Cortical Imaging as Seen at Ultrahigh Field MRI

Dissertation submitted for degree of Doctor of Philosophy

Submitted by

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Declaration

I, Dr Rasha Abdel-Fahim confirm that the work presented in this thesis is my own. Where information has been contributed from other sources and by colleagues, I indicate this in the References and Acknowledgements sections.

Abstract

Multiple Sclerosis (MS) has long been considered as White matter (WM) disease. The last decade, the significance of cortical lesions (CL) and their contribution to MS pathology has been intensely investigated. They have been shown to play a major role in physical and cognitive impairment in MS patients. CL detection has proven to be challenging, mainly due to poor contrast between cortical lesion and surrounding normal grey matter (GM) tissue. Various magnetic resonance imaging (MRI) sequences have been used to improve cortical lesion detection in MS patients. In recent years, Double inversion recovery (DIR), Phase sensitive inversion recovery (PSIR) and 7 Tesla T2* have been found to improve CL detection. Magnetization Transfer Imaging (MTI) has the advantage over conventional imaging as it reflects tissue myelin content. In this thesis, I present our studies using MTI at 7 Tesla to study cortical pathology in MS.

1) For a pilot study aiming to validate the use of magnetization transfer ratio (MTR) to detect cortical lesions, We examined the sensitivity of MTR to detect cortical lesions in comparison with 3 T DIR, 7 T PSIR, and 7 T T2* in 18 MS patients and 9 healthy controls.
2) A further 42 patients (11 clinically isolated syndrome (CIS), 11 relapsing remitting MS (RRMS), 10 primary progressive MS (PPMS), and 10 secondary progressive MS (SPMS)) and 8 healthy controls were scanned at baseline, 23 of these patients had a follow up scan at 12 months.

MTR at 7 Tesla has increased sensitivity to detect cortical lesions compared to 3T DIR, 7T PSIR and 7T T2*. CL myelin content as measured by the mean MTR lesional values were the lowest in SPMS patients in comparison with the rest of MS phenotypes. CL mean MTR values, more than volume was associated with the degree of physical and cognitive disability in MS patients.

When MTR was studied in a longitudinal study, we have seen more changes in average MTR of cortical lesions in SPMS and CIS patients compared to RRMS and PPMS patients.

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- Abdel-Fahim R, Mistry N, Mougin O, Blazejewska A, Pitiot A, Retkute R, Gowland P, Evangelou N. (2014). Improved detection of focal cortical lesions using 7T magnetisation transfer imaging in patients with multiple sclerosis. Mult Scler Relat Disord. 2014 Mar, 3(2):258-65.

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Abbreviations

ADC	Apparent Diffusion coefficient
AMPA	α-amino-3-hdroxy-5-methyl-4-isoxazolepropionic
BBB	Blood Brain Barrier
BICAMS	Brief International Cognitive Assessment for MS
BPF	Brain Parenchyma Fraction
BRB	Rao Brief Repeatable Battery
BVMT-R	Brief Visuospatial Memory Test-revised
CDMS	Clinically Definite MS
cGMR	Cortical Grey Matter Ribbon
CIS	Clinically Isolated Syndrome
CL	Cortical Lesion
CNR	Contrast Noise Ratio
CNP	2,3-cyclinc nucleotide 3-phosphodiesterase
CNS	Central Nervous System
CSF	Cerebrospinal Fluid
CVLT-II	California Verbal Learning Test, Second Edition
CW	Continuous Wave
DIR	Double Inversion Recovery
DIS	Dissemination in Space
DIT	Dissemination in Time
DTI	Diffusion Tensor Imaging
DWI	Diffusion-Weighted Imaging
EAE	Experimental Autoimmune Encephalomyelitis
EBV	Epstein - Barr Virus
EDSS	Expanded Disability Status Scale
FA	Fractional Anisotropy
FLAIR	Fluid Attenuated Inversion Recovery
GM	Grey Matter
GMF	Grey Matter Fraction
GML	Grey Matter Lesion
HQoL	Health Related Quality of Life
IC	Intracortical
IFN-Ɣ	Interferon-Gamma
IgGOBs	IgG Oligoclonal Bands
IQR	Interquartile Ratio
MACFIMS	Minimal Assessment of Cognitive Function in MS
MBP	Myelin Basic Protein
MD	Mean Diffusivity
MHC	Major Histocompatibility Complex
MOG	Myelin Oligodendrocyte Glycoprotein
MPRAGE	Magnetisation-Prepared-Rapid Acquisition-Gradient-Echo
MR	Magnetic Resonance
MRI	Magnetic Resonance Imaging
MTI	Magnetization Transfer Imaging
MTR	Magnetization Transfer Imaging

MXL	Mixed Lesion
NAA	N-acetyleaspartate
NABT	Normal Appearing Brain Tissue
NAGM	Normal Appearing Grey Matter
NAWM	Normal Appearing White Matter
NCV	Neocortical Volume
NGM	Normal Grey Matter
NMO	Neuromyelitis Optica
NMR	Nuclear Magnetic Resonance
NO	Nitric Oxide
NP	Neuropsychological
NWM	Normal White Matter
PASAT	Paced Auditory Serial Addition Task
PH	Peak Height
PL	Peak Location
PPMS	Primary Progressive Multiple Sclerosis
PSIR	Photosensitive Inversion Recovery
QoL	Quality of Life
RF	Radiofrequency
RRMS	Relapsing Remitting Multiple Sclerosis
SAR	Specific Absorption Rate
SDMT	Single Digit Modality Test
SNR	Signal to Noise Ratio
SP	Subpial
SPM2	Statistical Parameter Mapping 2
SPM8	Statistical Parameter Mapping 8
SPMS	Secondary Progressive Multiple Sclerosis
SRT	Selective Reminding Test
TFE	Turbo Field Echo
TNF-α	Tumour necrosis Factor-Alpha
T1w	T1-Weighted
WLG	Word List Generation Task
WM	White Matter
WMF	White Matter Fraction
WML	White Matter Lesions

Chapter 1

Introduction

1.1 Background:

Multiple sclerosis (MS) is a chronic inflammatory condition that affects the central nervous system (CNS). It was first described in 1838. It is classified into subtypes depending on the pattern of presenting symptoms. MS shows different patterns of evolution and disability accumulation across different phenotypes. The main four disease subtypes are relapsing remitting (RRMS), primary progressive (PPMS), secondary progressive (SPMS) and progressive relapsing multiple sclerosis. Lublin *et al* in 2014 recommended [1] another classification, although it is not widely used. They suggested that RRMS diagnosis does not always reflect the level of disease activity, hence recommended annual assessment of disease activity by clinical and brain imaging. According to their recommendations SPMS patients required annual clinical monitoring without brain imaging to assess disease activity. The degree of disability in MS is widely assessed using Expanded Disability Status Score (EDSS).[2] This scale was first recommended in 1983, and it assesses the degree of neurologic impairment using a combination of different scores reflecting function of different parts of the nervous system. The median age from the onset of the disease till reaching EDSS score of 4 and 6 is around 8 and 30 years respectively.[3]

1.2 Epidemiology:

MS is the second most common cause of disability in young adults in the western world.[4] Surveys have estimated the incidence of MS in the United Kingdom started since 1931, and show continuous increase in disease

frequency over time. The numbers reported from different surveys across the United Kingdom vary depending on which classification they use. The rates of MS are higher in northern United Kingdom (> $180/10^5$) compared with the south (< $160/10^5$). It is generally believed that the northeast of Scotland has the highest frequency of MS in the UK. The incidence of MS in migrants in the UK varies depending on how old they were when they moved to the UK. Individuals tend to keep the same risk of MS as their place of origin if they moved to the UK after the age of 15 years old, but acquire the UK incidence of MS if they move to the UK before the age of 15.

MS is normally more common in females than males. The reported figures vary but the ratio of females to males is usually 2-1.[4] This ratio applies to the young age of onset. MS presenting in or after the fifth decade more commonly affects males and normally is of primary progressive type.

1.3 Etiology:

MS is caused by inflammation in the CNS. The triggers of the inflammatory process causing the disease have been widely investigated. Environmental, genetic and infectious causes are possible factors that have been suggested to influence the development of disease. A genetic cause was suggested with the increase concordance in monozygotic twins compared to dizygotic twins than general population. MS susceptibility is associated with the major histocompatibility complex (MHC) region, mainly the MHC class II DRB1 gene.[6] Vitamin D deficiency is another factor suggested to contribute MS risk,[7] with disease incidence/prevalence MS significantly decreasing with increasing levels of 25-hydroxyvitamin D in whites.[8] Epstein-Barr virus (EBV) has been implicated in MS. A large study showed that all subjects who were EBV

seronegative at baseline, had seroconverted prior to MS onset, in comparison with only 35.7% of controls over the same period.[9] Smoking has also been shown to be associated with susceptibility to developing MS.[10]

1.4 Pathology:

Demyelination means loss of the myelin sheath in MS brains, and it is mainly due to a process that targets the oligodendrocytes, the cells that make and maintain the myelin sheath. This is called sometimes primary demyelination to distinguish it from secondary degeneration or Wallerian degeneration in which demyelination is secondary to axonal loss.[11]

MS is characterized by the presence of multifocal plaques. The first macroscopic demonstration of the lesions was by Carswell (1838) and Cruveilhier (1841).[12]

MS plaques evolve from acute stage in which activated mononuclear cells including lymphocytes, microglia and macrophages destroy myelin and oligodendrocytes. Within a few weeks, plaques contain a mixture of demyelinating axons and glial scar tissue. Depending on the degree of inflammation in lesions, attempts of remyelination start. If inflammation is arrested at early stage while gliosis in the plaques is not prominent, attempts of remyelination starts by remaining oligodendrocytes[13]

Initially MS was considered to be a white matter (WM) disease but with advances in immunohistochemical stains, demyelinated plaques have been observed in the grey matter (GM) as well.

Cortical lesions are difficult to visualise using conventional MR imaging. For pathological examination, immunohistochemical stains are necessary to identify cortical lesion (CL). The difference between pathology of WM and

GM lesions has been suggested to be due to the variation in the degree of inflammatory infiltration.[13]

This variation in the degree of infiltration could be due to the relatively smaller lesions size, hence the tissue needs to be removed from the CL tissue in comparison with white matter lesions (WML), hence probably less inflammation is needed to induce demyelination in the GM.[13]

Inflammation in MS is not restricted to demyelinated plaques, but extends to the regions surrounding the lesions, as well as WM and GM areas that are distant from established MS plaques.[15]

MS is known as a neuroinflammatory disease; on the other hand axonal loss is frequently observed and correlated with permanent disability. The traditional view is that inflammation is the main cause of neuronal degeneration and axonal loss.

1.5 Pathogenesis:

Inflammation and further neurodegeneration is thought to be the main driving factor in MS pathology. The primary cause of this inflammation is still unknown. Disease heterogeneity amongst individual MS patients suggests various pathogenic mechanisms across patients, but observed to be similar within the same patient.[16] These processes include

1) Demyelination which is mainly immune-mediated

2) Axonal loss which could be due to inflammation and glutamate excitotoxicity[17] as observed in active MS lesions, or due to Wallerian degeneration in remote regions that are anatomically related to MS lesions

3) Primary oligodendrocyte dystrophy induced probably by toxin induced demyelination rather than autoimmunity[16].

It is likely that interaction between these processes determines the inter individual variation among MS phenotypes and presentations.

MS is considered an immune mediated disease, involving both the cellular and the humoral parts of the immune system. It is initiated by CD4 T cell auto activation in the peripheral immune system which triggered via bacterial or viral antigens or possibly due to molecular mimicry with other pathogenic proteins.[18,19] The peripherally activated cells recognize autoantigens within the CNS parenchyma. CD4 T cells are then either polarized to Th1 with subsequent production of specific cytokines mainly interferon-gamma (IFN-Ɣ) or tumour necrosis factor-alpha (TNF-α), or polarised to Th2 with subsequent production of IL-13. It is recognised that Th1 CD4+ T cells are inflammatory while Th2 CD4+T cells are regulatory. Activated Th1 cells cause myelin disruption and the release of new potential CNS autoantigens. Moreover, the secreted proinflammatory cytokines, such as IFN-Ɣ and TNF-α recruit antimyelin antibody-forming B cells that further potentiates tissue injury.[18] The interaction between the upregulating proinflammatory cytokines and the immune response down regulating cytokines decides the severity and extent of MS lesions.[20]

IFN- Ɣ and TNF-α induce production of nitric oxide (NO), and osteopontin by macrophages, microglial cells and astrocytes. Nitric oxide synthase was found in demyelinating lesions. NO in turn is involved in oligodendroglial cells destruction by microglia. Osteopontin induces more T_H1 cytokines including

IFN- Ɣ and IL-12 and down-regulated T_H2 cytokines such as IL-10.[21] TNF is also expressed in MS lesions and it was found that down-regulating the expression of TNF and up-regulating Th2 cytokines can decrease the size of new lesions in the WM.[21]

In chronic MS, α-amino-3-hdroxy-5-methyl-4-isoxazolepropionic acid (AMPA) mediates toxicity induced by glutamate secretion. AMPA is present on oligodendroglial cells and neurons. In MS, lymphocytes, microglia and macrophages release excessive amounts of glutamate, which in turn activates AMPA receptors. This causes calcium influx through ion channels, causing necrotic damage to oligodendrocytes and axons. Blocking AMPA receptors with AMPA-kainate antagonist has been shown to ameliorate relapses in EAE,[17] it does not influence the immune response to myelin antigens but protects oligodendroglial cells and axons from immune mediated damage.[21]

A pathology study in RRMS patients showed that the largest infiltration of T cells was observed in active MS lesions indicating active inflammation, followed by less marked infiltration in slowly expanding lesions. Relatively few T cells were found in inactive lesions and NAWM, while no T cells in the cortex. The majority of the infiltrating T-cells were CD8+ (i.e cytotoxic T cells).[22] The number of B cells was 10 times lower than the T cells but B cells, HLA-D positive macrophages and microglia cells had the same pattern of distribution across tissue types.[22]

It has been suggested that T-cells have beneficial as well as destructive effects. The variation in effect was put at least partially to genetic predisposition. After optic nerve injury in Experimental Autoimmune Encephalomyelitis (EAE), it

was observed that the number of surviving neurons in the optic nerve was 60% lower in the strains that are resistant to CNS autoimmune disease than in those susceptible mainly due to lacking mature T-cells (due to thymectomy at birth),[23] which suggests that CNS injury initiates a protective response mainly T-cell dependent.[23]

1.6 Neuroinflammation versus neurodegeneration:

MS is usually considered as inflammatory disease that affects the CNS. However, axonal loss is seen in the early stages of the disease particularly in acute WM MS lesions. The frequency of transected axons correlates with the degree of inflammation within MS lesions, with more axonal loss in active lesions, while axons more preserved within hypocellular lesions.[24] The extent of axonal injury in MS patients spinal cords correlated with the degree of permanent disability.[25]

Inflammation cannot be solely responsible for axonal loss. Degeneration of axons in the absence of inflammatory cells may be due to the degeneration of chronically demyelinated axons, due to the loss of myelin trophic support, which in turn leads to progressive swelling and cytoskeletal disorganisation of chronically demyelinated axons, especially in the chronic phase of the disease. In animal models, axonal injury is detected within inactive MS lesions to a less but still significant extent in comparison with active MS plaques.[26] Moreover, a significant degree of axonal loss was observed on examining the NAWM of corpus callosum of post-mortem MS brains, which seem to be developing independently from MS lesions.[27] The significance of disease modifying treatment in the early stages of the disease and its lack of effect in the progressive stages of the disease supports the view that axonal loss cannot be

solely due to associated tissue inflammation.[28] The continuous chronic axonal loss in the cortex of progressive MS brains with no inflammatory cells infiltration[29] is further evidence supporting an independent neurodegenerative process that occurs either simultaneously with or in the absence of inflammatory drive. In the progressive stage of the disease, no correlation was detected between patients age or the disease duration and the extent of inflammatory infiltration.[22]

Acute axonal injury was found to be more frequent in active MS lesions, to less extent in slowly expanding lesions, then in the inactive lesions, NAWM and the cortex.[22] On comparing lesions of similar stages across patients of different disease phenotypes, there was no significant difference in the density of axonal density.[22] The degree of axonal injury in progressive MS patient's WM lesions correlated significantly with the degree of inflammation. However, this study did not include any patients with early or relapsing stage of the disease. A similar correlation was also detected in the NAWM tissue.[22]

There is a big degree of inter patient variability in terms of the density of inflammatory infiltrates either in the lesions or in NAWM. In a pathology study Frischer *et al* divided their sample of progressive MS patients who died in the late progressive stage of the disease into two categories, patients with pathologically active progressive disease (with classical active or slowly expanding lesions) and patients with pathologically inactive disease (showed inactive plaques only). They found that patients who belonged to the latter category were significantly older, and had longer disease duration, while the degree of their disability at the time of death was similar.[22] Interestingly, significantly higher amounts of meningeal plasma cell and B-cell densities

were observed in pathologically inactive patients in comparison with normal controls. Patients who died during the pathologically active progressive phase had more acute axonal injury than patients who died during the inactive phase of the disease. Patients who died in the pathologically inactive stage of the disease had similar density of acute axonal injury to age matched normal controls.[22] Patients who died during the inactive stage of the disease had three additional pathological features, which are thick demyelinated axons within inactive plaques, synaptic pattern of SPY immunoreactivity within lesions, and axonal or neuronal injury.[22]

It has been suggested that the ageing process may contribute its own pathological changes in the MS brain. A pathology study compared post-mortem brains of progressive MS patients who had pathologically inactive disease with and without Alzheimer's disease. Patients with associated Alzheimer's disease pathology, showed more acute axonal injury in the cortex and in the WM compared to with those who had pathologically inactive disease in the absence of Alzheimer's disease pathology. This suggests that in progressive MS, neurodegenerative changes may progress due to age related associated disease, even after cessation of MS related inflammation and axonal loss.[22]

The above studies suggest that it is difficult to explain the pathogenesis of MS due to either pure inflammatory or neurodegenerative process. It is more likely to be explained by a combination of these two processes.

1.7 Clinical Presentation:

The hallmark of MS diagnosis is dissemination in time and space.[1] The main MS phenotypes depend on their clinical course divided into RRMS, SPMS,

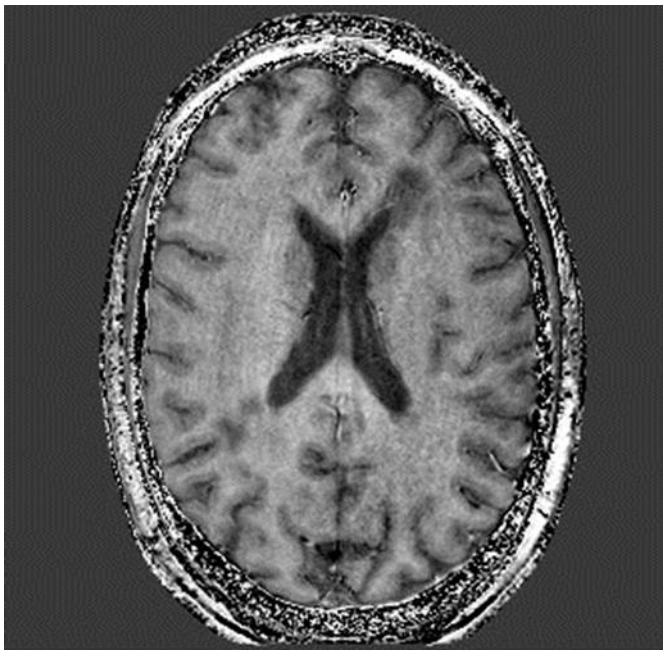
PPMS and progressive relapsing MS.[30] Benign MS is another phenotype that describes very minimal disability and lesion load during the disease course. The disease course and severity are highly variable amongst individual patients.

Around 85% of patients present with RRMS. Patients experience relapses at different frequencies and eventually enter the secondary progressive stage of the disease at variable rate. While PPMS constitutes around 15% of the patients, a percentage of those patients may later be revealed to have progressive relapsing MS when they start experiencing relapses.

1.8 Role of Imaging in MS:

Magnetic resonance imaging (MRI) has played a major role in the last 3 decades as the main tool helping understand MS, and as a surrogate biomarker in managing the disease and assessing treatment response. Initially conventional imaging focused on WM plaques.[31] The main sequences used are T1-weighted, T2-weighted and fluid attenuated inversion recovery (FLAIR). WM lesions appear hypointense on T1 images and hyperintense on T2 and FLAIR images. The diagnosis of MS is made based on dissemination of lesions in time and space. The presence of enhancing lesions, representing disease activity with break of the blood brain barrier (BBB), can be demonstrated using gadolinium chelates with T1 weighted acquisition. Diagnostic criteria based on white matter lesions (WML) on T2 weighted images have been proposed and revised over the years to facilitate diagnosis based on dissemination in time and space.[32]

(Figure 1.1) Example for MTR maps



The figure illustrates MTR maps for MS patients. The image is a result of reconstruction of M_{sat} and M_{nonsat}

1.9 Diagnosis:

The diagnosis of MS deploys the use of clinical and paraclinical assessments focusing mainly on demonstrating dissemination in time (DIT) and dissemination in space (DIS) and excluding any alternative diagnoses. Diagnostic criteria have been in development since 1965.[33] Diagnostic criteria are mainly based on clinical relapses and WM lesions on MRI. The most recent criteria were revised in 2010[32] aiming to simplify diagnostic criteria while preserving sensitivity and specificity levels.

Diagnosis of RRMS is usually suggested based on criteria as illustrated in (Table 1.1), while diagnosis of PPMS is mainly made with insidious onset of progressive neurological symptoms for at least 1 year plus 2 of 3 criteria: evidence of DIS (≥ 1 T2 lesions in periventricular, juxtacortical or infratentorial regions), evidence of DIS (≥ 2 T2 lesions in the cord), positive cerebrospinal fluid (CSF) for either oligoclonal bands or high IgG index or both.

Those diagnostic criteria were based on studies on European and North American populations, hence their applicability on other ethnic origins and paediatric populations remains under debate. Among Asian populations, neuromyelitis optica (NMO) is more frequent than MS. Sometimes the MRI criteria in patients can overlap between NMO and MS. Some MRI findings which are considered atypical for MS patients according to McDonald criteria, such as long spinal MRI lesions and cord swelling, are seen frequently in Asian patients with MS. The term opticospinal MS is used to present a mix of MS and NMO.[34]

The McDonald criteria are mainly based on detecting non cortical brain lesions and spinal cord lesions on 1.5T scanners. In the last few years, with the increase in work involving CL detection using several sequences, it was reported that the presence of at least 1 CL, using double inversion recovery (DIR), in patients with clinically isolated syndrome (CIS) can predict the development of clinically definite MS (CDMS) in those patients.[35] On the other hand CL are still not part of the McDonald diagnostic criteria.

In 85% of MS patients the onset is in the form of CIS. This is a presentation with an acute attack of neurological dysfunction including (but not limited to) optic neuritis, an isolated brain, brainstem or partial spinal cord involvement.[36]

The presence of MRI changes in CIS patients at time of presentation carries a higher risk of progression to clinically definite MS within 5 years.[37]

Typical MS plaques have been detected in autopsy of individuals who were not symptomatic, and lesions were mainly located in the periventricular regions, hence probably the silent presentation.[38]

On comparing MRI characteristics in sporadic versus familial MS, it was shown that they only show difference in the T1 lesion volume with patients with familial MS having higher T1 lesion load.[39]

Radiologically isolated syndrome (RIS) is an isolated MRI finding with no associated clinical presentation. Its actual incidence is difficult to accurately estimate as it is incidentally discovered by scanning patients due to other reasons.[40]

A cross sectional study[41] showed that WM lesions were detected in around 7% of first degree relatives of MS patients, with higher frequency in familial MS

relatives (around 10 %) than in sporadic MS relatives (around 4%). A longitudinal study was performed on 22 patients with RIS for a period of between 1 and 10 years.[41] They found that 8 patients went on to develop MS within one year; six patients were followed for 5 years without developing MS, while 3 patients were followed for 10 years without developing MS. The rest of the study subjects were followed for variable periods between 1 and 3 years without developing MS.

(Table 1.1) Revised 2010 McDonald diagnostic criteria for MS

Clinical Presentation		***Additional criteria for establishing MS diagnosis***
Clinical attacks	**Objective evidence**	
≥ 2 Attacks	≥ 2 WM Lesions Or 1 Lesion and history of prior attack	None
≥ 2 Attacks	1 Lesion	***DIS*** demonstrated by: ≥ 1 T2 lesion in at least 2 regions (periventricular, juxtacortical, infratentorial or spinal cord) **Or** Await another attack affecting a different CNS site
1 Attack	≥ 2 Lesions	***DIT*** demonstrated by: Presence of Gd enhancing and non-Gd enhancing lesions at the same time **Or** new T2 or Gd enhancing lesion on follow up scan in comparison with baseline scan
1 Attack	1 Lesion	***DIS*** as well as ***DIT*** (as mentioned above)

(DIS = dissemination in space, **DIT**= dissemination in time, **WM**= white matter,

CNS= central nervous system)

1.10 Anatomy of the cerebral cortex:

The volume of the adult human cerebral cortex varies between 197 and 331 cm^3 in females and between 242 and 358cm^3 in males. Cortical volume of the right hemisphere is frequently larger than the left which is mainly due to the frontal lobe being usually slightly wider in the right than the left hemisphere. There are 2 main types of cortices;[42]

1- Neocortex (Isocortex) which constitutes most of the cortex, is mainly found in the cerebral hemispheres, contains between 10 and 14 billion neurons, and is formed mainly of 6 layers numbered with Roman numerals from superficial to deep. The depth of Isocortex varies depending on the cortical area but generally is between 1.5 and 5.0 mm. Similarly cortical thickness vary, but mean neocortical thickness is 2.72± 0.24 mm in male and 2.61±0.21 mm in female brains.[44]

2- The Archicortex (Allocortex) is mainly found in the limbic system cortex and contains on average 3 layers. The Allocortex can be further divided into paleocortex (olfactory bulb, piriform regions and part of the amygdala) and Archicortex (Hippocampus, cortical part of cingulate gyrus).

The 6 layers constituting the cortical cytoarchitecture[45] are:

1) Molecular layer (Plexiform layer) (I) which is the outermost layer and contains dense network of tangentially oriented nerve fibers which are mainly derived from the apical dendrites of the pyramidal cells. This layer of the cortex is where large numbers of synapses between neurons take place.

2) External granular layer (II) which is a dense layer that is composed of small pyramidal and stellate cells. Dendrites of these cells terminate in the molecular layer, while the axons pass on to the WM.

3) External pyramidal layer (III) contains pyramidal cells, with their dendrites passing into the molecular layer while the axons enter the WM.

4) Internal granular layer (IV) contains mainly stellate cells. Fibers in this layer are horizontally arranged and known as the external band of Baillarger

5) Ganglionic layer (Internal pyramidal layer) (V) which contains pyramidal cells. There are a large number of horizontally arranged fibers that form the inner band of Baillarger. In the motor cortex of the precentral gyrus, the pyramidal cells of this layer are very large and known as Betz cells and account for around 3% of the corticospinal tract projection fibers.

6) Multiform layer (Layer of polymorphic cells) (VI) which is mainly composed of fusiform cells with many nerve fibers either entering or leaving the underlying WM.[45]

Not all areas of the cortex typically composed of 6 layers. The agranular part of the cortex contains poorly developed granular layers 2 and 4, hence the pyramidal cells in layers 3 and 5 are densely packed, and those are present in the precentral gyrus, and give efferent fibers associated with motor function. The granular part of the cortex has well developed granular layers 2 and 4 and these cells receive thalamocortical fibers and found mainly in the postcentral gyrus.

In terms of functional organization, the cerebral cortex is organized into vertical units measuring about 300 to 600 μm wide.

Brain tissue is composed of the neurons and the neuroglia.[46] Neurons vary according to the number, length, and the mode of branching of their neurites into unipolar, bipolar and multipolar neurons. Neuroglia are smaller than neurons and 5-10 times more prevalent. The four main types of neuroglial cells are astrocytes, oligodendrocytes, microglia and ependymal cells. Astrocytes serve as "electrical insulators", preventing axon terminals from influencing nearby neurons, as well as preventing spread of neurotransmitter substances released at synapses, they also serve as phagocytes for degenerating neurons, proliferate after the death of neurons to fill in the gaps (gliosis), secrete cytokines that regulate the immune cells entering the nervous system, and also play a significant role in the structure of the BBB. Oligodendrocytes are responsible for the formation of the myelin sheath. Microglia are found scattered throughout the CNS. They are normally inactive in the normal brain but in MS they become the immune effector cells and they migrate to the site of the lesion. At the inflammation site, they proliferate and become antigen presenting cells which together with the invading T lymphocytes react to the disease. They also work as phagocytic cells.

The cerebral cortex constitutes mainly of 3 main types of neurons arranged in layers. These cells are pyramidal cells, stellate neurons and fusiform neurons. In a smaller number two types of cells are also present which are horizontal cells of Cajal and cells of Martinotti. The apices of the pyramidal cells are oriented toward the pial surface of the cortex. An apical dendrite extends from the apex of each cell to the pia, while dendrites from the basal angles pass

laterally to synapse with surrounding axons. The axons terminate either in the deeper cortical layers or in the underlying WM. Stellate cells have short axons and usually terminate on a nearby neuron. The fusiform cells are located mainly in the deepest layers of the cortex and they have their long axis vertical to the surface. Their axons pass on to the underlying WM while their dendrites ascend toward the surface of the cortex. The horizontal cells of Cajal are small horizontally oriented cells located in the most superficial layers of the cortex, and their axons pass parallel to the surface of the cortex, making contact with the dendrites of the pyramidal cells. The cells of Martinotti are small cells present throughout all levels of the cortex. Their axons are directed toward the pial surface of the cortex and end usually in the most superficial layer of the cortex.[44]

The nerve fibers of the cerebral cortex are arranged either radially or tangentially. The radial fibers run at right angles to the cerebral cortex and they are either leaving the cortex (e.g axons from pyramidal, stellate and fusiform cells) to the WM tracts, or afferent fibers terminating into the cortex. The tangential fibers run parallel to the cortical surface and mostly located in layers IV and V, where they are referred to as the inner and outer bands of Baillarger. They are mostly collateral and terminal branches of afferent fibers. The bands of Baillarger are well developed in the sensory areas due to the high concentration of the thalamocortical fibers endings, as well as in the visual cortex.[44]

1.11 Role of non-conventional imaging in MS:

1.11.1 Diffusion weighted imaging:

Diffusion – weighted imaging (DWI) is considered a quantitative technique that has advantage over the conventional MRI sequences in that it provides information for the microscopic changes in the normal appearing brain tissue. Initially spin echo T2-weighted sequence was used for acquisition, but such sequence was very sensitive to motion producing lots of artifacts. Hence this was substituted with echo-planar spin-echo T2-weighted sequence. Other techniques are also being used such as single-shot gradient and spin-echo or single-shot fast spin-echo techniques. Diffusion is defined as the microscopic random motion of molecules in fluid. In the brain tissue this random movement is influenced by the microstructural changes in abnormal brain tissue. This causes the diffusion coefficient in brain tissue to be lower than the diffusion coefficient in free water. Only the apparent diffusion coefficient can be measured, as the motion of the molecules cannot be differentiated if it is due to concentration gradient or any other cause such as pressure gradients or thermal gradients. There is no correction for increase in distance travelled due to tortuous routes, hence only apparent diffusion coefficient (ADC) can be calculated. Measuring the diffusion depends on the direction of the diffusion of molecules. Hence measuring diffusion can provide information about tissue integrity. The apparent diffusion in the brain is anisotropic. Usually ADC values of grey and WM are similar, so there is no contrast between the grey and WM on an ADC map, and the contrast between these two types of tissue is seen on the DW images due to the T2-weighted contrast.[47] Diffusion tensor imaging (DTI) is one of the methods developed from DWI to provide more detailed description of water molecules motion and microstructural isotropic

and anisotropic tissues[48]. This particularly measures the magnitude, or mean diffusivity (MD) and the direction (fractional anisotropy (FA)) of motion of water molecules with FA values ranging from 0 (no directional dependence for diffusion) and 1 (diffusion along a certain direction). FA value usually increase with motion of molecules in a specific direction (anisotropy). Usually in GM, diffusion is isotropic whereas in WM it is anisotropic due to directionality of myelin fiber tracts.

Diffusion studies have been used to investigate MS brains for decades. MS lesions have been shown to have higher ADC and reduced anisotropy in comparison with NAWM. Gad- enhancing lesions and T1 hypointense lesions particularly have the highest ADC values. The different lesions ADC values do not differentiate between different MS phenotypes.[50]

Diffusivity was also found to be higher in lesions with lower mean magnetization transfer ratio (MTR) values.[51] Diffusivity is higher in NAWM and normal appearing grey matter (NAGM) in comparison with controls.[52] Areas with high diffusivity were correlated with lower MTR peak histograms.[53] A reduction in FA was noted in NAWM regions with well-formed tracts.[54] Reduced diffusion anisotropy could result from either axonal damage or replacement of axonal fibers with glial cells.[55] An increase in ADC in areas of NAWM preceded the development of lesions. There was simultaneous but milder increase in ADC in NAWM regions contralateral to the developed lesions suggesting structural damage to remote NAWM regions that are connected to lesions.[56] Postmortem studies in MS brains suggested that FA and MD are affected mainly by myelin content and to a lesser extent by axonal count.[57] As for cortical lesions, 9 patients and 9 healthy controls were

recruited in a study where DIR, PSIR and DTI were acquired.[58] They found that mean FA values of cortical lesions were significantly higher in comparison with cortical GM in healthy controls.[58] The increase in the FA values was suggested to be due to the increased activation of microglia with extended processes that ensheath neuronal cell bodies and neurites, and contribute to increased local organization of the cortical microstructure.[58]

Although FA is commonly used in the literature, there are concerns that it might not be specific to demyelination. However, comparing diffusion parallel to the principal axis of diffusion to diffusion perpendicular to this axis has proven helpful to differentiate demyelination to axonal loss..[59]

1.11.2 Magnetisation Transfer Imaging:

Although conventional magnetic resonance imaging remains the gold standard for the diagnosis of demyelinating diseases, it lacks pathological specificity in detecting the changes in histopathological findings in MS, which range from oedema and inflammation to severe demyelination, axonal loss, gliosis and remyelination.

Magnetization transfer (MT) scans can be interpreted in various ways. MTR values could be presented as normalised mean values for a region of interest or as histograms. Histograms of MTR values could be calculated for a whole brain (excluding the very low values as a measure of excluding the non-brain tissue such as skull and orbital tissue) or for a region of interest such as MS plaques or a selected region of normal appearing tissue. For each histogram the peak height (PH) and location (PL) could be presented, which represents the most common MTR values of the brain, and the MTR_{25}, MTR_{50}, MTR_{75}, which are the MTR values corresponding to the 25^{th}, 50^{th} and 75^{th} percentiles of the

histogram, and this indicates the MTR at which the integral of the histogram is 25%, 50% and 75% of the total respectively.

Using MT acquisitions in MS patients, brain tissue shows different features depending on its pathologic characteristics. Lesions with no MT, which usually appear hyperintense on T2W and hypointense on T1W sequences, represent almost complete tissue damage resulting in open fluid-filled spaces.

Studies in animal models showed that MTR decreased by 5%-8% in oedematous tissue in comparison with the same region before the onset of any pathology, while MTR values in demyelinated regions was as low as 26%.[60] This was consistent with Kimura *et al* findings on correlating MTR and spectroscopy, where they showed that the moderate reduction in MTR values in MS plaques correlate with the reduced N- acetylaspartate (NAA)/creatine (Cr) ratio, which is mainly caused by myelin degradation, which suggesting mainly axonal loss in those plaques.[61] NAA levels could also be influenced by metabolic dysfunction or changes in water content. Strong correlation was found between the tissue myelin content and MTR values in post mortem brains.[62]

Magnetization transfer imaging (MTI) offers a good potential, through its semi quantitative analysis in distinguishing different pathologies that involves the WM and look similarly on conventional MR imaging.[63] Hence MT imaging superiority over conventional MR sequences, which lack pathological specificity in distinguishing disease characteristics.

