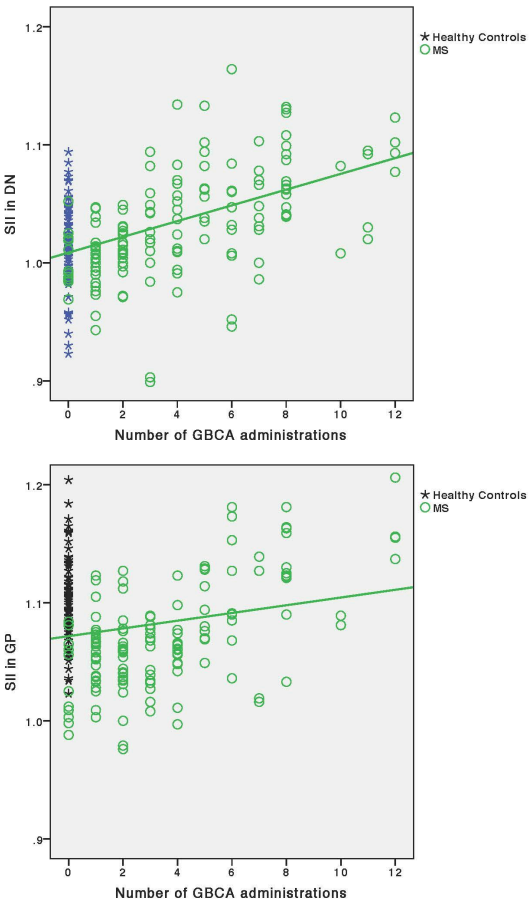
Larger LCL volumes were significantly associated with lower information processing speed (SDMT scores) on both conventional (β=-0.62, P=0.003, adjusted R^2=0.35) and synthetic PSIR (β=-0.55, P=0.010, adjusted R^2=0.26). A similar association was found with lower verbal fluency performance and larger volumes of LCL on both conventional (β=-0.51, P=0.019, adjusted R^2=0.22) and synthetic PSIR (β=-0.43, P=0.054, adjusted R^2=0.14). When combining the volumes of LCL with WM lesions from conventional MRI sequences, the associations with information processing speed (β=-0.66, P=0.001, adjusted R^2=0.41) and verbal fluency (β=-0.52, P=0.015, adjusted R^2=0.24) were both positively affected. The corresponding associations for synthetic PSIR were similarly positively affected for both information processing speed (β=-0.58, P=0.005, adjusted R^2=0.31) and verbal fluency (β=-0.47, P=0.030, adjusted R^2=0.18). When looking at LCL volumes and EDSS scores or fatigue severity scores, no significant associations were found for neither conventional (EDSS: β=0.45, P=0.18; fatigue: β=0.04, P=0.88) nor synthetic PSIR (EDSS: β=0.60, P=0.12; fatigue: β=-0.03, P=0.89).

4.3 STUDY III

4.3.1 Signal intensity index and GBCA-administrations

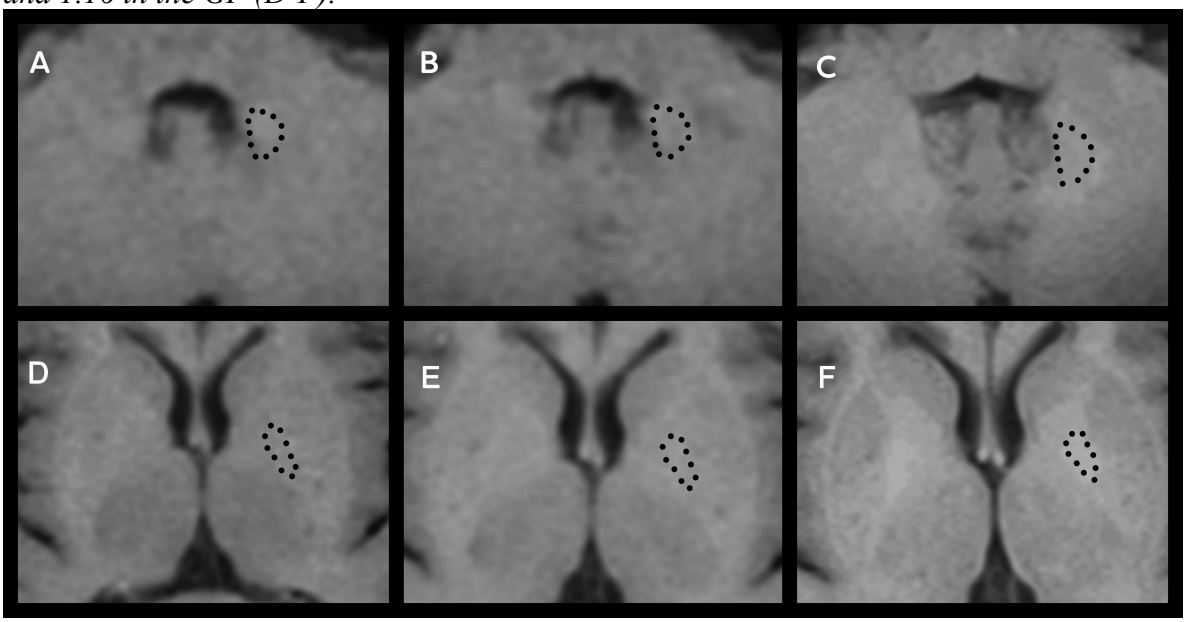
The DN (P<0.001), but not the GP (P=0.19), was found to have higher SII in MS patients than in healthy controls, as detailed in Table 5. When looking at the MS patients only, there was an association between higher number of linear GBCA-administrations and higher SII in the DN (β=0.45, P<0.001) and GP (β=0.58, P<0.001) when correcting for age, MRI scanner and the type of T1-weighted sequence. This association is illustrated in Figure 15. The association remained also after adding EDSS and disease duration as correction variables both for the DN (β=0.43 P<0.001) and GP (β=0.58, P<0.001). For the latter two time points, 3D imaging allowed volumetric tissue segmentations of the lesion volumes and atrophy (brain parenchymal fraction), which could then be added as additional correction factors in the regression analyses (to broaden the characterization of the severity MS disease burden). After doing so, the multiple regression still showed an association with higher SII in the DN and GP (β=0.39, P=0.007 and β=0.64, P<0.001, respectively). The mean change of SII, between the first and last MRI scan (spanning over 18 years), was 0.06 (6.0%) in the DN and 0.07 (6.7%) in the GP. This equaled a change of 0.009 (~1%) per

