Understanding cognitive dysfunction in secondary progressive multiple sclerosis using functional and structural MRI

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Declaration

I, Anisha Doshi, confirm that the work presented in this thesis is my own. Where information has been derived from other sources, I confirm that this has been indicated in the thesis.

The Multiple Sclerosis Secondary Progressive Multi-arm Randomisation Trial (MS-SMART) trial team, led by Professor Jeremy Chataway, included several clinical research fellows who assisted with the recruitment and consent of secondary progressive multiple sclerosis (SPMS) subjects in this thesis study. Dr Floriana De Angelis undertook these tasks from the onset of the study in September 2015 until follow-up timepoint, consenting 40% of subjects. Dr Domenico Plantone assisted from September 2015 to April 2016, consenting 40% of subjects. Professor Chataway consented the remaining 20% of subjects. I independently developed the study hypotheses, methodology including the batteries of tests used, and undertook all of the baseline visits, and scoring and interpretation of all assessments. Dr Nevin John, joined the team in April 2017, and contributed to 60% of the follow-up timepoint clinical assessments for the SPMS group until April 2018, whilst I was on maternity leave. The Research Nurses Tiggy Beyene, Vanessa Bassan, Laura Brockway, and Alvin Zapata aided with subject retention in the study and patient reminders of appointments.

Dr Nils Muhlert, Lecturer in Cognitive Neuroscience at the University of Manchester, provided mentorship, and guidance for the thesis study, particularly with regard to my understanding of cognitive outcomes and their interplay with MRI metrics for the study. Dr Dan Altmann (retired 2019), and associate Professor Jennifer Nicholas, at the London School of Hygiene and Tropical Medicine provided guidance for the statistical analysis in **chapters 4 to 6**. Drs Adnan Alahmadi and Gloria Castellazzi provided training for the rs-fMRI methodology in **chapters 5 and 6**. I undertook the rs-fMRI analysis pipeline and processed the results independently. Drs Arman Eshaghi and Ferran Prados advised on, and Mr Jonathan Stutters completed the image segmentation and parcellation pipeline used in **chapters 5 and 6**.

The MS-SMART trial, alongside which SPMS subjects in this study were recruited, this was designed by the UK Multiple Sclerosis Society Clinical Trials Network and led by Professor Jeremy Chataway as the Chief investigator of the study. The trial co-applicants were: Professor David H. Miller (later emeritus), Professor Sue H. Pavitt, Professor Gavin Giovannoni, Professor Claudia Gandini Wheeler-Kingshott, Professor Clive Hawkins, Professor Basil Sharrack, Mr Roger Bastow, Professor Christopher J Weir, Professor Nigel Stallard, and Professor Siddharthan Chandran. The MS-SMART trial was a project funded by the Efficacy and Mechanism Evaluation (EME) Programme, a Medical Research Council (MRC) and National Institute for Health Research (NIHR) partnership. It was also supported by the UK Multiple Sclerosis Society, the NIHR University College London Hospitals Biomedical Research Centre and University College London (UCL). In September 2015, I joined the trials team at the UCL site (n=176) as a clinical research associate, and contributed to MS-SMART with telephone screening (40%) and data collection (70%) by performing the MS-SMART clinical assessments of the enrolled SPMS patients (i.e. baseline, week 48 and week 96 visits), baseline T2 lesion filling (30%), and analysing the number of persistent new T1 lesions on MRI at follow-up (100%) for the exploratory outcome.

Abstract

This thesis concerns a 2 year follow-up study of people with secondary progressive multiple sclerosis (SPMS). I investigate: (1) cognitive performance of SPMS and changes over time, (2) the classification of cognitive impairment and predictors of this, (3) mechanisms underlying the SPMS phenotype with and without cognitive impairment using functional and structural MRI.

The literature has highlighted the input of executive dysfunction in the cognitive profile of SPMS over and above that seen in other multiple sclerosis (MS) phenotypes. I looked at cognitive performance in SPMS, and predictors of this in this pure SPMS cohort study. I found that being employed, having higher IQ, more premorbid leisure interests, and higher qualifications mitigate against negative cognitive outcomes in SPMS. Additionally, anxiety, even when not reaching clinically diagnostic levels, impacts on tests of information processing speed, verbal working memory, and executive function in SPMS. The symbol digit modality test (SDMT) at baseline is predicted by MS lower limb disability outcome measures; the Expanded Disability Status Scale (EDSS) and timed 25 foot walk (T25FW) which emphasises the role of the SDMT as an adjunctive measure of clinical disability prediction in studies. I show that decline on the SDMT at follow-up is purely predicted by cognitive measures of information processing speed and working memory at either timepoint, supporting, and furthering, the evidence for the SDMT as a sentinel assessment of cognitive performance in SPMS. These findings inform future longitudinal cognitive studies in SPMS, particularly with regards to the importance of tests of executive function, and important associations with clinical outcomes in a highly disabled cohort.

I also considered the threshold for classifying cognitive impairment, and its implications. There is marked heterogeneity in these thresholds due to the lack of current consensus on a diagnostic criteria. Using a higher threshold for cognitive impairment in my studies strengthened the associations with clinically relevant outcomes. Additionally, unemployment showed the greatest association with cognitive impairment regardless of criteria used. I found that assessments of

information processing speed, verbal memory, and executive function had the greatest input to cognitive impairment in SPMS. These findings indicate the importance of these cognitive domains and demographic factors when evaluating cognitive status in SPMS. These results will guide the international consensus on how best to measure cognitive impairment in SPMS, and in MS more broadly.

Posterior and deep resting state networks (RSNs) have been shown to be altered in resting state functional MRI (rs-fMRI) studies of progressive MS phenotypes. I confirm this using functional connectivity (FC) and highlight that this is mainly in terms of cognitive RSNs in SPMS versus healthy controls using a global rs-fMRI analysis technique. Additionally, with cognitive impairment in SPMS, I show that there are key attentional RSN FC reductions. I further highlight the importance of more stringent classification criteria of cognitive impairment to allow for more detailed evaluation of dynamic FC changes, that are missed when using a lenient criteria. Over time, the development of cognitive impairment in SPMS from a preserved state appears to relate to reduced FC in working memory, posterior default mode (DMN) and visual RSNs, and increased FC in the executive control, and more anterior DMN hubs at baseline. Therefore, alterations in posterior cognitive and executive RSNs may inform cognitive status in SPMS. These results provide, to my knowledge, the first longitudinal rs-fMRI study of cognitive status in SPMS.

Regional grey matter atrophy has been shown to be greater in SPMS then in other MS phenotypes. I found that SPMS cognitive impairment is associated with grey matter volume, cortical grey matter volume, and deep grey matter and regional deep grey matter atrophy. I also highlighted that proportionally, within the cerebellum, there are greater percentage changes in FC versus volume in those with SPMS with cognitive impairment versus in SPMS overall. These findings therefore show the importance of deeper grey matter atrophy in SPMS underlying cognitive impairment, and indicate the need for a longitudinal study of rs-fMRI and regional grey matter MRI metrics to understand the interplay of underlying mechanisms in more detail.

Acknowledgements

This research would not have been possible without the dedication and support of the people who took part. I appreciate the distances travelled, and commitment of everyone involved who gave up their time to take part and gave me inspiration for this study.

I would like to thank my supervisors. Professor Jeremy Chataway was supportive of my hypothesis and desire to undertake a project looking at cognition and imaging in SPMS. He has been a source of constant encouragement during my research period, clinical career and in my personal journey of becoming a parent during this project. Professor Olga Ciccarelli has provided so much motivation and energy towards my research. I felt enthused after every meeting with her. Both have also given me influential clinical teaching and supervision during my time as a clinical research associate at the National Hospital for Neurology and Neurosurgery.

I am thankful to the Mark Williams Foundation for their funding of this study. Professor Chataway would also like to add his personal thanks to the family of Mark and his memory. This was alongside that provided by the MS-SMART study funded by the Efficacy and Mechanism Evaluation (EME) Programme, a Medical Research Council (MRC) and National Institute for Health Research (NIHR) partnership, the UK Multiple Sclerosis Society, the NIHR University College London Hospitals Biomedical Research Centre and University College London.

The Queen Square MS Centre team have made this work possible. The physics team, led by Professor Claudia Gandini Wheeler-Kingshott were essential to the development of the MRI techniques and pipelines used in this study. In particular, I would like to acknowledge the postdoctoral students; Adnan Alahmadi and Gloria Castellazzi who guided me in terms of the rs-fMRI acquisition protocol, analysis, and post-processing (**chapters 5-6**). Professor Frederik Barkhof gave further motivation and guidance for the rs-fMRI project. The support and expertise of Jonathon Stutters, Arman Eshaghi, and Ferran Prados were integral to the structural MRI

atrophy analysis, pipeline, and tissue segmentation work (**chapters 5-6**). David McManus and his team provided training and support for the T2 lesion load evaluation using lesion filling at baseline and follow-up (**chapters 5-6**).

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External to the department, I received guidance for my statistical methodology from Professor Dan Altmann who retired in Autumn 2019, and Associate-Professor Jenny Nicholas at the London School of Hygiene and Tropical Medicine, to whom I am indebted. They took time to support my use of Stata and had patience in answering my questions. Mr David Blundred has kindly mentored my interest in higher education and supported my successful application for the AFHEA this year.

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I would like to thank my husband, Angad, for his patience, critical review, and constant source of encouragement and support throughout this work. To our daughter Ava, I cannot thank you enough for your smiles and laughter which have given me joy and motivated me to progress with this project. I do not think there are many 3 year olds who know what a chapter is, and you

celebrated each time I ticked one of the list. To our unborn son, you have given me more inspiration towards this study than you realise, and I thank you for this. I would especially like to thank my mother for her unwavering support with this academic work, there has been no task too large to help us with, particularly over the last few months. To our siblings, nephews, and my parents-in-law, I thank you for the visits, food, and encouragement with the write up.

Finally, I would like to thank my father who does not know how much inspiration he has given me. He had a persistent drive and energy to support research into MS, despite having disabling SPMS, and this will always inspire me.

