INVESTIGATING COGNITIVE IMPAIRMENT IN MULTIPLE SCLEROSIS USING MYELIN WATER IMAGING

by

Shawna Lynn Abel

B.A., hons. Simon Fraser University, 2009

M.Sc. The University of British Columbia, 2012

A THESIS SUBMITTED IN PARTIAL FULFILLMENT OF

THE REQUIREMENTS FOR THE DEGREE OF

DOCTOR OF PHILOSOPHY

in

THE FACULTY OF GRADUATE AND POSTDOCTORAL STUDIES

(Experimental Medicine)

THE UNIVERSITY OF BRITISH COLUMBIA

(Vancouver)

November 2020

The following individuals certify that they have read, and recommend to the Faculty of Graduate and Postdoctoral Studies for acceptance, the dissertation entitled:

Investigating Cognitive Impairment in Multiple Sclerosis Using Myelin Water Imaging

submitted by Shawna Lynn Abel in partial fulfillment of the requirements for the degree of Doctor of Philosophy in Experimental Medicine

Examining Committee:

Dr. Shannon Kolind, Associate Professor, Department of Neurology, UBC
Supervisor

Dr. Anthony Traboulsee, Professor, Department of Neurology, UBC
Supervisory Committee Member

Dr. Silke Appel-Cresswell, Associate Professor, Department of Neurology, UBC
University Examiner

Dr. Liisa Galea, Professor, Department of Psychology, UBC
University Examiner

Additional Supervisory Committee Members:

Dr. Susan Forwell, Professor & Department Head, Department of Occupational Therapy, UBC
Supervisory Committee Member

Dr. Lara Boyd, Professor, Department of Physical Therapy, UBC
Supervisory Committee Member

Abstract

Cognitive impairment is a common symptom in multiple sclerosis (MS) that presents in up to 70% of patients. Cognitive symptoms in MS typically manifest as deficits in attention, memory and/or processing speed, with processing speed being most frequently affected. MS-related cognitive impairment represents a major burden as it can significantly lower quality of life and is a main contributor to unemployment.

Conventional magnetic resonance imaging (MRI) with T1-weighted and T2-weighted contrast is the mainstay of MS diagnosis and monitoring. However, conventional MRI is limited in that it is qualitative, lacks biological specificity and correlates poorly with clinical and cognitive status. In contrast, myelin water imaging (MWI) is an advanced MRI technique that measures the signal from water in the myelin bilayers, providing a quantitative myelin-specific measurement (myelin water fraction, MWF). The aim of this thesis is to investigate the relationship between myelin damage and cognitive performance in MS using MWI.

First, we demonstrate that MWF in normal appearing white matter (NAWM) was significantly associated with processing speed performance in MS in 3 *a priori* selected white matter tracts associated with cognition. Next, we show that the relationship between NAWM MWF and cognitive performance extends to additional cognitive domains in a larger cohort. Finally, rather than selecting brain regions *a priori*, we employed an assumption-free data driven approach using permutation testing to show that myelin damage extent and anatomical location is unique to the cognitive domain being investigated, with greater myelin damage in these regions in cognitively impaired versus cognitively preserved patients. Further, we demonstrate that the severity and spatial extent of myelin damage in cognitive domain-specific white matter regions is strongly associated with cognitive performance.

This thesis demonstrates that there is a strong relationship between the location and severity of myelin damage and MS-related cognitive impairment. As the treatment landscape for MS moves toward the development of remyelination therapies, understanding the role of myelin pathology in cognitive symptoms is critical for translating findings to clinical trials. These results also highlight the promise of MWI for monitoring myelin changes and their relationship to cognitive worsening and improvement when investigating new therapies.

Lay Summary

Up to 70% of people living with MS experience problems with memory, attention and problem solving, which is called cognitive impairment. Cognitive impairment can have a negative impact on a person's quality of life, it makes it difficult to perform everyday tasks (like paying bills), and is the leading cause of unemployment in people who have MS. MS damages myelin, a fatty substance that covers and protects nerve fibers in the brain and spinal cord. Our research team has developed an advanced imaging technique that uses special MRI scans to calculate the amount of myelin throughout the brain. We looked at the relationship between the amount of myelin in the brain and how well people score on different cognitive tests. We found that the two are closely linked, which tells us myelin damage plays a role. This information is helpful when we are developing new treatments for MS.

Preface

Chapter 2

A version of chapter 2 has been published as **Abel, S., et al. (2020). Myelin Damage in Normal Appearing White Matter Contributes to Impaired Cognitive Processing Speed in Multiple Sclerosis. Journal of Neuroimaging, 30(2), 205-211.** This work was also presented as a poster at the 2019 Americas Committee for Treatment and Research in Multiple Sclerosis (ACTRIMS) Forum in Dallas, Texas, USA. Dr. Shannon Kolind designed and conceptualized the study, supervised the overall study and obtained funding. Dr. Susan Forwell guided the selection of the appropriate cognitive tests. Drs. Anthony Traboulsee and Lara Boyd guided the design of the data intake form. All supervisory members provided guidance and valuable feedback throughout each project. I assisted with recruitment for this study by designing and displaying recruitment posters in the UBC MS Clinic, coordinating recruitment advertisements on local health authority websites, presenting the study and distributing recruitment materials at the Langley MS support group, distributing recruitment flyers at MS fundraisers (annual MS walk, annual Women Against MS Luncheon), distributing recruitment packages to the neurologists at the UBC MS Clinic and recruiting controls from my social network. The neurologists at the UBC MS Clinic (Drs. Anthony Traboulsee, Robert Carruthers, Virginia Devonshire and Ana-Luiza Sayao) provided the majority of patient recruitment referrals. I coordinated the study with the assistance of Poljanka Johnson, who I trained. This included calling referrals to confirm interest in participating and ensuring inclusion/exclusion criteria were met, MRI safety screening, scheduling the research appointment (booking the MRI appointment, cognitive testing room and neurologist for EDSS assessment) and preparing all materials and equipment for the MRI appointment and cognitive and clinical testing. The MRI scanning procedures were performed by the technologists at the UBC MRI Research

Centre. I performed the cognitive and clinical testing with the assistance of Poljanka Johnson. The EDSS assessments were performed by Drs. Jillian Chan, Alice Schabas, Nathalie Ackermans, Robert Carruthers and Anthony Traboulsee. I built the project SPSS database, scored the cognitive and clinical data and performed the project data entry with the assistance of Stephen Ristow. Dr. Jeffrey Wilken and his colleagues at Neuropsychology Associates of Fairfax scored the BVMT-R data. I coordinated the transfer of the BVMT-R data. Lisa Lee assisted with the creation of myelin water fraction maps. I created the MRI data processing and analysis pipeline with the assistance of Dr. Irene Vavasour. I developed the data analysis methodology, analyzed the data and wrote the final manuscript. Coauthors assisted with valuable feedback on the interpretation of findings and editing the manuscript. This work was approved by the UBC Clinical Research Ethics Board (H17-00866, "Establishing an imaging biomarker for disease progression in multiple sclerosis"). I assisted with the completion and submission of the ethics application and oversaw its renewal for the first 2 years of the study.

Chapter 3

A version of chapter 3 has been published as **Abel, S., et al. (2020). Associations Between Myelin Imaging and Cognitive Performance in Multiple Sclerosis. JAMA Network Open, 3(9), e2014220.** This information is embargoed until the date of publication. This project was also presented in 2019 as a poster at the 35th Congress of the European Committee for Treatment and Research in Multiple Sclerosis (ECTRIMS) in Stockholm Sweden where it won a Top Poster Award. My contributions as well as those of my coauthors are listed above in the preface for chapter 2. In addition, Dr. Helen Cross performed lesion masking for this study.

Chapter 4

A version of chapter has been submitted for publication. My contributions as well as those of my coauthors are listed above in the preface for chapter 2. In addition, Sarah Morris provided guidance on the use of FSL Randomise and Adam Dvorak created the healthy control myelin water fraction atlas used in this project.

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List of Abbreviations

10/36 SPART	10/36 Spatial Recall Test
9-HPT	9-Hole Peg Test
BiCAMS	Brief International Cognitive Assessment for MS
BRB-N	Brief Repeatable Battery of Neuropsychological Tests
BVMT-R	Brief Visuospatial Memory Test-Revised
CIS	clinically isolated syndrome
CNS	central nervous system
COWAT	Controlled Oral Word Association Test
CVLT-II	California Verbal Learning Test II
DST	Delis-Kaplan Executive Function System (D-KEFS) sorting test
DTI	diffusion tensor imaging
JOLO	Judgment of Line Orientation
MACFIMS	Minimal Assessment of Cognitive Function in MS
MRI	magnetic resonance imaging
MS	multiple sclerosis
MSFC	Multiple Sclerosis Functional Composite
MTR	magnetization transfer imaging
MWF	myelin water fraction
MWI	myelin water imaging
NAWM	normal appearing white matter

PASAT	paced auditory serial addition test
PPMS	primary progressive MS
PST	Processing Speed Test
RIS	radiologically isolated syndrome
ROI	region of interest
RRMS	relapsing remitting MS
SD	standard deviations
SDMT	symbol digit modalities test
SPMS	secondary progressive MS
SRT	Selective Reminding Test
T25-FW	Timed 25-Foot Walk

Acknowledgements

A heartfelt thank you to my supervisor, Dr. Shannon Kolind. I could not have asked for a better match in a mentor. I've learned so much from you as a scientist and as a leader. The research environment you've cultivated for all of us, where both hits and misses are celebrated and learned from, allows innovative science to happen.

I extend my sincerest thanks to Dr. Irene Vavasour for being my unofficial second supervisor. Thank you for teaching me much of what I know about advanced imaging and for using interpretive dance to convey some of these lessons.

A huge thank you to my supervisory committee members, Drs. Anthony Traboulsee, Susan Forwell and Lara Boyd. The projects that form this thesis have greatly benefited from your guidance and input. Thank you for your support, encouragement, your investment in my success and for being as enthusiastic about the work as I was. I genuinely looked forward to our meetings. You made this fun.

I'd like to recognize Dr. Cornelia Laule for her guidance on this work. Your editing significantly elevates the writing herein. Thank you for your advice on visually representing data. A figure is worth a thousand words.

Thank you to Dr. Shelly Au for your guidance, advice and support. I really appreciated our scientific discussions and that you always gave me your time in being my practice audience for talks.

A thank you to my wonderful lab mates. To Poljanka Johnson for helping me coordinate this study. I literally couldn't have done it without you. To Lisa Lee for helping with MRI data transfer and creation of myelin maps. Also, a thanks to Stephen Ristow for your assistance with data scoring and entry. A special thanks to Adam Dvorak for always doing an excellent job at responding to "ok, please explain <enter physics concept> to me like I'm a 5-year-old".

A project is nothing without good, clean data. A huge thank you to our incredible MRI technologists.

This work was made possible by funding from the Multiple Sclerosis Society of Canada.

These projects would not have been possible without the time and dedication of our MS volunteers. Meeting you all, spending time with you and hearing your stories was one of the best parts of this process. Thank you.

Dedication

For my husband, Kevin. This one's for you.

Chapter 1

1. Introduction

Cognitive impairment in multiple sclerosis (MS) was noted in Charcot's original description as "marked enfeeblement of the memory" with "conceptions [that] are formed slowly".[1] Cognitive dysfunction in MS was largely ignored for much of the 20th century but is now recognized as a feature of the disease that presents in up to 70% of patients.[2] MS-related cognitive impairment can have a severe impact on quality of life,[3] including: the ability to perform tasks of daily living,[4] fitness to drive,[5] and social functioning.[4] It is a major contributor to unemployment in MS patients.[3,6] Undoubtedly, cognitive impairment presents a major burden to those living with MS. An improved understanding of the anatomy and myelin pathology involved in MS-related cognitive impairment is essential for translating research results to clinical trials that target and test therapies and interventions for cognitive decline. Demyelination, a hallmark of MS,[7] is likely to play a major role in cognitive impairment, but a quantitative, sensitive and myelin-specific measure is required to characterize this type of tissue damage. Myelin water imaging (MWI), a quantitative magnetic resonance imaging (MRI) technique, measures signal from water in the myelin bilayers, providing a specific measure of myelin (myelin water fraction (MWF)).[8] At present, myelin water imaging is the most direct means of non-invasively assessing alterations in myelin.[9] The overall aim of this thesis is to investigate the role of myelin damage in MS-related cognitive impairment.

1.1 Multiple Sclerosis

1.1.1 Overview of MS

MS is an inflammatory, neurodegenerative disease of the central nervous system (CNS)[10] that affects over 2 million people globally, rendering it the most prevalent chronic neuroinflammatory CNS disease worldwide.[11] Estimates from individual provinces indicate that the prevalence of MS in Canada is one of the highest in the world.[12] MS is more prevalent in women[13] and it typically presents between the ages of 20 to 45.[14] Its etiology is thought to involve a complex combination of several genetic and environmental factors.[15] Pathologically, MS is defined by inflammation, demyelination with partial remyelination, neuronal and axonal damage, and glial scarring.[16]

1.1.2 MS pathology, demyelination and remyelination

MS is primarily characterized by demyelination.[7] The main function of myelin—a fatty substance that insulates axons—is to maximize conduction velocity of action potentials, reduce axonal energy consumption, and supply energy metabolites to axons.[17] Demyelination often leads to axonal loss, neurodegeneration and irreversible neurological disability.[7] While there is debate as to whether or not the root cause of MS is intrinsic or extrinsic to the CNS, it is generally accepted that adaptive immunity plays a critical role.[18] The current consensus is that autoreactive T cells and B cells enter the CNS from peripheral circulation inducing an inflammatory cascade that leads to demyelination and axonal loss.[19] More specifically, autoreactive peripheral B cells and helper T cells cross the compromised blood brain barrier and attack myelin peptides as they recognize them as foreign.[15] Axonal injury is also present and appears to be an important contributor to permanent

disability.[20] Other mechanisms driving axonal and neuronal damage include glutamatergic excitotoxicity,[21] cytokine release,[22] and generation of reactive oxygen species.[23,24] The aforementioned phenomena lead to calcium influx into axons and neurons, mitochondrial dysfunction and oxidative stress.[23]

Microglia, specialized myeloid cells that serve as the resident macrophages of the CNS,[25] play an important role in demyelination (M1 state: pro-inflammatory) and remyelination (M2 state: anti-inflammatory/immunoregulatory) in MS.[26] Activated microglia present antigens and secrete proinflammatory molecules that damage myelin and oligodendrocytes.[26] Activated microglia are found in lesions and are diffusely present in normal appearing white matter (NAWM, discussed further below) as well as grey matter.[27] Diffuse injury in NAWM and grey matter associated with microglia activation is present in the early phases of the disease and increases in severity with disease progression.[28] The cortical demyelination associated with microglial activation is an important pathological substrate that correlates with disease progression, irreversible disability and cognitive impairment.[27]

Following demyelination, remyelination can occur; a process in which microglia are also involved. Remyelination is initiated by activation and migration of oligodendrocyte precursor cells (OPCs), which differentiate into myelinating oligodendrocytes.[29] Microglia play an important role in the initiation of remyelination via phagocytosis of myelin debris from the area of damage. Further, they enhance oligodendrocyte proliferation through cytokine production and encourage oligodendrocyte differentiation in their M2 state.[26]

Though the remyelination process typically results in thinner myelin, it is thought to restore metabolic support to the axon, thereby limiting the axonal degeneration associated with progressive disability.[29] Further, the nodes of Ranvier required to facilitate axonal conduction are

thought to be restored resulting in restoration of function.[29] Enhancing myelin regeneration has the potential to be neuroprotective,[30] therefore, there is broad consensus that remyelination is the most important treatment goal for future MS pharmacotherapy.[31]

1.1.3 Clinical features of MS

The majority of MS cases (~85%) begin with a relapsing-remitting (RRMS) disease course, which is characterized by episodes of new focal demyelination in a clinically eloquent path (clinical relapse) followed by periods of clinical remission with either full or partial recovery. Within 25 years, most patients with RRMS (~60-70%) transition to secondary progressive MS (SPMS), a clinical disease stage during which disability gradually accumulates independent of relapses. Roughly 10% of patients present with a progressive disease course at the outset, termed primary progressive MS (PPMS) (Figure 1.1).[14,32] Clinically isolated syndrome (CIS) refers to the first episode of neurologic symptoms lasting more than 24 hours caused by inflammation and demyelination, which is now recognized as the initial presentation of RRMS.[33] Not considered an MS subtype per se but raising suspicions of potential MS is radiologically isolated syndrome (RIS).[33] RIS is identified with incidental imaging findings suggestive of inflammatory demyelination with an absence of clinical symptoms.[33]

Relapses are the result of demyelination and conduction block caused by inflammatory aggregates in the CNS associated with blood brain barrier breakdown. These inflamed areas appear as gadolinium enhancing lesions on MRI.[34] Typical relapse symptoms include monocular vision loss due to optic neuritis (inflammation and demyelination of the optic nerve), limb weakness or sensory loss due to transverse myelitis (inflammation and demyelination in the spinal cord), double vision and ataxia due to a brainstem and cerebellar lesions.[35] During the progressive phase, there is typically fewer new inflammatory demyelinating lesions but chronic demyelination may lead to

a loss of axons.[32] The clinical transition from RRMS to SPMS is hypothesized to occur when axonal loss exceeds the compensatory capacity of the CNS, with continued axonal loss resulting in the steady progression of permanent neurological disability. However, pathological mechanisms of progressive MS are believed to be present at the earliest clinical stages of MS.[32] MS symptoms extend beyond those listed above to neurogenic bladder/bowel, spasticity, sexual dysfunction, fatigue, depression and cognitive impairment,[36] all of which have a major impact on a patient's daily functioning.[37]

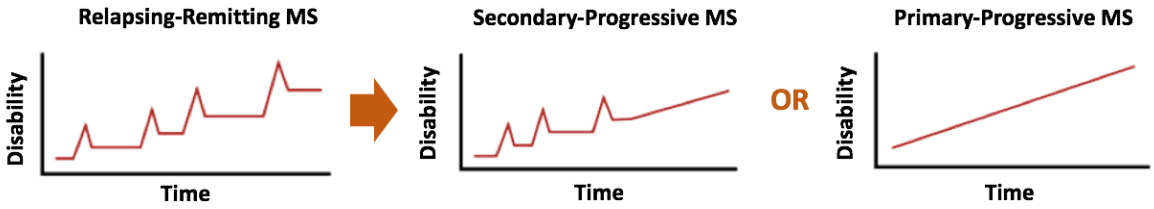

Figure 1.1: MS Subtypes. The majority of MS cases present with a relapsing-remitting course, which may then transition into a secondary-progressive phase. Some MS cases start with a primary-progressive course, in which the patients gradually accumulate disability from disease onset independent of relapses. (image source: ref 38)

1.1.4 Measuring disease severity in MS

In MS, disability is often quantified using the Kurtzke Expanded Disability Status Scale (EDSS) (Figure 1.2).[39] The EDSS measures disability with eight Functional System Scores (FSS), which focus heavily on physical disability and mobility. However, the EDSS does not adequately measure cognitive function.[40,41] Another common tool used for measuring MS diseases severity is the Multiple Sclerosis Functional Composite (MSFC).[42] The MSFC measures upper limb function with the 9-Hole Peg Test (9-HPT), lower limb function with the Timed 25-Foot Walk (T25-FW), and cognition originally with the Paced Auditory Serial Addition Test (PASAT)[43] which was recently replaced by the Symbol Digit Modalities Test (SDMT).[44] However, while the SDMT is considered the most sensitive singular cognitive test for detecting cognitive impairment in MS

patients,[45] it may not capture all cognitive phenotypes.[46] A battery of multiple individual cognitive tests that explore different cognitive domains is commonly used when quantifying cognitive performance in a research setting.

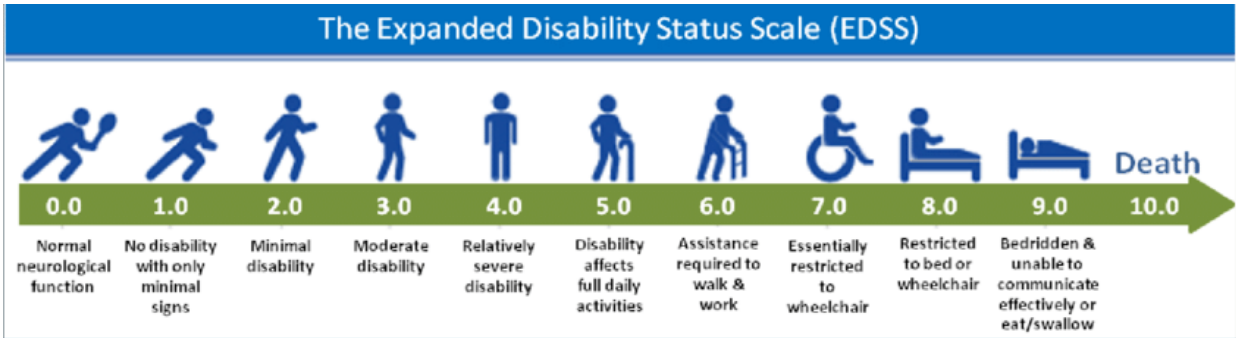

Figure 1.2: EDSS. A common disability measure in MS. (Image source: Ref 39)

1.2 Cognitive impairment in MS

1.2.1 Overview of cognitive impairment in MS

Cognitive impairment is common in MS, affecting 40-70% of patients.[2,47] Cognitive symptoms in MS typically manifest as deficits in attention, memory and processing speed,[2] with processing speed being most frequently affected.[48] Executive dysfunction and loss of verbal fluency may also be involved.[2] Cognitive dysfunction can present in newly diagnosed patients,[49] patients with CIS,[50] RIS,[51] and in people with so-called benign MS (EDSS of 3.0 or lower at least 10 years after diagnosis).[52] However, cognitive impairment in MS is more prevalent and typically more severe in progressive phenotypes.[53] Both short[54,55] and long-term follow up studies[56] conclude cognitive impairment to be progressive in nature, with little evidence for improvement once it emerges. Much of what we know about the underlying pathology associated with MS-related cognitive impairment comes from MRI studies, which will be discussed later in this chapter.

