MAGNETIC RESONANCE IMAGING ASSESSMENT OF BRAIN LESIONS FOR MULTIPLE SCLEROSIS IN RELATION TO DIAGNOSIS AND DISABILITY PROGRESSION

by

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DECLARATION

I, Mariaan Jaftha, declare that the contents in this thesis represent my own unaided work, and that this thesis has not been submitted for academic examination towards any examination. Furthermore, it represents my own opinions and not necessarily those of the Cape Peninsula University of Technology.

31st October 2024

_____ _____

Signed **Date**

ABSTRACT

INTRODUCTION

Multiple sclerosis (MS) is a major cause of neurological disability amongst young adults, with the global number diagnosed annually numbering almost 2.3 million people worldwide. Disability factors often found with MS are associated with lifestyle factors, genetics and biochemistry (Nelson, 2013). As monitoring the disease process over time has its own challenges, in such a context the need arises for a reliable biomarker/tool, like magnetic resonance imaging (MRI). Advances made with MRI technology and increased availability, since 2012, have enabled enhanced understanding and monitoring of specific pathological processes related to MS(Filippi et al., 2019). Recent studies show that 80% of MS patients have more MRI-detectable lesions when advanced sequences are used with either 1.5T or 3T magnets(Cahalane et al., 2018).

METHOD

Twenty-five participants with a confirmed diagnosis of MS and 25 age-matched controls enrolled for the study, with 7 participants (3 patients with MS and 4 controls), being unable to have the MRI scan, due to concerns related to either claustrophobia or illness. All participants had an MRI scan of the brain using a 3-T Siemens Skyra (Erlangen, Germany) scanner. The protocol used included fluid-attenuated inversion recovery (FLAIR) in both axial and sagittal planes, proton density (PD), T2 (in the coronal plane), magnetisation-prepared rapid acquisition gradient echo (MPRAGE), susceptibility-weighted imaging (SWI), diffusion tensor imaging (DTI), and readout segmentation of long variable echo-trains (RESOLVE) images were utilised for the lesion count. Other variables included were the genetic profiles of each participant and the assessment of disability, using the Expanded Disability Status Scale (EDSS), as well as the compiling of their biochemical, dietary and lifestyle information.

RESULTS

Although brain white matter lesions (WMLs) were found in both groups, an increased number of lesions was found in the MS group. Despite the WML volumes being significantly associated with the EDSS ($p<0.02$), they were not found to be so with the use of age, disease duration or disease-modifying therapies (DMT). The Sequence-Adaptive Multimodal SEGmentation (SAMSEG) software identified WMLs in some control participants, with such spaces possibly accounting for vascularity and Virchow-Robin spaces, among others, since the age group represented was varied.

CONCLUSION

As an imaging modality, MRI is regularly evolving, with it having proven itself to continue playing an integral and pivotal role in the diagnosis and monitoring of brain pathologies.

KEYWORDS

multiple sclerosis, magnetic resonance imaging (MRI), Expanded Disability Status Scale (EDSS), biochemistry, genetics.

PUBLICATIONS

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DEDICATION

Werp jou brood op die water, eendag kry jy dit terug (*Prediker*, 11:1).

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ABBREVIATIONS AND ACRONYMS

ADC	apparent diffusion coefficient
ANOVA	analysis of variance
ANCOVA	analysing of covariance
ARA	arachidonic acid
ATP	adenosine triphosphate
BBB	blood–brain barrier
BMI	body mass index
BSA	body surface area
CAD	coronary artery disease
CC	corpus callosum
CCI	corpus callosum index
CIS	clinically isolated syndrome
CNS	central nervous system
COPD	chronic obstructive airway disease
COR/CP	coronal plane
CPUT	Cape Peninsula University of Technology
CRP	C-reactive protein
CSF	cerebrospinal fluid
CUBIC	Cape Universities Body Imaging Centre
DHA	docosahexaenoic acid
DICOM	Digital Imaging and Communications in Medicine
DIR	double inversion recovery
DIS	dissemination in space
DM	diabetes mellitus
DMT	disease-modifying treatment
DNA	deoxyribonucleic acid
DTI	diffusion tensor imaging
DWI	diffusion-weighted imaging
EBV	Epstein-Barr virus
EDSS	Expanded Disability Status Scale
EPA	eicosapentaenoic acid

ESR	erythrocyte sedimentation rate
eTIV	estimated total intracranial volume
FLAIR	fluid-attenuated inversion recovery
FLOSS	Free and Libre Open Source Software
FO	fish oil
GBCAs	gadolinium-based contrast agents
GM	grey matter
GSH	Groote Schuur Hospital
GWASs	genome-wide association studies
HCY	homocysteine
HDACs	histone deacetylases
HDL	high-density lipoprotein
He	helium
HF	heart failure
HHcy	hospital information system
HIS	homocysteinemia
HLA	human leukocyte antigen
HPCSA	Health Professions Council of South Africa
HREC	Human Research Ethics Committee
HTN	hypertension
IFN	interferon
IL	interleukin
IL2RA	interleukin-2 receptor alpha chain
LSD	least significant difference
MHC	major histocompatibility complex
MPRAGE	magnetisation-prepared rapid acquisition gradient echo
MRI	magnetic resonance imaging
MS	multiple sclerosis
MSIF	Multiple Sclerosis International Federation
MSSA	Multiple Sclerosis Society of South Africa
MSSS	Multiple Sclerosis Severity Score
NK	natural killer
PACS	picture archiving and communication system
PCR	polymerase chain reaction

PD	proton density
P4	participatory, personalised, predictive and preventive
PI	Progression Index
PPMS	primary progressive multiple sclerosis
PRMS	progressive relapsing multiple sclerosis
PSGT	pathology-supported genetic testing
PSIR	phase-sensitive inversion recovery
PUFAs	polyunsaturated fatty acids
QoL	quality of life
RCTs	randomised control trials
RESOLVE	readout segmentation of long variable echo-trains
RFs	radiofrequency fields
RIS	radiology information system
RNS	reactive nitrogen species
ROS	reactive oxygen species
RRMS	relapsing-remitting multiple sclerosis
sbTIV	segmentation-based estimate of total intracranial volume
SCFAs	short-chain fatty acids
SNP	single nucleotide polymorphism
SPMS	secondary progressive multiple sclerosis
SWI	susceptibility-weighted imaging
SWI3D	three-dimensional susceptibility-weighted imaging
3D	three-dimensional
T1WI	T1-weighted imaging
T2WI	T2-weighted imaging
Tf	transferrin
Th	T helper
TIRM	turbo inversion recovery magnitude
TRA	trans axial plane
TSE	turbo spin echo
UVB	ultraviolet B
UVR	ultraviolet radiation
WHO	World Health Organization
WHR	waist–to–hip ratio
WM	white matter

WMHs	white matter hyperintensities
WML	white matter lesion

CHAPTER 1
OVERVIEW OF THE STUDY

1.1 Introduction

Multiple sclerosis (MS) is an inflammatory demyelinating disease, presenting in young to middle-aged adults, but which is also known to have developed in the elderly. According to the McDonald criteria (McDonald et al., 2001), the diagnosis of MS needs the objective assessment of white matter (WM) lesions, as they disseminate through both space and time (Cahalane et al., 2018). Magnetic resonance imaging (MRI) is an important diagnostic tool for detecting MS, especially in terms of the demonstration of the spatial and temporal distribution of the disease (Chen et al., 2016). MRI has an increased sensitivity to disease pathological substrates, namely neuroaxonal loss, inflammation and demyelination, particularly with the monitoring of disease progression and the efficacy of treatment options(Filippi et al., 2019). Accordingly, MRI is a highly sought-after imaging modality, especially in relation to the initial diagnosis of MS and the evaluation of therapeutic responses(Hemond & Bakshi, 2018). MRI is seen as the best imaging tool for monitoring response to therapies, since MRI has proven to be advantageous due to its high sensitivity (for highlighting WM lesions), and its ability to identify clinically silent lesions (Mclaughlin, 2017). As this modality can provide highly reproducible measures on ordinal scales (the ability of a system to provide organized data that can be ranked or ordered), it allows for the adoption of a powerful statistical approach to the analysis of lesions. Assessments of the MRI scan can be performed at the highest degree of blinding, with the data concerned being retrievable for both new and preplanned analysis(Bar-Zohar et al., 2008).

In the current study, MS lesions were assessed by means of using highly sophisticated imaging software. Images were obtained with a 3T MR scanner, which has proven to be a very sensitive imaging tool for the analysis of WM lesions. The present chapter will demonstrate the rationale for the study, specifically focusing on the use of MRI. MRI's impact as a favoured imaging tool in the assessment of disease progression and its role in the early diagnosis of MS will also be demonstrated.

1.2 Rationale for the Study

In this study we aim to determine/assess brain lesion formation as analysed with MRI imaging. Concurrently, we will assess the relation between MS diagnosis and disability progression by assessing data from the genetics, biochemical factors, MRI images and disability statuses of the participants.

1.3 MRI as a tool for measuring disease progression

MRI is a non-invasive, versatile medical imaging technique that has been in use for over 25 years as a diagnostic imaging tool (Kanal et al., 2013). Previous studies have shown that the number of enhancing lesions, and the rate of GM atrophy (on MRI) are very good indicators of disease progression, especially during the early stages of the disease (Lavorgna et al., 2014). While it remains difficult to make an initial definitive diagnosis, we have come to rely upon a set of diagnostic criteria, updated in 2001 by the International Panel on the Diagnosis of Multiple Sclerosis, namely the McDonald criteria. These criteria, which include specific guidelines (the revised McDonald criteria), consist of a set of clinical, radiological and laboratory rules, used to make a definitive diagnosis of MS (Harris & Sadiq, 2009). According to the revised McDonald criteria of the International Panel of Diagnosis of MS, with the use of MRI of the central nervous system (CNS), clinical criteria of MS can be supplemented, supported or even replaced, as the disease is both sensitive and specific, often resulting in an earlier diagnosis, thereby helping to facilitate enhanced treatment outcomes for patients (Polman et al., 2011). In addition, there are contemporary postprocessing tools, such as regional voxel-wise approaches, lesion-inpainting algorithms and composite MRI scales, all of which enable a more accurate assessment of both global and regional white and grey matter damage. Such methods offer a new pathway, linking MS with its clinical status (Ceccarelli et al., 2012).

1.4 Appearance of MS on MRI

Enhanced lesions are normally associated with active plaques, although such association does not necessarily indicate a relapse, or the exhibiting of any clinical symptoms (Kutzelnigg & Lassmann, 2014). Active plaques can be visualised as either a homogenous pattern or in a solid form (Cahalane et al., 2018). Characteristics of the different forms of lesions include the Dawson's fingers (lesions which are often found firmly in contact with the ventricles, as can be seen in Figure C), the juxtacortical lesions (best seen on FLAIR imaging and contiguous with the cortex, as can be seen on Figure D), the brainstem lesions (which can only be considered to be MS if they border on the subarachnoid space or a ventricle, as can be seen on Figure A), and the corpus callosal lesions (best seen on sagittal FLAIR imaging, and which should border on the callosal septal interface, as can be seen on Figure B (Filippi et al., 2019).

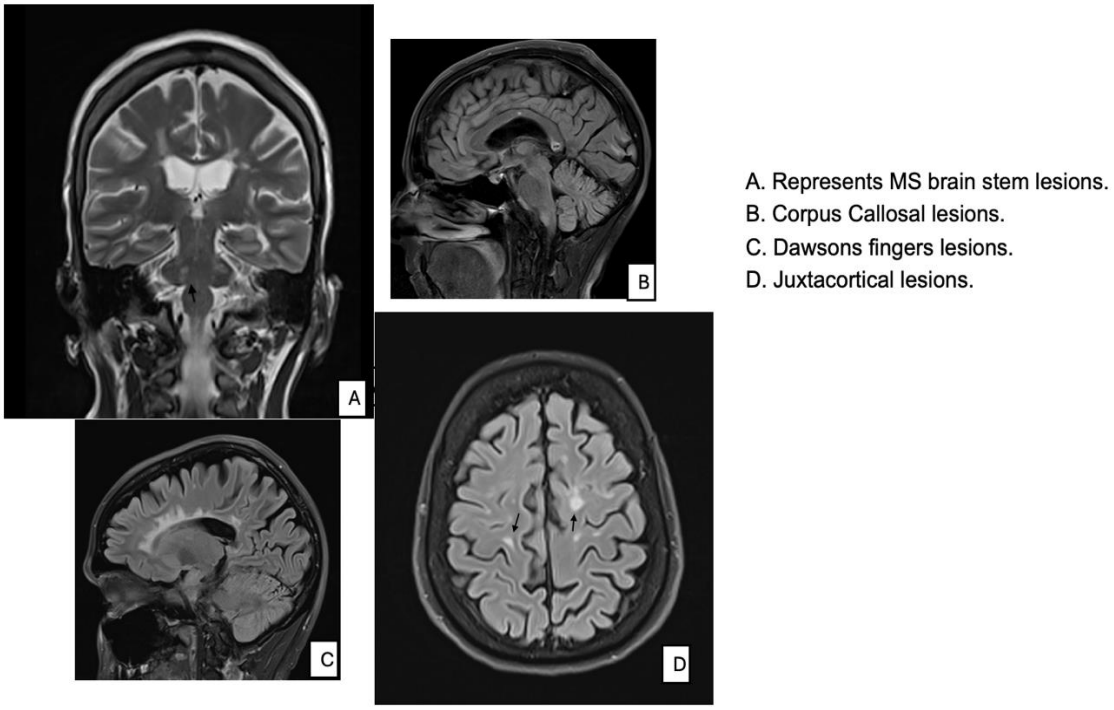


Figure 1.1: Various lesion forms (A–D) found in MS.
(Permission to use the images was granted by the participants concerned.)

In recent studies, with the advancements made in MRI and immunopathology techniques, the fundamental involvement of GM in, and its contributing role to, the diagnosis of MS has been demonstrated, including how such involvement and contribution correlate directly with seizure activity, cognitive dysfunction and physical disability, as well as with disease progression(Cahalane et al., 2018). Brain atrophy in MS is a global process that is consistent with both WM and GM pathology. Unfortunately, with MS patients, the rate of atrophy has tended to be faster (0.5%–1% per year) than in age-matched healthy controls (0.1%–0.3% per year). Patients with clinically isolated syndrome (CIS) suggestive of MS, which later converts to MS, usually show more brain atrophy than do those who do not (Giorgio & De Stefano, 2013). Modern techniques in MRI, namely the double inversion recovery (DIR) technique, phase-sensitive inversion recovery (PSIR) and high-resolution three-dimensional (3D) magnetisation-prepared rapid acquisition gradient echo (MPRAGE), are all imaging methods with improved sensitivity and classification of cortical lesions(Cahalane et al., 2018). Subsequent studies have shown that 80% of MS patients have more detectable lesions when advanced sequences are used on either 1.5T or 3T magnets(Cahalane et al., 2018). Generally, the benefits are greater when scanning on a 3 Tesla (representing the magnetic field strength) scanner rather than on a 1.5 Tesla. A 3 Tesla scanner has improved image quality, lesion contrast, and resolution (sharper images), and increased signal-to-noise ratio (comparing the desired signal to the level of background noise (Runge & Heverhagen, 2022). Imaging of GM lesions remains a challenge, due to their small size, the minimal associated inflammatory response, and the partial volume averaging caused by the adjacent cerebrospinal fluid (CSF). With GM lesions, 95% of lesions tend to be missed on the 1.5T magnets, whereas ultra-high MR field strength

MRIs (7 Tesla) tend to detect more cortical lesions than does the 3T magnet MRI scanner (Cahalane et al., 2018). Lesion volumes do not necessarily correlate with clinical disability status, which could explain why, even though patients may have a high number of lesions, their doing so does not necessarily correlate with their clinical disability progression (Van Rensburg, Van Toorn, et al., 2021)**.**

1.5 Focus of the research

The focus of the current study was on examining the number of enhancing lesions found on the MR images of the research participants. The hypointense lesions present in the brain were compared with the Expanded Disability Status Scale (EDSS) of subjects identified as being at extreme of the EDSS score spectrum. Comparisons were made with the results of the two subsets, with the biochemical markers (obtained from the blood results) and the EDSS scores of the participants presenting at the extremes of the disease outcome scale spectrums concerned. Whether the lesion number (derived from the MRI sequences) was sufficient for aiding the prediction of the disability of MS was also considered. The lifestyle questionnaires administered provided insight into the behavioural patterns of the participants concerned. Genetic testing and the blood results were measured against MS-free participants (the control group) to enable the assessment of any anomalies. Ultimately, the aim was to gain enhanced understanding of the disease processes involved that might facilitate improved disease outcome.

1.6 Problem statement

The problem to be addressed is to investigate the disparity of clinical outcomes between different people with the same diagnosis.

1.7 Aim of the study

The present prospective cross-sectional case-control study was aimed at using brain MRI images , biochemical parameters, genetic and lifestyle factors in a sample of MS patients to determine all or any risk factors causal or associated to disease progression. Furthermore, the intention of the research was to evaluate the MRI scans of female subjects diagnosed with MS, at either of the two extremes of the EDSS score spectrum, this in turn will assess the role of MRI with early diagnosis (to enable better disease management), and if we can monitor the disease progression over time. Following such procedures might help improve understanding of the pathophysiological processes associated with MS. Additionally, the results involved could potentially aid in the implementation of the safer and more effective treatment strategies developed, which might lead to the slowing down of disability progression and improve the quality of life (QoL) of those living with MS**.**

1.8 Research questions

The research questions asked were the following:

- How does the number of lesions present throughout the brain relate to the EDSS at the two extremes of the EDSS score spectrum?
- How do the results of the EDSS score and lesion number affect disability?
- What role does MRI play as a disease marker, and how can it aid in the prediction or/ and monitoring of disease progression?

1.9 Research objectives

The research objectives were the following:

- to confirm MS diagnosis, using McDonald criteria, as revised in 2017 (Thompson et al., 2018).
- to evaluate disability, as assessed in terms of the EDSS.
- to determine the relationship between lesion load and disability progression, in association with biochemical markers in MS.
- to develop a lesion analysis MRI protocol encompassing several related neuro-imaging measures, so as accurately to characterise lesion load in MS; and
- to use MRI images to establish the correlation between the status of the corpus callosum (CC) and MS disease progression, specifically focusing on its dimensions and lesion distribution within the part of the brain concerned.

1.10 Summary

Chapter 1 gave a brief overview of the disease and the rationale of the present study. Of particular interest, is the use of MRI as a preferred method for identifying MS activity. The discussion, which further elaborated on the appearance of MS in MRI images, included supporting evidence in the use of MRI as the imaging method of choice, for the visualisation of lesions in those with MS. Chapter 2 will provide an in-depth discussion of the literature on MS, pathophysiology, morbidity, mortality, and treatment strategies. The application of dedicated MR sequences is discussed, in an effort to facilitate the assessment of MS and to gain enhanced understanding of the aetiology of this disorder, so as to aid with treatment options and facilitate improved outcomes for people with multiple sclerosis (PwMS).

CHAPTER 2
LITERATURE REVIEW

2.1 Introduction

MS, which is a disease with unexplained heterogeneity in terms of outcomes and survival, affects over 2.5 million persons worldwide (Public Health and Primary Care Division, 2015). Demyelination and neuroaxonal damage in the CNS are common destructive features of MS. The origin of the processes concerned is complex and not yet fully understood (Olsson et al., 2021). The current study was aimed at reviewing the nature of MS by means of discussing the disease classification and its pathogenesis, as well as by applying a theoretical framework for evaluating MRI as the preferred imaging tool. The focus of the research was on the disease pathways of MS, and their prevalence. The relationship between MRI and MS was also explored, as well as was its role in imaging disease functionality and pathogenesis. Currently, there is still only a limited amount of knowledge of the exact aetiology of MS; therefore, an endeavour was made to increase the understanding of such aetiology, which is hoped to facilitate the development of a more favourable disease outcome than is currently available.

2.2 Spatial epidemiology

MS is one of the most prevalent demyelinating disorders in high-income countries, with heterogeneous prevalence occurring worldwide(Leray et al., 2016). Dean et al., 1967,reported the results of a survey on the annual incidence, prevalence and mortality of MS in the white South African population. The pervasiveness of the disease was found to be high among white South Africans, with it tending to be higher among English-speaking than among Afrikaans-speaking white South Africans. Simultaneously, the disease was reported to be less common among the coloured and Asian communities in South Africa, and rare among black South Africans, according to Dean et al. (1967). In a follow-up publication, Modi et al. (2008) describe the prevalence of MS in South Africa, with an estimation made by the Multiple Sclerosis Society of South Africa (MSSA) of there being about 5000 sufferers with the disease, with the Society finding it to be more commonly present in the white community, and rare in the black communities. The distribution of the disease was found to be spread out over the disease types concerned, although it was found to be predominantly present in the 40–49-year-old age group, and mostly afflicting women (F:M=3:1). Worldwide, the highest occurrence of MS has been found in North America (140/100 000 individuals) and Europe (108/100 000 individuals), with the lowest occurrence being present in East Asia (2.2/100 000 individuals) and sub-Saharan Africa (2.1/100 000 individuals) (Leray et al., 2016). The Multiple Sclerosis International Federation (MSIF) reported an increase in the global median of MS from 30/100 000 individuals in 2008 to 33/100 000 individuals in 2013 and, more recently, an estimation found there were

2,221,188 (95% Uncertainty Interval 2,033,866–2,436,858) persons living with MS in 2016. Data were retrieved from a survey compiled by the MSIF and the Atlas of MS, which is an open-source global compendium of data regarding the epidemiology of MS, also reflected a similar increase (see Figure 2.1) (Leray et al., 2016). The first edition of the Atlas, which was produced in 2008 in collaboration with the World Health Organization (WHO), was updated in 2013. The survey involved included responses from each WHO region (i.e. the Americas, Southeast Asia, Europe, the Eastern Mediterranean and the Western Pacific), apart from the continent of Africa, which represented only 56% of the total population. Previous editions of the Atlas of MS has reported poorer response of the availability and quality of incidence data than for the prevalence data, and only 75 countries(65% of the survey responders), actually responded .(Walton et al., 2020).

The following conclusions were drawn from the study:

- The estimated number of MS diagnoses worldwide increased to 2.8 million in 2020. (If one applies the same statistical method as was utilised in 2013, then the estimated surge is 30% higher than before.)
- In 2020, the global prevalence was 35.9 [95% CI:35.87, 35.95] per 100 000 people.
- The worldwide prevalence of MS has increased since 2013 (refer to Figure 2.1). Only 14% (11/81) of the countries with data accessible for both time points (2013 and 2020) reported stable or declining prevalence of the disease.
- The pooled incidence rate across the 75 reporting countries was 2.1 (95% CI:2.09, 2.12) per 100 000 persons/year. Someone in the world is estimated as being diagnosed with MS every five minutes.
- The paediatric onset of MS has also increased substantially with ⩾30 000 cases of MS diagnosed in individuals under 18 years of age being reported by 47 countries. In 2013, 7000 cases were reported in 34 countries.
- Globally, women are two-thirds as likely to have MS as are men (most likely due to the hormonal involvement concerned), which is consistent with the data provided by both prior editions of the Atlas. However, the ratio of women with MS to men with the same disease is as high as 4:1 in some countries, with it having doubled in other countries since 2013.
- If the analysis were to be restricted to countries that reported prevalence data in 2013 and 2020, the increase in global prevalence would be 50%.

