Cape Peninsula University of Technology

Magnetic Resonance Imaging and Biochemical markers to assess disability in female subjects with Multiple Sclerosis.

by

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Dissertation submitted in fulfilment of the requirements for the degree:

Master of Science Radiography

in the Faculty of Health & Wellness Sciences

at Cape Peninsula University of Technology

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Bellville
August 2016

DECLARATION

I, **Estelle Penelope Herbert**, declare that the contents of this dissertation/thesis represent my own unaided work, and that the dissertation/thesis has not previously been submitted for academic examination towards any qualification. Furthermore, it represents my own opinions and not necessarily those of Cape Peninsula University of Technology.

Signed **Date**

ABSTRACT

INTRODUCTION: Multiple sclerosis (MS) affects the central nervous system (CNS) and is characterized by multiple demyelinating lesions. It is in this context that a need arises for reliable biomarkers such as Magnetic Resonance Imaging (MRI), which could lead to the early diagnosis and therapeutic intervention when maximum potential impact is possible.

This study examines the impact of MRI as a marker and the sequences that give the best images to aid in evaluation of disease progression (which can indirectly be seen as disability) and the early diagnosis of MS which will, in turn, lead to more effective management of the disease.

METHOD: Sixteen subjects underwent a neurological examination, the Expanded Disability Status Scale (EDSS), blood tests for iron parameters and a 3Tesla Magnetic Resonance Imaging (MRI) scan. In a study of MS, 11 had MRI data that could be analysed by using tract-based spatial statistics (TBSS). Subjects were divided according to the EDSS score (8 of the subjects had an EDSS score of ≤ 3 while 3 subjects had scores of ≥ 6). Diffusion tensor imaging (DTI), the fused Proton Density and Fluid Attenuation Recovery (FLAIR) was utilised to compute the lesion numbers and standard laboratory procedures were used to measure other biochemical markers (serum iron, % transferrin saturation, ferritin, haemoglobin) in subjects with disability and simultaneously assess the disease process.

RESULTS: The FA of white matter tracts (WMTs) as a parameter of myelin integrity was lower in subjects with MS only in those who had high EDSS scores. An association between FA and iron parameters, especially percentage transferrin saturation (% Tf) sat were observed, which suggests that iron availability to the WM may be a requirement for optimal myelin functionality.

CONCLUSION: The FA of WMTs as a parameter of myelin integrity was lower only in those MS subjects who had high EDSS scores. Subjects who had EDSS scores < 3 (i.e. who had a “benign” disease outcome) had FA values similar to control values and this finding was not related to their age or disease duration. The association found between FA and iron parameters, especially % Tf sat, suggests that iron availability to the WM may be a requirement for optimal myelin functionality. Results also suggest that serum iron concentration, ferritin and % Tf sat had an effect on myelination. The lack of association between FA and Hb suggests that the iron in this protein is not available for WM function.

Keywords: Magnetic Resonance Imaging (MRI), Multiple Sclerosis (MS), Diffusion tensor imaging (DTI), Demyelination, Magnetic Resonance Spectroscopy (MRS)

ACKNOWLEDGEMENTS

I wish to thank:

- Professor Penelope Engel-Hills, my academic supervisor, for her guidance, support and always having time to help. I am thankful for her encouragement throughout.
- Professor Susan Janse van Rensburg, my co-supervisor, without whom I would not have finished, for her continued help throughout my research as well as sharing her expertise and knowledge with me. I am very grateful to have had her knowledge as a guide.
- JP Fouche, for sharing his expertise and his willingness always to help and share ideas.
- My husband Bradley and sister Eliza for their on-going support, encouragement and unconditional love.
- My family and friends for their constant encouragement.
- Professor Kidd for doing the statistical analysis.
- Coenie Hattingh for analysing the lesion numbers and for his valuable input.
- Professor Christine Lochner for the use of her controls.
- Helouise Avenant for giving contrast and doing the Disability testing.
- The NRF for providing me with the funding to do the research.
- Shafick Hassan, our faculty head of department at Radiography, for his innitial support in starting this research project.

Opinions expressed in this dissertation and the conclusions arrived at, are those of the author, and are not to be attributed to the University or the University Research Fund.

DEDICATION

To God be the Glory for the wisdom to complete this thesis.

For Bradley & Eliza with love and thanks for your support and encouragement.
Luke and Eli, Mommy loves you dearly.

“The price of success is hard work, dedication to the job at hand, and the determination that whether we win or lose, we have applied the best of ourselves to the task at hand.”

Vince Lombardi

ABBREVIATIONS/ACRONYMS:

ACR	**- anterior corona radiata**
ALIC	**- anterior limb of internal capsule**
ANOVA	**- analysis of variance**
CC_body	**- corpus callosum body**
CC_genu	**- corpus callosum genu**
CC_splenium	**- corpus callosum splenium**
Cg	**- cingulum**
Cg (HPC)	**- cingulum (hippocampus)**
CP	**- cerebral peduncle**
CPUT	**- Cape Peninsula University of Technology**
CNS	**- central nervous system**
CRP	**- C-reactive protein**
CSF	**- cerebrospinal fluid**
CST	**- corticospinal tract**
CT	**- computed tomography**
DICOM	**- digital imaging and communication in medicine**
DTI	**- diffusion tensor imaging**
DTPA	**- diethylenetriamine Pentaacetic Acid**
EC	**- external capsule**
F	**- fornix**
FA	**- functional anisotropy**
FLAIR	**- fluid attenuation recovery**
F/stria terminalis	**- fornix/stria terminalis**
FSL	**- FMRIB Software Library**
Gd	**- gadolinium**
GM	**- grey matter**
GPC+PCh	**- glycerophosphocholine + phosphocholine**
ICP	**- inferior cerebellar peduncle**
LH	**- left hemisphere**
ML	**- medial lemniscus**
MP	**- middle cerebellar peduncle**
MRI	**- magnetic resonance imaging**
MRS	**- magnetic resonance spectroscopy**
MS	**- multiple sclerosis**
NAA	**- n-acetyl-aspartate**
NAA+NAAG	**- n-acetyl-aspartate + n-acetyl-aspartyl-glutamate**

NADH	**- nicotinamide adenine dinucleotide**
PACS	**- picture archiving and communication systems**
PCR	**- posterior corona radiata**
PCT	**- pontine crossing tract**
PD	**- proton density**
PET	**- positron emission tomography**
PLIC	**- posterior limb of internal capsule**
PTR	**- posterior thalamic radiation**
RLIC	**- retrolenticular internal capsule**
RF	**- radiofrequency**
RH	**- right hemisphere**
SCP	**- superior cerebellar peduncle**
SCR	**- superior corona radiata**
SFOF	**- superior fronto occipital fasciculus**
SLF	**- superior longitudinal fasciculus**
SPECT	**- single-photon emission computerized tomography**
SS	**- sagittal stratum**
T	**- tapetum**
TBSS	**- tract-based spatial statistics**
TE	**- time to echo**
TR	**- time to repeat**
UF	**- uncinated fasciculus**
VOI	**- voxel of interest**
WM	**- white matter**
WMTs	**- white matter tracts**
2D CSI 1H-MRS	**- two-dimensional chemical shift imaging magnetic resonance spectroscopy**

GLOSSARY

The following terms and definitions are relevant to this study:

Affine – technique used in diffusion imaging that aligns images in 12 dimensions.

Allele – one of two or more alternative forms of a gene that occupy corresponding loci on homologous chromosomes. Each allele encodes a feature or a certain inherited characteristic. Humans normally have two alleles, one from each parent

Anisotropic – directionally dependent

Apoptosis – a natural process of self-destruction or damage in certain cells, such as epithelial cells and erythrocytes, which are genetically programmed to have a limited life span. Apoptosis can be induced either by a stimulus, such as irradiation or toxic drugs, or by removal of a repressor agent

Atrophy – a wasting or decrease in size or physiologic activity of a part of the body because of disease or other influences. Cells of the brain may atrophy in old age because of restricted blood flow to those areas

Concatenated – linked together in a chain or series

Demyelination – myelin damage

Disseminated – spread widely

Dysarthria – poorly articulated speech resulting from damage to a central or peripheral nerve

Leucoaraiosis - white matter hyperintensities

Locus – a specific place or position, e.g. a locus of a particular gene on a chromosome

Porphyria – abnormally increased production of porphyrins which could lead to a series of genetic diseases

Tumefactive demyelination – aggressive form of demyelination, usually manifesting as a solitary lesion greater than 2 cm that may mimic a neoplasm on imaging

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CHAPTER ONE
AN OVERVIEW OF THE STUDY

1.1 Introduction

Multiple sclerosis (MS) is a chronic disorder related to myelin damage affecting the central nervous system (CNS) and is characterized by multiple areas of demyelinating inflammatory lesions (Reilmann et al., 2012).

It is in this context that a need arises for reliable biomarkers such as Magnetic Resonance Imaging (MRI), which could lead to the early diagnosis and therapeutic intervention when maximum potential impact is possible.

This chapter will broadly examine the rationale for the study, the impact that MRI has as a marker and what sequences give the best images to aid in evaluation of disease progression (which can indirectly be seen as disability) and the early diagnosis of MS that will, in turn, lead to more effective management of the disease.

1.2 Rationale

Current evaluations of MRI scans in the diagnosis of MS involve the appearance of hyperintense lesions on T2 imaging. However, it has been noted that these lesions do not correlate with disability (Simon & Miller, 2007; Davis, 2013). This concurs with Barkhof's opinion when he speaks about the clinico-radiological dissociation being a paradox. The occurrence of axonal damage and white matter changes can be seen prior to being clinically evident (Barkhof, 2002). It was found that conduction was blocked in axons when the demyelinating lesion was around 5mm in length whereas when the lesion was shorter conduction was slowed or still apparent (the lowest impulse train frequency failure was observed at 290 Hz). This would then explain why even though patients have a high number of lesions their clinical disability does not necessarily correlate (Davis, 2013).

1.3 MRI to measure disease progression

There are diverging views in the literature on the methods used to assess disease progression, and several problems have been identified with regard to MRI as a marker of disease progression. It has been shown that the number of enhancing lesions and the rate of grey matter (GM) atrophy (on MRI) are a very good predictor of disease progression especially in the early stages of the disease (Lavorgna *et al*., 2014).

A definite diagnosis of MS is often very difficult; therefore a set of diagnostic criteria, updated in 2001 by the International Panel on the Diagnosis of Multiple Sclerosis, has been

incorporated. These criteria included specific guidelines (the revised McDonald criteria) and consist of a set of clinical, radiological and laboratory rules used to make a definitive diagnosis for MS (Harris & Sadiq, 2009). According to the revised McDonald criteria (Appendix A, that illustrates the MS criteria based on clinical presentation [McDonald, 2001]), clinical criteria can be supported, supplemented or even replaced through the use of MRI as it is both sensitive and specific, often resulting in an earlier diagnosis (Polman et al., 2011).

Even though Grossman (1999) said that MRI is by far the best imaging technique available for the detection of MS lesions, there are constraints with regard to conventional MRI sequences. Conventional MRI sequences such as T1 weighted (T1W), T2 weighted (T2W) and Fluid attenuation inversion recovery (FLAIR) images give poor lesion histopathology, do not demonstrate the full extent of lesions, do not detect microscopic lesions and cannot correlate lesion quantification with regard to clinical findings especially when considering progression of disease.

Barkhof (2002) stated that there should not be a reliance on single MRI sequences but that the multidimensional information provided by MRI should be used for maximum benefit.

Diffusion Tensor Imaging (DTI) does not only provide information on white matter (WM) pathology (Budde et al., 2009) but like Magnetic Resonance Spectroscopy (MRS) it also provides data on axonal injury in MS patients (Bjartmar & Trapp, 2003; Budde et al., 2009). Due to the fact that DTI provides information on the microscopic Brownian motion of water molecules hindered by cell membranes and axonal cytoskeleton, this property makes it sensitive to microstructure integrity disruption often seen in MS (Llufriu et al., 2012). MRS and DTI form an important part of imaging MS pathogenesis and suggest that axonal degeneration may be a determinant of the progressive disability in MS (Bjartmar & Trapp, 2003).

Advanced techniques like DTI and MRS can be used in conjunction with conventional MRI sequences to bridge the gap in order for MRI to truly be recognised as a marker of disease progression in order to aid in the diagnosis of MS and in the management of the disease (Grossman, 1999).

1.4 Focus of the research

The study sought to examine the number of enhancing lesions as well as the hypointense lesions in the brain and compare the enhanced lesions with the expanded disability status scale (EDSS) of subjects at the two extremes of the EDSS score spectrum (subjects with an EDSS of ≤ 3 and those with an EDSS of more than ≥6) in individuals with MS. In addition the

study evaluated the DTI and MRS results of the 2 subsets and compared them with biochemical markers (obtained from blood results) and EDSS scores at the extremes of disease outcome scale spectrums. The thesis also examined whether the lesion number and enhancement of lesions (derived from the conventional MRI sequences) were sufficient in aiding with the prediction of disability of MS, or whether DTI and MRS provided a better aid in predicting disability by evaluating the WMTs of MS subjects ultimately to provide better treatment and monitor disease course of MS. Additionally the DTI parameters were compared to the iron parameters of MS subjects compared to healthy controls.

1.5 Research Problem

One of the diagnostic criteria for MS is that patients have white matter lesions in their brains, caused by inflammation of which cytokines and chemokines are major regulators. Chemokines and cytokines determine the degree of inflammatory activity and play a role in the immunopathogenesis of MS (Harris & Sadiq, 2009). This study on the use of MRI of the brain aimed to identify other imaging indicators together with conventional sequences in order to contribute to predicting the severity of disability by evaluating the WMTs of MS subjects.

The overall aim of the study was to evaluate the MRI scans (conventional MRI sequences as well as DTI and MRS) of female subjects diagnosed with MS for 4 years and more who are at the two extremes of the EDSS score spectrum (those with an EDSS score of < 3 and those with EDSS scores of > 6). Additionally the MRI results were also correlated with laboratory findings (full blood count, serum iron, ferritin, transferrin, Trans fat saturation, C-reactive protein (CRP), cholesterol, serum folate, Vitamin B12, 25-OH Vitamin D, fibrinogen and D-dimer count) as well as clinical outcomes (measured by means of EDSS scores).

1.6 Research Question

Main study questions:

1. Can advanced MRI techniques such as DTI and MRS (together with conventional sequences) in the WMTs of the brain be used to achieve a better understanding of WM function?
2. Do DTI parameters exhibit associations with disability and blood chemistry (ferritin, haemoglobin, % Tf, CRP) in persons with MS?

1.6.1 Research sub questions

- Does lesion number in the whole brain correlate with the EDSS at the two extremes of the EDSS score spectrum and does it correlate with disability?

- Does lesion enhancement in the whole brain correlate with the EDSS at the two extremes of the EDSS score spectrum and does it correlate with disability?
- Do DTI findings (fractional anisotropy, mean diffusivity, axial diffusivity and radial diffusivity) correlate with myelin patency and function between MS subjects and healthy controls in the white matter tracts (WMTs) of the brain?
- Do DTI parameters correlate with EDSS in the two extremes of the EDSS score spectrum?
- Do MRS findings (N-acetyl-aspartate) correlate with disability (as measured by EDSS) at the two extremes of the EDSS spectrum?
- Are DTI and MRS superior in their evaluation of WM integrity and disability compared to conventional sequences?
- Can DTI be used to measure the effect of iron parameters on the WMTs of MS subjects?

1.7 Research Objectives

1. To evaluate the lesion load in the whole brain by counting the visible number of lesions, to assess lesion enhancement, to assess T1 hyper intensity and to compare it to EDSS score.
2. To compare myelin patency and function in the brain between MS patients and healthy control subjects using the DTI parameters, fractional anisotropy (FA), axial diffusivity (AD), radial diffusivity (RD) and mean diffusivity (MD).
3. To correlate DTI parameters (FA, MD, RD, AD) in the WMTs with the EDSS in MS subjects and controls.
4. To correlate MRS outcomes with EDSS and biochemical markers (full blood count, serum iron, ferritin, transferrin, Trans fat saturation, CRP, cholesterol, serum folate, Vitamin B12, 25-OH Vitamin D, fibrinogen and D-dimer).
5. To evaluate whether iron parameters influence the functioning of WMTs by comparing MS subjects to a control group.

1.8 Summary

Subjects with MS have various episodes of relapse and remission. The use of MRI continues to be an effective, non-invasive modality to monitor response to treatment and disease progression in MS patients. Even though conventional sequences like T2W, T1W, FLAIR and Proton Density (PD) are routinely used for screening, MRS and DTI may represent a potentially more effective approach. Thus, the objective of this study is to correlate MRI results (DTI and MRS), biochemical markers and clinical results in subjects with MS at the 2 extremes of the EDSS score spectrum (those with benign outcome of MS - scoring < 3, and those with malignant outcome of MS scoring > 6 on the EDSS scale). DTI and MRS

measures provide a non-invasive approach to the state of myelin and axonal integrity and may provide better correlation with the above markers (Hasan et al., 2005).

1.9 Overview of thesis

A brief overview of the thesis is presented.

In Chapter 2 an in-depth literature review follows – starting with a description of Multiple sclerosis including the different causes of the disease. The chapter concludes with the role of MRI in visualizing the disease and its progression.

In Chapter 3 the research design and methodology of the study will be explained. The data measurement tools, sample design, method of sampling, data collection and analysis as well as the limitations will be discussed.

In Chapter 4 the results will be discussed in detail. Details of the results will be presented in graphs, tables and diagrams. The different data pertaining to the objectives will be outlined.

Chapter 5 presents a discussion of the findings and concludes with a brief summary and relevant links between the results and literature will be reviewed. Recommendations for future research will be provided.

CHAPTER TWO
LITERATURE REVIEW

2.1 Introduction

In this chapter a review of relevant literature is presented as a theoretical framework to enhance the understanding of issues surrounding MRI, MS and how they relate to one another.

The chapter starts with an overview of MS and the MRI protocols used in the study. This thesis aims to give an account of how the selected imaging parameters contribute to the study of WM functionality and pathology, as a measure of demyelination and axonal damage in the various WMTs thus improving the long term management of MS. Special mention is made of the CC as it is the largest fibre bundle that connects the two hemispheres of the brain (Hasan *et al*., 2005) and a common target in the early stages of the disease frequently showing demyelinating lesions and atrophy. Therefore the CC will give insight into the impact MS has on cognitive and physical disability (Llufriu *et al*., 2012). The CC transfers auditory, sensory and motor information and is often associated with human behaviour, cognition, aging and different pathologies (Hasan *et al.*, 2005).

2.2 Multiple Sclerosis

Multiple sclerosis (MS) is a chronic disease of the CNS with an exacerbating pattern. It normally affects young adults, and is twice as common among women as it is among men (Byun *et al.*, 2008; Hitti, 2005; Chilcott *et al*., 2003).

There is common agreement that lesions are heterogeneous and differentiated by inflammation, demyelination, and variable extent of axonal and oligodendrocyte (cells responsible for myelin formation) damage (e.g. Lassmann, 1998; Trapp *et al.*, 1999; Bjartmar *et al*., 2003). The 4 clinical forms of MS were proposed by Lublin and Reingold in 1996: relapsing remitting, secondary progressive, primary progressive and progressive. However, it remains difficult to diagnose patients into subtypes due to the heterogeneity of the disease.

2.2.1 Neuropathology of MS

The lesions of MS are foci of demyelination secondary to inflammation (Trapp *et al*., 1999) and can be observed with Magnetic Resonance Imaging (MRI) scans (Lassmann, 1998; Trapp *et al.*, 1999) (Figure 2.1). The progressive loss of the myelin sheath enfolding the axons results in slow or complete loss of nerve impulse transmission (Reipert, 2004).

“The plaque is the hallmark of MS” (Vogel & Bouldin, 1995:737). Plaques are characterized by loss of myelin in regions where axons might still be preserved. Other features include a

few lymphocytes that are present that cluster around small veins and arteries, an influx of macrophages as well as considerable oedema. Due to the fact that these plaques age, they can damage astrocytes after a while and cause scarring which may impair the structural integrity of the axons (Vogel & Bouldin, 1995:738).

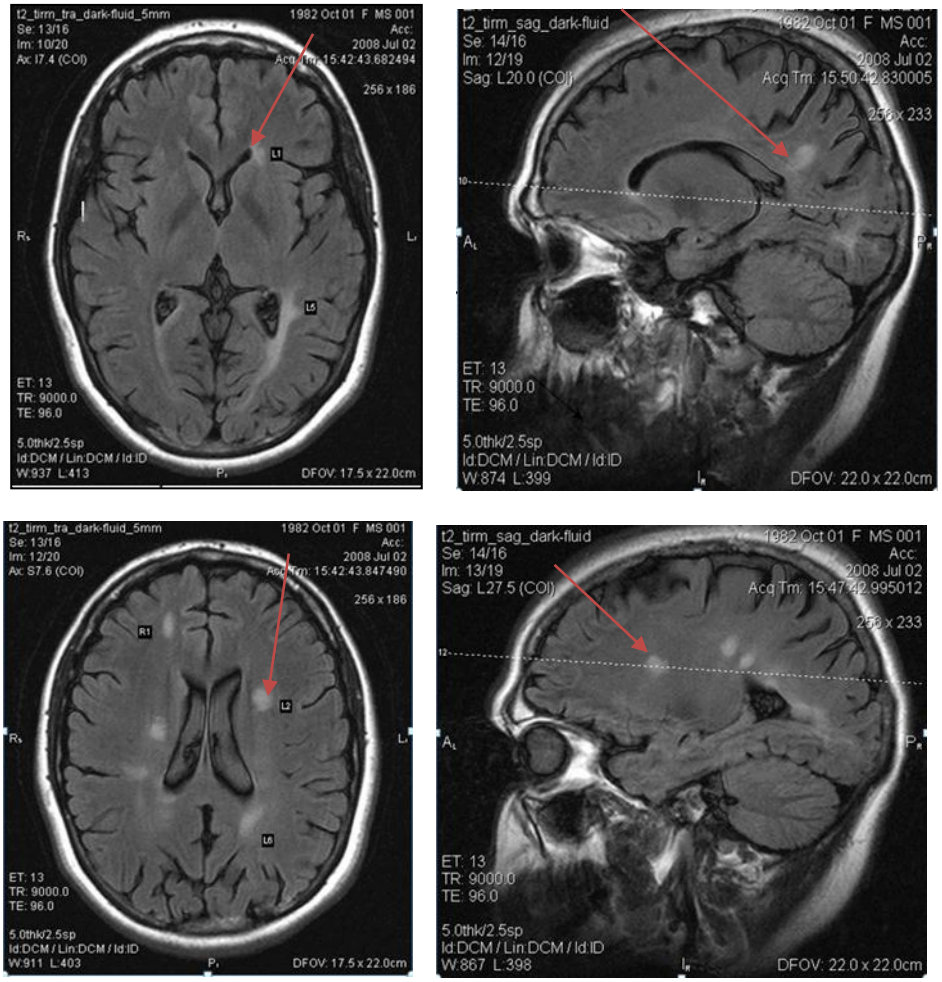


Figure 2.1: MRI Scan of a patient with relapsing remitting Multiple Sclerosis. The arrows indicate lesions formed due to demyelination of the axons and the regions with inflammation. Achieved by T2 FLAIR MRI scans

(Scans with permission of Van Rensburg, 2006)

2.2.2 Pathophysiology

MS is characterized by episodes of progression interspersed by periods of stability. However, some patients do not experience any periods of stability and the disease follows a relentless course without any improvements. Each attack on the myelin is an indication that new plaques are forming. Peripheral nerves are not affected and the areas most vulnerable to the disease are the visual system (Ozturk *et al.,* 2010) and the paraventricular areas. The patient with MS therefore may present with double vision, blurred vision and loss of vision in one eye and/or vertigo. These symptoms are normally associated with plaques in the brain stem and

optic nerve. When plaques occur in the spinal cord it will cause weakness in one or both legs and sensory symptoms in the form of numbness in the lower extremities. Most patients experience a chronic relapsing and remitting course. Functional impairment ranges from minor disabilities to severe incapacity which includes paralysis, dysarthria, severe visual defects, incontinence and dementia (Vogel & Bouldin, 1995:738). Dähnert (2007:310-311) gave a more extended list of symptoms that includes: fatigue, numbness, burning sensation, walking imbalance and co-ordination problems, signs of brain neoplasm (headache, seizures, dizziness, nausea and altered mental status), sexual dysfunction, emotional changes, depression and spasticity.

According to the autoimmune hypothesis on MS, peripheral immune cells penetrate the brain and attack normal myelin. Improvement in disease condition does not restore these damaged cells, nor does it restore function loss, nor disability acquired in the wake of this damage (Ozawa *et al*., 1994).

This view was challenged by Barnett and Prineas (2004) who showed that apoptotic oligodendrocytes are phagocytised by microglia which strip away myelin from axons. After this process, new oligodendrocytes appear which then remyelinate the axons. The blood brain barrier (BBB) becomes compromised if the inflammation caused by the microglia is not kept under control, which in turn activates the peripheral immune system to infiltrate the brain. This ultimately leads to the formation of lesions which can be detected by MRI (Van Rensburg *et al.,* 2006a).

2.3 Disease Classification

Several subgroups of MS have been classified as the disease affects each individual with a different combination of symptoms, differing in severity (and also at different times). The four clinically distinct subgroups include relapsing remitting, secondary progressive, progressive relapsing, and primary progressive MS (Lublin & Reingold, 1996). Symptoms such as impaired movement and disability become apparent in the early stages aiding in disease diagnosis (Miller & Leary, 2007).

2.3.1 Relapsing-remitting Multiple Sclerosis

Relapsing remitting MS can be described by means of patients experiencing episodes or relapses of the disease. Each stage may set off new symptoms or exacerbate the existing ones. Subjects may also undergo remission which fluctuates greatly in this time period as well as the level of severity (Millefiorini *et al*., 1997). During this stage, which may proceed for days to months, the symptoms often decrease or become absent (Lublin & Reingold, 1996; Pittock & Rodriquez, 2008).

2.3.2 Primary-progressive Multiple Sclerosis

In progressive Multiple Sclerosis patients experience a slow progressive, downhill course without any clinical episodes and tend to have fewer MRI lesions (Hafler, 1999). It has been suggested by Rovaris *et al*. (2006) that the median time between onset of disease and progression to the secondary progressive stage is 19 years. One third of relapsing-remitting disease patients go on to have progressive disease (Hafler, 1999).

2.3.3 Secondary-progressive Multiple Sclerosis

This phase is characterised as a progression from the initial relapsing-remitting phase. There are no occasional relapses, plateaus or remissions (Lublin & Reingold, 1996).

2.3.4 Progressive Multiple Sclerosis

A small amount of patients (10%) begin with the progressive form of the disease without ever having any previous episodes of the relapsing-remitting events (Hafler, 1999). Clear acute relapses occur with or without a full recovery (Lublin & Reingold, 1996).

2.4 Epidemiology

MS is a complex disease driven by several factors rather than a single cause. The epidemiology is underpinned by genetics and contributing environmental factors. Other theories concerning the cause of MS include viral infection and autoimmunity (Donati & Jacobson, 2002).

2.4.1 Genetics and hereditary aspects of MS

The major histocompatibility complex (MHC), a group of genes, plays an important role in immune responses and autoimmune diseases such as MS. In MS susceptibility is linked to the HLA gene complex (HLA-DR2 haplotype DRB1*1501, DQB1*0602). It is thus clear that MHC and in particular the HLA-DRB1 locus plays an important role in MS and contributes to disease activity (De Luca *et al.,* 2007).

According to widespread research done on genetic association with MS in Northern Europeans the HLA gene complex was the most consistent genotype found. This gene was also responsible for survival in the pre-antibiotic age, since it has an effect on the enhanced response to infection (Van Rensburg *et al*., 2006b).

2.4.2 MS and the environment

It is known that environmental factors also contribute to MS. Factors such as deficiency in Vitamin D from UV radiation (the prevalence of MS decreases in regions along the equator as exposure to daily sunshine increases) and cigarette smoking have been indicated to be

risk factors for MS development in individuals (Ascherio & Munger, 2007). In a study done by Cullen *et al.* (2012) on smoking in schizophrenia subjects they reported that there was a reduction in FA (a measure of WM integrity). This finding corroborates Ascherio and Munger's (2007) indications about cigarette smoking being a risk factor for MS as this also causes the WM integrity to be compromised. It has also been hypothesised that MS is an infectious disease, caused by Chlamydia pneumonia, human herpes virus-6 (HHV-6) (Korn, 2008) and Epstein-Bar virus (Ascherio & Munger, 2007; Korn, 2008), which was based on genetic material and proteins of microbial agents derived from MS lesions. However, the significance of this association is unclear (Korn, 2008).

Ascherio and Munger (2007) even proposed the poliomyelitis hypothesis, suggesting that a virus increases the risk of MS if acquired in late childhood as opposed to infancy. This led to the hygiene hypothesis which postulates that multiple exposure to infections at a young age is protective against MS. At this stage it was suggested that MS was an autoimmune reaction triggered in individuals as a result of infection by multiple organisms, with an increasing risk at age of infection. In many ways this hypothesis was more acceptable as it explained the latitude gradient issue and individuals born in low-risk areas (Asia, Africa and West Indies) migrating to high-risk areas (Canada, Europe and North America) (Dean *et al*., 1967) as well as MS being more prevalent in those with higher educational backgrounds and income (Ascherio & Munger, 2007). However, in a study done by Ramagopalan *et al*. (2008), an analysis of genes related to autoimmunity that might influence the prognosis of disease outcome found no evidence that any of these genes, in the 45 participants, had an effect on disability in MS.

2.5 Nutrients and myelination

Selzer *et al.* (2003) believe that minerals and nutrients are essential for functioning of many pathways in the body. According to Hallgren and Sourander (1958) and Ropele *et al.* (2011), the brain has been indicated as one of the most iron rich organs in the human body. Iron is needed for energy in the mitochondria as a production mechanism. In order to produce myelin, oligodendrocytes need iron which are essential for the functioning of certain groups of cells (Gally *et al.,* 2005). Therefore according to Connor *et al*. (1993) and Levine (1991) oligodendrocytes are rich in iron as it is found abundantly in brain tissue. Oligodendrocytes located in white matter regions of the brain, are primarily responsible for myelin production and regeneration and require iron both directly and indirectly (Connor & Menzies, 1996). Iron is also an important component of biosynthesis in order to produce cholesterol and lipids that are key components of the myelin sheath (Larkin & Rao, 1990).

Iron-requiring enzymes and coenzymes, which catalyse the two pathways both for synthesis and degradation of lipids and cholesterol, are enriched in oligodendrocytes (Cammer, 1984; Tansey & Cammer, 1988). Oligodendrocytes carry out the biosynthesis of cholesterol and lipids at a higher rate than any other cells in the brain (Cammer, 1984). Enzymes such as glucose-6-phosphate dehydrogenase, succinic dehydrogenase, NADH (Nicotinamide adenine dinucleotide) and cytochrome oxidase also require iron for their catalytic action in oxidative metabolism (Cammer, 1984).

The above highlights the importance of iron in the myelination process. Its deficiency affects activities being carried out in the oligodendrocytes and thus myelin production.

In a study done on a single patient with MS who took iron supplements daily including other nutrients, she experienced stabilisation of her disease as well as no further degeneration (Van Rensburg *et al*., 2006a).

Van Rensburg *et al.* (2006b) also report that vascular damage has been indicated in MS subjects who have high homocysteine levels, and iron deficiency may also be linked to increased homocysteine levels. This concurs with what Davis et al. (2014) say in their article, which is that raised homocysteine levels are caused by various factors such as obesity (with a body mass index (BMI) of 27kg/m2 at age 20 years), age (increases with age), alcohol, smoking, impaired renal function and certain drugs. The authors also mention that elevated homocysteine levels are often associated with dysfunction of the CNS, neurodegenerative diseases and cerebrovascular diseases and could thus have a link with cognitive dysfunction. The elevated homocysteine levels cause an increase in cholesterol levels as it stimulates the secretion of cholesterol in the hepatic cells.

If iron deficiency occurs over a long period of time it could result in gastritis followed by atrophy of glands in the stomach that produce intrinsic factor. The outcome of this is the reduction of vitamin B12 uptake (Van Rensburg et al., 2006b). The folate vitamin B12-methyl transfer cycle needs to function effectively as it is necessary for the production and maintenance of myelin (Van Rensburg et al., 2006b). Therefore the folate-vitamin B12 methylation pathway needs essential co-factors like folate (vitamin B9), pyridoxine (vitamin B6) and cobalamin (vitamin B12) for functioning of several enzymes, otherwise it can also give rise to increased homocysteine levels which is a marker of dysfunctional methylation (Davis et al., 2014).

In contrast to what Van Rensburg *et al.* (2006a) said, Zamboni (2006) found in his research that iron deposition in the brain may cause MS. This author states that periventricular iron

deposits formed in and around the brain, resemble those seen in and around the veins in chronic deep vein thrombosis, causing oxidative damage.

Zamboni (2006) called this chronic cerebrospinal venous insufficiency (CCSVI), which could be improved by cerebral venous drainage. Even though the medical community rejected this hypothesis it generated much optimism among those with MS, as cerebral venous drainage was said to rapidly improve disability in MS subjects. Considering that, Van Rensburg *et al.* (2012) mentioned that standard treatments like interferon-β might cause worsening effects in patients due to the fact that even though it reduces relapses, it has a limiting effect on disability. Also opposing the explanation of Zamboni, Rooney *et al.* (1999) found porphyria-like symptoms and a history of iron deficiency anaemia in his study of a family of Scottish descent. Additionally Van Rensburg *et al.* (2006a) found low serum iron concentrations in 50% of patients with MS. This could mean that neurodegenerative diseases which cause demyelination, like MS, could be affected by iron deficiency.

In addition to iron, one of the pathways involved in myelin production, is the folate-vitamin B12-methyl transfer pathway. Several polymorphisms in this pathway have been found in MS patients. Therefore deficiency in these nutrients could play an important role in myelin production (Selzer *et al*., 2003) which in turn has an effect on poor cognition in school going children (Jahanshad *et al*., 2012). In the absence or deficiency of minerals such as iron, vitamin D and folate-vitamin B12 genetic defects can be triggered resulting in the onset of the disease. Additionally, inhibition of these pathways through mutation, the use of nitrous oxide during anaesthesiology and increase in the level of homocysteine may result in demyelination and neurodegeneration (Van Rensburg *et al*., 2006a).

It is also not known whether blood iron parameters correlate with FA; however, Mahajan *et al.* (2011) found that a Tf sat of <16% was associated with behavioural deficits in Indian children with attention deficit hyperactivity disorder (ADHD). Piñero et al. (2000) showed that when systemic iron delivery is altered in brain regions of developing rats they regulate their iron concentration in response to the environment, as changes in the levels of dietary iron affect the metabolism of brain iron.

2.6 Fatty acids and their role in myelination

According to Peters et al. (2012) polyunsaturated fatty acids (PUFAs) contribute to myelination and are the building blocks of all cell membranes and the oligodendrocyte membranes which form myelin sheaths around the axons.

In studies done in rats it was determined that a PUFA deficiency changes the myelin structure, but when these rats were supplemented with linoleic acid (LA - omega-6 fatty acid) they showed marked protective effects.

Peters *et al*. (2012) say a diet supplemented with omega 3-fatty acids will promote the stimulation of myelin proteins in rat brains and decrease the disability rate in humans whose WM integrity is compromised or those that have MS.

Erythrocyte membranes have been used extensively as a model for brain cell membranes (Van Rensburg *et al*., 2009). Peters *et al*. (2012) previously reported a relationship between total erythrocyte membrane PUFA concentration and WM integrity, which leads to psychosis and first-episode (early onset) schizophrenia which also happens in MS.

Peters *et al*. showed that there was a decrease in radial diffusivity (RD) which was an indication of disturbances in myelination. Moreover, PUFA concentration was reduced which could be related to the WM integrity being compromised as a result of inflammation or oxidative stress. Nervonic acid (NA - mono-unsaturated omega-9 fatty acid) proved to be an important building block of myelin membranes during the first years of brain development, indicating that a decrease in NA concentrations could negatively affect myelination (Peters *et al*., 2012).

2.7 MS in Africa

In South Africa the prevalence of MS is relatively low. According to a study done by Modi *et al*. (2008), South Africa is known as a medium-frequency region with 5-30 MS patients per 100,000 population. In a report done by Rosati he found that in 1960, after an extensive survey among clinicians and hospitals, there was a prevalence of 13 English speaking white South Africans diagnosed with MS and 4 Afrikaners, with no Black people diagnosed, suggesting that there are differences in susceptibility (Rosati, 2001). Previously Dean in 1960 reported six MS cases in Black South Africans and in a study done in Cape Town in 1985, 3 coloured South Africans were reported.

Furthermore, contrary to the claims by physicians that MS does not exist among Blacks in Africa (Rosati, 2001), in 1987 Dean reported 12 patients in total with 7 in South Africa and 5 in Zimbabwe (Dean *et al*., 1994). Subsequent research has shown that there are only a few cases in Sub-Saharan Africa (Rosati, 2001).

In Modi *et al.*'s (2008) study it was reported that the respondents were predominantly female and white which correlates well with literature. In patients with mixed ancestry and of Indian

descent only 3 of the population studied were reported as affected (Modi *et al*., 2008). In the Canary Islands MS was reported in 6 patients in Las Palmas and 15 in Lanzarote, lower than reported in Spain in 1990. This suggests that there is a significant influence of location on the risk of MS for people of the same ethnicity (Rosati, 2001). Researchers found that there are some uncertainties surrounding the aetiology of MS with regard to migration studies. People who move from high risk areas like Europe to low risk areas like South Africa carry with them their original risk factors, which are more prevalent after the age of 15 years (Brassington & Marsh, 1998). This was interpreted by Ascherio and Munger (2007) as signifying that viral infections early in life could protect an individual from MS (**see 2.4.2**).

2.8 Disability

Disability in MS is measured by an objective instrument, the Expanded Disability Status Scale (EDSS; Kurtzke, 1983), which is used in all clinical studies as a "gold standard". The EDSS scores range from 0 to 10, with higher scores indicating higher disability. Thus 0 (for no disability) to ≤ 3 is regarded as "benign", while 5 to 6 indicates moderate impairment and > 6 indicates more severe impairment. The EDSS is divided into eight functional systems: pyramidal, cerebellar, brainstem, sensory, bowel and bladder, visual, cerebral and "other". Half point scores are also allocated from 0.0, 0.5, 1, 1.5, 2,….9.5 and 10 for death with MS as a cause (Appendix B). With a score of 0.0–4.0, people are able to walk without assistance and scores from 4.0–7.5 are determined by how far the person can walk and whether assistance is necessary. Point 6 on the scale is allocated to persons walking with a cane. When a patient has a score of 7.5–10.0 it mainly determines the person's ability to transfer from a wheelchair and to take care of him-/herself (Jelinek, 2010).

The associations between lesion load on T2W MRI and clinical disability are often weak, therefore DTI has been investigated by many authors to determine whether this modality would provide improved associations between structural WM changes and disability (Lyksborg *et al*., 2014; Hasan *et al*., 2005).

Liu *et al*. (2012) found significant inverse correlations between diffusion metrics and the EDSS in the CC splenium, left cingulum bundle and bilateral corticospinal tracts. Llufriu *et al.* (2012) found lower average FA in MS patients than controls in the CC; however, no correlations were observed with the EDSS. Memory deficits correlated with DTI in the fornix (Koenig *et al*., 2013). Altered cognitive scores were significantly associated with decreased FA and increased MD in all examined WMTs (Cesar *et al*., 2015).

2.9 Magnetic Resonance Imaging

MRI is a non-invasive imaging modality which provides information on tissue structure and function and is an important adjunct to clinical assessment and monitoring of MS that manifests in the brain and spinal cord. Quantitative MRI technique sequences are highly sensitive for the definition of lesions and, in addition, have an excellent ability to detect multifocal lesions, diffuse disease and macroscopic atrophy. MRI has also become an important part of clinical imaging to assess disease progression (Gauthier *et al*., 2009).

MRI is based on the principle that positively charged hydrogen nuclei (protons) emit signals that produce images. The presence of hydrogen atoms available in intra- and extracellular water, lipids and other molecules, found in MS plagues in the body facilitates the use of MRI. In response the magnetic field, the protons in an individual align with the longitudinal or z-axis of the magnet in a state of equilibrium. The protons spin around the z-axis in a state called precession. There is a proportional relationship between precession frequency and magnetic field strength that can be calculated by using a constant known as the Larmor equation. In order to disturb the protons from their state of equilibrium a radiofrequency (RF) pulse equal to the precession frequency is applied. The RF pulse rotates the protons from the longitudinal/ z-axis to the transverse (XY) axis. A 90º RF pulse rotates the protons into the XY plane whereas a 180º RF pulse produces twice the degree of rotation (Stone et al., 2005).

T1 weighted image (also referred to as T1WI or "spin-lattice" relaxation time) is one of the basic pulse sequences in MRI and demonstrates differences in the T1 relaxation times of tissues. A T1WI relies upon the longitudinal relaxation of a tissue's net magnetisation vector (NMV). This means that spins aligned in an external field (B0) are put into the transverse plane by an RF pulse. They then slide back toward the original equilibirum of B0. Not all tissues get back to equilibirum equally quickly, and a tissue's T1 reflects the amount of time its protons' spins realign with the main magnetic field (B0). T1 weighting tends to have short TE and TR times (Greenberg, 2010:129-134; Westbrook, 2015:21-34).

Fat quickly realigns its longitudinal magnetization with B0, and it therefore appears bright on a T1 weighted image. Conversely, water has much slower longitudinal magnetization realignment after an RF pulse, and therefore has less transverse magnetization after a RF pulse. Thus, water has low signal and appears dark (Westbrook, 2015:21-34).

If T1WIs did not have short TRs, then all the protons would recover their alignment with the main magnetic field and the image would be uniformly intense. Selecting a TR shorter than the tissues' recovery time allows one to differentiate them, for example tissue contrast. T1-weighted sequences provide the best contrast for paramagnetic contrast agents (e.g. gadolinium-containing compounds) (Westbrook, 2015:21-34).

T1-weighted sequences include:

- T1W spin echo (SE)
- T1W gradient echo (GRE)
- gadolinium postcontrast sequences (gradient echo sequences)
- time of flight 2D or 3D MR angiography sequences
- contrast-enhanced MR angiography
- dual echo sequence (in-phase and out-of-phase) (Westbrook, 2015:21-34).

T2 weighted image (also referred to as T2WI "T2 weighted image") is one of the basic pulse sequences in MRI. The sequence weighting highlights differences in the T2 relaxation time of tissues. A T2WI relies upon the transverse relaxation (also known as "spin-spin" relaxation) of the net magnetisation vector (NMV). T2 weighting tends to require long TE and TR times (Greenberg, 2010:129-134; Westbrook, 2015:21-34).

One way to think about T2 relaxation is as follows:

- after an RF excitation pulse, there is relaxation of the spins from the transverse plane toward the main longitudinal magnetic vector (B0). This is T1 weighting.
- At the same time, spins are decaying from their aligned precession in the transverse plane. Differences in this decay are captured in T2 weighting (Greenberg, 2010:129-134; Westbrook, 2015:21-34).

The amounts of T2 decay a tissue experiences depends on multiple factors. Each tissue has an inherent T2 value, but external factors (such as magnetic field inhomogeneity) can increase the T2 relaxation time. This additional effect is captured in T2*. The refocusing pulse in spin-echo sequences helps to mitigate these extraneous influences on the T2 relaxation time, trying to keep the image T2 weighted rather than T2* weighted. Paramagnetic contrast agents (e.g. gadolinium-containing compounds) do not cause the same bright tissue contrast as they do on T1WI. Gadolinium shortens T2 relaxation time and actually results in hypointense signal. If the TE is extended out over a very long time, only tissues with a very long T2 relaxation time will retain signal (Westbrook, 2015:21-34).

2.9.1 MRI in MS

A substantial amount of literature exists around MRI and its role in the diagnosis of MS.
Before the advent of MRI, diagnosis of MS was based solely on clinical indicators. This resulted in delayed diagnosis as well as misdiagnosis. For example, acute porphyrias may mimic MS (Rooney *et al*., 1999). Therefore, the development of MRI which identified lesions in the brain is seen as a breakthrough in MS diagnosis and follow-up. Additionally advanced

approaches like DTI and MRS have been extensively utilised in assessing WMT analysis in the brains of MS subjects (Liu *et al*., 2012).