1.2.2 Impact of cognitive impairment

MS-related cognitive impairment has a severe impact on quality of life,[3] including: the ability to perform tasks of daily living,[4] fitness to drive,[5] and social functioning.[4] Cognitively impaired patients are less likely to participate in social activities, are more likely to report reductions in self-esteem and have higher rates of divorce.[4,37] In addition, MS patients with cognitive impairment have higher rates of emotional distress and psychiatric disturbances.[2] Several studies have indicated that cognitive impairment is the strongest predictor of who will require a reduction in work responsibilities and become unemployed in MS patients,[57,3,58] this prediction is independent of the level of physical disability.[56]

1.3 Cognitive assessments in MS

1.3.1 Processing speed

1.3.1.1 Paced Auditory Serial Addition Test (PASAT)

Processing speed is measured as the amount of work completed correctly within a specific time limit, which can be measured by the Paced Auditory Serial Addition Test (PASAT). During the administration of the PASAT, a series of single digit numbers are presented via a speaker and the two most recent digits must be summed. For example, if the digits '4', '6' and '2' are presented, the participant must answer "8". If the subsequent number presented is '2', the correct answer would be "4".[59] Thus, the participant must perform addition while keeping the former number in mind and listening for the next number. The speed at which the numbers are announced can be at the rate of 3 seconds or 2 seconds to increase the level of difficulty of the test. The PASAT was previously the most commonly used cognitive assessment in MS research. However, it has now fallen out of favour as it is not well tolerated by patients and has only moderate sensitivity (mean

d = 0.63).[60] Further, the test's reliability is limited by practice effects and its sensitivity is limited by pre-existing math ability as well as test anxiety.[61] The PASAT is not recommended for clinical trials with more than one time point and is not recommended for cognitive monitoring in clinical practice.[61]

1.3.1.2 Symbol Digit Modalities Test (SDMT)

The Symbol Digit Modalities Test (SDMT), also a measure of processing speed, is the most sensitive cognitive assessment validated for use in MS.[60] It is thus recommended instead of the PASAT, and has replaced the PASAT as the cognitive test in the MSFC. The SDMT[62] (Figure 1.3) contains a reference key with the numbers 1-9, each corresponding to different geometric symbols. The answer key contains only symbols to which the participant must match the corresponding number according to the key. In the oral version of the SDMT, the subject responds orally with the digit associated with symbol as quickly as possible; in the written version, the corresponding digit must be written under the symbol in the answer key. The test is scored by tallying the total number of correct responses achieved in 90 seconds.[63]

The SDMT has very high sensitivity (d = 1.11), good to excellent reliability, is well tolerated by patients and there is evidence that a 3-4 point change is clinically meaningful.[60] Conversely, the SDMT is limited by its requirement for visual scanning and therefore may not be suitable for patients with visual impairment. The SDMT is highly recommended as a cognitive assessment tool for both cross-sectional and longitudinal studies as multiple forms are available.[61] It is also highly recommended for cognitive monitoring in clinical practice.[61] A tablet based version of the SDMT that can be self-administered, the Processing Speed Test (PST), has now been validated for use in MS.[64] The PST provides a practical tool for routine screening of cognitive

status in the MS clinic and allows for efficient administration and scoring, which can potentially be integrated into medical records and research databases.[64]

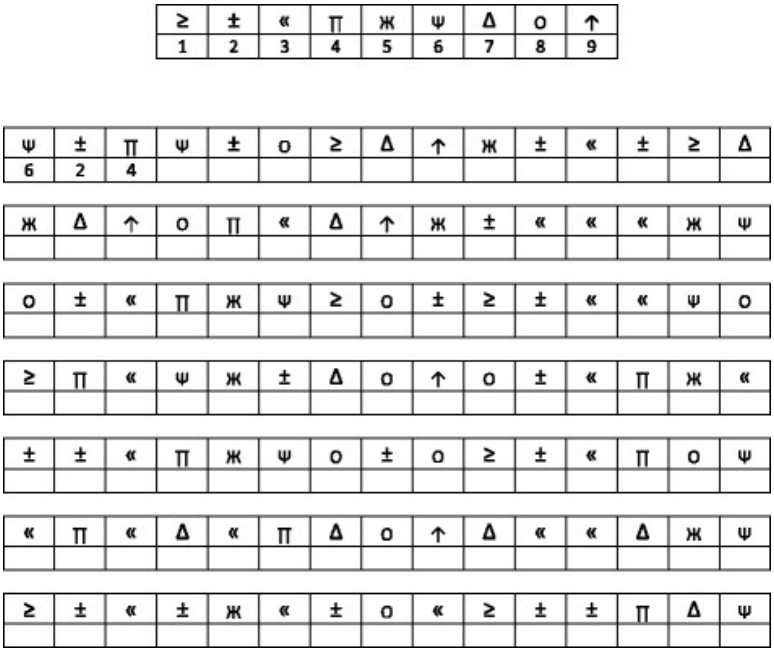

Figure 1.3: The Symbol Digit Modalities Test (SDMT) example stimuli. The SDMT is a tool used to measure processing speed performance in MS patients. It is a sensitive measure of cognitive impairment in MS.

1.3.2 Verbal memory

1.3.2.1 Selective Reminding Test (SRT)

Verbal memory, the storage and recall of phonological information, can be assessed with the SRT.[65] During administration of the SRT, the participant is read aloud a list of 12 words that they are asked to repeat back immediately. After they have repeated all words they can remember, the participant is read back only the words they have missed and asked to repeat all 12 words again. This procedure is repeated for 6 rounds. The participant is again asked to repeat all 12 words subsequent to a delay (approximately 30 minutes). The SRT is scored by tallying the total correct recalled words.

The SRT has high sensitivity (mean d = 0.86), many alternative forms and several sets of published norms. It is recommended as a verbal memory test for research use and clinical trials, particularly when more than one time point is planned. Unfortunately, the SRT is not an adequate candidate for future unsupervised assessment as a tablet based version would assess verbal recognition rather than auditory recall, which is less sensitive to verbal memory deficits in MS.[61]

1.3.2.2 California Verbal Learning Test II (CVLT-II)

The CVLT-II is also used to assess verbal memory in MS.[66] Similar to the SRT, during the CVLT-II the participant is read a list of 16 words (list A) and is asked to repeat back as many as they can remember for 5 learning trials. List A is composed of words drawn from 4 semantic categories (e.g., spices, tools, fruit, clothing). Subsequent to the 5 learning trials, a distractor list (list B) that shares 2 categories with list A (e.g., tools, fruit) is read. After the distractor list is recited, the participant is asked to recall list A immediately (immediate recall) as well as after a 20-minute delay (long term recall).

The CVLT-II has high sensitivity (mean d = 0.89), a standard and alternative form and normative data from a large sample from the United States of America (USA). It is recommended for clinical trial and research use when no more than 2 administrations are required. As with the SRT, the CVLT-II is a poor candidate for tablet based self-administered assessment.[61]

1.3.3 Visuospatial memory

1.3.3.1 Brief Visuospatial Memory Test-Revised (BVMT-R)

Visuospatial memory—the ability to remember objects and their location in space—can be measured in MS with the BVMT-R.[67] During administration of the BVMT-R, the participant is presented with a display of 6 geometric figures for 10 seconds and then asked to reproduce the display by drawing it exactly as it was seen on a blank page. The participant is given 3 opportunities to view and reproduce the display. Subsequent to a delay (approximately 25 minutes), the participant is asked to draw the display from memory without a prompt. Finally, the participant is shown a series of figures that appeared on the display as well as distractor figures (not on the display) and responds "yes" or "no" as to whether or not they have seen them previously.

The BVMT-R has very high sensitivity (mean d = 1.03), good reliability, 6 well-validated alternative forms and normative data from a large USA sample. The BVMT-R is recommended for research as well as clinical trial use and is appropriate for both cross-sectional and longitudinal studies. The BVMT-R excludes patients with severe upper limb disability who are unable to draw, however, it is a good candidate for a tablet based test should one be developed and validated for MS in the future.[61]

1.3.3.2 10/36 Spatial Recall Test (10/36 SPART)

Visuospatial memory can also be measured using the 10/36 SPART.[68] To administer the 10/36 SPART, the participant is shown a checkerboard with a pattern using 10 checkers for 10

seconds and the participant is required to reproduce the pattern of checkers on a blank board. This process in repeated 2 more times during the immediate recall portion. For the delayed recall portion, the participant is required to reproduce the checker pattern without a prompt.

The 10/36 SPART has lower sensitivity (mean d = 0.48) relative to the BVMT-R, is less reliable and acceptable normative data is lacking. Due to this low sensitivity, questionable reliability and lack of appropriate normative data, the 10/36 SPART is not recommended for use in clinical practice or clinical trials. While it is acceptable for research purposes, the BVMT-R is recommended over the 10/36 SPART.[61]

1.3.4 Visuospatial processing

1.3.4.1 Judgment of Line Orientation (JOLO)

The JOLO (Figure 1.4)[69] measures the participants ability to judge the angle and orientation of lines. The participant is asked to identify 2 lines from a fan-like arrangement at the bottom of the page that match the 2 lines at the top of the page. The JOLO has lower sensitivity (d = 0.49) relative to other cognitive tests used in MS. However, visuospatial processing isn't often impaired in MS. The JOLO can be used as a valid and reliable task for comprehensive cognitive evaluations in a research setting. It is not recommended for use in clinical practice or clinical trials unless the target of the intervention is visuospatial function.[61]

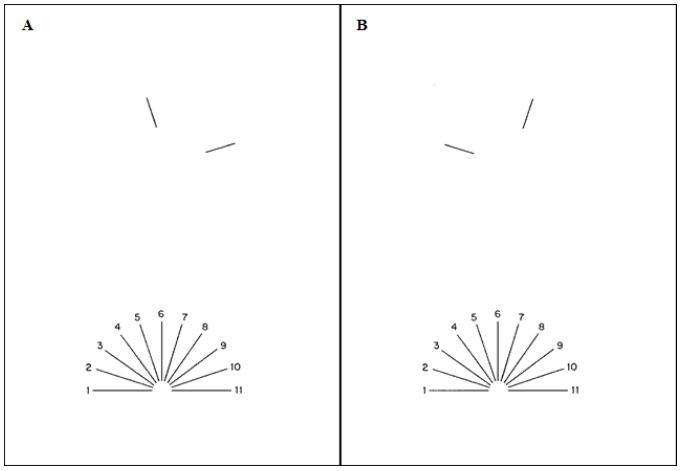

Figure 1.4: Judgment of Line Orientation (JOLO): Example stimuli for the JOLO, an assessment of visuospatial processing in MS. (Image source: Ref 70)

1.3.5 Executive function

1.3.5.1 Delis-Kaplan Executive Function System (D-KEFS) sorting test (DST)

Executive function refers to an assemblage of related abilities including abstract reasoning, conceptual flexibility, and planning and organizing behaviour. The DST can be used to measure conceptual reasoning in MS.[71] Sensitivity of the DST is moderate (d = 0.67) and reliability of the test is low, which is typical of executive function tests. This test is not recommended for monitoring in clinical practice as the testing procedures are long and cumbersome but may be useful for comprehensive clinical evaluation. It is also not recommended for clinical trials unless executive function is the target of the intervention.[61]

1.3.6 Verbal fluency

1.3.6.1 Controlled Oral Word Association Test (COWAT)

The ability to summon the correct word when needed—word retrieval—is the most common language problem encountered in MS.[72] Word retrieval can be assessed using the COWAT. [63,73] During the COWAT, the participant is given a letter of the alphabet (e.g., "F") and

asked to list as many words as they can recite beginning with that letter in 1 minute's time. The test typically includes 3 letter prompting categories and an animal category, for which the participant names as many animals as they can recall. The COWAT is scored by tallying the total number of permissible answers—proper names (e.g., cities) and variations of the same word (e.g., runs, running, ran) are not permitted.

The COWAT has moderate sensitivity (d = 0.54) but limited validity given that performance is very much related to level of education and vocabulary rather than MS disease burden. The COWAT is not recommended for monitoring in clinical practice or for clinical trials (unless verbal fluency is the target of the intervention). However, the COWAT is a reasonable component of a comprehensive neuropsychological assessment for both research and practice.[61]

1.4 Neuropsychological batteries used in MS

The above described cognitive tests are grouped together to form comprehensive neuropsychological batteries for assessing cognition in MS. Until recently, the most widely used battery in MS was the Brief Repeatable Battery of Neuropsychological Tests (BRB-N) developed by Rao in 1991 (Table 1). The BRB-N is highly sensitive (sensitivity= 71%) and specific (specificity=94%) in distinguishing cognitively impaired and intact patients and can be administered in 1 hour.[63] However, the BRB-N lacks tests of visuospatial processing and executive function. Subsequent to a consensus meeting in 2001, a panel of neuropsychologists with expertise in the field of cognitive impairment in MS developed the Minimal Assessment of Cognitive Function in MS (MACFIMS),[71] which is similar to the BRB-N but includes tests of visuospatial processing and executive function (administration time=1.5 hours). Evaluation of the MACFIMS concludes it to be a valid and reliable measure for cognitive impairment in MS, with all tests

discriminating MS patients from healthy controls and RRMS from SPMS patients.[73] Unfortunately, both the BRB-N and MACFIMS are limited by their administration time and are not applicable to routine clinical practice.[74] This limitation led to the development of the Brief International Cognitive Assessment for MS (BiCAMS),[75] which can be completed in 15 minutes; it is based on the MACFIMS but is less comprehensive. It should be noted that the most sensitive tests currently available for cognitive monitoring in MS are the SDMT, SRT, CVLT-II and BVMT-R,[61] three of which form the BiCAMS.

Table 1.1: Neuropsychological batteries commonly used in MS

Batteries				
Cognitive Domain	**BRB-N**	**MACFIMS**	**BICAMS**	**Thesis Project**
Attention/information processing speed	SDMT	SDMT	SDMT	SDMT
Working Memory/processing speed	PASAT	PASAT	x	x
Executive Function	x	D-KEFS	x	x
Verbal memory	SRT	CVLT-II	CVLT-II	SRT
Visuospatial memory	SPART (10/36)	BVMT-R	BVMT-R	BVMT-R
Word retrieval	COWAT	COWAT	x	COWAT
Spatial processing	x	JLO	x	x

SDMT = Symbol Digits Modalities Test; PASAT = Paced Auditory Serial Addition Test; D-KEFS = Delis-Kaplan Executive Function System; WCST = Wisconsin Card Sorting Test; SRT = Selective Reminding Test; CVLT-II = California Verbal Learning Test-2nd edition; SPART = 10/36 Spatial Recall Test; BVMT-R = Brief Visuospatial Memory Test-Revised; COWAT = Controlled Oral Word Association Test; JLO = Judgment of Line Orientation

1.5 Defining cognitive impairment

The above described tests all yield quantitative values to assess performance (high or low), however, investigators often prefer to classify study participants as cognitively "impaired" or "preserved". Definitions of impairment have varied widely across studies, but the general consensus is that performance of 1.5 standard deviations (SDs) below normative scores on a test is considered impaired on that test.[61] An important caveat regarding this definition is that clinicians and scientists are often observing cognition at a single time point. When a patient reports a cognitive issue, they are describing a decrease in function from a previous level. Premorbid or point of diagnosis cognitive assessments are rarely documented. Thus, a patient who previously performed well above the 50th percentile can experience significant cognitive decline but still be characterised as cognitively intact. Similarly, a patient whose premorbid cognitive function is below the 50th percentile may experience a mild decline and be classified as cognitively impaired (Figure 1.5). This is important to consider for cross-sectional studies as it increases variability in the cognitive data.

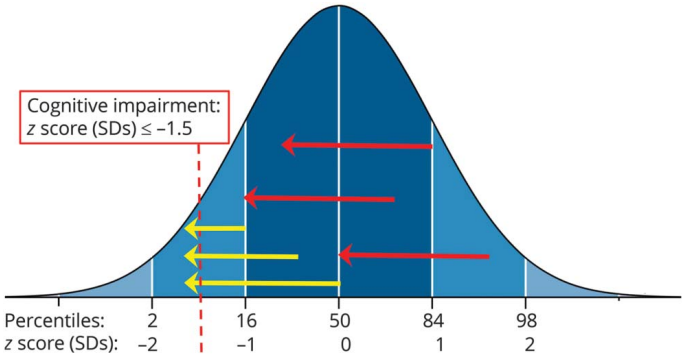

Figure 1.5: Limitations of defining cognitive impairment in MS. Premorbid or point of diagnosis cognitive assessments are rarely documented. A patient who previously performed well above the 50th percentile can experience significant cognitive decline but still be characterised as cognitively intact. Similarly, a patient whose premorbid cognitive function is below the 50th percentile may experience a mild decline and be classified as cognitively impaired. (Image source: Ref. 61)

1.6 Imaging cognitive impairment in MS

1.6.1 Lesions and limitations of conventional imaging

MRI is currently the best available method for the diagnosis and monitoring of MS. Conventional MRI is qualitatively useful for identifying lesions on T1 and T2-weighted images, with the dissemination of lesions in space and time being a core component of the MS diagnostic criteria.[38] Some studies have reported associations between white matter lesion burden and cognitive dysfunction in MS[76–80] while others have failed to find significant relationships.[81–83] Further, a recent meta-analysis of 32 studies (n=2050) investigating the relationship between T2 lesion burden and MS-related cognitive impairment found that the aggregate correlation was moderate at best (r = -0.30; 95%CI: -0.26 - -0.34).[84] The limited association between focal lesion burden and the severity of MS symptoms indicates that MS pathology extends beyond lesions visible on conventional clinical MRI to grey matter and white matter that appears normal on conventional MRI (NAWM). In addition, multiparametric MRI studies have consistently demonstrated that focal lesions play only a partial role in MS-related cognitive impairment relative to the contribution attributed to damage in NAWM and grey matter.[85–87]

1.6.2 Cognitive impairment, brain atrophy and grey matter damage

Measures of brain atrophy have been shown to correlate better with physical disability than lesion volume.[88,89] Likewise, studies suggest that grey matter atrophy may be a superior predictor of cognitive impairment relative to focal white matter lesion burden.[82,90] In terms of cognition, brain volume tends to correlate with processing speed performance,[82,91,92] and atrophy of deep grey matter structures, particularly the thalamus, has been associated with impairment in several cognitive domains.[82,93,94,95,96] While grey matter damage has been shown to be more strongly

correlated with cognitive measures than lesion load,[51,82,97] grey matter metrics are not biologically specific to what components within the tissue are damaged. Studies investigating lesions, grey matter damage and abnormalities in NAWM concurrently have reported that abnormalities in NAWM accounted for 36-51% of the variance in performance on attention and memory tasks (SRT and SDMT), with grey matter damage contributing less than 5% of the variance;[98] while another observed that damage to NAWM was the only statistically significant factor distinguishing cognitively impaired patients from cognitively intact patients.[99]

1.6.3 Normal appearing white matter (NAWM)

In MS, NAWM has several pathologic features that are not detectable by conventional clinical MRI examinations. These include gliosis, inflammation, macrophage infiltration, demyelination and axonal damage.[100,101] Damage to NAWM has often been investigated with diffusion tensor imaging (DTI), an advanced MRI technique used to visualize white matter fiber structure,[102] or magnetization transfer imaging (MTI), which estimates the exchange of magnetization between mobile protons in water and nonaqueous protons associated with macromolecules.[103] Previous DTI[104,105] and MTI[86] studies have demonstrated that abnormalities in NAWM are associated with cognitive deficits in MS.

While DTI and MTI studies highlight the utility of advanced imaging techniques in exploring the role of non-lesional white matter in MS-related cognitive impairment, these techniques are not myelin specific. Therefore, though we know that damage to NAWM is an important contributing factor to MS-related cognitive impairment, the exact nature of the damage remains unknown. DTI measures are sensitive to a large number of biological changes that occur in MS including demyelination, axonal loss, edema, inflammation and gliosis and thus have low biological specificity. Furthermore, DTI measures are confounded by white matter architecture

(e.g., crossing fibres, fibre orientation coherence), which often leads to misinterpretation of findings.[106] Abnormalities in the MTI derived magnetization transfer ratio (MTR) are often interpreted as damage to myelin,[107] however, MTR is heavily influenced by inflammation and edema[108] as well as axonal density.[109]

1.7 Myelin water imaging

To improve our understanding of the changes detected in the MS brain related to cognition, a quantitative and biologically specific measure should be used. As a principle pathology in MS is demyelination, it is reasonable to hypothesize that cognitive dysfunction in MS is associated with damage to myelin in white matter. An advanced MRI technique that is a sensitive and specific measure of myelin is myelin water imaging (MWI). Pioneered at the University of British Columbia (UBC), MWI provides quantitative measurements specific to myelin.[110,111] The MWI technique separates the MRI signal into contributions from the distinct water pools within a voxel based on the MRI property known as T_2 relaxation time. In CNS tissue, these water pools generally correspond to (i) a long relaxation time component, which arises from cerebrospinal fluid (T_2=~ 2 s); (ii) an intermediate component, due to intra- and extra-cellular water (T_2=~ 40–200ms); and (iii) a short component, stemming from water trapped between the myelin bilayers (T_2=~15-40ms).[8] The fraction of MRI signal arising from the myelin water divided by the total water signal is the myelin water fraction (MWF) (Figure 1.6). MWF has been histologically validated as a specific marker for myelin using post-mortem human tissue [112,113] and animal models of myelin damage [114]. At present, myelin water imaging is the most direct means of non-invasively assessing alterations in myelin.[9]

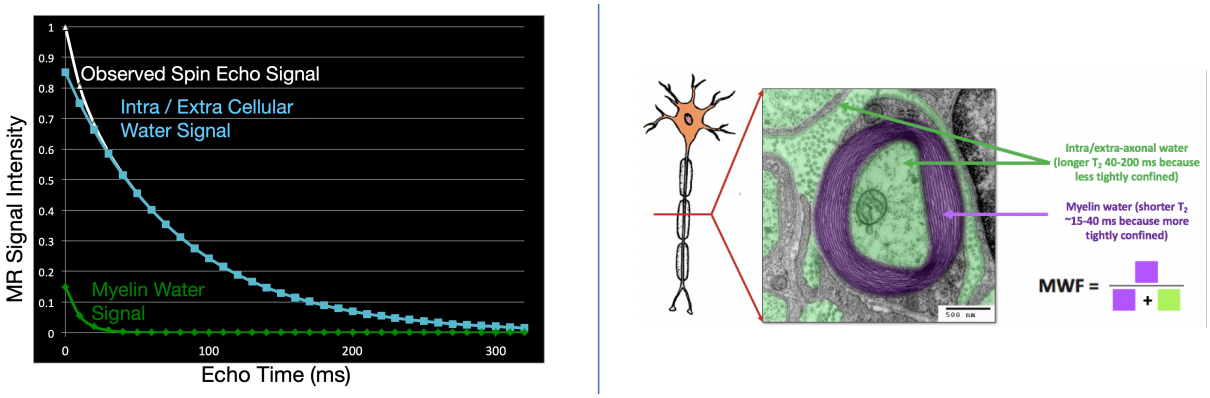

Figure 1.6: The myelin water fraction (MWF). In CNS tissue, T_2 signal has (i) a long relaxation time component, which arises from cerebrospinal fluid (T_2=~ 2 s); (ii) an intermediate component, due to intra- and extra-cellular water (T_2=~ 40–200ms); and (iii) a short component, stemming from water trapped between the myelin bilayers (T_2=~15-40ms)(left panel). The fraction of MRI signal arising from the myelin water divided by the total water signal is the MWF (right panel). (Image source: Ref 115)

1.7.1 Myelin water imaging in MS

When used to investigate individuals with MS, as would be expected, MWF is found to be significantly reduced (~50%) in MS lesions relative to NAWM.[116] In addition, while damage to NAWM is undetectable via conventional clinical MRI examinations, MWF is sensitive to diffuse myelin loss in NAWM in the brain (6-37%)[116–118] as well as the spinal cord (11-25%)[119,120] of MS patients relative to controls. Moreover, reductions in MWF in NAWM are predictive of increased clinical disability as measured by the EDSS[118,121] and can differentiate patients with CIS, RRMS, SPMS and PPMS.[121] Preliminary evidence also indicates a link between the anatomical location of MWF reductions and their functional consequences. Specifically, reductions in MWF near the sensory cortex corresponded to worse sensory subsystem scores, and reductions in MWF in the

anterior regions of the brain (frontal lobes and genu of the corpus callosum) related to worse cognitive subsystem clinical scores.[118]

1.7.2 Myelin water imaging and cognition

Variations in MWF have also been shown to be related to cognition outside of MS. Increased MWF has been associated with indices of more advanced cognition in preadolescents,[122] young children,[123,124] and babies and toddlers.[125] Children who exhibit slower but more prolonged myelin development, resulting in overall increased MWF at age 3 also demonstrate above average cognitive ability.[125] Greater MWF values have also been associated with measures of IQ and years of education in healthy adults.[126] When exploring a clinical population, decreased MWF values have been associated with cognitive impairment in people with amyotrophic lateral sclerosis.[127] Taken as a whole, the literature indicates that MWF is well suited for investigating differences in myelin in people with MS and its relationship to cognitive impairment.