Figure 15. *Higher number of GBCA-administrations were associated with increased SII in DN and GP.*



received GBCA-administration. The repeated measures ANOVA with pairwise comparisons showed that SII, in patients who had received 0 and 5-12 administrations of GBCA, were significantly different from each other (DN: 1.01±0.24 vs. 1.08±0.032, P<0.001 and GP: 0.96±0.128 vs. 1.09±0.53, P=0.013). The comparison between the patients who had 0 and 1-4 GBCA administrations showed a similar trend (DN P=0.049. GP P=0.064). An example of the change of signal intensity in one MS patient is illustrated in Figure 16.

Figure 16. *A female MS patient who at baseline (A & D) had received 0 GBCA, and then increasing amount of received GBCA (1 and 8 doses at B & E and C & F respectively). The SII increased gradually from 1.03 to 1.05 and 1.09 in the DN (A-C); and from 1.00 to 1.12 and 1.16 in the GP (D-F).*



4.3.2 Associations to cognition

There was an association between high SII and low verbal fluency performance in the GP (β=-0.45, P<0.001) and a trend in the DN (β=-0.25, P=0.03), which remained after correction for EDSS and disease duration both in the GP (β=-0.49, P<0.001) and the DN (β=-0.28, P=0.012). When looking at auditory verbal learning, a similar association was seen only in GP, although both before (β=-0.35, P=0.003) and after (β=-0.33, P=0.006) correction for EDSS and disease duration. A trend was seen for SDMT after correction for EDSS and disease duration (β=-0.24, P=0.043). No associations were found for ROCFT. At the later time points, brain parenchymal fraction and lesion volumes were available. When additionally adding those factors as correction variables, there was only one significant association remaining – between high SII in the DN and low verbal fluency performance (β=-0.40 P=0.013), and a trend in the GP (β=-0.36 P=0.034).

4.4 STUDY IV

4.4.1 Repeatability

The median scan-rescan difference in all regions was 2.0% with a range of 0.8-2.1% in the DN, CN and thalamus. As illustrated in Table 12, the GP showed a median change of 4.1% and 4.2% respectively for the R1- and R2-measurements.

Table 12. *The change for the R1 and R2 between first and second repeated MRI scan.*

	Dentate Nucleus	Globus Pallidus	Caudate Nucleus	Thalamus
ΔR_1 %	2.0 (3)	4.1 (5)	2.1 (3)	1.9 (3)
ΔR_2 %	2.0 (4)	4.2 (6)	0.9 (2)	0.8 (1)

The repeatability of the scans is reported as the median (interquartile range) % difference between the scans (the absolute difference between the scans divided by their mean).

4.4.2 Group differences

One-way ANOVA showed a significant difference of R1 and R2 in all brain regions between the groups, except R2-values in the thalamus. Hochberg post-hoc analysis showed that the overall differences consisted of significantly different values between the linear and mixed subgroup in comparison with the healthy controls. These results are detailed in Table 13.

4.4.3 GBCA-administrations and R1- and R2-values

Within both the linear and mixed group, there was an association between higher number of administered linear GBCA and higher R1 in the DN (linear: β=0.52, P=0.002; mixed: β=0.57, P<0.001), GP (linear: β=0.51, P=0.004; mixed: β=0.43, P<0.001), CN (linear: β=0.41, P=0.020; mixed: β=0.45, P<0.001) and thalamus (linear: β=0.23, P=0.21; mixed: β=0.39, P<0.001). After correcting for age, and for the macrocyclic group also the number of received macrocyclic GBCA, the associations remained significant for these two groups in the DN (linear: β=0.50, P=0.005; mixed: β=0.54, P<0.001), GP (linear: β=0.50, P=0.009; mixed: β=0.37, P<0.001); but only significant for the mixed group in the CN (linear: β= β=0.33, P=0.07; mixed: β=0.39, P=0.001) and thalamus (linear: β=0.24, P=0.22; mixed: β=0.41, P<0.001).

There was only an association between higher number of linear GBCA and higher R2-values in the mixed group: in the DN (β=0.30, P=0.006), GP (β=0.43, P<0.001) and CN (β=0.50, P<0.001), but not thalamus (P=0.32). These associations remained after age and number for macrocyclic GBCA in the GP (β=0.42, P<0.001) and CN (β=0.36, P<0.001), but not the DN (P=0.14).

