ADVANCED MRI TECHNIQUES IN STUDYING MULTIPLE SCLEROSIS PATHOLOGY AND LESION PROGRESSION

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Declaration

I declare that the studies undertaken for this thesis were conducted by me. Where other sources have been used, they are acknowledged by reference. The data used for the analysis in chapter 9 were obtained by another researcher prior to this PhD study as I acknowledged in the chapter but the data analysis and interpretation was conducted by me. No part of this work has been previously submitted or accepted for any other award.

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Abstract

There is an intimate spatial and functional relationship between cerebral microvasculature and the neuroglial tissues. It is known that both cerebrovascular and neuroglial alterations occur in multiple sclerosis (MS), but the pathophysiological relationships between these alterations, and the functional consequences, are not well characterised in vivo. Evidence from previous literature indicates that there are subtle changes in blood brain barrier (BBB) in normal appearing white matter (NAWM) of MS. However, lesion development due to subtle BBB leakage remains unclear. Additionally, the haemodynamic alteration and microstructural changes in grey matter (GM) is a well-known feature in MS. Nevertheless, the structural and functional relevance of GM perfusion and diffusion necessitate more exploration.

The main objective of this study was further interpretation of lesion development in MS, using advanced MRI techniques. Moreover, the study also aimed to provide valuable understandings into the association of different MRI measures with structural changes and clinical performance in MS, using advanced image analysis methods. The study has focused on the following goals: to determine BBB permeability in NAWM of MS and its correlation with microstructural damage, the relationship of subtle BBB leakage to lesion development, the association of grey matter perfusion with structural changes and functional performance and finally, the cortical diffusion alteration and its relevance with functional performance. Therefore, the main hypothesises of this thesis are: firstly, MS patients have impaired BBB permeability in NAWM compared to healthy controls. Secondly, that mean diffusivity (MD) in NAWM will correlate with BBB permeability in MS. Thirdly, that subtle BBB leakage

in NAWM may precede lesion progression in MS. Fourthly, that cortical and deep GM perfusion is reduced in MS patients compared to healthy controls. Fifthly, that cortical perfusion correlates inversely with cortical atrophy in MS and finally, pattern of regional cortical MD variability will explain performance in different functional domains in MS.

All MS and healthy participants included in this study underwent an MRI scan at 3T. Functional and cognitive assessment was performed on MS. Dynamic contrast enhance-MRI was used to generate perfusion and permeability maps. The microstructural changes were measured using diffusion imaging. The cortical perfusion and diffusion changes were explored by Surface based analysis approach.

Increased BBB permeability in NAWM of MS was detected when compared to healthy controls ($p<0.05$) and it revealed an association with MD in NAWM ($r= 0.48$, $p = 0.01$). The findings also revealed subtle BBB breakdown indicated by an increase in the permeability parameter (Ktrans) in prelesion NAWM. GM hypoperfusion was shown in MS when compared to healthy controls ($P<0.05$). However, surface based analysis revealed no correlation between cortical hypoperfusion with cortical atrophy and microstructural damage in MS. Furthermore, there was no association between GM perfusion and clinical scores. In addition, the cortical diffusion alteration revealed vertex-wise correlation with functional scores in MS ($P<0.05$).

In conclusion, advanced MRI and advanced image analysis in this study has delivered novel insights on lesion development and other pathological changes affecting GM and WM in MS, which might have prognostic and therapeutic importance in MS.

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List of abbreviations

ADC	Apparent diffusion coefficient
AIF	Arterial input function
ASL	Arterial spin labelling
BBB	Blood brain barrier
BDI	Beck Depression inventory II
BOLD	Blood oxygen level dependent
BVMTR	Brief visuospatial memory test revised
BVRT	Benton Visual Retention Test
CASL	Continuous arterial spin labelling
CNS	Central nervous system
CBF	Cerebral blood flow
CBV	Cerebral blood volume
CEC	Cerebral endothelial cells
CSF	Cerebral spinal fluid
CIS	Clinically isolated syndrome
CTC	Concentration time curve
CVLT-II	California verbal learning test-II
COWAT	Controlled oral work association test
DGM	Deep grey matter
DAWM	Diffusely abnormal white matter
DTI	Diffusion tensor imaging
DIS	Dissemination in space
DIT	Dissemination in time
DCE-MRI	Dynamic contrast-enhanced MRI
DSC-MRI	Dynamic susceptibility contrast-MRI
DWI	Diffusion weighted imaging
D-KEFS	Delis Kaplan Executive Function system

EBV	Epstein-Barr virus
EAE	Experimental allergic encephalomyelitis
EES	Extravascular extracellular space
EDSS	Expanded Disability Status Scale
ET-1	Endothelin 1
FA	Fractional anisotropy
FDR	False discovery rate
FLAIR	Fluid attenuated inversion recovery
fMRI	Functional MRI
FSPGR	Fast spoiled gradient echo
T25FW	Timed 25-Foot walk
Gd	Gadolinium
GGT	ɤ-glutamyl transpeptidase
GM	Grey matter
HLA	Human leukocyte antigen
9HPT	9-hole peg test
ICAM-1	Intercellular adhesion molecules
IFN-γ	Interferon gamma
IL-1	Interleukin 1
IL-12	Interleukin 12
JLO	Judgement of line orientation
MACFIMS	Minimal assessment of cognitive function in multiple sclerosis
MD	Mean diffusivity
MMSE	Mini mental status exam
MoCA	Montreal Cognitive Assessment
MRI	Magnetic resonance imaging
MRS	Magnetic Resonance Spectroscopy
MS	Multiple sclerosis
MSFC	Multiple sclerosis functional composite

MT	Magnetisation transfer
MTR	Magnetisation transfer ratio
MTT	Mean transit time
NAGM	Normal appearing grey matter
NAWM	Normal appearing white matter
NFI-MS	Neurological Fatigue Index
PASAT	Paced Auditory Serial Addition Test
PASL	Pulsed arterial spin labelling
PCASL	Pseudo-continuous arterial spin labelling
PD	Proton density
PS	Permeability-surface area
PET	Positron emission tomography
PPMS	Primary progressive MS
PRMS	Progressive relapsing MS
PV	Partial volume
PVC	Partial volume correction
RCFT	Rey complex Figure Test
RF	Radio frequency
ROI	Region of interest
RRMS	Relapsing-remitting MS
SDMT	Symbol digital modalities test
SNR	Signal to noise ratio
SPMS	Secondary progressive MS
TBSS	Tract based spatial statistics
TM	Tofts model
TNF	Tumor necrosis factors
VB	Voxel based
WM	White matter

Part 1: Introduction

Chapter 1 Introduction to Multiple Sclerosis

1.1 Epidemiology

Multiple sclerosis (MS) is a neurological disease of young adults in the developed world, it affects the central nervous system (CNS) and it is the main cause of neurological disability in young age (Koch-Henriksen and Sørensen 2010). Progressive disability was detected in around 80% of MS patients. Furthermore, it was found that within 10 years of diagnosis 40% of MS patients need a wheelchair (Scolding 2012). Approximately 400.000 people in Europe have MS and possibly 2.5 million people are affected worldwide (Scolding 2012). In the UK, about 100.000 people are diagnosed with MS (NHS Choices 2016). Studies have shown that the approximate prevalence of MS in: England is 165/100,000, Wales is 140/100,000, whereas in Northern Ireland it is around 174/100,000 and 212/100,000 in Scotland (Multiple Sclerosis Trust 2015) . It was found that number of people affected by MS in the UK is increasing by 2.4% per year (Mackenzie et al. 2013; Multiple Sclerosis Trust 2015). Furthermore, it was found that females are 2-3 times more commonly affected than males (Orton et al. 2006; Krökki et al. 2011).

1.1.1 Geographical Distribution

The prevalence of MS differs widely among geographical regions. It was found that prevalence of MS in North America and Northern European Countries is approximately 100 per 100,000 of the population, whereas in China, MS is very uncommon. Moreover, the disease appears to be rare in several Asian

countries. Interestingly, the prevalence of MS in various regions of the world can be affected by latitude. It was found that lower rates of the disease were noticed in areas closer to the equator, while MS rates in certain countries rises with increasing latitude (Scolding 2012).

1.2 Risk factors

1. Genetics risk factors

Previous studies have reported that individuals who have familial aggregation of MS are at an increased risk to develop the disease (Robertson et al. 1996; Ebers et al. 2004). Interestingly, it was found that the risk of having MS is linked to the kinship degree, for example; the risk of developing the disease is 10-25 times higher in first degree relatives than the general population (Robertson et al. 1996; Ebers et al. 2004).

The human leukocyte antigen (HLA) is considered the first known genetic association with MS (Scolding 2012). It is located on chromosome 6, and it is the most powerful genetic predisposition factor for MS. HLA gene has a role in response to pathogens by presenting antigens to immune cells, recognising self from non-self and coordinating complement activation and cytokine release (Scolding 2012). The immunological disturbance seen in MS makes the involvement of HLA genes expected. The theory is that change in the antigen presentation results in alteration in immunological recognition of self-antigen. However this aetiological theory has yet to be proven. A great degree of polymorphisms are seen in the HLA region and specific alleles have been linked to the susceptibility to MS. The DRB1*1501 is a notable allele found to

be associated with MS in most populations, and especially those of north European origins. However, most of DRB1*1501 allele carriers will not develop MS, and around 45% of MS patients do not have this allele indicating that the gene susceptibility to the disease is very complex (Scolding 2012).

2. Environmental risk factors

- Epstein-Barr virus

Studies found that around 99% of MS patients have history of Epstein-Barr virus (EBV) infection compared with almost around 94% of age-matched controls (Ascherio and Munger 2007a; Ramagopalan et al. 2010). In addition, individuals with elevated titres of anti-EBV antibodies are at high risk to develop MS (Sundström et al. 2004; Levin et al. 2005).

- Smoking

Evidence shows a history of smoking may increase the risk of MS by 40-50%. Furthermore, a two fold increase in the risk of MS is seen in long term smokers when compared to non-smokers (Hernan et al. 2001; Ascherio and Munger 2007b; Simon et al. 2011).

- Vitamin D

The association between incidence of MS and latitude may have been explained by exposure to sunlight and vitamin D (Acheson et al. 1960). It was found that there is an inverse relation between sunlight exposure and MS (Van der Mei et al. 2003; Ramagopalan et al. 2010). A cohort study found that the risk of developing MS can be reduced around 40% with the intake of supplements that contain vitamin D (Munger et al. 2004).

- Month of birth

A study carried out by Willer and colleagues found that individuals from north of the equator who were born in May are at high risk to develop MS. While, individuals born in November are at lower risk to develop the disease. Data from Great Britain, Denmark, Canada and Sweden revealed that 9.1 % of MS patients were born in May, while 8.5 % of MS patients were born in November (Willer et al. 2005).

- Latitude

The prevalence and incidence of MS in several regions of the world can be affected by latitude. It was found that regions near the equator have low incidence of MS (Kurtzke 1995). Therefore, the incidence of MS rises with increase in latitude (Ramagopalan et al. 2010).

1.3 Pathophysiology

1.3.1 General

The main characteristic features of demyelinating diseases, of which MS is part of, is the formation of sclerotic plaques, inflammation and the destruction of the myelin sheaths of neurons (Compston and Coles 2008). These features interact in a complex manner, which to date is still not fully understood. The end results of this is the breakdown of nerve tissue and the production of the signs and symptoms of the disease. The initial process of the disease involves the increased movement of autoreactive lymphocytes across the blood brain barrier (BBB) (Compston and Coles 2008). The transformation from a

physiological cascade arises with the disruption in the balance between cells that cause nerve tissue damage and demyelination also known as activated effector T cells and the cells that are capable of suppressing the self-activated T cells also known as regulatory T cells (Zozulya and Wiendl 2008). Lymphocytes from patients with MS have been found to have a diminished capability in supressing the effector cells (Viglietta et al. 2004).

Inflammation has been found to be provoked by a T lymphocyte subtype that produces Interleukin (IL) 17 which is under the control of Interleukin 23 (Langrish et al. 2005). Blood brain barrier disruption caused by IL 17 and IL 22, which in turn leads to the infiltration of Th 17 cells in to the brain tissue and therefore causing neuron damage (Kebir et al. 2007; Tzartos et al. 2008).

1.3.2 Blood brain barrier

1.3.2.1 Blood brain barrier in health

The Blood brain barrier (BBB) is a vital complex structure, which plays an important role in adjusting flow of components through the CNS. It also reduces the effect of pathogens and toxic composites to the CNS (Cardoso et al. 2010; Tam and Watts 2010). The BBB consists of cerebral endothelial cells (CEC), pericytes and encircled by perivascular macrophages and astrocytes. The BBB creates a solute impermeable barrier due to the numerous distinctive functional and structural features of the CEC (Minagar and Alexander 2003). To allow small level of BBB exchange it requires several transporters to be maintained via CEC for amino acids and glucose (Minagar and Alexander

2003). The CEC also express surface enzymes such as alkaline phosphatase, monoamine oxidase, cholinesterase and aromatic decarboxylases; in addition to ɤ-glutamyl transpeptidase (GGT), which are considered as specific markers for the CEC (Minagar and Alexander 2003). Further cells in the BBB such as astrocytes, glial cells and pericytes support the permeability barrier formed by CEC via secreting trophic factors. (Burns et al. 2000; Abbott 2002). Astrocytes are considered as the main cell in supporting the BBB integrity, as astrocytes cover more than 90% of the outer surface of CEC. This contact between astrocytes and CEC is important to preserve the BBB markers such as GGT (Demeuse et al. 2002). The damage of this communication will eliminate the BBB phenotype (Bondan et al. 2002). The restrictive features of the BBB are supported by subcellular structures such as the tight and adherence junctions. The tight junctions are pericellular structures that are extremely organized, they are placed in the apical part of adjacent cells (Petty and Lo 2002). The gate function of the BBB is also mediated by tight junctions. It consists of transmembrane and cytoplasmic proteins complex, which create a seal to inhibit paracellular diffusion. Furthermore, it controls lateral diffusion among the basolateral and apical membrane areas (Gumbiner 1987; Brown and Stow 1996). Adherens junctions comprise of cadherins and catenins. The cadherin connect to the actin microfilaments via the catenins (Dejana et al. 2001) . The adherens junction plays a role in supporting the BBB, as it interacts with the tight junction (Minagar and Alexander 2003).

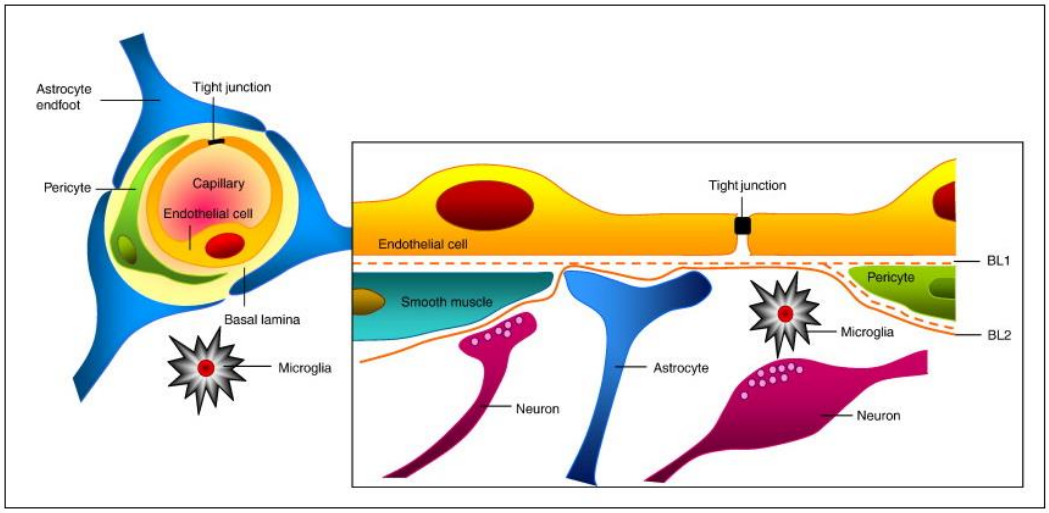


Figure 1.1 Illustrates blood brain barrier
Image was adapted from (Abbott et al. 2010)

1.3.2.2 Blood brain barrier in multiple sclerosis

The main pathophysiological events in MS are microvascular inflammation and demyelination (Hemmer et al. 2002). One of the likely mechanisms of BBB disruption in MS is the release of pro inflammatory cytokines and chemokines, which induce endothelial cell adhesion molecules expression; it also leads to shedding of endothelial micro-particles which have been used as a marker for endothelial injury (Minagar and Alexander 2003). Furthermore, tight junction alteration may occur due to pro-inflammatory effect (Minagar and Alexander 2003). However, BBB permeability disturbance was noticed in MS patients on magnetic resonance imaging (MRI) scans (Miller et al. 1998). Previous studies found that inflammatory cytokines such as tumour necrosis factors (TNF) and interleukin 1 (IL-1) are elevated in experimental allergic encephalomyelitis (EAE) which is an animal model for MS, the elevation of cytokines in EAE models corresponding with the changes in BBB integrity

(Gijbels et al. 1990). Interestingly, these BBB mediators are elevated in cerebrospinal fluid (CSF) of MS (Sharief et al. 1993; Shaw et al. 1995). Furthermore, the endothelial and astrocytic compartment in EAE models, demonstrates a significant up-regulation in intercellular adhesion molecules (ICAM-1) (Wilcox et al. 1990). It was noticed that symptom progression and regression correspond to the ICAM-1 levels. Additionally, ICAM-1 was found in the circulating soluble form in the serum and cerebral spinal fluid (CSF) of MS patients (Hartung et al. 1995a). It was also found that BBB leakage illustrated with contrast enhanced MRI scans is correlated with ICAM-1 levels (Hartung et al. 1995b; Petty and Lo 2002). Additionally, the level of tight junction alteration also plays a role in BBB permeability. Some studies noticed that MS brains show abnormal ZO-1 and occludin which results in junctional integrity disruption (Plumb et al. 2002) .This abnormality in tight junction will permit higher influx of cytokines and blood borne cells and, therefore augmenting inflammation and more parenchymal injury (Petty and Lo 2002).

1.4 Clinical presentation

- Optic neuritis

Optic neuritis is one of the most common presenting features of MS, which is usually unilateral, with subacute onset. Central scotoma is the characteristic visual defect. About 90% of MS patients will complain from pain around the eye in addition to photophobia (McDonnell 2007).

- Sensory Symptoms

Around 90% of MS patients present with sensory symptoms. MS patient can present with dysesthesia and paraesthesia. The bodily region presenting with sensory symptoms depends on the CNS lesion location (McDonnell 2007).

- Cerebellar disturbance

Cerebellar disturbance consisting of gait ataxia, nystagmus, dysarthria and intention tremors. Scanning speech is a late manifestation of MS (McDonnell 2007).

- Eye movement disturbance

The most common eye movement disruption reported is nystagmus. It can be seen in different forms such as pendular nystagmus and horizontal jerking nystagmus. (McDonnell 2007). Other eye movement disorder include internuclear ophthalmoplegia, it is a condition which affects MS due to lesion in the medial longitudinal fasciculus. The patient will present with abnormal conjugate gaze, which is a restricted adduction of the affected eye and nystagmus in the contralateral abducted eye (Javalkar et al. 2016).

- Brainstem disturbance

This will involve facial sensory disturbance or eye movement disorders. Approximately 1-4% of MS patients will present with Bell palsy due to involvement of the facial nerve. Another symptom is facial myokymia around the eye, which is a rapid contraction of the facial muscles. Around 16% of MS patients have vertigo as a presenting symptom and about 50% of patients can have attacks at different stages of the disease (McDonnell 2007). Approximately 1% of MS patient describe reduced taste sensation. Another common symptom is hearing problems; however permanent hearing loss is rare. MS patient can present with dysphagia during acute relapse at any phase of the disease. In addition, pseudobulbar type of dysarthria can occur (McDonnell 2007)

- Motor symptoms

Limb weakness is a common motor deficit. The common form of weakness in MS starts with involvement of both legs; however single leg or ipsilateral leg and arm involvement could occur. Spasticity is another motor symptom, which can occur in MS patients at different degree (McDonnell 2007).

- Sphincter disturbance

Approximately 5% of MS patients have micturition disturbance. Most of MS patients presented with urinary bladder symptoms such as urgency, nocturia, frequency, retention and incontinence. General bowel symptoms are presented in around 46-68% of patients, constipation was documented in around 43% of MS patients (McDonnell 2007). Sexual difficulties were noticed in around 56-72% of women with MS, and erectile dysfunction was documented in around

91% of men with MS. The sexual difficulties also could be due to loss of libido, fatigue, bladder problems and spasticity (McDonnell 2007).

- Pain

Pain is a common symptom in MS, it classified as paroxysmal and chronic pain. The paroxysmal pain involves trigeminal neuralgia, Lhermitte phenomenon and tonic spasms. The most common paroxysmal pain in MS is trigeminal neuralgia. The Lhermitte phenomenon is associated with forward flexion of the neck; the patient will feel electric like shock travelling down the spine and into the upper and lower limbs. The tonic spasms are usually painful, it is brief contraction which last from few seconds to few minutes (McDonnell 2007). Chronic pain in MS can be due to many factors such as contractures, spasm, poor posture and lumbar pain.

- Fatigue

Around 84% of MS patient considered fatigue as significant problem (McDonnell 2007). The forms of fatigue are variable, depending on several factors such as lack of sleep, depression and it can also depend on the ordinary activity. However, in many MS patients fatigue is persistent symptom and does not relieve with either rest or sleep (McDonnell 2007).

- Mood disorder and neuropsychiatric disturbances

One of the common features in MS patient is mood disorders such as euphoria, depression and psychosis. Around 50% of patients have depression which can increase the risk of suicide in MS patients. Psychotic episode is a rare feature which may affect MS patients at early stage (McDonnell 2007).

- Cognitive impairment

According to many researches, it was showed that cognitive dysfunction is a common associated feature in MS (Chiaravalloti and DeLuca 2008). The disease adversely affects different features of cognition such as attention (Litvan et al. 1988; Beatty et al. 1996), information processing effectiveness (Litvan et al. 1988; Diamond et al. 1997), speed of processing (DeLuca et al. 2004), executive function (Foong et al. 1997; Denney et al. 2005) and memory (Brassington and Marsh 1998). The most common affected cognitive domain in MS is processing speed, memory and visual learning (Rao et al. 1991; Benedict et al. 2006). However, verbal skills and simple attention such as repeating numbers are less affected (Rao et al. 1991)

Long term memory is the capability to acquire new information, and remember this information later (Chiaravalloti and DeLuca 2008). Around 40-65 % of MS patient have impaired memory (Rao et al. 1993). Previous studies showed that the main memory concern in MS is in early learning of information (DeLuca et al. 1994; Thornton et al. 2002), more information repetitions are required for MS patients to recall the information (DeLuca et al. 1994; Chiaravalloti and DeLuca 2008). The abilities of decision making will be poor as a result of insufficiency in learning new information (Nagy et al. 2006). Poor learning has been related also with slow processing speed and executive dysfunction (Chiaravalloti and DeLuca 2008).

Another impaired cognitive area in MS is information processing effectiveness, involving working memory and processing speed. Working memory (Baddeley 1992) is the capability to retain and operate information over a short period of

time, whereas, processing speed is the speed to process the information in the brain (Chiaravalloti and DeLuca 2008).

Processing speed reduction is one of the common cognitive impairments in MS (DeLuca et al. 2004). It was found that processing speed assessment has the ability to predict long term deterioration of cognitive function (Bergendal et al. 2007). Studies revealed a positive correlation between memory and processing speed impairment. Working memory impairment has been reported in early MS (Audoin et al. 2005). Findings from previous studies with large cohort of MS demonstrate a high incidence of processing speed and work memory impairment, especially in secondary progressive-MS (SPMS) (DeLuca et al. 2004).

Executive function is defined as the skills required for goal-directed behaviour and the ability to adapt to the environmental demands. The executive functions include planning, fluency, organising and conceptual reasoning. In MS executive function impairment occurs, however to a lesser extent than efficiency of information processing and memory (Chiaravalloti and DeLuca 2008).

Visual perception is the ability to recognise a visual stimulus and to identify the characteristics of that stimulus (Chiaravalloti and DeLuca 2008). Around quarter of MS patients could have visual perception impairment. The primary visual processing problems reported in MS such as optic neuritis might have an adverse influence on visual perception (Vleugels et al. 2000).

1.5 Types of multiple sclerosis

There are four different types of MS according to clinical course, which are clinically isolated syndrome (CIS), relapsing-remitting (RRMS), secondary progressive MS (SPMS) and primary progressive MS (PPMS) (Lublin 2014). Clinically isolated syndrome (CIS) is described as the single episode in which clinical manifestations suggest inflammatory demyelinating disease of CNS (Miller et al. 2008). It usually affects young adult with an acute or sub-acute onset. Furthermore, CIS patient is usually isolated in space and always isolated in time. They usually present with signs that suggests lesions in optic nerve, cerebellum, brainstem, spinal cord and rarely the cerebral hemisphere (Miller et al. 2012).

RRMS is the most common type; around 55% of MS patients are RRMS type. Relapses followed by symptoms recovery either totally or partially is the main clinical feature of RRMS. The damage is partially reversible in this type, since the focal regions of inflammation and demyelination resolve spontaneously, which explains symptoms recovery (Zuvich et al. 2009). The CIS and RRMS can be classified as active or not active. The activity of the disease is based on clinical relapses or MRI demonstrating a new/enlarged T2 lesions or enhancing lesions (Lublin 2014).

The second common type of MS is SPMS; approximately 30% of patients are documented as a SPMS type. It initiated with RRMS attacks which demonstrate reversible disability, and then over the disease course it substituted by progressive disability due to axonal degeneration which results in irreversible damage (Zuvich et al. 2009). Around 10% of MS patients are

PPMS, in this type there is no remitting phase and progressive disability is the main feature (Zuvich et al. 2009). The SPMS and PPMS has four sub classification according to the disability level which are: 1) Active with progression. 2) Active without progression. 3) Not active with progression. 4) Not active and without progression. The disease progression is measured through clinical assessment at least once a year (Lublin 2014)

1.6 Diagnostic criteria for multiple sclerosis

Clinical and laboratory assessments are required for MS diagnostic criteria, accentuating the necessity to illustrate dissemination of lesions in time (DIT) and space (DIS) (Polman et al. 2011). Therefore, MRI is essential to support these clinical criteria. The McDonald criteria (Table 1.1) are the diagnostic criteria which are used to diagnose MS and it characterise by its high specificity and sensitivity (Polman et al. 2011).

Table 1.1 McDonald criteria for MS diagnosis. Adapted from (Polman et al. 2011)

McDonald Criteria	
Clinical Presentation	**Further evidence for MS diagnosis**
- Two or more attacks - Objective clinical evidence of two or more lesions **or** one lesion with evidence of history of a previous attack	None
- Two or more attacks - Objective clinical evidence of one lesion	DIS [1]
- One attack - Objective clinical evidence of two or more lesions	DIT [2]
- One attack - Objective clinical evidence of one lesion (CIS)	DIS and DIT
Neurological progression suggestive of MS (PPMS)	One year of disease progression with two or three of the subsequent criteria: -DIS in the brain -DIS in the spinal cord based on more than two T2 lesions in the cord -CSF positive for oligoclonal bands and/or elevated IgG index
1 DIS: Dissemination in space = One or more T2 lesion in at least two of four regions of the CNS (Juxtacortical, Infratentorial, Periventricular, Spinal cord). * In DIS lesions Gadolinium enhancement is not essential. 2 DIT: Dissemination in time= a) On follow-up MRI there is a new T2 and/or gadolinium-enhancing lesion(s) with reference to a baseline scan, regardless of the baseline MRI timing. b) "Simultaneous presence of asymptomatic gadolinium-enhancing and non-enhancing lesions at any time" (Polman et al. 2011)	

1.7 Pathological and Magnetic resonance imaging findings in multiple sclerosis

MS is described as a disease of inflammation and demyelination that disturbs the CNS by developing focal lesions in the grey and white matter, furthermore it causes diffuse changes in normal appearing tissue (Filippi et al. 2012). Histopathological studies have assisted in describing different substrates of this disease. Even though pathological evaluation is considered as gold standard for characterisation of tissue injury, it is normally performed on post-mortem samples when disease is at advanced stages or as biopsies in atypical cases (Filippi et al. 2012). Moreover, pathological assessment does not provide assessment of changes over time. Therefore, MRI is considered to be a significant method not only to diagnose MS, but also to characterise pathological changes in patients, due to its non-invasiveness, reproducibility and sensitivity to multiple sclerosis related abnormalities (Filippi et al. 2012).

1.7.1 Focal lesions in Multiple sclerosis

Focal lesions/plaques are considered as one of the pathological features in MS patients, characterised pathologically by demyelination, inflammation, axonal injury and gliosis (Filippi et al. 2012). On MRI scans focal lesions are displayed as hyperintense lesions on T2-weighted images and fluid attenuated inversion recovery (FLAIR) (Figure 1.2). The lesions are usually ovoid or round and their size varies from few millimetres to more than 1 cm (Filippi et al. 2012). Lesion location is one of the important characteristic features, as lesions in MS have high tendency to develop in brain stem, periventricular

white matter and cerebellum (Fazekas et al. 1999). Lesions in MS have non-specific appearances, and may require other sequences or follow-up scans to differentiate from other pathologies. It was found that about 10-30% of T2 hyperintense lesions are displayed as lesions of low intensity on T1 weighted images (Filippi et al. 2012). During the acute stage, the hypointense signal of T1 is possibly due to demyelination and oedema, and once the inflammation subsides it can totally disappear. On the other hand, the T1 hypointense lesion (black holes) which is seen during the chronic phase, suggests regions with severe tissue damage (Van Waesberghe et al. 2001). Acute and chronic lesion distinction is based on the blood brain barrier breakdown which can be identified by using contrast enhanced MRI; however there are numerous factors that may alter contrast enhancement such as the contrast dose, steroids, image acquisition delay and MRI sequence characteristics (Filippi 2000a).

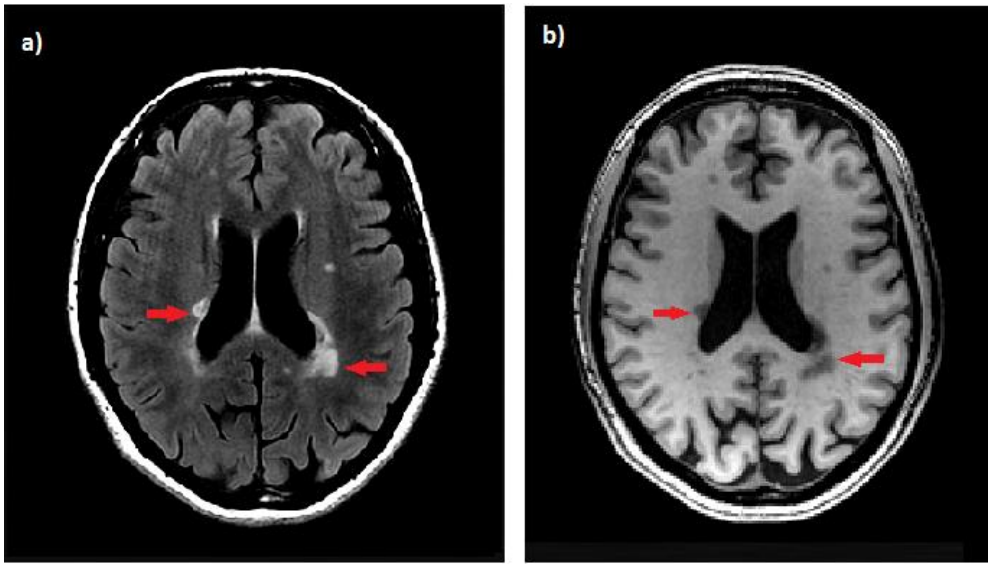


Figure 1.2 Demonstrate lesions of RR-MS patient.
This patient is one of the participants in this PhD study.
a) Hyperintense lesions on FLAIR. b) Hypointense lesions on T1 weighted image

1.7.2 Grey matter in Multiple sclerosis

During the chronic phase of MS, demyelination of grey matter (GM) can be profoundly extensive (Bø et al. 2003a; Kutzelnigg et al. 2005). The histopathological picture of grey matter lesions shows T-cell inflammation to a small extent (Bø et al. 2003b) and leakage of plasma protein due to blood brain barrier destruction (Van Horssen et al. 2007). A previous pathological study has examined a large number of biopsies, and they found that demyelination of the cortex occurs at early stage in MS, and they noticed that meningeal inflammation is related to these early cortical lesions (Lucchinetti et al. 2011). It was found that demyelination can be distributed randomly and commonly in the cortex (Peterson et al. 2001; Filippi et al. 2012), furthermore the distribution can heavily involve deep grey matter, hippocampus (Vercellino et al. 2005) and cerebellar cortex (Kutzelnigg et al. 2007). The degree of damage in the grey matter differs considerably between the different types of the disease. The damage is less in RR-MS while it is severe in progressive MS. (Bozzali et al. 2002; Agosta et al. 2006). Normally cortical lesions are not clearly visible on conventional MRI due to small size of lesion and the poor contrast with the adjacent normal GM (Filippi et al. 2012). Therefore, dedicated sequences have been developed to improve detection of cortical lesions, such as double inversion recovery sequences (Calabrese et al. 2010a). The cognitive deficit in MS could be partially explained by assessing the grey matter involvement and the functional restructuring which can be examined via functional MRI (Filippi and Rocca 2011).

1.7.3 White matter abnormalities in multiple sclerosis

Normal appearing white matter (NAWM) was described as normal white matter macroscopically, normal myelination is demonstrated microscopically, and the distance of the NAWM from a plaque's edge is more than 1 cm away (Allen and McKeown 1979; Filippi et al. 2012). This NAWM should be distinguished from diffusely abnormal white matter (DAWM), which comprises of myelin paleness with borders that are ill defined (Filippi et al. 2012). Lymphocytes infiltration, demyelination and macrophages are the main abnormal histopathological findings in NAWM. Moreover, knots of microglial cells which indicates microglial activation is a noticeable characteristic (Filippi et al. 2012). These microglial cell clusters were demonstrated as hyperintense lesions on T2 on post-mortem MRI (Vos et al. 2005). Around 12 –42% reduction of axonal density has been demonstrated in NAWM of corpus callosum and spinal cord (Evangelou et al. 2000a; Bjartmar et al. 2001). The histopathological picture of DAWM comprises reduction in myelin density, chronic fibrillary gliosis and axonal loss. These findings are abnormal when compared to NAWM and also differ from the pathology of the focal white matter lesions (Seewann et al. 2009). Previous radiological studies using MRI found that the signal intensity on T2 images in DAWM is higher than adjacent NAWM; however the signal intensity is lower in DAWM than in the white matter focal lesions (Seewann et al. 2009). Furthermore, the DAWM display intermediate abnormalities on diffusion tensor imaging (DTI) and magnetic transfer imaging (Vrenken et al. 2010).

Chapter 2 Introduction to Advanced MRI techniques

Advanced MRI techniques have the ability to detect structural and functional features which could not be identified on conventional MRI (Pagani et al. 2008). They provide more information and quantitative measures for different neurological diseases. This chapter will provide a brief introduction to some of the advanced MRI modalities including perfusion, diffusion-weighted, spectroscopy, magnetization transfer and functional MRI.

2.1 Magnetic resonance perfusion imaging

Perfusion is defined as the transfer of arterial blood to capillary bed (Sourbron 2010). Dynamic susceptibility contrast imaging (DSC), Dynamic contrast-enhanced MRI (DCE-MRI) and Arterial spin labelling (ASL) are the widely used techniques to describe perfusion via MRI (Barbier et al. 2001; Luypaert et al. 2001).

2.1.1 Dynamic contrast MRI

Dynamic imaging is based on the transient alterations which occur in the magnetic field of adjacent tissue influenced by contrast bolus tracer, passing in capillary network of an organ (Petrella and Provenzale 2000).

This alteration in local magnetic field could be measured as MRI signal changes. Ultrafast imaging such as echo-planar MRI (Edelman et al. 1994) is used to provide a precise measurement of the rapid signal changes which

occurs during the first pass of contrast with sufficient temporal resolution. Then the signal time curve could be converted to concentration time curve (Rosen et al. 1989). Subsequently, the concentration time curve is analysed to derive the parameter of haemodynamic tissue which include the following (Petrella and Provenzale 2000):

- Cerebral blood flow (CBF): it quantifies the blood volume flowing through a certain brain tissue amount per time unit (ml/100g/min).
- Cerebral blood volume (CBV): it quantifies the blood volume in a certain brain tissue amount (ml/100g).
- Mean transit time (MTT): the typical time it requires blood to pass through a certain brain tissue amount (Sec).

To obtain these parameters; arterial input function (AIF) deconvolution from tissue concentration time curve is required. AIF is essential to generate CBF maps, and it could be attained from the imaging data (Ostergaard et al. 1996; Petrella and Provenzale 2000).

Dynamic susceptibility contrast MRI is performed with T2* weighted images, this sequence leads to drop in the signal intensity due to the contrast agent susceptibility effects. The susceptibility effect displays short relaxation time of T2* weighted images, in which it leads to decrease signal on T2* weighted images (Petrella and Provenzale 2000). Extravasation of tracer into the interstitial space will affect T2* weighting and this is considered as a problematic issue when using DSC-MRI (Boxerman et al. 2006; Sourbron et al. 2009a).

Dynamic contrast enhanced MRI is performed using T1-weighted sequence to measure haemodynamic parameters, it requires small amount of contrast agent

and it has better temporal resolution compared to T2* weighted imaging. It measures the relaxivity effects of the contrast agent, which displays short relaxation time of T1, in which it leads to increase signal on T1 weighted images (Petrella and Provenzale 2000).

The T1-weighted technique is not adversely affected by tracer extravasation, DCE-MRI can be used for permeability quantification (Sourbron 2010). The aim of DCE-MRI analysis is to obtain quantitative parameters that describe the microcirculation condition. Therefore, a tracer kinetic model is performed, in which the measured data and relevant physiological parameters are linked. (Sourbron and Buckley 2011). There are different types of models such as Tofts model, modified Tofts model (Tofts 1997; Tofts et al. 1999), and the two compartment model.

2.1.1.1 Tofts model

The Tofts model (TM) is the most commonly used model, which describes the concentration time curve (CTC) in tissue (Chikui et al. 2012), this model consist of one compartment, in which two parameters are derived the (Ktrans and Ve). The Ktrans is constant and it is the volume transfer between extravascular extracellular space (EES) and plasma, while Ve is the EES volume per unit volume of tissue (Tofts et al. 1999). In the Tofts model the plasma volume is negligible (Tofts et al. 1999).

$C(t) = K\text{trans}\, e^{-tk\text{ep}} * Ca(t)$

- C(t) is the tissue concentration time curves
- Ca(t) is the Concentration time curves in the arterial plasma

- "*" is convolution.
- The Ktrans = EFp where, E is the extraction fraction and Fp is the plasma flow into the capillary bed.
- $k$ep = EFp/ve where, ve is the EES volume fraction.

The modified Tofts model was created to concede intravascular contribution; it suggests that contrast is derived from plasma and EES. Three parameters are derived from the modified Tofts model which are Ktrans, Ve and Vp (Tofts 1997; Tofts et al. 1999) The Vp is volume of vascular plasma.

$C(t) = v\text{p}\, C\text{a}(t) + K\text{trans}\, e^{-tk\text{ep}} * C\text{a}(t)$

2.1.1.2 Two compartment exchange model

In the two compartment models it is assumed that blood plasma and the EES are two compartments, with Vp and Ve volumes respectively (Sourbron and Buckley 2011). The plasma compartment has a single inlet and outlet. It is supposed that there is symmetric tracer exchange across the barrier between the EES and plasma; in which is measured via the permeability-surface area product (PS). The PS quantifies the plasma volume flowing through the capillary wall of 100g tissue per time unit (Sourbron 2010).

In addition, the plasma flow (Fp) via the arterial inlet and venous outlet should be the same due to the stationary state of the tissue. Therefore, the model is determined via four parameters, which are Vp, Ve, Fp and PS (Sourbron and Buckley 2011).

2.1.2 Arterial spin labelling

Arterial spin labelling is non-invasive imaging technique; it uses an endogenous tracer which is the water in the arterial blood, a radiofrequency pulses are used to invert blood magnetisation. Images are obtained for both labelled blood and static brain tissue water, however a time delay is necessary prior acquiring images, as this delay will permit sufficient time for the labelled blood to flow into the brain. Moreover, control images are obtained before labelling the blood. The difference between both labelled and control images will provide perfusion map. The relaxation time of blood governs the life time of the labelled blood. The labelling delay in ASL application is effected by arterial transient time (ATT), prolonged delay leads to strong T1 decay and reduce signal to noise ratio while short delay will not allow adequate time for labelled blood to flow into the tissue (Alsop et al. 2015)

There are two ASL approaches: Continuous ASL (CASL) and Pulsed (PASL), both techniques differ in their labelling time and spatial degree. In CASL, prolonged labelling time is applied, around 1-3 seconds while blood passes through the labelling plane (Alsop et al. 2015). There are two different types of CASL, the first method is CASL which requires one signal and long label (Williams et al. 1992); the second type is pseudo-continuous ASL (PCASL) where multiple radio frequency pulses are performed at a rate of one per millisecond (Dai et al. 2008). On the other hand, PASL is acquired via a single or small number of pulses; a short duration, around 10- 20 milliseconds, is required to invert arterial spin in the thick slab (Kim 1995; Wong et al. 1997; Alsop et al. 2015). The advantage of PCASL over the PASL is the better signal to noise ratio (SNR), due to the long labelling duration which is relative to the

amount of labelled blood transported to the tissue, which leads to increase SNR.

