MRI biomarkers in multiple sclerosis

Perfusion weighted imaging and restriction spectrum imaging

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Thesis submitted for the degree of Philosophiae Doctor

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Preface

“All people by nature desire knowledge.”

– Aristotle

“Everything is theoretically impossible, until it is done.”

– Robert A. Heinlein

Magnetic resonance imaging (MRI) has made impressive developments in the last years. The vast perspectives of this technique have given me motivation to perform the work presented in this dissertation.

The work was carried out at the Department of Radiology and Nuclear Medicine, Oslo University Hospital, Norway in the period from 2013 to 2016. It investigates advanced MRI techniques – perfusion weighted imaging (PWI) and restriction spectrum imaging (RSI) – in multiple sclerosis.

I hope you enjoy the reading.

Piotr Sowa

Oslo, 2nd of May 2017
Scientific environment

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I would also like to thank my family and friends for their support and encouragement during the project.

Last but not least I would like to thank all the patients who volunteered to participate in this project.
List of publications

Paper I
Piotr Sowa, Atle Bjørnerud, Gro O. Nygaard, Soheil Damangir, Gabriela Spulber, Elisabeth G. Celius, Paulina Due-Tønnessen, Hanne F. Harbo, Mona K. Beyer

Reduced perfusion in white matter lesions in multiple sclerosis.

Paper II
Piotr Sowa, Gro O. Nygaard, Atle Bjørnerud, Elisabeth G. Celius, Hanne F. Harbo, Mona K. Beyer

Magnetic resonance imaging perfusion is associated with disease severity and activity in multiple sclerosis.
Neuroradiology. Revised version submitted, April 2017

Paper III

Restriction spectrum imaging in multiple sclerosis
Manuscript ready for submission.
Abbreviations

ADC  Apparent diffusion coefficient
ARMSS Age related multiple sclerosis severity
CBF  Cerebral blood flow
CBV  Cerebral blood volume
CIS  Clinically isolated syndrome
CNS  Central nervous system
CT  Computed tomography
DIR  Double inversion recovery
DIS  Dissemination in space
DIT  Dissemination in time
DSC  Dynamic susceptibility contrast
DWI  Diffusion weighted imaging
EDSS  Expanded disability status scale
EWF  Extracellular water fraction
FA  Fractional anisotropy
FLAIR  Fluid attenuated inversion recovery
fADC  Fast apparent diffusion coefficient
FWF  Free water fraction
GCA  Global cortical atrophy
GM  Grey matter
<table>
<thead>
<tr>
<th>Term</th>
<th>Definition</th>
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<tr>
<td>MAGNIMS</td>
<td>Magnetic resonance imaging in multiple sclerosis</td>
</tr>
<tr>
<td>MRI</td>
<td>Magnetic resonance imaging</td>
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<tr>
<td>MS</td>
<td>Multiple sclerosis</td>
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<td>MSSS</td>
<td>Multiple sclerosis severity score</td>
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<td>MTT</td>
<td>Mean transit time</td>
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<td>NAWM</td>
<td>Normal appearing white matter</td>
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<td>ND</td>
<td>Neurite density</td>
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<tr>
<td>NMR</td>
<td>Nuclear magnetic resonance</td>
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<tr>
<td>nCBF</td>
<td>Normalized cerebral blood flow</td>
</tr>
<tr>
<td>nCBV</td>
<td>Normalized cerebral blood volume</td>
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<td>nMTT</td>
<td>Normalized mean transit time</td>
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<tr>
<td>PML</td>
<td>Progressive multifocal leukoencephalopathy</td>
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<tr>
<td>PPMS</td>
<td>Primary progressive multiple sclerosis</td>
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<tr>
<td>PSIR</td>
<td>Phase-sensitive inversion recovery</td>
</tr>
<tr>
<td>PWI</td>
<td>Perfusion weighted imaging</td>
</tr>
<tr>
<td>rFA</td>
<td>Restricted fractional anisotropy</td>
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<tr>
<td>RIS</td>
<td>Radiologically isolated syndrome</td>
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<td>RRMS</td>
<td>Relapsing remitting multiple sclerosis</td>
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<td>RSI</td>
<td>Restriction spectrum imaging</td>
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<tr>
<td>sADC</td>
<td>Slow apparent diffusion coefficient</td>
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<td>SPMS</td>
<td>Secondary progressive multiple sclerosis</td>
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<td>WM</td>
<td>White matter</td>
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<td>WML</td>
<td>White matter lesions</td>
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Abstract

Background

Magnetic resonance imaging (MRI) has a well-established role in diagnosis and follow-up of multiple sclerosis (MS) patients. MRI is under constant development, offering new advanced methods with promising prospects for diagnostic accuracy, establishing disease prognosis and monitoring patients with MS. Even though advanced MRI is still not recommended for routine clinical use in MS, its potential in this disease and a need for new imaging approaches are well recognized.

Objectives

The purpose of this thesis was to investigate advanced MRI techniques with focus on perfusion weighted imaging (PWI) and restriction spectrum imaging (RSI) to identify new imaging biomarkers of brain tissue damage, disease severity, disease activity and neurological disability in MS.

Materials and methods

The study was performed using two different patient cohorts.

1) MRI brain scans with a dynamic susceptibility contrast PWI sequence were performed on a 1.5 T scanner in 69 newly diagnosed MS patients. The patients underwent detailed neurological and neuropsychological examination at baseline and at approximately one-year follow-up within an ongoing neurological project “Imcogen” initiated by dr Gro Owren Nygaard. Perfusion parameters: cerebral blood flow (CBF), cerebral blood volume and mean transit time (MTT) were compared between white matter lesions (WML) and normal appearing white matter (NAWM), and their baseline normalized values were compared between subgroups of relapsing-remitting (RR) MS patients (n=66) defined according to disease severity and disease activity at one-year follow-up.
2) MRI brain scans with a RSI sequence were acquired on a 3.0 T scanner in 80 MS patients in different stages of the disease. Diffusion parameters derived from RSI: fast apparent diffusion coefficient, slow apparent diffusion coefficient (sADC), fractional anisotropy, restricted fractional anisotropy, neurite density (ND), cellularity, extracellular water fraction and free water fraction were compared between WML and NAWM, and between patient subgroups defined according to neurological disability.

**Results**

CBF was lower \( (p<0.001) \) and MTT was higher \( (p<0.001) \), consistent with reduced perfusion, in WML compared to NAWM in newly diagnosed MS patients. Baseline normalized MTT was lower in newly diagnosed RR MS patients with higher disease severity \( (p=0.016) \), patients with presence of disease activity \( (p=0.041) \) and patients with both higher disease severity and presence of disease activity \( (p=0.032) \) one year later. Of the diffusion parameters derived from the RSI sequence, the sADC in WML discriminated best between disability subgroups \( (p=0.006) \), while ND in NAWM correlated best with disability \( (\rho=-0.38, p=0.011) \).

All tested parameters derived from the RSI sequence differed significantly when obtained in WML and in NAWM \( (p<0.001 \) for all pairwise comparisons).

**Conclusions**

Both PWI and RSI showed promising results in MS and may become useful in clinical practice in the future.
1 Introduction

1.1 MRI

1.1.1 History, principles and medical applications

History

Magnetic resonance imaging (MRI) is an imaging technique based on the physical phenomenon of nuclear magnetic resonance (NMR). The phenomenon was first described by Rabi in 1938 in molecular beams (Rabi, Zacharias, Millman, & Kusch, 1938) (Nobel prize in physics in 1944) and by Bloch and Purcell in 1946 in liquids and solids (Bloch, Hansen, & Packard, 1946; Purcell, Torrey, & Pound, 1946) (shared Nobel prize in physics in 1952). These works gave a new and powerful method to chemistry where NMR found its first application – from the beginning of the 1950s it has been used for spectroscopic analysis to study the composition of complex chemical substances. A big advantage of this technique in chemistry was that the investigated substance was not destroyed or degraded during the analysis. The use of NMR in medicine dates back to 1971 when Damadian reported that tumors and normal tissue could be distinguished by NMR (Damadian, 1971). In 1973 Lauterbur published a first image based on NMR (Lauterbur, 1973), and in the late 1970s Mansfield developed mathematical methods for faster imaging that enabled MRI to be performed within minutes rather than hours and made this imaging method feasible in medicine (Mansfield, 1977) (shared Nobel prize in medicine in 2003 for Lauterbur and Mansfield). Nowadays, MRI has emerged as one of the most important methods in radiology and medical imaging along with x-ray, computed tomography and ultrasound, and it has become an especially important imaging technique in neuroradiology.

Principles

The principle of NMR is that atomic nuclei of a specimen placed in an external magnetic field and simultaneously exposed to radio waves at the nuclear resonance frequency will absorb the radio frequency energy and emit it back when the radio waves are switched off (Bloch et al.,
1946; Purcell et al., 1946). This emitted energy (or response) is dependent on the chemical composition of the specimen and on the parameters of the radio waves applied, and can be measured as a signal and presented in a form of images after computational processing (Lauterbur, 1973). The radio waves are applied as a sequence (or pulse sequence) which is a collection of wave blocks with different time intervals (Bitar et al., 2006). By changing the parameters of the sequence it is possible to receive various signal response and generate different contrasts between the tissues of a biological specimen. Thereby, different sequences can extract different information from the tissues and can address different clinical questions (Berger, 2002). The development of MRI in medicine to a large degree has consisted in creating new sequences that could provide more specific information about the tissues analyzed (Bitar et al., 2006; Dong, Andrews, Xie, & Yokoo, 2015).