MTR values for GM are generally higher than WM, with values of between 23% (0.23) and 24% (0.24) in the GM, and between 30% (0.3) and 33% (0.33)

in the WM.[64] The variation in MTR values is also dependant on location. Gass *et al* found MTR values of around 30% (0.3) in the occipital lobe, 31.8%(0.31) in the frontal lobes and 33% (0.33) in the genu of corpus callosum.[64]

The mean MTR values of WM MS lesions are lower than values in WM lesions in ischemic patients.[64] Mean MTR values of MS plaques are higher in benign MS patients than in SPMS patients.[64] MTR values of NAWM were found to be higher with increase distance from WM lesions.[65]

On comparing the MTR values of MS lesions at various stages of the disease according to their appearances on T1 sequence, it was noted that MTR values were lower in hypointense lesions than for isointense lesions. The MTR values for MS lesions varied with the change in their intensity on conventional MR sequences.[66] It was also reported that very low MTR values in MS lesions at initial enhancement was predictive of a persistent T1-weighted hypointensity and lower MTR values 6 months later.[66] In post-mortem brains hypointense T1 lesions showed lower MTR values and reduced myelin content in comparison with isointense lesions while no significant difference in axonal count and gliosis was noted.[62]

Filippi et al noted that mean lesion MTR, MTR PH and PL were lower in SPMS patients than in other MS phenotypes. CIS, benign MS patients and controls had the same MTR parameters.[67]

MTR values are known to be affected by several factors. Oedema and inflammation are known to cause mild reduction in MTR values, while demyelination and axonal loss cause a more severe reduction.[68,69] MTR values below the mid-20% range is highly suggestive of demyelination.[69] MTR values

were found to be higher in ischemic WM lesions (mean 34%) than in demyelinating MS plaques (mean 22.5%) and in oedema (mean 30.2%).[69]

In a longitudinal study GM PH MTR was found to be lower on comparing SPMS patients with RRMS, RRMS with CIS and MS patients versus controls.[70] On the other hand as quantitative MRI methods are not made for quantifying the extent of GM pathology at individual level, a very interesting study used individual GM MTR mapping to assess the degree of GM pathology in individual MS patients and its distribution. They noticed that 51% of their patients showed decrease in GM MTR in comparison to controls, and GM injury was very variable across patients from early stages of the disease.[71]

MT imaging has several advantages over conventional MR sequences as it provides an opportunity to better understand MS pathology as it is more specific than the water dominated signals of conventional T-2 weighted MRI sequences.[72] MT also provides better understanding of the invisible disease burden in the so called normal appearing brain tissue, as those regions which appear normal on conventional scans were found to have lesions on acquiring a high resolution sequences.[65,73]Derakhshan et al[74] used two-dimensional parametric surface models of the cortex to examine the spatial extension of subpial reduction in MTR values in MS patients in the cortical GM. They found MTR was abnormal mainly in the cingulate cortex, insula and in the depth of the sulci which is consistent with the known pathological topographic subpial lesions distribution. Moreover the distribution of the subpial located abnormal MTR values was more prevalent in the SPMS patients than in the RRMS patients.[74] CIS patients showed abnormally low GM MTR in at least

one brain region, most commonly in the temporal cortex, then frontal cortex and limbic cortex, while the parietal and occipital cortex were the least frequently involved.[75] On examining the cerebral cortex of CIS patients who presented with optic neuritis, a significant reduction of GMMTR was noted bilaterally in the visual cortex. Interestingly patients who did not have any T2 WM lesions did not show reduction in visual cortex MTR values in comparison with those who had T2 lesions.[76] Clinical correlations at base line such as visual acuity and EDSS were significant with visual cortex MTR but not with T2 lesion load.[76]

The major advantages of MT imaging and the MTR are that they are relatively easy to implement as MT is available from all major manufacturers. They also can be applied to both spin-echo and gradient echo sequences and are easy to post process. MT is a relatively high signal to noise ratio (SNR) technique and can be performed in clinically acceptable times.[59]

Physics of Magnetization transfer imaging:

MT was first discovered accidentally by Wolff and Balaban in 1989.[77] They were trying to perform a spin transfer experiment by selective saturation of urea looking for small signal suppression in water. Instead, they found a significant loss of image intensity from tissue which was explained by exchange of magnetization.[78]

MT is the nuclear magnetic resonance (NMR) phenomenon in which spinning of different materials causes exchange of their magnetization. The hydrogen nuclei (protons) of water are the main source of signal in conventional MRI as they have a high MR sensitivity, they are prevalent, and have sufficiently long relaxation times to allow easy detection. Hence image contrast is dependent on

the water content (proton density), characteristic spin-lattice (T1) and spin-spin (T2) relaxation times, and imaging sequence parameters. Proton MRI detects signal only from mobile protons which have T2 relaxation times greater than 10 ms, so that spatial encoding gradients can be played out between excitation and acquisition before the signal has completely decayed. The T2 relaxation times of macromolecules are less than 1 ms and hence are too short to be detected directly in MRI, resulting in lack of visibility on MRI, hence they are MRI invisible. However, coupling between the maromolecular protons and the mobile (liquid) protons allow the spin state of the immobile protons to influence the spin state of the liquid protons through exchange processes.[78] The macromolecular spins have a much broader absorption lineshape than the liquid spins, hence they are around 10^6 times more sensitive to an appropriately placed off-resonance irradiation.[78] So it is possible to selectively saturate the macromolecular spins using an off-resonance radio frequency pulse.[78] This selective saturation can be transferred to the liquid spins, with a variable rate of exchange depending on the nature of tissue, then the signal can be detected with MRI.[78] (Figure 1.1) The effect of the off- resonance irradiation on this system is different for the two pools. For example for pool B, the protons in the macromolecules are strongly coupled to each other resulting in a homogeneously broadened absorption lineshape. In MT imaging, the intent is to manipulate the liquid pool indirectly by saturating the macromolecular pool. When an on-resonance excitation pulse is applied after an off-resonance pulse, the produced MR signal will be lower than without that prepulse.

The exchange that transfers macromolecular saturation to the liquid pool, results in decreased longitudinal magnetization being available for imaging.[78]

Hence the greater the exchange rate, the higher the MTR. In tissues with no semi-solid component, such as CSF, the water magnetization is unaffected. So MT imaging provides an additional form of contrast that is a function of the relative semi-solid/water pool size and the strength of interaction between them.[72]

Off-resonance irradiation in MRI can be applied in a continuous wave (CW) mode or in a pulsed mode. In MT imaging CW is the first used pulse mode as it provides the cleanest separation between the amounts of saturation in the two pools. However as a result of their long duration, they are considered time consuming and result in high-energy deposition. The radio frequency energy from an imaging sequence can cause heating of the tissues of the body. Exposure to RF energy should be limited. The specific absorption rate (SAR) is the limiting measure. The recommended SAR varies in different parts of the body. For the head it must be less than 3.2w/kg. So, any pulse sequence must not rise the temperature by more than 1°C. The pulsed MT technique, involves selective saturation of the immobile pool using either brief off-resonance RF pulses (at a frequency that is off-set from the free water resonance) or on-resonance (near the free water resonance). The on-resonance pulsed saturation technique employs low flip angle saturation pulses to achieve selective saturation of the macromolecular pool. MTR maps are calculated via the equation $MTR = (M_{nonsat} - M_{sat}) / M_{nonsat} \times 100$. M_{nonsat} provides the intensity of a voxels without applying the saturation pulse while M_{sat} provides the intensity of the same voxel after applying the saturation pulse. MS lesions appear hypointense on the MTR maps and they have lower MTR values than the surrounding normal appearing tissue.[60]

(Figure1.2) Layers of the cerebral cortex

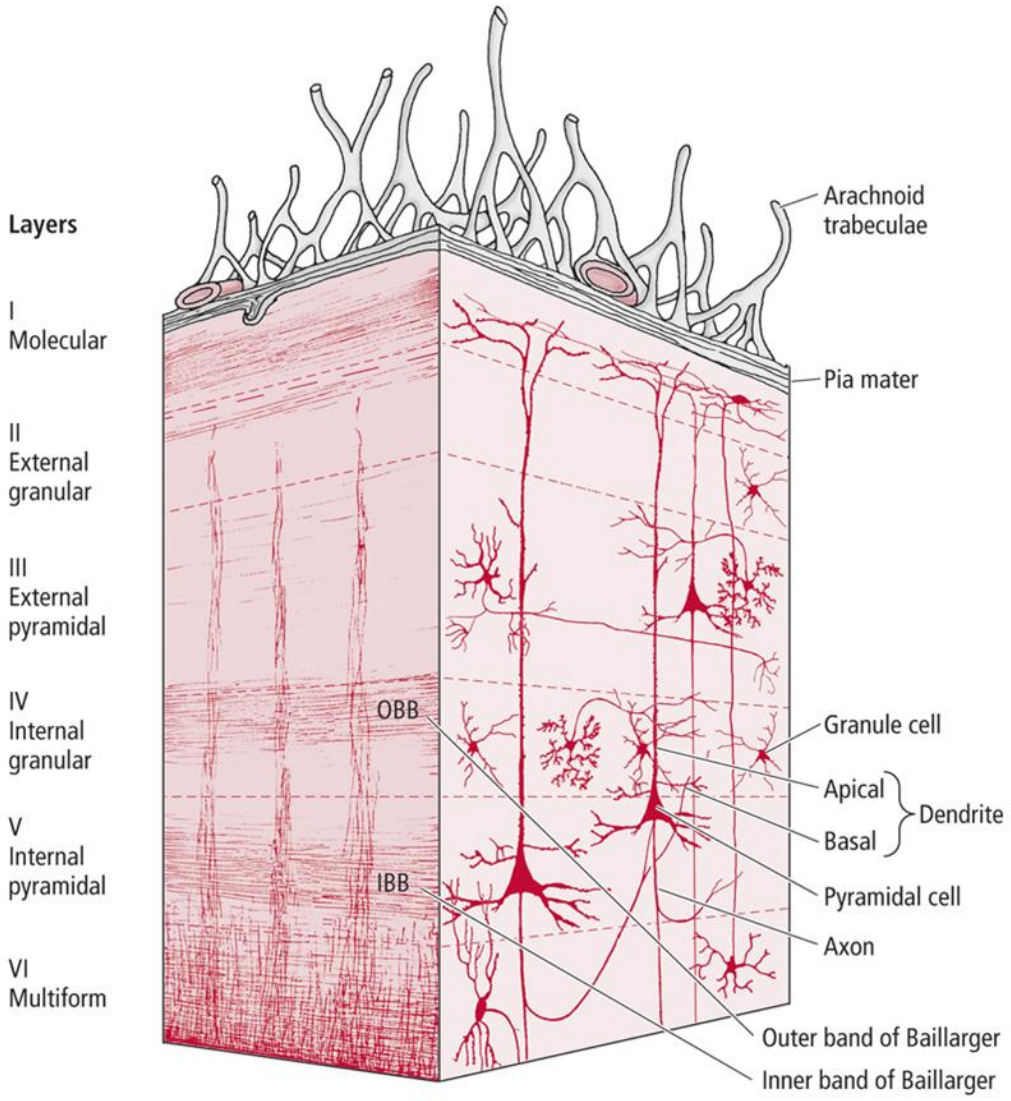
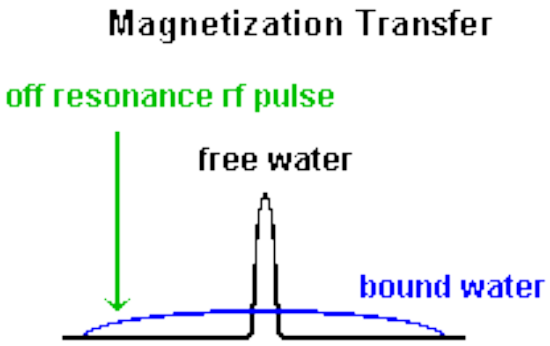


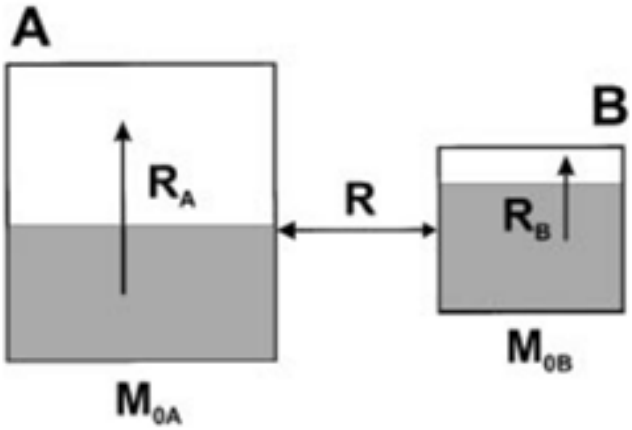
Diagram illustrates layers of the cerebral cortex (I to VI)

(Figure1.3) Variation in absorption lineshape causing macromolecular selective saturation



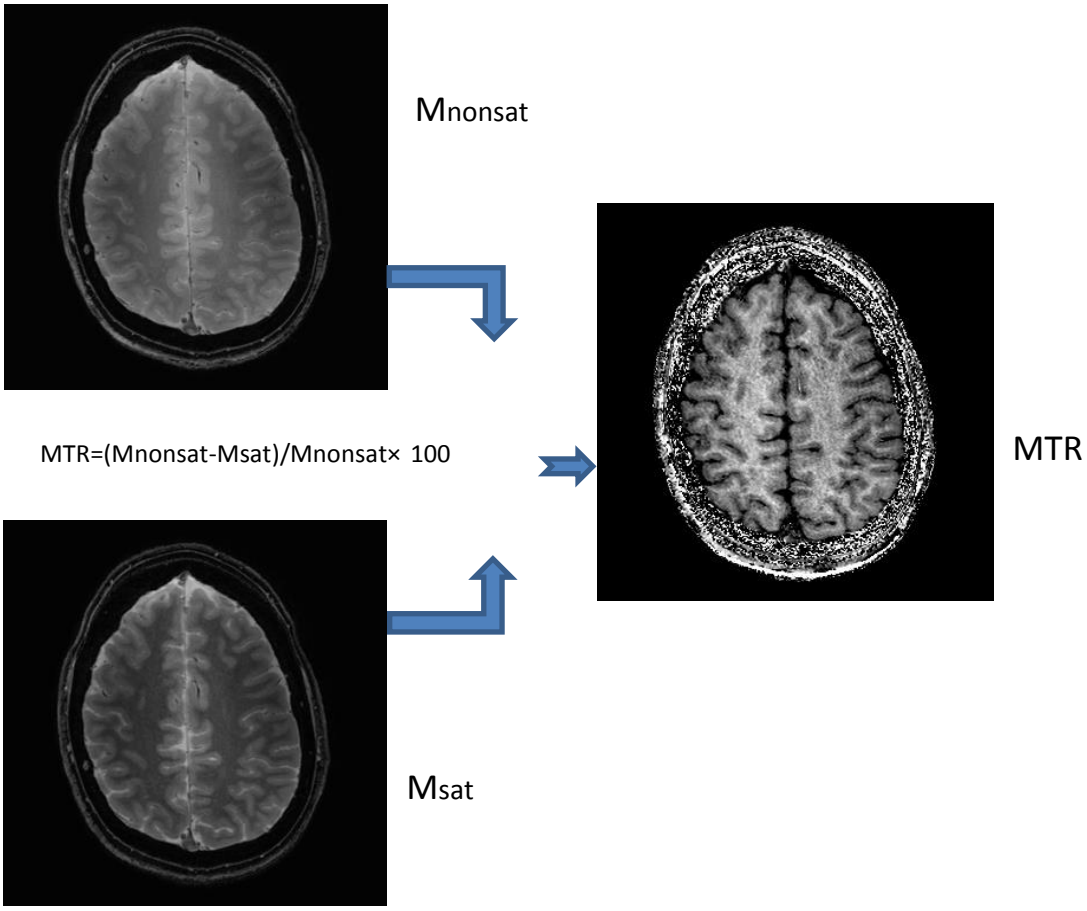
This figure illustrated the macromolecular spins having broader absorption lineshape than the liquid protons, and can selectively be saturated using a specific off-resonance RF pulse.

(Figure1.4) Model of MTR two pool exchange



This figure illustrates a two pool model of magnetization transfer exchange. Pool A represents the liquid pool, while pool B represents the macromolecular pool. The shaded area in each pool represents saturated spins, while unshaded area is the spins in the longitudinal orientation. R_A and R_B represent the longitudinal relaxation rates. R is the magnetization transfer exchange rate between the two pools.[78] The number of spins in each pool is represented by M_{0A} and M_{0B}. The number of macromolecular spins is much less than the liquid spins.

(Figure1.5) MTR formulation from MT_{sat} and MT_{nonsat}



This figure illustrates the formation of MTR image from Msat and Mnonsat images

1.12 Cortical Grey Matter as focus of interest:

Originally MS was thought to be a disease of the WM; however the GM involvement was noted over a hundred years ago in early pathological studies. In some MS patients, the pathological process starts in the cortex months and sometimes years before involving the WM.[79] The advances in immunohistochemical staining techniques sensitive to myelin has advanced our understanding of the contribution of GM to the disease process.[15] Recently the contribution of cortical injury to the diagnosis and prognosis of MS has become more widely appreciated.[80] CL were found to be more prevalent in males and more associated with the presence of oligoclonal bands (IgGOBs) in the CSF, with patients who had IgGOBs having twice as many CL than those without IgGOBs .[81] Interestingly the number of WML did not vary across patients with the presence or absence of IgGOBs in CSF.[81] CIS patients with CL and intrathecal synthesis of Ig had higher risk of conversion to MS, moreover RRMS patients with CLs and intrathecal Ig synthesis showed more disease activity.[82]

CL load was found to correlate with physical disability and cognitive impairment, and better explain them than WM lesion load.[83,84] Cortical involvement and its correlation with cognition was demonstrated in early stages of the disease, as CIS patients with cognitive impairment showed increased cortical recruitment and connectivity patterns when compared with CIS cognitively preserved patients or healthy controls.[85] Fillipi et al suggested that the presence of at least one intracortical lesion (IC) at the baseline DIR scans of CIS patients has a high accuracy for identifying patients at risk of developing clinically definite MS.[86] MRI studies showed that CL and cortical

atrophy are present in early stages of the disease,[81] and become more prominent during the progressive phase.[83] The rate of CL accumulation was found to be similar in RRMS and SPMS forms of the disease.[87] RRMS patients with epilepsy were found to have more severe and rapidly accumulating CL as well as cortical atrophy in comparison with RRMS patients without epilepsy.[88] The pathological changes in the cortex are reported from very early stages of the disease. Patients with higher CL load were found to have higher WM lesion load, and smaller brain parenchymal fraction in comparison with patients without cortical lesions.[81] Longitudinal studies showed that CL volume at baseline correlated with baseline disability, and the rate of CL accumulation was higher in those patients who showed worsening disability than those who were clinically stable.[87]

Buownell and Hughes reported in 1960 that 26% of MS lesions affect the GM.[89] Cortical demyelination was found to be slightly more extensive in the frontal and temporal lobes (17% and 19% respectively) compared with the parietal and occipital lobes (12% and 8% respectively), while the insular cortex was found to be the mostly demyelinated with an average of 26%.[90]

CL could vary in size between a few millimetres and 1 cm. The correlation between WM demyelination and cortical pathology are conflicting. Some studies[15] suggest very small correlation between the WM inflammation and microglial activation with the degree of cortical demyelination, while others suggest that GM demyelination is an independent process from WM pathology.[91] Studies in EAE showed that cortical inflammation is an early transient phenomenon in cortical demyelination.[92] Diffuse meningeal

inflammation was strongly associated with cortical demyelination, whereas focal perivascular meningeal inflammation was less strongly associated with cortical lesions. Topographically moderate to marked meningeal inflammation was associated with subpial plaques, while focal perivascular meningeal inflammation was more associated with intracortical or leukocortical plaques.[93] (Figure 1.5)

1.12.1 Role of imaging in cortical lesions:

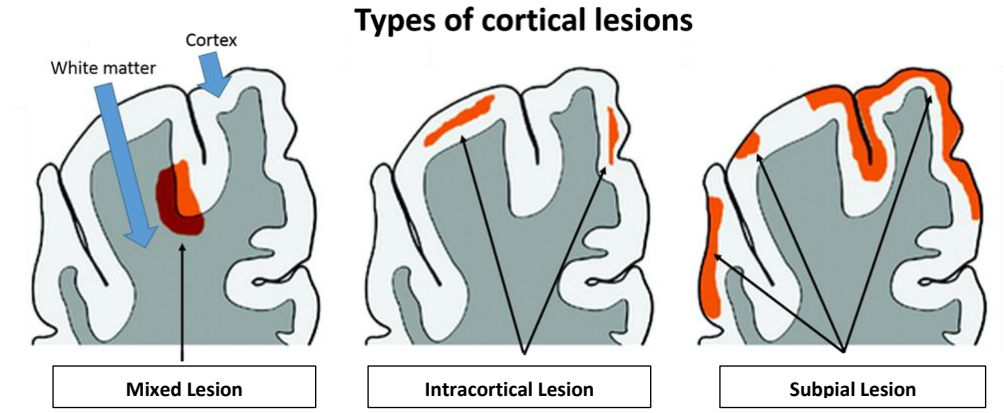
The above mentioned conventional imaging techniques are not optimal for cortical lesion detection, partially as GM lesions have longer relaxation times than normal WM which causes poor contrast between GM and lesions in the GM in comparison with WM lesions,[94] but also because partial volume effect caused by the CSF surrounding the cortical GM plays a significant role as well.[94] Another theory is that the high cellular density in the CL does not permit a sufficient expansion of the extracellular space to allow an increase in relaxation times in CL, as is seen in WML.[94] Over the last few years, a few imaging sequences have been developed to improve CL detection. DIR is an MR sequence which involves suppression of the signals from both the WM and CSF, giving better delineation of the GM. This contrast is mainly due to the differences in T1 relaxation times between GM and CSF, as well as between GM and WM.[95] Using 3D DIR showed an average increase of IC lesions detection by 152% per patient on comparison with 3D FLAIR,[96] and was useful in differentiating MS from other similar presentations such as NMO.[97] On the other hand combined 3D DIR and FLAIR still failed to detect most of CL that were observed in histopathology.[98] Moreover 3D DIR failed to detect subpial lesions (SP) and was difficult to differentiate between mixed

juxtacortical (MXL) or IC due to the difficulty in delineation of the cortex, and WM borders. It was also shown to have flow artifacts mainly in the occipital lobe.[81] In 2011, recommendations were published to improve CL detection using DIR. On using the proposed recommendations, full agreement was reached by all teams only on 19 % of lesions.[99] 3D-FLAIR at 7 Tesla was found to detect more CL than 3D-DIR, mainly mixed lesions.[100] On comparing 7 Tesla 3D-MP-FLAIR and 3D DIR with the same sequences at 3T, it was found that 7T sequences showed higher number of lesions, with better classification, however 7T DIR images showed poorly attenuated peri-vascular spaces in the WM which can be mistaken for lesions.[101] PSIR is a T1 sequence that showed superior GM-WM contrast with clear lesion delineation at shorter scan time in comparison with FLAIR.[102] It provides higher signal intensity than conventional T1-weighted inversion recovery sequences by adding combined negative and positive longitudinal magnetization in the image. Not only the number of CL identified on PSIR was higher than 3D DIR but it was able to classify lesions more accurately.[103] On comparing 3T DIR, 3T FLAIR and 7T MPRAGE, it was observed that each imaging sequence was sensitive to different lesions in the region of the cortex, on the other hand, high resolution scan (7T MPRAGE) had clearly improved the classification of CL and better classified artifacts.[104] 3D-FLAIR, 3D T1-weighted and 2D T2-weighted at 7Tesla were found superior to the same sequences at 3 Tesla in CL detection and classification.[105]

Currently available MRI sequences are not sensitive to depicting all CL subtypes especially SP. Hence any firm conclusions regarding the association between the degree of cognitive impairment or physical disability and the

extent of cortical demyelination are difficult to draw giving that most the disease burden is invisible.

(Figure 1.6) Cortical Lesions Classification



The figure illustrated different types of cortical lesions: to the left mixed lesions with the lesion mainly in the cortex with partial extension into the white matter. In the middle intracortical lesions which are usually small lesions extending into the middle layers of the cortex. To the right the subpial lesions which usually extend to involve the whole cortex from the pia to the white matter junction without extending into the white matter[106]

Chapter 2

Detection of Focal Cortical Lesions Using 7T Magnetisation Transfer Imaging In Patients with Multiple Sclerosis

2.1 Abstract

Background:

Cortical lesions account for a larger proportion of brain demyelination than WM lesions. Recent studies reported improved cortical lesions detection using DIR, T2*, MPRAGE and PSIR.

Hypothesis:

Our hypothesis is that high resolution MTI at 7 T performed within reasonable scanning time is able to detect cortical lesions in MS patients in comparison with other currently used conventional MRI sequences.

Methods:

Eighteen patients with MS and nine healthy controls were scanned using 7T MTR maps, 7T MPRAGE, 7T T2* and 3T 3D DIR.

Results:

We detected 365, 289 and 231 lesions with 7T MTR, 7T MPRAGE and 7T T2* respectively in the MS group. Few cortical abnormalities were detected in the controls. In 8 MS patients who had 3T DIR on the same day, a total of 136 lesions were detected as opposed to 171, 147 and 126 lesions with 7T MTR, 7T MPRAGE and 7T T2* respectively .

Conclusion:

We found that 7T MTR, in less than 10 mins scanning time, was very sensitive in detecting cortical lesions in comparison with the other sequences.

As MTR is more pathologically specific than other sequences in detecting tissue myelination, it raises the possibility that high resolution MTR will be able to demonstrate cortical remyelination in vivo.

2.2 Introduction

2.2.1 Pathology of cortical lesions:

A previous report suggested that inflammation of CL is less than that of white matter lesions,[107] and lesions tended to arise in the deepest point of the sulci. In another study WM lesions contained 6 times more CD68-positive cells and 13 times more CK3-positive lymphocytes than cortical lesions.[108] Parts of the leukocortical lesions that appeared within the WM were no different to other white matter lesions. It was also observed that most of the cortical lesions arise within the territory of the principal vein V_5, hence cortical lesions were classified into 7 subtypes depending on the cortical layer and venous territory they involve. The size of cortical lesions may vary depending on the size of the cortical vein involved, for example large type 7 lesions which might involve a whole gyrus are likely to reflect involvement of the central vein of the gyrus while smaller intracortical lesions (type 3 or 6), may arise as a result of involvement of small branch veins. [94]

A simpler classification has been proposed by Peterson et al. They classified cortical lesions identified in several postmortem studies depending on their location in the cortex.[109,108] The first type is leukocortical lesions (type I), which extends from the cortex to the WM and usually accounts for about 34% of lesions. The second type is the intracortical type (type II) which is small confined to the cortical ribbon and forms around 16% of lesions. The third and fourth types are called subpial (type III &IV), they involve different layers of the cortex or sometimes the entire cortex without invading the subcortical WM tissue and they form around 50% of cortical lesions.

Although the previous classification is the most commonly used nowadays, it excludes some types of cortical lesions. Other research groups consider cortical lesions that are affecting all 6 cortical layers with only marginal involvement of the WM as subpial, rather than leukocortical lesions.[110] The classification of cortical lesion that involves the entire thickness of the cortex as well as a considerable region of the sub adjacent white matter is even more challenging, as few possibilities are possible: it could be a subpial lesion that extends into the WM; a white matter lesion that extends into the cortex until it reaches the pia; or finally it could be merging of a subpial lesion with a leukocortical lesion.[111]

Cortical lesions can be classified according their stage of inflammation and the density and distribution of MHC class II positive cells into active, chronic active, and chronic inactive lesions.[108] Active lesions have a distinct border of MHC class II positive cells and also high density at the cores, while chronic active lesions had MHC class II positive cells at the borders not in their cores. Chronic inactive cortical lesions had no clear borders but their cores had similar positive cells density similar to this of normal appearing cortex. Inflammation level was similar in all types of cortical lesions. Compared to active WM lesions, active cortical lesions contained 4 times fewer CD68-positive cells at their borders and in their cores, while chronic active cortical lesions had 10 and 13 times fewer CD3-positive cells at their border and core respectively compared to chronic active white matter lesions.

On comparing different types of cortical lesions, it was found that intracortical lesions were the least inflammatory and lacked the lymphocytic and macrophagocytic inflammatory infiltrates and complement deposition, while

leukocortical lesions were more inflammatory than subpial and intracortical lesions.[107]

There is a growing interest in type III subpial lesions. It has been noticed that the depth of the demyelinated area is constant, and a subpial lesion can extend over multiple gyri. It is believed that demyelination or oligodendrocyte death in subpial lesions may be associated with elements that spread from the surface of the brain, hence the mechanisms of demyelination in subpial lesions may be different from those in intracortical and mixed lesions.[108]

Popescu *et al* reported the presence of a type of inflammatory subpial cortical lesion associated with MRI gadolinium contrast enhancement that preceded the white matter lesions dissemination which suggests that inflammation – induced cortical blood-brain barrier damage and demyelination may be an early pathogenic event in MS. [110]

2.2.2 Contribution of MRI to cortical lesion detection:

MRI plays a major role in cortical lesion detection in MS patients. GM lesions are difficult to detect on conventional MRI scans, mostly due to the relatively low myelin content of the cortex, causing a smaller increase in T2 relaxation times in cortical lesions compared to that observed with WM lesions.[98] This has hindered the in vivo assessment of the effect cortical demyelination has on the physical and neuropsychological status of MS patients. [94] New MRI techniques have emerged over the last few years that have improved cortical lesion detection.

Different types of cortical lesions behave differently in terms of their histopathological characteristics. This heterogeneity means that they have

different sensitivity to MR imaging, hence the different rates of cortical lesions detection by different MR sequences depending on their type as well as their stage.

DIR is one of the more recently developed, yet commonly used sequences to help with improved identification of cortical lesions. This technique combines two inversion pulses that suppress the signals of both CSF and WM, providing better cortical delineation and cortical lesion prominence.[112] Three dimensional (3D) MRI improves spatial resolution by allowing small voxel acquisition while maintaining a good SNR.[113] A postmortem study compared the sensitivity of 3D DIR to detect cortical lesions in comparison with 3D FLAIR. They found the rate of cortical lesion detection using 3D DIR was 1.6 folds higher than 3D FLIAR and this increased to 2 folds with retrospective scoring. 3D DIR had a particular higher sensitivity to intracortical lesions with 3D DIR having specificity of 90% to detect cortical lesions.[114]

Because of the prominent increase in the SNR, high –magnetic fields (higher than 1.5 Tesla) offer several advantages in terms of higher sensitivity to cortical lesions detection as well as better classification. An in vivo study has been performed to examine the sensitivity of 3D DIR, T2- weighted and FLAIR sequences at both 1.5 T and 3T field strengths. This showed that the sensitivity to cortical lesion detection was definitely improved by the high field strength.[115] The total number of lesions detected was higher at 3T compared with 1.5 T across all pulse sequences. More infratentorial, periventricular and juxtacortical WM lesions were detected using T2-weighted and FLAIR sequences at 3T in comparison with 1.5T.[115] However, one of the main draw backs for DIR at 1.5 or 3T is the presence of artefacts due to vessel and CSF

pulsation which was more observed than any other sequences such as T2-weighted and FLAIR.[115]

3T PSIR is another emerging sequence that has shown improved cortical lesion detection. In a direct comparison between 3T DIR and 3T PSIR in terms of their ability in detecting cortical lesions, it was shown that 3T PSIR was able to detect a significantly higher number of cortical grey matter lesions.[103] 7T FLASH T2* is another sequence that improves cortical lesion detection. In a recent study that directly compared the sensitivity of 3D 3T DIR and 7T FLASH T2* in detecting cortical lesions, it was shown that 7T FLASH –T2* detectd more cortical (especially subpial) lesions than 3T DIR. [116]

MT MR imaging is a tool that has been used to assess demyelination and remyelination in MS. Mougin and colleagues have previously reported the use of ultra-high field MTR, which benefits from improved signal to noise ratio, allowing better spatial resolution compared to lower field MTR.[117]

In this study we examine the use of ultra-high field (7T) MTR for the detection of cortical lesions in MS and validate it with other sequences reported previously at ultra-high field. We tried to strike a balance between high quality scans and reasonable scanning times. Therefore, we employed sequences that cover the whole brain in less than 10 minutes each. In addition, a subset of patients was also scanned on the same day with 3 Tesla 3D DIR; to compare our findings with the most common clinically used method for cortical MS lesion detection.

2.3 MATERIALS AND METHODS

Subjects

Eighteen patients with MS were recruited from the neurology outpatient clinic at Nottingham University Hospitals NHS Trust. These include 12 women and 6 men (16 relapsing remitting, 1 secondary progressive, 1 primary progressive (median Expanded Disability Status Scale (EDSS) score 3.0 (range 0-7.5); mean age 48 years (range 32-65 years); mean disease duration 7.25 years (range 2-19 years). Nine healthy controls subjects (6 men, 3 women); of mean age, 36.5 years (range 24-50 years) were also scanned. All subjects gave informed consent prior to examination and the study had received prior ethical approval from the local research ethics committee.

Data Acquisition

The 7T images were acquired using a 7T Achieva scanner (Philips Medical Systems), equipped with whole body gradient coils, head only quadrature transmit radiofrequency (RF) coil and a NOVA 32 channel receive coil equipped with SENSE reconstruction. The 3T images were acquired on a 3T Achieva scanner (Philips Medical Systems) equipped with a whole body gradient coil, a 32 channel SENSE receive coil and a whole-body transmit coil. All the patients and healthy volunteers were scanned on the 7T scanner. Eight patients were also scanned on the 3T scanner.

Sequences

(1) High resolution MPRAGE images were acquired with a tailored inversion pulse to reduce effects of B1 inhomogeneities. [99] Acquisition parameters are TI=1070ms, FA=8˚, TE/TR=7/15ms for a total of 280 slices at a resolution

of 0.5x0.5x0.5mm3 isotropic, for a total FOV of 205×215×140mm. The sequence was optimized to provide the maximum contrast between the different tissue types, as usually produced for subsequent segmentation procedure.

(2) The MTR maps were obtained with a magnetisation transfer turbo field echo (MT-TFE) acquisition, giving two images (MTsat and MTnosat): the MT sat was acquired by applying 20 off-resonance pulses (sinc pulses with bandwidth of 300 Hz and off-resonance by 1.0 kHz (-3.4ppm), 20 ms between each pulse) before each TFE readout train, chosen to maximize the GM/WM contrast in the MTR maps. The sequence used a turbo-field-echo readout (TR/TE=12/6.4ms, flip angle=8˚, 0.5×0.5×1 mm3 , FOV 205×175×80mm, centre-out sampling, TFE factor=450, shot to shot interval (SSi) of 10s, acquisition time=8min 50s). Images were then coregistered and high resolution MTR maps were calculated from $(MT_{noSat}-MT_{Sat})/MT_{noSat}$ on a pixel by pixel basis.

(3) T2*-images were also acquired with a 3D FLASH-T2*w spoiled Gradient Echo sequence with the following parameters: TR/TE=150/20ms, flip angle=14˚, FOV of 216×216×85mm, with a resolution of $0.5\times0.5\times0.5mm^3$ and acquisition time of 9min. The contrast in the T2* images was optimized to provide good WM/GM contrast with a sufficient TR for 3D imaging. It was also checked that the optimal sequences gave similar contrast to the sequences presented in the literature. [118]

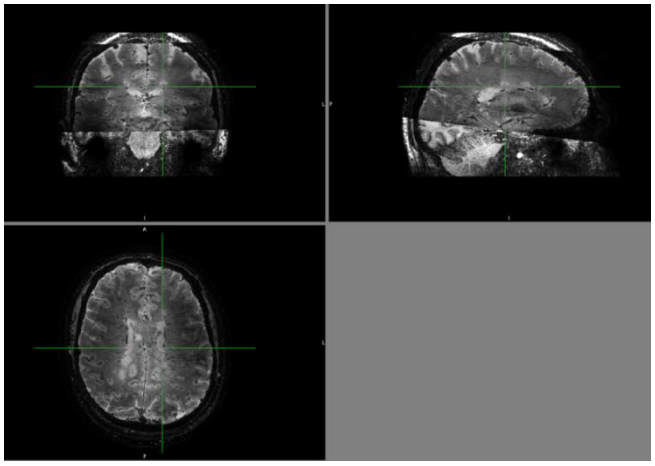
(4) 3T DIR images were acquired on a 3T Philips Achieva system in eight of the 18 patients using a standard 3D Turbo Spin Echo sequence. Parameters for the DIR were TI1= 2600ms, TI2=625ms, Flip angle=90°, Refocusing

angle=180°, TE/TR=250/5500ms, FOV of 250x250x195mm^3 with a resolution of 1x1x0.65mm^3 acquired sagittally, Number of Average=2 for a total acquisition time of 6.30min.