2.2 Diffusion Imaging

2.2.1 Diffusion weighted MRI

Molecular diffusion is the random motion of water molecules in fluid due to thermal energy, known as Brownian motion (Beaulieu 2002). In human tissue, this molecular motion is restricted and limited by different cell membranes, compartments and boundaries, which could have an effect on molecular mobility such in neurons, axons, extracellular space and intracellular space (Beaulieu 2002). The diffusion coefficient (D) is a constant that is correlated with the root mean square of molecular movement over time (Beaulieu 2002). The weight of molecule, temperature and viscosity are factors which could have an impact on diffusion (Beaulieu 2002). The directions in which molecules move and displaced are different. A medium with no obstacles such as fluid or structures with disorganised barriers all molecules will move the same in all directions and it is called isotropic diffusion, whereas in structures with highly aligned barriers, molecular diffusion is based on direction and it is termed anisotropic diffusion (Beaulieu 2002).

Diffusion weighted imaging (DWI) provides information about molecular diffusion in the tissue (Koh and Collins 2007). The concept of DWI is performed by applying spin-echo T2 weighted sequence and two gradient echo pulses which leads to dephasing and rephasing of water molecules (Stejskal

and Tanner 1965). In the absence of molecular motion the first gradient pulse will dephase the molecules while the second gradient pulse will cause rephasing, which leads to strong signal. In contrast, when there is a molecular movement the second gradient pulse will lead to incomplete rephasing, due to molecular displacement, which leads to signal loss. Therefore, DWI detects water diffusion via reduction of signal intensity (Koh and Collins 2007). Some factors could affect DWI sensitivity which includes gradient amplitude, the time between the two gradient pulses and the gradient pulse duration. The b value is a parameter that is proportionate to the gradient amplitude. DWI is usually applied with two b values (b=0 s/mm² and another b value ranging from 0-1000 s/mm². Signal attenuation is higher with higher b value (Koh and Collins 2007).

2.2.2 Diffusion tensor imaging

Diffusion tensor imaging (DTI) is a non-invasive technique which identifies microstructural organisation in tissue (Jones and Leemans 2011). As described earlier some tissue has an isotropic diffusion such as brain grey matter, in which diffusion could be identified via single apparent diffusion coefficient (ADC) (Basser and Jones 2002). Nevertheless, tissue demonstrates an anisotropic diffusion such as white matter, in which their diffusion relies on tissue orientation (Basser and Jones 2002). In order to determine the fiber orientation, measurements of independent diffusion along the three axes (X, Y and Z) are obtained. However, due to the fact that fibre orientation is not always aligned to one of these axes, measurements confined to only these three may not be enough. In fact, in reality fiber orientation is nearly always oblique

to these axes (Mori and Zhang 2006). Therefore, in order to measure accurately the fiber orientations with the largest ADC, a more profound approach would have to be adopted, where diffusion measurements would have to be acquired along thousands of axes and this is not practical. In the 1990's a model of diffusion tensor was introduced which helped simplify this issue (Basser et al. 1994a; Basser et al. 1994b). In this case, the measurements of diffusion along the different axes were fitted into a three dimensional ellipsoid shape, which represents the average distance of diffusion in each direction (Mori and Zhang 2006). The resulting properties of this three dimensional ellipsoid can be defined via six parameters, which include the longest, middle and shortest axes which are called eigenvalues (λ_1, λ_2 and λ_3) and their orientations named eigenvectors (v1, v2 and v3) (Mori and Zhang 2006). It is therefore possible to calculate the ellipsoid by measuring ADC along six axes. In order to convert the measurement results obtained which represents more than six ADC to these defined entities, a 3 × 3 symmetric matrix named tensor is used (diffusion tensor imaging). At each pixel, many important contrasts can be calculated once these six parameters are generated (Mori and Zhang 2006).

Different parameters are derived from DTI which include mean diffusivity (MD) and fractional anisotropy (FA). The MD is defined as the average motion of molecules and it is not dependent on tissue direction (Basser et al. 1994a; Pierpaoli et al. 1996). Increased MD indicates tissue damage due to increase in free diffusion (Soares et al. 2013). FA defines the diffusion directionality amount. Great FA value in a voxel suggests that diffusion occurs mainly on one axis, whereas small FA indicates that diffusion happens on all three axes (Rovira et al. 2013). Furthermore, FA reflects the amount of cellular structures

arrangement in the fiber tracts (Cercignani et al. 2001). Reduced FA indicates coherence loss in the ideal diffusion direction (Soares et al. 2013).

2.3 Other advanced MR modalities

2.3.1 Magnetic Resonance Spectroscopy

Magnetic Resonance Spectroscopy (MRS) is a technique used to identify the amount of metabolites in tissue. It has the ability to provide chemical and physiological information regarding the tissue structure. The spectra are acquired from various nuclei such as phosphorus, fluorine, sodium and carbon. The most applied nuclei in the clinical field are proton (^{1}H). Since the spectrum of proton is disturbed in most neurological disease. Weak signals from metabolites could be identified via great field strength, which could provide a high signal to noise ratio, improved resolution and short acquisition time (Bertholdo et al. 2013).

Proton spectroscopy is a technique that relies on a phenomena called atoms' chemical shift in which an interaction of the electric shell surrounding the nuclei with the adjacent molecules causes an alteration in the local magnetic field, which leads to a change in the spinning frequency of the atom. The chemical shift is expressed in parts per million (ppm), furthermore, the shift for nucleus is the same with different field strength. The spectrum consist of x axis which represent the frequency for different metabolites and the y axis relates to the amplitude (Bertholdo et al. 2013).

2.3.2 Magnetisation transfer

Magnetisation transfer (MT) is a technique which is based on the interaction between protons bound to macromolecules and protons in in free fluid (Wolff and Balaban 1994). The application of off resonance radiation causes the magnetised bound protons to become saturated. This magnetisation is conveyed from these protons to the more mobile protons, resulting in tissue signal reduction (Filippi and Agosta 2007). Therefore, MT imaging via a gradient echo sequence with and without RF saturation pulse, allows calculation of MT ratio (MTR) (Filippi and Rocca 2011). MTR reduction suggests that free water have low capacity to exchange magnetisation with the surrounding tissue (Filippi and Agosta 2007). In CSF the MTR approaches zero, since there is nearly no macromolecule and hence no exchange occurs (Horsfield et al. 2003). Estimation of degree of tissue damage could be identified from the MTR index (Filippi and Agosta 2007). Evidence that MTR reflects the extent of tissue damage has derived from previous post-mortem studies, in which a robust correlation was observed between MTR and axonal loss and the extent of myelin loss (Van Waesberghe et al. 2001; Schmierer et al. 2004a).

2.3.3 Functional MRI

Functional MRI (fMRI) is a technique which is based on identifying small changes in the brain connected to neuronal brain activity. It has the ability to identify changes in blood oxygen level dependent (BOLD) in MRI signals which occurs when neuronal activity changes subsequent to brain state

changes, due to task or stimulus (Gore 2003). Increase in neuronal activity in the cortex leads to increased CBF, due to increase in oxygen demand. Once perfusion in tissue exceeds the metabolic demand the deoxyhaemoglobin will reduce, this reduction has an influence on the signals used to generate MR images, since deoxyhaemoglobin is paramagnetic so signals from the magnetic properties of deoxygenated blood varies from other adjacent tissues. Consequently, MRI signals from deoxygenated blood decay faster, which leads to low MRI signal, whereas the magnetic properties of oxygenated blood is similar to the adjacent tissue, therefore signal from oxygenated blood shows slower decay which leads to high MRI signal (Gore 2003).

In fMRI, a snapshot imaging method is performed using echo planar imaging, (Cohen and Weisskoff 1991) and a comparison of set of images achieved through different conditions via statistical analysis (Gore 2003).

Chapter 3 Advanced MRI in multiple sclerosis

Conventional MRI techniques such as T1 weighted and T2 weighted images are characterised by their high sensitivity in identifying MS lesions and assessing lesion load (Rovira and León 2008). However, the relation between the degree of lesions noticed on MRI and clinical appearance in MS is weak, which has been referred as a clinical-radiological paradox (Barkhof 2002), this indicates that conventional MRI is not sufficient to characterise the full range of the disease progression. Therefore, advanced MRI techniques have been widely used in MS to overcome this issue and to provide better understanding of the disease process, lesion progression, microstructural changes in the brain and furthermore to assess treatment efficacy (Rovira and León 2008; Rovira et al. 2013) via DCE-MRI, DSC-MRI, ASL, diffusion imaging, fMRI and spectroscopy.

3.1 Perfusion measurement in multiple sclerosis using MRI techniques

Perfusion measurement in MS was a field of concern for many researchers, to examine the haemodynamic changes and lesion development (Ge et al. 2005), since perfusion quantification in past reflects the brain tissue metabolic activity (Rashid et al. 2004). Numerous studies were carried out. However, variable methods were performed using different MRI techniques and various clinical MS groups were included.

Several studies used DSC-MRI to investigate perfusion in MS patients. One of these studies was held by Ge et al. (2005), they examined the features of perfusion in MS lesions and explored the haemodynamic changes in NAWM in MS by using DSC-MRI (Ge et al. 2005). The study was performed on 17 MS patients with RRMS, in which 14 participants were on disease modifying treatment; the median period of the disease was 2.7 years. The patients were scanned using 1.5 Tesla MRI, non-enhanced T1 weighted, FLAIR, transverse dual echo T2 weighted images and DSC-MRI were obtained. CBF, CBV and MTT were measured in the NAWM of healthy subjects and in non-enhanced lesions, enhanced lesions and contralateral NAWM of MS patients. They found reduced perfusion in NAWM and lesions in MS patients when compared to healthy subjects. CBF was significantly high in enhancing lesions in comparison to contralateral NAWM. Furthermore they found reduction in perfusion in around 63% of non-enhancing lesions compared to contralateral NAWM, while around 36% demonstrated high perfusion compared to contralateral NAWM. They suggested that increase perfusion in some of non-enhancing lesion could be due to new lesion formation and new inflammation which occur around chronic lesions (Ge et al. 2005).

Findings from a previous longitudinal study by Wuerfel et al (2004) on 20 RRMS patients using DSC-MRI suggested that alteration in NAWM perfusion is an early sign of lesion formation, and that increase in perfusion occurs prior to BBB breakdown, furthermore they indicated that perfusion is increased prior to diffusion elevation. The early increase in perfusion within lesions was explained by vasodilatation which occurs during the inflammatory process, while perfusion reduction in lesions in late stage is due to hypo-metabolism at

the lesion area (Wuerfel et al. 2004). This interpretation was further supported by a previous MR spectroscopy study in which lower N acetyl aspartate was reported indicative of hypometabolism in hypointense lesions (Li et al. 2003).

Perfusion abnormalities in grey matter (GM) were explored previously in cortical GM and deep GM in different MS subtypes. A study performed by Inglese and colleagues examined the perfusion in the deep grey matter (Inglese et al. 2007a). Patients with PPMS and RRMS were compared with healthy subjects, using DSC-MRI. Measurements of CBF, CBV and MTT were selected from Putamen, thalamus and caudate nuclei. It was found that there was reduction in CBF in patients with PPMS and RRMS in comparison to healthy subjects. CBF was lower in PPMS patients than RRMS patients. In addition, there was no significant difference in CBV when RRMS patients were compared with healthy subjects. Patients with PPMS demonstrated a significant difference in CBV when compared to healthy subjects. Perfusion reduction in deep GM of MS patients was explained by the metabolic impairment and microstructural damage found in the basal ganglia and thalamus. Furthermore, demyelination, Wallerian degeneration and iron deposition are all factors which might lead to low metabolic activity and subsequently to low CBF in deep GM (Inglese et al. 2007a).

An ASL study was performed on different MS subtypes. They explored the perfusion variations in these different groups; and also examined perfusion in grey matter and white matter (WM). The study involved 60 patients (21 RR, 12 PPMS, 14 SPMS, 13 Benign MS) and 34 healthy subjects (Rashid et al. 2004). Continuous ASL images were acquired. T2 and T1 weighted images were achieved from all MS patients and analysis was performed using voxel based

analysis. It was found that perfusion was decreased in the cortical and deep GM regions in MS patients when compared to normal subjects. Furthermore, the reduction in GM perfusion was more obvious in patients with PPMS and SPMS. There was an increase in white matter perfusion in SPMS and RRMS, which possibly indicates a high metabolic activity in this region (Rashid et al. 2004).

Moreover, perfusion has been investigated in early RRMS, in which it was hypothesised that perfusion reduction in early MS could occur prior to volume loss (Debernard et al. 2014). The study by Debernard and colleagues, using ASL techniques has found global and regional GM perfusion reduction in early MS using voxel based technique. They suggested that GM hypo-perfusion could be due to primary vascular mechanism rather than secondary CBF reduction due to axonal loss (Debernard et al. 2014).

Another point of interest for researchers was perfusion in NAWM of MS patients. It was shown that, the axonal injury which involves the lesion is not only limited to that lesion (Trapp et al. 1998), however, it also spreads to the NAWM at variable levels (Evangelou et al. 2000b). In addition, a study carried out by Adhya et al (2006). They investigated the areas of reduced perfusion in MS, mainly in the NAWM of PPMS patients , RRMS patients and healthy subjects (Adhya et al. 2006). DSC MRI was performed on all subjects and CBF, CBV and MTT measurement were taken from the periventricular, occipital, frontal NAWM and the corpus callosum particularly from the splenium (Adhya et al. 2006). They found a significant reduction in CBF and CBV in all NAWM areas in PPMS and RRMS, additionally, when they compared PPMS to RRMS patients they noticed a significant reduction in CBF

in NAWM of periventricular area and furthermore, a significant reduction in the frontal and periventricular NAWM (Adhya et al. 2006). A study by Law et.al (2004), using DSC-MRI has explored the perfusion in NAWM of RRMS in periventricular region at the level of lateral ventricles, their results showed reduced CBF in NAWM. On the other hand, Rashid et al (2004) demonstrated high perfusion in WM of SPMS and RRMS, in which they suggested that increase perfusion in WM could indicate increase in metabolic activity and increase cellularity (Rashid et al. 2004).

Perfusion in CIS patients was a field of interest for many researchers, to identify in which stage of the disease perfusion alterations ensue. Therefore, Varga and colleagues (2009) have studied perfusion in 12 CIS patients and compared it to controls and MS patients, the perfusion parameters were assessed in the subcortical GM and NAWM for CIS, RR-MS and controls using DSC-MRI. They found that the CBF was reduced significantly in periventricular NAWM of RR-MS and CIS patients when compared to healthy subjects. Moreover, the CBF was significantly decreased in the putamen of RR-MS in comparison to healthy controls. Interestingly, it was noticed that there was no perfusion reduction in the subcortical GM of CIS patients. Signifying a range of cerebral perfusion reduction in MS commencing in periventricular region and spreading to further white matter, in addition to GM regions during the progression of the disease (Varga et al. 2009).

Additionally, a study performed by Papadaki et al. (2012) showed that the haemodynamic alterations in NAWM and deep grey matter (DGM) in CIS patients, using DSC-MRI (Papadaki et al. 2012). The study involved 30 CIS patients, 30 RR-MS and 30 healthy subjects. CBF, CBV and MTT were

obtained from subcortical frontal white matter, periventricular, centrum semiovale, putamen, caudate nuclei and thalamus, as well as on the contralateral side. It was found that there was a significant increase in CBV and MTT in NAWM and DGM in CIS patients. Moreover, there was a significant decrease in CBF in DGM in comparison to healthy group. In RR-MS patients it was found that there was a reduction in CBF in NAWM and DGM when compared to healthy group. There was a reduction in CBF and CBV in NAWM and DGM in RRMS patients when compared to CIS patients. These results suggested that DGM and WM demonstrates haemodynamic alterations at initial stages of MS (Papadaki et al. 2012). Even though, the findings in Papadaki et al. study, regarding the CBF in DGM of CIS contradicts with the results that were found in study performed by Varga et al. which suggest that there is no reduction of CBF in CIS patients (Varga et al. 2009).

Collectively most of these studies showed reduced cerebral perfusion in different types of MS in different brain compartments.

Table 3.1 Summarize Perfusion studies in multiple sclerosis using MRI techniques

Studies	Subjects number	MS Type	Technique	Region	Findings	Clinical correlation
Debernard et al. (2014)	25 MS 25 HC[1]	RRMS	ASL	Global grey matter (GM)	↓ CBF in global GM compared to control	Impaired memory scores associated with low CBF in GM.
Papadaki et al. (2012)	60 MS 30 HC	CIS, RRMS	DSC-MRI	Periventricular & subcortical frontal WM. Deep GM(thalamus, putamen, caudate nucleus)	CIS=↑CBV&MTT in NAWM,DGM. ↓CBF in averaged DGM. RRMS=↓CBF & ↓CBV in NAWM,DGM when compared to CIS.	
Varga et al. (2009)	24 MS 12 HC	CIS RRMS	DSC-MRI	Periventricular & frontal WM. Subcortical GM (Thalamus , putamen nuclei)	CIS=↓CBF in PV NAWM. No perfusion reduction in subcortical GM. RRMS=↓CBF in PV NAWM, putamen.	
(Inglese et al. 2007a))	22 MS 11 HC	PPMS RRMS	DSC-MRI	Putamen, thalamus, caudate nucleus	PPMS=↓CBF,↓CBV RRMS=↓CBF, No differences in CBV between RRMS & HC.	↓CBF,↓CBV in DGM associated with severity of fatigue.
Adhya et al (2006)	22 MS	PPMS RRMS	DSC-MRI	NAWM (Periventricular , occipital, frontal) corpus callosum.	PPMS=↓CBF,↓CBV in NAWM. RRMS=↓CBF, ↓CBV in NAWM.	EDSS significantly correlate with PV CBF and PV, frontal CBV
Ge et al. (2005)	17 MS	RRMS	DSC-MRI	NAWM	MTT in NAWM & lesions. ↑ CBV, ↑ CBF enhancing lesions.	

Law et.al (2004)	17 MS	RRMS	DSC-MRI	NAWM	↓CBF in NAWM of RRMS	
Wuerfel et al (2004)	20 MS	RRMS	DSC-MRI	NAWM	↑ CBF in pre lesion NAWM	
Rashid et al (2004)	60 MS 34 HC	RRMS PPMS SPMS Benign MS	ASL	WM GM	↓ Perfusion in cortical & deep GM. ↑ Perfusion in WM of SPMS & RRMS.	

3.1.1 Perfusion and clinical performance

Around 43%-65% of MS patients have cognitive impairment (Rao et al. 1991). Numerous MRI studies have demonstrated a relationship between cognitive dysfunction in MS and whole brain lesion burden (Inglese et al. 2007b). However, the T2 weighted lesion volumes do not correlate strongly with neuropsychological impairment (Rao et al. 1989; Foong et al. 1997; Rovaris et al. 1998). The relationship between MS pathology in normal appearing brain tissue and cognitive function have revealed better correlation when using MRI quantitative techniques such as magnetisation transfer, atrophy measures, proton MR spectroscopy and diffusion tensor imaging (Rovaris et al. 2002; Deloire et al. 2005; Dineen et al. 2009). The production of toxic metabolites and vascular dysfunction during inflammation might lead to ischemia due to microcirculation defect (Wakefield et al. 1994). Although many other mechanisms of tissue injury are likely to contribute to cognitive dysfunction seen in MS, microcirculatory dysfunction is an ,as yet, poorly understood mechanism that may also be contributary. Previous heamodynamic studies have illustrated decrease in blood flow and cerebral oxygen consumption in white matter, cortical grey matter and subcortial grey matter in MS patients using single-photon emission computed tomography and positron emission tomography (PET) and they corelated with cognitive dysfunction (Brooks et al. 1984; Paulesu et al. 1996).

A recent DSC-MRI study by Hojjat and colleagues has explored the differences in perfusion (CBF, CBV and MTT) between cognitivly impaired and non-cognitivly impaired RRMS patients, after contolling for structural differences (Hojjat et al. 2016a). They used minimal assessment of cognitive

function in multiple sclerosis (MACFIMS), which is a cognitive battery that includes Paced Auditory Serial Addition test (PASAT), Brief visuospatial memory test revised (BVMT-R), California verbal learning test-II (CVLT-II), symbol digital modalities test (SDMT), Delis Kaplan Executive Function system (D-KEFS), Judgement of line orientation test (JLO) and Controlled oral work association test (COWAT). Their results showed significant reduction in cortical perfusion of cognitively impaired RRMS compared to non cognitively impaired MS (Hojjat et al. 2016a). The same research group have investigated perfusion difference in cognitively impaired and non cognitively impaired RRMS, using PCASL. The MACFIMS battery was used for cognitive assessment, and their results found a sigificant difference in cortical perfusion between healthy and RRMS patients with cognitive impairment, while no difference in cortical perfusion was noticed between healthy group and non-cognively impaired RRMS (Hojjat et al. 2016b).

Debernard et al (2014) found no correlation between GM perfusion and EDSS, MSFC, MoCA and PASAT scores, while a significant correlation was observed between GM perfusion and memory scores in patients with early RRMS in the frontal, cingulate, postcentral and precentral gyri (Debernard et al. 2014). In contrast, another study revealed significant correlation between EDSS and reduced CBF and CBV, in periventricular and frontal NAWM, respectively (Adhya et al. 2006). In another study no correlation reported between perfusion and clinical status (MSFC and EDSS) (Rashid et al. 2004). Moreover, a significant correlation was observed between deep GM perfusion and fatigue scores in MS patients (Inglese et al. 2007a).

A correlation between cerebral perfusion and neuropsychological impairment in MS was studied previously by Inglese et al (2007b). The study was carried on RRMS and PPMS patients using DSC-MRI. They found a correlation between reduced CBF in the deep GM and the Rey Complex Figure Test (RCFT) scores, additionally, they showed a significant correlation between reduced CBV in the deep GM and the D-KEFS score with the CBV of the subcortical GM (Inglese et al. 2007b).

The highest incidence of cognitive impairment was reported in SPMS (Huijbregts et al. 2004). Therefore, a previous cerebral perfusion study by Francis et al. (2012) have examined the effect of cerebral perfusion on cognitive function in SPMS via MRI at the voxel level, using DSC-MRI (Francis et al. 2012). The cognitive function was assessed using a standard cognitive battery. SPMS patients with impaired cognitive function revealed a significant decrease in CBV in the superior medial frontal cortex and subcortical GM in compare to SPMS patients with non impaired cognitive function, these findings indicate that SPMS patients with cognitive impairment reveal vigorous perfusion deficit in subcortical and cortical GM (Francis et al. 2012). In a previous study for the same reseach group Aviv et al (2012) have studied the cerebral perfusion and cognitive impairment of SPMS whereas the subcortical and cortical grey matter was examined at region of interest level (Aviv et al. 2012). And they revealed a significant reduction in grey matter CBV in the left superior, middle and inferior frontal regions and bilateral medial superior frontal regions of SPMS with impaired cognitive function when compared to non cognitive impaired SPMS (Aviv et al. 2012).

3.2 Permeability measurement in multiple sclerosis using MRI techniques

The BBB is an essential structure which involves around 99% of brain capillaries (Vries et al. 1997). It guards the neuronal microenvironment from the systemic circulation (Bradbury 1993). BBB impairment is a pathological feature in different neurological diseases such as MS, infection and stroke (Zlokovic 2008; Farrall and Wardlaw 2009).

Examining the pathological changes in vivo via MRI techniques has been very valuable to understand the history of MS (Miller et al. 1998). Therefore, DCE-MRI is used to identify any focal alteration in the BBB via injecting Gadolinium contrast agent. Gadolinium contrast is able to identify focal pathology which occurs due to BBB destruction and associated with inflammation in acute and chronic reactivated lesions (Grossman et al. 1986; Katz et al. 1993). Slowly growing lesions probably show lack of enhancement in the progressive stages (Filippi et al. 2012). Furthermore, pathological studies have shown that tissue injury in the progressive stages in MS may occur without evidence of BBB leakage. (Hochmeister et al. 2006; Filippi et al. 2012). Visual grading of lesion enhancement was used by clinical studies as an indicator of overt BBB permeability alterations, this approach is considered a reasonably non sensitive technique and its more susceptible to subjective bias (Taheri et al. 2011).

The BBB abnormality in NAWM of MS has been studied recently by Cramer et al (2014) who investigated the BBB permeability (Ktrans) in periventricular NAWM and thalamus of MS and compared it with healthy controls, using

DCE-MRI (Cramer et al. 2014). They also correlated permeability (Ktrans) in lesions and NAWM with clinical manifestations including disability, treatment status and recent relapse. The study included 27 MS patients and 24 healthy controls. Their results demonstrated increase permeability in periventricular NAWM and thalamus compared to healthy subjects. Furthermore, it was found that recent relapses correspond with high permeability in NAWM, lesions and thalamus. They revealed gradual reduction in BBB leakage after relapses, which might be due to the effect of immunomodulatory treatment. In addition, no correlation was demonstrated between permeability and EDSS (Cramer et al. 2014).

A previous study held by Ingrisch and colleagues used a 3D T1 DCE-MRI with combination of 2-compartment modelling to examine the possibility of this technique to quantify perfusion and permeability parameters in MS patients (Ingrisch et al. 2012). Therefore, cerebral CBV, CBF and PS were measured in NAWM and lesions of MS patients (Contrast enhancing and non-enhancing lesions). The study was performed on 19 MS patients (7 males and 12 females), age range from 18 to 61. The MS patients were not receiving therapy at the time of study, except one patient. T1 weighted, FLAIR, T1 weighted DEC-MRI and T1 post contrast were carried out on a 3T scanner. It was found that CBF, CBV and PS in contrast enhancing lesions were significantly higher than in NAWM (P value <0.001). In addition, the CBV in non-enhancing lesions were significantly higher than in NAWM (P value <0.05).

Some studies have investigated BBB abnormalities in NAWM and lesions via changes in the rate of longitudinal relaxation following administration of gadolinium.

A study by Lund et al (2013) has explored permeability in NAWM of 59 RRMS patients and 9 healthy subjects. All MS patients were on immunomodulatory treatment. Permeability was assessed using mean T1 values. Their results reveal no global disruption of BBB in NAWM of RRMS patients (Lund et al. 2013). Nevertheless, variation coefficient of T1 values was higher in NAWM of RRMS compared to WM healthy controls.

A study performed by Silver and colleagues on MS patients, investigated if there is any subtle alteration in BBB (Silver et al. 2001a). Quantitative analysis performed on images of contrast enhanced MRI, where the signal intensity changes in the time profile was examined and compared between healthy controls and MS patients, to detect the subtle alteration in the BBB in both non enhancing lesions and NAWM. The study was carried out on 33 MS patients and 5 healthy subjects. Twelve patients had RR-MS; five patients had benign MS, Nine with SPMS and Seven with PPMS. Imaging was performed on 1.5 Tesla Scanner. T1-weighted images were achieved before and subsequently after contrast injection at 5, 20 and 40 min. Data analysis was performed on focal non enhancing lesions, focal enhancing lesions, diffuse non enhancing lesion and NAWM. For the focal non enhancing lesions the Region of interest was sited at the lesion and the contralateral NAWM. Subsequently, a comparison was made before and after contrast administration at 5, 20, 40 min. The changes in signal intensity for each time point after contrast injection was used to calculate the median percentage for each patient. The same analysis was performed on diffuse non enhancing lesions. For the NAWM analysis, a signal intensity comparison was made at 5, 20, 40 min post contrast, between MS Patients and healthy controls. For focal enhancing lesions the signal

changes was measured in all T1 weighted images. It was found that, there were significant increase in signal intensity of focal non enhancing lesions and diffuse non enhancing lesions in comparison to the contralateral NAWM. In focal enhancing lesions there was a significant increase in signal intensity in all lesions, and it was highest in patients with SPMS, as the maximum signal intensity was at 40 min. Signal intensity changes in NAWM were compared between MS patients and healthy subjects and there were no significant differences noticed, which contradict the previous histopathological research which indicate vascular damage and BBB alteration in NAWM of MS patients.

It has been proposed that subtle and extensive alteration in the BBB may occur in most of non-enhanced MS lesions (Soon et al. 2007). This was tested by Soon and colleagues by examining BBB in non-enhanced lesions of MS patients. The study was performed on 19 MS patients (10 with RRMS and 9 with SPMS). Scans were performed on 1.5 Tesla with proton density (PD) and T2* weighted images were obtained for lesion identification. T1 weighted spin echo was acquired prior to contrast administration and 2 min after gadolinium (Gd) administration, to identify the hypointense lesions on the T1 pre contrast and enhanced contrast lesions on T1 post contrast. PD and T1 gradient echo sequence were obtained at variable time points after Gd contrast administration (Soon et al. 2007). For T1 maps were obtained at (20 min, 40min and 60min) post contrast. Image analysis was achieved by identifying the visible non enhancing lesions. Subsequently, the visible non enhancing lesions were chosen as regions of interest (ROI). Each lesion was compared with another ROI selected on the contralateral normal appearing brain tissue. The two regions of interest were corresponded to compare the leakage from the visibly

non enhancing lesion with the normal appearing brain tissue. The cross sectional area for each lesion was recorded. The alteration in BBB was measured by using ΔR1/ Δt, since R1 is the rate of longitudinal relaxation (1/T1). Thus, R1 change subsequent to Gd injection was considered as Δ R1. The slope of Δ R1 overtime onwards ΔR1/ Δt. It was found that there was an increase in ΔR1/ Δt lesions compared to the corresponded normal appearing brain tissue at all-time points. In T1 hypointense lesions the ΔR1/ Δt was greater than in isointense lesions. In addition, ΔR1/ Δt were larger in RRMS compared to SPMS at the early time points. In the visible enhanced lesions the ΔR1/ Δt was extensively greater than visible non enhanced lesions. Furthermore, larger ΔR1/ Δt were noticed in lesions with small cross sectional area (CSA) in comparison to lesions with large CSA. Therefore in the previous mentioned study, they found that subtle alteration in BBB is typical of visible non enhancing lesions (Soon et al. 2007).

3.3 Diffusion in multiple sclerosis

DTI is considered as an important technique for exploring different pathological features in MS. Abnormal patterns of diffusion have been demonstrated in MS in lesions, NAWM and normal appearing grey matter (NAGM), (Rocca et al. 2000; Guo et al. 2002; Ge et al. 2004; Inglese and Bester 2010). Numerous studies have explored diffusion abnormalities in NAWM and NAGM in MS patients, which provide better understanding of pathophysiology of the disease.

In a serial diffusion MRI study held by Werring et al. (2000) on five MS patients their results revealed that moderate increase in apparent diffusion coefficient (ADC) may lead to development of new lesions, and that microstructural changes could precede BBB leakage. Their results found an increase in ADC in prelesion NAWM, and suggest that BBB leakage is not the initiating event of lesion formation in MS. However, they did not investigate the subtle changes in BBB at pre lesion NAWM, therefore BBB breakdown could not be excluded (Werring et al. 2000). Furthermore, many diffusion studies have revealed that injury in NAWM is more prominent with clinical disability and long disease duration (Rovaris et al. 2002; Rovaris et al. 2005; Pulizzi et al. 2007), which indicates that diffusion sensitivity, will be more with severe pathological progressions (Inglese and Bester 2010).

Few studies have used histogram analysis to measure DTI on global basis, as MS is considered a diffuse disease, in which high average MD and low FA were reported in MS compared to healthy controls (Cercignani et al. 2000; Nusbaum et al. 2000). Nusbaum and colleagues studied the global brain

diffusion and compared it between different MS subtypes. They found that SPMS patients have higher ADC values than RRMS patients, while no significant difference was demonstrated between RRMS and controls (Nusbaum et al. 2000). Furthermore, DTI alterations were reported in NAGM (Cercignani et al. 2000; Bozzali et al. 2002; Rovaris et al. 2002). High MD was demonstrated in NAGM of RRMS compared to healthy controls, while SPMS and PPMS demonstrated higher MD in NAGM than in RRMS patients (Bozzali et al. 2002; Rovaris et al. 2005; Inglese and Bester 2010).

Diffusion measures have been used to study pathological features of MS lesions (Inglese and Bester 2010). High MD and low FA have been reported in previous studies in different MS lesions, and it showed higher MD than in NAWM, these values various which suggest different degrees of tissue injury in lesions (Christiansen et al. 1993; Filippi et al. 2001b; Inglese and Bester 2010). Also, high MD values were reported in areas with irreversible tissue injury such in T1 hypointense lesions (Van Walderveen et al. 1998).

A longitudinal study over 3 years by Calabrese et al. (2011) was performed on 168 MS patients and 45 healthy controls; they combined DTI MRI with double inversion recovery to study microstructural changes in MS, they investigated cortical NAGM and cortical lesions (Calabrese et al. 2011). They revealed high FA in cortical NAGM of MS patients compared to healthy controls. In addition, high FA was reported in cortical NAGM of patient with cortical lesions compared to patients without cortical lesions. After 3 years, FA and MD values were the same in healthy controls while increase diffusion measures were observed in MS compared to baseline scan. Another study by Yaldizli et al (2015) was performed on 46 RRMS and 26 SPMS; they

compared FA and MD of cortical lesions and cortical NAGM. High FA was reported in cortical lesions compared to cortical NAGM. Furthermore, high MD values and low FA of cortical NAGM was demonstrated in MS compared to healthy controls(Yaldizli et al. 2015).

3.3.1 Diffusion and clinical performance

Many studies have correlated diffusion MRI with clinical disability in MS. They suggest that DTI could be used as a predictor for clinical outcome. These studies have explored cognitive and clinical scores with different diffusion indices in WM and GM using different analysis approaches. Rovaris et al. (2002) has studied the correlation between diffusion metrics and cognitive function on 34 RRMS with mild disability, using neuropsychological test battery which examines (memory, attention and language), histograms of MD and FA were calculated in NAWM, NAGM, brain tissue (BT) and normal appearing brain tissue (NABT). Also average MD and FA in lesions were measured. Their results demonstrated moderate significant correlation between MD and neuropsychological scores. However, no correlation was demonstrated between neuropsychological scores and FA in lesions and BT (Rovaris et al. 2002). Another diffusion study performed on 60 MS patients (RRMS and SPMS), patients were assessed for executive function, processing speed, visuospatial and auditory/verbal memory. Histogram analysis was applied to obtain diffusion indices, their results found a significant correlation between diffusion and all cognitive domains (Benedict et al. 2007). In a diffusion follow up study by Rovaris and colleagues it was revealed that high MD values in

NAGM in PPMS could predict increase of clinical disability after five years (Rovaris et al. 2006).

Since DTI has the ability to describe WM tracts, therefore were corresponded in many studies with voxel based (VB) analysis approach. VB analysis is useful to describe subtle alterations in cerebral structure and correlated with neurological dysfunction (Mechelli et al. 2005). Regional abnormalities in NAWM and correlation with cognitive impairment have been detected in previous studies using VB analysis. The correlation between corpus callosum and cognitive scores assessing memory and attention was explored in benign MS patients using DTI tractography, and a significant relationship was reported between FA/MD and cognitive scores (Mesaros et al. 2009). Furthermore, a diffusion study using voxel based analysis correlated cognitive scores using Paced Auditory Serial Addition Test (PASAT) with DTI measures in MS patients with mild and moderate cognitive dysfunction, the diffusion measures were correlated with PASAT in different WM regions namely temporal, parietal and frontal areas (Hecke et al. 2010). A tract based spatial statistics (TBSS) approach has been used in previous studies, which allows voxel based analysis of multi subject DTI (Smith et al. 2006). The use of TBSS technique in MS has showed an association between impaired cognitive domains (working memory, proceeding speed and attention) and FA in corpus callosum and further tracts which have prefrontal cortical connections (Dineen et al. 2009). Furthermore, the neuropsychological abnormalities detected on TBSS were corresponded partially with low probability of lesions which indicates that abnormalities in NAWM have a role in cognitive dysfunction (Dineen et al. 2009).

3.4 Cortical atrophy in multiple sclerosis

Cortical thickness may be measured from 3D T1 weighted images, using various software packages (Daams et al. 2013). Numerous studies demonstrated the correlation between cortical atrophy and clinical disability, cognitive dysfunction and progression of the disease, it was found that grey matter atrophy relates better than measures of white matter such as atrophy and lesion load (Chen et al. 2004; Sanfilipo et al. 2005; Fisniku et al. 2008; Roosendaal et al. 2011).

Cortical thinning was observed in MS patients using surface based analysis; the cortical thinning was demonstrated in frontal and temporal cortex (Sailer et al. 2003). In a study held by Calabrese and colleagues on 100 RRMS patients with mild and severe cognitive dysfunction have demonstrated cortical thinning in the frontal and temporal regions compared to healthy controls (Calabrese et al. 2010b). Furthermore, a significant correlation was observed between cognitive scores and cortical thickness (Calabrese et al. 2010b). In another study, focal cortical thinning was demonstrated in early RRMS at primary sensory areas and it was correlated with cognitive scores at superior temporal gyrus. (Achiron et al. 2013).

3.5 Clinical radiological paradox

MRI is considered as a surrogate measure in many clinical trials and also as a predictor in the assessment of disease progression. This will assume that there is a proximate relation between MRI abnormalities and clinical disability. In fact, the relation between radiological measures and clinical disability is weak, and this has been referred to as a clinical-radiological paradox (Barkhof 2002). Therefore, some progress was made to improve this association. Several factors have an effect on the clinical radiological correlation (Barkhof 2002) such as unsuitable clinical assessment, lack of histopathological specificity, not accounting for spinal cord involvement, NAWM/NAGM damage underestimation and cortical adaptation

One of these factors is the unsuitable clinical assessment such as Expanded Disability Status Scale (EDSS). It provides inadequate description of CNS domains. Therefore, a more inclusive scale was developed, namely Multiple sclerosis Functional Composite (MSFC), which describes three different clinical domains: Timed 25-Foot walk (T25FW), 9 hole-peg test (9HPT) and Paced Auditory Serial Addition Test (PASAT). The relation between MSFC and T2 lesion load has been studied previously, and it was found that the correlation between MSFC and T1 and T2 lesion load is better than the correlation between EDSS with lesion load (Kalkers et al. 2001). Furthermore, many studies found that cognitive scores have a strong correlation with different MRI measures (Edwards et al. 2001; Nocentini et al. 2001; Sperling et al. 2001; Zivadinov et al. 2001).

Another factor which could have an effect on the clinical radiological association is the lack of histopathological specificity. There is an increase in MRI sensitivity due to improvement in sequences; however the histopathological specificity of discovering T2 hyperintense lesions is still low. Although there is a good correlation demonstrated between elevated T2 signal and demyelination (Moore et al. 2000), the extent of axonal damage remains unknown with T2 weighted images. Therefore, numerous MRI techniques have been used to deliver further description regarding axonal pathology, such as MR spectroscopy, T1 hypointense lesions and cerebral atrophy measure (Barkhof 2002).

Furthermore, spinal cord involvement has been neglected in many studies and this could have an influence on the clinical radiological correlation. Although, the correlation between cerebral lesion location and clinical symptoms might not be clear, the lesions site in the spinal cord is clinically obvious; however, some silent lesions could be identified in the brain (Silver et al. 2001b). Furthermore, it was reported that spinal cord pathology including spinal cord atrophy and axonal loss shows strong correlation with clinical disability (Bjartmar et al. 2000).

In addition, NAWM damage should be considered in many studies, since it could have an impact on the clinical radiological association. MRI techniques have the ability to detect NAWM abnormalities. Although the cause of these NAWM abnormalities is ambiguous, a certain amount of changes were identified (Filippi et al. 2001b).

Histopathological involvement of GM has been described previously, and cortical lesions were identified microscopically (Barkhof 2002). It is extremely difficult to visualise cortical lesions using conventional MRI. Different MRI techniques have been used to visualise lesions in GM, which demonstrated cortical and subcortical GM involvement (Dehmeshki et al. 2001; Kapeller et al. 2001; Filippi et al. 2001a). The irregular cortical anatomy and small cortical lesions, might affect MRI studies of cortical NAGM, due to some undetectable cortical lesions and also due to the partial volume effect which may lead to errors with MRI results (Barkhof 2002).