Medical applications

For physical and biological reasons, MRI in medicine is based on protons (proton nuclei or hydrogenium nuclei) – protons produce good NMR signal and they are abundant in almost all body tissues (Henderson, 1983). Brain was the first anatomic structure to be studied with MRI due to its high content of water and lipids with large amount of protons and due to its static position that helped reduce motion artifacts and increase image quality (Henderson, 1983). In addition, most pathology in the brain contains more water than the surrounding healthy tissues, and it is therefore easily detectable with MRI (McKinstry, 1986). For these reasons, MRI in medicine was first used in neuroradiology but since the eighties of the 20th century it has been also used in musculoskeletal, abdominal, oncologic and pediatric imaging, supplying the clinical fields of medicine with key information for diagnosis and treatment monitoring (Berger, 2002; McKinstry, 1986). The field of MRI is under constant development. Examples of advanced MRI techniques in neuroradiology are perfusion weighted imaging (PWI) (Essig et al., 2013; Petrella & Provenzale, 2000) and restriction spectrum imaging (RSI) (White, Leergaard, D'Arceuil, Bjaalie, & Dale, 2013).
1.1.2 Perfusion weighted imaging (PWI)

Principles of PWI

Perfusion, or flow, in its most general term means a forced motion (or displacement) of molecules in gas or liquids, and it requires a pressure gradient. In the human organism, perfusion refers to the delivery of blood to tissues generated by pressure gradient from the heart beat that makes the blood circulate through the vessels of all calibers, including the capillaries (RN, 2011). The role of perfusion in living organisms is to supply the tissues with nutritional substances and with oxygen (RN, 2011). MRI perfusion weighted imaging (PWI) is based on measuring circulatory parameters in tissues at the capillary level using the phenomenon of NMR (Essig et al., 2013; Thomas, Lythgoe, Pell, Calamante, & Ordidge, 2000). Beyond MRI there are also other techniques that enable perfusion imaging, e.g. computed tomography and isotope perfusion. These techniques and MRI PWI are complementary to each other. There are three main MRI PWI techniques: dynamic contrast susceptibility (DSC) MRI known also as bolus-tracking MRI (Ostergaard, 2005), dynamic contrast-enhanced (DCE) MRI known also as “permeability MRI” (Tofts & Kermode, 1991) and arterial spin labeling (ASL) (Petersen, Zimine, Ho, & Golay, 2006). The DSC and DCE MRI require intravenous injection of a paramagnetic contrast medium while ASL is performed without use of external contrast (circulating blood itself is used as a endogenous contrast medium in this technique). The DSC and DCE methods provide more perfusion parameters than ASL. Of the MRI perfusion methods, DSC MRI is best established, most often used in neuroradiology and it is sometimes itself referred to as PWI. This method uses echoplanar acquisition technique and enables estimation of cerebral blood flow (CBF), cerebral blood volume (CBV) and mean transit time (MTT). In order to quantify these metrics, the arterial input function (AIF) has to be identified and mathematical deconvolution of the AIF and tissue response has to be applied (Petrella & Provenzale, 2000). Use of deconvolution in the processing of perfusion data ensures that the resulting perfusion metrics are less dependent on non-physiological factors like contrast agent injection rate and contrast agent dose administered (Ostergaard, 2005). Even when applying AIF deconvolution there are, however, still many confounding factors and the obtained perfusion related maps are generally semi-quantitative in nature. Figure 1. shows schematically the steps for calculation of perfusion parameters from the DSC perfusion data.
Figure 1. Principles of PWI.

DSC EPI MRI perfusion sequence is acquired after intravenous gadolinium-based contrast bolus injection. The perfusion data are processed using arterial input function, resulting in parametric perfusion maps of CBF, CBV and MTT. In our study the perfusion analysis was performed with the nordicICE software. Reprinted with permission from American Journal of Roentgenology.

CBF: cerebral blood flow; CBV: cerebral blood volume; DSC: dynamic susceptibility contrast; EPI: echoplanar imaging; MRI: magnetic resonance imaging; MTT: mean transit time; PWI: perfusion weighted imaging.

**Medical applications of PWI**

PWI is now an established method in medical imaging in certain clinical applications. Due to large intra- and inter-patient variations in perfusion measures caused by biological and technical factors (Shin et al., 2007), only normalized perfusion values can be reliably compared across the subjects, calculated as the ratio of a perfusion measure from two different brain regions in the same subject (Jafari-Khouzani et al., 2015). Such normalization has been extensively used in neuroradiology (Copen, Schaefer, & Wu, 2011; Emblem & Bjornerud, 2009; Ge et al., 2005; Law et al., 2008). Normalized CBV, as defined by the CBV
in tumor tissue divided by the CBV in extratumoral white matter, has been proven to have a prognostic value for progression and survival in patients with cerebral gliomas (Law et al., 2008). In cerebral ischemic disease the perfusion parameters are compared between different brain regions and used to identify the infarcted brain tissue and tissue at risk from the healthy tissue (Copen et al., 2011). Such normalization has also been used in MS for investigation of perfusion in lesions normalized to manually defined regions in contralateral normal appearing white matter (NAWM) (Ge et al., 2005), and for exploring the association between clinical data and perfusion in different brain regions normalized to hippocampi (Papadaki et al., 2014).

1.1.3 Restriction spectrum imaging (RSI)

Principles of RSI

Diffusion weighted imaging and diffusion tensor imaging

Restriction spectrum imaging (RSI) is a more advanced form of classic MRI diffusion weighted imaging (DWI) and diffusion tensor imaging (DTI). Contrary to perfusion, diffusion is a spontaneous motion (or displacement) of molecules and does not require any force gradient to take place. Diffusion is present in all gases and liquids in temperatures above absolute zero (-273° C). In DWI the MRI sequence is made sensitive to the random diffusion of water molecules by applying special magnetic field gradient so that the observed signal change is directly related to the average diffusion-induced molecular displacement. The MRI diffusion experiment was first performed by Stejskal and Tanner in 1965 (Stejskal & Tanner, 1965). Classic DWI provides the apparent diffusion coefficient (ADC), a global parameter that models the diffusion process in tissue by assuming the mean displacement of protons to be normally distributed (Gaussian probability function assumption). This assumption implies that the rate of diffusion is constant over time. In clinical practice, the ADC is estimated by using the diffusion-weighting factor (commonly referred to as the “b-value”), and comparing the signal at nonzero b-value with the baseline signal at a b-value of zero (i.e. measured without diffusion weighting) (Le Bihan et al., 1986). DTI is based on a tensor model and is an extension of the ADC concept to three-dimensional space, enabling measurement of ADCs along three orthogonal directions, thereby providing additional information about diffusion anisotropy which reflects the directionality of the diffusion process and which can be
quantified by parameters like the fractional anisotropy (FA) (Basser, Mattiello, & LeBihan, 1994; Le Bihan et al., 2001). The main limitation of ADC and tensor model as measures of tissue diffusion is their relatively unspecific character due to the dependence on a single Gaussian function for the displacement of molecules, while the underlying tissue microstructure may consist of multiple compartments with completely different diffusion properties (White et al., 2014).

**Restriction spectrum imaging**

Recent advances in software and hardware have enabled a greater range of b-values, diffusion directions and diffusion times to be acquired, and have led to further development of DWI techniques such as RSI. This technique acquires MRI data over an extended b-value range and thereby provides the ability to quantify complex non-Gaussian diffusion in tissue. The current clinical implementation of RSI acquires data with b-values of 500, 1,500, and 4,000 s/mm² and multiple diffusion directions at each b-value at a fixed intermediate diffusion time of approximately 90 ms (White, Leergaard, et al., 2013; White et al., 2014). As a result, the technique collects diffusion signals across a range (or “spectrum”) of length scales and different geometries in hindered and restricted water compartments providing quantitative estimates of tissue microstructure in a more detailed and specific way. Hindered diffusion refers to the delay of passage of water molecules as they navigate around cellular obstacles (as in brain extracellular space). Restricted diffusion refers to the trapping of water molecules within an enclosed compartment (i.e. within the cell membrane). In brain tissue the diffusion restriction can be spherical (limited by cell bodies) or cylindrical (limited by axons and dendrites). The sequence enables calculation of “classic” DWI and DTI parameters as well as a number of more specific parameters: restricted fractional anisotropy (rFA), neurite density (ND), cellularity, extracellular water fraction (EWF) and free water fraction (FWF). Figure 2. illustrates the water diffusing in different tissue compartments probed by RSI.
The idea of RSI is to separate various water compartments in tissue. RSI separates high mobility hindered water fraction from the low mobility restricted water fraction stemming from both spherical (cells) and cylindrical (axons and dendrites) geometries. Probing of this compartmental diffusion requires a more advanced, non-Gaussian diffusion model which is offered by RSI. Printed with permission from prof. Nathan S. White, Multimodal Imaging Laboratory, Department of Radiology, University of California San Diego Medical School, La Jolla, CA, USA.

RMS: root-mean-square; RSI: restricted spectrum imaging.

**Medical applications of RSI**

“Classic” DWI that uses a simple diffusion model providing the relatively unspecific ADC value still plays an important role in early detection of ischemic regions in stroke and can differentiate between various pathological conditions (Schaefer, Grant, & Gonzalez, 2000). DTI, due to its ability to measure orientation of the diffusion process have enabled identification of the fiber tracts and showed promising results in differentiation of pathological tissue from normal brain (Trivedi, Rathore, & Gupta, 2008). Emerging diffusion techniques such as RSI offer a new class of imaging biomarkers and open new prospects in
medical imaging. To what extent these new imaging biomarkers can help solve current clinical issues, is still an unanswered question. So far, RSI has been shown to improve tumor delineation in cerebral gliomas compared to DTI (White, McDonald, et al., 2013), used for recovering white matter tracts in regions with edema (C. R. McDonald et al., 2013) and shown to improve prostate cancer detection (McCammack et al., 2016). Recently, RSI showed reduced ND in frontotemporal WM in patients with temporal lobe epilepsy, which was more profound than changes in FA, and revealed an association between reduced ND and disease duration while FA was not associated with any clinical variables in these patients (Loi et al., 2016). RSI has not been applied in multiple sclerosis (MS) before.

1.2 Multiple sclerosis (MS)

1.2.1 Definition and historical background

Multiple sclerosis (MS) is a chronic inflammatory demyelinating disease of the central nervous system (CNS) primarily affecting young adults and often resulting in severe neurological disability and cognitive impairment (Jongen, Ter Horst, & Brands, 2012; Nicholas & Rashid, 2012). The name of the disease comes from the first descriptions of pathological changes characterized by scattered (hence multiple) hard lesions (hence sclerosis) in the CNS in patients with MS symptoms. Jean Cruveilhier and Robert Carswell are usually credited with having first described and illustrated the MS lesions in 1835 and 1838 while Jean-Martin Charcot is credited with having identified MS as a distinct disease in 1868 (Compston, 1988).

1.2.2 Etiology and epidemiology

Etiology

The cause of MS is still unknown. The leading hypothesis is that MS develops in genetically predisposed individuals exposed to common environmental factors like certain infections (especially Epstein-Barr virus), vitamin D insufficiency, smoking, antigens or salt intake (O’Gorman, Lucas, & Taylor, 2012). Genetic susceptibility studies have shown that MS is a complex, multifactorial disease, where several common genetic variants each exert a
relatively small effect. By now, more than 100 common genetic variants associated with MS have been identified (Sawcer, Franklin, & Ban, 2014).