Image analysis

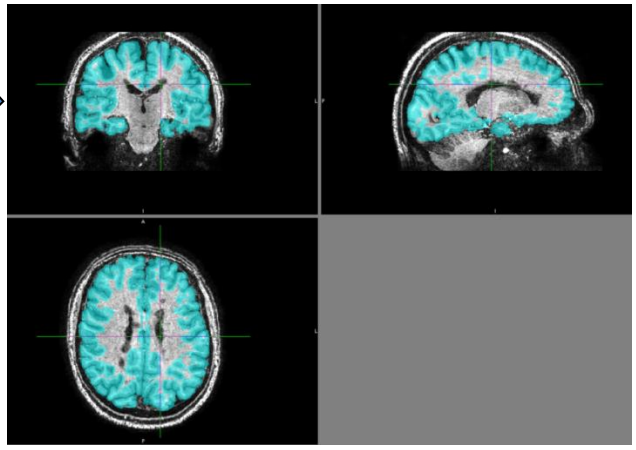
Cortical lesions were detected manually in patients and healthy controls by 2 independent observers. To avoid any bias by the presence of WM lesions in the MS group, and to ensure robust blinding in the detection of cortical lesions, the WM was masked. The cortical grey matter ribbon (cGMR) mask was produced from the MPRAGE sequence. The bias in the MPRAGE image was first removed using spm (http://www.fil.ion.ucl.ac.uk/spm/) before segmenting the GM ribbon volume via freesurfer. (Figure 2.1) (http://surfer.nmr.mgh.harvard.edu). As the segmentation produced by freesurfer is not optimized for high-resolution MPRAGE scans acquired at 7T, extra care was taken to produce the grey matter ribbon mask (cGMR). [119] The mask of the cGMR was first dilated by one pixel to compensate for any loss of GM volume due to classification and registration errors. The masked images were then manually checked for accuracy of classification and manually edited if necessary by an experienced independent observer. The mask was then projected on each of the volumes (MPRAGE, MTR, DIR and T2*w) which were previously registered to the MPRAGE scan using FLIRT FSL (http://www.fmrib.ox.ac.uk/fsl/), using mutual information for the cost function for the inter-modal registration. All the subsequent ribbon modalities were randomised, before a segmentation consensus was reached between three independent reviewers on four subjects. The DIR scans were segmented according to the recent recommendations proposed by the MAGNIMS group.

Lesions had to be hyperintense compared with the surrounding GM and occupy more than three pixels. [118] Similar consensus was reached for the other modalities: cortical lesions had to be hypointense on MTR and MPRAGE, hyperintense on T2* and DIR scans, had to be visible on a minimum of two consecutive slices and to have a diameter of at least three pixels. Manual cortical lesion detection was performed by consensus to all the slices in all the scans using imageJ. Retrospective analysis of all the original unmasked scans was then performed to identify cortical lesions undetected by the blinded assessment. Classification of the type of cortical lesions was performed retrospectively for all lesions on the original images. Lesions were classified according to their location as intracortical (IC, located within the cerebral cortex), Leukocortical, also known as mixed[120] (present mainly in the cortex with some (~25%) extending into the WM), and subpial (extending from pial surface through the entire width of the cortex without involving subcortical WM) lesions by consensus between all modalities. Examples of cortical lesions in different sequences (2.4 and 2.5), and artifacts on MTR sequences are illustrated in figure 2.2 and figure 2.3 respectively.

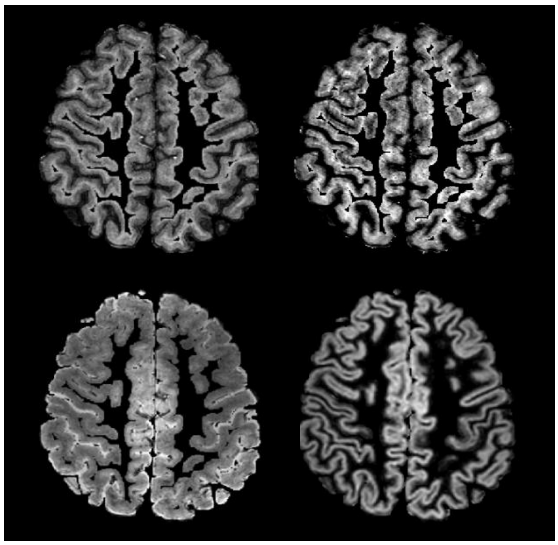


(Figure 2.1) Scans analysis and cortical ribbon segmentation

Registration of all images on MPRAGE

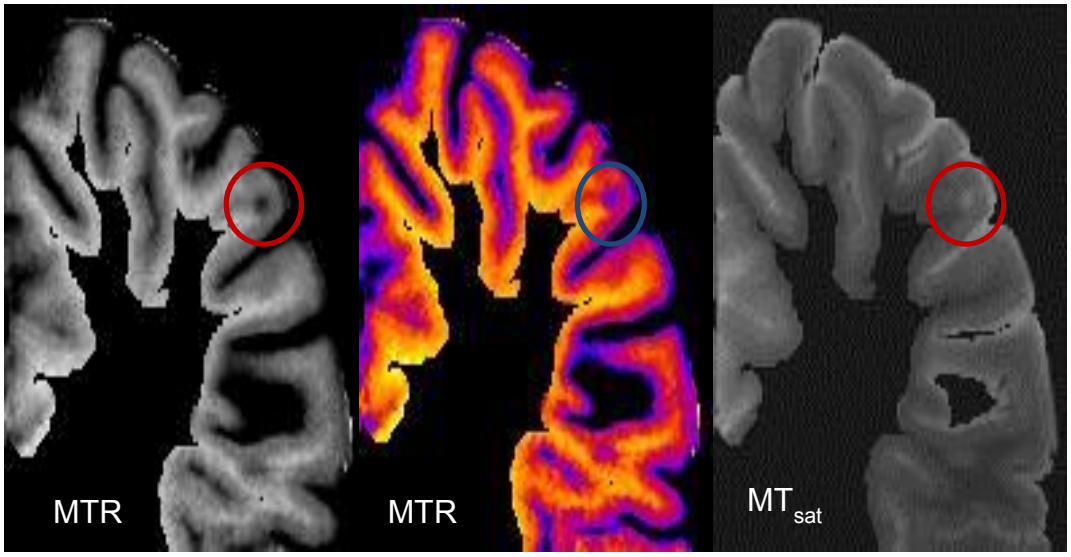


Segmentation of cortical ribbon via free surfer

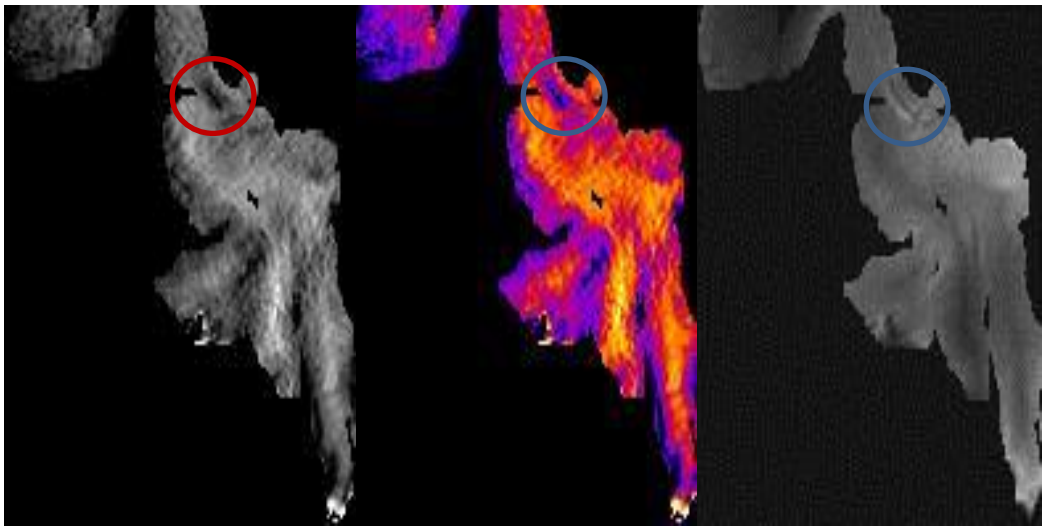


Ribbon mask applied to all modalities

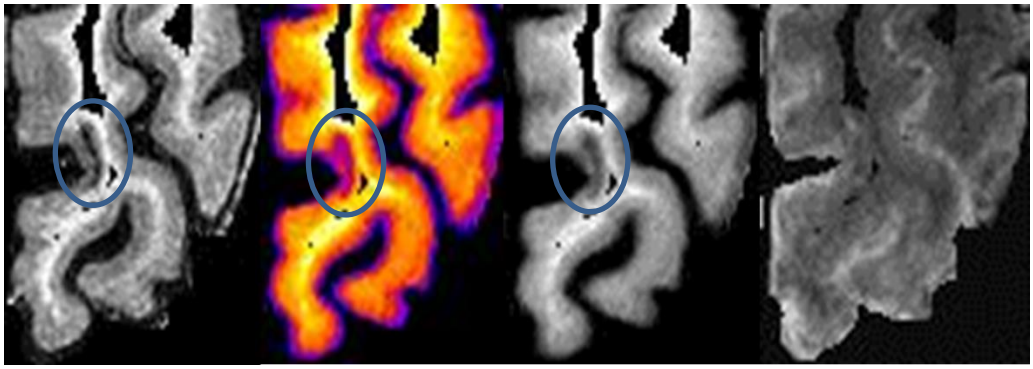
(Figure 2.2) Cortical ribbon of MS patient with mixed cortical lesion appearing as hypointense in the MTR images (left and middle) and hyperintense in the MTsat (Right)



(Figure 2.3) An artefact in the MT images in the form of a vein appearing as hypointense in the MTR images (left and middle)and hypointense in the MT sat (Right) as well

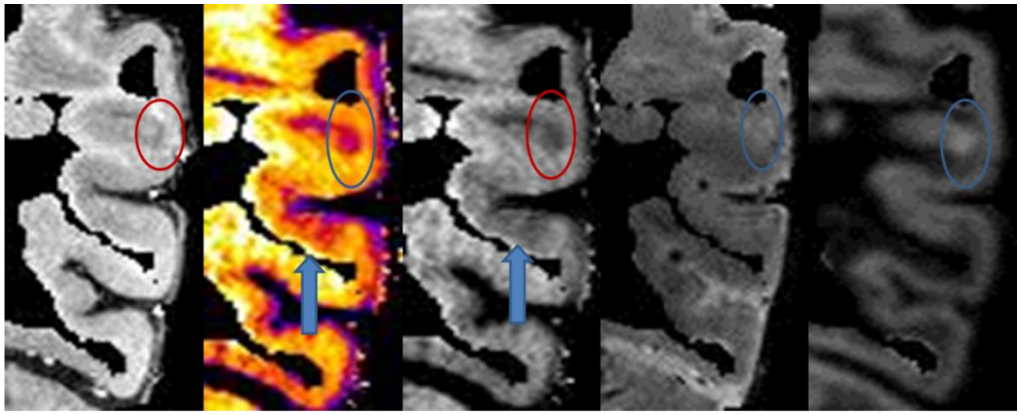


(Figure 2.4) Mixed cortical lesion in different MR sequences



7T MPRAGE | **7T MTR** | **7T MTR** | **7T T2***

(Figure 2.5) Intracortical lesions in different MRI sequences



MPRAGE *MTR* *MTR* *FLASH T2** *DIR*

Figure illustrates intracortical lesion (small lesion within the cortex, not extending to pial surface or the WM/GM junction) in different MRI sequences

(Figure 2.6) Subpial lesion in different MRI sequences

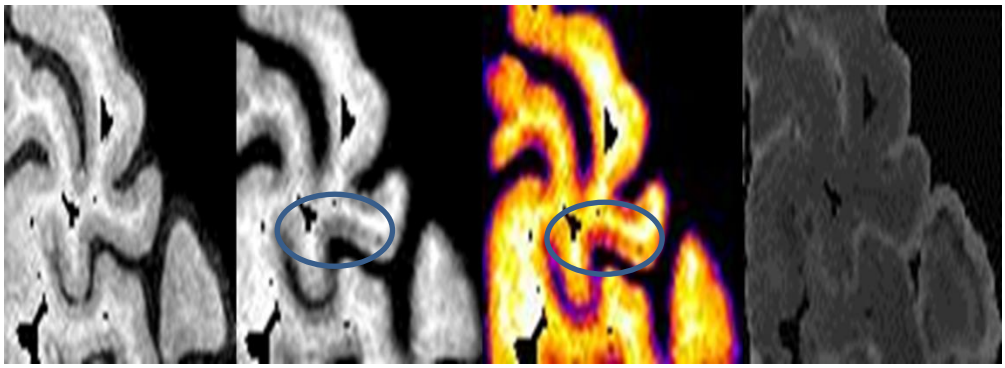


Figure illustrates subpial lesion detected on 7T MTR (middle grey scale and coloured versions), but is not detected on 7T MPRAGE (left) or on 7T FLASH T2* (right)

2.4 Statistics

Statistical analysis was performed using GraphPad Prism software by non-parametric ANOVA (Friedman test) with post hoc Dunn's multiple-comparisons. As the analysis was done twice (comparing lesion counts for 7T MTR, 7T MPRAGE, and 7T FLASH T2* from all patients and then comparing 7T MTR, 7T MPRAGE, 7T FLASH T2* and 3T DIR, for a subset of patients), p-values (for non-parametric ANOVA) and adjusted p-values (for multiple comparisons) of less than 0.025 were considered to indicate a significant difference. For multiple comparisons analysis, firstly I compared mean lesion counts from 7T MTR versus mean lesion counts from 7T MPRAGE and 7T FLASH T2* for all patients. Then I compared mean lesion counts from 3T DIR versus mean lesion counts from 7T MPRAGE, 7T MTR and 7T FLASH T2* for a subset of patients who had 3T DIR acquired on the same day. Bootstrap method was used to construct 95% confidence intervals for the proportion of number of lesions seen on two sequences.

The correlation between the numbers of lesions detected using different sequences was assessed using the Spearman correlation coefficient rho.

Intraobserver reproducibility was assessed for MTR cortical lesion detection on 200 slices using agreement for positive rating measure, while interobserver reproducibility was assessed using kappa coefficient test.

2.5 Results

Imaging at both field strengths (7T and 3T) was well tolerated in all our subjects. None of the subjects reported any discomfort within either of the scanners, beyond the occasional vertigo few of the subjects had as they were

entering or leaving the 7T scanner. Examples of MS lesions in different modalities are shown in figures 2.4 and 2.5

In order to assess scan reproducibility, four patients (2 CIS and 2 RRMS) were scanned twice on the same day, using the same protocol described before. In the first MTR scans, four mixed leukocortical lesions, three intracortical lesions; six subpial GM lesions were detected. The same scans acquired an hour later demonstrated the same lesions.

Intraobserver reproducibility for MTR sequence segmentation was estimated by agreement for positive rating as 0.91, while interobserver reproducibility was estimated by kappa coefficient as 0.8.

On prospective analysis, 335 cortical GM lesions in total were detected with 7T MTR in the patient group while 14 GM abnormalities were detected in the MTR scans of the control group in our blinded assessment. Full data on the prospective review of lesions can be found in table 2.1.

Statistical analysis of retrospective scoring of 7T MTR, MPRAGE and 7T FLASH T2* indicated statistically significant differences between lesion counts ($p<0.0001$). The mean number of lesions detected with 7T MTR was significantly higher (1.26-fold) than the mean number of lesions detected by 7T MPRAGE (adjusted $p=0.001$) and higher (1.6-fold) than the mean number of lesions detected by 7T FLASH T2* (adjusted $p<0.0001$). (Table 2.2)

Using retrospective review of all the sequences for each patient 365 GM abnormalities were detected using MTR scans in all subjects and seven in healthy controls. The lesions detected on MTR, when visualised along the

MPRAGE scans that provided better WM-GM contrast, were classified into 201 leukocortical lesions, 39 intracortical lesions and 125 subpial lesions.

When we compared lesions in different sequences, a significant overlap was found confirming that the lesions detected represent genuine cortical abnormalities. Fifty-nine per cent of MTR lesions were also (95% confidence interval 35% to 80%) visible on 7T MPRAGE, and 52% (95% confidence interval 32% to 70%) on 7T T2*. Sixty nine percent (95% confidence interval 56% to 79%) of lesions were visible on 7T MPRAGE and 7T T2*.

In those patients who have had 3T DIR acquired on the same day (n=8), 133 DIR lesions out of 136 were also detected in MTR, but DIR did not detect 38 lesions seen on 7T MTR. Similarly 89.7% of DIR lesions were seen on 7T MPRAGE (122 out of 136) and 84.6% (115 out of 136) seen on 7T T2*. (Table 2.3)

The total number of lesions detected in each patient with 7T MTR significantly correlated with the lesions detected on 7T MPRAGE (*rho*=0.84, $p<0.001$) and on 7T FLASH T2*(*rho*= 0.73, $p<0.001$). Only weak correlation was observed between the total number of lesions detected on 7T FLASH T2* and 7T MPRAGE (*rho*= 0.45, $p=0.049$).

In those patients who also had high resolution 3T 3D DIR scan, the number of lesions on DIR was significantly correlated with the number of lesions on MTR (*rho*= 0.75, $p<0.001$), the number of 7T MPRAGE lesions (*rho* = 0.82, $p<0.001$) and the number of 7T FLASH T2* lesions (*rho* = 0.76, $p<0.001$).

Leukocortical lesions constituted the highest percentage of cortical lesions detected by all modalities. (Table 2.4)

In this small cohort of mainly RRMS patients, we did not find any significant correlation between the lesion load on any of the sequences and EDSS or the disease duration of the subjects in the patients group using Spearman correlation coefficient rho.

(Table 2.1)

Prospective Grey Matter Lesion Detection comparison between sequences

	7T MTR	*7T MPRAGE*	*7T FLASH T2**
Total lesion number in 18 patients.	335	234	51
Mean no. of lesions per patient.(range)	18.6 (1-49)	13 (1-43)	2.8 (1-10)
Mean lesion volume. In patients	1412 mm^3	538 mm^3	162 mm^3

(Table 2.2)

Retrospective Grey Matter Lesion Detection in Different sequences.

	7T MTR	*7T MPRAGE*	*7T FLASH T2**
Total lesion number in 18 patients.	365	289	231
Mean no. of lesions per patient.(range)	20.28 (1-49)	16.06 (1-43)	12.83 (1-10)

(Table 2.3)

Retrospective Lesion Detection in the Subgroup of Patients who had an Additional 3T DIR Imaging on the same day. (*n*=8)

	7T MTR	*7T MPRAGE*	*7T FLASHA T2**	*3T 3D DIR*
Total lesion number. (range)	171 (4-49)	147 (2-43)	126 (1-10)	136 (0-29)
Mean no. of lesions per patient.	21.38	18.38	15.75	17

(Table 2.4)

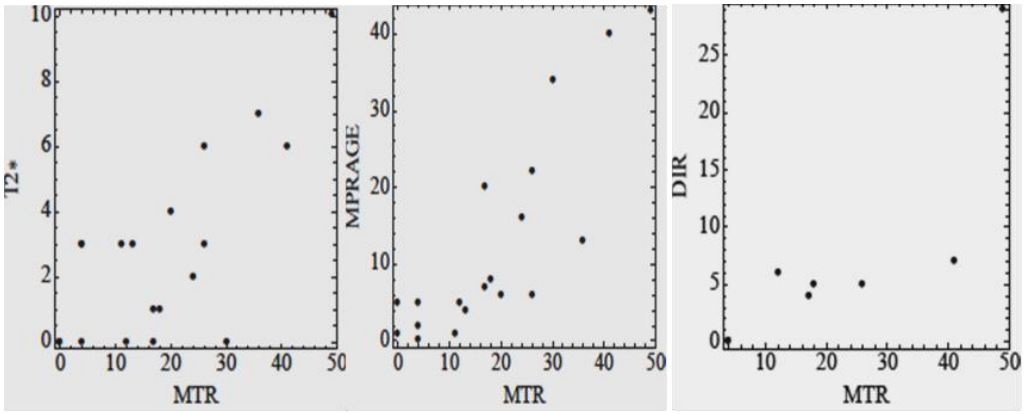
Location of Cortical Lesions in Different Sequences

Sequences examined	*Intracortical Lesion*	*Mixed Leukocortical*	*Subpial*
7T MTR (n = 365)	10.41 %	55.06 %	34.53 %
7T MPRAGE (n = 289)	7.28 %	63.66 %	29.06 %
7T T2* (n = 231)	4.76 %	68.39 %	26.85 %
3T DIR (n = 136)	17.65 %	82.35 %	0

Location of lesions detected in each of the four sequences. Note that the DIR sequence was used in a subset of patients, as described in the methods section.

(Figure 2.7)

Correlation between lesion numbers on different sequences



The figure illustrates significant correlation between the numbers of cortical lesions detected on different sequences. The highest correlation was between 7T MTR and 7T MPRAGE (*rho* = 0.84), while correlation between 7T MTR and 7T T2* was slightly weaker (*rho* = 0.73), and similar to correlation between 7T MTR and 3T 3D DIR (*rho* = 0.75)

2.6 Discussion

Ultra-high field MTR has proven very sensitive in detecting GM lesions in MS patients. The aim was to test a semiquantitative "myelin" sequence that would be sensitive enough to depicting cortical lesions within reasonable acquisition time and full brain coverage. In this study, 7T MTR demonstrated a good ability to detect cortical lesions of different types.

The ability to detect GM lesions with MTR opens exciting avenues in MS research, as it provides a semi quantitative measure of the myelination status of those lesions. Accumulating evidence suggest that remyelination is important in MS and a number of remyelinating treatments are in the pipeline. In longitudinal studies, high resolution MTR is a promising method to assess remyelination in vivo at least in the research setting.

In this "less than 10 minutes per sequence" study 7T MTR appears to improve GM lesion detection compared to other 7T sequences (MPRAGE and T2*) and 3T DIR. We believe that the MTR abnormalities detected correspond to cortical lesions, as only a few cortical MTR abnormalities were detected in healthy controls, and the MTR lesions overlap significantly with lesions detected with other sequences. Furthermore, MRI pathological studies showed that MTR lesions correspond to GM demyelination. [120,]

As with all sequences in our blinded assessment, we detected few GM signal abnormalities in the healthy controls. This is a very common finding in the WM,[122] and with the increasing resolution of GM imaging might become more frequent also in the GM. This is not surprising considering the high resolution of our imaging.

Ultra-high field MRI increases the signal to noise ratio and hence has the potential to enhance the detection of GM lesions. Mainero *et al* [118] found increased sensitivity of 7T T2* MRI in detecting cortical lesions, with more than 50% of the detected lesions of subpial type III or IV. [116] I did not aim to compare the sensitivity of MTR with T2* used previously at 7T. The 7T FLASH T2* we used had lower in-plane resolution but provided larger coverage of the brain in significantly shorter acquisition time. In addition, the sequence that was used in this study is 3D, thereby reducing partial volume effects and the risk of tissue misclassification. [116]

DIR despite being the most commonly used sequence for GM lesion detection has been criticised for being prone to artifacts. In a recent consensus paper for the detection of cortical lesions using DIR, there was agreement by all five teams involved on only 19.4% of the lesions. Mixed WM/GM lesions were visualised with more consistency. [99] In this study reassuringly, our use of DIR, detected lesions that correlated strongly with the total number of GM lesions seen at 7T high-resolution imaging. The better performance of DIR than previously reported, [116] might be due to the higher resolution 3T scans, whole brain coverage and 3D acquisition. Still with 3T DIR, we could not detect any subpial lesions with confidence.

There are of course many other sequences, which we have not been able to study in our cohort. We had not optimised and not performed 7T FLAIR at the time of this study, although a recent publication[100] suggests that 3D-FLAIR at 7T is sensitive to depict GM lesions compared to DIR,T1 and T2. It is possible that the moderate but not perfect overlap between sequences in the

cortical lesions may reflect the different sensitivity of sequences to different pathologies as it has been found in the WM lesions.[100,123] This is a promising avenue to follow in a larger study focusing mainly on GM lesions.

Prospective blinded analysis of all the scans did not detect as many lesions as the retrospective analysis of all the sequences simultaneously. Review of all scans particularly helped identify lesions missed initially on T2* and MPRAGE. Some of the lesions still, could not be detected on any modality other than MTR, even with careful retrospective examination.

Previous studies using MT MR measured the MTR globally in the brain or regionally through histogram analysis. This proved extremely informative in providing a global reflection of GM pathology but has not separated focal from diffuse GM damage. [71,123] As MTR better reflects tissue myelin content than other commonly used MRI sequences, it could also allow in vivo detection of lesion remyelination. [124]

MTR maps are prone to artifacts, especially at high field strengths. For example, typical dropout of signal near the sinuses and in the temporal lobes is common due to B0 and B1 inhomogeneites. The protocol described in this paper has been optimized to reduce these effects to a minimum,[125] but a dropout in the contrast to noise ratio (CNR) was visible in some of the maps. As two different images necessary to compute the MTR maps are acquired separately, movement artefacts can contaminate the images. Despite registration of those images, intra-scan movement could degrade the quality of the final MTR maps. It is worth noting that unlike MTR in white matter lesions, MTR in cortical lesions has not been validated in post mortem studies so far.

Using MTR alone, we were able to classify most of the cortical lesions. As MTR has a lower WM-GM contrast compared to MPRAGE, we found that combining those sequences improved significantly the GM lesion subtype classification.

As MTR at 7T was shown to detect cortical lesions bettern than other currently research applicable imaging sequences, we wanted to employ it to examine the correlation between the degree of damage in cortical lesions reflected by their mean MTR values and the degree of physical disability.

In the next chapter we examined a larger group of patients of different MS phenotypes aiming to assess the variation in the degree of damage in cortical lesions among the groups. I also aim to examine the correlation between patients' degree of physical disability and the degree of damage in their cortical lesions.

Chapter 3

Cortical lesions in secondary progressive MS are more destructive compared to other MS phenotypes

3.1 Abstract

Introduction:

The presence and significance of focal GM lesions is undisputed, but it is unclear if GM lesions exhibit the same pathological heterogeneity as is found in the WM. Cortical lesions are more prevalent in progressive than in relapsing multiple sclerosis. MTI is useful in depicting WM and GM lesions. MTR values in the brain have been shown to reflect tissue myelin content.

Hypothesis:

We hypothesise that:

1- There is a difference in the level of myelin loss in focal GM lesions as determined by MTR among different MS phenotypes.

2- There is a correlation between the degree of focal cortical lesion demyelination and the degree of physical disability in patients with MS.

Methods:

Forty two patients with demyelination were examined along with eight healthy controls, using MTI at 7Tesla. Cortical lesions were segmented and their individual mean MTR values were calculated. Clinically I estimated their clinical disability using EDSS.

Results:

Focal cortical lesions in patients with SPMS had lower mean MTR values compared to PPMS, RRMS or CIS. Larger cortical lesions had lower MTR. Mixed lesion volumes and mean MTR values correlated with the level of physical disability, while the other lesion types did not play an important role.

Conclusion:

These findings suggest that different MS phenotypes exhibit differences in their CL characteristics. Similarly different types of cortical lesions might be correlated to a different degree to the level of clinical symptoms. Leukocortical/mixed lesions are the main lesion type associated with physical disability in MS patients.

3.2 Introduction:

3.2.1 MS Pathology in Different MS Phenotypes

White matter lesion pathology in different MS phenotypes:

MS phenotypes usually present with different courses of a similar spectrum of symptoms. The pathology seems to vary between these phenotypes as described in previous reports.[126] The pathology of RRMS consists mainly of lesions disseminated in time and space. Lesions are normally in different stages of chronicity. WM and cortical lesions vary in stages from 1) acute plaques with active inflammatory infiltration and macrophages loaded with myelin degeneration products to 2) plaques that are only active at their margins and finally 3) chronic inactive demyelinated lesions. Lassman *et al.* [127] described four main types of lesions, of which the first 3 types are mainly present in RRMS patients, while the fourth type is only seen in PPMS patients. The classification of these lesion types was mainly based on the presence of inflammation and active demyelination. Lesions types were as follows:

(1) Plaques with inflammation and macrophages containing early myelin debris.

(2) Plaques with inflammation in the absence of early myelin degradation products in the macrophages.

(3) Plaques with early myelin degradation products in the absence of perivascular infiltrate.

(4) Plaques without inflammatory infiltrates and without myelin degradation products.

The main question for MS pathogenesis is whether the presence of several plaques types indicates that, MS might be caused by several different immunological pathways, probably acting at different times, causing demyelination in individual patients. The other theory is that probably the

mechanisms of demyelination may vary between patients, due to the variation in immunological mechanisms involved. This could explain the clinical and genetic heterogeneity of the disease.[127]

SPMS patients are most likely to have chronic inactive or occasionally active plaques showing demyelination and /or degeneration of multiple individual fibers. Degenerative changes in SPMS were shown to involve normal appearing tissue and were not restricted to lesions. Normal appearing tissue involvement is usually independent of current inflammatory demyelination activity. This might explain why disease modifying treatment and immunosuppressive drugs do not have an effect on SPMS patients' disability. This has led to suggestions that SPMS is not caused by continuous immune processes, but by demyelination affecting axonal transport and ultimately axonal loss and what is called "dying back process". This process affects long axons first such as the pyramidal tracts which may explain the significant impact on EDSS.[128]

The distribution of lesions varies between different types of MS. PPMS lesions have predilection for the spinal cord, whereas SPMS patients are more likely to have brain involvement compared to other disease phenotypes. In a report by Revesz et al.[129] they found that in the PPMS patients, 54% of the lesions were found in the cerebral hemispheres, 14% in the brainstem and 32% in the cord, while the distribution in the SPMS was 75% in the hemispheres, 17% in the brainstem and 8% in the cord. There were areas of prominent perivascular cuffs and increased cellular density in lesions of the SPMS compared to PPMS patients. Also the chronic hypocellular lesions had a hypercellular edge more often in the SPMS than in the PPMS group.

Cortical lesion pathology in different MS phenotypes:

It has been difficult to detect cortical lesions in the past due to the lack of sensitivity of classical myelin histochemical staining methods (luxol fast blue) that have always been used in the past. Over the last few years, the appearance of immunohistochemical markers improved the detection of cortical demyelination.

Cortical demyelination is classified into type I (leukocortical) lesions which extend between the cortex and white matter and they account for around 34% of cortical lesions, type II (intracortical) lesions which are small confined to the cortical ribbon and often have a vessel in their centre and account for around 17% of cortical lesions, and types III and IV which account for the remaining 50% and extend from the pial surface into the cortex without invading the subcortical WM.[108] Leukocortical lesions, also called mixed lesions (MXL) are the most common type of lesions in early MS, constituting around 50% of cortical lesions detected while subpial and intracortical lesions constitute around 34% and 16% consequetively.[93]

Cortical involvement varies in different stages of the disease. Leukocortical and intracortical lesions are generally found in all disease subtypes, including acute MS, RRMS, PPMS and SPMS.[130] However, these lesions constitute only a small percentage of the total cortical lesion load generally; they are the dominant subtype in patients with RRMS. Subpial lesions are mainly found in patients with PPMS or SPMS. In chronic patients the subpial lesions could extend to involve multiple adjacent gyri and penetrate into the cortex in variable depth.[130] In an immunohistochemical study by Lucchinetti *et al*.[93] that included tissue from 138 patients with early MS, cortical demyelination was

found in 38% of the patients. All three types of cortical lesions were observed with leukocortical lesions being the commonest type of lesions detected. Sixty six percent of lesions had foamy macrophages, indicating ongoing demyelination in all types of cortical lesions. Perivascular CD3+ T cells inflammation were observed in 77% of patients. Leukocortical lesions were highly inflammatory. Most of the intracortical and subpial plaques contained perivascular CD3+ and CD8+ Tcell infiltrates. Patients with cortical demyelination especially subpial lesions were more likely to have diffuse meningeal inflammation than patients without cortical demyelination. Oligodendrocyte density was reduced in a subset of lesions, as compared with adjacent, nondemyelinted cortex.

Similarities were reported in the CL volume and their topographical distribution between RRMS and PPMS patients.[131] Patients with benign MS did not show cortical lesions accumulation over a one year period in comparison with RRMS patients.[132]

In a large autopsy study, including patients of different disease phenotype, clinical and pathologic characteristics with varying extents of cortical demyelination and different types of CLs were examined. One of the main findings was the paucity of CLs generally and inflammatory changes particularly in PPMS patients. They found around 50% of all patients of all phenotypes harboured extensive subpial demyelination, however there was no difference in the age at death, or disease duration between those who had or did not have CLs. They found that some of the CL had a rim of active microglia hence called RAM-CL versus non-RAM-CL. In the RAM-CL group, a significantly higher proportion of the cerebral cortex was demyelinated

compared with that in the non-RAM-CL group. They also found a lower mean age of death, and shorter disease duration in the RAM-CL group than in the non-RAM-CL group, but no difference of the age of death was found between the non-RAM-CL group and the non-CL group. In this study most of the PPMS patients autopsy included (16 PPMS patients) did not have CLs. Furthermore, the presence of chronic active WMLs was associated with a higher load of RAM-CLs in the RAM-CL group. Most importantly they found that leukocortical lesions were more prominently present in the RAM-CL group and to a smaller extent in the non-RAM-CL group. The exact pathogenic role activated microglia play in CLs is still under defined, on the other hand the strong correlation with chronic active WMLs may suggest more pronounced activity of the innate immune system in disease progression in a subset of patients with MS. Based on the pattern of their neuropathological data, they suggested that CLs with RAM may similarly contribute to MS disease progression and disease severity.[133]

Intracortical lesions were reported to be prevalent in 36.2% of patients with CIS, while SPMS patients had higher numbers of IC lesions than those with RRMS or CIS patients.[81] In histopathology, biopsies of early diagnosed MS patients showed that 38% of patients showed cortical demyelination while 14 % of the patients who did not show evidence of cortical demyelination showed cortical inflammation.[93]

It was also reported that the functional loss produced by the lesions of MS depends on the phase of pathological evolution; the different phases look alike on conventional unenhanced MRI. Regardless lesion count and volume must also have a relevant role.[134]

The extent of cortical demyelination was found to be significantly higher in patients with a disease duration of more than 10 years.[90]

The pathology of cortical lesions has been examined using focal cortical EAE model, by injecting pro-inflammatory mediators in adult female Lewis` rats that were immunized with myelin oligodendrocyte glycoprotein (MOG). This generated highly reproducible demyelinated lesions in the neocortex with very high histological similarities to cortical lesions in MS. This focal cortical EAE model led to typical intracortical and subpial demyelination, inflammatory cell infiltration, complement deposition, acute axonal damage and neuronal cell death. They also found that the extensive cortical inflammation had largely resolved within 2 weeks and demyelination was compensated rapidly by remyelination. It is believed that the transient and rapidly reversible nature of cortical inflammation and demyelination in early MS may prevent the development of larger demyelinated lesions, hence making their clinical or radiographic detection more challenging in MS patients.[110]

The pathology of cortical demyelination in early MS differs substantially from that which happens at the chronic stages of the disease. Although the variation in cortical lesion characteristics among different MS phenotypes has been previously explored, the variation in their myelination status has not yet been widely investigated. This will help improve our understanding to the different pathology in different MS disease phenotypes.

3.3 Patients and methods:

Forty two patients with MS (11 CIS, 11 RRMS, 10 PPMS, and 10 SPMS), were recruited from the multiple sclerosis outpatient clinics, at Nottingham University Hospitals: 18 were male and 24 female. Eight healthy volunteers

(two male and six female) without neurological disease were also recruited. Patients and volunteers were between 18 and 65 years of age, with no other reported neurological disease. The MS patients had to be free from recent clinically overt disease activity (in the form of a relapse) for at least three months prior to scanning. The study was approved by the local ethics committee and all subjects gave written informed consent. The MS patients underwent a neurological examination and their EDSS score was determined.