For the macrocyclic group, there was unexpectedly an association with higher R2 in the CN (before: β=0.36, P=0.03 and after correction for age: β=0.40, P=0.006), but not in either of the subgroups in the other brain regions (P=0.08-0.82).

The relationship between the number of administered GBCAs and relaxation values in the different subgroups are illustrated in Figure 17 and all the results from these regression analyses are also presented in detail in Table 14.

Table 13. *Hochberg post-hoc group comparisons after one-way ANOVA.*

	Dentate nucleus		**Globus pallidus**		**Caudate nucleus**		**Thalamus**	
	R_1	R_2	R_1	R_2	R_1	R_2	R_1	R_2
Linear	1.28 ±0.05	16.74 ±1.96	1.31 ±0.05	20.52 ±1.74	0.96 ±0.03	14.44 ±0.62	1.14 ±0.05	14.45 ±0.54
Mix	1.29 ±0.05	16.59 ±1.21	1.29 ±0.06	20.89 ±1.47	0.95 ±0.04	14.75 ±0.83	1.15 ±0.05	14.64 ±0.50
Macrocyclic	1.26 ±0.04	15.87 ±1.14	1.27 ±0.04	20.50 ±1.101	0.93 ±0.02	14.18 ± 0.74	1.15 ±0.03	14.55 ±0.48
Controls	1.23 ±0.05	15.86 ±0.96	1.24 ±0.06	19.63 ±1.24	0.91 ±0.03	13.88 ± 0.50	1.12 ±0.04	14.64 ± 0.47
Linear vs. Controls	**0.026**	0.32	**0.012***	0.52	**0.006***	0.28	0.41	0.87
Mix vs. Controls	**>0.001***	0.14	**0.004***	**0.004***	**<0.001***	**<0.001***	**0.020***	1.0
Macrocyclic vs. Controls	0.13	1.0	0.55	0.41	0.69	0.82	0.25	1.0
Linear vs. Mix	0.99	0.99	0.89	0.97	1.0	0.79	1.0	0.82
Linear vs. Macrocyclic	0.98	0.44	0.42	1.0	0.27	0.95	1.0	1.0
Mix vs. Macrocyclic	0.64	0.32	0.77	0.93	0.18	0.07	1.0	0.99

Results by Hochberg post hoc test performed after One-Way ANOVA.
*R_1 and R_2 are presented as the subgroup mean in s^{-1} ± the standard deviation. *=$p<0.023$*

Figure 17. *The number of linear (A and C), but not macrocyclic (E), GBCA was associated with higher R1-values in the dentate nucleus, globus pallidus and caudate nucleus. The mixed group (D) also showed significant association with higher R1 for thalamus as well as R2 in the globus pallidus and caudate nucleus, the linear or macrocyclic group (B and F) did not show. The healthy controls (with GBCA administrations: N=0) are represented in each group in the scatter plot.*