Epidemiology

The prevalence of MS varies from high levels in North America and Europe (>100/100,000 inhabitants) to low rates in Eastern Asia and sub-Saharan Africa (2/100,000 inhabitants) (Leray, Moreau, Fromont, & Edan, 2016). Prevalence of MS in Norway is among the highest reported worldwide and is estimated as 203/100,000 (Berg-Hansen, Moen, Harbo, & Celius, 2014). The disease affects predominantly young adults, and women are affected more often than men with reported incidence gender ratio (women/men) from 2/1 to 3/1 (Leray et al., 2016). Excess mortality in patients with MS starts relatively late and there is only a moderate decrease in life expectancy in MS patients compared to general population which may be due to recent improvements in treatment and in care of the chronically disabled (Leray et al., 2015).

1.2.3 Clinical manifestations, diagnosis and treatment

Clinical manifestations

The clinical subtypes of disease course in MS were formally defined in 1996 and revised in 2014 (Lublin & Reingold, 1996; Lublin et al., 2014) as relapsing-remitting (RR), secondary progressive (SP) and primary progressive (PP) MS. Clinically isolated syndrome (CIS) is the first episode of MS-suggestive neurological symptoms lasting at least 24 hours, it can be an indicator of future conversion to clinically definite MS, and is now recognized as an element of the MS phenotype spectrum (Lublin et al., 2014). The most frequent clinical course of MS is RR MS, i.e. with relapsing and remitting neurological symptoms (named as relapses, attacks or exacerbations). The RR MS usually progresses into SP MS which is characterized by gradual accumulation of disability, without distinct relapses. The PP MS occurs in approximately 10% of patients (Ontaneda & Fox, 2015) and is characterized by gradual accumulation of disability from the onset.
**Diagnosis**

Diagnostic criteria for MS include both clinical and paraclinical assessments and emphasize the need to demonstrate dissemination in space (DIS) and time (DIT) (Polman et al., 2011; Alex Rovira et al., 2015). Although the diagnosis can be made on clinical basis alone, MRI of the CNS can support or replace some clinical criteria and has now become the most important paraclinical test in MS. The use of MRI for demonstration of dissemination of lesions in CNS in space and time was recently revised and published as “Magnetic Resonance Imaging in MS (MAGNIMS) guidelines” in 2016 (Filippi et al., 2016). Presence of neurological symptoms is essential for the diagnosis – individuals with accidentally detected MS-like lesions on MRI are referred to as having radiologically isolated syndrome (RIS) which is not classified as MS.

**Treatment**

There is still no curative treatment for MS but during the last years different disease modifying medications have been approved for RR MS that can speed recovery from attacks, slow the progression of the disease and help manage the symptoms (Torkildsen, Myhr, & Bo, 2016). The disease modifying treatment (DMT) is divided into three categories: first line (interferon, glatiramer acetate, teriflunomide, dimethylfumarate), second line (natalizumab, fingolimod, alemtuzumab) and third line (autologous haematopoietic stem cell transplantation). In Norway there are currently registered 11 different medications for MS (as per April 2017).

**1.2.4 Clinical measures in MS**

**Neurological disability**

The expanded disability status scale (EDSS) proposed by Kurtzke in 1983 as a modification of a previously used system is now an established method to quantify neurological disability in MS patients (Kurtzke, 1983). The scale is based on neurological assessment of eight functional systems (pyramidal, cerebellar, brainstem, sensory, bowel and bladder, visual, cerebral, other) and spans between zero and 10 with 0.5 unit increments that represent higher levels of disability. In the scale, zero is ascribed to normal neurological status and 10 is
ascribed to death due to MS. The EDSS scores of up to 4.5 refer to patients who are fully ambulatory, while scores of 5.0 to 9.5 refer to patients with impairment of ambulation.

**Disease severity**

Disease severity in MS is a term closely related to disease progression, and refers to the grade of neurological disability in relation to disease duration. The disease is more severe if a patient develops higher disability within shorter period of disease duration. Multiple sclerosis severity score (MSSS) was created as a method for determining progression of disability when a patient only had a single clinical assessment in the course of the disease, and can be used as a reference table for comparing groups of patients (Roxburgh et al., 2005). Newly, an alternative approach to disease severity has been proven feasible, where age was used instead of disease duration to calculate age-related multiple sclerosis severity (ARMSS) score (Manouchehrinia et al., 2017). In this approach the disease is more severe if a patient develops higher disability at lower age. Since age is typically unbiased and easily obtained, and since the ARMSS and MSSS were comparable, the ARMSS may provide a valuable tool for group comparisons with disease severity.

**Disease activity**

Disease activity in MS can be assessed using the concept of evidence of disease activity (EDA) introduced in recent years in clinical trials where the absence of EDA – no evidence of disease activity (NEDA), or disease-activity free status, was used as an outcome measure in efficacy evaluation of DMT (Bevan & Cree, 2014; Giovannoni et al., 2011; Rotstein, Healy, Malik, Chitnis, & Weiner, 2015). The EDA concept combines clinical and radiological components and is a composite measure of new relapses (i.e. new symptoms of more than 24 hours duration), disability progression (increase in EDSS score of one or more) and radiological progression (new or enlarging WM lesions). Even though the EDA concept is new, still evolving and not quite optimal due to its limitations and not including other important clinical and paraclinical parameters (e.g. neuropsychological or atrophy measurements), it has been reported as a powerful measure in prediction of long-term prognosis in MS and has emerged as a new goal in MS treatment (Rotstein et al., 2015). Currently, newer concepts of EDA are under development, where e.g. atrophy measurements (EDA-4) are added to the EDA components (Kappos et al., 2016).
1.2.5 Prognostic factors

There have been several attempts to identify prognostic factors for benign and severe disease course (G. S. Ramsaransing & De Keyser, 2007; Vukusic & Confavreux, 2007). Benign MS has usually been defined as EDSS of three or less at least 10 years from disease onset (Hawkins & McDonnell, 1999; G. Ramsaransing, Maurits, Zwanikken, & De Keyser, 2001). To date, no predictors for benign or severe MS have been validated, partly because an accurate assessment of disease severity requires observation over a long period of time. However, some studies have suggested a few possible clinical and paraclinical “candidates” for such predictors. Ramsaransing et al. reported that RR disease course, low EDSS score at baseline and low relapse rate at five years were predictors for benign MS at 10 years (G. S. Ramsaransing & De Keyser, 2007). Miller et al. reported that disability from MS was less likely in patients with optic neuritis or isolated sensory onset symptoms, low number of lesions on MRI, a long period to the first relapse, and no disability after the first five years (Miller, Barkhof, Montalban, Thompson, & Filippi, 2005). Also volumetric measures of the brain have been previously reported as predictors for disease severity in MS in some studies: whole brain and central atrophy assessed over two years were shown to predict EDSS at ten years (V. Popescu et al., 2013), baseline grey matter (GM) volume was reported to predict disease progression (Lavorgna et al., 2014) and disability accumulation (Filippi et al., 2013), and GM atrophy showed a stronger association with disease progression than white matter (WM) atrophy over 5-year and 10-year follow-up (Jacobsen et al., 2014). However, using volumetric measures as predictors has to be done with caution due to possible confounding factors and pseudoatrophy phenomenon (De Stefano & Arnold, 2015; Wattjes et al., 2015; Zivadinov et al., 2008). This phenomenon refers to a paradoxical acceleration of brain volume loss following the initiation of anti-inflammatory therapy and is generally assumed to be due to the resolution of inflammation (De Stefano & Arnold, 2015).

1.2.6 Pathological findings in MS

MS is a disease of the whole CNS and the underlying pathological mechanism in MS is damage of the myelin with subsequent axonal degeneration (Wu & Alvarez, 2011). Traditionally, demyelinating white matter lesions (WML) have been regarded as the most important pathological feature in MS, but recent pathological and imaging studies confirmed also substantial changes in GM and in normal appearing white matter (NAWM) (Wegner,
Previous histopathological studies have revealed that WML in MS are characterized by variable extent of inflammation, demyelination, gliosis and neurodegeneration that may vary in different disease subgroups (Popescu, Pirko, & Lucchinetti, 2013) while in NAWM diffuse microglia activation and parenchymal T cell infiltration were observed, especially in progressive phase of the disease (Kutzelnigg et al., 2005; Stadelmann, Wegner, & Bruck, 2011).

1.3 MRI in MS

1.3.1 Historical background

Early after first clinical installations of MRI machines it was observed that MRI is more sensitive than computed tomography (CT) in detecting brain abnormalities in MS: in 1981 Young et al. found only 19 lesions on CT but as many as further 112 lesions on MRI in a cohort of ten MS patients scanned at the same time with CT and MRI (Young et al., 1981). These signal abnormalities have been referred to as MS plaques, MS lesions or white matter lesions (WML). Moreover, the MRI lesions in MS seemed to follow a different distribution pattern than lesions in other conditions, affecting mainly periventricular and infratentorial regions (Runge et al., 1986; Young et al., 1981). In further studies it was observed that WML in MS tended to involve periventricular region, u-fibers (i.e. juxtacortical region), infratentorial structures, corpus callosum, temporal lobes and deep subcortical WM, and some lesions showed contrast enhancement. These observations suggested that MRI could be helpful in the diagnosis of MS and led to development of MRI criteria for MS in 1988 by Paty et al. (Paty et al., 1988) and Fazekas et al. (Fazekas et al., 1988), and in 1997 by Barkhof et al. (Barkhof et al., 1997). In 2001 the International Panel criteria (so called McDonald criteria) for the diagnosis of MS were published (Poser & Brinar, 2001) based on the results of previous research, introducing MRI findings as surrogates for the criterion of dissemination in time and space when clinical data are lacking. The criteria were revised in 2005 (Polman et al., 2005) and 2011 (Polman et al., 2011), and were newly confirmed and revised by the MAGNIMS group (“Magnetic resonance imaging in multiple sclerosis”) in 2015 (A. Rovira et al., 2015; Wattjes et al., 2015) and 2016 (Filippi et al., 2016). Figure 3 shows typical MRI lesions in an MS patient.
Figure 3. Lesions in MS on MRI scans.

Typical MS lesions on axial FLAIR images of the brain: left – periventricular (p), middle – juxtacortical (j), right – infratentorial (i).

FLAIR: fluid attenuated inversion recovery; MRI: magnetic resonance imaging; MS: multiple sclerosis.