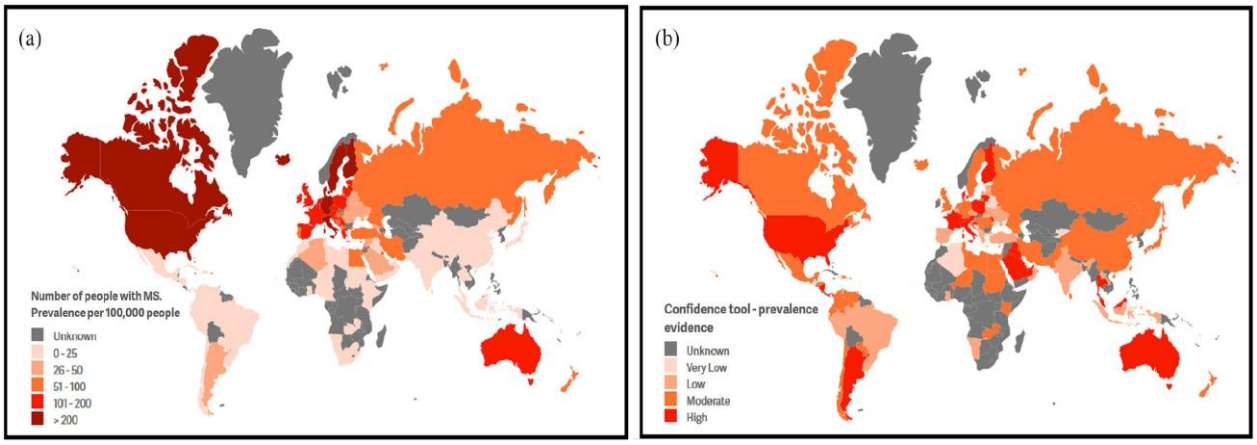


Figure 2.1: Geographical map showing variations in MS prevalence by country. The data confidence scores for MS prevalence per 100 000 population by country are shown in shades of orange and red as per key (Figure 1a); the grey areas highlight countries without any prevalence data. Figure 1b shows the confidence scores assigned to each country, based on the prevalence data sources provided (Walton et al, 2020).

In sub-Saharan Africa, regarding the global burden in terms of the disease study, the latest estimation of MS cases was reported as being ~49 000, with ~2800 new cases being reported annually. An increase of approximately 6.1% (95%CI 4.6–7.5%) in the incidence of MS was also repeated between 2007 and 2017(Heine et al., 2020). Aarli et al. (2014) described, according to the Atlas of MS survey conducted in 2008 and 2013, a 19.5% increase in the prevalence of MS over the previous five years. According to a review conducted by Heine et al. (2020), the most recent reports on the prevalence of MS in the Southern African subregion, as far back as 2007 (per 100 000), were 25.6 in Caucasians, 7.6 in Indians, 1.9 in mixed-ancestry, and 0.2 in black patients (Bhigjee et al., 2007). The researchers concerned noted this value to be much higher than that which was previously reported by Dean in 1967 (9.1/100 000) (Dean et al., 1967). Modi et al. (2008) also reported on a specific region on which the prevalence estimates made by Bhigjee et al. (2007) were based (namely, KwaZulu-Natal), and which may reflect a smaller proportion (12%) of patients with MS relative to other regions (e.g. the Western Cape [28%] and Gauteng [40%]) (Modi et al., 2008;Heine et al., 2020).

2.3 Mortality and morbidity

Over the last few decades, according to the Public Health and Primary Care Division, noticeable changes have occurred in the epidemiological features of MS(Public Health and Primary Care Division, 2015). Manouchehrinia et al. (2016), furthermore, also reported on a much higher mortality rate for MS patients than for the general population. According to Marrie et al. (2015), feedback from previous studies has shown an improved survival rate for MS over the past 40–50 years, although most of the studies concerned suggest the survival rate to be lower than expected for an age- and sex-matched population without MS. They also allude to an increase in the burden of comorbidity and complexity of the disease over the past five years and advocate the modification of the comorbidities for MS to improve outcomes. The reasons

for inequality in survival rates are not yet fully understood, due to disease-related complications and competing causes of mortality. Such complications as infection can play a key role in the mortality concerned, with other unrelated causes, like cardiovascular disease, seeming to contribute to higher-than-expected mortality, as reported in other studies. Little is known about the changes in MS mortality over time and regarding the drawing of a comparison of the changes thereto occurring within the general population.

2.4 Disease processes

Inflammatory demyelination underpins the effects of MS on the CNS(Sand, 2015). This chronic autoimmune inflammatory and demyelinating process destroys the fatty substance (myelin) that surrounds and insulates the nerve fibres, as well as the nerve fibres themselves(Taylor et al., 2014). Any damage done to the myelin sheath or the nerve fibre can disrupt and/or distort nerve impulses travelling to and from the brain, which, in turn, produces a variety of symptoms found in MS patients. The damaged myelin then forms scar tissue, termed 'sclerosis' (Chaiel & Abass, 2022).

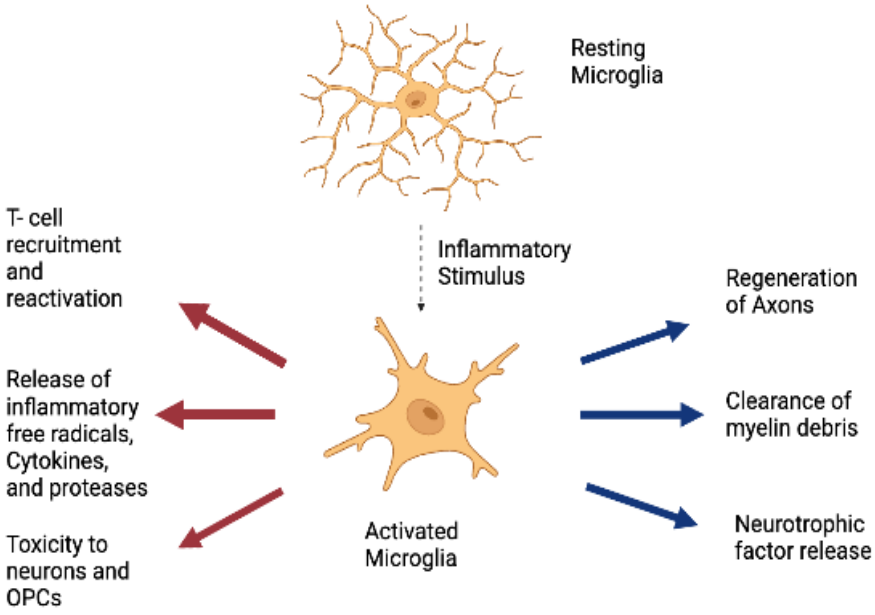


Figure 2.2: The demyelinating/remyelinating process
Adapted from Rawji and Yong (2013)and created using BioRender.com (2023).

A diagrammatic illustration showing the dichotomy of inflammatory processes occurring in the macrophages/microglia is displayed in Figure 2.2. Macrophages/microglia, which recruit and reactivate the T-cells in the CNS, release many detrimental molecules, like proteases, inflammatory cytokines and free radicals. Through the latter molecules and other mechanisms, macrophages/microglia have been reported to contribute to toxicity in neurons, as well as oligodendrocyte precursor cells. Conversely, they have also been observed to aid in axonal regeneration and remyelination, as well as to assist in the clearance of inhibitory myelin debris.

In addition, the cells concerned release various neurotrophic factors. Therefore, macrophages/microglia possess an array of detrimental and beneficial functions, with the balance involved being dictated by the temporal and spatial specifications following on CNS injury. Several radiological reports have described the re-enhancement of established MS lesions, and, therefore, the presence of any inactive, or mixed active/inactive, lesions. Lesions may also be infiltrated by a second wave of macrophages/microglia, resulting in the development of an active lesion(Kuhlmann et al., 2017).

2.5 Classification of multiple sclerosis

MS can be described as an inflammatory disease of the CNS that affects both white and grey matter. The most dominant feature of MS is the presence of focal demyelinated lesions, which tend to be scattered throughout the WM(Lassmann et al., 2012). The early onset of MS presents as active bouts of demyelination and periods of remission (as described in Table 1). Relapses are associated with new lesions, or with the reactivity of old lesions found primarily in the brain and spinal cord(Lassmann, 2019).

The exact aetiology and pathogenesis of MS still remain unknown. In early pathological investigations, both white and grey matter were mentioned, although the former has tended to be studied more extensively(Mike et al., 2011). MS was classified in 1996 as relapsing-remitting multiple sclerosis (RRMS), primary progressive multiple sclerosis (PPMS), secondary progressive multiple sclerosis (SPMS) and progressive relapsing multiple sclerosis (PRMS)(Lublin, 2014).

Table 2.1: Adaptation of the McDonald criteria (2010) for the diagnosis of multiple sclerosis

Clinical presentation	**Additional data needed for MS diagnosis**
Participants should have had at least two attacks. Objective clinical evidence of at least two lesions, or objective clinical evidence of one lesion, with reasonable historical evidence of prior attacks.	None.
Participants should have had at least two attacks. Objective clinical evidence of one lesion.	Evidence of dissemination in space (DIS), demonstrated by more than one T2 lesion in at least two of four MS-typical regions of the CNS (infratentorial, periventricular, juxtacortical or spinal cord). Alternatively, awaiting a further clinical attack, implicating a different CNS site.
Participants with at least one attack, and with objective clinical evidence of at least two lesions.	Dissemination in time (DIT) is demonstrated by the simultaneous presence of asymptomatic gadolinium-enhancing, and no enhancing lesions at any time.

	New T2 and/or gadolinium-enhancing lesion(s) on follow-up MRI, irrespective of its timing regarding a baseline scan. Awaiting a second clinical attack.
Participants with one attack. Objective clinical evidence of one lesion (CIS).	DIS and DIT are to be demonstrated by the following: In dissemination in space, there should be at least one T2 lesion in at least two of four MS-typical regions of the CNS (juxtacortical, periventricular, infratentorial, or spinal cord). Awaiting a second clinical attack, implicating a different CNS site for DIT: the simultaneous presence of asymptomatic gadolinium-enhancing, and no enhancing lesions at any time. New T2 and/or gadolinium-enhancing lesion(s) on follow-up MRI, irrespective of timing regarding a baseline scan. Awaiting a second clinical attack.
Insidious neurological progression, suggestive of MS PPMS	A disease progression of one year (retrospectively or prospectively determined), along with two of the following three criteria: Evidence of DIS in the brain, based on the presence of at least one T2 lesion in the MS-characteristic (juxtacortical, periventricular or infratentorial) regions. Evidence of DIS in the spinal cord, based on more than two T2 lesions in the cord-positive CSF (with isoelectric-focusing evidence of oligoclonal bands), and/or elevated immunoglobulin (G index).

Source: Adapted from Sand (2015).

2.5.1 Relapsing-remitting stage

The initial stage of the disease is characterised by recurrent and reversible neurological deficits (as referred to in Table 2.1). Women are twice as likely to experience a relapsing-remitting phase (RRMS) as are men. Reversible disability in RRMS is caused by focal areas of inflammatory demyelination, in which myelin, myelin-forming cells (oligodendrocytes) and axons are destroyed. Once such cells enter the brain, they compromise the blood–brain barrier (BBB), with the lesion areas becoming oedematous. Relapses normally last only a few months, after which time the patients concerned tend to regain their neurological function(O'Loughlin et al., 2017).

2.5.2 Primary progressive stage

Rather than this phase normally not being considered a separate entity, it tends to be seen as a part of the spectrum of progressive disease. Pathologically, inflammatory changes can be observed throughout the CNS in all forms of MS, with quantitative, rather than qualitative, differences being seen to occur between the phenotypes. The progressive-relapsing MS phenotype will, by now, have been replaced, with it being characterised as PPMS with activity(Lublin, 2014).

2.5.3 Secondary progressive stage

The relapsing-remitting stage is followed by a phase of uninterrupted disease progression, termed ‘secondary progressive multiple sclerosis’ (SPMS}. On average, 10–20% of patients do not present with RRMS, but instead experience unremitting disease progression (i.e. PPMS). Additionally, during the transition from RRMS to PPMS, continuous clinical deterioration can be interspersed with new diseases (reflecting the presence of relapsing progressive MS)(Lassmann et al., 2012).

2.5.4 Progressive relapsing stage

During the progressive stage of this disease (see Table 2.2), active tissue is associated with inflammation, as is present in the relapsing-remitting phase. At this stage, inflammation occurs only partially behind the BBB, thereby complicating the treatment process (Lassmann et al., 2012).

2.5.5 New Guidelines for the Classification of Multiple Sclerosis

Since the most recent classification of MS in 1996, an increased understanding of the disease pathology process has led to the re-examination of clinical phenotypes. New recommendations for the 2013 revisions have been made, as follows:

1. Patients with CISs should be included.
2. Patients with PRMS should be excluded from the study.
3. All forms of MS should be subcategorised as either active or nonactive (with active MS, defined as the occurrence of clinical relapse or the reappearance of T2 or gadolinium-enhanced lesions, occurring over a specific period, preferably one year).
4. The addition of a new subcategory for patients with progressive MS should help differentiate between those who are symptomatic over a certain period and those who present as stable.

5. Patients who showed signs of disease advancement, for any reason, were noted as `worsening', whereas the term `disease progression' was used to describe those with progressive disease who showed true progression.
6. Van Rensburg et al. (2021) observed another subtype of MS, namely 'benign MS', which describes a dormant period within the disease process, with the disability concerned not having occurred for over a period of 15 years (Banwell et al., 2013). Care should be taken when using the term` benign` because, with MS, a relapse can occur at any time throughout the disease process, even after the patient's condition has been stable for a long time(Lublin, 2014).

2.6 Disease symptoms

Multiple sclerosis affects the CNS, neural damage which occurs and can impair signal transfers within different parts of the nervous system and in turn this may cause mobility issues, spasticity, tremors, imbalances, etc. MS also affects other systems in the body (Hunter et al., 2021), for instance visual impairment, difficulty in swallowing and even bladder/ bowel/ sexual dysfunction. A positive MS diagnosis not only has an impact on the persons physical health, but it also affects their mental abilities. Due to the lack of control over the disease trajectory, this can bring all kinds of uncertainty to both the individual affected and their wider family. Those in the role of caregiver often would have to prioritise the PwMS treatment regimens and emotional and physical needs over their own. Thus, the impact of MS can cause tremendous emotional distress and can contribute to conflict in other relationships(Hunter et al., 2021).
With some of the most common symptoms being as follows:

- mobility issues
- spasticity
- tremors and imbalances
- pressure sores
- bladder/bowel/sexual dysfunction
- speech difficulties
- cognitive difficulties
- depression
- pain
- difficulty in swallowing
- dizziness and vertigo
- fatigue
- paroxysmal symptoms
- mobility issues
- numbness – cold feet and swollen ankles
- impaired vision

- emotional changes

2.7 Clinical trajectory

Lassmann (2019) postulates that, in many instances, the development of MS will start with the RRMS stage, whereafter it transforms into the SPMS stage, and, only in the minority of cases, will the RRMS stage be skipped, with the patient immediately progressing to the PPMS stage. Furthermore, the evidence suggests the presence of major differences between RRMS and progressive MS, but no essential difference between SPMS and PPMS, except for a lower incidence in the global load of focal WM lesions, and in the presence of classical active plaques in PPMS (Lassmann, 2019). During the relapsing-remitting stage of MS, both the clinical course and the disease appear to be driven by the formation of new inflammatory demyelinating lesions in the CNS. Irreversible neurological symptoms are largely determined by the number of lesions, the severity of relapse and the location of lesions. After years of disease onset, patients tend to reach a threshold for irreversible neurological symptoms. With the exhaustion of functional compensation, changes are encountered in the clinical features of the disease, signalling the onset of SPMS. Any further clinical deterioration occurs at a regular and continuous rate, irrespective of any previous disease path, relapse occurrence or severity(O'Loughlin et al., 2017).

The rate of clinical decline is normally predictable in patients with PPMS who do not experience a relapse-remitting phase. Noteworthy is that, the conversion from RRMS to PPMS normally occurs in the age window of 35–50 years, which is also the typical disease onset time for patients with PPMS. Previous evidence from families with MS does not support the view that PPMS is a disease entity that is separate from either RRMS or SPMS. Pathological studies highlight similar alterations in the CNS in RRMS, PPMS and SPMS, with the findings involved suggesting that MS is a single disease entity, with distinct clinical phenotypes. The conversion of MS from the relapse-remitting phase to the secondary progressive stage, which involves prolonged chronic inflammation in the CNS, are influenced by patient age and disease duration (Lassmann, 2019).

The data obtained suggests that the pathological hallmarks of MS include inflammation, demyelination, remyelination, neurodegeneration and glial scar formation. The processes involved can occur either focally or diffusely, involving both white and grey matter in the brain and spinal cord (Lassmann, 2018). All of these features, which are present during all phases of the MS journey, may vary over time, both qualitatively and quantitatively, between the three stages of MS, and among individuals with the same form of the disease(Dobson & Giovannoni, 2019).

Table 2.2: Demonstration of the clinical course of MS, in terms of definitions added to the new course description

Disease-course definitions, 1996	Disease-course definitions, 2013
N/A	'Clinically isolated syndrome' (CIS) indicates the first episode of the inflammatory demyelinating process in the CNS, which becomes MS if additional activity occurs.
'Relapsing-remitting multiple sclerosis' (RRMS) refers to episodes of acute worsening of the neurological functioning, with total or partial recovery, which may exclude any apparent disease progression.	RRMS refers to episodes of acute worsening of the neurological functioning, with total or partial recovery, which may exclude any apparent disease progression. RRMS can either be active, indicating new relapses, new gadolinium-enhancing lesions and/or new, or enlarging, T2 lesions on the MRI over a specified period, or it can be inactive, accordingly showing no disease activity. The RRMS can also manifest as worsening, which process is defined by increased disability, confirmed over a specified period following a relapse. When the RRMS is stable, no evidence of increased disability will be present over a specified period following a relapse.
Primary progressive MS (PPMS) signifies a steadily worsening neurologic function from the beginning, with no distinct relapse or remission phase.	Primary progressive MS (PPMS) signifies a steadily worsening neurologic function from the beginning, with no distinct relapse or remission phase. When it is active, this phase shows evidence of new relapses, with new gadolinium-enhancing lesions and/or new or enlarging T2 lesions on the MRI over a specified period. During the inactive phase, no evidence of disease activity is shown. During the progressive phase, there is evidence of disease worsening in relation to an objective measure of change confirmed over a specific period. Without progression, no evidence exists of disease worsening in relation to an objective measure of change over a specific period.
Secondary progressive multiple sclerosis (SPMS) signifies progression with or without relapse(s).	SPMS follows an initial relapse-remitting course, with the disease becoming more steadily progressive, with or without any relapse(s). During the active phase, evidence exists of new relapses, new gadolinium-enhancing lesions and/or new or enlarging T2 lesions on MRI over a specified period. The inactive phase shows no evidence of disease activity. With progression, evidence of disease worsening is present, with an objective measure of change being confirmed over a

	specified period, which can include/exclude any relapse(s) involved. Without disease progression, no evidence of disease worsening is shown in relation to an objective measure of change over a specified period.
Progressive relapsing MS (PRMS) shows a steady worsening of the neurologic function from the start, with occasional relapses.	Description of the PRMS course was eliminated, with individuals who were previously diagnosed with progressive relapsing MS now coming to be considered as primary progressive in terms of the course of their disease. Diagnosed as active (at the time of relapse or the appearance of new MRI lesions) or inactive.

Source: Adapted from Klineova and Lublin (2018).

2.8 Summation of inflammatory processes

All phases of MS are associated with inflammatory processes mediated by B- and T-cells, macrophages and activated microglia (Lassmann et al., 2012). Klineova et al. (2018) suggest MS to be an autoimmune disease mediated by autoreactive T helper (Th) 1 and Th17 cells. They also predicted that initial contact with an unknown antigen might lead to the production of proinflammatory cytokines interleukin (IL)-1 and interferon (IFN)-γ by Th1 cells and of IL-17 by Th17 cells. The production of cytokines can lead to further Th cell upregulation, the production of certain metalloproteinases, and the destruction of the BBB, thereby enabling Th cells to migrate into the CNS (Klineova & Lublin, 2018). Contrary to such a view, the autoantigen has still not been found after years of searching for it, both in animal models of MS and in clinical trials for MS patients. Ultimately, antigen-specific therapy for MS can lead to a more effective therapy for MS, by means of tolerance induction to a wide range of myelin-derived antigens, without hampering the normal surveillance and effector function of the immune system concerned(Derdelinckx et al., 2021). Pathological alterations in the brain tend to occur between the relapsing and progressive phases of multiple diseases, but their extent increases during the progressive phase. The progressive phase in MS involves various mechanisms that contribute to neurodegeneration. These mechanisms include a lack of trophic support, chronic microglial activation and altered expression of ion channels in demyelinated axons (Lassmann et al., 2012). A significant cause of mitochondrial injury-induced oxidative stress could be a major contributing factor to the pathological features of MS lesions. Such pathological changes include demyelination, oligodendrocyte apoptosis, the destruction of thin-calibre axons, and the lack of remyelination. Iron accumulation in the human brain, especially age-related iron release found in lesioned tissues, can also amplify the oxidative process, particularly in progressive MS. Any treatment option during the progressive stage of MS might be thwarted by the presence of inflammation being ‘trapped’ behind the BBB. Therefore, it might be preferable to utilise a combination therapy approach, including anti-

inflammatory and neuroprotective strategies(Lassmann et al., 2012). MS is responsible for such pathological processes as the dysregulation of the BBB and of the coagulation system, alterations in the gut microbiome, defects in remyelination, dysfunction of the adenosinergic signalling pathways, defective autophagy, changes in cellular metabolism and microbial metabolites, and increased apoptosis. Disease generation, especially that which is driven by T and B lymphocytes and pathogenic T-cells, plays a pivotal role in disease progression via such effector mechanisms as licensing myeloid cells, which infiltrate the CNS and cause damage. Inflammatory processes and demyelination occur due to the damage that is caused to oligodendrocytes (Dobson & Giovannoni, 2019)**.** Sand (2015) suggests that relatively new research demonstrates that axonal and neuronal loss in the early stages of the disease process tends to result in cognitive impairment and in other early disabilities. In addition, patients with a more progressive clinical phenotype than usual can show evidence of ongoing inflammatory activity, through either clinical relapses or new MRI lesions. Distinctions such as these are important at the clinical level, as they influence treatment considerations and eligibility for clinical trials(Sand, 2015).

MS involves various pathophysiological processes, all of which are involved in disease progression(Miljković & Spasojević, 2013). Subsequent sub studies have found that risk factors contributing towards a diagnosis for MS and its progression tend to fall into one of two categories: (1) *deficiencies*: (vitamin D, iron, antioxidants, vitamin B12, unsaturated oils, decreased CNS blood flow) and (2) *aggravators*: (smoking, oxidation/inflammation, dietary saturated fat, infections, increased cholesterol/homocysteine/obesity, allergies/food sensitivity and psychological stress) (Van Rensburg et al., 2021; Johannes et al., 2023). To gain enhanced understanding and to improve the management of the condition for future therapeutic targets, pathological assessments need to be performed. MRI, which is an important imaging tool, particularly in terms of the in vivo evaluation of this pathology(Shenton et al., 2012), can assist in the monitoring of disease progression and can exhibit treatment efficacy. As MRI is an evolving modality, continuous verification and improvement of technique specificity is required in the detection of pathological findings (Wattjes et al., 2015). Therefore, MRI or any other imaging modality with a high specificity for MS can be applied longitudinally, after verifying its accuracy (Filippi, Preziosa et al., 2019).