Impact Statement

The work in my PhD has contributed both to academic and non-academic work into SPMS, a chronic neurological condition with no current cure.

Academically, the current understanding and definition of cognitive function in MS and the measurement are very variable. This work provides a large cohort study of SPMS patients, and adds to the knowledge of significant executive dysfunction in SPMS than in other MS phenotypes. This study also suggests that more stringent grouped criteria are required to understand cognitive status in SPMS. The implication of these findings are that they can guide future cognitive batteries used to study SPMS and cognition in the literature and also to better define when a person with SPMS is cognitively impaired or not. This is necessary to accelerate the discovery of underlying pathological substrates and pathways using MRI metrics. This study shows that in SPMS overall there is increased FC in attentional resting state networks, but FC reduces here when cognitive impairment develops. This is vital to furthering the use and understanding of multi-modal MRI techniques in the diagnosis and management of SPMS cognition.

Outside academia, there has been a lack of urgency to discuss the topic of cognition in the MS community. I have highlighted that there is a culture of greater unemployment in those with SPMS who are cognitively impaired and that this has a causative association. This suggests a need to develop more public health and social support for this in a young adult population for health economic benefits. In terms of individuals, the topic of cognition and SPMS has been of interest to those with SPMS and I have undertaken social engagement in terms of discussing this topic via blog posts for the MS Society UK, a radio podcast for RealMS, talks for occupational medicine consultants who support healthcare workers with health conditions, general neurologists, and a Facebook live event. I am publishing the results of my work in scholarly journals.

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Abbreviations

β	Beta
25[OH]D	25-hydroxyvitamin D
2D	Two dimensional
3D	Three dimensional
9HPT	Nine-hole peg test
ACC	Anterior cingulate cortex
ACE-R	Addenbrooke's Cognitive Examination-Revised
ADEM	Acute Disseminated Encephalomyelitis
aDMN	Anterior default mode network
AHSCT	Autologous haemopoietic stem cell transplantation
AN	Auditory network
ANTS	Advanced normalisation tool software
ASCEND	Effect of natalizumab on disease progression in secondary progressive multiple sclerosis trial
ASIC	Acid-sensing ion channel
ATP	Adenosine triphosphate
AUROC	Area under the receiver operator curve
BBSI	Brain boundary shift integral
BDI-II	Beck's Depression Index
BET	Brain extraction tool
BGN	Basal ganglia network
BICAMS	Brief International Cognitive Assessment for Multiple Sclerosis
BJLO	Benton Judgement of Line Orientation Test
BOLD	Blood oxygen level dependent
BPI	Brief Pain Index
BRNB	Brief Repeatable Neuropsychological Battery
Ca^{2+}	Calcium
CBF	Cerebral blood flow
CBLN	Cerebellar network
CBV	Cerebral blood volume
CDP	Confirmed disability progression
CGM	Cortical grey matter
Chi^2	Chi squared
CI	Cognitively impairment
CI	Confidence interval
CIS	Clinically isolated syndrome
CLQ	Cognitive leisure questionnaire
$CMRO_2$	Cerebral metabolic rate of oxygen consumption
CMV	Cytomegalovirus
CNS	Central Nervous System
COGEx	Improving Cognition in People With Progressive Multiple Sclerosis Using Aerobic Exercise and Cognitive Rehabilitation study
COGIMUS	COGnitive Impairment in MUltiple Sclero-Sis trial
COPOUSEP	Oral versus intravenous high-dose methylprednisolone for treatment of relapses in patients with multiple sclerosis trial
COWAT	Controlled Oral Words Association Test
CP	Cognitively preserve

CRAMMS	Cognitive Rehabilitation for Attention and Memory in people with Multiple Sclerosis study
CSF	Cerebrospinal fluid
CVLT-II	California Verbal Learning Test version 2
DGM	Deep grey matter
DIS	Dissemination in space
DIT	Dissemination in time
DKEFS	Delis-Kaplan Executive Function System Sorting Test
DMT	Disease modifying treatment
DNA	Deoxyribonucleic acid
EAE	Experimental autoimmune encephalomyelitis
EBV	Epstein-Barr Virus
ECM	Eigenvector Centrality Measure
EDSS	Expanded disability status scale
EMA	European Medicines agency
EME	Efficacy and Mechanism Evaluation
EN	Executive network
EPI	Echo planar imaging
EQ-5D	Health-state questionnaire
EXPAND	Exploring the Efficacy and Safety of Siponimod in People with SPMS trial
FAB	Frontal Assessment Battery
FC	Functional connectivity
FCN	Frontal cortex network
FDA	Food and Drug Administration
FLAIR	Fluid Attenuated Inversion Recovery
fMRI	functional MRI
FSL	FMRB Software Library
FWHM	Full-width-at-half-maximum
GIF	Geodesic Information Flow
GLM	General Linear Model
GM	Grey matter
GWAS	Genome-wide association studies
H-MRS	Proton magnetic resonance spectroscopy
HADS	Hospital Anxiety and Depression Scale
Hayling	Hayling Sentence Completion task
HC	Healthy control
HHV-6	Human herpes virus-6
HLA	Human leukocyte antigen
HRQoL	Health Related Quality of Life
IC	Independent component
ICA	Independent component analysis
IMPACT	International MS Secondary Progressive Avonex Controlled Trial
IQ	Intelligence Quotient
JIM	Jacobian Integration method
K^+	Potassium
LDST	Letter digit substitution test
LLT	Location Learning Test
LR	Likelihood ratio
LVAN	Left ventral attentional network

LVN	Lateral visual network
MACFIMS	Minimal Assessment of Cognitive Function In Multiple Sclerosis
MAMS	Multi-arm multi-stage
MELODIC	Multivariate Exploratory Linear Optimized Decomposition into Independent Components
MHC	Major histocompatibility complex
MND	Motor neurone disease
MNI	Montreal Neurological Institute
MR	Magnetic resonance
MRC	Medical Research Council
MRI	Magnetic resonance imaging
MS	Multiple Sclerosis
MS-SMART	Multiple Sclerosis Secondary Progressive Multi-arm Randomisation Trial
MS-STAT1	Clinical trial of simvastatin in patients with secondary progressive multiple sclerosis
MSFC	Multiple Sclerosis Functional Composite
MSIS29v2	Multiple Sclerosis Impact Scale v2
MSNQ	Multiple Sclerosis Neuropsychological Questionnaire
MSQoL-54	Multiple Sclerosis quality of life questionnaire
MSVQ-7	Multiple Sclerosis Vision Questionnaire
MSWSv2	Multiple Sclerosis Walking Scale v2
MTR	Magnetisation transfer ratio
MVN	Medial visual network
Na^+	Sodium
NART	National Adult Reading Test
NAWM	Normal appearing white matter
NBV	Normalised brain volume
NEDA	No evidence of disease activity
NFI	Neurological Fatigue Index
NGMF	Normalised grey matter fraction
NICE	National Institute of Clinical Excellence
NIHR	National Institute for Health Research
NMO	Neuromyelitis Optica
NMO-SD	Neuromyelitis Optica Spectrum disorders
NMR	Nuclear magnetic resonance
NO	Nitric oxide
NSBMS	Neuropsychological Screening Battery for MS
OCB	Oligoclonal bands
OCT	Optical coherence tomography
OR	Odds ratio
OVN	Occipital visual network
P-value	Exact probability value
PASAT	Paced Auditory Serial Additions Test
PASAT3	Paced Auditory Serial Additions Test 3 seconds
PBVC	Percentage brain volume change
PCC	Posterior cingulate cortex
PD	Proton Density
pDMN	Posterior default mode network
PET	Positron emission tomography

PN	Precuneus network
PPMS	Primary progressive multiple sclerosis
pRNFL	Peripapillary retinal nerve fibre layer
PROM	Patient Reported outcome measure
PST	Processing Speed Test
R^2	R-squared
RCI	Reliable change index
RCT	Randomised controlled trial
REC	Research Ethics Committee
RIS	Radiologically isolated syndrome
RIVITaLISe	Rituximab by Intravenous and Intrathecal Injection Versus Placebo in Patients with Low-Inflammatory Secondary Progressive Multiple Sclerosis
ROC	Receiver operator curve
ROCF	Rey-Osterrieth Complex Figure
ROI	Region of interest
ROS	Reactive oxygen species
RQF	Regulated Qualifications Framework
RR	Relative risk
RRMS	Relapsing remitting multiple sclerosis
Rs-fMRI	Resting-state functional MRI
RSN	Resting state network
RVAN	Right ventral attentional network
S1P	Sphingosine 1-phosphate
SD	Standard deviation
SDMT	Symbol digit modalities test
SELs	Slowly evolving lesion
SF-36	Short form 36
SIENA	Structural Image Evaluation, using Normalization of Atrophy
SIENAX	Cross-sectional Structural Image Evaluation, using Normalization of Atrophy
SMN_M2	Sensorimotor network M2
SMN_S2a	Sensorimotor network S2a
SMN_S2b	Sensorimotor network S2b
SN	Salience network
sNfL	Serum neurofilament light
SNR	Signal to noise ratio
SPART	Spatial Recall Test
SPM	Statistical parametric mapping
SPMS	Secondary Progressive Multiple Sclerosis
SPRINT-MS	Safety, Tolerability and Activity Study of Ibudilast in PwPMS
SRT	Selective Reminding Task
SST	Short story test
Stroop	Stroop Colour-Word interference task
T	Tesla
T1	T1-weighted
T2	T2-weighted
T2*	T2 star
T25FW	Timed 25-foot Walk
T2LL	T2 lesion load
T2LV	T2 lesion volume

TE	Variable echo delay time
TEA	Test of Everyday Attention
TFCE	Threshold-free cluster enhancement
TIV	Total intracranial volume
TMT-B	Trail making test-B
TR	Repetition time
VAF	Visual analogue of fatigue
VBM	Voxel based morphometry
VF	Verbal Fluency
VFT	Verbal Fluency Test
WBV	Whole brain volume
WCST	Wisconsin card sorting test
WLG	Word list generation
WLT	Word learning test
WM	White matter
WMN (LNp)	Working memory network

Publications associated with this thesis

Solanky BS, John NA, DeAngelis F, Doshi A…, Barkhof F, Gandini Wheeler-Kingshott CAM, Chataway J and for the MS-SMART Investigators. NAA is a Marker of Disability in Secondary-Progressive MS: A Proton MR Spectroscopic Imaging Study. AJNR Am J Neuroradiol 41:2209–18 Dec 2020.