2.9.2 Routine Sequences for MS

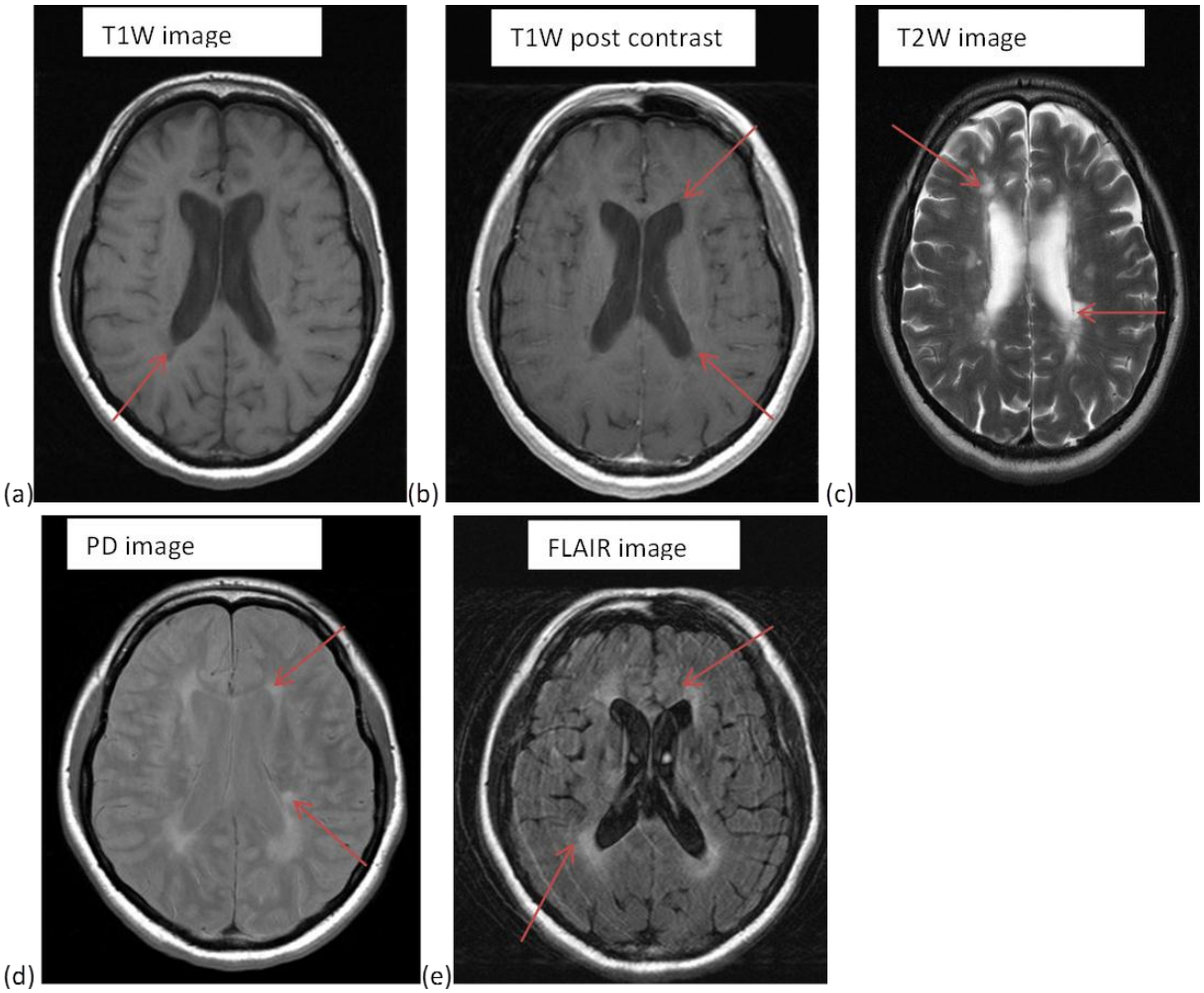


Figure 2.2 MRI of MS lesions. Arrows point to demyelinating lesions. (a) Axial T1-weighted, lesions has decreased signal intensity. (b) T1-weighted post contrast image. (c) The axial T2-weighted, lesions oriented perpendicular to the lateral ventricle is an example of Dawson's fingers. (d) MS lesions are hyperintense on PD images.
(e) Axial T2-weighted FLAIR (fluid-attenuated inversion recovery) image. Note that the free fluid (cerebrospinal fluid) appears dark with FLAIR.

(Permission given by subject MS05)

2.9.2.1 Proton density

Image contrast where the T1 and T2 weighting is minimised so that the contrast of the image is determined by the number of proton spins (amount of water). Requires a short TE and long TR. Proton density or PD (unlike T1 and T2) is not measured routinely. Proton-density weighted images maybe used for native tissue T1 correction in contrast enhanced data. They also demonstrate the white and grey matter differences in the brain. At long TR (12 s), CSF

appears bright (100% PD), followed by grey matter (78%) and white matter (70%). PD may differ from true water content due to short T2 components, which are not seen in MRI (Westbrook, 2015:21-34).

2.9.2.2 Fluid attenuated inversion recovery (FLAIR)

FLAIR imaging has an increased sensitivity for MS lesions especially white matter abnormalities. The majority of MS patients have the typical MRI scan findings of oval lesions with well-defined margins on either side of the two ventricles. This pattern is known as Dawson's fingers in and around the CC or in the subcortical white matter, middle cerebellar peduncle, pons, or medulla (Stone *et al*., 2005) and can be clearly seen on FLAIR sequences (Figure 2.2).

2.10 Advanced metrics

The use of advanced MRI sequences in MS is becoming more common especially with the increasing use of higher strength magnets.

For neurodegenerative diseases like MS a standard set of sequences (T1, T2 weighted, PD and FLAIR) are generally used (Stone *et al*., 2005). Song *et al.* (2005) suggest that conventional techniques are not sufficient to differentiate between axonal and myelin pathology in WM disorders. These authors advocate that other advanced technologies like DTI should be used to assess WM and axonal damage as it is highly sensitive to microscopic structural changes in tissue. Abe *et al*. (2010) agree that DTI is the neurological imaging sequences of choice as it gives information on the physiologic state of lesions not seen on T1W, T2W or PD images, while MRS provides information on the biochemistry of selected brain tissue (Ponnada & Narayana, 2005).

2.10.1 Diffusion tensor imaging (DTI)

DTI, a derivative of Diffusion weighted imaging (DWI), is a form of MR imaging based upon measuring the random Brownian motion of water molecules within a voxel of tissue. A great deal of confusion exists in the way the clinicians refer to diffusion restriction. The first problem is that the term "diffusion weighted imaging" is used to denote a number of different things:

- isotropic diffusion map (what most radiologists will refer to as DWI)
- sequence which results in generation of DWI, b=0 and ADC maps
- a more general term to encompass all diffusion techniques including diffusion tensor imaging (Hagmann *et al*., 2006; Moritani *et al*., 2009:1-43).

DTI, specifically, has been widely applied in imaging of the CNS for tissue morphology and pathology (Song *et al*., 2002) as it provides important insight into the neurobiological basis

for normal development, aging and disease processes of the brain and spinal cord (Abe *et al*., 2010) based on the diffusion of water (Price *et al*., 2005).

The normal direction of flow in the brain tends to be parallel to the nerve fibre tracts. When the myelin and axons undergo destruction, the rate of diffusion and direction of flow are altered significantly (Stone *et al*., 2005).

The interaction of water molecules with sub cellular structures is called “diffusion anisotropy”, where diffusion occurs parallel rather than perpendicular to an axon or myelin sheath (Abe *et al*., 2010), as it is easier to move along the fiber axis than traveling across (Song *et al*., 2002).

The characteristics of diffusion are also influenced by several CNS tissue components, cell membranes and organelles. Therefore if there is any pathological process causing restrictive barriers it could lead to an increase of diffusivity and decreased directionality (implying more space for diffusion to occur; Ge, 2004). As the diffusivity is dependent on the magnitude of the direction in which it is measured, the diffusion tensor (a matrix correlating the molecular displacement along an orthogonal direction), is the only manner in which to obtain its full character. From the diffusion tensor value the mean diffusivity (MD) can be derived, which is independent of the tissue. This can also be done for other indices like anisotropic diffusion which includes fractional anisotropy (FA) (Rovaris *et al*., 2008), the most commonly studied diffusion parameter (Imfeld *et al*., 2009). FA values are an indication of the damaged lesions (Shen *et al.,* 2014) and an index of decreasing WM integrity (Kochunov *et al.,* 2007; Imfeld *et al*., 2009) that is often utilised to separate the WM from the non-WM tissue. A decrease in FA values could indicate demyelination of the neuronal tissue (Shen *et al*., 2014) as it indicates the directional selectivity of water molecule movement within tissue. High FA values (maximum value 1.0) will be observed in heavily myelinated WM tracts. In CSF and grey matter (GM) water motion is random and can thus be described as isotropic diffusion (FA values is close to 0). Therefore when water motion is restricted anisotropic diffusion occurs. Anisotropic diffusion can be represented by FA, relative anisotropy (RA) and volume ratio (VR), which are eigenvalues that can be applied mathematically to produce formulas and calculations on which diffusion tensor measurements are based in order to make 3-dimentional images (Liu *et al.,* 2007). Therefore, as mentioned, when FA values decline it is indicative of degenerative changes in the structure of WM tracts. Based on the latter, FA is sensitive to various progressive WM disorders such as MS that result in axonal loss and the destruction of myelin (Kochunov *et al*., 2007) and thus has the potential to detect WM integrity early on in the disease course (Hasan *et al*., 2005).

Other indices of diffusivity are axial diffusivity (AD – diffusion along the axons) and radial diffusivity (RD – diffusion perpendicular to the axons). In this instance FA is based on the relation between RD and AD (Imfeld *et al*., 2009). Mean diffusivity (MD), a third index used when tracing a tensor, is a reflection of the magnitude of the diffusion (Cassol *et al.,* 2004). In order to investigate the changes in WM integrity in the brain Tract Based Spatial Statistics (TBSS) was employed to analyse the data. TBSS is an innovative technique for whole brain analysis. It uses voxel-wise statistics in conjunction with conventional voxel-based analysis on diffusion metrics, minimising the effects of misalignment (Liu *et al.,* 2012).

2.10.1.1 DTI in the assessment of WMTs of the brain

The cerebellum communicates with other brain areas via the superior, middle and inferior cerebellar peduncles and is connected to the cerebral cortex, limbic system and thalamus. According to Wang (2003) the cerebellum has always been a region which coordinates motor function. It was also discovered that the cerebellum could be the main focus of higher cognitive processes (Wang *et al.,* 2003). According to Martini (2006), the brainstem fibres are involved in programming and fine-tuning movements controlled at conscious and subconscious levels.

Association fibres include the superior longitudinal fasciculus (SLF), inferior longitudinal fasciculus, inferior fronto-occipital fasciculus (IFOF) and the uncinate fasciculus (UF) (Wakana *et al*., 2004).

The SLF is association fibre bundles connecting the frontal, occipital, parietal, and temporal lobes (Wakana *et al.,* 2004) and is especially important as it has a link with expressive language areas (Bernal & Altman, 2010).

These fibres connect the temporal areas of the brain that are involved in lower and higher order hearing processing, with frontal brain areas involved in the control of several important functions (including memory and attention). The SLF is also known as the primary pathway for processing and production of speech and music (Imfeld *et al.*, 2009).

The UF is the major fibre tract connecting the inferior frontal and anterior temporal lobes (of which the amygdala is a part). The UF plays a role in the formation and retrieval of memories (Diehl *et al*., 2008). Together all these association fibres interpret incoming information coordinating a motor response (Martini, 2006).

Projection fibres connect many areas of the cortex with the brainstem and include the internal capsule, posterior thalamic radiation, cingulum and optic radiation (Wakana *et al*.,

2004). These fibres control autonomic functions which integrate conscious and unconscious sensory information as well as motor commands (Martini, 2006).

Limbic System Fibres include the cingulum (Cg), cingulum (hippocampus), fornix (F) and stria terminalis (Wakana *et al.,* 2004). The limbic system controls the emotional state, linking conscious intellectual functions of the cerebral cortex with the unconscious and autonomic functions of the brainstem (Martini, 2006).

Callosal Fibres connect and permit communication between the corresponding areas of the opposite hemispheres (Martini, 2006). This includes the corpus calossum (genu and splenium); forming strong projections that collectively form the tapetum (Wakana *et al*., 2004).

DTI is becoming increasingly available and provides neuroradiologists and other investigators with the ability to assess white matter abnormalities and characterise white matter fibre bundles (Mori *et al.,* 2005). The 48 WMTs are based on a WM atlas by Mori et al. (2005). The atlas was used to identify the tracts in FSL (FMRIB Software Library) and to extract values that could be statistically analysed using Tract Based Spatial Statistics (TBSS) to extract values that could be statistically analysed. Therefore these 48 WMTs were selected for inclusion in this study due to their involvement with movement and cognition. These are also the only tracts that can be accurately identified with the DTI resolution currently available at CUBIC.

Through better understanding of the WMTs the clinical management of patients with specific neurological or psychological deficits can be improved (Hasan et al., 2009).

2.10.2 Magnetic resonance spectroscopy (MRS)

MRS has been widely used to assess WM integrity and axonal loss. Concentrations of N-acetylaspartate and N-acetylaspartylglutamate (NNA), creatine and phosphocreatine (tCr), choline-containing compounds (Cho), myo-inositol (mi) and lactate (Lac) could indicate metabolic disturbances of neuro-axonal damage (Dreha-Kulaczewski *et al.,* 2009).

The brain spectrum on MRS studies shows 3 peaks representative of the components of creatine (CR), which gives an idea of cellular energy metabolism, choline (Cho), associated with cell membranes and N-acetyl-aspartate (NAA), a marker of neuronal integrity as this metabolite is localised in the neurons and neuronal processes of the mature brain (Grossman, 1999; Ramin *et al.,* 2003; De Stefano *et al*., 1998). According to Ramin *et al.* (2003) the NAA peak in spectroscopy will be decreased if there is neuronal loss. The

presence of NAA resonates at 2.02 parts per million (ppm). CR peaks at 3.02ppm/ 3.94ppm and is mainly present in the grey matter. Cho is concentrated in the white matter and peaks at 3.2ppm.

Arnold and Matthews (1996) reported a rise in Cho resonance early in the demyelinating process followed by a drop in NAA concentration if the lesion was severe. Literature states that low levels of NAA normally reflect axonal damage in the brain, associated with clinical disability that occurs secondary to inflammation and demyelination (Richert & McFarland, 1998; De Stefano *et al*., 1998). Therefore most recent studies suggest MRS may provide indices that are more closely linked to neurological impairment in MS patients and as such would be a good marker for disease progression (Narayana, 2005).
Even though contrast enhanced images are of great value when used with conventional imaging, techniques like MRS have the specificity to detect and differentiate between neuronal and glial abnormalities in the absence of visible injury (Alam et al., 2011), and it is also strongly correlated with disability (Blamire et al., 2007). By looking at the Cho/Cr and Cho/NAA ratios lesions can be characterised as neoplastic and non-neoplastic (Alam et al., 2011).

2.11 MRI and contrast

Simon *et al*. (2006) recommend that gadolinium-chelate always be used for suspected MS patients, because a diagnosis may be less well visualised or even missed without contrast administration. According to Ge (2006) contrast enhanced T1W images are able to detect disease activity more often than clinical evaluations when relapses occur. This could mean that most of the lesions that enhance are silent during the clinical examination. Gass and Richards (2013) agreed with Ge, stating that grey and white matter that appears normal on conventional MRI may be affected in the early phases of the disease and therefore only enhance with contrast.

The brain normally contains paramagnetic substances such as hydrogen, and when a contrast medium is administered it shortens the relaxation times of the protons (thus changing the signal intensity), an effect called proton relaxation enhancement. The MRI agent most often used to enhance visualisation of lesions is gadolinium-chelate. Gadolinium as a contrast agent is said to be much safer than iodine based contrast media as it is non-toxic due to the fact that it is bound to DTPA (diethylene triamine pentaacetic acid) in a process called chelation. However it should be noted that Gadolinium is toxic in its free state, as it is a rare earth substance (Schild, 1990).

It was established that enhancement of active lesions are due to breakdown of the blood brain barrier (BBB) that occurs at sites of inflammation (Kermode *et al*., 1990) allowing Gadolinium to cross the BBB from the blood into brain tissue. This correlates with impaired function shown by neuropathological studies where Gadolinium was administered (Kappos *et al.,* 1999). Breakdown of the BBB is the first event that can be detected on conventional imaging and is seen as a measure of inflammatory activity (Ge, 2006).

A common hypothesis of MS is that it is due to an acute onset of inflammation, and it has always been the focus of MRI to measure the inflammatory process. It is also a known fact that when contrast agents are administered to patients with MS it could indicate new lesions as the contrast enhancement is related to acute inflammation (Ge, 2006). Ge also stated that the T1 lesion load (enhancing lesions and non-enhancing lesions) correlated better with disability than T2 lesions.

2.12 MRI lesion number

According to Barkhof (1999) MRI is one of the imaging modalities that sensitively shows how lesions are spread in the CNS. Lesion number is known as the number of T1W Gadolinium enhancing lesions, new T2 lesions or active (which are new or enlarging T2 lesions). Location of the lesion is critical; lesions that occur in the spinal cord contribute disproportionately to disability as measured by EDSS (McFarland, 1998). However according to Arnold and Matthews (1996) clinical measures alone reflect lesion load poorly and underestimate disease activity. Llufriu (2012) reported that abnormally reduced FA within the motor part of the CC may be found even without the presence of lesions. MS lesion load (i.e. volume of lesions) is determined with computerised techniques that reflect an objective and precise measure of counting the extent of disease abnormality (Ge, 2006). This does not necessarily relate to the physical lesion number. The lesion number involves manually counting the number of enhancing lesions and non-enhancing lesions and is a sensitive way of assessing MS activity. Lesions that enhance upon administration of contrast (clinically silent lesions) occur five times more often than clinical relapses and may not always accompany symptoms (Barkhof, 1999; Rovaris & Filippi, 2000). The poor relation between clinical disability and lesion loads seen on T2-weighted MRI has motivated the search for other MRI markers which may reveal the disease-related structural changes leading to disability (Lyksborg, 2014).

2.13 Limitations of MRI

MRI is the imaging method of choice in MS (Ge, 2006). However Levine *et al.* (2007) raised awareness of the risks associated with MRI. This generally arises from the three components used to produce images: a static magnetic field pulsed RF fields and the time-varying

gradient electromagnetic fields (EMF). For large static magnetic fields, the hazards are mainly biological effects, projectiles, implant malfunction and movement, and monitoring device malfunction and movement. The RF hazards are tissue heating and implant heating. For the time-varying gradients, the main concerns are peripheral nerve stimulation and acoustic noise, as well as potential implant or monitoring device interference (Levine *et al*., 2007).

Therefore screening of the patient before scanning is of utmost importance in order to minimise the risk to the patient. This involves questioning patients on whether they have implants such as aneurism clips, cochlear implants and metallic prosthetic joint implants. Dental implants or orthodontic wires and tattoos, body rings or even mascara could cause heating effects, but would not be life threatening, whereas jewellery and hair accessories will cause artefacts on the images. More seriously, drip stands, wheelchairs, scissors and any weapons have the potential to become projectiles. Patients are therefore instructed to disrobe and put on hospital clothing before they are imaged (Bushong, 2003).

2.14 Summary

In this chapter MRI as a tool in predicting the severity of disability in the WMTs of MS subjects was discussed. The main focus was on how imaging sequences like DTI and MRS complement conventional sequences in making a better diagnosis. The literature suggests that using advanced MRI approaches in conjunction with conventional imaging will provide valuable information about the similarities and differences between the MRI imaging results, biochemical tests and EDSS scores. All of these tools are useful in studying the structural and functional changes associated with the clinical characteristics found in MS.

Chapter 3 will focus on the methods used for this study.

CHAPTER THREE
MATERIALS AND METHODS

3.1 Introduction

This chapter describes the research process for this study. To contribute to the management of MS in South Africa this study sought to determine the role of conventional MRI measures, DTI (fractional anisotropy, radial diffusivity, mean diffusivity and axial diffusivity) and MRS in the WMTs of MS subjects as well as the effect of the different iron parameters on these tracts.

3.2 Ethical consideration

This study contributes to an existing study done by the University of Stellenbosch. A protocol on MS, including MRI scans, was approved by the Ethics Committee of the University of Stellenbosch (N07/09/203) (see approval letter N10/01/021 – **Appendix C**). The details of the MRI procedures as outlined in the protocol were also sent for approval to the Health and Wellness Science Research Ethics Committee of Cape Peninsula University of Technology (CPUT). Ethics approval was granted in September 2009 by CPUT according to the ethical guidelines provided by the Declaration of Helsinki [(World Medical Association, 2013), (approval letter – **Appendix D**)]. Permission, in writing, to scan the MS subjects at Cape Universities Brain Imaging Centre (CUBIC) has been granted (**Appendix E**).

Informed written consent was obtained from all study subjects (see informed consent form – **Appendix F**) for undergoing MRI examinations and for review of their records and images. All forms were translated from English into Afrikaans and Xhosa to ensure that the information was clearly understood by all the subjects. However, all of the subjects were Afrikaans or English speaking so the Xhosa version was never used.

All subjects in the study had adequate information regarding the research, were capable of comprehending the information and were capable of making an autonomous choice, thus enabling them to consent to or decline participation in the research voluntarily. Only those who signed the consent form and completed the screening form were included in the study. The subjects had the right to decide at any point to terminate their participation, or to refuse to give information, or to ask for clarification about the purpose of the study or specific procedures. Both the MRI and the administering of contrast media pose a low risk to the subjects (Shellock & Spinazzi, 2008) and were done by registered professionals who explained the procedures and conveyed all the risks involved. Together with the consent form subjects also had to fill in an MRI screening form (**Appendix G1** [master] and **Appendix G2** [example of completed form]). The subjects underwent a thorough screening procedure as they should not have any metallic implants and devices that would interfere with the

magnetic field or cause harm to them (Shellock & Spinazzi, 2008) (see screening form – **Appendix G1**). Most important to the issue of patient safety during MRI examinations is the maxim that no harm be done (Shellock & Spinazzi, 2008). If there was any question as to the safety of the MRI study, the examination was not done and the subject was excluded. The subjects could make a voluntary decision on their participation in the study, without the risk of harm or prejudicial treatment (Berg & Latin, 2004).

Individual results were confidential and the information and results were locked away in a password protected cabinet in the research centre. The names of subjects were only known to the researcher for quality assurance purposes. Personal information will only be released with the subjects' consent should the need arise.

3.3 Overview of the study

This study was conducted to add the dimension of MRI to an existing study on MS, biochemistry and EDSS done by the University of Stellenbosch. The biochemistry and EDSS data was collected and made available to the researcher, who added the MRI component. According to estimates, 80% of all new MS lesions seen on MRI are clinically silent; therefore the researcher drew on the EDSS and blood chemistry of the existing study in order to do comparisons. A delay in the definitive diagnosis of MS has a detrimental effect on the long term outcome of the disease; therefore early diagnosis and appropriate timely therapeutic intervention is critical (Harris & Sadiq, 2009).

3.4 Study design

An "extremes of outcome" study design was employed to enhance the statistical power of the present pilot study. Two sub-sets, within the same sample, with EDSS scores at the lower and higher ends of the disease spectrum (EDSS scores of ≤ 3 and ≥ 6) were compared. This therefore considered the two opposite extremes of the distribution of long-term outcome (DeLuca *et al*., 2007).

Women diagnosed with MS that met the entry criteria were between the ages of 37 and 57 years. Subjects were recruited from the existing study that had been referred by neurologists at an academic hospital in the Western Cape.

Subjects had to be willing to undergo an MRI examination on the 3Tesla (3T) research Unit at Stellenbosch Medical School. Prior to the collection of MRI data all procedures regarding the examination were explained to the participants by the researcher and informed consent was obtained.

Control MRI scans were sourced from another research study at the research centre and used for comparison of DTI results.

3.5 Research Question

The main research question was: Can advanced MRI techniques such as DTI and MRS (together with conventional sequences) in the WMTs of the brain, be used to achieve an improved correlation with disability and blood chemistry (serum ferritin, haemoglobin, % Tf, CRP, serum iron) in persons with MS?

3.5.1 Research sub questions

- Do lesion numbers and lesion enhancement in the whole brain correlate with the EDSS at the two extremes of the EDSS score spectrum and does it have an effect on disability?
- Do DTI findings (fractional anisotropy, mean diffusivity, axial diffusivity and radial diffusivity) correlate with myelin patency and function between MS subjects and healthy controls in the white matter tracts (WMTs) of the brain?
- Do DTI parameters correlate with EDSS in the two extremes of the EDSS score spectrum?
- Do MRS findings (N-acetyl-aspartate) correlate with disability (as measured by EDSS) at the two extremes of the EDSS spectrum?
- Are DTI and MRS superior in their evaluation of WM integrity and disability as opposed to conventional sequences?
- Do iron parameters have an effect on DTI findings in the WMTs of MS subjects compared to controls?

3.6 Study Sample

The study population consisted of 114 unrelated Caucasian MS patients, 98 females and 16 males. For this study the sample consisted of subjects between 37 and 57 years of age at the two extremes of the EDSS score spectrum in subjects diagnosed with MS for four years and more. Because of the small number of subjects and to obtain homogeneity in the study group, only female subjects were recruited.

3.6.1 Sampling

The study sample included subjects recruited from an academic hospital in the Western Cape. The sample size calculation was done by a qualified statistician using the free software called GPower.

Exact - Proportion: Difference from constant (binomial test, one sample case)

Analysis: Post hoc: Computer achieved power

Input: Tail(s) = One

Effect size g = 0.19

α err prob = 0.05

Total sample size = 14

Constant proportion = 0.01

Output: Lower critical N = 2.0000000

Upper critical N = 2.0000000

Power (1-β err prob) = 0.8020879

Actual α = 0.008401244

Therefore a sample size of 14 will provide 95% confidence (less than 99%) and an effect size of 0.19, which will give a power of 80%.

3.6.2 Inclusion criteria

- Women diagnosed with MS according to the criteria of MacDonald
- Subjects diagnosed with MS for 4 years and more

3.6.3 Exclusion criteria

- Male MS subjects
- MS patients with Neuromyelitis Optica or other neurological diseases
- Standard MRI exclusion criteria

3.7 Biochemistry and Disability measures

3.7.1 Biochemical Analysis

Laboratory analysis comprised intravenous blood collection in the morning between 9h00 and 10h30 to standardize for diurnal variation for evaluation of full blood count, serum iron, ferritin, transferrin, % transferrin saturation, CRP, cholesterol, serum folate, Vitamin B12, 25-OH Vitamin D, fibrinogen and D-dimer by using a Siemens Advia 1800 auto-analyser. Plasma homocysteine levels were measured using a Siemens Centaur XP auto-analyser. Blood for homocysteine determination was collected on ice and was centrifuged as soon as possible to ensure the stability of the homocysteine. The tests were performed by a qualified technician at the National Health Laboratory Service (NHLS), Tygerberg Hospital.

The above data were obtained from the main study and were disclosed to the researcher after the MRI scans were completed.

3.7.2 Disability status

The disability status of subjects was assessed by participating clinicians using the Expanded Disability Status Scale (EDSS). The patients were in remission when their EDSS scores were measured, so that the scores reflected the residual disability when patients were not in relapse.

3.8 MRI data collection

All subjects were scanned with a Siemens, Magnetom Allegra 3.0 Tesla (T) MRI at CUBIC, Tygerberg scanner. A standard head coil was used for all subjects. The MRI scans were carried out by trained radiographers and each examination lasted for about 40 minutes.

3.9 Statistical Analysis

One-way ANOVA was used to compare mean FA values between the groups. Fisher least significant difference (LSD) tests were used for post hoc comparison. Non-parametric Mann-Whitney U and Kruskall-Wallis tests were also conducted parallel to the ANOVA (F-tests), but in general the data was found to conform reasonably to the normality assumptions, and interpretations were done using the ANOVA F-test p-values. Spearman correlations were used for testing relationships between biochemical variables and various brain WMTs. No adjustments were made for multiple testing across analyses of FA variables, and this in conjunction with the small samples size is recognized as a restriction in the interpretation of the results.

3.10 MRI Protocol for this study

3.10.1 Routine Sequences

The MRI sequences performed included the following routine sequences, which is standard protocol at CUBIC:

T1 and T2 relaxation sequences were done in the sagittal plane (with TR = 600ms, TE = 11ms and 5mm slice thickness). A T2W sequence in the axial plane was done (with TR = 4200ms, TE = 95ms and 5mm slice thickness) and an axial T1 sequence. A T1 axial was also done 5 minutes after a 10 ml gadolinium (DTPA) injection administration.

The PD sequence had the following parameters - TR 2000ms, TE 12ms, 5mm slice thickness in the axial plane.

The FLAIR with TR 9000ms, TE 96ms and 5mm slice thickness was done in the axial plane.

3.10.2 Software used in analysis of lesions

The MRI scans were analysed for lesion load on the T1, T2 and FLAIR image by a consultant clinical neuroanatomist who has extensive experience in clinical neuroimaging.

OsiriX is developed as a stand-alone application for the MacOSX operating system. The program is an image processing software dedicated to DICOM images (".dcm" / ".DCM" extension) produced by imaging equipment (MRI, CT, PET, PET-CT, SPECT-CT, Ultrasounds). It is fully compliant with the DICOM standard for image communication and image file formats.

OsiriX is able to receive images transferred by DICOM communication protocol from any PACS or imaging modality (C-STORE SCP/SCU, and Query/Retrieve: C-MOVE SCU/SCP,

C-FIND SCU/SCP, C-GET SCU/SCP, WADO). OsiriX has been specifically designed for navigation and visualization of multi-modality and multidimensional images: 2D Viewer, 3D Viewer, 4D Viewer (3D series with temporal dimension) and 5D Viewer (3D series with temporal and functional dimensions). The 3D Viewer offers all modern rendering modes: Multiplanar reconstruction (MPR), Surface Rendering, Volume Rendering and Maximum Intensity Projection (MIP).

All these modes support 4D data and are able to produce image fusion between two different series (PET-CT and SPECT-CT display support). OsiriX is at the same time a DICOM PACS workstation for imaging and image processing software for medical research, functional imaging, 3D imaging, confocal microscopy and molecular imaging. The OsiriX software was developed based on an open architecture built on existing open-source components and offers all the basic image manipulation functions of zoom, pan, intensity adjustment, and filtering with real-time performance.

Additional functions such as multiplanar projection, convolution filters, variable slice thickness adjustments, volume rendering, minimum and maximum intensity projections, and surface rendering are also accessible in quasi-real-time.The program enables the rapid review of very large sets of images and does not rely on pre-loading of the images from the disk to memory before review. As soon as an image series is selected, images will automatically be displayed on the screen in a cine loop using a ‘‘streaming’’ technique, enabling direct display of the images at a very rapid pace.

3.10.3 Lesion Analysis and Characterisation of Lesion Load

To accurately characterise the lesion load of patients with MS, a specific protocol was developed to accurately identify lesions, and quantify their extent. The protocol follows several steps to (1) increase lesion visibility and conspicuity by fusing various sequences, (2) determine the lesion load by quantifying the number of lesions per anatomical lobe of the brain for each hemisphere, (3) characterise the extent of white matter lesions by assessing lesion confluence, (4) assess enhancement to determine whether lesions were undergoing active demyelination, and (5) assess hypointensity of lesion on T1WI to characterise the extent of demyelination in existing lesions. The sagittal midline on T1WI was also evaluated to assess for midbrain atrophy.

a) Image Fusion:

T2W axially acquired images were used as a standard for identifying white matter hyperintense (WMH) lesions. T2WI were preferred to FLAIR due to its superiority in spatial resolution on our acquisitions. However, we encountered difficulty to accurately identify

lesions in the subcortical arcuate fibers on T2WI due to visual interference of the high CSF signal intensity. To overcome this problem, proton density weighted images (PDWI) were fused with the T2WI so that lesions became more conspicuous. As a consequence of demyelination, gliotic scarring occurs. Collagen is used in the process of astrogliosis, which contains higher quantities of hydrogen protons. PDWI are exquisitely sensitive in picking up these changes in hydrogen proton concentration in WMH. The effect of this is demonstrated in Figure 3.1.

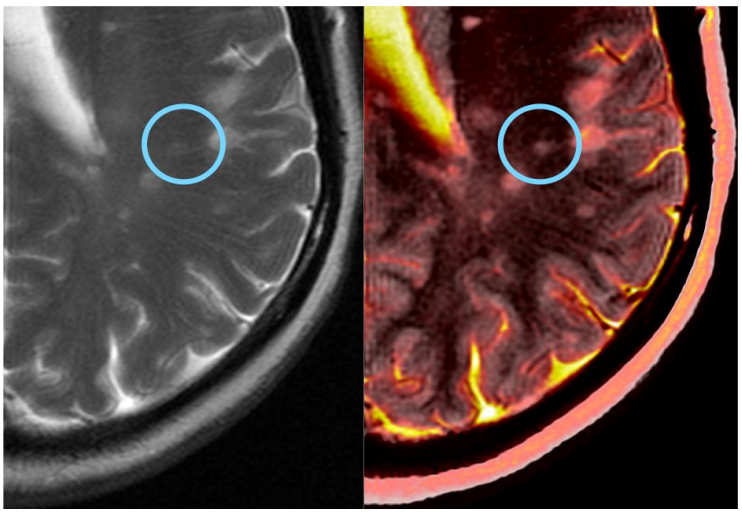


Figure 3.1: Left: T2WI in the axial plane at the level of the splenium of the corpus callosum. Blue circle indicates a subtle WMH. Right: Fusion of T2WI and PDWI. Blue circle indicates the same lesion as seen in the left image, however the visualization of the lesion is greatly enhanced.

(Permission granted by patient MS05)

b) Lesion Number:

The number of lesions was counted for each of the anatomical lobes of the brain and cerebellum as well as the brainstem. Lesions lateralization was also noted.

c) Confluence:

MS presents on T2WI as multiple punctate hyperintense lesions distributed within the white matter of the brain and spinal cord. As the disease progresses, these punctate lesions become confluent. As confluence and bridging of lesions occur, the number of distinct punctate lesions apparent on imaging gives way to large solitary confluent lesions. The appearance on imaging, if only the lesion number was being quantified, is that the lesion number is actually decreasing. It was therefore important to determine lesion confluence as a measure of charactering lesion load and severity. The Fazekas-scale (Wahlund *et al.,* 2001) provides an overall impression of the presence of WMH in the entire brain.

It characterizes lesions as follows:

Fazekas 0: None or a single punctate WMH lesion

Fazekas 1: Multiple punctate lesions

Fazekas 2: Beginning confluency of lesions (bridging)

Fazekas 3: Large confluent lesions

The Fazekas score was determined for each lobe of the cerebrum, the cerebellum and brainstem per hemisphere. This process is demonstrated in Figure 3.2.

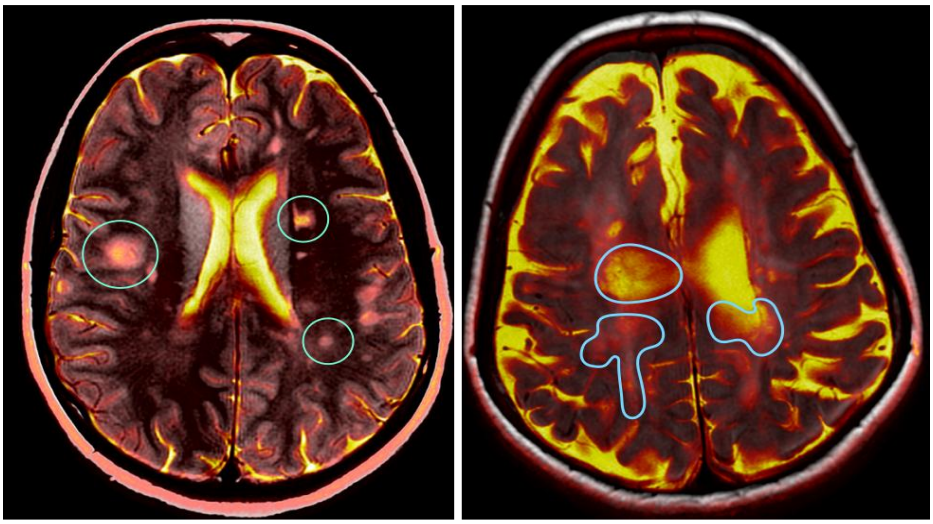


Figure 3.2: The image on the left demonstrates a fused PDWI/T2WI in the axial plane, with several circumscribed white matter lesions indicated. The lesions in the image on the left represent solitary discrete lesions. The image on the right in a different patient demonstrates three large confluent lesions. Though the lesions appear distinct, they are comprised of several confluent solitary white matter lesions. The image on the right therefore represents more advanced disease and lesion load.

(Permission granted by patient MS05)

c) Enhancement:

Due to the biphasic nature of MS, it was important to determine the extent of active demyelination in the current data set. Classically, to determine whether a lesion is active or inactive, post contrast administration images are evaluated for enhancement of lesions. During active demyelination, the inflammation localised to a specific area of active demyelination and various other pathophysiological cascades result in intravascular and interstitial enhancement of the lesion. Post contrast images were evaluated for the number of enhancing lesions in all of the lobes of the brain, per hemisphere as well as for the cerebellum and brainstem.

d) Hypointensity:

In a recent paper by Tam et al. (2011), white matter lesions in MS that demonstrated greater hypointense signal on T1WI were positively correlated to severity in clinical presentation. In order to fully characterize the lesion load in the current dataset, lesion hypointensity was also determined (demonstrated in Figure 3.3) for each lobe per hemisphere including the cerebellum and brainstem. Hypointensity was characterized as follows:

HIY 0: Isointense to surrounding parenchyma

HIY 1: Slightly hypointense to surrounding parenchyma

HIY 2: Moderately hypointense to surrounding parenchyma

HIY 3: Starkly hypointense to surrounding parenchyma

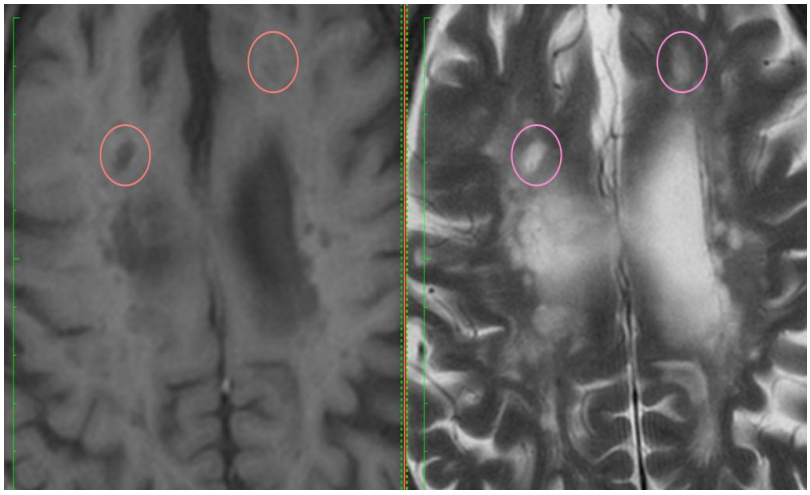


Figure 3.3: The left and right images present two lesions in the same patient but on different sequences (left T1WI and right T2WI). The lesions encircled on the T2WI appear quite similar in their signal intensity; however the lesion on the left appears demonstrably more hypointense on the T1WI than the lesion on the right.

(Permission granted by patient MS05)

3.11 Advanced technologies done in this study

3.11.1 Diffusion tensor imaging (DTI)

Brain imaging protocol for MS study:

Diffusion-weighted images were acquired in axial orientation on a four-channel transmit-receive RF head coil with the following paramaters: 2x2x2 mm^3 resolution, field-of-view of 220 mm, TR = 9500 ms, TE = 88 ms, 70 slices, distance factor of 0, 2x GRAPPA acceleration, 30 b=1000 mm/s^2 directions and 3 b=0 mm/s^2 reference images. The scan was repeated 3 times.

3.11.1.1 Imaging analysis

Data was imported into FSL (FMRIB Software Library) 5.0.1 (Smith *et al.,* 2004) and MATLAB R2012b for initial pre-processing and analysis. Data was corrected for eddy current distortions and the three acquisitions were affine registered to the b=0 mm/s^2 of the first acquisition. For each acquisition, outliers were calculated at a Z-value of the 25th and 75th percentile of the tensor estimate. Values further than three standard deviations from the mean estimate were excluded. The three acquisitions were concatenated (linked) and averaged for export to the tract-based spatial statistics (TBSS) toolbox of FSL. Maps of fractional anisotropy (FA) were created by fitting a linear tensor model to the 30 b = 1000 mm/s^2 directions. Brain extraction was performed with FSL BET at a threshold of 0.2. Images were then processed with the TBSS pipeline, which has been described previously (Smith *et al.,* 2006). After processing with TBSS 48 white matter regions-of-interest were extracted from the FA data, by utilizing the built-in atlas of Mori and colleagues (Mori *et al*., 2005). The mean FA for each of these regions was exported to SPSS 21.0 for further statistical analysis. This resulted in a three-dimensional image of the brain to detect subtle abnormalities (Le Bihan *et al*., 2000).

3.11.2 Magnetic Resonance Spectroscopy

In this study additional proton magnetic resonance spectroscopy (MRS) sequences were performed on the most prominent lesions to look at the biochemical structure of the tissue.

A 2D CSI ^{1}H-MRS slice was acquired (PRESS, TE = 30 msec, TR = 1700 msec, Hamming filter, 3 averages, delta = -2.85 ppm delta frequency, weighted phase encoding, FOV = 160 x 160 mm, VOI = 80 x 80 mm, voxel size 10 x 10 mm, thickness 15 mm, automated CHESS water suppression, scan time 6:53). The ^{1}H-MRS 2D slice was first positioned with standard reconstruction of the sagittal plane, finer positioning of the slice was achieved with the axial and coronal reconstructions to ensure the slice was positioned above the underlying ventricles. Additional saturation bands were applied vertically to the brain slice. Midline grey matter and white matter voxels were extracted bilaterally. For grey matter three voxels were extracted for each hemisphere, and for the white matter six voxels were extracted for each hemisphere to provide a more robust average of grey and white matter for each hemisphere as depicted in Figure 3.4.

3.11.2.1 Analysis

The chemical shift imaging (CSI) spectra were performed in LCModel (Provencher 1993), which fits spectra in relation to basis spectra provided by Provencher (PRESS TE30). Default parameters within LCModel were used for the CSI processing, which applies Bayesian learning from the central VOI of the 2D-slice. The relative concentrations of metabolites are

reported in relation to creatine containing metabolites (PCr+Cr). Only metabolites with a Cramér-Rao of %SD < 20% are reported, indicative of greater confidence. Relative metabolite concentrations reported include NAA and its metabolites (NAA, NAA+NAAG), choline metabolites (GPC+PCh), and *myo*-Inositol (mI).

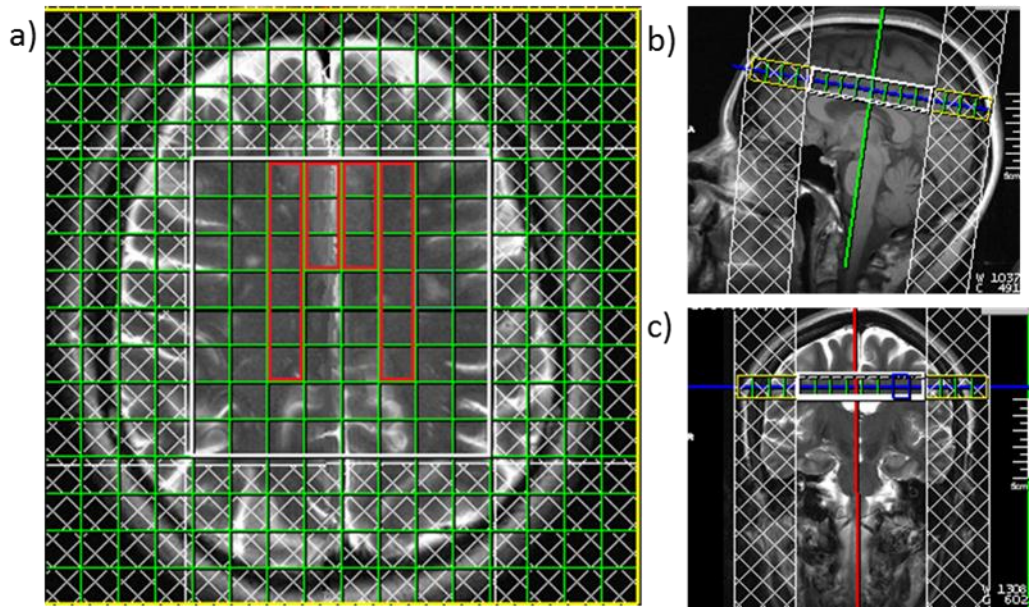


Figure 3.4: Two-dimensional chemical shift imaging magnetic resonance spectroscopy (2D CSI ^{1}H-MRS) representative brain slice performed in 7 individuals with multiple sclerosis. Three voxels bilaterally were extracted to determine average midline grey matter relative metabolites. Six voxels bilaterally were extracted to determine average white matter relative metabolites.

(Permission granted by patient MS02)

3.12 Conclusion

This chapter outlined the research method that was followed as well as the ethical considerations for the study. The MRI (research tool) sequences and protocol were discussed in detail in order to give the reader information on what was done and what was expected of the participants. The following chapter will provide the results for the study.

CHAPTER FOUR
RESEARCH RESULTS

4.1 Introduction

Initially 20 participants were recruited. Of the original 20 patients, 16 presented for MRI scans. Fourteen of the 16 were used for lesion number count. Five of the 16 scans could not be used for DTI analysis due to subject movement causing illegible data, two were claustrophobic, one decided against having the scan and one did not arrive. Eight of the sixteen MRI scans could be used for the MRS analysis (see Figure 4.1).