1.8 White matter and cognition

Traditionally, cortical grey matter is seen to have the most important role in cognitive function, with less attention devoted to the contributions of other parts of the brain—a view that has been termed "cortico-centric myopia".[128] The advent of MRI has helped to elucidate the essential role white matter plays in cognition.[129] As described above, myelination during childhood is associated with cognitive development,[130,131] and individual differences in white matter structure correlate with normal variation in reading,[132] working memory[132] and musical skill.[133] Myelin plays an essential role in processing speed, memory and learning (plasticity) by coordinating the precision of synaptic signals.[134] Myelin controls the speed and synchrony of signal conduction through axons between distant cortical regions, which is required for optimal mental performance

and learning.[134] In terms of learning, "synapses that fire together wire together". Timing-dependent plasticity shows that synapses that fire simultaneously prior to action potential initiation become strengthened while those that don't are weakened.[135] If two presynaptic neurons are located at different distances from the postsynaptic neuron, the conduction velocity of these axons must be precisely controlled for the synaptic signals to arrive simultaneously.[134] Myelin can influence conduction velocity by regulating the thickness of the sheath, the number and spacing of the nodes of Ranvier and the composition of the ion channels in the nodes.[134] Therefore, compromised myelin can lead to failure in these processes and lead to cognitive impairment.

1.8.1 Region of interest (ROI) selection

White matter tracts can be categorized as commissural, association or projection fibers.[136] Commissural fibers connect the left and right hemispheres of the brain, association fibers connect cortical regions within the same hemisphere and projection fibers connect the cortex with other parts of the CNS (deep grey matter, cerebellum, brainstem and spine).[136] The corpus callosum is the primary commissural tract of the brain[137] and serves an important role in cognitive function.[138,139] Demyelination of the corpus callosum occurs early on in MS[140,141] and abnormalities in white matter microstructure in the corpus callosum are often associated with cognitive impairment in MS.[142,143]

The superior longitudinal fasciculus (SLF) is an association tract that connects the occipital, parietal, and temporal lobes with the frontal cortex.[144] The SLF is involved in attention,[145] working memory,[146] executive function and language.[147] Abnormalities in the SLF are often associated with cognitive dysfunction in MS.[104,148–150]

The cingulum is mainly an association tract with many cortico-cortical connections that interlink medial parts of the frontal, parietal and temporal lobes.[151] Variations in cingulum

microstructure are associated with attention, language, memory and visuospatial function in healthy adults.[152] In addition, microstructural abnormalities in the cingulum of MS patients are associated with episodic memory and processing speed performance.[153] For these reasons, we initiated our investigation focusing on these three tracts (corpus callosum, SLF and cingulum). However, being aware that additional or alternative tracts may be involved, we expanded our search in the final research chapter.

1.9 Overview of thesis

Participant recruitment for this thesis project took place from August 23, 2017 to February 20, 2019. Analyses were performed incrementally throughout the recruitment process. Therefore, participant numbers increased for each project, however, the participants were drawn from the same sample. Participants underwent cognitive testing (Table 1 above) and MWI at 3 Tesla with a multi-echo 3D Gradient and Spin Echo sequence. The overall aim of the thesis was to investigate the role of myelin damage in MS-related cognitive impairment.

The relationship between myelin damage, measured by MWI, and cognitive performance in MS had yet to be investigated using cognitive assessments validated for use in MS. First, in chapter 2, we explored whether myelin damage in NAWM in 3 *a priori* selected white matter regions of interest (ROIs) known to be involved in cognition was associated with processing speed performance (SDMT)—one of the most commonly affected cognitive domains in MS. We hypothesized that increased myelin heterogeneity (MWF variance) in NAWM would be associated with decreased SDMT performance in participants with MS. This study included 27 MS patients and 13 age, sex and education matched controls.

In chapter 3, using the same 3 *a priori* selected regions as the previous chapter but a much larger sample size (73 MS patients and 22 age, sex and education matched controls), we extended our investigation of the relationship between myelin and cognitive performance to include several additional cognitive domains known to be affected in MS. In addition to processing speed performance (SDMT), these included verbal memory (SRT), visuospatial memory (BVT-R) and word retrieval (COWAT). In this chapter, rather than looking only at myelin heterogeneity, we employed a novel metric that characterizes the entire MWF distribution by combining both the average amount and heterogeneity of myelin within the region as the Coefficient of Variation (standard deviation / mean), termed the myelin heterogeneity index (MHI). We hypothesized that an increased MHI would be associated with decreased cognitive performance in participants with MS.

In chapter 4, rather than selecting white matter regions *a priori*, we employed an assumption-free data driven approach using permutation testing to determine the anatomical location and spatial extent of myelin damage associated with the different cognitive domains (processing speed (SDMT), verbal memory (SRT), visuospatial memory (BVMT-R), and word retrieval (COWAT)). We hypothesized that these white matter areas would be overlapping but the pattern would be distinct for each cognitive domain/test. Second, we used these cognitive domain-specific white matter regions to investigate differences in myelin damage between cognitively impaired and preserved groups. We hypothesized that the mean MWF would be significantly lower in the cognitive domain-specific white matter areas in cognitively impaired patients relative to cognitively preserved patients. Third, we investigated the strength of the relationship between mean MWF in each cognitive domain-specific white matter mask and scores on the respective tests. Finally, we explored the relative roles of the spatial extent and the magnitude of severity of

myelin damage in cognitive performance. We did this by producing an MWF z-score map for each patient relative to a control MWF atlas created from 100 neurologically healthy individuals. We then investigated the relationship between the number of voxels that fell certain standard deviations from the control atlas mean (severely damaged voxels: $z \leq -2$; moderately damaged: z between -2 and -1; normal range: z between -1 and +1) in the cognitive domain-specific white matter areas and performance on the respective cognitive tests. This study included 76 MS participants and 22 age, sex and education matched controls.

1.10 Specific aims and hypotheses

Chapter 2

Aim: To determine if myelin damage in NAWM in 3 *a priori* selected white matter regions of interest (ROIs) known to be involved in cognition (corpus callosum, SLF and cingulum) was associated with processing speed performance (SDMT) in MS patients.

Hypothesis: Increased myelin heterogeneity (MWF variance) in NAWM will be associated with decreased SDMT performance in participants with MS.

Chapter 3

Aim: To determine if myelin damage in NAWM in 3 *a priori* selected white matter ROIs is associated with additional cognitive domains known to be affected in MS (verbal memory, visuospatial memory and word retrieval).

Hypothesis: We hypothesized that an increased MHI would be associated with decreased cognitive performance in participants with MS.

Chapter 4

Aims:

- To employ an assumption-free data driven approach using permutation testing to determine the anatomical location and spatial extent of myelin damage associated with the different cognitive domains,
- To use the cognitive domain-specific white matter regions identified by permutation testing to investigate differences in myelin damage between cognitively impaired and preserved groups.
- To investigate the strength of the relationship between mean MWF in each cognitive domain-specific white matter region and scores on the respective tests.
- To explore the relative roles of the spatial extent and the magnitude of severity of myelin damage in cognitive performance.

Hypotheses

- White matter regions with MWF associations with cognitive performance will be overlapping but the pattern will be distinct for each cognitive domain/test.
- Mean MWF will be significantly lower in the cognitive domain-specific white matter regions in cognitively impaired patients relative to cognitively preserved patients.

- We made no hypothesises with regards to the strength of the relationships between mean MWF and cognitive domain-specific white matter regions and test performance.
- We made no hypotheses with regards to the relationships between the spatial extent and severity of myelin damage in cognitive domain-specific white matter regions and test performance.

Chapter 2

2. Myelin damage in normal appearing white matter contributes to impaired cognitive processing speed in multiple sclerosis

This chapter contains published work: **Abel, S., et al. (2020). Myelin Damage in Normal Appearing White Matter Contributes to Impaired Cognitive Processing Speed in Multiple Sclerosis. Journal of Neuroimaging, 30(2), 205-211.** This work was also presented as a poster at the 2019 Americas Committee for Treatment and Research in Multiple Sclerosis (ATRIMS) Forum in Dallas, Texas, USA. Dr. Shannon Kolind designed and conceptualized the study, supervised the overall study and obtained funding. Dr. Susan Forwell guided the selection of the appropriate cognitive tests. Drs. Anthony Traboulsee and Lara Boyd guided the design of the data intake form. All supervisory members provided guidance and valuable feedback throughout each project. I assisted with recruitment for this study by designing and displaying recruitment posters in the UBC MS Clinic, coordinating recruitment advertisements on local health authority websites, presenting the study and distributing recruitment materials at the Langley MS support group, distributing recruitment flyers at MS fundraisers (annual MS walk, annual Women Against MS Luncheon), distributing recruitment packages to the neurologists at the UBC MS Clinic and recruiting controls from my social network. The neurologists at the UBC MS Clinic (Drs. Anthony Traboulsee, Robert Carruthers, Virginia Devonshire and Ana-Luiza Sayao) provided the majority of patient recruitment referrals. I coordinated the study with the assistance of Poljanka Johnson, who I trained. This included calling referrals to confirm interest in participating and ensuring inclusion/exclusion criteria were met, MRI safety screening, scheduling the research appointment

(booking the MRI appointment, cognitive testing room and neurologist for EDSS assessment) and preparing all materials and equipment for the MRI appointment and cognitive and clinical testing. The MRI scanning procedures were performed by the technologists at the UBC MRI Research Centre. I performed the cognitive and clinical testing with the assistance of Poljanka Johnson. The EDSS assessments were performed by Drs. Jillian Chan, Alice Schabas, Nathalie Ackermans, Robert Carruthers and Anthony Traboulsee. I built the project SPSS database, scored the cognitive and clinical data and performed the project data entry with the assistance of Stephen Ristow. Dr. Jeffrey Wilken and his colleagues at Neuropsychology Associates of Fairfax scored the BVMT-R data. I coordinated the transfer of the BVMT-R data. Lisa Lee assisted with the creation of myelin water fraction maps. I created the MRI data processing and analysis pipeline with the assistance of Dr. Irene Vavasour. I developed the data analysis methodology, analyzed the data and wrote the final manuscript. Coauthors assisted with valuable feedback on the interpretation of findings and editing the manuscript. This work was approved by the UBC Clinical Research Ethics Board (H17-00866, "Establishing an imaging biomarker for disease progression in multiple sclerosis"). I assisted with the completion and submission of the ethics application and oversaw its renewal for the first 2 years of the study.

In this chapter, we sought to determine if MWF in NAWM was associated with the most commonly affected cognitive domain in MS: processing speed. A relationship between MWF in NAWM and SDMT performance had yet to be established in the literature. This work contributes a novel finding that increased myelin heterogeneity in 3 *a priori selected* ROIs known to be involved in cognition is correlated with worse SDMT performance in MS patients. In a hierarchical regression model, white matter volume, number of T_2 lesioned voxels, age and disease duration

did not contribute significantly to the variability explained in SDMT scores. No significant correlations were observed in controls.

2.1 Introduction

Cognitive impairment is a disabling and common symptom in multiple sclerosis (MS), affecting up to 70% of patients.[2] Diffusion Tensor imaging (DTI) and Magnetization Transfer Imaging (MTI) studies have demonstrated that damage to normal appearing WM (NAWM) is a significant contributor to cognitive impairment in MS.[86,154] While sensitive to MS-related biological changes, DTI and MTI metrics are not specific to a particular pathology. In contrast, myelin water imaging (MWI), can be used to calculate the myelin water fraction (MWF), which has been histologically validated as specific for myelin.[155]

Reduced mean MWF in NAWM is commonly found in MS and is associated with increased disability (for review see Mackay and Laule (2016)[8]). Between healthy individuals, the mean MWF can be highly variable,[156] while MWF values within a healthy white matter tract are relatively homogeneous.[157] Myelin heterogeneity (increased variance of the MWF within a region of interest) has been shown to be increased in MS reflecting myelin damage,[157] and may provide a more sensitive marker for myelin changes than the mean MWF in cross-sectional group studies.

Further investigation into how MWF heterogeneity relates to MS symptoms, in particular, cognitive impairment, is warranted. The cognitive domain most consistently affected in MS is processing speed,[48] which can be measured using the Symbol Digit Modalities Test (SDMT).[63] The aim of this study was to determine if greater myelin heterogeneity in NAWM is associated with decreased SDMT performance in MS.

2.2 Methods

2.2.1 Participants

Participants were recruited through the University of British Columbia Hospital MS clinic and via online recruitment advertisements on local health authority websites. Twenty-seven participants with clinically definite MS fulfilling the 2010 revised MacDonald's criteria for diagnosis[158] (11 relapsing-remitting MS (RRMS); five primary progressive MS (PPMS); and 11 secondary progressive MS (SPMS)) and 13 age, sex and education matched healthy volunteers without neurological disease were included in the study. Participant characteristics are displayed in Table 1. All participants provided written informed consent. This study was approved by the University of British Columbia Clinical Research Ethics Board (H17-00866).

Table 2.1: Clinical and demographic characteristics

	Controls (n = 13)	MS (n = 27)	*P*
Age, years, mean (min-max)	43.4 (28-63)	50 (26-65)	.12
Education, years, median (min-max)	16 (15-20)	16 (11-23)	.078
Females, *n* (%)	9 (69)	19 (70)	.941
SDMT (mean ± SD)	53 ± 10.1	46 ± 14.4	.079
EDSS, median (min-max)		3.5 (1-8.5)	
Disease duration, years, median (min-max)		11 (0-42)	
RRMS, *n* (%)		11 (40)	
PPMS, *n* (%)		5 (20)	
SPMS, *n* (%)		11 (40)	

min = minimum; max = maximum; *n* = number; SD = standard deviation; EDSS = Expanded Disability Status Scale; RRMS = relapsing remitting MS; PPMS = primary progressive MS; SPMS = secondary progressive MS.

2.2.2 Clinical and neuropsychological assessments

In addition to a full neurological examination including the Kurtzke Expanded Disability Status Scale (EDSS),[39] participants performed the written version of the SDMT.[63] The SDMT contains a reference key with the numbers 1-9 each corresponding to different geometric symbols. The answer key contains only symbols to which the participant must match the corresponding number according to the key. The test is scored by tallying the total number of correct responses achieved in 90 seconds, with lower scores indicating worse processing speed.

2.2.3 MRI data acquisition

MRI scans were conducted on a 3T scanner (Philips Achieva; Best, Netherlands). Sequences included a 3DT1 anatomical scan (whole-brain 3D magnetization-prepared rapid gradient-echo (MPRAGE), repetition time [TR]=3000ms, inversion time [TI]=1072ms, 1x1x1mm voxel, 160 slices) for registration and segmentation of WM ROIs and a 3D 48-echo Gradient and Spin Echo (GRASE) T2 relaxation sequence with an EPI factor of 3 (TR=1073ms, echo spacing=8ms, 20 slices acquired at 1x2x5mm reconstructed to 40 slices at 1x1x2.5mm) for MWF determination.[159] A spin echo PD/T2 scan (TR=2900ms, TE=8.4/80ms, 0.94x0.94x3mm, 54 slices) was also acquired for lesion identification.

2.2.4 MRI image registration and analysis

Voxel-wise signal decay curves obtained from the T2 relaxation (GRASE) sequence were modelled by multiple exponential components, with no a priori assumptions about the number of contributing exponentials. Analysis used a regularized non-negative least squares algorithm with the extended phase graph and flip angle estimation to correct for stimulated echo artifacts (in house software, MATLAB® R2013b, The MathWorks, Inc., Natick, MA).[160] Voxel-wise MWF maps

were computed as the ratio of the area under the T2 distribution with times of <40ms to the total area under the distribution.[159]

MWF maps were aligned with the anatomical images for each individual by linearly co-registering the 3DT1 to the first echo of the GRASE scan using FLIRT (9 degrees of freedom),[161] part of FMRIB's Software Library (FSL, www.fmrib.ox.ac.uk/fsl).[162] Non-brain parenchyma signal was removed using an automated approach[163] with Brain Extraction Tool (BET, part of FSL). Normal appearing WM masks were generated from the 3DT1 image using the automated brain segmentation algorithm, FAST[164] (part of FSL), followed by in-plane erosion by 2 voxels. The JHU tract atlas[165] in MNI (standard template) space was used to segment 3 WM regions of interest (ROIs) using FSL (cingulum, superior longitudinal fasciculus (SLF), corpus callosum; selected a priori based on their known involvement in MS-related cognitive impairment[149]). The 3DT1 image was non-linearly warped to MNI space using FNIRT and the ROIs were then transformed onto the MWF map in native space. Each ROI mask was multiplied by the participant's global NAWM mask to eliminate GM, CSF and lesioned tissue, then manually checked and edited as needed. Mean MWF and myelin heterogeneity were computed for the cingulum, SLF and corpus callosum NAWM.

To investigate the potential confound that lesions bordering NAWM ROIs were driving the relationship between myelin heterogeneity and SDMT performance, analysis was conducted with and without lesions. To identify lesions in each MS participant, a three-class segmentation was performed using FAST (FSL) on the T2 image to separate lesions from WM and GM. The same procedure was performed on the 3DT1 image to separate lesions from CSF and WM. These images were combined to create a lesion mask, which was manually checked and corrected as needed, and then added to the WM mask for each ROI to create a mask that included all WM

(NAWM and lesions). Myelin heterogeneity (variance of the MWF within a region of interest) was computed for all WM in the cingulum, SLF and corpus callosum.

To investigate the potential confounds of WM volume, age, disease duration and number of T2 lesioned voxels driving the relationship between myelin heterogeneity and SDMT performance, analyses incorporating these variables were conducted. The WM (NAWM and lesions) masks were used to calculate the WM volume in each ROI, which was defined as the total number of voxels. Disease duration was defined as time since symptom onset.

2.3 Statistical analysis

All statistical procedures were performed using IBM SPSS Statistics for Mac, Version 25.0. (Armonk, NY: IBM Corp). An independent samples t-test was used to determine if there was a significant difference in age between groups. A Mann-Whitney U test was used to investigate a difference in years of education between groups. A chi-square test was used to determine if the groups were matched for sex. Differences between people with MS and controls in myelin heterogeneity, mean MWF and SDMT performance were explored with independent samples t-tests for parametric data, whilst Welch's t-test or Mann-Whitney U tests were employed for nonparametric data. Associations between myelin heterogeneity and SDMT performance, mean MWF and SDMT performance, and WM volume and SDMT performance were explored with Pearson's correlation. Hierarchical regression models were used to correct for potential covariates and determine the amount of variability WM volume, age, disease duration and number of T2 lesioned voxels contributed to SDMT performance relative to myelin heterogeneity in each ROI. While all p-values < 0.05 are reported, significance thresholds were set with the most conservative Bonferroni correction.

2.4 Results

The clinical and demographic characteristics of participants with MS and controls are shown in table 1. People with MS and controls did not differ significantly in age, sex or level of education. On average, controls performed better on the SDMT (mean=53) relative to people with MS (mean=46), however, this difference was not significant (p=0.079).

Figure 2.1 illustrates the correlations between myelin heterogeneity (MWF variance) in NAWM and SDMT scores in our cohort. Increased myelin heterogeneity is associated with worse SDMT performance in participants with MS. In the cingulum, myelin heterogeneity was significantly correlated with SDMT in people with MS ($r = -0.654$, $p = 0.0002$) but this relationship was not significant in controls ($r = -0.094$, $p = 0.760$). A similar pattern was observed for the SLF, where myelin heterogeneity and SDMT scores were significantly correlated in MS ($r = -0.584$, $p = 0.001$) but not controls ($r = 0.174$, $p = 0.569$). And once again in the corpus callosum, myelin heterogeneity was significantly correlated with SDMT performance in participants with MS ($r = -0.561$, $p = 0.002$) but not controls ($r = -0.466$, $p = 0.108$). All p-values for the correlations in patients were less than the Bonferroni corrected p-value of <0.016. Figure 2.2 depicts illustrative myelin maps and MWF distributions for high, moderate and low myelin heterogeneity and the associated SDMT scores in three people with MS.

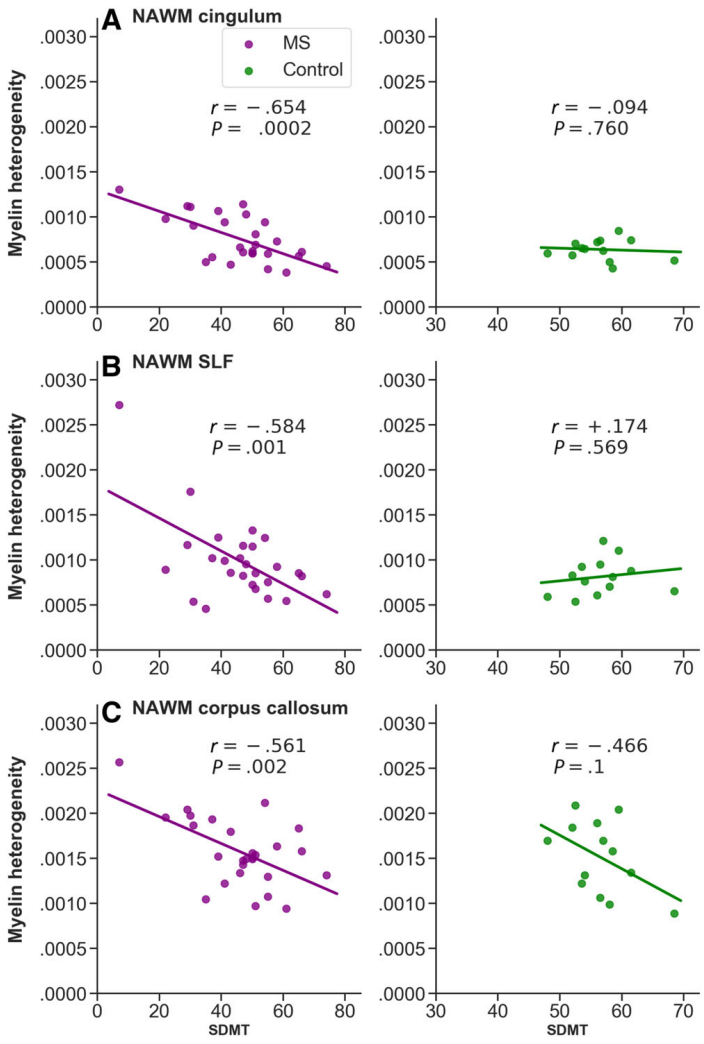

Figure 2.1: Correlations between myelin heterogeneity in NAWM and SDMT scores in MS and controls in three ROIs. Correlations between myelin heterogeneity in NAWM and SDMT scores in MS (purple) and controls (green). (A) Heterogeneity in NAWM in cingulum vs SDMT scores, (B) heterogeneity in NAWM in SLF vs SDMT scores, (C) heterogeneity in NAWM in corpus callosum vs SDMT scores. Correlations were only significant in MS. ROI: region of interest; NAWM: Normal Appearing White Matter; SDMT: Symbol Digit Modalities Test; SLF: Superior Longitudinal Fasciculus.