Data acquisition:

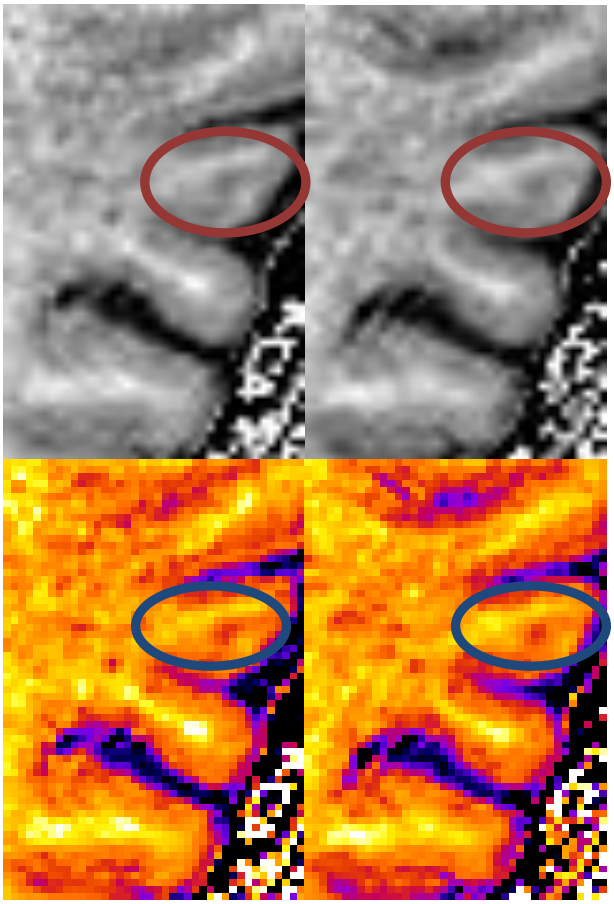
MRI scans were acquired using a 7T Achieva Philips scanner, with a 32 channel receive coil. PSIR data was acquired from each patient using a 3D interleaved PSIR sequence,[135,136] with imaging parameters: TI1=780ms (first TFE read out), TI2=2380ms (second TFE readout), shot to shot interval (SSi) =5000ms, flip angle (FA)=8°, TE/TR=6/13ms, 0.6mm^3 isotropic resolution, field of view 200x180x120 mm^3, acquisition time 12 minutes. These parameters were chosen to ensure PSIR images with good grey-matter/white-matter contrast in a reasonable scanning time. MTR data was acquired with a MT-TFE sequence as described by Mougin *et al*.[125] and consisted of a presaturation at a specific offset frequency, followed by a 3D Turbo Field-Echo (TFE) readout consisting of gradient echoes sampling k-space radially in a centre-out fashion. The presaturation consisted of 20 off-resonance pulses (B1avg of 1.02 μT, BW=250 Hz, T=50 ms) applied at offset frequencies of $\Delta\omega$= 3.3 ppm, chosen from the results of Mougin *et al*,[125] to give high sensitivity to NOE with reasonable insensitivity to variations in RF amplitude and B0 inhomogeneity, and setting B1peak 25% higher than optimum to

compensate for overestimation of nominal B1 compared to actual B1. The pulse train was followed by a 3D TFE readout with resolution of 0.6x0.6x0.6 mm^3, TR/TE=13/6 ms, SSi= 12 s, a = 8°, TFE factor= 500, FOV=192x180x120 mm^3; the acquisition time was 13 min.

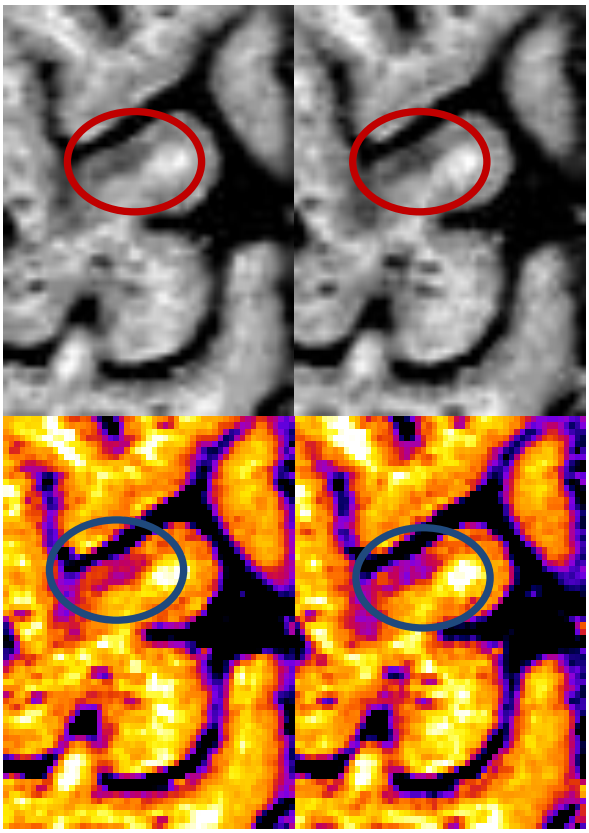
Data analysis:

MTR maps were registered to the PSIR images using FSL FLIRT (http://www.fmrib.ox.ac.uk/fsl/). Mutual information was used as the cost function to handle this inter-modal registration task. The relative effect of B1 field inhomogeneity on MTR values was corrected using the method described by Ropele *et al*,[137] which models the relationship between the B1 error and the MTR error as linear (a reasonable assumption, both in theory and in practice). Two independent observers (RAF and CMA) manually segmented all cortical lesions in the anonymised scans of both patients and healthy controls using the MIPAV medical images processing, analysis and visualization software package (http://mipav.cit.nih.gov/). The segmentation criteria were as follow: lesions had to be hypointense compared with the surrounding GM and occupy more than three pixels; they also had to be visible on a minimum of two consecutive slices.[99] Lesions were classified according to their location as intracortical, as illustrated in figure 3.1, leukocortical, also known as mixed, and subpial lesions, as illustrated as in figure 3.2, by consensus between both modalities. Finally, the mean MTR values for each lesion were computed. WML were also segmented and their MTR values were computed similarly to cortical lesions.

(Figure 3.1)



Example of intracortical lesion in MTR images in two consecutive slices in an MS patient brain

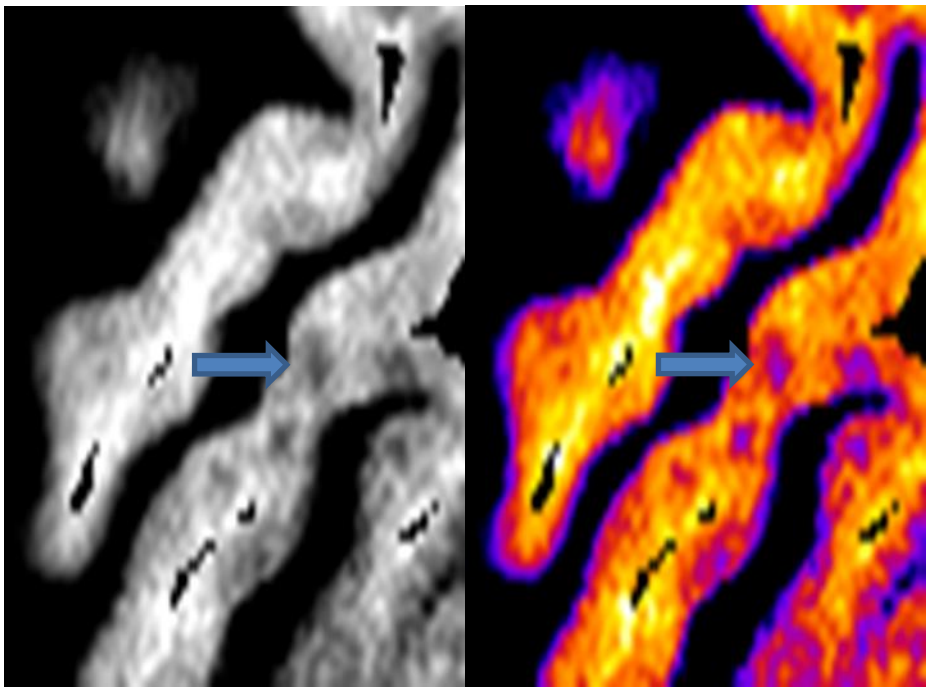


(Figure 3.2)

Example of subpial lesion in MTR images in two consecutive slices in an MS patient brain

(Figure 3.3)

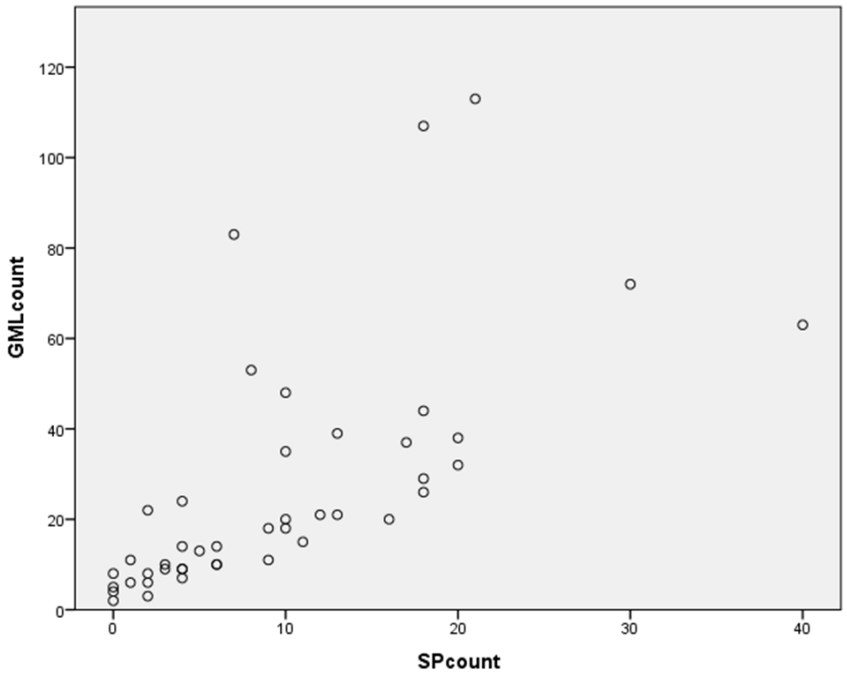
Example of mixed lesion in MTR images in MS brain



The figure illustrates a mixed lesion in MTR images (grey scale on the left and coloured on the right) with the lesion mainly in the grey matter but extending to involve the white matter as well.

(Figure 3.4)

Correlation between total grey matter count and subpial lesion



The figure illustrates the strong positive correlation between subpial lesion count and total grey matter lesion count. (*rho* = 0.83, $p < 0.05$)

3.4 Statistics:

Intraobserver reproducibility was assessed using agreement for positive and negative rating measures, as well as variation coefficients while interobserver reproducibility was assessed using kappa coefficient. Spearman's correlation coefficients between the variables, indicated as *rho*, were used to investigate correlations between the CL and WML count, volumes, and their MTR values with the level of patient's physical disability as measured by EDSS scores. We also tested the correlations between different CL and WML parameters.

As mean GML MTR values and NAGM MTR are normally distributed, one way ANOVA was used to assess significant variations across MS phenotypes. An ordered logit regression was used to identify the association between MRI measurements as independent variable with EDSS as the dependant variable. This method takes into account the ordinal, rather than continuous nature, of the EDSS score. EDSS was estimated as a function of cortical, WML volume, mean lesion MTR value, disease duration, age and gender, as independent variables.

As individual focal lesions MTR values in all subjects are normally distributed, linear regression was used to assess the association between individual lesion MTR (dependent variable) and their volume controlling for disease duration, age and gender.

3.5 Results:

Descriptive statistics:

***Clinical Data*:**

Patients' demographics are shown in table 3.1. Intra-observer reproducibility for cortical lesion detection: agreement for positive rating and negative rating as 0.91 and 0.09 respectively, while kappa coefficient was 0.8, (95%CI 0.46-1.07). Inter-observer reproducibility was estimated by kappa coefficient as 0.8 (95% CI 0.6-1.02), while variation coefficient was 3.5%.

Cortical lesions (CL): (Table 3.2)

Descriptive statistics for all MS subtypes are shown in table 3.2. The total number of cortical lesions (of all types) was the highest in the SPMS group in comparison with other MS subtypes. Total CL count was 6 times higher in SPMS than in CIS patients. Mixed lesions (MXL) were higher in SPMS compared to CIS, RRMS and PPMS by 18 times, 6 times and 4 times respectively. Patients with SPMS showed higher median total CL volume in comparison with the other MS disease subtypes. This was mainly due to the higher MXL volume in SPMS compared to the other groups. SPMS patients had the highest subpial lesion load. Focal CL mean MTR values were different between disease subtypes. SPMS patients had the lowest focal CL mean MTR values, while CIS patients had the highest values. (Table 3.2)

***MRI metrics correlations*:**

GML count and volume correlated with WML count and volume (*rho* = 0.5, *rho* =0.6 with *p* = 0.00 and 0.00 respectively). GML count and volume correlated the highest with SP lesion count and volume (*rho* = 0.83 and *rho* = 0.71 with *p* = 0.00 and 0.00 respectively). In terms of WML count and volume,

they had the strongest correlation with MXL count and volume (p = 0.62 and 0.71 with p = 0.00 and 0.00 respectively). Individual correlations are demonstrated in table 3.5).

Correlations between Disability and different clinical parameters and MRI metrics:

A significant positive correlation was detected between EDSS and patient age (*rho* = 0.55, p = 0.01). Similarly, a positive correlation was seen between EDSS and disease duration (*rho* = 0.59, $p < 0.05$), while no significant correlation was detected between EDSS and gender (rho = - 0.21, p = 0.15). A significant correlation was found between the total CL volume and disease duration (*rho* = 0.4, p = 0.01). Subject age correlated with CL volumes (*rho* = 0.3, p = 0.04) and their mean MTR values (*rho* = - 0.36, p = 0.02). EDSS correlated with total CL volume (*rho* = 0.6, p = 0.01). The strongest correlation was found between MXL volume and EDSS (*rho* = 0.5, p = 0.01), while a smaller correlation was detected between EDSS and intracortical (IC) lesion volume (rho = 0.34, p = 0.02). There was no significant correlation between subpial lesion volume and EDSS (*rho* = 0.23, p = 0.13).

There was a significant correlation between EDSS and total CL count (*rho* = 0.53, p = 0.00) with highest correlation with MXL subtype (*rho* = 0.6, p = 0.00), a lower correlation with subpial lesion count (*rho* = 0.36, p =0.01), while the correlation with IC lesion count was insignificant (*rho*= 0.2, p = 0.1).A significant positive correlation was found between EDSS and WML volume (*rho* = 0.5, $p < 0.05$).

There was no significant correlation between age and WML volumes or WML mean MTR values. I did not find any correlation between gender and CL volumes, CL mean MTR values, WML volumes or WML mean MTR values.

Regression analyses of disability and clinical parameters to MRI measurements:

In this regression analysis, disease duration was a significant predictor (p = 0.01) of disability as measured by EDSS. MXL volumes and MXL mean MTR values were significant parameters correlated with disability with p values of 0.03 and 0.02 respectively. MXL mean MTR values appeared to associate with the degree of disability more than MXL volumes. EDSS was not associated with either WML volume or their MTR values. (Table 3.6)

Focal CL MTR values in SPMS patients was the lowest in comparison with CIS, RRMS and PPMS (p = 0.00, 0.01 and 0.02 respectively). There was no significant effect of chronicity of the disease on the NAGM MTR values.

Individual CL and WML volume influenced lesion MTR values, with larger volume lesions having lower mean MTR values (p = 0.045), after controlling for age, gender and disease duration. There was a trend for the disease duration influencing the individual CL mean MTR values (p = 0.09), while the patients' age and gender were not significant. (Table 3.6)

(Table 3.1) Patient's demographics*:*

	CIS	*RRMS*	*PPMS*	*SPMS*	*Controls*
Female, n (%)	*6(54%)*	*8(72%)*	*4(40%)*	*6(60%)*	*6(75%)*
Mean age *	*38 ± 14*	*43 ± 7*	*50 ± 8*	*51 ± 6*	*36.7 ± 10*
Mean disease duration *	*0.9 ± 0.5*	*5 ± 3*	*6.9 ± 3*	*12 ± 6*	*NA*
Median EDSS (IQR)	*1 (1-1)*	*1.5 (1-2)*	*6 (4-6)*	*6 (6-6.5)*	*NA*

CIS =Clinically Isolated Syndrome, RRMS = Relapsing Remitting Multiple Sclerosis, PPMS = Primary Progressive Multiple Sclerosis, SPMS = Secondary Progressive Multiple Sclerosis, IQR = Interquartile ratio* Mean and standard deviation

(Table 3.2) Median count and volume of different cortical lesions subtypes in MS patients and controls

	CIS	*RRMS*	*PPMS*	*SPMS*	*Control*
Median CL count (IQR)	*8 (5 – 9)*	*15 (13 – 35)*	*19 (10 – 38)*	*49 (22 – 83)*	*0*
Median CL volume in mL (IQR)	*215 (0.168 – 0.367)*	*504 (0.274 – 0.837)*	*413 (0.153– 0.1232)*	*1,797 (0.767– 0.2182)*	*0*
Median MXL count (IQR)	*1 (0-3)*	*3 (1-6)*	*4 (2-11)*	*18 (6-60)*	*0*
Median MXL volume in mL (IQR)	*44 (0- 0.088)*	*66 (0-0.03)*	*638 (0.033- 0.723)*	*1659 (0.405- 1.733)*	*0*
Median IC lesion count (IQR)	*3 (1-5)*	*6 (2-11)*	*3 (1-10)*	*9 (4-14)*	*0*
IC lesion volume in mL(IQR)	*50 (0.025 – 0.075)*	*50 (0.025 – 0.158)*	*51 (0.007 – 0.177)*	*95 (0.062 – 0.174)*	*0*
Median subpial lesion count (IQR)	*3 (2-4)*	*10 (5-14)*	*10 (1-18)*	*16 (7-20)*	*0*
Median subpial lesion volume in mL(IQR)	*122 (0.016 – 0.3)*	*244 (0.097 – 0.483)*	*148 (0.057- 0.459)*	*368 (0.09 – 0.533)*	*0*

CIS = Clinically Isolated Syndrome, RRMS = Relapsing Remitting MS, PPMS = Primary Progressive MS, SPMS = Secondary Progressive MS, CL = Cortical Lesion, MXL = Mixed Lesion, IC = Intracortical Lesion, IQR= Interquartile Ratio

Table 3.3: Mean MTR values of different cortical lesions subtypes in MS patients and controls

	CIS	*RRMS*	*PPMS*	*SPMS*	*Controls*
CL mean MTR (std)	*0.31 (0.04)*	*0.29 (0.05)*	*0.27 (0.03)*	*0.21 (0.05)*	*0*
MXL mean MTR (std)	*0.17 (0.16)*	*0.28 (0.06)*	*0.27 (0.05)*	*0.17 (0.1)*	*NA*
IC lesion mean MTR (std)	*0.28 (0.1)*	*0.28 (0.05)*	*0.27 (0.04)*	*0.22 (0.05)*	*NA*
Subpial lesion mean MTR (std)	*0.28 (0.1)*	*0.26 (0.1)*	*0.24 (0.09)*	*0.22 (0.05)*	*NA*
NAGM mean MTR (std)	*0.38 (0.04)*	*0.35 (0.06)*	*0.36 (0.06)*	*0.31 (0.05)*	*0.35 (0.1)*

CL = Cortical Lesion, MXL = Mixed Lesion, IC = Intracortical Lesion, NAGM = Normal Appearing Grey Matter

(Table 3.4) White matter lesions median count, median volume and mean MTR values for patients of different MS phenotypes and controls

	CIS	*RRMS*	*PPMS*	*SPMS*	*Controls*
Median WML count (IQR)	*11 (7 -13)*	*26 (12 – 34)*	*23 (17 – 47)*	*40 (26 – 65)*	*0*
Median WML volume in mL (IQR)	*1,161 (0.526–6.649)*	*3,010 (0.838 – 5.648)*	*7,764 (2.102 – 29.816)*	*18,416 (5.013 – 34.226)*	*0*
WML mean MTR (sd)	*0.35 (0.06)*	*0.34 (0.09)*	*0.33 (0.06)*	*0.23 (0.07)*	*NA*

WML = White Matter Lesion

(Table 3.5) Correlations between different lesions types count and volumes

	WML count	***WML volume***	***GML count***	***GML volume***	***MXL count***	***MXL volume***	***IC count***	***IC volume***	***SP count***	***SP volume***
WML count	-	-	-	-	-	-	-	-	-	-
WML volume	*0.58 (0.00)*	-	-	-	-	-	-	-	-	-
GML count	*0.53 (0.00)*	*0.49 (0.001)*	-	-	-	-	-	-	-	-
GML volume	*0.58 (0.00)*	***0.59 (0.00)***	*0.79 (0.00)*	-	-	-	-	-	-	-
MXL count	*0.62 (0.00)*	***0.81 (0.00)***	***0.62 (0.00)***	*0.65 (0.00)*	-	-	-	-	-	-
MXL volume	*0.53 (0.00)*	***0.71 (0.00)***	*0.51 (0.00)*	*0.67 (0.00)*	*0.91 (0.00)*	-	-	-	-	-
IC count	*0.29 (0.05)*	*0.11 (0.45)*	*0.74 (0.00)*	*0.43 (0.00)*	*0.19 (0.21)*	*0.12 (0.42)*	-	-	-	-
IC volume	*0.25 (0.09)*	*0.16 (0.29)*	*0.59 (0.00)*	*0.44 (0.00)*	*0.20 (0.18)*	*0.155 (0.31)*	*0.81 (0.00)*	-	-	-
SP count	*0.38 (0.01)*	*0.27 (0.07)*	***0.83 (0.00)***	*0.66 (0.00)*	*0.29 (0.05)*	*0.21 (0.15)*	*0.63 (0.00)*	*0.48 (0.00)*	-	-
SP volume	*0.3 (0.04)*	*0.26 (0.08)*	*0.49 (0.00)*	***0.71 (0.00)***	*0.1 (0.51)*	*0.11 (0.45)*	*0.26 (0.08)*	*0.24 (0.10)*	*0.71 (0.00)*	-

Spearman's correlations presented with rho and (p value)

(Table 3.6) Regression analysis of the association between focal cortical lesions MTR values as a dependant variable and lesion volume, disease duration, gender and age as independent variables

	Coefficient	*P value*	*95% conf. Interval*	
Lesion Volume	*- 5.82*	*0.045*	*- 0.0000115*	*- 1.26*
Disease Duration	*- 0.0022198*	*0.89*	*- 0.0047936*	*0.0003541*
Gender	*- 0.0070501*	*0.763*	*- 0.0541456*	*0.0400454*
Age	*0.000643*	*0.63*	*- 0.0020354*	*0.0033214*

Regression illustrates that individual cortical lesions correlate significantly with the degree of damage in those lesions. With bigger lesions show more damage reflected in their mean MTR values. Disease duration, gender, and age don't show as significant association.

3.6 Discussion:

In this study, individual CL in SPMS patients had lower mean MTR values than those in CIS and RRMS patients, suggesting a higher degree of tissue destruction and demyelination. In agreement with previous studies I also found that patients with progressive MS had higher CL load than those with non-progressive phenotypes.[138]

In agreement with previous reports, I found that SPMS patients had the highest CL count and volume, especially Mixed lesions (MXS).[84] Subpial lesion volume was larger in the progressive forms of the disease, although the difference was to a smaller extent. This could be due to the shorter disease duration for our SPMS patients, as previous pathology studies suggested that subpial lesions tend to accumulate in later stages of the disease.[139]

Early MS patients may have more extensive capacity to repair; while in the later stages of the disease there may be a reduction in the effectiveness of remyelination.[140] Alternatively, irrespective of remyelination, more chronic lesions in the progressive stage could simply show the result of continuing progressive demyelination. Only longitudinal studies will be able to determine the contribution of demyelination and remyelination processes to the final degree of tissue damage in CL.

The individual lesion damage appears to have clinical significance. In this study, MTR of CL was independently associated with the degree of disability in MS patients, a finding that we could not replicate when we examined the MTR value of WM lesions. This is additional evidence in support of the contribution of CL to the degree of disability over WML. However, despite the

significant association between CL mean MTR values and physical disability, we cannot assume causality as other factor could contribute as significantly. For example, we did not take into account various elements, such as NAWM volume or topographical distribution of cortical lesions, which could be relevant.

In a previous longitudinal study[138], it was found that development of new DIR CL was associated with deterioration of EDSS. Nielsen *et al*[141] found that using 7T T2* scanning, mixed and subpial CL correlated with disability. In our study, using regression analysis and taking into account disease duration and other clinical parameters, I found that among all CL subtypes; only the volume and MTR values of mixed lesions were significantly associated with disability. It is possible that the proximity of mixed lesions to the cortex, hence the cell soma of long projecting axons, such as the corticospinal neurons, might play a more important role compared to more distant WM lesions.[142]

In several previous studies[53, 143], whole GM MTR was found to be lower in progressive than in early MS patients. I did not find a difference between NAGM MTR among the groups, nor compared to healthy controls. We cannot exclude low grade tissue damage in the NAGM but our findings are in agreement with previous pathology reports[144] suggesting that demyelination is most pronounced in CL surrounded by normal appearing cortical tissue. Our results suggest that the low cortical GM MTR found in other studies may have been caused by undetected focal CL[145] rather than diffuse GM disease.[103]

In this study, the duration of the disease seems to be correlated to the degree of destruction within lesion tissue. On the other hand, the tissue destruction observed with time might not be linear. Agosta *et al*. ,who studied the whole GM rather than just focal lesions, found that after 12 months, the reduction in GM MTR was higher in the progressive than in the early stages of the disease.[146] As reported previously, I found that cortical lesions are more common in late disease, possibly reflecting accumulation of cortical lesions with increasing disease duration.[147] I, like others,[138] found increasing WM lesion load with longer disease duration.

Directly comparing the mean MTR values for all lesion subtypes in this cohort, subpial lesions had the lowest MTR values compared to IC and mixed lesions. This is not surprising in view of the lower myelin content in the outermost layers of the cortex. Great care has been taken during manual segmentation of subpial lesions, which was done on the MTR sequence. An element of compromise of the mean MTR values due to involvement of CSF voxels cannot be completely excluded.

Although this study focused mainly on GM pathology, my findings related to the WM lesions were similar to those reported previously in the literature. The mean MTR values in SPMS patients were lower than PPMS, RRMS and CIS.[148]

It is difficult to explain the variation of lesion load as well as the difference of the degree of demyelination between different MS groups solely by the different disease duration or other demographic imbalances.[149] Bramow *et al* found that WM plaque types, as well as the plaque density, were similar

between PPMS and SPMS patients, although there was a difference between lesions in both groups in the degree of demyelination and remyelination. [149] Hence the variation in the lesions mean MTR values in our study might reflect different response to the usual mechanism of demyelination pronounced in the variation in the degrees of demyelination and remyelination rather than different mechanisms of the plaques formation in different types of the disease.

In this chapter we showed there is variation in the degree of damage in cortical lesions and its association with the degree of physical disability. As the contribution of normal appearing tissue damage in the degree of disability is well established, we wanted to explore the effect of cortical lesions on surrounding normal appearing tissue in the same patient cohort.

Chapter 4

Correlation of Normal Appearing Grey Matter and Normal Appearing White Matter to Cortical Lesions in MS: Cross Sectional Study

4.1 Abstract

Background

Pathological and MRI studies have shown that WM lesions cause axonal loss in the NAWM but the role that focal GM demyelination plays in determining NAWM or NAGM pathology has not yet been systemically explored yet, mainly due to the difficulties in detecting cortical demyelination using conventional MR imaging. MTR at ultra-high field benefits from increased signal to noise ratio, which makes it possible to study small variations across both WM and GM.

Hypothesis

We hypothesize that focal GM/WM lesions influence nearby NAWM/NAGM in patients with MS. We will test this hypothesis using ultra high resolution MT imaging.

Methods

42 MS patients (18 males/24 females; age= 46±10 years; EDSS= 3.5±2.4; disease duration= 6.3±5.7 years) and 10 age-matched controls were scanned on a Philips 7T platform. For each participant, high-resolution, 0.63 mm3, PSIR and MTR images were acquired, on which GM and WM lesions were manually segmented by 2 trained operators. Tissue maps were estimated using SPM8 and mean MTR ratios for NAWM and NAGM MTR were calculated as

a function of the distance to the nearest GM or WM lesion. The ratios were obtained by normalising each MTR values for the average MTR value in the corresponding normal appearing tissue. When considering NAWM, we computed a fibre tract-weighted mean rather than an arithmetic mean using the JHU tractography atlas, to model the influence of fibres. In order to distinguish between natural spatial variations in MTR and those induced by the presence of lesions, the above analysis was repeated, substituting control scans for the patient ones.

Results

In agreement with the literature, NAWM MTR was significantly reduced in the vicinity of WM lesions compared to controls, up to 10mm away from the closest lesions. There was also an effect of cortical lesions on both NAGM and NAWM but at shorter range (up to 3mm).

Discussion

The observed distance effect between GML, WML and NAWM suggests that WM lesions are important in the pathogenesis of NAWM damage and play a more significant role than GM lesions. This is probably due to the arborisation of cortical neurons in comparison with the much simpler pathways of WM tracts. It is also possible that GM lesions cause neuronal dysfunction rather than tissue loss.

Conclusion:

GM lesions appear to cause much less prominent long distance tissue damage in the NAWM and NAGM compared to WM lesions as detected with MTR.

4.2 Introduction:

4.2.1 Pathology on Normal Appearing Grey Matter in Multiple sclerosis

Early quantitative studies showed that there were lower MTR values in the cortical GM in different MS phenotypes in comparison with controls.[53] With the emergence of higher resolution MRI enabling the detection of very small lesions, as well as the development of more sophisticated quantitative MR techniques, studying of NAWM and NAGM has become even more promising. Understanding the abnormalities in the NAWM and NAGM is very important to help us understand the clinical progression in MS patients that is not fully explained by macroscopically visible pathology.[150]

In a study on a 102 patients of all MS subtypes, GM lesions were segmented using a diffusion anisotropy thresholding based technique. They observed a higher mean diffusivity in SPMS than other MS patients and controls; however no difference was noticed between both RRMS or PPMS patients and controls.[151] The difference in the GM diffusivity could be partially due to the small cortical lesions that went undetected with conventional MR scanners.

Another study showed a reduction of the average MTR as well as increase in the diffusivity in the NAGM in MS patients compared to controls after using manual as well as thresholding techniques to get rid of the lesions, Significantly these changes correlated with the T2 lesion load which suggests a role that lesions might play in inducing pathology in NAGM structure.[53]

On using MTR maps, it was noticed that the majority of patients show significant regional GM MTR decrease, although the extent of the regional GM injury in these regions did not exceed 25% of the volume of this region.[71] In a longitudinal study which followed PPMS patients over 1 year, it was noted

that cortical GM mean MTR value decreased more than rate of reduction in NAWM , it was suggested that this could be due to the development of new cortical lesions which went undetected with conventional imaging.[152]

The variation of GM MTR across groups could be due to cortical lesions going undetected which is supported by the lower GM mean MTR values on comparing the RRMS with the PPMS and the SPMS patient groups.[151] Another theory is that WM lesions cause retrograde degeneration of the GM neurons which could be explained by the detected correlation between WM T2 lesion volume and MTR of the GM.[151]

4.2.2 Pathology of Normal Appearing White matter in Multiple Sclerosis

Focal cortical and WM demyelination has long been the main feature of MS pathology. Physical and cognitive disability cannot be fully explained by the focal cortical and WM demyelination. Hence interest has grown over the last few years in exploring the extent of normal appearing tissue involvement in patients with MS.

Studies focusing on the NAWM in MS started as early as 1978 in a combined histological, biochemical and histochemical study, which showed widespread changes in the NAWM, mainly astrogliosis, microglial activation, vascular hyalinisation, blood brain barrier breakdown, reduced myelin density, axonal loss and remyelination.[153,154] Similar findings were found in WM tissue of patients with mild and mainly spinal varies of MS.[155] NAWM is the term used to describe parts of the WM in MS that appear normal on some MRI sequences but have been found to be abnormal[153] using different imaging methods.[156,65] Unlike MS plaques, NAWM tissue is normally myelinated, however it shows some axonal changes.[24] Diffuse NAWM involvement can start early in the

disease course. Widespread gliosis and astrocytic proliferation in the WM away from the WM lesions has been described.[157] Recently pathology studies described changes in NAWM, in the form of diffuse inflammatory reaction that consisted of perivascular cuffs of mononuclear cells and a diffuse infiltration of the tissue by T-lymphocytes and microglia activation. This widespread injury was more pronounced in patients with progressive forms of the disease than those with relapsing MS. Moreover MRS studies showed that reversible and irreversible neuronal injury in NAWM shows different patterns in RRMS from PPMS.[158]

Reduction of axonal density and volume by around 34% and 66%, respectively, was found in the corpus callosum of MS patients, and the reduction in axonal density correlated with regional white matter lesion load in the brains of individual patients.[159]

The evidence of the contribution of WM lesions to NAWM damage is conflicting. A correlation was established between the extent of NAWM injury and cortical demyelination, while only a weak correlation was detected between WM lesion load and NAWM injury. Further analysis of individual cases in that study further supported the view that diffuse WM injury and cortical lesions develop independent from WM lesions.[15] On the other hand, MTR values were significantly lower in the NAWM surrounding a WM lesion and increased gradually with increasing distance from the lesion.[160] The pathological component in NAWM with subtle MTR changes vary depending on their proximity to WM lesions.[161] Axonal pathology and microglial activation seem to be responsible for the slight changes in NAWM MTR values in NAWM tissue that is close to WM lesions, while microglial

activation alone was noticed in NAWM regions far from the WM lesions. NAWM changes are apparent in all MS phenotypes even in patients at first presentation with CIS.[162]

Contribution of MRI in Assessing Normal Appearing Tissue Damage in MS

GM MTR not NAWM MTR in normal controls was found to decrease with increase in age.[145] Studies investigated the early involvement of normal appearing tissue in patients with MS using MTI and DTI giving their tissue quantitative values.[163] They found that involvement of the WM starts earlier than the GM. Compared to healthy volunteers, patients with early MS (mean disease duration of 2 years) had significantly higher MD and FA peak height and lower average FA of the NAWM. On the other hand, there was neither MTR nor diffusion differences in the GM between the groups.

Proton magnetic resonance spectroscopy (H-MRS) is a non-conventional MR technique that has the potential to assess the microstructural damage in the normal appearing tissue in MS brains in vivo. In RRMS patients, NAA, Glx and Cho were all reduced in CGM in comparison with healthy controls, whilst the changes in the NAWM were more limited than for CGM, moreover Ins was significantly elevated and modestly related to T2 lesion loads.[164] T2 relaxation time was used to compare normal grey matter (NGM) tissue to NAGM tissue and similarly normal white matter (NWM) tissue to NAWM tissue. They found that NAGM did not show any significant difference form NGM, while average myelin content was around 15% less in NAWM than in NWM.[165] Another study showed that NAWM mean MTR and PL was lower on comparing MS patients versus controls, RRMS and SPMS versus CIS but not RRMS versus SPMS, while GM PH MTR was significantly lower on

comparing MS patients versus controls, SPMS and RRMS versus CIS and SPMS versus RRMS.[70] Similar observation was noted using DTI, with GM pathology more prominent in in SPMS and PPMS than in those with other forms of the disease.[151] In another study, each of the RRMS and SPMS patient groups had a significantly smaller MTR histogram mean values in NAGM and NAWM than in controls.[166] NAA was also found to be reduced and Cr increased in NAWM in RRMS and PPMS patients suggesting that axonal dysfunction extends beyond focal lesions.[158]

MTI was used to examine MS patients in a longitudinal study and showed that NAWM MTR values correlated highly with the length of time since the patient's initial clinical presentation. Moreover NAWM MTR values showed gradual steady decline over 12 months period.[167]

NAWMMTR and GMMTR at baseline in early MS patients are lower than those in controls. Moreover progressive decline of cortical GMMTR was noted in early MS patients when followed up over a year. Similar decline was observed in the NAWMMTR in comparison with controls.[168] The rate of change in GMMTR was significantly greater than the rate of change in NAWM.[168] No correlations were found between the rate of change and EDSS, cognitive decline, or T2 lesion load in the patient group.[168] More interestingly, on assuming the gradient of change in controls was zero and in patients was linear at all times, NAWM MTR abnormality began 2.9 years before clinical onset, while NAGM MTR changes began 0.4 years after clinical onset.[168]

A study on 12 RRMS patients using 1.5 T scanner compared diffusion tensor MR and MT imaging to assess the variation in pathological entity of different

regions in NAWM amongst patients and between patients and controls.[169] Interestingly, the FA values and mean MTR values were significantly different between WM plaques, perilesional NAWM, and remote NAWM regions, with the values being the lowest in the WM plaques, followed by perilesional NAWM regions, while remote NAWM regions had the highest values.[169] On the other hand, the correlation between FA and mean MTR values of individual lesions was poor.[169]

T1 and T2 relaxation times were found to be longer in NAWM tissue in MS patients compared to controls.[170] The difference in the relaxation times from values in the healthy controls was ascribed partially to the abundance of invisible lesions in the WM.[171] T1 relaxation time of cortical GM was significantly longer in patients than in controls which was ascribed to the possibility of partial volume effect induced by the global cortical atrophy in MS patients.[172]

Correlation of Normal Appearing Tissue Damage with Clinical Parameters in MS Patients

Conventional MR imaging has been found to be invaluable in examining the extent of the disease and its evolution via examining the extent of macroscopic WM abnormalities. On the other hand, microstructural changes in the NAWM reflected by low MTR values were found to correlate highly with the level of physical disability and cognitive impairment in MS patients.[173,174,175] NAWM MTR was shown to correlate highly with the disease duration,[167] and was found to prospectively predict the worsening of clinical disability in individual MS patients.[175]

A cross sectional study on 31 RRMS patients showed a significant negative correlation between EDSS and N-Acetyl Aspartate/creatine (NAA/Cr ratio) levels in NAWM in these patients, however there was no significant correlation with disability measured by MSFC.