1.3.2 Role of MRI in diagnosis of MS

The MRI criteria allow earlier diagnosis by replacing some clinical criteria for DIS and DIT with MRI criteria. The last MAGNIMS criteria from 2016 (Filippi et al., 2016) define five types of MS specific lesions: periventricular, cortical/juxtacortical, infratentorial, in optic nerve and intramedullary. Periventricular lesions are lesions adjacent to the lateral ventricles. Cortical/juxtacortical lesions are lesions located in the white matter next to the cortex and/or in the cortex – this terminology is a new concept that combines juxtacortical (adjacent to cortex), leukocortical (affecting both white matter and cortex) and intracortical (located purely in cortex) lesions in one type of lesions. Infratentorial lesions are lesions in the brain stem or cerebellum. Optic nerve lesions are located in the optic nerves while intramedullary lesions are lesions in the spinal cord. Figure 4 shows schematic representation of the supratentorial MS lesions.
Figure 4. Supratentorial MS lesions.

Schematic representation of the supratentorial MS lesions according to the MAGNIMS criteria from 2016. Juxtacortical (adjacent to cortex), leukocortical (affecting both white matter and cortex) and intracortical (located purely in cortex) lesions are combined in one type of lesions: cortical/juxtacortical lesions.

MS: multiple sclerosis.

According to the last MAGNIMS criteria from 2016 DIS on MRI can be demonstrated by the involvement of at least two out of five areas of the CNS as follows: a) at least three periventricular lesions; b) at least one cortical/juxtacortical lesion; c) at least one infratentorial lesion; d) at least one optic nerve lesion and e) at least one spinal cord lesion, while DIT can be demonstrated by at least one of the following: a) a new or enlarging T2 or gadolinium-enhancing lesion compared to previous scan; b) simultaneous presence of gadolinium-enhancing and nonenhancing lesion on one scan (Table 1).

Table 1. The 2016 MAGNIMS MRI criteria for DIS and DIT.

<table>
<thead>
<tr>
<th>DIS can be demonstrated by the involvement of at least 2 out of 5 areas of the CNS as follows:</th>
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<tr>
<td>• ≥ 3 periventricular lesions</td>
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<tr>
<td>• ≥ 1 cortical/juxtacortical lesion</td>
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</table>
≥ 1 infratentorial lesion

≥ 1 optic nerve lesion

≥ 1 spinal cord lesion

DIT can be demonstrated by at least one of the following:

- A new or enlarging T2 or gadolinium-enhancing lesions compared to previous scan

- Simultaneous presence of gadolinium-enhancing and nonenhancing lesion(s) on one scan.

CNS: central nervous system; DIS: dissemination in space; DIT: dissemination in time; MAGNIMS: Magnetic resonance imaging in multiple sclerosis; MRI: magnetic resonance imaging.

Another important role of MRI in diagnosis of MS is exclusion of other conditions that can mimic this disease. In the diagnostic work-up of patients with suspected MS, also MRI of spinal cord can provide important information for differential diagnosis and should be considered if the brain scans are not conclusive (A. Rovira et al., 2015). A number of other neurological disorders can simulate MS (Trojano & Paolicelli, 2001). For a systematic approach a mnemonic “VITAMIN” rule used in other diseases (Zabidi-Hussin, 2016) can also be useful in differential diagnosis of MS, pointing to common etiologies of MS mimics: vascular (cerebral autosomal dominant arteriopathy with subcortical infarctions and leukoencephalopathy – CADASIL, age-related hyperintensities, lacunar infarctions), infectious (boreliosis, progressive multifocal leukoencephalopathy (PML)), traumatic (cervical spondylotic myelopathy), autoimmune (acute disseminated encephalomyelitis – ADEM, sarcoidosis, neuromyelitis optica spectrum disorders, systemic lupus erythematosus), metabolic/toxic (osmotic demyelinating syndrome, vitamin B12 deficiency), idiopathic/genetic (leukodystrophies) and neoplastic (lymphoma, metastases).

1.3.3 Role of MRI in disease monitoring of MS

MRI monitoring of MS patients refers to three main aspects: monitoring of disease course, evaluating of response to treatment and detection of side effects (Wattjes et al., 2015). The role of MRI monitoring is to detect new or enlarged or contrast-enhancing focal lesions in order to determine MRI activity. The use of intravenous contrast in MRI monitoring of MS is not strictly obligatory, but it is recommended, since it facilitates detection of new or enlarged
lesions especially in patients with multiple lesions or advanced disease (Wattjes et al., 2015). The frequency of routine MRI scans is recommended yearly or at any unexpected clinical event. Detection of side effects is also an important role of MRI monitoring of MS patients who are on treatment. In the last years, along with introduction of new immunomodulatory medicines that are highly effective but can provoke serious complications, the MRI differentiation between early PML and new MS lesions has become especially important (Bloomgren et al., 2012). The frequency of MRI scanning should be adjusted according to the individual’s risk of PML.

1.3.4 MRI in MS research

Advanced MRI techniques, understood as any techniques other than “classic” T1, T2 and FLAIR sequences are still not recommended for routine use (Alex Rovira et al., 2015; Wattjes et al., 2015) but they are extensively applied in MS research in attempt to improve diagnosis, establishing prognosis and monitoring of MS patients. Examples of new MRI techniques that can be important for the diagnosis of MS are FLAIR*, double inversion recovery (DIR) and phase-sensitive inversion recovery (PSIR). The FLAIR* technique is a combination of overlaid FLAIR and T2* images which facilitates identification of “central vessel sign” found more frequently in MS lesions than in other type of lesions, indicating perivenular inflammation typical of MS (Kilsdonk et al., 2014). The DIR and PSIR sequences help identify GM lesions typical of MS which can improve the diagnostic accuracy (Favaretto et al., 2015). Image subtraction techniques are promising methods that can help identify new lesions, however technical challenges still remain to be solved in these methods (Moraal et al., 2010). A number of different MRI techniques are used to analyze changes in lesions, WM or GM in correlation with clinical data, in order to try to establish disease prognosis or improve monitoring of patients. Volumetric measurements are an example of such techniques; they are used in clinical studies and as end points in clinical trials, but confounding factors like pseudoatrophy still limit the use of these techniques (Wattjes et al., 2015). Other examples of advanced MRI in MS research are perfusion and diffusion techniques like DSC PWI and RSI, analyzed in this thesis.
2 Objectives

The general aim of the thesis was to investigate advanced MRI techniques in MS to identify new imaging biomarkers of brain tissue damage, disease severity, disease activity and neurological disability in the disease. The project focused on two MRI techniques: a perfusion technique: dynamic susceptibility contrast (DSC) perfusion weighted imaging (PWI) and a diffusion technique: restriction spectrum imaging (RSI). In particular, the aims of the papers included in the thesis were:

**Paper I**

To investigate DSC perfusion measures: cerebral blood flow (CBF), cerebral blood volume (CBV) and mean transit time (MTT) in white matter lesions (WML) and in normal appearing white matter (NAWM) in newly diagnosed MS patients, using semi-automated and fully automated segmentation methods.

**Paper II**

To compare baseline normalized (n) DSC perfusion measures: nCBF, nCBV and nMTT in subgroups of newly diagnosed RRMS patients defined according to disease severity and disease activity assessed one year later.

**Paper III**

To assess diffusion parameters derived from the RSI sequence: fast apparent diffusion coefficient (fADC), slow apparent diffusion coefficient (sADC), fractional anisotropy (FA), restricted fractional anisotropy (rFA), neurite density (ND), cellularity, extracellular water fraction (EWF) and free water fraction (FWF), in relation to neurological disability.
3 Summary of results

3.1 Paper I

“Reduced perfusion in white matter lesions in multiple sclerosis.”

The perfusion changes in white matter lesions (WML) in MS have in general not been well documented. In this paper, we compared the dynamic susceptibility contrast (DSC) perfusion measures: cerebral blood flow (CBF), cerebral blood volume (CBV) and mean transit time (MTT) obtained in the whole volume of white matter lesions (WML) and in normal appearing white matter (NAWM) in newly diagnosed MS patients (n=69), using semi-automated and fully automated segmentation methods of the brain tissue and lesions. In addition, the perfusion measures in contrast enhancing lesions were analyzed in relation to non-enhancing lesions and in relation to NAWM. When using semi-automated segmentation method with visual control for errors in automatic lesion detection (“gold standard” method), the CBF was lower \( p<0.001 \) and MTT was higher \( p<0.001 \) in WML compared to NAWM, while the CBV did not differ \( p=0.627 \) between WML and NAWM. This finding is consistent with reduced perfusion in WML compared to NAWM. The results remained similar using a fully automated segmentation method (with grey matter (GM) exclusion mask). There was no significant difference between perfusion measures in contrast enhancing lesions and non-enhancing lesions or NAWM, but the total number of enhancing lesions in our material (n=7) was too small to draw definite conclusions from these findings. In this paper we concluded that WML as a whole showed reduced perfusion compared to NAWM in newly diagnosed MS patients, and that fully automated segmentation methods are a promising tool in perfusion analysis of WML in MS and shows the same results as the “gold standard”.

3.2 Paper II

“Magnetic resonance imaging perfusion is associated with disease severity and activity in multiple sclerosis.”

The utility of PWI in clinical work-up of MS patients is not well investigated. This paper is based on baseline perfusion data from our previous study reported in Paper I and on clinical
data from a neurological study performed at approximately one-year follow-up (Nygaard, Celius, et al., 2015). We compared baseline normalized (n) perfusion measures: nCBF, nCBV and nMTT in subgroups of newly diagnosed relapsing remitting (RR) MS patients (n=66) defined according to disease severity and disease activity assessed one year later. The normalized perfusion measures were calculated by dividing each perfusion parameter obtained in the whole volume of WML by the same parameter obtained in NAWM. The disease severity was assessed using multiple sclerosis severity score (MSSS), and the disease activity was assessed using evidence of disease activity (EDA) approach, based on the neurological examination and MRI performed at baseline and at follow-up approximately one year later. Baseline nMTT was lower in patients with higher severity as defined by MSSS>3.79 (ANCOVA: $p=0.016$, $F(1,62)=6.12$, $\eta^2=0.09$), in patients with EDA (ANCOVA: $p=0.041$, $F(1,62)=4.35$, $\eta^2=0.07$) and in patients with both MSSS>3.79 and EDA (ANCOVA: $p=0.032$, $F(1,62)=4.81$, $\eta^2=0.07$) at one-year follow-up, controlled for disease modifying treatment (DMT). Baseline nCBF and nCBV did not differ between these groups. In addition, we also compared volumetric data between these groups and we found that the baseline whole brain volume was lower (ANCOVA: $p=0.043$, $F(1,62)=4.28$, $\eta^2=0.07$) in patients with higher severity, controlled for age and DMT, while no differences in other comparisons of the volumetric data were found. Thus, the results showed that lower baseline nMTT was associated with higher disease severity and with presence of disease activity one year later in newly diagnosed RR MS patients and suggested that nMTT can be a promising imaging biomarker in MS. The lower baseline whole brain volume was associated with higher disease severity only.