Figure 4.1 illustrates a flow diagram of the recruitment of subjects and data analysis

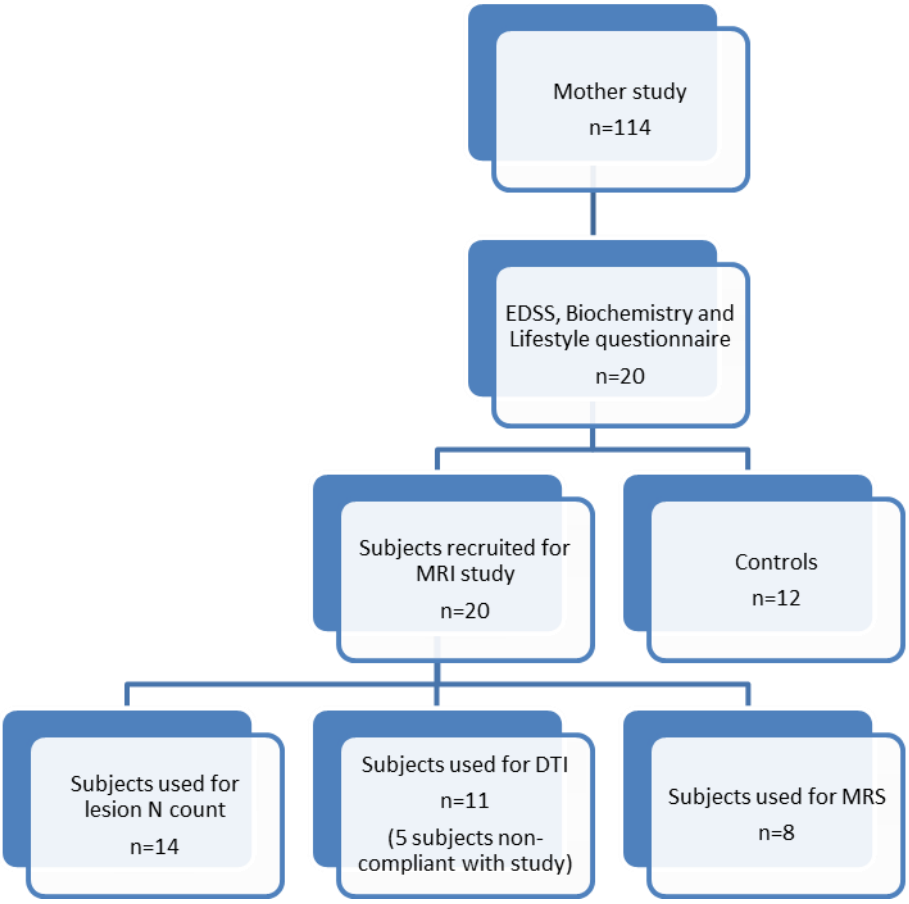


Figure 4.1 Flow diagram for recruitment of subjects for data analysis

This chapter presents the findings of data in order to give meaning to the data that has been collected so that the following objectives can be satisfied:

- Do lesion numbers and lesion enhancement in the whole brain correlate with the EDSS at the two extremes of the EDSS score spectrum?
- Do DTI findings (FA, MD, AD and RD) correlate with myelin patency and function between MS subjects and healthy controls in the WMTs of the brain?
- Do DTI parameters correlate with EDSS in the two extremes of the EDSS score spectrum?
- Do MRS findings (N-acetyl-aspartate) correlate with disability (as measured by EDSS) at the two extremes of the EDSS spectrum?
- Are DTI and MRS superior in their evaluation of WM integrity and disability as opposed to conventional sequences?
- Do DTI findings have an impact on iron parameters in the WMTs of MS subjects compared to controls?

4.2 Characteristics of the study sample

The group consisted of 16 female subjects with a mean age of 47.69 years (37-49 years) (see Table 4.1). They were categorized, into those with high EDSS scores and those with low EDSS scores [9 (64%) - low EDSS scores and 5 (36%) - had high EDSS scores]. Twelve healthy control subjects were used for comparisons. Characteristics are presented as 95% confidence interval (CI).

Table 4.1: Demographics of MS subjects

Subjects		**Controls**	
Number	16	Number	12
Age, mean (range)	47 (37-55)		27.6 (18-43)
Sex	females		females
Race	15 Caucasian, 1 Mixed ancestry		12 Caucasian
Low EDSS (≤ 3)	11		NA
High EDSS (≥ 6)	5		NA

(EDSS= Expanded Disability status Scale. NA= not applicable)

4.3 EDSS compared to Lesion Number (N)

The number of lesions in the whole brain was assessed by using the T1WI, T2WI and PD images and dividing the brain into the lobe regions. The disability status, assessed with the EDSS, correlated significantly with the number of lesions in the whole brain (Spearman rank correlation coefficient p=0.01). Only 14 of the 16 subjects were used for the lesion number count, as 2 sets of data could not be used.

A summary of the MS subjects and their total lesion numbers in the various lobes of the brain are presented in Table 4.2.

Table 4.2 Characteristics of MS subjects (n=14) lesion numbers in the various lobes of thebrain

Lesion N	MS01	MS02	MS03	MS05	MS06	MS07	MS08	MS09	MS10	MS11	MS13	MS14	MS15	MS16
Frontal lobe	43	67	5	47	35	18	26	29	9	13	10	55	NA	96
Parietal Lobe	15	10	0	8	25	7	7	10	0	9	14	15	NA	26
Temporal Lobe	11	41	3	7	13	13	4	1	0	1	3	32	NA	61
Occipital Lobe	3	5	0	5	6	5	1	0	0	4	6	13	NA	18
Pons	1	12	0	10	1	0	1	1	0	5	1	6	NA	21
Medulla Oblongata	0	7	0	3	0	0	0	0	0	0	0	0	10	8
Cerebellum	6	3	1	1	0	0	0	0	0	0	0	0	2	0
TOTAL	**79**	**145**	**9**	**81**	**80**	**43**	**39**	**41**	**9**	**32**	**34**	**121**	**12**	**230**
EDSS	**2.0**	**6.0**	**1.5**	**6.5**	**1.5**	**1.5**	**3.5**	**3.0**	**1.5**	**2.5**	**1.0**	**7.5**	**7.5**	**7.5**

(EDSS= Expanded Disability status Scale. MS= Multiple Sclerosis. NA= not applicable)

However, even though it was expected that the subjects with high EDSS scores would have a high lesion N, there were 2 subjects with low EDSS scores who also had a high lesion N (see Table 4.2 [MS01 and MS06]). Most of the lesions of all subjects were found in the frontal lobes with MS15 being the only exception to this.

4.4 EDSS compared to Hypointensity of lesions

The Spearman rank correlation coefficient was used to assess disability compared to the number of lesions that were seen as hypointense (dark holes) lesions. Subjects with a high EDSS score showed a high number of dark holes in the frontal lobes, seen in graphic form in Table 4.3. and Figure. 4.2.

Table 4.3 Characteristics of the Hypointensive lesions in the MS subjects (n=14) in the various lobes of the brain.

Hypointensity	MS 01	MS 02	MS 03	MS 05	MS 06	MS 07	MS 08	MS 09	MS 10	MS 11	MS 13	MS 14	MS 15	MS 16
Frontal lobe	3	4	0	4	3	2	2	4	0	2	2	3	6	6
Parietal Lobe	3	2	0	4	0	0	2	2	0	3	0	3	6	4
Temporal Lobe	0	2	1	0	0	0	0	0	0	0	0	0	6	4
Occipital Lobe	0	3	**0**	**2**	0	0	0	0	0	0	0	0	6	4
Pons	2	0	0	2	0	0	0	0	0	1	0	4	4	4
Medulla Oblongata	0	0	0	1	0	0	0	0	0	0	0	0	2	4
Cerebellum	0	2	0	0	0	0	0	0	0	0	0	0	4	0
TOTAL	**8**	**13**	**1**	**13**	**3**	**2**	**4**	**6**	**0**	**6**	**2**	**10**	**34**	**26**
EDSS	**2.0**	**6.0**	**1.5**	**6.5**	**1.5**	**1.5**	**3.5**	**3.0**	**1.5**	**2.5**	**1.0**	**7.5**	**7.5**	**7.5**

(EDSS= Expanded Disability Status Scale)

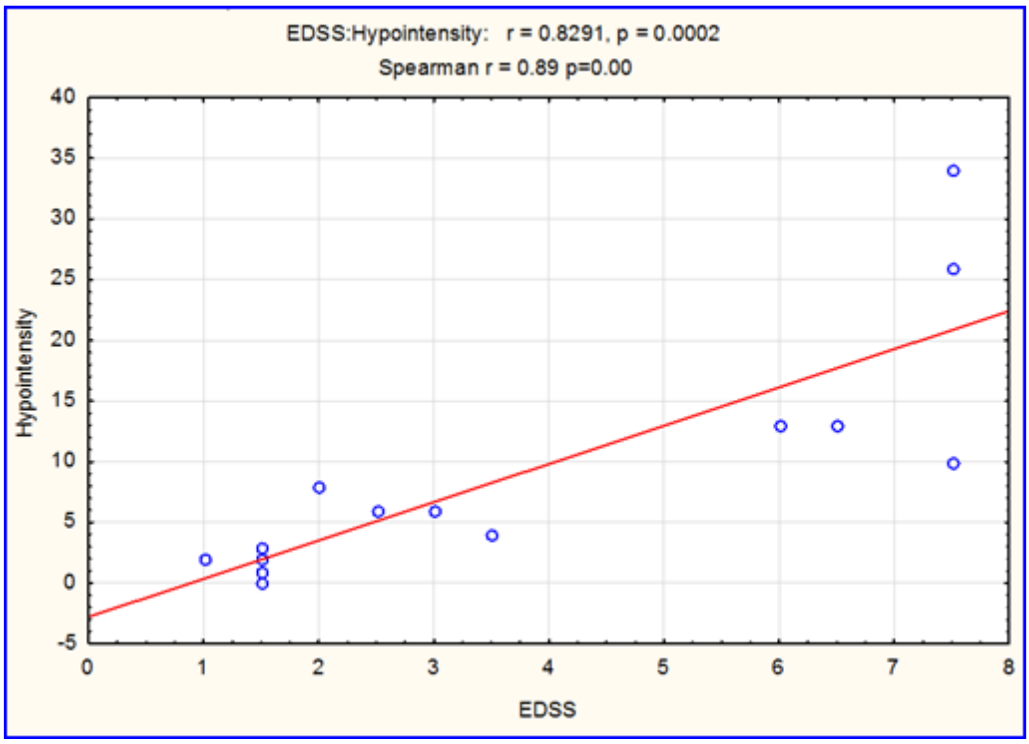


Figure 4.2 Relation between the EDSS and Hypointensity of MS subjects (Spearman rank correlation p=<0.01). (EDSS= Expanded Disability Status Scale)

4.5 EDSS compared to Fazekas score

The Spearman rank correlation was used to compare the EDSS to the Fezekas score (r=0.68; p=0.01) in the different lobes of the brain. Table 4.4 and Figure 4.3 illustrate a significant correlation between the Fezakas score and the EDSS (Spearman rank correlation p=0.01). Those subjects with high EDSS have a high Fezekas score and vice versa. It is only MS 01 with an EDSS of 2 that also has a high Fezekas score.

Table 4.4 Fezekas score of MS subjects in the various lobes of the brain

Fazekas	MS 01	MS 02	MS 03	MS 05	MS 06	MS 07	MS 08	MS 09	MS 10	MS 11	MS 13	MS 14	MS 15	MS 16
Frontal lobe	4	2	1	6	4	2	2	2	2	3	2	4	6	6
Parietal Lobe	3	2	0	2	2	2	2	2	0	2	2	4	6	4
Temporal Lobe	2	2	1	2	2	2	1	0	0	0	1	2	6	4
Occipital Lobe	1	2	0	1	2	2	0	0	0	2	2	2	6	4
Pons	0	0	0	0	0	0		0	0		0	0	0	0
Medulla Oblongata	0	2	0	1	0	0	0	0	0	0	0	0	4	2
Cerebellum	2	2	0	0	0	0	0	0	0	0	0	0	0	0
TOTAL	**12**	**12**	**2**	**12**	**10**	**8**	**5**	**4**	**2**	**7**	**7**	**12**	**28**	**20**
EDSS	**2.0**	**6.0**	**1.5**	**6.5**	**1.5**	**1.5**	**3.5**	**3.0**	**1.5**	**2.5**	**1.0**	**7.5**	**7.5**	**7.5**

(EDSS= Expanded Disability Status Scale)

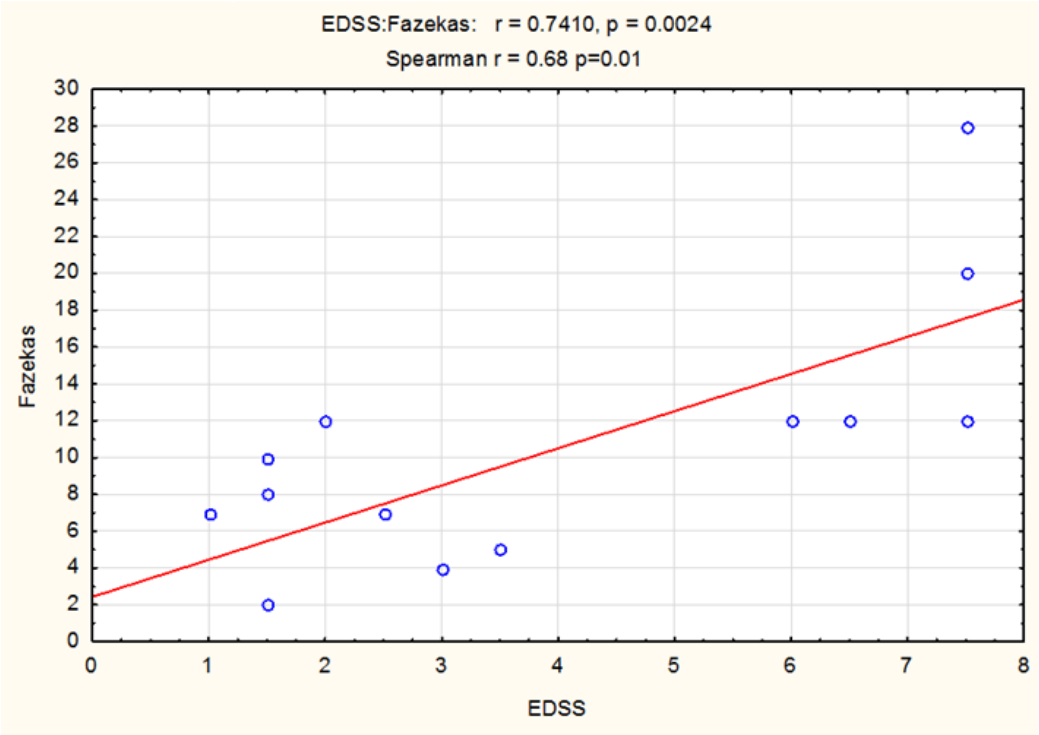


Figure 4.3 Significant correlation between the EDSS and Fazekas score (p=0.01). EDSS= Expanded Disability Status Scale.

4.6 Lesion Enhancement with Gadolinium

Of the 11 subjects participating in the study six subjects (MS01, MS02, MS03, MS09, MS15 and MS16) had enhancing lesions. Three of the six subjects that had lesion enhancement had high EDSS scores (MS02, MS15 and MS16).

The lesion enhancement is illustrated in Figure 4.4.

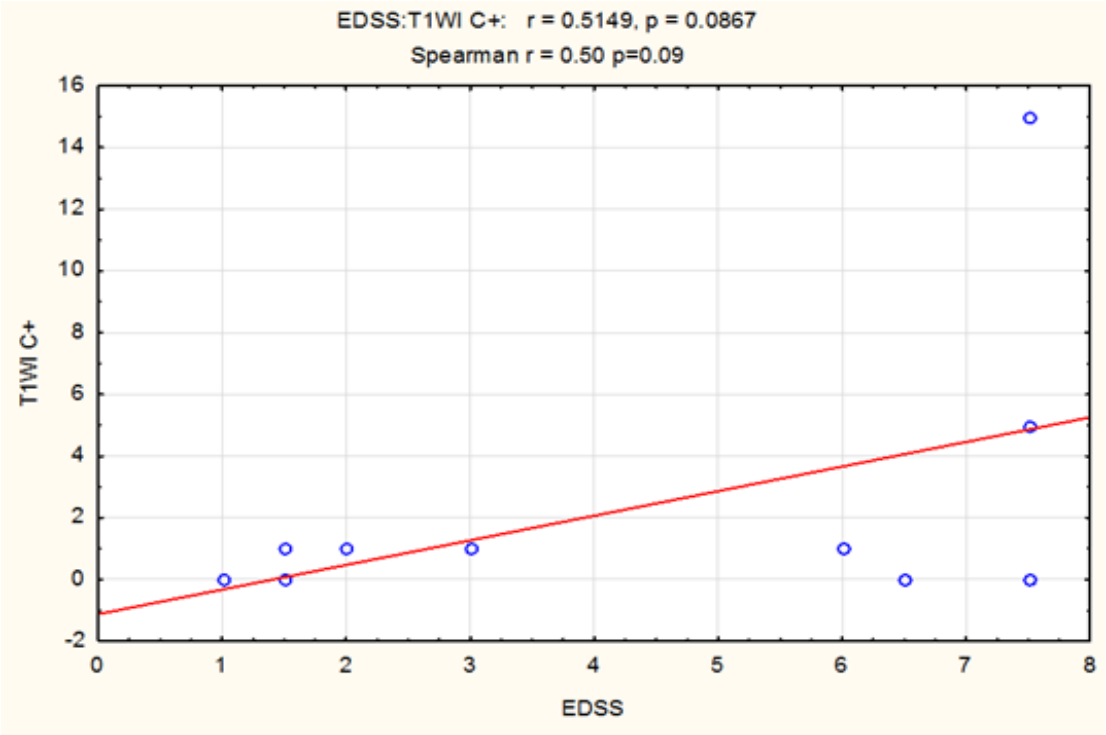


Figure 4.4 EDSS vs T1WI C+ (p=0.09). (EDSS= Expanded Disability Status Scale. T1WI= T1 weighted image. C+= contrast added)

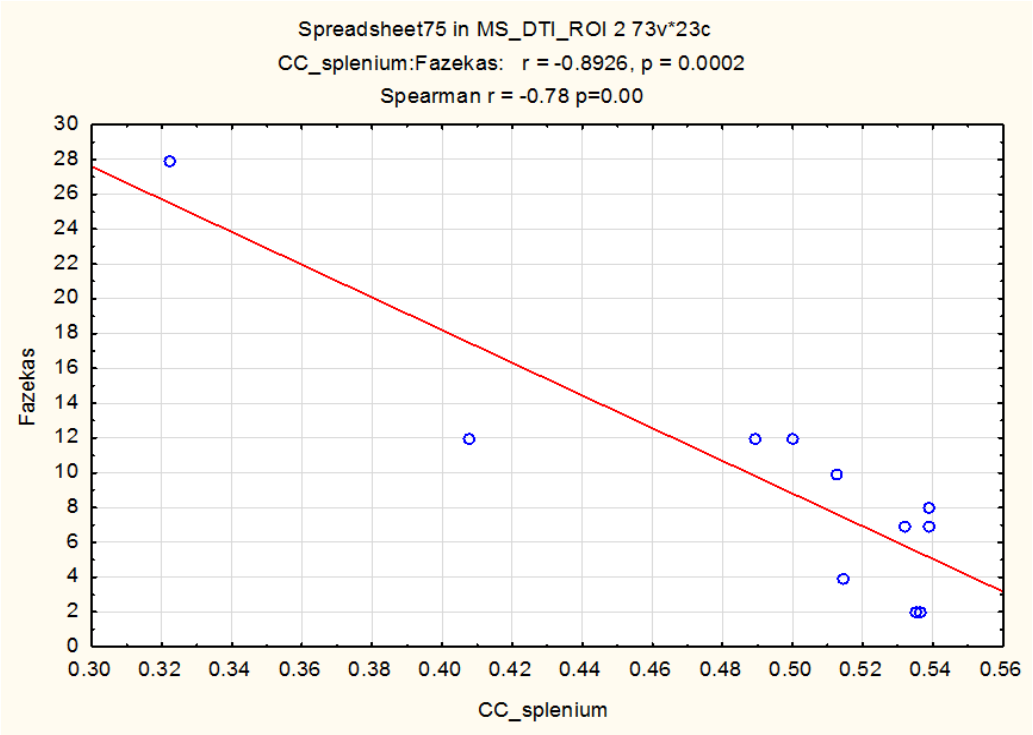
4.7 Lesion number, Fezekas score and hypointensity of lesions in the Corpus callosum:

CC Genu

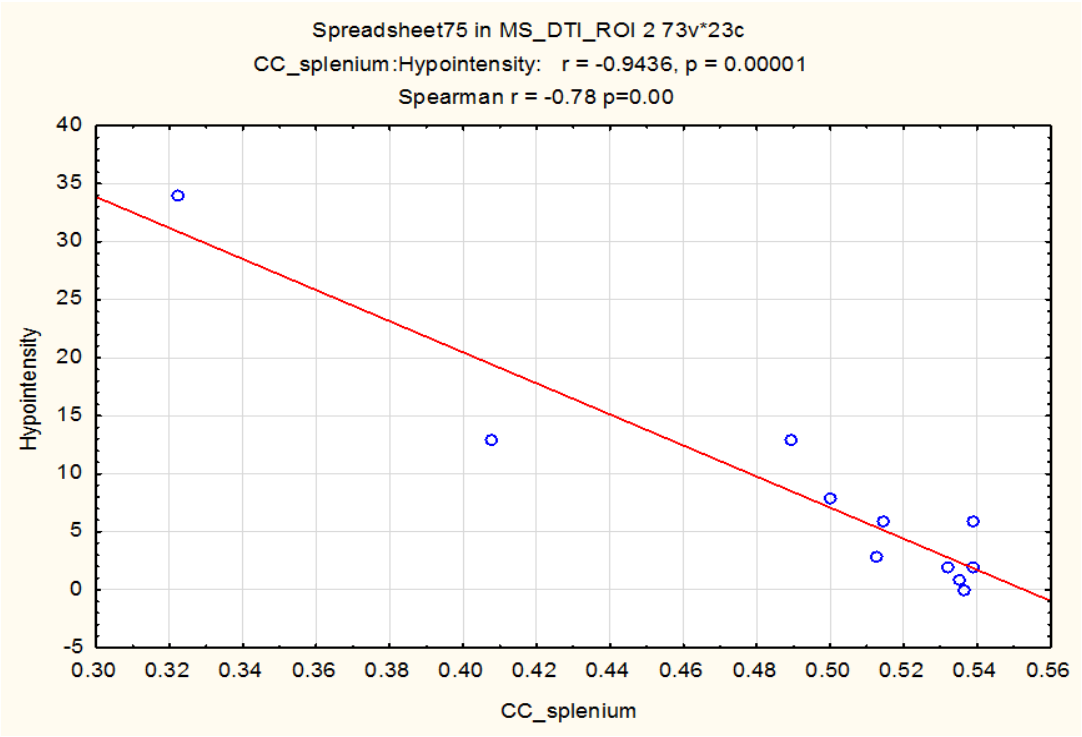
There was no significance found in the CC genu for lesion N analysis (p=0.84), Fezekas (p=0.10) and hypointensity of lesions (p=0.10).

Figures 4.5 (a) and (b) illustrate no significant associations between Fezekas score and hypointensity of lesions seen in the CC splenium.

CC Splenium



(a)

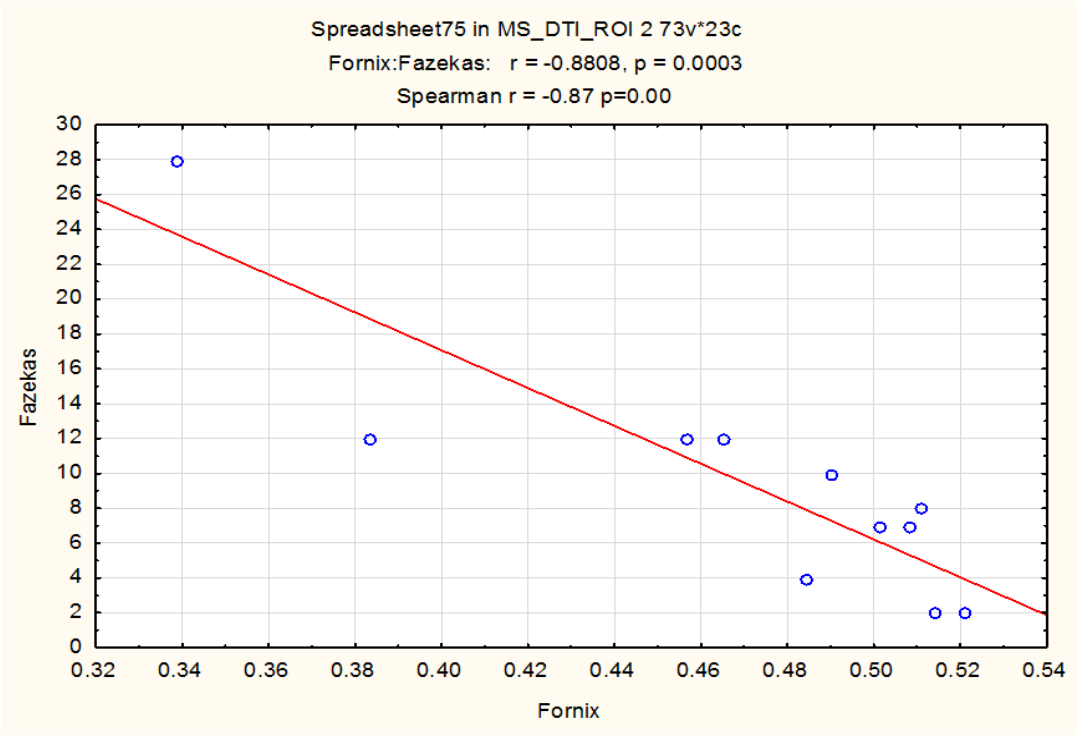


(b)

Figure 4.5 (a) CC_splenium vs Fazekas (p=<0.01). (b) CC_splenium vs Hypointensity (p=<0.01) (CC=corpus callosum)

Figures 4.6 (a) and (b) illustrate no significant associations between Fezekas score and hypointensity of lesions seen in the CC fornix.

CC Fornix



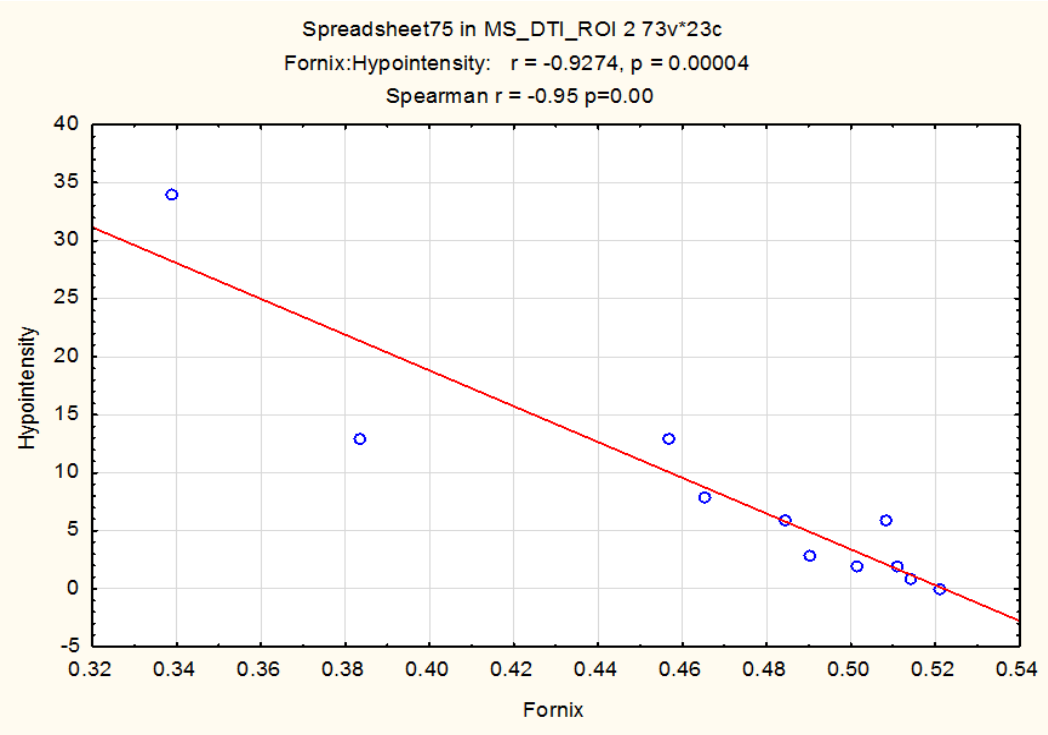


Figure 4.6 (a) Fornix vs Fazekas (p=<0.01). (b) Fornix vs Hypointensity (<p=0.01)

4.8 MRI Diffusion Tensor Imaging (DTI)

DTI calculations were done in 48 White Matter Tracts. The measures were Fractional Anisotropy (FA), Radial Diffusivity (RD), Mean Diffusivity (MD) and Axial Diffusivity (AD). For the RD, MD and AD the threshold was lowered to include only highly significant. The 48 WMTs are based on an atlas by Mori et al. (2005). The atlas was used to identify the tracts in FSL and to extract values that can be analysed statistically. These are the only tracts that can be identified with the present DTI resolution used at CUBIC. A list of the White Matter Tracts is given in the appendices (**Appendix H**).

4.8.1 Fractional anisotropy (FA): MS Total Group versus Control group

Fractional anisotropy is a measure of the axonal loss and functionality of the myelin sheath. Higher FA reflects a more efficient function, while lower FA is related to dysfunction (Benedetti *et al*., 2011). It is therefore to be expected that the FA would be higher in the Controls than in the MS group.

Below is a table from the statistics as an example of the results that were found (a threshold of less than 0.01 was used). The FA assessments of the EDSS were done in 48 WMTs of each subject as well as controls in the study. Between group differences were measured using the Mann-Whitney U test. DTI comparisons were done in controls (n=12) and MS subjects (11). In 20 of the 48 tracts assessed, the white matter FA was significantly ($p< 0.05$) lower in the MS subjects than in the controls. Of these, 4 were highly significantly different ($p< 0.01$). All the results for the 48 tracts are depicted in Table 4.5 and Figure 4.7 shows FA in the CST of the MS subjects versus controls.

Table 4.5 Fractional anisotropy (FA) in MS patients and Controls. Values are given as the means (± SD). FA was lower in MS patients than controls in all the tracts except in the CC splenium and Fornix where the values were similar. FA was significantly lower in patients than controls in 20 of the 48 tracts measured (significant-values in bold).

White Matter Tract	Controls	Patients	F-test
Middle_cerebellar_peduncle	0.45(0.04)	0.42(0.05)	F(1,21)=2.20, p=0.15
Pontine_crossing_tract	0.52(0.04)	0.48(0.06)	F(1,21)=2.92, p=0.10
CC_genu	0.42(0.06)	0.40(0.05)	F(1,21)=0.64, p=0.43
CC_body	0.41(0.05)	0.39(0.05)	F(1,21)=0.65, p=0.43
CC_splenium	0.49(0.05)	0.49(0.07)	F(1,21)=0.00, p=0.97
Fornix	0.47(0.05)	0.47(0.06)	F(1,21)=0.00, p=1.00
Corticospinal_tract_RH	0.58(0.04)	0.54(0.06)	F(1,21)=5.59, **p=0.03**
Corticospinal_tract_LH	0.58(0.04)	0.54(0.05)	F(1,21)=4.63, **p=0.04**
Medial_lemniscus_RH	0.50(0.03)	0.47(0.05)	F(1,21)=2.98, p=0.10
Medial_lemniscus_LH	0.49(0.03)	0.46(0.06)	F(1,21)=1.93, p=0.18
Inferior_cerebellar_peduncle_RH	0.58(0.03)	0.53(0.04)	F(1,21)=8.29, **p<0.01**
Inferior_cerebellar_peduncle_LH	0.37(0.04)	0.35(0.05)	F(1,21)=1.12, p=0.30
Superior_cerebellar_peduncle_RH	0.57(0.03)	0.54(0.03)	F(1,21)=6.30, **p=0.02**
Superior_cerebellar_peduncle_LH	0.51(0.03)	0.46(0.04)	F(1,21)=11.45, **p<0.01**
Cerebral_peduncle_RH	0.52(0.04)	0.47(0.06)	F(1,21)=5.59, **p=0.03**
Cerebral_peduncle_LH	0.42(0.04)	0.37(0.07)	F(1,21)=5.74, **p=0.03**
Anterior_limb_of_internal_capsule_RH	0.42(0.04)	0.36(0.07)	F(1,21)=5.09, **p=0.03**
Anterior_limb_of_internal_capsule_LH	0.44(0.03)	0.39(0.06)	F(1,21)=6.62, **p=0.02**
Posterior_limb_of_internal_capsule_RH	0.44(0.03)	0.39(0.06)	F(1,21)=9.55, **p<0.01**
Posterior_limb_of_internal_capsule_LH	0.43(0.03)	0.38(0.06)	F(1,21)=7.62, **p=0.01**
Retrolenticular_internal_capsule_RH	0.42(0.03)	0.36(0.07)	F(1,21)=7.20, **p=0.01**
Retrolenticular_internal_capsule_LH	0.53(0.05)	0.46(0.07)	F(1,21)=8.04, **p<0.01**
Anterior_corona_radiata_RH	0.51(0.04)	0.45(0.09)	F(1,21)=4.46, p=0.05
Anterior_corona_radiata_LH	0.51(0.05)	0.45(0.07)	F(1,21)=5.98, **p=0.02**
Superior_corona_radiata_RH	0.49(0.04)	0.43(0.05)	F(1,21)=7.79, **p=0.01**
Superior_corona_radiata_LH	0.44(0.04)	0.39(0.06)	F(1,21)=6.81, **p=0.02**
Posterior_corona_radiata_RH	0.35(0.02)	0.32(0.06)	F(1,21)=2.73, p=0.11
Posterior_corona_radiata_LH	0.36(0.03)	0.33(0.05)	F(1,21)=4.07, p=0.06
Posterior_thalamic_radiation_RH	0.42(0.04)	0.40(0.06)	F(1,21)=0.69, p=0.41
Posterior_thalamic_radiation_LH	0.45(0.04)	0.43(0.07)	F(1,21)=1.05, p=0.32
Sagittal_stratum_RH	0.31(0.05)	0.29(0.04)	F(1,21)=1.70, p=0.21
Sagittal_stratum_LH	0.30(0.05)	0.29(0.04)	F(1,21)=0.64, p=0.43
External_capsule_RH	0.41(0.05)	0.35(0.08)	F(1,21)=4.27, p=0.05
External_capsule_LH	0.56(0.03)	0.50(0.08)	F(1,21)=5.42, **p=0.03**
Cingulum_RH	0.43(0.06)	0.37(0.08)	F(1,21)=4.49, **p=0.05**
Cingulum_LH	0.42(0.04)	0.39(0.04)	F(1,21)=3.47, p=0.08
Cingulum(hippocampus)_RH	0.42(0.04)	0.39(0.04)	F(1,21)=4.67, **p=0.04**
Cingulum(hippocampus)_LH	0.44(0.05)	0.40(0.07)	F(1,21)=1.48, p=0.24
Fornix/stria_terminalis_RH	0.42(0.04)	0.37(0.08)	F(1,21)=3.57, p=0.07
Fornix/stria_terminalis_LH	0.40(0.04)	0.38(0.05)	F(1,21)=1.03, p=0.32

Superior_longitudinal_fasciculus_RH	0.39(0.04)	0.38(0.05)	F(1,21)=0.49, p=0.49
Superior_longitudinal_fasciculus_LH	0.35(0.04)	0.32(0.08)	F(1,21)=1.64, p=0.21
Superior_fronto_occip_fasciculus_RH	0.26(0.03)	0.24(0.05)	F(1,21)=1.82, p=0.19
Superior_fronto_occip_fasciculus_LH	0.65(0.05)	0.61(0.08)	F(1,21)=2.61, p=0.12
Uncinate_fasciculus_RH	0.35(0.05)	0.28(0.09)	F(1,21)=5.82, **p=0.03**
Uncinate_fasciculus_LH	0.44(0.04)	0.42(0.06)	F(1,21)=0.88, p=0.36
Tapetum_RH	0.44(0.04)	0.42(0.05)	F(1,21)=1.18, p=0.29
Tapetum_LH	0.52(0.04)	0.49(0.04)	F(1,21)=2.73, p=0.11
Average FA of WMTs	**0.45(0.035)**	**0.41(0.056)**	

(CC= corpus callosum. EDSS= Expanded Disability Status Scale. RH= right hemisphere. LH= left hemisphere)

Figure 4.7 shows that the normal probability plot indicated deviation from normality, and subsequent Mann-Whitney U test indicated significant differences (p=<0.01).

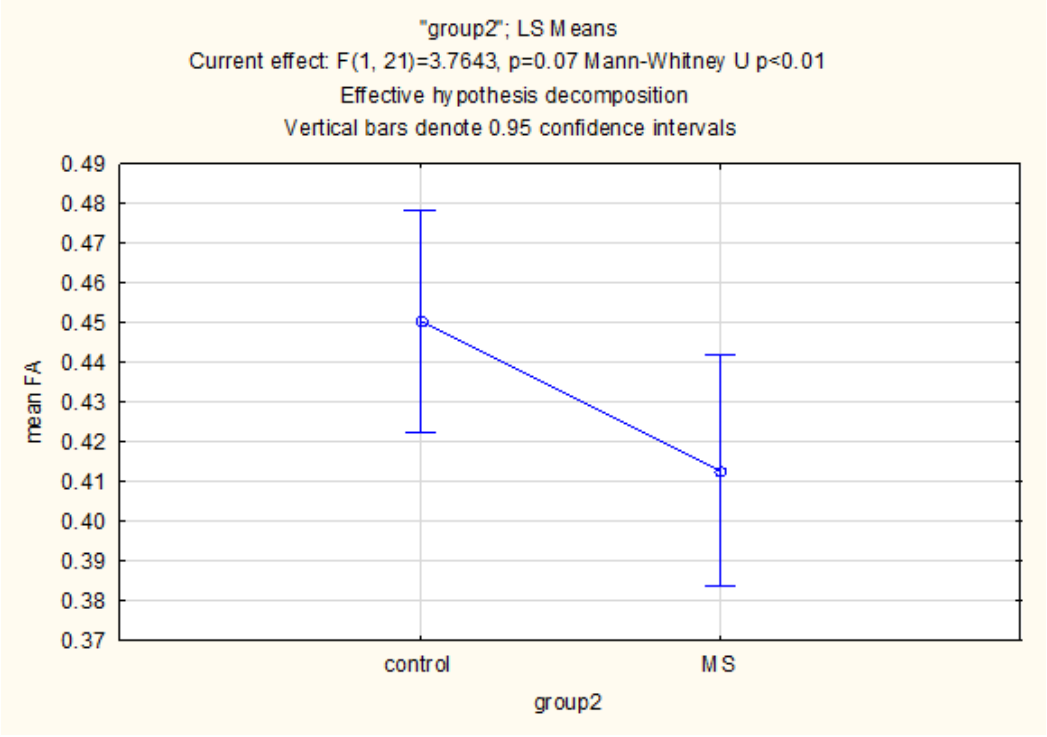


Figure 4.7 The mean FA between the MS subjects and controls (Mann-Whitney U test p=<0.01). FA= Fractional anisotropy. MS= Multiple Sclerosis.

No differences were found in FA between MS subjects and controls in the following tracts: middle cerebellar peduncle, pontine crossing tract, CC (genu), CC (body), CC (splenium), CC (fornix), medial lemniscus (RH and LH), inferior cerebellar peduncle (LH), anterior corona radiate (RH), posterior corona radiate (RH and LH), posterior thalamic radiation (RH and LH), sagittal stratum (RH and LH), external capsule (RH), cingulum (RH and LH), cingulum hippocampus (LH), fornix/stria terminalis (RH and LH), superior longitudinal fasciculus (RH and LH), superior fronto-occipital fasciculus (RH and LH), uncinate fasciculus (LH), tapetum (RH and LH) depicted in Table 4.5.

4.8.2 Fractional anisotropy (FA): MS divided into Low EDSS and High EDSS versus Control group

Further analysis examined associations between FA in the three cohorts (controls, MS high and MS low groups) by using the Kruskal-Wallis ANOVA test. When the MS subjects were divided into 3 groups, low EDSS, high EDSS and controls, the EDSS was inversely associated with the FA, indicating that higher disability resulted in greater impaired FA which suggests an increased likelihood of white matter dysfunction. However, unexpectedly, while the patients with high EDSS had FA values significantly lower than controls (average 0.34 ±, p =<0.001), the patients with low EDSS had FA values similar to controls (average 0.44 ±, p =0.5), demonstrating that subjects with high EDSS had significantly lower FA associations in scrutinized white matter tracts except the sagittal stratum (bilaterally). This is depicted in Table 4.6. The superior longitudinal fasciculus (see Figure 4.8), inferior fronto-occipital fasciculus and the inferior longitudinal fasciculus are 3 major WMTs that run through the sagittal stratum, that contribute to the limbic-cortical neural network and visual system (Kieseppä *et al.*, 2010).

Figure 1 shows the FA in the CC (which is most heavily affected by MS) indicating that the patients with low disability had FA values similar to controls (CC body; p=0.802, CC genu; p=0.816 and CC splenium; p= 0.115) while there were significant differences between patients with high EDSS and controls (CC body; p = 0.015, CC genu; p=0.017 and CC splenium; p=0.007) and between high EDSS and low EDSS (CC body; p = 0.013, CC genu; p=0.016 and CC splenium; p=0.0007).

In the Fornix, which is related to cognitive function and memory, MS patients with low EDSS also had similar FA values to controls (p = 0.135), while there were significant differences between patients with high EDSS and controls (p = 0.008) and between patients with high EDSS and low EDSS (p = 0.001). (Figure 4.9)

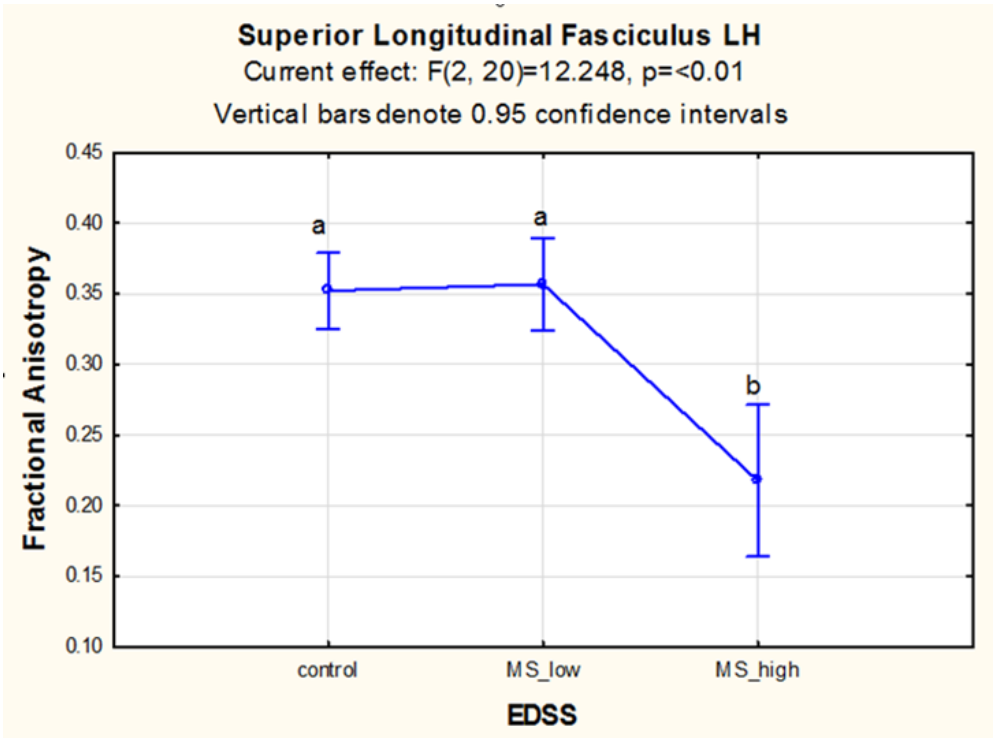


Figure 4.8 FA values in MS patients with high EDSS and low EDSS compared to controls in the Superior Longitudinal Fasciculus Mann Whitney U test (p=<0.01) Significant differences between a and b (Kruskal-Wallis p < 0.01). EDSS=Expanded disability Status Scale. MS= Multiple Sclerosis. LH= left hemisphere.

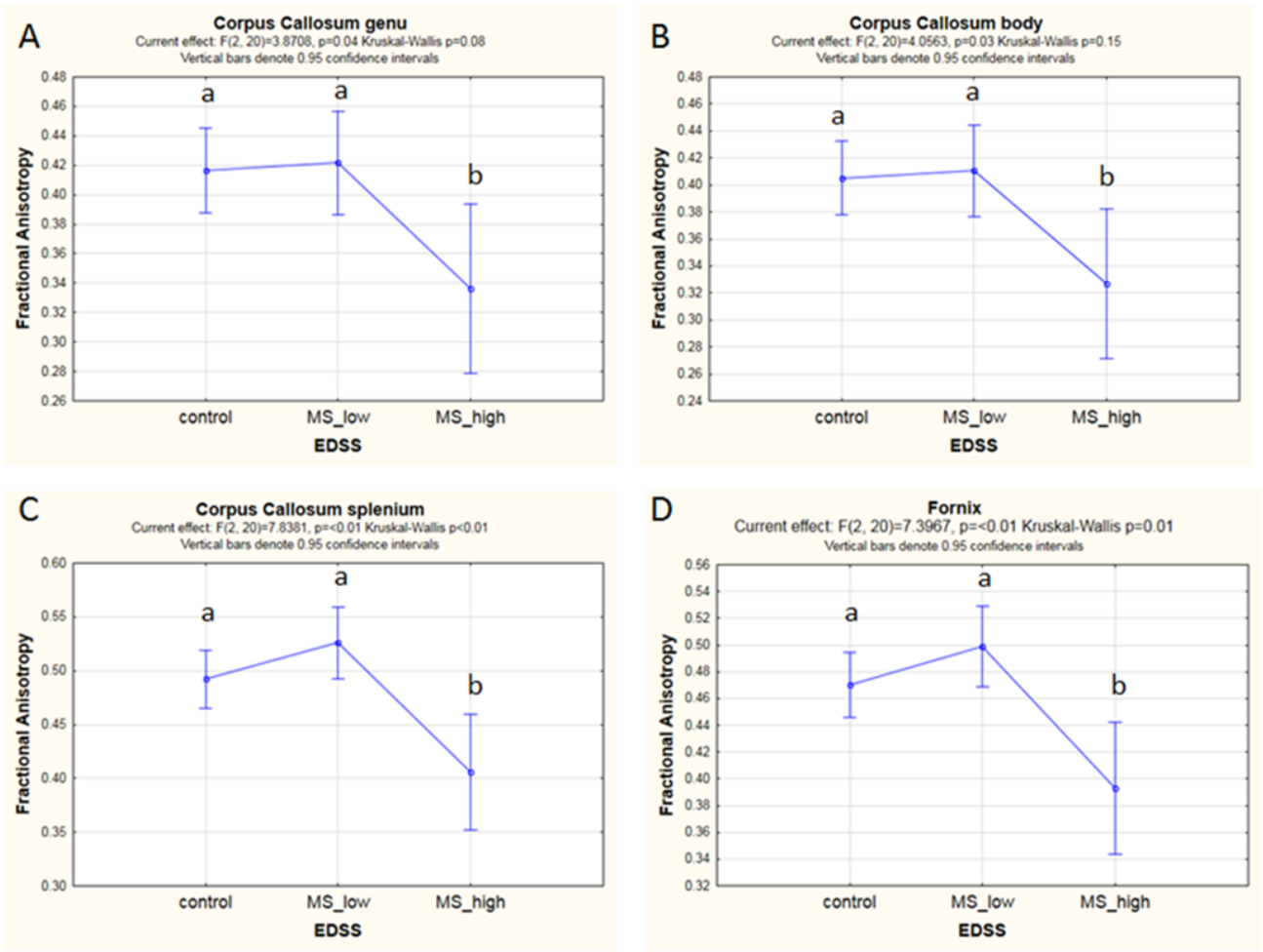


Figure 4.9 FA of CC genu (A), body (B) and splenium (C) and Fornix (D) Significant differences between a and b (Kruskal-Wallis p= 0.01). EDSS=Expanded disability Status Scale. MS= Multiple Sclerosis. CC= corpus callosum.