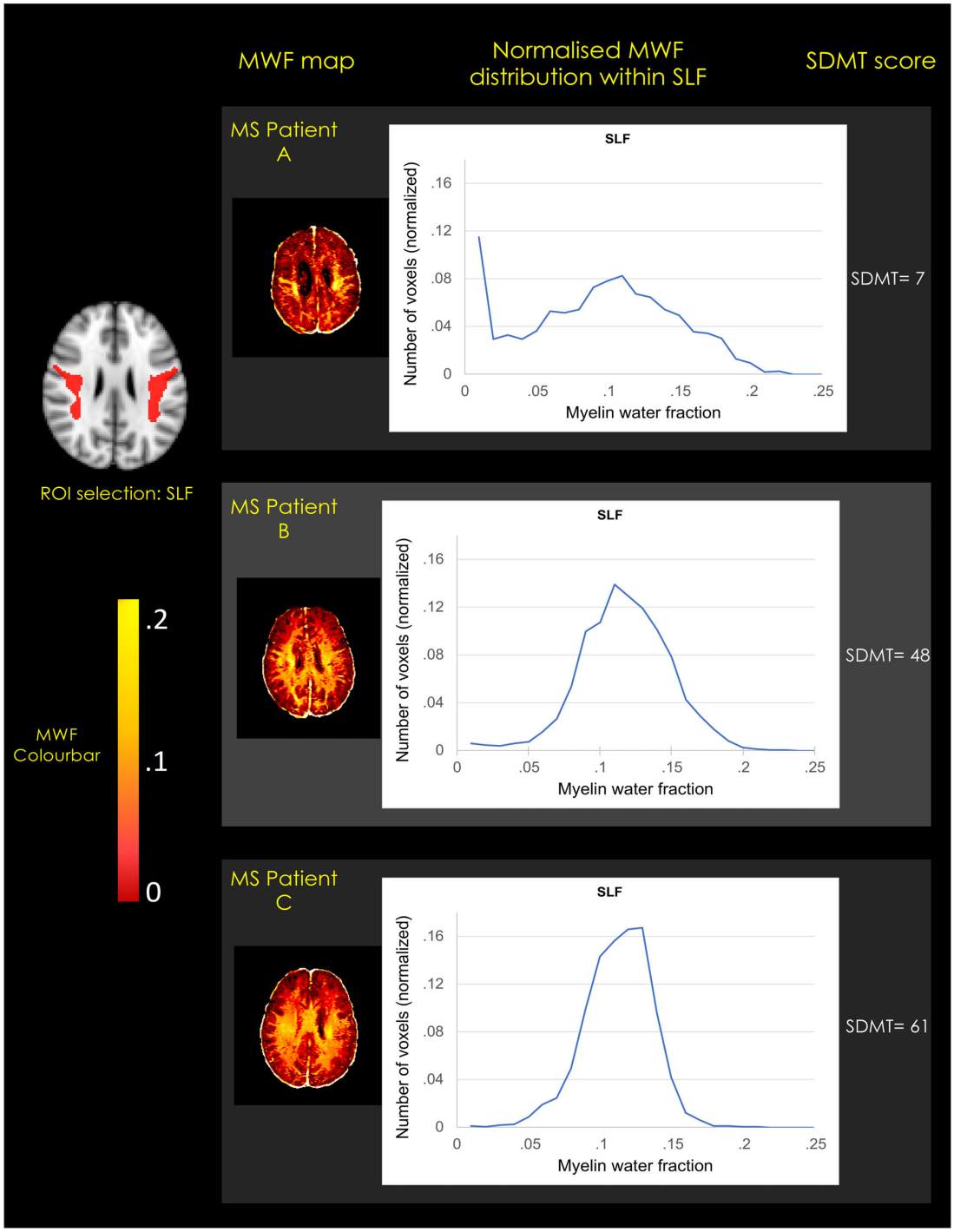

Figure 2.2: Axial map of MWF values, MWF histograms in SLF and SDMT scores for three MS patients. Axial maps of MWF values (left), normalised histograms of MWF values in the SLF (middle) and SDMT scores (right) of three MS patients. The MWF map and histogram of MS Patient A (top) both demonstrated a high degree of myelin heterogeneity, matching the patient's low SDMT score. MS Patient B exhibited a moderate degree of myelin heterogeneity and a moderate SDMT score. MS Patient C had low myelin heterogeneity and a high SDMT score. MWF: Myelin Water Fraction; SDMT: Symbol Digit Modalities Test; SLF: Superior Longitudinal Fasciculus.

The associations between myelin heterogeneity in NAWM and SDMT performance did not change appreciably when lesioned tissue was included in the ROIs. Including lesions resulted in an r value of -0.533 ($p = 0.004$) in the cingulum, an r value of -0.598 ($p = 0.001$) in the SLF, and an r value of -0.578 ($p=0.002$) in the corpus callosum.

Figure 2.3 shows the relationships between WM volume and SDMT scores. The correlation between WM volume in the cingulum and SDMT scores was not significant ($r = 0.338$, $p = 0.085$). In contrast, the associations between WM volume in the SLF and corpus callosum and SDMT scores demonstrated a positive trend towards significance ($r = 0.430$, $p = 0.025$ and $r = 0.433$, $p = 0.024$ respectively; Bonferroni corrected $p<0.01$ required for significance).

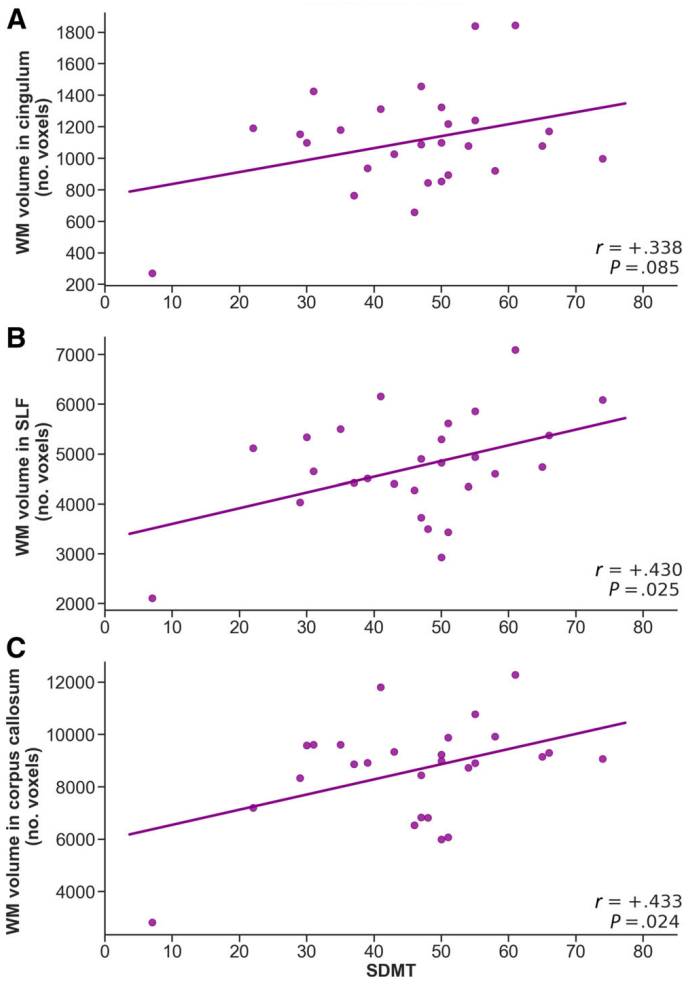

Figure 2.3: Correlations between WM volume and SDMT scores in MS in three ROIs. Correlations between WM volume and SDMT scores in (A) cingulum, (B) SLF, and (C) corpus callosum. No correlation reached significance with Bonferroni correction. ROI; region of interest; no: number; WM: White Matter; SDMT: Symbol Digit Modalities Test; SLF: Superior Longitudinal Fasciculus.

Hierarchical regression analysis was performed for each ROI to determine if the addition of WM volume improved prediction of SDMT scores over and above myelin heterogeneity alone (model 1 and 2). The addition of WM volume did not lead to a statistically significant increase in R2 in any ROI ($p > 0.187$). Model 3 added age, disease duration and number of T2 lesioned voxels, which also failed to lead to a statistically significant increase in R2 in any ROI ($p > 0.534$). Table 2.2 summarizes the R2 and p-values for each regression model.

Table 2.2: Hierarchical regression models predicting SDMT performance from myelin heterogeneity, WM volume, age, disease duration and number of T2 lesioned voxels.

	SDMT Performance		
	Cingulum	**SLF**	**Corpus Callosum**
Model 1[a]			
R^2	.428	.342	.315
P	.0002	.001	.002
Model 2[b]			
R^2	.434	.350	.364
P	.001	.006	.004
ΔR^2	.006	.009	.049
$\Delta R^2 P$	.627	.579	.187
Model 3[c]			
R^2	.469	.399	.425
P	.015	.044	.030
ΔR^2	.035	.049	.062
$\Delta R^2 P$	.710	.639	.534

[a]Myelin heterogeneity.
[b]Myelin heterogeneity, WM volume.
[c]Myelin heterogeneity, WM volume, age, disease duration, number of T2 lesioned voxels.

Myelin heterogeneity was higher in MS participants (cingulum=0.0008; SLF=0.0009; corpus callosum=0.0016) compared to controls (cingulum=0.0006; SLF=0.008; corpus callosum=0.0015). However, these differences were not significant ($p > 0.054$). Mean MWF was lower in MS (cingulum=0.069; SLF=0.116; corpus callosum=0.106) relative to controls (cingulum=0.071; SLF=0.117; corpus callosum=0.108), but these differences were not significant ($p > 0.54$). Unlike myelin heterogeneity, no significant relationships were found between mean MWF and SDMT scores ($p > 0.26$).

2.5 Discussion:

Our results demonstrate that increased myelin heterogeneity in ROIs associated with cognition are predictive of slower processing speed (i.e., lower SDMT scores) in people with MS. Our findings build on a previous study reporting that myelin heterogeneity in NAWM is increased in people with MS and correlates with EDSS severity.[157] Myelin heterogeneity has also been shown to be significantly greater in participants with schizophrenia—another disease with pervasive WM abnormalities—relative to controls.[166]

We observed moderate to moderately strong correlations between increased myelin heterogeneity in the cingulum, SLF and corpus callosum and worse SDMT performance in people with MS. These findings are supported by a number of previous quantitative MRI studies, including reports of extensive fractional anisotropy (FA) reductions along with increased radial diffusivity (RD) and/or decreased magnetization transfer ratio (MTR) in participants with MS who demonstrate processing speed impairments. The cingulum demonstrates decreased FA as well as increased diffusivity measures which are related to slower processing speed (as measured by the Paced Auditory Serial Addition Test (PASAT)),[167] and increased cingulum RD is associated with worse performance on the SDMT.[168] Decreased FA[148] and MTR[169] in the SLF are associated with impaired processing speed (worse PASAT scores), and reduced corpus callosum FA has been associated with worse performance on the SDMT in both adult[170] and pediatric MS.[171] In addition, lower MTR in the corpus callosum is significantly correlated with slower processing speed (PASAT).[172]

While our results are consistent with previous DTI and MTI studies, MWI offers greater biological interpretability. DTI measures reflect a large number of biological changes that occur in MS, including demyelination, axonal loss, edema, inflammation and gliosis and are confounded

by WM architecture (i.e., fibre orientation and coherence).[106] Similarly, MTR estimates of macromolecular-bound water include, but are not limited to myelin, and are heavily influenced by inflammation and edema.[108] In contrast, MWI has been validated with both human histology[113,155] and animal models[114] as a specific measure of myelin. Our study provides evidence that MS-related processing speed deficits are related to abnormalities in myelin in NAWM in WM tracts associated with cognition.

While we observed significant associations between myelin heterogeneity and SDMT scores, we did not find significant associations between mean MWF and SDMT scores. Discussing our results within the context of previous literature proves difficult as, to our knowledge, no previous study has investigated the relationship between MWF and SDMT performance. Earlier studies have reported significant relationships between mean MWF and clinical measures. However, these studies explored the association between mean MWF in whole brain NAWM and EDSS scores,[121,157] rendering it difficult to draw comparisons to the present work on MWF in WM tracts associated with cognition and processing speed scores. One possible reason for the lack of significant associations between mean MWF and SDMT performance may be due to the fact that mean MWF measurements vary considerably, even between healthy individuals, though they are reproducible and reliable over time.[173] Given this variability in the population, the mean MWF may not be the most sensitive indicator of myelin damage within a ROI as normal variation can dilute mean differences and clinical associations. Conversely, myelin heterogeneity is comparably homogenous in controls but varies in people with MS and therefore may be a more sensitive measure for clinically relevant myelin damage, particularly in cross-sectional studies and when sample size is limited.

As a possible limitation, we examined how lesions bordering our NAWM ROIs and the WM volume of our ROIs might influence the relationship between myelin heterogeneity and SDMT performance. As expected, the myelin heterogeneity increased slightly with lesion inclusion. However, the observed relationships between myelin heterogeneity in NAWM and SDMT performance did not change considerably when lesions were included. This finding is consistent with several studies reporting the limited contribution of lesions to cognitive performance in MS.[81] Conversely, we observed a positive trend with ROI WM volume and SDMT performance. This observation is not surprising given that loss of myelin decreases brain volume, and brain volume is established as a strong predictor of cognitive impairment in MS.[174] However, regression analysis indicated that myelin heterogeneity explains a significant amount of the variability in SDMT scores and including WM volume contributed very little to the models. A third regression model incorporating the additional confounds of age, disease duration and number of T2 lesioned voxels also failed to lead to a significant increase in variability explained. This suggests that these confounds are not driving the relationship between myelin heterogeneity and SDMT scores.

Our investigation of a relationship between myelin heterogeneity and cognitive performance in MS was restricted to processing speed as measured by the SDMT. Though the SDMT is considered to be the most sensitive and reliable test for detecting cognitive impairment in MS,[61] the inclusion of additional measures probing other cognitive domains known to be affected in MS would lead to more robust results.

Our results indicate that myelin heterogeneity in NAWM due to MS-related injury is associated with processing speed deficits in people with MS. Myelin heterogeneity may be a more

sensitive and appropriate measure for investigating cognition in MS beyond the assessment of mean MWF values.

Chapter 3

3. Myelin damage in normal appearing white matter extends to additional cognitive domains beyond processing speed

A version of this chapter is accepted for publication as: **Abel, S., et al. (In Press). Associations Between Myelin Imaging and Cognitive Performance in Multiple Sclerosis. JAMA Network Open** (accepted June 9th, 2020). This information is embargoed until the date of publication. This project was also presented in 2019 as a poster at the 35th Congress of the European Committee for Treatment and Research in Multiple Sclerosis (ECTRIMS) in Stockholm Sweden where it won a Top Poster Award. My contributions as well as those of my coauthors are listed above in the preface and opening paragraph for chapter 2. In addition, Dr. Helen Cross performed lesion masking for this study.

With an association between MWF in NAWM and the most commonly affected cognitive domain (processing speed) in MS established in chapter 2, our aim for this next chapter was to extend our findings to a larger sample and several additional cognitive domains know to be affected in MS. We used a novel MWF measure that incorporates both the mean and spread of the MWF distribution—MHI—in NAWM in the same 3 *a priori* selected ROIs known to be involved in cognition. We report, for the first time, that MWF in NAWM is significantly associated with verbal memory (SRT) and word retrieval (COWAT) in addition to processing speed (SDMT) in MS. These findings appear to be specific to cognition rather than a proxy for overall disability as no significant correlations were found between MWF in NAWM in the 3 ROIs and upper (9-HPT)

or lower (T25-FW) limb function. No associations between MWF and cognitive performance were observed in controls.

3.1 Introduction

Multiple sclerosis (MS) is an inflammatory, neurodegenerative disease of the central nervous system (CNS)[10] that affects over 2 million people globally, rendering it the most prevalent chronic neuroinflammatory disease of the CNS worldwide.[11] Cognitive impairment is a core symptom in MS that presents in up to 70% of patients.[2] Cognitive symptoms in MS typically manifest as deficits in attention, memory and processing speed,[2] with processing speed being most frequently affected.[48] MS-related cognitive impairment has a severe impact on quality of life[3] including the ability to perform tasks of daily living;[4] fitness to drive;[5] and social functioning.[4] It is also a major contributor to unemployment in MS patients.[3,6] Undoubtedly, cognitive impairment presents a major burden to those living with MS and an improved understanding of its underlying pathology would be of great benefit to patients and clinicians.

MS is characterized by demyelination,[7] with the radiological hallmark of MS being focal areas of myelin loss, referred to as lesions.[175] Lesions are visible on conventional magnetic resonance images (MRI) with T1-weighted and T2-weighted contrast and are the mainstay of MS diagnosis and disease monitoring.[38] However, the relationship between focal lesion burden with physical and cognitive disability is limited; this is known as the clinico-radiological paradox.[84,176] One possible cause of this paradox may be because MS pathology extends beyond lesions that are visible on conventional MR images.[177] Post-mortem histopathology studies demonstrate that normal appearing white matter (NAWM)—areas that appear normal on standard imaging—is diffusely demyelinated in MS.[178,179] Investigating the contribution of demyelination within NAWM

to clinical outcomes such as cognition requires advanced imaging techniques that are quantitative, sensitive, and biologically specific to MS pathology.

Quantitative characterization of myelin in vivo is feasible using myelin water imaging (MWI). MWI separates the MRI signal into contributions from the distinct water pools within a voxel (a 1mm3 of brain) based on the MR property known as T2 relaxation time. In CNS tissue, these water pools generally correspond to (i) a long relaxation time component, which arises from cerebrospinal fluid (T2=~ 2 s); (ii) an intermediate component, due to intra- and extra-cellular water (T2=~ 60–80ms); and (iii) a short component, stemming from water trapped between the myelin bilayers (T2=~ 20ms).[110] The fraction of MR signal arising from the myelin water divided by the total water signal is the myelin water fraction (MWF). The MWF has been histologically validated as a specific marker for myelin using human tissue[113,155] and animal models of myelin damage.[114] At present, MWI is the most direct means of assessing alterations in myelin non-invasively.

Here, we used MWI to investigate the role of myelin damage in NAWM and cognitive function in MS. MWF results are typically described by the mean value within a region of interest (ROI), or by the heterogeneity (variance). To increase sensitivity to disease-associated changes, we characterized the entire MWF distribution by combining both measures as the Coefficient of Variation (standard deviation / mean), termed the myelin heterogeneity index (MHI), with an increased MHI indicating more myelin damage. To assess cognitive deficits, we utilized measures from widely used and validated cognitive batteries for MS.[63,71] Processing speed and attention was measured using the oral version of the Symbol Digit Modalities Test (SDMT); verbal memory with the Selective Reminding Test (SRT); word retrieval with the Controlled Oral Word Association Test (COWAT); and visuospatial memory with the Brief Visuospatial Memory Test

Revised (BVMT-R). We selected 3 white matter (WM) regions of interest (ROIs) a priori: the cingulum, superior longitudinal fasciculus (SLF) and corpus callosum, based on their known involvement in MS-related cognitive impairment[149]). We hypothesized that an increased MHI would be associated with worse cognitive performance in MS.

3.2 Methods

3.2.1 Participants

This study was approved by the University of British Columbia Clinical Research Ethics Board. All participants provided written informed consent. Participants were recruited through the University of British Columbia Hospital MS clinic and via online recruitment advertisements on local health authority websites. Study appointments took place from August 23, 2017 to February 20, 2019. Seventy-three participants with clinically definite MS fulfilling the 2017 revised MacDonald's criteria for diagnosis[38] (38 relapsing-remitting MS (RRMS); 12 primary progressive MS (PPMS); 23 secondary progressive MS (SPMS)) and 22 age, sex and education matched healthy volunteers without neurological disease were included in the study. All MS phenotypes were recruited to capture varying levels of cognitive disability and MWF values. This study followed the Strengthening the Reporting of Observational Studies in Epidemiology (STROBE) reporting guideline for cross-sectional studies.

3.2.2 Clinical and neuropsychological assessments

To characterize overall disability, participants were examined with the Kurtzke Expanded Disability Status Scale (EDSS).[39] To investigate whether our MWI findings were specific to cognition rather than a proxy for physical disability, MS patients performed the Timed 25 Foot

Walk (T25-FW) as a measure of lower limb function and the Nine-Hole Peg Test (9-HPT) as a measure of upper limb function. Participants performed a battery of neuropsychological assessments validated for use in MS. The oral version of the SDMT[63] was used as a measure of processing speed. One control participant performed the written version with instructions provided by a translator as they were non-English speaking. This test contains a reference key with the numbers 1-9 each corresponding to different geometric symbols. The answer key contains only symbols to which the participant must match the corresponding number according to the key. The subject responds orally with the digit associated with symbol as quickly as possible. The test is scored by tallying the total number of correct responses achieved in 90 seconds. The SRT[63] was used to assess verbal memory. The participant is read aloud a list of 12 words that they are asked to repeat back immediately. After they have repeated all words they can remember, the participant is read back only the words they have missed and asked to repeat all 12 words again. This procedure is repeated for 6 rounds. The participant is again asked to repeat all 12 words subsequent to a delay during which they perform other cognitive tests in the battery. The SRT is scored by tallying the total correct recalled words. Word retrieval was assessed using the COWAT.[63,71] The participant is given a letter of the alphabet (e.g., "F") and asked to list as many words as they can produce beginning with that letter in 1 minute's time. The version of the test employed in this study included 3 letter prompting categories and an animal category, for which the participant names as many animals as they can recall. The COWAT is scored by tallying the total number of permissible answers—proper names (e.g., cities) and variations of the same word (e.g., runs, running, ran) are not permitted. Visuospatial memory was evaluated with the BVMT-R.[71] The participant is presented with a display of 6 geometric figures for 10 seconds and then asked to reproduce the display by drawing it exactly as it was seen on a blank page. The participant is given

3 opportunities to view and reproduce the display. In the present study, the total recall t-score was used, which is sum of all valid items generated across learning trials 1–3, corrected for age. Lower scores indicate worse performance on all tests. All participants completed the SDMT at a minimum. However, due to the time constraints of the research appointment, not all participants completed every test. Sixty-six patients completed the SRT; sixty-five patients completed the COWAT; and sixty-three patients completed the BVMT-R.

3.2.3 MRI data acquisition

MRI scans were conducted on a 3T scanner (Philips Achieva; Best, Netherlands). Sequences included a 3DT1 anatomical scan (whole-brain 3D magnetization-prepared rapid gradient-echo (MPRAGE), repetition time [TR]=3000ms, inversion time [TI]=1072ms, 1x1x1mm voxel, 160 slices) for registration and segmentation of WM ROIs and a 48-echo 3D Gradient and Spin Echo (GRASE) T2 relaxation sequence with an EPI factor of 3 (TR=1073ms, echo spacing=8ms, 20 slices acquired at 1x2x5mm reconstructed to 40 slices at 1x1x2.5mm) for MWF determination.[159] A spin echo PD/T2 scan (TR=2900ms, TE=8.4/80ms, 0.94x0.94x3mm, 54 slices) was also acquired for lesion identification.