3.3 Paper III

“Restriction spectrum imaging in multiple sclerosis.”

Restriction spectrum imaging (RSI) is a relatively novel MRI sequence, and not investigated in MS before. The sequence was validated histologically and theoretically in 2013 (White, Leergaard, et al., 2013). In this paper we explored diffusion parameters derived from RSI: fast (fADC) and slow apparent diffusion coefficient (sADC), fractional anisotropy (FA), restricted fractional anisotropy (rFA), neurite density (ND), cellularity, extracellular water fraction
(EWF) and free water fraction (FWF) in relation to clinical measures, with focus on neurological disability, in MS patients \((n=80)\) in different stages of the disease. The diffusion parameters were calculated in the whole volume of WML and NAWM. The neurological disability was assessed according to expanded disability status scale (EDSS) and the patients were divided into three subgroups: with minimal, low or substantial disability (EDSS<2.5, 2.5–3 and >3, respectively). All tested diffusion parameters were significantly different when obtained in WML and in NAWM \((p<0.001\) for all pairwise comparisons). The diffusion parameter that differentiated best between disability subgroups was sADC in WML (ANOVA: \(p=0.006, F(1,77)=5.5, \eta^2=0.13\)). Compared to patients with minimal disability, patients with substantial disability had higher fADC \((p=0.009)\), sADC \((p=0.005)\) and FWF \((p=0.031)\), and lower ND \((p=0.018)\) and cellularity \((p=0.015)\) in WML. In NAWM patients with substantial disability had higher fADC \((p=0.021)\), sADC \((p=0.024)\) and FWF \((p=0.033)\), and lower FA \((p=0.027)\), rFA \((p=0.030)\) and ND \((p=0.015)\) than patients with minimal disability. The parameter that correlated best with neurological disability was ND in NAWM \((\varrho=-0.38, p=0.011)\). In conclusion, sADC in WML differentiated best between disability subgroups, while ND in NAWM showed best correlation with disability and may become useful for disease monitoring in MS patients.
4 Methodological considerations

4.1 Study design and participants

The thesis is based on two different MS patient cohorts that were studied using two MRI techniques: PWI and RSI. These two parts of the study are hereafter referred to as the \textit{PWI study} and \textit{RSI study}. The whole study is observational – no intervention was applied to the subjects, other than usual disease modifying treatment administered according to the current guidelines.

4.1.1 PWI study

In the PWI part of the study baseline imaging data and longitudinal clinical data were used. Sixty-nine patients were included in the final analysis in \textit{Paper I} and 66 patients (only RRMS patients) in \textit{Paper II} (Fig. 3). The patients were diagnosed with MS according to the 2010 McDonald Criteria (Polman et al., 2011) in the period from January 2009 to October 2012. They were identified from the hospital registry in 2011 and 2012 or reported by the treating clinicians, and recruited to this study within an ongoing neurological project in our institution (Imcogen study, dr Gro O. Nygaard (Nygaard, Walhovd, et al., 2015)). The inclusion criteria were: age 18–50 years, three years or less since MS diagnosis, at least six weeks since the last relapse or steroid treatment. Patients with prior neurological disease, head injury or substance abuse were excluded. Also pregnant or breastfeeding patients and patients with a previous adverse reaction to gadolinium injection were excluded. We also had to exclude eight patients from the original group of 77 patients due to technical reasons, as the images were not suitable for image analysis (image acquisition incomplete or of inadequate quality). All patients in the PWI study were scanned at the Rikshospitalet location of the Oslo University Hospital on a 1.5 T scanner. The clinical data in this cohort were acquired systematically and prospectively by a dedicated neurologist (dr Gro O. Nygaard) within the above mentioned ongoing neurological project (Imcogen).
4.1.2 RSI study

In the RSI part of the study 80 patients were included in the analyses. This was a separate cohort scanned at a different location (Ullevål) of Oslo University Hospital on a 3 T scanner where the novel RSI sequence was installed by our project collaborators from Multimodal Imaging Lab of the University of California San Diego. The RSI study was started in order to evaluate the usefulness of RSI sequence in MS. The patients were recruited by treating neurologists especially for this RSI project, they were in different stages of the disease, and were previously diagnosed with MS according to the current diagnostic criteria. The inclusion criteria were: age of 18 or more, no prior neurological, neurovascular or psychiatric disease, no current pregnancy or breastfeeding, no contraindication for MRI and no allergy to gadolinium-based contrast media. Patients completely restricted to bed or wheelchair and unable to move themselves onto the scanner table were excluded, since the study was performed in an ambulatory setting. Of 109 patients that met the inclusion criteria 29 were excluded, mainly due to technical reasons related to image processing (Fig. 3). The clinical data in this cohort were obtained retrospectively from the patients’ hospital record.
4.2 Clinical data

4.2.1 PWI study

In the PWI study all patients underwent a detailed neurological and neuropsychological examination at baseline within 14.2 ± 9.6 months (range 1–34) from the diagnosis. The follow-up neurological examination was repeated approximately one year later (on average 14 ± 1.7 months). The following demographical and clinical measures were obtained in all patients at baseline: age, sex, age at disease onset, time since diagnosis, disease duration, neurological disability as measured by expanded disability status scale (EDSS) (Kurtzke, 1983), number of relapses, disease modifying treatment (DMT), and multiple sclerosis severity score (MSSS) (Roxburgh et al., 2005). At follow-up the EDSS was reassessed, the MSSS was calculated, the number of new relapses was recorded and the EDA/NEDA status was determined based on the clinical and radiological progression.
4.2.2 RSI study

In the RSI study the clinical and paraclinical data were collected retrospectively from the patients’ electronic hospital record: age, sex, age at disease onset, disease duration, disease subtype, symptom type at disease onset, neurological disability assessed with EDSS, and type of disease modifying treatment. EDSS was collected for the date closest to the MRI acquisition: the median difference between EDSS date and MRI acquisition date was two months (range 0–8). A multiple sclerosis disability score (MSSS) (Roxburgh et al., 2005) was determined, and progression index (defined as EDSS divided by disease duration in years) and age-related disability (defined as EDSS divided by age in years) were calculated. There were no longitudinal clinical data in this part of the project.

4.3 Image acquisition

4.3.1 PWI study

In the PWI study all MRI scans were acquired on the same 1.5 Tesla scanner (Avanto, Siemens Medical, Erlangen, Germany) equipped with a 12-channel head coil. The imaging protocol included the following sequences in all subjects: (1) post-contrast DSC perfusion sequence (19 axial slices; FOV: 230 x 230 mm; slice thickness: 5 mm; voxel size: 1.8 x 1.8 x 5 mm; TR: 1400 ms; TE: 30 ms; flip angle: 90°); i.v. contrast agent (Dotarem, Laboratoire Guerbet, Paris, France) was administered at a dose 0.2 ml/kg and injection rate 5 ml/sec.; (2) pre-contrast sagittal 3D T1 MPRAGE (FOV: 240 x 240 mm; slice thickness: 1.2 mm; voxel size: 1.3 x 1.3 x 1.2 mm; TR: 2400 ms; TE: 3.61 ms; TI: 1000 ms; flip angle: 8°; (3) pre-contrast sagittal 3D T2 FLAIR (FOV: 260 x 260 mm; slice thickness: 1 mm; voxel size: 1 x 1 x 1 mm; TR: 6000 ms; TE: 333 ms; TI: 2200 ms; and (4) post-contrast sagittal 3D T1 MPRAGE, with parameters identical to those of pre-contrast 3D T1, acquired approximately 7 minutes after contrast agent injection following the DSC acquisition.

4.3.2 RSI study

In the RSI study all MRI scans were acquired on the same 3 Tesla scanner (Signa Optima HDxt, General Electric, Fairfield CT, USA). Seventy-two patients were scanned using an 8-
channel head coil and 10 patients using a 12-channel head coil, due to damage of the coil in the period of the study. All patients were included in final analysis since the distribution of clinical parameters was not significantly different in the two groups. The imaging protocol included the following sequences in all subjects: (1) pre-contrast axial single-shot spin-echo diffusion-weighted echo-planar multi-shell RSI sequence (TE=96-289 ms; TR=17 s; FA=90°; FOV=24 cm; matrix=96 x 96 mm; slice thickness=2.5 mm, acquired with b=0, 500, 1500 and 4000 s/mm² with 6, 6 and 15 unique gradient directions for each nonzero b-value, respectively); (2) pre-contrast sagittal 3D T1 FSPGR (TE=3-12 ms; TR=7.8 ms; TI=450 ms; FA=12°; FOV=25.6 cm; matrix=256 x 192 mm; slice thickness=1.2 mm); (3) pre-contrast sagittal 3D T2 FLAIR CUBE (TE/TR=126.5/6000 ms; TI=1861 ms; FOV=25.6 cm, matrix=256 x 256 mm, slice thickness=1 mm); and (4) post-contrast sagittal 3D T1 FSPGR, with parameters identical to those of pre-contrast 3D T1, acquired approximately 5 minutes after i.v. contrast agent injection at a dose of 0.2 ml/kg (Dotarem).

4.4 Image analysis

4.4.1 PWI study

In the PWI study the image analysis included perfusion analysis, tissue segmentation, co-registration, visual inspection, and region of interest (ROI) analysis. These steps were necessary to obtain perfusion parameters in visually affected and visually normal brain tissue for further analysis.

Perfusion analysis was applied to the imaging data from the DSC perfusion sequence, to obtain parametric perfusion maps of CBV, CBF and MTT. A population based average arterial input function was used as this has been previously shown to provide more stable inter-patient perfusion estimates (Mouridsen, Emblem, Bjørnerud, Jennings, & Sorensen, 2011). The perfusion analysis was performed using the nordicICE software package (www.nordicneurolab.com).