Table 4.6 FA in MS patients with low EDSS, high EDSS and Controls. Values are given as the means ± SD. FA was significantly lower in patients with high EDSS (b) compared to low EDSS and controls (a) in all the tracts except the Sagittal stratum RH and Sagittal stratum LH. Patients with low EDSS had similar FA values as controls in all the tracts (a) except in the Superior cerebellar peduncle LH and the Posterior limb of internal capsule RH. Average FA was calculated over all WMTs measured.

Variable	control	EDSS(low)	EDSS(high)	F-test
Middle_cerebellar_peduncle	0.45(0.04)a	0.45(0.02)a	0.36(0.06)b	F(2,20)=8.49, p<0.01
Pontine_crossing_tract	0.52(0.04)a	0.50(0.04)a	0.42(0.06)b	F(2,20)=6.14, p<0.01
CC_genu	0.42(0.06)a	0.42(0.02)a	0.34(0.06)b	F(2,20)=3.87, p=0.04
CC_body	0.41(0.05)a	0.41(0.03)a	0.33(0.06)b	F(2,20)=4.06, p=0.03
CC_splenium	0.49(0.05)a	0.53(0.01)a	0.41(0.08)b	F(2,20)=7.84, p<0.01
Fornix	0.47(0.05)a	0.50(0.02)a	0.39(0.06)b	F(2,20)=7.40, p<0.01
Corticospinal_tract_RH	0.58(0.04)a	0.57(0.02)a	0.46(0.06)b	F(2,20)=15.62, p<0.01
Corticospinal_tract_LH	0.58(0.04)a	0.56(0.02)a	0.48(0.05)b	F(2,20)=9.73, p<0.01
Medial_lemniscus_RH	0.50(0.03)a	0.49(0.02)a	0.40(0.07)b	F(2,20)=8.94, p<0.01
Medial_lemniscus_LH	0.49(0.03)a	0.49(0.02)a	0.39(0.07)b	F(2,20)=12.47, p<0.01
Inferior_cerebellar_peduncle_RH	0.58(0.03)a	0.55(0.01)a	0.48(0.03)b	F(2,20)=17.75, p<0.01
Inferior_cerebellar_peduncle_LH	0.37(0.04)a	0.37(0.03)a	0.31(0.04)b	F(2,20)=4.43, p=0.03
Superior_cerebellar_peduncle_RH	0.57(0.03)a	0.56(0.01)a	0.50(0.03)b	F(2,20)=9.62, p<0.01
Superior_cerebellar_peduncle_LH	0.51(0.03)c	0.48(0.02)b	0.41(0.06)a	F(2,20)=11.99, p<0.01
Cerebral_peduncle_RH	0.52(0.04)a	0.49(0.01)a	0.40(0.08)b	F(2,20)=9.27, p<0.01
Cerebral_peduncle_LH	0.42(0.04)a	0.40(0.02)a	0.28(0.08)b	F(2,20)=14.35, p<0.01
Anterior_limb_of_internal_capsule_RH	0.42(0.04)a	0.40(0.02)a	0.27(0.07)b	F(2,20)=18.13, p<0.01
Anterior_limb_of_internal_capsule_LH	0.44(0.03)a	0.42(0.02)a	0.32(0.06)b	F(2,20)=19.97, p<0.01
Posterior_limb_of_internal_capsule_RH	0.44(0.03)c	0.41(0.02)b	0.32(0.07)a	F(2,20)=18.13, p<0.01
Posterior_limb_of_internal_capsule_LH	0.43(0.03)a	0.41(0.02)a	0.29(0.07)b	F(2,20)=20.23, p<0.01
Retrolenticular_internal_capsule_RH	0.42(0.03)a	0.39(0.02)a	0.27(0.08)b	F(2,20)=18.63, p<0.01
Retrolenticular_internal_capsule_LH	0.53(0.05)a	0.49(0.02)a	0.39(0.11)b	F(2,20)=9.24, p<0.01
Anterior_corona_radiata_RH	0.51(0.04)a	0.49(0.03)a	0.33(0.10)b	F(2,20)=17.78, p<0.01
Anterior_corona_radiata_LH	0.51(0.05)a	0.48(0.02)a	0.36(0.08)b	F(2,20)=13.67, p<0.01
Superior_corona_radiata_RH	0.49(0.04)a	0.46(0.02)a	0.36(0.06)b	F(2,20)=13.98, p<0.01
Superior_corona_radiata_LH	0.44(0.04)a	0.42(0.02)a	0.32(0.07)b	F(2,20)=11.22, p<0.01
Posterior_corona_radiata_RH	0.35(0.02)a	0.35(0.01)a	0.26(0.09)b	F(2,20)=7.84, p<0.01
Posterior_corona_radiata_LH	0.36(0.03)a	0.35(0.01)a	0.27(0.08)b	F(2,20)=9.89, p<0.01
Posterior_thalamic_radiation_RH	0.42(0.04)a	0.43(0.03)a	0.33(0.08)b	F(2,20)=7.38, p<0.01
Posterior_thalamic_radiation_LH	0.45(0.04)a	0.46(0.03)a	0.34(0.09)b	F(2,20)=7.80, p<0.01
Sagittal_stratum_RH	0.31(0.05)	0.30(0.02)	0.26(0.06)	F(2,20)=1.82, p=0.19
Sagittal_stratum_LH	0.30(0.05)	0.30(0.02)	0.25(0.04)	F(2,20)=1.74, p=0.20
External_capsule_RH	0.41(0.05)a	0.38(0.03)a	0.26(0.11)b	F(2,20)=9.46, p<0.01
External_capsule_LH	0.56(0.03)a	0.54(0.03)a	0.41(0.10)b	F(2,20)=13.54, p<0.01
Cingulum_RH	0.43(0.06)a	0.40(0.02)a	0.28(0.10)b	F(2,20)=9.32, p<0.01
Cingulum_LH	0.42(0.04)a	0.41(0.02)a	0.34(0.05)b	F(2,20)=5.98, p<0.01
Cingulum(hippocampus)_RH	0.42(0.04)a	0.40(0.02)a	0.34(0.05)b	F(2,20)=6.56, p<0.01
Cingulum(hippocampus)_LH	0.44(0.05)a	0.44(0.02)a	0.32(0.10)b	F(2,20)=7.30, p<0.01
Fornix/stria_terminalis_RH	0.42(0.04)a	0.41(0.02)a	0.28(0.10)b	F(2,20)=11.70, p<0.01

Fornix/stria_terminalis_LH	0.40(0.04)a	0.41(0.03)a	0.33(0.06)b	F(2,20)=4.92, p=0.02
Superior_longitudinal_fasciculus_RH	0.39(0.04)a	0.40(0.02)a	0.32(0.06)b	F(2,20)=4.26, p=0.03
Superior_longitudinal_fasciculus_LH	0.35(0.04)a	0.36(0.03)a	0.22(0.09)b	F(2,20)=12.25, p<0.01
Superior_fronto_occip_fasciculus_RH	0.26(0.03)a	0.26(0.03)a	0.18(0.06)b	F(2,20)=8.88, p<0.01
Superior_fronto_occip_fasciculus_LH	0.65(0.05)a	0.64(0.02)a	0.51(0.10)b	F(2,20)=10.57, p<0.01
Uncinate_fasciculus_RH	0.35(0.05)a	0.31(0.06)a	0.19(0.09)b	F(2,20)=8.10, p<0.01
Uncinate_fasciculus_LH	0.44(0.04)a	0.45(0.03)a	0.34(0.05)b	F(2,20)=10.39, p<0.01
Tapetum_RH	0.44(0.04)a	0.45(0.02)a	0.35(0.05)b	F(2,20)=8.03, p<0.01
Tapetum_LH	0.52(0.04)a	0.51(0.03)a	0.43(0.02)b	F(2,20)=7.25, p<0.01
Average FA of WMTs	**0.45(0.04)**	**0.44(0.01)**	**0.34(0.07)**	**F(2.20)=11.72, p<0.01**

4.9 Correlations of EDSS, biochemical data and FA

Biochemical data was available for all MS subjects used in the study. The Spearman rank correlation was used to show significant associations. The EDSS was inversely associated with the FA in all of the tracts, indicating that higher disability resulted in measurable myelin dysfunction. FA values were not associated with age or disease duration in any of the WMTs (Table 4.7).

4.9.1 Effects of Iron on Myelin: % Transferrin Saturation (TF sat) and FA

Positive associations were also found between the % Tf sat and FA in all 48 WMTs (Table 4.8); the r-values ranged between 0.22 for the CC genu to 0.84 for the external capsule RH. The associations were significant ($p < 0.05$) in 25 of the 48 WMTs assessed and highly significant in 14 of these tracts ($p < 0.01$; Table 4.8), namely, the medial lemniscus (left), cerebellar peduncle (left), anterior limb of the internal capsule (right), retrolenticular limb of the internal capsule (right), anterior corona radiate (right), superior corona radiate (right and left). As an example of the FA values obtained, the FA for the superior fronto occipital fasciculus (LH) of MS patients ranged from 0.42 to 0.68 and the association with the % Tf saturation was highly significant ($r = 0.83$, $p<0.01$; Figure 4.10).

Of the 5 blood iron parameters measured (Hb, ferritin, serum iron, Tf and % Tf saturation), the serum iron and the % Tf sat showed significant associations with FA measures, while ferritin showed significant associations in some of the tracts after adjusting for high CRP. CRP is an index of infection/inflammation, and ferritin is falsely elevated as a result of the inflammatory response. Therefore, ferritin has to be viewed in conjuction with CRP. The Hb showed no associations with FA (Table 4.8).

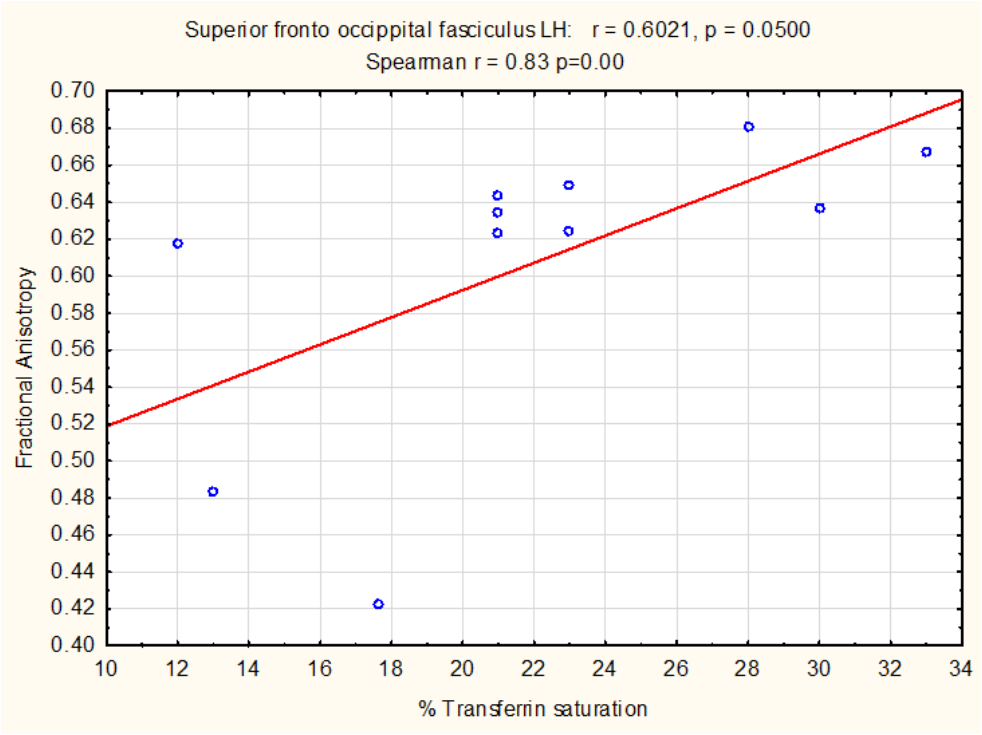


Figure 4.10 Association of FA with the % Tf saturation for the superior fronto occipital fasciculus (LH) (r = 0.83, p<0.01).

Table 4.7 Associations of FA with serum iron, % TF saturation, haemoglobin and ferritin (corrected for high CRP) in the 48 WMTs

FRACTIONAL ANISOTROPY (n=11)	Serum Iron		% Tf saturation		Hemoglobin		Ferritin corrected	
	r	p	r	p	r	p	r	p
Middle_cerebellar_peduncle	0.57	0.07	0.71	0.01	0.46	0.15	0.69	0.04
Pontine_crossing_tract	0.55	0.09	0.57	0.07	0.01	0.97	0.4	0.28
CC_genu	0.15	0.65	0.22	0.52	-0.15	0.65	0.26	0.5
CC_body	0.44	0.18	0.47	0.15	-0.17	0.61	0.25	0.51
CC_splenium	0.52	0.11	0.69	0.02	0.3	0.37	0.43	0.25
Fornix	0.51	0.11	0.69	0.02	0.44	0.18	0.61	0.08
Corticospinal_tract_RH	0.40	0.23	0.59	0.06	0.46	0.16	0.47	0.2
Corticospinal_tract_LH	0.43	0.19	0.59	0.05	0.37	0.26	0.56	0.12
Medial_lemniscus_RH	0.47	0.15	0.69	0.02	0.52	0.1	0.75	0.02
Medial_lemniscus_LH	0.54	0.09	0.74	<0.01	0.46	0.15	0.64	0.06
Inferior_cerebellar_peduncle_RH	0.68	0.03	0.65	0.03	0.12	0.73	0.79	0.01
Inferior_cerebellar_peduncle_LH	0.49	0.13	0.4	0.23	0.05	0.89	0.59	0.09
Superior_cerebellar_peduncle_RH	0.39	0.24	0.44	0.17	0.26	0.43	0.6	0.09
Superior_cerebellar_peduncle_LH	0.14	0.69	0.3	0.38	0.43	0.18	0.29	0.44
Cerebral_peduncle_RH	0.25	0.47	0.44	0.18	0.54	0.08	0.38	0.31
Cerebral_peduncle_LH	0.59	0.06	0.78	<0.01	0.28	0.41	0.41	0.27
Anterior_limb_of_int_capsule_RH	0.60	0.06	0.75	<0.01	0.18	0.59	0.31	0.42
Anterior_limb_of_int_capsule_LH	0.61	0.05	0.55	0.08	0.05	0.89	0.72	0.03
Posterior_limb_of_int_capsule_RH	0.50	0.12	0.56	0.08	0.29	0.38	0.61	0.08
Posterior_limb_of_int_capsule_LH	0.71	0.02	0.65	0.03	0	1	0.83	<0.01
Retrolenticular_int_capsule_RH	0.74	0.01	0.76	<0.01	0.14	0.69	0.79	0.01
Retrolenticular_int_capsule_LH	0.50	0.12	0.69	0.02	0.53	0.09	0.63	0.07
Anterior_corona_radiata_RH	0.63	0.04	0.79	<0.01	0.39	0.24	0.82	<0.01
Anterior_corona_radiata_LH	0.40	0.22	0.41	0.22	0.04	0.92	0.92	<0.01
Superior_corona_radiata_RH	0.71	0.02	0.78	<0.01	0.19	0.58	0.49	0.19
Superior_corona_radiata_LH	0.63	0.04	0.79	<0.01	0.44	0.17	0.27	0.49
Posterior_corona_radiata_RH	0.63	0.04	0.58	0.06	0.21	0.53	0.52	0.15
Posterior_corona_radiata_LH	0.72	0.02	0.74	<0.01	0.22	0.51	0.54	0.14
Posterior_thalamic_radiation_RH	0.54	0.09	0.77	<0.01	0.58	0.06	0.73	0.03
Posterior_thalamic_radiation_LH	0.55	0.09	0.8	<0.01	0.61	0.05	0.49	0.18
Sagittal_stratum_RH	0.48	0.14	0.63	0.04	0.51	0.11	0.4	0.28
Sagittal_stratum_LH	0.12	0.73	0.32	0.34	0.37	0.26	0.7	0.03
External_capsule_RH	0.67	0.03	0.84	<0.01	0.45	0.17	0.81	<0.01
External_capsule_LH	0.54	0.09	0.75	<0.01	0.61	0.05	0.44	0.24
Cingulum_RH	0.59	0.06	0.68	0.02	0.34	0.3	0.66	0.05
Cingulum_LH	0.60	0.06	0.64	0.03	0.18	0.6	0.57	0.11
Cingulum(hippocampus)_RH	0.43	0.19	0.47	0.15	0.24	0.47	0.59	0.09
Cingulum(hippocampus)_LH	0.55	0.09	0.72	0.01	0.51	0.11	0.44	0.24
Fornix/stria_terminalis_RH	0.30	0.37	0.49	0.12	0.5	0.12	0.58	0.1
Fornix/stria_terminalis_LH	0.45	0.17	0.51	0.11	0.26	0.45	0.48	0.19
Sup_longitudinal_fasciculus_RH	0.65	0.03	0.61	0.05	0.05	0.88	0.6	0.09
Sup_longitudinal_fasciculus_LH	0.74	0.01	0.78	<0.01	0.21	0.55	0.79	0.01
Sup_fronto_occip_fasciculus_RH	0.55	0.09	0.52	0.1	0.03	0.93	0.79	0.01
Sup_fronto_occip_fasciculus_LH	0.65	0.03	0.83	<0.01	0.43	0.19	0.62	0.08
Uncinate_fasciculus_RH	0.41	0.21	0.56	0.07	0.52	0.1	0.52	0.15
Uncinate_fasciculus_LH	0.30	0.37	0.31	0.36	-0.21	0.54	0.45	0.22
Tapetum_RH	0.29	0.39	0.37	0.27	0.07	0.83	0.57	0.11
Tapetum_LH	0.43	0.19	0.47	0.15	-0.03	0.93	0.58	0.1

4.9.2 Serum Iron compared to FA

Spearman correlations indicated significant positive associations between serum iron concentrations and the FA in all 48 WMTs, while the associations were significant ($p < 0.05$) in 13 of the 48 WMTs (Table 4.8): in the inferior cerebellar peduncle (right), anterior limb of the internal capsule (left), posterior limb of the internal capsule (left), retrolenticular internal capsule (right), anterior corona radiate (right), superior corona radiate (right and left), posterior corona radiata (right and left), external capsule (right), superior longitudinal fasciculus (right and left) and superior fronto-occipital fasciculus (left).

When assessing the FA values of EDSS compared to age diagnosed there were 4 significant associations in the cortico spinal tract RH (p=<0.01), superior cerebellar peduncle RH (p=0.04) and LH (p=<0.01) and posterior cerebellar peduncle LH (p=0.04). This is depicted in Table 4.8.

Table 4.8 FA correlations of EDSS and age diagnosed in the 48 WM tracts (significant p values in bold).

FRACTIONAL ANISOTROPY (n=11)	EDSS		Age Diagnosed	
	r	p	r	p
Middle_cerebellar_peduncle	**-0.69**	**0.02**	0.51	0.11
Fornix	**-0.84**	**<0.01**	0.50	0.12
Corticospinal_tract_RH	**-0.81**	**<0.01**	**0.74**	**<0.01**
Corticospinal_tract_LH	**-0.87**	**<0.01**	0.53	0.09
Medial_lemniscus_RH	**-0.73**	**0.01**	0.37	0.26
Medial_lemniscus_LH	**-0.79**	**<0.01**	0.52	0.10
Superior_cerebellar_peduncle_RH	**-0.88**	**<0.01**	0.59	0.05
Superior_cerebellar_peduncle_LH	**-0.89**	**<0.01**	0.39	0.23
Cerebral_peduncle_RH	**-0.84**	**<0.01**	0.47	0.14
Anterior_limb_of_int_capsule_LH	**-0.85**	**<0.01**	0.47	0.15
Superior_corona_radiata_LH	-0.61	0.05	**0.75**	**<0.01**
Posterior_corona_radiata_RH	**-0.77**	**<0.01**	0.59	0.05
Cingulum_LH	**-0.83**	**<0.01**	0.31	0.36
Cingulum(hippocampus)_RH	**-0.95**	**<0.01**	0.36	0.28
Cingulum(hippocampus)_LH	**-0.71**	**0.01**	0.40	0.22
Fornix/stria_terminalis_RH	**-0.81**	**<0.01**	**0.68**	**0.02**
Fornix/stria_terminalis_LH	**-0.85**	**<0.01**	0.46	0.15
Sup_longitudinal_fasciculus_RH	**-0.82**	**<0.01**	0.34	0.30
Uncinate_fasciculus_RH	**-0.83**	**<0.01**	0.27	0.41
Tapetum_RH	**-0.84**	**<0.01**	0.14	0.68

(Complete graph can be seen as **Appendix I**)

4.9.3 Haemoglobin, Ferritin and FA

There were no significant associations between the FA and Hb in any of the WMTs assessed (Table 4.7). Since blood ferritin values are increased during inflammation, CRP values higher than 10 were used as a cut-off to adjust for falsely high ferritin values. Some of the WMTs then showed significant associations between ferritin and FA with r-values ranging from 0.69 to 0.92 (Table 4.7).

4.10 Radial diffusivity (RD)

RD is diffusivity perpendicular to the WMTs. RD is often increased when myelin breakdown is experienced in diseases like MS (Benedetti et al., 2011).

4.10.1 RD compared to disability

When the RD was compared to EDSS in the MS subject and control subjects there were significant associations in 33 of the 48 tracts of which 19 were highly significant with a p=<0.01.

Figure 4.11 illustrates a significant association of RD values in the fornix of the 3 groups (Kruskal-Wallis, p=<0.01).

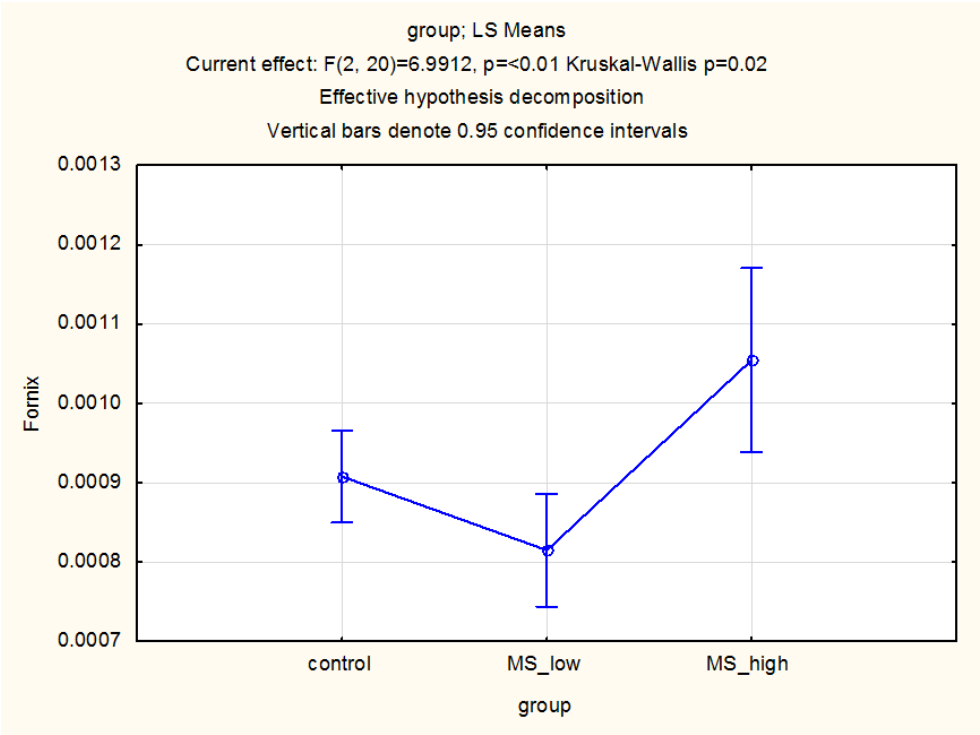


Figure 4.11: RD in the fornix between subjects with high EDSS, low EDSS and controls. CC= corpus callosum. MS= Multiple Sclerosis.

4.10.2 Effects of Iron on Myelin (RD results)

When assessing RD values of % Tf sat there were significant associations in 22 of the 48 WMTs of which 11 were highly significant with p=<0.01 (see Table 4.11). Haemoglobin and ferritin showed no significant associations.

Table 4.9 Radial diffusivity of MS subjects. RD correlations of EDSS, % Tf saturation, Haemoglobin, Ferritin in the WM tracts (only those with a threshold of < 0.01 were considered in all categories, significant p values in bold).

RADIAL DIFFUSIVITY (n=11)	EDSS		% Tf saturation		Haemoglobin		Ferritin	
	r	p	r	p	r	p	r	p
Middle_cerebellar_peduncle	**0.77**	**<0.01**	-0.38	0.24	-0.23	0.49	-0.45	0.16
CC_genu	**0.83**	**<0.01**	-0.14	0.69	0.02	0.96	-0.32	0.33
CC_splenium	**0.71**	**0.01**	-0.54	0.08	-0.44	0.17	-0.15	0.65
Fornix	**0.68**	**0.02**	**-0.68**	**0.02**	-0.49	0.12	-0.17	0.61
Corticospinal_tract_RH	**0.79**	**<0.01**	-0.39	0.23	-0.20	0.55	-0.19	0.58
Corticospinal_tract_LH	**0.77**	**<0.01**	-0.38	0.24	-0.37	0.26	0.01	0.98
Medial_lemniscus_RH	**0.76**	**<0.01**	-0.61	0.05	-0.46	0.16	0.01	0.98
Medial_lemniscus_LH	**0.84**	**<0.01**	**-0.65**	**0.03**	-0.46	0.16	-0.08	0.82
Inferior_cerebellar_peduncle_RH	**0.84**	**<0.01**	-0.58	0.06	-0.23	0.49	-0.29	0.38
Superior_cerebellar_peduncle_RH	**0.83**	**<0.01**	-0.49	0.12	-0.26	0.43	-0.26	0.45
Superior_cerebellar_peduncle_LH	**0.82**	**<0.01**	-0.50	0.12	-0.42	0.20	-0.13	0.71
Cerebral_peduncle_RH	**0.66**	**0.03**	-0.55	0.08	-0.12	0.73	-0.32	0.34
Cerebral_peduncle_LH	0.57	0.07	**-0.90**	**<0.01**	-0.33	0.32	-0.01	0.98
Anterior_limb_of_int_capsule_RH	0.55	0.08	**-0.80**	**<0.01**	-0.11	0.75	-0.20	0.55
Anterior_limb_of_int_capsule_LH	**0.67**	**0.03**	**-0.74**	**<0.01**	-0.22	0.51	-0.30	0.37
Posterior_limb_of_int_capsule_RH	**0.64**	**0.03**	-0.57	0.07	-0.33	0.32	-0.26	0.45
Posterior_limb_of_int_capsule_LH	0.53	0.10	**-0.85**	**<0.01**	-0.20	0.55	-0.21	0.54
Retrolenticular_int_capsule_RH	0.47	0.14	**-0.79**	**<0.01**	-0.24	0.47	-0.13	0.70
Retrolenticular_int_capsule_LH	0.24	0.47	**-0.74**	**<0.01**	-0.20	0.55	-0.03	0.93
Anterior_corona_radiata_RH	**0.62**	**0.04**	**-0.81**	**<0.01**	-0.34	0.30	-0.15	0.66
Superior_corona_radiata_RH	**0.62**	**0.04**	**-0.79**	**<0.01**	-0.08	0.82	-0.29	0.38
Superior_corona_radiata_LH	0.49	0.13	**-0.83**	**<0.01**	-0.06	0.85	-0.33	0.32
Posterior_corona_radiata_RH	**0.84**	**<0.01**	**-0.67**	**0.02**	-0.39	0.24	-0.17	0.62
Posterior_corona_radiata_LH	**0.65**	**0.03**	**-0.68**	**0.02**	-0.18	0.59	-0.33	0.32
Posterior_thalamic_radiation_RH	**0.72**	**0.01**	**-0.71**	**0.01**	-0.55	0.08	-0.07	0.85
Posterior_thalamic_radiation_LH	**0.83**	**<0.01**	**-0.65**	**0.03**	-0.36	0.28	-0.18	0.59
Sagittal_stratum_RH	0.56	0.07	**-0.65**	**0.03**	-0.56	0.07	-0.15	0.66
Sagittal_stratum_LH	0.53	0.10	-0.56	0.07	-0.29	0.39	-0.21	0.54
External_capsule_RH	**0.61**	**0.05**	**-0.66**	**0.03**	-0.06	0.85	-0.41	0.22
External_capsule_LH	0.52	0.10	**-0.64**	**0.03**	-0.27	0.42	-0.37	0.26
Cingulum_RH	**0.61**	**0.05**	-0.54	0.09	-0.10	0.78	-0.34	0.31
Cingulum_LH	**0.59**	**0.05**	**-0.63**	**0.04**	0.01	0.98	0.04	0.92
Cingulum(hippocampus)_RH	**0.76**	**<0.01**	-0.31	0.36	-0.30	0.37	-0.17	0.61
Cingulum(hippocampus)_LH	**0.76**	**<0.01**	**-0.78**	**<0.01**	-0.44	0.17	0.00	1.00
Fornix/stria_terminalis_RH	**0.77**	**<0.01**	-0.43	0.19	-0.37	0.26	-0.40	0.23
Fornix/stria_terminalis_LH	**0.84**	**<0.01**	-0.59	0.06	-0.49	0.12	-0.02	0.95
Sup_longitudinal_fasciculus_RH	**0.91**	**<0.01**	-0.60	0.05	-0.32	0.35	-0.26	0.45
Sup_longitudinal_fasciculus_LH	0.38	0.25	**-0.73**	**0.01**	-0.30	0.38	-0.13	0.71
Sup_fronto_occip_fasciculus_RH	**0.62**	**0.04**	-0.53	0.09	-0.45	0.17	-0.08	0.81

Sup_fronto_occip_fasciculus_LH	0.54	0.09	**-0.80**	**<0.01**	-0.39	0.24	-0.18	0.61
Uncinate_fasciculus_RH	**0.83**	**<0.01**	-0.51	0.11	-0.46	0.15	-0.15	0.66
Uncinate_fasciculus_LH	**0.74**	**<0.01**	-0.14	0.69	0.05	0.89	-0.23	0.49
Tapetum_RH	**0.85**	**<0.01**	-0.40	0.23	0.07	0.84	-0.30	0.36
Tapetum_LH	**0.65**	**0.03**	-0.35	0.29	0.10	0.77	-0.11	0.75

Age diagnosed showed 5 associations in the fornix (p=0.03), cortico spinal tract RH (p=<0.01), inferior cerebellar peduncle RH (p=0.04), posterior cerebellar peduncle RH (p=0.03) and posterior thalamic radiation LH (p=0.03). See **Appendix J**.

4.11 Mean diffusivity (MD)

MD together with FA are two diffusion metrics often applied as indices of tissue pathology. It measures the magnitude of water molecule diffusion (Benedetti *et al*., 2011).

4.11.1 MD compared to disability

Of the 48 tracts assessed only 1 [medial lemniscus LH (p=<0.01)] was highly significant for the whole MS group as there were mostly trends seen.

Figure 4.12 illustrates a significant association when measuring disability between groups in the medial lemniscus LH (Kruskal-Wallis, p=<0.01).

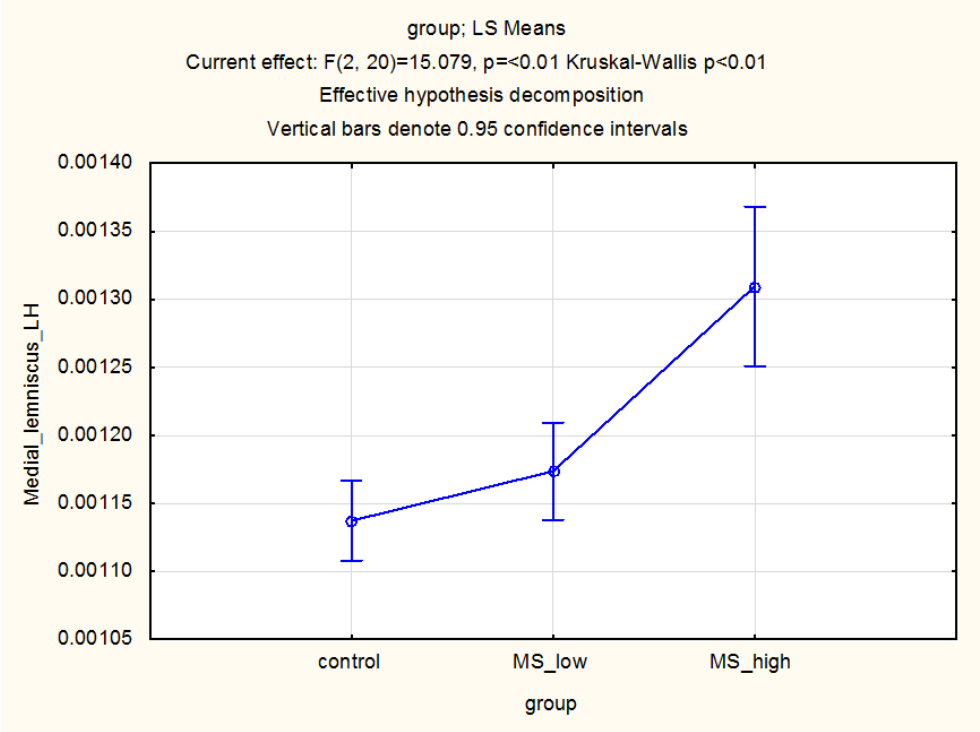


Figure 4.12 MD between groups in the medial lemniscus LH. MS= Multiple Sclerosis. LH= left hemisphere

4.11.2 Effects of Iron on Myelin (MD results)

When MD values of EDSS were compared to % Tf saturation, 8 WMTs were highly significantly associated [cerebellar peduncle LH, anterior limb of internal capsule RH and LH, posterior limb of internal capsule LH, retrolenticular internal capsule RH, anterior corona radiate RH, cingulum (hippocampus) LH, superior fronto-occipital fasciculus LH all with p values of <0.01 (see **Appendix K**)]. Haemoglobin showed 1 highly significant association in the SLF_RH (p=<0.01). Ferritin showed no significant associations (see **Appendix K:** Table 4.13). MD correlations of EDSS compared to age diagnosed in the 48 WM tracts showed no significant associations (see **Appendix L**).

4.12 Axial diffusivity (AD)

AD is diffusivity parallel to the primary fiber orientation. Axonal damage is often reflected by a decrease in AD (Benedetti *et al*., 2011).

4.12.1 AD compared to disability

There were no significant differences between groups with AD except for the medial lemniscus LH and RH.

Figure 4.13 illustrates a significant association when measuring disability between the controls, MS high and MS low groups in the medial lemniscus LH (Kruskal-Wallis, p=<0.01).

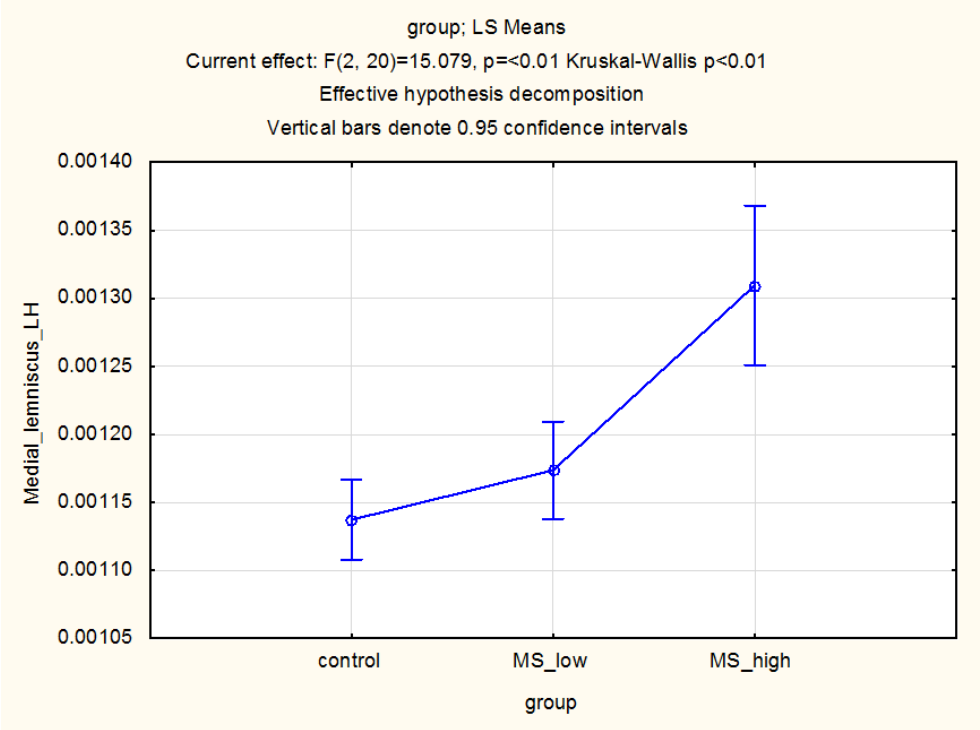


Figure 4.13 AD between group differences in the medial lemniscus LH.

4.12.2 Effects of Iron on Myelin (AD)

In subjects where the % Tf sat was low significance was shown in the cerebellar peduncle LH, anterior limb of internal capsule RH and LH, posterior limb of internal capsule LH, retrolenticular internal capsule RH, anterior corona radiate RH, cingulum (hippocampus) LH, superior fronto-occipital fasciculus LH all with p values of <0.01 (see Table 4.12). Haemoglobin showed significance in the superior longitudinal fasciculus (right; p=<0.01). Ferritin showed no significance. The above results for AD are an exact replica of MD for the MS subjects. (See Table 4.10).

Table 4.10 Axial Diffusivity of MS subjects. Correlation between EDSS, % Tf saturation, Haemoglobin and Ferritin in the WM tracts (only those with a threshold of < 0.01 were considered in all categories).

AXIAL DIFFUSIVITY (n=11)	EDSS		% Tf saturation		Haemoglobin		Ferritin	
	r	p	r	p	r	p	r	p
Middle_cerebellar_peduncle	0.67	0.02	-0.2	0.55	0.03	0.94	-0.49	0.13
CC_genu	0.52	0.10	0.04	0.9	-0.09	0.80	-0.69	0.02
Medial_lemniscus_LH	0.74	<0.01	-0.31	0.36	-0.31	0.35	-0.05	0.87
Superior_cerebellar_peduncle_LH	0.67	0.02	-0.53	0.1	-0.31	0.35	-0.27	0.42
Cerebral_peduncle_RH	0.64	0.04	-0.64	0.03	-0.12	0.73	-0.34	0.31
Cerebral_peduncle_LH	0.54	0.09	-0.84	<0.01	-0.26	0.45	-0.17	0.62
Anterior_limb_of_int_capsule_RH	0.61	0.05	-0.79	<0.01	-0.18	0.59	-0.31	0.35
Anterior_limb_of_int_capsule_LH	0.37	0.27	-0.75	<0.01	-0.19	0.58	-0.06	0.86
Posterior_limb_of_int_capsule_LH	0.25	0.45	-0.84	<0.01	-0.30	0.37	0.16	0.64
Retrolenticular_int_capsule_RH	0.45	0.17	-0.76	<0.01	-0.28	0.40	-0.11	0.75
Anterior_corona_radiata_RH	0.43	0.18	-0.86	<0.01	-0.28	0.41	-0.22	0.51
Superior_corona_radiata_LH	0.42	0.20	-0.66	0.03	-0.04	0.90	-0.45	0.16
Sagittal_stratum_LH	0.61	0.05	-0.63	0.04	-0.22	0.52	-0.29	0.39
Cingulum(hippocampus)_LH	0.59	0.05	-0.8	<0.01	-0.28	0.41	-0.05	0.89
Fornix/stria_terminalis_RH	0.72	0.01	-0.36	0.28	-0.20	0.55	-0.45	0.16
Sup_longitudinal_fasciculus_RH	0.07	0.85	-0.35	0.3	-0.89	<0.01	0.25	0.46
Sup_fronto_occip_fasciculus_LH	0.39	0.24	-0.76	<0.01	-0.13	0.70	-0.41	0.21
Uncinate_fasciculus_RH	0.70	0.02	-0.54	0.08	-0.49	0.13	-0.2	0.55
Tapetum_RH	0.62	0.04	-0.19	0.57	-0.20	0.56	-0.45	0.16

(Complete graph can be seen as **Appendix M**)

AD correlations of EDSS compared to age diagnosed in the 48 WM tracts showed no associations (**Appendix N**).

4.13 Correlation of MRS outcomes with EDSS and biochemical markers

In the assessment of the MRS results only 8 (n=8) of the subject data could be successfully used. There was a normal distribution of all numerically valued variables.

Table 4.11 Correlations (MS MRS EDSS & Biological) Marked correlations are significant at p < .05000 (n=8)

	N=8 (Casewise deletion of missing data)					
	Age Symptoms	Age Diagnosed	Age at scan time	Duration of symptoms	Duration of diagnosis	EDSS SCORE
right midline grey	-0.348194	0.064699	-0.085054	0.420584	-0.182378	-0.458648
right midline grey matter NAA	-0.481173	-0.227591	-0.397305	0.337986	-0.090404	-0.544281
right midline grey	-0.482279	-0.062392	-0.474732	0.272244	-0.409218	-0.566424
right midline grey	-0.469476	-0.146866	-0.490565	0.240296	-0.304439	-0.653887
left midline grey	0.261570	0.557438	0.566091	0.120646	-0.206320	-0.079364
left midline grey	-0.448793	-0.399369	-0.323531	0.356136	0.234028	-0.557438
left midline grey	-0.119409	0.352320	-0.006079	0.164308	-0.512791	-0.385982
left midline grey	-0.556802	-0.644686	-0.439332	0.408862	0.464936	-0.363518
right white matter Ins	-0.105041	0.141009	0.221070	0.341379	0.029645	0.545304
right white matter NAA	-0.509759	-0.123799	-0.202436	0.548001	-0.034800	-0.670491
right white matter	-0.000773	0.050710	-0.206751	-0.178647	-0.290162	-0.008574
right white matter	-0.569951	-0.086394	-0.084406	0.736102	0.035475	-0.398115
left white matter Ins	-0.059943	-0.214249	0.320850	0.364075	0.645129	0.730716
left white matter NAA	-0.341510	0.067913	0.143457	0.609753	0.053146	0.121364
left white matter	0.053550	0.016710	-0.218457	-0.265977	-0.253595	0.229426
left white matter	-0.252675	0.358869	0.091541	0.438450	-0.419615	-0.575297

Pearson's analysis proved a significant association between the EDSS and the white matter insula in the left hemisphere of the brain (r= 0.73; p=0.39).

NAA+ NAAG showed a significant correlation with duration of disease in the Right WM of the brain (r=0.73; p=0.37).

Upon closer inspection of the significant correlations the WM variables (GPC+PCh) left and right proved to have a strong positive relationship with % Tf (right- r=0.81, p=0.026; left- r=0.81, p=0.028) and HB (right- r=0.96, p=0.001; left- r=0.88, p=0.009). See Table 4.11.

4.14 Conclusion

The results of this chapter will be discussed in chapter 5 within the study context. The conclusions that were drawn from the findings will be discussed and the limitations and recommendations for future studies will be presented.

CHAPTER FIVE
DISCUSSION AND CONCLUSION

5.1 Introduction

In the present study Diffusion tensor imaging (DTI), and Magnetic Resonance Spectroscopy (MRS) were the techniques used to obtain high quality images that would allow the investigation of a possible association between lesion load, disability and biochemical markers (serum iron, % transferrin saturation, ferritin, haemoglobin, etc.) in the MS disease process.

Previously published studies have reported conflicting results with regards to correlations between disability and MRI (e.g. Giorgio *et al*., 2010). There are many reasons that could explain the varied result but one probable explanation is that the MRIs did not make use of advanced metrics like DTI and MRS. For this reason the study reported on in this thesis was designed to examine whether there is a role for DTI and MRS as the most accurate markers in monitoring disability and disease progression in MS. Following is a discussion of the association between disability, measured by EDSS, lesion number and DTI. The role of biochemical marker values, in particular iron, in subjects diagnosed with MS is also discussed.

5.2 EDSS compared to Lesion Number (N) in MS subjects

The T2 hyperintense lesions demonstrate some of the total WM damage. These lesions are a reflection of focal demyelination and axonal loss (De Groot *et al*., 2001). The current study demonstrated that subjects with high EDSS scores had high lesion N's, high Fezeka's scores as well as a high number of enhanced lesions. It is noted that the measure of lesion number in some subjects was affected by the fact that the lesions were so profound and confluent that they could not be characterised individually. This was possibly an indication that active demyelinating disease was present; however it could also be representative of many other pathological conditions such as blood-brain barrier breakdown, inflammatory demyelination, fibrillary astrocytosis and remyelination (Mostert*et al.,* 2010). This could not be clearly assessed, probably due to the variance in the disabilities compared to the lesion numbers in MS subjects.