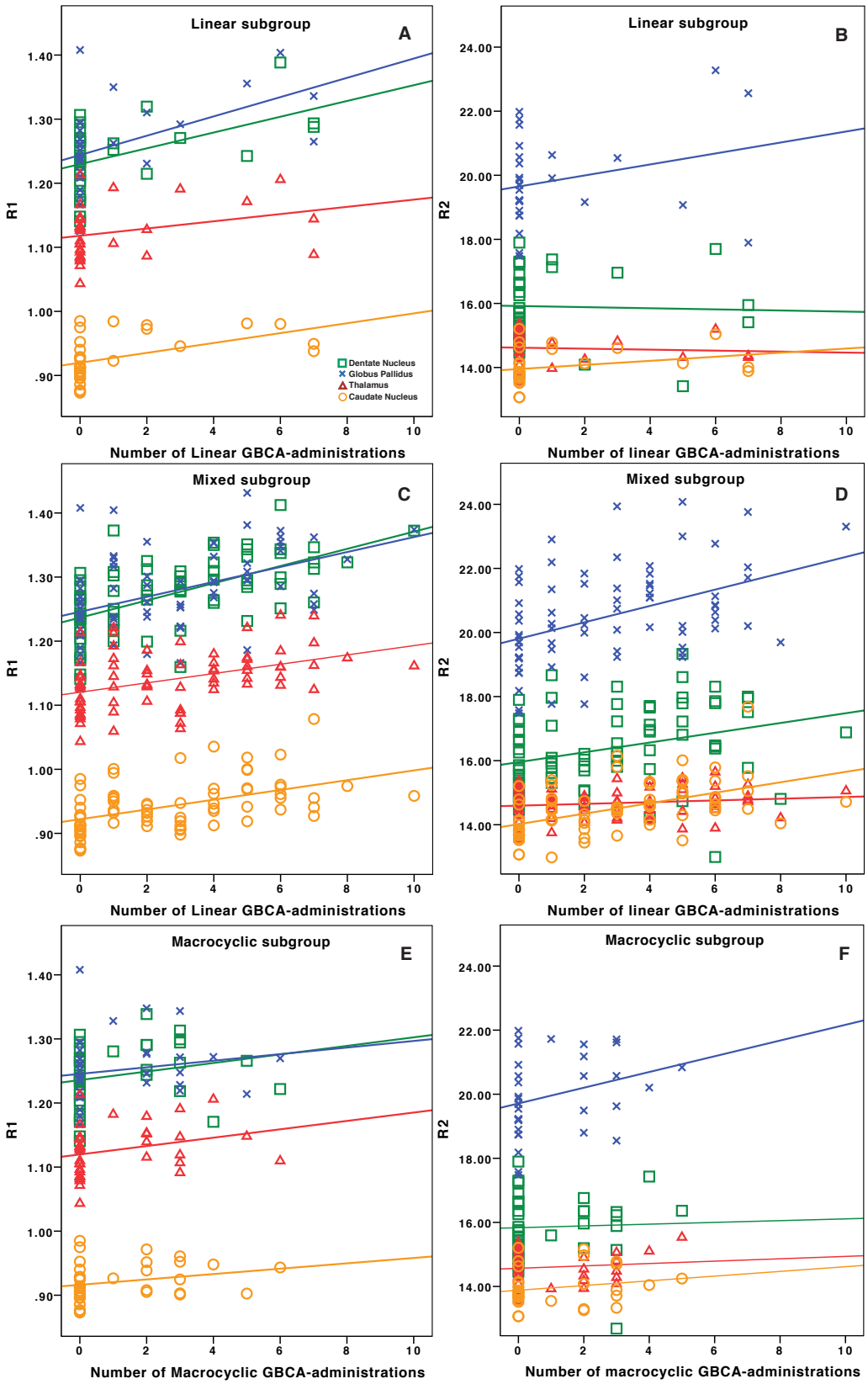


Table 14. Associations between number of linear or macrocyclic GBCA and relaxation rates

	Linear group		Mixed group		Macrocyclic group	
	Uncorrected	**Corrected****	**Uncorrected**	**Corrected*****	**Uncorrected**	**Corrected****
DN R_1	**0.52, P=0.002***	**0.50, P=0.005***	**0.57, P<0.001***	**0.54, P<0.001***	0.23, P=0.19	0.24, P=0.16
DN R_2	0.14, P=0.45	0.04, P=0.83	**0.30, P=0.006***	0.17, P=0.14	0.04, P=0.82	0.07, P=0.64
GP R_1	**0.51, P=0.004***	**0.50, P=0.009***	**0.43, P<0.001***	**0.37, P=0.002***	0.16, P=0.36	0.17, P=0.33
GP R_2	0.28, P=0.14	0.26, P=0.19	**0.43, P<0.001***	**0.42, P<0.001***	0.29, P=0.10	0.29, P=0.10
CN R_1	**0.41, P=0.020***	0.33, P=0.07	**0.45, P<0.001***	**0.39, P=0.001***	0.24, P=0.16	0.27, P=0.11
CN R_2	0.21, P=0.25	0.06, P=0.72	**0.50, P<0.001***	**0.36, P<0.001***	0.36, P=0.032	**0.40, P=0.006***
Thalamus R_1	0.23, P=0.21	0.24, P=0.22	**0.39, P<0.001***	**0.41, P<0.001***	0.28, P=0.10	0.27, P=0.12
Thalamus R_2	-0.21, P=0.26	0.15, P=0.44	0.11, P=0.32	0.16, P=0.17	0.10, P=0.59	0.08, P=0.65

All association results are given as beta coefficients. The healthy controls (with N=0 GBCA administrations) are included in regression analyses for each group.

**P<0.023. **Corrected for the age. ***Corrected for age and number of macrocyclic GBCA administrations.*

CN = Caudate Nucleus, DN = Dentate Nucleus, GP = Globus Pallidus.

4.4.4 Associations to cognition and global motor function

In the mixed group, as detailed in Figure 18 and Table 15, but not the macrocyclic group, higher R1 and R2 in the CN and R1 in the thalamus remained significantly associated with lower verbal fluency performance after correction for disease duration and lesion volumes (CN: R1/R2: β=-0.63/-82, P=0.007/0.010; thalamus R1: β=-0.51; P=0.011).

R1 in the DN and R1 and R2 in the thalamus were the only remaining significant associations to lower information processing scores (SDMT) after correction for disease duration and lesion volumes (DN: R2: β=-0.37; P=0.014; thalamus R1/R2: β= -0.39/-0.45, P=0.011/0.002).

When similarly assessing associations with the Fatigue Severity Scale (P=0.09–0.96) in the mixed group and global motor function (EDSS) (P=0.12–0.99), no significant associations were found for any of the brain regions in any of the groups.

Figure 18. *Associations between R1 and neuropsychological tests in the mixed group. Higher R1-values in the thalamus were associated with lower information processing speed/symbol digit modalities test (SDMT) (A) and caudate nucleus and thalamus with lower verbal fluency performance (B) after correction for multiple sclerosis disease duration and lesion volumes.*