Tissue segmentation was performed to obtain binary masks representing WM, GM and WML. The WM and GM masks were created from the volumetric T1 series using the Matlab-based Statistical Parametric Mapping toolbox (SPM8; http://www.fil.ion.ucl.ac.uk/spm). WML segmentation was performed in collaboration with our partners from Karolinska Institute,
Stockholm, Sweden, who made available their CASCADE segmentation software (ki.se/en/nvs/cascade). The segmentation was performed with both T1 and FLAIR series as input sequences. CASCADE was also used to obtain volumetric measurements. For these measurements the software uses a "brain tissue segmentation refinement" method: first GM voxels that are bright on either FLAIR or T2 images (top 15% voxels of GM intensity histogram) are labeled as suspicious voxels, and then the suspicious voxels surrounded by mostly WM are labeled as WM and voxels surrounded by mostly GM are labeled as GM (Damangir et al., 2016). The method should be at least equally accurate as “lesion filling” approaches (Valverde, Oliver, & Lladó, 2014).

In the next step, co-registration of the structural series (T1 and FLAIR) and binary masks to the perfusion maps was performed. As a result the structural series and binary masks were aligned into the coordinate system of the perfusion maps to enable perfusion measurements in the next steps. SPM8 software was used for the co-registration.

Visual inspection was performed to check image and co-registration quality, and to control for possible errors in automatic lesion detection. The automatically generated WML mask was manually edited and modified (if necessary) in comparison of the mask with the FLAIR series. Manual editing and modification of the WML mask was performed in NordicICE software.

ROI analysis was finally performed to derive perfusion parameters (CBF, CBV and MTT) from the corresponding volumes of WML and NAWM. This step was done both using the corrected WML mask (semi-automated “gold standard” method) and non-corrected WML mask (fully automated method). In Paper I both semi-automated and fully automated methods were used for comparisons. In Paper II only perfusion data from semi-automated method were used.

**4.4.2 RSI study**

In the RSI study, image analysis included diffusion analysis, co-registration, tissue segmentation with visual inspection, and region of interest (ROI) analysis. The steps were performed to obtain diffusion parameters (derived from the RSI sequence) in visually affected and visually normal brain tissue for further analysis, with a similar concept as in the PWI study.
The RSI diffusion data were corrected offline for spatial distortions due to susceptibility and eddy currents and postprocessed in native space. FA was calculated from all b-values: $b=0$, 500, 1500 and 4000 s/mm$^2$, fADC was calculated from $b=500$ data and sADC from the $b=4000$ data. rFA was calculated from a tensor fit to the restricted water signal derived from the RSI model, with optimal sensitivity to cylindrically restricted diffusion. Also ND, cellularity, EWF and FWF were calculated. The acquisitions and the processing of the RSI data were performed in collaboration with our partners from Multimodal Imaging Laboratory at the University of California, San Diego, USA, who contributed and helped at all steps of image analysis. Details concerning RSI processing are provided elsewhere (White, Leergaard, et al., 2013; White et al., 2014).

Co-registration was performed using Matlab software (Matlab Works, Natick MA, USA). Structural scans, including T2-weighted FLAIR series and pre- and postgadolinium T1-weighted series were corrected for distortions due to gradient nonlinearities and were rigidly registered to each other. The derived RSI images were resampled and co-registered to the atlas computed from the structural series. The image data from each participant were visually inspected, resulting in exclusion of eight datasets due to insufficient registration accuracy and five datasets due to severe scanner artifacts on the RSI series.

Semi-automated WML segmentation was performed by two radiologists in all subjects using in-house software (Segmentation Editor, Multimodal Imaging Laboratory, University of California, San Diego, La Jolla CA, USA) and MIPAV software (version 7.2.0, Center for Information Technology, National Institutes of Health, Bethesda MD, USA). The 3D FLAIR series served as a basis for the WML segmentation. As a result, a WML mask representing the whole volume of all lesions was created for each patient.

Freesurfer software (www.freesurfer.net) was used to segment the 3D T1 series to obtain WM and GM masks and volumes. The masks were visually inspected and corrected for segmentation errors. The WML volume was calculated from the WML mask using NordicICE software (www.nordicneurolab.com). WM volume was calculated using a “lesion filling” approach – first the normal appearing white matter (NAWM) volume was calculated (using WM mask as inclusion mask and WML mask as exclusion mask) and then the volumes of NAWM and WML were added.
Finally, the diffusion parameters were extracted from the whole volume of WML and from the NAWM. For each patient we calculated fADC, sADC, FA, rFA, ND, cellularity, EWF and FWF mean values in WML and in NAWM. Matlab R2012a (MathWorks, Natick, MA, USA) was used for the region of interest analysis.

4.4.3 Visual assessment

The MRI images of all patients in both cohorts were visually assessed using a self-designed radiological assessment form (see below) which was partly based on the concept published recently in Swedish consensus guidelines on the use of MRI in MS (Vågberg et al., 2017). The following radiological data were noted: total number of WML (as a score), localization and distribution of lesions, changes in lesions and contrast enhancement, fulfilling of 2010 MRI McDonald criteria for dissemination in space and dissemination in time (Polman et al., 2011), radiological appearance and assessment of atrophy. The total number of lesions was graded as follows: none, fewer than 10, 10–20, and more than 20. Assessment of atrophy was performed according to global cortical atrophy (GCA) scale: 0 = none, 1 = mild (wide sulci), 2 = moderate (matter loss in gyri), 3 = severe (sharp gyri) (Pasquier et al., 1996).

Table 2. MS visual assessment form.

<table>
<thead>
<tr>
<th>MS VISUAL ASSESSMENT FORM</th>
<th>Assessed by ………………</th>
</tr>
</thead>
<tbody>
<tr>
<td>PATIENT (SCAN) ID</td>
<td>Sex</td>
</tr>
<tr>
<td></td>
<td>Born (year)</td>
</tr>
<tr>
<td>Scan date</td>
<td>Date compared with</td>
</tr>
<tr>
<td></td>
<td>Review date</td>
</tr>
<tr>
<td></td>
<td>Visit nr</td>
</tr>
</tbody>
</table>

**WHITE MATTER LESIONS**

<table>
<thead>
<tr>
<th>Total number of lesions</th>
<th>none</th>
<th>&lt;10</th>
<th>10-20</th>
<th>&gt;20</th>
</tr>
</thead>
<tbody>
<tr>
<td>- total number of lesions</td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
</tbody>
</table>

<table>
<thead>
<tr>
<th>Localization / distribution of lesions</th>
<th>No</th>
<th>Probably</th>
<th>Yes</th>
</tr>
</thead>
<tbody>
<tr>
<td>- periventricular&quot;</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>- juxtacortical&quot;</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>- subcortical&quot;</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>- infratentorial</td>
<td></td>
<td></td>
<td></td>
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</tbody>
</table>

<table>
<thead>
<tr>
<th>Changes in lesions and contrast enhancement</th>
<th>No</th>
<th>Probably</th>
<th>Yes</th>
</tr>
</thead>
<tbody>
<tr>
<td>- new lesion(s)²</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>- enhancing lesion(s)²</td>
<td></td>
<td></td>
<td></td>
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</tbody>
</table>

<table>
<thead>
<tr>
<th><strong>Fulfilling of McDonald criteria (2010)</strong></th>
<th>No</th>
<th>Probably</th>
<th>Yes</th>
</tr>
</thead>
<tbody>
<tr>
<td>- for dissemination in space</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>- for dissemination in time</td>
<td></td>
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</tbody>
</table>

<table>
<thead>
<tr>
<th><strong>Radiological appearance</strong></th>
<th>No</th>
<th>Probably</th>
<th>Yes</th>
</tr>
</thead>
<tbody>
<tr>
<td>- radiating lesion(s)</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>- lesion(s) in temporal lobe(s)</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>- involvement of corpus callosum³</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>- confluent lesions</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>- general appearance consistent with MS⁴</td>
<td></td>
<td></td>
<td></td>
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</tbody>
</table>

<table>
<thead>
<tr>
<th><strong>Global cortical atrophy</strong></th>
<th>0</th>
<th>1</th>
<th>2</th>
<th>3</th>
</tr>
</thead>
<tbody>
<tr>
<td>GCA scale⁵ (0-3)</td>
<td></td>
<td></td>
<td></td>
<td></td>
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</tbody>
</table>

**Comments / other findings:**

1. periventriculær = adjacent ventricles, juxtacortical = affecting U-fibers, subcortical = other supratentorial
2. specify where (in the “comments area”)
3. focal lesions, diffuse high T2 signal or atrophy of corpus callosum
4. subjective assessment
5. atrophy scale: 0-none, 1-mild (wide sulci), 2-moderate (matter loss in gyri), 3-severe (sharp gyri)

### 4.5 Statistical analyses

Statistical analyses were performed in SPSS Statistics for Windows v. 21 (*Paper I*) and v. 22 (*Paper II* and *Paper III*) (IBM Corp., Chicago IL, USA). In *Paper III* we used also the “R” statistical software (v3.1.1, www.r-project.org) to calculate adjusted $p$-values according to Benjamini-Hochberg false discovery rate method. The significance level was set to 0.05 for all tests.
4.5.1 Descriptive statistics and normality tests

In the descriptive statistics the data were presented as n (%) for qualitative variables, mean ± standard deviation for normally distributed quantitative variables, or median (interquartile range or range) for non-normally distributed quantitative variables. To assess the distribution of the variables the Kolmogorov-Smirnov normality test was used when the sample size was over 50 or the Shapiro-Wilk normality test when the sample size was below 50. According to the results of the normality tests, an appropriate statistical test was chosen for further analysis: parametric or non-parametric.

4.5.2 Group comparisons

In Paper 1 the differences in perfusion measures between the whole volume of WML and NAWM were analyzed using parametric paired samples t-test. Additional statistical analysis was performed in subjects with contrast enhancing lesions (n=7). In this dataset some of the variables were normally distributed and some non-normally distributed, but since significance of the results in parametric and non-parametric tests remained the same, the results were presented from parametric tests only, in the sake of simplicity and clarity.

In Paper 2 normalized perfusion measures and volumetric parameters were compared in two subgroups of patients stratified according to disease severity (as defined by MSSS) and disease activity (as defined by EDA/NEDA status). One-way between-group analysis of covariance (ANCOVA) test was used in these comparisons, with disease modifying treatment (DMT) as covariate. DMT was included as covariate to control for its possible influence on the results – as we previously have shown that the EDA patients included in this study cohort used more first line treatment and less second line treatment than NEDA patients (Nygaard, Celius, et al., 2015). Analyses of volumetric parameters were also performed with age as covariate.

In Paper 3 first a comparison of diffusion parameters was performed between the whole volume of WML and NAWM using non-parametric Wilcoxon signed rank test since the data were non-normally distributed in each pair. Then the diffusion parameters were compared between three clinical subgroups stratified according to neurological disability as defined by EDSS. For this comparison the parametric one-way analysis of variances (ANOVA) test was used (corrected for multiple comparisons with post-hoc Bonferroni test) when the data were
normally distributed; otherwise the non-parametric Kruskal-Wallis $H$ test was used (with post-hoc Mann-Whitney $U$ test and Bonferroni correction for multiple comparisons).