Kidd et al. (1999) stated that fewer enhancing lesions are an indication of clinically benign disease whereas more extensive lesions are an indication of a secondary progressive disease course. In contrast with this statement the current study had 2 subjects with low EDSS who had high lesion numbers. MS01 (EDSS=2) had a lesion number of 79 and MS06

(EDSS=1.5) had a lesion number of 80. On the other hand in MS15 (EDSS=7.5) the supratentorial lesions in this patient were too numerous and confluent to individually charactersise. Loss of the normal midbrain convexity and tectal atrophyas well as significant cerebellar atrophy were noted. Therefore the lesion count was low: not because there were few lesions, but because they were too numerous to characterise. This corroborates with what has been reported in longitudinal studies done by Blinkenberget al. (2000), that evidence of multiple lesions in the brains of some subjects did not necessarily relate to the degree of disability. It could be possible that the lesions seen on the scans are merely due to leucoaraiosis associated with small vessel disease. Therefore as stated by Kidd *et al.* (1999), the correlation of lesion number to disability will never be perfect as T2 lesions are heterogeneous in nature.

5.3 DTI findings compared to EDSS

By applying various diffusion indices across the brain different patterns of FA, RD, AD and MD changes become visible indicating that various WM pathology occurs in MS (Lui *et al*., 2012).

The present study found negative associations of the FA with the EDSS in all the WMTs investigated, and these associations were significant ($p < 0.05$) in 32 (67%) of the 48 WMTs. Previous studies (Bodini*et al.,* 2013; Onu*et al.,* 2012; Caligiuri*et al.,* 2014) had a similar finding of association, while other studies found no significant correlations between FA and EDSS values (Hasan*et al*., 2005; Temel*et al*., 2013; Bethune *et al*., 2011; Giorgio *et al*., 2010). Furthermore in the current study, significantly decreased FA was found in several brain regions of MS patients when compared to the control group (Table 4.5), which is a similar finding to previous studies (Onu*et al*., 2012; Hasan*et al.,* 2005; Asaf*et al.,* 2015; Temel*et al.,* 2013; Liu *et al*., 2012; Bethune *et al.,* 2011).

A novel finding of this study was that when the MS patients were grouped according to their EDSS scores, subjects with low EDSS had similar FA values to the controls. Subjects with an EDSS < 3 showed white matter function similar to control subjects extending over the whole of the brain, this effect can be seen in 46 (96%) of the 48 WMTs investigated (Table 4.6). Examples of this novel finding are depicted in Figures 4.8 and Figure 4.9, showing that subjects with low EDSS had decreased FA and RD in the WMTs of the brain investigated effect. This can especially be seen in the CC genu, body and splenium, the fornix and the superior longitudinal fasciculus which is WMTs involved with cognition and movement. It appears to be possible that the three patients with high disability scores masked the favorable FA outcome of the other 8 patients who had low disability, so that the MS group as a whole seemed to have significantly lower FA values, and by implication worse myelin function compared to controls. Improved myelin function in patients with low EDSS may be

associated with factors that have an impact on myelin synthesis and maintenance: avoidance of risk factors such as inhalation of cigarette smoke. The intake of dietary factors such as 5 fruits and vegetables (Davis *et al.,* 2014), epigenetic factors such as iron, essential fatty acids and vitamin B12 (Bartzokis, 2011; Van Rensburg *et al*., 2012) as well as sufficient levels of physical activity (Fjeldstad*et al.,* 2015; Nelson *et al*., 2014).

In the study group an increase of MD was noted in certain areas of the brain due to the fact that the average diffusion increased. This could also happen if there is a loss in WM integrity because water can only move across membranes. Song *et al*. (2002; 2005) have shown in their studies that if there is an increase in RD and AD stays the same in an area then so-called “demyelination” takes place. In this study there was no change in AD, only the medial lemniscus LH showed a highly significant AD result. According to Song *et al*. (2002; 2005) when AD decreases in an area it is due to the loss of axons in structures, however the latter was only done on animals and not on humans.

In this study it showed that for disability (where the EDSS was increased) there was decreased FA and RD in most of the WMTs whereas AD and MD were increased.

The study shows that there is an abnormal DTI which correlates positively with an increased disability. This is usually a good indication of pathological changes.

5.4 Effects of Iron on Myelin

Iron is one of the most important metals in the brain and oligodendrocytes are the major iron containing cells found within the brain mainly involved in myelination (Sjöbeck&Englund, 2003; Bartzokis*et al.,* 2004). As a result, lack of iron may inversely affect some of the processes being carried out in the brain such as demyelination. Additionally, dysregulation of iron in the body has been found in some of the neurodegenerative diseases such as Alzheimer’s disease (AD) (Bartzokis*et al*., 2007) Parkinson’s disease and Hemochromatosis. Due to the fact that previous studies had shown that iron affects myelination negatively by causing effects like oxidative stress (Qian&Shen, 2001; Pinero & Connor, 2000), we assessed whether negative effects of blood iron parameters can be seen with FA.

The second novel finding of this study is that FA was positively associated with specific blood iron parameters (Table 4.7), while no negative associations were found, except with Hb; however, these associations were not significant and had very low r-values. While Hb was not associated with FA in any of the WMTs, serum iron concentration showed significant positive associations ($p < 0.05$) in WMTs, serum ferritin (after correction for increased CRP) in WMTs and with % Tf sat in 25 (52%) of the 48 tracts. Highly significant associations ($p< 0.01$) of FA with % Tf sat were found in 14 (29%) of the tracts (Table 4.7). These results may suggest that iron which is transported into the brain is of functional significance for white matter maintenance and that these iron parameters had no negative effects on WM function.

Due to the fact that oligodendrocytes have a high dependency on iron for normal function it is expected that there should be a requirement for iron in the WMTs (Barzokis, 2011). In MS, % Tf sat may be reduced due to inflammation, reduced iron uptake or increased iron loss (Khumalo, 1998). Especially in areas like the thalamus (of which the corona radiate, internal capsule, cingulum and cerebral peduncles are a part (Martini, 2006). Table 1 shows that only % Tf saturation is significantly associated with FA which could mean that risk of MS diagnosis may be related to factors other than iron overload as mentioned by Khalil *et al.* (2011). In addition, the study showed that in areas like the fornix (p=0.02) and CC splenium (p=0.02) the % Tf sat also had a significantly positive association with the FA. These areas are involved in emotional regulation as they also form part of the limbic system (Martini, 2006). Since patients with MS experience an increased incidence of depression as well as symptoms of fatigue, there may be an association of these conditions with decreased iron concentration and myelin function. The lack of significant associations of FA values with Hb suggests that the iron in this protein may not be available for WM synthesis (see Table 4.7). In MS patients with iron deficiency, Hb is the last parameter to decrease, since the iron concentration in Hb for oxygen delivery is preferentially protected (Van Toorn *et al*., 2010). Measuring Hb alone may therefore not provide a dependable estimate of iron availability for myelin functionality.

When the threshold was lowered to include only highly significant associations between FA and % Tf sat ($p < 0.01$) the WMTs that still showed an association were the medial lemniscus, cerebellar peduncle, and anterior limb of the internal capsule, retrolenticular internal capsule, anterior corona radiata and the superior corona radiate (Table 4.7). All these tracts are involved in automatic processing (Martini, 2006).

Serum iron concentration was also significantly ($p < 0.05$) positively associated with FA in 13 (27%) of the 48 WM tracts that were assessed, the inferior cerebellar peduncle, anterior limb of the internal capsule, posterior limb of the internal capsule, retrolenticular internal capsule, anterior corona radiata, superior corona radiata, posterior corona radiata, external capsule, superior longitudinal fasciculis and superior fronto-occipital fasciculis. All of these areas mentioned above are involved in the control of automatic functions (Martini, 2006).

Serum Tf concentration only showed 2 significant associations in the superior longitudinal fasciculus (right; p=0.02) and the F/striaterminalis (right; p=0.01) while Hb did not show significant associations (Table 4.7).

After adjusting for high CRP, serum ferritin showed significant ($p<0.05$) positive associations with FA in 4 WMTs (Middle cerebellar peduncle, Medial lemniscus RH, Anterior limb of internal capsule LH, Retrolenticular internal capsule RH), while 3 tracts were highly significantly associated ($p<0.01$) namely the Posterior limb of internal capsule LH, Anterior

corona radiata RH and Anterior corona radiata LH. This finding is in keeping with the work of Schonberg *et al*. (2013) who found that ferritin stimulated genesis of oligodendrocytes in the spinal cord, and Hulet *et al*. (2000) and Todorich *et al.* (2011) who found ferritin receptors on oligodendrocytes, which suggested that ferritin may present a delivery route of iron for myelin production in oligodendrocytes. In the present study, principal component analysis indicated that serum ferritin values co-segregated with CRP (results not shown).

5.5 Limitations and Recommendations

A limitation of this study is the small number of patients of whom the DTI could be used for analysis. In the "high EDSS" group, the FA data of only 3 patients could be used for analysis, although 4 patients with EDSS values > 6 were included in the study. Nevertheless, the FA data from these 3 patients needed to be analysed separately since the true outcome of FA in the whole cohort was masked by the highly significant decreased FA in these 3 patients. We acknowledge the limitation of 11 subjects but this was accommodated for by examining the 48 WMTs of the 11 subjects which provided a total of 528 WMTs. To increase the statistical power of the study, an "extremes of outcome" approach was used to evaluate the effect of disability status and iron parameters on FA.

The strength of the study was the availability of blood iron parameters and EDSS measurements, which presented the opportunity to evaluate the association of systemic iron concentrations with FA, and therefore potentially with WM function and disease outcome.

Future studies should address these limitations and include a larger sample size in order to verify the results.

5.6 Conclusion

Although other MRI metrics may have stronger clinical correlates it can be concluded that the relationship between EDSS and lesion number count has an impact on cerebral neural activity and is capable of being logically proved.

This study made 2 important discoveries: First, the FA of WMTs as a parameter of myelin integrity was lower only in those MS subjects who had high EDSS scores. Subjects who had EDSS scores < 3 (i.e. who had a "benign" disease outcome) had FA values similar to control values and this finding was not related to their age or disease duration. These findings may argue against the assumption that people diagnosed with MS inevitably experience increasing myelin degeneration over time. With the results of Wake *et al.* (2015) taken into account, concerted efforts by patients to improve their brain function by employing activities to increase neuronal function may prevent the regression of WM.

Second, the association found between FA and iron parameters, especially % Tf sat, confirms the hypothesis that iron availability to the WM may be a requirement for optimal

myelin functionality. The association between FA as a parameter of myelin integrity and iron availability to the white matter, confirms previous perceptions that iron is a requirement for myelin maintenance. The energy needed to create myelin is extremely high, thus the increased requirement for iron in the brain; conversely, if there is a lack of iron, myelination cannot occur. The comparisons between the FA and the % Tf sat of the patients and the controls showed the most significant differences. Therefore these results suggest that serum iron concentration, ferritin and % Tf sat had an effect on myelination. The lack of association between FA and Hb suggests that the iron in this protein is not available for WM function.

This was echoed in the results of the radial diffusivity whereas axial diffusivity and mean diffusivity showed significant negative associations between FA, EDSS and % Tf. It is noted that these findings are in contrast to what many authors found surrounding FA and iron in the brain (Qian&Shen, 2001; Piñero& Connor, 2000; Larkin &Rao, 1990). It is thus imperative that medical professionals measure iron and supplement those with low blood values as it is may be related to myelin function.

The results of the present study, if confirmed in larger studies, should be an indication that measurement of iron parameters in patients with MS could provide important information regarding myelin function.

REFERENCES

Abe, O., Takao, H., Gonoi, W., Sasaki, H., Murakami, H., Kabasawa, H., Kawaguchi, H., Goto, M., Yamada, H., Yamasue, H., Kasai, K., Aoki S. and Ohtomo, K. 2010. Voxel-based analysis of the diffusion tensor. *Neuroradiology*, 52:699-710.

Alam, M.S., Sajjad, Z., Hafeez, S. and Akhter, W. 2011. Magnetic resonance spectroscopy in focal brain lesions. *Journal of Pakistan Medical Association,* 61(6):540-543.

Ames, F.R. and Bowen, R.M. 1979. First coloured South African with multiple sclerosis confirmed by necropsy. *Journal of Neurology, Neurosurgery and Psychiatry,* 42:731-733.

Annapurna, A.A., Kumar, K.V., Mohan, M.R.P., Someswara, R.K. and Rai, A.J. 2002 Multiple sclerosis: the disease and its treatment. *Indian Journal of Pharmacology,* 34:3-15.

Arnold, D.L. and Matthews, P.M. 1996. Practical aspects of clinical applications of MRS in the brain. In: Young, I.R. and Charles, H.C. (eds). *MR spectroscopy clinical applications and techniques,* United Kingdom: Cambridge University Press: 147-148.

Asaf, A., Evans, S. and Anat, A. 2015. Injury to white matter tracts in relapsing-remitting multiple sclerosis: A possible therapeutic window within the first 5 years from onset using diffusion-tensor imaging tract-based spatial statistics. *Neuroimage Clinical*, 8:261-266.

Ascherio, A. and Munger, K.L. 2007 Environmental risk factors for multiple sclerosis. Part I: the role of infection. *Annals of Neurology,* 61(4):288-99.

Barkhof, F. 2002. The clinico-radiological paradox in multiple sclerosis revisited. *Current Opinion in Neurology,* 15:239-245.

Barnett, M.H. and Prineas, J.W. 2004. Relapsing and remitting multiple sclerosis: pathology of the newly forming lesion. *Annals of Neurology,* 55:458-468.

Bartzokis, G. 2011. Neuroglialpharmacology: white matter pathophysiologies and psychiatric treatments. *Frontiers in Bioscience* (Landmark Edition), 16:2695-733.

Bartzokis, G., Tishler, T.A., Lu, P.H., Villablanca, P., Altshuler, L.L., Carter, M., Huang, D., Edwards, N. and Mintz, J. 2007. Brain ferritin iron may influence age- and gender-related risks of neurodegeneration. *Neurobiology of Aging*, 28:414-423.

Benedetti, F., Absinta, M., Rocca, M.A., Radaelli, D., Poletti, S., Bernasconi, A., Dallaspezia, S., Pagani, E., Falini, A., Copetti, M., Colombo, C., Comi, G., Smeraldi, E. and Filippi, M.

2011. Tract-specific white matter structural disruption in patients with bipolar disorder. *Bipolar disorders,* 13:414-424.

Benedict, R.H., Hussein, S., Englert, J., Dwyer, M.G., Abdelrahman, N., Cox, J.L., Munschauer, F.E., Weinstock-Guttman, B. and Zivadinov, R. 2008. Cortical atrophy and personality in multiple sclerosis. *Neuropsychology*, 22(4):432-441.

Berg, K.E. and Latin, R.W. 1994. *Essentials of Research Methods in Health: Physical Education, Exercise Science, and Recreation*, 3rd edition, Philadelphia: Lippincott Williams & Wilken: 15-20.

Bernal, B. and Altman, N. 2010. The connectivity of the superior longitudinal fasciculus: a tractography DTI study. *Magnetic resonance Imaging*, 28: 217-225.

Bethune, A., Tipu, V., Sled, J.G., Narayanan, S., Arnold, D.L., Mabbott, D., Rockel, C., Ghassemi, R., Till, C. and Banwell, B. 2011. Diffusion tensor imaging and cognitive speed in children with multiple sclerosis. *Journal of Neurological Science*, 309(1-2):68-74.

Bjartmar, C. and Trapp, B.D. 2003. Axonal degeneration and progressive neurologic disability in multiple sclerosis. *Neurotoxicity Research,* 5:157-164.

Bjartmar, C., Wujek, J.R. and Trapp, B.D. 2003. Axonal loss in the pathology of MS: consequences for understanding the progressive phase of the disease. *Journal of Neurological Science,* 206:165-71.

Blamire, A.M., Cader, S., Lee, M., Palace, J. and Matthews, P.M. 2007. Axonal damage in the Spinal Cord of Multiple Sclerosis Patients detected by Magnetic resonance Spectroscopy. *Magnetic Resonance in Medicine*, 58:880-885.

Blinkenberg, M., Rune, K., Jensen, C.V., Ravnborg, M., Kyllingsbæk, S., Holm, S., Paulson, O.B., and Sørensen, P.S. 2000. Cortical cerebral metabolism correlates with MRI lesion load and cognitive dysfunction in MS. *Neurology*, 54(3):558.

Bodini, B., Cercignani, M., Khaleeli, Z., Miller, D.H., Ron, M., Penny, S., Thompson, A.J. and Ciccarelli, O. 2013. Corpus callosum damage predicts disability progression and cognitive dysfunction in primary-progressive MS after five years. *Human Brain Mapping*, 34(5):1163-72.

Brassington, J.C., and Marsh, N. 1998. Neuropsychological aspects of multiple sclerosis. *Neuropsychology Review,* 8(2):43-77.

Budde, M.D., Xie, M., Cross, A.H. and Song, S. 2009. Axial Diffusivity Is the Primary Correlate of axonal Injury in the experimental autoimmune Encephalomyelitis Spinal Cord: Quantitative Pixelwise Analysis. *The Journal of Neuroscience*, 29(9):2805-2813.

Bushong, S.C. 2003. Magn*etic Resonance Imaging: Physical and Biological Principles*, 3rd edition, Texas: Mosby: 403-416.

Byun, E., Cailliers, S., Montalban, X., Villoslada, P., Fernandez, O., Brassat, D., Comabella, M., Wang, J., Barcellos, L.F., Baranzini, S.E. and Oksenberg, J.R. 2008. Genome-wide harmacogenomics analysis of the response to interferon beta therapy in multiple sclerosis. *Archives of Neorology,* 64(3):337-344.

Caligiuri, M.E., Barone, S., Cherubini, A., Augimeri, A., Chiriaco, C., Trotta, M., Granata, A., Filippelli, E., Perrotta, P., Valentino, P. and Quattrone, A. 2014. The relationship between regional microstructural abnormalities of the corpus callosum and physical and cognitive disability in relapsing-remitting multiple sclerosis. *Neuroimage Clinical*, 7:28-33.

Cammer, W. 1984. Oligodendrocyte associated enzymes. In: *Oligodendroglia.* Norton, W.T. (ed). New York: Plenum Press: 199-232.

Cassol, E., Ranjeva, J., Ibarrola, D., Mékies, C., Manelfe, C., Clanet, M. and Berry, I. 2004. Diffusion tensor imaging in multiple sclerosis: a tool for monitoring changes in normal-appearing white matter. *Multiple Sclerosis*, 10:188-196.

Ceccarelli, A., Rocca, M.A., Pagani, E., Colombo, B., Martinelli, V., Comi, G. and Fillipi, M. 2008. A voxel based morphometry study of grey matter loss in MS patients with different clinical phenotypes. *Neuroimaging,* 42(1):315-322.

Ceccarelli, O., Brex, P.A., Thompson, A.J. and Miller, D.H. 2002. Disability and lesion load in MS: a reassessment with MS functional composite score and 3D fast FLAIR. *Journal of Neurology*, 249:18-24.

Cercignani, M., Inglese, M., Pagani, E., Comi, G. and Filippi, M. 2001. Mean Diffusivity and Fractional Anisotropy Histograms of Patients with Multiple Sclerosis. *American Journal of Neuroradiology*, 22:952-958.

Chilcott, J., McCabe, C., Tappenden, P., O'Hagan, A., Cooper, N.J., Abrams, K. and Claxton, K. 2003. Modeling the cost effectiveness of interferon beta and glatiramer acetate in the management of multiple sclerosis. *British Medical Journal*, 326:1-6.

Clark, K.A., Nuechterlein, K.H., Asarnow, R.F., Hamilton, L.S., Phillips, O.R., Hageman, N.S., Woods, R.P., Alger, J.R., Toga, A.W., and Narr, K.L. 2011. *Journal of Psychiatry Res*. 45(7):980-988.

Compston, A. and Coles, A. 2002. Multiple sclerosis. *Lancet,* 359(9313):1221-31.

Connor, J.R. and Menzies, S.L. 1996. Relatioship iron to oligodendrocytes and myelination. *Glia,* 17:83-93.

Connor, J.R., Roskams, A.J.I., Menzies, S.L. and Williams, M.E. 1993. Transferrin in the central nervous system of the shiverer mouse myelin mutant. *Journal of Neuroscience Research*, 36(5):501-507.

Cullen, K.R., Wallace, S., Magnotta, V.A., Bockholte, J., Ehrlich, S., Gollub, R.L., Manoach, D.S., Ho, B.C., Clark, V.P., Lauriello, J., Bustillo, J.R., Schulz,S.C., Andreasen, N.C., Calhoun, V.D., Lima, K.O. and White, T. 2012.Cigarette smoking and white matter microstructure in schizophrenia. *Psychiatry Research: Neuroimaging,* 201(2):152-158.

Dähnert, W. 2007. *Radiology Review Manual*, 6th edition, USA: Lippencott Williams & Wilkins; 310-311.

Davis, F.A. 2013. The clinic-radiological paradox in multiple sclerosis: novel implications of lesion size. *Multiple Sclerosis Journal*, DOI: 10.1177/1352458513498949. [13 November 2014]

Davis, W., Van Rensburg, S., Cronje, F.J., Whati, L., Fisher, L.R., Van der Merwe, L., Geiger, D., Hassan, M.S., Matsha, T., Erasmus, R.T. and Kotze, M.J. 2014. The fat mass and obesity-associated FTO rs9939609 polymorphism is associated with elevated homocysteine levels in patients with multiple sclerosis screened for vascular risk factors. *Metabolic Brain Diseases*, 29:409-419. DOI 10.1007/s11011-014-9486-7. [13 February 2015]

Dean, G., Bhigjee, A.I., Bill, P.L., Fritz, V., Chikanza, I.C., Thomas, J.E., Levy, L.F. and Saffe, D. 1994. Multiple sclerosis in black South Africans and Zimbabweans. *Journal of Neurological Neurosurgery and Psychiatry*, 57:1064-1069. doi:10.1136/jnnp.57.9.1064. [04 September 2014]

De Groot, C.J.A., Bergers, E., Kamphorst, W., Ravid, R., Polman, C.H., Barkhof, F. and Van der Valk, P. 2001. Post-mortem MRI-guided sampling of multiple sclerosis brain lesions, Increased yield of active demyelinating and (p) reactive lesions. *Brain*, 124:1635-1645.

De Luca, G.C., Ramagopalan, S.V., Herrrera, B.M., Dyment, D.A., Lincoln, M.R., Montpetit, A., Pugliatti, M., Barnardo, M.C.N., Risch, N.J., Sadovnick, A.D., Chao, M., Sotgiu, S., Hudson, T.J. and Ebers, G.C. 2007. An extremes of outcome strategy provides evidence that multiple sclerosis severity is determined by alleles at the HLA – DRB1 locus. *Proceedings of the National Academy of Sciences,* 104(52):20896-20901.

De Stefano, N., Matthews, P.M., Fu, L., Narayanan, S., Stanley, J., Francis, G.S., Antel, J.P. and Arnold, D.1998. Axonal damage correlates with disability in patients with relapsing-remitting multiple sclerosis. Results of a longitudinal magnetic resonance spectroscopy study. *Brain*, 121: 1469-1477.

Diehl, B., Busch, R.M., Duncan, J.S., Piao, Z., Tkach, J. and Lüders, H. 2008. Abnormalities in diffusion tensor imaging of the uncinate fasciculus relate to reduced memory in temporal lobe epilepsy. *Epilepsia*, 49(8):1409-1418.

Donati, D. and Jacobson, S. 2002. Viruses and multiple sclerosis. In Brogden, K.A. & Guthmiller, J.M. (eds). *Polymicrobial diseases*. Washington (DC): American Society for Microbiology. http://www.ncbi.nlm.nih.gov/books/NBK2475/ [18 March 2013]

Dreha-Kulaczewski, S.F., Helms, G., Dechent, P., Hofer, S., Gärtner, J. and Frahm, J. 2009. Serial proton MR spectroscopy and diffusion tensor imaging in infantile Balo's concentric sclerosis. *Neuroradiology,* 51:113-121.

Duddy, M., Haghikia, A., Cocco, E., Eggers, C., Drulovic, J., Carmona, O., Zéphir, H. and Gold, R. 2011. Managing MS in a changing treatment landscape. *Journal of Neurology*, 258:728-739. DOI10.1007/s00415-011-6009-x. [3 March 2014]

Ebers, G.C. 2008. Analysis of 45 candidate genes for disease modifying activity in multiple sclerosis. *Journal of Neurology*, 255(8):1215-1219.

Farlax. 2013. *Apoptosis*. http://www.thefreedictionary.com [15 March 2010].

Fjeldstad, C., Fjeldstad, A.S. and Pardo, G. Use of Accelerometers to Measure Real-Life Physical Activity in Ambulatory Individuals with Multiple Sclerosis: A Pilot Study. 2015. *International Journal of MS Care*, 17(5):215-20.

Gally, B., Ferring, D. and Hentze, W.M. 2005. Generation of conditional alleles of the murine iron regulatory protein (IRP)-1 and -2 genes. *Genesis,* 43(4):181-8.

Gass, A. and Richards, T.L. 2013. Serial proton magnetic resonance spectroscopy of normal- appearing gray and white matter in MS. *Neurology*, 80(1):17-18.

Gauthier, S.A., Berger, A.M., Liptak, Z., Duan, Y., Egorova, S., Buckle, G.J., Glanz, B.I., Khoury, S.J., Bakshi, R. and Weiner, H.L. 2009. Rate of brain atrophy in benign vs early multiple sclerosis. *Archives of Neurology,* 66(2):234-237.

Ge, Y. 2006. Multiple Sclerosis: The Role of MR Imaging. *American Journal of Neuroradiology*, 27:1165-1176.

Ge, Y., Law, M., Johnson, G., Herbert, J., Babb, J.S., Mannon, L.J. and Grossman, R.I. 2004. Preferential Occult Injury of Corpus Callosum in Multiple Sclerosis Measured by Diffusion Tensor Imaging. *Journal of Magnetic Resonance Imaging*, 20:1-7.

Giorgio, A., Palace, J., Johansen-Berg, H., Smith, S.M., Ropele, S., Fuchs, S., Wallner-Blazek, M., Enzinger, C. and Fazekas, F. 2010. Relationships of Brain White Matter Microstructure with Clinical and MR Measures in Relapsing Remitting Multiple Sclerosis. *Journal of Magnetic Resonance Imaging,* 31(2):2309-16.

Greenberg, M.S. 2010. Handbook of Neurosurgery. Thieme, ISBN:1604063262: 129-134. [29 July 2016]

Grossman, R.I. 1999. Magnetic resonance techniques in clinical trials in MS. In Filippi, M., Grossman, R.I. and Comi, G. (eds). *Topics in Neuroscience,* Milano: Springer: 85-94.

Hafler, D.A., 1999. The distinctive blurs between an autoimmune versus microbial hypothesis in multiple sclerosis. *The Journal of Clinical Investigation*, 104(5):527-529.

Hagmann, P., Jonasson, L., Maeder, P., Thiran, J., Wedeen, Van J., and Meuli, R. 2006. Understanding diffusion MR imaging techniques: from scalar diffusion-weighted imaging to diffusion tensor imaging and beyond. *Radiographics,* 26 Suppl 1:S205-23. doi:10.1148/rg.26si065510. [29 July 2016]

Hallgren, B. and Sourander, P. 1958. The effect of age on the non-haemin iron in the human brain. *Journal of Neurochemistry*, 3(1):41-51.

Harris, V.K. & Sadiq, S.A. 2009. Disease biomarkers in multiple sclerosis: potential for use in therapeutic decision making. *Molecular Diagnosis & Therapy,* 13(4):225-244.

Hasan, K.M., Gupta, R.K., Santos, R.M., Wolinsky, J.S. and Narayana, P.A. 2005. Diffusion Tensor Fractional Anisotropy of the Normal-Appearing Seven Segments of the Corpus

Callosum in Healthy Adults and Relapsing-Remitting Multiple Sclerosis Patients. *Journal of Magnetic Resonance Imaging*, 21:735-743.

Hasan, K. M., Iftikhar, A., Kamalia, A., Kramer, L. A., Ashtari, M., Cirino, P.T., Papanicolaou, A.C., Fletcher, J.M. and Ewing-Cobbs, L. 2009. Development and aging of the healthy human brain uncinate fasciculus across the lifespan using diffusion tensor tractography. *Brain Research*, 1276:67-76. doi:10.1016/j.brainres.2009.04.025. [5 September 2015]

Hellman, M. A., Steiner, I. and Mosberg-Galili, R. 2011. Sudden sensorineural hearing loss in multiple sclerosis: clinical course and possible pathogenesis. *Acta Neurologica Scandinavia*, 124(4):245-249. [4 March 2015]

Hitti, M. 2005. Why more women get multiple sclerosis. *WebMD Health News*, http://women.webmd.com/news/20050127/ why-more-women-get-multiple-sclerosis [15 March 2010]

Hulet, S.W., Heyliger, S.O., Powers, S. and Connor, J.R. 2000. Oligodendrocyte progenitor cells internalize ferritin via clathrin-dependent receptor mediated endocytosis. *Journal of Neuroscience Research*, 61(1):52-60.

Imfeld, A., Oechslin, M.S., Meyer, M., Loenneker, T. and Jancke, L. 2009. White matter plasticity in the corticospinal tract of musicians: A diffusion tensor imaging study. *NeuroImage*, 46:600-607.

Jahanshad, N., Kohannim, O., Hibar, D.P., Stein, J.L., McMahon, K.L., de Zubicaray, G. I., Medland, S.E., Montgomery, G.W., Whitfield, J.B., Martin, N.G., Wright, M.J., Toga, A.W. and Thompson, P.M. 2012. Brain structure in healthy adults is related to serum transferrin and the H63D polymorphism in the HFE gene. *PNAS PLUS*, E851-E859. www.pnas.org/cgi/doi/10.1073/pnas.1105543109. [12 January 2014]

Jelinek, G. 2010. *Overcoming multiple sclerosis*. Australia: Allen & Unwin.

Jellison, B.J., Field, A.S., Medow, J., Lazar, M., Salamat, S. and Alexander, A.L. 2004. Diffusion Tensor Imaging of Cerebral White Matter: A Pictorial Review of Physics, Fiber Tract Anatomy, and Tumor Imaging Patterns. *American Journal of Neuroradiology* 25:356-369.

Kappos, L., Moeri, D., Radue, E.W., Schoetzau, A., Schweikert, K., Barkhof, F., Miller, D., Guttmann, C.R.G., Weiner, H.L., Gasperini, C. and Filippi, M. 1999. Predictive value of gadolinium-enhanced magnetic resonance imaging for relapse rate changes in disability or impairment in multiple sclerosis: a meta-analysis. *The Lancet*, 353:964-969.

Kermode, A.G., Thompson, A.J., Toft, P., McManus, D.G., Kendall, B.E., Kingsley, D.P.E., Moseley, I.F., Rudge, P. and McDonald, W.I. 1990. Breakdown of the blood-brain barrier precedes symptoms and other MRI signs of new lesions in multiple sclerosis. *Brain*, 113:1477-1489.

Khalil, M., Teunissen, C. and Langkammer, C. 2011. Iron and Neurodegeneration in Multiple Sclerosis. *Multiple Sclerosis International*, 2011, Article ID 606807, 6 pages. http://dx.doi.org/10.1155/2011/606807. [5 September 2015]

Khumalo, H., Gomo, Z.A.R., Moyo, V.M., Gordeuk, V.R., Saungweme, T., Rouault, T.A. and Gangaidzo, I.T. 1998. Serum transferrin receptors are decreased in the presence of iron overload. *Clinical Chemistry*, 44(1):40-44.

Kidd, D., Barkhof, F., McConnell, R., Algra, P.R., Allen I.V. and Revesz, T. 1999. Cortical lesions in multiple sclerosis. *Brain*, 122:17-26.

Kieseppä, T., Eerola, M., Mäntylä, R., Neuvonen, T., Poutanen, V., Luoma, K., Tuulio-Henriksson, A., Jylhä, P., Mantere, O., Melartin, T., Rytsälä, H., Vuorilehto, M. and Isometsä, E. 2010. Major depressive disorder and white matter abnormalities: A diffusion tensor imaging study with tract-based spatial statistics. *Journal of Affective Disorders,* 20(1-3):240-244. [28 August 2015]

Kochunov, P., Thompson, P.M., Lancaster, J.L., Bartzokis, G., Smith, S., Coyle, T., Royall, D.R., Laird, A. and Fox, P.T. 2007. Relationship between white matter fractional anisotropy and other indices of cerebral health in normal aging: Tract-based spatial statistics study of aging. *NeuroImage*, 35: 478-487.

Korn, T. 2008. Pathophysiology of multiple sclerosis. *Journal of Neurology*, 255(6):2-6.

Kotze, M., Rooney, R., de Villiers, J. and Hillerman, R. 1999. Multiple Sclerosis, Porphyria-Like Symptoms, and a History of Iron Deficiency Anemia in a Family of Scottish Descent. *American Journal of Medical Genetics*, 86:194-196.

Kutzke, J.F. 1983. Rating neurological impairment in multiple sclerosis: an expanded disability status scale (EDSS). *Neurology,* 33(11):1444-1452.

Larkin, E.C. and Rao, G.A. 1990. Importance of fetal and neonatal iron: adequacy for normal development of central nervous system. In Dobbing, J. (ed). *Brain, Behavior and iron in the infant diet*, 3rd ed, London: Springer-Verlag: 43-63.

Lassmann, H., 1998. Neuropathology in multiple sclerosis: new concepts. *Multiple Sclerosis Journal*, 4:93-98.

Lavorgna, L., Bonavita, S., Ippolito, D., Lanzillo, R., Salemi, G., Patti, F., Valentino, P., Coniglio, G., Buccafusca, M., Paolicelli, D., d'Ambrosio, A., Bresciamorra, V., Savettieri, G., Zappia, M., Alfano, B., Gallo A., Simone, I.L. and Tedeschi G. 2014. Clinical and magnetic resonance imaging predictors of disease progression in multiple sclerosis: a nine-year follow-up study. *Multiple Sclerosis Journal*, 20(2):220-226. DOI: 10.1177/1352458513494958. [13 February 2015]

Le Bihan, D., Mangin, J., Poupon, C., Clark, C., Pappata, S., Molko, N. and Chabriat, H. 2000. Diffusion tensor imaging: concepts and applications. *Journal of Magnetic Resonance Imaging*, 13(4):534-546.

Levine, G.N., Gomes, A.S., Arai, A.E., Bleumke, D.A., Flamm, S.D., Kanal, E., Manning, W.J., Martin, E.T., Smith, J.M. and Wilke, N. 2007. Safety of magnetic resonance imaging in patients with cardiovascular devices. *Circulation*, 116:2878-2891.

Levine, S.M. 1991. Oligodendrocytes and myelin sheaths in normal, quaking and shiverer brains are enriched in iron. *Journal of Neuroscience*, 29:413-419.

Liu, T., Li, H., Wong, K., Tarokh, A., Guo, L. and Wong, S.T.C. 2007. Brain tissue segmentation based on DTI data. *NeuroImage*, 38: 114-123.

Liu, Y., Duan, Y., He, Y., Yu, C., Wang, J., Huang, J., Ye, J., Parizel, P.M., Li, K. and Shu, N. 2012. Whole brain white matter changes revealed by multiple diffusion metrics in multiple sclerosis: A TBSS study. *European Journal of Radiology*, 81:2826-2832. DOI: 10.1016/j.ejrad.2011.11.022. [15 September 2015]

Llufriu, S., Blanco, Y., Martinez-Heras, E., Casanova-Molla, J., Gabilondo, I., Sepulveda, M., Falcon, C., Berenguer, J., Bargallo, N., Villoslada, P., Graus, F., Valls-Sole, J. and Saiz, A., 2012. Influence of corpus callosum damage on cognition and physical disability in Multiple Sclerosis: A multimodal study. *PLoS ONE*. 7(5): 37167. www.plosone.org. [05 June 2014]

Loseff, N.A., Wang, L. and Lai, H.M. 1996. Progressive cerebral atrophy in multiple sclerosis: a serial MRI study. *Brain*, 119:2009-2019.

Lublin, F.D. and Reingold, S.C. 1996. Defining the clinical course of multiple sclerosis: results of an international survey. *Neurology*, 46(4):907-11.

Lyksborg, M., Siebner, H.R., Sørensen, P.S., Blinkenberg, M., Parker, G.J., Dogonowski, A.M., Garde, E., Larsen, R. and Dyrby, T.B. 2014. Secondary progressive and relapsing remitting multiple sclerosis leads to motor-related decreased anatomical connectivity. *PLoS*

One, 9(4):e95540. doi: 10.1371/journal.pone.0095540. eCollection 2014. [05 September 2015]

Martini, F. 2006. Chapter 14 - The brain and cranial nerves. 451-494. In: *Fundamentals of anatomy & physiology,* 7th ed, San Francisco, CA: Pearson Benjamin Cummings.

McDonald, W. I., Compston, A., Edan, G., Goodkin, D., Hartung, H.P., Lublin, F.D., McFarland, H.F., Paty, D.W., Polman, C.H., Reingold, S.C., Sandberg-Wollheim, M., Sibley, W., Thompson, A., Van den Noort, S., Weinshenker, B.Y. and Wolinsky, J.S. 2001. Recommended Diagnostic Criteria for Multiple Sclerosis: Guidelines from the International Panel on the Diagnosis of Multiple Sclerosis: *Annals of Neurology*, 50:121-127.

McFarland, H.F. 1998. The lesion in multiple sclerosis: clinical, pathological, and magnetic resonance imaging considerations. *Journal of Neurology, Neurosurgery and Psychiatry*, 64 Suppl 10S26-30.

Mendes-Ribeiro, J.A., Soares, R., Simões-Ribeiro, F. and Guimarães, M.L. 1998. Reduction in temporal n-acetylaspartate and creatine (or choline) ratio in temporal lobe epilepsy: does this 1H-magnetic resonance spectroscopy finding mean poor seizure control? *Journal of Neurology Neurosurgery Psychiatry*, 65 (4):518-522.

Millefiorini, E., Gasperini, C., Pozzilli, C., D'Andrea, F., Bastianello, S., Trojano, M., Morino, S., Morra, V.B., Bozzao, A., Calo', A., Bernini, M.L., Gambi, D. and Prencipe, M. 1997. Randomized placebo-controlled trial of mitoxantrone in relapsing-remitting multiple sclerosis: 24-month clinical and MRI outcome. *Journal of Neurology*, 244(3):153-9.

Miller, D.H. and Leary, S.M. 2007. Primary-progressive multiple sclerosis. *Lancet Neurology*, 6(10):903-12.

Mitchell, D.G. and Cohen M. MRI principles. Saunders. ISBN:0721600247.

Modi, G., Mochan, A., du Toit, M. and Stander, I. 2008. Multiple sclerosis in South Africa. *South African Medical Journal*, 98(5):391-393.

Mori, S., Wakana, S., Nagae-Poetscher, L.M. and Van Zijl, P.C.M. 2005. MRI Atlas of Human White Matter. *American Journal of Neuroradiology*, 27:1384-1387.

Moritani, T., Ekholm, S. and Westesson, P.L. 2009. Diffusion-Weighted MR Imaging of the Brain. Springer. ISBN:3540787852.

Mosby's dictionary of medicine, nursing & health professions. 7th ed. 2006. Mosby: Elsevier: St Louis.

Mostert, J.P., Koch, M.W., Steen, C., Heersema, D.J., de Groot, J.C. and de Keyser, J. 2010. T2 lesions and rate of progression of disability in multiple sclerosis. *European Journal of Neurology,* 17: 1471-1475. doi:10.1111/j.1468-1331.2010.03093.x [04 September 2015]

Narayana, P. 2005. Magnetic Resonance Spectroscopy in Monitoring of Multiple Sclerosis. *Journal of Neuroimaging*, 15 (4 supplementary):46S-57S.

Nelson, M., Isaacs, F., Hassan, M.S., Kidd, M., Cronje, F.J. and Van Rensburg, S.J. 2014. Prevalence of abnormal bloodflow patterns and effects of biochemistry and lifestyle factors on the major neck vessels in patients with Multiple Sclerosis in the Western Cape, South Africa*. Medical Technology SA*, 28(1):28-33.

Onu, M., Roceanu, A., Sboto-Frankenstein, U., Bendic, R., Tarta, E., Preoteasa, F. and Bajenaru, O. 2012. Diffusion abnormality maps in demyelinating disease: Correlations with clinical scores. *European Journal of Radiology,* 81: e386-e391. DOI:10.1016/j.ejrad.2011.12.014. [20 October 2015]

Ozawa, K., Suchanek, G., Breitschopf, H., Brück, W., Budka, H., Jellinger, K. and Lassmann, H. 1994. Patterns of oligodendroglia pathology in multiple sclerosis. *Brain* 117(6):1311-22.

Ozturk, A., Smith, S.A., Gordon-Lipkin, E.M., Harrison, D.M., Shiee, N., Pham, D.L., Caffo, B.S., Calabresi, P.A. and Reich, D.S. 2010. MRI of the corpus callosum in multiple sclerosis: association with disability. *Multiple Sclerosis*, 16(2):166-77.

Peters, B.D., Machielsen, M.W., Hoen, W.P., Caan, M.W.A., Malhotra, A.K., Szeszko, P.R., Duran, M., Olabarriaga, S.D. and de Haan, L. 2012. Pollyunsaturated fatty acid concentration predicts myelin integrity in early-phase psychosis. *Schizophrenia Bulletin*, 39(4):830-838.

Piñero, D.J. and Connor, J.R. 2000. Iron in the Brain: An Important Contributor in Normal and Diseased States. *The Neuroscientist*, 6(6):435-453.

Pittock, S.J. and Rodriguez, M. 2008. Benign multiple sclerosis: a distinct clinical entity with therapeutic implications. *Current Topics in Microbiology and Immunology*, 318:1-17.

Polman, C.H., Reingold, S.C., Banwell, B., Clanet, M., Cohen, J.A., Filippi, M., Fujihara, K., Havrdova, E., Hutchinson, M., Kappos, L., Lublin, F.D., Montalban, X., O'Connor, P., Sanberg-Wollheim, M., Thompson, A.J., Waubant, E., Weinshenker, B. and Wolinsky, J.S.

2011. Diagnostic criteria for multiple sclerosis: 2010 revisions to the McDonald criteria. *Annals of Neurology*, 69(2):292-302.

Poonawalla, A.H.,Datta, S., Juneja, V., Nelson, F., Wolinsky, J.S., Cutter, G. and Narayana, P.A. 2010. Composite MRI scores improve correlation with EDSS in multiple sclerosis. *Multiple Sclerosis*, 16(9): 1117-1125.

Price, G., Bagary, M.S., Cercignani, M., Altmann, D.R. and Ron, M.A. 2005. The corpus callosum in first episode schizophrenia: a diffusion tensor imaging study. *Journal of Neurolological Neurosurgy & Psychiatry* 76:585-587. doi:10.1136/jnnp.2004.042952. [29 January 2015]

Provencher, S.W. 1993. Estimation of metabolite concentrations from localized in vivo proton NMR spectra. *Magnetic Resonance in Medicine: Official Journal of the Society of Magnetic Resonance in Medicine / Society of Magnetic Resonance in Medicine* 30(6):672-679.

Puri, B.K., Bydder, G.M., Chaudhuri, K.R., al Saffar, B.Y.S., Currati, W.L. and White, S.J. 2001. MR changes in MS following treatment with Lofepramine and L-Phenylalanine. *Neuroreport*, 12(9):1821-24.

Qian, Z.M. and Shen, X. 2001. Brain iron transport and neurodegeneration. *Trends in Molecular Medicine.* 7(3)103-108.

Ramagopalan S.V., Deluca, G.C., Morrison, K.M., Herrera, B.M., Dyment, D.A., Lincoln, M.R., Orton, S.M., Chao, M.J., Degenhardt, A., Pugliatti, M., Sadovnick, A.D., Sotgiu, S. and Ebers, G.C. 2008. Analysis of 45 candidate genes for disease modifying activity in multiple sclerosis. *Journal of Neurology*, 255(8):1215-1219.

Reilmann, R., Holtbernd, F., Bachmann, R., Mohammadi, S., Ringelstein, E.B. and Deppe, M. 2012. Grasping multiple sclerosis: do quantitative motor assessments provide a link between structure and function? *Journal of Neurology*, 260:407-414.

Reipert, B. 2004. Multiple sclerosis: a short review of the disease and its differences between men and women. *The Journal of Men's Health & Gender*, 1(4):334-340.

Rosati, G. 2001. The prevalence of multiple sclerosis in the world: an update. *Neurological Science*, 22:117-139.

Rovaris, M., Confavreux, C., Furlan, R., Kappos, L., Comi, G. and Filippi, M. 2006. Secondary progressive multiple sclerosis: current knowledge and future challenges. *Lancet Neurology*, 5(4):343-54.

Rovaris, M. and Filippi, M. 2000. The role of magnetic resonance in assessment of multiple sclerosis. *Journal of Neurological Sciences*, 172:3-12. www.elsevier.com/locate/jns [11March 2014]

Rovaris, M., Judica, E., Cecarelli, A., Ghezzi, A., Martinelli, V., Comi, G. and Filippi, M. 2008. A three year diffusion tensor MRI study of grey matter damage progressing during the earliest clinical stage of multiple sclerosis. *Journal of Neurology*, 255:1209-1214.

Sanchez, M.P., Nieto, A., Barroso, J., Martin, V. and Hernandez, M.A. 2008. Brain atrophy as a marker of cognitive impairment in mildly disabling relapsing-remitting multiple sclerosis. *European Journal of Neurology*, 15(10):1091-9.