3.2.4 MRI image registration and analysis

Voxel-wise signal decay curves obtained from the T2 relaxation (GRASE) sequence were modelled by multiple exponential components, with no a priori assumptions about the number of contributing exponentials. Analysis used a regularized non-negative least squares algorithm with the extended phase graph algorithm and flip angle estimation to correct for stimulated echoes (in house software, MATLAB® R2013b, The MathWorks, Inc., Natick, MA).[160] Voxel-wise MWF

maps were computed as the ratio of the area under the T2 distribution with times of <40ms to the total area under the distribution.[159]

MWF maps were aligned with the anatomical images for each individual by linearly co-registering the 3DT1 to the first echo of the GRASE scan using FLIRT (9 degrees of freedom),[161] part of FMRIB's Software Library (FSL, www.fmrib.ox.ac.uk/fsl).[162] Non-brain parenchyma signal was removed using an automated approach with Brain Extraction Tool (BET, part of FSL).[163] WM masks were generated from the 3DT1 image using the automated brain segmentation algorithm, FAST[164] (part of FSL), followed by in-plane erosion using a 2D kernel and 3x3x1 box centered on the target voxel to eliminate grey matter and CSF voxels. The JHU tract atlas[165] in MNI (standard template) space was used to segment 3 WM ROIs using FSL (cingulum, superior longitudinal fasciculus (SLF), corpus callosum; selected a priori based on their known involvement in MS-related cognitive impairment[149]). The 3DT1 image was non-linearly warped to MNI space using FNIRT and the ROIs were then transformed onto the MWF map in native space. Each ROI mask was multiplied by the participant's global WM mask to eliminate GM, CSF and most lesioned tissue, then manually checked and edited as needed. Lesion masks produced by a neurologist were subtracted from the ROI masks to eliminate any remaining lesioned tissue. The MHI was computed for the cingulum, SLF and corpus callosum NAWM for each individual by dividing the standard deviation of MWF values by the mean MWF.

3.3 Statistical analysis

All statistical procedures were performed using IBM SPSS Statistics for Mac, Version 25.0. (Armonk, NY: IBM Corp). Assumptions of normality were tested with Shapiro-Wilk test for normality. The assumption of homogeneity of variance was tested with Levene's test of equality

of variance. If the assumptions for a parametric test were violated, we proceeded with the appropriate nonparametric test. A Welch's t-test was used to determine if there was a significant difference in age and years of education between groups. A chi-square test was used to determine if the groups were matched for sex. Associations between MHI and performance on each cognitive test were explored with Pearson's correlation. While all p-values < 0.05 are reported, significance thresholds were set with the Bonferroni correction for the 3 brain regions assessed, with each cognitive domain treated separately ($p < 0.016$). All tests were two-sided.

3.4 Results

3.4.1 Participant characteristics

Participants with MS had a mean age of 50.2 (range: 26-65) years, were 66% female and had a mean of 14.7 (range: 12-22) years of education. MS patients had a median EDSS of 3.5 (range: 1.0-8.5) and median disease duration of 12 (range: 0.3-48) years. Controls had a mean age of 46.4 (range: 27-65) years, were 64% female and had a mean of 15.8 (range: 12-22) years of education. MS participants and controls did not differ significantly in age, sex or education. The clinical and demographic characteristics of participants with MS and controls are shown in Table 3.1.

Table 3.1: Clinical and demographic characteristics

	Controls (n=22)	MS (n=73)	*p*
Age, y, mean (min-max)	46.4 (27-65)	50.2 (26-65)	0.24
Females, n (%)	14 (64)	48 (66)	0.85
Education, y, mean (min-max)	15.8 (12-22)	14.7 (12-22)	0.06
EDSS, median (min-max)		3.5 (1.0-8.5)	
Disease duration, y, median (min-max)		12.0 (0.3-48)	

3.4.2 SDMT

MS patients had a mean SDMT score of 56 (range: 7-88). The mean SDMT score for controls was 62 (range: 41-79). Figure 3.1 illustrates the correlations between the MHI in NAWM and SDMT scores. In MS, an increased MHI in the SLF ($r = -0.490$, 95% CI: -0.697 - -0.284 ; $p = 0.00001$), corpus callosum ($r = -0.471$, 95% CI: -0.680 - -0.262; $p = 0.00002$) and cingulum ($r = -0.419$; 95% CI: -0.634 - -0.205 $p = 0.0002$) was associated with worse performance on the SDMT. In controls, MHI was not associated with SDMT performance in any ROI ($p > 0.2$).

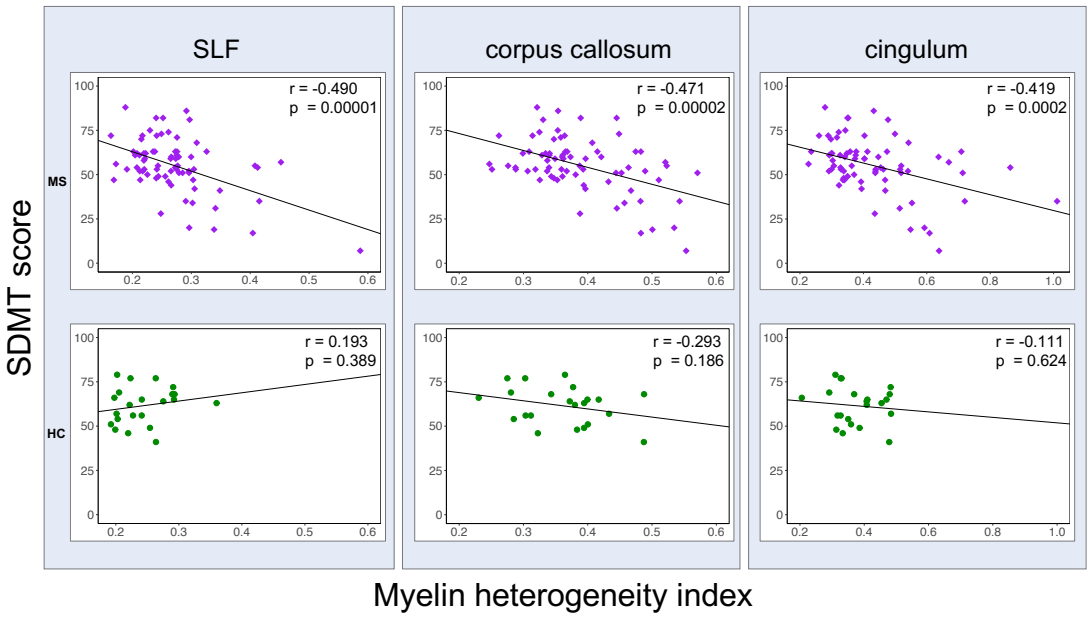

Figure 3.1: Correlations between SDMT performance and MHI. Correlations between the myelin heterogeneity index in NAWM (x axis) and SDMT scores (y axis) in MS (purple dots) and controls (green dots) in three ROIs. Lines = line of best fit. NAWM: normal appearing white matter; ROI: region of interest; MHI: Myelin heterogeneity index; SDMT: Symbol Digit Modalities.

3.4.3 SRT

The mean SRT score was 55 (range: 23-83) for MS participants and 62 (range: 46-78) for controls. Increased MHI in the SLF (r = -0.444, 95% CI: -0.660 - -0.217; p = 0.0002), corpus callosum (r = -0.411, 95% CI: -0.630 - -0.181; p = 0.001) and cingulum (r = -0.361, 95% CI: -0.602 - -0.130; p = 0.003) was significantly correlated with worse SRT performance in MS. MHI was not associated with SRT performance in controls ($p > 0.2$) (Figure 3.2).

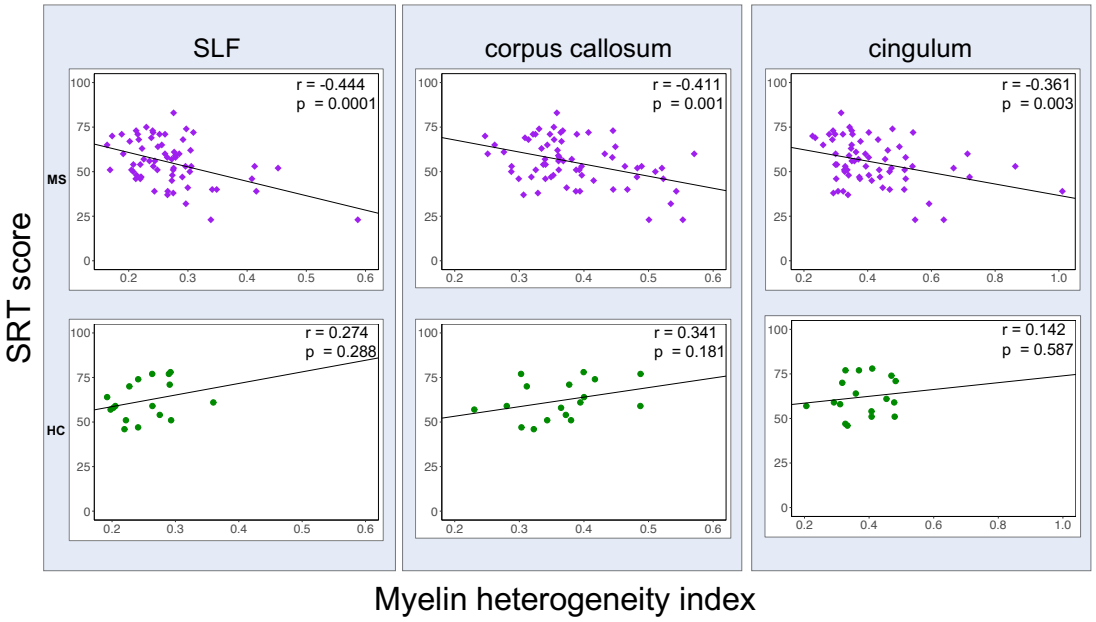

Figure 3.2: Correlations between SRT performance and MHI. Correlations between the myelin heterogeneity index in NAWM (x axis) and SRT scores (y axis) in MS (purple dots) and controls (green dots) in three ROIs. Lines = line of best fit. NAWM: normal appearing white matter; ROI: region of interest; MHI: Myelin heterogeneity index; SRT: Selective Reminding Test.

3.4.4 COWAT

MS participants had a mean score of 60 (range: 19-103) on the COWAT while controls had a mean score of 69 (range: 55-88). Increased MHI in MS NAWM was significantly correlated with worse COWAT scores in the SLF ($r = -0.317$, 95% CI: -.549 - -0.078; $p = 0.010$) and cingulum ($r = -0.335$, 95% CI: -0.658 - -0.113; $p = 0.006$), however, the corpus callosum ($r = -0.294$, 95% CI: -0.535 - -0.053; $p = 0.017$) did not pass Bonferroni correction for multiple comparisons. No significant correlations were observed between MHI and COWAT scores in controls ($p > 0.1$) (Figure 3.3).

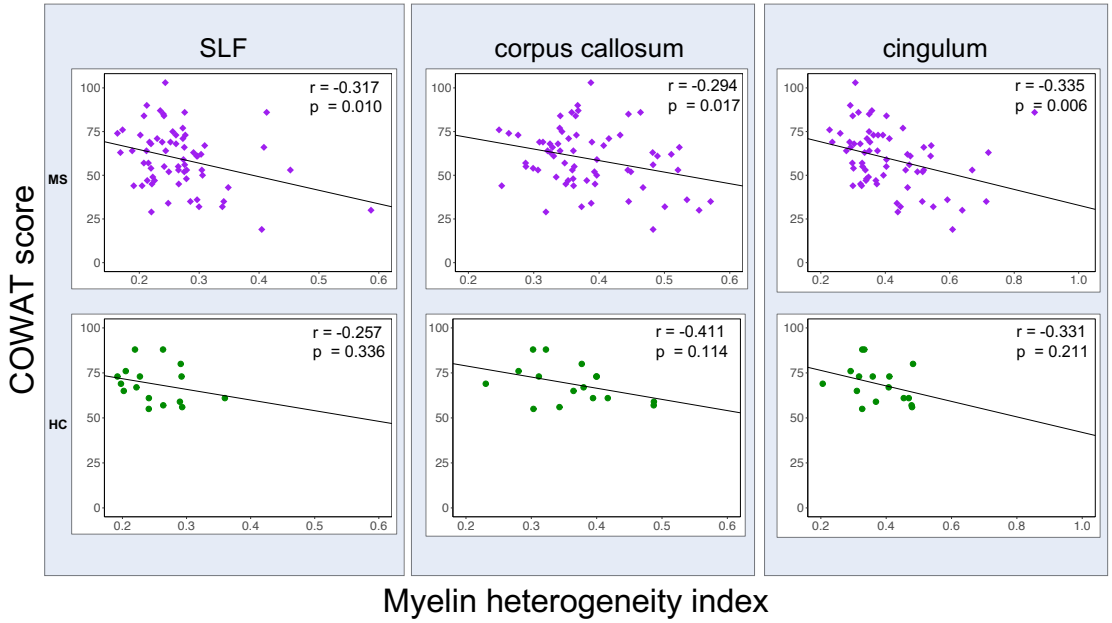

Figure 3.3: Correlations between COWAT performance and MHI. Correlations between the myelin heterogeneity index in NAWM (x axis) and COWAT scores (y axis) in MS (purple dots) and controls (green dots) in three ROIs. Lines = line of best fit. NAWM: normal appearing white matter; ROI: region of interest; MHI: Myelin heterogeneity index; COWAT: Controlled Oral Word Association Test.

3.4.5 BVMT-R

The mean BVMT-R score for MS patients was 49 (range: 1-71) while controls had a mean score of 56 (range: 25-68). Increased MHI in MS NAWM showed correlations with worse BVMT-R scores in the SLF ($r = -0.257$, 95% CI: -0.582 - -0.011; $p = 0.042$), corpus callosum ($r = -0.250$, 95% CI: -0.505 - -0.002; $p = 0.048$) and cingulum ($r = -0.266$, 95% CI: -0.515 - -0.019; $p = 0.035$); however these relationships do not reach significance after Bonferroni correction. No significant correlations were observed between MHI and BVMT-R scores in controls ($p > 0.3$) (Figure 3.4).

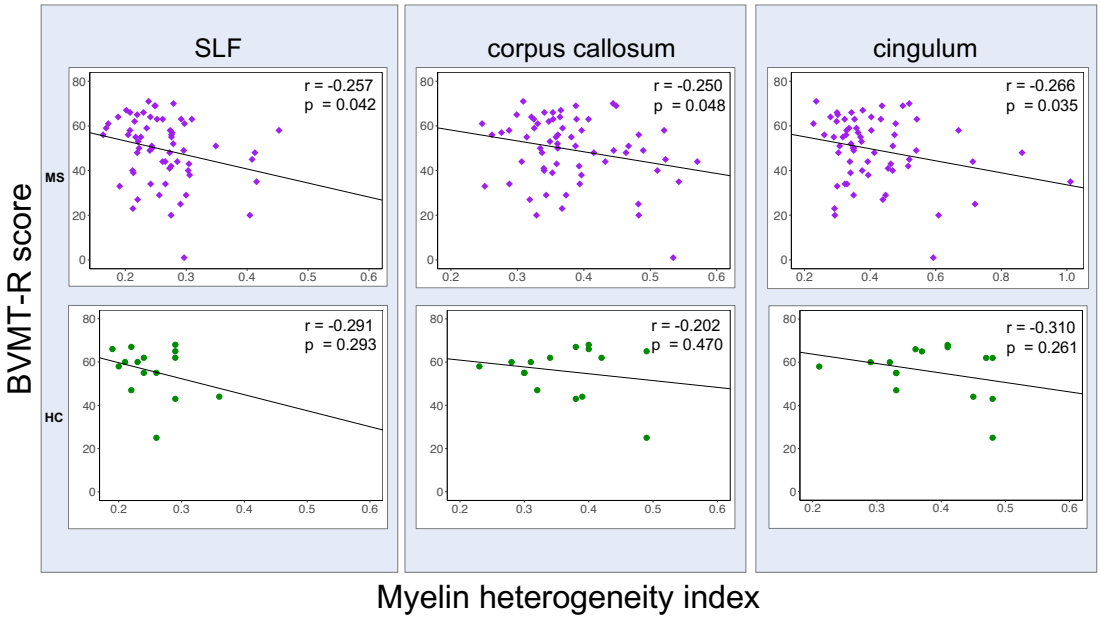

Figure 3.4: Correlations between BVMT-R performance and MHI. Correlations between the myelin heterogeneity index in NAWM (x axis) and BVMT-R scores (y axis) in MS (purple dots) and controls (green dots) in three ROIs. Lines = line of best fit. NAWM: normal appearing white matter; ROI: region of interest; MHI: Myelin heterogeneity index; BVMT-R: Brief Visual Spatial Memory Test – Revised.

3.4.6 Myelin heterogeneity index and cognitive performance

To illustrate the relationship between MHI and cognitive performance, Figure 3.5 depicts MWF maps and the distribution of MWF values within the SLF for high, moderate and low MHI and the associated range of cognitive z-scores in three people with MS. The SLF was selected for this example as it exhibited the strongest relationship with cognitive test scores in MS of the three ROIs. A range of cognitive z-scores obtained from the 4 tests for each of the three patients are displayed as they are more informative than raw cognitive scores for this illustrative purpose. The z-scores were calculated using the mean and standard deviation for each cognitive test from our control sample. Patient A had a high MHI (0.59) in the SLF, matching their low cognitive scores

relative to controls (z = -3.6 – -5.1). Patient B had both moderate MHI (0.28) in the SLF and cognitive test scores (z = -0.9 – -1.6). Patient C had a low MHI (0.22) in the SLF and performed at and above the level of controls on the cognitive tests (z = -0.08 – 0.9).

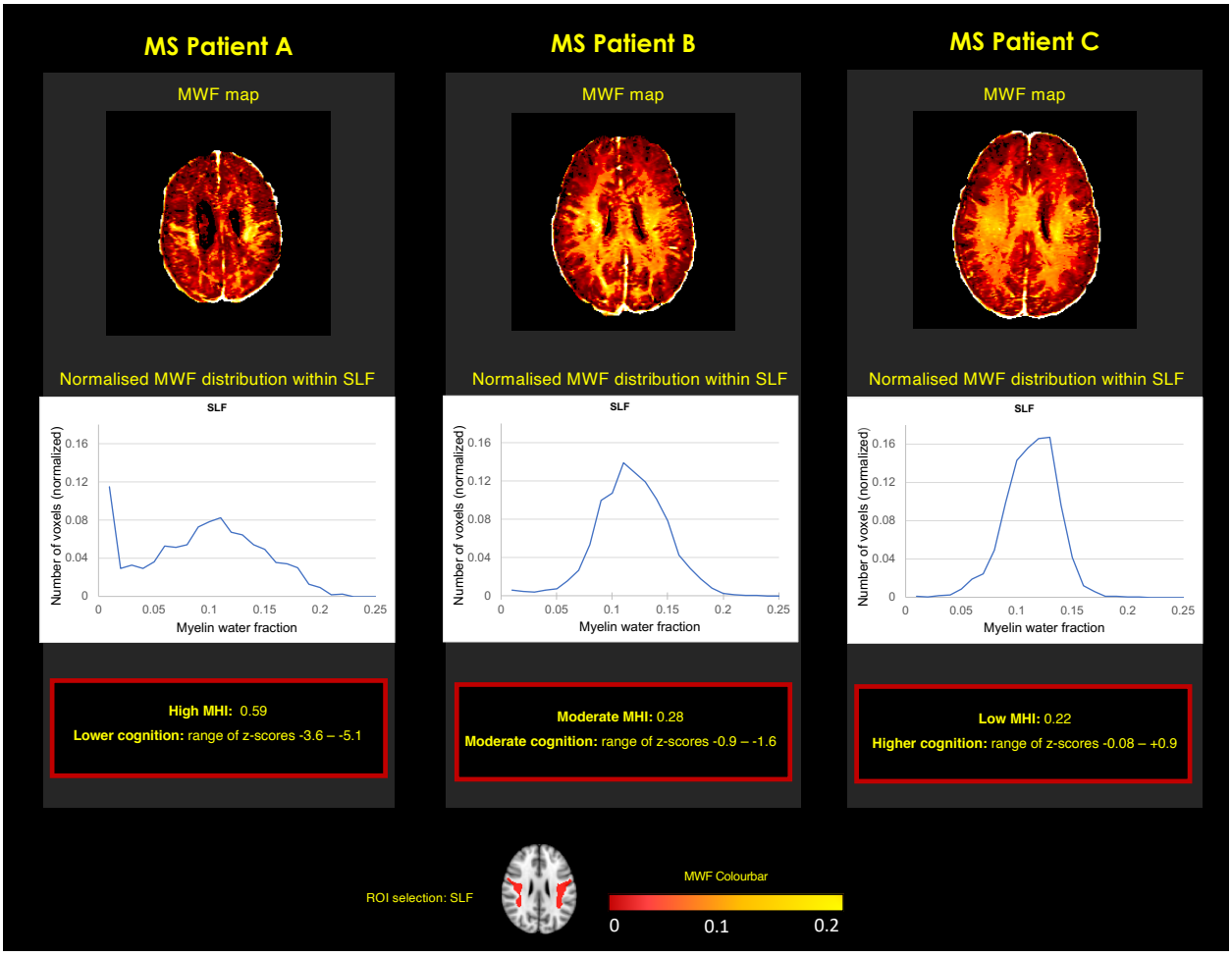

Figure 3.5: Axial map of MWF values, MWF distributions in SLF, MHI in SLF and cognitive z-scores in three MS participants. Axial maps of MWF values (top), normalized histograms of MWF values in the SLF (middle), MHI in the SLF and cognitive z-scores scores (bottom) of three MS patients. Patient A had a high MHI in the SLF (0.59), matching their low cognitive scores relative to controls (range of z-scores -5.1– -3.6). Patient B had both moderate MHI in the SLF (0.28) and cognitive test scores (range of z-scores -1.6 – -0.9). Patient C had a low MHI in the SLF (0.22) and performed at and above the level of controls on the cognitive tests range of z-scores (-0.08 – +0.9). The z-scores were calculated using the mean and standard deviation for each cognitive test from the control sample. MWF: Myelin Water Fraction; MHI: Myelin heterogeneity index; SLF: Superior Longitudinal Fasciculus.

3.4.7 Physical disability (T25-FW and 9-HPT)

MHI was not associated with lower limb disability, as measured by the T25-FW, in corpus callosum ($r = -0.018$; $p = 0.9$), SLF ($r = -0.007$; $p = 1.0$) or the cingulum ($r = 0.068$; $p = 0.6$). In addition, MHI in the corpus callosum ($r = 0.07$; $p = 0.6$), SLF ($r = 0.226$; $p = 0.08$) and cingulum ($r = 0.184$; $p = 0.2$) was not associated with the 9-HPT.

3.5 Discussion

Increased MHI in the MS cohort indicative of diffuse myelin abnormalities was found in NAWM of tracts that are associated with cognition. Furthermore, the MHI abnormalities correlated with cognitive deficits that are common in MS. Recent histopathological studies suggest that myelin in NAWM is affected in MS[178,179] and previous MWI reports found that decreased mean MWF and increased MWF heterogeneity (increased MWF variance in the brain) in NAWM is associated with worse physical disability.[157] The MHI incorporates both MWF mean and variance; a decrease in MWF mean or an increase in MWF heterogeneity would result in an increased MHI. Thus, the MHI is sensitive to gross changes in MWF that shift the entire distribution, as well as smaller changes that broaden the distribution more subtly yet are clinically relevant.