2.9 MS and the environment

Environmental factors that affect MS onset include iron and vitamin deficiency, physical activity, diet, obesity and smoking (Van Rensburg et al., 2006; Ghasemi et al., 2017). Previous studies have supported the coherent view that MS is a multifactorial disease resulting from complex interactions between susceptibility genes and one or more environmental factors. None of the previously examined environmental factors or any single gene can be unambiguously identified as the causative agent, with such cumulative factors as the influence of genes and environmental factors potentially leading to onset of the disease(Akkad et al.,

2015). Environmental factors most likely to be associated with susceptibility to MS are sunlight exposure (mediation of vitamin D synthesis and ultraviolet radiation [UVR]) and infectious agents (like the Epstein-Barr virus [EBV]), although more evidence is required for its causal relationship with MS (Milo & Kahana, 2010).

2.9.1 Iron

Iron, which is an element that is found most abundantly in the body, is involved in numerous biological processes (e.g. immune system regulation and adenosine triphosphate [ATP] generation). Its functioning is fundamental to the maintenance of homeostasis. Iron dyshomeostasis has been implicated in the development of pathogenic T lymphocytes(Duarte-Silva et al., 2023). H-ferritin, which has been identified as the iron delivery protein for oligodendrocytes, is a critical micronutrient. Iron is, therefore, a crucial cofactor in the synthesis and maintenance of myelin (Connor & Menzies, 1996; Todorich et al., 2009). Increased iron concentration levels in the brains of patients with MS have shown global alterations that are noticeable in both MRI and histological studies. However, what remains unclear is the origin of this influx of iron into the tissue, or a relative reduction in tissue compartments in the absence of much iron(Schweser et al., 2021). In the researchers' study, the most obvious changes were noted in the iron stored by macrophages and microglia (Hametner et al., 2013). Although excessive free iron can be toxic, the presence of so much iron in such a form would only be likely to occur if the iron-binding capacity of the iron transport protein, Tf, is exceeded (>70%). Tf, which binds very strongly to iron, tends to mop up any free iron in the environment. In a biochemical study of 76 female MS patients, Tf saturation was found to exceed 40% in only 4 patients, with one-fifth of the patients having a Tf saturation of less than 20% (Herbert, 2016). Iron, which serves to maintain the integrity of oligodendrocytes and myelin, facilitates their regeneration following injury. The extracellular matrix, which is a key regulator of remyelination, can also serve to modulate iron levels(Stephenson et al., 2014). Overall, the research suggests that women tend to have lower iron levels than do their male counterparts, which may also be a contributing factor to the ratio inequality involved (4:1 [F:M]; Walton et al., 2020) in MS prevalence(Dobson & Giovannoni, 2019). MRI is found to be useful in identifying ischaemic lesions. In addition, more novel sequences, like susceptibility-weighted imaging (SWI), utilise the magnetic susceptibility variances of different tissues, including blood, iron and calcification in the diagnosis of neurodegenerative disease(Rahma et al., 2022).

2.9.2 Vitamin D

MS has long been thought to be an immune-mediated disorder of the CNS(McGinley et al., 2021). The functioning of the immune system is influenced by vitamin D, which acts as a modulator of immune responses, and which also plays a role in autoimmune diseases, including MS(Clark & Mach, 2016). Vitamin D deficiency, or variations in the deoxyribonucleic acid (DNA) sequence (polymorphism) of the vitamin D receptor gene, diminish its optimal

functioning within the immune system and, consequently, can lead to an increased risk of MS(Harandi et al., 2014). Vitamin D is a lipid-soluble vitamin, which acts like a hormone. Normal vitamins are essential organic compounds that cannot be synthesised by the body and must be ingested. However, vitamin D, which is also known as 1,25-dihydroxy vitamin D (1,25[OH]2VD) and calcitriol, can be synthesised (Sintzel et al., 2018). Vitamin D has chemical similarities to typical hormones, like oestrogen, testosterone and cortisol. The active form of vitamin D plays a crucial role in lymphocyte activation and proliferation, as well as in tissue-specific lymphocyte homing, T-helper cell differentiation, the production of specific antibody isotopes, and regulation of the immune response (Umar et al., 2018). According to Sintzel et al. (2018), the main sources of vitamin D are sunlight (ultraviolet B [UVB] in the 290–315 nm range), diet and dietary supplements. Photolysis of 7-dehydrocholesterol occurs in the skin to form pre-vitamin D3, which tends to isomerise into vitamin D3 or cholecalciferol. Vitamin D-enriched foods include fatty fish (e.g. mackerel, salmon), egg yolk, cod liver oil and shiitake mushrooms. The plant form of vitamin D is also known as vitamin D2 or ergocalciferol. Cholecalciferol and ergocalciferol are also available in fortified foods (e.g. milk, cereal, orange juice and cheese) and vitamin supplements(Sintzel et al., 2018). The main mechanisms of action of vitamin D in MS appear to be immunomodulatory, involving various categories of T and B lymphocytes in the general immune system; however, neuroprotector and neurotrophic mechanisms could also be exerted at the CNS level (Pierrot-Deseilligny & Souberbielle, 2017). The most important neuroprotective mechanisms are those preventing demyelination, increasing new myelin formation, preserving axons and promoting regeneration of transected axons. Questions remain regarding when, where and how hypovitaminosis D might be capable of influencing putative MS risk gene function, epigenetic markings, thymic selection, and deregulation of the effector T-cell-regulatory T-cells (Hayes & Nashold, 2017).

2.9.3 Vitamin B

The eight B vitamins are B1 (thiamine), B2 (riboflavin), B3 (niacin), B5 (pantothenic acid), B6 (pyridoxine), B7 (biotin), B9 (folate) and B12 (cobalamin). Although they have different functions in the human body, they often occur naturally in food and are water-soluble (Calderón-Ospina & Nava-Mesa, 2020). As mammals are unable to synthesise B vitamins on their own, they must ingest them in sufficient quantities with their diet. Instead of being produced by plants, vitamin B12 is produced by bacteria that colonise the foregut of ruminants or the colon of humans. The vitamin is found in red meat, dairy products, eggs, fish and liver (Calderón-Ospina & Nava-Mesa, 2020). Vitamin B12 is important in the normal functioning of the nervous system, due to the role that it plays in the synthesis of myelin. Any deficiency can lead to demyelination, axonal degeneration, and, eventually, irreversible damage, due to axonal death(Najafi et al., 2012), especially in the spinal cord, where it causes irreparable damage, referred to as subacute combined degeneration of the spinal cord (Xiao et al., 2016). Vitamin B12 requirements increase in patients with chronic immune reactions or chronic myelin repair,

which may explain why CSF and vitamin B12 levels can be suppressed, even when serum levels are normal. A negative correlation exists between serum vitamin B12 levels and disease activity, which supports the relationship between vitamin B12 and demyelination(Kocer et al., 2009). Vitamin B12 deficiency is usually observed in middle-aged to elderly patients, whereas MS commonly occurs in young adults. Both diseases are autoimmune in origin, with their geographical and racial distributions being similar; however, the probability of coincidence is low. Patients with vitamin B12 deficiency may be more sensitive to the viral and immunological mechanisms of MS, due to vitamin B12 deficiency, folate deficiency and hyperhomocysteinemia of the T and B lymphocytes, natural killer (NK) cells, cytokines and adhesion molecules (Najafi et al., 2012).

Van Rensburg et al. (2006) concur that the folate vitamin B12-methyl transfer cycle needs to function effectively, as it is necessary for the production and maintenance of myelin. The folate -vitamin B12 methylation pathway requires essential cofactors, like pyridoxine (vitamin B6), folate (vitamin B9), and cobalamin (vitamin B12) for the functionality of several enzymes, which can also cause increased homocysteine levels, indicating dysfunctional methylation (Davis et al., 2014).

2.9.4 The Epstein-Barr virus and MS

Although the aetiology of MS remains unknown, according to Lemprière (Zhang et al., 2022), a strong association seems to exist between the EBV and MS. Bar-Or et al. (2020)also stress the importance of focusing on B-cells, reporting that B-cell depletion by antibodies can be a controlling factor in MS. The EBV is a viral infection affecting 90% of the human population, with their seeming to be a strong suggestion that the disease is linked to the experiencing of stressful episodes (Latour & Winter, 2018). The EBV, which is also referred to as ‘kissing disease’, explains the role played by disease transmission and typical infection sites, including the lymph nodes and throat(Barnes, 2022). MS involves the immune system in the demyelinating process, and, therefore, the destruction of neurons within the brain and spinal cord. The immune system affects the demyelination process, suggesting that the process could be initiated by viral infection (Lassmann & Bradl, 2017). In humans, such infection can remain dormant for years, and, once in remission, it can reinfect both immune and normal cells, and reprogramme their functioning. The EBV has been found in MS demyelinated lesions, suggesting that demyelination can be initiated by such viral infection (Barnes, 2022). However, latent EBV infection occurs in 90% of the general population, with it not being specific to MS(Burnard et al., 2017).

2.9.5 Physical activity and obesity

Motl (2014) found that regular exercise is beneficial for persons with MS who have mild-to-moderate disabilities. The researcher based his evidence on meta-analyses indicating that exercise training improves fatigue, walking disability and QoL among individuals with MS(Motl,

2014). With increasing disability, a noticeable decline occurs in physical activity, which can result in depression, apathy and reduced QoL. A decrease in physical activity in people with MS is perpetuated by a decrease in activities of daily living (e.g. daily grooming, dressing and the performing of household chores) and recreational activities(Backus, 2016). People with MS may not experience the necessary/minimum amount of daily exercise required to achieve sedentary adulthood without neurological injury or disease. Such a cycle of decline in mobility and activity can lead to progressive deterioration in normal functioning persons, leading to the risk of such secondary adverse health conditions as diabetes, obesity and cardiovascular disease (Backus, 2016). Decreased physical activity leads to deconditioning, problematising participation in physical activity, which, in turn, exacerbates physical inactivity. Apathy, depression and reduced QoL may ultimately result from a lack of exercise(Klaren et al., 2013). Exercise training has been identified as one of the best therapeutic strategies for inclusion in comprehensive MS care (Motl, 2014), as exemplified in the case study of an MS participantt who remained a marathon runner 15 years after her MS diagnosis (Johannes et al., 2023).

2.9.6 Diet (nutrition)

MS is thought to result from a combination of genetic predisposition, environmental influences and established environmental risk factors, including low vitamin D levels, sun exposure, smoking and viral exposure. Diet is a major source of environmental interaction, with dietary metabolites exerting far-reaching systemic effects, rendering diet an attractive candidate as a potential environmental mediator in MS(Katz Sand, 2018). Obesity, due to the spread of the westernised diet, has resulted in adverse effects and in an increased potential for developing MS. Other diets, such as the Mediterranean, calorie-restricted, McDougall, Swank, Wahls and intermittent fasting diets, all emphasise the intake of vegetables and fruits (Wahls et al., 2021). Such eating patterns contain calorie restrictions, together with many different vitamins and nutrients that may synergistically affect MS (Evans et al., 2019). Previous surveys have indicated that the vast majority of PwMS are interested in, or already implementing, dietary modifications (Brenton & Goldman, 2016; Evans et al., 2019). However, effective treatment options for fatigue are limited. Emerging evidence suggests that diet and vascular risk factors, including obesity and hyperlipidemia, may influence MS progression and improve QoL (Yadav et al., 2016). Evans et al. (2019) also suggest the following of a specific, non-westernised diet, which may be useful as an adjunct therapy for the modifying of outcomes among PwMS.

2.9.7 Smoking

Smoking is an established environmental risk factor associated with MS, with both the duration and the intensity of the habit contributing independently to the risk of MS (Hedström et al., 2016). According to Manouchehrinia et al. (2013), regular smoking is associated with more severe disease and faster disability progression, whereas smoking cessation, whether before

or after the onset of the disease, can slow down the progression of disability. MS patients with smoking habits tend to have far worse long-term prognoses, an increased rate of brain atrophy, and a greater disability burden. This is a common environmental hazard, with severe health consequences(Rosso & Chitnis, 2020). Poorolajal et al. (2017) suggested a potential relationship between certain genotypes and smoking habits. The researchers further referred to experimental studies showing that exposure to cigarette smoking can increase genetic susceptibility attributable to established genetic factors (DRB1 and HLA-A). Smoking is an immunosuppressive agent in the lungs, which can result in a proinflammatory cascade, culminating in autoimmunity. Inflammation may increase the risk of MS in some individuals, in a process that is driven by antigen cross-reactivity between lung and myelin antigens. Additionally, cyanates, free radicals and carbon monoxide in cigarette smoke can be directly toxic to neurons (Rosso & Chitnis, 2020).

2.10 Biochemical factors and MS

2.10.1 Fatty acids and their role in myelination

Fatty acids play a fundamental role in the biology of all living organisms. Fatty acids restore and provide energy, influence the properties of biomembranes, and are also involved in the cell signalling process. Long-chain polyunsaturated fatty acids (PUFAs), like eicosapentaenoic acid (EPA), docosahexaenoic acid (DHA) or arachidonic acid (ARA), are involved in many (patho)physiological processes. These eicosanoids and other oxylipins are highly potent lipid mediators regulating, for example, inflammation, vasoconstriction or the experiencing of pain (Koch et al., 2021). The first study of MS and fatty acids was conducted by Roy Swank in the 1940s in Norway. The researcher found a lower incidence of MS, and lower occurrence of disability, in people living near the sea and eating fish, than in people who lived in mountainous areas and who ate comparatively more red meat (Jagannath et al., 2018). Swank deduced that eating saturated fat was detrimental, and that ingesting unsaturated fatty acids instead was beneficial, in terms of the progression of MS. He conducted an intervention study over 35 years, in which he demonstrated that MS patients eating <20 grams of saturated fat per day, together with unsaturated oils, tended to suffer less from disability and tended to have a longer lifespan than did those patients who ate >20 grams of saturated fat per day (Chenard et al., 2019). Jiang et al. (2019)also reported from their study, which was undertaken in relation to mice, that the administration of omega-3 polyunsaturated fatty acids (n-3 PUFAs) after the onset of ischemia tended to promote histological and neurological functioning in both young and aged mice, especially when DHA and fish oil (FO) dietary supplementation were combined. The changes in short-chain fatty acids (SCFAs) concentration were reported as being observable in various cardiovascular diseases, including heart failure (HF), in both experimental animals and humans. The relevant literature suggests that SCFAs can play a

significant role in the pathology of HF, possibly through having an agonistic effect on G-protein-coupled receptors, histone deacetylases (HDACs) inhibition, the restoration of mitochondrial function, the amelioration of cardiac inflammatory response, its utilisation as an energy source, and remote effect, attributable to a protective effect on the other organs concerned. All such possible mechanisms have not yet been entirely clarified and still require further investigation (Yukino-Iwashita et al., 2022).

2.10.2 Gut microbiota

Olsson et al. (2021) claim that the accumulating evidence suggests that human gut microbiota and the associated metabolites tend to influence MS pathogenesis. Yukino-Iwashita et al. (2022) also lean towards the theory of gut microbiota-derived metabolites, namely SCFAs, which might influence the pathology of a variety of diseases (Johannes et al., 2023).

2.10.3 Homocysteine

Smith et al. (2021) claim to have found over 100 diseases that are implicated by raised levels of concentration of plasma total homocysteine. In addition, they also highlight five conditions that can be prevented by means of lowering the total homocysteine, namely impaired childhood cognition, macular degeneration, primary stroke, neural tube defects and cognitive impairment in the elderly. Homocysteine (HCY) can be described as a neurotoxic amino acid that accumulates in patients with neurodegenerative disorders. The amino acid concerned, which contains a thiol group, derives from methionine metabolism that increases the vulnerability of the neuronal cells to both excitotoxic and oxidative damage(Thaimory et al., 2021). Homocysteine, which is produced during methionine metabolism, can be removed either by conversion or by remethylation to methionine. Methylation is vital for normal cell function, due to the key roles that it plays in protein, lipid and DNA metabolism, gene expression and myelination. Homocysteine can also cause damage to CNS cells or influence macrophage activation, with both processes being important aspects of MS pathology (Zhu et al., 2011). Clinically, the importance of such associations becomes relevant where the lowering of plasma total homocysteine by means of vitamin B treatment can serve to achieve the prevention of disease and the improvement of health outcomes (Smith et al., 2021). Increased levels of homocysteine, also known as homocysteinemia (HHcy), are a major, yet under-recognised, risk factor for vascular contributions to cognitive impairment. Commonly, in the elderly, vitamin B deficiency is the main contributor to such condition(Price et al., 2018). Hyperhomocysteinemia (>15 μmol/l) can also lead to the enhancement of the adverse effects of such risk factors as hypertension (HTN), lipid and lipoprotein metabolism, as well as the promotion of inflammation development (Ganguly & Alam, 2015). Davis et al. (2014) describe the association between an increased level of homocysteine levels and vascular damage in PwMS. Epidemiological studies previously undertaken indicate that high levels of homocysteine may be a risk factor for the development of neurodegenerative disorders. The

presence of elevated blood homocysteine levels in disorders of the CNS and the identification of vitamin B or folate deficiencies in PwMS have prompted investigation into the contribution made by homocysteine levels and MS(Zhu et al., 2011). However,Shemirani et al. (2023)found that lowering homocysteine levels with vitamin B12 supplementation did not improve mood in their MS patients.

2.10.4 Cholesterol

Cholesterol, which is known as a major component of myelin, is an important element in maintaining homeostasis in the brain (Murali et al., 2020). The immune response following on injury or disease entails the clearing out of excess debris from the cells involved by means of the action of phagocytes. The phagocytes concerned serve to metabolise the cholesterol components and to transport them from the cells onto lipoprotein particles (Deng et al., 2023). MS is well-known for its inflammatory processes, including the loss of myelin and axons. Cholesterol is an essential component of mammalian cellular and myelin membranes. The cholesterol in myelin membranes is manufactured by the oligodendrocytes themselves and is not obtained from the blood(Todorich et al., 2011). Dyslipidaemia or abnormal cholesterol levels, namely decreased high-density lipoprotein (HDL) cholesterol and increased low-density lipoprotein (LDL) cholesterol levels, play an accepted role in terms of cardiovascular disease and atherogenesis. According to Boshra et al. (2022), MS is associated with an increased risk of stroke and myocardial infarction, especially after the first year of diagnosis. Furthermore, Kemp et al. (2023) postulate the risk factors for coronary artery disease (CAD) to be the same as are those for cerebrovascular disease. All the major lipoprotein classes, which represent a heterogeneous group, differ in particle size, densities, apoprotein content and migration characteristics, as well as playing its own role in relation to the disease. Therefore, the different subfractions may vary in their risk profile, with patients with similar, or with even normal, HDL and LDL levels may vary in their degree of risk for the development of cardiovascular disease(Radikova et al., 2018). Such pathological features translate into clinical disability, and highly variable and unpredictable disease course(Murali et al., 2020). Currently, the need exists to develop readily available biomarkers that will relate to disease evolution and outcomes, which should serve to enhance understanding of the disease process. Cholesterol and markers of cholesterol turnover, which may contribute to the pathogenesis of MS, may prove to be useful in terms of the biomarkers of disease activity and treatment efficacy(Zhornitsky et al., 2016).

2.11 Disability and MS

MS, which is a debilitating disease affecting the CNS, often affects the cognitive domains, including memory, visual impairment, the processing of information speed, and acquisitive difficulties(De Caneda & De Vecino, 2016). The EDSS is a neurological examination that has been widely accepted as the gold standard measurement for the severity of MS (Cao et al.,

2013). The scale, which quantifies the extent of disability in MS patients, plots the available data according to the person's signs and symptoms during their neurological assessments (Kurtzke, 2015). Such plotting is complicated, with, according to Ellenberger et al. (2020), uncertainty remaining about whether it is acceptable to rely solely on the EDSS score for disease severity. One solution to the situation has been to derive a ratio of the EDSS and time from onset (in years), in the form of the Progression Index (PI). However, the fact remains that, as no linear approach exists for disability when measured in terms of the EDSS, such an approachis limited (Roxburgh et al., 2005). The Multiple Sclerosis Severity Score (MSSS) corrects the EDSS regarding the duration of the disease, by means of utilising an arithmetically simpler method for comparing the individual`s disability with the distribution of scores, in cases with equivalent disease duration(Zhou et al., 2020). Zhou et al. (2020) found differences in the disability accrual rate between progressive- and relapsing-onset of MS, which had a significant effect on the MSSS. Further recommendations were to use an onset-specific MSSS, when comparing the rate of disability progression among progressive-onset cases and for mixed cohorts(Zhou et al., 2020).

2.12 Genetics and hereditary aspects of MS

The past decade has witnessed an expansion in the understanding of MS genetics, from a handful of replicated variants to over 200 robust associations (Kim & Patsopoulos, 2022). More than 2.3 million people worldwide are currently affected by the disease, often resulting in a high socioeconomic burden. The prevalence of MS varies between countries, from 1 to 100 cases per 100 000 individuals. Such heterogeneous prevalence is believed to be due to a combination of differences in healthcare infrastructure and environmental differences and degree of access. Western countries at higher latitudes tend to have a higher prevalence of MS, with it having been suggested that the extent of sun exposure, and the resultant vitamin D levels, could be the main causal factorof such variance (Kim & Patsopoulos, 2022). In the 1970s an association was formed between MS and the extent of variations in the genes encoding human leukocyte antigens (HLAs) within the major histocompatibility complex (MHC). However, most recently with the advent of genome-wide association studies (GWASs), over 100 genetic variants were revealed, which enabled the identifying of immunogenetic markers (e.g. interleukin-2 receptor alpha chain [IL2RA], interleukin-7 receptor subunit alpha [IL7RA]). Studies of the natural history of MS suggest it to be a two-phase disease: during the first phase, inflammation is focal, with flares, whereas, during the second phase, the disability involved tends to progress independently of focal inflammation(Leray et al., 2016). As neuroinflammation is almost always present within the pathogenesis of MS, histopathological studies have helped to formulate an association between neurodegeneration and demyelination, with the production of inflammatory molecules by both blood-derived immune cells recruited to the CNS and by the activated resident microglia. Prolonged ongoing processes of damage/degeneration of cytokines reactive oxygen species (ROS), reactive

nitrogen species (RNS) and chemokines all play an essential role in disease onset and progression(Olla et al., 2021). According to Muñoz-Culla et al. (2013), the HLA locus, which was one of the first MS-related genetic factors to be discovered during the 1970s, lies in the short arm of chromosome 6, in the region of the MHC. The region concerned is also known for the presence of gene-encoding highly polymorphic cell-surface glycoproteins, which are key components of the immune system(Muñoz-Culla et al., 2013). HLA-DR2+, HLA-DQ6, DQA 0102 and DQB1 0602, HLA-DRB1, DR15, DRB1*1501 and DRB1*1503 are all genes that are susceptible to the onset of MS(Ghasemi et al., 2017). Populations of European ancestry with GWAS have shown that susceptibility to MS is associated with 32 independent signals at the MHC, of which there are 200 additional autosomal loci and one X-chromosome locus. The existence of such signals collectively explains up to ~50% of the estimated genetic heritability of MS. However, additional research is required, as such findings alone fail to explain the genetic architecture of MS in other ancestral populations(Jacobs et al., 2019). Future definitive studies of endophenotypes and disease progression have yet to be performed, with the majority of the identified MS variants still requiring functionally characterisation. Such shortcomings notwithstanding, accessing an understanding of the causal mechanisms of MS, is still possible (Kim & Patsopoulos, 2022).

2.13 Magnetic resonance imaging: components and physics of the magnet system

MRI consists of both electrical and mechanical components, with the main hardware of the MRI scanner including the following: the superconducting magnet; the gradient and radiofrequency coils; signal/image creation/formation; and basic MR sequences.