Schilld, H.H. 1990. *MRI made Easy*. Berlin: Schering.

Schmierer, K., Scaravilli, F., Altman, D.R., Barker, G.J. and Miller, D.H. 2004. Magnetization Transfer Ratio and Myelin in Postmortem Multiple Sclerosis Brain. *Annals of Neurology,* 56:407-415.

Schonberg, D.L., Goldstein, E.Z., Sahinkaya, F.R., Wei, P., Popovich, P.G. and McTigue, D.M. 2012. Ferritin stimulates oligodendrocyte genesis in the adult spinal cord and can be transferred from macrophages to NG2 cells in vivo. *Journal of Neuroscience*, 32(16):5374-84.

Selzer, R.R., Rosenblatt, D.S., Laxova, R. and Hogan, K. 2003. Adverse effect of nitrous oxide in a child with 5,10-methylenetetrahydrofolate reductase deficiency. *The New England Journal of Medicine*, 349:45-50.

Shellock, F.G. and Spinazzi, A. 2008. MRI Safety Update 2008: Part 1, MRI Contrast Agents and Nephrogenic Systemic Fibrosis. *American Journal of Radiology,* 191:1129-1139.

Shen, Y., Bai, L., Gao, Y., Cui, F., Tan, Z., Tao, Y., Sun, C. and Zhou, L. 2014. Depressive symptoms in Multiple sclerosis from an In Vivo Study with TBSS. *Biomed Research International*, 148465: 1-8. http://dx.doi.org/10.1155/2014/148465 [5 August 2014]

Simon, J.H., Li, D., Traboulsee, A., Coyle, P.K., Arnold, D.L., Barkhof, F., Frank, J.A., Grossman, R., Paty, D.W., Radue, E.W. and Wolinsky, J.S. 2006. Standardized MR imaging protocol for multiple sclerosis: consortium of MS centres consensus guidelines. *American Journal of Neuroradiology*, 27:455-461.

Simon, J.H. & Miller, D.E. 2007. Multiple Sclerosis Therapeutics. In Cohen, J.A. and Rudick, R.A. (eds). Measures of gadolinium enhancement, T1 black holes and T2 hyperintense lesions on magnetic resonance imaging. Boca Raton (FI). [15 December 2014]

Sjöbeck, M. and Englund, E. 2003. Glial levels determine severity of white matter disease in Alzheimer's disease: a neuropathological study of glial cells. *Neuropathology and Applied Neurobiology,* 29:159-169.

Smith, S.M., Jenkinson, M., Johansen-Berg, H., Rueckert, D., Nichols, T.E., Mackay, C.E., Watkins K.E., Ciccarelli, O., Cader, M.Z., Matthews, P.M. and Behrens T.E.J. 2006. Tract-based spatial statistics: Voxelwise analysis of multi-subject diffusion data. *NeuroImage*, 31: 1487-1505.

Smith, S.M., Jenkinson, M., Woolrich, M.W., Beckman, C.F., Behrens, T.E.J., Johansen-Berg, H., Bannister, P.R., De Luca, M., Drobnjak, I., Flitney, D.E., Niazy, R., Saunders, J., Vickers, J., Zhang, Y., De Stefano, N., Brady, J.M. and Matthews, P.M. 2004. Advances in structural and functional MR image analysis and implementation as FSL. *NeuroImage,* 23 (S1):208-219.

Song, S.K., Sun, S.W., Ramsbottom, M.J., Chang, C., Russel, J. and Cross, A.H. 2002. Dysmyelination revealed through MRI as increased radial (but unchanged axial) diffusion of water. *NeuroImage*, 17:1429-1436.

Song, S., Yoshino, J., Le, T.Q., Lin, S., Sun, S.W., Cross, A.H. and Armstrong. R.C. 2005. Demyelinatio increases radial diffusivity in corpus callosum of mouse brains. *NeuroImage*, 26:132-140.

Stone, L.A., Richert, N. and McFarland, H.F. 2005. Neuroimaging and the use of magnetic resonance in multiple sclerosis. In: Cook, S.D. (ed). *Handbook of multiple sclerosis*, 3rd ed., New York: Marcel Dekker: 403-423.

Tam, R. C., Traboulsee, A., Riddehough, A., Sheikhzadeh, F., and Li, D.K.B. 2011. The impact of intensity variations in T1-hypointense lesions on clinical correlations in multiple sclerosis. *Multiple Sclerosis* (Houndmills, Basingstoke, England), 17(8), 949-957. doi:10.1177/1352458511402113. [13 February 2015]

Tansey, F.A. and Cammer, W.1988. Acetyl-CoA carboxylase in rat brain. I. Activities in homogenates and isolated fractions. *Developmental Brain Research*, 43:123-130.

Temel, S., Keklikoğlu, H.D., Vural, G., Deniz, O. and Ercan, K. 2013. Diffusion tensor magnetic resonance imaging in patients with multiple sclerosis and its relationship with disability. *Neuroradiology Journal*, 26(1):3-17.

Todorich, B., Zhang, X. and Connor, J.R. 2011. H-ferritin is the major source of iron for oligodendrocytes. *Glia*, 59(6):927-35.

Trapp, B.D., Bo, L., Mork, S. and Chang, A. 1999. Pathogenesis of tissue injury in MS lesions. *Journal of Neuroimmunology*, 98:49-56.

Van Rensburg, S.J., Hon, G.M., Smuts, C.M., Kidd, M., Van der Merwe, S., Myburgh, C., Oosthuizen, P. and Emsley, R.A. 2009. Changes in erythrocyte membrane fatty acids during a clinical trial of eicosapentaenoic acid (EPA) supplementation in schizophrenia. *Metabolic Brain Disease*, 24:659-672.

Van Rensburg, S., Kotze, M.J. and Haug, P. 2006b. Researchers lead study on the development of a comprehensive gene-based, pathology supported intervention program for improved quality of life in patients diagnosed with multiple sclerosis. Cape Town: University of Stellenbosch.

Van Rensburg, S.J., Kotze, M.J., Hon, D., Haug, P., Kuyler, J., Hendricks, M., Botha, J., Potocnik, F.C.V., Matsha, T. and Erasmus, R.T. 2006a. Iron and folate-vitamin B12-methylation pathway in multiple sclerosis. *Metabolic Brain Disease*, 21:121-137.

Van Rensburg, S., Kotze, M. and Van Toorn, R. 2012. The conundrum of iron in multiple sclerosis – time for an individualised approach. *Metabolic Brain Disease*, 27:239-253.

Van Toorn, R., Schoeman, J.F., Solomons, R., Rensburg, M.A. and Van Rensburg, S.J. 2010. Iron status in children with recurrent episodes of tumefactive cerebral demyelination. *Journal of Child Neurology*, 25 (11):1401-1407. http://jcn.sagepub.com/content/25/11/1401. [13 May 2015]

Vogel, F.S. & Bouldin, T.W. 1995. The nervous system. In: Rubin, E. & Farber, J.L. (eds). *Pathology*. Philadelphia: Lippencott.

Wahlund, L. O., Barkhof, F., Fazekas, F., Bronge, L., Augustin, M., Sjögren, M., et al. (2001). A new rating scale for age-related white matter changes applicable to MRI and CT. Stroke; a *Journal of Cerebral Circulation*, 32(6), 1318-1322.

Wakana, S., Jiang, H., Nagae-Poetscher, L.M., Van Zijl, P.C.M. and Mori, S. 2004. Fiber Tract–based Atlas of Human White Matter Anatomy. *Radiology*, 230(1): 77-87. DOI: http://dx.doi.org/10.1148/radiol.2301021640

Wake, H., Ortiz, F.C., Woo, D.W., Lee, P.R., Angulo, M.C. and Fields, R.D. 2015. Nonsynaptic junctions on myelinating glia promote preferential myelination of electrically active axons. *Nature Communications*, 6:7844. DOI:10.1038/ncomms8844. [20 September 2015]

Wang, F., Sun, Z., Du, X., Wang, X., Cong, Z., Zhang, H., Zhang, D. and Hong, N. 2003. A diffusion tensor imaging study of middle and superior cerebellar peduncle in male patients with schizophrenia. *Neuroscience*, 348:135-138. DOI:10.1016/S0304-3940(03)00589-5. [5 September 2015]

Westbrook, C. 2015. MRI at a glance. Wiley-Blackwell: 21-34.

World Medical Association Declaration of Helsinki, 2013. http://www.ub.edu/recerca/Bioetica/doc/Declaracio_Helsinki_2013.pdf. [5 September 2015]

Zamboni, P. 2006. The big idea Iron-dependent inflammation in venous disease and proposed parallels in multiple sclerosis. *Journal of the Royal Society of Medicine*, 99:589-593. [25 August 2014]

Zapata-Arriaza, E. and Dìaz-Sánchez, M. 2013. Clinico-radilogical dissociation in multiple sclerosis: Future prospects. *World Journal of Neuroscience*, 3:147-153.

APPENDICES

Appendix A: Diagnostic criteria for Multiple Sclerosis

Clinical Presentation	Additional Data Needed for MS Diagnosis
Two or more attacks; clinical evidence of 2 or more lesions	None[a]
Two or more; clinical evidence of 1 lesion	Dissemination in space, demonstrated by MRI[b] *or* Two or more MRI-detected lesions consistent with MS plus positive CSF[c] *or* Await further clinical attack implicating a different site
One attack; clinical evidence of 2 or more lesions	Dissemination in time, demonstrated by MRI[d] *or* Second clinical attack
One attack; clinical evidence of 1 lesion (mono-symptomatic presentation; clinically isolated syndrome)	Dissemination in space, demonstrated by MRI[b] *or* Two or more MRI-detected lesions consistent with MS plus positive CSF[c] *and* Dissemination in time, demonstrated by MRI[d] *or* Second clinical attack
Insidious neurological progression suggestive of MS	Positive CSF[c] *and* Dissemination in space, demonstrated by 1) Nine or more T2 lesions in brain *or* 2) or more lesions in spinal cord, *or* 3) 4-8 brain plus 1 spinal cord lesion *or* abnormal VEP[e] associated with 4-8 brain lesions, or with fewer than 4 brain lesions plus 1 spinal cord lesion demonstrated by MRI *and* Dissemination in time, demonstrated by MRI[d] *or*
MRI criteria for brain abnormality	MRI criteria for dissemination in time
3 or 4 of the following: 1. One Gd – enhancing lesion or nine T2 – hyperintense lesions if there is no Gd enhancing lesions, 2. At least 1 infratentorial lesion, 3. At least 1 juxtacortical lesion, 4. At least 3 periventricular lesions.	1. If a first scan occurs 3 months or more after the onset of the clinical event, the presence of a gadolinium-enhancing lesion is sufficient to demonstrate dissemination in time, provided that it is not at the site implicated in the original clinical event. If there is no enhancing lesion at this time, a follow-up scan is required. The timing of this follow-up is not crucial, but 3 months is recommended. A new T2- or gadolinium- enhancing lesion at this time then fulfils the criterion for dissemination in time. 2. If the first scan is performed less than 3 months after the onset of the clinical event, a second scan done 3 months or more after the clinical event showing a new gadolinium-enhancing lesion provides sufficient evidence for dissemination in time. However, if no enhancing lesion is seen at this second scan, a further scan not less than 3 months after the first scan that shows a new T2 lesion or enhancing lesion will suffice.

(Adapted from 2010 McDonald criteria, Polman *et al*., 2011: 297)

APPENDIX B: Example of Kurtzke's expanded disability status scale (EDSS) used to assess disease progression in MS patients

Score	Level of Disability in Multiple Sclerosis
0.0	Normal neurological exam
1.0	No disability, single sign present
1.5	No disability, minimal signs present
2.0	Minimal disability
2.5	Mild disability
3.0	Moderate disability
3.5	Fully ambulatory despite some disabilities
4.0	Fully ambulatory without aid despite relatively severe disability
4.5	Fully ambulatory with relatively severe disability
5.0	Disability impairs full daily activities
5.5	Disability precludes full daily activities
6.0	Requires intermittent or unilateral constant assistance to walk
6.5	Requires constant bilateral support to walk
7.0	Unable to walk and essentially restricted to a wheelchair
7.5	Restricted to wheelchair and unable to take more than a few steps
8.0	Essentially restricted to a wheelchair with generally effective use of arms
8.5	Essentially restricted to bed much of day with some effective use of arms
9.0	Confined to bed, still able to communicate and eat
9.5	Bedridden and unable to communicate effectively or eat and swallow
10.0	Death due to MS

(Kurtzke, 1983)

APPENDIX C: Letter of approval of study (Stellenbosch University)

UNIVERSITEIT·STELLENBOSCH·UNIVERSITY
jou kennisvennoot · your knowledge partner

07 June 2010 **MAILED**

Ms E Arendse
Department of Radiography
Cape Peninsula University of Technology
PO Box 1906
Bellville
7535

Dear Ms Arendse

"Magnetic Resonance Imaging as a tool to predict the severity of disability in female subjects with multiple sclerosis."

ETHICS REFERENCE NO: N10/01/021

RE : FINAL APPROVAL

At a meeting that was held on 03 March 2010 the Health Research Ethics Committee considered your application for the registration and approval of the abovementioned project. The Committee referred the project back to you awaiting further information that was required.

This information was supplied and the project was finally approved at a meeting on 02 June 2010 for a period of one year from this date.

Please quote the above-mentioned project number in ALL future correspondence.

Please note that a progress report obtainable on www.sun.ac.za/rds/ should be submitted to the Committee before the year has expired. The Committee will then consider the continuation of the project for a further year (if necessary). Annually a number of projects may be selected randomly and subjected to an external audit.
Translations of the consent document in the languages applicable to the study participants should be submitted.

Federal Wide Assurance Number: 00001372
Institutional Review Board (IRB) Number: IRB0005239
The Health Research Ethics Committee complies with the SA National Health Act No.61 2003 as it pertains to health research and the United States Code of Federal Regulations Title 45 Part 46. This committee abides by the ethical norms and principles for research, established by the Declaration of Helsinki, the South African Medical Research Council Guidelines as well as the Guidelines for Ethical Research: Principles Structures and Processes 2004 (Department of Health).

Please note that for research at a primary or secondary healthcare facility permission must still be obtained from the relevant authorities (Western Cape Department of Health and/or City Health) to conduct the research as stated in the protocol. Contact persons are Ms Claudette Abrahams at Western Cape Department of Health (healthres@pgwc.gov.za Tel: +27 21 483 9907) and Dr Hélène Visser at City Health (Helene.Visser@capetown.gov.za Tel: +27 21 400 3981). Research that will be conducted at any tertiary academic institution requires approval from the relevant hospital manager. Ethics approval is required BEFORE approval can be obtained from these health authorities.

Fakulteit Gesondheidswetenskappe · Faculty of Health Sciences

Verbind tot Optimale Gesondheid · Committed to Optimal Health
Afdeling Navorsingsontwikkeling en -steun · Division of Research Development and Support
Posbus/PO Box 19063 · Tygerberg 7505 · Suid-Afrika/South Africa
Tel.: +27 21 938 9075 · Faks/Fax: +27 21 931 3352

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Approval Date: 02 June 2010

Expiry Date: 02 June 2011

Yours faithfully

MR FRANKLIN WEBER
RESEARCH DEVELOPMENT AND SUPPORT
Tel: +27 (0)21 938-9657 / E-mail: fweb@sun.ac.za
Fax: +27 (0)21 931-3352

Fakulteit Gesondheidswetenskappe · Faculty of Health Sciences

Verbind tot Optimale Gesondheid · Committed to Optimal Health
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APPENDIX D: Letter of approval of study (CPUT)

Cape Peninsula
University of Technology

10 September 2009
CPUT/HW-REC 2009/H012

P.O. Box 1906 • Bellville 7535 South Africa •Tel: +27 21 442 6162 • Fax +27 21 447 2963
Symphony Road Bellville 7535

OFFICE OF THE CHAIRPERSON:
HEALTH AND WELLNESS SCIENCES RESEARCH ETHICS COMMITTEE (HW-REC)
Registration Number NHREC: REC- 230408-014

At the meeting of the Health and Wellness Sciences-REC on 7 August 2009 approval was granted to Estelle Arendse, pending amendments now received. This approval is for research activities related to an M Tech: Radiography at this institution.

TITLE:
Magnetic Resonance Imaging as a tool to predict the severity of disability in female subjects with Multiple Sclerosis.

INTERNAL SUPERVISOR: Prof P Engel-Hills

EXTERNAL CO-SUPERVISOR: Dr S Janse Van Rensburg
Dr M Grobbler

Comment:
This ethics approval is supported by permission from the research site where the study will be conducted.

Research activities are restricted to those detailed in the proposal and application submitted in September 2009.

Approval will not extend beyond 9 September 2010. An extension must be applied for should data collection for this study continue beyond this date.

Prof PENELOPE ENGEL-HILLS
CHAIR: HEALTH AND WELLNESS SCIENCES RESEARCH ETHICS COMMITTEE

e-mail: *engelhillsp@cput.ac.za*

APPENDIX E: Permission to do research

54 Riverton Crescent
Elsies River
7490

The Head of Department
3 T Brain Imaging Centre
University of Stellenbosch
Bellville
7500

Dear Sir/ Madam

This serves to confirm that I **Estelle Arendse**, student number: **194081214** am registered for the M Tech Degree (Radiography) at Cape Peninsula University of Technology (CPUT).

The topic of my research project is entitled: **STUDY THE USE OF MAGNETIC RESONANCE IMAGING AND BIOCHEMICAL MARKERS TO ASSESS THE SEVERITY OF DISABILITY IN FEMALE SUBJECTS WITH MULTIPLE SCHLEROSIS.**

This project would not require any name of persons to be recorded; and I give my solemn undertaking that the information of each participant will be treated confidentially. I undertake not to reveal the names and/or hospital numbers of patients during writing up of my research, or during presentation of my results. All data will be handled by me as the researcher.

I would appreciate it if you could grant me consent to undertake the project at your organization/institution/company.

Thank you for your participation and cooperation in this regard

_____	_____ **(Consent Granted)**
Estelle Arendse	**Bruce Spottiswood**
Lecturer	**Manager (CUBIC)**

APPENDIX F: Consent form

MAGNETIC RESONANCE IMAGING AS A TOOL TO PREDICT THE SEVERITY OF DISABILITY IN MULTIPLE SCLEROSIS
INFORMED CONSENT

Participant's File no: **Date:**

I, ESTELLE ARENDSE, am currently a registered Masters student in Radiography at Cape Peninsula University of Technology. My study involves Magnetic Resonance Imaging as a tool to predict the severity of disability in Multiple Sclerosis. This study will involve participants undergoing an MRI scan of the brain.
If you are willing to participate in the study I will need your permission before I am able to commence with the examination. Confidentiality will be maintained at all times.
By signing below you give permission for an MRI scan to be performed on your brain, and consent to the data obtained through the examination to be used in this research study as well as any publications or presentations linked to this study **in SA or abroad**. If however you need the information in the future it would be made available to you on request.

What is a MRI of the brain?

An MRI scan is a method of producing detailed pictures of any body tissue / organs.
It makes use of electromagnetic energy that is released when exposing a patient to radiofrequency waves in a strong magnetic field. This information is then measured and analysed by a computer.
It is a painless and harmless procedure to assess the brain. A special dye will however be injected intravenously to enhance the appearance of blood vessels.

NOTE:
Patients with metal implants, cochlear implants and cardiac pacemakers are prevented from having an MRI scan due to the effects that the magnetic field have on these devices.

Side effects**: Nausea and vomiting as well as allergic type dermal and mucosal reactions have occasionally been observed after the administration of Gadovist**.

Please tell us if you have any of these devices or if you experience any of the above side effects.

I declare that:

I understand what the procedure will be for this MRI scan.
I voluntarily agree to take part in this study voluntarily.
I have read and/or someone has read the information pertaining to the study to me.
The information is in a language with which I am fluent and comfortable.
I was given an opportunity to ask questions and all my questions have been answered to my satisfaction.
I understand that taking part in this study is voluntary.
I may choose to leave the study at any time.

_____	At Bellville (TBH)	_____
Signature of participant		on this day
_____	at Bellville (TBH)	_____
Signature of witness		on this day

Declaration by investigator:

I (name)…….declare that:-

I have explained the information in this document to this participant. I encouraged him/her to ask questions and took adequate time to answer all the questions. I am satisfied that he/she understands the information. The information obtained from this study, will ONLY be used for the purpose of this study and academic publications/presentations emanating from it. The information will be available to the participant on request.

_____	At Bellville (TBH)	_____
Signature of investigator		on this day

APPENDIX G1: MRI Screening form (master)

Cape Universities Brain Imaging Centre (CUBIC)

MRI Patient Screening Form

Patient Information:

Name	Ward
Folder Number	Clinic
Date of Birth	Weight

The following information is very important to ensure your safety and to prevent any interference during the MR procedure.

Please answer the following questions (mark with an X):	**Yes**	**No**	**Don't know**
Pacemaker			
Aneurism clips			
Artificial heart valve			
Vena cava filter			
Prosthesis (e.g. eye, breast etc)			
Shrapnel in eye or body			
Neurostimulator			
Cochlear implant (ear) or hearing aid			
? Diabetic			
? Renal impairment			
? Asthma			
? Allergies			
? Any other implants (e.g. Screws, plates, joint replacements)			
? Pregnant			
? Previous MRI investigation with intravenous contrast			
? Previous Surgery			
Is there any other device implanted or are there any other ailments that you think that we should be aware of?			

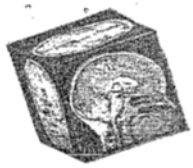
I hereby acknowledge that the potential risks of the examination have been explained to me and that during the course of the investigation it may be necessary for the intravenous injection of a contrast agent.

Attention: It is the policy of this institution not to discuss results of the MR investigation with the patients for ethical reasons. All enquiries in this regard should be directed to the referring physician.

Signature: **Date:**

Cape Universities Brain Imaging Centre
Fisan Building, Faculty of Health Sciences, University of Stellenbosch, Tygerberg, 7505
Tel: 27-21-938-9646 Fax: 27-21-938-9728 www.sun.ac.za/cubic

APPENDIX G2: MRI screening form (completed example)



Cape Universities Brain Imaging Centre (CUBIC)

MRI Patient Screening Form

[redacted]

Patient Information:

Name	Ward
Folder Number	Clinic
Date of Birth [redacted]	Weight

The following information is very important to ensure your safety and to prevent any interference during the MR procedure.

Please answer the following questions (mark with an X):	**Yes**	**No**	**Don't know**
Pacemaker		✓	
Aneurism clips		✓	
Artificial heart valve		✓	
Vena cava filter		✓	
Prosthesis (e.g. eye, breast etc)		✓	
Shrapnel in eye or body		✓	
Neurostimulator		✓	
Cochlear implant (ear) or hearing aid		✓	
? Diabetic		✓	
? Renal impairment		✓	
? Asthma		✓	
? Allergies		✓	
? Any other implants (e.g. Screws, plates, joint replacements)		✓	
? Pregnant		✓	
? Previous MRI investigation with intravenous contrast ✓			
? Previous Surgery	tonsils		
Is there any other device implanted or are there any other ailments that you think that we should be aware of?		✓	

I hereby acknowledge that the potential risks of the examination have been explained to me and that during the course of the investigation it may be necessary for the intravenous injection of a contrast agent.

Attention: It is the policy of this institution not to discuss results of the MR investigation with the patients for ethical reasons. All enquiries in this regard should be directed to the referring physician.

Signature: [redacted] **Date:** 22/6/2011

Cape Universities Brain Imaging Centre
Fisan Building, Faculty of Health Sciences, University of Stellenbosch, Tygerberg, 7505
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Appendix H: A list of the WM tracts (LH-left hemisphere / RH-right hemisphere)

MCP - Middle_cerebellar_peduncle
PCT - Pontine_crossing_tract
CC_genu
CC_body
CC_splenium
F – Fornix
CST - Corticospinal_tract_RH
CST - Corticospinal_tract_LH
ML - Medial_lemniscus_RH
ML - Medial_lemniscus_LH
ICP - Inferior_cerebellar_peduncle_RH
ICP - Inferior_cerebellar_peduncle_LH
SCP - Superior_cerebellar_peduncle_RH
SCP - Superior_cerebellar_peduncle_LH
CP - Cerebral_peduncle_RH
CP - Cerebral_peduncle_LH
ALIC - Anterior_limb_of_internal_capsule_RH
ALIC - Anterior_limb_of_internal_capsule_LH
PLIC - Posterior_limb_of_internal_capsule_RH
PLIC - Posterior_limb_of_internal_capsule_LH
RLIC - Retrolenticular_internal_capsule_RH
RLIC - Retrolenticular_internal_capsule_LH
ACR - Anterior_corona_radiata_RH
ACR - Anterior_corona_radiata_LH
SCR - Superior_corona_radiata_RH
SCR - Superior_corona_radiata_LH
PCR - Posterior_corona_radiata_RH
PCR - Posterior_corona_radiata_LH
PTR - Posterior_thalamic_radiation_RH
PTR - Posterior_thalamic_radiation_LH
SS - Sagittal_stratum_RH
SS - Sagittal_stratum_LH
EC - External_capsule_RH
EC - External_capsule_LH
Cg - Cingulum_RH
Cg - Cingulum_LH
Cg (HPC) - Cingulum(hippocampus)_RH
Cg (HPC) - Cingulum(hippocampus)_LH
F/stria terminalis - Fornix/stria_terminalis_RH
F/stria terminalis - Fornix/stria_terminalis_LH
SLF - Superior_longitudinal_fasciculus_RH
SLF - Superior_longitudinal_fasciculus_LH

SFOF - Superior_fronto_occip_fasciculus_RH
SFOF - Superior_fronto_occip_fasciculus_LH
UF - Uncinate_fasciculus_RH
UF - Uncinate_fasciculus_LH
T - Tapetum_RH
T - Tapetum_LH

Appendix I: FA correlations of EDSS and age diagnosed in the 48 WM tracts.

FRACTIONAL ANISOTROPY (n=11)	EDSS		Age Diagnosed	
	r	p	r	p
Middle_cerebellar_peduncle	-0.69	0.02	0.51	0.11
Pontine_crossing_tract	-0.49	0.13	-0.03	0.94
CC_genu	-0.49	0.12	-0.05	0.87
CC_body	-0.30	0.37	-0.06	0.86
CC_splenium	-0.68	0.02	0.45	0.17
Fornix	-0.84	<0.01	0.50	0.12
Corticospinal_tract_RH	-0.81	<0.01	0.74	<0.01
Corticospinal_tract_LH	-0.87	<0.01	0.53	0.09
Medial_lemniscus_RH	-0.73	0.01	0.37	0.26
Medial_lemniscus_LH	-0.79	<0.01	0.52	0.10
Inferior_cerebellar_peduncle_RH	-0.70	0.02	0.47	0.15
Inferior_cerebellar_peduncle_LH	-0.71	0.01	0.24	0.48
Superior_cerebellar_peduncle_RH	-0.88	<0.01	0.59	0.05
Superior_cerebellar_peduncle_LH	-0.89	<0.01	0.39	0.23
Cerebral_peduncle_RH	-0.84	<0.01	0.47	0.14
Cerebral_peduncle_LH	-0.57	0.07	0.39	0.23
Anterior_limb_of_int_capsule_RH	-0.55	0.08	0.47	0.14
Anterior_limb_of_int_capsule_LH	-0.85	<0.01	0.47	0.15
Posterior_limb_of_int_capsule_RH	-0.65	0.03	0.56	0.07
Posterior_limb_of_int_capsule_LH	-0.66	0.03	0.41	0.21
Retrolenticular_int_capsule_RH	-0.62	0.04	0.40	0.23
Retrolenticular_int_capsule_LH	-0.40	0.23	0.22	0.51
Anterior_corona_radiata_RH	-0.65	0.03	0.31	0.35
Anterior_corona_radiata_LH	-0.66	0.03	0.29	0.39
Superior_corona_radiata_RH	-0.62	0.04	0.63	0.04
Superior_corona_radiata_LH	-0.61	0.05	0.75	<0.01
Posterior_corona_radiata_RH	-0.77	<0.01	0.59	0.05
Posterior_corona_radiata_LH	-0.67	0.03	0.63	0.04
Posterior_thalamic_radiation_RH	-0.67	0.03	0.42	0.19
Posterior_thalamic_radiation_LH	-0.67	0.02	0.61	0.05
Sagittal_stratum_RH	-0.27	0.43	0.16	0.63
Sagittal_stratum_LH	-0.54	0.09	0.13	0.70
External_capsule_RH	-0.55	0.08	0.37	0.26
External_capsule_LH	-0.60	0.05	0.56	0.08
Cingulum_RH	-0.50	0.12	0.42	0.19
Cingulum_LH	-0.83	<0.01	0.31	0.36
Cingulum(hippocampus)_RH	-0.95	<0.01	0.36	0.28
Cingulum(hippocampus)_LH	-0.71	0.01	0.40	0.22
Fornix/stria_terminalis_RH	-0.81	<0.01	0.68	0.02
Fornix/stria_terminalis_LH	-0.85	<0.01	0.46	0.15
Sup_longitudinal_fasciculus_RH	-0.82	<0.01	0.34	0.30
Sup_longitudinal_fasciculus_LH	-0.55	0.08	0.40	0.23
Sup_fronto_occip_fasciculus_RH	-0.60	0.05	0.24	0.48
Sup_fronto_occip_fasciculus_LH	-0.53	0.09	0.38	0.25
Uncinate_fasciculus_RH	-0.83	<0.01	0.27	0.41
Uncinate_fasciculus_LH	-0.63	0.04	-0.10	0.78

Tapetum_RH	-0.84	<0.01	0.14	0.68
Tapetum_LH	-0.58	0.06	-0.11	0.74

Appendix J: RD correlations of EDSS and age diagnosed in the 48 WM tracts.

RADIAL DIFFUSIVITY (n=11)	EDSS		Age Diagnosed	
	r	p	r	p
Middle_cerebellar_peduncle	0.77	<0.01	-0.43	0.18
Pontine_crossing_tract	0.42	0.19	0.06	0.85
CC_genu	0.83	<0.01	-0.38	0.24
CC_body	0.59	0.06	-0.22	0.52
CC_splenium	0.71	0.01	-0.58	0.06
Fornix	0.68	0.02	-0.66	0.03
Corticospinal_tract_RH	0.79	<0.01	-0.76	<0.01
Corticospinal_tract_LH	0.77	<0.01	-0.54	0.09
Medial_lemniscus_RH	0.76	<0.01	-0.48	0.14
Medial_lemniscus_LH	0.84	<0.01	-0.49	0.13
Inferior_cerebellar_peduncle_RH	0.84	<0.01	-0.62	0.04
Inferior_cerebellar_peduncle_LH	0.49	0.13	0.06	0.86
Superior_cerebellar_peduncle_RH	0.83	<0.01	-0.56	0.07
Superior_cerebellar_peduncle_LH	0.82	<0.01	-0.56	0.07
Cerebral_peduncle_RH	0.66	0.03	-0.33	0.32
Cerebral_peduncle_LH	0.57	0.07	-0.54	0.08
Anterior_limb_of_int_capsule_RH	0.55	0.08	-0.44	0.18
Anterior_limb_of_int_capsule_LH	0.67	0.03	-0.63	0.04
Posterior_limb_of_int_capsule_RH	0.64	0.03	-0.51	0.11
Posterior_limb_of_int_capsule_LH	0.53	0.10	-0.46	0.16
Retrolenticular_int_capsule_RH	0.47	0.14	-0.20	0.55
Retrolenticular_int_capsule_LH	0.24	0.47	-0.12	0.72
Anterior_corona_radiata_RH	0.62	0.04	-0.29	0.39
Anterior_corona_radiata_LH	0.55	0.08	-0.20	0.55
Superior_corona_radiata_RH	0.62	0.04	-0.48	0.14
Superior_corona_radiata_LH	0.49	0.13	-0.39	0.24
Posterior_corona_radiata_RH	0.84	<0.01	-0.65	0.03
Posterior_corona_radiata_LH	0.65	0.03	-0.53	0.09
Posterior_thalamic_radiation_RH	0.72	0.01	-0.47	0.15
Posterior_thalamic_radiation_LH	0.83	<0.01	-0.66	0.03
Sagittal_stratum_RH	0.56	0.07	-0.14	0.69
Sagittal_stratum_LH	0.53	0.10	0.00	0.99
External_capsule_RH	0.61	0.05	-0.26	0.43
External_capsule_LH	0.52	0.10	-0.44	0.17
Cingulum_RH	0.61	0.05	-0.21	0.54
Cingulum_LH	0.59	0.05	-0.26	0.45
Cingulum(hippocampus)_RH	0.76	<0.01	-0.37	0.26
Cingulum(hippocampus)_LH	0.76	<0.01	-0.38	0.25
Fornix/stria_terminalis_RH	0.77	<0.01	-0.44	0.18
Fornix/stria_terminalis_LH	0.84	<0.01	-0.57	0.07
Sup_longitudinal_fasciculus_RH	0.91	<0.01	-0.54	0.09
Sup_longitudinal_fasciculus_LH	0.38	0.25	-0.10	0.78
Sup_fronto_occip_fasciculus_RH	0.62	0.04	-0.10	0.78
Sup_fronto_occip_fasciculus_LH	0.54	0.09	-0.36	0.27
Uncinate_fasciculus_RH	0.83	<0.01	-0.23	0.49
Uncinate_fasciculus_LH	0.74	<0.01	0.01	0.97

Tapetum_RH	0.85	<0.01	-0.30	0.37
Tapetum_LH	0.65	0.03	0.09	0.79

Appendix K: MD correlations of EDSS, % TF sat, Haemoglobin and Ferritin in the 48 WM tracts.

MEAN DIFFUSIVITY (n=11)	EDSS		% Tf saturation		Haemoglobin		Ferritin	
	r	p	r	p	r	P	r	p
Middle_cerebellar_peduncle	0.67	0.02	-0.20	0.55	0.03	0.94	-0.49	0.13
Pontine_crossing_tract	-0.44	0.17	0.60	0.05	0.26	0.44	-0.03	0.94
CC_genu	0.52	0.10	0.04	0.90	-0.09	0.80	-0.69	0.02
CC_body	0.56	0.07	-0.08	0.81	-0.03	0.94	-0.49	0.13
CC_splenium	0.41	0.21	-0.42	0.19	-0.27	0.42	-0.06	0.85
Fornix	0.44	0.18	-0.48	0.14	-0.14	0.68	-0.05	0.87
Corticospinal_tract_RH	0.36	0.28	0.28	0.41	0.25	0.47	-0.05	0.88
Corticospinal_tract_LH	0.21	0.53	0.24	0.48	0.13	0.70	0.05	0.89
Medial_lemniscus_RH	0.59	0.06	-0.36	0.28	-0.17	0.62	0.07	0.84
Medial_lemniscus_LH	0.74	<0.01	-0.31	0.36	-0.31	0.35	-0.05	0.87
Inferior_cerebellar_peduncle_RH	0.44	0.17	-0.26	0.45	-0.27	0.41	0.30	0.37
Inferior_cerebellar_peduncle_LH	-0.26	0.44	0.10	0.76	0.14	0.68	0.05	0.89
Superior_cerebellar_peduncle_RH	0.61	0.05	-0.38	0.25	-0.24	0.47	-0.17	0.62
Superior_cerebellar_peduncle_LH	0.67	0.02	-0.53	0.10	-0.31	0.35	-0.27	0.42
Cerebral_peduncle_RH	0.64	0.04	-0.64	0.03	-0.12	0.73	-0.34	0.31
Cerebral_peduncle_LH	0.54	0.09	-0.84	<0.01	-0.26	0.45	-0.17	0.62
Anterior_limb_of_int_capsule_RH	0.61	0.05	-0.79	<0.01	-0.18	0.59	-0.31	0.35
Anterior_limb_of_int_capsule_LH	0.37	0.27	-0.75	<0.01	-0.19	0.58	-0.06	0.86
Posterior_limb_of_int_capsule_RH	0.48	0.14	-0.56	0.08	-0.27	0.42	-0.12	0.72
Posterior_limb_of_int_capsule_LH	0.25	0.45	-0.84	<0.01	-0.30	0.37	0.16	0.64
Retrolenticular_int_capsule_RH	0.45	0.17	-0.76	<0.01	-0.28	0.40	-0.11	0.75
Retrolenticular_int_capsule_LH	0.03	0.92	-0.61	0.05	-0.05	0.87	-0.15	0.65
Anterior_corona_radiata_RH	0.43	0.18	-0.86	<0.01	-0.28	0.41	-0.22	0.51
Anterior_corona_radiata_LH	0.46	0.15	-0.36	0.28	0.31	0.36	-0.49	0.12
Superior_corona_radiata_RH	0.57	0.07	-0.44	0.18	0.05	0.89	-0.38	0.25
Superior_corona_radiata_LH	0.42	0.20	-0.66	0.03	-0.04	0.90	-0.45	0.16
Posterior_corona_radiata_RH	0.59	0.05	-0.35	0.29	-0.22	0.51	-0.06	0.85
Posterior_corona_radiata_LH	0.61	0.05	-0.41	0.21	-0.10	0.78	-0.29	0.39
Posterior_thalamic_radiation_RH	-0.33	0.33	0.33	0.32	0.22	0.51	0.10	0.77
Posterior_thalamic_radiation_LH	-0.31	0.35	0.47	0.14	0.34	0.30	-0.09	0.79
Sagittal_stratum_RH	0.47	0.14	-0.28	0.41	-0.38	0.25	-0.54	0.09
Sagittal_stratum_LH	0.61	0.05	-0.63	0.04	-0.22	0.52	-0.29	0.39
External_capsule_RH	0.47	0.15	-0.38	0.24	0.15	0.66	-0.57	0.07
External_capsule_LH	0.54	0.09	-0.55	0.08	-0.21	0.53	-0.46	0.16
Cingulum_RH	0.53	0.09	-0.49	0.13	-0.05	0.88	-0.29	0.39
Cingulum_LH	0.04	0.90	-0.58	0.06	0.06	0.85	0.01	0.98
Cingulum(hippocampus)_RH	0.25	0.46	-0.32	0.33	-0.42	0.19	-0.22	0.51
Cingulum(hippocampus)_LH	0.59	0.05	-0.80	<0.01	-0.28	0.41	-0.05	0.89
Fornix/stria_terminalis_RH	0.72	0.01	-0.36	0.28	-0.20	0.55	-0.45	0.16
Fornix/stria_terminalis_LH	-0.19	0.57	-0.05	0.87	-0.46	0.16	0.08	0.82
Sup_longitudinal_fasciculus_RH	0.07	0.85	-0.35	0.30	-0.89	<0.01	0.25	0.46
Sup_longitudinal_fasciculus_LH	0.20	0.55	-0.48	0.13	-0.14	0.69	0.07	0.83
Sup_fronto_occip_fasciculus_RH	0.57	0.07	-0.40	0.23	-0.47	0.15	0.02	0.95
Sup_fronto_occip_fasciculus_LH	0.39	0.24	-0.76	<0.01	-0.13	0.70	-0.41	0.21
Uncinate_fasciculus_RH	0.70	0.02	-0.54	0.08	-0.49	0.13	-0.20	0.55
Uncinate_fasciculus_LH	0.55	0.08	-0.29	0.38	-0.14	0.68	-0.37	0.26

Tapetum_RH	0.62	0.04	-0.19	0.57	-0.20	0.56	-0.45	0.16
Tapetum_LH	-0.39	0.24	0.33	0.32	0.31	0.36	-0.26	0.44

Appendix L: MD correlations of EDSS and age diagnosed in the 48 WM tracts.

MEAN DIFFUSIVITY (n=11)	EDSS		Age Diagnosed	
	r	p	r	p
Middle_cerebellar_peduncle	0.67	0.02	-0.21	0.54
Pontine_crossing_tract	-0.44	0.17	0.32	0.34
CC_genu	0.52	0.10	-0.23	0.49
CC_body	0.56	0.07	-0.46	0.15
CC_splenium	0.41	0.21	-0.58	0.06
Fornix	0.44	0.18	-0.55	0.08
Corticospinal_tract_RH	0.36	0.28	-0.17	0.62
Corticospinal_tract_LH	0.21	0.53	-0.06	0.86
Medial_lemniscus_RH	0.59	0.06	-0.17	0.61
Medial_lemniscus_LH	0.74	<0.01	-0.23	0.50
Inferior_cerebellar_peduncle_RH	0.44	0.17	-0.24	0.48
Inferior_cerebellar_peduncle_LH	-0.26	0.44	0.20	0.56
Superior_cerebellar_peduncle_RH	0.61	0.05	-0.47	0.15
Superior_cerebellar_peduncle_LH	0.67	0.02	-0.45	0.17
Cerebral_peduncle_RH	0.64	0.04	-0.43	0.19
Cerebral_peduncle_LH	0.54	0.09	-0.42	0.20
Anterior_limb_of_int_capsule_RH	0.61	0.05	-0.39	0.24
Anterior_limb_of_int_capsule_LH	0.37	0.27	-0.29	0.38
Posterior_limb_of_int_capsule_RH	0.48	0.14	-0.39	0.23
Posterior_limb_of_int_capsule_LH	0.25	0.45	-0.31	0.36
Retrolenticular_int_capsule_RH	0.45	0.17	-0.19	0.58
Retrolenticular_int_capsule_LH	0.03	0.92	0.16	0.64
Anterior_corona_radiata_RH	0.43	0.18	-0.27	0.41
Anterior_corona_radiata_LH	0.46	0.15	-0.09	0.79
Superior_corona_radiata_RH	0.57	0.07	-0.32	0.33
Superior_corona_radiata_LH	0.42	0.20	-0.36	0.28
Posterior_corona_radiata_RH	0.59	0.05	-0.24	0.47
Posterior_corona_radiata_LH	0.61	0.05	-0.36	0.28
Posterior_thalamic_radiation_RH	-0.33	0.33	0.26	0.43
Posterior_thalamic_radiation_LH	-0.31	0.35	0.28	0.40
Sagittal_stratum_RH	0.47	0.14	0.06	0.85
Sagittal_stratum_LH	0.61	0.05	-0.05	0.89
External_capsule_RH	0.47	0.15	-0.14	0.68
External_capsule_LH	0.54	0.09	-0.51	0.11
Cingulum_RH	0.53	0.09	-0.16	0.65
Cingulum_LH	0.04	0.90	-0.19	0.57
Cingulum(hippocampus)_RH	0.25	0.46	-0.15	0.67
Cingulum(hippocampus)_LH	0.59	0.05	-0.24	0.48
Fornix/stria_terminalis_RH	0.72	0.01	-0.26	0.43
Fornix/stria_terminalis_LH	-0.19	0.57	-0.17	0.61
Sup_longitudinal_fasciculus_RH	0.07	0.85	-0.57	0.07
Sup_longitudinal_fasciculus_LH	0.20	0.55	0.10	0.77
Sup_fronto_occip_fasciculus_RH	0.57	0.07	-0.07	0.84
Sup_fronto_occip_fasciculus_LH	0.39	0.24	-0.36	0.28
Uncinate_fasciculus_RH	0.70	0.02	-0.21	0.54
Uncinate_fasciculus_LH	0.55	0.08	-0.41	0.21

Tapetum_RH	0.62	0.04	-0.23	0.50
Tapetum_LH	-0.39	0.24	0.25	0.46

Appendix M: AD correlations of EDSS, % Tf saturation, haemoglobin and ferritin in the 48 WM tracts.