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1 Overview of Multiple Sclerosis and cognitive impairment

1.1 Introduction

This thesis is based on a 2-year clinical, functional MRI, and structural MRI imaging longitudinal follow-up study of cognition in secondary progressive multiple sclerosis (SPMS). The first 3 chapters of my thesis provide information on; multiple sclerosis (MS) and specifically SPMS, cognition in MS, and a background summary of the quantitative and functional MRI methodologies used in this study including their role in cognition in MS. As is shown in the background chapters; there is clearly need for more clarity about the cognitive profile of SPMS and how we define and investigate this using MRI (Sumowski *et al.*, 2018). The main experimental body of this thesis focuses on cognitive performance in SPMS at baseline and changes over time, using clinical metrics and MRI modalities; resting state functional MRI (rs-fMRI), and grey matter regional atrophy. The rationale of these experiments was to add to the literature in terms of the phenotype of SPMS cognitive function, and to provide suggestions of suitable outcomes for use in the clinical and research setting for measuring potential therapeutic measures (Benedict *et al.*, 2020).

1.2 Aims of this thesis

Chapter 4 aims to define the cognitive profile of SPMS versus healthy controls specifically and investigate what predicts this. In SPMS, the literature suggests executive deficits, in addition to the working memory and information processing speed impairment known about in all MS phenotypes. To investigate this further I chose a battery of tests, including tests for this domain. Adding to the cross-sectional snapshot of cognitive function in SPMS, I investigated changes over time. The final section of this chapter looks at cognitive impairment in SPMS. How we define and characterise cognitive impairment in MS is not uniform currently, and so I explore two key classification strategies for this in this SPMS cohort. The benefit of a longitudinal cohort study is that it allows study of the development of cognitive impairment in those preserved at baseline.

The final aim of this chapter is therefore to look at independent predictors of the development of cognitive impairment from a preserved state using the two earlier defined criteria.

Rs-fMRI allows a window into resting brain function and so provides an ideal tool for investigating conditions where active MRI modalities could be impacted upon by physical disability. **Chapter 5** of this thesis aims to investigate the rs-fMRI changes of SPMS in a large cohort versus healthy controls and how these change over time. Thereafter, in the second section, I looked at how FC changes according to the classification of cognitive impairment in SPMS using the two composite criteria defined in **chapter 4**. In the final part of this chapter I aim to investigate FC alterations in those developing cognitive impairment using the most dynamic criteria from a preserved state at baseline.

The final results chapter, **chapter 6**, looks at the relationship of regional grey matter atrophy and cognitive status in SPMS in terms of group differences. I also investigate predictors of cognitive impairment at the follow-up timepoint in terms of core GM volumes and atrophy metrics. In particular there is a focus on a posterior anatomical structure shown to be relevant in terms of rs-fMRI FC changes relating to cognitive impairment; the cerebellum, and proportional comparisons of volume and FC measures in SPMS overall and with cognitive impairment.

To summarise, the major aims of this thesis are:

1. To explore the cognitive phenotype of SPMS and the classification thresholds and development of cognitive impairment cross-sectionally and over time (**chapter 4**).
2. To review global resting FC changes in SPMS, and SPMS with cognitive impairment cross-sectionally and over time, as well as in those developing cognitive impairment from a preserved state (**chapter 5**).
3. The associations of regional grey matter volume and atrophy metrics with cognitive status in SPMS (**chapter 6**).

1.3 Multiple Sclerosis

Multiple Sclerosis (MS) is a chronic neurological disease predominantly of young adults (Filippi *et al.*, 2018, Thompson *et al.*, 2018*b*; Dobson and Giovannoni, 2019), affecting approximately 2.3 million people worldwide (Wallin *et al.*, 2019*b*). It is thought to have immune-mediated aetiology which causes both inflammation and neurodegeneration (Lassmann *et al.*, 2007; Mahad *et al.*, 2015) and has been shown to correlate with disability outcome measures (Absinta *et al.*, 2019). The mechanisms by which this occurs, and the interplay of factors underlying these pathological processes is not yet certain. It is thought that inflammation is driven by the influx of lymphocytes and microglial activation. This is followed by the development of compartmentalised inflammation with less blood brain barrier disruption. These lymphoid aggregates may lead to more cortical or grey matter inflammation (Howell *et al.*, 2011). A recent pathological study has highlighted a specific subpial ribbon-like demyelination pattern in MS that is not present in other demyelinating central nervous system (CNS) diseases such as Neuromyelitis Optica Spectrum disorders (NMO-SD) or Acute Disseminated Encephalomyelitis (ADEM) (Junker *et al.*, 2020). Further demyelination of axons and redistribution of sodium channels leads to the neurodegeneration of chronically demyelinated axons, which dominates progressive MS. MS affects the entire central nervous system (CNS) and results in multiple pathognomonic lesions in the white matter (Frischer *et al.*, 2015; Kuhlmann *et al.*, 2017). Additionally, normal appearing white and grey matter has been shown to be affected by MS pathology. These changes result in a heterogeneous array of cognitive and locomotor symptoms (Compston and Coles, 2008; Sumowski *et al.*, 2018). Up to 70% of those with MS are unemployed with half being due to factors related to the disease itself (Mitchell *et al.*, 2005; Langdon, 2011)

There is up to a 40% 5-year conversion rate to SPMS from early, or relapsing remitting MS (RRMS) in those not treated with disease-modifying treatment (DMT) (Brown *et al.*, 2019). Those treated had lower hazards of conversion to SPMS than untreated patients. With glatiramer acetate or interferon beta the 5-year absolute risk of SPMS was 12% versus 27% in untreated respectively; fingolimod 7% versus 32%; natalizumab 19% versus 38%; alemtuzumab 10%

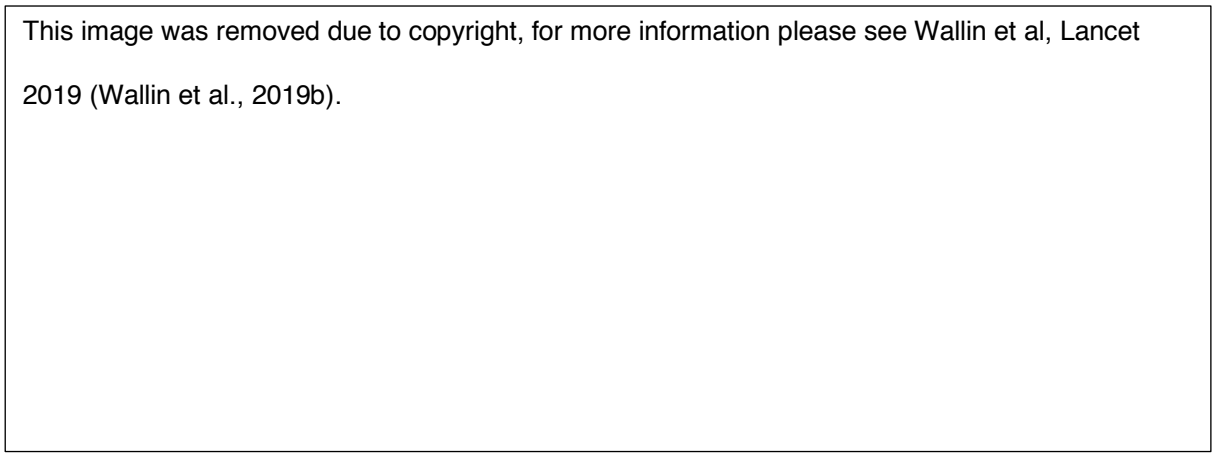
versus 25%. Lower conversion rates were seen in those treated with higher efficacy DMT and within 5-years of disease onset (Brown et al., 2019). 10-15% of people with MS present with a progressive course from onset with slowly accumulating symptoms and signs, known as primary progressive MS (PPMS). It is estimated that about 75,000 people have progressive MS (SPMS and PPMS) in the UK costing £3 billion per annum (McCrone *et al.*, 2008). Siponimod has been licensed in the USA as the first disease modifying therapy (DMT) for SPMS and is being reviewed by NICE in the UK, due to a 21% reduction in 3-month confirmed disability progression (Kappos *et al.*, 2018). Ocrelizumab is licensed for use in PPMS.

1.3.1 Epidemiology

In 1868 Charcot first described patients with signs of a demyelinating nervous system disease likely to have been MS (Charcot, 1885). However, almost 30 years earlier, Robert Carswell (1793–1857), a British professor of pathology, and Jean Cruveilhier (1791–1873), a French professor of pathologic anatomy had already described many of the clinical details of MS, but did not identify it as a separate disease. Despite this, it was probably not until the mid-20th century that MS became widely-known as a discrete diagnosis (McAlpine *et al.*, 1955).

MS predominantly affects young adults, with the first symptoms of RRMS typically occurring between 20-35 years old, and in PPMS at approximately 40 years old (Compston and Coles, 2008; Filippi *et al.*, 2018, Thompson *et al.*, 2018*b*; Dobson and Giovannoni, 2019). However, the diagnosis of MS is often delayed by around 8 years on average in the UK (Thompson *et al.*, 2017). MS does occur in the paediatric setting, and worldwide, 3-10% of people with MS have their first presentation in childhood (Cappa *et al.*, 2017). Overall, the incidence of MS is higher in females than in males in RRMS with an approximate ratio of 2:1, but the ratio plateaus in PPMS to 1:1 (Orton *et al.*, 2006). In the UK there are 130,000 people with MS, with 286 women and 111 men per 100,000 people. 7,000 people are diagnosed with MS each year (Multiple Sclerosis Society UK, 2020).