2.13.1 The superconducting magnet

This magnet system, which is currently the most widely used, is known for generating the strong homogeneous static magnetic fields that are required for clinical magnetic resonance imaging(Tadic & Fallone, 2012). Each magnet consists of miles of superconducting wire, which are wound in rings and immersed in a liquid bath of helium (He), as can be seen in Figure 2.3. The helium concerned is kept at extremely low temperatures (approximately -269°C), to cool down the relevant wires(Lvovsky et al., 2013). Such systems are capable of enhanced field strengths, improved temporal stability, and higher field uniformity than are their permanent and resistive magnet counterparts(Tadic & Fallone, 2012). Such highly advantageous features permit faster imaging, greater spatial resolution and superior image quality than do comparative systems (Setsompop et al., 2016).

2.13.2 The gradient coils

An MRI image is normally created by means of imposing linear gradient fields and the encoding of spatial information from an object, in the frequency and phase of the acquired MR signal(Hamilton et al., 2017). Gradient coils tend to generate gradient fields by means of

carrying coils of wire which, in turn, produce low-frequency currents that play out a myriad of pulse sequences. Gradient fields, which must be accurately linear and intense, can switch rapidly between signals (Poole et al., 2012). Each scanner has three sets of gradient coils, with one in each (x, y, z) plane, whose function it is to produce deliberate variations in the main equilibrium magnetic field, or B0. Such variation allows for the localisation of the different image slices, namely axial, coronal and sagittal, without moving the subject, as well as for phase and frequency encoding (Holmes et al., 2019).

2.13.3 The radiofrequency coils

Radiofrequency fields (RFs) are used to introduce additional energy (in the form of radio waves) to the static magnetic field, thereby causing the deflection of the magnetic vector(Keevil, 2016). After the excitement of the hydrogen protons in the body, the subject emits an RF signal, which is received and measured by the surface coil. The RF antennae are responsible for transmitting excitation pulses and for signal reception (Gruber et al., 2018). After some time, the magnetisation vector returns to equilibrium, resulting in two main imaging parameters, known as T1 and T2 (weighting), which directly relate to the image contrast. The antennae concerned are commonly referred to as RF coils, with the main RF coil, which is also known as the 'body coil', being situated within the bore of the magnet (Westbrook, 2016).

2.13.4 Signal/image creation/formation

The human body consists of an abundance (approximately 63%) of hydrogen atoms (water molecules). Image formation in MRI primarily depends on the inherent magnetic properties of human tissue, which are used to create tissue contrast (Westbrook, 2016). Atoms are made up of a nucleus, which consists of protons and neutrons, and a shell, which consists of electrons. Protons, which have a positive electrical charge, possess a spin, the movement of which results in the creation of an electrical current, with an electrical current inducing magnetic force, or a magnetic field (Westbrook, 2016).

Lerch et al. (2017) identify the following four basic steps in image formation:

- Step one: The patient is placed within the magnetic field. Random moving protons in the patient's body align and process themselves along the external magnetic field. Longitudinal magnetisation forms along the Z-axis.
- Step two: On the emission of an RF pulse, all the precessing protons pick up/absorb the energy from the RF signal, which they process to a higher energy level. The resultant effect is a reduction in longitudinal magnetisation and the formation of transverse magnetisation in the X-Y planes.
- Step three: The transverse magnetisation (in the transverse plane) generates a current that is received as a signal by the RF coil.

- Step four: The MR signal received by the coil is then transformed into an image, by means of using a mathematical process, like the computerised Fourier Transformation.

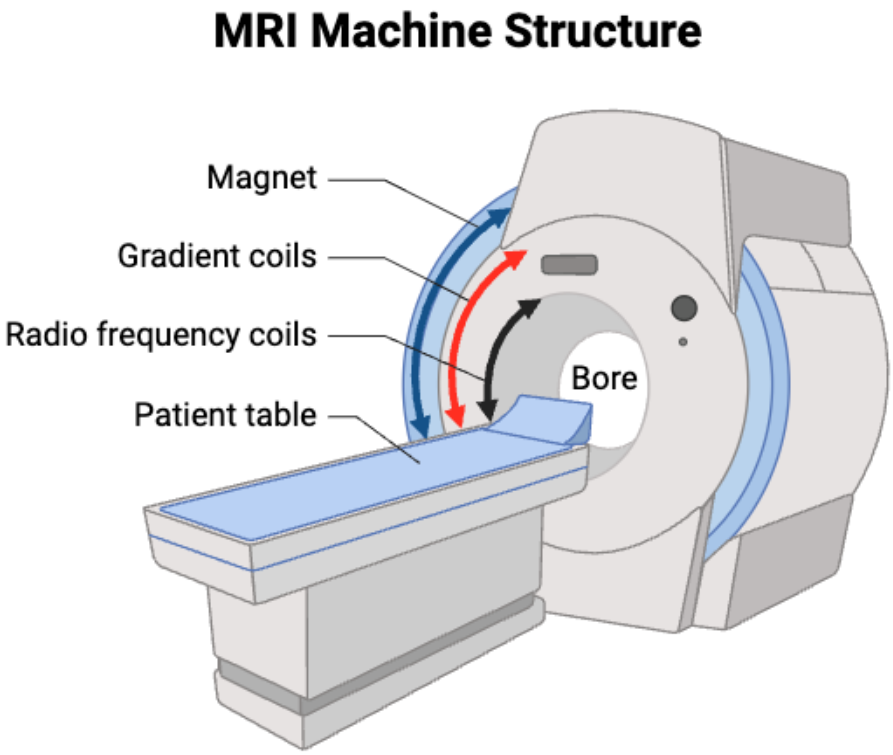


Figure 2.3: The magnet system in terms of the MRI machine structure
Source: Adapted from the MRI machine structure by Biorender.com (2023). Retrieved from https://app.biorender.com/bioremder-templates.

2.13.5 Basic MR sequences

A T1WI sequence refers to tissue with a short T1, which appear as bright images (like fat), from their regaining most of their longitudinal magnetisation during the repetition time (TR) interval, resultantly producing a relatively strong MR signal, while tissues with a long T1 appear darker (like muscle), as they do not regain their longitudinal magnetisation (Weishaupt, Kochli, & Marincek, 2006; Bushong, 2015). The T2WI sequences are generated through echo time (TE), which is the interval falling between the application of the excitation pulse and the collection of the MR signal. Tissues with a short T2 image appear darker (like a hematoma) on T2WIs, whereas tissues with a long T2 appear bright (like a tumour) (Weishaupt, Kochli, & Marincek, 2006; Bushong, 2015). PD-weighted images are determined by means of the density of the protons that are present in the tissues. The T1 effect is reduced by means of keeping the TR long, with the T2 effect being reduced by means of keeping the TE, short. A PD-weighted image has a long TR and a short TE, with the number of protons in the tissues available determining the signal intensity concerned (Westbrook, 2016).

2.14 MRI and MS

The McDonald criteria, as revised in 2010, is the method of choice in the diagnosis of MS(McDonald et al., 2001 Polman et al., 2011). The criteria concerned are based on the evaluation of clinical symptoms (as at presentation with CIS and/or the patient's history) and on the MRI of the CNS(Giorgio & De Stefano, 2013). The diagnosis of MS, which is founded on clinical evaluation, can offer clinicians a range of applications for the management of MS, including insights into pathogenesis, and enable the development of understanding of prognostic relevance to individual patients. Furthermore, such a diagnosis also assists in monitoring the effects of therapy(Lövblad et al., 2010). The relevance of MRI as a tool for examining MS, especially during early diagnosis, has proven to be valuable as a non-invasive method of obtaining images, which is readily available, and has proven to be very effective for disease monitoring. MRI can also differentiate between old, new and active lesions (Filippi, Brück et al., 2019)**.** Recently, significant advances have been made on the technological front for MRI, in terms of it offering the prospect of heightened specificity including in measurements of the brain(Ozturk et al., 2010). The advances made include the application of such MRI techniques as DIR imaging and dynamic contrast-enhanced imaging, among others. Multimodal MRI techniques and combined histopathologic-MR studies have helped shed light on the diverse pathological mechanisms underlying MRI changes, as seen in both white and grey matter (Bakshi et al., 2014). Also, the use of such innovative postprocessing tools as lesion-inpainting algorithms, regional voxel-wise approaches, and composite MRI scales have facilitated the assessment of both global and regional white and grey matter damage resulting from MS. Such methods offer a new pathway, linking MS with its clinical status (Bakshi et al., 2014). MRI, which is an important tool for diagnosis, clearly demonstrates both the temporal and the spatial distribution of lesions (Chen et al., 2016). In recent times, increasingly refined MRI sequences have been developed that have come vastly to improve the characterisation of brain volumes and focal WM lesions(Ceccarelli et al., 2012). Classically, focal hyperintense T2 and FLAIR sequences tend to reflect varying degrees of inflammation, gliosis, demyelination and axonal injury. The characteristic of plaques on MRI reflects the heterogeneity of MS (Cahalane et al., 2018). To improve understanding of the mechanism of MS, it is important to elucidate those strategies that can be used to help repair the damaged myelin and axonal structures involved (Lemus et al., 2018). Therefore, special interest has been shown in the CC, which is known to be the largest fibre bundle in the brain, with it connecting both hemispheres, and being an essential element for investigating the impact of MS on cognition and physical disability(Llufriu et al., 2012).

MRI sequences used for imaging multiple sclerosis patients, consists of the following:

Table 2.3: Differences between the conventional and the newly developed protocols.

	Conventional protocol	**Scan time**	**New protocol**	**Scan time**
	t2_tirm_tra_dark fluid_fs	1 min 54 sec	t2_tirm_tra_dark fluid	14 min 08 sec
	t2_tirm_sag_dark fluid_fs	1 min 52 sec	pd_tse_tra_3mm	7 min 08 sec
	T2_tse_tra	1 min 42 sec	t2_tse_cor_3mm	5 min 35 sec
	Dwi_tra_b0,1000_adc	1 min 37 sec	t1_mprage_tra	7 min 49 sec
	t2_swi3d_tra_p2_1.5mm	2 min 28 sec	t2_swi3d_tra_p2_1.5mm	4 min 20 sec
	t1_sag	2 min 17 sec	BME_DTI_30gr_4b0_2mm_ISO_52sl_AP	5 min 23 sec
	t1_tra_5mm	6 min 39 sec	resolve_4scan_trace_tra_p2_192	6 min 29 sec
	t1_tra_mprage_pre	4 min 39 sec	t2_tirm_sag_dark fluid (optional)	4 min 32 sec
	Gadolinium injection			
MRI sequences	t1_tra_mprage_post	4 min 39 sec		
Total scan time		25 min 47 sec		54 min 04 sec

Important to be aware off is that every institution/ department have their own protocols set-up, and this is dependent on the specific Radiologist/s involved and based on their preferences.

Table 2.4: Comparison between Conventional protocols and the New Protocol used within this study.

MRI Sequences for MS	Conventional Protocol	New Protocol
t2_tirm_tra_dark fluid_fs (In both the Sagittal and axial planes)	Suppresses the fat signal and highlights water rich tissues. Shorter scan time involved.	Provide excellent contrast between white and grey matter and better spatial resolution

		between adjacent structures. Longer scan time though.
T2_tse_tra	Performed in the axial plane, and has a shorter scan time, but an increase in the slice thickness.	Performed in the coronal plane, the scan time is significantly longer, the slice thickness is smaller hence better image quality.
pd_tse_tra_3mm (Proton Density) Displays the hydrogen atoms in any given area/tissue.	N/A	
t2_swi3d_tra_p2_1.5mm	Displays 3D imaging, highlighting the differences in tissue magnetization.	Allows for imaging of active lesions. The time is slightly longer(smaller slice thickness and no gaps in-between slices).
t1_sag (Performed in the sagittal and the axial plane)	The longitudinal relaxation time, measures how long it takes for protons in a tissue to realign with the external magnetic field.	N/A
Dwi_tra_b0,1000_adc		
BME_DTI_30gr_4b0_2mm_ISO_52sl_AP		
t1_tra_mprage (Magnetization Prepared Rapid Gradient Echo)	Performed before and after Gadolinium administration. A 3D image providing excellent anatomical detail.	Performed only once and no Gadolinium needed. Scan has high quality resolution, due to the smaller slice thickness and matrix values.
Gadolinium Administration	Performed to enhance existing and newly formed white matter lesions and other pathologies.	Not administered, minimises adverse reactions one can have during these injections.

2.15 Conclusion

MS is a complex disease that is influenced by various factors, including environmental components, biochemical factors, lifestyle choices and genetics, with all such factors playing a pivotal role in the disease's onset and progression. The current chapter discussed the

increasing prevalence of MS and the need to enhance understanding of the disease pathogeneses, to aid with improving treatment plans and disease outcomes. The literature reviewed suggests that adopting advanced approaches to MRI provides valuable diagnostic information for studying the structural and functional changes associated with the clinical characteristics found in MS. However, the need still exists for further research to be undertaken, to enable the identifying of the causes, and the development and disability progression, of the disease, which should come to inform advanced treatment methods for helping to ensure the improved QoL in MS patients. Chapter 3 describes the research design and methodology used. In addition, the ethical considerations relevant to the present study, the recruitment strategy and sampling techniques employed, the data acquisition process, and the data analysis undertaken will be discussed.

CHAPTER 3
RESEARCH METHODOLOGY

3.1 Introduction

Chapter 3 provides a comprehensive discussion of the research methodology used in the current study. Firstly, it is necessary to state that this was a discreet imaging study that formed a component of a larger research project on MS, which received ethical approval, titled: “The implementation of a comprehensive gene-based, pathology-supported intervention program in patients with disorders that includes a component of demyelination such as multiple sclerosis (MS)” NO7/09/203 (Division of Chemical Pathology, Tygerberg Hospital / Stellenbosch University). Reliability of the study results was ensured by supervision provided by experts in the field of MRI Dr C.J. Hatting, a neuropsychologist and Associate Professor F Robertson (a Neuroimaging Researcher) and our analysis was closely monitored by a Professor Martin Kidd a, statistician. According to Professor Martin Kidd (statistician, University of Stellenbosch) a minimum of 25 patients is required for this study to be of significance. The prevalence of MS within South Africa is very low. Out of a South African population of approximately 56 million, an approximate 5 000 persons suffer from MS. This accounts for less than 1% of the South African population.

3.2 Research study design

The research concerned contributed to a retrospective, cross-sectional study. MRI brain scans of 25 participants clinically diagnosed with MS, according to the McDonalds criteria as revised in 2017 (Thompson et al,2018), and brain scans of 25 MS-free participants, whom had demographic, biochemical, and lifestyle data were all assessed looking for brain lesions. All the scans were performed at the University of Cape Town MRI Unit (Cape Universities Body Imaging Centre [CUBIC]), situated at Groote Schuur Hospital (GSH), Cape Town, by the principal investigator concerned. A Siemens MAGNETOM Skyra 3.0 Tesla scanner was used to perform the scans, which involved a variety of scanning sequences, which will be discussed later on in the present chapter. This design were chosen to develop a new MRI protocol, which will assist in the future with MS diagnosis(without the use of contrast),as well as provide the best possible MRI imaging.

3.3 Participant recruitment and enrolment.

The participants were recruited from the database of the main study, as well as via word of mouth, media and the MS Care Trust. The scanning involved was performed voluntarily, with consent given by completing a MRI safety questionnaire, before commencing the scan. All the

participants were given codes(as not to reveal their real identities), and only the principal investigator and the study coordinator were privy to those details.

3.4 Research questions

The research questions asked were the following:

- How does the number of lesions present throughout the brain relate to the EDSS at the two extremes of the EDSS score spectrum?
- How do the results of the EDSS score and lesion number affect disability?
- What role does MRI play as a disease marker, and how can it aid in the prediction or/ and monitoring of disease progression?

3.4 Aim of the study

This case controlled study was aimed at developing an MRI protocol of high resolution and at utilising the data to compare the association between lifestyle factors, biochemical results and genetic tests, so as to be able to identify the risk factors associated with disease progression, so as to demonstrate the value of MRI in MS.

3.5 Research objectives

The objectives of the present study were the following:

- to confirm MS diagnosis, using the McDonald criteria, as revised in 2017 (Thompson et al., 2018).
- to evaluate disability, as assessed in terms of the EDSS;
- to determine the relationship between lesion load and disability progression, in association with biochemical markers in MS;
- to develop a lesion analysis MRI protocol encompassing several related neuro-imaging measures, so as accurately to characterise lesion load in MS; and
- to use MRI images to establish the correlation between the status of the corpus callosum (CC) and MS disease progression, specifically focusing on its dimensions and lesion distribution within the part of the brain concerned.

3.6 Sample

3.6.1 Study sample

A cohort of sporadic MS cases (n=25) was included from a distribution of the severity scale, the disability progression, and imaging features, so as to determine the specific factors that are involved in disability progression.

3.6.2 Sample size

A minimum of 25 participants with MS was required, so as to be able to demonstrate statistically significant results. Twenty-five (25) age-matched controls without any neurological diagnosis, were recruited for comparison and to further highlight the differences for potential risk factors. The formula used for the calculation of the sample size was as follows:

$n = 4(p)(1-p)/\text{interval}^2$ $n = 4(0.5)(1-0.5)/(0.2)^2$

$= 4(0.5)(0.5)/0.04 = 1/0.04$

$= 25$

n = sample size

p = observed population (0.5=50%)

confidence interval -0.2 (20%)

Clinical data of 22 pwMS and 21 Controls whom had a 3-T MRI scan were assessed. However,7 of the participants within the control group could not be scanned due to claustrophobia and other illnesses. Although we could not gain any imaging information from these participants, we still had other data samples from their biochemical markers (inflammatory markers, lipids, vitamins, cardiac markers / risk factors, and haematinics). Genetic analyses was conducted focussing on the extent of variations in the genes encoding human leukocyte antigens (HLAs) within the major histocompatibility complex (MHC). Single nucleotide polymorphism were conducted in the following metabolic pathways: lipid and lipoprotein metabolism, homocysteine and folate metabolism (Davis et al., 2014) ; hypertension (Davis et al., 2014); obesity and insulin resistance (Johannes et al., 2023).; and inflammation, detoxification, oxidative stress(van Rensburg et al., 2016),haemostasis and thrombophilia; iron overload and iron deficiency (van Rensburg et al., 2019).

Genetic testing, biochemical results aligned with the environmental factors allows for preventative medicine by influencing clinically relevant metabolic pathways (Johannes et al., 2023).Genotyping was performed by real-time polymerase chain reaction (PCR) assays on the Roche Lightcycler LC480-II using hydrolysis probe TaqMan® single nucleotide polymorphism (SNP) genotyping assays. The HLA DRB1*1501 was investigated by use of a haplotype tagging SNP, rs9271366 (Field et al., 2010).

Lifestyle and disability factors were assessed by filling out a questionnaire. The disability status of the MS participants was assessed by a clinician using the Expanded Disability Status Scale (EDSS) (Kurtzke, 1983).

Participants diagnosed with MS had a voluntary option to follow a PSGT Program or not .The PSGT program was offered to the participants during the study and the program provides a method for implementing a personalised risk reduction plan and can be used by referring

clinicians to monitor and manage disease treatments|. Of the 22 pwMS, 13 had followed the Program for more than 10 years, while 9 had not followed the program (Jaftha, 2024).

3.6.3 The inclusion criteria for participation in the study

The inclusion and exclusion factors were discussed and agreed upon by clinicians and other referrers present at the initial meetings discussing the strategies for the upcoming study.
The following persons were included in the study:

- women between the ages of 18 and 60, with a confirmed diagnosis of MS (relapsing/remitting);
- MRI-compatible individuals;
- individuals undertaking MS disease-modifying therapies (DMTs);
- nonsmokers, or individuals who had refrained from smoking for five years prior to the date of enrolling for the current study; and
- normotensive individuals (including participants undergoing medical management for HTN).

3.6.4 The exclusion criteria for participation in the study

The following persons were excluded from participation in the study:

- those with a body mass index (BMI) of >30;
- those with any factors excluding them from undergoing an MRI scan (e.g. mechanical devices, metal fragments, etc.);
- those with smoking-related medical pathology, like chronic obstructive pulmonary disease (COPD);
- those with a previous medical history of cardiovascular disease and/or diabetes;
- those with an erythrocyte sedimentation rate (ESR) of >20mm/hour;
- those with other existing neuropathology, like meningitis or epilepsy;
- those with a creatinine level of >97µmol/L; and
- those with other comorbidities, like HTN (uncontrolled) or/and diabetes mellitus (DM).

3.6.5 Participant care and safety

The risks involved when undergoing MRI are minimal, provided that one adheres to the relevant safety precautions when preparing the research/study participants(both MS confirmed and control groups). To minimise such risks, an MRI safety questionnaire was required to be completed in each case, so as to ensure that the participants did not have any implants that could be affected by, or that could affect, the magnetic field involved (Appendix A). Although, quite often, MRI studies require the administration of contrast media, in the present study no contrast medium was administered, due to the possible side-effects associated with gadolinium administration(Hao et al., 2012). As the participants in the study might have

experienced a heating effect, due to the energy produced from the activated gradient coils, all clothing items with metal clips and containing unnatural fibres had to be removed, and replaced with a cotton gown, where necessary. Each participant received a panic button, which they could squeeze, should they have experienced any discomfort during the scan. In such an instance, those affected were to be attended to by the researcher, with the remainder of the required treatment being conducted keeping in mind the nature of their complaint(s). Should the participants have required any further, or urgent, medical attention, they could have been transferred to the medical emergency department at GSH.

3.7 MS diagnosis contributing factors

According to Waubant et al. (2019), more recent findings highlight the combined contributions made by all the multifaceted risk factors to the onset of MS. Chronic dysregulation of immune homeostasis resulting from complex interactions between infectious exposures, genetic predispositions and factors that lead to pro-inflammatory states, including smoking, obesity and low degrees of sun exposure, appear to be the precursor of such a disease(Waubant et al., 2019). Diagnosis can be made based on a combination of such signs and symptoms as those detected in radiographical findings (e.g. T2 lesions found on an MR scan), genetics and laboratory findings (e.g. in a sample taken of the cerebrospinal fluid), which are all components of McDonald (2017) criteria, as illustrated in Figure 3.1 (McGinley et al., 2021).