AXIAL DIFFUSIVITY (n=11)	EDSS		% Tf saturation		Haemoglobin		Ferritin	
	r	P	r	p	r	P	r	p
Middle_cerebellar_peduncle	0.67	0.02	-0.2	0.55	0.03	0.94	-0.49	0.13
Pontine_crossing_tract	-0.44	0.17	0.6	0.05	0.26	0.44	-0.03	0.94
CC_genu	0.52	0.10	0.04	0.9	-0.09	0.80	-0.69	0.02
CC_body	0.56	0.07	-0.08	0.81	-0.03	0.94	-0.49	0.13
CC_splenium	0.41	0.21	-0.42	0.19	-0.27	0.42	-0.06	0.85
Fornix	0.44	0.18	-0.48	0.14	-0.14	0.68	-0.05	0.87
Corticospinal_tract_RH	0.36	0.28	0.28	0.41	0.25	0.47	-0.05	0.88
Corticospinal_tract_LH	0.21	0.53	0.24	0.48	0.13	0.70	0.05	0.89
Medial_lemniscus_RH	0.59	0.06	-0.36	0.28	-0.17	0.62	0.07	0.84
Medial_lemniscus_LH	0.74	<0.01	-0.31	0.36	-0.31	0.35	-0.05	0.87
Inferior_cerebellar_peduncle_RH	0.44	0.17	-0.26	0.45	-0.27	0.41	0.3	0.37
Inferior_cerebellar_peduncle_LH	-0.26	0.44	0.1	0.76	0.14	0.68	0.05	0.89
Superior_cerebellar_peduncle_RH	0.61	0.05	-0.38	0.25	-0.24	0.47	-0.17	0.62
Superior_cerebellar_peduncle_LH	0.67	0.02	-0.53	0.1	-0.31	0.35	-0.27	0.42
Cerebral_peduncle_RH	0.64	0.04	-0.64	0.03	-0.12	0.73	-0.34	0.31
Cerebral_peduncle_LH	0.54	0.09	-0.84	<0.01	-0.26	0.45	-0.17	0.62
Anterior_limb_of_int_capsule_RH	0.61	0.05	-0.79	<0.01	-0.18	0.59	-0.31	0.35
Anterior_limb_of_int_capsule_LH	0.37	0.27	-0.75	<0.01	-0.19	0.58	-0.06	0.86
Posterior_limb_of_int_capsule_RH	0.48	0.14	-0.56	0.08	-0.27	0.42	-0.12	0.72
Posterior_limb_of_int_capsule_LH	0.25	0.45	-0.84	<0.01	-0.30	0.37	0.16	0.64
Retrolenticular_int_capsule_RH	0.45	0.17	-0.76	<0.01	-0.28	0.40	-0.11	0.75
Retrolenticular_int_capsule_LH	0.03	0.92	-0.61	0.05	-0.05	0.87	-0.15	0.65
Anterior_corona_radiata_RH	0.43	0.18	-0.86	<0.01	-0.28	0.41	-0.22	0.51
Anterior_corona_radiata_LH	0.46	0.15	-0.36	0.28	0.31	0.36	-0.49	0.12
Superior_corona_radiata_RH	0.57	0.07	-0.44	0.18	0.05	0.89	-0.38	0.25
Superior_corona_radiata_LH	0.42	0.20	-0.66	0.03	-0.04	0.90	-0.45	0.16
Posterior_corona_radiata_RH	0.59	0.05	-0.35	0.29	-0.22	0.51	-0.06	0.85
Posterior_corona_radiata_LH	0.61	0.05	-0.41	0.21	-0.10	0.78	-0.29	0.39
Posterior_thalamic_radiation_RH	-0.33	0.33	0.33	0.32	0.22	0.51	0.1	0.77
Posterior_thalamic_radiation_LH	-0.31	0.35	0.47	0.14	0.34	0.30	-0.09	0.79
Sagittal_stratum_RH	0.47	0.14	-0.28	0.41	-0.38	0.25	-0.54	0.09
Sagittal_stratum_LH	0.61	0.05	-0.63	0.04	-0.22	0.52	-0.29	0.39
External_capsule_RH	0.47	0.15	-0.38	0.24	0.15	0.66	-0.57	0.07
External_capsule_LH	0.54	0.09	-0.55	0.08	-0.21	0.53	-0.46	0.16
Cingulum_RH	0.53	0.09	-0.49	0.13	-0.05	0.88	-0.29	0.39
Cingulum_LH	0.04	0.90	-0.58	0.06	0.06	0.85	0.01	0.98
Cingulum(hippocampus)_RH	0.25	0.46	-0.32	0.33	-0.42	0.19	-0.22	0.51
Cingulum(hippocampus)_LH	0.59	0.05	-0.8	<0.01	-0.28	0.41	-0.05	0.89
Fornix/stria_terminalis_RH	0.72	0.01	-0.36	0.28	-0.20	0.55	-0.45	0.16
Fornix/stria_terminalis_LH	-0.19	0.57	-0.05	0.87	-0.46	0.16	0.08	0.82
Sup_longitudinal_fasciculus_RH	0.07	0.85	-0.35	0.3	-0.89	<0.01	0.25	0.46
Sup_longitudinal_fasciculus_LH	0.20	0.55	-0.48	0.13	-0.14	0.69	0.07	0.83
Sup_fronto_occip_fasciculus_RH	0.57	0.07	-0.4	0.23	-0.47	0.15	0.02	0.95
Sup_fronto_occip_fasciculus_LH	0.39	0.24	-0.76	<0.01	-0.13	0.70	-0.41	0.21
Uncinate_fasciculus_RH	0.70	0.02	-0.54	0.08	-0.49	0.13	-0.2	0.55
Uncinate_fasciculus_LH	0.55	0.08	-0.29	0.38	-0.14	0.68	-0.37	0.26

Tapetum_RH	0.62	0.04	-0.19	0.57	-0.20	0.56	-0.45	0.16
Tapetum_LH	-0.39	0.24	0.33	0.32	0.31	0.36	-0.26	0.44

Appendix N: AD correlations of EDSS and age diagnosed in the 48 WM tracts.

AXIAL DIFFUSIVITY (n=11)	EDSS		Age Diagnosed	
	r	P	r	P
Middle_cerebellar_peduncle	0.67	0.02	-0.21	0.54
Pontine_crossing_tract	-0.44	0.17	0.32	0.34
CC_genu	0.52	0.10	-0.23	0.49
CC_body	0.56	0.07	-0.46	0.15
CC_splenium	0.41	0.21	-0.58	0.06
Fornix	0.44	0.18	-0.55	0.08
Corticospinal_tract_RH	0.36	0.28	-0.17	0.62
Corticospinal_tract_LH	0.21	0.53	-0.06	0.86
Medial_lemniscus_RH	0.59	0.06	-0.17	0.61
Medial_lemniscus_LH	0.74	<0.01	-0.23	0.50
Inferior_cerebellar_peduncle_RH	0.44	0.17	-0.24	0.48
Inferior_cerebellar_peduncle_LH	-0.26	0.44	0.20	0.56
Superior_cerebellar_peduncle_RH	0.61	0.05	-0.47	0.15
Superior_cerebellar_peduncle_LH	0.67	0.02	-0.45	0.17
Cerebral_peduncle_RH	0.64	0.04	-0.43	0.19
Cerebral_peduncle_LH	0.54	0.09	-0.42	0.20
Anterior_limb_of_int_capsule_RH	0.61	0.05	-0.39	0.24
Anterior_limb_of_int_capsule_LH	0.37	0.27	-0.29	0.38
Posterior_limb_of_int_capsule_RH	0.48	0.14	-0.39	0.23
Posterior_limb_of_int_capsule_LH	0.25	0.45	-0.31	0.36
Retrolenticular_int_capsule_RH	0.45	0.17	-0.19	0.58
Retrolenticular_int_capsule_LH	0.03	0.92	0.16	0.64
Anterior_corona_radiata_RH	0.43	0.18	-0.27	0.41
Anterior_corona_radiata_LH	0.46	0.15	-0.09	0.79
Superior_corona_radiata_RH	0.57	0.07	-0.32	0.33
Superior_corona_radiata_LH	0.42	0.20	-0.36	0.28
Posterior_corona_radiata_RH	0.59	0.05	-0.24	0.47
Posterior_corona_radiata_LH	0.61	0.05	-0.36	0.28
Posterior_thalamic_radiation_RH	-0.33	0.33	0.26	0.43
Posterior_thalamic_radiation_LH	-0.31	0.35	0.28	0.40
Sagittal_stratum_RH	0.47	0.14	0.06	0.85
Sagittal_stratum_LH	0.61	0.05	-0.05	0.89
External_capsule_RH	0.47	0.15	-0.14	0.68
External_capsule_LH	0.54	0.09	-0.51	0.11
Cingulum_RH	0.53	0.09	-0.16	0.65
Cingulum_LH	0.04	0.90	-0.19	0.57
Cingulum(hippocampus)_RH	0.25	0.46	-0.15	0.67
Cingulum(hippocampus)_LH	0.59	0.05	-0.24	0.48
Fornix/stria_terminalis_RH	0.72	0.01	-0.26	0.43
Fornix/stria_terminalis_LH	-0.19	0.57	-0.17	0.61
Sup_longitudinal_fasciculus_RH	0.07	0.85	-0.57	0.07
Sup_longitudinal_fasciculus_LH	0.20	0.55	0.10	0.77
Sup_fronto_occip_fasciculus_RH	0.57	0.07	-0.07	0.84
Sup_fronto_occip_fasciculus_LH	0.39	0.24	-0.36	0.28
Uncinate_fasciculus_RH	0.70	0.02	-0.21	0.54
Uncinate_fasciculus_LH	0.55	0.08	-0.41	0.21
Tapetum_RH	0.62	0.04	-0.23	0.50

Tapetum_LH	-0.39	0.24	0.25	0.46

Appendix O: Submitted article.

Fractional Anisotropy of White Matter, Disability and Blood Iron Parameters in Multiple Sclerosis

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Abstract

Multiple sclerosis (MS) is a disorder related to myelin damage, which can be investigated by means of novel brain imaging techniques such as fractional anisotropy (FA). Since FA is a measure of microstructural white matter integrity, high FA values are considered to relate to functional myelin. The present study compared FA in 11 MS patients with 12 healthy control subjects. The objectives of the study were to investigate (1) the relationship between FA and disability in MS patients as measured by the Expanded Disability Status Scale (EDSS), with high versus low disability scores in the whole brain and in individual white matter tracts, and (2) whether blood iron parameters would have an impact on FA, since there is a controversy about whether iron is beneficial or harmful to people with MS. Results: (1) The mean FA was significantly lower in MS patients with high EDSS scores (0.34±0.067) compared to controls (0.45±0.036; $p = 0.04$), while subjects who had low EDSS scores had mean FA values similar to control values (0.44±0.014). This was not related to their age or disease duration, suggesting that people with MS in this cohort did not inevitably experience increasing myelin degeneration over time. (2) There was a significant positive association between mean FA and %transferrin saturation ($p<0.01$), suggesting that iron availability to oligodendrocytes may be a requirement for optimal myelin functionality. The association between the FA of the superior fronto occipital fasciculus (LH) and the %transferrin saturation was highly significant ($r = 0.83$, $p<0.001$). (3) Significant inverse associations of disease duration with haemoglobin ($p = 0.04$) and %transferrin saturation ($p = 0.02$) suggest that blood iron concentrations may decrease over time in MS and that it may be important to assess iron parameters regularly in MS patients.

Introduction

Multiple sclerosis (MS) is a chronic inflammatory disease of the central nervous system (CNS). It preferentially affects young adults, and is more common among women than men [1-3]. White matter (WM) lesions of the brain and spinal cord, identified with magnetic resonance imaging (MRI), reflecting a variable extent of myelin and/or neuronal damage, are considered to be one of the diagnostic features of MS [4-6]. Concurrent with the lesions are

clinical symptoms that vary considerably between patients. For some the clinical manifestations are a “lifelong occasional nuisance”, while others experience severe incapacity [7,8]. This disparity in outcome seems to be unrelated to the use of immune modulating therapies [9-13]. Rather, disability progression seems to be related to a failure of functional connectivity in the CNS [14,15]. Connectivity is in turn strongly related to white matter (WM) function [16] and remyelination of axons which have experienced myelin damage or demyelination. Remyelination is accomplished by oligodendrocyte precursor cells (OPCs), adult stem cells resident in the CNS that mature into oligodendrocytes [17]. The risk factors that prevent OPCs from remyelinating the axons have not yet been fully established, presenting an exciting field of scientific investigation in MS. It is unlikely that increased disability is related to a single factor, and is more likely to result from an interaction of the various risk factors that have already shown such associations, such as vitamin D deficiency [18,19], smoking [20,21], cardiovascular risk factors [21-24], saturated fat intake [25], low intake of fruits and vegetables [21] and presence of the HLA DRB1*1505 genetic variant [7], This field of research is greatly aided by objective instruments for measuring disability, such as the Expanded Disability Status Scale (EDSS) as described by Kutzke [26]. The EDSS is used in all clinical studies as a ‘gold standard’ and ranges between 0 and 10, with higher values indicating greater disability. Patients having EDSS values below 3 for extended periods of time are regarded as having a ‘benign disease course’ [13]. Besides benign MS, several other subtypes of MS have been described: relapsing-remitting (RRMS), secondary progressive (SPMS) and primary progressive (PPMS), although the diagnosis remains confusing due to the heterogeneity of the disease [27,28]. Clinically Isolated Syndrome (CIS) refers to a first clinical episode with features suggestive of MS.

Since the association between the MRI lesion load and clinical disability is weak, another MRI modality, Diffusion Tensor Imaging (DTI), has been investigated as a method to provide improved associations between structural WM changes and disability [15]. DTI measures diffusion of water molecules in white matter tracts (WMTs) which normally tends to be parallel to the direction of the nerve fibres [29], as it is easier for water molecules to move

along the fiber axis than traveling across a fiber [30]. When the myelin or axons are damaged, the rate of diffusion and direction of flow are altered significantly [31]. Therefore if there is any pathological process causing restrictive barriers it could lead to a decrease of diffusivity. The most commonly studied diffusion parameter is the fractional anisotropy (FA), which is a mathematical combination of the “eigenvalues” of diffusivity [32-34]. FA varies between 0, representing isotropic (random) diffusion, and 1 representing diffusion taking place along one plane or direction (anisotropic diffusion). Low FA values could therefore be indicative of myelin damage or demyelination as an impediment in the directional selectivity of water molecule movement within tissue [35]. Conversely, high FA values are observed in heavily myelinated WMTs [36], for example in specific brain areas in musicians such as concert pianists and string players, as a result of the development of repetitive fine motor skills [36-38] and may be associated with greater effectiveness in neural communication [16,39]. In cerebrospinal fluid (CSF) and grey matter (GM), water motion is random and can thus be described as isotropic diffusion (FA values close to 0).

Considering the fact that MS is primarily related to myelin damage, FA is expected to be lower in MS patients than controls, while lower FA values in MS patients are considered to be related to dysfunction and disability [36,40]. Significantly lower DTI measures, including FA, have been found in MS patients compared to controls in several studies, in the whole brain [41,42] and in specific WMTs such as the corpus callosum (CC) [43,44], fornix [45] and in the inferior longitudinal and fronto-occipital fasciculi [41]. Hasan et al. found that while the FA was significantly decreased in the CC anterior, posterior and midbody, the FA in the CC genu and splenium were similar to controls [43]. Significant FA differences have also been detected between MS lesions compared to normal appearing white matter NAWM [46,47] while subtle abnormalities were detected in early RRMS [48]. Liu et al. found significant inverse correlations between diffusion metrics and disability (EDSS) in the CC splenium, left cingulum bundle and bilateral corticospinal tracts [42]. Llufriu et al. found lower average FA in MS patients than controls in the CC; however, no correlations were observed with the EDSS [44]. Another study found that memory functions correlated with DTI in the fornix [45], while

cognitive deficits were significantly associated with decreased FA in all examined WMTs in a study by Cesar et al. [41]. Some authors also found that the FA was inversely associated with disease duration [33,42,49], while other studies showed no such associations [43,48]. It has not yet been established whether lower FA as a measure of myelin damage is persistent in all patients with MS, irrespective of their disability status.

Controversy regarding the presence of iron in the brain of MS patients

Several studies have investigated associations of FA with brain iron concentrations, since a controversy has arisen regarding whether iron is detrimental or beneficial to patients with MS. High iron levels are present within the microglia / macrophages and the lesion perimeters in autopsy brain samples of MS patients [50]. Detection of brain iron deposits in MS patients has raised concern, and some have advocated reduced iron intake or removal of iron via chelation therapy in order to reduce oxidative free iron [51]; however, clinical trials of iron chelators in MS have not been successful [52]. Others feel that iron is a prerequisite for myelin synthesis and maintenance, and that iron *supplementation* should be considered instead as an intervention strategy in patients with proven iron deficiency [27].

The controversy is compounded by the fact that iron in the body exists in different compartments with different functions, so that all forms of iron are not equal. Haemoglobin (Hb) is often used as a first line blood test for determining anaemia or iron deficiency. Hb measures the iron which is covalently bound inside red blood cells (RBC), which deliver oxygen to tissues. If the blood-brain barrier (BBB) is compromised, RBCs may enter the brain parenchyma as micro-bleeds (haemorrhages), and heme-iron may be deposited as insoluble hemosiderin. These iron deposits, detectable by MRI, have been hypothesised to be the primary cause of MS [53]. However, this theory has not been confirmed in subsequent studies [54-56].

Heme-iron is not available for other biochemical reactions such as myelin production. The oligodendrocytes, the cells which produce myelin, need iron delivered to them by the iron carrier protein transferrin (Tf) [57,58]. This form of iron is used by the mitochondria for energy

production [59]. Another form of iron in the brain is found as hydrate ferric iron-oxide crystals encased by ferritin, a storage protein [60]. One ferritin molecule can store up to 4500 iron atoms [61]. Ferritin is synthesized in the brain as an antioxidant to “mop up” any iron that is freed up from haemorrhages [54]. Ferritin therefore has an iron buffering function in order to protect the CNS from toxic free iron. However, although the iron in ferritin is a normal part of brain iron metabolism and is available for iron delivery to oligodendrocytes [58], the iron crystals in ferritin can also be registered as “iron deposits” by some MRI techniques [62,63]. Since the matter has not yet been resolved, several research methods, including FA, are being employed for further investigation.

Results from studies of FA and iron in the brain have been contradictory [62]. Xu et al found that the effect of iron deposition was opposite to that of aging: while FA decreased with aging, elevated iron concentration was associated with increased FA [62]. Syka et al. also found that increased iron as estimated by relaxometry (the T2 relaxation rate RR) showed a strong positive relationship with FA [63]. Both studies have interpreted increased FA in the presence of increased iron as an artefact due to the magnetic properties of iron [62], specifically in ferritin or haemosiderin [63]. However, a study investigating FA in WM of patients with Huntington’s disease, interpreted increased FA in the presence of higher iron as improved myelin function. Patients in the pre-symptomatic stages of Huntington’s disease experienced demyelination together with a reduction in brain iron content in the CC, while there was a proliferation of oligodendrocytes and increased myelination together with increased iron levels in the corticospinal tract and deep white matter structures [64]. This was interpreted as a repair mechanism which ensured adequate function in the early stages of the disease process. Oligodendrocytes are known to have the highest iron concentrations of all cells in the brain under normal conditions [57].

In MS, iron concentration in the brain determined by relaxometry showed that short term changes in iron concentration were not linked to disease or changes in disability [65]. Furthermore, MRI-identified iron did not necessarily reflect pathology and was also seen in apparently normal tissue [61]. When comparing MS patients with CIS and healthy controls,

there was an increase in the iron levels in the brain of patients with MS, but the iron levels of the CIS group were lower than those of the healthy controls, suggesting that excess iron is a by-product of MS and not the initial cause of the disease [66]. Lower brain iron could even play a role in perpetuating the disease process, since Hametner et al. found a significant decrease of iron in the NAWM in chronic MS, which corresponded with disease duration [67].

As postulated by Van Rensburg et al., if increased iron in the brain is indicative of increased morbidity it should correlate with disability [27]. However Ceccarelli et al. stated that patients with a benign course of MS and SPMS had similar iron levels in the brain, suggesting that disability in MS could be due to other causative factors and not iron deposition [68]. Other authors emphasised the importance of functional iron concentrations in oligodendrocytes for energy production during myelin synthesis and maintenance, as under normal circumstances the oligodendrocytes accumulate iron for the extremely high energy requirements of producing and maintaining the myelin sheath [57,16]. Energy production in mitochondria is dependent on iron availability [59]. Therefore, when there is a deficiency of iron delivery to mitochondria the viability of oligodendrocytes could be compromised. Khalil et al. suggest that it is not clear whether increased iron can be implicated in MS or whether it is as a result of another process happening simultaneously [66].

As far as we are aware, associations of FA with blood iron parameters at the time of MRI have not been determined before. Since blood iron parameters are the only means for clinicians to evaluate the iron status of patients, it could be expedient to evaluate the associations of these parameters with FA as a measure of WM function, and disease outcome in MS.

Aims of the present study

The present study was done to investigate (1) the relationship between FA and disability in MS patients with high versus low disability scores as determined by the EDSS and (2) whether blood iron parameters would have an impact on FA as a measure of microstructural white matter integrity.

Methods

The MS study, including MRI, was approved by the Ethics Committee of the University of Stellenbosch (N07/09/203 and N10/01/021) and the Health and Wellness Science Research Ethics Committee of the Cape Peninsula University of Technology (REC-230408-014), in line with the ethical guidelines provided by the Declaration of Helsinki [69]. Control results were obtained from a concurrent study (Stellenboch University Ethics Committee approval number 99/013). Informed consent was processed for all participants; they also all completed an MRI screening form.

Twenty patients with neurologically confirmed MS [70] were enrolled in the study. Biochemical test results were available for all 20 patients. EDSS assessments were performed at the time of MRI by a specially trained clinician [26]. To increase the statistical power in this small sample and to obtain homogeneity in the study group, only female patients were recruited. Of the original 20 patients, 16 presented for MRI scans; however 5 of the scans could not be used for DTI analysis due to subject movement or illegible data, leaving 11 patients with useable DTI data. Of the four who did not have the scans, two were claustrophobic, one decided against having the scan and one did not arrive. The 11 patients were between 37 and 57 years of age, with an EDSS range of 1.0 – 7.5 and diagnosed with MS for more than 4 years. The control group consisted of 12 healthy females matched as far as possible for age.

Biochemical Analysis

Laboratory analysis comprised blood collection in the morning between 9h00 and 10h30 to standardize for diurnal variation. The following blood iron parameters were measured: Hb, serum iron, Tf, ferritin and % Tf saturation (Tf sat). C-reactive protein (CRP) was also measured to control for inflammation. Full blood counts were determined on a Beckman Coulter autoanalyser, while serum iron, ferritin, Tf and % Tf sat as well as CRP were determined using a Siemens Advia 1800 auto-analyser. The tests were performed by an accredited pathology laboratory.

Acquisition of DTI data

The present study measured DTI, including FA, in 48 WMTs, selected due to their involvement with movement and cognition relevant to MS and which could be accurately identified with the DTI resolution available. A WM atlas by Mori et al. was used to identify the tracts with Oxford Centre for Functional MRI of the Brain (FMRIB) Functional Software Library (FSL) and to extract values that could be statistically analysed using Tract Based Spacial Statistics (TBSS) [71].

All patients were scanned with a Siemens Magnetom Allegra 3.0 Tesla (T) MRI at the Cape Universities Brain Imaging Centre (CUBIC), Tygerberg, South Africa. A standard 8-channel head coil was used for all patients. The MRI scan was carried out by trained radiographers according to standard protocol.

Brain imaging protocol

Diffusion-weighted images were acquired in axial orientation on a four-channel transmit-receive RF head coil with the following parameters: 2x2x2 mm3 resolution, field-of-view of 220 mm, TR = 9500 ms, TE = 88 ms, 70 slices, distance factor of 0, 2x GRAPPA acceleration, 30 b=1000 mm/s2 directions and 3 b=0 mm/s2 reference images. Scan acquisition was repeated 3 times.

Imaging analysis

Data was imported into FSL (FMRIB Software Library 5.0.1; Smith et al.) and MATLAB R2012b for initial pre-processing and analysis [72]. Data was corrected for eddy current distortions and the three acquisitions were affine registered to the b=0 mm/s2 of the first acquisition. For each acquisition, outliers were calculated at a Z-value of the 25th and 75th percentile of the tensor estimate. Rigorous criteria were applied regarding scan quality; values further than three standard deviations from the mean estimate were excluded. The three acquisitions were averaged for export to the TBSS toolbox of FSL. Maps of FA were

created by fitting a linear tensor model to the average of the diffusion-weighted acquisitions. Brain extraction was performed with FSL BET (Brain Extraction Tool) at a threshold of 0.3. Images were then processed with the TBSS pipeline, which has been described previously [73]. After processing with TBSS 48 white matter regions-of-interest were extracted from the FA data, by utilizing the built-in atlas of Mori and colleagues [71].

Statistical Analysis

DTI data from 11 MS patients were analysed. FA was measured in 48 WMTs for each of the 11 patients, which gave a total of 528 WMTs for analysis. An exploratory factor analysis (using parallel analysis to indicate the number of factors) was used to demonstrate the viability of using average FA over all WMT's as a summary measure of brain function.Factor analysis indicated that one underlying factor (FA) explained 85% variance in the data, that the FA effects occurred in all the WMT's assessed and that it was therefore appropriate to calculate the average FA values over all the WMTs. The data were analysed using Statistica 12. One-way ANOVA was used to compare mean FA values between the groups. Fisher least significant difference (LSD) tests were used for post hoc comparison. Non-parametric Mann-Whitney U and Kruskall-Wallis tests were also conducted parallel to the ANOVA (F-tests), but in general the data was found to conform reasonably to the normality assumptions, and interpretations were done using the ANOVA F-test p-values. Spearman correlations were used for testing relationships between biochemical variables and FA. Since the controls were younger than the patients and FA decreases with age, an analysis of covariance (ANCOVA) was done to determine age effects. Multiple testing across analyses of FA variables in conjunction with the small sample size is recognized as a restriction in the interpretation of the results, and that the results should be regarded as exploratory. Patients were grouped according to their EDSS scores to evaluate associations between FA and disease outcome. P-values were considered significant at <0.05.

Results

Demographic and clinical characteristics of MS patients and healthy controls are reported in Table 1. Patients were divided into two groups with high and low disability according to the EDSS: of the 11 MS patients, 3 had EDSS scores of 6.0 – 7.5 while 8 had scores of 1.0 – 4.5 (Table 1)

Table 1: Demographic and clinical characteristics of MS patients and healthy controls

Characteristics	Controls	MS high EDSS	MS low EDSS
No of patients (%)	12	3 (27)	8 (72)
Age at MRI, mean (range)	27.6 (18-43)	52.7 (48-57)	47 (37-55)
EDSS score, mean (range)	NA	6.7 (6.0 - 7.5)	1.4 [a] (1.0 - 4.5)
Disease duration, y, mean (range)	NA	16.7 (11 – 23)	8.5 (4 – 19)
Immunomodulatory therapy, n (%)	NA	2 (67)	2 (25)
Betaferon	NA		1 (13)
Avonex	NA	1 (33)	1 (13)
Novantrone	NA	1 (33)	
Untreated	NA	1 (33)	6 (75)
Mean (±SD) FA of WMTs	0.45 (±0.036)	0.34(±0.067) [c]	0.44 (±0.014)

Age at MRI of MS patients significantly different from controls, $p < 0.01$

[a] Significantly different from high EDSS, $p = 0.04$

[c] Significantly different from controls and from low EDSS, $p = 0.04$

Fractional Anisotropy (FA) values in different brain areas in MS versus Controls

The mean FA in all MS patients (n = 11) was lower than the mean FA in the controls (0.41 ± 0.056 vs 0.45 ± 0.036 respectively) (S1 Table). The mean FA in patients with high EDSS (0.34 ± 0.067) was significantly lower (p = 0.04) compared to both the controls (0.45 ± 0.036) and low EDSS group (0.44 ± 0.014) (Table 1). No difference was found between controls and low EDSS. An ANCOVA done on the three groups (controls, high EDSS and low EDSS) still indicated a significantly lower mean FA in the EDSS high group, an indication that age did not affect the results.

Mean FA in MS patients was lower than controls in all 48 tracts except the CC splenium and the fornix, where the FA values were similar. The difference in FA values between cases and controls had p-values < 0.05 in 20 of the 48 tracts (41.6%) assessed. Of these, 4 were highly significantly different (p< 0.01): the inferior cerebellar peduncle RH, the superior cerebellar peduncle LH, the posterior limb of the internal capsule RH and the rentolenticular internal capsule RH (S1 Table).

Associations between FA and the EDSS

The EDSS was inversely associated with the FA in all of the tracts, suggesting that higher disability was associated with measurable myelin dysfunction (S2 Table). Unexpectedly, however, when the MS patients were grouped as low EDSS vs high EDSS, the FA for patients with low EDSS was similar to control values in all the tracts except the sagittal stratum RH and sagittal stratum LH which had similar FA values for all three groups (low EDSS, high EDSS and controls). While the average FA of patients with high EDSS was significantly lower (0.34 ± 0.067) than controls (0.45 ± 0.036, p = 0.04), the patients with low EDSS had FA values similar to controls (0.43 ± 0.014, p = 0.5) (Table 1 and S3 Table)

Figures 1 and 2 show examples of these associations. Figure 1 A – C: FA in the CC (which is regarded as the first brain area to suffer myelin damage in MS) indicated that the patients with low disability had FA values similar to controls (CC body; p = 0.802, CC genu; p = 0.816 and CC splenium; p = 0.115), while there were significant differences between patients with

high EDSS and controls (CC body; p = 0.015, CC genu; p = 0.017 and CC splenium; p = 0.007) and between high EDSS and low EDSS (CC body; p = 0.013, CC genu; p = 0.016 and CC splenium; p = 0.0007). Figure 1 D: In the fornix, which is related to cognitive function and memory, MS patients with low EDSS also had similar FA values to controls (p = 0.135), while there were significant differences between patients with high EDSS and controls (p = 0.008) and between patients with high EDSS and low EDSS (p = 0.001). Similar associations were seen in the superior longitudinal fasciculus LH (Figure 2).

Fig 1. FA of CC genu (A), CC body (B) and CC splenium (C) and Fornix (D) in patients with low EDSS, high EDSS and controls. * denotes significant differences (see text). EDSS = Expanded Disability Status Scale. MS_low = MS patients with EDSS scores below 4.5, i.e. low disability. MS_high = MS patients with EDSS scores> 6.0, i.e. high disability.

Fig 2. FA in MS patients with high EDSS and low EDSS respectively compared to controls in the Superior Longitudinal Fasciculus LH. There was no difference between MS with low EDSS and controls (p = 0.82). .

* Significant differences between MS patients with high EDSS and low EDSS (p = 0.0002) and MS with high EDSS and controls (p = 0.0001). EDSS = Expanded Disability Status Scale. MS_low = MS patients with EDSS scores below 4.5, i.e. low disability. MS_high = MS patients with EDSS scores> 6.0, i.e. high disability. LH = Left Hemisphere.

As mentioned above, the same pattern was seen in 46 of the 48 WMTs (95.8%), that patients with low EDSS scores had similar FA values as controls (S3 Table).

Associations between FA, Disease Duration and Blood Iron Parameters

A significant inverse association was found between the mean FA and disease duration (r = -0.79, p<0.01). No associations were found between age of first symptoms, age at diagnosis, or age at MRI (results not shown). Significant inverse associations were found between

disease duration and Hb (r = - 0.64, p = 0.04) and % Tf sat (r = - 0.69, p = 0.02) (Table 2), suggesting that blood iron parameters may decrease over time.

Table 2. Associations between Disease Duration and Iron Parameters

	Iron parameter	Spearman r	Spearman p
Disease duration	Haemoglobin	-0.64	**0.04**
Disease duration	Serum iron	-0.52	0.1
Disease duration	% Tf saturation	-0.69	**0.02**
Disease duration	Serum Transferrin	-0.1	0.77
Disease duration	Serum Ferritin	-0.28	0.47

Positive Spearman correlations (r-values) were found between the mean FA and all iron parameters tested, ranging from no correlation for Tf (r = 0.07), moderate correlation for Hb (r = 0.37) to strongly correlated for % Tf sat (r = 0.77) (Table 3). The association between the mean FA and % Tf sat was highly significant (p < 0.01). No negative associations were found, suggesting that higher blood iron parameters were not detrimental to FA, and by implication, WM function.

Table 3. Associations between mean FA and Iron Parameters.

	Iron parameter	Spearman r	Spearman p-value
Mean FA	Haemoglobin	0.37	0.26
Mean FA	Serum iron	0.59	0.06
Mean FA	% Tf saturation	0.77	**<0.01**
Mean FA	Serum Transferrin	0.07	0.84
Mean FA	Serum Ferritin	0.59	0.09

No significant associations were found between EDSS values and blood iron parameters. However, Spearman r-values were negative in each case, with a trend towards a significant

inverse association in the case of ferritin, corrected for high CRP (p=0.09), suggesting that there was a trend for higher iron parameters to be associated with improved outcome: (Hb: r = - 0.21, p = 0.53; serum iron r = - 0.23, p = 0.50; % Tf sat: r = - 0.30, p = 0.38; ferritin, corrected for high CRP: r = - 0.59, p = 0.09). The EDSS is not a precise measurement and is influenced by many factors (see Introduction); however, comparing these results with the FA results, the outcomes are similar, suggesting that higher blood iron parameters are not indicative of a worse outcome, which is a confirmation of the FA results.

Blood iron parameters and FA in individual WMTs

When the individual 48 tracts were assessed, both the serum iron and the % Tf sat showed significant associations with FA measures in many of the tracts, while ferritin also showed significant associations in some of the tracts after adjusting for high CRP. Hb showed no associations with FA (S4 Table).

Serum Iron and FA

Significant positive associations were found between serum iron concentrations and the FA in all 48 WMTs, while the associations were significant ($p < 0.05$) in 13 of the 48 WMTs (27%) (S4 Table): in the inferior cerebellar peduncle (RH), anterior limb of the internal capsule (LH), posterior limb of the internal capsule (LH), retrolenticular internal capsule (RH), anterior corona radiate (RH), superior corona radiate (RH and LH), posterior corona radiata(RH and LH), external capsule (RH), superior longitudinal fasciculus (RH and LH) and superior fronto-occipital fasciculus (LH). Spearman correlations ranged from weakly correlated (r = 0.12) for the sagittal stratum, to strongly correlated (r = 0.74) for the retrolenticular internal capsule RH (S4 Table).

% Transferrin Saturation and FA

Positive associations were found between the % Tf sat and FA in all 48 WMTs (S4 Table); Spearman correlation r-values ranged between 0.22 (weakly correlated) for the CC genu to 0.84 (strongly correlated) for the external capsule RH. The associations were significant (p <

0.05) in 25 of the 48 WMTs assessed and highly significant in 14 of these tracts ($p < 0.01$; S4 Table). Overall, the mean FA of all the WMTs correlated significantly with the % Tf sat ($r = 0.77$, $p = 0.01$). Mean FA values for all WMTs ranged from 0.28 to 0.46 (Figure 3A). An example of the association between FA and the % Tf sat in a single WMT is shown in Figure 3B: the superior fronto occipital fasciculus (LH). FA in individual patients ranged from 0.42 to 0.68 and the association with the % Tf sat was highly significant ($r = 0.83$, $p<0.001$; Figure 3B). More patients would need to be included in a follow-up study to establish whether a cut-off value of more than 20 % for Tf sat could be suggestive of optimal FA.

Fig 3A. Association of mean FA with the % Tf saturation for all the WMTs ($r = 0.77$, $p = 0.01$).
Fig 3B. Association of FA with the % Tf saturation for the superior fronto occipital fasciculus (LH) ($r = 0.83$, $p<0.01$).

Hemoglobin, Ferritin and FA

There were no significant associations between the FA and Hb in any of the WMTs assessed (S4 Table). Since blood ferritin values are increased during inflammation, CRP values higher than 10 were used as a cut-off to adjust for falsely high ferritin values. Some of the WMTs then showed significant correlations between ferritin and FA with r-values ranging from 0.69 to 0.92 (strongly correlated) (S4 Table).

Serum Transferrin and FA

There were no significant associations between the FA and serum Tf in any of the WMTs assessed (S5 Table)

Discussion

When all the MS patients were grouped together, significantly decreased FA was found in many WMTs of MS patients compared to controls (S1 Table), similar to results found in other studies [33, 42, 43, 49,74,75]. Significant associations were also found between mean FA and disease duration ($p < 0.01$) (Table 2). Additionally, negative associations were found between the EDSS and FA in all the WMTs, while the differences were significant in some of the tracts (S2 Table) in agreement with some previous studies [33,76,77] while other studies found no significant associations between FA and EDSS values [43,74,75,78].

A novel finding of the present study was that when the MS patients were grouped according to their EDSS scores, patients with EDSS scores below 4.5 had similar FA values as controls. This effect was seen in 46 of the 48 WMTs investigated, suggesting that patients with an EDSS < 4.5 had white matter functionality similar to control subjects extending over the whole of the brain (S3 Table). Examples are depicted in Figures 1 and 2, showing this effect in the CC genu, body and splenium, the fornix and the superior longitudinal fasciculus, which represent WMTs involved with cognition and movement. This suggests that the three patients with high disability scores masked the favorable FA outcome of the other 8 patients who had low disability, so that the MS group as a whole seemed to have significantly lower FA values, and by implication worse myelin function compared to controls. Further research should be done to determine whether the adequate myelin functionality in the patients with low EDSS was associated with previously described factors that have an impact on myelin synthesis and maintenance. Previous studies showed associations between improved MS outcome and environmental factors such as avoidance of inhalation of cigarette smoke, dietary factors such as fruit and vegetable intake [21], epigenetic factors such as adequate availability of iron, essential fatty acids and vitamin B12 to oligodendrocytes [16,27] as well as physical activity [79]. Wake et al. demonstrated that myelin synthesis by OPCs was initiated by neuronal firing of the axons touching the OPCs and suggested that their results may have implications for MS [80]. Further research should be done to establish whether stimulation of neuronal activity in specific brain areas could increase WM synthesis and

maintain high FA in these WMTs in MS patients, similar to the increased FA found for musicians (see Introduction) [36].

The second novel finding of this study is that FA was positively associated with blood iron parameters (Table 3 and S4 Table). While Hb was not significantly associated with FA in any of the WMTs, FA showed significant positive associations with serum iron, % TF sat and serum ferritin after correction for increased CRP. Significant associations ($p < 0.05$) of FA with % Tf sat were found in 25 of the 48 WMTs. When the threshold was lowered to include only highly significant associations between FA and % Tf sat ($p< 0.01$) 14 of the tracts still showed significant associations (S4 Table). Spearman correlations showed that the FA in 25 of the tracts was strongly correlated ($r = 0.63$ to $r = 0.84$) with the % Tf sat. The positive associations found may suggest that iron which is transported into the brain is of functional significance for white matter synthesis and maintenance. Tf in the blood delivers iron to the barrier cells of the BBB and the choroid plexus, where the iron is transferred to Tf that had been synthesized by oligodendrocytes [81,82]. Due to the fact that oligodendrocytes have a high dependency on iron for normal function it is expected that there should be a requirement for iron in the WMTs [16]. In MS patients, iron absorption may be lower due to inflammation, genetic variations that cause reduced iron uptake or increased iron loss [28], while the significant negative associations between disease duration and Hb ($p = 0.04$) and disease duration and %Tf sat ($p = 0.02$) suggest that iron parameters may decrease over time in MS patients (Table 2). The lack of significant associations of FA values with Hb suggests that the iron in this protein may not be available for WM synthesis (Table S3). In MS patients with iron deficiency, Hb is the last parameter to decrease, since the iron concentration in Hb for oxygen delivery is preferentially protected [83]. Measuring Hb alone may therefore not provide a dependable estimate of iron availability for myelin functionality.

After adjusting for high CRP, serum ferritin showed significant ($p<0.05$) positive associations with FA in 4 WMTs (middle cerebellar peduncle, medial lemniscus RH, anterior limb of internal capsule LH, retrolenticular internal capsule RH), while 3 tracts were highly significantly ($p<0.01$) associated (posterior limb of internal capsule LH, anterior corona

radiata RH and anterior corona radiate LH). This finding is in keeping the work of Schonberg et al. who found that ferritin stimulated genesis of oligodendrocytes in the spinal cord [84], as well as Hulet et al. and Todorich et al. who found ferritin receptors on oligodendrocytes [85,58], which suggested that ferritin may present a delivery route of iron for myelin production in oligodendrocytes. While ferritin is often used as a surrogate marker for iron concentration [81], ferritin levels in serum are regulated by several factors including inflammation; it is therefore not sufficient to measure ferritin levels to determine the iron status of a patient. In the present study, principal component analysis indicated that serum ferritin values co-segregated with CRP (results not shown).

As far as we are aware, the present study is the first to investigate the associations of FA of WMTs with blood iron parameters done concurrently with the MRI. Jahanshad et al. determined FA in adult twins who had serum Tf measured when they were adolescents. They found increased FA in several brain regions (external capsule, superior longitudinal fasciculus, and the cingulum bilaterally) associated with lower Tf. Since there is generally an inverse correlation between serum iron levels and Tf, these authors concluded that the adolescents who had higher iron levels had better myelin function when they became adults. In the present study there were no significant associations of FA with serum Tf levels [82]. The difference could be explained by the different times of Tf measurement in the two studies, or by the fact that Tf concentrations may be variable in MS patients due to inflammation [86], since Tf is a negative acute phase protein.

Limitations and strengths of the study

A limitation of this study is the small number of patients of whom the DTI could be used for analysis. In the “high EDSS” group, the FA data of only 3 patients could be used for analysis, although 4 patients with EDSS values > 6 were included in the study. Nevertheless, the FA data from these 3 patients needed to be analysed separately since the true outcome of FA in the whole cohort was masked by the highly significant decreased FA in these 3 patients. A second limitation of the study was multiple testing across analyses of FA variables. However, examining 48 WMTs in the 11 patients provided a total of 528 WMTs and found similar

associations across all the WMTs investigated. Future studies should address these limitations and include a larger sample size in order to verify the results.

The strength of the study was the availability of blood iron parameters and EDSS measurements, which presented the opportunity to evaluate the association of systemic iron concentrations with FA, and therefore potentially with WM function and disease outcome.

Conclusions

This study had 3 important findings: First, the FA of WMTs as a parameter of myelin integrity was lower in subjects with MS only in those who had high EDSS scores. Subjects who had EDSS scores < 4.5 (i.e. who had a favorable disease outcome) had FA values similar to control values and this finding was not related to their age or disease duration. If these findings are confirmed in other larger cohorts, the findings may argue against the assumption that people diagnosed with MS inevitably experience increasing myelin degeneration over time. Further research should be done to determine whether concerted efforts by patients to improve their brain function by employing activities to increase neuronal activity together with lifestyle improvements could stimulate myelin recovery and prevent progression of disability.

Second, the association found between FA and iron parameters, especially % Tf sat, supports the hypothesis that iron availability to the WM may be a requirement for optimal myelin functionality. In the present study no negative associations were found between FA and the blood iron parameters serum iron, % Tf sat and ferritin, suggesting that higher blood iron parameters were not detrimental to WM function. The association between FA as a parameter of myelin integrity and iron availability to the white matter, confirms previous findings that iron is a requirement for myelin synthesis and maintenance. The energy needed to create myelin is extremely high, thus the increased requirement for iron in the brain; conversely if there is a lack of iron (together with other requirments of the electron transfer chain [28]) myelination cannot occur. The most significant associations were seen between the FA and the %Tf sat. Therefore these results suggest that higher serum iron concentration, ferritin and % Tf sat had a positive effect on myelination.

Third, the lack of association between FA and Hb suggests that the iron in this protein is not available for WM synthesis. However, the significant inverse association found between Hb and disease duration suggests that iron concentrations may decrease over time and that it may be important to assess iron parameters regularly in MS patients. The results of the present study, if confirmed in larger studies, would be an indication that measurement of all the iron parameters in patients with MS could provide important information regarding myelin function and hence could influence management of these patients.

Supporting Information

Table S1. Fractional anisotropy (FA) in MS patients and Controls.

Table S2. Negative association between FA and EDSS in 48 WMTs.

Table S3. FA in MS patients with low EDSS, high EDSS and Controls.

Table S4. Associations of FA with serum iron, % TF saturation, haemoglobin and ferritin (corrected for high CRP) in the 48 WMTs

Table S5. Associations between FA and serum Transferrin in the 48 WMTs.

Author Contributions

Wrote the paper: EH, SJVR. Interpreted the data: JPF, MK, SJVR, EH. Revised the manuscript: PEH, CL. Performed the DTI and Collected the data: EH, CL. Analyzed the data: JPF, MK.

Acknowledgements

We wish to thank Dr Heloise Avenant for the EDSS measurements. We also thank Mr Shafick Hassan for his involvement with the project. The study was funded by the National Research Foundation (NRF).

References:

Byun E, Cailliers S, Montalban X, Villoslada P, Fernandez O, et al. (2008) Genome-wide harmacogenomics analysis of the response to interferon beta therapy in multiple sclerosis. Archives of Neorology 64(3):337-344.

Hitti, M. 2005. Why more women get multiple sclerosis. WebMD Health News, http://women.webmd.com/news/20050127/ why-more-women-get-multiple-sclerosis

Chilcott J, McCabe C, Tappenden P, O'Hagan A, Cooper, NJ, et al. (2003) Modeling the cost effectiveness of interferon beta and glatiramer acetate in the management of multiple sclerosis. British Medical Journal 326:1-6.

Lassmann H, (1998) Neuropathology in multiple sclerosis: new concepts. Multiple Sclerosis Journal, 4:93–98.

Trapp BD, Bo L, Mork S, Chang A, (1999) Pathogenesis of tissue injury in MS lesions. Journal of Neuroimmunology 98:49–56.

Bjartmar C, Wujek JR, Trapp BD, (2003) Axonal loss in the pathology of MS: consequences for understanding the progressive phase of the disease. Journal of Neurological Science 206:165–71.

De Luca GC, Ramagopalan SV, Herrrera BM, Dyment DA, Lincoln MR, (2007) An extremes of outcome strategy provides evidence that multiple sclerosis severity is determined by alleles at the HLA – DRB1 locus. Proceedings of the National Academy of Sciences 104(52):20896-20901.

Vogel FS, Bouldin TW, (1995) The nervous system. In: Rubin, E. & Farber, J.L. (eds). Pathology. Philadelphia: Lippencott.

Tremlett H1, Zhu F, Petkau J, Oger J, Zhao Y, (2012) Natural, innate improvements in multiple sclerosis disability. Multiple Sclerosis 18(10):1412-21.

Karim ME, Gustafson P, Petkau J, Zhao Y, Shirani A, Kingwell E, Evans C, van der Kop M, Oger J, Tremlett H, (2014) Marginal Structural Cox Models for Estimating the Association Between β-Interferon Exposure and Disease Progression in a Multiple Sclerosis Cohort. American Journal of Epidemiology 180(2):160–171.

Shirani A, Zhao Y, Karim ME, Evans C, Kingwell E, van der Kop ML, Oger J, Gustafson P, Petkau J,
Tremlett H, (2012) Association Between Use of Interferon Beta and Progression of Disability in Patients
With Relapsing-Remitting Multiple Sclerosis. Journal of the American Medical Association 308(3):247-256.

Zhang T, Shirani A, Zhao Y, Karim ME, Gustafson P, Petkau J, Evans C, Kingwell E, van der Kop M, Zhu F, Oger J, Tremlett H, (2015) Beta-interferon exposure and onset of secondary progressive multiple sclerosis.European Journal Neurology 22(6):990-1000.

Pittock SJ, Rodriguez M, (2008). Benign multiple sclerosis: a distinct clinical entity with therapeutic implications. Current Topics in Microbiology and Immunology 318:1–17.

Faivre A, Robinet E, Guye M, Rousseau C, Maarouf A, et al. (2016) Depletion of brain functional connectivity enhancement leads to disability progression in multiple sclerosis: A longitudinal resting-state fMRI study. Multiple Sclerosis pii: 1352458516628657.

Lyksborg M, Siebner HR, Sørensen PS, Blinkenberg M, Parker GJ, et al. (2014) Secondary progressive and relapsing remitting multiple sclerosis leads to motor-related decreased anatomical connectivity. PLoS One 9(4):e95540.