Previous quantitative MRI studies using diffusion tensor imaging (DTI) or magnetization transfer imaging (MTI) have reported associations between abnormalities in NAWM and cognitive impairment in MS in the same brain regions as our study. DTI studies use fractional anisotropy (FA) as a general measure of microstructural WM tissue integrity. In WM, diffusion of water is restricted by micro-structural components like myelin. This causes the diffusion to be parallel, rather than perpendicular, to the direction of the axonal fibres, and yields a high FA value in

healthy WM. When micro-structural damage to WM occurs, restrictions on the movement of water molecules are reduced, and diffusion becomes more isotropic. This results in a reduction in the FA.[180] DTI also provides the mean diffusivity (MD) metric (equal to the magnitude of diffusion) with increased MD thought to represent microstructural abnormalities in WM.[181] MTI studies typically report the magnetization transfer ratio (MTR), a semi-quantitative metric which estimates the exchange of magnetization between non-aqueous tissue and water and is proposed to decrease with myelin loss.[108]

In the current study, we found a highly significant correlation between increased MHI in NAWM in the SLF, corpus callosum and cingulum and slower processing speed performance as measured by the SDMT. Similarly, decreased FA and MTR as well as increased MD have been correlated with worse processing speed in the SLF,[104,169,182] corpus callosum,[104,105,169–171,182] and cingulum NAWM.[104,182] Likewise, we observed an association between increased MHI in NAWM in the SLF, corpus callosum and cingulum and decreased verbal memory (worse SRT scores). Correlations between decreased FA in NAWM in these same 3 brain regions and impaired verbal memory have also been reported.[104] In contrast to a previous study that failed to find significant relationships between FA in NAWM and COWAT scores,[104] we found significant correlations between MHI in NAWM and performance on the COWAT in MS patients. This may be due to the fact that MHI is a more specific measure of myelin damage than mean FA values. We noted a trend between increased MHI in NAWM in SLF, corpus callosum and cingulum and decreased visuospatial memory as measured by the BVMT-R, though it did not pass multiple testing correction. However, it is worth noting that increased FA and decreased MD in these brain regions and a significant relationship with decreased visuospatial memory has been observed in studies

that did not employ the conservative Bonferroni correction.[104,182] It is possible that investigating visual pathway ROIs would lead to more significant correlations.

It was observed that there was a subset of patients with particularly low cognitive scores and high MHI values. Not surprisingly, these tended to be progressive MS patients.[2] This finding emphasizes the importance of including both relapsing-remitting and progressive phenotypes, with a broad spectrum of cognitive ability and severity of myelin damage representative of the multiple sclerosis population as a whole, to comprehensively characterize the relationship between cognitive ability and severity of myelin damage in correlation studies.

To determine if the observed associations between MHI values and cognitive performance were specific to cognition rather than a proxy for overall disability, we investigated the relationship between MHI in the 3 white matter tracts we selected and performance on the T25-FW and the 9-HPT. We found no correlation between MHI in the corpus callosum, SLF and cingulum and upper and lower limb disability. Therefore, we believe our findings are specific to cognitive function.

While our results are consistent with previous DTI and MTI studies, MWI offers greater biological interpretability. DTI measures reflect a large number of biological changes that occur in MS and caution is warranted when interpreting diffusion anisotropy changes as myelin changes.[183] DTI measures are influenced by biological factors other than myelin, such as the directionality of fibre bundles, tortuosity, fibre crossings and fibre orientation coherence.[106,183–185]

Similarly, MTI estimates of macromolecular-bound water include, but are not limited to myelin, and are heavily influenced by inflammation and edema,[108] which are often present in MS. In contrast, MWI has been validated with both human histology[113,155] and animal models[114] as a specific measure of myelin. We acknowledge that the denominator in the MWF is total water, therefore, increases in edema and inflammation can influence changes in MWF. However, if the

MWF decreases in MS reported in the literature were due to only edema rather than myelin loss, dilution of the MWF from edema would require such significant swelling in the brain that it would result in a lethal increase in intracranial pressure.[177] The use of MWI in the current study provides evidence that cognitive symptoms in MS result, at least in part, from myelin abnormalities in NAWM. This is of major clinical importance as it not only provides insight into the underlying pathology contributing to cognitive symptoms in MS, but also offers a non-invasive, tissue specific biomarker for monitoring treatment efficacy, particularly for therapies geared towards remyelination.[186]

3.5.1 Study limitations

The study was limited to one hospital site, using a single scanner, which might restrict the generalizability of the results. However, the findings substantiate the use of MWI, and specifically MHI, to quantify MS-related demyelination and the association with cognitive impairment. This would be feasible for multicenter studies as MWI has excellent inter-site[156] and inter-vender[187] reproducibility.

3.6 Conclusions

This study implements a myelin specific imaging technique to demonstrate that otherwise normal appearing brain tissue is diffusely damaged in MS. We have also shown that these changes are a significantly associated with disease related cognitive symptoms. These findings contribute to a better understanding of the underlying pathology involved in MS-related cognitive impairment; myelin damage in NAWM is likely playing a role. The MHI metric offers an in-vivo marker feasible for use in clinical trials investigating cognitive symptoms in MS, for which a reliable, quantitative biomarker is sorely needed.

Chapter 4

4. Myelin water imaging provides evidence for unique anatomical-functional relationship between myelin damage and different cognitive domains in MS

A version of chapter 4 has been submitted for publication. My contributions as well as those of my coauthors are listed above in the preface and opening paragraph for chapter 2. In addition, Sarah Morris provided guidance on the use of FSL Randomise and Adam Dvorak created the healthy control myelin water fraction atlas used in this project.

In the 2 preceding chapters, we established that MWF in NAWM was associated with performance on the SDMT (processing speed), SRT (verbal memory) and COWAT (word retrieval) with various correlation strengths. The goal of our next investigation was to identify regions with relevant anatomical-functional relationships between MWF and cognitive symptoms, and the strength of these relationships, without imposing *a priori* assumptions about their size and location. This chapter contributes an exciting and novel finding that MWF values within specific white matter tracts, which we'll refer to as cognitive domain-specific white matter regions, are associated with distinct cognitive domains. The spatial extent and anatomical location of MWF relationships with cognitive performance are overlapping but they are unique to each cognitive domain. This finding highlights the benefit of investigating specific voxel clusters within a tract rather than entire white matter tracts. This chapter presents 3 additional novel findings: 1) mean MWF in cognitive domain-specific white matter regions was significantly lower in cognitively impaired patients relative to cognitively preserved patients; 2) the relationship between MWF in

cognitive domain-specific white matter regions was moderately strong and highly significant; 3) the spatial extent and severity of myelin damage was associated with poor cognitive performance in MS whilst preserved myelin in cognitive domain-specific white matter regions was associated with maintained cognitive performance.

4.1 Introduction

Multiple sclerosis is an inflammatory, demyelinating and neurodegenerative disease of the central nervous system (CNS)[10] that affects over 2 million people globally, rendering it the most prevalent chronic neuroinflammatory CNS disease worldwide.[11] Cognitive impairment is a common symptom of multiple sclerosis that presents in up to 70% of patients.[2] Cognitive symptoms in multiple sclerosis typically manifest as deficits in attention, memory and most frequently processing speed.[248] Multiple sclerosis related cognitive impairment has a severe impact on quality of life,[3] including the ability to perform tasks of daily living,[4] fitness to drive,[5] and social functioning.[4] Cognitive dysfunction is also a major contributor to unemployment in multiple sclerosis patients.[3,6] Given the major burden cognitive impairment presents to those living with multiple sclerosis, an improved understanding of the underlying myelin pathology driving these deficits would be beneficial for targeting and testing new therapies aimed at slowing cognitive decline.

Damage to myelin may be a key factor contributing to cognitive dysfunction in multiple sclerosis. Investigating the role of demyelination in clinical outcomes, such as cognition, requires imaging techniques that are quantitative, sensitive, and biologically specific to myelin. Myelin water imaging is a magnetic resonance imaging (MRI) technique that separates the MRI signal into contributions from the distinct water pools within a voxel based on the MRI property known

as T_2 relaxation time. In CNS tissue, these water pools correspond to (i) cerebrospinal fluid (long T_2=~ 2 s); (ii) intra- and extra-cellular water (intermediate T_2=~ 60–80ms); and (iii) and water trapped between the myelin bilayers (short T_2=~10- 20ms).[8] The fraction of MRI signal arising from the myelin water divided by the total water signal is the myelin water fraction (MWF), which has been histologically validated as a specific marker for myelin using human tissue[112,155] and animal models of myelin damage.[114]

Myelin water imaging has been used to investigate myelin damage in multiple sclerosis and other demyelinating diseases,[8] although very few have considered cognitive symptoms. Our recent work[188,189] demonstrated that MWF measures in three white matter tracts, selected *a priori,* were associated with cognitive performance in multiple sclerosis. However, examining only pre-defined specific brain regions limits the scope and broader applicability of these findings. We aimed to build on our previous work by identifying regions with relevant anatomical-functional relationships between myelin damage and cognitive symptoms, and the strength of these relationships, without imposing *a priori* assumptions about their size and location.

Therefore, our goal was to determine how cognitive deficits are associated with changes in specific brain structures, a research direction recently identified by experts in the field as a critical future priority.[61] We hypothesized that performance in different cognitive domains would be associated with MWF in distinct white matter tracts. We used a cohort of relapsing remitting and progressive MS patients and first employed an assumption-free, data driven approach using permutation testing[190] to determine the existence, and anatomical location of, myelin damage associated with different cognitive domains. We then investigated differences in myelin damage in these cognitive domain-specific white matter regions between cognitively impaired and

nonimpaired groups. Finally, we explored the relative roles of the spatial extent and the magnitude of myelin damage in cognitive performance in multiple sclerosis.

4.2 Materials and methods

4.2.1 Participants

This study was approved by the University of British Columbia Clinical Research Ethics Board. All participants provided written informed consent. Participants were recruited through the University of British Columbia Hospital MS clinic and via online recruitment advertisements on local health authority websites. Seventy-six participants with clinically definite multiple sclerosis fulfilling the 2017 revised MacDonald's criteria for diagnosis [38] (40 relapsing-remitting; 13 primary progressive; 23 secondary progressive) and 22 age, sex, and education matched healthy volunteers without neurological disease were included in the study.

4.2.2 Clinical and neuropsychological assessment

In addition to neurological examination with the Kurtzke Expanded Disability Status Scale (EDSS) [39], participants underwent a thorough neuropsychological assessment using tests drawn from cognitive batteries validated for use in multiple sclerosis. [61] The oral version of the Symbol Digit Modalities Test (SDMT) [63] was used as a measure of processing speed. One control participant performed the written version with instructions provided by a translator as they were non-English speaking. The test contains a reference key with the numbers 1-9 each corresponding to different geometric symbols. The answer key contains only symbols to which the participant must match the corresponding number according to the key. The subject responds orally with the digit associated with the symbol as quickly as possible. The test is scored by tallying the total

number of correct responses achieved in 90 seconds. The Selective Reminding Test (SRT) [63] was used to assess verbal memory. The participant is read aloud a list of 12 words that they are asked to repeat back immediately. After they have repeated all words they can remember, the participant is read back only the words they have missed and asked to repeat all 12 words again. This procedure is repeated for 6 rounds. The participant is again asked to repeat all 12 words subsequent to a delay during which they perform other cognitive tests in the battery. The SRT is scored by tallying the total correct recalled words. Verbal fluency was assessed using the Controlled Oral Word Association Test (COWAT). [63,73] The participant is given a letter of the alphabet (e.g., "F") and asked to list as many words as they can recite beginning with that letter in 1 minute's time. The version of the test employed in this study included 3 letter prompting categories and an animal category, for which the participant names as many animals as they can recall. The COWAT is scored by tallying the total number of permissible answers—proper names (e.g., cities) and variations of the same word (e.g., runs, running, ran) are not permitted. Visuospatial memory was evaluated with the Brief Visuospatial Memory Test-Revised (BVMT-R). [71] The participant is presented with a display of 6 geometric figures for 10 seconds and then asked to reproduce the display by drawing it exactly as it was seen on a blank page. The participant is given 3 opportunities to view and reproduce the display. In the present study, the total recall t-score was used, which is sum of all valid items generated across learning trials 1–3, corrected for age. Lower scores indicate worse performance on all tests. All participants completed the SDMT at a minimum. However, due to the time constraints of the research appointment, not all participants completed every test. The sample size for each test is indicated in the Results section.

Raw cognitive test scores were used for all association analyses. For between-group analyses, raw scores for each individual for each test were converted to Z-scores in order to categorize

participants into cognitive groups. Canadian normative data [191] was used for the SDMT and the BVMT-R. American normative data [192] was used for the SRT, as Canadian norms were not available. For each test, participants whose scores were $Z \leq -1.5$ relative to normative test scores were classified as having impaired performance on that test; [61] those with scores $Z > -1.5$ to < -1 were classified as mildly impaired; and participants with scores $Z \geq -1$ were classified as cognitively preserved, based on previously validated methodology.[193]

4.2.3 MRI data acquisition

MRI scans were conducted on a 3T scanner (Philips Achieva; Best, Netherlands) using an 8-channel head coil. Sequences included: a 3DT1 anatomical scan (whole-brain 3D magnetization-prepared rapid gradient-echo (MPRAGE), repetition time [TR]=3000ms, echo time [TE] = 8ms, inversion time [TI]=1072ms, 1x1x1mm voxel, 160 slices) for region of interest (ROI) identification and to facilitate anatomical alignment between individual participants and a reference template; and a 48-echo 3D Gradient and Spin Echo (GRASE) T2 relaxation sequence (EPI factor = 3, TR=1073ms, echo spacing=8ms, 20 slices acquired at 1x2x5mm reconstructed to 40 slices at 1x1x2.5mm) for MWF determination.[159]

4.2.4 Creation of MWF maps

Voxel-wise signal decay curves obtained from the T2 relaxation GRASE sequence were modelled by multiple exponential components, with no a priori assumptions about the number of contributing exponentials. Analysis used a regularized non-negative least squares algorithm with the extended phase graph algorithm and flip angle estimation to correct for stimulated echoes (https://mriresearch.med.ubc.ca/news-projects/myelin-water-fraction/ MATLAB® R2013b, The

MathWorks, Inc., Natick, MA).[160] Voxel-wise MWF maps were computed as the ratio of the area under the T2 distribution with 15ms < T2 <40ms relative to the total area under the distribution.[159]

4.2.5 Aligning individual MWF fraction maps with a study-specific template

Each individual's 3DT1 image was linearly registered to the first echo image of their GRASE scan using FLIRT (9 degrees of freedom),[161] part of FMRIB's Software Library (FSL, www.fmrib.ox.ac.uk/fsl).[162] Non-brain parenchyma signal in the registered 3DT1 was removed using an automated approach (FSL Brain Extraction Tool).[163] Then, each brain extracted registered 3DT1 image was registered to a normative 3DT1 study-specific anatomical template created from 100 healthy control scans (mean age 41 years, range 20-78 years; 58 female) performed at the UBC MRI Research Centre (including the 22 healthy controls from the current study))[194] the study-specific template using FLIRT (9 degrees of freedom).[161] Finally, the resulting transformation was used to align each patient and control MWF map with the study-specific template, in a common space. This facilitated permutation testing (described in the following section) and calculation of MWF Z-score maps comparing multiple sclerosis patient MWF maps with those of controls.

4.2.6 Permutation testing

The anatomical location of associations between MWF and cognitive test scores was investigated using non-parametric permutation testing on the MWF maps, aligned with the normative template, with the FSL Randomise tool.[195] 1000 permutations were used for each cognitive test. Threshold-Free Cluster Enhancement and family-wise error correction was used to correct for multiple comparisons.[196] The threshold for significance was set to $p = 0.01$. For each cognitive test, all voxel clusters where MWF was associated with cognitive test performance at $p \leq 0.01$ were included in the cognitive test specific mask. For cognitive group comparisons and

individual association analysis, the mean MWF within each cognitive test specific mask (region) was extracted for each individual.

4.2.7 Creation of multiple sclerosis MWF z-score maps from a healthy control atlas

To investigate how the severity of myelin damage related to cognitive test performance, a MWF z-score map was produced to compare each patient with a healthy control MWF atlas. To create the healthy control MWF atlas, the MWF map from each study-specific template participant was aligned in template space, where voxel-wise mean and standard deviation atlases were calculated.[194] To produce an MWF z-score map, the healthy control mean MWF atlas corresponding to the 100 subject normative study-specific template was subtracted from each patient's MWF map and then divided by the healthy control MWF standard deviation atlas. The number of voxels falling within certain z-scores from the control atlas mean (e.g., $Z \leq 4$, $Z = -4$ to -3, $Z = -3$ to -2, etc.) were exported from each cognitive test-specific white matter mask for each patient.

4.2.8 Statistical analysis

All statistical procedures were performed using IBM SPSS Statistics for Mac, Version 25.0. (Armonk, NY: IBM Corp). Assumptions of normality were tested with Shapiro-Wilk test for normality. The assumption of homogeneity of variance was tested with Levene's test of equality of variance. If the assumptions for a parametric test were violated, we proceeded with the appropriate nonparametric test. A Mann-Whitney U test was used to determine if there was a significant difference in age and years of education between patients and controls. A chi-square test of homogeneity was used to determine if patients and controls were matched for sex.

Differences in mean MWF in task-specific white matter regions between cognitive groups were assessed with Welch's ANOVA and Games-Howell post hoc pairwise comparisons. Associations between mean MWF in cognitive task-specific white matter regions and cognitive test performance were explored with Pearson's correlation. Associations between the severity of myelin damage (number of voxels within certain z-scores from the healthy control atlas mean) and cognitive test scores were also assessed with Pearson's correlation. All tests were two sided.

4.2.9 Data availability

Deidentified demographic, clinical and MWF data can be made available to qualified researchers with a reasonable request. For researchers interested in running myelin water imaging at their site, manufacturer specific acquisition files and MATLAB analysis code is available on a project specific github. Please visit https://mriresearch.med.ubc.ca/news-projects/myelin-water-fraction/ for details.

4.3 Results

4.3.1 Participant characteristics

The clinical and demographic characteristics of participants with multiple sclerosis and healthy controls are displayed in Table 4.1. Age, sex and years of education did not differ significantly between patients and controls.

Table 4.1. Demographic and clinical characteristics

	Controls (n=22)	Multiple Sclerosis (n=76)	*p*
Age, years, median (min-max)	48 (27-65)	54 (26-65)	0.81
Females, n (%)	14 (63.6)	50 (65.8)	0.85
Education, years, median (min-max)	16 (12-22)	14 (12-22)	0.1
EDSS, median (min-max)		3.2 (1.0-8.5)	
Disease duration, years, mean (min-max)		15.5 (0.3-48.0)	
Disease phenotype, RRMS/PPMS/SPMS		40/13/23	

4.3.2 Anatomical location of myelin content associated with cognitive performance

Permutation testing revealed that MWF in several white matter tracts was significantly associated with scores on the SDMT (processing speed), the SRT (verbal memory) and the BVMT-R (visuospatial memory) in participants with multiple sclerosis ($p \leq 0.01$). No significant associations were observed between MWF and scores on the COWAT (word retrieval) in patients ($p > 0.07$). There were no white matter areas where MWF was correlated with cognitive test scores in control participants ($p > 0.2$). Table 4.2 lists the white matter regions with voxels significantly associated with each test at $p \leq 0.01$ accompanied by images demonstrating the anatomical location of the significantly correlated voxels in each cognitive test-specific white matter region (Figure 4.1).

Table 4.2 White matter regions with MWF significantly associated with each cognitive test

Cognitive Test (domain)	**Projection tracts**	**Association tracts**	**Commissural tracts**	**Number of voxels $p \leq 0.01$**
SDMT (processing speed)	• Corona radiata - superior, anterior, posterior (bilateral) • Internal capsule - anterior limb (right) - posterior limb (bilateral) - retrolenticular (bilateral, right dominant) • Posterior thalamic radiation (bilateral)	• Superior longitudinal fasciculus (bilateral) • Inferior longitudinal fasciculus (right) • Anterior cingulum (bilateral)	• Corpus callosum - genu and body dominant, less splenium	118 932
SRT (verbal memory)	• Corona radiata - superior, anterior, posterior (bilateral)	• Superior longitudinal fasciculus (bilateral)	• Corpus callosum - Body dominant,	53 336

	• Posterior thalamic radiation (bilateral)		less splenium and genu	
BVMT-R (visuospatial memory)	• Corona radiata - posterior (bilateral, left dominant) • Posterior thalamic radiation (left) • Internal capsule - posterior limb (left) - retrolenticular (left)		• Corpus callosum - splenium	8 714

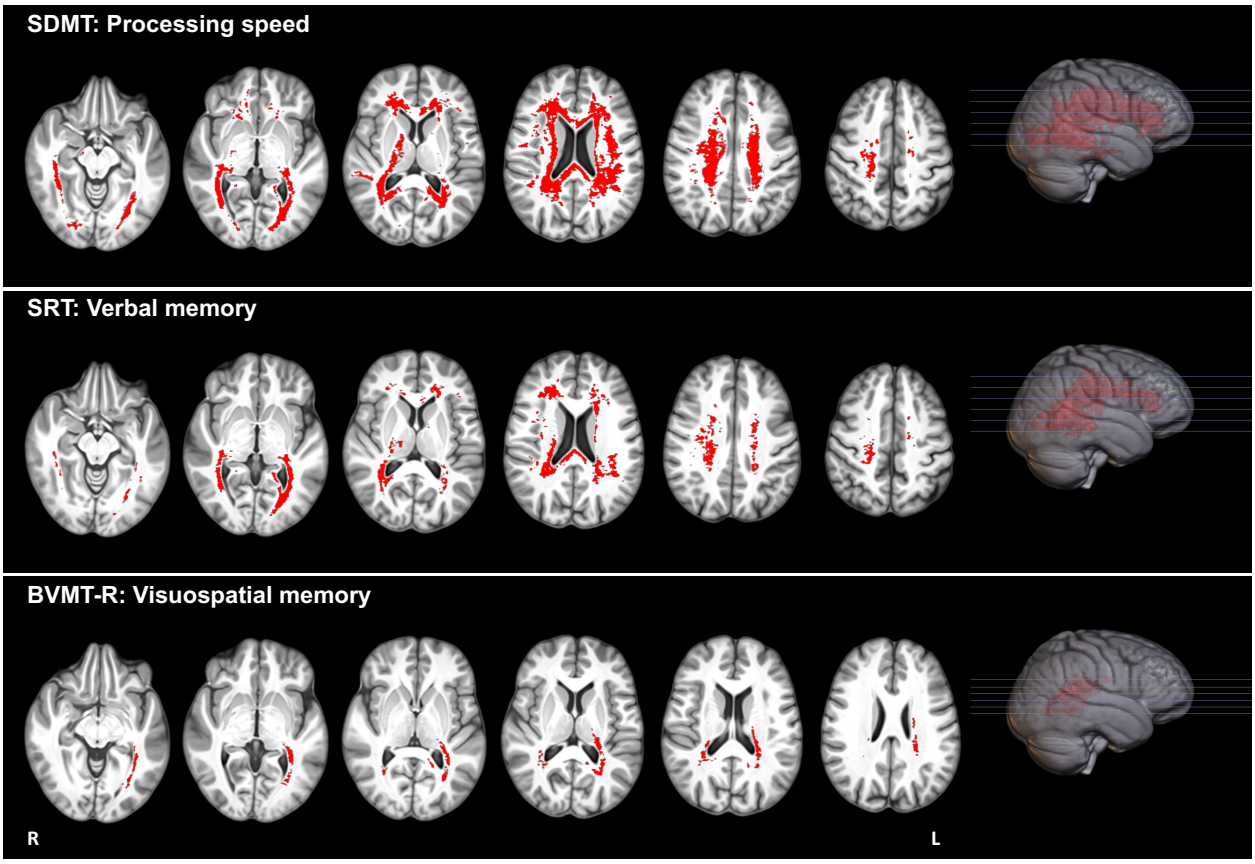

Figure 4.1: Cognitive domain-specific white matter regions identified by Randomise. Selected axial images demonstrating the anatomical location of the significantly correlated voxels in each cognitive test-specific white matter region (left) and a 3D rendering of each cognitive test-specific white matter region with location of axial slices indicated by horizontal lines (right). SDMT: Symbol Digit Modalities Test; SRT: Selective Reminding Test; BVMT-R: Brief Visuospatial Memory Test Revised.