### 4.5.3 Correlations

Correlation analysis was used in Paper III to assess correlations between clinical measures and the diffusion parameters in WML and NAWM. The Spearman’s $\rho$ (rho) was used for assessing the correlations as the data had a non-normal distribution; otherwise the Pearson’s $r$ was used for assessing partial correlations (controlled for age). The Benjamini-Hochberg false discovery rate method was applied to control for multiple correlations, using R statistical software (v3.1.1, www.r-project.org).

### 4.6 Ethical and safety issues

#### 4.6.1 Ethical approvals

The study was performed in accordance with the ethical standards laid down in the 1964 Declaration of Helsinki with its later amendments, and was approved by the data inspectorate representative at the hospital and by the Regional Committee for Medical and Health Research Ethics for South-Eastern Norway. Written informed consent was obtained from all study participants.

#### 4.6.2 MRI scanning

The MRI acquisitions were performed according to current safety procedures at Oslo University Hospital and did not have any increased risk for the patients. Adding research sequences to the scanning protocol that prolongs the total scanning time could, however, slightly increased the discomfort for the patients.

#### 4.6.3 Contrast media

All scans within this study were performed with the use of intravenous contrast media which was in line with current recommendations and is also confirmed in newest guidelines on
monitoring of MS patients (Wattjes et al., 2015). A gadolinium-based, macrocyclic and ionic contrast medium (gadoterate meglumine) was injected in all participants, which is confirmed safe to use in patients without renal failure or known allergy (Davenport, Cohan, & Ellis, 2015).

Recently more focus is given to increased T1 signal and gadolinium concentration in brain tissue in patients with repeated contrast injections (R. J. McDonald et al., 2015), a finding confirmed also on MRI scans in our cohort (manuscript in preparation, Abstract P478, ECTRIMS 2016). Even though the clinical importance of this finding is still unknown, it has raised concerns and suggested more caution in the use of contrast media. The study participants were scanned as part of their routine clinical work-up and the MRI scans were clinically indicated. However, since patients in the PWI study were scanned and clinically followed at two different locations we could not avoid that some patients could undergo more scans than strictly necessary.
5 General discussion

This chapter summarizes and discusses the results presented in chapter 3, obtained using materials and methods as discussed in chapter 4.

5.1 Perfusion measures in WML and NAWM

The main finding of Paper I was lower CBF and higher MTT in WML compared to NAWM in patients newly diagnosed with MS, consistent with reduced WML perfusion. This finding indicates a general hemodynamic impairment of the visually affected brain tissue in MS and brings more information about vascular aspects in MS. This type of perfusion changes (lower CBF and prolonged MTT) can be theoretically explained either by dominant hypoxia-like changes or by increased stiffness in the blood vessel wall in the lesions which in theory is in line with histopathological changes reported previously in WML in MS: perivascular inflammation, gliosis and subsequent alterations in the blood vessel structure (Lassmann, 2003; Lassmann, Bruck, & Lucchinetti, 2007; Lucchinetti et al., 2000; B. F. Popescu et al., 2013). These pathological changes around and in lesional vessel walls are likely to influence hemodynamic properties of WML. Interestingly, the reduced perfusion in WML occurs early in the disease course, as all the patients were newly diagnosed.

Previous perfusion studies differ in methodology. The automated segmentation methods were not easily available for perfusion analysis before and previous reports are mainly based on perfusion in manually defined regions of the brain. However, the main result of our study is partly in accordance with a previous study of this type, where perfusion measures in manually segmented WML were analyzed in comparison to manually defined regions in the contralateral NAWM in 17 MS subjects (totally 75 lesions) (Ge et al., 2005). The authors reported lower CBF and higher MTT (in line with our findings) but lower CBV in the majority of non-enhancing lesions.

We performed a separate analysis for contrast enhancing lesions and we did not find significant difference in perfusion parameters between enhancing lesions and the non-enhancing lesions, nor between enhancing lesions and NAWM. In the latter comparison only a trend towards lower CBF in enhancing lesions was found \((p=0.069)\). However, the total
number of enhancing lesions in our material was very low (n=7) so definite conclusions concerning perfusion in enhancing lesions cannot be drawn from our study as the power of this test is too low.

Another important aspect of analyses in Paper I was the comparison of semi-automated (“gold standard”) and fully automated segmentation methods in the perfusion analysis. The semi-automated method included manual editing and visual control of the automatically generated WML mask to quality-check the fully automated method: the automatically detected lesions were visually inspected and modified (if needed), ensuring the lesion segmentation to be as correct as possible. Both methods gave similar results (lower CBF and higher MTT in WML compared to NAWM), if GM exclusion mask was used with the fully automated method, although in the fully automated method the CBV in WML showed a trend to be lower (p=0.088) in WML compared to NAWM.

The main limitations of this part of the study are inclusion of newly diagnosed patients only (so that no conclusion can be drawn on perfusion properties of WML in MS in general) and lack of control group which makes it difficult to conclude whether the revealed perfusion changes are typical of WML in MS or are a general feature of WML. The strengths are relatively large patient cohort (n=69) compared to previous perfusion studies in MS, and the novel methodological approach that we used in this study – the perfusion properties were analyzed in the whole volume of WML using semi-automated and fully automated analysis methods, not in manually defined regions of the brain. This approach should minimize user bias, and was possible to implement owing recent advances in hardware and software.

The results presented in Paper I help to understand vascular aspects of MS, suggest that reduced perfusion in lesions occurs early in the disease course, and indicate the usefulness of automated and semi-automated segmentation methods for perfusion analysis in MS.

5.2 Perfusion measures in clinical subgroups

In Paper II we looked at baseline normalized perfusion measures in clinical subgroups of MS patients defined according to disease severity and disease activity one year later. The main finding in this paper was that baseline nMTT (measured on average 14 months after
diagnosis) was lower in patients with higher disease severity and in patients with presence of disease activity at one-year follow-up.

The results suggest that the baseline microvascular properties of lesions (related to NAWM) in newly diagnosed MS patients seem to be associated with future disease severity. Lower nMTT in patients with higher disease severity can be interpreted as less reduced (or relatively increased) WML perfusion, possibly due to ongoing inflammatory activity in WML in these patients, compared to the patient group with lower severity. The nMTT differed significantly also between groups defined according to presence of disease activity: it was lower in the EDA group at one-year follow-up, and in the group defined as patients with both higher disease severity and EDA at follow-up. The fact that higher disease severity and presence of activity were both associated with lower nMTT, suggests that the biological background, at least in its vascular aspect, can be similar in both patient groups.

The observed variations in nMTT in our study were not accompanied by a corresponding variation in nCBV or nCBF which may seem surprising, given that MTT is defined as the ratio CBV/CBF (Ostergaard, 2005). This observation may be due to technical reasons: nCBV and nCBF are estimated globally from the ratio of mean values in WML and NAWM whereas MTT is estimated pixel-wise prior to normalization. Hence, nMTT may reveal variations, not reflected in the global nCBV and nCBF measures. Recently the MTT parameter has also gained more attention in vascular diseases: changes in MTT have been reported to improve cerebral infarct prediction (Carrera, Jones, Alawneh, et al., 2011) and to be useful in assessment of penumbra (Carrera, Jones, Iglesias, et al., 2011).

In our analyses the nMTT showed more significant results than atrophy measurements in all performed comparisons. The baseline total brain volume was significantly lower in patients with higher severity but no volumetric measures were different between groups defined according to disease activity in our study. The findings are partly in line with previous reports where whole brain atrophy (V. Popescu et al., 2013) and baseline GM volumes (Filippi et al., 2013; Lavorgna et al., 2014) were associated with future disability or disease progression in MS.

The use of normalized perfusion measures, not absolute values, in our analyses provides reliability for comparisons across subjects The absolute MRI DSC perfusion measures are subject to technical and physiological variations (such as selection of arterial input function,
non-linear dose response, gender and age-related variations) and are often reported in arbitrary units (Bjornerud & Emblem, 2010; van Osch, Vonken, Bakker, & Viergever, 2001). For this reason only normalized perfusion values can reliably be compared across individuals (Shin et al., 2007), calculated as the ratio of a perfusion measure from two different brain regions in the same subject. Such normalization has been extensively used in neurooncology and cerebral ischemic disease (Copen et al., 2011; Emblem & Bjornerud, 2009; Law et al., 2008), but in some studies also in MS (Ge et al., 2005; Papadaki et al., 2014).

Normalization of perfusion measures in lesions to NAWM in MS which is a disease of the whole brain may seem questionable but since the MS patients included in this study consist of newly diagnosed patients who have not yet reached the progressive phase, it can be assumed that pathological changes in NAWM would be subtle in these patients (Kutzelnigg et al., 2005). Furthermore, a newer perfusion study comparing perfusion measures in NAWM in MS patients and in healthy controls has suggested normal perfusion in NAWM in MS patients (Ingrisch et al., 2016).

The main limitations of the part of the study presented in Paper II are short observation time which was 14 months on average, and the MRI acquisition on a 1.5T scanner which gives a lower signal to noise ratio compared to 3.0T. Also, even though this is one of the largest perfusion studies in MS the number of patients (n=66) could ideally be larger to increase the power of the test, since the effect size was moderate for our findings. The main strengths are 1) use of a well-established perfusion sequence that does not prolong the scanning time substantially and does not require more intravenous contrast than the routinely used dose; 2) use of semi-automated segmentation methods that are becoming increasingly available and facilitate the analysis; 3) use of relative perfusion measures that are more reliable for comparisons across the subjects.

This study may help develop new imaging perfusion-based biomarkers to identify patients with higher disease severity and disease activity in early stage of the disease. Such biomarkers could help optimize the treatment.
5.3 RSI-derived parameters in WML and NAWM

In Paper III we analyzed eight diffusion parameters derived from RSI sequence: fADC, sADC, FA, rFA, ND, cellularity, EWF and FWF. The parameters were calculated in WML and in NAWM, which resulted totally in 16 variables – eight parameters in WML and eight parameters in NAWM. Of these eight parameters five are “novel” (rFA, ND, cellularity, EWF and FWF) as they were not possible to obtain with traditional diffusion-based MRI sequences like DWI or DTI.