Previous studies have demonstrated the ability of the brain to relocate functions from one area to another in children with acute lesions, this finding was reported also in adults using functional MRI (Barkhof 2002). Cortical adaptation to compensate for cerebral damage has been described previously using MR spectroscopy, they studied the relation between motor activity lateralisation and axonal destruction, it was found that activation of the ipsilateral hemisphere is increased in patients with axonal destruction (Reddy et al. 2000). This compensatory brain mechanism could affect the relation between some MRI measures and clinical disability.

Chapter 4 Aims and Objectives

Advanced MRI techniques including perfusion, permeability and diffusion imaging have been widely used in MS. Since they have the ability to detect and provide better understanding of microstructural changes, haemodynamic alteration, lesion development and progression in different brain compartments comprising WM and GM (Revenaz et al. 2016). Even though, conventional MRI has a crucial role in MS diagnosis, the ability of conventional MRI to identify the underlying pathology and microstructural changes in MS remains restricted. Moreover, the correlation between conventional MRI findings and clinical status is still limited, which might be due to limitations of conventional MRI to deliver a precise estimation of the extent of tissue damage in the brain (Filippi and Rocca 2011). Therefore, using structural and functional MRI methods will provide an insight about the pathological mechanisms affecting various brain regions.

Despite the evidence in previous literature that subtle changes in BBB integrity occur in NAWM of MS, the relationship between permeability alteration with microstructural changes in NAWM and the sequence behind lesion development relative to subtle BBB leakage is still not clear. Furthermore, the haemodynamic alteration in GM in MS was observed previously using different imaging and analysis techniques. However, the structural and functional relevance of GM perfusion is still controversial, and requires more exploration using advanced analysis techniques. Previous studies have revealed microstructural tissue damage in MS using diffusion measures, however these studies have used voxel based and histogram analysis to investigate cerebral

cortex. The vertex wise approach is considered as a feasible method to study cortical pathology (Sailer et al. 2003). Nevertheless, measurement of diffusion alteration in MS using this approach has not been applied previously.

The main purpose of this research study was the clarification of MS pathology in different brain compartments, as well as the mechanism behind lesion development in MS. In addition to correlate different MRI measures with clinical performance in MS cohort, using advanced MRI techniques and advanced image analysis methods.

In this piece of work it was hypothesised in Chapter 6 that MS patients have an impaired NAWM BBB permeability when compared to healthy control subjects. Furthermore, it is suggested that the degree of BBB leakage correlates with NAWM micro-structural alterations, and that the extent of BBB impairment is linked with the clinical status seen in MS patients. The aim of this chapter is to compare the BBB permeability in MS patients and controls via permeability maps generated from DCE-MRI images. Moreover, correlation between diffusion measure and permeability parameter in regional NAWM is illustrated. Additionally, the correlation between permeability parameter in regional NAWM with functional and clinical scores in MS cohort was explored.

It is also hypothesised in Chapter 7 that minor changes in the BBB permeability in NAWM of MS patients results in lesion development and progression and that this disruption occurs prior to NAWM tissue destruction. This is achieved by comparing permeability parameters in prelesion NAWM

and regional NAWM. Furthermore, MD is measured in prelesion NAWM and compared to regional NAWM.

It is further hypothesised in Chapter 8 that MS patients have a reduction in grey matter perfusion when compared to healthy controls, and that a reduction in cortical perfusion in MS is associated with cortical atrophy and clinical disability. This is applied by using surface based analysis approach to compare cortical perfusion between MS and healthy controls. The same approach is used to correlate cortical perfusion with clinical scores and cortical atrophy.

Finally, Chapter 9 hypothesised that cortical diffusion alteration is linked to functional performance and cortical atrophy in MS. This is applied by using surface based analysis techniques to correlate cortical diffusion with clinical scores and cortical atrophy.

Part 2: Experiments

Chapter 5 General Methods and Patient Recruitment

5.1 Introduction

This chapter will describe subject recruitment for Multiple sclerosis (MS) participants and healthy controls, inclusion and exclusion criteria for all participants, MRI protocol and neurological assessment. This study was approved by Nottingham Research Ethics Committee East Midlands - Nottingham 2 (10/H0408/37).

5.2 Methods

5.2.1 Subject recruitment

5.2.1.1 Multiple sclerosis patient selection

MS patients and clinically isolated syndrome (CIS) patients were recruited from Neurology clinics based in Queens Medical Centre campus of Nottingham University Hospitals NHS Trust and from the neurological clinics database. Potential participants were contacted via telephone or email, and information sheets were sent to them. Those who expressed a desire to participate were assessed for their eligibility to participate in the study according to the criteria below, following which they were given an appointment to attend for the study. All participants gave written informed consent prior to their participation in the study.

Inclusion criteria for MS patients

- Diagnosis of clinically definite MS or CIS
- Able to provide informed consent

Exclusion criteria for MS patients

- Age < 18.
- Pregnant.
- Other neurological or neurovascular condition or previous neurosurgery.
- Currently involved in MS therapeutic drug trial
- Taking MS disease modifying drug or has taken any MS disease modifying drug in the past 3 months.
- Received systemic steroids in the past 60 days.
- Contraindication to MRI.
- Previous reaction to MRI contrast agent.

5.2.1.2 Healthy controls selection

Healthy controls were recruited by placing a poster on notice boards approved for this purpose at different sites at University of Nottingham. All healthy controls were assessed for their eligibility to participate in the study according to the criteria below, and all gave written informed consent prior to participation.

Inclusion criteria for healthy volunteer

- Healthy adults
- Able to provide informed consent

Exclusion criteria for healthy volunteer

- Age < 18
- Pregnant
- MS or history of clinically isolated syndrome suggestive of MS
- Other neurological or neurovascular condition, or previous neurosurgery
- Other significant medical condition
- Received systemic steroids in the past 60 days
- Contraindication to MRI
- Previous reaction to MRI contrast agent
- Currently participating in another study using MRI or trial medications

5.2.2 Sample size justification

The sample size for permeability analysis was calculated using G power version 3.1.9.2 software (Faul et al. 2007), the calculation was based on a previous paper by Ingrisch et al (2012), in which they measured permeability difference in contrast enhanced lesions and NAWM in MS (Ingrisch et al. 2012), since there was no previous study measured permeability group difference in MS and healthy, at the start of the study. Therefore to achieve an 80% power with alpha of <0.05, a number of 7 subjects per group is required. During patient recruitment in this current study, sample size calculation was re-calculated again based on a published paper by Cramer et al (2014), as they measured the Ktrans difference in healthy and MS (Cramer et al. 2014). Based on this paper the required number of subjects to attain an 80% power with alpha of <0.05 a number of 8 subjects per group is needed.

The sample size for perfusion analysis was calculated by the Chief investigator of this study Dr. Robert Dineen, using previous Arterial spin labelling (ASL) data acquired at 3T and analysed to give grey matter region-of-interest arterial CBF values. Variation Coefficient for CBF was 10.4%. To detect a 10% change at 80% power with alpha of <0.05, a number of 22 subjects per group is required. Non-usable perfusion data due to patient and / or technical factors were considered in 10% of cases, a sample size of 25 subjects per group would be sufficient to produce the required data of 22 usable datasets per group.

Therefore, a sample size of 25 per group is required to measure permeability and perfusion in MS and healthy controls.

5.2.3 MRI Scan Protocol

MRI safety questionnaire was administered to all participants by a qualified radiographer. An intravenous cannula was inserted prior to the scan by the radiographer or one of the doctors. The MRI scan was performed on all subjects using a 3T scanner (General electric, MR750 3.0T), with a 32-channel head coil. Administration of an intravenous gadolinium (Gd) -chelate based contrast agent (administration schedule described below) was performed during the scan in the presence of one of the radiologists or neurologists. The MRI sequence protocol comprised the following:

- Pre-Gd sagittal 3D Fast spoiled gradient echo (FSPGR) (TE = 3.1 ms, TR = 8.1 ms, TI = 900 ms, Flip angle 8°, acquisition matrix 256 x 256, voxel size 1x1x1, FOV = 25.6 cm^2).
- Axial T2 FLAIR (TE = 120 ms, TR = 8000 ms, TI = 2250 ms, acquisition matrix 512x512, number of slices= 46, Slice thickness 3 mm, pixel size = 0.46x0.46, FOV = 24 cm^2).
- DCE-perfusion (TR= 3.8 mm, TE= 1.3, flip angle 19°, matrix size 256x256, Slice thickness 3mm, pixel size 0.87x0.87, FOV=22.4 cm^2). The sequence was commenced 20 seconds before contrast agent injection of 0.1 mmol/kg body weight of Gadobutrol (Gadovist, Bayer, Berlin, Germany). The contrast was injected in two half doses (Roberts et al. 2006; Sourbron et al. 2009b) and each dose was followed by 20 ml flush of saline using automated injection pump (Medrad Spectris Solaris EP MR injection system). There was a pause of 50 seconds between injection of the two half doses (Ingrisch et al. 2012). The

double-bolus injection technique was used to decrease the peak arterial concentration, while the full contrast dose was maintained, and to prevent arterial concentration underestimation by reducing the risk of arterial signal saturation during the bolus passage (Ingrisch et al. 2012).

- Post-Gd sagittal 3D FSPGR (TE = 3.1 ms, TR = 8.1 ms, TI = 900 ms, Flip angle 8, acquisition matrix 256 x 256, voxel size 1x1x1, FOV = 25.6 cm^2).
- DTI (TE = 81.3 ms, TR = 5600 ms, 30 directions, voxel size 2.2x2.2x2.2, FOV = 22.4 cm^2, acquisition matrix 102 x 102, number of slices 46, one b0 image).

Subjects were pseudo-anonymised by giving each participant a unique study participant number. After all sequences were acquired, the images from the scanner were copied on to DVD in DICOM format. All images were then loaded onto the secure departmental server. All data were imported in the following directory: home/msxae2/. Subsequently, the images were converted from DICOM format to ANALYSE format using in house software dtoa programme (http://www.nottingham.ac.uk/~msapm1/software/dtoa.html). MRIcro version 1.4 software (Rorden and Brett 2000) was used to convert the Pre-Gd sagittal FSPGR 3D and post-Gd sagittal FSPGR 3D to axial orientation.

5.2.4 Neurological Assessment

5.2.4.1 Expanded Disability Status Scale (EDSS)

EDSS is a score by Kurtzke (1983) to measure the degree of disability in multiple sclerosis, during the course of the disease (Kurtzke 1983). Higher EDSS scores represent higher disability. The scale gives a rating from 0-10 and it is based on 8 functional systems (FS) and ambulation. The 8 functional systems include: Pyramidal, Cerebellar, Brainstem, Sensory, Bowel and Bladder functions, Visual, Mental and other functions. All MS participants in this study had EDSS scores, provided by a neurologist.

5.2.4.2 The Montreal Cognitive Assessment (MoCA)

The MoCA (Nasreddine et al. 2005) test is used to assess mild cognitive impairment and it has been widely used in different neurological diseases. It has the ability to detect mild cognitive impairment in MS patients, with 90% sensitivity while the mini mental status exam (MMSE) shows only 18% sensitivity (Nasreddine et al. 2005; Zadikoff et al. 2008). A score under 26 is considered to be indicative of cognitive impairment. Different cognitive domains are measured including concentration, attention, memory, language, executive functions, orientation, calculations, conceptual thinking and visuoconstructional skills. The MOCA test consists of 11 parts which include the following: Alternating Trail Making, visuoconstructional Skills (Cube), visuoconstructional Skills (Clock), naming, memory, attention, sentence repetition, verbal fluency, abstraction, delayed recall and orientation. The test

was performed by me face to face with all MS participants during their MRI scan visit.

5.2.4.3 Beck Depression inventory II

The BDI II score was introduced by Beck et al (1996) as an update of the original Beck Depression Inventory for measuring depressive symptoms (Beck et al. 1996). It consists of 21 items and each item has scale of four points from 0 to 3. Increased BDI II scores indicates increased severity of depressive symptoms. A total score of 0-13 indicates no or minimal depressive symptoms, 14-19 indicates mild depressive symptoms, 20-28 indicates moderate depressive symptoms while total scores from 29-63 suggest severe depressive symptoms (Beck et al. 1996).

The BDI measure is self-administered. All participants in this study completed the BDI II on the day of their visit.

Raw scores for BDI-II were calculated out of total 63.

5.2.4.4 Neurological fatigue index for multiple sclerosis (NFI-MS)

The NFI-MS is a test to assess fatigue in MS patients, which was developed from Rasch measurement model on 635 MS subjects (Mills et al. 2010). The index consists of 12 items which include physical and cognitive fatigue. It also includes a sleep related fatigue scale (relief by diurnal sleep and abnormal nocturnal sleep). The summary of the scale is derived from the cognitive and physical fatigue scales.

The NFI-MS form was completed by each participant during their visit. Higher scores indicate increased fatigue.

Raw scores of NFI-MS for each subject were calculated from a summary scale by adding values of Items 1 to 7, 9, 11 &12 in the questionnaire.

5.2.4.5 Multiple sclerosis functional composite (MSFC)

MSFC is a clinical outcome measure which was suggested by National Multiple Sclerosis Clinical Outcomes Assessment Task Force (Rudick et al. 1997; Cutter et al. 1999). It is a multidimensional measure which consists of lower extremity function, upper extremity function and cognitive function. These functions are assessed by Timed 25-Foot walk (T25FW), 9-hole peg test (9HPT) and the Paced Auditory Serial Addition Test (PASAT), respectively.

MSFC scores were expressed as Z-scores based on the normative data (Cutter et al. 1999); hence the lower the score, the poorer the functional performance. The Z-score for MSFC was calculated for each subject using the equation given in the MSFC manual (Fischer et al. 2001).

MSFC score = [{Average (reciprocal 9HPT) - 0.0439}/0.0101+ {- Average T25FW - 9.5353 /11.4058}+{ PASAT-3 – 45.0311 /12.0771}] /3

- ***Timed 25-Foot walk (T25FW)***

This measures the lower extremity function / ambulation. The test consists of two trials. In the first trial the participant was asked to walk a 25 foot distance as quickly as possible and safely. In the second trial the participant was asked to walk back the same distance to the starting point. An aid device could be used during the test. The time limit for this test is 3 minutes per trial. The results are expressed in seconds, hence the higher the score the poorer the performance. All participants with MS in this study performed the T25FW test during their visit, except for 2 patients, as they were in wheel chairs and unable to undergo the assessment.

The average score of the two trials was calculated for each subject. T25FW scores were expressed as Z-scores based on the normative data from the Task force Database. The recommended Z-score for patients who were not able to perform the T25FW was (-13.7) based on Task Force database (Cutter et al. 1999; Fischer et al. 2001). Therefore, this Z-score was used for two MS patients in this study.

Lower Z scores indicate worse performance.

- ***9 hole peg test***

This test is used to assess arm function / fine motor control. It was first administered by Goodkin et al. (1988). The test was performed on both the dominant and non-dominant hands with 2 trials for each hand. Using a device consisting of 9 holes and 9 pegs, the patient was asked to insert 9 pegs in each hole one at a time in any order, and then take out all the pegs one at a time as quickly as possible. The time limit for this test is 5 minutes per trial. All participants in this study had performed the 9HPT successfully during their visit. The average 9HPT for the right and left hands were calculated. The results are expressed in seconds, so the higher the score the poorer the performance.

The average score of the two trials was calculated for dominant and non-dominant hand for each subject.

- ***Paced Auditory Serial Addition Test (PASAT)***

This is a test to provide a quick measure of cognitive function that is commonly used in MS research and clinical practice. It provides a summary measure of processing speed, working memory, calculation ability and sustained attention. It was first administrated by Gronwall (1977) in patient with sustained mild head injuries to observe their recovery (Gronwall 1977). The test includes a 3 second PASAT record form and MP3 which presents a serial of single digit number at a rate of 3 seconds. The participant was asked to add the first two digits they have heard; subsequently they have to add the

following number to the last number that they heard. All participants with MS in this study performed the PASAT. Before the test was performed a practice trial was performed to make sure that the test is clear to the participants. All subjects completed the test successfully during their visit. The number of correct responses was recorded; hence, the lower the score the poorer the performance.

Normative data on 101 healthy adults found that educational level has a significant influence on PASAT performance (Rao et al. 1991). Therefore, PASAT Z scores were calculated, using this normative data, to adjust for educational level.

5.3 Results

This study involved 28 participants with clinically definite MS or clinically isolated syndrome (19 RRMS, 7 SPMS and 2 CIS) patients and 24 age and gender matched healthy controls (Table 5.1). The number of subjects for healthy controls is less than the aimed number, however during sample size estimation a 10% of patient and / or technical factors was put in consideration and a sample size of 25 subjects per group would produce the required data of 22 usable datasets per group, based on previous ASL data.

Two RR-MS patients have had an incomplete scan, as the first patient had a history of anaphylaxis, therefore no gadolinium was administrated and the second patient felt unwell during gadolinium administration.

The EDSS range for MS participants was (0-7) and the median was 2.5 with interquartile range (1.5-5.8). Kurtzke indicates that score 2.5 associates with '*minimal disability in two functional systems'* , score 7 associates with '*Unable to walk 5 meters even with aid, essentially restricted to wheelchair; wheels self in standard wheelchair and transfers alone; up and about in wheelchair some 12 hours a day'*(Kurtzke 1983).

The MOCA scores range for MS participants was (21-30), median 28 with interquartile range (26-29), and this suggests that most MS participants demonstrate normal to mild cognitive impairment, according to MOCA test.

The MSFC Z scores range for MS participants was from (-5.98-0.75), median -0.09, with interquartile range (-0.8 – 0.12), a score of -0.09 indicates increased disability.

The NFI-MS range for MS was from (0-30); the median was 20 with interquartile range (11.25-22.75).

The BDI-II range for MS participants was (0-40); the median was 7.5 with interquartile range (3-17.75), which indicates that majority of MS participants had minimal to mild depression.

Table 5.1 Demographic and clinical variables of multiple sclerosis patients and healthy participants.

	MS (N=28)	**Healthy (N=24)**
Age (Year)	43.39±10.45	41.5±13.19
Gender M:F	10:18	8:16
Disease duration	9 IQR (5-17)	N/A
EDSS (Median)	2.5 IQR (1.5-5.8)	N/A
MSFC(Z-scores)(Median)	-0.09 IQR (-0.8 – 0.12)	N/A
9HPT (DH)	35.01±30.19	N/A
9HPT(NDH)	31.95±19.19	N/A
T25FW (Zscores)(Median)	0.3 IQR (-0.036-0.38)	N/A
PASAT (Zscores)(Median)	-1.17 IQR (-2.18-0.16)	N/A
BDI-II (Median)	7.5 IQR (3-17.75)	N/A
NFI-MS (Median)	20 IQR (11.25-22.75)	N/A
MOCA (Median)	28 IQR (26-29)	N/A

5.4 Conclusion

This chapter will be referred to in the method sections of the subsequent studies in this thesis. The study involved 28 MS participants and 24 age and gender matched healthy controls and they all met the eligible criteria. The majority of the MS cohort demonstrated normal to mild cognitive impairment, according to the MOCA test. Furthermore, the range of disability in patient cohort falls between minimal to moderate disabilities.

Chapter 6 Blood brain barrier integrity in multiple sclerosis using dynamic contrast-enhanced MRI at 3T

6.1 Introduction

In multiple sclerosis (MS), blood brain barrier (BBB) disturbance is considered as a vital stage in lesion progression (Harris et al. 1991; McFarland et al. 1992). BBB disruption in MS occurs as a result of alteration in endothelial cells due to the release of pro-inflammatory cytokines released by activated macrophages, and these cytokines also lead to tight junction abnormalities (Minagar and Alexander 2003). The changes in the cerebral endothelial cells and BBB facilitate migration of leukocytes into the central nervous system (Minagar and Alexander 2003). Several imaging studies using contrast enhanced imaging revealed BBB alteration in active MS lesions (Grossman et al. 1986; McFarland et al. 1992; Smith et al. 1993; Minagar and Alexander 2003).

Interestingly, previous histopathological studies revealed that BBB breakdown could occur in normal appearing white matter (NAWM). It was found that 15% of blood vessels in the NAWM have abnormal tight junctions, while MS lesions showed 40% of abnormal tight junctions (Plumb et al. 2002). Kirk and colleagues measured the irregular distribution of tight junction abnormalities and its relation with BBB permeability, finding that these tight junctions abnormalities were detected in all blood vessels calibres and accompanied with leakage of fibrinogen (Kirk et al. 2003). These previous histopathological

studies suggest that lesion pathogenesis in multiple sclerosis is related to blood brain barrier leakage, in which tight junction abnormalities are considered to play a major role in BBB breakdown.

In the last decades, few MRI studies were carried out to investigate BBB disruption in MS using DCE-MRI technique, and most of their findings were suggestive of BBB alteration in multiple sclerosis when comparing MS lesions to NAWM (Silver et al. 2001a; Ingrisch et al. 2012).

Based on previous literature, BBB disruption in MS plays a key role in the inflammatory process in NAWM; this in turn may lead to microstructural changes in NAWM. Nevertheless, the relationship between BBB impairment and microstructural alteration in the NAWM has not been studied yet. Numerous studies have correlated NAWM changes with clinical status (Filippi et al. 2000c; Schmierer et al. 2004b; Lund et al. 2012). However, the influence of BBB alteration in NAWM on clinical status and cognitive impairment in MS is still not clear.

NAWM abnormalities have been demonstrated in most MS subtypes, although the extent of the damage varies among MS patients according to disease severity (Sbardella et al. 2013). NAWM changes can be detected by diffusion tensor imaging (DTI); this technique has the ability to detect alterations in the WM, which is more obvious as the disease progress. Mean diffusivity (MD) is a recognized DTI index measuring microstructural changes in brain tissue. (Sbardella et al. 2013). An increase in MD indicates axonal and myelin loss in the NAWM (Filippi et al. 2000b). The MD was explained in more detail in chapter 2 (section 2.2.2).

In this chapter the blood brain barrier integrity was examined in NAWM, DAWM and lesions. Furthermore, the correlation between BBB impairment and microstructural alteration in the NAWM was investigated. Additionally, the correlation between clinical status and blood brain barrier permeability was inspected.

6.2 Hypothesis and aims

Hypothesis:

- That MS patients have impaired (NAWM) BBB permeability in comparison to healthy subjects.
- That MD in normal appearing white matter will correlate with BBB permeability in MS.
- That functional performance and clinical status correlate with impaired BBB permeability in MS.

The aim of this study was to test the first hypothesis by measuring BBB permeability using DCE-MRI at 3T, in MS patients and healthy controls. The second hypothesis was approached via measuring mean diffusivity values in the NAWM ROIs, which were then correlated with the permeability values in the same ROIs. The third hypothesis in this chapter was investigated through correlating Expanded Disability Status Scale (EDSS***),*** Multiple sclerosis functional composite (MSFC), Montreal Cognitive Assessment (MoCA), Beck Depression inventory II (BDI-II) and Neurological fatigue index for multiple sclerosis (NFI-MS) scores with permeability parameter (Ktrans) in multiple sclerosis.

6.3 Methods

6.3.1 Subject recruitment

Twenty eight MS patients (7 Secondary progressive-MS, 19 Relapsing remitting MS, 2 clinically isolated syndrome (CIS)), and 24 age- and gender-matched healthy controls were included in this study. All participants met the inclusion criteria for the study. Subject recruitment is described in more detail in Chapter 5. Two RRMS patients were excluded, as the first patient had a history of anaphylaxis, therefore no gadolinium was administrated. The second patient felt unwell during gadolinium administration, therefore had incomplete scan.

6.3.2 MRI protocol

All subjects underwent 3T MRI scan (Discovery MR750, GE Healthcare). The image protocol is described in full detail in chapter 5.

6.3.3 Clinical and functional assessment

Following MRI scan, MS patients and CIS patients were assessed for their clinical status by MSFC and EDSS. Cognitive status was assessed by MoCA test. Symptoms of fatigue were assessed using NFI-MS, and symptoms of depression were assessed by BDI-II. The clinical, cognitive and functional assessments are described in more detail in chapter 5.

6.3.4 Image analysis

6.3.4.1 Permeability maps

The DCE-MRI images were processed to generate permeability maps. JIM 5.0 software (Xinapse system, http://www.xinapse.com) was used. The permeability maps were created via DCE-MRI option on JIM software. The haematocrit value that was used in the analysis was 0.45. The arterial input function (AIF) was obtained; it measures the gadolinium concentration in the artery. It was identified manually via drawing one ROI at the middle cerebral artery. The time of contrast arrival was identified at the 2nd bolus, and analysis end time was identified from the AIF graph (Figure 6.1) .The AIF output file was saved, to be used in the analysis. Subsequently, modified Tofts model (Tofts 1997) was used, the model was described in more detail in chapter 2 (section 2.1.1.1). Pixel by pixel analysis was performed, which provides full parameter estimation for the whole image (Figure 6.2). Output images for three parameters were generated automatically (http://www.xinapse.com/Manual/):

- Volume transfer constant (Ktrans, ml/ml/min) (Figure 6.2)
- Extra vascular extra cellular space fraction volume (Ve)
- Plasma fraction volume (Vp)

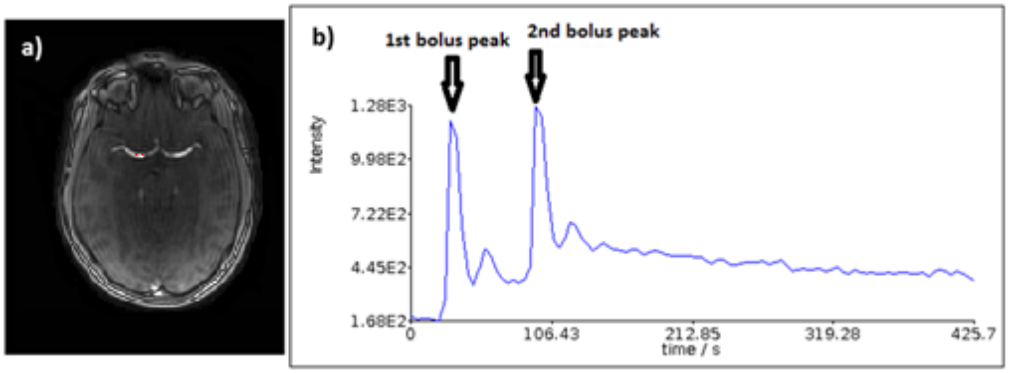


Figure 6.1 DCE-MRI with region of interest (ROI) to measure AIF, b) AIF graph demonstrates the 1st and 2nd bolus peak

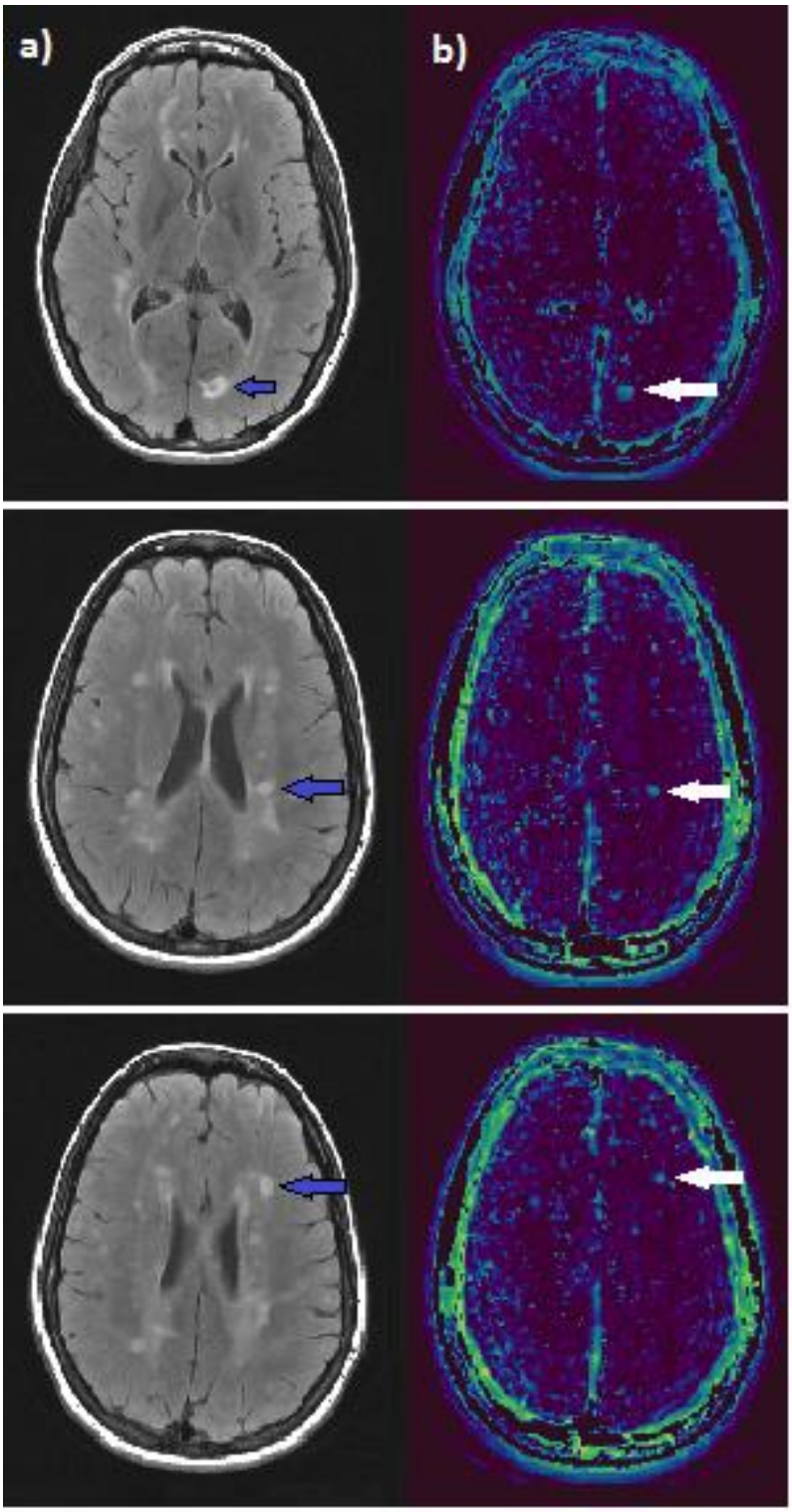


Figure 6.2 a) Axial FLAIR with hyperintense lesions. b) Permeability (Ktrans) maps, the white arrows demonstrate high permeability in hyperintense lesions

6.3.4.2 T2 hyperintense lesion segmentation

FLAIR images were used to segment hyperintense lesions. This was achieved using NeuRoi image analysis software (Dr. C.Tench, Division of Clinical Neuroscience, University of Nottingham, http://www.nottingham.ac.uk/research/groups/clinicalneurology/neuroi.aspx). Semi-automated method was used to identify the region of interest (ROIs), via Sobel edge detection which is a method of identifying boundaries in images, using non maximum suppression (Niepel et al. 2006). T2 hyperintense lesions were identified as areas of increased signal intensity with clear well defined borders. The window settings of the image were adjusted to provide an optimal contrast between the lesion and WM. Auto ROI option was used and seed was applied on the lesion with adjusting the size and shape of the ROI via a tool implemented in the Auto ROI option. Subsequently, manual editing was applied to lesions with low intensity borders. The ROI was performed on all hyperintense lesions demonstrated on all slices. Consequently all the ROIs were saved as binary masks.

6.3.4.3 Diffusely abnormal white matter segmentation

Diffusely abnormal white matter (DAWM) ROIs were created from FLAIR images via NeuRoi software the analysis was performed as described above. The DAWM was defined as areas with slight diffuse increase in signal intensity when compared to the normal appearing white matter (García-Lorenzo et al. 2013). Voxels already included in the lesion segmentation described above in section 6.3.4.2 were later subtracted out from the DAWM

segmentation in a step described below in section 6.3.4.5. Segmentation was performed on all MS patients.

6.3.4.4 Brain segmentation

T1 weighted images were used for brain segmentation. Prior to brain segmentation brain extraction was performed using the brain extraction tool (BET) (Smith 2002) with a default extraction factor of 0.5, a tool implemented in FSL (FMRIB Software Library v 5.0, Analysis group, Oxford, UK). Consequently, the extracted brain images were segmented via FSL using FMRIB's Automated Segmentation Tool (FAST) segmentation (Zhang et al. 2001). Three output images were generated WM, GM and CSF.

A probabilistic threshold of ≥90% was applied on the WM segmentation, using fslmaths to remove the remaining parts of the deep grey matter. This step was applied on all subjects in this study.

6.3.4.5 Image registration

Image co-registration was performed using FMRIB's Linear Image Registration Tool (FLIRT) (Jenkinson and Smith 2001; Jenkinson et al. 2002) via FSL; Affine 12 parameter model was used. FLAIR image was registered to T1 weighted reference image, while the DAWM and hyperintense lesion segmentation images were co-registered as secondary images using the same transformation matrix as the main FLAIR image. The WM segments were registered to T1 weighted image. The generated registered images with DAWM were thresholded (threshold 50, to remove unwanted not DAWN

pixels) and binarised, while the registered images with the WM were binarised only, through FSL maths tools. Furthermore, DCE-MRI was co-registered to T1 weighted image (FSPGR) and the Ktrans maps were used as secondary images. Prior to Ktrans registration, orientation correction was made on all Ktrans maps performed by JIM. The orientation adjustment was performed using fslswapdim, a tool in FSL.

The NAWM segment for MS patients was achieved by subtracting the co-registered DAWM image and lesions segments from the co-registered WM image, using FSL maths. Also, DAWM segment only was created, through subtracting the co-registered lesions segments from the co-registered DAWM image (Figure 6.3).

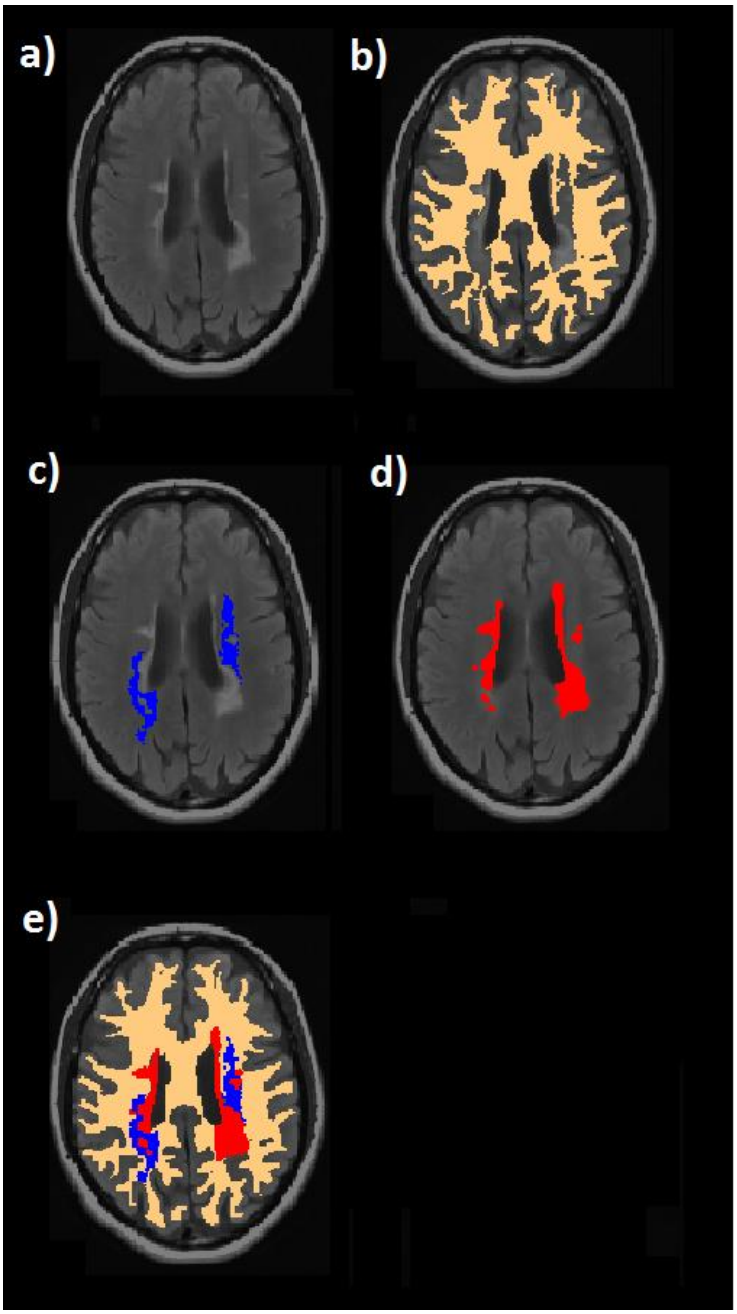


Figure 6.3 Illustrates images from patient with RRMS.
a) Co-registered FLAIR image to T1 weighted image, b) NAWM segmentation, c) DAWM segmentation, d) T2-hyperintense lesions and e) All segmentation maps on co-registered FLAIR image.

6.3.4.6 Regional Normal appearing white matter

NAWM regions-of-interest (ROIs) were created, using the 'create mask' a tool in fslview. Six square ROIs of 5x5 pixels were generated on the NAWM image, three ROIs on each hemisphere. The ROIs were selected in the frontal and parietal lobes, as more NAWM tissue were preserved, which provide better creation of ROIs (Figure 6.4). Later, the ROIs mask was saved. This method was applied on all subjects included in this study.

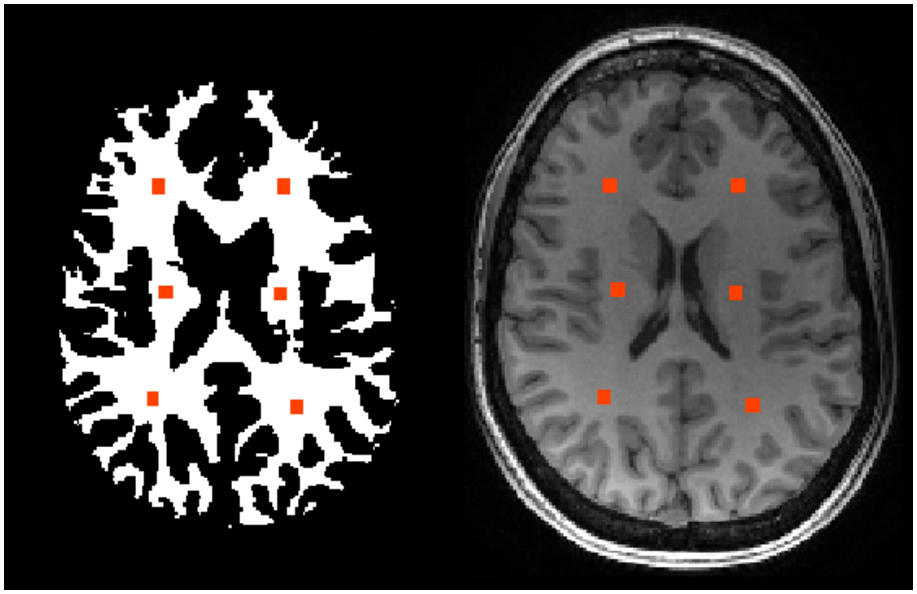


Figure 6.4 Illustrates the ROI's in the NAWM.

6.3.4.7 Measuring Ktrans values

The regional NAWM, DAWM and hyper-intense lesions were masked to the co-registered Ktrans images, through fslmaths. Finally, fslstats was used to work out the means of Ktrans values from the masked images.

6.3.4.8 Diffusion image analysis

Diffusion tensor imaging data were analysed using FSL version 5 (Jenkinson et al. 2012). Following eddy current correction and removal of non-brain voxels, MD maps were generated using the FSL Diffusion Toolbox (FDT). All MD images were registered to the brain extracted FSPGR, via FLIRT linear registration (Jenkinson and Smith 2001; Jenkinson et al. 2002) in FSL; Affine 12 parameter model was used. Subsequently, the regional NAWM were masked to the registered MD images; this step was performed using fslmaths. Later, fslstats was used to extract all the mean MD values from the regional NAWM.

6.3.5 Statistical analysis

Statistical analysis was performed using SPSS statistics (IBM SPSS Statistics for Windows, Version 21.0. Armonk, NY: IBM Corp). Descriptive analysis was performed for both MS and healthy controls. The data for Ktrans, MD and clinical/functional scores (NFI, BDI-II, MOCA, EDSS, 9HPT, PASAT and T25FW) were tested for normality of distribution, using Shapiro-Wilk test.

Analysis of covariance (ANCOVA) was used to compare Ktrans mean differences between MS group and healthy control group, while controlling for age. This approach was also used to compare MD values between MS and healthy control groups. Furthermore, non-parametric Wilcoxon signed-rank test was performed to compare Ktrans in NAWM and DAWM, and between NAWM and lesions for MS patients. Partial correlation was used to explore the correlation between MD and Ktrans in MS, with control the effect of age. Non parametric Spearman correlation was applied to examine the relationship between Ktrans and functional scores. Although not a novel analysis, as an exercise to confirm previous studies we additionally performed Spearman correlation to investigate the relationship between MD and functional scores. Significance was set at p value < 0.05.