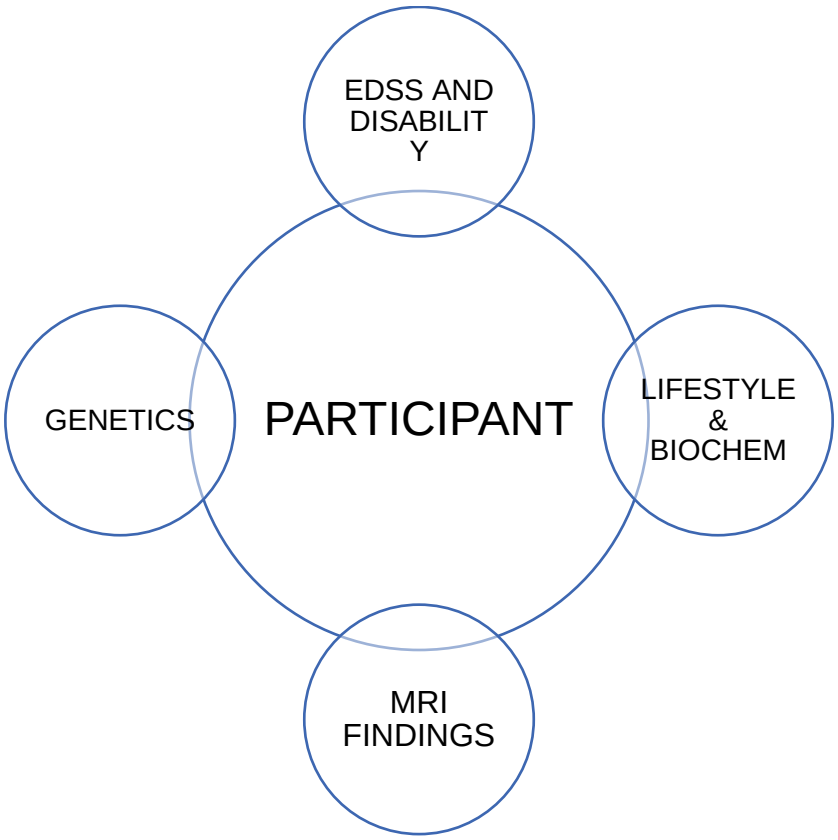


Figure 3.1: Diagram of the contributing factors influencing MS diagnosis and outcome status

3.7.1 EDSS and disability

The EDSS has been employed as a clinical scale, so as to enable the evaluation of the different levels of MS, as the disorder can disrupt the regulation of balance (Cao et al., 2013). The scale involved quantifies disability in MS patients, plotting the data concerned according to the person's signs and symptoms, during their neurological assessments (Kurtzke, 2015). In the present instance, the quantified data were acquired from eight functional systems in the body, each of which was assigned a score called the Functional System Score. The functional systems involved consist of the pyramidal, cerebellar, brainstem, sensory, bowel and bladder, visual and cerebral systems (see Appendix B). In the current study, a clinician (Dr Clint Johannes, an internal medicine registrar with neurological training in the department of internal medicine at Tygerberg Academic hospital, also in the process of studying towards his MMED degree), used the EDSS platform to assess all the MS participants involved. The EDSS protocol followed consisted of a group of questions that each participant was asked, with the attendant score being allocated according to their responses thereto. The steps ranged from 0.0 (no neurological fallout) to 10.0 (death due to MS).

3.7.2 Lifestyle and disability

All the participants involved were required to complete a medical and lifestyle questionnaire, with the questions asked pertaining to their eating habits (over three months), and their consumption of certain foods. Other information required included their family and medical history, their clinical symptoms and their treatment plans. Their physical activity, which was self-reported, was categorised into high (with exercise being undertaken four or more times a week), moderate (with exercise being undertaken two to three times a week), or low (with exercise being undertaken occasionally, or a complete lack of exercise) (see Appendix C).

3.7.3 Biochemical analysis

Blood samples were drawn for biochemical and genetic testing in the morning between 09h00 and 10h30, so as to facilitate the standardisation of any diurnal variation. Blood tests were performed by an accredited pathology laboratory in the department of chemical pathology at Tygerberg Hospital. The blood test selection were made to provide clinical information of the health of the participants.

The biochemical parameters were determined using the following methods:

- immunoturbidimetry
- chemiluminescence
- enzymatic method
- glycerol phosphate oxidase

- accelerator selective detergent method
- measuring liquid selective detergent

The blood parameters analysed included the following:

- **Iron parameters**
  Haemoglobin (l/l)
  Serum iron (µmol/l)
  Transferrin (Tf)
  Tf saturation %
  Ferritin (µg/l)

- **Methylation metabolism**
  Vitamin B12 (pmol/l)
  Serum folate (nmol/l)
  Homocysteine (µmol/l)

- **Inflammation**
  C-reactive protein (CRP)
  Vitamin D (ng/ml)

- **Lipid metabolism**
  Total cholesterol (mmol/l)

3.7.4 Genetic analysis

Genetic testing were performed at the Pathology Research facility(PRF), at Tygerberg Hospital, This data helped to inform us of any pre-existing pathologies, and if any association was formed between MS and the extent of variations in the genes encoding human leukocyte antigens (HLAs) within the major histocompatibility complex (MHC). All the Genomic DNA was extracted from anticoagulated whole blood samples using the Omega Bio-Tek E.Z.N.A® Blood DNA Mini Kit (Spin Protocol Norcross, GA, USA). Genotyping of single nucleotide variants was performed by high-throughput real-time PCR on the Roche LightCycler® 480II instrument (Indianapolis, IN, USA), using TaqMan® SNP Genotyping Assays (Thermo Fisher Scientific, MA,USA). To ensure accuracy, of the high-throughput genotyping was verified by direct DNA sequencing as the gold standard. Haplotype tagging by rs9271366 was employed to investigate the presence of the HLA-DRB1*1501 allele associated with increased risk for MS diagnosis (Johannes et al., 2023).

3.7.5 Fazekas Scale and lesion load

The Fazekas scale assisted with identifying the presence of WM hyperintensities (WMHs) found on the T2 MRI. The use of such a measuring tool helped in accurate characterisation of the lesion load of patients. Employing the Fazekas Scale provided an overall impression of the presence of WMH in all parts of the brain so affected (Wahlund et al., 2001). The Fazekas score can be ascertained for each lobe of the cerebrum, the cerebellum, and the brainstem, per hemisphere. According to the Fazekas Scale, lesions are characterised as can be seen in Figure 3.2 below.

3.7.5.1 Fazekas Scale for white matter abnormalities

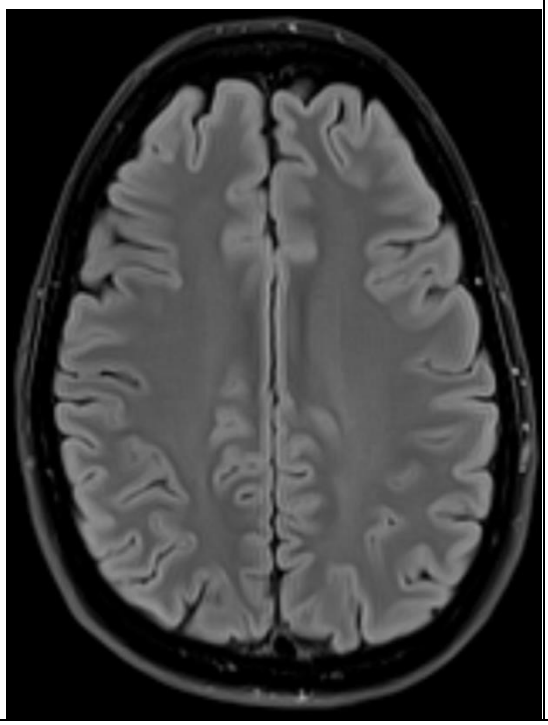
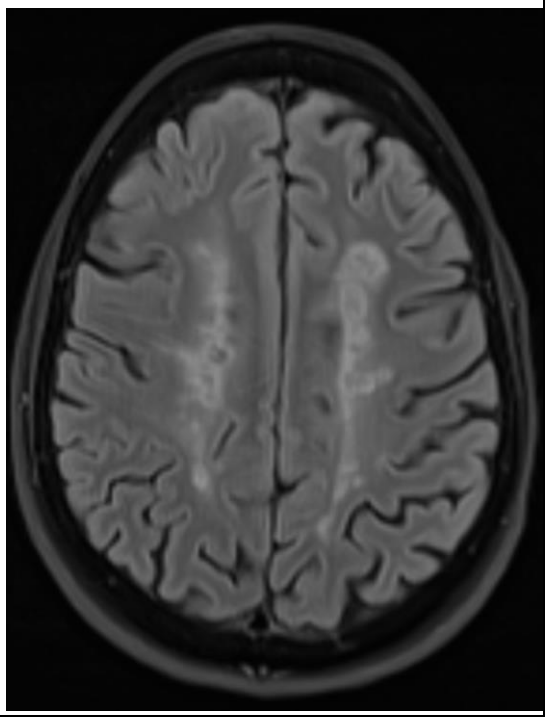
Fazekas 0 – None, or a single punctuate WMH lesion	
Fazekas 1 – Multiple punctuate lesions	
Fazekas 2 – Beginning confluency of lesions (bridging)	
Fazekas 3 – Large confluent lesions	
Example 1: Fazekas Scale 0	Example 2: Fazekas Scale 3
	

Figure 3.2: MRI brain images of the Fazekas Scale (reproduced from https://www.quantib.com), highlighting ranges from 0 to 3, indicating less and more severe WMH lesions, respectively**.**
(Permission to use the images was granted by the participant concerned.)

Tumefactive demyelinating lesions were also included in the imaging aspect of the extremes of outcome measure. According to the protocol of the current study, the spinal cord was not

assessed due to the new study protocol used, the enhanced images required extra time spent on the MRI scanner, with only brain lesions being included in the analysis.

3.7.6 Corpus callosum and its dimensions

Lassmann et al. (2018) report MS as being classified as a demyelinating disease affecting the brain and spinal cord by means of the accumulation of lesions, in both white and grey matter, and turning into diffuse neurodegeneration throughout the whole brain. The CC is described as being the largest fibre tract in the brain and the connecting component between the two cerebral hemispheres. The part of the brain concerned is involved in the performance of complex tasks (Ozturk et al., 2010). Yaldizli et al. (2014) define the CC as a region in the brain where MS lesions can most commonly be found. The CC is of particular interest, considering its structure, consisting of up to 250 million transversely oriented nerve fibres (Sotgui et al., 2022). The WM tract, which contains transversely connecting oriented axons, serves as the interface between the two cerebral hemispheres. It is divided into the sagittal plane from the anterior to the posterior aspect, consisting, namely, of the rostrum, the genu, the body and the splenium(Garg et al., 2015). Callosal lesions, which often occur in up to 95% of all MS patients, may strongly contribute to the development of both physical and cognitive impairment (Sotgui et al., 2022). Novel studies highlight the relationship between disease progression and brain volume loss in terms of the corpus callosum index (CCI) (Van Rensburg et al., 2021).

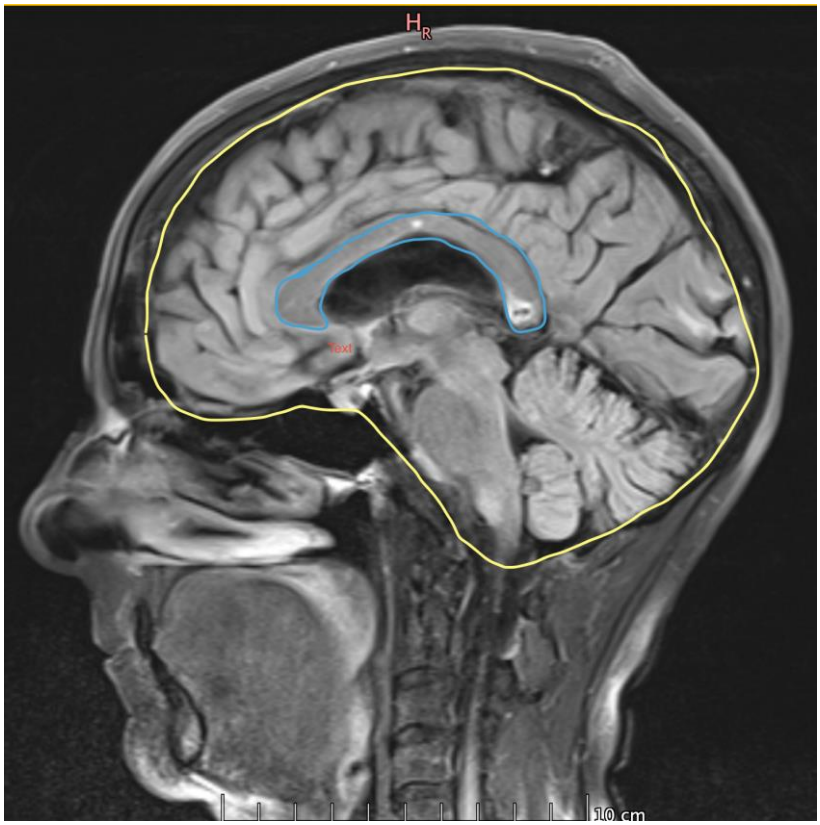


Figure 3.3: A T2-weighted MRI image of the midsagittal plane, outlining the corpus callosum (CC) area. The area outlined in yellow represents the whole brain, with the area outlined in blue indicating the CC.

(Permission to use the image was granted by the participant concerned.)

In the current study, the correlation of the CCI with both brain volumes and lesion load, and the scores of physical and cognitive disability in patients with MS were assessed. MRI imaging was used to measure various dimensions of the CC, including its diameter, size, age morphology and biological sex-related differences, as indicated in many different studies (Mohammadi et al., 2010).

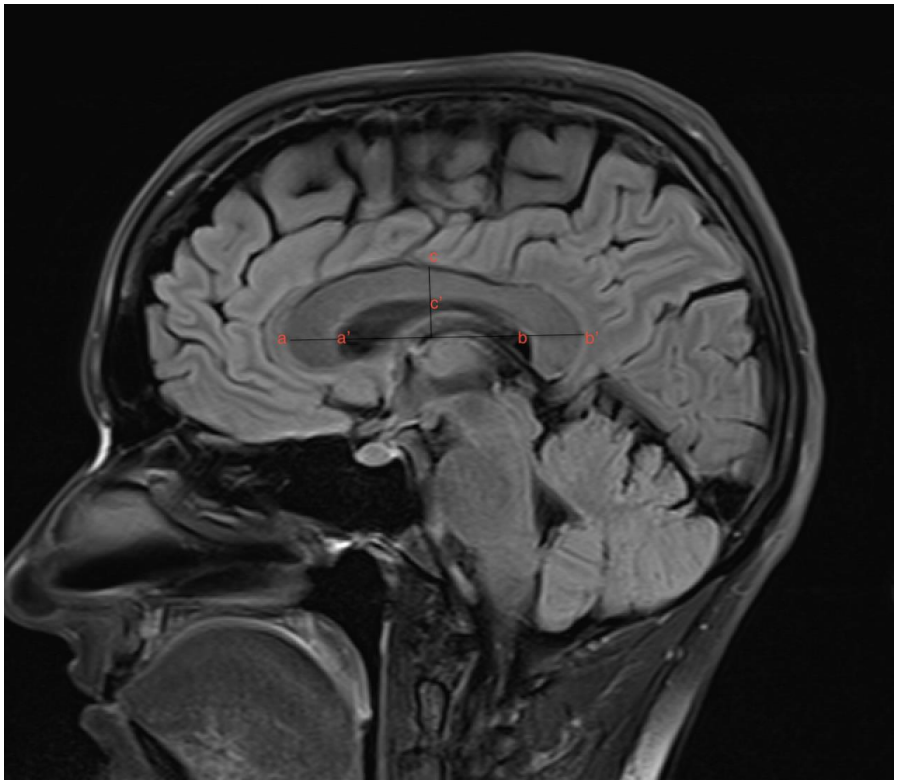


Figure 3.4 : An adapted image of the midsagittal T1 MRI view, demonstrating the measurement technique of the CCI, as proposed by Figueira et al. (2007)
CCI = aa'+bb'+cc'/ab
(Permission to use the image was granted by the participant.)

The CCI technique included making a calculation using three different measuring points on the CC, as shown above, in Figure 3.4, points aa' (anterior), bb' (medium) and cc' (posterior) segments of the CC. Together, the segments are normalised by the greater anteroposterior diameter of the CC (segment ab), yielding the CCI (Gonçalves et al., 2018).

3.7.7 Magnetic resonance imaging

Magnetic resonance images were planned to be obtained from 50 different participants (with 25 participants having already been clinically diagnosed with MS, and with 25 participants being from a control group). MRI brain scans were conducted at the CUBIC, University of Cape Town, and at the MRI Unit of GSH. The participants were scanned on a 3 Tesla, Siemens,

MAGNETOM Skyra scanner, using a 32-channel transmit–receive (RF) head coil. Hour-long, on average, scans were performed by the researcher concerned, who is also an experienced MRI radiographer. The participants' briefing, which transpired outside the scan room, included an explanation of the procedure, with it being aimed at ensuring that the participants were at ease with the process. On the completion and signing of the MRI safety questionnaire (Appendix A) by both the participants and the radiographer, the former replaced their clothing with a cotton gown. Accessories such as jewellery, hairclips and personal belongings were removed and locked away for safekeeping. All the participants were asked to empty their bladder before the commencement of the scan, so as to minimise disruption during the image acquisition. The participants were scanned in the head-first, supine position, after a pillow was placed under their head and a wedge under their knees, so as to lessen their discomfort during the scan. To ensure privacy, the participants were covered with a sheet or light blanket. A panic ball was handed to all participants, for use as an alarm, in case they required any assistance during the procedure. MRI-compatible headphones were placed over their ears, so as to reduce the acoustic noise of the scanner, and so as to ensure that the participants could hear the instructions that were issued to them during the scan.

3.7.7.1 MRI protocol used

The scanning protocol included the following sequences:

- Localisers in the axial, coronal and sagittal planes were used to determine the participants' position inside the gantry.
- The t2_tirm_tra_dark-fluid followed, and was scanned in, the axial plane. The sequence concerned was specifically developed for its high sensitivity to MS lesions and increased lesion visibility and conspicuity.
- The PD sequence, pd_tse_tra_3mm, demonstrated white and grey matter differences in the brain.
- The following sequence, which is a t2 weighted sequence (t2_tse_cor_3mm) scanned in the COR, provided contrast for the paramagnetic contrast agents**.**
- The t1_mprage_tra sequence was acquired in the axial plane, with the sequence concerned providing contrast enhancement between the grey and white matter**.**
- The following sequence, which is a susceptibility-weighted image (t2_swi3d_tra_p2_1.5mm), was used for detecting blood products and calcium.
- The next scan consisted of the DTI sequence (BME_DTI_30gr_4b0_2mm_ISO_52sl_AP), which was also conducted in the axial plane, with the image involved being used in the CNS for obtaining the tissue morphology and pathology.

- The following scan, which is a diffusion-weighted image called resolve_4scan_trace_tra_p2_192, was also acquired in the axial plane. The sequence involved is useful in evaluating small lesions.
- The last sequence, which was acquired in the sagittal plane, was the inversion recovery sequence (t2_tirm_sag_dark-fluid), with it being used to highlight the pathology and specific scan plane necessary for the localisation of lesions.

Table 3.1: The MRI sequences and parameters used in the current study.

MRI sequences	Repeti-tion time	Echo time	Slice thick-ness	Field of view	Matrix %	Distance factor
S1 = t2_tirm_tra_dark-fluid	9400,0 ms	78,0 ms	3,0 mm	256 mm	256 x 100	0%
S2= pd_tse_tra_3mm	2200,0 ms	10,0 ms	3,0 mm	75%	256 x 100	0%
t2_tse_cor_3mm	4500,0 ms	10,0 ms	3,0 mm	256 mm	256 x 100	0%
t1_mprage_tra	1720,0 ms	2,47 ms	2,0 mm	100%	256 x 100	50%
t2_swi3d_tra_p2_1.5mm	27,0 ms	20,0 ms	1,50 mm	20,00 %	224 x 96	91,10%
BME_DTI_30gr_4b0_2mm_ISO_52sl_AP	8700,0 ms	82,0 ms	2,1 mm	100%	128 x 100	30%
resolve_4scan_trace_tra_p2_192	5700,0 ms	65/107 ms	4,0 mm	100%	192 x 100	30%
t2_tirm_sag_dark-fluid	9000,0 ms	81,0 ms	4,0 mm	100%	320 x 70	30%

3.8 MS and gadolinium

3.8.1 Note on post-contrast imaging

Recent evidence suggests that gadolinium-based contrast agents (GBCAs) have shown a tendency to accumulate within the brain, along with associated cognitive deficits (Forslin et al., 2017; Gulani et al., 2017). Though much uncertainty still exists in the literature, the risk to

participants was regarded as being too great to enable the inclusion of contrast-enhanced imaging in the present study. The project, consequently, relied on such ancillary measures as the clinical presentation of the patient during the neurological examination and associated imaging features, such as susceptibility fallout on the SWI, restricted diffusion on high b-value diffusion-weighted imaging (DWI), to detect active disease processes in MS, without the need to administer GBCAs.

3.9 Data collection

The study participants diagnosed with MS underwent EDSS measurements that were performed by the attending clinician for the purposes of the present study. The examination concerned consisted of a questionnaire and certain body function tests, which were scored according to the answers given. After the clinician had plotted all the responses on an answer sheet, the final values were included on a spreadsheet, together with the values for the other measurements taken for the current study.

The participants completed a medical history and lifestyle questionnaire (Appendix C) developed in collaboration with a registered dietician and approved by the Human Research Ethics Committee (HREC) at Stellenbosch University (references NO7/09/203 and N09/08/224) (Appendix D). The questionnaire (Appendix C) was used to document any relevant information, including personal and family medical history, sociodemographic and anthropometric data, and lifestyle and dietary habits, as well as medication use. Blood pressure, body weight and height, as well as waist and hip circumference, were measured and documented, in addition to being used to calculate BMI, as well as the waist–to–hip ratio (WHR).

Physical activity was categorised into none/occasionally, low (once/week), moderate (2–3 times/week), or high (>4 times/week). The type and regularity of alcoholic beverage consumption were documented, and differentiated into abstain (0 units/week), 1 to 2 units/week,1 to 13 units/week, 14 to 21 units /week, and >22 units/week, so as to determine the optimal cutoff levels for both men and women, based on genotype–phenotype association studies. The number of days per week that patients had consumed certain foods rich in saturated fat, antioxidants and folate for the preceding three months was also documented and used to derive a fat, fruit and vegetable, as well as folate, score. The biochemical data collected included a full blood count, focusing on inflammatory markers, lipids, vitamins, cardiac markers / risk factors, and haematinics. Genetic data were extracted from the password-protected website, Gknowmix.com, which allows access only by registered users. All data retrieved was captured on the Microsoft Excel research database. The CCI data were collated by the research radiographer (whom are working in a research environment and are familiar with doing data analyses and measurements in the field of MRI), using a Digital Imaging and

Communications in Medicine (DICOM) program, called Weasis, to enable the measurement of the CC, with it focusing on three different data points (namely the genu, the midbody and the splenium), as proposed by Figueira et al. (2007). Weasis is a standalone/multipurpose DICOM viewer, equipped with multilanguage cross-platform, Free and Libre Open Source Software (FLOSS), allowing for flexible integration to the picture archiving and communication system (PACS), the radiology information system (RIS) and the hospital information system (HIS). This popular software program is used in healthcare by hospitals, health networks, multicentre research trials and patients. The necessary MRI data were collected and recorded by the research radiographer concerned. The collated data were plotted on a spreadsheet, with the key points concerned including the participants' details (with their actual names being replaced with study numbers, and together with their date of birth), the scan dates, the number of lesions present and their CCI values.

3.10 Statistical analysis

Statistical analysis was conducted by the researcher concerned, and overseen by the supervisors and statistician involved. The necessary data were collated for statistical analysis by a qualified biostatistician.

- Lesion volumes were determined using the FLAIR and T1 images with Sequence Adaptive Multimodal SEGmentation (SAMSEG) software, a part of FreeSurfer 7.2 (Puonti et al., 2016; Cerri et al., 2021).
- Relationships between biochemical measures and EDSS were examined, using Spearman correlations. The Spearman correlations were used for testing the relationships existing between the biochemical variables and lesion load.
- An exploratory factor analysis (using parallel analysis to indicate the number of factors involved) was used to demonstrate the variability of using lesion load over disability and biochemical measure, as a summary measure of the influence of lesion load in disability progression. The data were analysed using Statistica 13.
- Fisher's least significant difference (LSD) test was used for purposes of post hoc comparison.
- Since extremes of outcome approach were adopted, the age of subjects varied, leading to an analysis of covariance (ANCOVA) being performed, so as to determine the related age effects. Patients were grouped according to their EDSS scores, so as to enable the evaluation of associations between lesion load, biochemistry and disease outcome. One-way analysis of variance (ANOVA) was used to compare the neuroimaging parameters existing between the groups.
- Non-parametric Mann-Whitney U and Kruskal-Wallis tests were conducted parallel to the ANOVA (F-tests) and this was to ensure robustness and validate the findings. If the results of the tests agree, then the conclusion is stronger.