NAWM as well as GM demyelination have been found to correlate to the clinical outcome, with GM having more influence than NAWM.[70]

In PPMS patients a correlation was found between GMMTR, but not NAWMMTR and EDSS.[145] On the other hand, in a longitudinal study on PPMS patients it was noted that low baseline NAWM MTR values predicted EDSS and MSFC deterioration in patients 1 year later.[152]

The PSIR sequence [102,176,103] was introduced to provide T1-weighted (T1w) images of the brain with increased contrast to noise ratio (CNR) compared to other anatomical sequences such as MPRAGE, in a fashion similar to the MP2RAGE modality.[135,177] In a typical PSIR acquisition, two images are collected during a single inversion recovery and the phase images are used to restore the sign of the inversion recovery signal in the magnitude images, thereby doubling the dynamic range in the reconstructed image. In turn, this increase in range can be exploited by increasing image resolution, reducing scanning time or, crucially, increasing image contrast. PSIR has been shown to improve classification of cortical lesions.[176] A recent large study indicated that combining PSIR and DIR at 3T increased the rate of detection of cortical GM lesions.[103]

MTR at 7T benefits from increased signal to noise ratio and increased sensitivity to MT due to the increase in longitudinal relaxation times with field

strength. Overall this provides a two-fold increase in the CNR between GM and WM in MT images at 7T compared to 3T.[178] This gain in sensitivity has allowed acquiring high spatial resolution MT data to study variations in MT across the cortex.[117] Although ultra-high field MRI is more prone to the effects of RF and static field inhomogeneities we have overcome these effects with the use of Ropele et al. approach mainly by field mapping and post-processing.[137,117] Indeed MTR has been shown to be sensitive in detecting focal and diffuse abnormalities in the GM.[179]

The aim from this analysis is to explore the relationship between focal GM/WM lesions and NAWM/NAGM in patients with MS, using ultra high resolution MT imaging.

Previous work outlining the effect of different types of lesions on normal appearing tissue is illustrated in Table 4.1

(Table 4.1) Literature illustrating the effect of different types of lesions on various normal appearing tissues

Lesions type effect	Publications	Nature of the study	Effect
WM lesions to NAWM NAWM to GM	"Regional axonal loss in the corpus callosum correlates with cerebral white matter lesion volume and distribution in multiple sclerosis." Brain 123.9 (2000): 1845-1849.	Pathology	Total number of axons crossing the corpus callosum (CC) was reduced in MS brains in comparison with controls. WML load in the anterior, middle and posterior cerebral WM volumes correlated with both axonal density and total number of axons crossing the CC in the corresponding region. This correlation remained strong on testing the data on data from individual patients.
	"A longitudinal study of MR diffusion changes in normal appearing white matter of patients with early multiple sclerosis" Magn Reson Imaging. 2002 Jun;20(5):383-8	MRI acquired in 19 CIS patients (16 converted to MS during the study period)at baseline and 12 months later	No significant difference in trace of apparent diffusion coefficient (TADC) was found between NAWM in CIS patients and controls at baseline but significant difference was noticed at the end of 12 months period. WML load in T2 correlated with NAWM TADC at the end of the study.

Lesions type effect	Publication	Nature of the study	Effect
WM lesions to NAWM NAWM to GM	"Meningeal T cells associate with diffuse axonal loss in multiple sclerosis spinal cords" Ann Neurol. 2010 Oct;68(4):465-76. doi: 10.1002/ana.22054	Pathology on 13 SPMS, 5PPMS	CD3- T, not B cells, were observed in similar density in the NAWM and NAGM of the spinal cords of MS patients' not in controls. Axonal density is around 24% less in the NAWM of MS patients in comparison of controls.[70]
	"Absolute quantification of brain metabolites by proton magnetic resonance spectroscopy in normal appearing white matter of multiple sclerosis patients" Brain (1999), 122, 513–521	1.5 T MRI. 27 RRMS, 13 SPMS, 12 healthy controls	A significant decrease in the N-acetyl-aspartate concentration in NAWM in MS patients than control and in SPMS than in RRMS. The decrease in N-acetyl-aspartate correlated with WM lesion load and EDSS.
	"Assessment of normal-appearing white and gray matter in patients with primary progressive multiple sclerosis A Diffusion-Tensor Magnetic Resonance Imaging Study" Arch Neurol. 2002 Sep;59(9):1406-12	MRI DTI 1.5T.	Average diffusivity higher in SPMS than in PPMS in WM lesions, NAWM and NAGM.

Lesions type effect	Publication	Nature of the study	Effect
WM lesions to NAWM NAWM to GM	"Axonal injury in the cerebral normal-appearing white matter of patients with multiple sclerosis is related to concurrent demyelination in lesions but not to concurrent demyelination in normal-appearing white matter" NeuroImage 29 (2006) 637 – 642	MRI spectroscopy	The fractional size of the restricted pool in NAWM (F-NAWM) was significantly smaller in patients than controls but not different between RRMS and SPMS. Mean F within Tw lesions was lower than in NAWM
	"Water diffusion is elevated in widespread regions of normal-appearing white matter in multiple sclerosis and correlates with diffusion in focal lesions Mult Scler April 2001 vol. 7 no. 2 83-89	1.5 T PD, EPI diffusion imaging.	Water diffusion is elevated in NAWM and correlated with diffusion in WM lesions. No significant difference between MS subtypes but was higher than controls. WML had the highest ADC. WML ADC correlated with NAWM ADC values, with correlation higher in PPMS and benign MS than in RRMS and SPMS.

Lesions type effect	Publication	Nature of the study	Effect
WM lesions to NAWM NAWM to GM	"Axonal loss in normal-appearing white matter in a patient with acute MS" NEUROLOGY 2001;57:1248–1252	Pathology	Spinal cord ventral column in MS patients showed a 22% axonal loss despite the absence of MS lesions in the spinal cord post-mortem, with normal axonal numbers in the posterior column. This could be explained by the remote effect of the lesion at the cervicomedullary junction.
	"The impact of isolated lesions on white-matter fiber tracts in multiple sclerosis patients" NeuroImage: 8 (2015) 110–116	MRI, DTI	Directly at the lesion site, FA values decreased in the ipsilesional fibers in the 21 patients with lesions affecting the ICP compared to the Corresponding site in HC and contralesional NAWM. A decrease in FA was also observed in the contralesional NAWM relative to HC

Lesions type effect	Publication	Nature of the study	Effect
WM lesions to NAWM NAWM to GM	"Brain metabolite changes in cortical grey and normal-appearing white matter in clinically early relapsing remitting multiple sclerosis" Brain. 2002 Oct;125(Pt 10):2342-52	MRI, 27 RRMS patients, 29 Controls	Reduced NAA, Cho and Glx in CGM in MS pts in comparison with controls and this was unrelated to WM lesion load. Metabolite changes in NAWM more limited than for CGM with Ins significantly elevated and modestly related to T2 lesion load.
	"Cortical demyelination and diffuse white matter injury in multiple sclerosis" Brain (2005), 128, 2705–2712	Pathology 52 MS patients, 30 controls	Diffuse WM injury in part correlates with cortical demyelination but not with focal WMLs. Diffuse axonal injury in NAWM more pronounced in PPMS and SPMS than in early MS.
	"Unravelling the relationship Between Regional Gray Matter Atrophy and Pathology in Connected White Matter Tracts in Long-Standing MS" Hum Brain Mapp. 2015 ;36(5):1796-807	Conventional and DTI on 3T was performed in 208 pts. And 60 controls.	Cortical atrophy prominent in frontal and temporal regions. Tractography using robabilistic atlas based on healthy controls. Pathology in connected WM tracts y explained 82% of cortical GM atrophy in RRMS.

Lesions type effect	Publication	Nature of the study	Distance measured	Effect
WM lesions to NAWM NAWM to GM	"Normal-appearing white matter changes vary with distance to lesions in multiple sclerosis" AJNR Am J Neuroradiol. 2006 Oct;27(9):2005-11.	MRI (MTR) 63 RRMS	1×1×2 (4 layers)	MTR histograms of distant NAWM in controls similar to patients. 2 Layers near the lesions had low MTR
	"Multiple sclerosis normal-appearing white matter: pathology-imaging correlations" Ann Neurol. 2011 Nov;70(5):764-73	Pathology and 1.5 Tesla postmortem MTR, DTI in 4 SPMS brains.	NAWM close (1-4mm), NAWM far (≥5mm)	Abnormal looking voxels 0.2mm distance from lesion low MTR. Sa-WM close and far similar mean MTR. All sa-WM Far as well as many sa-WM Close ROIs were located in close proximity to the cortex.
	"Evaluation of Normal Appearing White Matter in Multiple Sclerosis Comparison of Diffusion Magnetic Resonance, Magnetization Transfer Imaging and Multivoxel Magnetic Resonance Spectroscopy Findings with Expanded Disability Status Scale" Clin Neuroradiol. 2011 Nov;21(4):207-15	MTR, DTI. 30 pts, 30 controls	WML, perilesions, NAWM (1cm from lesions)	MTR lowest in lesions than perilesional then NAWM

Lesions type effect	Publication	Nature of the study	Effect
WM lesions to NAGM	"Magnetisation transfer ratio and mean diffusivity of normal appearing white and grey matter from patients with multiple sclerosis". J Neurol Neurosurg Psychiatry 2001;70:311-317	MRI, MTR	Correlation between T2 lesion load and NAGM MTR histogram
GM lesions to NAWM	"Cortical lesion load correlates with diffuse injury of multiple sclerosis normal appearing white matter" Mult Scler. 2014 Feb; 20(2):227-33.	MTR at MRI 7T	Cortical lesion volumes and counts all had significant correlation with NAWM mean MTR. The strongest correlation was with cortical lesion volumes obtained using MTR images. WML volume had no significant correlation with NAWM mean MTR.

4.3 Patients and Methods:

Forty-two patients with MS (18 males, 24 females) between 18 and 65 years of age were recruited from the Multiple Sclerosis outpatient clinics, at Nottingham University Hospitals. The patients included 11 with CIS, 11 RRMS, 10 PPMS, 10 SPMS. This is the same cohort of patients that was recruited for the study in chapter 3. None of the subjects had any other comorbidities (such as diabetes, uncontrolled hypertension, major depression, or epilepsy) apart from MS. Patients included did not have a relapse within 3 months from the scanning. All patients underwent a neurological examination and were scored on the Expanded Disability Status Scale. Ten healthy controls (4 males and 6 females) were also recruited.

Acquisitions:

We acquired on all subjects PSIR and MTR as per protocol described in Chapter 3.

Image Analysis:

All MR images were inspected for artifacts that could affect image analysis, such as movement noise, susceptibility or Gibbs effects.

Inhomogeneity correction and co-registration:

The relative effect of B1 field inhomogeneity on MTR values was corrected using Ropele *et al* approach,[137] which models the relationship between the B1 error and the MTR error as linear, a reasonable assumption both in theory and in practice. The PSIR images were inherently corrected for inhomogeneities. For each participant (patient and control), the PSIR image was linearly registered onto the MTR image using SPM8's "Coregister" approach.[180] The default parameters were used and normalised mutual information was selected

as the objective function since the registration task was multimodal. Tissue maps were estimated using SPM8 and the mean MTR ratios for NAWM and NAGM were calculated as a function of the distance to the nearest GM or WM lesion. The ratios were obtained by normalising each MTR values by the average MTR value in the corresponding normal appearing tissue. When considering NAWM, a fibre tract-weighted mean rather than an arithmetic mean was computed using the JHU tractography atlas, to model the influence of fibres. In order to distinguish between natural spatial variations in MTR and those induced by the presence of lesions, the above analysis was repeated by substituting control scans for the patient ones. To remove the influence of WM lesion on WM MTR, all WM tissue closer than 5 voxels (5mm) from the nearest WM lesion was excluded. Then the same analysis was repeated examining the effect of WML on mean NAWM MTR values as a function of a distance to the nearest WM lesion.

Statistical Analysis:

Since the NAWM mean MTR values and NAGM mean MTR values for all disease subtypes are not normally distributed, I used Kruskall Wallis to compare values across groups. Pair wise comparison was performed and correction for multiple comparisons was applied. Pearson's coefficient was used to examine the relationship of NAWM mean MTR values and NAGM mean MTR values with the WML volume and CL volume. For each patient, we estimated the parameters for a robust linear regression of the average NAWM MTR value on the distance to the nearest GM lesion using iteratively reweighted least squares. Similarly we examined the effect of WM lesions on mean NAWM MTR values as a function of distance to the nearest lesion.

Spearman's rank correlation analysis was used to explore the relationship of clinical parameters (mainly EDSS and disease duration) with NAWM mean MTR values and NAGM mean MTR values.

4.4 Results:

The mean NAGM MTR and mean NAWM MTR values for patients of all disease phenotypes are displayed in table 4.2.

Mean NAGM MTR and mean NAWM MTR values were lower with the chronicity of the disease. Mean MTR values were also lower in NAGM than in NAWM. The variation in mean MTR values of NAGM and NAWM across groups did not reach statistical significance after correcting for multiple comparisons.

NAWM mean MTR values and NAGM mean MTR values correlated significantly with WML volume (*rho* = -0.54, *p* = 0.001 and *rho* = -0.39, *p* = <0.05 respectively), not with cortical lesions volume (*rho* = - 0.23, *p* = 0.17 and rho = - 0.15, *p* = 0.34 respectively).

A one sample Wilcoxon signed rank for the association between cortical lesions and mean NAWM MTR values, showed that the regression slopes were not significant. NAWM MTR was significantly reduced in the vicinity of WM lesions with respect to controls, up to 10mm away from the closest lesions. We also found an effect of cortical lesions on both NAGM and NAWM but at shorter range (up to 3mm). Figure 4.1&4.2

A significant correlation was demonstrated of disease duration with NAWM mean MTR values and NAGM mean MTR values with *rho* = -0.64. *p* = 0.00 and *rho* = - 0.36, *p* = 0.25 respectively. A negative correlation was noted

between NAWM mean MTR values and age (*rho* = -0.41, *p* = 0.01) while no correlation was detected between NAGM mean MTR values and age. No correlation was detected between NAWM mean MTR values, NAGM mean MTR values and gender. EDSS correlated significantly with both NAWM mean MTR values and NAGM mean MTR values, although the correlation with the former was higher (*rho*= - 0.57, *p*= 0.00 and *rho* = - 0.33, *p* = 0.03 respectively).

(Table 4.2) Mean NAGM and NAWM MTR values in different MS phenotypes and healthy controls

	CIS	*RRMS*	*PPMS*	*SPMS*	*Control*
NAGM MTR mean (std)	***0.318 (0.05)***	***0.297 (0.036)***	***0.310 (0.064)***	***0.250 (0.044)***	***0.322 (0.056)***
NAWM MTR mean (std)	***0.449 (0.018)***	***0.438 (0.034)***	***0.406 (0.027)***	***0.372 (0.063)***	***0.481 (0.056)***

NAGM = Normal Appearing Grey Matter, NAWM = Normal Appearing White Matter

(Figure 4.1) Mean MTR values as a function of distance from cortical lesions

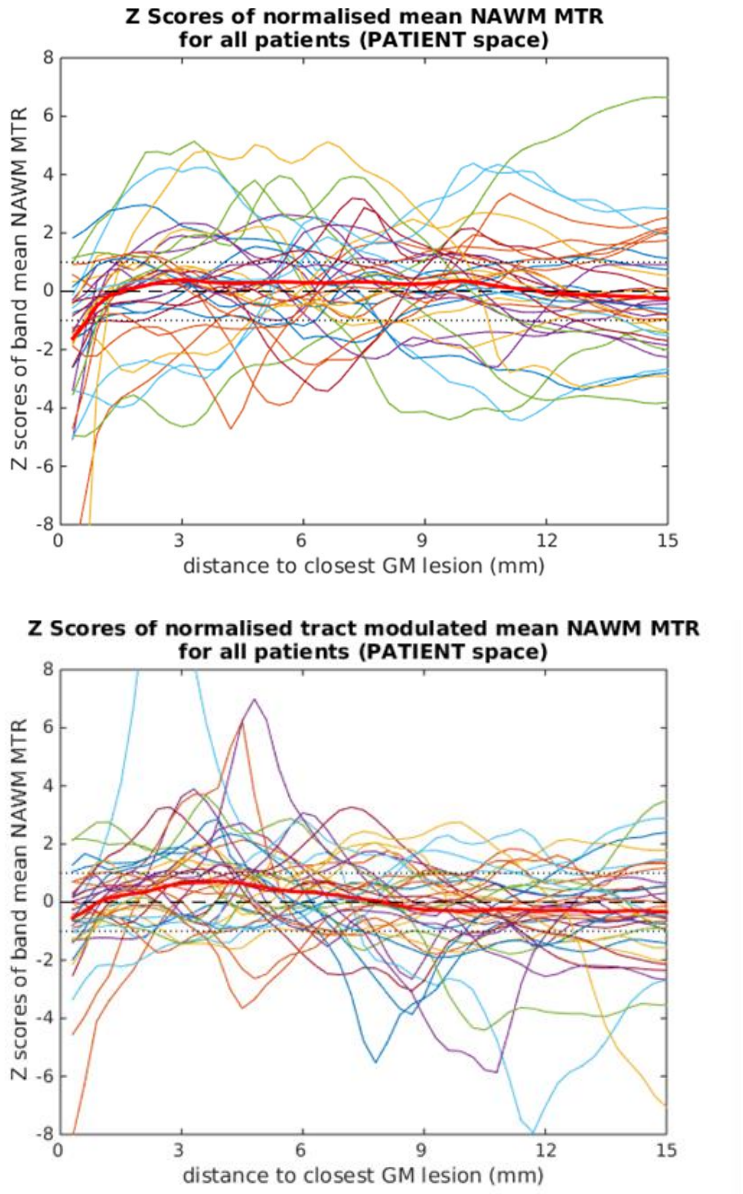


Figure illustrating mean MTR values of NAWM tissue as a function of the distance to the nearest GM lesion. Arithmetic mean values are presented in the upper panel, while the lower panel shows fibre tract weighted means. Each line represents a patient, with the red line representing the mean for all patients. Both figures show that mean MTR values are the lowest in NAWM tissue just outside the GM lesions. The meat MTR values increase with the distance from the lesion. The effect of GM lesion on NAWM tissue extends to 3mm from the GM lesion before reaching a plateau.

(Figure 4.2) Mean MTR values as a function of distance from white matter lesions

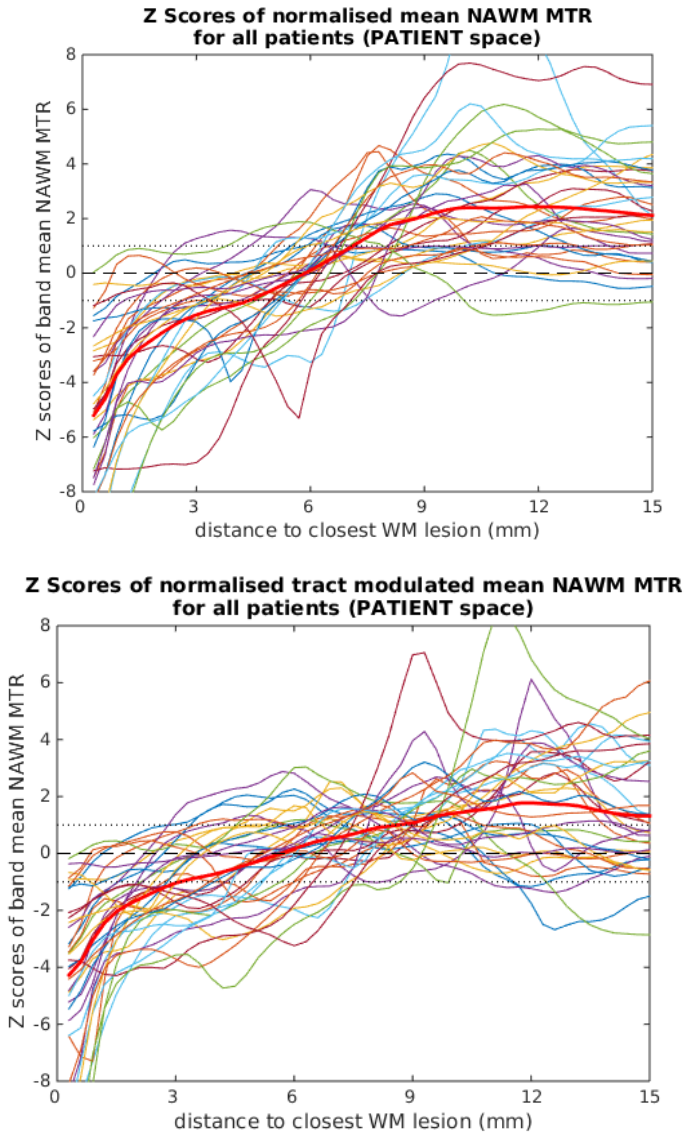
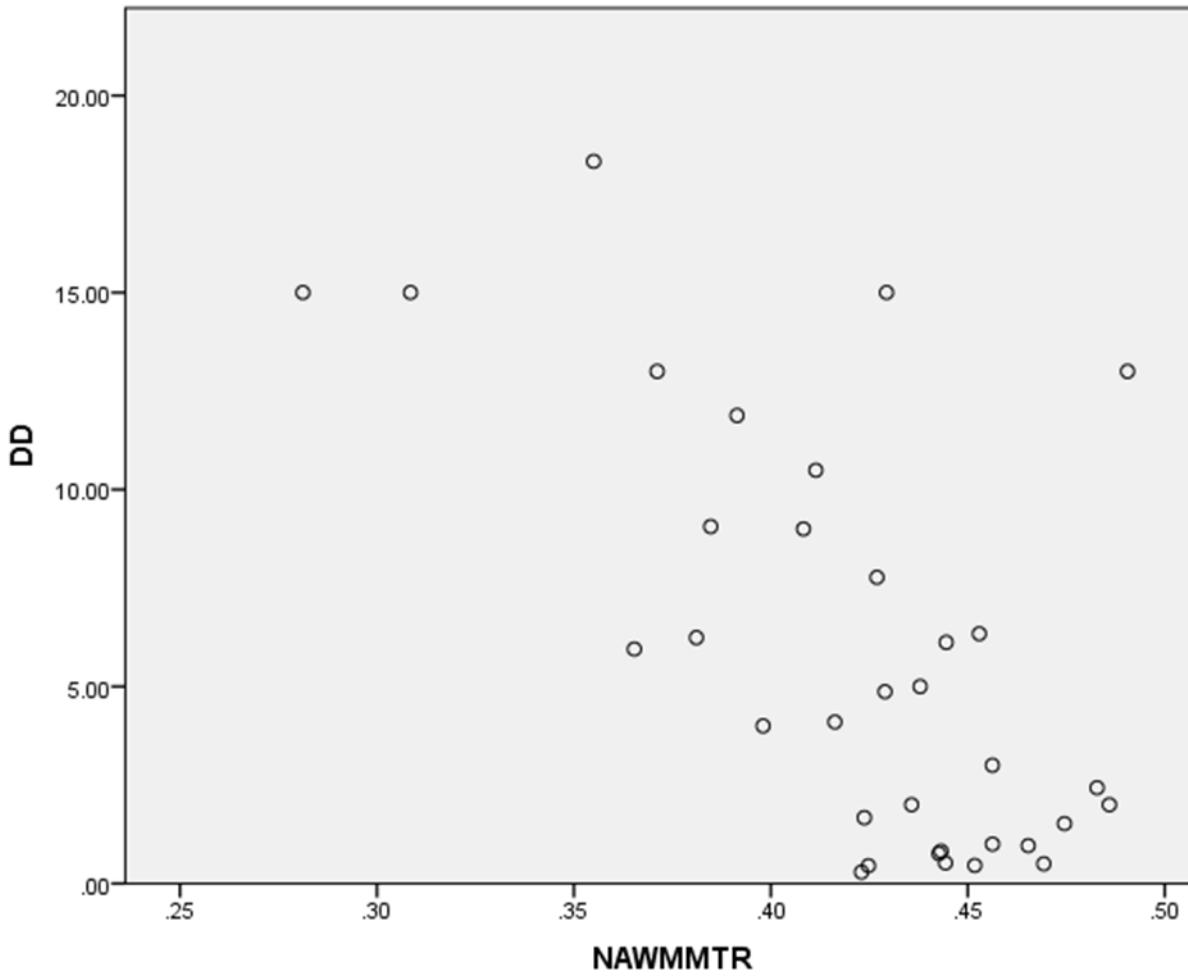


Figure illustrating mean MTR values of NAWM tissue as a function of a distance to the nearest WM lesion. In the upper panel arithmetic mean values are shown, while the bottom panel shows fibre tract weighted means. Each line represents a patient and the red line represents the mean for all patients. Both figures show that mean MTR values are the lowest in NAWM tissue just outside the WM lesions. Mean MTR values increase with the distance from the lesion. The effect of WM lesion on NAWM tissue extends to 10 mm from the WM lesion before reaching a plateau.

(Figure 4.3) Correlation between disease duration and NAWM mean MTR values



The figure illustrates the significant negative correlation between disease duration and NAWM mean MTR values in MS patients. (*rho* = - 0.64, P< 0.05)

4.5 Discussion:

Cortical demyelination and diffuse WM injury are more prominent in patients with progressive forms of the disease than in RRMS or CIS patients. On the other hand, the pathogenesis of the diffuse injury in the NAWM is controversial. The observed distance effect between NAWM and WM lesions suggests white matter lesions are important in the pathogenesis of NAWM damage. Two possible pathogenic mechanisms have been proposed for the diffuse axonal loss in the NAWM. The first mechanism is due to diffuse axonopathy which independent from the focal demyelinating changes, and this could simply apply to PPMS patients who have diffusely spread NAWM changes despite very small lesion load. On the other hand axonal loss in NAWM could be secondary to retrograde wallerian degeneration of the axons transecting demyelinated lesions. Supporting the second theory is the work by Evangelou *et al*, who reported that the WML load correlated with the axonal density and the number of axons crossing the corpus callosum in the corresponding region, and this correlation held strong on applying on individual patients.[159] The low MTR values of the NAWM regions close to the WM lesions or the cortical demyelinated lesions suggests that either WM or cortical lesions may contribute to these changes.[161]

CD3+ T cells (CD4+ &CD8+ with predominance of CD8+ T cells), not B cells, were observed in the NAWM and NAGM of the spinal cords of MS patients' not in controls.[181] The density was similar in NAWM and NAGM. Axonal density was around 24% less in the NAWM of MS patients compared to controls.[181] Interestingly, the extent of diffuse axonal loss in NAWM

correlated with the density of MHC II^+ microglia in the NAWM as well as with $CD3^+$ T cells in meninges..[181] Post mortem studies showed reduction in axonal density in NAWM in SPMS brains by around 34%.[27]

Axonal injury in early stages of the disease can cause significant axonal loss in NAWM distal to the lesion while sometimes sparing myelin in these areas.[157] The spinal cord ventral column in MS patients showed a 22%axonal loss despite the absence of MS lesions in the spinal cord post-mortem, with normal axonal numbers in the posterior column.[157] This could be explained by the remote effect of a lesion at the cervicomedullary junction.[157]

An interesting longitudinal MRI study followed 5 cases over a year. They found that T2-hyperintense lesions develop along the ipsilateral corticospinal tract following an initial lesion developing proximally in the corona radiata.[182] They suggested that axonal injury can occur distally as a result of a proximal acute MS lesion, which in turn can cause subsequent T2-hyperintense lesions that are temporally and spatially dissociated from inflammatory activity.[182] In this study, they measured Wallerian degeneration by the presence of T2-hyperintense abnormality developing along the course of the corticospinal tract inferior and contiguous to an initial acute T2-hyperintense lesion.

The density of T cells, B cells, and CD68+ macrophages is higher in the spinal cord meninges of MS patients in comparison with controls.[181] The density of lymphocytes in the meninges was strikingly higher than in the NAWM and NAGM.[181]

Significant reduction in MTR was found in NAWM regions up to 3 months prior to the appearance of gadolinium enhancing lesion, which could explained

by the susceptibility of these regions of NAWM to the development of new lesions, or possibly that new lesions form in NAWM before the disruption of the BBB as indicated by the gadolinium enhanced lesions.[183] This view is supported by observing the NAWM uptake of PK11195, a positron emission tomography radioligand which is a marker of activated microglia.[184] Another possibility is the presence of a degree of leakage in the BBB with trafficking of pathogenic T cells into the CNS.[185] It is also possible that the pathology in the NAWM plays a significant role in influencing the pathological response to a lesion within different areas of the brain.[170] This is supported by the reduced frequency of T1 hypo-intense regions in certain areas of the brain. The degree of axonal loss in the corpus callosum was found to correlate with the lesion load of the corresponding projection areas which suggested mutual local and collateral effects on the changes in NAWM.[27] De Stefano *et al* found normal NAA/Cr ratio in normal appearing tissue in the hemisphere contralateral to the lesion in the acute phase. However, the ratio was reduced in the same region 1 month later. They suggested that the abnormalities in NAWM could be due to long tract injury with remote effect on connected brain regions. Of note, in all patients there was a lesion detected near the corpus callosum s.[186] Only modest correlation was detected between normal appearing tissue MTR histograms and T2 lesion load suggesting a more widespread pathology rather than mainly the influence of the lesions.[187]

In a previous report NAWM mean MTR value was found to vary depending on the distance to WM lesions, with the regions close to the WM lesions showing more axonal swelling than areas further away.[161] They found that NAWM regions with reduced mean MTR values were often located close to cortical

lesions as well.[161] It was suggested that cortical lesions might contribute indirectly to the degree of damage in the NAWM that is reflected in their mean MTR values.[161] In this study the proximity to WM lesions, rather than to cortical lesions influenced the mean MTR values in the NAWM. On taking into account the fiber tracts going between GM and WM, the NAWM regions located in the vicinity of the fiber tracts course were more prone to have lower mean MTR values than those located in the surrounding areas.

There are several factors that could influence mean MTR values in the NAWM, such as the location in relation to the ventricular CSF interface. Mean MTR values were found to be lower in the NAWM regions close to the ventricles in MS patients.[188]

D Pelletier *et al* found that changes in NAWM expressed in the form of reductions of mean NAA:Cr were found in PPMS patients with high and low lesion load in comparison with controls. More importantly, there was no significant difference between the high and low lesion load groups in the mean level of NAA:CR.[189] This in turn supports the view that widespread NAWM damage happens independently of focal tissue demyelination, resulting in diffuse NAWM injury in PPMS patient despite the small load of focal tissue demyelination.

Previous studies showed that T2 lesion load had negative correlation with cortical GM mean MTR values in RRMS and SPMS patients.[166]

The variation in the degree of damage of NAGM as indicated by their MTR values unlike other studies was not statistically significant, which could be either due to segmenting the cortical lesions out, which probably went

undetected on using previous conventional scans, and hence contributed to the previously reported high degree of damage in progressive forms of the disease. Another possibility is the small number of subjects in each of our patients groups making comparison between values in each group difficult. On the other hand on comparing the CIS and RRMS patients as one group against the PPMS and SPMS in one group, we still could not find a significant difference between the two groups in the NAGM mean MTR values.

One of the advantages of this work is the ability to study both GM lesions and the NAWM using a semiquantitative imaging methods that has been correlated previously with tissue integrity, and more specifically with myelin both in the grey and WM. In this study WML segmentation was done using a 7T T1 based sequence which was shown previously to be more sensitive than clinical FLAIR. In this way we have possibly detected most of the small white matter lesions that have been considered a possible cause of contamination when NAWM was examined in previous studies.

We showed previously that the variation in the degree of damage in cortical lesions as reflected by their mean MTR values has a significant role in the degree of physical disability. However, cognitive decline is considered one of the most disabling MS symptoms as disease progresses; therefore, in the next chapter I will explore the contribution of the degree of damage in cortical lesions as indicated by mean MTR values to patients' cognitive performance.

Chapter 5

The contribution of different cortical lesions' parameters and neocortical volume to physical and cognitive performance in MS patients

5.1 Abstract

Background

Cognitive impairment has long been described in association with MS. The role GM plays in MS generally and cognitive impairment specifically has come under more scrutiny during recent years. Recently, it was reported that cognitive impairment correlates better with cortical lesion load than with cortical atrophy.

Hypothesis:

We hypothesise that the degree of damage in cortical lesions as well as in NAGM as reflected by their mean MTR values plays an important role in cognitive decline in MS patients. We aim to examine the contribution of cortical lesion volume and their MTR values, as well as cortical volume and NAGM MTR values to the degree of cognitive impairment using a simple practical cognitive test, the Brief International Cognitive Assessment for Multiple Sclerosis (BICAMS).

Methods:

42 MS patients were included (11 CIS, 11 RRMS, 10 PPMS and 10 SPMS). Patient demographics were as described in chapter (3). BICAMS was used to assess cognitive performance in subjects. 7T PSIR and 7T MT images were acquired on all patients. The images were then coregistered, then cortical and white matter lesions were segmented out manually. Neocortical grey matter

was segmented using SPM8. Cortical grey matter fraction (GMFc) was computed for all subjects.

Results:

GML volume and mean MTR values are presented in chapter (3). Single digit modality test (SDMT) was associated with cortical lesion mean MTR values (with the lower SDMT scores associating with lower cortical lesions mean MTR values), and not with volume. SDMT was also associated with WM lesion volume and mean MTR values with mean MTR values having a stronger association. Brief visuospatial memory test-revised (BVMT) was associated with cortical lesion volume, mean MTR values and WM lesion volume. GMFc was only associated with BVMT.

Discussion:

The significant association between different cognitive scores with cortical lesions, and WML mean MTR values could reflect the disruption in the cortical-subcortical tract connections. The observed contribution of lesions' mean MTR values over their volume is an emphasis of the previously described impact of the degree of damage of demyelination and axonal loss in MS plaques over simple lesion load.

Conclusion:

I found that the cognitive performance in MS patients correlates not only with lesion volume but more importantly with the degree of damage in these lesions indicated by the mean MTR values. Cortical and WM lesions contributed more to the degree of cognitive impairment than neocortical volume in MS patients.

5.2 Introduction:

5.2.1 Cognitive Impairment in Multiple Sclerosis:

Cognitive impairment has long been described in association with multiple sclerosis. The frequency of cognitive dysfunction is relatively high in MS patients, occurring in more than four in 10 patients.[190] It is frequently overlooked, as intact language skills, especially early in the disease process, could mask some deficits in concentration, memory and reasoning.[191] It has been reported that information processing speed[192] rather than working memory is the primary deficit underlying poor cognitive performance in patients.[193] It has also been suggested that memory impairment in MS patients could be a result of inadequate initial learning and not a function of impaired retrieval.[194] However, there was a relative sparing of verbal learning amongst MS groups.[195]

Although MS is more prevalent in women than men, reports of variation of the risk of cognitive impairment according to gender in MS patients are rather conflicting. Savettieri *et al* reported that cognitive impairment is more frequent among men than women. In men, an older age, longer disease duration, a higher EDSS score, a lower education level, and the APOE ε4 allele are significant risk factors for developing severe cognitive impairment. Among women, no association was found between any level of cognitive deterioration and the other variables.[196,197] On the other hand meta-analysis[198] by Prakash and colleagues showed that studies which recruit mainly females show more cognitive impairment than those recruiting males.

Cognitive impairment affects patients' functional status. It could be predictive of functional status in MS.[199] It can affect patients at any stage of disease, including CIS patients, but is more marked in patients with progressive forms.

Studies of the relationship between cognitive impairment and Quality of Life (QoL) have been contradictory. In some studies, cognitively impaired MS patients showed lower quality of life scores than cognitively preserved patients.[200] Even in early stages of the disease, it was found that patients with mild cognitive impairment and slowed processing speed tend to have reduced health related quality of life (HQOL). This was ascribed to the fact that slowed information processing may impact an individual's ability to complete tasks and to cope in demanding work and social situations.[201]

Various batteries of cognitive tests have been used to test cognitive impairment in MS patients. The most commonly used is the Rao Brief Repeatable Battery (BRB), which includes tests of verbal memory acquisition and delayed recall using selective reminding test (SRT), spatial memory acquisition, delayed recall, speed and accuracy in visual search and scanning with the Symbol Digit Modalities Test (SDMT), sustained attention, concentration and processing speed using Paced Auditory Serial Addition Task (PASAT), and verbal fluency on semantic stimulus using the Word List Generation Task (WLG).

Several studies have examined the differences in cognitive impairment of RRMS, PPMS and SPMS. One such study included 108 RRMS, 71 SPMS, 55 PPMS patients and 67 healthy controls. SPMS patients performed worse than PPMS patients on SRT and WLG. RRMS patients performed generally better than SPMS patients except on WLG. RRMS patients performed better than

PPMS patients on the PASAT.[202] It was noticed that SRT and WLG distinguished RRMS and SPMS patients from PPMS patients. The authors hypothesized that CNS inflammation in different MS phenotypes might selectively affect some working memory operations more than others.