Figure 1.1 Prevalence of multiple sclerosis globally standardised by age per 100,000 population.



The green arrow indicates a general reduction in prevalence with decreasing latitude. Adapted from the 2016 summary of the global burden of multiple sclerosis by Wallin et al, Lancet 2019 (Wallin et al., 2019b).

MS occurs worldwide, and had a prevalence of 2.2 million cases globally in 2016 (**Figure 1.1**). Since 1990 this is an increase of approximately 10% which may be partially due to a change in diagnostic and investigative criteria (Wallin *et al.*, 2019*b*). Globally, MS is most prevalent in north America (164.6 per 100,000) and western Europe (127 per 100,000); the prevalence is lowest in eastern sub-Saharan Africa (3.3 per 100,000) and Oceania (2.0 per 100,000) (Baranzini and Oksenberg, 2017, Wallin *et al.*, 2019*b*). Although this indicates a greater incidence gradient related to increasing latitude, there are pockets where the incidence of MS is unexpectedly high (**Figure 1.1**) (Wallin *et al.*, 2019*a*). These geographical differences likely underlie key differences in genetic and environmental factors. An example is south west Sardinia with a high overall incidence in females of 11.88 per 100,000 females and in males 4.32 per 100,000, but regional differences are also present here indicating additional environmental factors are at play (Cocco *et al.*, 2011). Caution is required when interpreting these epidemiological studies due to differences in completeness of case ascertainment relating to infrastructure availability.

There is an apparent increase in the frequency of MS with an increase in latitude (Kurtzke, 1995), but in the northern hemisphere, this gradient is not so apparent (Koch-Henriksen and Sorensen,

2011; Simpson *et al.*, 2011). Considerations have to be made in terms of diagnostic variations and ascertainment bias (Ascherio and Munger, 2016). There is likely to be an underlying genetic susceptibility with an interplay of environmental factors such as sunlight and vitamin D levels (Ghareghani *et al.*, 2018).

1.3.2 Aetiology

The underlying cause of MS is currently not known. The geographical patterns of MS distribution worldwide support the aetiology of MS being a combination of both genetic susceptibility and environmental factors. The gene loci found by genome-wide association studies (GWAS) suggests that there is a strong link to the immune system and overlap with other autoimmune disorders (Sawcer et al., 2012)(Baranzini and Oksenberg, 2017). Therefore MS is likely to develop in genetically predisposed individuals exposed to one or more environmental triggers (Ascherio and Munger, 2016).

Genetic factors

Familial studies have shown a concordance rate of 20-30% in monozygotic twins versus 2-5% in dizygotic twins (Kemppinen *et al.*, 2011; Baranzini and Oksenberg, 2017). In the early 1970s the discovery of the effects of certain human leukocyte antigen (HLA) genes, HLA-DRB1 in the class II region of the major histocompatibility complex (MHC), 6p21.3 explained some of the genetic susceptibility described in addition to the geographical tendencies (Kemppinen *et al.*, 2011; Sawcer *et al.*, 2012). The greatest risk HLA haplotype was further elicited to be the class II HLA-DRB1*1501-DQA1*0102-DQB1*0602 with a rs3135388 single nucleotide polymorphism (Kemppinen *et al.*, 2011). HLA genes play a role in antigen presentation for $CD4^+$ (HLA class II) and $CD8^+$ T lymphocytes (HLA class I) therefore having a direct role in the pathogenesis of MS. Overall, the class II HLA variant is a risk factor for MS (odds ratio (OR) 3), and the class I haplotype is protective (OR 0.6) (Olsson *et al.*, 2016).

The development of GWAS allowed the investigation of MHC, and non-MHC genetic loci for MS (Kemppinen *et al.*, 2011). Further fine mapping of GWAS signals has confirmed 103 discrete genetic loci outside of the MHC which describe some of the heritability of MS (International Multiple Sclerosis Genetics Consortium (IMSGC) *et al.*, 2013). Currently, GWAS has resulted in an increase in the number of potential susceptibility loci for MS, with over 200 gene loci variants identified outside of the MHC, further associations within the extended MHC, and one X chromosome variant (International Multiple Sclerosis Genetics Consortium (IMSGC) *et al.*, 2013; Baranzini and Oksenberg, 2017).

There is likely overlap of gene loci associations between MS and other autoimmune conditions confirming the role of autoimmunity in the aetiology of MS (Baranzini and Oksenberg, 2017). Multiple gene loci also support the hypothesis of a role of further genetic or environmental factors underlying MS (Isobe *et al.*, 2015). It is likely that there are common genetic loci for MS, but none of these have been shown to fully contribute via GWAS and so other factors must play a role.

Environmental factors

Studies completed in the 1970s showed that immigrants from the West Indies and east Asia, where there is a lower risk of MS development, to the UK maintain their low risk of developing MS (Dean *et al.*, 1976). In parallel, those migrating from high risk areas to lower risk areas maintain this high risk (Dean, 1967), unless they move under the age of 16 and probably up to 20 years (Dean and Kurtzke, 1971; Ascherio and Munger, 2016). Finally the children of immigrants take on the risk of the country of birth confirming that there is more to MS aetiology than genetic susceptibility alone (Elian and Dean, 1987).

Several environmental factors have been investigated, including putative viruses using molecular mimicry, low vitamin D, distance from the equator in early childhood, diet, smoking and toxins (Compston and Coles, 2008; Ramagopalan *et al.*, 2010; MSIF, 2013). Meta-analyses suggest that the strongest evidence of association is related to Epstein-Barr virus (EBV) biomarker positivity, infectious mononucleosis and smoking (Belbasis *et al.*, 2015). Interactions of several

factors together have been investigated and this suggests that perhaps there is a grouped effect of environmental factors (Ghareghani *et al.*, 2018).

Smoking

The odds of developing MS is higher if there is a background of smoking, and a metanalysis suggested that the pooled OR is 1.51(95% CI 1.24-1.83)(Hawkes, 2004). Whether this is mediated via a direct immunological or is a secondary effect of other health related factors is not clear (Ramagopalan *et al.*, 2010; Ascherio and Munger, 2016). The risk of smoking on MS development appears to be greater in men (relative risk (RR) 1.6-2.0) than women (RR 1.0-1.4) (Ascherio and Munger, 2016).

Smoking has two further roles in MS. A history of smoking at the time of diagnosis has been shown to lead to a more progressive disease course and a greater severity as measured by the expanded disability status scale (EDSS) (Manouchehrinia *et al.*, 2013). This may be due to the fact that smoking also has a negative effect on brain lesion load and brain volume (Olsson *et al.*, 2016). An additional aspect maybe the role of smoking on the risk of developing neutralising antibodies to DMTs; beta-interferon (Hedström *et al.*, 2014*b*) and natalizumab (Hedström *et al.*, 2014*a*).

Viral infections

Childhood viral infections have been described as a possible aetiological factor for MS. This is due to the tendency for a higher viral antibody titre in MS versus healthy controls indirectly giving evidence for a viral agent. Confounding factors for these results include similar levels in siblings, those with other neurological, and non-neurological inflammatory conditions. However, a direct viral causal agent has not been identified. Additionally, case control studies have not shown an increased frequency of childhood infections such as measles, mumps or varicella (Marrie, 2004; Ascherio and Munger, 2007). Human herpes virus 6 (HHV-6), retrovirus, and endoretroviruses have as yet an unclear role in MS aetiology (Ascherio and Munger, 2007).

EBV antibody has been shown to have a higher incidence of 99% in MS versus 94% in controls (Ramagopalan *et al.*, 2010). The type of EBV antibody has different roles in MS. IgM Viral capsid antigen antibodies are at high titre at the onset of MS symptoms. IgM decreases and IgG viral capsid antigen antibodies persist throughout the MS course. IgG to EBV nuclear antigen-2 occurs in the acute disease phase, and decreases during convalescence, whereas IgG to EBV nuclear antigen-1 increases with MS convalescence (Ascherio and Munger, 2007, 2016). Additionally, people with a history of infectious mononucleosis due to EBV show an increased rate of MS versus controls (Belbasis *et al.*, 2015). Overall the risk of MS is 20 times higher in those with previous EBV infection and 10 times higher in those with a history of infectious mononucleosis than controls (Ascherio and Munger, 2007; Belbasis *et al.*, 2015).

Cytomegalovirus (CMV) is part of the same herpes family as EBV. However, studies of association of CMV seropositivity and MS risk have not consistently shown a link. Pooled analyses suggest that CMV may have a protective effect on MS risk reducing the OR to 0.7, but the differences in study design and size make this conclusion unreliable (Belbasis *et al.*, 2015; Olsson *et al.*, 2016).

Studies of vaccinations and MS risk have not shown an association with MS (Belbasis *et al.*, 2015). Hepatitis B vaccination was suspected of possible CNS demyelination, however analyses suggested a lower OR in cases versus controls (Ascherio *et al.*, 2001).

Vitamin D

A large case control study of over 7 million US military subjects performed by Munger and colleagues in 2006 first indicated a link between low serum 25-hydroxyvitamin D (25[OH]D) and MS. They highlighted reduced odds of developing MS in white military staff for a 50nmol/L increase in vitamin D level (OR 0.59 (95% CI 0.36-0.97)). There was no association between vitamin D levels and MS risk in hispanic or black ethnicity subjects (Munger *et al.*, 2006). The greatest impact of vitamin D deficiency appears to occur in childhood (Munger *et al.*, 2006).

A further role of 25[OH]D was evaluated from the Betaferon/Betaseron in Newly Emerging MS For Initial Treatment (BENEFIT) trial of early versus delayed interferon beta-1b in CIS (Kappos *et al.*, 2006). Increased 25[OH]D was shown to inversely decrease relapse rate, new lesions, T2 lesion load, and brain volume loss. This supports a putative role of vitamin D in CIS conversion to MS, and MS progression and activity (Ascherio *et al.*, 2014). Current guidance therefore supports the supplementation of vitamin D in MS if baseline 25[OH]D levels are not supranormal given the evidence of vitamin D as a potential risk factor for MS development and also prognosis, and the benefit of fracture risk reduction (Ascherio *et al.*, 2014; Dobson *et al.*, 2018).