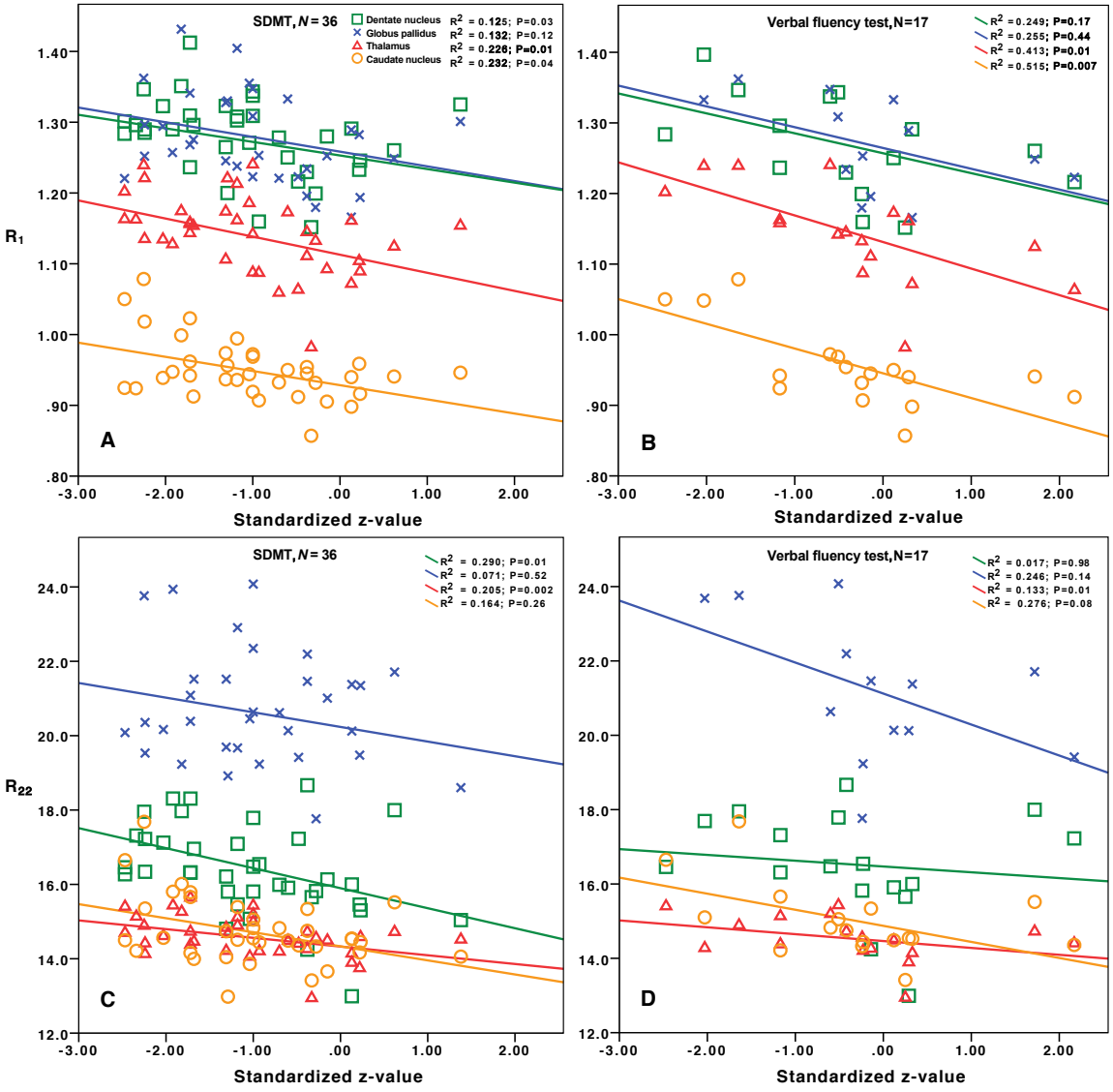


Table 15. *Associations between relaxation values (R1 and R2) and information processing speed (SDMT) and verbal fluency performance (F-A-S test) in DN, GP, CN and thalamus.*

	SDMT *N*=36		**Verbal Fluency test *N*=17**	
	Uncorrected	Corrected**	Uncorrected	Corrected**
DN R_1	-0.34, P=0.04	-0.35, P=0.026	-0.38, P=0.18	-0.31, P=0.17
DN R_2	-0.41, P=0.012*	-0.37, P=0.014*	-0.06, P=0.82	-0.004, P=0.98
GP R_1	-0.32, P=0.07	-0.29, P=0.12	-0.43, P=0.16	-0.21, P=0.44
GP R_2	-0.18, P=0.32	-0.11, P=0.52	-0.37, P=0.23	-0.37, P=0.14
CN R_1	-0.46, P=0.005*	-0.33, P=0.04	-0.63, P=0.009*	-0.63, P=0.007*
CN R_2	-0.39, P=0.018*	-0.21, P=0.26	-0.52, P=0.04	-0.82, P=0.010*
Thalamus R_1	-0.45, P=0.005*	-0.39, P=0.011*	-0.59, P=0.017*	-0.51, P=0.011*
Thalamus R_2	-0.42, P=0.011*	-0.45, P=0.002*	-0.44, P=0.09	-0.35, P=0.08

*The associations are displayed as coefficient beta with each p-value. *P<0.023. **Corrections were made for lesion volume and disease duration. CN=Caudate Nucleus, DN=Dentate Nucleus, GP=Globus Pallidus, SDMT=Symbol Digit Modalities Test.*