In a separate longitudinal study over 2 years 25 PPMS, 30 SPMS and 33 controls were examined. Foong and colleagues found that cognitive performance of both patient groups remained largely stable during the study period.[202] Although their patient sample was very small, which require cautious interpretation of their results.

5.2.2 Cognitive impairment and MRI metrics in MS patients:

The role GM plays in MS generally and cognitive impairment specifically has come under more scrutiny over the last decade, especially after it was reported that GM atrophy predicts physical and cognitive disabilities better than WM damage.[203] Trying to correlate the degree of cognitive impairment with the severity and site of lesions on MRI has proven difficult and showed conflicting results. This is usually due to the difficulty in assessing the contribution of focal pathology in the presence of widespread brain abnormalities. Foong *et al* [204] reported that the contribution of frontal lobe pathology to cognitive impairment is difficult to delineate, and according to their results, is less significant than previously reported. In their study they showed moderate correlations between MRI frontal lesion load and some of the neuropsychological scores, such as verbal fluency, and working memory. But when controlled for the total WML load, all previous correlations were lost. On the other hand, they found some similarities in the pattern of performance on the neuropsychological tests between their MS patients and those with

frontal lobe excisions (mainly due to brain tumour or arteriovenous malformation), but patients with frontal lobe excisions had more impairment in the planning tasks.[205]

Swirsky-Sacchetti *et al* reported that the combination of several MRI measures which are total lesion load, ventricular-brain ratio and the size of the corpus callosum are predictive of cognitive impairment in patients with MS. They used a total lesion load of > 30 cm^2 as an arbitrary cut off point for the best sensitivity and specificity for overall cognitive impairment in their sample. This supports the notion that the left frontal lobe is significantly involved with word fluency and abstract, nonverbal problem solving, the right parieto-occipital region involvement is more prominent in measures of sustained attention and visual scanning (Symbol Digit Modalities) and the left parieto-occipital region is most predictive of verbal learning repeated over several trials, while memory for complex figures is predicted by right parietal lobe involvement.[206]

Patients with RRMS who showed cognitive impairment on the BRB also had a lower neocortical volume (NCV) than those who were cognitively unimpaired. The degree of atrophy in these patients significantly correlated with their cognitive scores.[83] Another study found that corrected GM volume (corrected for tissue segmentation misclassification) was the only MRI variable that best predicted the short and long-term auditory/verbal memory performance, as assessed by California Verbal Learning Test - second edition (CVLT-II), total learning and delayed recall. On the other hand, corrected WM volume was the best predictor for mental processing speed and working memory as assessed by the subject's performance on SDMT and BVMT-R delayed recall, which is

consistent with the notion that manipulation of new information requires communication between different parts of the brain through WM tracts.[207] GM and WM volume were more related to patients neuropsychological performance in comparison with lesion burden.[207]

In a longitudinal study[208] that followed patients with early MS for 2years, significant cognitive impairment was demonstrated, starting early on in the disease course and steadily progressing. Furthermore, those patients who demonstrated cognitive deterioration by the end of the study also showed a more conspicuous decrease of brain parenchymal volumes than an increase of T2 and T2 lesion loads, although there was a significant increase in the T1 and T2 lesions load detected. It was also shown that the cognitive impairment was independently predicted over 2 years only by the mean absolute change of brain parenchymal volumes. This supports the view that the rapid development of brain atrophy determines substantial cognitive decline in MS patients.

Some studies have looked at the effect of cortical lesions on cognitive function. Histopathology studies [130] showed the distribution of subpial cortical demyelination in MS patients to involve areas known to be engaged in information processing, and this may play a role in cognitive impairment in some MS patients. It is not surprising that a number of studies examined the effect of cortical lesions on cognitive functions. Nelson et al [120] compared the correlation between cortical lesion location, number and type with the different degrees of cognitive impairment. They used DIR and PSIR at 3T for lesion detection and classification. The authors found that the presence of intracortical lesions has less impact on cognitive functions than mixed and

juxtacortical lesions. This finding was explained by either the bigger size of mixed lesions over the intracortical lesions, or the inability of current MRI techniques to capture all the intracortical lesions due to their relatively small size. There was a significant increase in the total IC lesions count in the patients with mild and moderate cognitive impairment, however a stronger association was found with the mixed cortical lesion type. There was a very strong association between CL load and information processing speed (using Wechsler Adult Intelligence Scale III) in particular. In another study using 3D FLAIR and 3D T1-weighted IR-SPGR in 26 MS patients, information processing speed and working memory (SDMT), visuospatial memory (BVMT-R) scores as well as verbal learning and memory (CVLT-II) scores were correlated with cortical lesion count and volume.[209] Cortical lesions on DIR in RRMS patients were found to increase significantly in patients over a period of 3 years and were associated with worsening performance on neuropsychological measures at follow up whilst WM lesion load did not change significantly,[210] which suggests that GM lesions play a more important role in cognitive functions. In agreement with these results, a longitudinal study on early RRMS patients over 2.5 years, found that neocortical volume changes were significantly more pronounced in MS patients who had a deterioration in their cognitive performance (using Rao Brief Repeatable Battery) than in those with stable or even improving cognitive performance, while the changes in T2 WM lesion load was not signifcant.[211]

On comparing the results of 1.5T scans with 3T scans in terms of the cortical lesion load, different MR parameters and their correlation with variable neuropsychological tests, they found that the higher magnetic field improved

the sensitivity of the scans to depict WM and GM lesions in the whole brain. Significant association was found between the lesion load on 3T scans and cognitive tests scores specially SDMT, CVLT DR, CVLT TL, BVMT DR and PASAT2, while on the 1.5T scans, only SDMT and CVLT DR were significantly associated with lesion load.[212]

Previously mentioned studies used conventional MR imaging. These imaging sequences do not have any pathological specificity. An early study by van Buchen and colleagues suggested both that structural macroscopic atrophy and microscopic structural damage in the form of MTR values have complementary role in influencing the neuropsychological functioning in MS patients. They found that neuropsychological tests correlated with MTI measures of brain volume. Also variation of the MTI values between severely impaired, moderately impaired, and cognitively normal patients was observed.[213] Comi *et al* further explored the correlation between MTR values and cognitive impairment, demonstrating that the average MTR, peak height and location of overall brain and frontal lobe histograms were significantly lower for cognitively impaired than for cognitively intact patients.[214] In a more detailed analysis, Filippi *et al* found a significant correlation between cognitive scores and average WML mean MTR as well as normal appearing brain tissue peak height and location MTR histograms. They found that average lesion MTR was significantly lower in the patient group with more cognitive impairment than those with mild cognitive impairment. This indicated that patients with severe cognitive impairment not only have higher lesion load but most of these lesions seem to have more severe tissue damage.[215]

Significant correlations have also been observed between lower PASAT scores and lower mean MTR values in the right inferior parietal cortex and right inferior occipital gyrus. Regions that showed reduced mean MTR values without significant atrophy represent reversible changes without any irreversible neuro-axonal loss, but these areas were considered most vulnerable for atrophy in the future.[216]

5.2.3 Physical disability progression and cortical atrophy

Longitudinal studies showed that GMF at baseline correlates with EDSS in RRMS and SPMS patients with higher degree of GM atrophy in patients who experience EDSS progression over the period of the study, nevertheless a similar pattern was not observed for the change in WMF.[203] Neocortical volumes were found to correlate significantly with EDSS scores in MS patients but this correlation was much stronger in PPMS patients than in RRMS patients.[217] EDSS score and age at disease onset were found to be a significant predictors of GM atrophy (1- point increase in the EDSS score corresponded to a 0.54 % decrease of GMF), similarly GMF was found to significantly predict the level of patient disability.[218] Patients with EDSS score of < 3.5 also showed the lowest GM atrophy compared with those with higher EDSS score.[218] In a longitudinal study, the change in EDSS at follow-up correlated significantly with the cortical thickness of precentral gyrus and superior frontal gyrus.[219]

Regional cortical thinning in primary motor and visual cortex was found to correlate significantly with pyramidal and visual FSS scores consecutively, not EDSS scores in MS patients.[220] This is consistent with the view of the role that retrograde axonal degeneration plays in cortical atrophy.[220] Similarly another study showed average loss of 0.08 mm in cortical thickness per unit increase in

EDSS, with the most significant correlations found in bilateral middle and superior frontal gyri as well as the anterior pole of the left inferior temporal gyrus.[221] However this was not a reproducible finding in other longitudinal studies.[222]

In a study that acquired MTR and FSPGR at 1.5 Tesla on PPMS patients within 5 years from symptoms onset, a significant correlation was found between EDSS scores and reduced MTR values in the pre-central and the post-central gyri.[216]

5.2.4 Pathology of Cortical Atrophy in Multiple Sclerosis in Different Disease Phenotypes

Interest in brain atrophy generally and neocortical atrophy specifically has been growing over the last few years, hoping to improve our understanding of the pathophysiology of MS.[223]

It is still not known if cortical atrophy is due to the decrease in the size or the number of neuronal cell bodies in MS brains. In healthy individuals, age-related atrophy is more evident in the GM than in the WM, with average tissue loss around – 0.3% per year and -0.2 % per year respectively. Similarly, significant differences are seen in both GM and WM fraction between males and females, which points the importance of taking both age and gender into consideration when comparing degree of atrophy between different MS phenotypes.[224]

Brain atrophy starts at early stage in the disease course.[224] Neocortical volume is significantly lower in MS patients than controls,[217] and is more pronounced in progressive MS patients rather than RR patients, and in CIS patients who convert to RRMS compared to those who remain CIS.[224] Sensitivity (78.0%)

of dissemination in space of lesions in predicting the conversion to MS was lower in comparison to GM atrophy (89.8%) mainly in the cerebellum and motor cortex, while the specificity was 58.7%, and 52.2% for DIS, and grey matter atrophy respectively.[219]

However, not all studies show GM atrophy as the prominent feature. Some studies found that atrophy in the context of MS seems to affect the WM to a higher degree than the GM, hence the view that tissue loss early in the MS course does not simply reflect a simple acceleration of the age related atrophy.[224,225] On the other hand another study showed that the rate of GM loss over a period of two years was found to exceed the 95% limit of the change observed in the normal control subjects, while there was no significant change in the white matter fraction (WMF) in patients with low Gd enhancing lesions load observed over the same period.[226] It was suggested that atrophy may occur secondary to inflammation, possibly due to Wallerian degeneration from transected neurons which pass through acute lesions. Significantly, this study was conducted on early RRMS patients with short disease duration (2 years from symptom onset).

During the 19th and early 20th century, there was a common assumption that demyelination was the main pathologic substrate in MS pathology, resulting in loss of tissue volume, and contributing to the brain atrophy observed in MS brains. However, axonal loss has also been recognised to be a major component of lesions, especially in progressive disease, and thus may also play a key role in brain volume loss. Pathology studies have found no significant correlation between the mean cortical thickness and the mean degree of cortical demyelination observed in MS patients.[227] Several studies have

correlated brain volumes with other neuronal markers indicating that atrophy mainly reflects axonal loss.[228]

Topographical distribution of cortical atrophy:

According to pathology studies, cortical atrophy usually involves the motor, somatosensory, superior and middle frontal gyri, superior and middle temporal gyri of MS patients' brains. On the other hand the difference has mostly been shown to be significant in the motor cortex.[227] Using Tract based spatial statistics and Optimized voxel-based morphometry, GM atrophy in PPMS patients was found to be mainly pronounced in bilateral sensory motor cortex followed by the insula then the right superior temporal gyrus.[229] In another study which included PPMS patients within 5 years of symptom onset, MTI as well as 3D-FSPGR were acquired. SPM2 was used to compare the regions with lower MTR values and smaller volumes in the cortex in comparison with controls. It was reported that the neocortical volume was mostly reduced in the right pre-central gyrus, as well as right middle frontal gyrus, left post-central gyrus and left insula.[216] Using voxel based morphometry on 1.5T 3D MPRAGE images, cortical atrophy in RRMS patients mostly involved the precentral cortex, while postcentral gyrus involvement was more pronounced in SPMS patients.[230] Compared to RRMS and PPMS patients, SPMS patients had widespread neocortical volume loss in the occipital lobes bilaterally as well as in the anterior lobe of the cerebellum.[230] In RRMS patients, significant neocortical GM loss was predominantly noticed in the left dorso-lateral frontal lobe and bilateral anterior cingulate gyrus with a preferential involvement of the left hemisphere.[231]

Pathophysiology of atrophy:

The mechanisms underlying neocortical pathology in MS are not fully understood, nevertheless, it seems to be not driven by one single mechanism but a combination of several methods. Chard et al[224] assessed cortical thickness in a large sample of patients using 1.5T scanners in a multicentre study. Measuring cortical thickness is usually a more accurate approach than other techniques such as voxel based morphometry as it follows the curvature of the ribbon rather than using the surface blurring technique. A high correlation was detected between white matter lesion load on T2w and grey matter fraction (GMF) and not WMF, and more than 50% of the GMF reduction can be explained by the variations in T2 white matter lesion load. Total WM lesion load particularly correlated with cortical thinning in bilateral cingulate gyrus and bilateral posterior insular cortex.[221] GM atrophy could be explained partially by focal lesion genesis, but probably another more widespread process is involved. On the other hand, the anisotropic voxels and the lower spatial resolution in the images used in this study could influence the sensitivity to cortical thickness measurements. Some studies reported a significant reduction in neocortical volume in MS patients even with minimal WM lesion load,[217] and no correlation was found between white matter enhancing lesions, which was found to represent active inflammation,[232] and GMF.[226] Nevertheless, high Gd-enhancing lesion volume at baseline was associated with more WMF not GMF loss over the subsequent 2 years, which might indicate that the loss of WM tissue occur as a delayed effect following inflammation.[226] Wallerian degeneration (defined by the presence of newly developed T2 WM lesions in follow up MRI scans along the course of

corticospinal tract distal to initial acute T2 hyper intense lesion) was found in the corticospinal tracts that was temporally and spatially distant from focal demyelinating lesions.[182] This could explain white matter plaques causing retrograde injury to different areas of the cortex depending on the axons involved, hence the focal cortical thinning of the cortex that was detected by some studies.[233] Nevertheless, it was also reported that axonal atrophy can happen in the absence of inflammation or demyelination.[234] As cortical lesions are associated with demyelination and axonal damage, they in turn may contribute to tissue loss in the cortex.[94] Pathology studies showed that the mean neuronal density and mean neuronal cell size, as well as glial density is usually reduced in cortical lesions than in the surrounding normal-appearing MS neocortex.[227] The only significant difference between normal appearing neocortex and control brains was the shape of the neurons as they were more circular in the MS brains.[227]

Some studies suggest that the mechanisms contributing to GM atrophy change over the course of disease. So in the early stage of the disease, pathological changes in the WM might be the main culprit whilst latter in the disease course widespread GM involvement is the more responsible for the cortical atrophy.[203] This could explain the lack of correlation detected between WMF and GMF at baseline or follow-up scans in RRMS patients, while in SPMS patients GMF and WMF were moderately correlated at baseline and 4 years later.[203] Another theory is that the mechanism of cortical atrophy vary among different MS phenotypes since the disease onset, so neocortical atrophy in PPMS is more due to neurodegeneration and progressive axonal loss rather than due to WM lesion accumulation while in RRMS the reduction in neocortical volume is

explained to some extent with the WM lesion load from very early on in the disease course, and this could partly contribute to the clinicopathologic difference between these two disease types.[217,218] In PPMS and SPMS patients, regional GM atrophy in a given lobe correlated to the T2 lesion load within the same lobe.[230]

How early cortical atrophy starts in the disease course is still controversial. Some studies report no difference in neocortical volume between CIS patients and controls,[230] while others document cortical volume loss in CIS patients in early stage of the disease course, and evolves faster in the grey than in the white matter tissue.[235] One study involved 53 RRMS patients with short disease duration (between 1-5 years), following the patients for 2 years with 1.5T T1-w and T2 sequences. They analysed the whole brain volume, and reported the mean rate of brain atrophy to be 1.33% a year, indicating that brain atrophy could be one of the primary pathological events in early disease development and progression.[208] Chard and colleagues in a longitudinal study examined the difference of cortical atrophy in early MS patients and controls. Although there was no difference between the GM volume between early MS patients (mean disease duration 1.9 years) and controls at baseline, there was a significant difference in the rates of change between MS and healthy controls.[236] To investigate if there was a difference in the rate of cortical atrophy in patients depending on how long they had the disease, Zivadinov et al performed a longitudinal study including 181 RRMS patients. They classified the patients into two groups, one with short disease duration (less than 2 years) and the other group is with longer disease duration (longer than 2 years). Patients received a 2 monthly scans for 2 years. They found that patients with longer

disease duration at baseline had a smaller cortical volume; on the other hand there was no significant difference in the rate of cortical atrophy between the two groups.[237] Two hundred and sixty seven patients were scanned at baseline and 24 months later showed a mean reduction of GMF of 0.38 % in comparison with 0.21% in healthy controls, with higher GMF at baseline correlated with higher GMF variation over the period of the 24 months.[238] It also suggested that the decrease in GMF reduces over time.[238]

On comparing the neocortical volume between different groups of MS patients with similar disease duration (7 years), there was no significant difference between RRMS and PPMS patients in their normalized cortical volumes. On the other hand they were both lower than normal controls. There was no correlation between the neocortical volumes and the WML load, suggesting that a proportion of neocortical pathology could be due to WM involvement, however the extent of the changes suggests that an independent neurodegenerative process may also be active.[217] An alternative explanation would be a temporal delay between the process leading to T2 lesion formation and the subsequent GM atrophy.[226] Similarly, on comparing the neocortical volumes between SPMS and RRMS patients, they found that neocortical volume in SPMS patients was less than in RRMS patients.[218,239]

Cortical thickness is another way of estimating cortical atrophy in MS patients, and it is normally defined as the average of the distance between the GM-CSF boundary and the GM-WM boundary. Unfortunately, measuring cortical thickness has proved to be technically challenging mainly due to the complex folding patterns of the cortex. Mean cortical thickness negatively correlates

with age, with an average loss of 0.1 mm in cortical thickness per decade. Bilateral primary motor, somatosensory and visual areas are mostly involved.[221] A 25% reduction in cortical thickness of primary motor and visual areas was reported in MS patients in comparison with healthy controls.[220] The primary motor cortex is significantly thinner in CIS patients with pyramidal onset while no such correlation was found in PPMS or SPMS patients.[220] Cortical thickness was examined by Chen *et al* in a longitudinal cohort of RRMS and SPMS patients over a period of 1 year.[147] A correlation between cortical grey matter thickness and brain volume was observed. There was no difference in baseline cortical thickness between the patient's group who progressed and those who have not, however, the patients who showed progressive disability over the period of a year showed more progressive loss of cortical thickness involving especially the parietal and precentral areas than the stable group.[147]

Whether the abnormalities in the NAWM correlate with the diffuse cortical grey matter damage was investigated in PPMS patients using DTI.[229] They found a negative correlation between the FA in the corticospinal tract and the GM atrophy in the adjacent sensory-motor cortex. Also the reduction in the FA of the tracts adjacent to the left insula correlated with GM atrophy of the insula.[229] On the other hand, they found that some abnormal WM tracts did not show any corresponding GM atrophy, and similarly some areas of GM atrophy were not adjacent to any regions of reduced NAWM FA.[229] This suggests that the abnormalities in the GM, at least in some part, could develop independently from the abnormalities in the NAWM.

Overall, we think the mechanisms of atrophy are still poorly understood and in need for further exploration. As per previous studies the correlation between the degree of atrophy and either the WML or the GML load is only moderate, which suggests that further mechanisms need to be explored. As per pathological studies, diffuse pathological changes affect the NAWM which constitutes a significant proportion the brain by volume; hence the role the NAWM plays in the pathogenesis of cortical atrophy is worth exploring using quantitative imaging. Lower normal appearing brain tissue (NABT) MTR was associated with a greater rate of BPF change over a period of 4 years. On the other hand this effect was more pronounced in the RRMS than in the SPMS patients.[203]

The accuracy of volume measurements is mainly influenced by the image resolution in three dimensions and the tissue contrast. So it is always preferable to have a higher scan resolution which can be achieved using higher field strength, 3D acquisitions and small voxel sizes with high white matter, grey matter, CSF, and lesions tissue contrast.[240] MT imaging can be very sensitive to tissue damage in the early stages before any apparent brain volume loss. Reduced MTR values were reported in all regions where cortical atrophy was observed.[216] On the other hand, reduced MTR was also observed in the left pre-central and superior frontal gyri without significant atrophy.[216]

To what extent the degree of damage in cortical and white matter lesions contribute to cognitive impairment in comparison with cortical atrophy have not yet been fully investigated in patient samples that are representative of the total MS population. Small lesions that are not normally detected by conventional MRI may play a crucial role on the degree of cognitive

impairment in MS patients.[241] Structural high resolution imaging has proven to be effective for predicting cognitive impairment. On the other hand there have been no studies so far using high resolution MRI, exploring the contribution of the degree of damage in focal cortical lesions, using magnetisation transfer imaging, on cognitive impairment in MS patients of different disease phenotypes.

5.3 Hypothesis:

1- We hypothesise that cortical atrophy, cortical lesion subtype and white matter lesion load contribute to the degree of cognitive impairment to variable degrees in MS patients of different disease phenotypes.

2- We also hypothesise that there is an association between the degree of damage in focal cortical and white matter lesions, as indicated by their mean MTR values, and cognitive impairment in MS patients.

5.4 Methods:

We used the same cohort of patients as in chapter 3.

Neuropsychological evaluation:

A neuropsychological evaluation was implemented to all patients by the same assessor on the same day of the scan prior to acquisition. We used BICAMS, which is a simple neuropsychological (NP) assessment that consists of three sub-tests from the Minimal Assessment of Cognitive Function in Multiple Sclerosis (MACFIMS) battery. The BICAMS test consists of three tests SDMT, CVLT-II (first five trials), and BVMT-R (first three recall trials).

MRI acquisition:

As per chapter (3) MRI acquisition protocol

Analysis:

The relative effect of B1 field inhomogeneity on MTR values was corrected using Ropele *et al's* approach.[137] PSIR and MTR images were linearly coregistered for each subject using SPM8 "Coregister" tool.[180] Default parameters were used, and normalised mutual information selected as the objective function since the registration task was multimodal. Cortical and WM lesions were segmented out using MIPAV medical images analysis software. Cortical lesion segmentation criteria are the same as delineated in chapter 3. Tissue maps were created using SPM8 and the mean MTR ratios for NAWM or NAGM were created. MTR values were normalised by average MTR values in the corresponding normally appearing tissue. As the presence of lesions can potentially affect the ability of automated tissue classification methods to correctly handle normally appearing tissues, Chard et al.'s[242] approach has been used to inpaint the PSIR images. Inpainted PSIR images were then classified using SPM8's "New Segment" approach. GMFc was calculated as GM/ (GM+WM+CSF).

Statistics:

One way ANOVA was used to compare GMFc across different MS phenotypes. Pearson correlation coefficient was used to assess the association between different MRI parameters, mainly GML volume, mean MTR values, WML volume, mean MTR values with GMFc.

Multiple linear regressions were used to examine the association between different MRI metrics and cognitive performance. Each cognitive score such as SDMT, CVL and BVMT was indicated as dependent variable while CL

volume, their mean MTR values, WML volumes, their mean MTR values were considered as independent variables controlling for age. Ordinal logistic regression was used to assess the correlation between physical disability measured by EDSS and GMFc.

5.5 Results:

Descriptive statistics for the cortical and white matter lesions are described in chapter 3. Descriptive statistics for cognitive scores as well as GMFc are presented for all subjects by their MS phenotype in table 5.1.

GMFc was the smallest in the SPMS patients in comparison with other disease phenotypes. There was no significant difference in GMFc between the groups using one way ANOVA (p =0.45).

MRI metric correlations:

GMFc consistently showed stronger correlation with tissues mean MTR values than with simply lesion load. GMFc was significantly correlated with mean cortical lesion MTR (*rho*= 0.5, *p* = 0.002) and white matter lesion MTR (*rho*= 0.4, *p*= 0.02). Significant correlation was detected between GMFc and NAWM MTR values (*rho*= 0.4, *p*= 0.005) not with NAGM MTR values (*rho*= 0.2, p= 0.3). No significant correlation was detected between GMFc and CL volume (*rho*= -0.3, *p*= 0.07) or WML volume (r= -0.3, p= 0.1). No significant correlations were detected between cortical lesions volume and GMFc even on taking into account the cortical lesion subtypes.

Regression analyses of MRI metrics to cognitive performance and EDSS:

On taking into account individual lesions' mean MTR values, we used a multivariate regression with SDMT (as previously explained is consistently

found to be the most sensitive test for cognitive performance in MS patients) as a dependant variable and mean cortical lesion volume, their mean MTR values as independent variables controlling for age, we found a significant association between SDMT performance mean cortical lesion MTR values not CL volume (p= 0.00, p = 0.9 respectively). A significant association was demonstrated between SDMT as a dependant variable and WML volume and mean MTR values controlling for age (p=0.03, p = 0.00 respectively), with mean lesion MTR values having stronger effect than WML volume. There was no significant association between CVLL and either cortical or WML volumes or their mean MTR values. BVMT was significantly associated with CL volume, mean MTR values and WM lesion volume controlling for age (p=0.001, p = 0.001, p = 0.005 respectively), while WM lesion mean MTR values was not a significant predictor. Mean cortical lesion MTR values had the strongest effect on BVMT in comparison with the other variables.

The GMFc in SPMS was the lowest in comparison with the rest of the MS phenotypes. GMFc showed significant association with BVMT (p = 0.04) not with SDMT or CVLT controlling for age. NAWM MTR as well as NAGM MTR showed significant association with SDMT (p= 0.002 and 0.047 respectively), not with any of the other cognitive scores.

We used a linear model to describe the association between SDMT with the degree of damage in cortical lesions and NAGM. We modelled the NAGM MTR and the CL mean MTR values as independent variables and SDMT as the dependant variable, the NAGM MTR lost significance while cortical lesion mean MTR values remained the significant parameter associated with cognitive performance (p= 0.95 and 0.016 respectively). Similarly, on

modelling NAWM MTR and white matter lesion mean MTR values as independent variables while SDMT as dependant variable, the NAWM MTR not white matter lesions mean MTR values significantly associated with SDMT scores (p= 0.014 and 0.676 respectively). No significant association was seen between EDSS and GMFc.

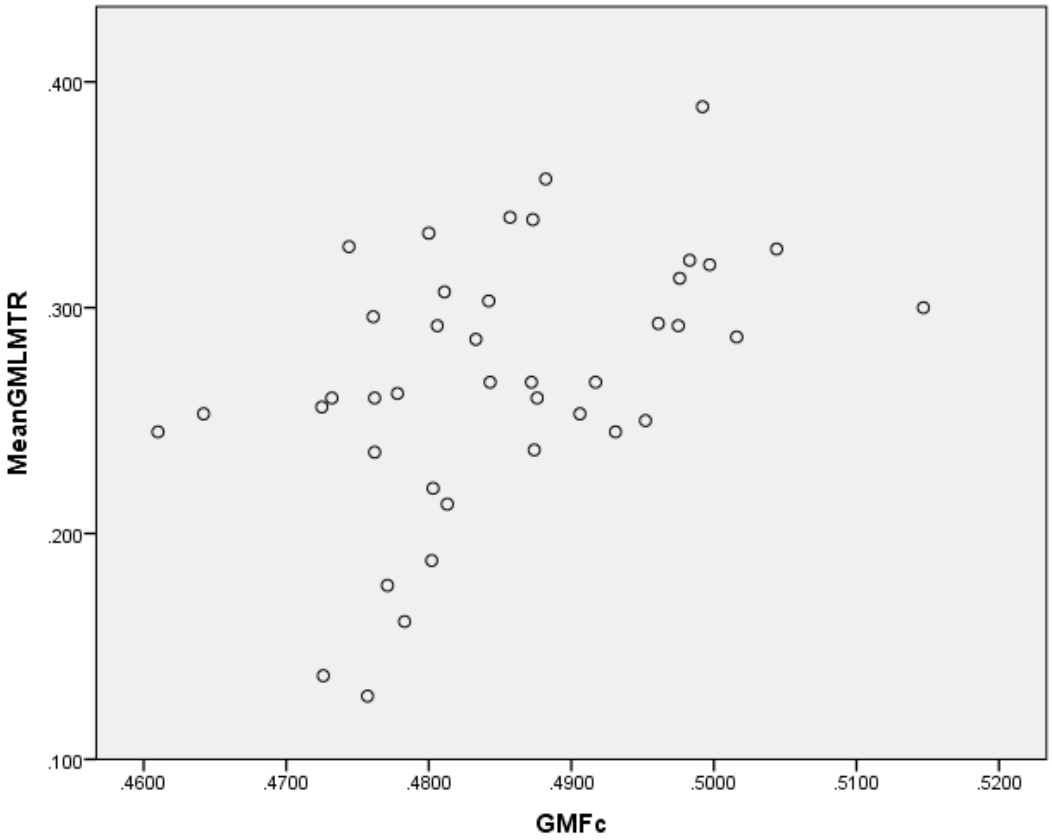
(Table 5.1) Descriptive statistics for cognitive performance and cortical volume in MS patients

	MS phenotypes			
	CIS	**RRMS**	**PPMS**	**SPMS**
	Mean (std)	Mean (std)	Mean (std)	Mean (std)
SDMT	56.56 (8)	50.86 (8)	41.63 (7)	35.13 (9.9)
CVLTTL	54.89 (6.5)	57.13 (9)	54.89 (11.4)	48.5 (13)
BVMTR	29.44 (4.9)	30.38 (2)	25.89 (6)	22 (6)
GMFc	0.489 (0.009)	0.490 (0.009)	0.482 (0.014)	0.479 (0.008)

Table illustrating the means and standard deviation for cognitive tests such as SDMT, CVLTTL, BVMTR and GMFc

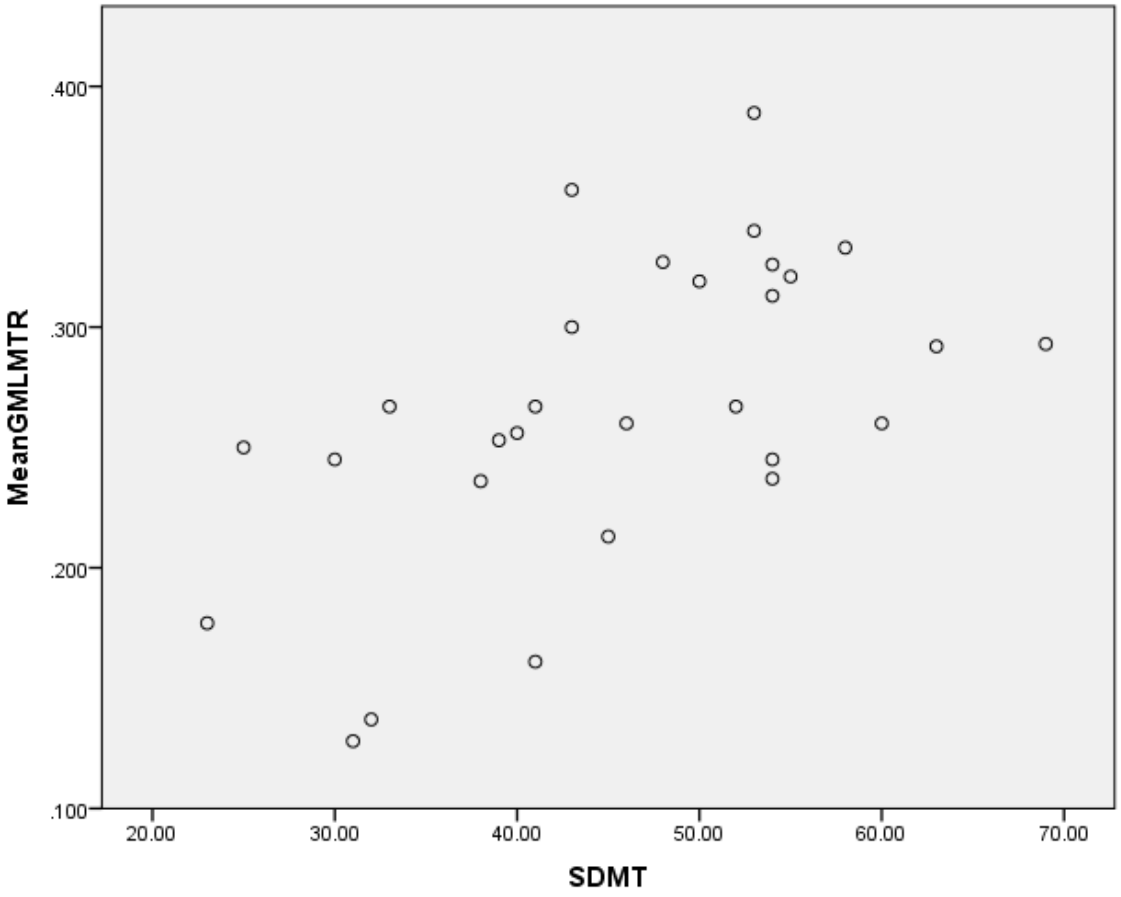
Std = standard deviation

(Figure 5.1) correlation between grey matter fraction and cortical lesion mean MTR values



The figure illustrates significant high positive correlation between cortical lesions mean MTR values and cortical grey matter fraction indicating the important contribution of the degree of damage in cortical lesions to nearby tissue volume.

(Figure 5.2) correlation between SDMT and cortical lesion mean MTR values



The figure illustrates the significant positive correlation between SDMT (single digit modality test) and cortical lesion mean MTR values in MS patients, suggestive of the important role the degree of damage in cortical lesions play in cognitive performance in MS patients.

5.6 Discussion:

There are a number of neuropsychological tests used to assess cognitive decline described in the literature. We aimed to use a neuropsychological battery test that was sensitive enough to detect cognitive decline, and at the same time not time consuming, and so could be implemented in clinical settings. The diagnostic accuracy of the BICAMS in comparison with other conventional NP batteries (MACFIMS) was assessed in a large group of MS patients by Dusankova et al.[243] They showed that despite the simplified application of the three included tests in the NP battery, it did not diminish its overall sensitivity or specificity. They found that SDMT and BVMT-R are the two most sensitive tests of the battery showing the largest differences between MS patients and controls. I similarly found in this study that SDMT and BVMT-R show the most prominent differences between different MS phenotypes. Like others I found that SDMT was the most sensitive measure for cognitive decline in MS patients.[244]

Cognitive decline in MS patients has been correlated with both macro structural and microstructural changes in MS brains. In this study, I assessed cognitive performance in different MS phenotypes and its correlation to different lesion parameters as well as with corrected neocortical volume. The NP battery of tests was interpreted by the same assessor without prior knowledge of the MRI findings. Similar to previous reports,[138] I found a significant association between lower cognitive scores and higher cortical lesion volume. Also a significant association was detected between different cognitive scores and cortical lesions, WM lesions mean MTR values. This could be explained by the disruption in the cortical subcortical tract

connections that is responsible for intact cognition. The noted contribution of lesions' mean MTR values over their volume is consistent with the previously suggested correlation of the degree of damage in MS plaques to clinical parameters over their volume. As conventional MRI sequences do not have pathological specificity to differentiate between MS plaques of various pathological entities from mild inflammation to demyelination and axonal loss, hence correlation between simple lesion load and cognitive decline does not reflect a full representation of the effect of these lesions, and correlation of lesions microstructure may provide a more accurate representation of the effect of such lesion pathology. Like others,[141] I found that cortical lesions and WM lesions have more influence on cognitive decline than GM atrophy.

SPMS patients had the smallest cortical GMF in comparison with the other MS phenotypes on the other hand; difference was not significant on controlling for age and correcting for multiple comparisons. In a previous study[138], a correlation between the GMF in MS patients and the cortical lesion volume was found. In this study we could not detect such a correlation, which could be due to the small sample included in comparison with the previous study which detected such a correlation. The higher magnetic field with increased resolution providing an advantage in increased accuracy in lesions detection and segmentation out of the cortex providing cleaner GM could be another reason for the lack of correlation unlike previous report.

In this study the variation in GMF was associated with the degree of demyelination and axonal loss in the cortical and WM lesions, with cortical lesions showing higher association than WM lesions.

GM atrophy was shown to correlate with worsening in cognition not EDSS[222], similarly in this study GMFc correlated with performance in BVMT but no correlation was found with physical disability. Various previous reports showed similar results to this study, with lack of correlation between physical disability and grey matter atrophy. [245,225] The discrepancy between my findings and some reports, which found such a correlation, is probably difference in sample size, as well as the use of disease modifying treatment through the disease course which could affect degree of atrophy amongst other parameters.