4.3.3 Differences in myelin damage between cognitive groups

4.3.3.1 SDMT – processing speed

Categorizing patients into cognitively impaired, mildly impaired and cognitively preserved on processing speed performance (SDMT) resulted in 14/76 impaired (18.4%), 7/76 mildly impaired (9.2%) and 55/76 preserved (72.4%). Levene's Test of Homogeneity of Variance was

significant ($p < 0.01$), therefore, we proceeded with Welch's ANOVA. The mean MWF in processing speed-specific white matter regions identified using randomise, was significantly different for different cognitive groups, Welch's $F(2, 12.7) = 6.17$, $p = 0.01$. Games-Howell post hoc contrasts revealed that mean MWF was significantly lower in the cognitively impaired group (0.076) relative to the cognitively preserved group (0.104) ($p = 0.008$). There were no significant differences in mean MWF between the mildly impaired group (0.096) and any other group ($p > 0.1$) (Figure 4.2).

4.3.3.2 SRT – verbal memory

Classifying patients into cognitively impaired, mildly impaired and cognitively preserved on verbal memory performance resulted in 16/69 impaired (23.2%), 12/69 mildly impaired (17.4%), and 41/69 preserved (59.4%). The mean MWF in verbal memory specific white matter regions was significantly different between the cognitive groups, Welch's $F(2, 21.2) = 5.95$, $p = 0.009$. The mean MWF in the verbal memory specific white matter regions was significantly lower in the cognitively impaired group (0.082) relative to the cognitively preserved group (0.108) ($p = 0.01$). There were no differences in mean MWF between the mildly impaired group (0.096) and any other group ($p > 0.2$) (Figure 4.2).

4.3.3.3 BVMT-R – visuospatial memory

There were 9/66 patients classified as impaired on visuospatial memory (13.6%), 11/66 classified as mildly impaired (16.7%), and 46/66 categorized as preserved (69.7%). The mean MWF in visuospatial memory specific white matter regions was significantly different between the cognitive groups, $F(2, 14.4) = 8.13$, $p = 0.001$. Mean MWF in the impaired group (0.078) was significantly lower than the preserved group (0.116) ($p = 0.002$). There were no differences in

mean MWF between the mildly impaired group (0.094) and any other group (p >0.05) (Figure 4.2).

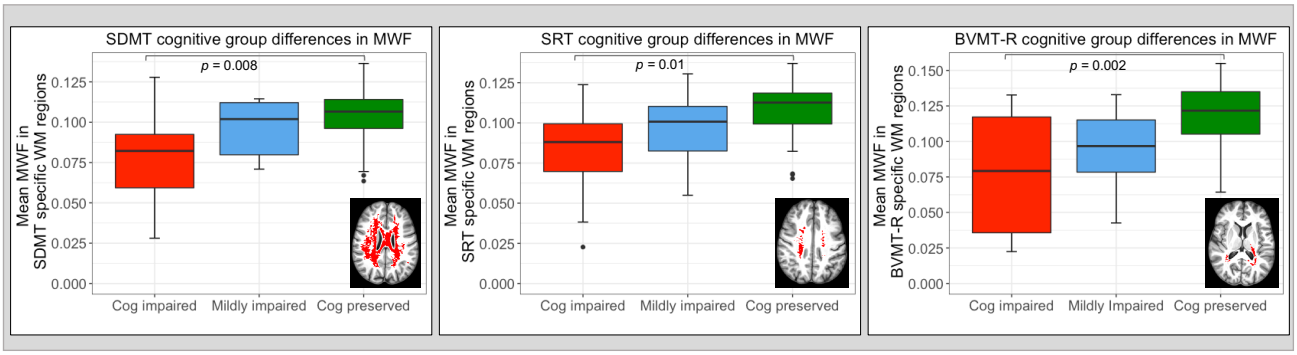

Figure 4.2: Differences in MWF between cognitive groups. Mean MWF in the cognitive test-specific white matter regions was significantly lower in the cognitively impaired groups (red) compared to the cognitively preserved groups (green). Mean MWF in the cognitive test-specific white matter regions in the mildly impaired group (blue) was not significantly different from any other group. SDMT: Symbol Digit Modalities Test; SRT: Selective Reminding Test; BVMT-R: Brief Visuospatial Memory Test Revised.

4.3.4 Correlations between MWF and cognitive performance

The mean MWF in white matter regions specific to processing speed was significantly correlated with scores on the SDMT in multiple sclerosis patients (r = 0.58, p = 4.11 x 10^{-8}). Likewise, mean MWF in verbal memory specific white matter regions was significantly correlated with performance on the SRT (r = 0.56, p = 4.14 x 10^{-7}). Finally, mean MWF in white matter regions specific to visuospatial memory was significantly correlated to BVMT-R scores (r = 0.56, p = 1 x 10^{-6}) (Figure 4.3).

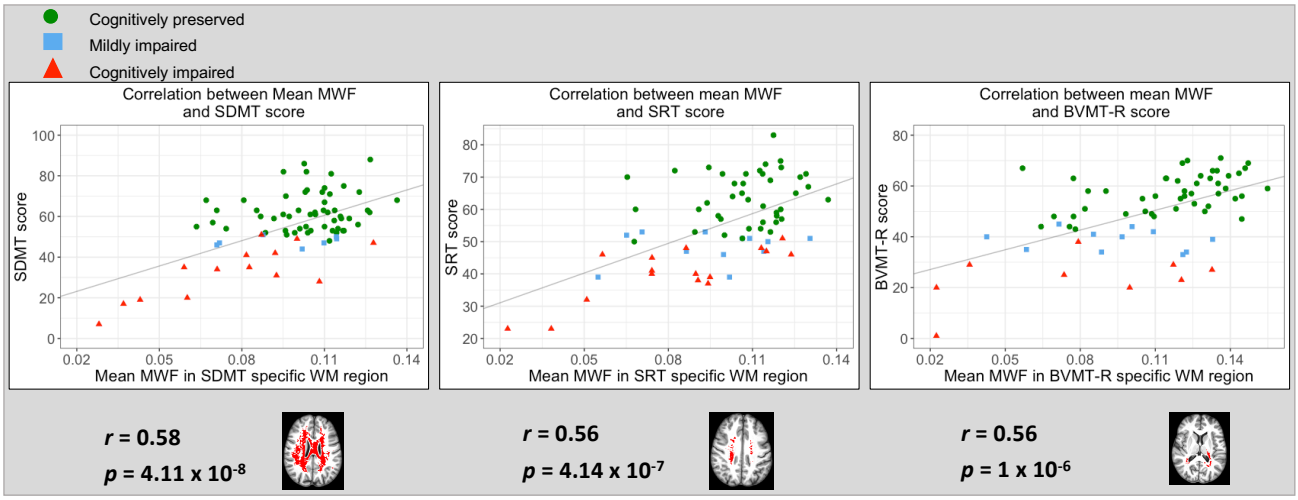

Figure 4.3: Correlations between MWF and cognitive test scores. Correlations between mean MWF in each cognitive test-specific white matter region and cognitive scores. Green circles: cognitively preserved; blue squares: mildly impaired; red triangles: cognitively impaired; SDMT: Symbol Digit Modalities Test; SRT: Selective Reminding Test; BVMT-R: Brief Visuospatial Memory Test Revised.

4.3.5 Relationship between severity of myelin damage and cognitive performance

4.3.5.1 SDMT – processing speed

Figure 4.4 depicts the relationship between the severity of myelin damage in white matter regions specific to processing speed and performance on the SDMT. The number of voxels with an MWF that falls Z ≤ -4 below the control MWF atlas mean was significantly correlated with worse processing speed performance (r = -0.61, p = 2.8 x 10^{-11}). Similarly, the number of voxels with a MWF between Z = -4 to -3 (r = -0.66, p = 5.4 x 10^{-11}) and Z = -3 to -2 (r = -0.59, p = 2.2 x 10^{-8}) was significantly correlated to lower scores on the SDMT. The number of voxels with less severe myelin damage, Z = -2 to -1 below the control atlas mean, was not related to processing speed performance (r = -0.17, p = 0.14). When considering the number of voxels with MWF values

more within normal healthy control range, the relationship was reversed. The number of voxels with MWF values Z = -1 to 0 and Z = 0 to 1 relative to the control atlas mean was positively correlated with SDMT scores ($r = 0.51$, $p = 2.0 \times 10^{-6}$ and $r = 0.43$ $p = 9.9 \times 10^{-5}$ respectively).

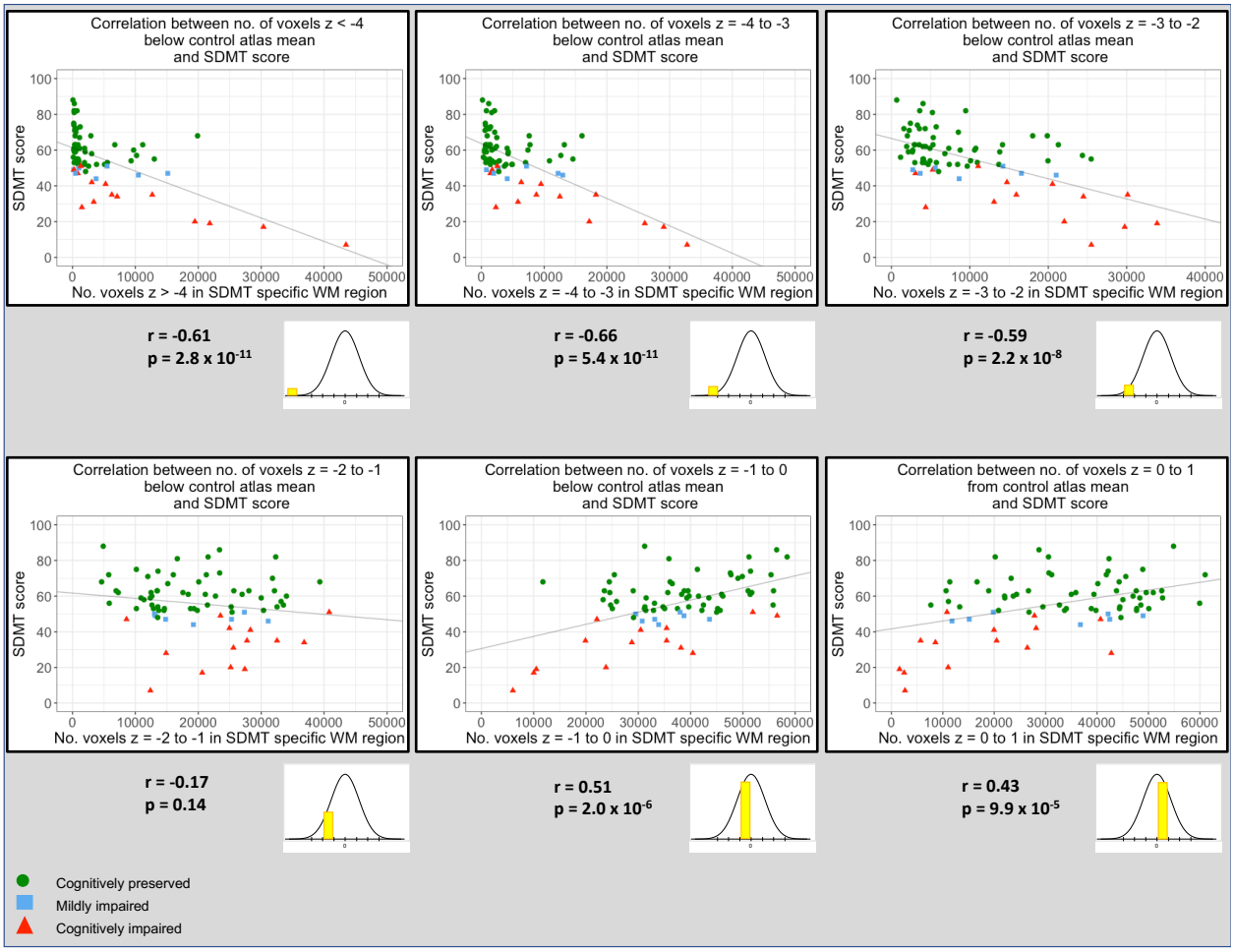

Figure 4.4: Correlations between the severity of myelin damage in white matter regions specific to processing speed and performance on the SDMT. Green circles: cognitively preserved; blue squares: mildly impaired; red triangles: cognitively impaired; SDMT: Symbol Digit Modalities Test.

4.3.5.2 SRT – verbal memory

Figure 4.5 illustrates the relationship between the severity of myelin damage in verbal memory specific white matter regions and performance on the SRT. The number of voxels in

verbal memory specific regions with severely damaged myelin—MWF Z $\leq$ -4 below the control atlas mean—was significantly correlated with worse SRT scores (r = -0.56, p = 5.3 x 10^{-7}). The case was similar for the number of voxels in verbal memory specific regions with MWF values Z = -4 to -3 and Z = -3 to -2 (r = -0.61, p = 3.5 x 10^{-8} and r = -0.55, p = 1.0 x 10^{-6}). As was seen for processing speed (SDMT scores), the number of voxels in verbal memory specific white matter regions with moderately damaged myelin (Z = -2 to -1 relative to the control atlas mean) demonstrated a much weaker relationship with SRT Scores (r = -0.24, p = 0.042). Again, the relationship reversed when investigating the number of voxels with MWF more within normal range, for which an increased number of voxels with normal MWF was correlated with higher SRT scores (Z = -1 to 0: r = 0.4, p = 0.001; Z = 0 to 1: r = 0.48, p = 2.8 x 10^{-5}).

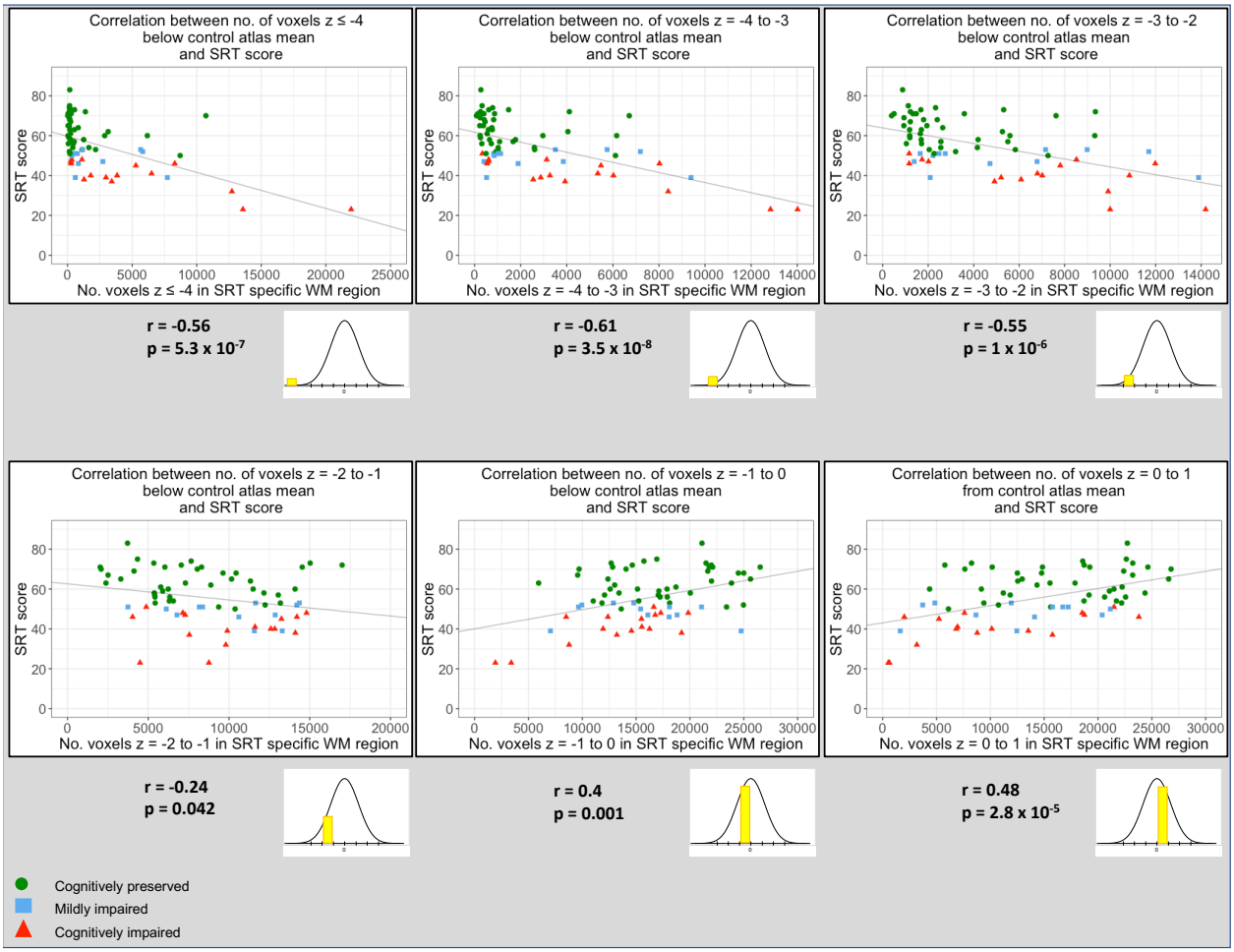

Figure 4.5: Correlations between the severity of myelin damage in verbal memory specific white matter regions and performance on the SRT. Green circles: cognitively preserved; blue squares: mildly impaired; red triangles: cognitively impaired; SRT: Selective Reminding Test.

4.3.5.3 BVMT-R – visuospatial memory

Figure 4.6 shows the relationship between the severity of myelin damage in visuospatial memory specific white matter regions and performance on the BVMT-R. The number of voxels in visuospatial memory specific white matter regions with markedly damaged myelin was significantly correlated with worse BVMT-R scores ($Z \leq -4$: $r = -0.55$, $p = 2.0 \times 10^{-6}$; $Z = -4$ to -3: $r = -0.53$, $p = 5.0 \times 10^{-6}$; $Z = -3$ to -2: $r = -0.37$, $p = 0.002$). Akin to processing speed and verbal

memory, the number of voxels in visuospatial memory specific white matter regions with moderately damaged myelin was not correlated with performance on the BVMT-R (Z = -2 to -1; r = -0.23, p = 0.06). Finally, the number of voxels with MWF values within normal range was correlated with better performance of the BVMT-R (Z = -1 to 0: r = 0.26, p = 0.032; Z = 0 to 1: r = 0.50, p = 1.8 x 10^{-5}).

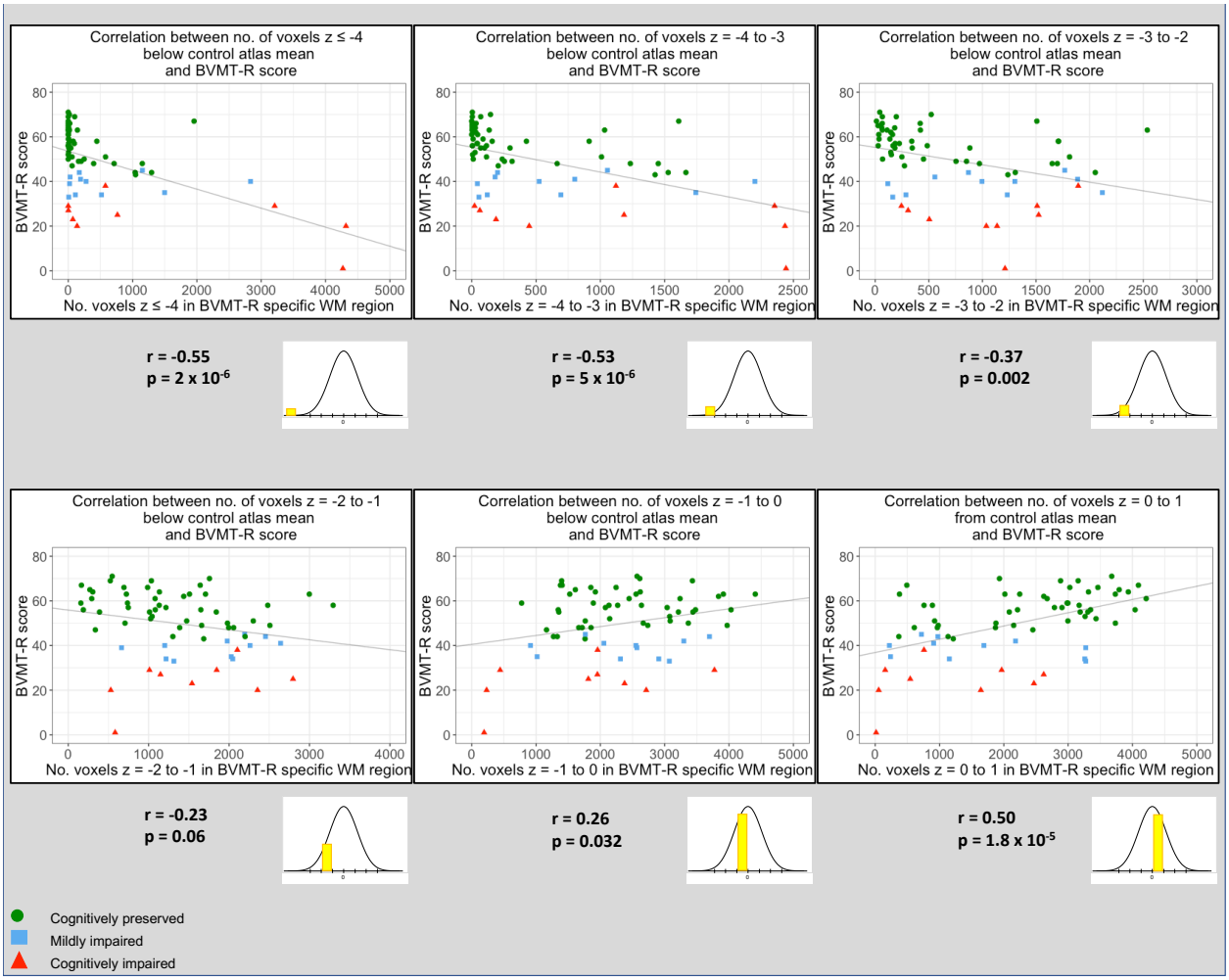

Figure 4.6: Correlations between the severity of myelin damage in visuospatial memory specific white matter regions and performance on the BVMT-R. Green circles: cognitively preserved; blue squares: mildly impaired; red triangles: cognitively impaired; BVMT-R: Brief Visuospatial Memory Test-Revised.