5 DISCUSSION

5.1 STUDY I

We performed a population-based study in Västmanland County in Sweden and found that RIS is a rather unusual finding with an incidence rate of 0.8 cases per 100,000 person-years in a high-incidence country for MS. This equals to approximately 1 RIS case per 10 diagnosed MS cases. Our study did not show any difference in incidence when applying the latest classification for DIS (2010 McDonald criteria) at the time of the study, in comparison with the older Barkhof classification for DIS (used in the 2005 McDonald criteria and Okuda's RIS criteria). It is, however, hard to generalize the results from one Swedish county because of many possible confounding factors, such as regional differences in MRI practices, availability of MRI scanners and technical differences in MRI parameters and sequences, as well as population-related risk differences. It is important to stress that these incidence numbers are the incidence of the clinical entity RIS as an incidental finding among patients referred for an MRI scan without neurological symptoms, and not the prevalence of RIS in the general population that would fulfill RIS criteria if they would have performed a brain MRI scan. It is also important to keep in mind that the DIS classification for MS is validated for use in patients with suspected MS and for WM anomalies that are consistent with MS and should not be applied to other patient categories without a second thought. A review of diseases that may mimic MS have summarized that 7-58% of patients with other conditions such as migraine, CNS vasculitis and Sjögren's syndrome fulfill the 2005 classification for DIS.[77] The radiological MS or RIS criteria should therefore be used with discretion and with consideration of possible MS mimics.

We know that at least some people with RIS go on to develop MS, about 33% in 5 years and 50% in 10 years.[41,43] It has been hard to differentiate those that will advance to MS and those that have other causes for the MRI findings with the concurrent RIS criteria. It is therefore challenging to know how to manage these patients clinically. The goal should be to give treatment as early as possible to those patients that have preclinical/subclinical MS, while not overtreating patients that will not convert to MS. The MAGNIMS group recommendations for RIS, however, states that there is currently lacking evidence to support treatment for RIS and that the recommendation instead is to actively monitor (clinically and radiologically) persons with RIS with a higher likelihood of having a subclinical form of MS.[44] The relatively high age of the RIS patients in our study was surprising, since the highest incidence of MS occurs at the age of 30 years in Sweden.[4] These results suggest that RIS could reflect a more benign MS entity that is not in the same need for prompt initiation of treatment. However, one of the patients in this study performed the MRI scan due to headache and additionally was found to have oligoclonal bands in the CSF. Moreover, there have been studies showing high prevalence of headaches in MS, especially in relation to the time of first neurological symptom.[130,131] Although headache is an unspecific symptom, the result of this study supports the idea to at least perform a primary neurological investigation of RIS patients in order to make a more informed decision on whether to perform further clinical follow-ups

The main limitation of the study is the relatively low sample size, as the number of detected RIS cases was few, which makes the estimation of the RIS incidence less robust. Another limitation was that many of the MRI protocols that were used for different indications for MRI did not correspond with an MS-dedicated protocol. This can most likely have reduced the sensitivity for especially smaller WM anomalies. This difference in MRI protocols did, however, represent the true variation present in the clinical praxis.

5.2 STUDY II

Recently, synthetic MRI has proved to be a time-efficient alternative to conventional MRI since multiple weightings, including synthetic PSIR, can be obtained from a single acquisition.[75] The main result of our prospective cohort study is that synthetic PSIR and conventional PSIR are comparable in their ability to detect leukocortical MS lesions. The study also showed that LCL volumes on both PSIR sequences were similarly associated with cognitive deficits, which indicate that using any of the PSIR sequences may add clinically valuable information.

Previous studies have shown that imaging of cortical MS pathology can increase the diagnostic accuracy for MS and that it may give prognostic information in borderline cases.[8,64,132] The recently updated diagnostic criteria for MS therefore included assessment of cortical lesions in the DIS classification (Table 2). The results in our study support this specific change to the DIS classification since detection of cortical pathology (that is associated with cognitive deficits), was possible even with a clinical 3 T MRI scanner. Imaging of cortical lesions have, however, been proven to be challenging, especially using MRI scanners with field strengths typically used in clinics, 1.5 or 3 T. A study validating the findings from a 3D DIR sequence with histopathology, showed a detection rate of intracortical lesions of merely 18%.[133] It has been suggested that PSIR is better suited than DIR for the task of detecting cortical lesions.[134] However, using a multi-modal approach with several different MRI sequences (as was used as a golden standard in Study II) was preferred by the raters of this study, especially as delineation of the WM-cortical boundary was facilitated. This suggested methodology with multiple sequences is also in line with some previously proposed approaches.[9,69,135,136] The significant association between LCL volume and cognitive dysfunction in combination with the lack of significant association to physical disability or fatigue, implies that the LCLs are primarily related to cognitive deficits.

There were some limitations of the study. The small sample size made it unsuitable to perform subgroup analyses among the different MS subtypes. Due to the *in vivo* character of the study, no histopathological validation was available. Availability of 7T would likely have improved the golden standard assessment but that is not yet available in the Stockholm region. To avoid artefacts due to inter-slice cross-talk, a slice gap of 0.5 (1.5 mm) had to be used, which emphasize the advantage to complement synthetic MRI with 3D-based MRI sequences, as used in the golden standard in this study. Recent developments also hold promise of a 3D-based synthetic MRI approach.[137]

5.3 STUDY III AND IV

Study III and IV both investigated associations between previously received GBCA administrations and relaxation rates in different brain structures. As a higher relaxation rate gives an increased signal intensity on conventional T1-weighted MRI sequences, Study III could semi-quantitatively estimate the effect on signal intensity by indexing the measurements with a region that supposedly had less gadolinium retained (i.e. SII). The study then utilized the longitudinal information from the 18-year follow-up of the patients to better confirm an existing association. Study IV instead used a quantitative MRI sequence to more directly estimate both the longitudinal (R1) and transversal (R2) relaxation rates, without the need to measure an adjacent reference structure that also may have some degree of gadolinium retained

and thereby a risk of confound the results. Study IV, however, did not have longitudinal data and had a purely cross-sectional design, which is less optimal, and therefore was compensated with a larger sample size. Study IV used the same MRI scanner with the same protocol in all patients, which was not possible with the longitudinal design of Study III, where both scanners and sequences had been upgraded in the hospital between the follow-up scans. Study IV also explored the repeatability of synthetic MRI within the same MRI scanner, which was shown to be robust for measurements in DN, CN and thalamus, but with larger variance in GP.