The correlation between GMFc and cortical lesions mean MTR values is supported by early findings of reduced MTR values in the grey matter where atrophy is observed [216], which could be explained by the impact of the high degree of damage, as expressed by low mean MTR values, in cortical lesions on causing retrograde probably irreversible neuroaxonal loss.

Early studies showed the correlation between neocortical grey matter mean MTR values and cognitive performance. [246] My finding that cognitive performance is significantly associated with cortical lesion mean MTR values not NAGM MTR values, when put in the same model, could be explained that the main effect in previous studies was of the unsegmented cortical lesions, hence in our study on segmenting those lesions out the effect of the NAGM has become negligible.

I observed that the contribution of NAWM MTR to cognitive performance was significant in comparison with white matter lesions mean MTR values. This supports previous report that microstructural NAWM damage contributes to cognitive decline regardless WML volume.[247] This could be explained by the

disruption of the widespread connections in the WM due to microstructural changes, as the integrity of WM connections is important for high brain functions.[248]

The consistent finding in this study is the contribution of the degree of damage in lesions and NAWM to the cognitive function. This prompts to the significance of looking beyond lesion load and tissue atrophy to determine patient's treatment or monitor response and treatment escalation.

In this study I did not assess correlations between cognitive performance and regional neocortical atrophy, but it was suggested in previous studies that cortical atrophy was found predominantly in the superior temporal gyrus and the superior and middle frontal gyri in early stages of the disease.[249] In MS patients with cognitive decline, mainly low scores on PASAT, neocortical atrophy was more pronounced in the frontal and temporal and parietal regions.[250]

In this study, I did not investigate deep grey matter and their contribution to cognitive decline. I did not correlate various cognitive tests scores with the topographical distribution of lesions. The correlation between individual cognitive tests scores with white matter lesions depending on their regional distribution has been previously explored; however potential association with cortical lesions has not yet been examined.

We illustrated the significant role the degree of damage in cortical lesions plays in patients' physical and cognitive disability in different MS phenotypes. The degree of demyelination and remyelination in patients determine their level of disability to a large extent. We wanted to examine if the degree of

demyelination and remyelination over time, reflected by lesions mean MTR values, vary between patients' phenotypes.

Chapter 6

The change in focal lesion MTR across time in patients with MS of different disease phenotypes

6.1 Abstract

Introduction:

Remyelination in MS appears to be significant, for protecting axonal integrity, restoring nerve conduction, and preventing long term physical disability. The degree of myelin loss in MS lesions (reflecting demyelination and remyelination) varies across disease subtypes, and probably this variation contributes to the level of disability different MS phenotypes manifest.

Objectives:

This is an exploratory study that aimed to follow a small group of patients of different disease phenotypes (5 CIS, 4 RRMS, 6 PPMS, and 5 SPMS) over a period of 1 year. We aim to examine the variation of myelin status and axonal loss in MS patients of different subtypes longitudinally, and the ability of MTR at 7 T to detect GM remyelination.

Methods:

A baseline 7T PSIR and 7T MTR were acquired (time point 0), and the same protocol was acquired 12 months later (time point 1). Cortical lesions were segmented, and lesion mean MTR values were computed for each visit. I compared the changes in mean MTR values in cortical lesions among patients of different disease phonotypes, and its association with clinical parameters.

Results:

In this exploratory study, most lesions either cortical or WM lesions in different MS subtypes remained unchanged across two time points irrespective of the chosen cut off. A significant percentage of lesions showed an increase in the average change in mean MTR values across the two time points while the rest of the lesions showed decrease in mean MTR values. The average change in the mean MTR values of individual CL over 12 months period was 0.053 ± 0.089 in CIS patients, 0.001 ± 0.074 in RRMS patients, 0.018 ± 0.127 in PPMS patients, while in SPMS patients was 0.061 ± 0.105. There was significant difference among the groups in the average change in mean MTR values across two time point using one way ANOVA ($p < 0.001$, F= 12.47).

Discussion:

In this exploratory study most CL showed unchanged mean MTR values over the 12 months' period, which may reflect the non-changing nature of mostly chronic cortical lesions over one year. Surprisingly, the frequency of cortical lesions showing increase and reduction in their mean MTR values was quite similar between CIS and SPMS patients; this was unexpected however as considering the progressive degree of disability in SPMS patients for which CL have substantial contribution, we would have expected possibly a more demyelinating picture in SPMS.

What is more difficult to explain is the lack of significant remyelination in RRMS patients which could be due to the small number of patients included. Large longitudinal studies needed to confirm or refute those findings.

Conclusion:

The frequency of lesions showing an increase of CL mean MTR values was similar in CIS and SPMS patients, which is counter intuitive. This conflicts with most pathology studies on SPMS patients. The average change in CL mean MTR values was higher in males than females. No correlation detected between the change in individual CL mean MTR values and disease duration or patients age.

6.2 Introduction

Remyelination was first described in 1961 by Bunge et al using CSF barbotage model, by repeated withdrawal and reinjection of CSF into cat's spinal cords. This has led to myelin loss which was followed by formation of new myelin with a slightly different staining quality.[251] Not long after this, remyelination was described in MS lesions.[252]

Early neuropathology examination of cortical lesions in MS brains proved to be challenging mainly as the cortex in general is much more cellular than the WM, which makes it more difficult to examine accurately. This in turn contributed to the difficulty to examine neuronal and axonal loss within the intracortical part of the lesion. [94] This has improved since the development of immunohistochemical preparations.

In MS, apoptotic neurons were found to be significantly higher in demyelinated cortex compared to myelinated cortex.[108] Axonal damage is also a well-known phenomenon in MS plaques, and it normally occurs at the peak of demyelination and inflammation.[92]

In terms of correlations and regional variations of GM and WM demyelination, Gilmore et al reported that the proportion of GM demyelination correlated significantly with the proportion of WM demyelination. The proportions of GM demyelination was found to be significantly reduced in the motor cortex, especially in comparison with the cingulate cortex.[253]

Merkler et al reported the transient nature of inflammatory infiltration and demyelination in the GM. EAE models targeted to the subcortical white matter (corpus callosum) were similar to targeted WM spinal cord EAE lesions in

terms of persisting anti-inflammatory cells infiltration, demyelination and axonal damage. [92]

Pathology studies indicated that remyelination by oligodendrocytes can occur in the CNS in MS patients.[252] Remyelination in the CNS is very significant, to protect axonal integrity, and restore nerve conduction. Hence, in theory inducing remyelination is important to prevent long term physical disability in MS patients. Encouraging therapies promoting remyelination requires a well-established imaging tool that could assess the degree of demyelination and remyelination in MS patients.

6.2.1 Pathogenesis of demyelination and remyelination:

MS is thought reflect oligodendrocyte pathology. So it could be that the regenerating oligodendrocytes have very short processes, hence the inability to wrap as efficiently as those with longer processes, however the problem could equally be due to defect with the regulatory signals causing restriction to the sheath thickness and the lengths of internodes and nodes in MS lesions.[254] Although demyelination mainly targets oligodendrocytes,[255] during remyelination oligodendrocytes remain very quiescent, while progenitor cells are mainly responsible for remyelination.[256]

Remyelination involves the formation of new oligodendrocytes. New mature oligodendrocytes normally originate from oligodendrocyte progenitor cells. Progenitor cells are widespread throughout the CNS. Macrophages, oligodendrocytes, OPCs all play important role in remyelination. Macrophages play an important role in the removal of the myelin debris that is generated from demyelination which is important as CNS myelin contains proteins that

could inhibit the differentiation of oligodendrocyte progenitors during remyelination.[257] Remyelination is generally dependant on timely interaction between astrocytes, macrophages, demyelinated axons and OPCs, so any delay in interaction between OPCs and demyelinated axons would be associated with temporal mismatch in the sequence of events for successful remyelination.[258]

6.2.2 Pathology of demyelinated and remyelinated Lesions

Pathological analysis of lesions showed the patterns of demyelination vary in subgroups of MS patients and at different stages of the disease.[16] Lesions were classified into 4 different patterns. With pattern 2 the most frequent. Pattern I and II were found in patients who presented with all different clinical subtypes of the disease before biopsy or death, while pattern III lesions were found mainly in patients with a disease of less than 2 months duration before biopsy or autopsy.[16] Pattern I and II show more perivenous distribution and tend to confluence resulting in large demyelinated plaques.

The characteristic feature of remyelination that makes it very distinguished from normally myelinated tissue is that the sheath is always thinner and shorter than expected for the diameter of the axon, on the other hand the composition of the myelin and the manner in which it is formed are similar to those in the originally myelinated intact axons.[259] On examining remyelination in demyelinated cortical lesions, it was noted that some cortical demyelinated lesions have very sharp lesions borders and those noted as demyelinated, whereas remyelinated cortical lesions have irregular, punctuate, less densely and less orderly arranged myelin under light microscopy.[90] Cortical lesions shows more extensive remyelination than white matter lesions even on case

per case basis, with no difference detected in the extent of cortical and WM remyelination noted between SPMS and PPMS patients.[90]

Remyelination in human and experimental tissue could be assessed using electron microscopy via measuring the quotient axon/fiber diameter which is known as g-ratio. Remyelinated fibers are characterised by thinner myelin sheaths, hence increased g-ratio indicating remyelination. In a pathology study on MS brains of chronic progressive patients using electron microscopy, myelin sheaths appeared thin in areas of normal appearing cortex, and g-ratios were significantly higher in comparison with controls, which suggests that part of the normal appearing cortex in MS brains may indeed be remyelinated.[90]

Albert et al examined myelin expression in oligodendroglial cells using immunohistochemistry.[90] Oligodendroglia numbers were low in the centre of demyelinated cortical lesions regardless if they have remyelinated borders or not. As expected cortical lesions without evidence for remyelination showed low numbers of myelin basic protein (MBP) positive oligodendrocytes at the lesion border but had 2, 3- cyclinc nucleotide 3-phosphodiesterase (CNP) positive cells were detectable (indicating the presence of cells of the oligodendrocyte lineage).[90] Interestingly they also found that the borders of remyelinated cortical lesions showed an increased number of both MBP and CNP positive cells even in comparison with normal appearing cortex.[90] The presentence of lipid-laden macrophages or microglia in contact with thinly remyelinated fivers suggests ongoing active remyelination and demyelination.[260]

6.2.3 Variation of demyelination and remyelination in different stages of the disease

The cycle of demyelination and remyelination is so important in determining the disease course in MS patients. Demyelination is necessary but not enough to cause permanent disability in MS patients. The lack of protective myelin causes axonal loss. Normally when demyelination is well established and remyelination is exhausted, this is when disability is progressive.[261]

The pattern of demyelination is different in different stages of the disease. In acute MS, myelin is usually lost and there is usually associated axonal loss as well. In early stages of chronic MS more than 50% of the demyelinated lesions have thin myelin sheath giving them shadow appearance hence called shadow plaques and these plaques are suggestive to remyelination throughout the entire lesion. In late stage of the chronic MS, remyelination is mainly restricted to the margins of the lesions, with still a degree of preservation of axons, although the density of axons in the middle of lesions is fairly reduced.[262] The degree of cortical demyelination was recognised to be much higher with disease duration longer than 10 years, although that was in a subset of patients.[90] Studies showed that younger patients have greater GM demyelination with no influence of disease duration.[253] On the other hand both younger age and long disease duration were associated with more extensive WM demyelination.[253]

The outcome of an MS relapse depends on the balance between the degree of demyelination and remyelination in active lesions, which is significant if a patient is going to develop SPMS. Even the pattern of remyelination in MS lesions varies depending on the stage of the disease. In chronic stage of the disease, remyelination is generally sparse and mostly involving the edges of

the lesions.[262] Another pathology study[149] described that PPMS patients show higher percentage of shadow plaques than SPMS patients and suggested that remyelination is more complete in PPMS patients than in SPMS patients which leads to larger WM and deep GM lesions in patients with SPMS phenotype. On the other hand the spinal cord did not show any variation in the rate of demyelination (as assessed by the volume of the slowly expanding and active lesions) and remyelination (as assessed by the volume of shadow plaques), although the size of each lesion has appeared to be bigger in the PPMS patients than in SPMS patients.[149]

6.2.4 Factors influencing demyelination and remyelination:

Bramow et al. [149] found demyelination to be more prominent in remyelinated tissue than in normal appearing tissue which they described as the 2nd hit theory. This was mainly explained by the effect of slowly expanding demyelination (which are more prevalent in SPMS than in PPMS patients) rather than active demyelination.

Early pathology studies suggest that recurrence of demyelination in the shadow plaques contributes to future failure of remyelination in this region.[263] . It was also shown that age influences the rate of lesions remyelination, not the extent of remyelination. [264] remyelination in the optic nerve following optic neuritis can continue for up to 2 years. [265] It is also noted that patients who die older show more shadow plaques in comparison with those who die at younger age.[266] On the other hand, remyelination is more efficient when it is closer (72 hours) to the demyelination which is thought to be due to the easier recruitment of remyelination progenitors at the time of demyelination,[267] as well as the role inflammation plays in efficient remyelination.

Individual variation across patients in the degree of remyelination and the frequency of shadow plaques is also described. [266] It is also noted that the degree of remyelination is influenced by the location of the lesion as the lesions located subcortically or in the deep white matter showed more complete remyelination than the periventricular plaques.[266]

Mixed cortical lesions, as they extend into both the cortex and the WM hence the assumption they are lesions of the same age, showed signs of more extensive remyelination (6.8 folds) in the cortical than in the WM portion of the same lesion.[268] Moreover, the oligodendrocytes observed in the WM portion of the lesion had a dystrophic appearance, characterized by fragmented processes and no myelin internodes.[268] It was suggested that white matter lesions expressed higher amounts of molecular inhibitors, such as GFAP, CD44, hyaluronan, and versican, which potentially inhibits brain repair and remyelination.[268]

Whether targeting demyelination and enhancing remyelination in early stages of the disease is beneficial in terms of preventing long term disability is controversial. In C57BL/6 mice that were fed cuprizone, complete remyelination after demyelination was found to restore the loco motor function is short term, on the other hand, it did not prevent late onset functional deficits.[269] It also showed that a low degree of axonal degeneration has continued even after complete remyelination which probably causes the long term disability in MS patients with increase disease duration. On the other hand they could not exclude the long term effect of the cuprizone on axonal loss.[269]

Remyelination varies across MS patients, partially because of the previously discussed lesion heterogeneity. The degree of remyelination varies between 0 and 96%.[266] Another explanation to the variation in the degree of remyelination is the chronicity of the disease course. It is documented that remyelination is very prominent in very early stages of the disease and it can start within a few weeks from the acute MS lesion evolution.[270] Repeated demyelination can contribute to persistent demyelination and failure of remyelination.[263]

6.2.5 Monitoring remyelination using MTR:

Deloire- Grassin et al in 2000 reported in a comparative study analysing MTR in vivo and histological data in a toxic demyelination model, and MTR changes in correlation with the histopathological modifications of myelin. Reduction of MTR values was correlated with tissue destruction mainly demyelination and axonal loss, on the other hand the differentiation between the two is difficult.[271] Several longitudinal MRI studies on WM lesions showed that MTR values drop initially with the enhancement of the lesion and then most lesions MTR values recover over subsequent months with most of the recovery within the first two months, while a very small subset of patients do not show MTR recovery.[272] This reflects the degree of repair in these lesions which could vary between lesions which reflects again the heterogeneity of the MS plaques. It was reported that there is an association between the WM lesion MTR signal inhomogeneity on baseline MTR sequences and the change in lesion MTR 2 months later which is likely to reflect the stages of demyelination and remyelination.[273]

5 year longitudinal study[274] (with a 6 monthly scans) of 30 MS patients showed focal MTR decline significantly in the WM tissue in prelesional phase up to 2 years prior to the appearance of lesions on T2 sequence. .[167] This could be explained by either increase susceptibility of these regions to lesion formation or the early abnormality of lesion formation and break down of blood brain barrier, another explanation is that these regions were influenced by nearby lesions causing disruption of the axons going through these apparently normal regions.

Due to the heterogeneous evolution of various MS plaques, Chen et al used voxel based analysis to quantify the change in MTR of individual voxels of MS lesions over a period of 6.5 months.[275] They found that after initial lesion enhancement, MTR values were low and stable in the lesion centre, while increased at the lesion border..[275] voxel based analysis of individual lesions [276] showed that over a period of 39 months some of the initially enhancing voxels showed ongoing decrease in MTR , some showed ongoing increase in the MTR and some showed low values that remained stable which is suggestive that heterogeneous demyelination and remyelination in individual lesions can carry on for years which has implications on the disease progress and the patients prognosis.

To our knowledge the variation in focal cortical lesions myelination status over time has not been studied so far among different MS phenotypes. We aimed to follow a group of patients of different disease subtypes over 12 month's period, to investigate the difference among different phenotypes in the degree of change in mean MTR values in focal cortical and WM lesions.

6.3 Patients and Methods:

The initial protocol applied in chapter 3 at baseline (time point 0), was repeated on a subgroup of patients cohort that was used in chapter 3 (4 CIS, 6 RRMS, 8 PPMS and 5 SPMS) 12 months later (time point 1). Figure 6.3

Mean patients age 41 ± 16 years for CIS group, 41 ± 7 years for RRMS, 50 ± 6 years for PPMS and 52 ± 6 years for SPMS. Mean disease duration for CIS patients was 0.93 ± 0.34, RRMS was 4.04 ± 2.45, and PPMS was 10.16 ± 3, while SPMS was 12.12 ± 7.86 years. None of those patients was on disease modifying treatment. There was no change in any of the patients EDSS between time point 0 and time point 1.

For each participant (patient and control), the GM maps extracted from the PSIR images at time point 1 were linearly and non-linearly registered onto the GM maps of the PSIR image at time point 0 image. The symmetric diffeomorphic (SyN) registration approach,[277] which is part of the Advanced Normalization Tools (ANTs) package, was used due to its excellent performance and its ability to handle large deformations (such as those required to register control scans with ordinarily sized ventricles to patient scans with enlarged ventricles). All registrations were manually quality controlled. The GM maps were registered instead of the PSIR or MTR images as that made for improved registration quality, both qualitatively (upon visual inspection) and quantitatively (in terms of similarity metric, when comparing the mutual information of the PSIR images directly registered with SyN against that of the PSIR images registered by applying the deformation field estimated from the registration of GM maps). The estimated non-linear deformation fields were then applied to the MTR scans.

Manual comparison of individual lesions histograms in a pilot sample:

Before exploring the mean change of lesion MTR, I attempted to demonstrate if histogram analysis can detect shifts of MTR values in lesions. In this first step a random sample of 23 segmented cortical and 10 WM lesions, of different subtypes, was selected in patients of different disease courses. I compared the histograms of those lesions over two time points of acquisitions. Lesion segmentation and histogram comparison was done manually as a sort of initial assessment. Due to the time consuming nature of the analysis, this methodology could not be applied on all cortical and WM lesions examined in all patients, hence an automated methodology was adopted as following. Examples of the histograms are demonstrated in figure 6.4.

Automated analysis of all cortical and WM lesions in all patients:

All cortical and WM lesions were segmented on the MTR images. Each lesion ROI was replaced by a small ball of radius 3 positioned at the lesion's centre and removed from it all voxels which might be CSF (i.e. all those voxels whose MTR is less than CSF mean + 1 CSF std). This provided a more consistent way of examining the core of each lesion, reducing partial volume effects and avoids assessing the lesions borders that might exhibit some degree of remyelination. Then the average MTR values in each GM and WM lesions (mean MTR time point 0 and time point 1) were computed and normalised them using a variety of techniques:

1- By the overall MTR GM average, with the percentage difference between time point 0 and time point 1

2- By the overall MTR GM & WM average

3- By the overall modulated MTR GM value. The modulation takes into account the amount of MTR variability calculated across all patients for each region in the Harvard/Oxford GM atlas, with the more variable regions counting less towards the modulated average.

Each of these normalisation techniques has their advantages and disadvantages. For instance, the modulated GM normalisation should be more precise for cortical lesions but perhaps too strict for mixed lesions whilst the GM & WM normalisation should be more suitable to mixed lesions subtype but less good for pure GM lesions. Hence, it seemed that the second method which involves normalising the cortical lesions mean MTR values by the overall MTR GM& WM average is the most appropriate method.

In order to set a threshold to classify the change in mean MTR values of each lesion into increasing, decreasing or no change, we tried 3 different methods:

1) We used 1 standard deviation of the average change in individual lesions mean MTR values as a cut off with lesions showing changes in their mean MTR values higher than that figure considered as having increase in their mean MTR values, while similar values meant no change, and lesions showing change in their mean MTR values less than that number considered with reduced mean MTR values across two time points. (Results reported in Table 6.1)

2) Trying in this pilot study to detect any signal of change of MTR over a period of a year we also used a low threshold of change (r 0.05) The results are reported in Table 6.3
3) The third threshold was also used in between the above 1and 2 And results reported in Table 6.2

   The first and third thresholding techniques have not detected many lesions showing change and probably reflect the non-changing nature of mostly chronic cortical lesions over one year. As this is an exploratory study, mainly trying to understand the changes in individual lesions mean MTR values in different MS subtypes, we also used the least conservative threshold allowing us more lesions to examine. I fully appreciate that the results using this threshold might not reflect real change of myelin tissue but wanted to explore if I can identify directions for future research.

6.4 Results:

Comparing cortical lesions histograms across two time points:

In a random sample of 23 cortical lesions (18 subpial, and 5 mixed lesions), histograms for those lesions were compared between two time points. (Illustrated in figure 6.4) Seven of those lesions showed stable MTR values histograms across the two time points, while 6 lesions showed shift of MTR histograms to the left, and 10 lesions showed MTR histograms shift to the right across the two time points.

Comparing white matter lesions histograms across two time points:

On comparing histograms of a subgroup of 10 white matter lesions, 2 out of the 10 lesions showed stable MTR histograms, 5 lesions histograms showed a shift to the right, while 3 showed shift to the left across time point 0 and time point 1.

Comparison between individual CL mean MTR values across time points: (automated analysis)

Cortical lesions

The collective number of cortical lesions in different MS subtypes and the percentage of lesions showing stable, increase or reduction in their mean MTR values across two time points is illustrated in Table 6.5.

A significant difference was found in the frequency of cortical lesions showing increase or decrease in their mean MTR values among MS subtypes across time points using Pearson Chi-Square. Patients with RRMS showed the highest frequency of cortical lesions with decrease in mean MTR values across two time points, while CIS patients showed the lowest frequency. SPMS and CIS patients had the highest percentage of cortical lesions showing increase in mean MTR values across two time points in comparison with other MS patients' subtypes. (Figure 6.1)

The average change in the mean MTR values of individual cortical lesions over 12 months in CIS patients was .053 ± 0.089, RRMS 0.001 ± 0.074, PPMS 0.018 ± 0.127, while SPMS was 0.061 ± 0.105.

As the average changes of mean MTR values of individual cortical lesions across different MS phenotypes are normally distributed, one way ANOVA was applied to test any significant difference among the groups. Significant

difference was observed among different MS phenotypes in the average change of mean MTR values of individual cortical lesions ($p < 0.001$, F= 12.47). On running a post hoc and correction for multiple comparisons, significant difference was detected between SPMS and RRMS ($p < 0.05$; 95% confidence interval 0.32 – 0.09), as well as between SPMS and PPMS (p <0.05; confidence interval 0.017 – 0.068). There was no significant difference between SPMS and CIS patients ($p = 0.9$; 95% confidence interval 0.05 - 0.07).

There was no significant difference among lesions subtypes in the degree of change in mean MTR values across two time points on using Pearson Chi Square ($p = 0.07$). Table 6.6

The average change of individual cortical lesions mean MTR values over 12 months' time was the highest for mixed lesions (mean 0.04 ± 0.109), followed by subpial lesions (mean 0.034 ± 0.104) while intracortical lesions (mean 0.025 ± 0.112) showed the smallest degree of changes over the 12 months period.

I could not detect any significant difference between cortical lesions subtypes in terms of the degree of change of mean MTR values over 12 months period on using one way ANOVA. (p=0.413, F= 0.886)

On comparing the degree of change in individual cortical lesions mean MTR values between male and female subjects, it was observed that males show significantly (p= 0.001) higher average change in their cortical lesions mean MTR values (mean 0.056 ± 0.124) in comparison with female subjects (mean 0.022 ± 0.097) on using Mann – Whitney U test.

There was no significant correlation between the changes in individual cortical lesions mean MTR values across time points and the disease duration, or age (*rho* = - 0.1, *p* = 0.08 and *rho* = -0.03, *p* =0.52 respectively) on using spearman correlation coefficient.

White matter lesions

There was a significant difference among the groups in the frequency of lesions showing increase or decrease in their mean MTR values using Pearson Chi – Square test ($p < 0.01$). Table 6.7

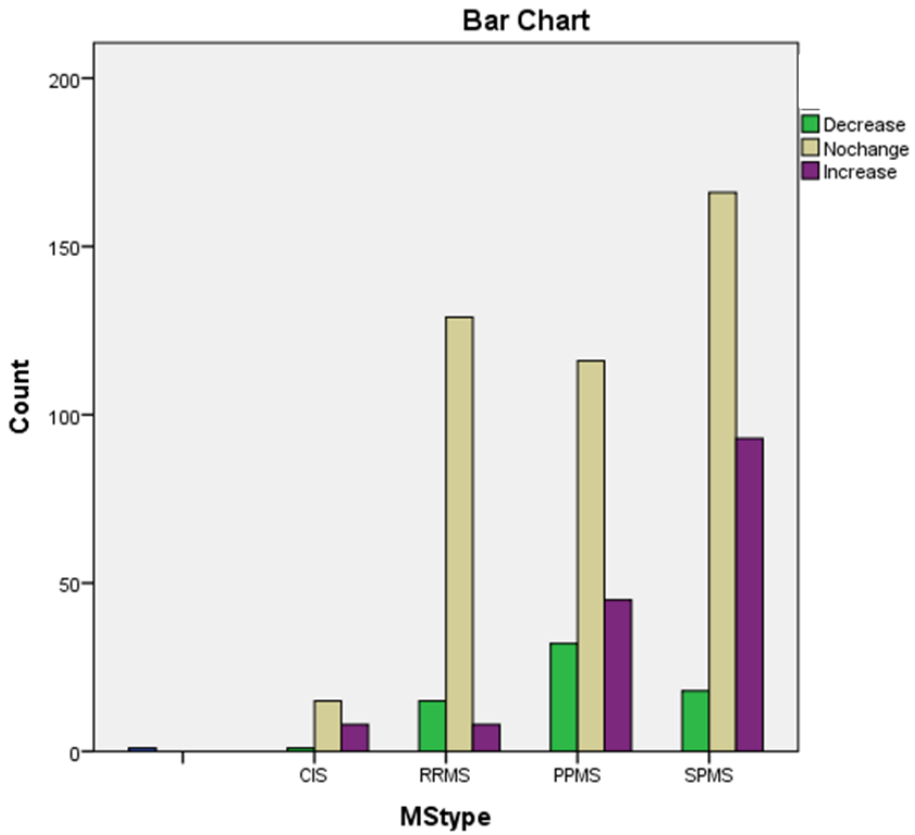
The average changes in the mean MTR values in individual WM lesions was the highest in RRMS patients (mean 0.134 ± 0.143), followed by CIS (mean 0.050 ± 0.131), and SPMS (mean 0.032 ± 0.193), while PPMS patients showed the lowest variation in mean MTR values of individual WML across time points (mean 0.001 ± 0.242).

As the changes in mean MTR values in individual WM lesions across the two time points was normally distributed, one way ANOVA was used to compare the distributions of these values among patients of different MS phenotypes. There was a significant difference in individual WML mean MTR values average change across time points. ($p < 0.001$, F = 10.4). On post hoc comparison among different phenotypes, significant difference in individual WML average change in mean MTR values between RRMS and PPMS patients. ($p < 0.05$, 95% confidence interval 0.069 and 0.202), as well as between RRMS and SPMS ($p < 0.05$, 95% confidence interval 0.039 and 0.164).

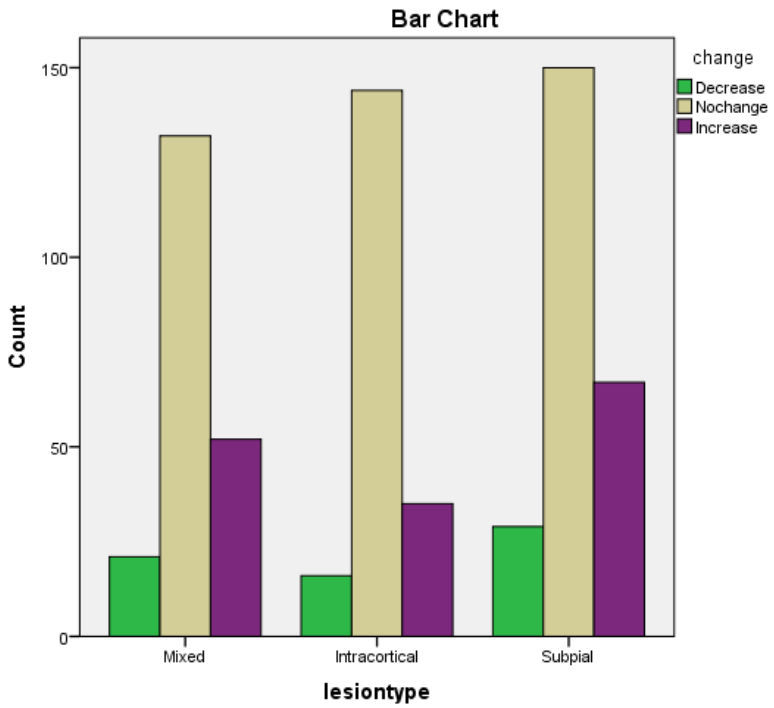
The average change in mean MTR values of individual WML across the two time points was significantly higher in female (mean 0.098 ± 0.171) than male subjects (mean 0.012 ± 0.218) using Mann Whitney U test ($p < 0.05$).

Using spearman's correlation coefficient to assess the association between the changes of mean MTR values of individual WML across two time points with the patients age and disease duration, a significant negative correlation was found with patients age (*rho* = -0.2, $p < 0.01$), while no significant association was detected with disease duration (*rho* = - 0.1, $p = 0.08$).

(Figure 6.1) The average variation in mean MTR values in all cortical lesions across time points in different disease phenotypes



(Figure 6.2) The average variation in mean MTR values in Mixed, Leukocortical, and Subpial lesions across time points



(Table 6.1) The change in cortical lesions mean MTR values in MS groups (Threshold 1 SDV of mean changes)

		MTR change			
		decrease	No change	Increase	Total
MStype	CIS	0	22	2	24
	RRMS	0	151	1	152
	PPMS	4	178	11	193
	SPMS	0	257	20	277
Total		4	608	34	646

The change in cortical lesions mean MTR values in MS groups across two time points using 1SDV of the mean changes in lesions MTR values between visits as a threshold to classify lesions.

(Table 6.2) The change in cortical lesions mean MTR values in MS groups (Threshold 0.07)

		MTR change			
		Decrease	No change	Increase	Total
MStype	CIS	1	20	3	24
	RRMS	6	140	6	152
	PPMS	18	147	28	193
	SPMS	7	213	57	277
Total		32	520	94	646

The change in cortical lesions mean MTR values in MS groups across two time points using 0.07 as a threshold to classify the mean changes in individual lesions MTR values between visits.

(Table 6.3) The change in cortical lesions mean MTR values in MS groups (Threshold 0.05)

		MTR change			
		Decrease	No change	Increase	Total
MStype	CIS	1	15	8	24
	RRMS	15	129	8	152
	PPMS	32	116	45	193
	SPMS	18	166	93	277
Total		66	426	154	646

The change in cortical lesions mean MTR values in MS groups across two time points using 0.05 as a threshold to classify the mean changes in individual lesions MTR values between visits.

(Table) 6.4 The number of lesions and the change in mean MTR across the two time points in different patients

Patient ID	Disease subtype	No of cortical lesions with mean MTR increase	No of cortical lesions with no change in mean MTR	No of cortical lesions with mean MTR decrease
3	CIS	0	4	0
4	CIS	2	1	0
20	CIS	4	4	0
26	CIS	2	6	1
5	RRMS	1	11	1
8	RRMS	1	10	1
10	RRMS	1	8	6
37	RRMS	4	30	1
41	RRMS	0	35	1
42	RRMS	2	35	3
6	PPMS	2	10	5
11	PPMS	2	19	6
15	PPMS	3	16	0
18	PPMS	13	14	12
23	PPMS	2	0	0
24	PPMS	0	5	4
28	PPMS	0	4	0
40	PPMS	23	19	5
9	SPMS	38	32	1
14	SPMS	21	35	4
25	SPMS	23	48	3

Patient ID	Disease subtype	No of cortical lesions with mean MTR increase	No of cortical lesions with no change in mean MTR	No of cortical lesions with mean MTR decrease
31	SPMS	7	31	6
45	SPMS	4	19	4

(Table 6.5) Cortical lesions change in different MS phenotypes:

Disease Phenotype (collective number of lesions in all patients)	% of CL showing increase in mean MTR values	% of CL showing reduction in mean MTR values	% of CL showing no change in mean MTR values
CIS (24)	33	4	63
RRMS (152)	5	10	85
PPMS (193)	23	16	61
SPMS (277)	33	6	61

The table illustrates the number of cortical lesions in different MS phenotypes and the percentage of lesions changing or showing stable mean MTR values over 12 months' time.

(Table 6.6) Cortical lesions subtypes change in different lesion subtypes

Cortical lesion subtype	% of lesions showing increase in mean MTR values	% of lesions showing reduction in mean MTR values	% of lesions showing stable mean MTR values
Mixed lesion	25.4	10.2	64.4
Subpial lesion	27.2	11.8	61
Intracortical lesion	18	8.2	73.8

The table illustrates the percentage of different cortical lesion subtypes showing stable, increase, or reduction in their mean MTR values over 12 months' time

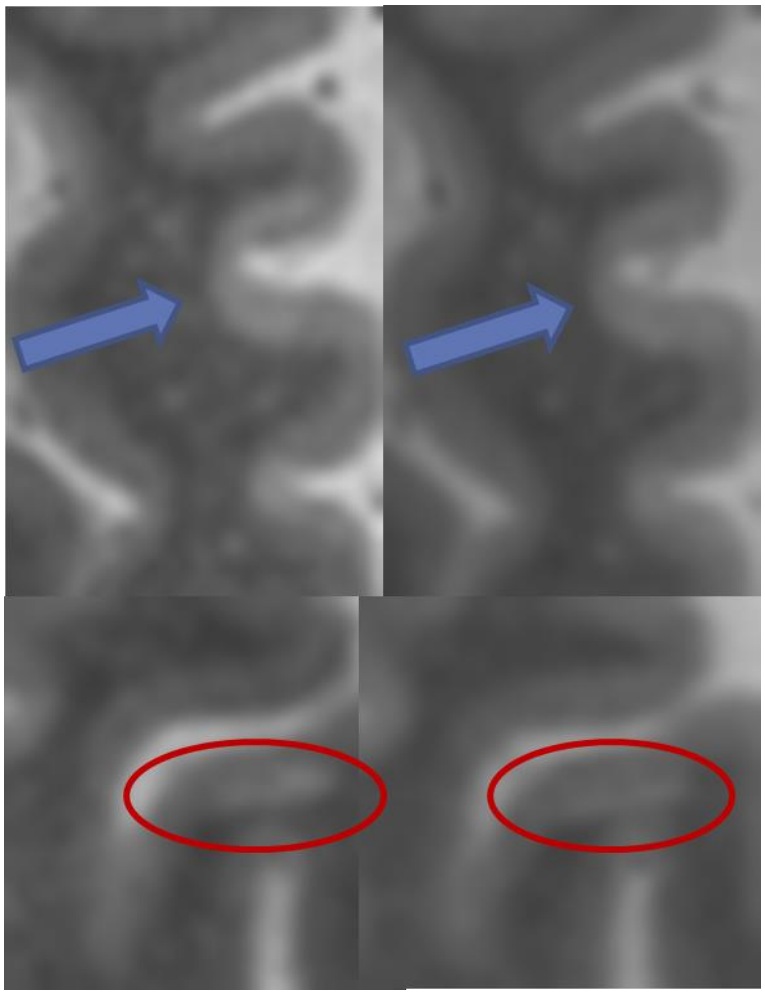
(Table 6.7) The percentage of white matter lesions changing over time

Different MS phenotypes (collective number of WML)	% of WML showing increase in their mean MTR values	% of WML showing reduction in their mean MTR values	% of WML showing no change in their mean MTR values
CIS (31)	32	13	55
RRMS (110)	53	3	44
PPMS (137)	33	27	40
SPMS (189)	34	25	41

WML = White matter lesion.