- The biochemical analysis included genetic studies of SNPs related to the vascular function, performed using DNA extraction from whole blood, PCR TaqMan technology, next-generation sequencing and genotype confirmation, using conventional Sanger sequencing

3.11 Ethical considerations

The current MRI study forms part of a larger, ethically approved study titled: “The implementation of a comprehensive gene-based, pathology-supported intervention program in patients with disorders that include a component of demyelination such as multiple sclerosis (MS)” NO7/09/203 (Division of Chemical Pathology, Tygerberg Hospital and Stellenbosch University) (Appendix D). Data collection for this discreet imaging study commenced once ethical approval was received from the Research Ethics Committee of the Faculty of Health and Wellness Sciences at the Cape Peninsula University of Technology (CPUT) (CPUT/HW-REC 2019/H27) (Appendix E and ethics renewal form, Appendix F). Permission to scan the MS study participants was obtained from the head of the CUBIC scanning department (Appendix G).

The following factors were taken into consideration during the undertaking of this medical research study (Appendix I). The same cohort of participants took part in a previous study for Ultrasound with ethics code, Research Ethics Committee of the Faculty of Health and Wellness Sciences at the Cape Peninsula University of Technology CPUT/HW-REC 2017/H4(renewal).

Privacy and Confidentiality

Personal information of all the participants were held private and treated with the utmost respect. Only researchers involved in the study had access to data and participant information. The principal researcher were responsible for inventory and organization of the data collection forms. All the participants were assigned a code, as to ensure anonymity, which were used on all study paperwork. All data was kept in a locked room and the electronic database was locked with coded access. Data with the patient code were only accessible to the supervisor and statistician.

Informed Consent

Every Participant had the right to withdraw from the study at any time. All the participants were asked to voluntarily read and sign an informed consent letter (Appendix H) after they were fully explained about the aims, purpose and benefits of the study (Participation letter Appendix I). The informed consent was available in 2 languages (English and Afrikaans). Consent in isiXhosa will not be available as MS is a very rare disease amongst Black Africans, however the incidence is highest amongst English-speaking white South Africans and lower in Afrikaans-speaking white South Africans (Dean,1976).

The study was conducted according to the code of ethics, as per instruction from the World Medical Association (Declaration of Helsinki), which was adhered to in the following ways:

- In keeping with the Declaration, the participation of all the participants was voluntary. No coercion or force was used to make any participant take part in the study. Informed consent was obtained from all the participants involved.
- The participants were also assured that they could withdraw at any time from the study without any consequences or penalties being imposed.
- All the participants were treated with respect and dignity, and all necessary precautions were taken to ensure the confidentiality and privacy of their personal information. Therefore, no actual names of participants were used, with every participant being assigned a unique identifying code, which was used in all the associated paperwork throughout the study.
- In addition to all data being kept in a locked room, electronic data were stored on a coded access database. Only those researchers who were involved in the study had access to the relevant data and to the participants' personal details and information.

The present study was conducted according to the ethical guidelines of the Health Professions Council of South Africa (HPCSA), in relation to which the following requirements were followed:

- All the participants had a full understanding of the study to which they were to contribute, and provided their signed informed consent to the research undertaken.
- The health professionals involved explained the objectives of the study and described the way in which it was to be conducted, as well as any risks associated with the MRI procedure.

3.12 Publication and dissemination

Before the commencement of the study, consent was obtained from all the participants for the publication of study-related information pertaining to themselves in journals and/or in a thesis (Appendix H). The results of the study were to be analysed and made available via peer-reviewed journals to others in the medical and scientific community.

The benefits of the study included obtaining a free MRI scan, and If any pathology or disease progression were identified then the participants was referred to an appropriate physician for further management and medical treatment. The study results were incorporated into a research article and this was shared with all the participants. In addition, the publication of the findings of this study would also allow further research to be conducted on the topic and would help to substantiate the results obtained. Knowledge gained through the study could be presented at conferences, seminars and congresses. All contributions would be acknowledged in publications resulting from this research project.

3.13 Conclusion

The present chapter has outlined a detailed plan/design of the mechanisms/processes used in the current research study. The aim of the study was to provide a clear and comprehensive description of the study design and methods used in the research, as well as to allow future researchers, wishing to pursue the same topic of study, easy transferability of the results for either replication or validation. Chapter 4 will provide the data accumulated during the present study and the analyses undertaken in these exercises, with the results obtained being presented in the form of graphs, tables and diagrams. The different data acquired about the set objectives will also be outlined in Chapter 4. Any limitations encountered during the aforementioned process and/or any effects that they might have had on the data analyses involved will also be revealed. The following chapter will present the findings made, the meaning of the data will be explained, with an eye on satisfying the set research objectives.

CHAPTER 4

RESULTS

4.1 Introduction

The present chapter includes the results of the current research study. The study was aimed at using brain MRI, biochemical parameters, and genetic and lifestyle factors in a sample of MS patients, so as to provide additional insights into the pathophysiological processes associated with MS. The chapter represents the findings made in terms of the data collected, in an effort to help give meaning to the results obtained, and, ultimately, to satisfy the set research objectives.

A 5% significance level ($p<0.05$) was used as a guideline for statistical significance. For the analyses, comparisons between two variables showing significant or nonsignificant differences are represented by means of the p-value (i.e. the probability that such a strength might occur by chance) and the correlation coefficient, r. Whereas a p-value of >0.05 is considered nonsignificant, significant correlations are associated with a p-value of <0.05. A p-value is described as being the product of hypothesis testing, using multiple statistical tests. The sign of the r shows the direction of the correlation involved. The r value ranges from +1.0, indicating a positive relationship, to -1.0, indicating a negative relationship.(Akoglu, 2018). The results of the current study are presented in a graphic and tabular format.

4.2 Sample characteristics

The baseline characteristics of all the MS participants and controls involved in the present study are illustrated in Table 4.1 below. Of the 51 participants in the study, 100% were women, representing both the MS-diagnosed and control groups, with the mean age of the study population being 42.84 ±13.84 years old in the MS-diagnosed group and 38.87 ± 12.31 years old in the control group. No significant differences were present in terms of age, height, weight, BMI, and body surface area (BSA) between the patients and the controls. The number of participants recruited for the study was n=51, with the number of nonarrivals being n=2, and the total number of subjects scanned, n=48. The number of those included in the analysis was n=44, with the number of claustrophobic cases and incomplete scans being n=1. No reported symptoms or comorbidities were present in the control group. MRI was performed at baseline, as well as after an interval period of at least a decade, or longer, in the case of 22 PwMS. WML volumes were determined using (SAMSEG) software, forming part of FreeSurfer 7.2. Other variables included age at MRI, disease duration, disability status and medication.

Table 4.1: Baseline characteristics of MS and control participants.

Parameter	MS participants (n=22)	Controls (n=21)
Females n	22	21
Age at MRI: years old, mean (range)	50.7 (23–71)	48.3 (25–72)
Disease duration: years, mean (range)	15.4 (1–29)	N/A
Disease-modifying therapy (DMT)	10	N/A
Untreated n (%)	12	N/A
BMI: mean (range)	25.3 (19–35)	27.7 (21.4–37.5)
Cholesterol mean (range)	5.4 (3.9–8)	5.6 (3.9–7.9)
Vitamin D (ng/ml)	30.6 (9.2–63.9)	27.4 (8.4–78.7)
Smokers n	0	0
Previous smokers n	6	2
Passive smokers n	9	7
Fruits/veg>5: days/week mean (range)	4.8 (0–7)	3.9 (0–7)
EDSS, median (range)	2.9 (1–8)	N/A

In the MS group (n=22), associations were assessed between WML volumes and age, disease duration (years with MS), and disability (EDSS) (see Table 4.2). Of the 22 PwMS, 10 were on disease-modifying treatment (DMT), of whom all were prescribed IFN-β (As shown in table 4.2). WML volumes were significantly associated with the EDSS ($p<0.02$), although not with age, disease duration or DMT use (as seen in Tables 4.3 and 4.4).

Table 4.2. Clinical data of 22 pwMS and 21 Controls who had 3-T MRI assessments.

Parameter	**PwMS on PSGT Program for >10 years**		**All pwMS (n=22)**	**Controls (n=21)**
	YES	NO		
Females n	13	9	22	21
Age at MRI: years, mean (range)	52 (36-65)	48.8 (23-71)	50.7 (23-71)	48.3 (25-72)
Disease duration: years, mean (range)	17.5 (11-29)	12.2 (1-27)	15.4 (1-29)	NA
MS Subtype: RR	13	6	19	NA
MS Subtype: SP	0	3	3	NA
Disease modifying therapy	3	7	10	NA
Untreated n (%)	10	2	12	NA
BMI kg/m², mean (range)	25.2 (21.5-35)	25.5 (19-32)	25.3 (19-35)	27.7 (21.4-43)
Cholesterol mmol/L, mean (range)	5.5 (3.9-8)	5.4 (4.4-6.8)	5.4 (3.9-8)	5.6 (3.9-7.9)
Vitamin D (ng/ml), mean (range)	28.8 (9.2-63)	33.2 (13.3-63.9)	30.6 (9.2-63.9)	27.4 (8.4-78.7)
Smokers n	0	0	0	0
Previous smokers n	4	2	6	2
Passive smokers n	4	5	9	7

Fruits/veg>5: days/week, mean (range)	5 (0-7)	4.4 (0-7)	4.8 (0-7)	3.9 (0-7)
EDSS, median (range)	2 (1-3.5)	4.2 (1-8)	2.9 (1-8)	NA

PwMS (n=13) who had followed the PSGT Program for more than 10 years, had significantly smaller WML volumes (mm3) compared to pwMS who did not adhere to the Program (n=9) (4950 ± 5303 vs 17934 ± 11139; p=0.002),(as seen in table 4.3).

Correlations were also made between the EDSS, DMT, years with MS and lesion volumes(As seen in table 4.3).

Table 4.3: Demonstrating lesion volumes versus DMTs, EDSS.

PT NO.	PT CODE	EDSS	YRS WITH MS	AGE @MRI	LESION VOL	PROGR >10yrs	DMT
1	MS01/NA	3	11	56	5185.406769	Y	Y
2	MS02/HF	2	13	60	9568.237849	Y	N
3	MS03/RC	1.5	11	40	676.390397	Y	N
4	MS04/KM	1.5	12	36	669.351986	Y	N
5	MS05/KB	3	25	58	16.724225	Y	N
6	MS06/CR	1	11	43	1599.882760	Y	N
7	MS07/ES	6.5	20	51	28666.566625	N	N
8	MS08/MS	6	15	71	14270.432243	N	Y
9	MS09/RL	2	29	56	3503.081392	Y	N
11	MS11/LK	8	21	61	32046.071361	N	N
12	MS/12	7	9	57	31198.412097	N	Y
13	MS/13	1	1	23	9235.214264	N	Y
15	MS/15	2	24	51	4145.498535	Y	Y
16	MS/16	1.5	3	41	15550.094281	N	Y
17	MS/17	2	27	45	21908.248539	N	Y
18	MS/18	1.5	23	34	11285.787152	Y	N
19	MS/19	1	6	40	1256.017690	N	Y
20	MS/20	2	16	51	2243.869319	Y	Y
21	MS/21	1	22	65	2281.200127	Y	N
22	MS/22	3.5	13	62	4565.013185	Y	N
23	MS/23	3.5	8	50	7277.680705	N	Y
25	MS/25	1.5	18	65	18603.050071	Y	N
		2.9285714		50.7272727			

Table 4.4: Associations of WML volumes in PwMS with age, disease duration, disability and medication use

Variable	Pearson correlation	P-value
Age at MRI	00.21	00.350
Disease duration	0.08	0.709
EDSS	0.73	<0.02
DMT use		0.70*

* ANOVA test for equal means.

Of the PwMS who had not followed the program (a Pathology-supported genetic testing (PSGT) Program) (n=4), two had to use a cane for walking and two were wheelchair-bound. Three of them had transitioned to SPMS.

Table 4.5: Correlation between environmental factors and lesion volumes

	5FOR_STATS_LESION VOLS MS&CONT_BLOCK&GENETICS\PAT&CONT\Spreadsheet						
	Variable 1	Variable 2	Pearson	Pearson p-value	Spearman	Spearman p-value	#cases
52	ng/ml VitD	LESION VOL	-0.44	0.06	-0.52	0.02	19

4.3 Correlations of lesion volumes with disability status, genetics and environmental factors in MS patients and controls

4.3.1 Lack of association between environmental factors and lesion volumes

The environmental factors concerned in the present study showed no association with the lesion volumes involved (as shown below in Table 4.5), except for in the case of vitamin D, with serum vitamin D concentrations showing a significant inverse association with lesion volumes in the controls.

Request to insert table with results of environmental factors v lesions and DMT v lesions. The database with participant results cannot be published due to ethical considerations but would be available upon reasonable request.

The EDSS showed a significant association with lesion volumes in MS, although number of years with MS nonsignificant (Table 4.6).

Table 4.6: Lesion volume influence on disability

	5FOR_STATS_LESION VOLS MS&CONT_BLOCK&GENETICS\PAT&CONT\Spreadsheet						
	Variable 1	Variable 2	Pearson	Pearson p-value	Spearman	Spearman p-value	#cases
1	EDSS	LESION VOL	0.73	<0.01	0.48	0.02	22
2	YRS WITH MS	LESION VOL	0.08	0.71	0.04	0.86	22

Patients who were on MS medications (DMTs) showed no difference in lesion volumes compared to those who were not on DMTs. Patients and controls who were passive smokers had larger lesion volumes than did those who were not, but not significantly.

4.4 Correlations between genetics and MS

4.4.1 Results

The findings made in relation to the correlations between genetics and MS were as follows:

1. MTHFR 677 C>T showed significant differences between MS patients who had the variant and those who did not, although no differences were perceived in the controls (see Figure 4.1; Table 4.7).
2. MTHFR 1298 A>C variants tended to be associated with increased lesion volumes in MS (p=0.09), although not in the controls.
3. HFE C282Y showed no difference in lesion volumes between MS patients with the variant and those without (p=0.77), which is significant because we found no effect of the ‘iron loading’ gene on lesion volumes.
4. HFE H63D also showed no difference in lesion volumes between those who had the variant and those who did not.
5. MS patients with the FABP2 variant showed increased lesion volumes (p=0.05) compared to those without, which is significant because it confirms the results found by Johannes et al. (2023), who showed that the FABP2 variant was associated with significantly higher EDSS (p=0.03). No differences were present in the controls.
6. MS patients with the TNF-alpha variant had smaller lesion volumes than those of patients without the variant, although not in the controls. Such a finding was interesting, because, in the literature, TNF-alpha is associated with lesion resolution (Ribeiro et al., 2019).
7. MS patients with GSTM1 deletion had higher lesion volumes than did patients without the deletion, although no difference was present in the controls. The finding is interesting, because GSTM1 is involved in detoxification (Aliomrani et al., 2019).

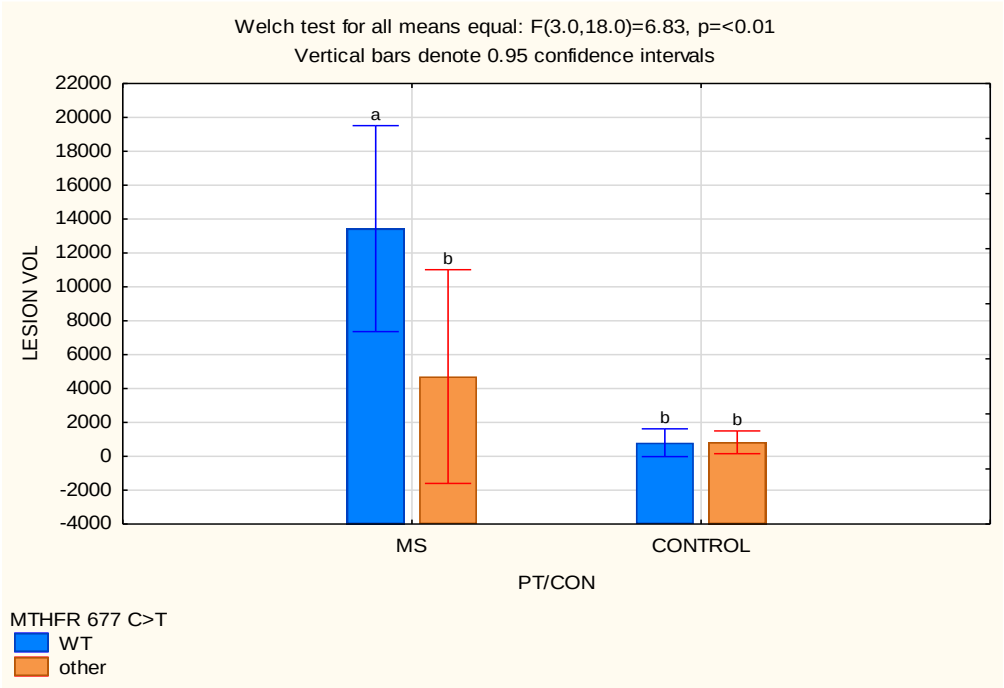


Figure 4.1: PT/CON*MTHFR 677 C>T-adjusted means plot.

Table 4.7: PT/CON*MTHFR 677 C>T post hoc table

	Welch test unadjusted post hoc table					
	PT/CON	MTHFR 677 C>T	{1}	{2}	{3}	{4}
Cell no.			13436.372	4705.379	799.405	828.493
1	MS	WT			<0.01	<0.01
2	MS	Other	0.04		0.19	0.19
3	CONTROL	WT	<0.01	0.19		0.95
4	CONTROL	Other	<0.01	0.19	0.95	

4.5 Correlation between the corpus callosum and MS disease progression

The CC, which is the primary commissural region between the two cerebral hemispheres, consists of WM tracts(Ajitomi et al., 2022). In terms of MS, according to Yaldizli et al., (2014), the CC is a region in the brain where MS lesions can most commonly be found. In the current study, the proportions of the CCI were calculated according to the method prescribed by Figueira et al. (2007), by means of utilising the sagittal T1-weighted MRI image. As the sample size calculated was 25 MS, and the controls were as previously reported, 25 was the number of patients with multiple sclerosis (PwMS) and of the controls randomly recruited; however, seven of those concerned (three PwMS and four controls) could not undergo MRI, due to concerns regarding claustrophobia or illness. In total, 22 women with MS and 21 female controls signed their consent to be included in the 3-T MRI assessment. In the research, three different regions of the CC were measured: the genu, the midbody and the splenium (see Figure 4.2), with the number of lesions in the CC being compared in relation to their assessments. The EDSS, which is the gold standard for the measuring of MS disability, ranges from 0 (no disability) to 10 (death due to MS) (Kurtzke, 2015).

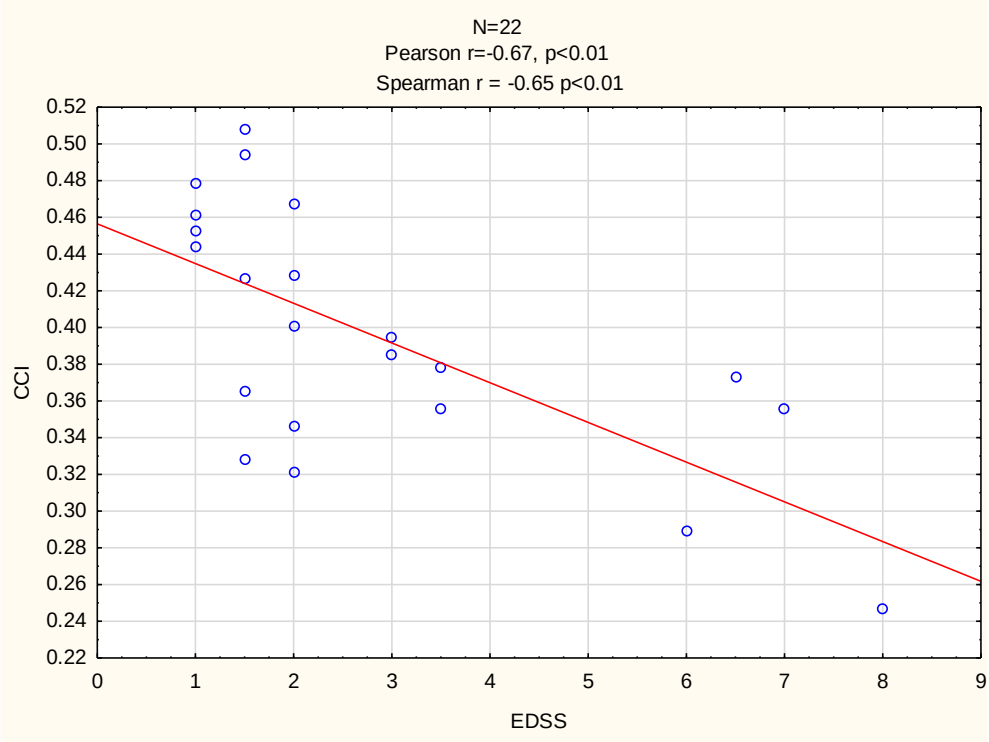


Figure 4.2: A comparative graph representing data of MS-diagnosed and control participants Measurement analyses of the corpus callosum, compared to the EDSS.
The corpus callosum index (CCI) consists of a straight line drawn across the greatest anteroposterior axis of the CC, with another straight line drawn across its craniocaudal axis at its midpoint, leading to points a, a, b, b' and c, c'. The anterior, middle and posterior segments of the CC are then measured and normalised to the greatest anteroposterior diameter of the CC, based on the formula CCI = (aa' + bb' + cc')/ab.