6.4 Results

6.4.1 Descriptive analysis

As mentioned above in the methods section the analysis involved 26 MS patients, as two subjects had an incomplete MRI scan. Therefore, the statistical analysis was performed on 50 subjects (26 MS and 24 healthy controls) (Table 6.1).

Table 6.1 Demonstrates all subject characteristics

	MS (N=26)	**Healthy (N=24)**
Age (Year)	43.2±10.8	41.5±13.19
Gender (M:F)	10:16	8:16
Disease duration (Median)	9 IQR (4.5-17)	N/A
EDSS (Median)	2.5 IQR (1.3-5.6)	N/A
MSFC Z score (Median)	-0.06 IQR (-0.8-0.15)	N/A
9HPT (Median)	22.4 IQR (19-31.1)	N/A
T25FW Z score (Median)	0.3 IQR (-0.02-0.39)	N/A
PASAT Z score (Median)	-1.17 IQR (-1.9-0.21)	N/A
MOCA (Median)	28 IQR (26-29)	N/A
BDI-II (Median)	7.5 IQR (3-17.2)	N/A
NFI-MS (Median)	20 IQR (10.5-22.2)	N/A

6.4.2 Permeability comparison between MS and controls in NAWM

The comparison between MS group and healthy controls demonstrates that the regional NAWM mean Ktrans 0.066 ml/ml/min (Age-adjusted mean; SEM= 0.007) was significantly higher ($p < 0.05$) in MS group than the mean Ktrans 0.046 ml/ml/min (Age-adjusted mean; SEM=0.007) in the control group, with control for age (Figure 6.5). There was no statistical significant difference demonstrated between mean Ve in MS 1.05 (SEM=0.3) and mean Ve in healthy controls 0.42 (SEM=0.31).

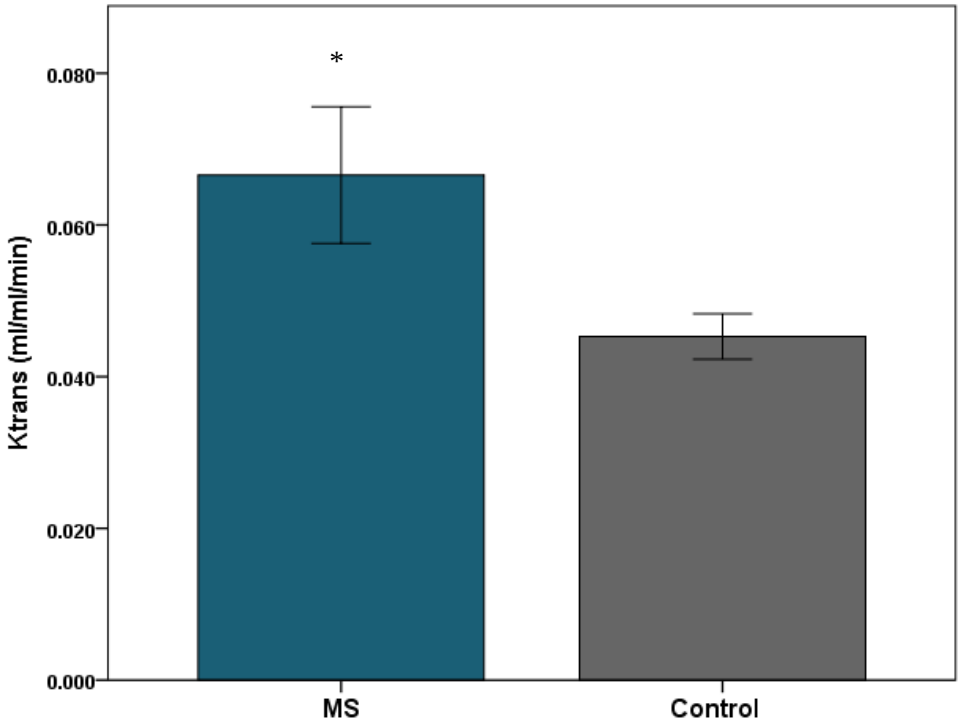


Figure 6.5 Bar chart illustrates significant difference in Ktrans in regional NAWM between MS and healthy control groups.
Data in this graph represent (means ± SEM). * ($p<0.05$) indicates a significant difference from control.

6.4.3 Ktrans comparison between regional NAWM and hyperintense lesions in MS

A statistically significant difference was demonstrated between median Ktrans in lesions 0.088 (Interquartile range 0.047-0.133) and NAWM 0.046 (Interquartile range 0.034-0.095), ($p< 0.001$). (Figure 6.6)

6.4.4 Ktrans comparison between regional NAWM and DAWM in MS

A statistically significant difference was demonstrated between median Ktrans in DAWM 0.069 (Interquartile range 0.045-0.12) and regional NAWM, ($p<0.05$). (Figure 6.6)

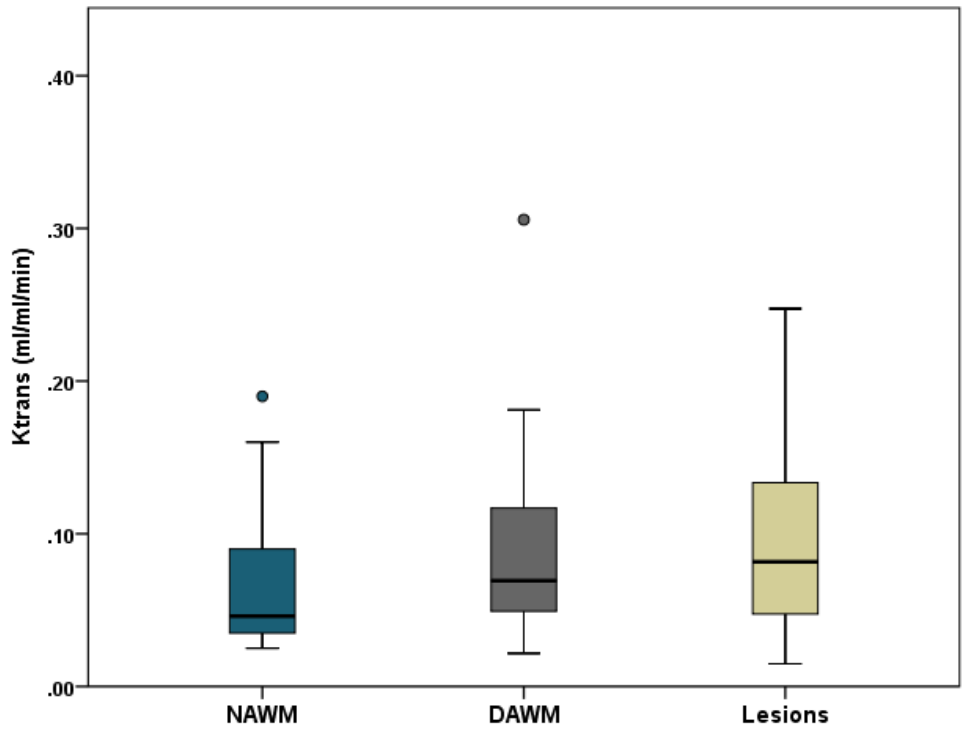


Figure 6.6 Box plot illustrates significant differences in NAWM Ktrans compared to Ktrans in DAWM and hyperintense lesions..
Data in this graph represent (medians and interquartile range).

6.4.5 MD comparison between MS and controls in regional NAWM

The comparison between the MS group and healthy controls demonstrates that the regional NAWM mean MD 0.83 $x10^{-3}$ mm^2/s (SEM= $0.13x10^{-4}$) was significantly higher ($p<0.001$) in MS group than the mean MD 0.76 $x10^{-3}$ mm^2/s (SEM=$0.66x10^{-5}$) in the control group, with control for age (Figure 6.7).

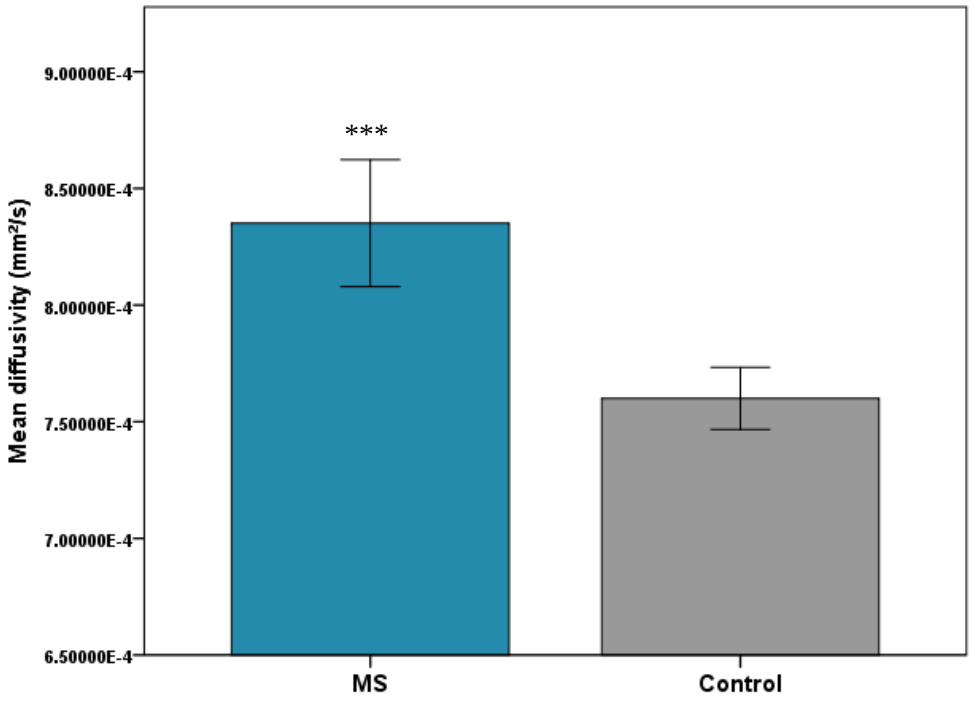


Figure 6.7 Bar chart illustrates NAWM mean diffusivity (mm^2/s) comparison between MS and control.
Data in this graph represent (means ± SEM). *** ($p<0.001$) indicates a significant difference from control.

6.4.6 Correlation between NAWM MD and NAWM permeability in MS

There was a statistically significant positive correlation between NAWM MD and NAWM Ktrans in MS group (r= 0.48, p = 0.01) with control the effect of age. Increase in NAWM Ktrans is associated with higher MD values (Figure 6.8). There was no correlation demonstrated between NAWM MD and NAWM Ve (r= 0.23, p= 0.26).

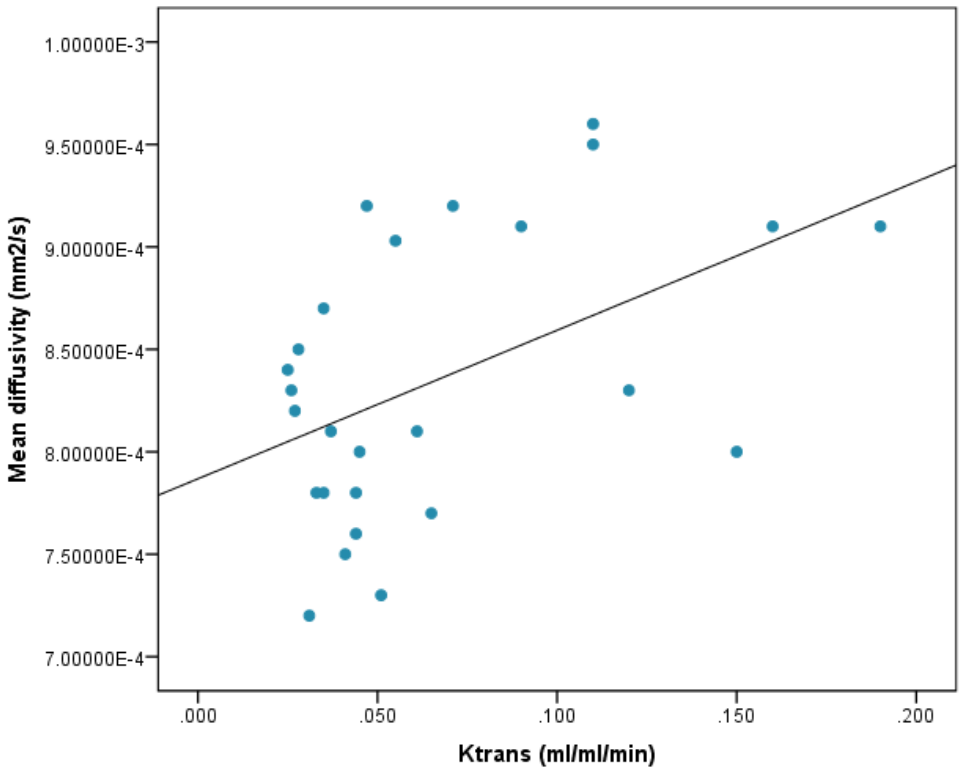


Figure 6.8 Scatter plot illustrates the correlation between regional NAWM mean diffusivity (mm^2/s) and regional NAWM Ktrans in MS.

- Represent MS patients.

6.4.7 Correlation between WM MD and WM permeability in healthy controls

There was no statistical significant correlation between WM Ktrans and WM MD in healthy controls (r= - 0.14, p= 0.52). Also, there was no correlation demonstrated between WM MD and WM Ve in healthy controls (r= 0.043, p=0.84).

6.4.8 Correlation between NAWM Ktrans and clinical scores in MS

There were no statistically significant correlations between NAWM Ktrans and functional scores (NFI-MS, BDI-II, MOCA, EDSS, MSFC Z scores, PASAT Z scores, T25FW Z scores and 9HPT), as illustrated in (Table 6.2).

Table 6.2 Association between NAWM Ktrans and (clinical and functional scores) in MS.

Ktrans NAWM	Spearman correlation	p value
EDSS	0.055	0.78
MSFC Z score	-0.151	0.46
9HPT	0.089	0.66
T25FW Z score	0.128	0.53
PASAT Z score	-0.266	0.18
MOCA	-0.150	0.46
BDI-II	-0.189	0.35
NFI-MS	-0.190	0.35

6.4.9 Correlation between NAWM MD and clinical scores in MS

There was no correlation demonstrated between NAWM MD and clinical/functional scores (NFI-MS, BDI-II, MOCA, EDSS, MSFC Z scores, PASAT Z scores and T25FW Z scores and 9HPT), as illustrated in (Table 6.3).

Table 6.3 Demonstrates correlations between NAWM MD and (clinical/functional scores) in MS

MD NAWM	Spearman correlation	p value
EDSS	0.045	0.82
MSFC Z score	-0.053	0.79
9HPT	0.109	0.59
T25FW Z score	0.111	0.59
PASAT Z score	-0.099	0.63
MOCA	-0.303	0.13
BDI-II	-0.249	0.22
NFI-MS	-0.04	0.84

6.5 Discussion

This study demonstrates subtle changes in BBB permeability in regional NAWM in MS patients compared to healthy controls. The findings support the first proposed hypothesis. The results show an increase in BBB permeability among MS patients compared to healthy controls in the regional NAWM. Furthermore, there is a significant increase in permeability in lesions in comparison to regional NAWM. Also a significant difference was demonstrated between DAWM and regional NAWM.

Previous histopathological studies demonstrated that BBB in the regional NAWM is disrupted with involvement of abnormal tight junction in MS patients (Plumb et al. 2002)

The fact that MS patients in this study were not on either disease modifying therapy or steroids at the time of their scan is considered as an important point as immunomodulatory treatment could decrease the possibility of detection of BBB disruption, due to reduction in the inflammatory process (Lund et al. 2013). The reason for considering regional NAWM instead of global NAWM in this study is to avoid contamination of NAWM masks with the vessels and the cortical ribbon during the registration process with the permeability maps, which may lead to overestimation of Ktrans values. The increase in permeability detected in the NAWM in MS supports previous studies (Cramer et al. 2014).

Furthermore, this study explores the association between BBB leakage and MD in the normal appearing white matter. The findings support the second hypothesis in that suggest that increase in permeability is associated with higher MD values. The positive correlation is as expected. This indicates that BBB alteration is associated with microstructural changes in NAWM. Also, no correlation demonstrated between MD and Ve as well as between Ve and Ktrans in MS. This may suggest that the correlation between MD and Ktrans is not driven by Ve, which represents the extravascular extracellular volume. The explanation of the correlation between MD and Ktrans, may be due to other pathological mechanisms which may have an influence on these two parameters. Both alterations in cellularity and endothelial structure in MS could have an effect on MD and Ktrans, respectively. The subtle structural changes in the NAWM have been detected in this study via MD. Diffusion alteration in NAWM might represent axonal loss and extracellular space expansion, which leads to high diffusivity (Evangelou et al. 2000a; Ciccarelli et al. 2003). No correlation was illustrated between MD and Ktrans in healthy controls. This supports that the correlation found in MS is influenced by pathological changes. Disruption of BBB is an early stage in lesion development in MS (Lund et al. 2013), therefore, one of the predisposing factors of ultrastructural damage in NAWM could be due to vascular changes which enhance infiltration of fibrinogen, gliosis and inflammation. The correlation between BBB permeability and MD values in this study may provide information regarding the extent of damage occurred in the NAWM. The high MD values in NAWM in MS patients compared to controls is in agreement with previous studies (Werring et al. 1999; Filippi et al. 2000b).

However, the relationship between BBB alteration and microstructural damage has not been previously explored. Although, the findings in this chapter demonstrate a strong correlation between BBB permeability and MD in regional NAWM, it remains unclear whether the tissue damage occurs before the BBB breakdown or vice versa.

There is an argument that the ROI analysis of DTI data should have included an analysis of the FA data. FA has been widely used to identify MS-related loss of white matter integrity. However, the reason for analysing MD data not FA is because we can observe FA values in NAWM in healthy individuals from 0.15-0.75, and this magnitude change is based purely on anatomy which will overwhelm any subtle changes detected based on pathology. This problem can be partially overcome by using a co-registration technique, ideally WM skeleton-based, such as TBSS, however that then will lead to different anatomical space location from that in which NAWM ROIs were drawn.

Moreover, this study investigates the correlation between functional/clinical performances and impaired blood brain barrier permeability in MS, as well as mean diffusivity. The third proposed hypothesis is not supported by the results, since no correlations were demonstrated between BBB leakage in NAWM and other functional/neuropsychological scores (EDSS, MOCA, MSFC Z-scores, PASAT Z-scores, 9HPT and T25FW Z-scores BDI-II and NFI-MS). Results of a previous study also failed to detect a correlation between BBB breakdown and clinical status (EDSS) in MS (Lund et al. 2013; Cramer et al. 2014).

Additionally, there were no correlations demonstrated between MD in regional NAWM and functional/neuropsychological scores (EDSS, MOCA, MSFC Z-

scores, PASAT, 9HPT and T25FW Z-scores BDI-II and NFI). Previous studies found a moderate correlation between EDSS and fractional anisotropy in NAWM in RR-MS and SP-MS (Ciccarelli et al. 2001). A previous tract-based spatial (TBSS) meta-analysis showed that the FA changes correlated with clinical disability in the callosal and deep white matter (Welton et al. 2015); however, the NAWM ROIs in this chapter were more in the peripheral NAWM.

A study held by Griffin and colleagues found that there is no correlation between DTI measures in regional NAWM and (MSFC scores and EDSS) in early relapsing remitting patients, which is in agreement with the results in this study (Griffin et al. 2001). Patients in this study may not have severe disability to detect the correlation between MD and MS, as the extent of damage in MS is not severe.

In conclusion, the DCE-MRI technique reveals subtle alteration of BBB in NAWM of MS patient in comparison to healthy controls. Furthermore, it reveals correlation between subtle BBB alteration and microstructural change in regional NAWM. Future larger studies may be required to establish a relationship between permeability in NAWM and clinical disability.

Chapter 7 The effect of blood brain barrier leakage on lesion progression: Longitudinal study for MS patients after 6 months

7.1 Introduction

Lesion formation is a characteristic feature in multiple sclerosis (MS), and the evolution of these lesions has been explored for decades. Since the aetiology that triggers the disease is unclear, many studies were carried out to explore structural changes in NAWM that precede lesion formation using magnetization transfer imaging, diffusion tensor imaging, perfusion imaging and gadolinium enhanced MRI (Filippi et al. 1998; Goodkin et al. 1998; Werring et al. 2000; Wuerfel et al. 2004). Understanding the sequence of events leading to lesion formation could have an impact when designing disease modifying treatments.

Blood brain barrier (BBB) disruption is considered as one of the main pathological events in lesion formation and progression in multiple sclerosis (MS). Previous *in vitro / in vivo* histopathological and radiological studies explored the involvement of BBB breakdown in lesion development and progression in MS, which in turn leads to cellular infiltration and tissue damage (Mahringer et al. 2014). Acute MS lesions show contrast enhancement, which is an obvious manifestation of BBB breakdown, and this is well established as a marker of active disease in clinical practice. Early studies such as that from Kermode et al (1990) looked at overt lesion enhancement, but did

not attempt to quantify subtle abnormalities in BBB integrity using DCE-MRI (Kermode et al. 1990).

Increased BBB permeability and immune cell infiltration have been studied as early event in lesion formation in MS (Floris et al. 2004). Many radiological studies were carried out using advanced MRI techniques to understand the pathology of the disease in lesions, white matter and grey matter in multiple sclerosis, such as mean diffusivity, DCE-MRI and magnetization transfer (MT). Advanced MRI modalities have the ability to detect microstructural abnormalities, even at the early stage of the disease, which provides better understanding of MS pathogenesis in vivo.

Few imaging studies have used quantitative MRI measures to detect subtle changes in BBB in normal appearing white matter (NAWM) in multiple sclerosis and their findings suggest BBB disruption in NAWM (Lund et al. 2013; Cramer et al. 2014), these studies were described in more detail in chapter 3 (section 3.2). Increased permeability among MS patients compared to healthy controls in regional NAWM was described in the results of previous chapter (chapter 6). Nevertheless, this subtle alteration in NAWM requires more MRI studies to explore their effect on lesion progression, as only limited number of studies, had investigated the impact of BBB leakage on lesion development. MRI studies performed on animal models have demonstrated that BBB leakage occurs before lesion development (Maggi et al. 2014).

An imaging study held by Floris and colleagues on experimental allergic encephalitis (EAE) found that BBB leakage occurred at early stage of the disease alongside with neurological sign, whereas no monocyte infiltration was

detected during BBB leakage based on immunohistochemical analysis. However, the monocyte infiltration was identified after leakage of BBB, using MRI. They concluded that monocyte infiltration and BBB permeability are two distinct processes in lesion formation (Floris et al. 2004). However, the sequence of lesion formation in MS remains controversial. It is not clear whether it is due to abnormalities in BBB which in turn enhance the inflammatory process through infiltration of immune cells and proteinaceous materials, or whether the inflammation process and the tissue destruction lead to leakage in the BBB.

In this chapter the BBB integrity was examined in pre-lesion NAWM using DCE-MRI at 3T. Furthermore, mean diffusivity (MD) at pre-lesion NAWM was inspected, as it reflects microstructural abnormalities.

7.2 Hypothesis and aims

Hypothesis:

- That subtle BBB leakage in NAWM may precede lesion progression in MS.
- BBB disruption occurs prior to tissue damage in MS.

The aim of this study is to test the first hypothesis by measuring BBB permeability using DCE-MRI at 3T, in the NAWM ROIs of the first MRI scan where new lesions developed at the second MRI scan. Furthermore, to test the

second hypothesis by measuring MD in the NAWM ROIs of the first MRI scan where patients developed new lesions at the second MRI scan and compare it to the regional and contralateral NAWM MD of the first scan.

7.3 Methods

7.3.1 Subject recruitment

The ethical amendment for longitudinal study was approved by the ethical committee. The MS participants were informed about the second visit scan which was 6 months after their first visit. Twenty MS patients (14 RRMS and 6 SPMS) out of 28 attended the second visit scan. The other 8 patients did not attend the second scan due to various reasons; one patient was pregnant, two patients had incomplete scan at the first visit, and it was not possible to contact the remaining 5 patients. The subjects were not on any disease modifying treatment at the time of the scan. Therefore, this study includes 20 MS participants, who have had the first and second scans.

7.3.2 MRI protocol

The twenty MS participants underwent 3T MRI scan (Discovery MR750, GE Healthcare) using 32-channel head coil. The scan was performed at the MRI department, School of Medicine, University of Nottingham. All participants had MRI safety assessment prior to the scan.

The MRI protocol for the first visit was described in full detail in chapter 5.

The MRI protocol for the second scan comprised the following:

- FSPGR 3D (TE = 3.1 ms, TR = 8.1 ms, TI = 900 ms, Flip angle 8°, acquisition matrix 256 x 256, voxel size 1x1x1, FOV = 25.6 cm^2).

- Axial T2 FLAIR (TE = 120 ms, TR = 8000 ms, TI = 2250 ms, acquisition matrix 512x512, number of slices= 46, Slice thickness 3 mm, pixel size = 0.46x0.46, FOV = 24 cm^2).
- DTI (TE= 81.3 ms, TR=5600 ms, 30 directions, voxel size 2.2x2.2x2.2, FOV 22.4, matrix 102x102, number of slices 46, one b0 image).

7.3.3 Image analysis

7.3.3.1 T2 hyperintense Lesion segmentation

FLAIR images from the second scan were used, to segment hyperintense lesions. This was performed using NeuRoi image analysis software (Dr. C.Tench, Division of Clinical Neuroscience, University of Nottingham, http://www.nottingham.ac.uk/research/groups/clinicalneurology/neuroi.aspx). The lesion segmentation was performed as described in chapter 6.

7.3.3.2 New lesion on second scan

Visual comparison was performed on both axial FLAIR images from first and second scan; new lesions were identified on the second scan, as illustrated in (Figure 7.1). Five MS patients had demonstrated new lesions on the second scan, which were not present on their first scan.

FLAIR image from the first scan was used to draw ROIs in the pre-lesion NAWM, which is the same area where the new lesion was developed on the second scan, using FSL view (tool in FSL).

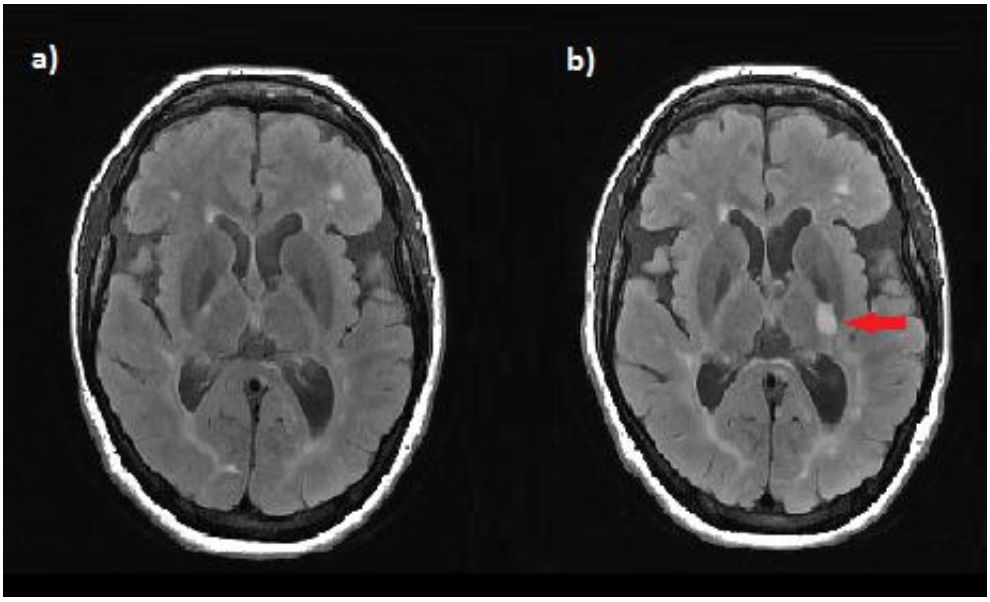


Figure 7.1 Image illustrating development of new lesion in MS patient.
a) Axial FLAIR for RRMS patient at the first scan. b) Axial FLAIR for the same patient after 6 months, demonstrates a new hyperintense lesion at the posterior limb of the internal capsule.

7.3.3.3 Normal appearing white matter mask

- The bilateral regional NAWM masks were described in full detail in chapter 6.
- The contralateral NAWM masks were created using FSL (FMRIB Software Library v 5.0, Analysis group, Oxford, UK), at the contralateral side, which anatomically corresponds with pre-lesion NAWM.

7.3.3.4 Image registration

Image registration was performed using FLIRT linear registration (Jenkinson and Smith 2001; Jenkinson et al. 2002) via FSL software; Affine 12 parameter model was used. FLAIR image from the first scan was registered to T1 weighted (first scan) images which was the reference image, while pre lesion masks were used as secondary images.

7.3.3.5 Permeability maps

The Ktrans map processing was described in detail in chapter 6.

7.3.3.6 Measuring Ktrans values in NAWM

The Ktrans was measured in pre-lesion NAWM, regional NAWM and contralateral NAWM via masking the ROIs to the permeability maps which were created from DCE-MRI of the first scan; it was performed via fslmaths

(FSL software). Subsequently, fslstats was used to extract the mean Ktrans from the pre-lesion NAWM, regional NAWM and contralateral NAWM.

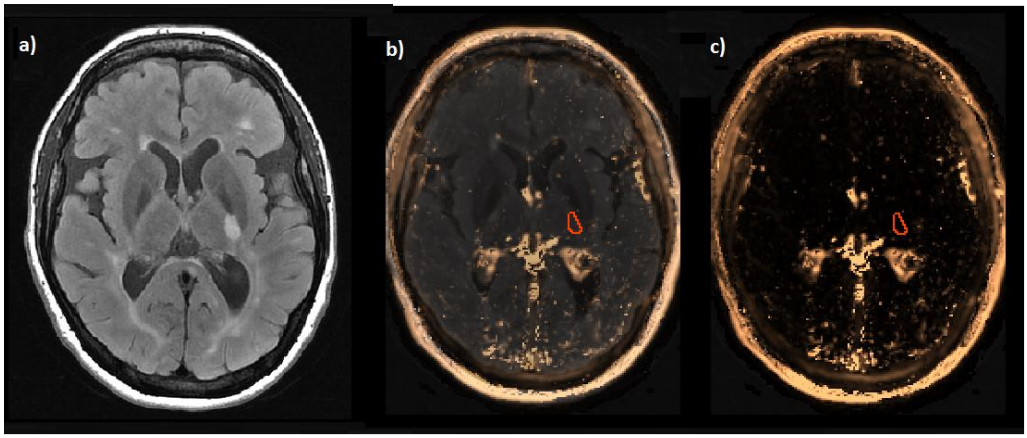


Figure 2 Image illustrating Ktrans map with prelesion ROI: a) Axial FLAIR for RR-MS patient on second scan b) Ktrans map with prelesion ROI overlaid axial FLAIR for same RRMS patient at the first scan. c) Ktrans map with prelesion ROI, even though prelesion foci is not visualised on Ktrans map**.**

7.3.3.7 Diffusion image analysis

All MD images from the first scan were registered to the brain extracted FSPGR, via FLIRT linear registration (Jenkinson and Smith 2001; Jenkinson et al. 2002) in FSL. The next step was to mask the pre-lesion NAWM, regional NAWM and contralateral NAWM to the co-registered MD, through fslmaths. Consequently, fslstats was used to work out the means of MD values from the masked ROIs.

7.3.4 Statistical analysis

Statistical analysis was performed using SPSS statistics (IBM SPSS Statistics for Windows, Version 21.0. Armonk, NY: IBM Corp). Non-parametric Wilcoxon signed-rank test was performed, due to the small sample size (5 subjects) to compare permeability (Ktrans) in pre-lesion NAWM with permeability in regional NAWM and contralateral NAWM. This approach was also used to compare diffusion (MD) in pre-lesion NAWM with MD in regional NAWM and contralateral NAWM.

7.4 Results

7.4.1 Descriptive analysis

Twenty MS patients were included in the second visit. It involved 14 RRMS and 6 SPMS, which were 8 males and 12 females with mean age (44.9±10.3) (Table 7.1). However, the statistical analysis was performed on five MS patients that had developed new lesions (3 RRMS and 2 SPMS) on the second scan.

Table 7.1 Demonstrates MS patients characteristics

	Number of subjects	Age (mean ±S.D)	Gender	MS subtype
MS	20	44.9±10.3	M=8 F=12	SPMS=6 RRMS=14

7.4.2 Ktrans comparison between pre-lesion NAWM and regional NAWM

The comparison between Ktrans in pre-lesion NAWM and regional NAWM, demonstrates that median Ktrans in pre-lesion NAWM (0.083 ml/ml/min) (Interquartile range 0.051-0.13) was significantly higher (p value <0.05) than the median Ktrans in regional NAWM (0.061 ml/ml/min) (Interquartile range 0.03-0.11) (Figure 7.3).

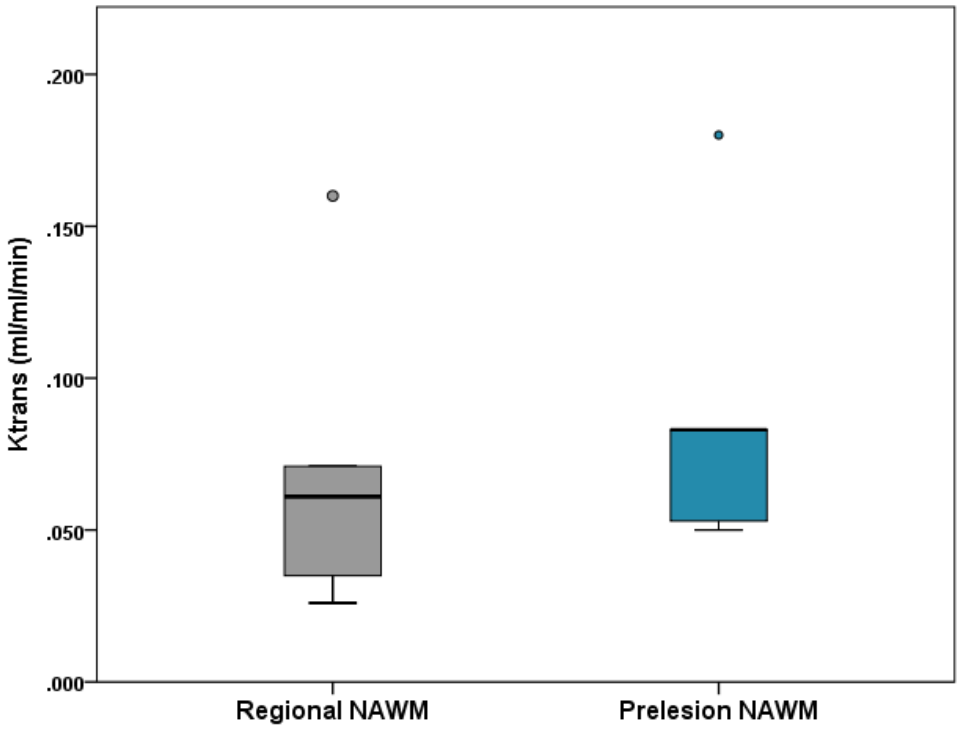


Figure 7.3 Box plot illustrates the Ktrans (ml/ml/min) difference between pre-lesion NAWM and regional NAWM in five MS patients (3 RRMS and 2 SPMS).
Data in this graph represent (medians and interquartile range).
● Outlier.

7.4.3 Ktrans comparison between pre-lesion NAWM and contralateral NAWM

The comparison between Ktrans in pre-lesion NAWM and contralateral NAWM (Figure 7.4), demonstrates that median Ktrans in pre-lesion NAWM (0.083 ml/ml/min) (Interquartile range 0.051-0.13) was significantly higher (p value <0.05) than the median Ktrans in contralateral NAWM (0.036 ml/ml/min) (Interquartile range 0.029-0.074).

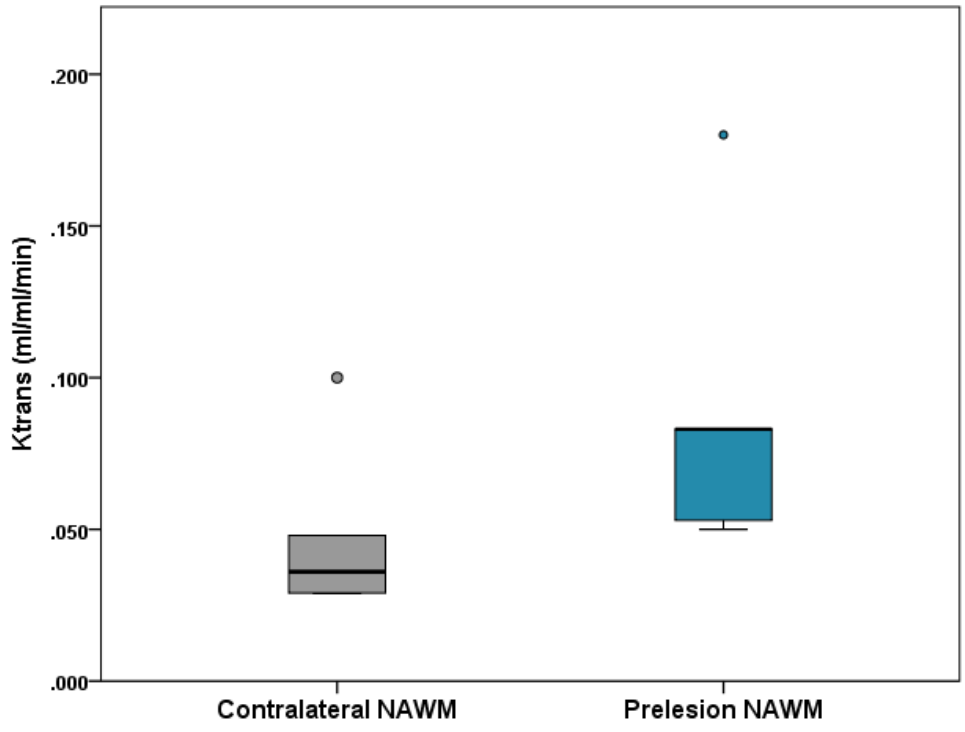


Figure 7.4 Box plot illustrates the Ktrans difference between pre-lesion NAWM and contralateral NAWM in five MS patients (3 RRMS and 2 SPMS)
Data in this graph represent (medians and interquartile range).
● Outlier.

7.4.4 MD comparison between pre-lesion NAWM and regional NAWM

There was no statistically significant difference demonstrated between MD in pre-lesion NAWM and regional NAWM (Figure 7.5). The median MD in pre-lesion NAWM is 0.76 $x10^{-3}$ mm^2/s (Interquartile range 0.7-0.89 $x10^{-3}$) and the median MD in regional NAWM is 0.83 $x10^{-3}$ mm^2/s (Interquartile range 0.79-0.91 $x10^{-3}$)

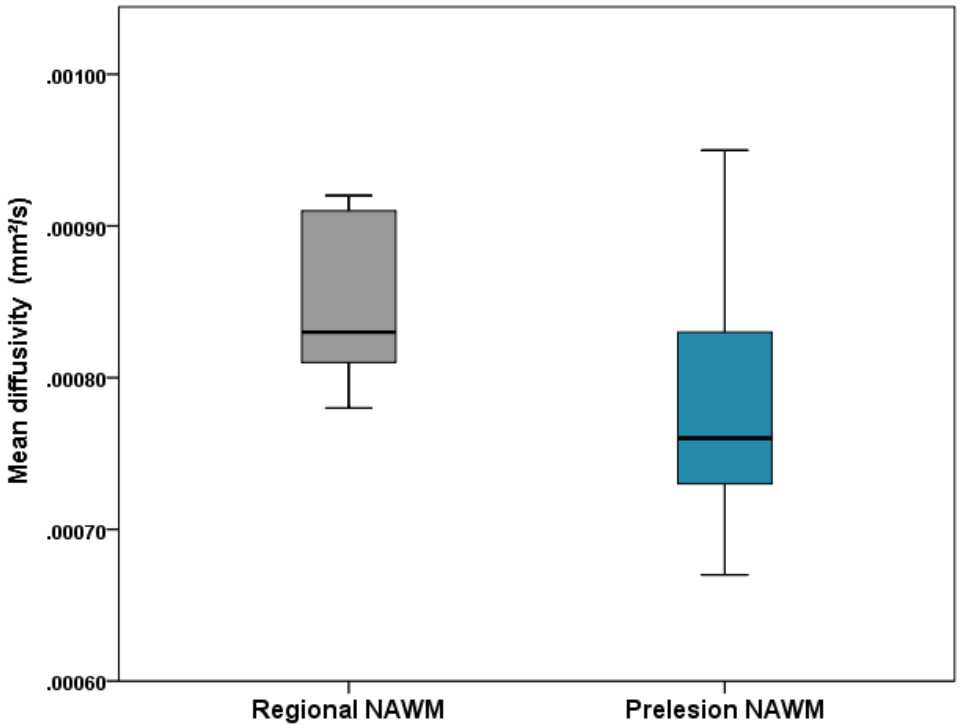


Figure 7.5 Box plot demonstrates a comparison of MD (mm^2/s) between pre-lesion NAWM and regional NAWM in five MS patients
Data in this graph represent (medians and interquartile range).