That fact that exposure to linear GBCA leads to retained gadolinium in the body and brain has been confirmed in numerous other studies.[96] Some of these studies indicate that the retained gadolinium from linear GBCAs are caused by dissociated gadolinium that binds to macromolecules in the brain, whereas macrocyclic GBCAs maintain their chelated form.[108,138] In line with the hypothesized pathological process (caused by dissociated Gd^{3+}) leading to nephrogenic systemic fibrosis - it is hypothetically more likely that the unchelated form of retained gadolinium (primarily caused by linear types of GBCA) may cause more pathological effects than macrocyclic GBCAs.[92] The results from Study IV is in line with previous studies, which show that the concentration of retained gadolinium from macrocyclic GBCAs is much less than for linear GBCAs and may therefore also be thought to be less harmful. There is, however, an uncertainty in this speculative statement as the potential toxic effect of retention by either one of the (linear and macrocyclic) GBCA types is not confirmed. The macrocyclic subgroup in Study IV was also relatively small (N=15) with few GBCA-administrations (median N=3), which may have been too few to demonstrate a possible association, although the even smaller linear group (N=11) did demonstrate an association in most brain regions.
At the moment, the most interesting and important questions regarding gadolinium retention are of how and to what degree the retained gadolinium may affect physiological functions in the tissue (especially in the brain). In both Study III and IV we looked at associations between SII/relaxation rates and neuropsychological test performance and in Study IV also global motor function and fatigue. In Study III, only a significant association between higher SII in DN and lower verbal fluency performance remained after correction for MS-related biomarkers. In Study IV, instead similar associations with lower verbal fluency remained with higher R1 in thalamus and CN (as well as R2 in CN), but not for the DN or GP. Study IV, but not Study III, showed a significant association between higher R1 in thalamus and R2 in both DN and thalamus and lower information processing speed that remained after correction for lesion volumes and disease duration. The discrepancy between the results of Study III and IV rises wariness of a possible type 1 error. Furthermore, both Study III and IV were performed in MS patients that to some degree suffer from the effects of their lesion burden and brain atrophy, leading to cognitive deficits, out of which information processing speed performance is especially affected.[23] Decline in verbal fluency performance is also confounded by MS disease, but may generally be less common in MS in comparison to information processing speed.[139] Study IV, however, showed that gadolinium retention seems to occur more or less in many parts of the brain that makes it more difficult to infer any relation to specific clinical deficit.
In summary, the statistically significant results from Study III and IV do not determine a causational relationship, but instead should encourage future studies on the subject for better confirmation or rejection of the null hypothesis. No association was found between relaxation rates and global motor function or fatigue in Study IV, which implies that future studies instead should focus on exploring potential effects in the cognitive domain.

6 CONCLUSIONS

Study I

RIS is an uncommon entity in a region with high incidence of MS. The sparsity of RIS highlights the need for large-scale and multi-center collaborative efforts to better characterize the entity. There may however be regional differences in the RIS incidence, and it may also change over time as the availability and usage of MRI increases. Updating the Okuda RIS criteria to harmonize with the (at the time of the study) concurrent McDonald 2010 MS criteria in terms of radiological classifications for DIS did not change the RIS incidence in our study.

Study II

Synthetic MRI can produce synthetic PSIR images comparable to conventional PSIR in terms of the detection rate of leukocortical lesions. Since synthetic MRI can produce PSIR on top of other spin-echo-based weightings, without extra scan time, this could be of value in the assessments of MS patients even at conventional field strengths. Furthermore, the detected leukocortical lesion volumes were associated with lower verbal fluency test scores and slower information processing in MS, suggesting that adding the assessment of leukocortical lesions into the radiological investigation practice is clinically valuable. However, combining different sequences to improve the certainty of the detection of cortical lesions was preferred by the raters, so ideally synthetic or conventional PSIR can be added as a valuable part of the "puzzle" together with other 3D MRI sequences in such a multi-parametric approach when evaluating cortical MS pathology.

Study III and IV

Gadolinium retention from linear GBCAs has a long-lasting effect on the signal intensity in the DN and GP, as well as in the CN and thalamus. Synthetic MRI can be used with good repeatability to extract R1 and R2 relaxometry values without the need for a reference region and thereby quantitatively detect changes related to gadolinium retention.

Shorter relaxation times were related to lower information processing speed and verbal fluency performance. However, conclusions regarding causality between gadolinium retention and possible cognitive effects can, however, not be made confidently as it may be impossible to fully correct for confounding factors, such as disease related cognitive deficits in MS. The results may, however, be used to inspire as well as power future studies that explore associations between GBCA-exposure and negative effects on cognition.