4.4 Discussion

Our study identified specific voxel clusters in the brain where myelin was associated with performance on cognitive tests in patients with multiple sclerosis. When categorizing patients into cognitive groups based on test performance, we observed that the cognitively impaired groups had a significantly lower MWF in cognitive test-specific white matter regions compared to the cognitively preserved groups. Finally, we showed that the number of severely damaged voxels provided the strongest predictive value for poor performance on cognitive tests.

4.4.1 White matter regions associated with cognitive tasks

The white matter regions we identified where MWF was associated with performance on different cognitive tests were overlapping but distinct, and in agreement with MRI studies examining cognitive performance in other neurological diseases. The SDMT had the largest volume of associated white matter, which aligns with the fact that information processing speed and attention requires coordination of several brain areas.[197] Similar brain regions were also implicated in traumatic brain injury, where decreased white matter fractional anisotropy (a diffusion tensor imaging MRI metric indicative of microstructural white matter integrity) in the corona radiata, superior longitudinal fasciculus and corpus callosum was associated with worse SDMT performance.[198] Verbal memory (SRT)-MWF associated regions identified in our multiple sclerosis cohort have also shown reduced fractional anisotropy in the corona radiata, corpus callosum and major forceps in bipolar depression[199] and people exposed to chemotherapy.[200] Finally, white matter regions with MWF associated with BVMT-R performance occupied voxels that were generally confined to visual processing areas,[201] in agreement with work showing an

association between visual memory and white matter fractional anisotropy of the posterior thalamic radiation and splenium of the corpus callosum in pre-term born adults.[201]

4.4.2 Myelin plays a role in cognitive symptoms

Cognitively impaired patients had significantly decreased MWF in cognitive task-specific white matter regions compared to cognitively preserved patients, which indicates that myelin damage plays a major role in multiple sclerosis-related cognitive symptoms. Our observation is in agreement with previous reports describing white matter damage in similar regions in cognitively impaired patients including decreased fractional anisotropy in the corona radiata, optic radiation, anterior limb of the internal capsule, cingulum, superior longitudinal fasciculus,[149] corpus callosum,[149,202] forceps major and minor, inferior fronto-occipital fasciculus, and thalamic tracts.[202] Increased mean diffusivity (another diffusion tensor imaging surrogate for microstructural damage in white matter) has also been observed in cognitively impaired vs. preserved patients in the corona radiata, optic radiation, anterior limb of the internal capsule, cingulum, superior longitudinal fasciculus,[149] and corpus callosum.[149,203] While diffusion tensor imaging metrics can clearly demonstrate these group differences, fractional anisotropy and mean diffusivity lack tissue specificity, making it difficult to pinpoint what exact microstructural damage is occurring. Our study allows for more tissue specific interpretations by employing a myelin-specific imaging technique.

The moderately strong, highly significant relationships between MWF in cognitive test-specific white matter regions and cognitive test performance indicates that the amount of myelin present in these functionally relevant regions exerts a strong influence on cognitive performance. Put simply, patients with lower myelin content in functionally relevant white matter regions perform worse cognitively whilst patients with higher myelin content perform better. Previous

work using a subset of the patients from the current study employed 3 *a priori* selected regions (cingulum, corpus callosum, superior longitudinal fasciculus) demonstrated significant relationships in participants with multiple sclerosis but not in controls between MWF measurements and processing speed (SDMT),[189,204] verbal memory (SRT) and world retrieval (COWAT), but no correlation was found with visuospatial memory (BVMT-R).[189] Using a data driven approach, our current SDMT and SRT results are consistent, albeit with stronger correlation values, with the anatomical areas chosen *a priori* in the previous studies. In contrast to the previous work, we did find highly significant correlations between MWF and BVMT-R scores. This discrepancy is likely because the visual processing areas selected by our data driven approach were not included in the previous analysis. The threshold-Free Cluster Enhancement with family-wise error correction[196] used in the present study involves several corrections for multiple comparisons whereas the ROI approach in the previous study required only three—one for each region. This could by why permutation testing for correlations between MWF and COWAT scores trended towards, yet failed to reach significance ($p > 0.07$).

4.4.3 Severity of myelin damage drives cognitive impairment

The severity of myelin damage appears to be a key driver of multiple sclerosis cognitive impairment. This is evidenced by the fact that we observed significant relationships between the number of voxels with MWF values that fall between $Z \leq -4$ to -2 below the control MWF atlas mean and poor performance on every cognitive test. On the other hand, moderately damaged voxels, Z = -2 to -1 below the control MWF atlas mean, demonstrate very weak to no relationship with cognitive scores. Notably, the number of voxels with MWF values that fall within normal range of the distribution (Z = -1 to +1) are positively related to cognitive performance, which indicates that preserved myelin is associated with maintained cognition. Recent work demonstrates

that higher FA in the longitudinal fasciculi, corticospinal tracts and corpus callosum was significantly associated with better recovery outcomes subsequent to visuomotor training, which supports the notion that maintained microstructural integrity in these regions relates to better functional outcomes.[205]

4.4.4 Strengths and limitations

The current work was strengthened by the inclusion of a patient group with a broad spectrum of cognitive ability and severity of myelin damage representative of the multiple sclerosis population as a whole. Our study also had the advantage of using a study specific template. Registration accuracy was vital for this study to draw appropriate conclusions regarding the anatomical location of myelin damage and its relationship to cognitive performance. The similarity metrics that guide image registration rely on correspondence between the study data and the anatomical template to which it is registered. A template (generated from image data with similar image field-of-view, resolution, contrast, etc) provides improved anatomical alignment between each subject and the template. The current study was limited to one hospital site, using a single scanner, which may impact the generalizability of the results. However, the findings substantiate the use of MWI to quantify multiple sclerosis-related demyelination and its association with cognitive impairment. This would be feasible for multicenter studies as MWI has excellent inter-site[156] and inter-vender[187] reproducibility.

Taken as a whole, the current work using a quantitative, myelin-specific imaging technique demonstrated that myelin damage is an important contributor to the underlying pathology of multiple sclerosis-related cognitive symptoms. Myelin content in functionally relevant white matter regions was significantly lower in cognitively impaired relative to cognitively preserved patients and correlated strongly with cognitive test performance. Finally, the severity of myelin

damage also played an important role in impaired cognitive performance while preserved myelin is associated with maintained cognition. This study contributes to an improved understanding of how myelin pathology plays a role in multiple sclerosis related cognitive impairment and highlights the promise of MWI for monitoring changes in myelination and its relationship to cognitive worsening and improvement during clinical trials.

Chapter 5

5. Conclusions

The work presented in this thesis provides evidence that myelin health throughout MS white matter may play an important role in cognitive performance and elucidates the nature of the relationship between the location and severity of myelin damage and cognitive symptoms. In chapter 2, using myelin specific MRI (MWI), we demonstrated that greater myelin damage (increased myelin heterogeneity) in NAWM in 3 ROIs involved in cognition was associated with decreased processing speed (SDMT) performance in MS patients. In addition, we showed that myelin heterogeneity explained a significant amount of the variability in SDMT scores in patients, while adding lesions, white matter volume, age and disease duration did not lead to a significant increase in variability explained. Once we had established that myelin damage was involved in the most commonly impaired cognitive domain in MS in a relatively small sample, we proceeded to expand our investigation to include a larger cohort and a more comprehensive cognitive battery, using the novel metric, MHI, that encompasses both mean MWF and myelin heterogeneity.

In chapter 3, we found that increased myelin damage (increased MHI) in NAWM was again associated with processing speed performance (SDMT), and also significantly correlated with verbal memory (SRT) performance as well as word retrieval (COWAT) performance. Conversely, MHI in the SLF, corpus callosum and cingulum trended towards significant ($p < 0.05$) correlations with visuospatial memory (BVMT-R) scores but did not reach significance after Bonferroni correction. Further, we demonstrated that these relationships are specific to cognition rather than a proxy for overall disability given that MHI in NAWM in the 3 *a priori* selected ROIs did not correlate with upper (9-HPT) or lower (T25-FW) limb function.

In chapter 4, using a hypothesis free data driven approach, we demonstrate that there is an anatomical-functional relationship between myelin damage and cognition in MS. The spatial extent and anatomical location of MWF relationships with cognition are overlapping but they are unique to each cognitive domain. This is an exciting and novel finding that shows, for the first time, that MWF values within specific white matter tracts are associated with distinct cognitive domains. It also highlights the benefit of investigating specific voxel clusters within a tract rather than the entire white matter tracts. Notably, there was minimal overlap between the white matter regions determined to be associated with BVMT-R scores via permutation testing and the ROIs selected in the previous chapters. The white matter regions determined to be associated with BVMT-R performance are involved in visual processing,[206,207] which may explain why a highly significant association was observed in chapter 4 but not chapter 3.

We showed that mean MWF in cognitive domain-specific white matter regions was significantly lower in cognitively impaired patients relative to cognitively preserved patients. Moreover, the relationship between MWF in cognitive domain-specific white matter regions was moderately strong and highly significant. The spatial extent and severity of myelin damage was associated with poor cognitive performance in MS. Conversely, preserved myelin in cognitive domain-specific white matter regions was associated with maintained cognitive performance. This finding suggests that protecting or restoring myelin may prevent or improve cognitive symptoms.

The current work employed 3 different MWF measures: myelin heterogeneity (MWF variance), MHI (MWF CV) and mean MWF. Mean MWF is the most commonly reported measure in the literature, however, it may not be the most sensitive in detecting myelin damage in a small sample using an ROI based analysis. There is considerable variability in mean MWF between individuals as well as intra-individual regional variability,[208] which can wash out associations with

mean MWF. Myelin heterogeneity and MHI provide a more sensitive measure for studies with small sample sizes employing an ROI approach. However, MHI may be the more sensitive of the two given that it characterizes the entire MWF distribution by taking both the mean and spread of the distribution into consideration. Mean MWF is appropriate for large sample sizes and data driven approaches. Further, mean MWF has the advantage of being more interpretable by a general scientific audience than MHI. However, MHI will yield similar results under these conditions.

5.1 Significance and potential applications

Results from this thesis further our understanding of the underlying myelin pathology of MS-related cognitive impairment. Myelin damage in white matter is likely playing a significant role. This finding is quite informative in light of the fact that cognition has been primarily investigated as a cortical function; an axiom that has been critically termed "corticocentric myopia".[128] A variety of injuries (e.g., leucotoxic, vascular, traumatic, hydrocephalic, genetic) can lead to a substantial burden of white matter pathology and cognitive dysfunction.[129] This entity has been termed white matter dementia[209] and shares several features with MS-related cognitive impairment: cognitive slowing, memory retrieval deficits and executive dysfunction.[129] This is in contrast to cortical dementias, which are dominated by amnesia, aphasia, apraxia and agnosia.[129]

Identifying the accurate underlying pathology is likely necessary to developing successful therapeutic approaches. A prime example of the disadvantages of corticocentric bias is the dominant cortical amyloid hypothesis in Alzheimer's disease (AD).[210] However, all efforts to treat AD by targeting brain amyloid have been unsuccessful thus far, suggesting amyloid may not be the appropriate target.[211] There is evidence supporting the "myelin model of AD", which proposes that amyloid and tau are by-products of homeostatic myelin repair processes.[212,213] Should the

myelin model of AD prove to have merit, myelin preservation as a therapeutic target could have profound implications for AD.[129] Similarly, it is well established that grey matter neurodegeneration (cortical and subcortical) contributes to cognitive impairment in MS.[214] Yet, it is unclear to what extent white matter demyelination drives grey matter atrophy via retrograde degeneration versus separate processes causing damage to white matter and grey matter independently.[215] Should white matter demyelination be the driving factor, therapeutic myelin preservation and remyelination may be key to safeguarding cognitive function from the effects of both white and grey matter damage. Results from recent clinical trials demonstrate that treatment with siponimod can maintain[216] or improve[217] processing speed function in SPMS patients, which may be associated with its pro-myelinating effects.[218] Further, MS patients with better remyelination profiles, as determined by positron emission tomography (PET), are more likely to have preserved thalamic volumes than patients with poor remyelination profiles.[219] This observation was taken as evidence of the neuroprotective function of remyelination.[219]

The current work also lends further support for the application of MWF as a myelin specific biomarker useful for monitoring changes in myelination and its relationship to worsening or improvement of cognitive symptoms. This is of particular importance as the MS treatment landscape moves towards remyelination therapies,[220,221] for which a quantitative, myelin specific, *in vivo* biomarker has been identified as an unmet need.[220] Thus, MWI meets the first step in FDA biomarker qualification, which is establishing an unmet drug development and medical need that may be addressed with the proposed biomarker.[222] The second step is identifying the biomarker's context of use. With the current level of evidence, MWI can be used as a monitoring biomarker. In fact, MWF has been previously used in this context to investigate remyelination and neuroprotection associated with alemtuzumab treatment.[223] Longitudinal analysis would be

required to establish MWF as surrogate endpoint (e.g., are changes in MWF associated with changes in cognitive status?). Longitudinal analysis would also be required to establish MWF as a predictive biomarker to investigate whether MWF values at a baseline time point were able to predict cognitive status at a follow up time point.

5.2 Strengths

The current work investigating the role of myelin damage and cognition in MS is strengthened by the application of MWF, which has been histologically validated as a specific marker for myelin using post-mortem human tissue[112,155] and animal models of myelin damage.[114] We investigated several cognitive domains and employed 3 of the most sensitive tests currently available for cognitive monitoring in MS: The SDMT (processing speed), SRT (verbal memory) and BVMT-R (visuospatial memory),[61] with the addition of the COWAT as a measure of verbal fluency/word retrieval. Though the current work is cross-sectional, we recruited a patient sample that included both relapsing-remitting and progressive MS phenotypes in order to represent a wide range of the MS population. In chapter 4, we had the advantage of a study specific template. Registration accuracy was vital for this study to draw appropriate conclusions regarding the anatomical location of myelin damage and its relationship to cognitive performance. The similarity metrics that guide image registration rely on correspondence between the study data and the template to which it is registered. A study specific template (generated from image data with similar image field-of-view, resolution, contrast, etc) provides improved anatomical alignment between each subject and the template.

5.3 Limitations

The study was limited to one hospital site, using a single scanner. This might limit the precision of power calculations generated from our results for multi-center and multi-vender trials. However, our results substantiate the use of MWI to quantify MS-related demyelination and its association with cognitive performance. This would be feasible for multicenter studies as MWI has excellent inter-site[156] and inter-vender[187] reproducibility. What's more, the use of a single scanner ensures our results reflect the true variability in our sample rather than variability due to scanner, site and image parameter differences.

While MWF demonstrates strong quantitative correspondence with myelin histology,[8] there are some potential confounding factors that warrant mentioning. Analysis of GRASE data with the T_2 decay curve approach does not account for the exchange of water between the myelin bilayers and inter/extracellular fluid. This approach assumes that water molecules remain in the myelin bilayers long enough to be captured during the T_2 decay time of myelin water. Studies of rodent spine suggest that movement of water from the myelin bilayers can occur at fast enough rates to artificially lower the MWF.[224,225] On the contrary, investigations in bovine brain and optic nerve conclude that exchange does not exert a large role on MWF measurements.[103,226] The effect of exchange on MWF measurements in humans is not well understood.

Myelin damage results in myelin debris, which is present until macrophages clear the debris from the site of injury.[227] Animal studies show that MWI is unable to distinguish intact myelin from myelin debris.[114,228,229] The time scale of myelin debris clearance in humans is unknown,[8] therefore, myelin debris may not be a relevant factor in MWF measurements in humans. However, the potential for the presence of myelin debris following recent demyelination to artificially increase MWF in humans should be acknowledged.

It should be recognized that the denominator of the MWF is total water, therefore, increases in edema and inflammation can influence changes in MWF. However, if the MWF decreases in MS reported in the literature were due to only edema rather than myelin loss, dilution of the MWF from edema would require such significant swelling in the brain that it would result in a lethal increase in intracranial pressure.[230] In addition, it has been shown that changes in water content have minimal effect on changes in MWF.[231]

There are additional factors that may contribute to variability in the data that need to be recognized. The first being compensatory functional reorganization.[232] Participants with MS may recruit ancillary neural resources to maintain cognitive performance.[232] This may result in MS patients with significant structural damage who still perform well on cognitive tasks, which would weaken the relationship between the two variables. Similarly, patients whose premorbid cognitive status was well above the 50th percentile may experience a significant decrease in cognitive performance as well as myelin while their current cognitive scores would still be considered within normal range.

Though the current work included cognitive tests deemed to be the most sensitive for detecting deficits in MS, we administered these tests under conditions that are designed to minimize destractions.[61] Monotasking under ideal testing conditions likely doesn't capture patient-reported real-word deficits. Difficulties with the everyday demands of multitasking (e.g., walking or preparing dinner while holding a conversation) are often reported by MS patients.[233] Correspondingly, they perform worse than controls during dual task performance (walking while performing a cognitive task)[234] and under noisy test conditions.[235] Dual task paradigms would likely be more sensitive to patient reported real-world deficits. However, they have yet to be validated for use in MS.[61]

5.4 Future directions

An important next step would be to determine the predictive value of baseline MWF for future cognitive decline. To this end, our lab is currently collecting follow up data 2 years after the baseline examination for the participants included in this thesis. Predicting cognitive decline before symptoms emerge is necessary for early intervention.[236] However, it could also be argued that cognitive interventions should be initiated at diagnosis as cognitive decline may begin at disease onset[237] and may even be apparent prior to MS diagnosis.[238]

It would be beneficial to replicate our findings in an independent population to increase confidence regarding future clinical utility. The ability to use mean MWF in cognitive domain-specific white matter regions as a surrogate marker for cognitive impairment would be a meaningful clinical advancement; particularly for patients with cognitive complaints whose premorbid cognitive status was well above the 50th percentile. These patients may perform within normal range on cognitive tests despite impactful cognitive decline and legitimate cognitive concerns. Their self-reported cognitive decline could be substantiated with low MWF regardless of normal cognitive scores. Imaging measures are also more stable than cognitive scores, which can vary depending on time of day, level of fatigue, recent relapse, etc. Therefore, a clinically meaningful change in MWF may be detectible sooner than a change in cognitive score. The newest techniques have reduced full-brain MWI scan time to less than 5 minutes,[239] rendering it ever more clinically feasible.

It would be informative to tease out the variability in cognitive scores explained by MWF and other previously associated variables, such as cortical and deep grey matter atrophy.[214] For one, this knowledge would assist in building more complete predictive models of cognitive decline. Further, it is important to determine the relative contributions of each tissue type for future

therapeutic approaches. There is evidence that white matter demyelination and axonal transection leads to grey matter atrophy via retrograde degeneration.[215] Should it be the case that white matter demyelination is the proceeding factor to grey matter damage, therapies targeted to myelin preservation and remyelination alone may protect cognition. Evidence also exists for the theory that white matter and grey matter demyelination are two, at least partially independent processes, and white matter damage alone does not cause neuronal loss.[215] If this alternate theory is accurate, a combination therapy approach would be required.

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Appendix: Data Table

Participant type	Age (years)	Sex (0:M; 1:F)	Education (years)	Disease duration (years)	EDSS score
Control	29.00	1	16	N/A	N/A
Control	27.00	1	14	N/A	N/A
Control	39.00	1	14	N/A	N/A
Control	31.00	1	18	N/A	N/A
Control	28.00	1	18	N/A	N/A
Control	35.00	0	18	N/A	N/A
Control	39.00	1	18	N/A	N/A
Control	55.00	1	16	N/A	N/A
Control	55.00	1	16	N/A	N/A
Control	63.00	1	14	N/A	N/A
Control	44.00	0	14	N/A	N/A
Control	60.00	1	18	N/A	N/A
Control	35.00	0	16	N/A	N/A
Control	52.00	0	14	N/A	N/A
Control	28.00	0	12	N/A	N/A
Control	41.00	0	16	N/A	N/A
Control	54.00	0	22	N/A	N/A
Control	60.00	1	16	N/A	N/A
Control	64.00	1	12	N/A	N/A
Control	65.00	0	18	N/A	N/A
Control	57.00	1	12	N/A	N/A
Control	60.00	1	16	N/A	N/A
MS	39.00	0	14	5.000	2.50
MS	57.00	1	18	42.000	7.50
MS	26.00	1	16	3.000	2.50
MS	37.00	1	16	7.000	4.00
MS	51.00	1	16	24.000	6.50
MS	64.00	1	16	29.000	8.50
MS	64.00	0	18	42.000	4.00
MS	60.00	1	16	21.000	6.00
MS	49.00	1	16	5.000	1.00
MS	31.00	0	14	1.000	3.00
MS	55.00	1	12	1.000	2.00
MS	34.00	0	14	0.300	1.50
MS	56.00	0	12	9.000	4.00
MS	45.00	1	12	11.000	1.50

MS	57.00	1	14	35.000	3.50
MS	36.00	0	19	1.000	2.00
MS	51.00	0	14	30.000	4.50
MS	65.00	1	14	15.000	3.50
MS	58.00	0	12	38.000	2.50
MS	56.00	0	16	8.000	2.00
MS	42.00	1	16	4.500	2.00
MS	55.00	1	16	16.000	3.50
MS	64.00	1	12	27.000	4.00
MS	50.00	1	14	8.000	2.50
MS	61.00	1	14	9.000	3.00
MS	62.00	1	12	20.000	6.00
MS	48.00	1	12	14.000	5.50
MS	53.00	1	16	11.000	4.00
MS	57.00	1	12	10.000	N/A
MS	60.00	0	12	13.000	5.00
MS	44.00	1	14	8.000	4.00
MS	60.00	0	16	12.000	N/A
MS	58.00	1	14	15.000	3.50
MS	47.00	1	14	6.000	N/A
MS	61.00	1	22	37.000	6.50
MS	27.00	0	16	1.000	N/A
MS	61.00	1	14	4.000	2.50
MS	57.00	0	12	17.000	4.00
MS	37.00	1	14	8.000	2.50
MS	55.00	1	18	18.000	3.00
MS	29.00	1	16	10.000	2.00
MS	31.00	1	16	12.000	1.00
MS	58.00	0	12	12.000	2.50
MS	57.00	0	14	28.000	4.00
MS	63.00	1	12	34.000	6.50
MS	60.00	1	14	43.000	3.50
MS	54.00	1	16	28.000	6.50
MS	47.00	1	14	1.600	2.00
MS	55.00	1	12	22.000	6.00
MS	58.00	0	14	25.000	2.00
MS	52.00	0	12	10.200	3.50
MS	63.00	0	16	33.000	4.00
MS	51.00	1	14	1.000	2.00
MS	31.00	0	16	1.000	2.50
MS	50.00	1	22	12.000	3.00

MS	26.00	1	16	12.750	1.50
MS	54.00	1	14	25.000	4.00
MS	57.00	1	12	12.000	3.00
MS	43.00	1	12	18.000	N/A
MS	53.00	1	16	27.000	3.50
MS	58.00	0	14	23.500	6.00
MS	43.00	1	16	14.000	3.00
MS	59.00	1	12	48.000	N/A
MS	37.00	1	16	5.000	N/A
MS	47.00	1	16	2.000	2.00
MS	40.00	0	14	5.000	2.00
MS	59.00	0	14	28.000	5.50
MS	49.00	1	12	2.500	3.50
MS	56.00	0	14	16.000	N/A
MS	44.00	0	14	5.000	2.50
MS	48.00	0	18	13.000	N/A
MS	54.00	1	16	13.000	3.50
MS	29.00	1	16	4.000	N/A