All diffusion parameters investigated in the study differed significantly in pair comparisons between WML and NAWM: fADC, sADC, rFA, and FWF were higher in WML than in NAWM, while FA, ND, cellularity and EWF were lower in WML than in NAWM. These results, considering the “traditional” ADC and FA parameters, are in accordance (despite somewhat different methodology) with previous research (Droogan et al., 1999; Gratsias et al., 2015) that reported higher ADC and lower FA in WML compared to segmented NAWM regions. Since this is the first study to investigate RSI in MS, no other reports are available to compare our findings with results from others. ND was found lower and FWF was found higher in WML than in NAWM, which can be explained by reduced axonal count in WML compared to NAWM, reported in pathological studies (Schmierer et al., 2007; Stadelmann et al., 2011). The rFA values in WML were significantly higher compared to NAWM as against to FA values that were lower in WML. The histological background for rFA is not certain and we cannot anticipate the rFA values in WML. Lower rFA values in NAWM could be partly caused by the large region of interest leading to partial voluming of gray matter as well as including areas of crossing fibers in the computation. Both of these factors will reduce the mean rFA value in NAWM compared with WML.

The parameters derived from RSI provide a more specific assessment of brain tissue injury in MS and can improve our understanding of pathophysiology of the disease.

5.4 RSI-derived parameters in clinical subgroups

In Paper III the diffusion parameters derived from RSI sequence were also analyzed in relation to clinical measures, with focus on neurological disability. The main finding in this
analysis was that the sADC in WML differentiated best between patients with substantial and minimal disability, while ND in NAWM showed best correlation with disability.

Higher sADC in WML in patients with substantial disability may be due to greater exchange of intracellular and extracellular water compartments perhaps caused by more pronounced demyelination in WML in these patients. ND represents the volume fraction of cylindrically restricted water and in theory describes the density of axons and dendrites in the brain tissue. The strong negative correlation of this parameter with disability ($\rho=-0.38$, $p=0.011$) when obtained in NAWM suggests that the damage to axons in NAWM (not only in WML) plays an important role for disability.

There is limited literature available on comparisons of diffusion parameters in WML or NAWM between clinical subgroups in MS, or on correlation of these parameters with clinical data. The previous reports have been partly inconsistent. Droogan et al. in a study from 1999 reported no significant correlation between ADC values in lesions and EDSS, contrary to our findings (Droogan et al., 1999). Gratsias et al. in a newer study from 2015 reported a correlation between ADC in NAWM and EDSS (in accordance with our findings) and no correlation between FA in NAWM and EDSS (contrary to our findings) (Gratsias et al., 2015). These differences may be at least partly due to different methodology used in the studies, e.g. different patient cohorts and different definition of NAWM. Our finding of negative correlation of ND in NAWM with disability in general supports recent reports by Brownlee et al. from 2016 who found an association between disability and lower ND values in NAWM calculated with a diffusion technique named “neurite orientation dispersion and density imaging” (NODDI) in relapse-onset multiple sclerosis patients (Brownlee et al., 2016).

Whole brain volume and WM volume normalized to intracranial volume correlated negatively with disability (controlled for age) which supports previous reports. Shiee et al. in a study from 2012 (Shiee et al., 2012) reported an association between lower WM volume and higher disability in multiple sclerosis patients. WML volume showed a moderate positive correlation with disability considering both absolute values and values normalized to WM or intracranial volume; this finding is in accordance with previous publications where disability in multiple sclerosis patients was reported to correlate with absolute WML volume (Mammi et al., 1996) and WML volume normalized to WM (Nygaard, Walhovd, et al., 2015).
The main limitations of our RSI study are retrospective clinical data collection and lack of healthy controls. The strengths are the use of the novel MRI technique, a large study cohort (n=80) and a small group of experienced neurologists involved in examining the patients which should counterbalance the drawbacks of retrospective clinical data collection.

Our results show that diffusion parameters derived from RSI are promising biomarkers for neurological disability in MS patients and can help in disease monitoring. The results also indicate the importance of changes in NAWM and can increase our understanding of the mechanisms underlying the accumulation of disability in MS patients.

5.5 PWI and RSI study – similarities and differences

5.5.1 Study design

The whole study presented in this thesis is a prospective observational study. The clinical data were collected somewhat different in PWI study versus RSI study. In the PWI study (paper I and II) the clinical data were collected by a dedicated neurologist prospectively as part of a research protocol. In the RSI study (paper III) the patients were examined at a regular clinical follow-up and were scanned routinely. However, all included patients were examined by a few experienced neurologists.

We did not include a healthy control group in either study part, since the analyses were performed within the patients group, comparing parameters in different parts of the brain. It is not feasible to establish a control group for the PWI study because of the necessity of intravenous contrast injection for the PWI sequence, which is not used in healthy individuals. Establishing a control group for the RSI study is possible and can be done in future RSI projects in MS. This could explain, for instance, whether there are differences in RSI-derived parameters between NAWM in MS patients and healthy controls.

5.5.2 The use of gadolinium-based contrast

We used DSC perfusion sequence in our PWI-study since it is a well-established MRI technique providing several perfusion parameters and the expertise for processing of DSC perfusion data was available at our institution. However, this sequence requires intravenous
injection of gadolinium-based contrast medium. In the RSI-study a diffusion-based MRI technique was used, and no intravenous contrast injection was needed in this sequence.

5.5.3 Choice of software for tissue segmentation

In both PWI and RSI study we used a similar segmentation approach. The analysis was focused on WM which was segmented into WML and NAWM. The MRI parameters were calculated in the whole volume of WML and NAWM. This segmentation approach is easily reproducible and such segmentation methods are available today. The WML segmentation was semi-automated and involved a visual inspection of the masks to assure quality check. In the PWI study the CASCADE software was used for lesion segmentation and NordicIce software was used for quality check, while in the RSI study the MIPAV software was used for lesion segmentation and for quality check. For the segmentation of WM and GM the SPM8 was used in the PWI study and Freesurfer was used in the RSI study. Volumetric WM measures were obtained in a little different way: with “brain tissue segmentation refinement” approach in the PWI study and “lesion filling approach” in the RSI study. The choice of the software at different stages of the whole project depended on the availability, own experience and on the experience of the collaborating institutions.
6 Conclusions and clinical implications

In this thesis we have investigated the MRI techniques PWI and RSI in MS. The main purpose of the thesis was to analyze parameters obtained with these sequences in WML and in NAWM and to explore their relation to clinical measures.

*Paper I* revealed reduced perfusion in the whole volume of WML compared to NAWM in newly diagnosed MS patients. The results elucidated vascular aspects of WML in MS and suggested that affection of the microvasculature may be an early feature of the disease. The paper showed also similar results using fully automated and semi-automated segmentation methods indicating that fully automated methods are promising tools in perfusion analysis in MS. Since the perfusion analysis in MS has been time-consuming and operator-dependent, it is interesting that the study points to a broader use of automated segmentation methods in MS. Such methods can facilitate inclusion of larger cohorts in perfusion analysis in MS in the future.

In *Paper II* we found that in newly diagnosed MS patients baseline nMTT was associated with disease severity and disease activity one year later. This finding indicates that nMTT may be a marker that can differentiate between more severe and more benign disease course. Baseline whole matter volume was associated with disease severity only, and to a lesser degree than nMTT. The findings in *Paper II* may help find new imaging biomarkers of disease severity and disease activity in MS, and help indicate which patients are likely to develop a severe disease course in the future. This will have impact on the choice of MS treatment. However, our findings need to be validated in longitudinal studies.

*Paper III* revealed differences in diffusion parameters derived from RSI between WML and NAWM and explored their relationship with neurological disability. All tested parameters differed significantly when obtained in WML and NAWM. The parameter that correlated best with disability was ND in NAWM, and the parameter that discriminated best between disability subgroups was ADC in WML. The results can improve our understanding of pathophysiology and mechanisms underlying disability in MS and suggest that RSI-derived parameters are promising biomarkers of neurological disability. This can improve disease
monitoring of MS patients. The results also indicate the importance of changes in NAWM for neurological disability in MS.

Both PWI and RSI showed promising results in our study. The techniques address different tissue properties and are therefore complementary to each other. Our results do not allow indicating clearly which technique used in our study, PWI or RSI, is more likely to be routinely applied in MS patients in the future.
7 Future perspectives

Perfusion analysis in MS is still under-investigated and more perfusion studies are needed in the future. The assessment of the hemodynamic properties of brain tissue in MS can provide additional radiological information beyond what is possible to obtain from other techniques. The previous technical barriers in perfusion analysis are becoming less important today due to constant developments in image analysis methods and in MRI technology. DSC perfusion sequence is also attractive as its time of acquisition is short (less than two minutes). Including of this sequence in the MRI protocol does not lead to an additional burden for the patient. The use of contrast agent in this sequence will probably not be a limitation, since most of the MS patients receive intravenous contrast during the MRI scans, at least at the time of diagnosis and at the first evaluations.

Better equipment and more efficient software along with more robust and fully automated segmentation methods will hopefully also result in better availability of perfusion analysis in clinical practice in the future. Today, the perfusion analysis has become a well-established method in neuroradiology in conditions that do not require precise tissue segmentation, but it not a routinely used technique in MS.

The RSI sequence is also interesting for future research in MS. This technique requires a longer acquisition time, but no intravenous contrast injection is needed. Emerging reports since 2014 have raised new safety questions concerning gadolinium depositions in brain tissue after use of MRI contrast agents (Adin et al., 2015). This shows the need for developing new contrast-free MRI techniques like RSI.

The RSI technique, by providing more detailed diffusion parameters that characterize the brain tissue in a more specific way, offers new imaging biomarkers and this sequence is therefore highly interesting in MS. Future studies with RSI, also in healthy controls, may provide more information about the character of tissue damage in MS. Parameters not available in other MRI techniques, like cellularity, density of neurites and water fraction in brain tissue microcompartments, may become new imaging parameters in the follow-up of MS patients.
Interestingly, the parameter that correlated best with disability (i.e. ND) in our study was obtained in NAWM, but not in WML. This finding suggests that the pathological processes in NAWM in MS may be important for development of disability, and future RSI studies of NAWM can help to explain the clinico-radiological paradox (Barkhof, 2002; Zapata-Arriaza & Diaz-Sanchez, 2013) understood as a lack of strong correlation between disability and radiological findings on conventional MRI.

MRI has become an important research tool in the study of MS, in addition to its established role in diagnosis and follow-up of MS patients. Future developments in MRI techniques will provide a more specific characterization of tissue injury in MS and will helpfully contribute to a better correlation between MRI and clinical findings. This can further improve clinical management of MS patients.
8 References


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