7 FUTURE ASPECTS

Although RIS is not classified as MS, it is important that persons with RIS are followed-up in a structured fashion, similar to how persons with Clinically Isolated Syndrome are managed. They should primarily be evaluated clinically by an MS-specialized neurologist and preferably go through a lumbar puncture to assess the presence of oligoclonal bands in the CSF. While the presence of oligoclonal bands seem to have a predictive role for the risk of converting from RIS to MS, there are also other promising biofluid markers whose role still remains to be studied. For example, neurofilament in CSF and serum, which is a marker of axonal injury,[140] is becoming more frequently used biomarker in clinical practice. Such laboratory tests and other biomarkers associated with neuroinflammatory diseases may be helpful in pointing out cases that have a subclinical form of MS.

It should also be possible to improve the clinical evaluation to better point out RIS cases that have a high risk of being constituted by subclinical MS. A recent study showed that an engineered glove was able to discriminate RIS-patients to have subtle relative finger movement dysfunction.[141] To use new methods to find subtle motor dysfunction or perhaps subclinical cognitive impairment that the patients themselves are not aware of, is an intriguing idea to use as aid in deciding which patients are of greatest need to be followed-up prospectively or in some cases directly start therapy. Furthermore, unspecific symptoms (such as headache) that traditionally have been thought not to be associated to MS, can be of value to reassess in that regard – especially if the patients fulfill the RIS criteria.[130] Due to the rareness of RIS, future epidemiological studies should include a larger study population, which preferably is achievable through multi-center collaborations. Future studies should preferably adapt to the MAGNIMS consensus recommendations for the diagnosis of RIS, to better align with the use of the latest version of DIS and DIT classifications specified in the 2017 revision of MS diagnosis. These recommendations also aim to better differentiate the RIS cases that are at risk to have a subclinical form of MS and may be of help to decide how to clinically investigate and follow-up the patients. Improved utilization of imaging techniques of cortical pathology and volumetric measurement of brain volumes and MS-lesions (and possibly also molecular and metabolic imaging by positron emission tomography)[142] will most likely play a larger role in differentiating and stratifying the patients for different treatments.

Quantitative MRI methods, such as Synthetic MRI that can synthesize several different image weightings from a single sequence, may be beneficial for the clinical diagnostics. Recently, a new MRI technology referred to as MR fingerprinting similarly provides the same type of output and post-processing capabilities, although with a completely different acquisition scheme.[143] However, the role of synthetic MRI and MR fingerprinting for MS diagnostics has not yet been fully crystalized. Future studies should further explore the possible diagnostic and prognostic advantages of synthesized images and fast volumetrics generated by these new quantitative MRI techniques. Further validation of the multi-parametric output across field strengths and vendors are also needed.

Future prospective studies aiming to investigate possible negative neurological effects of gadolinium retention should be performed in other patient groups than MS patients or patients with other neurological disease. The neurological pathologies are obvious confounders for both physical and cognitive deficits. Examples of more suitable cohorts are patients that undergo regular contrast-enhanced MRI as a surveillance for hereditary malignancies such

as breast cancer or pancreatic cancer. These patients are usually otherwise healthy and could feasibly undergo a brain MRI scan including a quantitative sequence (such as synthetic MRI) in conjunction with their routine MRI. Ideally, these patients should also undergo neurological and cognitive tests prior to entering their MRI screening program and then be followed longitudinally both neurologically, neuropsychologically and with MRI. Further investigation regarding cognitive outcomes after multiple administrations of GBCAs are very important for future guidelines of the use of GBCA.

The age with highest incidence of MS is around 30 years in Sweden,[4] and the national MRI guidelines for MS in Sweden currently recommends that GBCAs should be given at each follow-up, which at the beginning of the therapy initiation is fairly frequently. It is therefore important to conduct studies that explore both the benefits and risks, so that a well-weighted decision can be made for the patients regarding the use of GBCAs. In clinical practice, we can see that the benefits of using contrast agents is reduced when the patients are getting closer to the secondary progressive phase,[59] but the question is when and at what terms we should put the threshold to avoid the GBCAs for MRI follow-ups. Additionally, one study in RRMS patients showed that only 24% had enhancing lesions at follow-up, and that all of them also had either more or enlarged lesions in comparison with the previous MRI scan. The presence of lesion enhancement did therefore not have any significant effect on therapy choice.[119] However, such a retrospective study may not be fully generalized to other MS subgroups, and the need of GBCA administrations at follow-up MRI scans may have high clinical value only early after the diagnosis and therapy initiation and in patients that have not yet stabilized in their disease activity. Furthermore, in a master thesis from last year (2018), in our research group at Karolinska Institutet,[144] an unexpectedly low occurrence (5.9%) of GBCA enhancing MS-lesions were found during a period of three months on three MRI-scanners at Karolinska University Hospital in Huddinge. Larger studies are needed to confirm if these results reflect a general trend with lower frequency of GBCA enhancements in MRI-follow-ups of MS patients.

It is also possible that our current risk-benefit analysis for the use of GBCAs is altered when new diagnostic techniques and tools are emerging that could replace the use of GBCAs. In a longitudinal pilot study, follow-up MRI scans of glioblastoma brain tumors with quantitative T1-relaxometry, investigators were able to determine a T1-relaxation cut-off value that could predict which areas would be contrast-enhancing with a sensitivity of 86% and specificity of 80%.[145] This and other similar techniques that are able to compensate for not using GBCAs are encouraging if it means that we might be able to not use GBCAs and still preserve similar diagnostic accuracy.

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