UV light increases the production of dermal vitamin D, and sunlight or UV light exposure has been investigated both in tandem with vitamin D levels and without (Ghareghani *et al.*, 2018). An Australian case-control study highlighted a reduced OR of MS with higher levels of sunlight exposure (Van Der Mei *et al.*, 2003). It has also been postulated that UV light has immunosuppressive properties, but whether this is due to increases in levels of 25[OH]D is not clear (Marrie, 2004). Past sunlight exposure was inversely related to the odds of MS development (OR 0·32 (95% CI 0·11–0·88)) (Ramagopalan *et al.*, 2010).

Obesity in childhood

In Danish school children aged 7-13 years old, every unit increase in BMI increased the risk of MS by 15-20% (Ascherio and Munger, 2016). The risk of MS development was two times higher in women who were obese at age 18 years. Adult obesity rates do not seem to have an effect on MS risk. This has been reaffirmed in large population cohorts in Copenhagen, Italy, and Southern California (Ascherio and Munger, 2016). It is known that obesity gives rise to lower 25[OH]D levels, and so this may be the putative pathway. However, the risk is only apparent in females and therefore there may be a hormonal influence on this too (Olsson *et al.*, 2016).

Female sex

Epidemiologically, MS is more prevalent in women compared with men (Filippi *et al.*, 2018, Thompson *et al.*, 2018*b*; Dobson and Giovannoni, 2019). There has been a rise in the incidence

of MS in women over time suggesting that this may be related to other factors such as hormonal effects (Whitacre, 2001; Krysko *et al.*, 2020), or increased susceptibility to environmental factors (Orton *et al.*, 2006).

Gut barrier disruption and the microbiome

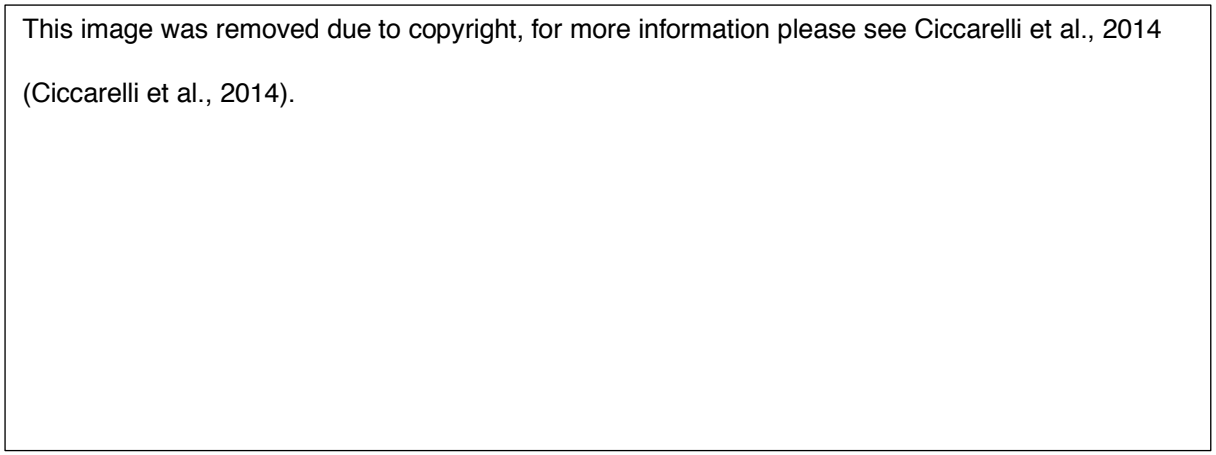
Changes in intestinal permeability have been shown to influence MS and neuroinflammation. Permeability variations allow the intestinal microbiome to transfer more circulatory endotoxins, which leads to a cascade of CNS inflammatory cytokines and subsequent neuroinflammation (Buscarinu *et al.*, 2019). Specific gastrointestinal diseases which cause gut barrier disruption, e.g. inflammatory bowel disease and coeliac disease, are linked with CNS demyelination and MS (Buscarinu *et al.,* 2018). However, even in the absence of intestinal disease, in MS there has been shown to be translocation of microbial organisms and toxins due to the changes within the gut microbiome and intestinal permeability resulting in microglial activation (Camara-Lemarroy *et al.*, 2018). In vitro studies have suggested that gut barrier breakdown leads to an increase in zonulin, interleukin 17, and interferon gamma which cause a rise in pro-inflammatory cytokines and blood brain barrier disruption. This, through microglial activation and astrocyte dysfunction, leads to demyelination and axonal damage (Rahman *et al.*, 2018).

Gut barrier changes have been associated with other risk factors for MS. An example is vitamin D deficiency which reduces intestinal calcium and causes gut stasis and alters intestinal permeability resulting in the release of endotoxins such as lipopolysaccharides (Ghareghani *et al.*, 2018). There is a similarity in the pattern of intestinal permeability in MS and depression which may underlie the high proportion of depressive and neuropsychiatric diagnoses in MS (Buscarinu *et al.*, 2019). Better understanding of the gut-brain axis could therefore help to develop treatments to target this potential neuroinflammatory pathway (Camara-Lemarroy *et al.*, 2018).

1.3.3 Pathophysiology

The characteristic hallmark of MS is the focal demyelinated CNS lesion. The disease is widespread in the brain and spinal cord and involves both white matter (WM) and grey matter (GM) (Lassmann, 2018). Controversially, it is still uncertain if tissue damage is primarily driven by inflammation, or whether underlying neurodegeneration is worsened by concurrent inflammation (Frischer *et al.*, 2009; Kuhlmann *et al.*, 2017). However, different types of CNS lesion show different degrees of inflammatory activity (Frischer *et al.*, 2015). Additionally, recent pathological studies have indicated that cerebral white matter demyelination can be absent, despite neurodegeneration in MS. In 12% of the analysed post mortem tissues from patients with MS there were only spinal cord and subpial lesions. This new MS subtype "myelocortical MS" is defined by demyelination of the spine and cerebral cortex in the absence of cerebral white matter demyelination (Trapp *et al.*, 2018). This might explain why the MRI white matter lesion load does not fully explain clinical disability (Fisniku *et al.*, 2008*a*; Chung *et al.*, 2020). There are different mechanisms of inflammation and tissue repair at different stages of MS which alter with disease progression (Reynolds *et al.*, 2011; Ciccarelli *et al.*, 2014; Dendrou *et al.*, 2015; Kuhlmann *et al.*, 2017). As with the presentation of MS, the mechanisms of tissue injury are also likely heterogeneous in MS. These underlying pathological changes may explain some of the differences in response to therapeutic strategies due to MS phenotype discussed in the subsequent sections (Lassmann *et al.*, 2007; Mahad *et al.*, 2015).

Figure 1.2 Pathological mechanisms in multiple sclerosis.



Schematic overview of the putative underlying pathological processes of MS. Adapted from Ciccarelli et al., 2014 (Ciccarelli et al., 2014).

There are key differences and similarities in the CNS lesion and pathophysiological processes in both early and later MS stages, indicating less of a dichotomy, and more of a continuous process (Ciccarelli *et al.*, 2014; Lassmann, 2018). Active and inactive CNS lesions occur in both stages, and the features of these are described below (Frischer *et al.*, 2015; Kuhlmann *et al.*, 2017).

MS lesional pathology

In the early relapsing stages of MS, key pathological processes underlie inflammation and the formation of new lesions (**figure 1.2**) . Autoreactive T-helper cells infiltrate the CNS by disrupting the blood brain barrier and migrate across it resulting in activated macrophages and microglia which lead to demyelination (Ciccarelli *et al.*, 2014; Lassmann, 2018). This process causes local expression of proinflammatory cytokines. MHC class I restricted CD8+ T cells dominate within lesions and undergo clonal expansion. CD8+ T cells display class I antigens. Inflammatory changes may be modulated by the immune system at these earlier active MS stages (Kuhlmann *et al.*, 2017; Lassmann, 2018). Whether these pathophysiological processes are driven by a primary CNS process which triggers activation of the peripheral nervous system and leads to progressive neuronal damage, or whether an external, peripheral immune activation triggers the CNS immune-mediated inflammatory process is not certain. However support from the latter comes from the presence of T and B cells in the inflammatory infiltrate as well as microglia and macrophages, in early MS lesions (Ciccarelli *et al.*, 2014; Lassmann, 2018). How these pathways result in neuroaxonal damage and death is described in the next sub-section.

The cellular infiltrate of the hallmark CNS plaque in MS varies according to the individual, and may contain various amounts of T cells, macrophages, immunoglobulins or complement (Dendrou *et al.*, 2015). This results in variable amounts of inflammation and demyelination. The presence of immunoglobulins and complement suggests that there is a role of pathogenic antibodies. Other lesions display tissue injury due to oxygen and nitric oxide radical production indicating mitochondrial dysfunction and histotoxic hypoxia (**figure 1.3**). Where there is mild inflammation, perilesional white matter shows profound oligodendrocyte degeneration likely due to immune-mediated processes. Remyelination occurs at the edges of the plaques or within the

whole lesion and is due to oligodendrocyte progenitor cell recruitment and occurs more in subcortical or deep white matter locations (Lassmann *et al.*, 2007; Kuhlmann *et al.*, 2017).

Early in progressive MS, inflammatory CNS lesions lead to acute axonal damage, and loss (Luchetti *et al.*, 2018). In both progressive phenotypes of MS, PPMS and SPMS, previous studies have suggested that inflammatory activity decreases in CNS plaques over time (Frischer *et al.*, 2015) (**table 1.1**). However, a post-mortem study by Luchetti et. al from the Netherland brain bank showed that at death, those with progressive disease had the presence of active inflammation and demyelination or mixed active and inactive lesions in 57% of demyelinated white matter and mixed grey–white matter lesions. Even in those with the greatest disease duration (42-64 years), 34% of lesions showed inflammatory activity. Lesion load was found to correlate inversely with remyelinated lesions, and with disease severity. Therefore there is considerable inflammatory lesion burden in long term progressive MS; this cohort included 100 SPMS and 56 PPMS subjects (Luchetti *et al.*, 2018). Absinta and colleagues showed that the presence of rim lesions which evidence of chronic active inflammation on histology was associated with clinical outcome measures in MS. Those with 4 or more rim lesions reached motor and cognitive disability at an earlier age (Absinta *et al.*, 2019).