7.4.5 MD comparison between pre-lesion NAWM and contralateral NAWM

There was no statistically significant difference between MD in pre-lesion NAWM and contralateral NAWM (Figure 7.6). The median MD in pre-lesion NAWM 0.76 $x10^{-3}$ mm^2/s (Interquartile range 0.7-0.89 $x10^{-3}$) while the median MD in contralateral NAWM was 0.75 $x10^{-3}$ mm^2/s (Interquartile range 0.73-0.88 $x10^{-3}$)

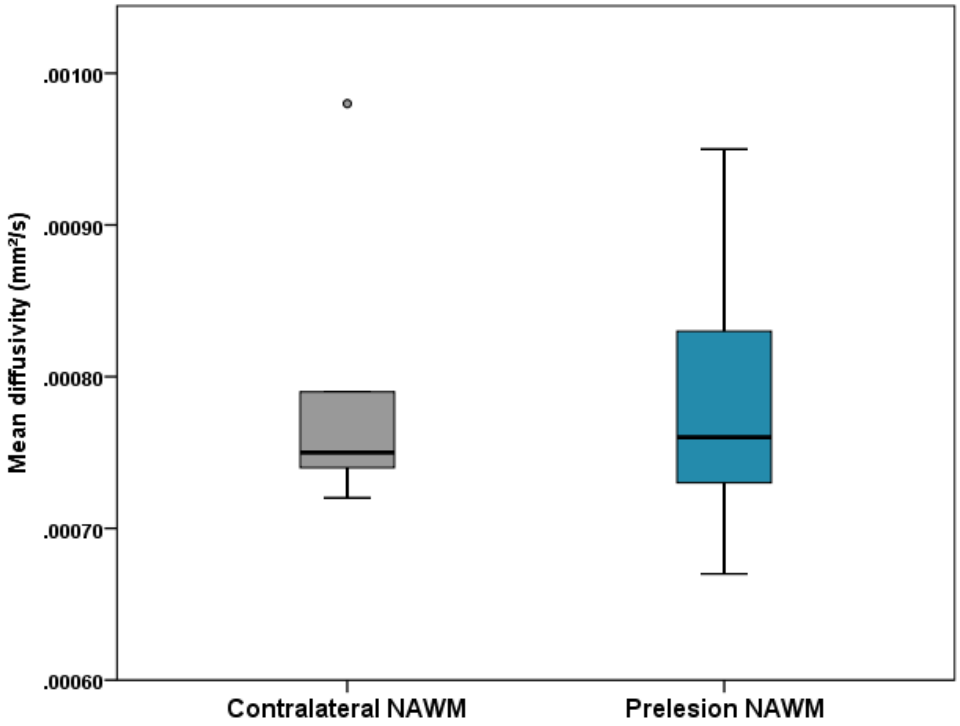


Figure 7.6 Box plot demonstrates a comparison of MD (mm^2/s) between pre-lesion NAWM and contralateral NAWM in five MS patients.
Data in this graph represent (medians and interquartile range).
● Outlier.

Table 7.2.2 Summarise the comparison between prelesion NAWM, regional NAWM and contralateral NAWM in MS.

	Median Ktrans (ml/ml/min)	Median MD (mm^2/s)
Pre-lesion NAWM vs Regional NAWM (P value)	0.083 vs 0.061 (<0.05)	0.00076 vs 0.00083 (0.34)
Pre-lesion NAWM vs Contralateral NAWM (P value)	0.083 vs 0.036 (<0.05)	0.00076 vs 0.00075 (0.89)

7.5 Discussion

This chapter explores whether subtle BBB leakage in NAWM may lead to lesion development in multiple sclerosis. The results show increase permeability at pre-lesion NAWM when compared to the regional NAWM. Also, it demonstrates an increase in permeability in pre-lesion NAWM when compared to the contralateral NAWM. Although only five MS patients (3 RRMS and 2 SPMS) out of twenty had developed new hyperintense lesions after 6 months, these findings may be suggestive of early and subtle BBB alteration in pre-lesion NAWM. A wide variation in Ktrans was noticed between one SPMS subject and the other four MS participants; this could be related to the type of MS. Since a study held by Leech and colleagues found that SPMS have tight junction abnormalities in normal appearing grey matter (Leech et al. 2007), furthermore abnormal tight junctions were detected in NAWM in SPMS (Plumb et al. 2002).

The early changes in BBB detected on MRI, are in good agreement with the literature which consider that BBB disruption occurs at early stage of lesion development (Alvarez et al. 2011). Histopathological studies found abnormal tight junction in BBB of the NAWM (Plumb et al. 2002) and this subtle abnormality in the BBB leads to infiltration of immune cells (Filippi et al. 2004) which in turn leads to lesion development. A series of MRI study on MS patients held by Goodkin et al. (1998) found abnormal quantitative MRI measures such as increase gadolinium enhancement in NAWM which developed new lesions compared to contralateral NAWM (Goodkin et al. 1998). These findings support the current results in this chapter. In addition, a

study performed on an EAE model in marmoset using MRI at 7T, found an increase in BBB permeability of white matter one month before lesion progression (Maggi et al. 2014).

Furthermore, this study investigates whether the subtle alteration in BBB is an early event that occurs prior tissue damage or is it secondary event due to tissue damage. The results demonstrate no significant difference between MD in the pre-lesion NAWM compared to the regional NAWM. Also, there is no difference in mean diffusivity between pre-lesion NAWM and contralateral NAWM. This may indicate that microstructural changes which follow the inflammatory process in the NAWM may occur subsequently to BBB breakdown, and that subtle leakage in the NAWM may be considered as the commencing stage in lesion formation. Increase in MD indicates tissue injury; this increase is not found in this study which suggests that BBB leakage might be detectable at early stage before tissue damage. However, a previous MRI study found an increase in the apparent diffusion coefficient (ADC) in pre-lesion NAWM and also slight increase of ADC in the contralateral NAWM, the follow up MRI scans in the Werring study was performed every month for a year (Werring et al. 2000). They found that increase in ADC occurs prior to GD enhancement, while subtle BBB leakage could not be excluded (Werring et al. 2000). The discrepancy between the results could be due to the different MRI protocol used and the interval between baseline and follow up MRI, as well as the characteristics of MS participants.

Using Ktrans as a quantitative MRI measure to quantify permeability has the ability to detect subtle BBB alteration in NAWM prior to lesion development. Therefore, permeability measures could be used as an indicator to detect BBB

leakage. A limitation of this study was the restricted number of patients who developed new hyperintense lesions. Therefore, patients with highly active MS and the seasonal variation in forming MS lesions (Auer et al. 2000) need to be considered when designing future longitudinal studies in multiple sclerosis. The choice of interval was set as 6 months. This choice was a compromise between having a long enough interval so that we would have a good chance of identifying new lesions in a number of patients, but not making the interval too long such that the relationship between initial imaging findings and subsequent lesion developing was lost.

In conclusion, the findings in this chapter suggest that BBB alteration in multiple sclerosis is detectable at early stage prior MD elevation. Advanced MRI has the ability to detect BBB leakage in NAWM; therefore, further lesion progression could be prevented, by providing a treatment that reduces BBB leakage. Moreover, tight junction abnormality should be put into consideration when designing disease-modifying therapy.

Chapter 8 Structural and functional relevance of grey matter perfusion alterations in multiple sclerosis

8.1 Introduction

Cerebral blood flow alteration in multiple sclerosis (MS) was reported previously in the WM and GM as discussed earlier in chapter 3. This alteration putatively reflects the cerebral metabolic demand and neuronal integrity in the brains of MS patients (Debernard et al. 2014). Many studies have been carried out using various imaging techniques to explore cerebral metabolic demand and cerebral perfusion in MS such as single photon emission tomography (SPECT), dynamic susceptibility contrast (DSC-MRI), arterial spin labelling (ASL) and positron emission tomography (PET) (Ota et al. 2013).

The fundamental basis of haemodynamic alteration in MS has not been established. It may possibly be linked to primary cause which leads to ischemia due to vascular abnormality; a process which is considered reversible (Saindane et al. 2007). Another possible cause, could be reduction in metabolic function as a result of axonal degeneration (Wallerian degeneration), which is an irreversible process (Saindane et al. 2007). It is essential to identify the cause of hypoperfusion in MS, since it could provide a future impact on treatment and outcome of the disease according to the reversibility process of hypoperfusion (Saindane et al. 2007).

Cortical thinning in multiple sclerosis has been reported in several previous studies and it was significantly correlated with clinical disability and cognitive

impairment (Sailer et al. 2003; Calabrese et al. 2010b; Narayana et al. 2013; Geisseler et al. 2016). However, the correlation between cortical atrophy and perfusion in MS has not been established.

Debernard and colleagues studied cortical perfusion in early RRMS prior to cortical atrophy using Pseudo-continuous arterial spin labelling (PCASL), and their results revealed reduced cortical perfusion without volume loss (Debernard et al. 2014). Mean diffusivity is a well-recognised index to measure microstructural alterations in the brain tissue. Previous study has explored the correlation between hypoperfusion and diffusion in NAWM (Saindane et al. 2007). However, the association between cortical diffusion and perfusion in MS has not been established.

A significant association between cortical pathology and clinical disability/cognitive impairment in MS has been reported in previous published studies (Horakova et al. 2012). Various imaging techniques have correlated haemodynamic alteration of the brain with neuropsychological impairment and clinical disability. Inglese and colleagues studied the correlation between regional CBF alteration and neuropsychological impairment in NAWM and deep GM of RRMS and PPMS using DSC-MRI. A significant correlation was reported between hypoperfusion in deep GM and a visuospatial memory test (Inglese et al. 2007b). In another study, no correlation was identified between hypoperfusion in WM/GM and clinical status, using ASL (Rashid et al. 2004), However, a further study found a significant correlation between NAWM CBF in periventricular areas and EDSS, using DSC-MRI (Adhya et al. 2006).

Previous research using (SPECT) has found significant correlation between GM and WM regional CBF and cognitive function in MS patients (Lycke et al. 1993). Moreover, reduced metabolic activity rate in the cortex was reported in MS using PET imaging, however there was no correlation between cortical metabolic activity and clinical disability demonstrated (Blinkenberg et al. 2000).

The results of these studies revealed correlation between hypoperfusion and cognitive impairment. However the correlation between perfusion and clinical disability is not fully understood and appears to be conflicting.

The previously mentioned studies have used voxel based analysis techniques to study perfusion alteration in MS. Only a few studies have used a surface based analysis technique to study cortical pathology in multiple sclerosis (Sailer et al. 2003; Narayana et al. 2013; Geisseler et al. 2016). It is considered a feasible technique to study cortical pathology and correlate it with clinical disability (Sailer et al. 2003). Surface based techniques provide better gyral / sulcal co-registration between subjects (Fischl et al. 1999a) and hence more specific localisation of significant alterations of the imaging metric such as CBF relative to cortical topography, either between groups or to functional scores.

To date no previous study has explored the structural and functional relevance of cortical perfusion alterations in multiple sclerosis using surface based analysis. In this chapter cortical perfusion alterations were examined in MS and correlated with cortical thickness and cortical diffusion, as well as functional scores, using surface based analysis.

8.2 Hypothesis and aim

Hypotheses:

- That cortical and deep grey matter perfusion is reduced in patients compared to healthy controls
- That cortical perfusion correlates with cortical thickness in MS.
- That cortical perfusion correlates inversely with cortical MD
- That patterns of regional cortical perfusion variability will explain performance in different functional domains in MS.

The aim of this study is firstly to demonstrate that surface based analysis can replicate the previous findings of reduced cortical perfusion in MS compared to controls. Also, a non-surface based technique is used to test whether the data shows the expected reduction in deep grey matter perfusion in MS patients compared to controls. The second hypothesis is then tested using cortical surface based analysis with a vertex-wise correlation between cortical CBF and cortical thickness in MS patients. As a secondary analysis surface based analysis is used to compare cortical thickness between MS patients and controls. Finally the third hypothesis is tested using the surface-based approach for a cohort of MS patients, with vertex-wise correlation between CBF and clinical status (MSFC, BDI-II and NFI-MS) and cognitive status (MoCA and PASAT).

8.3 Methods

8.3.1 Subject recruitment

Twenty six MS patients (7 Secondary progressive-MS, 17 Relapsing remitting MS, 2 clinically isolated syndrome) out of 28 MS were included in this study, since 2 MS patients were excluded as described in chapter 5, and 24 age- and gender- matched healthy controls were included in this study. All participants met the inclusion criteria for the study. Subject recruitment is described in more detail in chapter 5.

8.3.2 MRI protocol

All subjects underwent, 3T MRI (Discovery MR750, GE Healthcare). The image protocol is described in full detail in chapter 5.

8.3.3 Clinical and functional assessment

MS patients were assessed for their clinical status using the Multiple Sclerosis Functional Composite (MSFC) and Expanded Disability Status Scale (EDSS). Cognitive status was assessed by the Montreal Cognitive Assessment (MoCA) test. Symptoms of fatigue were assessed using the Neurological Fatigue Index for Multiple Sclerosis (NFI-MS), and symptoms of depression were assessed by the Beck Depression Inventory II (BDI-II). The clinical, cognitive and functional assessments are described in more detail in chapter 5.

8.3.4 Image analysis

8.3.4.1 Cerebral blood flow map

DCE-MRI images were processed to generate perfusion maps; JIM 5.0 software (Xinapse system, http://www.xinapse.com) was used. The perfusion maps were created via the brain perfusion analysis tool in JIM software. A haematocrit value of 0.45 was used in the analysis. The arterial input function (AIF) was obtained; it measures the gadolinium concentration in the artery. It was identified manually via drawing one ROI at the middle cerebral artery in all subjects. Time of contrast arrival was identified at the 1st bolus, and analysis end time was identified from the AIF graph at the 2nd bolus. The AIF output file was saved, to be used in the analysis. The output cerebral blood flow (CBF) image was generated automatically by Jim software: CBF ml/100 g /min. (http://www.xinapse.com/Manual/).

8.3.4.2 Surface based analysis

- **Cortical reconstruction**

Cortical reconstruction was performed on T1 weighted FSPGR images using FreeSurfer software package-linux-V 5.1.0(http://surfer.nmr.mgh.harvard.edu/) (Dale et al. 1999; Fischl et al. 1999b). The reconstruction was performed automatically as part of the FreeSurfer pipeline, which includes the following steps: 1) Talairach registration, in this step a matrix transformation was created from T1 weighted, which was coordinated with the Talairach. Subsequently, 2) Intensity normalization was performed in which intensity variations were corrected (Sled et al. 1998). The next step was 3) skull stripping; it removes the skull from the normalized image. 4) Segmentation is applied using geometric calculation of grey and white matter. After that sub cortical structure and the cerebral hemisphere were separated from cortical element using cutting planes (Fischl et al. 2002; Fischl et al. 2004a). 5) Surface Tessellation of the pial surface (grey matter/cerebral spinal fluid) and grey/white matter surface with a triangular mesh (Fischl et al. 2001; Ségonne et al. 2007). 6) Automated topology correction is applied. Next step is 7) Surface deformation to acquire an accurate and smooth demonstration of grey/white matter surface (Dale and Sereno 1993; Dale et al. 1999; Fischl and Dale 2000). Once the cortex is reconstructed, additional procedures are implemented, including, surface inflation (Fischl et al. 1999b), registration to a spherical atlas (Fischl et al. 1999a) and cortical parcellation (Fischl et al. 2004b; Desikan et al. 2006).

After cortical reconstruction, manual correction of the cortical surface for subcortical lesions was performed.

- **Registration of CBF maps**

CBF maps were registered (with manual correction if necessary) via the *bbregister* and *tkregister2* commands provided by FreeSurfer. The CBF maps were projected on the cortical surface using *mri_vol2surf* tool with a projection fraction of 0.5, which samples the CBF data in to the voxels that are halfway across the width of the cortical ribbon. A multi-intersubject topography-based co-registration was performed in spherical surface space implemented in FreeSurfer (Fischl et al. 1999a). The field of view covering the brain for DCE-MRI image extends from the level of cingulate cortex to the mid pons level. Therefore, the cortical perfusion did not include the whole cortical surface (Figure 8.1).

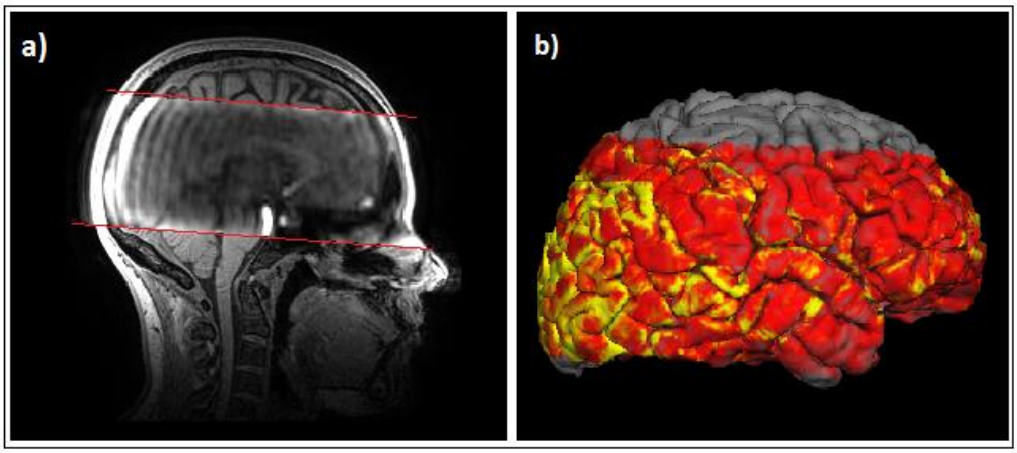


Figure 8.1 a) Field of view for DCE-MRI registered on T1 weighted image. b) Surface based map of cortical CBF.

- **Partial volume correction and registration of MD maps**

Diffusion tensor imaging data were analysed using FSL version 5 (Jenkinson et al. 2012). Following eddy current correction and removal of non-brain voxels, MD maps were generated using the FSL Diffusion Toolbox (FDT). Partial volume correction (PVC) was applied on the MD maps using the approach of Koo et al (2009). Prior to PVC, registrations of MD maps and b0 images to the MNI 152 standard space T1 weighted brain were performed, using FLIRT linear registration (Jenkinson and Smith 2001; Jenkinson et al. 2002) via FSL. Subsequently, tissue probability maps were calculated for CSF and GM from registered b0 images, using Statistical parametric mapping (SPM8) software (http://www.fil.ion.ucl.ac.uk/spm/software/spm8/). The remainder of the PV correction steps were locally implemented using in house Matlab code (Matlab Version 7.12.0.635, R2011a) based on (Koo et al. 2009) written by Dr.Yue Xing (a research fellow, Division of Clinical Neuroscience, University of Nottingham). The GM and CSF apparent signal fraction weightings (λapp) from the reference b0 scans (Pasternak et al. 2009), were estimated using tissue probability maps. Subsequently, the measured MD maps and modelling MD-CSF based on each individual eroded CSF mask were used to estimate MD-GM.

The PV corrected MD maps were registered (with manual correction if necessary) via the *bbregister* and *tkregister2* commands provided by FreeSurfer. The MD maps were projected on the cortical surface using *mri_vol2surf* tool with a projection fraction of 0.5.

8.3.4.3 Basal ganglia mask

For basal ganglia perfusion analysis a mask of the corpus striatum (the putamen and caudate nucleus) was created from MNI 152 standard space T1 weighted, using FSL (FMRIB Software Library v 5.0, Analysis group, Oxford, UK). Segmentation of the FSPGR image was performed on each subject, using FMRIB's Automated Segmentation Tool (FAST) segmentation (Zhang et al. 2001) via FSL. The grey matter map was registered to MNI 152 standard space T1 weighted brain using FLIRT linear registration (Jenkinson and Smith 2001; Jenkinson et al. 2002) via FSL software. Later, the basal ganglia were masked to the registered grey matter for each subject using fslmaths.

8.3.4.4 Measuring CBF values from basal ganglia

DCE-MRI was co-registered to MNI 152 standard space T1 weighted and the CBF maps were used as secondary images, using FLIRT linear registration (Jenkinson and Smith 2001; Jenkinson et al. 2002) via FSL (FMRIB Software Library v 5.0, Analysis group, Oxford, UK). Before applying CBF map registration, orientation correction was made on all CBF maps. The orientation adjustment was performed using fslswapdim, a tool in FSL. Later, the basal ganglia were masked to the co-registered CBF maps, using fslmaths (Figure 8.2). Finally, fslstats was used to work out the means of CBF values from basal ganglia

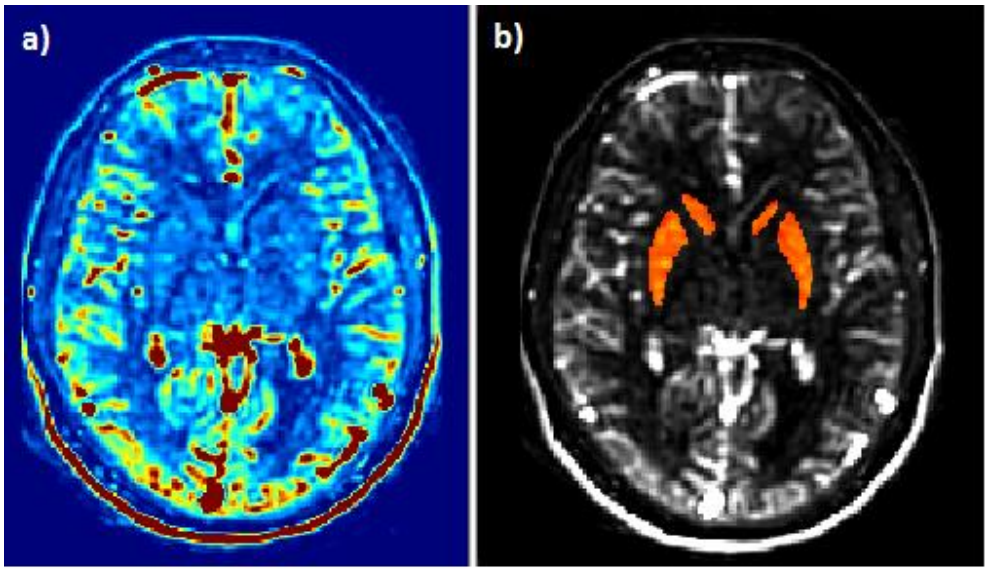


Figure 8.2 a) CBF map generated from (DCEMRI) using (Jim 5.0 software) b) Basal ganglia perfusion mask overlaid a CBF map.

8.3.5 Statistical analysis

Surface group statistical analysis was performed using the QDEC application in FreeSurfer to compare cortical perfusion between MS and healthy controls. Also to correlate surface based cortical perfusion with BDI-II, NFI-MS, MoCA, MSFC Z-scores, T25FW Z-scores, PASAT Z-scores and 9HPT. Correction for multiple comparisons was performed using false discovery rate (FDR) a tool implanted in QDEC. Significance was set at $p < 0.05$ (FDR-Corrected). For tests where no significant clusters remained at the $p < 0.05$ (FDR-Corrected), the data was further explored using a more lenient threshold of $p<0.01$ (uncorrected for multiple comparisons). The correlation between cortical perfusion and functional scores were controlled for age. Age was selected as a nuisance factor in QDEC.

Anatomical naming of clusters reaching significance as presented in the results was facilitated by use of the aparc.annot cortical atlas that is distributed with FreeSurfer (Desikan et al. 2006).

Statistical analysis was performed using SPSS statistics (IBM SPSS Statistics for Windows, Version 21.0. Armonk, NY: IBM Corp), descriptive analysis was applied. The data was tested for normality of distribution using Shapiro-Wilk test. Independent samples t-test for group comparison of BG CBF was performed. Spearman correlation was performed to correlate perfusion in the basal ganglia with BDI-II, NFI-MS, MoCA, MSFC Z-scores, T25FW Z-scores, PASAT Z-scores and 9HPT.

Pearson correlation was used to correlate regional cortical perfusion with cortical atrophy and MD measures. To measure cortical atrophy, the cortical thickness Z-score was calculated for the MS patients and ROIs were selected

from clusters illustrated in the group difference of cortical perfusion. Subsequently, the cortical thickness mean and standard deviation for the selected ROI were calculated in control and MS cohort using *mris_anatomical_stats*. Finally, to attain a measure of atrophy the Z score of cortical thickness in the ROI for MS patients were calculated.

CBF and MD values were also extracted from the selected ROI using *mris_anatomical_stats.*

8.4 Results

8.4.1 Descriptive analysis

The descriptive statistical analysis was performed on 26 MS patients (7 SPMS, 17 RRMS, 2 CIS) and 24 age- and gender- matched healthy controls (Table 8.1).

Table 8.1 Demographic and clinical variables of multiple sclerosis patients and healthy control.

	MS (N=26)	**Healthy (N=24)**
Age (Year)	43.2±10.8	41.5±13.19
Gender (M:F)	10:16	8:16
Disease duration (Median)	9 IQR (4.5-17)	N/A
EDSS (Median)	2.5 IQR (1.3-5.6)	N/A
MSFC Z score (Median)	-0.06 IQR (-0.8-0.15)	N/A
9HPT (Median)	22.4 IQR (19-31.1)	N/A
T25FW Z score (Median)	0.3 IQR (-0.02-0.39)	N/A
PASAT Z score (Median)	-1.17 IQR (-1.9-0.21)	N/A
MOCA (Median)	28 IQR (26-29)	N/A
BDI-II (Median)	7.5 IQR (3-17.2)	N/A
NFI-MS (Median)	20 IQR (10.5-22.2)	N/A

8.4.2 Group comparison of cortical perfusion between MS patients and controls

The group comparison involved 25 MS patients, as one MS patient was excluded due to persistent error occurring during the registration process of CBF maps that was not resolved by attempts at manual correction. Multiple cortical areas showing significant differences in cortical perfusion between MS and healthy controls were demonstrated in both right and left hemispheres, (P<0.05 FDR corrected). These areas, which are detailed in (Table 8.2), showed lower perfusion in MS patients compared to healthy controls (Figure 8.3).

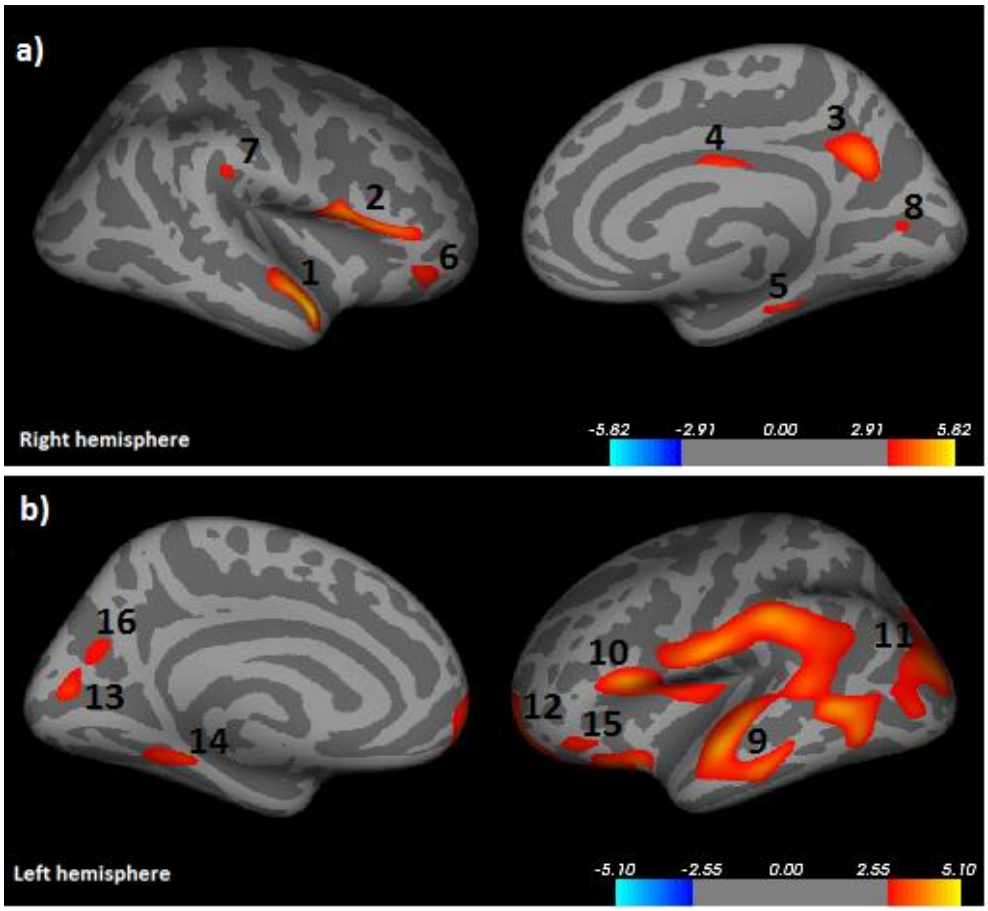


Figure 8.3 Cortical surface maps illustrating cortical perfusion group difference between MS and healthy controls in the right (a) and left (b) hemisphere, p <0.05 (FDR-corrected).

Table 8.2 Vertex-wise clusters of significant difference in cortical perfusion between MS and healthy controls.

Cluster	Size(mm²)	Tal X	Tal Y	Tal Z	Anatomy
Right hemisphere					
1	581.26	51.2	5.6	-21.1	Superior temporal gyrus
2	808.82	52.4	11.4	5.4	Parsopercularis
3	436.52	5.4	-58.7	28.3	Precuneus
4	67.79	3.6	-9.5	29.4	Posterior cingulate
5	110.12	34.7	-29.8	-17.7	Parahippocampal
6	218.70	41.6	38.8	-12.1	Parsorbitalis
7	65.98	50.7	-31.0	29.8	Supramarginal gyrus
8	47.06	16.7	-75.6	9.1	Perialcarine
Left hemisphere					
9	6038.84	-51.5	-12.3	-12.2	Superior temporal gyrus
10	1240.77	-48.9	16.9	11.4	Parsopercularis
11	1626.46	-31.9	-88.9	12.5	Lateral occipital
12	2089.05	-19.5	58.2	-12.4	Rostral middle frontal
13	242.76	-10.1	-79.3	12.4	Pericalcarine
14	276.38	-26.6	-39.0	-12.4	Parahipocampal
15	173.00	-40.6	33.1	-12.8	Parsorbitalis
16	131.84	-20.0	-65.8	25.0	Precuneus

8.4.3 Group comparison of Basal ganglia perfusion between MS patients and controls

A significant difference in perfusion between MS and healthy controls was demonstrated in the basal ganglia (p=0.001), with lower CBF identified in MS patients (42.9±11.5 ml/100g/min) compared to healthy controls (59.91± 20.4 ml/100g/min) (Figure 8.4).

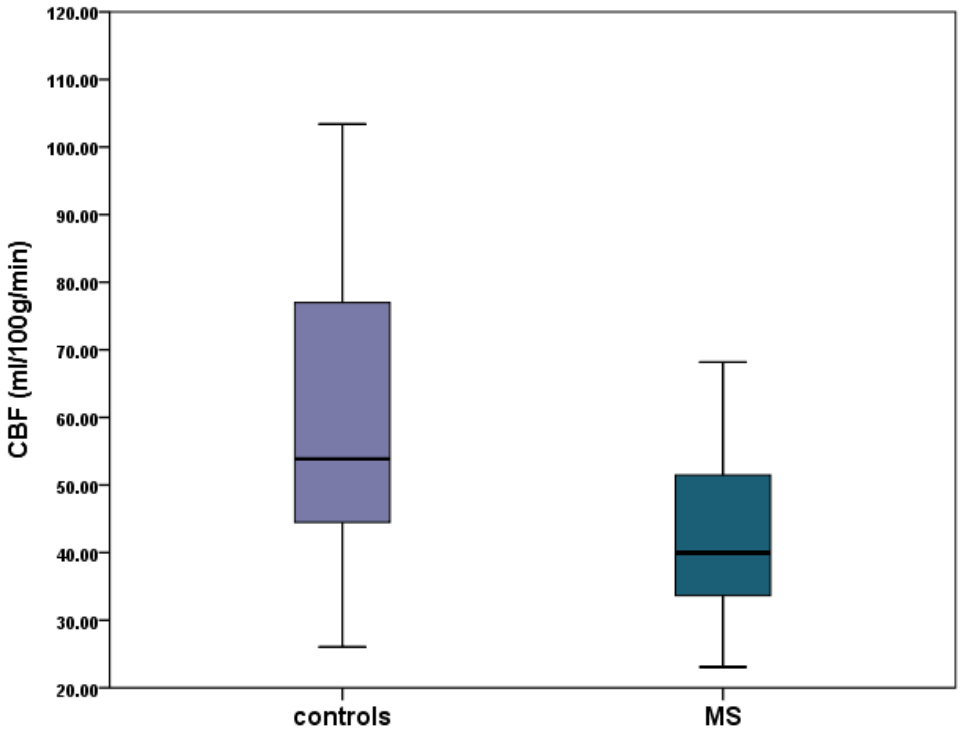


Figure 8.4 CBF comparison revealed significant difference in basal ganglia between MS and controls (p=0.001).

8.4.4 Group comparison of cortical thickness between MS patients and controls

Surface based analysis demonstrated no group differences in cortical thickness between MS and healthy controls.

8.4.5 Correlation between regional cortical perfusion in MS and cortical thickness

There were no statistical significant correlations demonstrated between cortical perfusion and cortical thickness (expressed as the cortical thickness Z-score) in the two selected test ROIs the superior temporal cluster of the left hemisphere (r=0.028, p=0.89) and the precuneus area of the right hemisphere (r=0.13, p=0.53), (Figure 8.5).

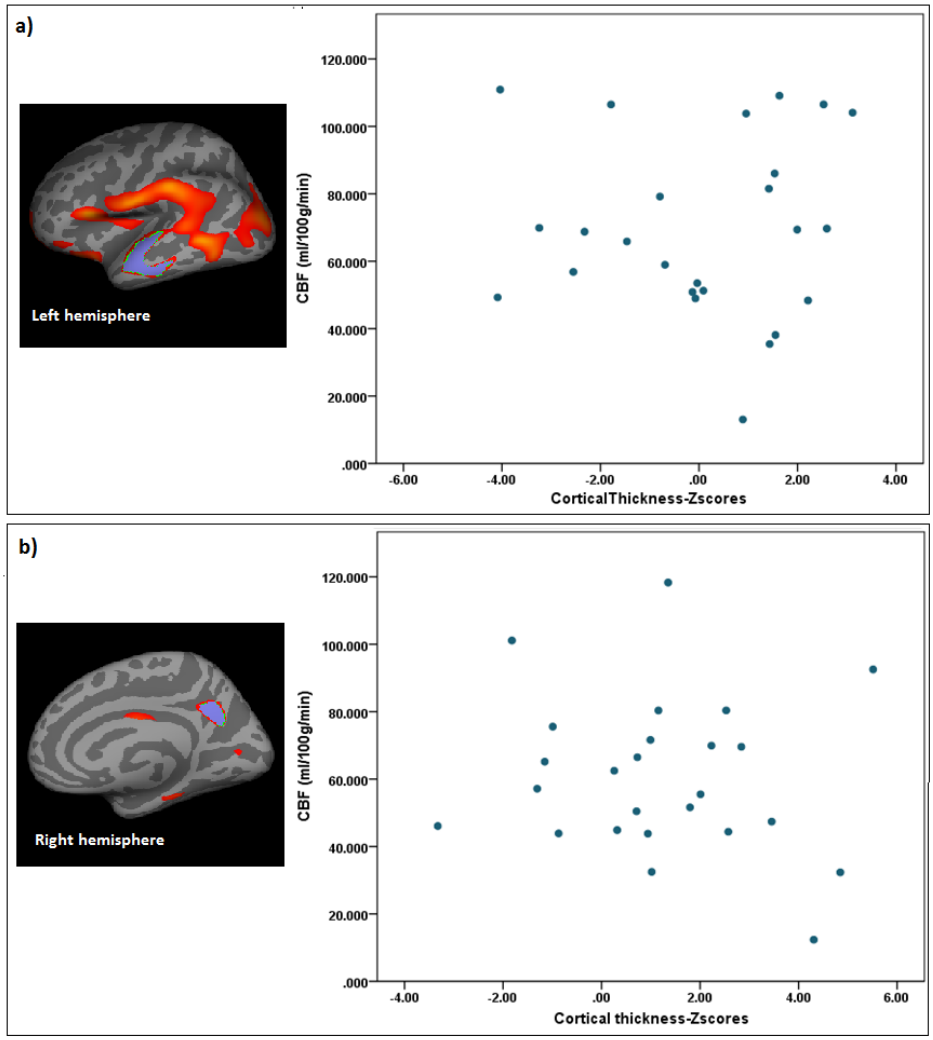


Figure 8.5 Scatter plot illustrating the correlation between regional cortical perfusion in MS and cortical atrophy
No correlation demonstrated between cortical thickness-Z scores and cortical CBF in a) Superior temporal ROI of left hemisphere and b) Precuneus ROI of right hemisphere.

8.4.6 Correlation between regional cortical perfusion in MS and regional cortical MD

The correlation involved 24 MS patients, as one MS patient was excluded due to persistent error occurring during the registration process of MD maps. There was no statistically significant correlation between cortical perfusion and cortical MD in the superior temporal cluster of the left hemisphere (r=0.31, p=0.13) and the precuneus area of the right hemisphere (r=0.18, p =0.39) (Figure 8.6).

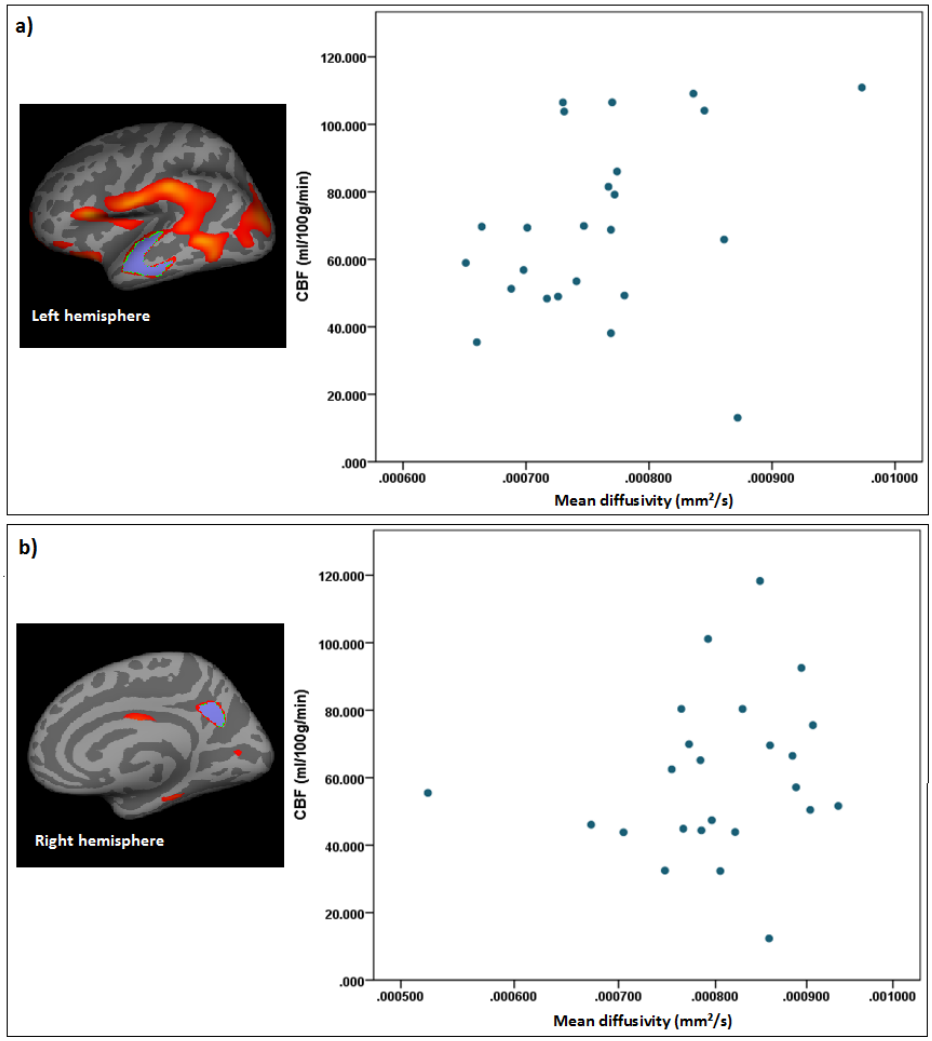


Figure 8.6 Scatter plot illustrating the correlation between regional cortical perfusion in MS and cortical diffusion
No correlation demonstrated between cortical CBF and cortical diffusion in a) Superior temporal ROI of left hemisphere and b) Precuneus ROI of right hemisphere.