Bartzokis G (2011) Neuroglialpharmacology: white matter pathophysiologies and psychiatric treatments. Frontiers in Bioscience (Landmark Edition) 16:2695-733.

Barnett MH, Prineas JW, (2004) Relapsing and remitting multiple sclerosis: pathology of the newly forming lesion. Annals of Neurology 55:458-468.

Ascherio A, Munger KL, White R, Köchert K, Simon KC, Polman CH, Freedman MS, Hartung HP, Miller DH, Montalbán X, Edan G, Barkhof F,Pleimes D, Radü EW, Sandbrink R, Kappos L, Pohl C. Vitamin D as an early predictor of multiple sclerosis activity and progression. JAMA Neurol. 2014;71: 306-314.

Thouvenot E, Orsini M, Daures JP, Camu W (2015) Vitamin D is associated with degree of disability in patients with fully ambulatory relapsing-remitting multiple sclerosis. European Journal of Neurology 22(3):564-9.

Manouchehrinia A, Tench CR, Maxted J, Bibani RH, Britton J, Constantinescu CS, (2013) Tobacco smoking and disability progression in multiple sclerosis: United Kingdom cohort study. Brain 136(Pt 7):2298-304.

Davis W, van Rensburg S, Cronje FJ, WhatiL, Fisher LR, et al. (2014) The fat mass and obesity-associated FTO rs9939609 polymorphism is associated with elevated homocysteine levels in patients with multiple sclerosis screened for vascular risk factors. Metabolic Brain Disease 29:409-419.

Moccia M, Lanzillo R, Palladino R, Maniscalco GT, De Rosa A, Russo C, Massarelli M, Carotenuto A, Postiglione E, Caporale O, Triassi M, Brescia Morra V, (2015) The Framingham cardiovascular risk score in multiple sclerosis. European Journal Neurolology 22(8):1176-83.

Tettey P, Simpson S Jr, Taylor BV, van der Mei IA, (2014) Vascular comorbidities in the onset and progression of multiple sclerosis. Journal of Neurological Science 15;347(1-2):23-33.

Nelson M, Isaacs F, Hassan MS, Kidd M, Cronje FJ, et al.(2014) Prevalence of abnormal bloodflow patterns and effects of biochemistry and lifestyle factors on the major neck vessels in patients with Multiple Sclerosis in the Western Cape, South Africa. Medical Technology SA 28(1): 28-33.

Swank R, (1953) Treatment of Multiple Sclerosis with Low-Fat Diet. Archives of Neurology & Psychiatry, 69(1):91-103.

Kurtzke JF, (1983) Rating neurological impairment in multiple sclerosis: an expanded disability status scale (EDSS). Neurology 33(11):1444-1452.

Van Rensburg S, Kotze M, van Toorn R, (2012) The conundrum of iron in multiple sclerosis – time for an individualised approach. Metabolic Brain Disease 27:239-253.

Van Rensburg SJ, van Toorn R, Moremi KE, Peeters AV, Oguniyi A, Kotze MJ, (2016) Multiple sclerosis-like diagnosis as a complication of previously treated malaria in an iron and vitamin D deficient Nigerian patient. Metabolic Brain Disease 31(1):197-204.

Abe O, Taka H, Gonoi W, Sasaki H, Murakami H, et al. (2010) Voxel-based analysis of the diffusion tensor. Neuroradiology 52:699-710.

Song SK, Sun SW, Ramsbottom MJ, Chang C, Russel J,et al.(2002)Dysmyelination revealed through MRI as increased radial (but unchanged axial) diffusion of water. NeuroImage 17:1429-1436.

Stone LA, Richert N, McFarland HF,(2005) Neuroimaging and the use of magnetic resonance in multiple sclerosis. In: Cook SD, (ed). Handbook of multiple sclerosis, 3rd ed. New York: Marcel Dekker: 403-423.

Imfeld A, Oechslin MS, Meyer M, Loenneker T, Jancke L, (2009) White matter plasticity in the corticospinal tract of musicians: A diffusion tensor imaging study. NeuroImage 46:600-607.

Onu M, Roceanu A, Sboto-Frankenstein U, Bendic R, Tarta E, et al. (2012) Diffusion abnormality maps in demyelinating disease: correlations with clinical scores.Europen Journal of Radiolology 81(3):e386-91.

Rovaris M, Judica E, Cecarelli A, Ghezzi A, Martinelli V, et al. (2008) A three year diffusion tensor MRI study of grey matter damage progressing during the earliest clinical stage of multiple sclerosis. Journal of Neurology 255:1209-1214.

Shen Y, Bai L, Gao Y, Cui F, Tan Z, et al. (2014) Depressive symptoms in Multiple sclerosis from an In Vivo Study with TBSS. Biomed Research International 148465: 1-8.

Schmithorst VFJ, Wilke M, (2002) Differences in white matter architecture between musicians and non-musicians: a diffusion tensor imaging study. Neuroscience 321(1-2):57-60.

Bengtsson SL, Nagy Z, Skare S, Forsman L, Forssberg H, et al. (2005) Extensive piano practicing has regionally specific effects on white matter development. Nature Neuroscience 8(9):1148-50.

Vollmann H, Ragert P, Conde V, Villringer A, Classen J, et al. (2014) Instrument specific use-dependent plasticity shapes the anatomical properties of the corpuscallosum: a comparison between musicians and non-musicians. Frontiers of Behavioural Neuroscience 8:245.

Kochunov P, Thompson PM, Lancaster JL, Bartzokis G, Smith S, (2007) Relationship between white matter fractional anisotropy and other indices of cerebral health in normal aging: Tract-based spatial statistics study of aging. NeuroImage 35: 478-487**.**

Benedetti F, Absinta M, Rocca MA, Radaelli D,Poletti S, et al.(2011) Tract-specific white matter structural disruption in patients with bipolar disorder. Bipolar disorders 13:414-424.

Cesar B. Dwyer MG, Shucard JL, Polak P, Bergsland N, et al.(2015) Cognitive and White Matter Tract Differences in MS and Diffuse Neuropsychiatric Systemic LupusErythematosus. American Journal of Neuroradiolology 36(10):1874-83.

Liu Y, Duan Y, He Y, Yu C, Wang J, et al. (2012) Whole brain white matter changes revealed by multiple diffusion metrics in multiple sclerosis: a TBSS study. European Journal of Radiolology 81(10):2826-32.

Hasan KM, Gupta RK, Santos RM, Wolinsky JS,Narayana PA,(2005) Diffusion tensor fractional anisotropy of the normal-appearing seven segments of the corpus callosum in healthy adults and relapsing-remitting multiple sclerosis patients. Journal of Magnetic Resonance Imaging 21(6):735-43.

Llufriu S, Blanco Y, Martinez-Heras E, Casanova-Molla J, Gabilondo I, et al. (2012) Influence of corpus callosum damage on cognition and physical disability in multiple sclerosis: a multimodal study. PLoS One 7(5):e37167.

Koenig KA, Sakaie KE, Lowe MJ, Lin J, Stone L, (2013) High spatial and angular resolution diffusion-weighted imaging reveals forniceal damage related to memory impairment. Magnetic Resonance Imaging 31(5):695-9.

Erb K, Bohner G, Harms L, Goektas O, Fleiner F, (2012) Olfactory function in patients with multiple sclerosis: a diffusion tensor imaging study. Journal of Neurological Science 316(1-2):56-60.

Gratsias G, Kapsalaki E, Kogia S, Dardiotis E, Tsimourtou V, (2015) A quantitative evaluation of damage in normal appearing white matter in patients with multiple sclerosis using diffusion tensor MR imaging at 3 T. Acta Neurologica Belgica115(2):111-6.

Rashid W, Hadjiprocopis A, Davies G, Griffin C, Chard D, et al. (2008) Longitudinal evaluation of clinically early relapsing-remitting multiple sclerosis with diffusion tensor imaging. Journal of Neurology 255(3):390-7.

Asaf A, Evans S, Anat A,(2015) Injury to white matter tracts in relapsing-remitting multiple sclerosis: A possible therapeutic window within the first 5 years from onset using diffusion-tensor imaging tract-based spatial statistics. Neuroimage Clinical 8:261-266.

Mehta V, Pei W, Yang G, Li S, Swamy E, et al. (2013) Iron Is a Sensitive Biomarker for Inflammation in Multiple Sclerosis Lesions. PLOS One 8(3): e57573.

Le Vine SM, Chakrabarty A, (2004) The Role of Iron in the Pathogenesis of Experimental Allergic Encephalomyelitis and Multiple Sclerosis. Annals of New York Academy of Sciences 1012:252-266.

Stephenson E, Nathoo N, Mahjoub Y, Dunn JF, Yong VW (2014) Iron in multiple sclerosis: roles in neurodegeneration and repair. Acta Neuropathologica 10(8):459-68.

Zamboni P, Galeotti R, Menegatti E, Malagoni AM, Tacconi G, et al. (2009) Chronic cerebrospinal venous insufficiency in patients with multiple sclerosis. Journal of Neurological Neurosurgery Psychiatry 80:392-399.

Worthington V, Killestein J, Eikelenboom MJ, Teunissen CE, Barkhof F, et al. (2010) Normal CSF ferritin levels in MS suggest against etiologic role of chronic venous insufficiency. Neurology 75: 1617-1622.

Williams R, Rohr AM, Wang WT, Choi IY, Lee P, Berman NE, Lynch SG, LeVine SM (2011) Iron deposition is independent of cellular inflammation in a cerebral model of multiple sclerosis. Bio Med Central Neuroscience. 23(12):59.

Tsivgoulis G, Faissner S, Voumvourakis K, Katsanos AH, Triantafyllou N, Grigoriadis N, Gold R, Krogias C (2015) "Liberation treatment" for chronic cerebrospinal venous insufficiency in multiple sclerosis: the truth will set you free. Brain and Behaviour 5(1):3-12.

Connor JR, Menzies SL, (1996) Relatioship iron to oligodendrocytes and myelination. Glia 17:83-93.

Todorich B, Zhang X, Connor JR,(2011) H-ferritin is the major source of iron for oligodendrocytes. Glia 59(6):927-35.

Lane DJR, Merlot AM, Huang ML-H, Bae D-H, Jansson PJ, et al. (2015) Cellular iron uptake, trafficking and metabolism: Key molecules and mechanisms and their roles in disease. BiochimicaetBiophysicaActa 1853(5):1130–1144.

Xu B, Chasteen ND, (1991) Iron oxidation chemistry in ferritin. Increasing Fe/O2 stoichiometry during core formation. Journal of Biololical Chemistry 266(30):19965-70.

Bagnato F, Hametner S, Welch EB (2013) Visualizing iron in multiple sclerosis. Magnetic Resonance Imaging 31(3): 376–384.

Xu X, Wang Q, Zhong J, Zhang M, (2015) Iron deposition influences the measurement of water diffusion tensor in the human brain: a combined analysis of diffusion and iron-induced phase changes. Neuroradiology 57(11):1169-78.

Syka M, Keller J, Klempíř J, Rulseh AM, Roth J, Jech R, Vorisek I, Vymazal J, (2015) Correlation between relaxometry and diffusion tensor imaging in the globus pallidus of Huntington's disease patients. PLoS One 10(3):e0118907.

Phillips O, Squitieri F, Sanchez-Castaneda C, Elifani F, Caltagirone C, Sabatini U, Di Paola M (2014) Deep white matter in Huntington's disease. PLoS One 9(10):e109676.

Khalil M, Langkammer C, Pichler A, Pinter D, Gattringer T, et al. (2015) Dynamics of brain iron levels in multiple sclerosis, A longitudinal 3T MRI study. Neurology 84:2396-2402.

Khalil M, TeunissenC, Langkammer C,(2011) Iron and Neurodegeneration in Multiple Sclerosis. Multiple Sclerosis International.

Hametner S, Wimmer I, Haider L, Pfeifenbring S, Brück W, Lassmann H, (2013) Iron and neurodegeneration in the multiple sclerosis brain. Annals of Neurolology 74(6):848-61.

Ceccarelli A, Filippi M, Neema M, Arora A, Valsasina P, et al. (2009) T2 hypointensity in the deep gray matter of patients with benign multiple sclerosis. Multiple Sclerosis 15: 678–686.

World Medical Association Declaration of Helsinki, 2013.
http://www.ub.edu/recerca/Bioetica/doc/Declaracio_Helsinki_2013.pdf

McDonald WI, Compston A, Edan G, Goodkin D, Hartung HP, Lublin FD, McFarland HF, Paty DW, Polman CH, Reingold SC, Sandberg-Wollheim M, Sibley W, Thompson A, van den Noort S, Weinshenker BY, Wolinsky JS, (2001) Recommended diagnostic criteria for multiple sclerosis: guidelines from the International Panel on the diagnosis of multiple sclerosis. Annals of Neurology 50(1):121–127.

Mori S, Wakana S, Nagae-Poetscher LM, van Zijl PCM (2005) MRI Atlas of Human White Matter. American Journal of Neuroradiology 27:1384-1387.

Smith SM, Jenkinson M, Woolrich MW, Beckman CF, Behrens TEJ, et al. (2004) Advances in structural and functional MR image analysis and implementation as FSL. NeuroImage 23 (S1):208-219.

Smith SM, Jenkinson M, Johansen-Berg H, Rueckert D, Nichols TE, et al. (2006) Tract-based spatial statistics: Voxelwise analysis of multi-subject diffusion data. NeuroImage 31: 1487-1505.

Bethune A, Tipu V, Sled JG, Narayanan S, Arnold DL, et al. (2011) Diffusion tensor imaging and cognitive speed in children with multiple sclerosis. Journal of Neurological Science 309(1-2):68-74.

Temel S, Keklikoğlu HD, VuralG, Deniz O,Ercan K,(2013) Diffusion tensor magnetic resonance imaging in patients with multiple sclerosis and its relationship with disability. Neuroradiology Journal 26(1):3-17.

Bodini B, Cercignani M, Khaleeli Z, Miller DH, Ron M, et al. (2013) Corpus callosum damage predicts disability progression and cognitive dysfunction in primary-progressive MS after five years. Human Brain Mapping 34(5):1163-72.

Caligiuri ME, Barone S, Cherubini A, Augimeri A, Chiriaco C, et al. (2014) The relationship between regional microstructural abnormalities of the corpus callosum and physical and cognitive disability in relapsing-remitting multiple sclerosis. Neuroimage Clinical 7:28-33.

Giorgio A, Palace J, Johansen-Berg H, Smith SM, Ropele S, (2010) Relationships of brain white matter microstructure with clinical and MR measures in relapsing-remitting multiple sclerosis. Journal of Magnetic Resonance Imaging 31(2):309-16.

Fjeldstad C, Suárez G, Klingler M, Henney HR, Rabinowicz AL, Pardo G (2015) Dalfampridine Effects Beyond Walking Speed in Multiple Sclerosis. International Journal of MS Care 17(6):275-83.

Wake H, Ortiz FC, WooDW, Lee PR, Angulo MC,et al.(2015)Nonsynaptic junctions on myelinating glia promote preferential myelination of electrically active axons. Nature Communications 6:7844.

Rouault TA1, Zhang DL, Jeong SY, (2009) Brain iron homeostasis, the choroid plexus, and localization of iron transport proteins. Metabolic Brain Disease 24(4):673-84.

Jahanshad N1, Kohannim O, Hibar DP, Stein JL, McMahon KL, de Zubicaray GI, Medland SE, Montgomery GW, Whitfield JB, Martin NG, Wright MJ,Toga AW, Thompson PM, (2012) Brain structure in healthy adults is related to serum transferrin and the H63D polymorphism in the HFE gene. Proceedings of the National Academy of Sciences of the U S A 109(14):E851-9.

Van Toorn R, Schoeman JF, Solomons R, Rensburg MA, Van Rensburg SJ (2010) Iron status in children with recurrent episodes of tumefactive cerebral demyelination. Journal of Child Neurology 25(11):1401-1407.

Schonberg DL, Goldstein EZ, SahinkayaFR, Wei P, Popovich PG, et al. (2012) Ferritin stimulates oligodendrocyte genesis in the adult spinal cord and can be transferred from macrophages to NG2 cells in vivo. Journal of Neuroscience 32(16):5374-84.

Hulet SW, Heyliger SO, Powers S, Connor JR (2000)Oligodendrocyte progenitor cells internalize ferritin via clathrin-dependent receptor mediated endocytosis. Journal of Neuroscience Research 61(1):52-60.

Zeman D, Adam P, Kalistová H, Sobek O, Kelbich P, Andel J, Andel M, (2000) Transferrin in patients with multiple sclerosis: a comparison among various subgroups of multiple sclerosis patients. Acta of Neurologica Scandinavica 101(2):89-94.

Fig 1 A, B, C, D

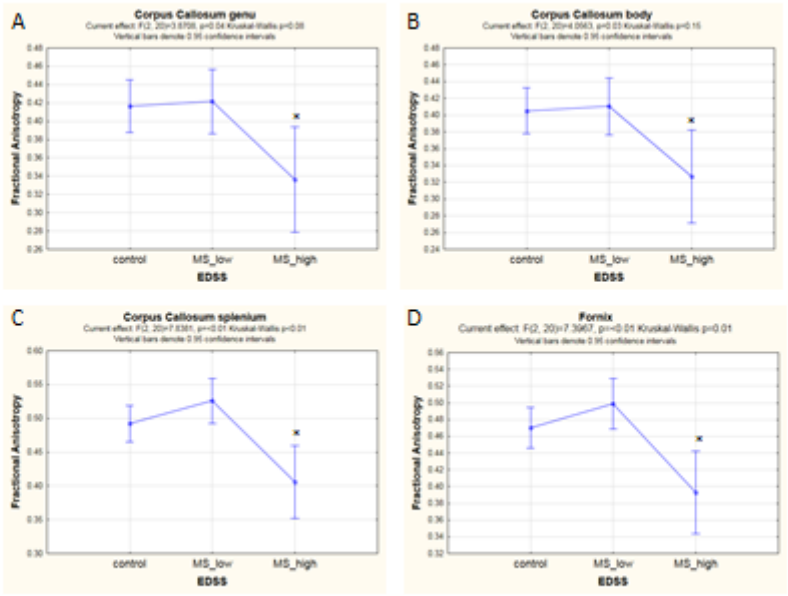


Fig 3A and 3B

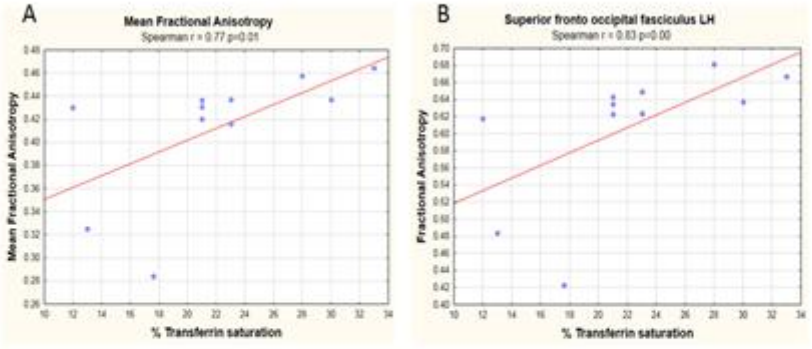
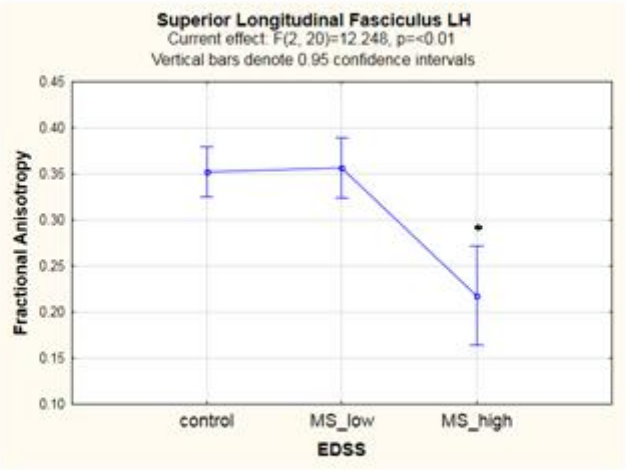


Fig 2


Superior Longitudinal Fasciculus LH
Current effect: F(2, 20)=12.248, p=<0.01
Vertical bars denote 0.95 confidence intervals
Fractional Anisotropy
0.45
0.40
0.35
0.30
0.25
0.20
0.15
0.10
control
MS_low
MS_high
EDSS

Suplementary Tables:

Table S1: Fractional anisotropy (FA) in MS patients and Controls. Values are given as the means (± SD). FA was lower in MS patients than controls in all the tracts except in the CC splenium and Fornix where the values were similar. FA was significantly lower in patients than controls in 20 of the 48 tracts measured (significant-values in bold).

White Matter Tract	Controls	Patients	F-test
Middle_cerebellar_peduncle	0.45(0.04)	0.42(0.05)	F(1,21)=2.20, p=0.15
Pontine_crossing_tract	0.52(0.04)	0.48(0.06)	F(1,21)=2.92, p=0.10
CC_genu	0.42(0.06)	0.40(0.05)	F(1,21)=0.64, p=0.43
CC_body	0.41(0.05)	0.39(0.05)	F(1,21)=0.65, p=0.43
CC_splenium	0.49(0.05)	0.49(0.07)	F(1,21)=0.00, p=0.97
Fornix	0.47(0.05)	0.47(0.06)	F(1,21)=0.00, p=1.00
Corticospinal_tract_RH	0.58(0.04)	0.54(0.06)	F(1,21)=5.59, **p=0.03**
Corticospinal_tract_LH	0.58(0.04)	0.54(0.05)	F(1,21)=4.63, **p=0.04**
Medial_lemniscus_RH	0.50(0.03)	0.47(0.05)	F(1,21)=2.98, p=0.10
Medial_lemniscus_LH	0.49(0.03)	0.46(0.06)	F(1,21)=1.93, p=0.18
Inferior_cerebellar_peduncle_RH	0.58(0.03)	0.53(0.04)	F(1,21)=8.29, **p<0.01**
Inferior_cerebellar_peduncle_LH	0.37(0.04)	0.35(0.05)	F(1,21)=1.12, p=0.30
Superior_cerebellar_peduncle_RH	0.57(0.03)	0.54(0.03)	F(1,21)=6.30, **p=0.02**
Superior_cerebellar_peduncle_LH	0.51(0.03)	0.46(0.04)	F(1,21)=11.45, **p<0.01**
Cerebral_peduncle_RH	0.52(0.04)	0.47(0.06)	F(1,21)=5.59, **p=0.03**
Cerebral_peduncle_LH	0.42(0.04)	0.37(0.07)	F(1,21)=5.74, **p=0.03**
Anterior_limb_of_internal_capsule_RH	0.42(0.04)	0.36(0.07)	F(1,21)=5.09, **p=0.03**
Anterior_limb_of_internal_capsule_LH	0.44(0.03)	0.39(0.06)	F(1,21)=6.62, **p=0.02**
Posterior_limb_of_internal_capsule_RH	0.44(0.03)	0.39(0.06)	F(1,21)=9.55, **p<0.01**
Posterior_limb_of_internal_capsule_LH	0.43(0.03)	0.38(0.06)	F(1,21)=7.62, **p=0.01**
Retrolenticular_internal_capsule_RH	0.42(0.03)	0.36(0.07)	F(1,21)=7.20, **p=0.01**
Retrolenticular_internal_capsule_LH	0.53(0.05)	0.46(0.07)	F(1,21)=8.04, **p<0.01**
Anterior_corona_radiata_RH	0.51(0.04)	0.45(0.09)	F(1,21)=4.46, p=0.05
Anterior_corona_radiata_LH	0.51(0.05)	0.45(0.07)	F(1,21)=5.98, **p=0.02**
Superior_corona_radiata_RH	0.49(0.04)	0.43(0.05)	F(1,21)=7.79, **p=0.01**
Superior_corona_radiata_LH	0.44(0.04)	0.39(0.06)	F(1,21)=6.81, **p=0.02**
Posterior_corona_radiata_RH	0.35(0.02)	0.32(0.06)	F(1,21)=2.73, p=0.11
Posterior_corona_radiata_LH	0.36(0.03)	0.33(0.05)	F(1,21)=4.07, p=0.06
Posterior_thalamic_radiation_RH	0.42(0.04)	0.40(0.06)	F(1,21)=0.69, p=0.41
Posterior_thalamic_radiation_LH	0.45(0.04)	0.43(0.07)	F(1,21)=1.05, p=0.32
Sagittal_stratum_RH	0.31(0.05)	0.29(0.04)	F(1,21)=1.70, p=0.21
Sagittal_stratum_LH	0.30(0.05)	0.29(0.04)	F(1,21)=0.64, p=0.43
External_capsule_RH	0.41(0.05)	0.35(0.08)	F(1,21)=4.27, p=0.05
External_capsule_LH	0.56(0.03)	0.50(0.08)	F(1,21)=5.42, **p=0.03**
Cingulum_RH	0.43(0.06)	0.37(0.08)	F(1,21)=4.49, **p=0.05**
Cingulum_LH	0.42(0.04)	0.39(0.04)	F(1,21)=3.47, p=0.08
Cingulum(hippocampus)_RH	0.42(0.04)	0.39(0.04)	F(1,21)=4.67, **p=0.04**
Cingulum(hippocampus)_LH	0.44(0.05)	0.40(0.07)	F(1,21)=1.48, p=0.24
Fornix/stria_terminalis_RH	0.42(0.04)	0.37(0.08)	F(1,21)=3.57, p=0.07
Fornix/stria_terminalis_LH	0.40(0.04)	0.38(0.05)	F(1,21)=1.03, p=0.32
Superior_longitudinal_fasciculus_RH	0.39(0.04)	0.38(0.05)	F(1,21)=0.49, p=0.49
Superior_longitudinal_fasciculus_LH	0.35(0.04)	0.32(0.08)	F(1,21)=1.64, p=0.21
Superior_fronto_occip_fasciculus_RH	0.26(0.03)	0.24(0.05)	F(1,21)=1.82, p=0.19
Superior_fronto_occip_fasciculus_LH	0.65(0.05)	0.61(0.08)	F(1,21)=2.61, p=0.12
Uncinate_fasciculus_RH	0.35(0.05)	0.28(0.09)	F(1,21)=5.82, **p=0.03**
Uncinate_fasciculus_LH	0.44(0.04)	0.42(0.06)	F(1,21)=0.88, p=0.36
Tapetum_RH	0.44(0.04)	0.42(0.05)	F(1,21)=1.18, p=0.29
Tapetum_LH	0.52(0.04)	0.49(0.04)	F(1,21)=2.73, p=0.11
Average FA of WMTs	0.45(0.036)	0.41(0.056)	

Table S2. Negative association between FA and EDSS in 48 WMTs.
Spearman correlations ranged from weakly correlated for Sagittal stratum_RH (r = -0.27; p = 0.43)
to strongly correlated for Cingulum (hippocampus)_RH (= -0.95; p = <0.01)

FRACTIONAL ANISOTROPY (n=11)	EDSS	
	Spearman r	Spearman p
Middle_cerebellar_peduncle	-0.69	0.02
Pontine_crossing_tract	-0.49	0.13
CC_genu	-0.49	0.12
CC_body	-0.30	0.37
CC_splenium	-0.68	0.02
Fornix	-0.84	<0.01
Corticospinal_tract_RH	-0.81	<0.01
Corticospinal_tract_LH	-0.87	<0.01
Medial_lemniscus_RH	-0.73	0.01
Medial_lemniscus_LH	-0.79	<0.01
Inferior_cerebellar_peduncle_RH	-0.70	0.02
Inferior_cerebellar_peduncle_LH	-0.71	0.01
Superior_cerebellar_peduncle_RH	-0.88	<0.01
Superior_cerebellar_peduncle_LH	-0.89	<0.01
Cerebral_peduncle_RH	-0.84	<0.01
Cerebral_peduncle_LH	-0.57	0.07
Anterior_limb_of_int_capsule_RH	-0.55	0.08
Anterior_limb_of_int_capsule_LH	-0.85	<0.01
Posterior_limb_of_int_capsule_RH	-0.65	0.03
Posterior_limb_of_int_capsule_LH	-0.66	0.03
Retrolenticular_int_capsule_RH	-0.62	0.04
Retrolenticular_int_capsule_LH	-0.40	0.23
Anterior_corona_radiata_RH	-0.65	0.03
Anterior_corona_radiata_LH	-0.66	0.03
Superior_corona_radiata_RH	-0.62	0.04
Superior_corona_radiata_LH	-0.61	0.05
Posterior_corona_radiata_RH	-0.77	<0.01
Posterior_corona_radiata_LH	-0.67	0.03
Posterior_thalamic_radiation_RH	-0.67	0.03
Posterior_thalamic_radiation_LH	-0.67	0.02
Sagittal_stratum_RH	-0.27	0.43
Sagittal_stratum_LH	-0.54	0.09
External_capsule_RH	-0.55	0.08
External_capsule_LH	-0.60	0.05
Cingulum_RH	-0.50	0.12
Cingulum_LH	-0.83	<0.01
Cingulum(hippocampus)_RH	-0.95	<0.01
Cingulum(hippocampus)_LH	-0.71	0.01
Fornix/stria_terminalis_RH	-0.81	<0.01
Fornix/stria_terminalis_LH	-0.85	<0.01
Sup_longitudinal_fasciculus_RH	-0.82	<0.01

Sup_longitudinal_fasciculus_LH	-0.55	0.08
Sup_fronto_occip_fasciculus_RH	-0.60	0.05
Sup_fronto_occip_fasciculus_LH	-0.53	0.09
Uncinate_fasciculus_RH	-0.83	<0.01
Uncinate_fasciculus_LH	-0.63	0.04
Tapetum_RH	-0.84	<0.01
Tapetum_LH	-0.58	0.06

Table S3. FA in MS patients with low EDSS, high EDSS and Controls. Values are given as the means ± SD. FA was significantly lower in patients with high EDSS (b) compared to low EDSS and controls (a) in all the tracts except theSagittal stratum RH and Sagittal stratum LH. Patients with low EDSS had similar FA values as controls in all the tracts (a) except in the Superior cerebellar peduncle LH and the Posterior limb of internal capsule RH. Average FA was calculated over all WMTs measured.

Variable	control	EDSS(low)	EDSS(high)	F-test
Middle_cerebellar_peduncle	0.45(0.04)a	0.45(0.02)a	0.36(0.06)b	F(2,20)=8.49, p<0.01
Pontine_crossing_tract	0.52(0.04)a	0.50(0.04)a	0.42(0.06)b	F(2,20)=6.14, p<0.01
CC_genu	0.42(0.06)a	0.42(0.02)a	0.34(0.06)b	F(2,20)=3.87, p=0.04
CC_body	0.41(0.05)a	0.41(0.03)a	0.33(0.06)b	F(2,20)=4.06, p=0.03
CC_splenium	0.49(0.05)a	0.53(0.01)a	0.41(0.08)b	F(2,20)=7.84, p<0.01
Fornix	0.47(0.05)a	0.50(0.02)a	0.39(0.06)b	F(2,20)=7.40, p<0.01
Corticospinal_tract_RH	0.58(0.04)a	0.57(0.02)a	0.46(0.06)b	F(2,20)=15.62, p<0.01
Corticospinal_tract_LH	0.58(0.04)a	0.56(0.02)a	0.48(0.05)b	F(2,20)=9.73, p<0.01
Medial_lemniscus_RH	0.50(0.03)a	0.49(0.02)a	0.40(0.07)b	F(2,20)=8.94, p<0.01
Medial_lemniscus_LH	0.49(0.03)a	0.49(0.02)a	0.39(0.07)b	F(2,20)=12.47, p<0.01
Inferior_cerebellar_peduncle_RH	0.58(0.03)a	0.55(0.01)a	0.48(0.03)b	F(2,20)=17.75, p<0.01
Inferior_cerebellar_peduncle_LH	0.37(0.04)a	0.37(0.03)a	0.31(0.04)b	F(2,20)=4.43, p=0.03
Superior_cerebellar_peduncle_RH	0.57(0.03)a	0.56(0.01)a	0.50(0.03)b	F(2,20)=9.62, p<0.01
Superior_cerebellar_peduncle_LH	0.51(0.03)c	0.48(0.02)b	0.41(0.06)a	F(2,20)=11.99, p<0.01
Cerebral_peduncle_RH	0.52(0.04)a	0.49(0.01)a	0.40(0.08)b	F(2,20)=9.27, p<0.01
Cerebral_peduncle_LH	0.42(0.04)a	0.40(0.02)a	0.28(0.08)b	F(2,20)=14.35, p<0.01
Anterior_limb_of_internal_capsule_RH	0.42(0.04)a	0.40(0.02)a	0.27(0.07)b	F(2,20)=18.13, p<0.01
Anterior_limb_of_internal_capsule_LH	0.44(0.03)a	0.42(0.02)a	0.32(0.06)b	F(2,20)=19.97, p<0.01
Posterior_limb_of_internal_capsule_RH	0.44(0.03)c	0.41(0.02)b	0.32(0.07)a	F(2,20)=18.13, p<0.01
Posterior_limb_of_internal_capsule_LH	0.43(0.03)a	0.41(0.02)a	0.29(0.07)b	F(2,20)=20.23, p<0.01
Retrolenticular_internal_capsule_RH	0.42(0.03)a	0.39(0.02)a	0.27(0.08)b	F(2,20)=18.63, p<0.01
Retrolenticular_internal_capsule_LH	0.53(0.05)a	0.49(0.02)a	0.39(0.11)b	F(2,20)=9.24, p<0.01
Anterior_corona_radiata_RH	0.51(0.04)a	0.49(0.03)a	0.33(0.10)b	F(2,20)=17.78, p<0.01
Anterior_corona_radiata_LH	0.51(0.05)a	0.48(0.02)a	0.36(0.08)b	F(2,20)=13.67, p<0.01
Superior_corona_radiata_RH	0.49(0.04)a	0.46(0.02)a	0.36(0.06)b	F(2,20)=13.98, p<0.01
Superior_corona_radiata_LH	0.44(0.04)a	0.42(0.02)a	0.32(0.07)b	F(2,20)=11.22, p<0.01
Posterior_corona_radiata_RH	0.35(0.02)a	0.35(0.01)a	0.26(0.09)b	F(2,20)=7.84, p<0.01
Posterior_corona_radiata_LH	0.36(0.03)a	0.35(0.01)a	0.27(0.08)b	F(2,20)=9.89, p<0.01
Posterior_thalamic_radiation_RH	0.42(0.04)a	0.43(0.03)a	0.33(0.08)b	F(2,20)=7.38, p<0.01
Posterior_thalamic_radiation_LH	0.45(0.04)a	0.46(0.03)a	0.34(0.09)b	F(2,20)=7.80, p<0.01
Sagittal_stratum_RH	0.31(0.05)	0.30(0.02)	0.26(0.06)	F(2,20)=1.82, p=0.19
Sagittal_stratum_LH	0.30(0.05)	0.30(0.02)	0.25(0.04)	F(2,20)=1.74, p=0.20
External_capsule_RH	0.41(0.05)a	0.38(0.03)a	0.26(0.11)b	F(2,20)=9.46, p<0.01
External_capsule_LH	0.56(0.03)a	0.54(0.03)a	0.41(0.10)b	F(2,20)=13.54, p<0.01
Cingulum_RH	0.43(0.06)a	0.40(0.02)a	0.28(0.10)b	F(2,20)=9.32, p<0.01
Cingulum_LH	0.42(0.04)a	0.41(0.02)a	0.34(0.05)b	F(2,20)=5.98, p<0.01
Cingulum(hippocampus)_RH	0.42(0.04)a	0.40(0.02)a	0.34(0.05)b	F(2,20)=6.56, p<0.01
Cingulum(hippocampus)_LH	0.44(0.05)a	0.44(0.02)a	0.32(0.10)b	F(2,20)=7.30, p<0.01
Fornix/stria_terminalis_RH	0.42(0.04)a	0.41(0.02)a	0.28(0.10)b	F(2,20)=11.70, p<0.01
Fornix/stria_terminalis_LH	0.40(0.04)a	0.41(0.03)a	0.33(0.06)b	F(2,20)=4.92, p=0.02
Superior_longitudinal_fasciculus_RH	0.39(0.04)a	0.40(0.02)a	0.32(0.06)b	F(2,20)=4.26, p=0.03
Superior_longitudinal_fasciculus_LH	0.35(0.04)a	0.36(0.03)a	0.22(0.09)b	F(2,20)=12.25, p<0.01
Superior_fronto_occip_fasciculus_RH	0.26(0.03)a	0.26(0.03)a	0.18(0.06)b	F(2,20)=8.88, p<0.01
Superior_fronto_occip_fasciculus_LH	0.65(0.05)a	0.64(0.02)a	0.51(0.10)b	F(2,20)=10.57, p<0.01
Uncinate_fasciculus_RH	0.35(0.05)a	0.31(0.06)a	0.19(0.09)b	F(2,20)=8.10, p<0.01
Uncinate_fasciculus_LH	0.44(0.04)a	0.45(0.03)a	0.34(0.05)b	F(2,20)=10.39, p<0.01
Tapetum_RH	0.44(0.04)a	0.45(0.02)a	0.35(0.05)b	F(2,20)=8.03, p<0.01
Tapetum_LH	0.52(0.04)a	0.51(0.03)a	0.43(0.02)b	F(2,20)=7.25, p<0.01
Average FA of WMTs	**0.45(0.036)**	**0.44(0.014)**	**0.34(0.067)**	**F(2.2)=11.7, p=0.04**

Table S4: Associations of FA with serum iron, % TF saturation, haemoglobin and ferritin (corrected for high CRP) in the 48 WMTs

FRACTIONAL ANISOTROPY (n=11)	Serum Iron		% Tf saturation		Hemoglobin		Ferritin corrected	
	r	p	r	p	r	p	r	p
Middle_cerebellar_peduncle	0.57	0.07	0.71	0.01	0.46	0.15	0.69	0.04
Pontine_crossing_tract	0.55	0.09	0.57	0.07	0.01	0.97	0.4	0.28
CC_genu	0.15	0.65	0.22	0.52	-0.15	0.65	0.26	0.5
CC_body	0.44	0.18	0.47	0.15	-0.17	0.61	0.25	0.51
CC_splenium	0.52	0.11	0.69	0.02	0.3	0.37	0.43	0.25
Fornix	0.51	0.11	0.69	0.02	0.44	0.18	0.61	0.08
Corticospinal_tract_RH	0.40	0.23	0.59	0.06	0.46	0.16	0.47	0.2
Corticospinal_tract_LH	0.43	0.19	0.59	0.05	0.37	0.26	0.56	0.12
Medial_lemniscus_RH	0.47	0.15	0.69	0.02	0.52	0.1	0.75	0.02
Medial_lemniscus_LH	0.54	0.09	0.74	<0.01	0.46	0.15	0.64	0.06
Inferior_cerebellar_peduncle_RH	0.68	0.03	0.65	0.03	0.12	0.73	0.79	0.01
Inferior_cerebellar_peduncle_LH	0.49	0.13	0.4	0.23	0.05	0.89	0.59	0.09
Superior_cerebellar_peduncle_RH	0.39	0.24	0.44	0.17	0.26	0.43	0.6	0.09
Superior_cerebellar_peduncle_LH	0.14	0.69	0.3	0.38	0.43	0.18	0.29	0.44
Cerebral_peduncle_RH	0.25	0.47	0.44	0.18	0.54	0.08	0.38	0.31
Cerebral_peduncle_LH	0.59	0.06	0.78	<0.01	0.28	0.41	0.41	0.27
Anterior_limb_of_int_capsule_RH	0.60	0.06	0.75	<0.01	0.18	0.59	0.31	0.42
Anterior_limb_of_int_capsule_LH	0.61	0.05	0.55	0.08	0.05	0.89	0.72	0.03
Posterior_limb_of_int_capsule_RH	0.50	0.12	0.56	0.08	0.29	0.38	0.61	0.08
Posterior_limb_of_int_capsule_LH	0.71	0.02	0.65	0.03	0	1	0.83	<0.01
Retrolenticular_int_capsule_RH	0.74	0.01	0.76	<0.01	0.14	0.69	0.79	0.01
Retrolenticular_int_capsule_LH	0.50	0.12	0.69	0.02	0.53	0.09	0.63	0.07
Anterior_corona_radiata_RH	0.63	0.04	0.79	<0.01	0.39	0.24	0.82	<0.01
Anterior_corona_radiata_LH	0.40	0.22	0.41	0.22	0.04	0.92	0.92	<0.01
Superior_corona_radiata_RH	0.71	0.02	0.78	<0.01	0.19	0.58	0.49	0.19
Superior_corona_radiata_LH	0.63	0.04	0.79	<0.01	0.44	0.17	0.27	0.49
Posterior_corona_radiata_RH	0.63	0.04	0.58	0.06	0.21	0.53	0.52	0.15
Posterior_corona_radiata_LH	0.72	0.02	0.74	<0.01	0.22	0.51	0.54	0.14
Posterior_thalamic_radiation_RH	0.54	0.09	0.77	<0.01	0.58	0.06	0.73	0.03
Posterior_thalamic_radiation_LH	0.55	0.09	0.8	<0.01	0.61	0.05	0.49	0.18
Sagittal_stratum_RH	0.48	0.14	0.63	0.04	0.51	0.11	0.4	0.28
Sagittal_stratum_LH	0.12	0.73	0.32	0.34	0.37	0.26	0.7	0.03
External_capsule_RH	0.67	0.03	0.84	<0.01	0.45	0.17	0.81	<0.01
External_capsule_LH	0.54	0.09	0.75	<0.01	0.61	0.05	0.44	0.24
Cingulum_RH	0.59	0.06	0.68	0.02	0.34	0.3	0.66	0.05
Cingulum_LH	0.60	0.06	0.64	0.03	0.18	0.6	0.57	0.11
Cingulum(hippocampus)_RH	0.43	0.19	0.47	0.15	0.24	0.47	0.59	0.09
Cingulum(hippocampus)_LH	0.55	0.09	0.72	0.01	0.51	0.11	0.44	0.24
Fornix/stria_terminalis_RH	0.30	0.37	0.49	0.12	0.5	0.12	0.58	0.1
Fornix/stria_terminalis_LH	0.45	0.17	0.51	0.11	0.26	0.45	0.48	0.19
Sup_longitudinal_fasciculus_RH	0.65	0.03	0.61	0.05	0.05	0.88	0.6	0.09
Sup_longitudinal_fasciculus_LH	0.74	0.01	0.78	<0.01	0.21	0.55	0.79	0.01
Sup_fronto_occip_fasciculus_RH	0.55	0.09	0.52	0.1	0.03	0.93	0.79	0.01
Sup_fronto_occip_fasciculus_LH	0.65	0.03	0.83	<0.01	0.43	0.19	0.62	0.08
Uncinate_fasciculus_RH	0.41	0.21	0.56	0.07	0.52	0.1	0.52	0.15
Uncinate_fasciculus_LH	0.30	0.37	0.31	0.36	-0.21	0.54	0.45	0.22
Tapetum_RH	0.29	0.39	0.37	0.27	0.07	0.83	0.57	0.11
Tapetum_LH	0.43	0.19	0.47	0.15	-0.03	0.93	0.58	0.1

Table S5. Associations between FA and serum Transferrin in the 48 WMTs. There were no significant associations.

FRACTIONAL ANISOTROPY (n=11)	SERUM TRANSFERRIN	
	r	p
Middle_cerebellar_peduncle	-0.11	0.74
Pontine_crossing_tract	0.40	0.23
CC_genu	0.27	0.41
CC_body	0.50	0.12
CC_splenium	0.07	0.84
Fornix	0.01	0.98
Corticospinal_tract_RH	-0.11	0.75
Corticospinal_tract_LH	0.06	0.86
Medial_lemniscus_RH	-0.15	0.66
Medial_lemniscus_LH	-0.04	0.90
Inferior_cerebellar_peduncle_RH	0.16	0.63
Inferior_cerebellar_peduncle_LH	0.39	0.24
Superior_cerebellar_peduncle_RH	0.01	0.98
Superior_cerebellar_peduncle_LH	0.00	0.99
Cerebral_peduncle_RH	-0.06	0.85
Cerebral_peduncle_LH	0.19	0.58
Anterior_limb_of_internal_capsule_RH	0.21	0.54
Anterior_limb_of_internal_capsule_LH	0.32	0.35
Posterior_limb_of_internal_capsule_RH	0.04	0.92
Posterior_limb_of_internal_capsule_LH	0.28	0.40
Retrolenticular_internal_capsule_RH	0.18	0.60
Retrolenticular_internal_capsule_LH	-0.04	0.90
Anterior_corona_radiata_RH	0.03	0.93
Anterior_corona_radiata_LH	0.02	0.96
Superior_corona_radiata_RH	0.29	0.39
Superior_corona_radiata_LH	0.17	0.62
Posterior_corona_radiata_RH	0.31	0.36
Posterior_corona_radiata_LH	0.23	0.50
Posterior_thalamic_radiation_RH	-0.13	0.70
Posterior_thalamic_radiation_LH	-0.08	0.81
Sagittal_stratum_RH	0.05	0.88
Sagittal_stratum_LH	-0.21	0.54
External_capsule_RH	-0.02	0.96
External_capsule_LH	-0.02	0.95
Cingulum_RH	0.03	0.94
Cingulum_LH	0.36	0.28
Cingulum(hippocampus)_RH	0.20	0.55
Cingulum(hippocampus)_LH	0.15	0.66
Fornix/stria_terminalis_RH	-0.18	0.59
Fornix/stria_terminalis_LH	0.22	0.51
Superior_longitudinal_fasciculus_RH	0.45	0.17
Superior_longitudinal_fasciculus_LH	0.12	0.73

Superior_fronto_occip_fasciculus_RH	0.16	0.63
Superior_fronto_occip_fasciculus_LH	0.04	0.90
Uncinate_fasciculus_RH	0.09	0.80
Uncinate_fasciculus_LH	0.35	0.30
Tapetum_RH	0.21	0.54
Tapetum_LH	0.27	0.42