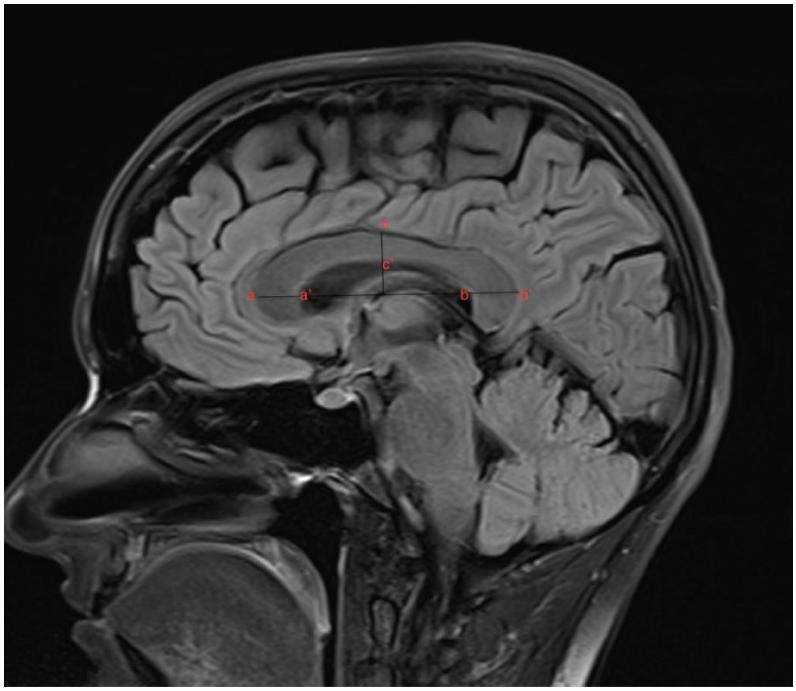
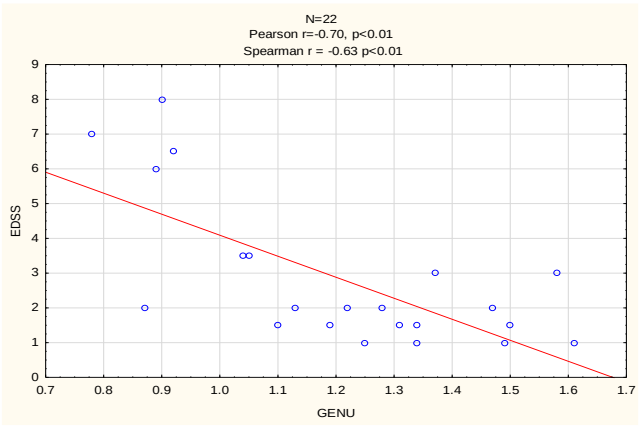
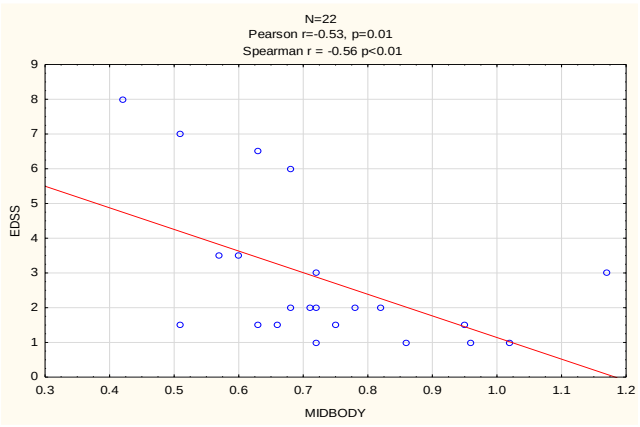


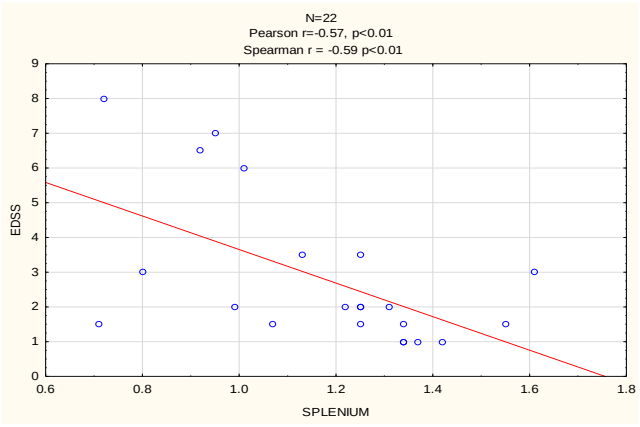
Figure 4.3: The CCI measurement, as proposed by Figuera et al. (2007).
(Permission to use the image was granted by the participant.)



Genu vs EDSS



Midbody vs EDSS



Splenum VS EDSS

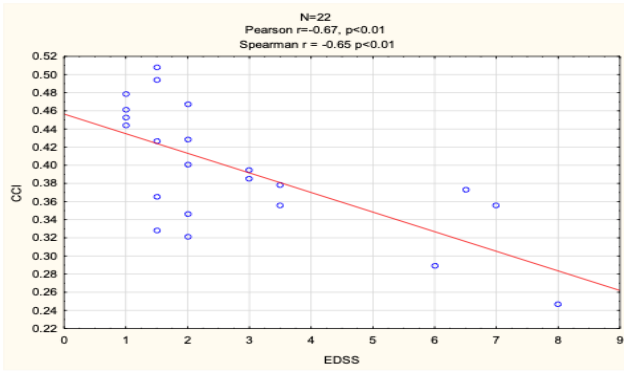


Figure 4.4: Graphs depicting the significant inverse association between the thickness of the CC and the disability concerned.

4.5.1 Results

The CCI was significantly lower in the MS patients than it was in the controls (p=0.03) (see Figure 4.2). Splenium thickness was also significantly lower in the MS patients than it was in the controls (p=0.01) (see figure 4.4). The EDSS values of the patients involved did not correlate with the number of lesions in the CC. In contrast, a significant inverse association (p<0.01) was found between the CCI and the EDSS, and between the EDSS and the thickness of the genu, midbody and splenium (p<0.01).

4.6 MRI results of two groups (MS-diagnosed and control groups)

For the pathological evaluation of any disease, including MS, it is of pivotal importance to identify disease manifestations and to provide guidance respecting the desired treatment options. Although this process has its limitations, MRI has proven itself to be instrumental, due to its high sensitivity to different pathological substrates of MS (demyelination, inflammation and neuroaxonal loss). Moreover, such imaging can be used in the conducting of longitudinal studies, geared towards helping to monitor the disease course(Filippi, Brück et al., 2019).

4.6.1 MRI protocol used

The protocol used in the current study allowed for the following procedures to be undertaken (as shown in tables 4.8 and 4.9):

- increased spatial resolution, revealing minute differences between adjacent structures on T1WI;
- a clear delineation between anatomical structures on FLAIR and ADC images;
- excellent contrast between white and grey matter, as well as excellent lesion visibility and conspicuity on FLAIR;
- good contrast and cortical lamination on T1-weighted MPRAGE; and
- the ability to image an active lesion during a relapse without gadolinium contrast, using SWI.

Table 4.8: Differences between the conventional and the newly developed protocol.

	Conventional protocol	**Scan time**	**New protocol**	**Scan time**
MRI sequences	t2_tirm_tra_dark fluid_fs	1 min 54 sec	t2_tirm_tra_dark fluid	14 min 08 sec
	t2_tirm_sag_dark fluid_fs	1 min 52 sec	pd_tse_tra_3mm	7 min 08 sec
	T2_tse_tra	1 min 42 sec	t2_tse_cor_3mm	5 min 35 sec
	Dwi_tra_b0,1000_adc	1 min 37 sec	t1_mprage_tra	7 min 49 sec
	t2_swi3d_tra_p2_1.5mm	2 min 28 sec	t2_swi3d_tra_p2_1.5mm	4 min 20 sec
	t1_sag	2 min 17 sec	BME_DTI_30gr_4b0_2mm_ISO_52sl_AP	5 min 23 sec
	t1_tra_5mm	6 min 39 sec	resolve_4scan_trace_tra_p2_192	6 min 29 sec
	t1_tra_mprage_pre	4 min 39 sec	t2_tirm_sag_dark fluid (optional)	4 min 32 sec
	Gadolinium injection			
	t1_tra_mprage_post	4 min 39 sec		

Total scan time		25 min 47 sec		54 min 04 sec

Table 4.9: Time-related comparison of the newly developed protocol and more conventional sequences.

MRI sequences			
Conventional protocol & scan time (ms)		**New protocol & scan time (ms)**	
t2_tirm_tra_dark-fluid	78	t2_tirm_tra_dark-fluid	256
pd_tse_tra_3mm	10	pd_tse_tra_3mm	75
t2_tse_cor_3mm	10	t2_tse_cor_3mm	256
t1_mprage_tra	2	t1_mprage_tra	100
t2_swi3d_tra_p2_1.5mm	20	t2_swi3d_tra_p2_1.5mm	20
BME_DTI_30gr_4b0_2mm_ISO_52 sl_AP	82	BME_DTI_30gr_4b0_2mm_ISO_52 sl_AP	100
resolve_4scan_trace_tra_p2_192	65/107	resolve_4scan_trace_tra_p2_192	100
t2_tirm_sag_dark-fluid	81	t2_tirm_sag_dark-fluid	100
Total scan time	**283**		**1007**

Limitations of the new protocol

The newly developed protocol, although advantageous, had its own impediments. The lengthy scan time (an increase in image resolution results in an increase of scan time), could prove to be uncomfortable for participants, especially those already suffering from other physical challenges. Furthermore, with an increase of time spent in the scanner has a direct impact on the specific absorption rates (SAR) levels, which refers to the amount of power deposited by a radiofrequency field into a mass of tissue (Allison & Yanasak, 2015), hence certain individuals might complain about experiencing a heating effect. Thus it is very important to make sure the participants are in the correct dress code(preferably cotton gowns), and continuous monitoring to prevent these unforeseen consequence. As a result of these limitations the spinal cord ws not imaged.

4.6.2 MRI results compared between MS-diagnosed and MS control participants

Lesion volumes were determined using the FLAIR and T1 images with SAMSEG software, part of FreeSurfer 7.2.

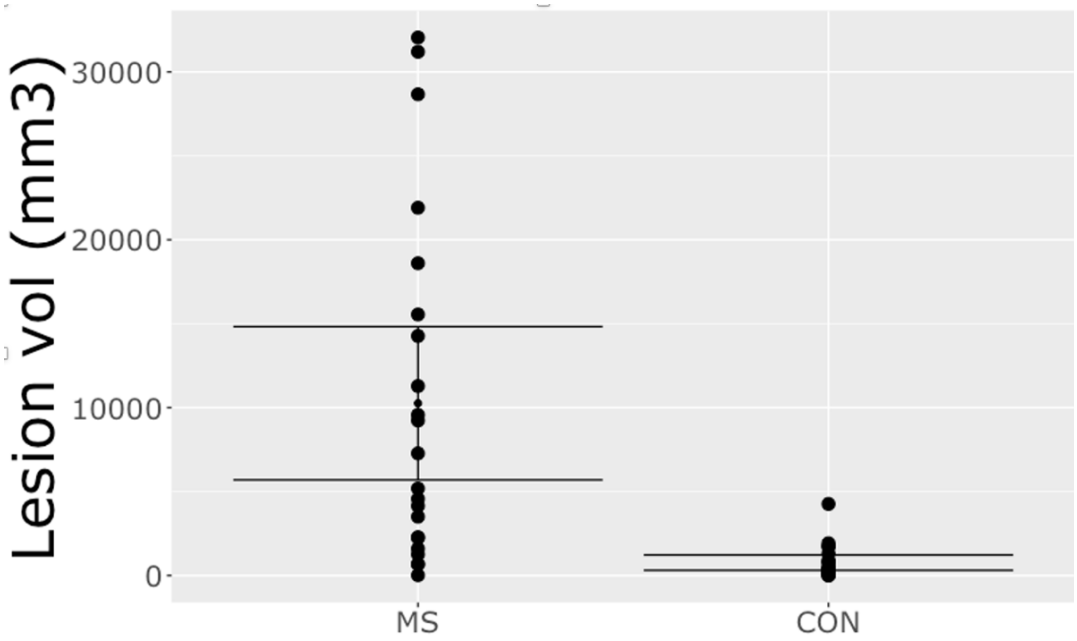


Figure 4.5: A comparative graph representing data of MS-diagnosed and control participants.

4.6.2.1 White matter hyperintensities in the control group

WMLs were also found in the control group (figure 4.5). The mean lesion volumes (mm^3) were found to be significantly greater ($p<0.001$) in PwMS (10262 ± 10294) than they were in the controls (768 ± 1001), with. the mean WML volumes of PwMS being 10.3 ml vs 0.76 ml in the controls. None of the controls had neurological symptoms, although the SAMSEG software identified WMLs in some control participants. The radiologist concerned (who was responsible for reporting on scans for the researchers at CUBIC) reported the presence of age-appropriate WM hyperintensities, which were identified as being WMLs by the software used.

Recent findings of the present study have been accepted for publication in the journal *Multiple Sclerosis International* (with the final author version being included at the end of this chapter).

4.7 Conclusion

WML volumes were significantly associated ($p=0.02$) with disability (EDSS) but not with age ($p=0.350$), disease duration ($p=0.709$), or Interferon-β treatment ($p=0.70$). In some of the pwMS WMLs showed resolution over time. Noteworthy is the influence of dietary- and lifestyle changes which may lower the risk of developing cerebral WMLs in pwMS and potentially slow disease progression. We also observed WM hyperintensities in some control participants which can be attributed to age-appropriate lesions or Virchow-Robin spaces. Larger studies are required to confirm the effectiveness of such interventions in pwMS. The results of the current chapter will be discussed in Chapter 5, within the context of the study. The focus in the next chapter will be on the discussion of the conclusions drawn from the findings obtained. In addition, the limitations of the study, and recommendations for future studies, will be presented.

CHAPTER 5
DISCUSSION

5.1 Introduction

The primary aim of the present study was to use brain MRI, biochemical parameters, and genetic and lifestyle factors, in a sample of MS patients (22 participants with MS and 21 control participants), so as to gain additional insights into the pathophysiological processes associated with MS. In addition, the intention was to evaluate the MRI scans of female subjects diagnosed with MS, who were at one of the two extremes of the EDSS score spectrum. MRI, and the outstanding advances made in the field concerned, have become an essential part of the treatment pathway for those living with MS. The modality concerned allows for longitudinal monitoring of the disease process and sensitive qualitative and quantitative assessments of macroscopic areas in the CNS. Furthermore, the clinical usage of MRI has increased, in parallel with technical innovations currently being made(Hemond & Bakshi, 2018). Following I will be discussing the value of MRI, in the assessment of brain lesions for MS, comparing with the biochemical parameters, genetics and lifestyle factors and, how all this data relates to the diagnosis and disability progression.

5.2 Disability, as assessed in terms of the EDSS

The EDSS was used to monitor and assess the participants' physical abilities, with the measuring tool concerned proving useful for monitoring changes in the level of one's disability levels over time. The MSSS, which is a functional composite, also assesses disability, although, in the current study, this method was not used. Instead, the focus was on cognition, in the form of a questionnaire assessment aimed at enabling the evaluation of the participants' activity levels and other daily routines, including their working schedules and any other activities in which they were involved. The results obtained reflected the assessment of WMLs concerning the age, disability (EDSS) and disease duration of the 22 PwMS studied, of whom ten were undergoing DMT, all of whom were prescribed IFN-β. Although the WML volumes were significantly associated with the EDSS ($p<0.02$), there was no assessents made in correlation with age, disease duration or DMT use (as can be seen in Table 4.2).

5.3 White matter lesion volumes of PwMS and control participants

The formation of WMLs tends to be the hallmark of the prognosis of MS. The early phase of lesion development is normally accompanied by focal inflammation and myelin destruction(Van den Elskamp et al., 2010). Gaetano et al. (2020) postulate the significance of lesion location to lie in its being an integral prognostic tool for the determining of disease progression and disability severity. Furthermore, the authors concerned highlight the importance of demonstrating lesion dissemination across both space and time (which form the basis of MS criteria). Gaetano et al. (2020) hypothesise that achieving enhanced understanding of lesion location could help to ascertain functional loss, by means of taking

neighbouring structures into account. Observing the presence and changes of both cortical and WM lesions can provide key metrics for the diagnosing and monitoring of MS, especially during the early stages of the disease (Todea et al., 2020). WMLs are strongly associated with disability, as well as with both motor and cognitive outcomes at long-term follow-up. Cortical lesion load, however, appears to have the strongest association with cognitive dysfunction and severe GM atrophy, related to disability progression in all MS phenotypes, with extensive cortical damage at the onset of the disease being associated with florid inflammatory clinical activity and with the rapid occurrence of the progressive phase (Todea et al., 2020).

In the current study, significant differences were found between the two cohort groups, in relation to WMLs. The mean lesion volumes (mm^3) were found to be significantly greater ($p<0.001$) in PwMS (10262 ± 10294) than they were in the controls (768 ± 1001), with the mean WML volumes of PwMS being 10.3 ml vs 0.76 ml in the controls, which can validate the effectiveness of MRI as an imaging tool for the diagnosis and monitoring of MS. Volume and surface-based approaches were used in the taking of volumetric measurements of the brain utilising intensity normalisation, bias field correction and skull stripping. FreeSurfer has an estimated total intracranial volume (eTIV), which is based on the relationship between the intracranial volume (ICV) and linear transfer to the MNI305 space (as defined in relation to the Montreal Neurological Institute spaces, which are regions in the brain that are divided by the main brain lobes [as defined by the Talaraichs atlas, referring to a three-dimensional coordinate system of the human brain, used to map the location of brain structures independent from individual differences in the size and overall shape of the brain]) (Gaetano et al., 2020). Although FreeSurfer uses such a tool as a normalisation measure, the relationship concerned can be seen as biased, with it possibly resulting in incorrect normalisation, with the SAMSEG tool, consequently, being used for the normalisation of FreeSurfer volumes. SAMSEG, which is a relatively novel software tool, uses a mesh-based probability atlas that requires no preprocessing steps to be taken. In the current study, the segmentation-based estimate of total intracranial volume (sbTIV) was used for purposes of normalisation, with SAMSEG forming part of the FreeSurfer package. SAMSEG and SynthSeg were the preferred choice, due to them having been developed specifically for their cross-adaptability between scanners and sequences, and, therefore, their ability to produce additional reproducible results**.** The multimodal technique involved uses both the T1 (which is excellent for showing cortical lamination) and FLAIR (which is known for remarkable lesion visibility) sequences to facilitate the segmentation of the brain and to display the lesion load, with the lesions found using the SAMSEG program being measured in mm^3 (Van Nederpelt et al., 2023). A few PwMS (n=13) took part in the pathology-supported genetic testing (PSGT) program, in terms of which the essential nutritional factors flowing into the brain and the determinants contributing to MS progression were identified. The assessments involved included data accessed from personal and family medical history, lifestyle data (gleaned from

questionnaire-based assessment of, for instance, the intake of saturated fats, fruits, vegetables and fibre, as well as the individuals' BMI, smoking habits, alcohol intake and physical activity, among others). Biochemical tests were performed to evaluate any deficiency in iron, vitamin D, B12, CRP (entailing increased inflammation), and increased cholesterol and homocysteine levels. The PSGT program enables observation of the transitioning of risk stratification from the study population to the individual (Okunola et al., 2023), with all the data concerned being integrated, so as to create an adaptable personalised report. PwMS (n=13) who had followed the PSGT program for longer than a decade tended to have significantly smaller lesion volumes (in mm^3) compared to PwMS who did not adhere to the program (n=9) (4950 ± 5303 vs 17934 ± 11139; p=0.002). WML volumes were significantly associated (p=0.02) with disability (in terms of the EDSS), but not with age (p=0.350), disease duration (p=0.709) or IFN-β treatment (p=0.70). Those who were on the PSGT program were found to experience outstandingly improved disease outcomes after having been on the program for over a decade, despite the small study sample used. For those who did not, follow the PSGT program (n=4), and whose disease duration was over a decade, two had to use a cane for walking and two were wheelchair-bound, with three of them having transitioned to SPMS. The five other PwMS not following the program had a disease duration of under ten years. Although the SamSeg software identified WMLs in some control participants, none had any neurological symptoms. The radiologist concerned reported the presence of WMLs as age-appropriate WM hyperintensities that were identified by the software. The findings made tentatively suggest the mitigation of any risk factors, so as to delay, or to alleviate, the anticipated disease outcomes.

5.4 Corpus Callosum Index (CCI) measurement association with disability (EDSS) in PwMS

The CCI, which is a normalised measurement that reflects changes in brain volume, is performed to measure three different regions of the CC: the genu, the midbody and the splenium. MRI scans of the brains of 22 adult females with MS, and of 21 female controls, were evaluated. In the MS patients, measurements of the CCI and the number of lesions that were present in the CC, were compared, along with their EDSS assessments, which is the gold standard for measuring MS disability. The CCI was found to be significantly lower in the MS patients than in the controls (p=0.03). Splenium thickness was also significantly lower in the MS patients than it was in the controls (p=0.01). The EDSS values of the patients did not correlate with the number of lesions present in their CC. In contrast, a significantly inverse association (p<0.01) was found between the CCI and the EDSS, and between the EDSS and the thickness of the genu, midbody and splenium (p<0.01). From the above results obtained, the conclusion was drawn that the CC thickness is inversely associated with disability (EDSS) in PwMS. Measurement of the CCI could, therefore, prove to be a useful tool, in terms of indicating disease progression. The findings of the present study confirm the previous results found that indicated brain volume loss as being one of the causes of MS disability progression,

which might come to facilitate the development of therapeutic methods that should serve to preserve brain volume, so as to delay the progressive neurological decline that is associated with some MS cases.

Ajitomi et al. (2022),postulate that any structural damage or disconnection of the CC, due to axonal damage, is thought to add to the development of cognitive dysfunction in MS. Furthermore, the researchers concerned note how the functioning of the CC has been widely appreciated in MS, and that it correlates with the level of cognitive impairment involved. Measurement of the CC could be a useful tool, as an indicator of disease progression(Bodini et al., 2013). The findings of the present study confirm the previous results obtained, which indicate brain volume loss as the primary cause of MS disability progression, and which might help to develop therapeutic methods that should aid in preserving brain volume, so as to prevent the progressive neurological decline associated with some MS cases.

5.5 Biochemistry and lifestyle assessments

Biochemical analysis of biomarkers (iron parameters, ferritin, C-reactive protein (CRP), total cholesterol, homocysteine, serum folate, vitamin B12 and 25-OH vitamin D) was performed by an accredited pathology laboratory, and blood was drawn for testing between 09h00 and 10h30, so as to standardise the diurnal variation. Biochemical tests were also performed, so as to allow for the identification of possible deficiencies of iron, vitamin D and vitamin B12, or excess cholesterol/homocysteine ratios and increased inflammation (CRP) (Davis et al., 2014). The lifestyle parameters of the participants were retrieved from the medical and lifestyle questionnaire, which was completed upon entry of the participants concerned to the study (Appendix C), so as to obtain information on their family/personal medical history, alcohol intake, smoking habits and physical activity. The saturated fat, folate and fruit/vegetable/fibre intake scores, as retrieved from the questionnaire, were based on the different types of food that the participants ate, and on the frequency of such intake per week. The results showed a negative trend association between WM hyperintensities and vitamin D concentrations in the controls ($r = -0.44$; $p=0.058$). Alcohol intake was not significantly associated with WML volumes, whether in the PwMS or the controls ($r=-0.27$, $p=0.22$ and $r=-0.32$, $p=0.18$, respectively). The data were complemented by genetic testing, so as to be able to identify any clinically relevant variants in the metabolic pathways, and the integration thereof with the demographic data, so as to generate personalised reports, enabling the clinicians concerned to address any imbalances revealed in the blood test investigations, so as to mitigate all the risk factors through nutritional supplementation and the modification of lifestyle.

5.6 Genetics and MS

All participants in the current study underwent genetic testing, as was previously published, so as to identify the SNPs present. Genetics plays an important role in identifying the elusive aetiological factors contributing to the complex development of MS. Patsopoulos (2018),

Sadovnick et al. (1997)and Sawcer et al. (2014) all characterise genetics and environmental influences as having a pivotal role to play in disease progression. Associations have already been formed between MS and variations in the genes encoding HLAs within the MHC. Neither were any of the genetic variants tested associated with WML volumes, nor was any form of physical activity.

5.7 Study limitations

The greatest limitations of the current study are the small sample size and the current absence of randomised control trials (RCTs). The strength of the study is the significantly smaller WML volumes and benign disease outcomes encountered in PwMS. A limiting factor of the WML volume measurement could be the requirement, in terms of automated methods, to specify a probability threshold for lesion identification. Too low a threshold could result in false positives, with too high a threshold possibly impeding sensitivity. However, since the data for the current study was acquired using a standardized protocol on the same scanner, a suboptimal threshold selection would be likely to result in a systematic over- or underestimation of lesion volume across subjects. There may be subjective bias due to the use of one clinician to evaluate the EDSS data. The CCI measurements were also conducted by one person, namely the research Radiographer, and this might also be subjective to bias within the study.