8.4.7 Correlation between cortical perfusion in MS and functional /Neuropsychological scores

There was no vertexwise correlation demonstrated between cortical perfusion and MoCA, MSFC Z scores, PASAT Z scores and 9HPT. However, positive correlation was observed between cortical perfusion and T25FW Z-scores ($p<0.01$, uncorrected) controlled for age in both hemispheres (Figure 8.7). Low T25FW Z-scores associated with low cortical perfusion were detected in the right hemisphere in the Precuneus, Pars-triangularis, Parsorbitalis, Supramarginal and Rostral middle frontal areas. In the left hemisphere the correlation was demonstrated in Supramarginal gyrus and Precuneus. However, these correlations did not survive FDR multiple test correction.

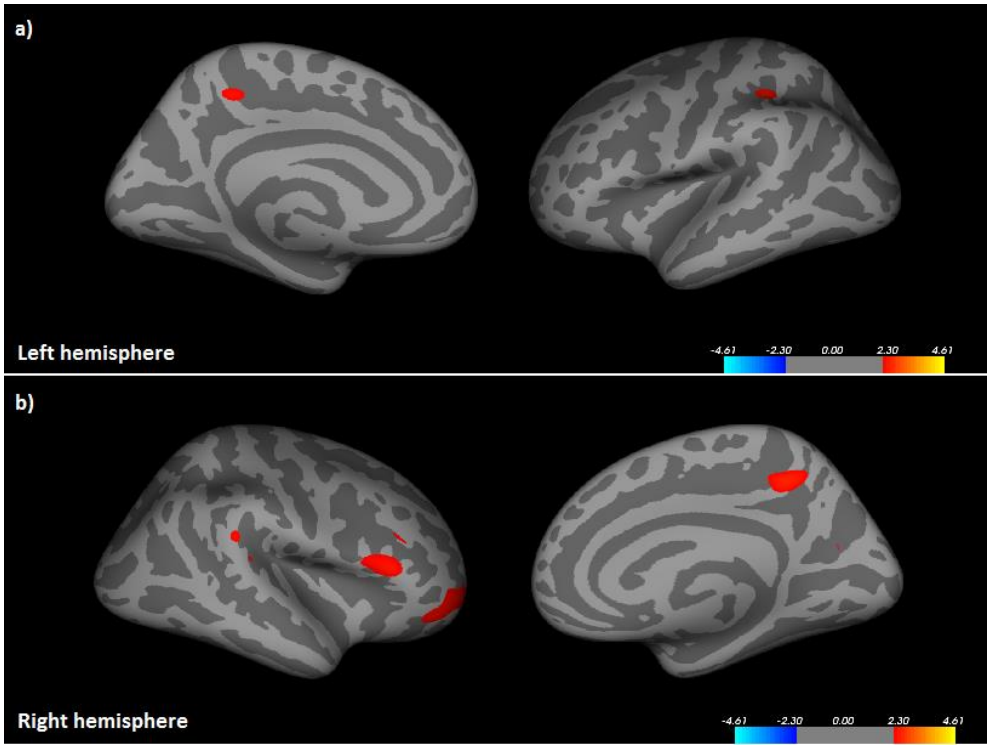


Figure 8.7 a) , b) Cortical surface maps illustrating correlation between T25FW Z- scores and cortical perfusion in MS patients, p <0.01 (uncorrected and controlled for age).

Furthermore, negative correlation was observed between cortical perfusion and BDI-II scores ($p<0.01$, uncorrected) controlled for age in right hemisphere (Figure 8.8). High BDI-II scores associated with low cortical perfusion were detected in the right hemisphere in the Caudal anterior cingulate, Medial orbitofrontal and Posterior cingulate. However, these correlations did not survive FDR multiple test correction.

There were no correlations between cortical perfusion and NFI-MS scores.

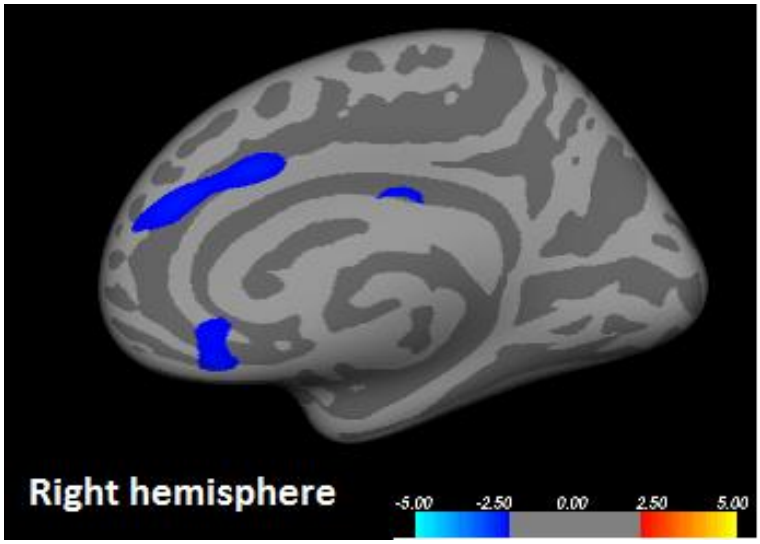


Figure 8.8 Cortical surface maps illustrating correlation between BDI-II scores and cortical perfusion in MS patients, p <0.01 (uncorrected and controlled for age).

8.4.8 Correlation between basal ganglia perfusion in MS and functional /Neuropsychological scores

There were no statistical significant correlations between basal ganglia perfusion and neuropsychological/functional scores (NFI-MS, BDI-II, MOCA, EDSS, MSFC Z scores, PASAT Z scores and T25FW Z scores and 9HPT), as illustrated in (Table 8.3)

Table 8.3 Demonstrates correlations between basal ganglia perfusion and (Neuropsychological/ functional scores) in MS

BG perfusion	Spearman correlation	p value
EDSS	0.109	0.59
MSFC Z score	-0.245	0.22
9HPT	-0.031	0.88
T25FW Z score	-0.264	0.19
PASAT Z score	-0.356	0.07
MOCA	-0.101	0.62
BDI-II	-0.167	0.41
NFI-MS	0.096	0.63

8.5 Discussion

This study explored the structural and functional relevance of cortical perfusion alterations in MS, using a surface based analysis technique. In addition, perfusion in basal ganglia was investigated in both groups, and correlation with functional and neuropsychological scores was performed.

The bilateral cortical surface maps demonstrate clusters of lower perfusion in MS patients, and furthermore hypoperfusion was reported in the basal ganglia compared to healthy controls, These findings regarding cortical and basal ganglia hypoperfusion in MS is in agreement with previous studies (Rashid et al. 2004; Debernard et al. 2014).

The relationship between cortical hypoperfusion and two measures of cortical damage (cortical atrophy and cortical MD) was assessed using an ROI analysis. There was no correlation illustrated between cortical hypoperfusion and cortical atrophy in MS in the two ROIs tested. To quantify cortical atrophy in the selected ROIs, the cortical thickness Z-score was calculated for the MS patients using the mean and standard deviation of cortical thickness in the control cohort. This was felt to provide a better measure of atrophy than using cortical thickness alone, which would not account for the normal regional variation in cortical thickness. Since MD is a well known index to measure microstructural changes in brain tissue the association between hypoperfusion and cortical MD was explored. However, no correlation between the CBF reduction and cortical MD in areas of cortical hypoperfusion (namely superior temporal of left hemisphere / precuneus of right hemisphere) was found.

Previous studies found that CBF reduction in MS is mediated by elevation of endothelin 1(ET-1) levels (D'haeseleer et al. 2015), which is a strong vasoconstrictor found in the CSF and peripheral venous circulation of MS patients (Speciale et al. 2000; Haufschild et al. 2001). The cause of high level of ET-1 in MS is still vague (D'haeseleer et al. 2013). D' Haeseleer and colleagues found around 20 % reduction in CBF measured by ASL in MS compared to controls, in centrum semiovale NAWM, frontoparietal cortex and cerebellum; interestingly, this CBF reduction in MS elevated significantly after ET-1 receptor antagonist was administered (D'haeseleer et al. 2013).

A previous study correlated normal appearing corpus callosum hypoperfusion with DTI measures (MD and FA) in MS, and they found a significant correlation between hypoperfusion and MD reduction (Saindane et al. 2007). The authors of this paper argue that the finding of hypoperfusion in the absence of MS related MD elevation indicate that the hypoperfusion is a primary phenomenon. Furthermore, Debernard and colleagues studied GM perfusion in early RRMS prior to GM atrophy using ASL, and their findings revealed reduced cortical perfusion without volume loss (Debernard et al. 2014).

These studies suggest that hypoperfusion in MS is an early phenomenon that occurs prior to axonal damage. This may possibly explain the findings in this chapter, which showed no correlation between hypoperfusion and cortical atrophy/MD.

The surface based analysis demonstrates no vertex wise correlation revealed between cortical perfusion and motor, cognitive and neuropsychiatric measures. Also, there was no correlation detected between perfusion in the basal ganglia and functional scores. This could be due to small sample size,

which is not sufficient to detect the correlation between CBF and functional scores. Another reason is that patients in this study may not have severe disability or cognitive impairment to detect such a significant correlation.

However, with more lenient threshold of $p<0.01$ (uncorrected for multiple comparisons), non-significant clusters was observed when correlated T25FW Z-scores with cortical perfusion, it showed positive correlation between cortical perfusion and scores where a lower value indicates worse motor performance. Bilateral clusters were identified in the Precuneus, Pars-triangularis, Parsorbitalis, Supramarginal and Rostral middle frontal areas; Precuneus would be expected to have a role in motor coordination (Wenderoth et al. 2005) and visuospatial function, hence potentially relevant for T25FW performance.

Furthermore, non-significant negative correlation between cortical CBF and depression was detected; it indicates that higher scores are associated with lower cortical CBF. Surface correlation maps illustrate regions of lower perfusion which relates to worse depression scores in the anterior cingulate gyrus and sulcus, an area which is known to be associated with depression. Previous imaging studies found reduction in perfusion in ACC in patients with depression (Mayberg et al. 1994; Ito et al. 1996).

Using a surface based analysis technique provides better intersubject registration which delivers best matching of corresponding cortical region (Fischl et al. 1999a).

To the best of my knowledge this is the first study that explores the structural and functional relevance of cortical perfusion alterations in multiple sclerosis,

using surface based analysis. Furthermore, the patients included in this study were not on any disease modifying therapy, since most of the previous perfusion studies included patients under treatment, which might have an effect on the haemodynamics of the brain.

Although some of the results in this study did not survive correction for multiple comparisons, however they show valuable information which can open avenues for future studies with larger sample size, to explore the functional relevance of cortical perfusion using surface based approach, and provide more understanding of cortical perfusion in different types of MS at different stages of the disease. One of the limitations of this study was the limited field of view of DCE-MRI, for that reason, global cortical perfusion could not be measured. Therefore, more studies are needed to explore global cortical perfusion using surface based analysis.

Chapter 9 Structural and functional relevance of cortical diffusion alterations in multiple sclerosis

9.1 Introduction

Functional performance in multiple sclerosis (MS) has been partially explained by several neuroimaging measures such as normal appearing white matter (NAWM) damage, white matter lesion volume, cerebral atrophy and spinal cord involvement (Ge 2006).

Few imaging studies have investigated the cortex in MS, using different methods such as measuring changes in the grey matter volume (Chard et al. 2002; Sailer et al. 2003) and magnetisation transfer (MT) to investigate the GM in primary progressive MS (Dehmeshki et al. 2003; Sailer et al. 2003).

Recent studies have focused on the influence of cortical pathology to functional status in MS; they have shown that cortical pathology has a greater association to clinical disability than whole brain atrophy, NAWM damage and white matter lesion volume (Geurts et al. 2012; Filippi et al. 2013). Good visualisations of focal cortical lesions are provided through various *in vivo* magnetic resonance imaging (MRI) modalities such as ultrahigh field T1-weighted acquisitions (Tallantyre et al. 2010) and double inversion recovery (DIR) (Geurts et al. 2005; Calabrese et al. 2007). In addition to cortical lesion identification, information on cortical atrophy and cortical myelin content has been provided via quantitative measures such as cortical volumetry / morphometry (De Stefano et al. 2003; Sailer et al. 2003; Amato et al. 2004)

and post-mortem MT imaging (Schmierer et al. 2010; Chen et al. 2013), respectively.

Diffusion tensor imaging (DTI) parameters such as mean diffusivity (MD) and fractional anisotropy (FA) have the ability to detect microstructural changes in white matter integrity (including NAWM). Therefore many studies have used DTI to explore the correlation of white matter pathology in MS and its relationship with cognitive and clinical status (Filippi et al. 2001b; Dineen et al. 2009; Roosendaal et al. 2009; Welton et al. 2015). Nevertheless, relatively few studies have used diffusion metrics to study cortical pathology in MS.

A longitudinal study held by Calabrese et al. (2011) investigated the relationship between cortical lesion load and cortical DTI in relapsing remitting MS (RRMS) patients compared to controls. They found that baseline MD, which quantifies the orientationally-averaged water displacement (Pierpaoli et al. 1996), was raised in the cortical lesions compared to normal appearing cortex. Additionally, over the 3-years interval there were no changes in the MD parameters in control subjects, while MD values were higher in normal appearing cortex in RRMS patients whose EDSS scores worsened (Calabrese et al. 2011). In another study, an elevated cortical MD was observed in MS patients compared to controls using a region-of-interest (ROI) approach (Vrenken et al. 2006). Notably, the appearances of the cortical MD histogram in relapsing remitting-MS were found to correlate with clinical disability (Cercignani et al. 2001) Moreover, high MD and low FA were reported in extra-lesional cortex in MS patients, and a correlation was demonstrated between these measures and clinical disability (Yaldizli et al. 2015).

One concern is that diffusion values at the cortex are susceptible to partial volume (PV) effects that arise from within-voxel averaging of the signals from cerebrospinal fluid and grey matter which in turn may lead to overestimation of mean diffusivity measures (Alexander et al. 2001; Koo et al. 2009). Therefore, to avoid partial volume errors (PVE) from CSF contamination, a partial volume correction (PVC) should be applied to attain an impartial cortical MD measure (Jeon et al. 2012), although these have not been applied in most MS studies to date.

Interestingly, the cortical thickness can be evaluated from MRI images using surface based methods (Clarkson et al. 2011), in which a triangulated mesh is created on both pial and white matter borders, followed by distortion to detect the contrasting borders. Sailer and colleagues have investigated cortical thickness in multiple sclerosis, using a surface based technique. It is considered an achievable method to study the cortical pathology in MS, and correlate it with the clinical status and disease progression (Sailer et al. 2003). None of the previous studies have used a vertex-wise approach to study functional relevance of MD changes in the whole cortex.

9.2 Hypothesis and aim

Hypothesis:

- That patterns of regional cortical MD variability will explain performance in different functional domains in MS.
- That cortical thickness correlates inversely with cortical MD in MS.

The aim of this study is to test the first hypothesis using cortical surface based analysis of partial volume corrected MD for a cohort of MS patients, with vertex-wise correlation between MD and functional performance in components of the MS functional composite (MSFC) (Fischer et al. 1999). Moreover, to investigate whether cortical thickness correlates with cortical diffusion in MS, via vertex-wise correlation.

9.3 Methods

9.3.1 Subject recruitment

The imaging and functional/cognitive data used in this chapter were from a previous study (Dineen et al. 2009; Dineen et al. 2012). Therefore, all the recruitment, data acquisition, cognitive and functional assessment was performed by Dr. Robert Dineen. Forty patients with clinically definite MS (37 RRMS, 3 SPMS) were included. The MS participants met the following inclusion criteria: i) age less than 60 years. ii) Diagnosed with MS. iii) In remission stage. The exclusion criteria: i) Doubt about MS diagnosis. ii) Age less than 18 years. iii) Acute relapses of MS in the previous 6 weeks. iv) Evidence of current infection. v) Other neurological conditions. vi) MRI contraindication or claustrophobia. vii) MS therapy trial or immunomodulatory therapy in the previous 2 months. viii) Pregnancy. ix) Unable to consent. A neurological examination including estimation of the expanded disability status scale (EDSS) (Kurtzke 1983) was performed on all participants.

9.3.2 Functional assessment

All participants underwent the three components of the MSFC (the 9 hole peg test (9HPT), Timed 25-Foot walk (T25FW) and Paced Auditory Serial Addition Test (PASAT)) according to the standard administration (Fischer et al. 2001) MSFC Z-scores were calculated using the equation given in the MSFC manual based on normative data; hence, the lower the score, the poorer the functional performance. Results from the 9HPT and T25FW, which measure arm function / fine motor control and lower extremity function /

ambulation respectively, are expressed in seconds; hence, the higher the score the poorer the performance. For 9HPT, both left and right hands were assessed. The average 9HPT for the right and left hands were calculated. For PASAT, a measure of processing speed, working memory and sustained attention, the number of correct responses was recorded; hence, the lower the score the poorer the performance. The PASAT Z-score was calculated from normative data to adjust for education level (Rao et al. 1991). In addition to the PASAT, participants underwent testing of two other cognitive domains: the Benton Visual Retention Test (BVRT), which is a test used to assess visuoconstructive ability and visual memory (Benton 1974), and the California Verbal Learning Test 2nd edition (CVLT-II), which is used to assess recall and verbal learning (Delis et al. 2000). For BVRT, Z- score was used in the analysis. For CVLT-II (short delay free recall) sub-score was included and presented as Z-score in the analysis. For both scores, lower scores indicate poorer performance.

9.3.3 MRI protocol

All participants had MRI safety assessment prior to the scan. All images were acquired using Philips Achieva (Philips, Eindhoven, NL) at 3 tesla.

The MRI protocol comprised the following sequences:

- DTI (voxel size 2 × 2 × 2.5 mm voxels, TR = 9700 ms, TE = 56 ms, b = 1000 mm2/s, 15 directions, 45 slices)
- MPRAGE (FOV = 205, Matrix 256 × 256, Flip angle = 8, voxel size = 0.8 × 0.8 × 0.8 mm, TR = 7.5, TE = 2.2).

9.3.4 Image analysis

Diffusion tensor imaging data were analysed using FSL version 3.3 (Jenkinson et al. 2012). Subsequent eddy current correction was performed and non-brain voxels were removed. MD maps were generated using the FSL Diffusion Toolbox (FDT). These MD maps were then PV corrected using the approach of Koo et al (Koo et al. 2009). Before applying the PV correction, MD maps and the b0 image were registered to MNI 152 standard space T1 weighted using FLIRT linear registration (Jenkinson and Smith 2001; Jenkinson et al. 2002) via FSL. Then, the correction was performed by calculating tissue probability maps for CSF and grey matter (GM) from registered b0 images, which were calculated using Statistical parametric mapping (SPM8) software (http://www.fil.ion.ucl.ac.uk/spm/software/spm8/). The remainder of the PV correction steps were locally implemented using in house Matlab code (Matlab Version 7.12.0.635, R2011a) based on (Koo et al. 2009) written by Dr.Yue Xing, (research fellow, Division of Clinical Neuroscience, University of Nottingham). The tissue probability maps were used to estimate the GM and CSF apparent signal fraction weightings (λapp) from the reference b0 scans (Pasternak et al. 2009). These were then used to estimate MD-GM using the measured MD maps and modelling MD-CSF based on each individual eroded CSF mask (Figure 9.1).

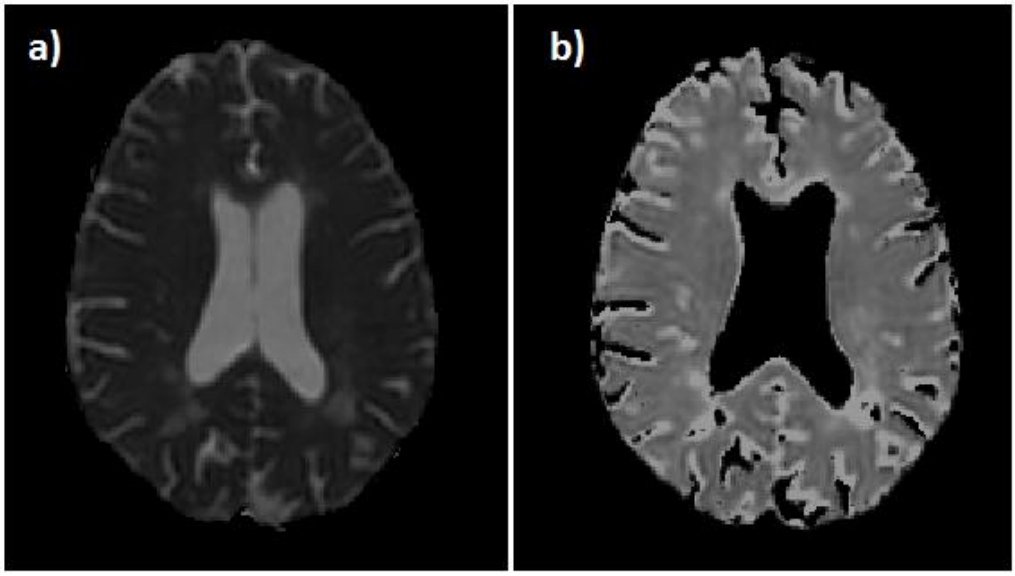


Figure 9.1 a) Illustrates MD map for MS patient. b) Illustrates partial volume corrected MD map

Cortical surface-based analysis was performed on MPRAGE data using the FreeSurfer software package, linux-V 5.1.0 (http://surfer.nmr.mgh.harvard.edu) for surface segmentation and reconstruction. Manual correction of the cortical surface for subcortical lesions was achieved (Dale et al. 1999; Fischl et al. 1999b). The PV-corrected MD maps were registered (with manual correction if necessary) via the *bbregister* and *tkregister2* programs provided by FreeSurfer. The PV-corrected MD maps were projected on the cortical surface using the *mri_vol2surf* tool with a projection fraction of 0.5, which samples the MD data in to the voxels that is halfway through the cortical ribbon. A multi-intersubject topography-based co-registration was performed in spherical surface space implemented in FreeSurfer (Fischl et al. 1999a)

9.3.5 Statistical analysis

Statistical analysis was performed using SPSS statistics (IBM SPSS Statistics for Windows, Version 21.0. Armonk, NY: IBM Corp). Descriptive analysis was performed to measure the means and standard deviation for MS patients.

The surface group statistical analysis was performed using the QDEC application in FreeSurfer to correlate surface based cortical diffusion with MSFC Z scores, 9HPT, T25FW, PASAT Z scores, BVRT Z scores and CVLT-II Z scores. Correction for multiple comparisons was performed using false discovery rate (FDR) tool implanted in QDEC. Significance was set at $p < 0.05$ (FDR-Corrected). For tests where no significant clusters remained at the $p < 0.05$ (FDR-Corrected), the data was further explored using a more lenient threshold of $p<0.01$ (uncorrected for multiple comparisons). Anatomical naming of clusters reaching significance as presented in the results was facilitated by use of the aparc.annot cortical atlas that is distributed with FreeSurfer (Desikan et al. 2006).

9.4 Results

9.4.1 Descriptive analysis

As mentioned above in the methods section the analysis involved 40 MS patients (37 RR-MS and 3SPMS). Demographic and clinical variables for the MS patients are given in (Table 9.1).

Table 9.1 Demographic and clinical variables of multiple sclerosis patients.

	MS (N=40)
Age (Year)	43.79 ± 8.09
Gender (M:F)	11:29
MS type (RR:SPMS)	37:3
Disease-duration(Median)	10 IQR (7.2-14.12)
EDSS (Median)	3 IQR (2.5-4)
MSFC Z scores (Mean±SD)	0.039 ± 0.56
9HPT (Median)	21.6 IQR (19-24.7)
T25FW (Median)	7 IQR(5.7-9.7)
PASAT Z scores (Median)	-0.45 IQR (-1.6-0.57)
BVRT Z scores (Mean±SD)	-0.44 ± 1.37
CVLT-II Z scores (Mean±SD)	-0.28 ± 1.22

9.4.2 Correlation between MSFC and PV-corrected cortical MD

There were significant negative correlations between MSFC and PV-corrected cortical MD of the left hemisphere, p <0.05 (corrected), controlled for age (Figure 9.2, Figure 9.3). Lower MSFC Z scores associated with higher cortical MD were noted in the isthmus cingulate. In the right hemisphere the significance was demonstrated in the isthmus cingulate p <0.001, controlled for age. However, this correlation did not survive FDR multiple test correction.

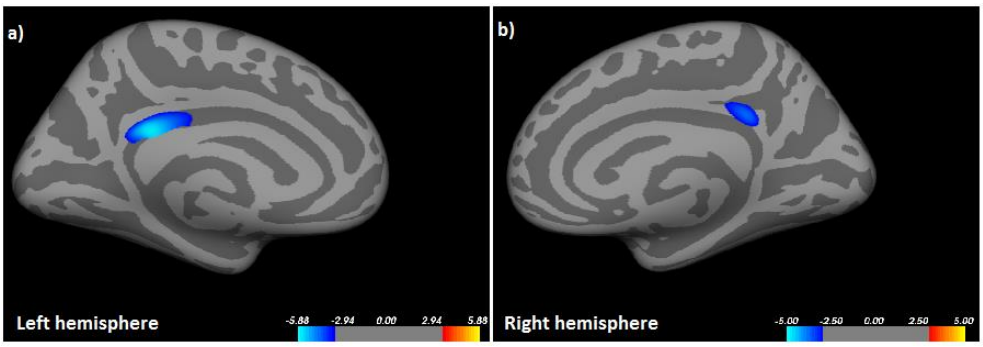


Figure 9.2 Cortical surface maps illustrate the correlation between MSFC scores and PV corrected cortical MD.
a) Significant negative correlation demonstrated in the left hemisphere (p<0.05 corrected, controlled for age) b) Negative correlation demonstrated in the right hemisphere (p<0.001 uncorrected, controlled for age)

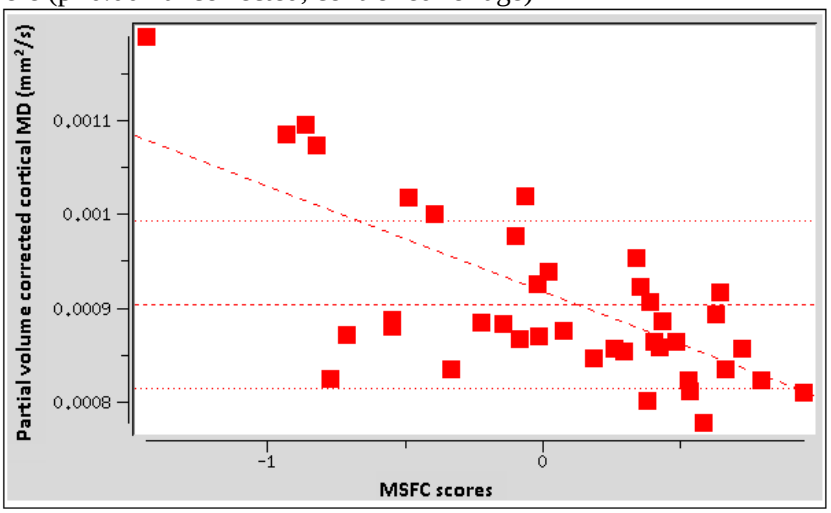


Figure 9.3 Scatter plot demonstrates the significant negative correlation at the Isthmus cingulate between the PV corrected cortical MD of left hemisphere and MSFC Z scores

9.4.3 Correlation between 9HPT and PV-corrected cortical MD

There were significant positive correlations between 9HPT and PV-corrected cortical MD of the left hemisphere, ($p < 0.05$ corrected) controlled for age (Figure 9.4). Higher 9HPT scores associated with higher cortical MD were noticed in the isthmus cingulate. The right hemisphere showed no significant correlation between 9HPT and cortical MD when corrected for multiple comparisons.

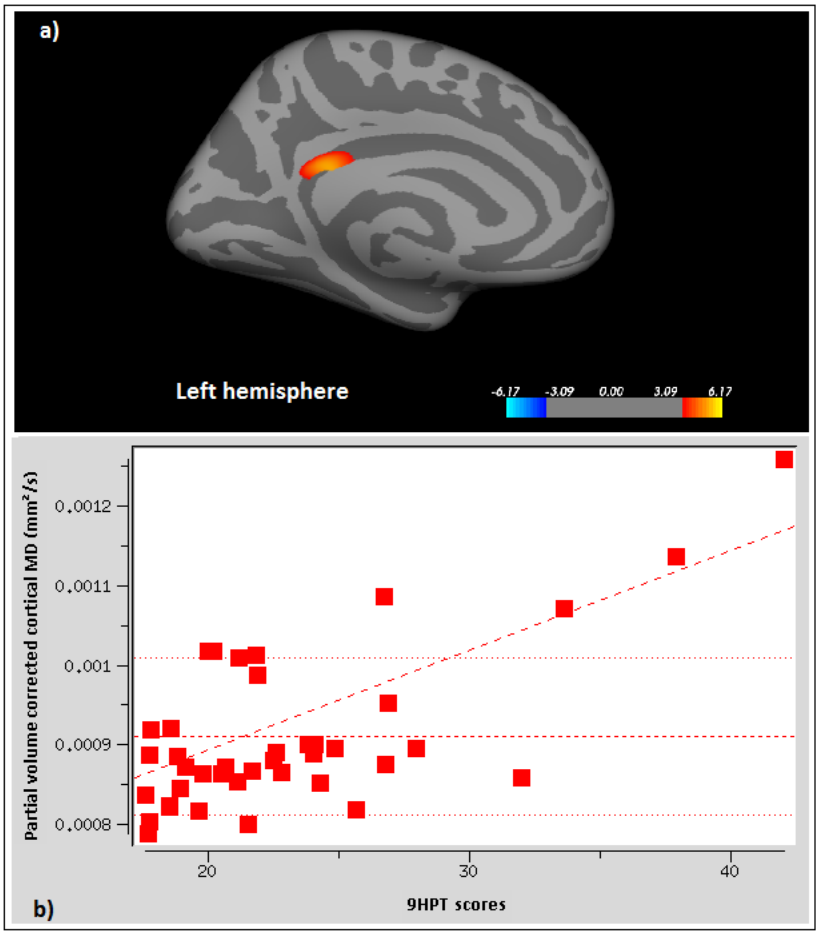


Figure 9.4 Demonstrates the correlation between 9HPT scores and PV corrected cortical MD of the left hemisphere
a) Cortical surface map illustrates a significant positive correlation ($p<0.05$ corrected), controlled for age. b) Scatter plot demonstrates the significant positive correlation at the Isthmus cingulate.

9.4.4 Correlation between T25FW and PV-corrected cortical MD

Significant positive correlations were demonstrated between T25FW scores and PV-corrected cortical MD of left hemispheres ($p < 0.05$ corrected), controlled for age. High T25FW scores associated with high cortical MD were detected in the left hemisphere in the isthmus cingulate (Figure 9.5 a, c). In the right hemisphere the correlation was demonstrated in the isthmus cingulate $p < 0.001$, controlled for age (Figure 9.5 b). However, this correlation did not survive FDR multiple test correction.

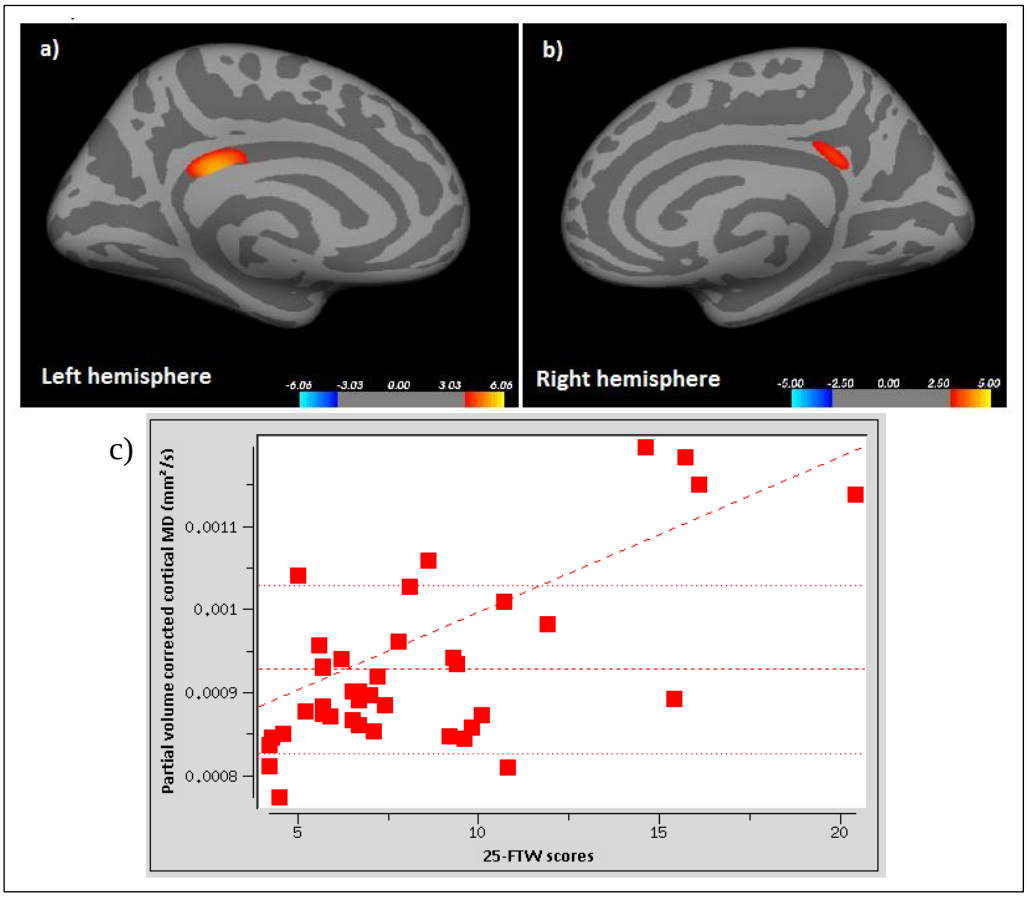


Figure 9.5 Cortical surface maps illustrating correlation between T25FW scores and PV corrected cortical MD.
a) Significant positive correlation was demonstrated in the left hemisphere ($p<0.05$ corrected, controlled for age). b) Positive correlation was demonstrated in the right hemisphere ($p<0.001$ uncorrected, controlled for age). c) Scatter plot demonstrates the significant positive correlation at the Isthmus cingulate between the PV corrected cortical MD of left hemisphere and T25FW scores.

9.4.5 Correlation between PASAT and PV-corrected cortical MD

Negative correlation was found between PASAT Z-scores and PV-corrected cortical MD of the left hemispheres, (p <0.01 uncorrected) and controlled for age, with low PASAT Z-scores associated with high cortical MD detected in the left hemisphere in pericallosal sulcus and anterior transverse collateral sulcus. In the right hemisphere the correlation was demonstrated in the posterior cingulate, isthmus cingulate and fusiform p <0.01, controlled for age. However, these correlations did not survive FDR multiple test correction (Figure 9.6).

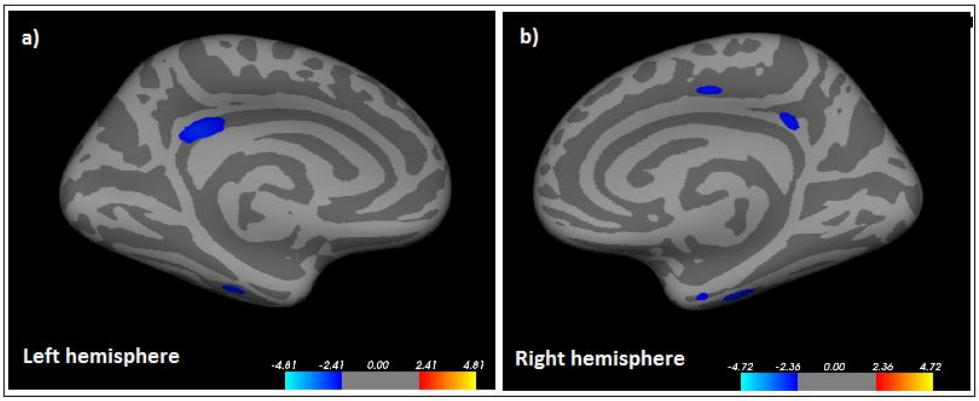


Figure 9.6 Cortical surface maps illustrating correlation between PASAT Z scores and PV corrected cortical MD.
a) Negative correlation was demonstrated in the left hemisphere (P<0.01 uncorrected, controlled for age). b) Negative correlation was demonstrated in the right hemisphere (P<0.01 uncorrected, controlled for age).

9.4.6 Correlation between BVRT and PV-corrected cortical MD

Negative correlation was demonstrated between BVRT Z-scores and PV-corrected cortical MD in the left hemisphere, ($p < 0.005$ uncorrected, controlled for age). Lower BVRT Z-scores associated with higher cortical MD were noticed in the isthmus cingulate and superior frontal areas. In the right hemisphere, significance was demonstrated in the superior frontal, superior temporal, parstriangularis and rostral middle frontal ($p < 0.01$, uncorrected, controlled for age). However, these correlations did not survive FDR multiple test correction (Figure 9.7).

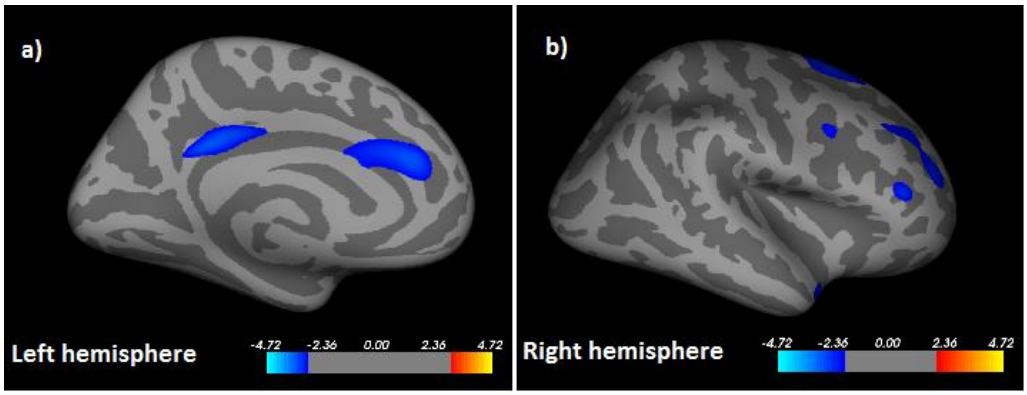


Figure 9.7 Cortical surface maps illustrating correlation between BVRT Z-scores and PV corrected cortical MD
a) Negative correlation was demonstrated in the left hemisphere (P<0.005 uncorrected, controlled for age). b) Negative correlation was demonstrated in the right hemisphere (P<0.01 uncorrected, controlled for age).

9.4.7 Correlation between CVLT-II and PV-corrected cortical MD

Negative correlation was illustrated between CVLT-II Z-scores and PV-corrected cortical MD of both right and left hemispheres, (p <0.005 uncorrected) and controlled for age (Figure 9.8). Low CVLT-II Z-scores associated with high cortical MD were noticed in the left hemisphere , in the following regions: superior temporal, parsopercularis, isthmus cingulate, caudal middlefrontal, postcentral, precuneus, rostral middlefrontal and supramarginal. In the right hemisphere, the correlation clusters are demonstrated in the isthmus cingulate, rostral middlefrontal, paracentral and rostral anterior cingulate. However, these correlations did not survive FDR multiple test correction.

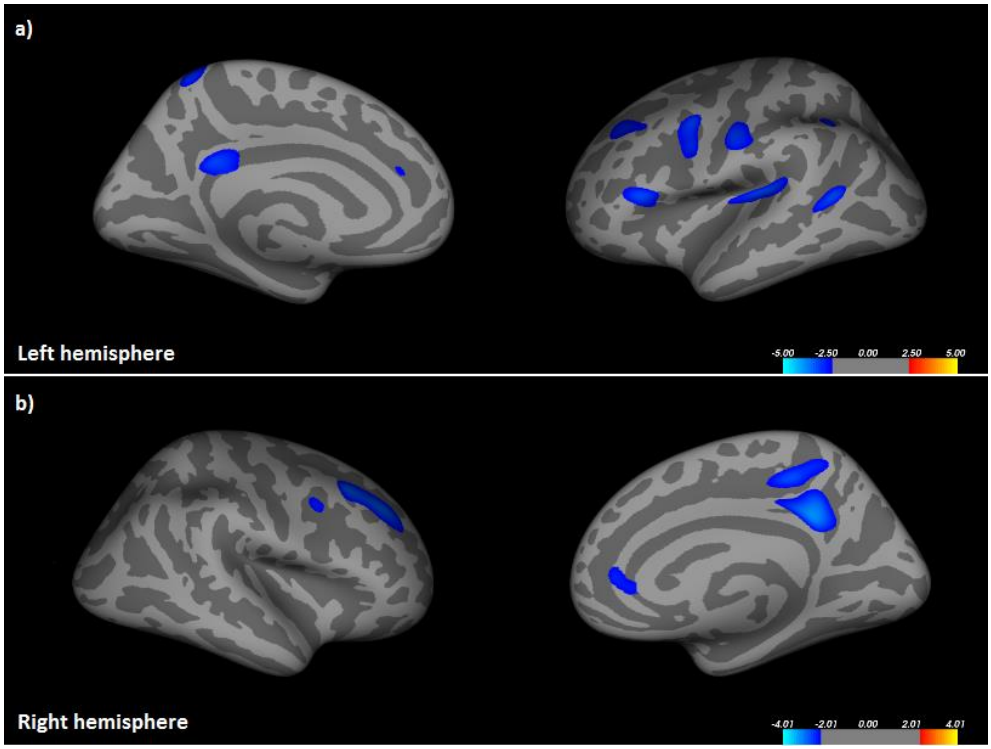


Figure 9.8 Cortical surface maps illustrating correlation between CVLT-II Z-scores and PV corrected cortical MD.
a) and b) Negative correlation was demonstrated in the left and right hemispheres (P<0.005 uncorrected, controlled for age).