Cortical lesions contain T and B cells, and plasma cells on their surface in the meninges which may drive the subpial demyelination (Lassmann *et al.*, 2007, 2012; Howell *et al.*, 2011; Reynolds *et al.*, 2011; Stadelmann *et al.*, 2011; Mahad *et al.*, 2015; Junker *et al.*, 2020) (**table 1.1**). The grey matter of the cortex shows extensive demyelination with leptomeningeal inflammatory infiltrates (Howell *et al.*, 2011). Cortical demyelination is common in the early stages of MS, but different to that in the late progressive MS stage. In chronic subpial and pial lesions, there are fewer T or B cells, and activated microglia dominate (Kuhlmann *et al.*, 2017; Lassmann, 2018) (**table 1.1**). This suggests less grey matter inflammation, but greater neuroaxonal damage as MS progresses over time.

Chronic MS lesions express cytokines and chemokines involved in homing of B cells and those which are active contain more plasma B cells then in early MS inflammatory lesions. However, pre-existing lesions show evidence of lesion margin expansion, slowly evolving lesions (SELs). This expansion is associated with moderate inflammatory T cell infiltrates, but enhanced microglial activation at the rim encompassing very slow demyelination. This slow axonal damage results in low remyelination rates in progressive MS (**table 1.1**).

Table 1.1 MS lesion pathology.

Lesion Type	**Classic active lesions**	**Classic Inactive lesions**	**Cortical Lesions** Type 1: cortico-subcortical. Type 2: perivenous intracortical lesions. Type 3: subpial.	**Slowly Expanding lesions (SELS)**
Features	Microglia activation at the lesion edge; macrophages are most pronounced in the lesion centre. Demyelination associated with phagocytosis (activated microglia and macrophages) and signs of acute axonal injury.	Demyelination, partial axonal damage, and reactive gliosis. A variable degree of microglia activation is present in the peri-plaque white matter.	Type 3 subpial lesions predominate, and are associated with inflammation in the meninges. In early MS there if more inflammatory infiltrate then in later stages where microglia prevail.	Inactive lesion centre, but activated microglia & macrophages containing myelin degradation products in the rim. Signs of acute axonal injury.
Inflammatory activity	High. When demyelination and tissue damage increase, T cells increase.	Low	Variable according to MS stage	Low – rim only
MS type – early or late	Early	Early and Late	Early and Late	Late

Adapted from (Frischer *et al.*, 2015; Kuhlmann *et al.*, 2017)

Progressive MS pathology

MS with a progressive onset from the outset is less affected by new lesion formation, but leads to diffuse atrophy in both normal-appearing and diseased grey and white matter. Activated microglia express nitric oxide radicals and myeloperoxidase resulting in subsequent Wallerian degeneration (Bramow *et al.*, 2010). The surrounding white matter is highly abnormal (normal appearing white matter (NAWM)) with diffuse inflammation made up of CD8+ T cells and activated microglia. Microglia express MHC antigens and radical production antigens leading to extensive axonal injury resulting in an abnormal appearance of the white matter not due to demyelination (Christensen *et al.*, 2013). In PPMS and SPMS perivenous inflammatory infiltrates are surrounded by rims of demyelination, astrocytic gliosis, microglia activation, and axonal degeneration (Bjartmar *et al.*, 2003; Kuhlmann *et al.*, 2017) (**figure 1.2**).

The key pathological hallmarks of progressive MS have been summarised by Mahad et. al and include; widespread abnormalities in both normal-appearing and lesional white and grey matter, cortical demyelination, neuronal degeneration of chronically demyelinated axons, and failure of remyelination (Mahad *et al.*, 2015) (**figure 1.2**).

With progression, it was thought there is less disruption of the blood brain barrier as in early MS. However, recent studies showed that there was greater total fibrin(ogen) deposition, a surrogate marker of blood-brain barrier disruption, in the progressive MS cortex compared with non-neurological controls (Bell *et al.*, 2018; Spencer *et al.*, 2018) and additionally fibrinogen deposition in cortical layers in progressive MS (Yates *et al.*, 2017). This may indicate more of a vascular, less myelin specific pathophysiological process in progressive MS. Meningeal inflammation, particularly where there is reduced CSF flow, leads to lymphoid aggregates and inflammatory changes becoming more compartmentalised or follicle-like and contain clusters of microglia, B cells and plasma cells. Lymphoid follicles have been found in 40% of cases of progressive MS on brain bank studies. This results in cortical demyelination and lesions (**figure 1.2**, **table 1.1**).

Mechanisms of neuroaxonal injury

Understanding the processes which contribute to neuroaxonal damage and loss especially in more chronic MS phenotypes, allows an appreciation of what to target therapeutically for neuroprotective and remyelination strategies (Ontaneda *et al.*, 2017). These are summarised in the schematic below (**figure 1.3**).

Figure 1.3. Mechanisms of neuroaxonal damage in multiple sclerosis.

This image was removed due to copyright, for more information please see Ciccarelli et al., 2014 (Ciccarelli et al., 2014).

Adapted from Ciccarelli et al., 2014 (Ciccarelli et al., 2014).

1. ***Oxidative stress***. Longstanding inflammation results in the accumulation of reactive oxygen (ROS) and nitric oxide (NO) species. ROS are released by macrophages, microglia, and mitochondria to activate kinases, express transcription factors and genes, and regulate apoptosis of phagocytosed cells and proliferation. When the oxygen species exceed the cellular antioxidant threshold there is histotoxic hypoxia leading to lipid dysfunction, protein misfolding, and cellular toxicity.

2. ***Excitotoxicity.*** Glutamate is an excitatory neurotransmitter which binds to post-synaptic receptors allowing transfer of sodium (Na+), potassium (K+), and calcium (Ca^{2+}) cations into the cell. Astrocytes, microglia, and oligodendrocytes control glutamate concentrations in the synaptic space, converting it to glutamine. Neuronal damage leads to excess excitatory glutamate accumulation in the synaptic space which is above the

threshold for the cellular reuptake processes to manage. Activated microglia and macrophages also produce copious glutamate. This glutamate excess over-activates signalling pathways leading to intracellular Ca^{2+} influx and phospholipase, endonuclease, and protease enzyme activation. This alters the membrane lipids, disrupts the cytoskeleton and damages deoxyribose nucleic acid (DNA) .

3. ***Mitochondrial Injury.*** Oxidative stress leads to the accumulation of mitochondrial DNA mutations and dysfunction. Neuroaxonal transport is affected by the resultant metabolic stress, protein misfolding in the endoplasmic reticulum, and energy deficiency. There is compensatory ion channel redistribution along the demyelinated axon; voltage-gated 1.2 and 1.6 Na+ channels which are normally situated in the nodes of Ranvier. This results in intra-axonal influx of Na+. This is compensated by reverse flow of the Na^+/Ca^{2+} above a certain concentration of axonal Na^+, approximately 20mM, to expel Na^+ back into the extracellular space. The Na^+/K^+ pump exchanges intra-axonal Na^+ for extracellular K^+, but this requires high adenosine triphosphate (ATP) concentrations. ATP production is reduced when inflammation is present due to the production of NO by activated microglia and macrophages, and the negative effect on the mitochondrial respiratory chain by causing oxidative phosphorylation. This reduction in ATP results in failure of Ca^{2+} homeostasis and subsequent axonal microtubule damage, protease activation and neurofilament fragmentation. Cellular cytoskeleton alterations, and changes in axonal transport result in neuroaxonal destruction. Further sources of Ca^{2+} include membrane acid-sensing ion channels (ASIC), e.g. (ASIC1). ASIC activation occurs due to prolonged acidity in the setting of prolonged inflammation and energy deficits. The resulting ion imbalance leads to more neuronal damage. Degeneration spreads towards the distal axon terminal (Wallerian degeneration or anterograde degeneration), or towards to cell body (retrograde degeneration). This results in cellular necrosis and apoptosis which can affect other neighbouring pre- and post-synaptic neurones.

4. ***Direct oligodendrocyte loss***. Direct neuronal cell body and axonal damage can be from the lymphocytic inflammatory processes, especially on denuded axons. Grey matter damage is more likely due to microglial activation, and this is also present in active and early MS stages.

5. ***Trophic Factors***. Buffering mechanisms upregulate in the presence of axonal damage. These include upregulation of pro-survival genes, and the cannabinoid system. However, a threshold is reached after which these are not sufficient.

1.3.4 Clinical features

MS is a disease of the entire CNS and therefore leads to differential involvement of the motor, sensory, visual, and autonomic systems (Gelfand, 2014, Thompson *et al.*, 2018*b*). No clinical feature is disease specific in MS, and the clinical presentation usually depends on the location of the demyelinating lesion.

In 85% of cases MS onset is typified by an initial clinical attack or relapse (Clinically Isolated syndrome or CIS). This is usually acute or subacute in onset, gradually worsens over days to weeks with a peak after 2-3 weeks. Improvement or remission is approximately 2-4 weeks after peak deficit. Recovery from a clinical relapse is spontaneous whether steroids are used or not, but can take several months. The degree of recovery from a clinical episode is an important determinant of whether the disease is progressive (Doshi and Chataway, 2016, De Angelis *et al.*, 2018*a*; Filippi *et al.*, 2018; Dobson and Giovannoni, 2019).