5.8 Conclusion

In the present study, a 3-Tesla MRI scanner at the CUBIC was used to supply quality detailed imaging data, which helped to improve the reliability of the statistical conclusions drawn. Higher field strength systems and receiver coils with additional channels provided enhanced signal–to–noise ratio, thereby improving the quality of the imaging involved. Use of such equipment was crucial to the methods employed, as MS and its homologous neuropathological presentation on imaging can be distinctly subtle, due to its multiphasic nature (Dr Coenie Hattingh, personal communication).

Significant results found in this case-control study was the quality of the MRI imaging clearly delineating WMLs, for associations to be drawn between WMLs($p=0.02$) and disability (EDSS), though not with age(0.350), disease duration(0.709) or Interferon-β treatment ($p=070$). Other results of note includes those whom had followed the PSGT Program for more than 10 years, had significantly smaller WML volumes (mm3) compared to pwMS who did not adhere to the Program (n=9) (4950 ± 5303 vs 17934 ± 11139; $p=0.002$. The CCI was significantly lower in the MS patients than it was in the controls ($p=0.03$) (see Figure 4.2). Splenium thickness was also significantly lower in the MS patients than it was in the controls ($p=0.01$) (see figure 4.4). The EDSS values of the patients involved did not correlate with the number of lesions in the CC. In contrast, a significant inverse association ($p<0.01$) was found between the CCI and the EDSS, and between the EDSS and the thickness of the genu, midbody and splenium ($p<0.01$). There were no significant findings in the correlation between environmental factors and lesion

volumes, although, the serum Vitamin D concentrations had a significant inverse association with lesion volumes in the controls.

5.9 Recommendations

- The new MRI protocol although lengthier, made it possible to differentiate between active and inactive lesions without the need for contrast administration.
- The measurement of the CC could be a useful tool as an indicator for disease progression/early detection and adding the results of the EDSS could prove to be helpful with an earlier diagnosis(and hopefully better disease outcome). This strategy might counterbalance the need to use contrast media to aid with the diagnosis of MS. The CC index is a scalar measurement easily accessible from conventional MRI without the need for specific software. This fact enhances the validity of the CCI and its potential use in clinical applicability.
- Of significands were the decrease in number of WMLs in pwMS who followed the PSGT Program for more than 10 years. A PSGT program is where associations can be made between diet, lifestyle, and biochemistry data, incorporated into a clinical risk assessment algorithm, integrated with genetic test results, which is used to write a report for each participant. This report can inform clinicians the steps that can be undertaken to reduce the risk of disability for participants.
- Possible side effects and cost effectiveness of only having a MRI scan is more conducive for lower income countries whose governments tend neither to pay for such drugs, nor to compensate the patients concerned for the side-effects that they suffer. Although medical aid companies might pay for some of the less expensive drugs, they tend not to pay for the more costly ones.
- Noticeable in the literature was the role of brain volume and its association with disability(De Stefano et al., 2014), though in this study we did not cover much of this topic, but for future research we can include brain volumes as our focus using the images generated during the present study. All MS research is geared towards preventing disability in MS. So brain research measurements need to be standardised for the purpose that different clinical studies can be compared to.

Further research studies on MRI and disease monitoring need to be undertaken, so as to be able to report and update the relevant literature consistently, since the technology involved is constantly evolving and relatively new techniques are continuously being developed.

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Zagon,I, Multiple sclerosis

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APPENDIX A

APPENDIX A

Patient Name: _____

Hospital/Clinic: _____

Date Of Birth: _____**Weight:**_____

Height:_____

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

Please answer the following questions (mark with a X)

		Yes	No	Don't Know
1.	Do you have a cardiac pacemaker/defibrillator?			
2.	Do you have a neuro-stimulator?			
3.	Do you have cochlea implant/surgery to your ears? (If yes, please specify)			
4.	Have you ever had heart surgery such as a valve replacement? (If yes, please specify)			
5.	Have you ever had any type of electronic, mechanical, or magnetic implant? (If yes, please specify)			
6.	Do you have any foreign body in your eyes/body? Eg:(Bullet fragments etc)			
7.	Do you have a vena cava filter?			
8.	Do you have a prosthetic limb, eye/ other artificial device not already mentioned? (If yes, please specify)			
9.	Are you pregnant or breast feeding?			
10.	Are you claustrophobic?			
11.	Do you have aneurism clips?			
12.	Do you have renal impairment?			
13.	Do you have asthma?			
14.	Do you have allergies? (If yes, please specify)			
15.	Do you have other implants? Eg: (screws, plates, joint replacements)			
16.	Other			

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

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

Patient Signature:_____Date:_____

Consented by:_____

APPENDIX B

KURTZKE EXPANDED DISABILITY STATUS SCALE (EDSS)

Source: http://www.nationalmssociety.org/pdf/research/EDSS_Form.pdf

Patient.....

Date.....

Examiner.....

Final score.....

The Kurtzke Expanded Disability Status Scale (EDSS) is a method of quantifying disability in multiple sclerosis (Kurtzke, 1983). The EDSS quantifies disability in eight Functional Systems (FS) and allows neurologists to assign a Functional System Score (FSS) in each of these. The Functional Systems are: pyramidal, cerebellar, brainstem, sensory, bowel and bladder, visual, and cerebral.

Note1: EDSS steps 1.0 to 4.5 refer to patients who are fully ambulatory and the precise step number is defined by the Functional System score(s). EDSS steps 5.0 to 9.5 are defined by the impairment to ambulation and usual equivalents in Functional Systems scores are provided.

Note 2: EDSS should not change by 1.0 step unless there is a change in the same direction of at least one step in at least one FS.

* Excludes cerebral function grade 1.

Disability score	Neurological status
0.0	Normal neurological examination (all grade 0 in all Functional System (FS) scores*).
1.0	No disability, minimal signs in one functional score (FS*)(i.e. grade 1).
1.5	No disability, minimal signs in more than one FS* (more than 1 FS grade 1).
2.0	Minimal disability in one FS (one FS grade 2, others 0 or 1).
2.5	Minimal disability in two FS (two FS grade 2, others 0 or 1).
3.0	Moderate disability in one FS (one FS grade 3, others 1 or 0), or mild disability in three or four FS (three or four FS grade 2, others 0 or 1) though fully ambulatory.
3.5	Fully ambulatory but with moderate disability in one FS (one grade 3) and one or two FS grade 2, or two FS grade 3 (others 0 or 1) or five grade 2 (others 0 or 1).
4.0	Fully ambulatory without aid, self-sufficient, up and about some 12 hours a day despite relatively severe disability consisting of one FS grade 4 (others 0 or 1), or combination of lesser grades exceeding limits of previous steps; able to walk without aid or rest some 500 meters.
4.5	Fully ambulatory without aid, up and about much of the day, able to work a full day, may otherwise have some limitation of full activity or require minimal assistance; characterized by relatively severe disability usually consisting of one FS grade 4 (others or 1) or combinations of lesser grades exceeding limits of previous steps; able to walk without aid or rest some 300 meters.

APPENDIX C

MEDICAL & LIFESTYLE QUESTIONNAIRE

Patient Name……..……..... **Date**…….……

Contact Tel Numbers…….....…..... **Email**……

How many times a week have you consumed the following foods over the past 3 months?
[0] Never [1] Once a week [2] Twice a week [3] Three times a week
[4] Four times a week [5] Five times a week [6] Six times a week [7] Everyday

Any hamburger		All legumes (beans, peas, lentils)	
Red meat (e.g. beef, lamb, mutton)		Potatoes with skin	
Fried chicken / cooked chicken with skin		Five portions fruits and vegetables (whole, salad, pure juice)	
Hot dogs/ sausages		Whole grain breads and cereals (e.g. wheat, oats, corn)	
Salad dressings (excludes 'Lite' versions)		**FRUIT / VEG / FIBRE SCORE (Office use)**	
Butter and margarine (excludes 'Flora olive/ pro-active versions)		Broccoli, cauliflower, mushrooms	
Eggs (excludes cooking and baking)		Turnips, artichokes	
Full cream milk and diary products (fresh, sour or powered)		Avocado, spinach	
Fried hot potato chips, potato crisps, corn chips, buttered popcorn		Oranges and grapefruits (pure fruit versions)	
Biscuits, cake, cookies, pastries		Organ meats (e.g. liver, kidney, giblets)	
SATURATED AND *TRANS*-FAT SCORE (Office Use)		**FOLATE SCORE (Office use)**	
Food allergy /			
Medication / supplements			

Which best describes your physical activity status?
(Includes walking, swimming, cycling, attending exercise classes, each lasting more than 30 minutes)

Recreational sport occasionally or complete lack of exercise
Recreational sport 1 time a week
Exercise 2-3 times a week
Exercise 4 or more times a week

Which best describes your day-time or occupational activity?

Sedentary (desk work, driving, etc.)
Moderate (housework, gardening, walk often)
Intense physical labour (building and construction work, etc.)

Weight…… kg Height…….....meters

Waist.....cm Hip measurement.....cm

Office Use:

Systolic blood pressure …….....mmHg

Diastolic blood pressure ……mmHg

Date…….....Time.....

Smoker?

Y x Current
x Previous

How many units of alcohol do you consume on average per week? One unit alcohol equals
250 ml beer, 1 glass (125 ml) of wine, 1 pub measure of spirits

Abstain
1-2 units occasionally only
1-13 units
14-21 units
22 units or more

(Females) Do you take?

Y x An oral contraceptive pill (OCP)
Y N Hormone replacement therapy (HRT)

Blood donor?

Y x Current
Y N Previous

Vegetarian/vegan?

Y x Current
Y x Previous

APPENDIX D

UNIVERSITEIT·STELLENBOSCH·UNIVERSITY
jou kennisvennoot • your knowledge partner

19 November 2007

Dr S Janse van Rensburg
Division of Chemical Pathology
Dept of Pathology

Dear Dr Janse van Rensburg

RESEARCH PROJECT: **"THE DEVELOPMENT AND COMMERCIALISATION OF A COMPREHENSIVE GENE-BASED, PATHOLOGY SUPPORTED INTERVENTION PROGRAM FOR IMPROVED QUALITY OF LIFE IN PATIENTS DIAGNOSED WITH MULTIPLE SCLEROSIS (MS)"**

PROJECT NUMBER : N07/09/203

At a meeting of the Committee for Human Research that was held on 3 October 2007 the above project was approved on condition that further information that was required, be submitted.

This information was supplied and the project was finally approved on 12 November 2007 **for a period of one year from this date**. This project is therefore now registered and you can proceed with the work. Please quote the above-mentioned project number in all further correspondence.

Please note that a progress report (obtainable on the website of our Division) 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.

Patients participating in a research project in Tygerberg Hospital will not be treated free of charge as the Provincial Government of the Western Cape does not support research financially.

Due to heavy workload the nursing corps of the Tygerberg Hospital cannot offer comprehensive nursing care in research projects. It may therefore be expected of a research worker to arrange for private nursing care.

Yours faithfully

CJ VAN TONDER
RESEARCH DEVELOPMENT AND SUPPORT (TYGERBERG)
Tel: +27 21 938 9207 / E-mail: cjvt@sun.ac.za
CJVT/pm

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Fakulteit Gesondheidswetenskappe • Faculty of Health Sciences

APPENDIX E

Cape Peninsula University of Technology

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

P.O. Box 1906 • Bellville 7535 South Africa
Symphony Road Bellville 7535
Tel: +27 21 959 6917
Email: simonsy@cput.ac.za

5 December 2019
REC Approval Reference No:
CPUT/HW-REC 2019/H27

Dear Ms Mariaan Jaftha

Re: APPLICATION TO THE HW-REC FOR ETHICS CLEARANCE

Approval was granted by the Health and Wellness Sciences-REC to Ms Mariaan Jaftha for ethical clearance on 5 December 2019. This approval is for research activities related to student research in the Department of Informatics of this Institution.

TITLE: **Magnetic resonance imaging assessment of lesions for multiple sclerosis in relation to diagnosis and disability progression**

Supervisor : Prof P Engel-Hills

Comment

Approval is granted with the following conditions:

1. Approval from the 'mother study'
2. Approval from CUBIC

Approval will not extend beyond 6 December 2020. An extension should be applied for 6 weeks before this expiry date should data collection and use/analysis of data, information and/or samples for this study continue beyond this date.

The investigator(s) should understand the ethical conditions under which they are authorized to carry out this study and they should be compliant to these conditions. It is required that the investigator(s) complete an **annual progress report** that should be submitted to the HWS-REC in December of that particular year, for the HWS-REC to be kept informed of the progress and of any problems you may have encountered.

Kind Regards

Dr. Navindhra Naidoo
Chairperson – Research Ethics Committee
Faculty of Health and Wellness Sciences

APPENDIX F

Cape Peninsula University of Technology

HEALTH AND WELLNESS SCIENCES RESEARCH ETHICS COMMITTEE (HWS-REC)
Registration Number NHREC: REC- 230408-014

P.O. Box 1906 • Bellville 7535 South Africa
Symphony Road Bellville 7535
Tel: +27 21 959 6917
Email: sethn@cput.ac.za

18 November 2020
REC Approval Reference No:
CPUT/HW-REC 2019/H27/Renewal

Dear Ms Mariaan Jaftha,

Re: APPLICATION TO THE HWS-REC FOR ETHICS CLEARANCE - RENEWAL

Approval was granted by the Health and Wellness Sciences-REC on 05 December 2019 to Ms Mariaan Jaftha for ethical clearance. This approval is for research activities related to student research at this Institution.

Title: Magnetic resonance imaging assessment of lesions for multiple sclerosis in relation to diagnosis and disability progression

Supervisor: Prof P Engel-Hills

Comment:

Approval will not extend beyond 19 November 2021. An extension should be applied for 6 weeks before this expiry date should data collection and use/analysis of data, information and/or samples for this study continue beyond this date.

The investigator(s) should understand the ethical conditions under which they are authorized to carry out this study and they should be compliant to these conditions. It is required that the investigator(s) complete an **annual progress report** that should be submitted to the HWS-REC in December of that particular year, for the HWS-REC to be kept informed of the progress and of any problems you may have encountered.

Kind Regards,

M. le Roes-Hill

Dr Marilize Le Roes-Hill
Deputy Chairperson – Research Ethics Committee
Faculty of Health and Wellness Sciences

APPENDIX G

APPENDIX G

Director, MRI: Cape Universities Body Imaging Centre
UCT Faculty of Health Sciences

Professor Ernesta Meintjes

Private Bag X3, Rondebosch, 7701, South Africa
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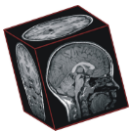

21 August 2020

Mariaan Jaftha
Senior Radiographer, CUBIC

Dear Ms Jaftha

Re: MR scanning at CUBIC as part of your research for the Master of Science in Radiography at the Cape University of Technology (CPUT)

This letter serves to confirm our support for the above study through access to the MRI facilities at the Cape Universities Body Imaging Centre (CUBIC) located at Groote Schuur Hospital, adjacent to the Faculty of Health Sciences of the University of Cape Town. The facility houses research-dedicated Siemens 3T Magnetom Skyra MRI that was commissioned in March 2015.

The MRI scanner room is equipped with an MR compatible 32-inch LCD screen, pair of slim design NordicNeuroLab (NNL) headphones, and left/right hand Lumina response pads for functional MRI experiments. In addition, a mock scanner and consulting rooms are available to prepare research participants for scanning and to collect biological specimens, if required.

Since the core focus of the centre is research, researchers are assured time on the scanners. All studies are required to have approval by the Institutional Review Board of the relevant institution. MRI scans of locally funded research are currently billed at an hourly rate of R3,407, as detailed on our website (www.cubic.uct.ac.za). Note that this is our current facility fee and that annual increases will be made in accordance with the South African Consumer Price Index (CPIX). Please contact us with regards any specific requirements or constraints.

CUBIC adheres to strict quality assurance standards and regular calibration scans are done to ensure optimal stability and performance of the imaging equipment. Three MRI technologists and three MRI physicists are available to run the scans and assist with technical aspects of the scan protocols. We look forward to your success with your research. Should you require any further information, please do not hesitate to contact me.

Yours sincerely

E. Meintjes

Ernesta Meintjes, PhD

cc. Daniel Doetz

APPENDIX H

CONSENT FORM FOR CASE REPORTS[1]

For a patient's consent to publication of information about them in a journal or thesis

Name of person described in article or shown in photograph:_Mrs Lizette Kotze_____

Subject matter of photograph or article:_Ultrasound and MRI scans_____

Title of article:__Preventing disability in multiple sclerosis (MS)_____

Medical practitioner or corresponding author:_Prof SJ van Rensburg_____

I_____ [insert full name] give my consent for this information about MYSELF, relating to the subject matter above ("the Information") to appear in a journal article, or to be used for the purpose of a thesis or presentation.

I understand the following:

1. The Information will be published without my name/child's name/relatives name attached and every attempt will be made to ensure anonymity. I understand, however, that complete anonymity cannot be guaranteed. It is possible that somebody somewhere - perhaps, for example, somebody who looked after me/my child/relative, if I was in hospital, or a relative - may identify me.
2. The Information may be published in a journal which is read worldwide or an online journal. Journals are aimed mainly at health care professionals but may be seen by many non-doctors, including journalists.
3. The Information may be placed on a website.
4. I can withdraw my consent at any time before online publication, but once the Information has been committed to publication it will not be possible to withdraw the consent.

Signed:_____ Date: _____

Signature of requesting medical practitioner/health care worker:

_____Date:_____

[1] Adapted from *BMJ Case Reports* consent form.

APPENDIX I

INFORMED CONSENT (English and Afrikaans)

CEREBROSPINAL VENOUS AND CAROTID ARTERY ULTRASOUND STUDY

INFORMED CONSENT /

INGELIGTE TOESTEMMING VIR SEREBROSPINAAL VENEUSE EN KAROTIS ARTERIE ULTRAKLANK STUDIE

INVESTIGATORS STATEMENT/ VERKLARENDE ONDERSOEK:

We are asking you to be part of this research study. The purpose of this consent form is to give you the information you will need to help you decide whether or not to join this study. Please read it carefully. You may ask questions about the purpose of the research, what you need to do, the possible risks and benefits, your rights as a volunteer and anything else about the research. Please ask if you do not understand any part of this form. When your questions have been answered you can decide if you want to join this study. This process is called 'informed consent'.

Ons vra dat U deel sal wees van hierdie navorsingstudie. Die doel van hierdie toestemmingsvorm is om inligting deur te gee wat help met die besluit om deel van die studie te wees of nie. Lees asseblief deeglik. Voel gerus on vrae to vra oor die doel van die navorsing, wat U as 'n pasient moet doen, die risiko's en voordele, U regte as 'n vrywilliger en enige iets met betrekking tot die navorsing.

Vra asseblief vrae as daar dele van die vorm is wat U nie verstaan nie. Na beantwoording van vrae kan u besluit of U wel aan die studie wil deelneem. Die proses staan bekend as ingeligte toestemming.

VOLUNTARY PARTICIPATION / VRYWILLIGE DEELNAME:

You have the right to withdraw from this study at any time. There will be no penalty or loss of benefits to which you are entitled.

U het die reg om enige tyd van die studie te onttrek. Daar sal geen verlies of penalisering van voordele wees nie.

BACKGROUND / AGTERGROND:

Previous research has shown that some of MS patients have narrowing of the neck veins (demonstrated with ultrasound) which cause reflux (backflow) of blood to the brain. Scientists' suggest that the narrowed veins in the neck are strongly associated with MS and could possibly be the cause of MS.

An ultrasound machine uses sound waves to image parts of the body including blood vessels. This study will use such a machine to look at the veins and arteries in your neck for narrowing and clot formation as well as plaque formation in the arteries of the neck.

Vorige navorsing het bewys dat pasiente met veelvuldige skleroise vernouing van die nek venes het en terugvloei van die bloed na die brein veroorsaak. Wetenskaplike vermoed dat die vernoude venes in die nek kan geassosieer word met VS.

‘n Ultraklankmasjien gebruik klankgolwe om dele van die liggaam te visualiseer insluitend bloedvate.
Hierdie studie gaan gebruik maak van so ‘n masjien om te kyk na die vene en arteries in die nek vir vernouing, terugvloei, klont- en plaakformasie.

THE PURPOSE OF THE STUDY / DIE DOEL VAN DIE STUDIE:
The purpose of this study is to use Ultrasound to investigate the presence abnormal blood flow in the veins and arteries of the neck. Furthermore, if any abnormal flow is detected then you will be referred to the appropriate medical practitioner for further management.

Die doel van hierdie studie is om ultraklank te gebruik om die teenwoordigheid van abnormal bloedvloei in die vene en arteries in die nek te ondersoek. Indien daar enige abnormale vloei opgespoor word sal U verwys word na die mees gepasde medies praktisyn vir verder behandeling.

ULTRASOUND EXAMINATION / ULTRAKLANK ONDERSOEK:
This study will require the patients to undergo one ultrasound examination of the arteries and veins of the neck. This test takes about 30-40 minutes to carry out. It is a painless examination and requires the patients to lie on their back while a small instrument called a probe is moved across the skin. A water based gel is first applied to the skin so the probe has good contact and can move smoothly. This examination is not painful. Some patients find this test relaxing.

RISK AND BENEFITS / RISIKO’S EN VOORDELE:
There are no known risks to an ultrasound examination. The most important benefit for this group of patients is that abnormal blood flow (if present) will be identified on ultrasound. If abnormal blood flow is identified then appropriate steps can be taken to manage the patient as necessary.

Daar is geen gevare aan ‘n ultraklank ondersoek verbande nie. Die mees belangrike voordeel vir hierdie groep pasiente is dat indien teenwoordig, abnormale bloedvloei geindentifiseer word, sal die nodige stappe in die behandeling van die pasient toegepas word.

OTHER INFORMATION / ANDER INLIGTING
Joining this study is voluntary. You can stop at any time. If you choose to join the study or not it will not affect your health care. Information about you is confidential. We will code the study records. The link between your name and code will be kept separate in a secure location. Only the study investigators will have access to that information. The link between your name and the code will be kept for 10 years then destroyed. Results of the study will be published where participants will be informed of the outcome.
Deelname aan hierdie studie is vrywillig. U kan enige tyd onttrek. Indien U kies om aan hierdie studie deel te neem sal dit nie U gesondheid affekteer nie. U informasie is vertroulik. Ons sal U studie rekords kodeer. Die skakel tussen U naam en kode sal op ‘n aparte en veilige bestemming gehou word. Slegs die persone wat vol mag het, sal toegang he tot die informasie. Die skakel tussen U naam en kode sal vir 10 jaar gehou word en dan vernietig word. Uitslae van hierdie studie sal gepubliseer word waar narvorsings onderwerpe ingelig sal word oor die resultate.

SUBJECT'S STATEMENT / ONDERWERP VERKLARING:

I understand what the procedure will be for this Ultrasound scan. I voluntarily agree to take part in this study. 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.

Die studie was aan my verduidelik. Ek bied aan om deel te neem aan hierdie navorsing. Ek het 'n kans gehad om vrae to vra en my vrae was beantwoord. Ek verstaan dat ek kan enige tyd van die studie kan onttrek.

Please complete the table indicating your voluntary participation in the study / Voltooi asseblief die table om U vrywillige deelname aan te dui.

SIGNED AT / GETEKEN TE	
DATE / DATUM	
NAME OF WITNESS / NAAM VAN GETUIE	
SIGNATURE OF WITNESS / HANDTEKENING VAN GETUIE	
NAME OF PARTICIPANT / NAAM VAN NAVORSINGS ONDERWERP	
SIGNATURE OF PARTICIPANT / HANDTEKENING VAN NAVORSINGS ONDERWERP	

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