9.4.8 Correlation between cortical thickness and PV-corrected cortical MD in MS

A Negative correlation was illustrated between cortical thickness and PV-corrected cortical MD of both right and left hemispheres, ($p < 0.005$ uncorrected) and controlled for age (Figure 9.9). Low cortical thickness associated with high cortical MD were noticed in the left hemisphere, in the following regions, the rostral middle frontal, caudal anterior cingulate, isthmus cingulate, middle temporal and supramarginal gyrus. In the right hemisphere, the correlation was demonstrated in the isthmus cingulate, medial orbito frontal, lingual, precuneus, inferior parietal. However, these correlations did not survive FDR multiple test correction.

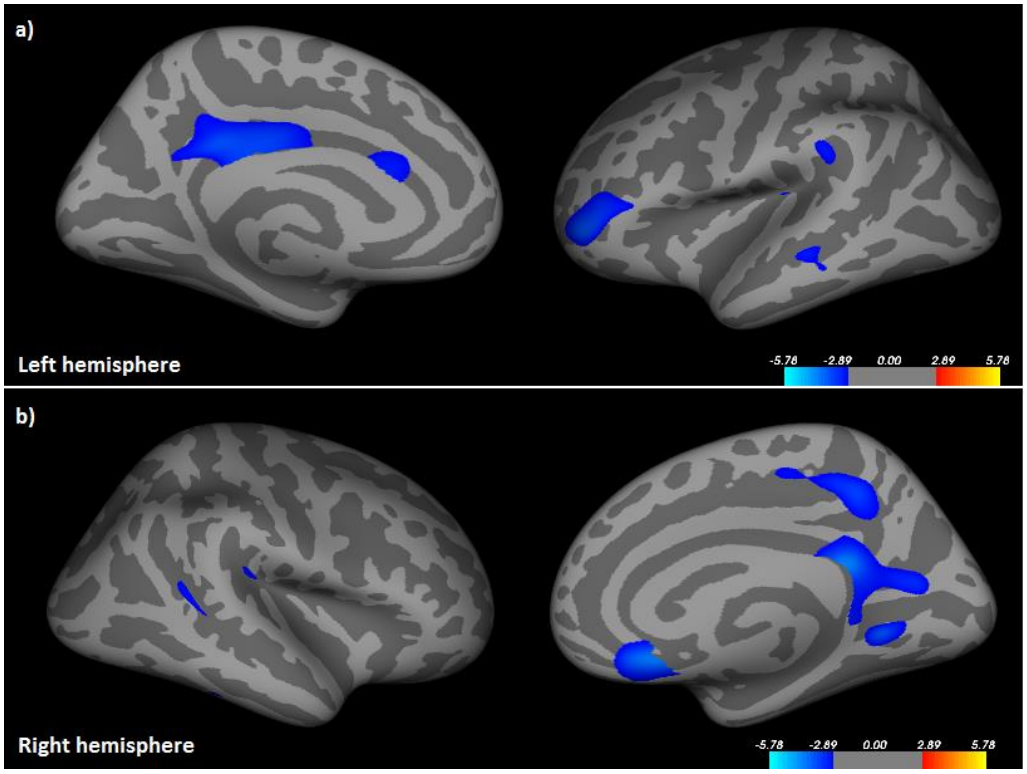


Figure 9.9 Cortical surface maps illustrating correlation between cortical thickness and PV corrected cortical MD.
a) and b) Negative correlation was demonstrated in the left and right hemispheres ($P<0.005$ uncorrected, controlled for age).

9.5 Discussion

This chapter illustrates different patterns of cortical diffusion alteration through the cerebral surface in MS relating to MSFC scores, its component parts (PASAT, T25FW and 9HPT), and other cognitive tests, using surface based analysis. The surface based analysis reveals positive correlations between cortical MD and motor function scores (9HPT and T25FW); this indicates that poor motor function performance is associated with higher cortical MD.

Negative correlations were detected between cognitive scores (PASAT Z scores, BVRT Z scores and CVLT-II Z scores) and cortical MD, however these correlations did not survive the multiple test correction.

The bilateral cortical surface map demonstrates clusters of higher MD correlated with worse 9HPT and T25FW performances in the isthmus cingulate gyrus (Ventral-Posterior cingulate gyrus). The cingulate gyrus is part of the non-primary motor group regions, and hence potentially relevant for motor performance (Picard and Strick 1996; Fink et al. 1997). Additionally, a TBSS meta-analysis study on MS patients, revealed that lower FA is correlated with disability in the posterior cingulate (Welton et al. 2015). Furthermore, part of the posterior cingulate gyrus has connections with primary motor areas (Schiffer et al. 2003). This could explain the correlation found between motor function scores and elevated MD in the cingulate gyrus. Additionally, negative correlation was detected at the isthmus cingulate gyrus between cortical thickness and cortical MD in MS, which indicates that higher MD is associated with cortical thinning. Studies found that the cingulate gyrus is one of the

cortical areas which is more prone to demyelination and cortical thinning (Bø et al. 2003a; Ramasamy et al. 2009).

The cognitive scores in this study were correlated with elevated MD at the posterior cingulate and isthmus cingulate, which are considered to be areas with cognitive roles such as memory and attention (Dineen et al. 2009; Leech and Sharp 2014).

Previous studies investigated the relationship between cortical pathology and cognitive function in MS. A study held by Calabrese et al, found an extensive cortical thinning in different cortical regions in MS patients with cognitive impairment, which include temporal, parietal and frontal regions (Calabrese et al. 2010b). Additionally, they found a significant correlation between frontal lobe atrophy of both hemisphere and PASAT scores.

The relationship between clinical status and cortical diffusion has been studied previously, where a significant correlation was found (Vrenken et al. 2006; Calabrese et al. 2011).

Cortical diffusion elevation was demonstrated during healthy aging, Alzheimer's disease, mild cognitive impairment and Creuzfelt-Jakob Disease (Liu et al. 2006; Ray et al. 2006; Abe et al. 2008; Jeon et al. 2012).

To the best of my knowledge, the surface-based approach that is used in this study to map the functional correlates of cortical MD change has not been applied previously.

Recently there is a large number of imaging studies that explored cortical pathology in MS, using various neuroimaging techniques and correlated with

clinical disability and cognitive impairment. (Filippi et al. 2013). Post-mortem histopathological studies found that elevated MD in the white matter reflects reduction in myelin and axonal density (Schmierer et al. 2007). However, there are only a small number of studies that investigate the histopathological correlation with cortical diffusion in MS. Future studies exploring cortical MD elevation and its association with other cortical pathology such as cortical lesions is required, to provide better understanding of the pathology behind cortical MD alteration in MS.

In this study partial volume effects have been considered, as they could lead to overestimation of cortical MD values due to inclusion of cerebral spinal fluid (CSF) in the cortical MD measurement. Therefore two main steps were performed to reduce this partial volume effect. Since cortical atrophy, which occurs in MS and has clinical and cognitive relevance (Sailer et al. 2003; Tekok-Kilic et al. 2007; Calabrese et al. 2010b), possibly elevates the risk of partial volume artefacts, and such artefacts would be likely to co-locate with areas of most severe cortical atrophy; therefore apparent correlations between elevated MD and worse functional scores could simply reflect cortical atrophy. Firstly, PV correction of MD maps was performed using a previously published method (Koo et al. 2009; Pasternak et al. 2009). Moreover, the PV-corrected MD for each subject was sampled onto the co-registered (and manually checked) cortical ribbon derived from the same subject's MPRAGE data prior to performing the multi-subject topography-based co-registration in spherical surface space implemented in FreeSurfer (Fischl et al. 1999a), intersubject averaging could be minimized and individual gyral anatomy could be preserved.

Using a surface-based analysis technique revealed differential patterns of functionally-relevant regional cortical diffusion elevation between the MSFC and its component scores in MS patients. Also, it revealed the correlation between cortical atrophy and cortical diffusion alteration. Future studies will be needed to establish the relative contribution of cortical MD alteration to functional status compared to other established correlates of functional status in MS, such as damage to cerebral white matter and deep grey matter structures.

Part 3: General Discussion

Chapter 10 General Discussion

10.1 Summary of the results and Implications

The main aim of this thesis was to explore the mechanism behind lesion development, haemodynamic alteration and microstructural changes in multiple sclerosis (MS) and the effect of these pathological changes on clinical manifestations using advanced MRI techniques and advanced image analysis methods.

In this thesis 28 participants with clinically definite MS and clinically isolated syndrome (19 RRMS, 7 SPMS and 2 CIS) patients and 24 age and gender matched healthy controls, were recruited and the data from this cohort was used in chapter 6, 7 and 8. All MS participants were not on any disease modifying treatment (DMT). The majority of the MS cohort demonstrated normal to mild cognitive impairment, according to MOCA test. Furthermore, the range of disability according to EDSSS in patient cohort falls between minimal to moderate disabilities. The imaging and functional/cognitive data used in chapter 9 were from a previous study held by (Dineen et al. 2009; Dineen et al. 2012).

The summaries of results of these studies are as following:

Subtle BBB leakage was demonstrated in regional normal appearing white matter (NAWM) of MS patients compared to healthy controls, which was detected by permeability maps generated from DCE-MRI. This finding is in agreement with previous study by Cramer et al (2014) in which increased

permeability (Ktrans) has been reported in NAWM compared to healthy subjects at the periventricular area. (Cramer et al. 2014). However other studies demonstrated different outcomes where no group differences were demonstrated in NAWM of MS and healthy controls (Lund et al. 2013). This discrepancy in the result might be due to the fact that the MS participants in these studies were on disease modifying treatment (DMT), which could regulate BBB integrity to further impermeable condition (Cramer et al. 2014). Recruiting MS participants who were not on DMT or steroids was challenging in the current study, but was considered to be an essential element of this study as it provided an opportunity to detect BBB alterations in MS in its unmodified state.

Furthermore, hyperintense lesions and diffusely abnormal white matter (DAWM) demonstrated increase in BBB permeability compared to regional NAWM. Previous histopathological studies have revealed BBB alteration in active and chronic lesions (Gay and Esiri 1991; Kirk et al. 2003; Vos et al. 2005), In addition, post-mortem study has shown BBB disruption in DAWM and lesions (Vos et al. 2005), and BBB leakage was demonstrated by Soon et al in most non-enhancing lesions (Soon et al. 2007). These findings are in agreement with findings of this study, which suggests that the extent of BBB dysfunction in both DAWM and lesions is more than in NAWM.

In spite of the low degree of BBB leakage found in NAWM compared to DAWM and lesion, an increase in BBB permeability was reported in NAWM of MS compared to healthy controls. BBB permeability is known to have an essential role in the inflammatory process through facilitating infiltration of immune cells which in turn lead to tissue destruction. Therefore, the current

study has investigated the association between permeability and measures of tissue damage in NAWM, which has not been previously investigated. The findings indicated that BBB alteration is associated with microstructural changes in regional NAWM. The fact that the BBB alteration occurs in the initial phase of lesion development in MS (Lund et al. 2013) it might be one of the influencing factors of microstructural destruction in NAWM. Thus, the suggested association between increase BBB leakage and microstructural damage might provide evidence regarding the degree of tissue damage in NAWM. Moreover, there was no association between MD and Ve as well as between Ve and Ktrans in MS. This may suggest that the relationship between MD and Ktrans is not provoked by an increase in extravascular extracellular volume (Ve). The interpretation of the association between MD and Ktrans, may be due to other pathological mechanisms which may have an influence on these two parameters. Both changes in cellularity and endothelial structure in MS could have an effect on MD and Ktrans, respectively. The subtle structural changes in the NAWM have been detected in this study via MD. Diffusion variation in NAWM might signify axonal loss and extracellular space expansion, which leads to high diffusivity (Evangelou et al. 2000a; Ciccarelli et al. 2003). There was no relationship between MD and Ktrans in healthy controls. This supports that the association found in MS is influenced by pathological mechanisms.

Moreover, the findings revealed that BBB alteration in NAMW were not correlated with functional/neuropsychological scores in MS cohort. Results of earlier studies also failed to identify a relationship between BBB alteration and clinical disability in MS (Lund et al. 2013; Cramer et al. 2014).

In addition, this study found no correlation between microstructural changes and functional/neuropsychological scores. This finding is not in agreement with previous literature, in which a high diffusion measure was associated with clinical disability and cognitive scores in NAWM of MS patients (Ciccarelli et al. 2001; Welton et al. 2015). According to previous meta-analysis study, low fractional anisotropy (FA) was demonstrated in the genu and anterior body of corpus callosum related to cognition, they also indicated that white matter injury is more linked to cognitive impairment than to clinical disability (Welton et al. 2015). The explanation for this discrepancy in the results could be due to the selected NAWM ROI used in the current study, moreover it might be due to the fact that MS cohort involved in this study revealed only mild cognitive impairment, which could have an influence on the results.

The high permeability in prelesion NAWM found in chapter 7, indicates that BBB leakage is an initial event in lesion development. The association between BBB leakage and microstructural changes in regional NAWM has been revealed in chapter 6. However, it was not known whether the tissue damage have occurred before the BBB breakdown or vice versa. Therefore, the results in chapter 7 have shown that subtle alteration of BBB in MS could be detected prior to tissue destruction.

A previous study by Werring et al indicated that inflammatory leakage of BBB is not essentially the commencing step in lesion development, suggesting that BBB leakage might be initiated by tissue integrity changes, since they reported increase in diffusion in prelesion NAWM before BBB alteration. (Werring et al. 2000), however, in their study they did not investigate subtle BBB leakage.

Another possible explanation for the current findings are that the active inflammatory process with infiltration of immune cells might have an effect on diffusion measures leading to reduction in diffusion (Yeh et al. 2016), hence masking any increase in diffusion through other mechanisms. This could explain why increase in diffusion was not detected in prelesion NAWM in chapter 7.

The current findings suggest that using a quantitative MRI measure such as Ktrans to measure permeability is a feasible method to identify subtle BBB changes in NAWM even before microstructural changes and lesion development. Hence, permeability measures might be employed as an indicator to detect BBB leakage, detecting leakage at early stage of lesion formation; this has the potential to guide treatments aimed at preventing development of the lesion, but clearly a large prospective validation study would be required to allow translation of the findings into clinical practice. According to a previous histopathological study, tight junction abnormality has been detected in NAWM, and they suggested that tight junction abnormality should be put into consideration when designing disease-modifying therapy (Plumb et al. 2002; Kirk et al. 2003). Also, more therapeutic studies are required to target the molecules responsible for BBB disruption in MS. Previous studies found that matrix metalloproteinases 9 (MMP-9) levels are high in the CSF of MS patients. MMP 9 has an effect on BBB breakdown in MS (Rosenberg 2012) . It was found that methylprednisolone could decrease the level of MMP 9 in CSF which in turn leads to BBB closure (Rosenberg et al, 1996). Nevertheless, treatment with repeated high dose of steroids might have numerous

complications. Therefore, other BBB breakdown treatment was used to provide more continued BBB alteration reduction in MS, using immunomodulatory treatment such as glatirmer acetate and interferon β (Filippi et al. 2011; Kala et al. 2011). The underlying mechanisms of BBB damage in MS and the effect of molecules leading to this BBB breakdown need to be explored further to provide new therapeutic trials to restore the BBB (Rosenberg 2012).

The study in chapter 8 provides a feasible technique to measure cortical cerebral blood flow in MS, and to the best of my knowledge this is a novel study that uses a surface based analysis approach to explore cortical haemodynamics. It provides an achievable measurement to understand the relation between cortical perfusion and cortical atrophy as well as functional scores in MS participants. In addition, the MS patients in this study were not on any steroids or disease modifying therapy which might have an influence on the cerebral circulation. The surface based analysis revealed cortical hypoperfusion in MS compared to healthy participants when corrected for multiple comparison. Furthermore, hypoperfusion was observed in basal ganglia of MS participants compared to controls. These findings are in agreement with previous imaging studies, in which low cerebral blood flow is identified among MS participants (Rashid et al. 2004; Debernard et al. 2014). The reduction in cortical perfusion noticed in MS was explored further, to understand the aetiology of the cortical perfusion reduction in MS patients. Therefore, cortical cerebral blood flow was correlated with cortical atrophy, and the results demonstrated no association between cortical hypoperfusion and cortical atrophy as well as microstructural changes, which indicated that the cause of cortical hypoperfusion might be due to primary vascular effect

rather than secondary cause due to axonal destruction. A previous study indicated that lower perfusion in MS could be induced by high endothelin-1 levels (D'haeseleer et al. 2015), and in another study hypoperfusion was associated with lower mean diffusivity in corpous callosum (Saindane et al. 2007) Furthermore, grey matter (GM) hypoperfusion was detected in early RRMS before GM atrophy (Debernard et al. 2014). All these findings are in agreement with the findings in the current study.

The vertex wise correlation revealed no correlation between cortical perfusion and functional/neuropsychological scores, when corrected for multiple comparisons. Although, the correlation of lower motor function score and depression with hypoperfusion did not survive multiple comparison correction. A correlation was observed with more lenient threshold ($p<0.01$). Some previous studies demonstrated no association between clinical status and GM hypoperfusion (Debernard et al. 2014). In another study no cortical perfusion difference was observed between non cognitively impaired MS and healthy controls (Hojjat et al. 2016b). Since, MS participants in this current study demonstrated normal to mild cognitive impairment, therefore, this might explain the absent of association between perfusion and cognitive scores.

Using surface based method, could provide an enhanced understanding of the mechanism behind perfusion alteration in the cortex, hence this might have an effect on patient progression and treatment through providing a therapy which regulate cerebral perfusion in MS.

The findings in chapter 9, revealed the different pattern of cortical diffusion alterations through the cortical surface in MS relating to MSFC scores and its

components (PASAT, 12-FTW and 9HPT), and other cognitive tests, using surface based analysis. The vertex wise correlation suggested that poor motor function performance is associated with higher cortical MD at the cingulate gyrus. Although, the correlation of cognitive scores (PASAT, BVRT and CVLT-II) with diffusion did not survive multiple comparison correction. A negative correlation was observed with more lenient threshold ($p<0.01$) in the posterior and isthmus cingulate gyrus, which are areas with cognitive function such as memory and attention (Dineen et al. 2009; Leech and Sharp 2014).

Furthermore, the consideration of partial volume effect of MD maps was an essential step in this study, to avoid overestimation of cortical diffusion. In addition, surface based analysis approach delivers improved intersubject registration and provide an accurate localisation of functional and structural brain features (Fischl et al. 1999a). The cortical diffusion alteration and its relationship with functional scores in MS using surface-based approach considered as a novel study in which has not been applied previously.

10.2 Limitations

Some limitations need to be considered, since it could have an influence on the results.

I acknowledge that healthy control participants have not been assessed for their cognitive and clinical performance. However, all healthy participants in this study did not have any neurological or neurovascular condition or other significant medical condition, which could affect their cognitive and clinical performance.

Most MS participants demonstrated mild to moderate disability and mild cognitive impairment according to EDSS and MOCA, respectively. Also, the BDI scores suggest that majority of MS participants had minimal to mild depression. This might explain why there was no correlation demonstrated between perfusion/ permeability parameter and clinical/ functional performance. A strong correlation might be observable with a cohort with more cognitive impairment and more disabilities.

Some factors could influence the measurement of BBB permeability and perfusion measures using DCE-MRI, such as motion effects during the scan which could occur due to patient movement or slight physiological movement. Therefore, prior acquiring the DCE-MRI sequence it was made sure that all participants were in a comfortable position, and they were reminded to keep still as much as possible during the sequence. Moreover, during the post processing analysis to generate perfusion and permeability maps, a threshold was set to avoid processing of pixels in the background noise.

Haematocrit value to calculate plasma concentration is required for permeability and perfusion analysis, and to achieve the ideal estimation of haematocrit, it need to be measured in each subject via blood sample, and this was not possible in this study. Therefore, a haematocrit of 0.45 was assumed as a standard value used in most pharmacokinetic models from previous studies (Sourbron and Buckley 2013; Heye et al. 2014).

Another methodological limitation was the limited field of view of DCE-MRI; therefore full brain coverage to measure cortical perfusion using surface based analysis was not possible.

The imaging and functional/cognitive data used in chapter 9 were from a previous study, as mentioned in section 10.1. Since, the diffusion data for the cohort used in chapter 6,7 and 8 has a small sample size. Furthermore, the spread of cognitive tests included in the second data set were more, since it includes (PASAT, CVLT and BVRT). However, an attempt of combining both data sets from the two different studies group was performed, however, the two mean diffusivity histogram for the two data sets demonstrated significant differences and on this basis it was felt that it would not be appropriate to combine the data.

T1 weighted images, deliver the greatest contrast for cortical reconstruction. However, cortical reconstructions for MS participants are susceptible to error. Since it might include subcortical white matter lesions in the cortical ribbon, as T1 lesions in MS are hypointense, therefore the software will assume that it's cortical grey matter due to the same contrast. Therefore, all the reconstructed cortices were assessed for their accuracy and manual correction was performed

to exclude these subcortical lesions from the cortical ribbon, and re analysis was performed.

In chapter 8 and 9 cortical lesions were not excluded from the cortex and measuring perfusion and diffusion in cortical normal appearing grey matter (NAGM) was not achievable, since the cortical lesions were not visualised on T2 FLAIR images and T1 weighted images which were acquired in these studies. Therefore, cortical lesion segmentation was not possible.

10.3 Future work

This research provides a better understanding about subtle changes in BBB integrity in NAWM in MS. It also provides insights to the mechanism behind lesion development in NAWM, which has been a point of concern for many researchers. Haemodynamic alteration and microstructural changes in grey matter, using different advanced MRI and advanced imaging analysis techniques, may further expand the knowledge into MS pathology.

This study explored the BBB integrity in NAWM in MS and it is role on lesion development and progression. However, a large prospective study would be required to investigate the value of permeability imaging for predicting lesion development in MS. The mechanism behind cortical lesion development is still not established. Therefore, future studies are required to study BBB integrity in NAGM and investigate cortical lesion development. In this study there was no correlation demonstrated between BBB permeability and cognitive/ clinical disability since MS participants have mild cognitive impairment, In the future, studies with cognitively impaired MS cohort is desirable to provide more information regarding this association, and to explore it further.

The surface based analysis approach was used to investigate cortical perfusion in MS, as mentioned above in the limitation section this study did not explore the cortical NAGM, since it was not possible to visualise cortical lesions on either FLAIR or T1 weighted images. Therefore, it requires further studies to explore the perfusion in cortical NAGM using surface base approach.

This could be achievable using MRI techniques such as double inversion recovery or 7 T magnetization transfer.

Even though, cortical perfusion correlation with cognitive and functional scores did not survive multiple comparison correction, nevertheless they demonstrate valuable information which can be used as a base for future studies with larger sample size, to determine the functional relevance of cortical perfusion using a surface based approach, and provide a further knowledge of cortical perfusion in different types of MS at different stages of the disease.

The findings in this study illustrated no correlation between cortical hypoperfusion and cortical atrophy; previous studies have shown increase in endothelin 1 (ET-1) in peripheral circulation and cerebral spinal fluid (CSF) of MS patients (Speciale et al. 2000; Haufschild et al. 2001), which might be the primary cause of hypoperfusion in MS. Hence, this requires further exploration by correlating cortical hypoperfusion with circulating vasoconstrictive peptides such, as ET-1, using surface based analysis approach, to provide more understanding regarding the leading mechanism of cortical perfusion in MS.

10.4 Conclusion

In conclusion, this present study provided evidence of subtle BBB alteration in NAWM of MS using advanced MRI approaches. This indicates an association between BBB permeability and microstructural tissue damage. It is concluded that changes in BBB integrity is the main and initial event in lesion development and progression, which occurs prior to microstructural tissue damage. The use of a feasible method such as surface based analysis approach provides better understanding of cortical pathology in MS. The outcome of this study suggested that cortical hypoperfusion may be due to a primary cause and not because of cortical atrophy. The vertex wise correlation, in this study, also provides an insight on the association between cortical diffusion with the patients cognitive and clinical status. These findings might provide more clarification of different pathologies in GM and WM and how it relates with functional performance, which may have prognostic and therapeutic values in MS patients. Nevertheless, while some novel outcomes have been concluded in this study; further investigations are required to understand the full picture of lesion development and the functional relevance with GM/WM pathologies in MS, and overcome some of the limitations observed in this study.

Part 4: References and Appendices

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Appendix

Appendix 1 Ethical committee Approval

The study approval has been granted by:

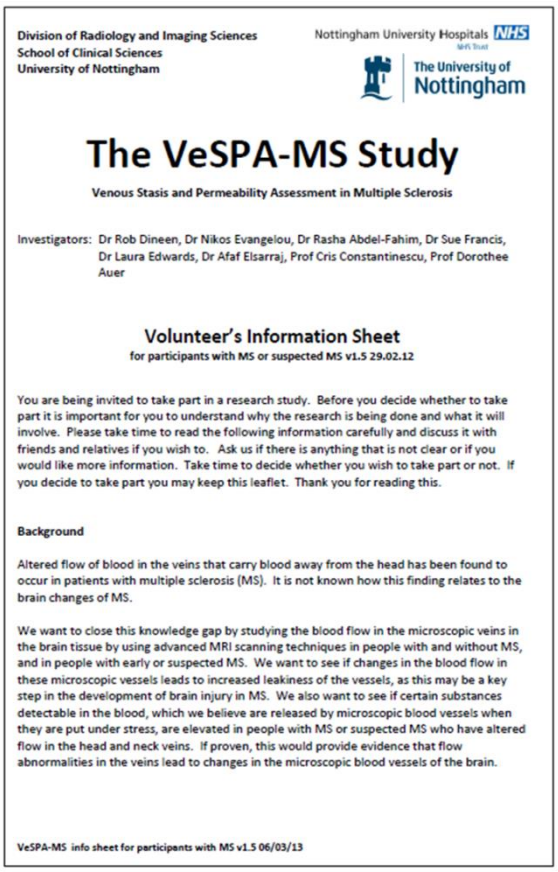
Nottingham Research Ethics Committee East Midlands - Nottingham 2

REC reference number: 10/H0408/37.

The ethical application and the ethical amendment were submitted by Dr. Robert Dineen the Chief investigator of the study "Venous Stasis and Permeability Assessment in Multiple Sclerosis" (VeSPA-MS).

Appendix 2 Participants information sheet and consent form

2.1 MS Participants information sheet



Division of Radiology and Imaging Sciences
School of Clinical Sciences
University of Nottingham

Nottingham University Hospitals NHS
The University of Nottingham

The VeSPA-MS Study

Venous Stasis and Permeability Assessment in Multiple Sclerosis

Investigators: Dr Rob Dineen, Dr Nikos Evangelou, Dr Rasha Abdel-Fahim, Dr Sue Francis, Dr Laura Edwards, Dr Afaf Elsarraj, Prof Cris Constantinescu, Prof Dorothee Auer

Volunteer's Information Sheet

for participants with MS or suspected MS v1.5 29.02.12

You are being invited to take part in a research study. Before you decide whether to take part it is important for you to understand why the research is being done and what it will involve. Please take time to read the following information carefully and discuss it with friends and relatives if you wish to. Ask us if there is anything that is not clear or if you would like more information. Take time to decide whether you wish to take part or not. If you decide to take part you may keep this leaflet. Thank you for reading this.

Background

Altered flow of blood in the veins that carry blood away from the head has been found to occur in patients with multiple sclerosis (MS). It is not known how this finding relates to the brain changes of MS.

We want to close this knowledge gap by studying the blood flow in the microscopic veins in the brain tissue by using advanced MRI scanning techniques in people with and without MS, and in people with early or suspected MS. We want to see if changes in the blood flow in these microscopic vessels leads to increased leakiness of the vessels, as this may be a key step in the development of brain injury in MS. We also want to see if certain substances detectable in the blood, which we believe are released by microscopic blood vessels when they are put under stress, are elevated in people with MS or suspected MS who have altered flow in the head and neck veins. If proven, this would provide evidence that flow abnormalities in the veins lead to changes in the microscopic blood vessels of the brain.

What does the study involve?

The study involves three visits to the Queen's Medical Centre, Nottingham.

During the **first visit** you will be asked to complete a brief **questionnaire** about symptoms that will take about 10 minutes to complete. You will then have an **ultrasound scan** to look at flow of blood in the veins of your head and neck. Ultrasound scanning is a safe and non-invasive way to look at the blood flowing in vessels using a small, hand-held probe, and is the same type of scan performed on pregnant women. For the scan you will be taken into the ultrasound room, which is darkened to allow the researcher to see the screen more clearly. A friend or relative will be allowed in with you if you wish. The researcher will scan the veins of your neck. A blob of ultrasound gel is put on the skin of your neck to help get clearer images. The researcher will also look at the blood flow in your head by placing a blob of gel and the ultrasound probe on your temple. The scan will be performed with you in the sitting and lying position. The scan will last up to 45 minutes. Finally a neurologist will carry out a brief **clinical examination**. This is important because MS can affect people in different ways, with a range of symptoms from very mild through to severe, and we need to know how significantly the participants in our study are affected by MS.

During the **second visit** you will have the **MRI scan** and a **blood test**. On arrival you will be asked to complete a safety questionnaire. This is to check that you can safely go into the MRI scanner, and is standard practice worldwide. You will then be taken to a preparation room where you will have the blood test. The blood test will be taken through a 'drip needle' or *canula* by one of the researchers. The canula is a small sterile plastic tube that is positioned in a vein in the arm and is kept in place by a sticky plaster. About 15 mls (three teaspoons) of blood will be taken, and will be analysed for substances which we are released when microscopic blood vessels become damaged.

As well as allowing us to take the blood sample, the canula will be used to give you an injection of MRI contrast agent during the MRI scan. The contrast agent is a harmless liquid that allows us to see blood vessels and detect blood flow more clearly on the MRI scans. For the MRI scan you will be required to lie in the MRI scanner for a period of no more than 1 hour while we acquire the brain scan images. The injection of contrast agent will be given towards the end of the scan.

While in the scanner you will have protective earplugs or earphones on as MRI scanners can be quite noisy. Most people undergo MRI scanning without any difficulty, but if you need to you will be able to contact the MRI technician at any time during the scan via an intercom or with an emergency buzzer.

The **third visit** will be six to nine months following your second visit. On this visit you will have another **MRI scan** but this will be shorter than the initial scan, lasting only up to 30 minutes, and will not require any injections or blood samples. You will also have a brief (10 minute) clinical assessment to check for any changes since your last visit. **Following this visit, your involvement in the study is complete.**

VeSPA-MS info sheet for participants with MS v1.5 06/03/13

As well as being useful for the research study, your MRI scans may also contain information that could contribute to your ongoing clinical care. For this reason, as well as being analysed by the researchers, your MRI scans will be transferred to the Nottingham University Hospitals secure computer system and reported by a qualified radiologist, so that the doctors looking after you will be able to access your MRI scans.

We are hoping to include 25 participants with MS or suspected MS who have altered blood flow in the neck veins (as shown on the ultrasound) and 25 participants with MS or suspected MS who have normal blood flow patterns. It is therefore possible that following your first visit we may not need you to come to for the second visit, if the group that you would be allocated to (based on the ultrasound scan) is already full.

Why have I been invited to take part?

You have been invited to take part because you have multiple sclerosis, or are being investigated for a possible diagnosis of multiple sclerosis. To carry out this study we need to identify 25 participants with MS or suspected MS who have altered blood flow in the neck veins (as shown on the ultrasound) and 25 participants with MS or suspected MS who have normal blood flow patterns. To do this, we are expecting to have to screen up to 100 people with MS with ultrasound.

Do I have to take part?

It is up to you to decide whether or not to take part. If you return the reply form indicating that you wish to enrol, we will leave it at least two days before contacting you to give you a chance to reflect on your decision. If you do decide to take part you will be given this information sheet to keep and be asked to sign a consent form. You will still be free to withdraw from the study at any time and without giving a reason.

What do I have to do?

Participation simply requires attendance at the three visits detailed above. Apart from the injection of contrast agent during the first MRI scan, the study does not involve taking any medication. You will not have to make any changes to your diet or medications.

What are the benefits of taking part?

There are no direct benefits for the individual in taking part in the study. However, the study aims to further our understanding of how altered blood flow patterns contribute to brain changes in MS. We hope this information will be useful in the future for helping to develop and test more effective treatments for MS.

What are the possible disadvantages and risks of taking part?

Provided you do not have a condition which prevents you from having an MRI scan or MRI contrast agent, there are no risks associated with this study. You will be screened for conditions preventing you from having an MRI scan or contrast agent before you have the scans.

The MRI scanner is a relatively enclosed space and occasionally participants can feel claustrophobic. During the scans you are able to speak to the researchers performing the scan. If you would like to come out of the scanner at any time, you can request this. If you know that you are claustrophobic, we would advise against you participating.

In the unlikely event that an unsuspected abnormality is identified on the scans by one of the researchers, the images will be reviewed by a qualified consultant neuroradiologist working within the Division of Radiological and Imaging Sciences. If the suspicion of a significant abnormality is confirmed, the neuroradiologist involved will contact your hospital consultant and GP to advise on appropriate measures which will need to be taken in light of the findings, which may include performing further scans or advising on referral to an appropriate hospital-based specialist. The finding of a significant unexpected abnormality may have benefits in that it may be possible to offer treatments earlier than would otherwise have been possible. However, participants should also be aware that the finding of significant abnormality may adversely effect employment and insurance status.

What if something goes wrong? Who can I complain to?

In case you have a complaint on your treatment by a member of staff or anything to do with the study, you can initially approach the lead investigator. If this route fails to achieve a satisfactory resolution and you still wish to complain about any aspect of the way you have been approached or treated during the course of this study, the normal National Health Service complaints mechanisms may be available to you. The Patient Advice and Liaison Service (PALS) can be contacted for further assistance at QMC by calling 0800 1830204

Will my taking part in this study be kept confidential?

Yes. All information which is collected about you during the course of the research will be kept on a password protected database and is strictly confidential. Any information about you which leaves the research unit will have your name and address removed so that you cannot be recognised from it. Your personal details will not be passed onto any third parties.

Your GP and hospital doctor will be made aware that you are taking part in this study. As described above, the clinically-useful parts of the MRI scans and a report of the scans will transferred to the secure Nottingham University Hospitals computer archive, to allow these scans to contribute to your clinical care. Regarding the results of the Doppler ultrasound scan of blood flow in the veins of the neck, we understand that some participants may be keen for these results to be made available to their hospital doctor as the results may be important for decisions about treatment in the future. Your permission to pass on this

information to your hospital doctor will be asked when you sign the consent form. Please be aware that at present the proposed treatments for altered blood flow in the head and neck veins in people with MS are unproven and are not currently provided by the Nottingham University Hospitals NHS Trust.

What will happen if I don't want to carry on with the study?

Your participation is voluntary and you are free to withdraw at any time, without giving any reason, and without your legal rights being affected. If you withdraw then the information collected so far cannot be erased and that this information may still be used in the project analysis. We would still like to use any blood samples that have been obtained, but will destroy these if you do not want these to be used.

What will happen to the results of the research study?

It is hoped that the overall results of the study will be published in a high-profile scientific journal and presented at an international scientific meeting. You will not be identified in any report or publication. If you would like to receive information about the final results of the study, please mention this to one of the researchers.

Who is organising and funding the research?

The study is being organised by members of the Division of Radiological and Imaging Sciences, University of Nottingham, in collaboration with researchers from the Division of Clinical Neurology.

Who has reviewed the study?

All research in the NHS is looked at by independent group of people, called a Research Ethics Committee, to protect your interests. This study has been reviewed and given favourable opinion by Nottingham NHS Research Ethics Committee 2.

Contact for Further Information

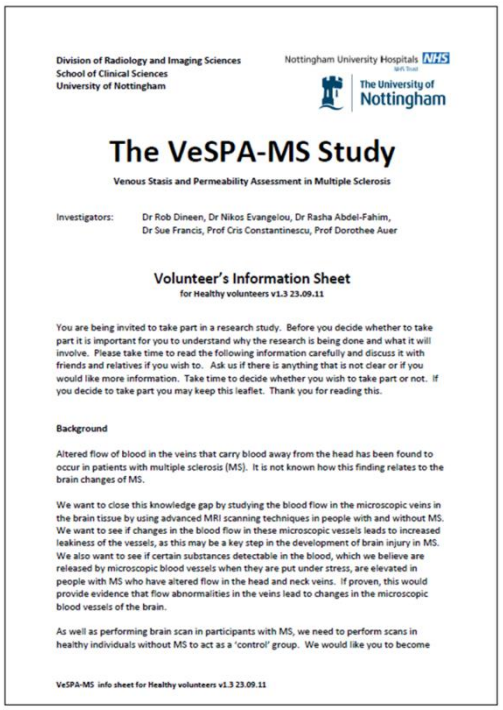
If you would like to discuss the study further or would like more information, please feel free to contact me:

Dr Rob Dineen,
Clinical Associate Professor,
Division of Radiological and Imaging Sciences,
Queen's Medical Centre,
Nottingham,
NG7 2UH

Phone:0115 8231173 Email: rob.dineen@nottingham.ac.uk

Further general information about getting involved in medical research is available from the INVOLVE website at http://www.invo.org.uk/Members_of_the_Public.asp

2.2 Healthy volunteer Participants information sheet



Division of Radiology and Imaging Sciences
School of Clinical Sciences
University of Nottingham

Nottingham University Hospitals NHS Trust

The University of Nottingham

The VeSPA-MS Study

Venous Stasis and Permeability Assessment in Multiple Sclerosis

Investigators: Dr Rob Dineen, Dr Nikos Evangelou, Dr Rasha Abdel-Fahim, Dr Sue Francis, Prof Cris Constantinescu, Prof Dorothee Auer

Volunteer's Information Sheet

for Healthy volunteers v1.3 23.09.11

You are being invited to take part in a research study. Before you decide whether to take part it is important for you to understand why the research is being done and what it will involve. Please take time to read the following information carefully and discuss it with friends and relatives if you wish to. Ask us if there is anything that is not clear or if you would like more information. Take time to decide whether you wish to take part or not. If you decide to take part you may keep this leaflet. Thank you for reading this.

Background

Altered flow of blood in the veins that carry blood away from the head has been found to occur in patients with multiple sclerosis (MS). It is not known how this finding relates to the brain changes of MS.

We want to close this knowledge gap by studying the blood flow in the microscopic veins in the brain tissue by using advanced MRI scanning techniques in people with and without MS. We want to see if changes in the blood flow in these microscopic vessels leads to increased leakiness of the vessels, as this may be a key step in the development of brain injury in MS. We also want to see if certain substances detectable in the blood, which we believe are released by microscopic blood vessels when they are put under stress, are elevated in people with MS who have altered flow in the head and neck veins. If proven, this would provide evidence that flow abnormalities in the veins lead to changes in the microscopic blood vessels of the brain.

As well as performing brain scan in participants with MS, we need to perform scans in healthy individuals without MS to act as a 'control' group. We would like you to become



one of the healthy control volunteers so that we can compare the results from your ultrasound scan, MRI scan and blood test with the scans from the participants with MS. As we are aiming to match our control group closely to our patient groups in terms of age and gender, it may be that we are unable to include you in the study. If this is the case, then we will be extremely grateful to you for having volunteered even if you do not get included in the study.

What does the study involve?

The study involves two visits to the Queen's Medical Centre, Nottingham.