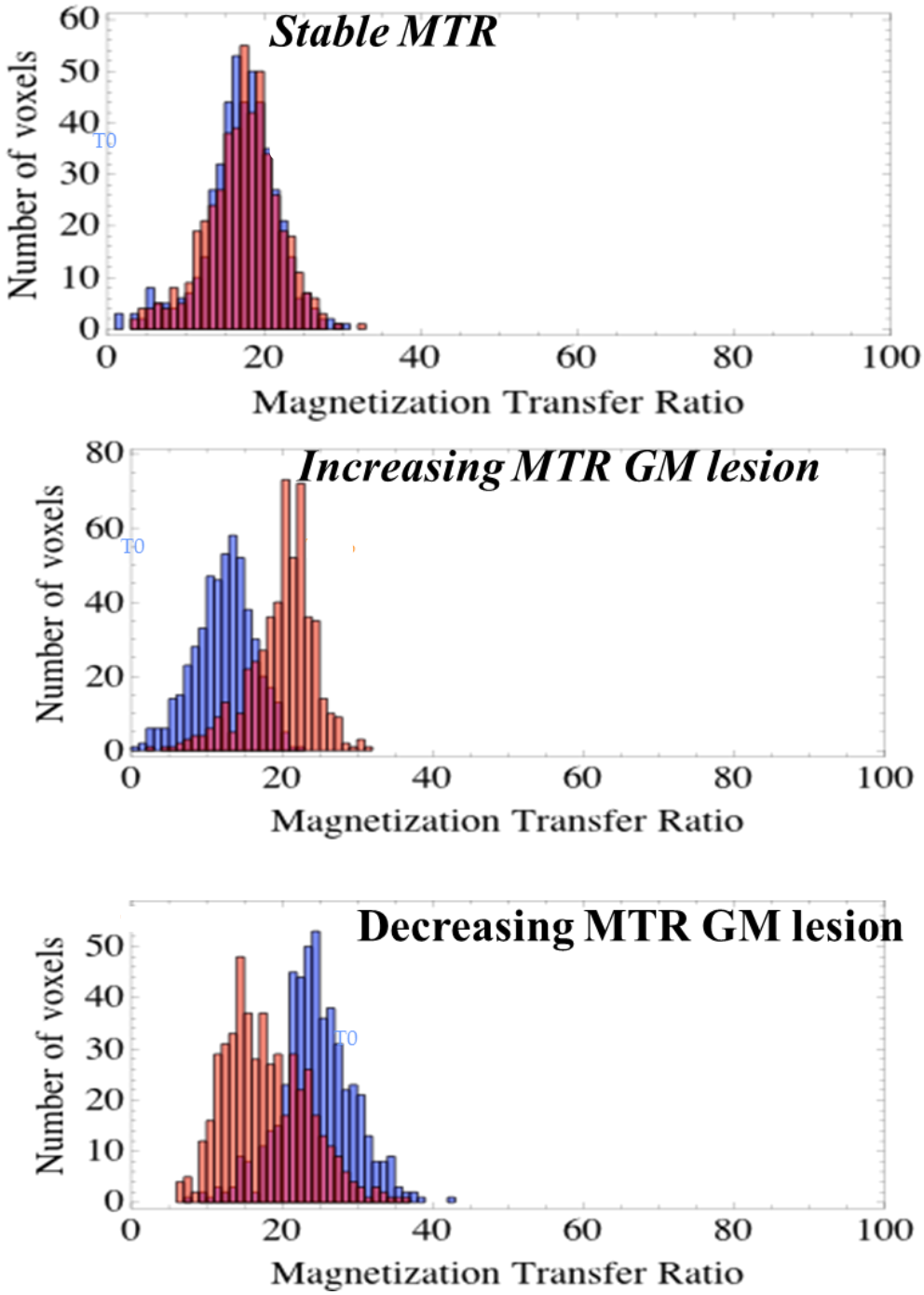
The table illustrates the collective number of white matter lesions in different disease subtypes and the percentage of lesions showing stable, increase, or reduction of their mean MTR values over 12 months' time.

(Figure 6.3) Subpial lesions on saturated MT images (MT sat) at time point 0 (left) and time point 1 (right)

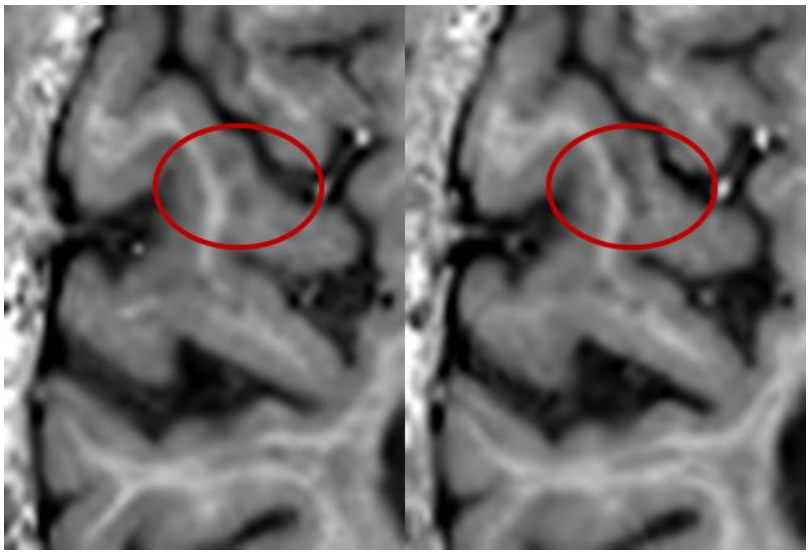


The figure illustrates two cortical lesions in two consecutive scans on MT_{sat} sequence. At the baseline visit (left) the lesion has a lower mean MTR (more demyelination), while at the follow up scan 12 months later (right), both lesions seem less bright, reflecting an increase in their mean MTR (remyelination with an element of resolved oedema).

(Figure 6.4) Three cortical lesions with one having stable MTR voxels (Top), one increasing MTR (middle) and one decreasing MTR (bottom) across time point 0 (Blue) and time point 1 (Orange)



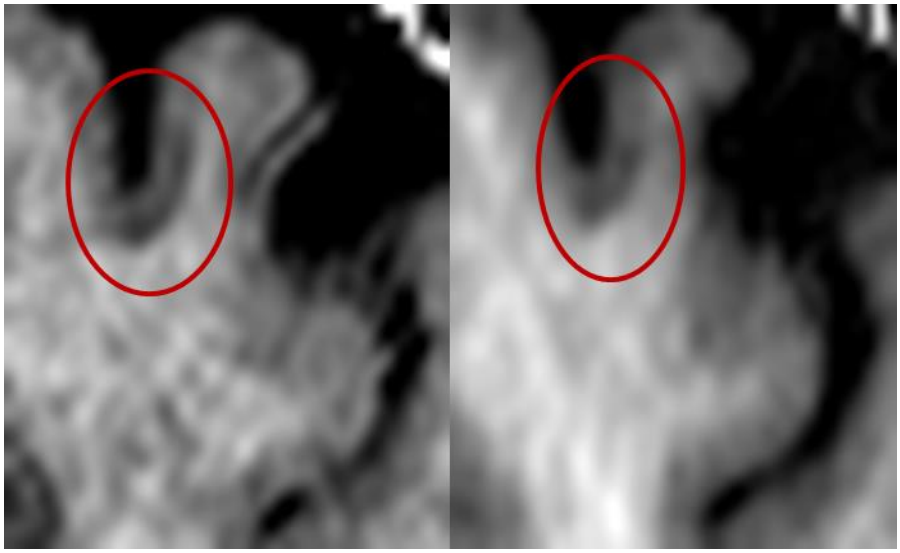
(Figure 6.5) Evolution of MTR values overtime



The figure illustrates the lesion at time point 0 (left panel) and at time point 1(right panel), showing a reduction in mean MTR value.

(Figure 6.6)

Evolution of MTR values overtime



The figure illustrates the change in a lesion's mean MTR value over time. A cortical lesion is shown at baseline (left panel) and at a follow up scan 12 months later (right panel), with increased mean MTR value, reflecting partial recovery of the lesion.

6.5 Discussion:

Studies in animal models showed that primary demyelination of axons following traumatic injury usually undergo complete remyelination, and long lasting demyelination of axons is unusual.[278] However, demyelination induced by immune mediated mechanism such as MS is associated with an unfavourable environment for oligodendrocytes which makes it difficult for remyelination in some cases to be complete.

Various imaging sequences have been used to examine the degree of demyelination.[279] In this exploratory study, we examined the variation in individual CL and WML mean MTR values over a period of a year among different MS subtypes, the frequency of those changes, their correlation with age, gender, and disease duration. Despite overlap of whole brain histograms of both time points as well as normalisation of lesions MTR values, a higher frequency of lesions in all patients' subtypes seemed to show increase in their mean MTR values over the study period irrespective of the threshold used.

Changes of lesions mean MTR values across MS subtypes

The heterogeneity of oligodendrocyte destruction and preservation pattern influences the degree of remyelination in different MS disease subtypes.[280] Remyelination is frequent in all stages of the disease, although it is usually very limited in chronic lesions. Surprisingly, in this study the frequency of cortical lesions showing increase or reduction in their mean MTR values was quite similar between CIS and SPMS patients. The high degree of disability known in SPMS patients for which CL are found to have substantial contribution, could be explained by remyelinated lesions, with associated depletion of OPCs around the demyelinated plaques, becoming more and more

of a target to demyelination[263] attacks rendering not only the degree, but the quality of remyelination in chronic MS lesions less and less conceivable. This could partially explain the smaller number of CL detectable in CIS patients with more efficient remyelination early on in the disease course in comparison with SPMS patients.

In this study most CL and WML mean MTR values over the 12 months' period remained unchanged. CIS and SPMS patients showed the highest frequency of CL exhibiting increase in their mean MTR values over the study period in comparison with the other groups, While RRMS patients had the highest frequency of WML showing increase in their mean MTR values across the study period in comparison with other disease subtypes. A histopathological study mainly on progressive MS patients' samples, showed cortical remyelination more extensive than WM remyelination.[90]

The frequency of WML showing reduction in their mean MTR values over the study period was higher than CL across all patients groups but was more pronounced in PPMS and SPMS patients. Despite the high impact of cortical lesions on patients disability, our findings emphasises the significant contribution of WML to disability progression in those disease subtypes.Disability progression in SPMS patients despite the high frequency of CL exhibiting increase in their mean MTR values, could be explained by the increase frequency ofWML experiencing reduction in their mean MTR values and probably disrupting long tract axons.

In this study, the average change in cortical lesions mean MTR values was higher in CL in comparison with WML in SPMS patients, which is consistent

with pathology data suggesting that remyelination in cortical MS lesions is more extensive than in the WM lesions in chronic progressive MS brains.[90]

Reduced degrees of remyelination seemed to have different mechanisms depending on the stage of the disease. In late stages of the disease it is mainly due to poor recruitment of oligodendrocytes, while in earlier stages of the disease where more oligodendrocytes are available, impaired maturation seem to be the main problem causing inefficient remyelination.[254] Failure in recruitment phase could be due to the presence of antibodies against OPCs [281] or repeated demyelination contributing to cells exhaustion in the affected region.[270] Also the genetic and environmental variation between patients such as epigenetic changes in oligodendrocytes, growth factors, expression of myelin inhibitors or abnormal composition of the chronically demyelinated axons remain to be significant factors influencing the degree of remyelination is a possibility needs further examination.[282,283]

Remyelination capacity in MS patients is variable not only between groups, but also within individual patients' brain depending on several parameters. The localization of lesions influences the degree of remyelination. Pathology studies suggested that higher degree of remyelination was observed as lesions localize closer to the cortex.[140] In this study, excluding RRMS patients, the average change in mean MTR values was higher in CL of MS subtypes in comparisons with WML. The frequency of lesions showing increase or reduction in mean MTR values was similar across mixed, intracortical, and subpial lesion subtypes.

In our SPMS patients, the average change in CL mean MTR values was higher than that of WML. Previous studies suggest that environment of chronically demyelinated cerebral cortex is more conductive to oligodendrogenesis and remyelination than the environment of chronically demyelinated white matter.[268] Pathology data suggests that the number of oligodendrocyte progenitor cells in CL is not less than that in nonlesion cortex, hence their ability to produce myelinating oligodendrocyte might contribute to the greater repair capacity of CL when compared with chronic WML.[268]

Previous pathology studies detected higher degree of WML remyelination and smaller degree of active demyelination in PPMS than in SPMS patients, although the remyelination was the significant effect even after correction for the effect of the active demyelination.[149] In this exploratory study, PPMS and SPMS patients had similar frequency of WML showing increase, or reduction in their mean MTR values across two time points. This could be due to the short interval between scans (12 months apart) with most lesions remaining unchanged, hence the difficulty of comparing our results with gold standard pathology.

Association of lesions mean MTR changes with disease duration

In this study, no association was found between the degree of change in individual lesions mean MTR values and disease duration, which could be partially explained by the short disease duration of our patients especially of progressive MS patients in comparison with samples included in pathology studies.[90] Histological analysis do not allow for longitudinal studies, and the

lack of robust imaging techniques measuring remyelination makes it difficult to determine the exact frequency and stages of remyelination in vivo.

Demyelination is known to be more abundant in progressive stages of the disease; however the correlation with disease duration is rather variable across different studies. Surprisingly in our study, the frequency of CL exhibiting reduction in their mean MTR values across time point was similar in CIS and SPMS groups. However, SPMS patients showed high frequency of WML with mean MTR reduction in comparison with CIS and RRMS patients. No association was detected between disease duration and either CL or WML mean MTR values changes. This is similar to a previous pathology study suggesting variation in the degree of remyelination between early and progressive MS subtypes with no obvious correlation with disease duration.[140] The lack of correlation with disease duration in our study could be due to the short disease duration of our subjects in comparison with other studies. Another possibility is the different techniques used assessing remyelination with pathology being the gold standard, in comparison with our study using MTR at 7T with the disadvantage of inhomogeneity probably affecting some of our measures.

We found that CL exhibiting increase in their mean MTR values across time points was prominent in all patients groups, with the lowest degree in PPMS patients. Patients with SPMS particularly had shorter disease duration, in comparison with other studies. In pathology studies, shadow plaques were more frequent in RRMS patients than in SPMS patients.[266] Remyelination in cortical lesions was evident in more than 95% of patients with long standing MS.[90] Another pathology study reported that remyelination is extensive in a

subset of SPMS patients with longer disease duration but it was clear that this was in a subset of patients while the rest showed low remyelinating capacity.[266] My results should be interpreted with caution due to the small sample number included.

Association of lesions mean MTR changes with age

Remyelination is regulated by multiple factors which constitute a very complex signalling network that has to be very tightly regulated in order to create an optimum environment for a successful remyelination. Views regarding age as a factor that could influence remyelination are conflicting. In this study, there was no association between age and the degree of change of mean MTR values in either cortical or white matter lesions. Age associated reduction in remyelination was reported with impairment of OPCs recruitment and differentiation into remyelinating oligodendrocytes in older animal.[284] The rate of colonisation of OPCs in depleted areas in animal models was found to show steady decline in their rate of depleted tissue colonisation by OPCs with age.[258] It was suggested that not only the age related changes in biological characters of these OPCs but also the age related variation in the environment of the demyelinated plaques influencing the rate of the OPCs migration, proliferation or differentiation into myelin sheath forming oligodendrocytes. [258] Macrophages are a significant source of growth factors [285] and important for clearance of myelin in the lesion, hence are very important for remyelination and it is noted that they are deficient in aged tissue due to observed delayed inflammatory response.[286] Pathology studies, showed active remyelination to be present in patients of all ages with no correlation between the extent of remyelinated lesions and age at death.[268] Shields et al suggested that age

influences the rate of remyelination rather than the degree of remyelination.[264] This was found on following the animals at 4 and 9 weeks. At 4 weeks young rats showed higher degree of remyelination than young rats while at 9 weeks, there was no difference in the total extent of remyelination between young and old animals.

Association of lesions mean MTR changes with gender

In this study, the average change in WML mean MTR values was significantly higher in females than males; a similar finding was suggested by pathology study on animal models. Similarly Goldscmidt et al [140] examined biopsies from MS brains of different disease duration. They found that WML in women brains showed more extensive yet not significant remyelination capacity than WML in male brains. However this was not replicated in CL, as the average change was found to be higher in males than in females. Studies support the view of sex dependent variation in the degree of remyelination. More oligodendrocyte progenitors were observed in cultures of female neonatal rodents' brains than males. Treatment with 17β- estradiol delayed the exit of oligodendrocyte progenitor cells from the cell cycle, while progesterone was found to enhance cellular branching, which suggests that sex hormones probably contribute to gender specific difference in repair.[287] Another study[288] suggested that the variation in remyelination capacity between male and female animal models is only apparent in old rats, although, they could not demonstrate a role for sex steroid hormones in influencing remyelination in either young or old animals.

Conclusion:

The percentage of CL exhibiting increase in their mean MTR values over 12 months period was similar in CIS and SPMS patients possibly suggesting the high frequency of remyelination at least in a subset of SPMS patient. The average increase in CL mean MTR values was the lowest in PPMS patients. The frequency of WML showing reduction in their mean MTR values over the study period was higher than CL across all patients groups but was more pronounced in PPMS and SPMS patients suggesting the significant contribution of WML on patient's disability.

It is significant to note that we cannot imply causality from this study. Although causality could be interpreted on occasion from human longitudinal studies, it is very difficult to control for various parameters. Animal studies would be required to help provide insights into the causal relationship of the mechanisms of damage in brain tissue in MS.

Limitations of this work:

There are a number of caveats to MT imaging. First, the effect of B1 inhomogeneities present at high field can reduce sensitivity (increase variance) in MT measures, although these are reduced to some extent in MTR maps due to the way MTR is calculated and corrected for B1 effects[137,178] Second, the MTR maps are sensitive to movement artefacts, which will be exacerbated by acquiring two MTR images separately. Despite registration of those images, intra-scan movement during the 3D acquisition could degrade the quality of the final MTR maps. Due to the reduced myelin content in the outermost layers

of the cortex, it could be difficult to use MTR alone to classify CL especially for the subpial lesions, but the raw MT images especially the MT_{sat} provide clearer contrast between the cortex and the CSF, and hence easier classification. This is an exploratory study and we are currently following our patients longitudinally, which will help to address some of the concerns raised.

In this study we used the variance in mean MTR values across two time points as a cut off to classify the degree of change in lesions mean MTR values, 94% of lesions did not show any change in their mean MTR values across the two time points. This is probably due to the short interval between scans, and we expect that with following those patients for longer period of time, more detectable changes would be observed. This is an exploratory study hence the reason we used a smaller cut off point rather than the standard deviation of the mean difference over time to explore the different degrees of change in individual lesions mean MTR values among MS groups.

There is great degree of variation in the MTR values due to multiple factors. The variation in relationship between different tissue types and MTR, and the difficulty in predicting the behaviour of individual tissues in different MR system provides another challenge. As previously mentioned the low MTR can be a result of reduction of the capacity of the macromolecules to exchange magnetization with the surrounding water molecules or due to the amount of these macromolecules. Also the use of off-resonance saturation pulse, can cause unwanted direct saturation effects on the liquid pool.[289]

Studying the degree of remyelination in MS lesions in vivo is challenging, mainly due to the lack of robust imaging techniques. Another aspect is that

remyelination has proven to be heterogeneous even in lesions within the same patients brain.[140]

Exact interpretation of these results is quite difficult, as the change in focal lesions mean MTR values is multifactorial. It reflects partially demyelination involving some of lesions voxels while other voxels may show remyelination or even failure of remyelination with static MTR values. Longitudinal voxel based studies for that number of lesions in such a number of patients does not seem practically plausible in terms of resources availability due to the lack of availability of automated method as yet. Also the changes in the MTR values are specific but not restricted to myelin pathology, as the reduction in MTR could be not only correlated to the myelin loss in brain tissue but to a degree with associated axonal loss as well, and the discrimination between both is often unachievable by imaging techniques. However when Schmierer et al [62] regressed MTR on axon count and myelin transmittance at the same time, the association between MTR and axonal count disappeared.

The calculation of MTR for individual voxels or for a region of interest depends on the accuracy of the registration of the saturated and the unsaturated images. A shift of 1mm or less due to patient motion could affect the calculated values. Also the partial volume effect between white and grey matter tissue or cortical tissue and CSF can affect the MTR values.

I did not assess exactly the mean MTR values in lesions but instead for practicality lesions were replaced with radius 3 small ball expanding from the centre of the lesions, hence the possibility that some of these voxels could have been contaminated by normal appearing brain tissue.

Finally, we examined a small sample of patients, 10-11 subjects of each phenotype. Future larger studies in attempt to replicate this project's findings will be necessary.

I did not assess the influence of topographical distribution of focal lesions on the variation in mean MTR values across time points. I measured mainly cortical volume although cortical thickness is a more robust measure of atrophy but its applicability could be difficult as it mainly requires the use of FreeSurfer, which is not adapted for use on 7T scans. Similarly. I did not obtain any fMRI data, which would have been very helpful to verify the association between various cognitive functions and cortical pathology. I did not take into consideration white matter volume or thalamic volume either. There were no spinal cord measures included into this study.

For cognitive assessment I used BICAMs, which does not take into consideration executive function or working memory tests.

Assessing the degree of demyelination and remyelination, and finding a feasible way to enable its application into clinical practice could assist determining patient treatment options and monitor response.

Chapter 7

General Discussion& Conclusion

Grey matter pathology in general and cortical lesions in particular have proven to be a very significant part of MS pathology. Cortical lesions have always been difficult to examine in vivo, but over the past few years various imaging sequences have emerged improving cortical lesions detection, hence expanding the horizon to explore the contribution of cortical lesions to physical and cognitive disability in MS patients. Various imaging studies take into account the cortical lesion load and its association with disability without taking into account the degree of damage in these lesions and its correlation with the different clinical parameters in those patients.

In this project we had several objectives:

We initially aimed to find a sequence with pathological specificity that is sensitive to depicting cortical lesions within a clinically acceptable acquisition time.

Magnetization transfer imaging has a good potential, through its semi quantitative properties which gives it superiority over conventional MR sequences, which lack pathological specificity in distinguishing different disease characteristics. Pathology studies showed the reduction of MTR values to correlate with tissue destruction mainly demyelination and axonal loss. We wanted to use one sequence type that would enable us to detect cortical lesions and provide us with information regarding lesional microscopical characteristics.

We initially explored the ability of MTR scans at 7T to detect cortical lesions. This sequence has the advantage of being a quantitative method, hence the capability to detect the degree of damage in tissue reflected in mean MTR values.

We examined 18 MS patients and 9 healthy controls using 7T MTR, 7T MPRAGE, and 7T T2*. On a subset (8 subjects) of the same patient cohort, 3T 3D DIR was acquired on the same session. We compared the ability of each of those sequences to detect cortical lesions.

7T MTR appeared to improve cortical lesion detection in comparison with other 7T sequences (MPRAGE and T2*) and 3T 3D DIR. The abnormalities that were found on MTR corresponded to cortical lesions on the other sequences. Ultra-high field MRI increases the signal to noise ratio, hence enhance the detection of GM lesions. In our study, the 7T T2* rate of cortical lesion detection was particularly low in comparison with previous studies, but this partly because we used lower in-plane resolution as a trade-off for larger coverage of the brain in shorter acquisition time.

Retrospective analysis in our study showed more lesions that were not detected in prospective study. In the retrospective study, we examined all sequences simultaneously which provided complementary information about cortical lesions using different sequences. We know from pathology and imaging studies that different lesions are sensitive to different imaging modalities.

We found that MTR was a useful sequence in depicting cortical lesions and can be acquired within clinically acceptable time. MTR is a semiquantitative myelin sequence that is sensitive to depicting cortical lesions of all subtypes in

comparison with the other examined sequences. MTR was superior to conventional imaging sequences due to its pathological specificity. MTR values correlate mainly to myelin content in brain tissue and to a lesser degree with axonal loss.

As MTR is a considered primarily a myelin sequence but with an element of axonal loss detection, we wanted to compare the degree of demyelination, and potentially axonal loss[290] in cortical lesions in different disease phenotypes, and assess the correlation between the degree of damage and physical disability.

I used a larger sample of patient representatives of all MS phenotypes (11 CIS, 11 RRMS, 10 PPMS, and 10 SPMS). We acquired 7T MTR, and 7T PSIR on each patient. I compared cortical lesions counts, volume, and lesions mean MTR values amongst the groups. Cortical lesion count and volume were higher in SPMS patients than in other MS phenotypes. This was mainly due to the high number of mixed lesion subtypes. Subpial lesion volume was larger in the progressive forms of the disease, although the difference was to a smaller extent in comparison with mixed lesions. The lower number of lesions in early stages of the disease is probably due to the higher capacity to repair in early MS brains, with reduced efficiency of repair later on in the disease.

Moreover the mean MTR values of individual cortical lesions were lower in SPMS patients in comparison with CIS and RRMS patients. On the other hand, the difference was not significant between PPMS and RRMS patients.

Individual lesion damage in our study appeared to be clinically significant as individual cortical lesions mean MTR values was independently associated

with the degree of disability in patients. We could not replicate the same finding for white matter lesions mean MTR values. This suggests the important contribution of cortical lesions over white matter lesions in the degree of patients physical disability. Out of all cortical lesions subtypes, mixed lesions mean MTR values were associated with the degree of physical disability, probably because they are the most abundant lesion subtype. Another explanation is the location of mixed lesions that could be disrupting long tracts before they end in the cortex.

Subpial lesions had the lowest mean MTR values in comparison with intracortical and mixed lesion subtypes. This could be due to the lower myelin content in the outermost layers of the cortex, or less likely due to contamination of cortical lesions segmentation with nearby CSF voxels.

The presence of cortical lesions is specific to MS, significant in the diagnosis of the disease, and very helpful to differentiate it from MS mimics. The variation in the mean MTR values of individual cortical lesions among different MS phenotypes potentially provides additional tool to help differentiate these subgroups, which could potentially help management plans.

Physical disability correlated with cortical lesion load as well as with mean MTR values of individual cortical lesions, but not white matter lesions. The main significant correlation was with mixed lesions volume and mean MTR values. The location of mixed lesions to the cortex, hence the cell soma of long projecting axons, such as the corticospinal neurons, may be a contributing factor to patients degree of disability over white matter lesions. Such a finding is very significant in developing future treatment options for MS. Monitoring

the response to these therapeutic options is very significant especially in clinical trials. In our correlations we did not take into consideration the spatial distribution of cortical lesions, hence we cannot account for the functional localization of these lesions.

It was clear in our study that the influence of cortical lesions load and mean MTR values is more prominent than the influence of white matter lesions. The difference of mean MTR values in individual cortical and white matter lesions among different MS phenotypes suggests the heterogeneity in the pathogenesis of different types of the disease.

After examining the contribution of individual cortical lesion mean MTR values to the degree of physical disability, we similarly wanted to explore the association between the degree of damage in cortical lesions as reflected by their mean MTR values and the degree of cognitive impairment.

I used the same patient cohort that was used for physical disability cortical lesion mean MTR values correlations. That group included 11 CIS, 11 RRMS, 10 PPMS, and 10 SPMS patients. I used BICAMS to assess patient's cognitive performance. It is mainly composed of 3 neuropsychometric subtests SDMT, CVLL, and BVMT tests. Those tests have been modified to be practically appealing for clinical use with no impact on the sensitivity or specificity to detect cognitive impairment.

As others reported[206], we found that SDMT and BVMT-R were the most sensitive tests for cognitive performance in different MS phenotypes.

In this study, we found a significant correlation between lower cognitive scores and higher cortical lesion volume. I also found a significant association between different cognitive scores and cortical lesions and white matter lesions mean MTR values. This could be explained by cortical-subcortical tracts being very significant for good cognitive performance.

The correlation between cognitive scores and cortical lesions mean MTR values rather than their volume is an emphasis of the significance of the degree of damage in individual lesions over the mere lesion size. This also supports the notion that the state of tissue microscopic structure is more significant representation to the patient's clinical state than the macroscopic changes.

I found that cortical grey matter fraction was the lowest in SPMS patients in comparison with the other phenotypes, although the difference did not reach statistical significance. In this study cortical grey matter fraction did not correlate with physical disability represented by EDSS but it correlated with cognitive decline mainly BVMT scores. Previous reports[291] showed that regional cortical thinning in primary motor cortex correlated significantly with pyramidal FSS scores. However, in our study we did not attempt to correlate topographical distribution of cortical volume loss to the degree of disability due to technical reasons. Cortical thickness is probably a more accurate measure than cortical volume but far more challenging technique to implement especially on high field scanning.

I found that SPMS patients had the smallest cortical grey matter fraction. Unlike other reports [83] there was no correlation between the cortical grey matter fraction and cortical lesion load. This could be partially due to better

quality of cortical lesion segmentation with the use of high resolution scans in comparison with lower resolutions used by other studies. Reassuringly, cortical grey matter fraction correlated with cortical lesions mean MTR values which supports our previous suggestion of the significance of the degree of damage in lesions over the volume and its contribution to various clinical and radiological parameters. This correlation could be explained by damage in lesions causing retrograde axonal loss hence causing higher degree of atrophy.

With the consistent finding of the contribution of the degree of damage in lesions over lesions volume to various clinical parameters, it seemed crucial to examine normally appearing tissue and explore the variation in the degree of damage in normal appearing tissue among different MS groups and its correlation to different clinical parameters.

The degree of damage in NAWM and NAGM indicated by their mean MTR values was the severest in SPMS patients. The differences in NAWM mean MTR values not NAGM mean MTR values was statistically significant. The less significant difference between patients phenotypes in their NAGM is probably due to the high resolution scans allowing cortical lesions segmentation in probably more accurate way than other studies using lower resolution scans with less chance of NAGM contamination by undetected cortical lesions.

Cognitive performance correlated more with NAWM mean MTR values than with white matter lesions mean MTR values. This suggests the importance of white matter tracts integrity for higher cognitive functions. This illustrates the

significance of looking beyond lesion load and tissue atrophy to determine patient's treatment or monitor response and treatment escalation.

The pathogenesis of diffuse injury in the NAWM is controversial. Diffuse axonopathy independently form the focal demyelinating lesions, which could explain diffuse spread changes in NAWM of PPMS patients. Alternatively it could be secondary to retrograde Wallerian degeneration of the axons transecting demyelinated lesions which is supported by pathology studies showing correlation of WML load with the axonal density and number of axons crossing the corpus callosum in the corresponding lesions.

To further understand the pathology of normal appearing tissue and the role cortical and white matter lesions play, we examined in the same cohort of patients the influence of cortical and white matter lesions on nearby normal appearing tissue. We found mean MTR values to be lower in the normal appearing tissue voxels for up to 10 mm around nearby white matter lesions while cortical lesions influence on nearby tissue was limited to a shorter distance of 3 mm. This suggests that lesions especially white matter lesions are significant in the pathogenesis of diffuse tissue injury, although the role of diffuse axonopathy which independent from the focal demyelinating changes could be equally significant.

Both mechanisms probably coexist in each MS phenotype with variable degree depending on the MS type, with the latter probably more prominent in PPMS patients while in other MS phenotypes retrograde wallerian degeneration of the axons transecting demyelinated lesions could be the main contributing factor to the diffuse normal appearing tissue injury. The degree of axonal loss in the

corpus callosum was previously found to correlate with the lesion load of the corresponding projection areas which suggested mutual local and collateral effects on the changes in NAWM.

Several other factors could influence mean MTR values in the NAWM, such as the voxel location in relation to the ventricular CSF interface.

Unlike other studies, we could not find significant difference between the degree of damage in NAGM as indicated by their mean MTR values among different MS phenotypes, which could be due to the high resolution of our scans; and the ability to segment cortical lesions which probably went undetected in previous studies using lower resolution conventional MRI scans.

Understanding normal appearing tissue integrity, its clinical correlations, the pathogenesis of microscopic abnormalities and its relation to white matter and cortical lesions is crucial for future better understanding of MS pathology.

Remyelination in MS is very significant for protecting axonal integrity, restore nerve conduction, and prevent long term physical disability.

We found a variation in the degree of damage in focal lesions among different MS phenotypes, which could reflect the variation in the rate of demyelination and remyelination in lesions in various MS phenotypes. Pathological analysis of MS lesions showed variation in the patterns of demyelination in MS patients subtypes and at different stages of the disease. Moreover, studies showed that cortical lesions generally showed more extensive remyelination in comparison with white matter lesions even on case by case basis.

We examined the variation in myelin status in MS patients of different MS subtypes longitudinally over a 12-month period. We found that the pattern of demyelination and remyelination is different in different MS phenotypes, which could be due to the heterogeneity of oligodendrocyte destruction and preservation pattern.

Studies [262] showed that remyelination is usually limited in chronic stages of the disease, in contrast with our study we found that frequency of cortical lesions showing increase or reduction in their mean MTR values was quite similar between CIS and SPMS patients. It is well reported [280] that the high degree of remyelination in early stages of the disease, which we found in this cohort, but in terms of the findings in SPMS patients, only a few pathology studies [292] showed a high degree of remyelination in cortical lesions in a subset of SPMS patients.

The high degree of disability in our SPMS patients who had similar degree of remyelination as our CIS patients could be explained by the remyelinated lesions in SPMS patients becoming increasingly the target of demyelination attacks rendering not only the degree, but also the quality of remyelination in chronic MS lesions gradually poorer. Pathology studies[262]suggest that in chronic stages of the disease, remyelination is generally sparse and mostly involving the edges of the lesions. Moreover, early pathology studies[263] suggest that recurrence of demyelination in shadow plaques contributes to future failure of remyelination in that region.

Most lesions in the longitudinal study did not show any change in their mean MTR values, which is expected with the short time frame of the study and the lack of change in any of the patients EDSS.

The average change in cortical lesions mean MTR values was higher than in WML mean MTR values in SPMS patients, which is consistent with pathology data suggesting that remyelination in cortical MS lesions is more extensive than in the WM lesions in SPMS patients. Other studies suggested that environment of chronically demyelinated cerebral cortex is more conducive to oligodendrogenesis and remyelination than the environment of chronically demyelinated white matter. Moreover, the number of oligodendrocyte progenitor cells in cortical lesions is not less than that in nonlesion cortex; hence their ability to produce myelinating oligodendrocyte might contribute to the greater repair capacity of cortical lesions when compared to chronic white matter lesions.

Remyelination capacity is variable not only among MS groups but even within individual patients depending on several factors. Lesion location could influence the degree of remyelination to a degree as higher degree of remyelination was reported in lesions closer to the cortex.[266] In our study we found that the degree of remyelination in cortical lesions was higher than the degree of remyelination in white matter lesions.

We found that generally cortical lesions showing increase in their mean MTR values across time was prominent in all patients groups, with the lowest degree in PPMS patients. This could be due to a technical issue with the scanner, images registration or analysis.

I could not detect a correlation between disease duration and the degree of remyelination in early or progressive MS subtypes. This could be due to the short disease duration in our subjects in comparison with other studies.

The balance between demyelination and remyelination is so important in determining the disease course in MS patients. When demyelination is well established and remyelination is exhausted, this is when disability occurs. Frequently the balance between the degree of demyelination and the degree of remyelination in active lesions determines the outcome of an MS relapse.

Heterogeneous demyelination and remyelination in individual lesions has implications on disease progression and the degree of disability. Being able to monitor the degree of damage in lesions over time in a practical way could prove to be crucial for future patient management and monitoring treatment response especially remyelinating agents.

Overall, we found that MTR at 7 T is a sensitive sequence to depicting cortical lesions in comparison with other conventional MRI sequences that are currently being used for cortical lesions detection. MTR at 7 T has the advantage over currently used MRI sequences of being pathologically specific to microscopic changes in brain tissue especially myelin content.

The degree of damage in SPMS cortical lesions were the highest in comparison with cortical lesions in other disease phenotypes. The degree of damage in cortical lesions as reflected in their mean MTR values correlated with the degree of physical disability as well as cognitive impairment in those patients.

The degree of demyelination and remyelination is variable in cortical lesions in different disease phenotypes. Developing MTR sequence at 7T could provide a crucial step in further understanding MS pathology, and help future management.

Future Work:

MTR at 7 Tesla has proven to be beneficial in understanding cortical lesions pathology, the variation between different MS phenotypes and the association with physical and cognitive disability. With this in mind we hope in our future studies to build on this work further:

1- Perform the baseline study in a bigger cohort using a powered study, with recruiting an extra arm, involving patients on disease modifying treatment.
2- Extend the longitudinal study to follow patients yearly for the next 4 years and then every 3 years for the next 20 years to explore short term variations and longer term variations in cortical lesions pathology, its association with physical and cognitive pathology in different MS groups.
3- Examine the sensitivity of MTR at 3Tesla to depict cortical lesions especially as a comparison between 7Tesla MTR and 3Tesla MTR. If MTR at 3 Tesla has proven to be capable of depicting cortical lesions, it will be crucial for practical applications as 7Tesla scanners so far are only available for research.

Chapter 8

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