Optic neuritis due to a demyelinating lesion in the optic nerve, is the first episode in 25% of patients and is associated with conversion to clinically definite MS in 34-75% 10-15 years later (Tintore *et al.*, 2015). 70% of patients with MS will experience optic neuritis at some point in their disease course. Symptoms include total or partial unilateral visual loss with central scotoma, dyschromatopsia, and pain worsened by eye movement. 43% of people with MS present with

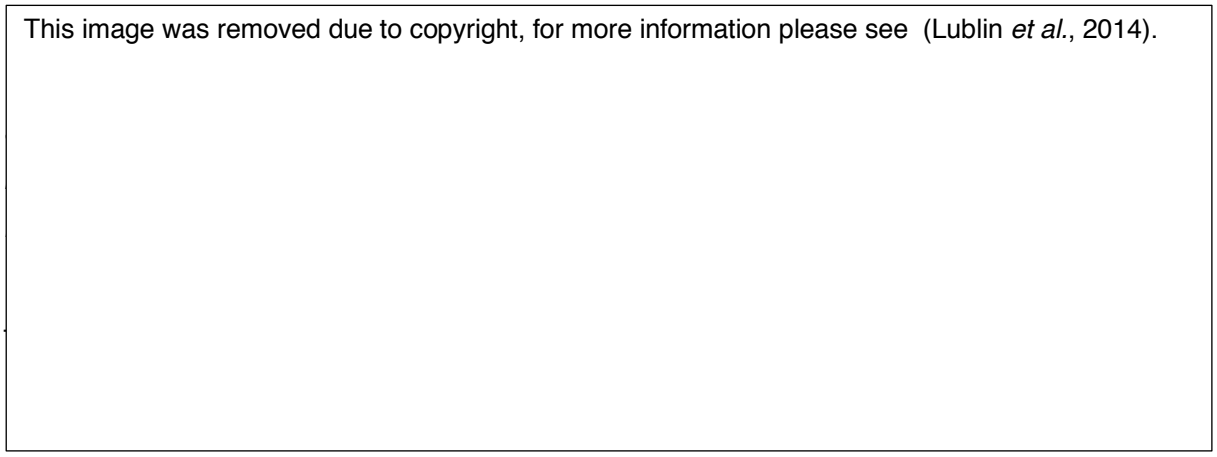
sensory symptoms, relating to spinal cord (myelitis), or brainstem demyelination episodes. Other typically characteristic symptoms and signs include; Lhermitte's phenomenon (an abnormal electric-shock like sensation down the spine or limbs on neck flexion) and Uhthoff's phenomenon (worsening of clinical symptoms during exercise or when in a hot environment) (Miller *et al.*, 2008; Brownlee *et al.*, 2016). Motor syndromes with paresis and spasticity are the presenting feature in 30-40% of cases (Filippi *et al.*, 2018, Thompson *et al.*, 2018*b*; Dobson and Giovannoni, 2019).

During the course of MS, 70% of patients will develop brainstem or cerebellar features, such as eye movement abnormalities e.g. internuclear ophthalmoplegia, ataxia, and tremor. Sphincter and sexual dysfunction is often correlated with motor impairment and affects up to 34-99% in later disease. Cognitive impairment affects 40-70% of those with MS and is covered in more detail in **chapter 2**. Although fatigue is often related to an acute relapse, it can persist in MS and up to 95% of those with MS experience this symptom. Fatigue might be related to sleep disorders which occur in 54% of cases. Pain has been reported in up to 43% of those with MS. Mood and affective disorders are common, with depressive symptoms being most frequently reported in up to 50% (Doshi and Chataway, 2016; Filippi *et al.*, 2018, Thompson *et al.*, 2018*b*; Grech *et al.*, 2019).

1.3.5 Classification of MS

In 1996 Lublin and colleagues initially classified MS into 4 distinct clinical courses after undertaking an international poll of MS specialists. These subtypes were; relapsing remitting MS (RRMS), primary progressive MS(PPMS), secondary progressive MS (SPMS) see **chapter 2**, and progressive relapsing MS. At this time clinical biomarkers and MRI were not readily available to aid diagnosis or subtype differentiation (Lublin and Reingold, 1996).

Figure 1.4. The 2013 revisions to the Lublin criteria for MS Phenotypes.



(Lublin *et al.*, 2014) (**figure 1.4**). Definition of the core phenotypes is reliant on clinical relapses, MRI activity, and disability progression. The main MS phenotypes are described below and summarised in **figure 1.5**.

Those presenting with a clinical relapse without MRI or clinical findings sufficient to make a formal MS diagnosis are classified as CIS (clinically isolated syndrome) (Polman *et al.*, 2011; Lublin, 2014). Clinically Isolated syndrome (CIS) and Relapsing Remitting MS (RRMS) are subtypes of the relapsing-remitting disease phenotype (Lublin and Reingold, 1996; Miller *et al.*, 2005, 2008; Lublin *et al.*, 2014).

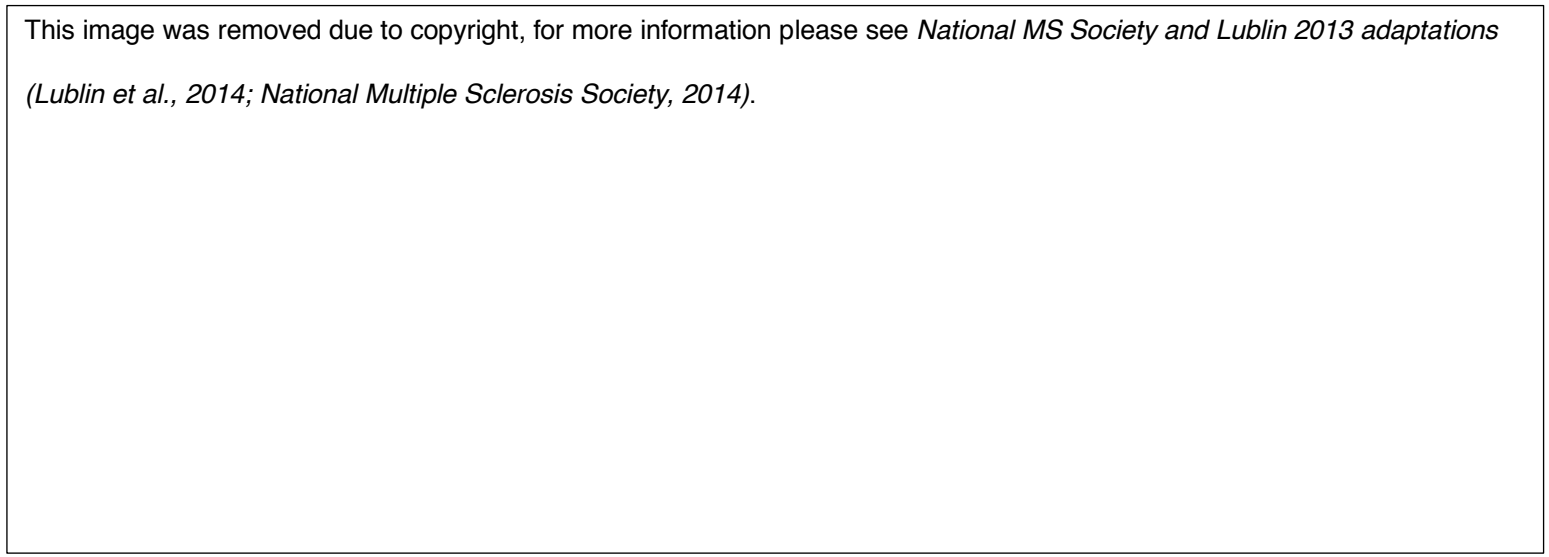
80% of MS patients present with CIS (Compston and Coles, 2008). CIS is classified as a clear central nervous system clinical episode, with (active) or without (not active) MRI activity (**figure**

1.5). This MRI activity is classed as gadolinium enhancing or, new or enhancing T2 lesions. CIS patients should be followed up to determine their disease course. If there are MRI T2 lesions at clinically unaffected sites the chance of fulfilling the RRMS diagnostic criteria are 50% at 2 years and 82% at 20 years, versus 21% at 20 years if no brain T2 lesions (Fisniku *et al.*, 2008*a*). Radiologically Isolated syndrome (RIS) is considered a separate entity to the MS phenotypes as MRI findings alone are not sufficient for a diagnosis of MS. However, overall a third of patients with RIS will convert to MS within 5 years (Thompson *et al.*, 2018*a*).

When further clinical episodes or relapses occur, RRMS is established (**figure 1.5**). Patients with RRMS are considered not active when there are no ongoing clinical relapses or MRI activity (gadolinium enhancing or new/enlarging T2 lesions) within a specified time frame, or active if these are present (Lublin *et al.*, 2014).

Over time, there is accumulation of disability and incomplete recovery from each relapse. Disease progression is the term used when patients have worsening disability over time compared with those who are worse because of a relapse. Disability progression can be defined clinically with objectively worsening neurological dysfunction or disability without unequivocal recovery or, on imaging (MRI) (See **section 1.3**). In those with a relapsing onset not treated with disease modifying therapy, there is a 25-38% 5-year conversion rate to SPMS (Lorscheider *et al.*, 2016; Brown *et al.*, 2019). SPMS is discussed in more detail in **section 1.3**.

Figure 1.5. Summary of the main MS phenotypes.



Adapted from the National MS Society and Lublin 2013 adaptations (Lublin et al., 2014; National Multiple Sclerosis Society, 2014).

10–15% of patients have progressive disability from the outset, usually due to spinal cord disease. This is defined as primary progressive MS (PPMS) and presents with slowly progressive symptoms from the outset (**figure 1.5**). The age of onset is 40 years, roughly a decade older than for relapse-onset MS, and there is no gender bias as with relapsing onset MS (Leary and Thompson, 2005; Miller and Leary, 2007). Usually the presentation is of a progressive myelopathy, however visual loss and dementia have been described (Miller and Leary, 2007; Thompson, 2015). Although relapses are less frequent than in relapse-onset MS, they decrease the time to a higher EDSS (Expanded Disability Status Scale) score, i.e. disease progression in both forms of progressive MS (Soldán *et al.*, 2015).