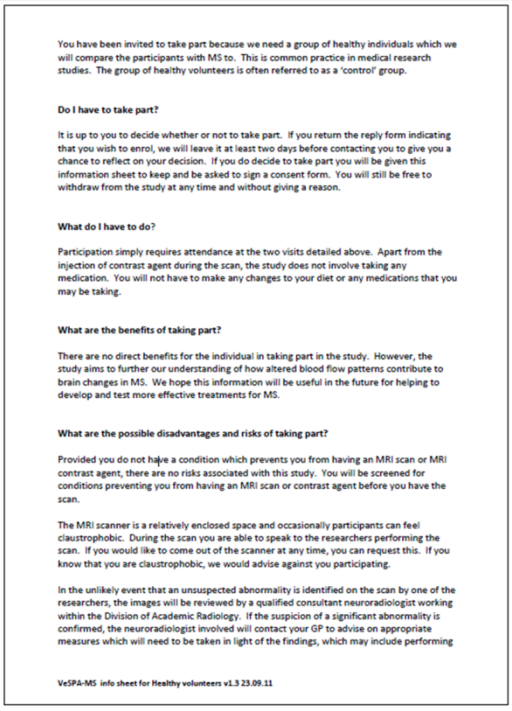
During the **first visit** you will be asked to complete a **brief questionnaire** about symptoms that will take about 10 minutes to complete. You will then have an **ultrasound scan** to look at flow of blood in the veins of your head and neck. Ultrasound scanning is a safe and non-invasive way to look at the blood flowing in vessels using a small, hand-held probe, and is the same type of scan performed on pregnant women. For the scan you will be taken into the ultrasound room, which is darkened to allow the researcher to see the screen more clearly. A friend or relative will be allowed in with you if you wish. The researcher will scan the veins of your neck. A blob of ultrasound gel is put on the skin of your neck to help get clearer images. The researcher will also look at the blood flow in your head by placing a blob of gel and the ultrasound probe on your temple. The scan will be performed with you in the sitting and lying position. The scan will last up to 45 minutes.

During the **second visit** you will have the **MRI scan** and a **blood test**. On arrival you will be asked to complete a safety questionnaire. This is to check that you can safely go into the MRI scanner, and is standard practice worldwide. You will then be taken to a preparation room where you will have the blood test. The blood test will be taken through a 'drip needle' or *canula* by one of the researchers. The canula is a small sterile plastic tube that is positioned in a vein in the arm and is kept in place by a sticky plaster. About 15 mls (three teaspoons) of blood will be taken, and will be analysed for substances which we are released when microscopic blood vessels become damaged.

As well as allowing us to take the blood sample, the canula will be used to give you an injection of MRI contrast agent during the MRI scan. The contrast agent is a harmless liquid that allows us to see blood vessels and detect blood flow more clearly on the MRI scans. For the MRI scan you will be required to lie in the MRI scanner for a period of no more than 1 hour while we acquire the brain scan images. The injection of contrast agent will be given towards the end of the scan.

While in the scanner you will have protective earplugs or earphones on as MRI scanners can be quite noisy. Most people undergo MRI scanning without any difficulty, but if you need to you will be able to contact the MRI technician at any time during the scan via an intercom or with an emergency buzzer. **Following this visit, your involvement in the study is complete.**

Why have I been invited to take part?



You have been invited to take part because we need a group of healthy individuals which we will compare the participants with MS to. This is common practice in medical research studies. The group of healthy volunteers is often referred to as a 'control' group.

Do I have to take part?

It is up to you to decide whether or not to take part. If you return the reply form indicating that you wish to enrol, we will leave it at least two days before contacting you to give you a chance to reflect on your decision. If you do decide to take part you will be given this information sheet to keep and be asked to sign a consent form. You will still be free to withdraw from the study at any time and without giving a reason.

What do I have to do?

Participation simply requires attendance at the two visits detailed above. Apart from the injection of contrast agent during the scan, the study does not involve taking any medication. You will not have to make any changes to your diet or any medications that you may be taking.

What are the benefits of taking part?

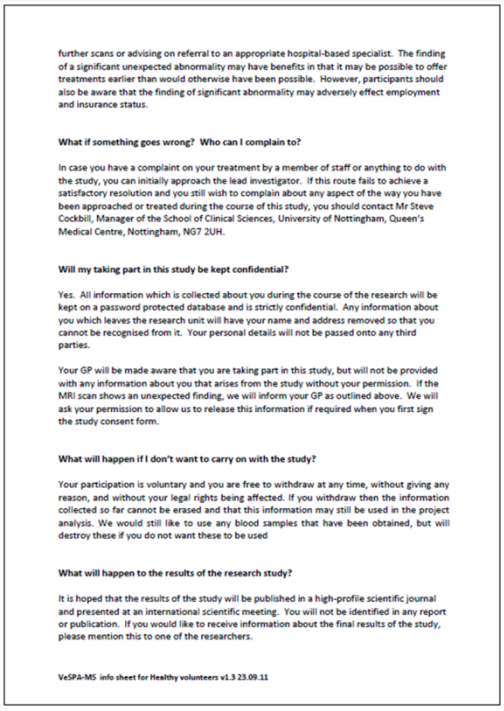
There are no direct benefits for the individual in taking part in the study. However, the study aims to further our understanding of how altered blood flow patterns contribute to brain changes in MS. We hope this information will be useful in the future for helping to develop and test more effective treatments for MS.

What are the possible disadvantages and risks of taking part?

Provided you do not have a condition which prevents you from having an MRI scan or MRI contrast agent, there are no risks associated with this study. You will be screened for conditions preventing you from having an MRI scan or contrast agent before you have the scan.

The MRI scanner is a relatively enclosed space and occasionally participants can feel claustrophobic. During the scan you are able to speak to the researchers performing the scan. If you would like to come out of the scanner at any time, you can request this. If you know that you are claustrophobic, we would advise against you participating.

In the unlikely event that an unsuspected abnormality is identified on the scan by one of the researchers, the images will be reviewed by a qualified consultant neuroradiologist working within the Division of Academic Radiology. If the suspicion of a significant abnormality is confirmed, the neuroradiologist involved will contact your GP to advise on appropriate measures which will need to be taken in light of the findings, which may include performing



further scans or advising on referral to an appropriate hospital-based specialist. The finding of a significant unexpected abnormality may have benefits in that it may be possible to offer treatments earlier than would otherwise have been possible. However, participants should also be aware that the finding of significant abnormality may adversely effect employment and insurance status.

What if something goes wrong? Who can I complain to?

In case you have a complaint on your treatment by a member of staff or anything to do with the study, you can initially approach the lead investigator. If this route fails to achieve a satisfactory resolution and you still wish to complain about any aspect of the way you have been approached or treated during the course of this study, you should contact Mr Steve Cockbill, Manager of the School of Clinical Sciences, University of Nottingham, Queen's Medical Centre, Nottingham, NG7 2UH.

Will my taking part in this study be kept confidential?

Yes. All information which is collected about you during the course of the research will be kept on a password protected database and is strictly confidential. Any information about you which leaves the research unit will have your name and address removed so that you cannot be recognised from it. Your personal details will not be passed onto any third parties.

Your GP will be made aware that you are taking part in this study, but will not be provided with any information about you that arises from the study without your permission. If the MRI scan shows an unexpected finding, we will inform your GP as outlined above. We will ask your permission to allow us to release this information if required when you first sign the study consent form.

What will happen if I don't want to carry on with the study?

Your participation is voluntary and you are free to withdraw at any time, without giving any reason, and without your legal rights being affected. If you withdraw then the information collected so far cannot be erased and that this information may still be used in the project analysis. We would still like to use any blood samples that have been obtained, but will destroy these if you do not want these to be used

What will happen to the results of the research study?

It is hoped that the results of the study will be published in a high-profile scientific journal and presented at an international scientific meeting. You will not be identified in any report or publication. If you would like to receive information about the final results of the study, please mention this to one of the researchers.

Who is organising and funding the research?

The study is being organised by members of the Division of Radiological and Imaging Sciences, University of Nottingham, in collaboration with researchers from the Division of Clinical Neurology.

Who has reviewed the study?

All research in the NHS is looked at by independent group of people, called a Research Ethics Committee, to protect your interests. This study has been reviewed and given favourable opinion by Nottingham NHS Research Ethics Committee 2.

Contact for Further Information

If you would like to discuss the study further or would like more information, please feel free to contact me:

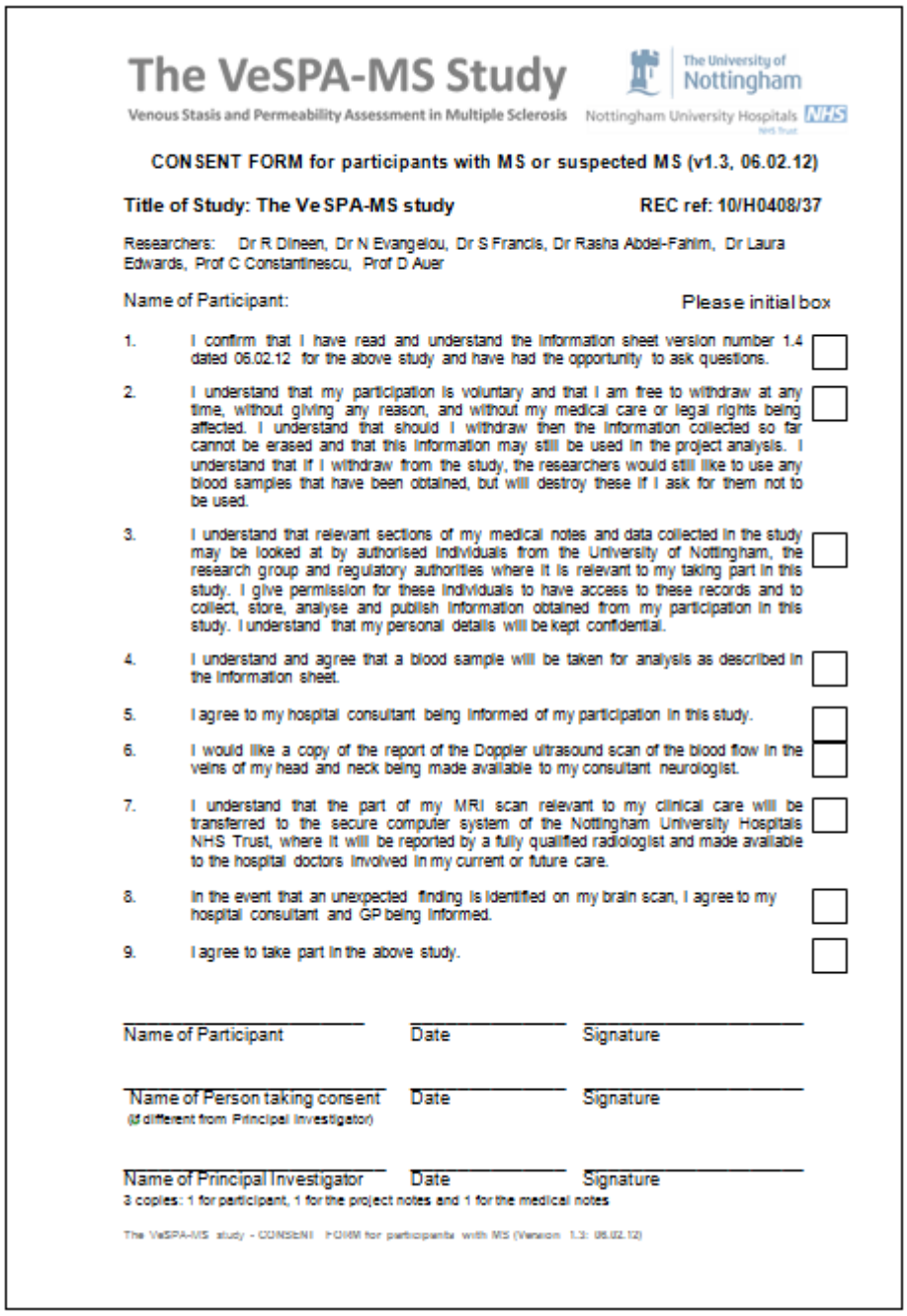
Dr Rob Dineen,
Clinical Associate Professor,
Division of Radiological and Imaging Sciences,
Queen's Medical Centre,
Nottingham,
NG7 2UH,

0115 8231173

rob.dineen@nottingham.ac.uk

Further general information about getting involved in medical research is available from the INVOLVE website at http://www.invo.org.uk/Members_of_the_Public.asp

2.3 Consent form for patients with MS



The VeSPA-MS Study

Venous Stasis and Permeability Assessment in Multiple Sclerosis

The University of Nottingham

Nottingham University Hospitals NHS

CONSENT FORM for participants with MS or suspected MS (v1.3, 06.02.12)

Title of Study: The Ve SPA-MS study **REC ref: 10/H0408/37**

Researchers: Dr R Dineen, Dr N Evangelou, Dr S Francis, Dr Rasha Abdel-Fahim, Dr Laura Edwards, Prof C Constantinescu, Prof D Auer

Name of Participant: Please initial box

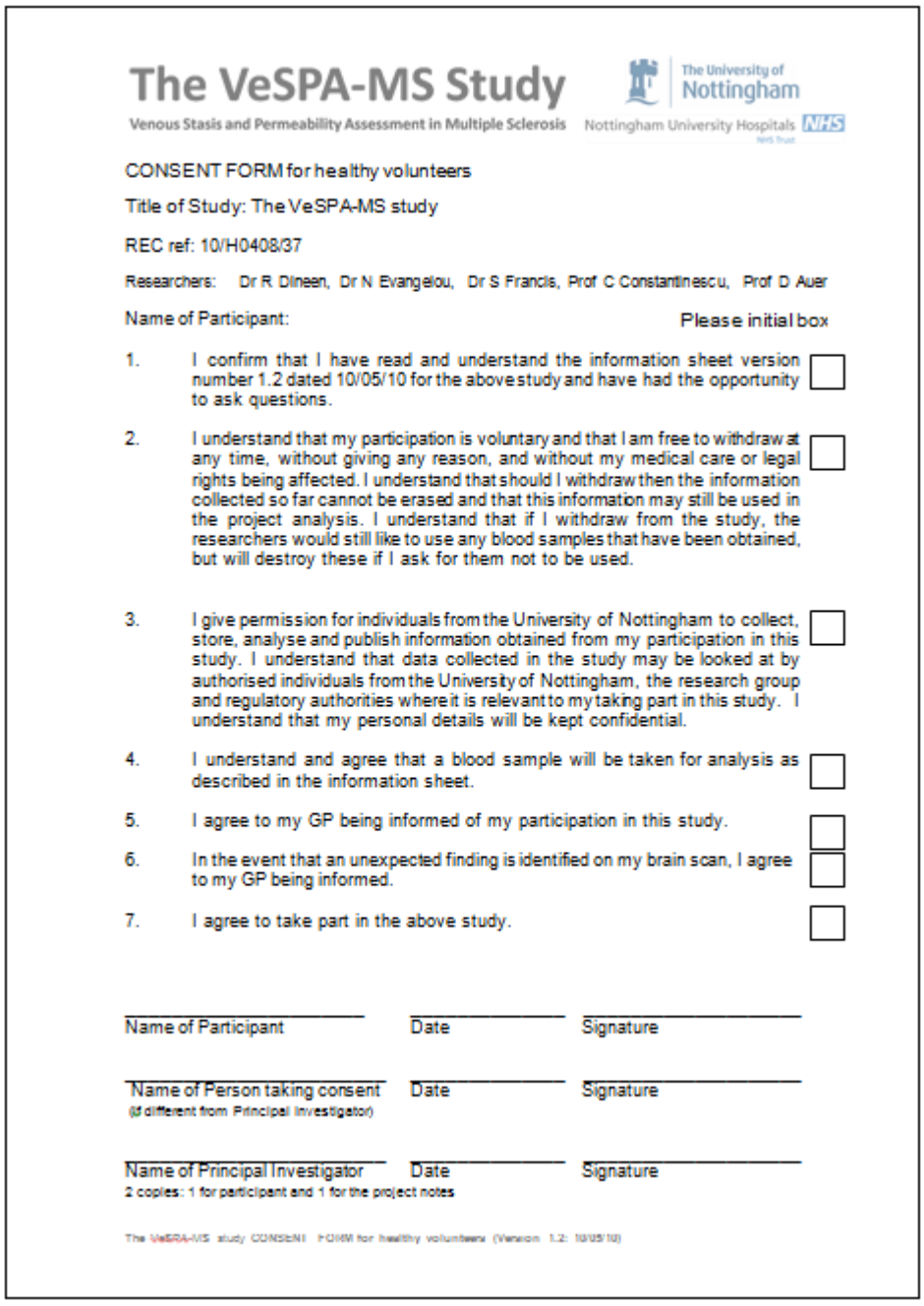
1. I confirm that I have read and understand the information sheet version number 1.4 dated 06.02.12 for the above study and have had the opportunity to ask questions. ☐
2. I understand that my participation is voluntary and that I am free to withdraw at any time, without giving any reason, and without my medical care or legal rights being affected. I understand that should I withdraw then the information collected so far cannot be erased and that this information may still be used in the project analysis. I understand that if I withdraw from the study, the researchers would still like to use any blood samples that have been obtained, but will destroy these if I ask for them not to be used. ☐
3. I understand that relevant sections of my medical notes and data collected in the study may be looked at by authorised individuals from the University of Nottingham, the research group and regulatory authorities where it is relevant to my taking part in this study. I give permission for these individuals to have access to these records and to collect, store, analyse and publish information obtained from my participation in this study. I understand that my personal details will be kept confidential. ☐
4. I understand and agree that a blood sample will be taken for analysis as described in the information sheet. ☐
5. I agree to my hospital consultant being informed of my participation in this study. ☐
6. I would like a copy of the report of the Doppler ultrasound scan of the blood flow in the veins of my head and neck being made available to my consultant neurologist. ☐
7. I understand that the part of my MRI scan relevant to my clinical care will be transferred to the secure computer system of the Nottingham University Hospitals NHS Trust, where it will be reported by a fully qualified radiologist and made available to the hospital doctors involved in my current or future care. ☐
8. In the event that an unexpected finding is identified on my brain scan, I agree to my hospital consultant and GP being informed. ☐
9. I agree to take part in the above study. ☐

Name of Participant	Date	Signature
Name of Person taking consent (if different from Principal Investigator)	Date	Signature
Name of Principal Investigator	Date	Signature

3 copies: 1 for participant, 1 for the project notes and 1 for the medical notes

The VeSPA-MS study - CONSENT FORM for participants with MS (Version 1.3: 06.02.12)

2.4 Consent form for healthy volunteers



The VeSPA-MS Study

Venous Stasis and Permeability Assessment in Multiple Sclerosis

The University of Nottingham

Nottingham University Hospitals NHS

CONSENT FORM for healthy volunteers

Title of Study: The VeSPA-MS study

REC ref: 10/H0408/37

Researchers: Dr R Dineen, Dr N Evangelou, Dr S Francis, Prof C Constantinescu, Prof D Auer

Name of Participant: Please initial box

1. I confirm that I have read and understand the information sheet version number 1.2 dated 10/05/10 for the above study and have had the opportunity to ask questions. ☐

2. I understand that my participation is voluntary and that I am free to withdraw at any time, without giving any reason, and without my medical care or legal rights being affected. I understand that should I withdraw then the information collected so far cannot be erased and that this information may still be used in the project analysis. I understand that if I withdraw from the study, the researchers would still like to use any blood samples that have been obtained, but will destroy these if I ask for them not to be used. ☐

3. I give permission for individuals from the University of Nottingham to collect, store, analyse and publish information obtained from my participation in this study. I understand that data collected in the study may be looked at by authorised individuals from the University of Nottingham, the research group and regulatory authorities where it is relevant to my taking part in this study. I understand that my personal details will be kept confidential. ☐

4. I understand and agree that a blood sample will be taken for analysis as described in the information sheet. ☐

5. I agree to my GP being informed of my participation in this study. ☐

6. In the event that an unexpected finding is identified on my brain scan, I agree to my GP being informed. ☐

7. I agree to take part in the above study. ☐

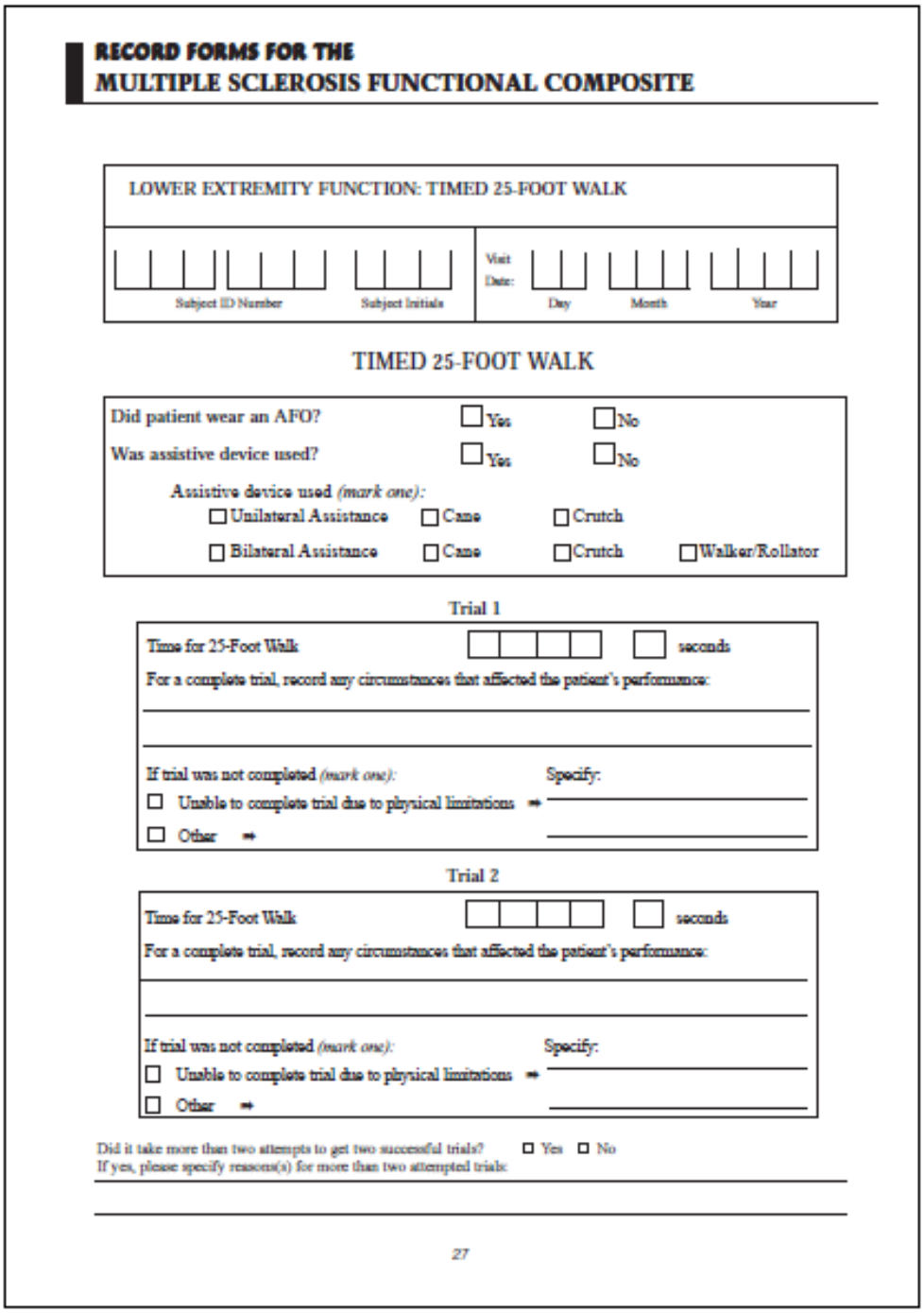
Name of Participant	Date	Signature
Name of Person taking consent (if different from Principal Investigator)	Date	Signature
Name of Principal Investigator	Date	Signature

2 copies: 1 for participant and 1 for the project notes

The VeSPA-MS study CONSENT FORM for healthy volunteers (Version 1.2: 10/05/10)

Appendix 3 Clinical assessments

3.1 Multiple Sclerosis Functional composite



RECORD FORMS FOR THE
MULTIPLE SCLEROSIS FUNCTIONAL COMPOSITE

LOWER EXTREMITY FUNCTION: TIMED 25-FOOT WALK

Subject ID Number | Subject Initials | Visit Date: Day | Month | Year

TIMED 25-FOOT WALK

Did patient wear an AFO? ☐ Yes ☐ No

Was assistive device used? ☐ Yes ☐ No

Assistive device used *(mark one)*:

☐ Unilateral Assistance ☐ Cane ☐ Crutch

☐ Bilateral Assistance ☐ Cane ☐ Crutch ☐ Walker/Rollator

Trial 1

Time for 25-Foot Walk ☐☐☐☐ ☐ seconds

For a complete trial, record any circumstances that affected the patient's performance:

If trial was not completed *(mark one)*: Specify:

☐ Unable to complete trial due to physical limitations ➡

☐ Other ➡

Trial 2

Time for 25-Foot Walk ☐☐☐☐ ☐ seconds

For a complete trial, record any circumstances that affected the patient's performance:

If trial was not completed *(mark one)*: Specify:

☐ Unable to complete trial due to physical limitations ➡

☐ Other ➡

Did it take more than two attempts to get two successful trials? ☐ Yes ☐ No

If yes, please specify reasons(s) for more than two attempted trials:

27

UPPER EXTREMITY FUNCTION: NINE-HOLE PEG TEST (9-HPT)

Subject ID Number | Subject Initials | Visit Date: Day Month Year

9-HOLE PEG TEST

DOMINANT HAND *(Check one)*: Right ☐ Left ☐

DOMINANT HAND

Trial 1

☐☐☐ ☐ seconds

For a complete trial, record any circumstances that affected the patient's performance:

If trial was not completed *(mark one)*:

☐ Unable to complete trial due to physical limitations ➡ Specify:

☐ Other ➡

Trial 2

☐☐☐ ☐ seconds

For a complete trial, record any circumstances that affected the patient's performance:

If trial was not completed *(mark one)*:

☐ Unable to complete trial due to physical limitations ➡ Specify:

☐ Other ➡

Did it take more than two attempts to get two successful trials? ☐ Yes ☐ No
If Yes, please specify reason(s) for more than two attempted trials:

NON-DOMINANT HAND

Trial 1

☐☐☐ ☐ seconds

For a complete trial, record any circumstances that affected the patient's performance:

If trial was not completed *(mark one)*:

☐ Unable to complete trial due to physical limitations ➡ Specify:

☐ Other ➡

Trial 2

☐☐☐ ☐ seconds

For a complete trial, record any circumstances that affected the patient's performance:

If trial was not completed *(mark one)*:

☐ Unable to complete trial due to physical limitations ➡ Specify:

☐ Other ➡

Did it take more than two attempts to get two successful trials? ☐ Yes ☐ No
If Yes, please specify reason(s) for more than two attempted trials:

COGNITIVE FUNCTION: PASAT - FORM A

Subject ID Number | Subject Initials | Visit Date: Day | Month | Year

RATE #1
(3 sec)

1 + 4 5____	8 12____	1 9____	5 6____	1 6____	3 4____	7 10____	2 9____	6 8____	9 15____
4 13____	7 11____	3 10____	5 8____	3 8____	6 9____	8 14____	2 10____	5 7____	1 6____
5 6____	4 9____	6 10____	3 9____	8 11____	1 9____	7 8____	4 11____	9 13____	3 12____
7 10____	2 9____	6 8____	9 15____	5 14____	2 7____	4 6____	8 12____	3 11____	1 4____
8 9____	5 13____	7 12____	1 8____	8 9____	2 10____	4 6____	9 13____	7 16____	9 16____
3 12____	1 4____	5 6____	7 12____	4 11____	8 12____	1 9____	3 4____	8 11____	2 10____

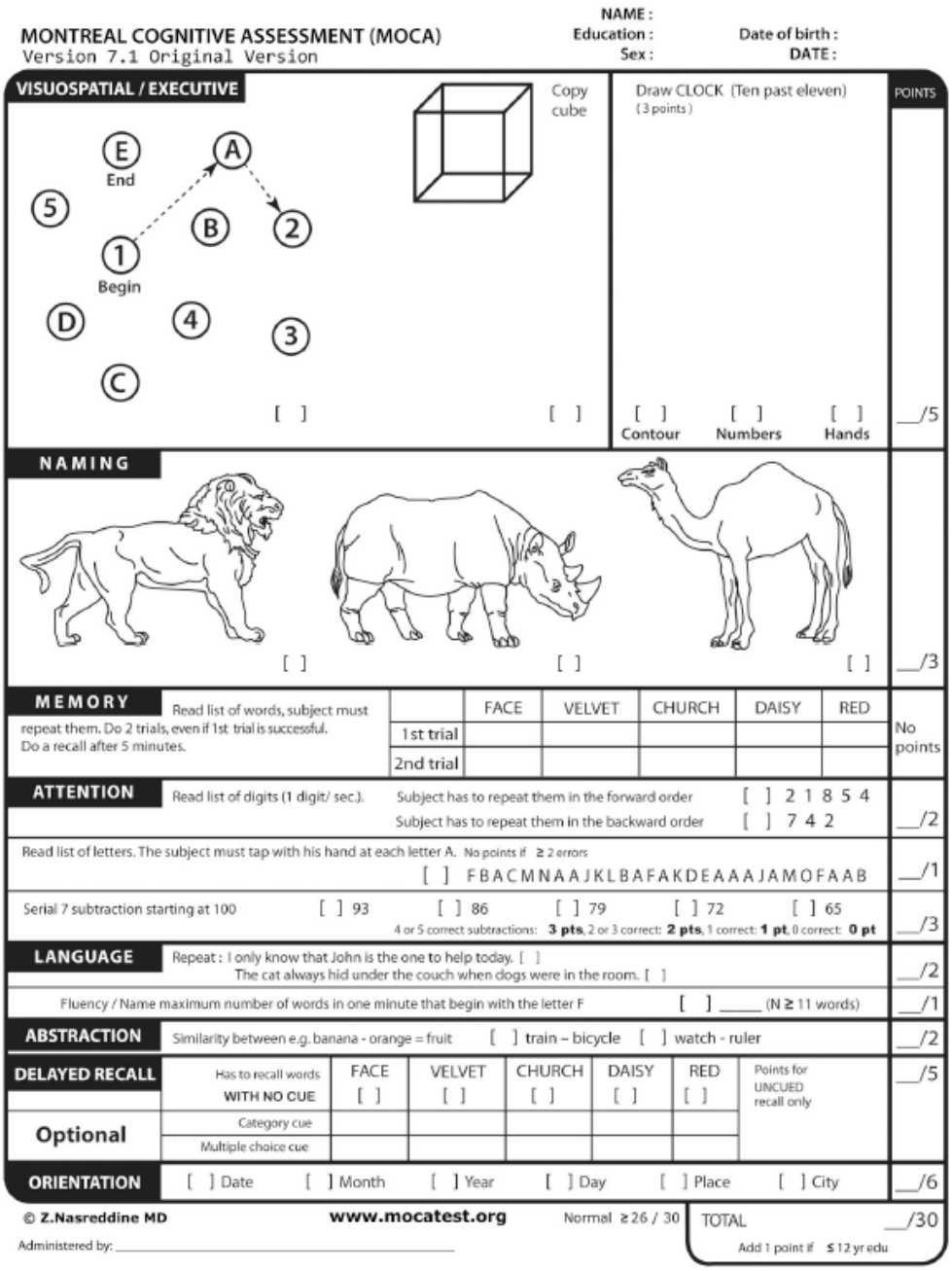
Total Correct (raw) = _____ Percent Correct = _____

RATE #2
(2 sec)

4 + 3 7____	7 10____	2 9____	5 7____	1 6____	8 9____	6 14____	9 15____	1 10____	7 8____
9 16____	4 13____	6 10____	3 9____	5 8____	8 13____	1 9____	6 7____	2 8____	7 9____
5 12____	9 14____	4 13____	5 9____	2 7____	6 8____	4 10____	8 12____	3 11____	5 8____
9 14____	7 16____	4 11____	2 6____	8 10____	5 13____	2 7____	1 3____	6 7____	4 10____
7 11____	3 10____	5 8____	9 14____	6 15____	4 10____	5 9____	3 8____	9 12____	4 13____
1 5____	8 9____	3 11____	1 4____	6 7____	8 14____	5 13____	4 9____	2 6____	6 8____

Total Correct (raw) = _____ Percent Correct = _____

3.2 Montreal Cognitive assessment (MoCA)



MONTREAL COGNITIVE ASSESSMENT (MOCA)
Version 7.1 Original Version

NAME :
Education : Date of birth :
Sex : DATE :

VISUOSPATIAL / EXECUTIVE — Copy cube — Draw CLOCK (Ten past eleven) (3 points) — POINTS

E End, A, 5, B, 2, 1 Begin, D, 4, 3, C

[ ] [ ] [ ] Contour [ ] Numbers [ ] Hands /5

NAMING /3

[ ] [ ] [ ]

MEMORY Read list of words, subject must repeat them. Do 2 trials, even if 1st trial is successful. Do a recall after 5 minutes. — No points

	FACE	VELVET	CHURCH	DAISY	RED
1st trial					
2nd trial					

ATTENTION Read list of digits (1 digit/ sec.). Subject has to repeat them in the forward order [ ] 2 1 8 5 4 /2
Subject has to repeat them in the backward order [ ] 7 4 2

Read list of letters. The subject must tap with his hand at each letter A. No points if ≥ 2 errors /1
[ ] F B A C M N A A J K L B A F A K D E A A A J A M O F A A B

Serial 7 subtraction starting at 100 [ ] 93 [ ] 86 [ ] 79 [ ] 72 [ ] 65 /3
4 or 5 correct subtractions: **3 pts**, 2 or 3 correct: **2 pts**, 1 correct: **1 pt**, 0 correct: **0 pt**

LANGUAGE Repeat : I only know that John is the one to help today. [ ] /2
The cat always hid under the couch when dogs were in the room. [ ]

Fluency / Name maximum number of words in one minute that begin with the letter F [ ] ____ (N ≥ 11 words) /1

ABSTRACTION Similarity between e.g. banana - orange = fruit [ ] train – bicycle [ ] watch - ruler /2

DELAYED RECALL /5

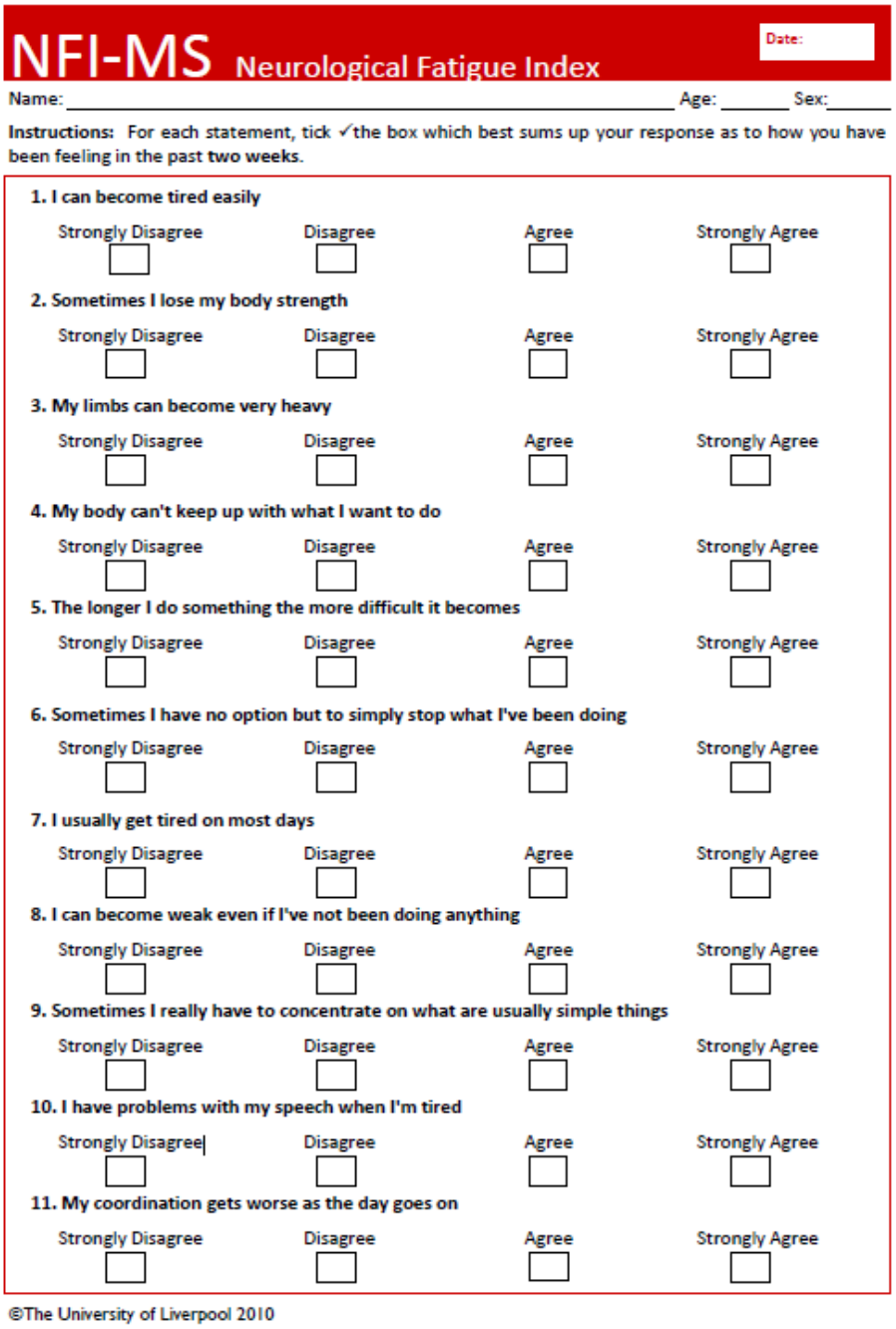
	Has to recall words WITH NO CUE	FACE [ ]	VELVET [ ]	CHURCH [ ]	DAISY [ ]	RED [ ]	Points for UNCUED recall only
Optional	Category cue						
	Multiple choice cue						

ORIENTATION [ ] Date [ ] Month [ ] Year [ ] Day [ ] Place [ ] City /6

© Z.Nasreddine MD www.mocatest.org Normal ≥ 26 / 30 TOTAL /30
Add 1 point if ≤ 12 yr edu

Administered by: _____

3.3 Neurological fatigue index



NFI-MS Neurological Fatigue Index

Date:

Name: _____ Age: _____ Sex: _____

Instructions: For each statement, tick ✓the box which best sums up your response as to how you have been feeling in the past **two weeks**.

1. I can become tired easily

Strongly Disagree ☐ Disagree ☐ Agree ☐ Strongly Agree ☐

2. Sometimes I lose my body strength

Strongly Disagree ☐ Disagree ☐ Agree ☐ Strongly Agree ☐

3. My limbs can become very heavy

Strongly Disagree ☐ Disagree ☐ Agree ☐ Strongly Agree ☐

4. My body can't keep up with what I want to do

Strongly Disagree ☐ Disagree ☐ Agree ☐ Strongly Agree ☐

5. The longer I do something the more difficult it becomes

Strongly Disagree ☐ Disagree ☐ Agree ☐ Strongly Agree ☐

6. Sometimes I have no option but to simply stop what I've been doing

Strongly Disagree ☐ Disagree ☐ Agree ☐ Strongly Agree ☐

7. I usually get tired on most days

Strongly Disagree ☐ Disagree ☐ Agree ☐ Strongly Agree ☐

8. I can become weak even if I've not been doing anything

Strongly Disagree ☐ Disagree ☐ Agree ☐ Strongly Agree ☐

9. Sometimes I really have to concentrate on what are usually simple things

Strongly Disagree ☐ Disagree ☐ Agree ☐ Strongly Agree ☐

10. I have problems with my speech when I'm tired

Strongly Disagree ☐ Disagree ☐ Agree ☐ Strongly Agree ☐

11. My coordination gets worse as the day goes on

Strongly Disagree ☐ Disagree ☐ Agree ☐ Strongly Agree ☐

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Instructions: For each statement, tick ✓the box which best sums up your response as to how you have been feeling in the past two weeks.

12. Mental effort really takes it out of me

Strongly Disagree ☐ Disagree ☐ Agree ☐ Strongly Agree ☐

13. I need to rest in the day

Strongly Disagree ☐ Disagree ☐ Agree ☐ Strongly Agree ☐

14. I need to sleep in the day

Strongly Disagree ☐ Disagree ☐ Agree ☐ Strongly Agree ☐

15. Sleep in the day can really help me

Strongly Disagree ☐ Disagree ☐ Agree ☐ Strongly Agree ☐

16. Resting allows me to carry on

Strongly Disagree ☐ Disagree ☐ Agree ☐ Strongly Agree ☐

17. I try to get everything done in the morning

Strongly Disagree ☐ Disagree ☐ Agree ☐ Strongly Agree ☐

18. I try to rest or sleep beforehand, if I know I've got to do something that requires a lot of effort

Strongly Disagree ☐ Disagree ☐ Agree ☐ Strongly Agree ☐

19. I get a feeling as if I have not slept for a couple of nights

Strongly Disagree ☐ Disagree ☐ Agree ☐ Strongly Agree ☐

20. I yawn a lot

Strongly Disagree ☐ Disagree ☐ Agree ☐ Strongly Agree ☐

21. I sometimes wake in the night for no reason

Strongly Disagree ☐ Disagree ☐ Agree ☐ Strongly Agree ☐

22. When I awake in the morning I feel unrefreshed

Strongly Disagree ☐ Disagree ☐ Agree ☐ Strongly Agree ☐

23. Often in the morning, I don't feel like getting out of bed

Strongly Disagree ☐ Disagree ☐ Agree ☐ Strongly Agree ☐