1.3.6 Diagnosis of MS

There is no definitive test for the diagnosis of MS, and current and previous criteria is reliant on the demonstration of CNS lesions with dissemination in time (DIT) and space (DIS) (Poser *et al.*, 1983; McDonald *et al.*, 2001; Polman *et al.*, 2005, 2011, Thompson *et al.*, 2018*a*). Primarily these criteria have been developed and revised to rule out alternative or differential causes of the symptoms and signs associated with MS (Brownlee *et al.*, 2016).

DIT is described as the simultaneous presence of contrast enhancing and non-enhancing lesions, or new T2- hyperintense or contrast enhancing lesions on subsequent imaging.

DIS describes one or more T2-hyperintense lesions in two of four areas; cortical, juxtacortical, periventricular, or infratentorial (Thompson *et al.*, 2018*a*).

The 2010 McDonald criteria for the diagnosis of MS have recently been replaced with the 2017 revisions by an international panel of MS experts to improve the speed and accuracy of diagnosis (Thompson *et al.*, 2018*a*) (**table 1.2**). The 2017 revisions to the McDonald diagnostic criteria have been adapted with the inclusion of cortical lesions and positive oligoclonal bands (OCBs) being representative of DIT (Thompson *et al.*, 2018). Finally, the distinction between clinically

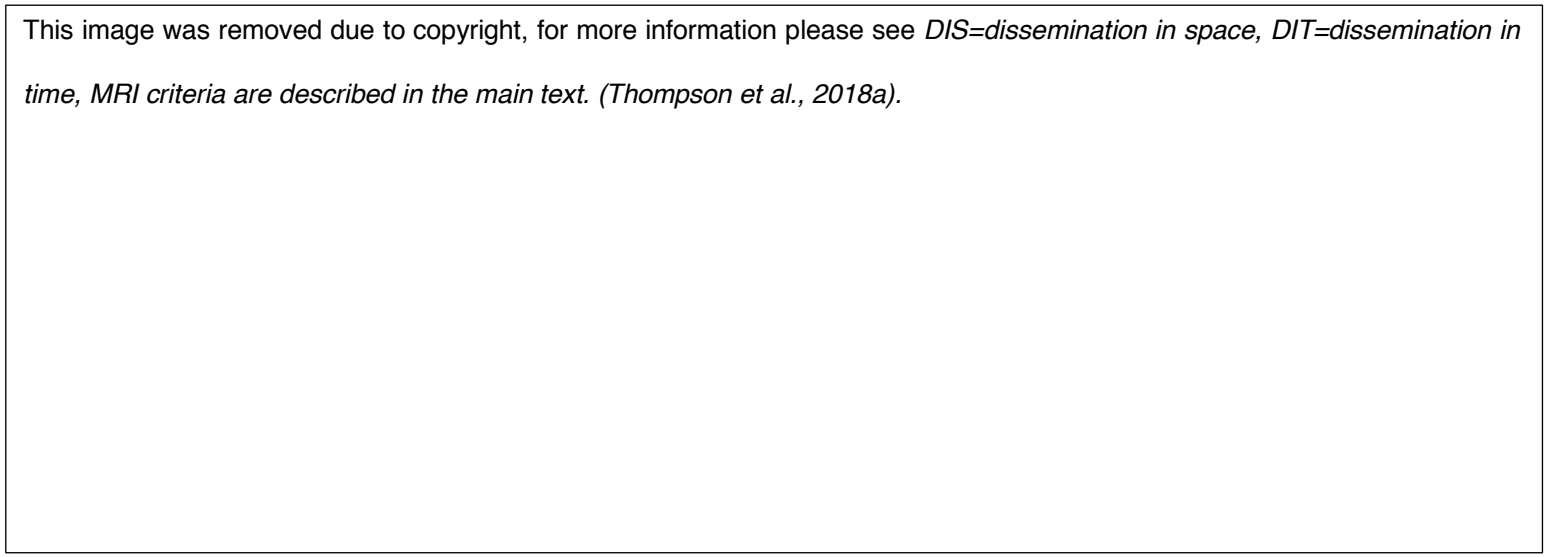
symptomatic and asymptomatic MRI lesions is removed (Thompson *et al.*, 2018*a*). A new anatomical location for DIS, the optic nerve, was suggested based on the MAGNIMS consensus and removed the need for distinction between symptomatic or asymptomatic lesions detected by MRI or visual evoked potentials, however this has not been adopted in the 2017 diagnostic criteria (Filippi *et al.*, 2016).

PPMS is defined by the 2017 criteria in those with at least a year of disability progression independent of relapse. This is in addition to at least two of; one or more characteristic T2-hyperintense lesions in the infratentorial, periventricular cortical or juxtacortical regions, two or more T2-hyperintense lesions in the spinal cord, or OCBs in the CSF.

MRI has revolutionised the diagnostic criteria by the detection of T2-weighted white matter lesions, and the exclusion of differential diagnoses (Miller *et al.*, 2008; Brownlee *et al.*, 2016). It has been shown that 82% of people with CIS and abnormal MRI went on to develop clinically definite MS (Fisniku *et al.*, 2008*a*). The presence of OCBs in the CSF can be useful for estimating prognosis, or where there is diagnostic uncertainty and are present in up to 80% of those with MS (Dobson *et al.*, 2013). Evoked potentials can be used to find dissemination in space by identifying demyelination in the anterior visual pathways, auditory or sensory pathways. Overall the sensitivity is lower than MRI and CSF (Deangelis and Miller, 2014). A large, multivariate observational study of over 1000 subjects with CIS showed that, greater conversion to clinically definite MS was associated with younger decade of onset, OCBs, and the presence of more than 10 brain lesions on MRI, with this being the most predictive factor (Tintore *et al.*, 2015). CIS presenting with optic neuritis had lower risk of clinically definite MS and disability progression than these factors, but uncertain effects on disability itself (Tintore *et al.*, 2015). Those with a symptomatic lesion on MRI were more likely to develop a second relapse and disease progression (Tintore *et al.*, 2016).

Table 1.2. The 2017 revisions of the McDonald diagnostic criteria.

DIS=dissemination in space, DIT=dissemination in time, MRI criteria are described in the main text. (Thompson et al., 2018a).

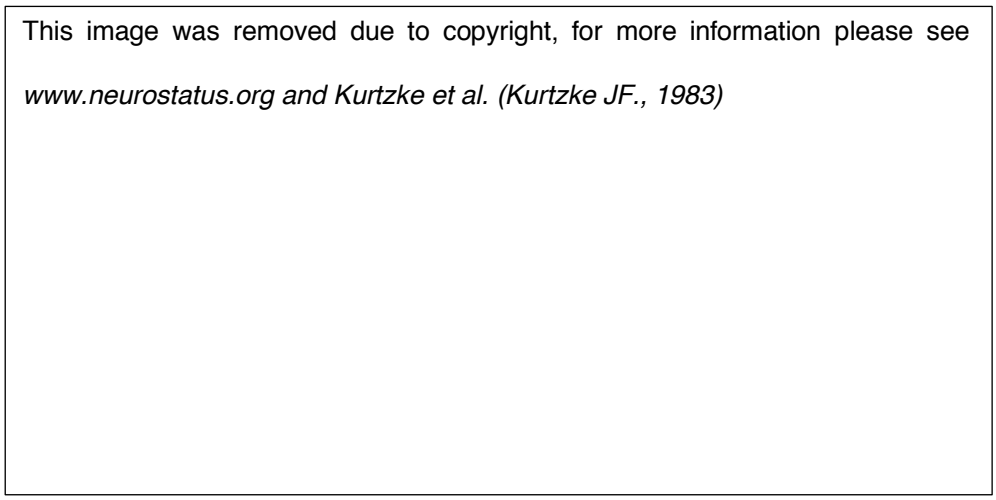


Currently there are still no reliable MRI or biomarker measures that can predict the individual disease course. Prior to the development of any type of MS, there appears to be a presymptomatic phase. Serum neurofilament light (sNfL) chain levels were higher in those who went on to have clinically definite MS a median of 6 years prior to the first clinical event versus matched controls. Additionally, an increase in sNfL produced a higher risk rate of MS at 7.50 (95% CI 1.72-32..80) for a ≥5pgml sNfL increase (Bjornevik *et al.*, 2019).

1.3.7 Measuring disability

The Kurtzke Expanded Disability Status Scale (EDSS) is a well validated physical disability scoring system which can be used to monitor physical disability change in MS (**figure 1.6**). The EDSS is non-linear and ranges from 0 (no disability) to 10 (death) (Kurtzke, John, 1983). EDSS is used as a research and clinical trial tool, but also in the clinical setting for monitoring change in MS function, and for elucidating entry into studies (Weinshenker *et al.*, 1991; Zajicek *et al.*, 2013; Liu *et al.*, 2016). Negatives to the EDSS are that it is very much guided by the ambulatory distance more than the functional non-motor scores, e.g. sensation, especially between the scores of above 4.0. Therefore, measures of other functional systems have less weight than ambulation to the overall score. Adaptations of the EDSS have allowed functional scores to play more of a role in the EDSS score. Disability progression on the EDSS scale is usually a 0.5- or 1-point worsening confirmed for 3-6 months as an example, but this consensus is variable (Cohen *et al.*, 2012; EMA, 2015).

Figure 1.6. The Expanded Disability Status Scale (EDSS).



Adapted from www.neurostatus.org and Kurtzke et al. (Kurtzke JF., 1983).

In the late 1990s, a task force evaluated a better compound disability measure of neuroimaging outcomes. The Multiple Sclerosis Functional Composite (MSFC) consists of the paced auditory serial additions test (PASAT3) of cognition, the 9 Hole Peg Test (9HPT) of upper limb function, and the timed 25-foot walk test (T25FW) of ambulation (Cutter *et al.*, 1999). The T25FW involves the patient walking 25 feet in their normal manner. The 9HPT consists of moving nine pegs into one of the nine holes on a peg board, then back into an open box twice per hand and then averaging per hand. PASAT3 involves serial additions using auditory numbers from a recording. Scores from the component tests are standardised to a reference population and z-scores are derived, these are then standardised to produce a single summative score. The MSFC has high inter-rater reliability, but has an initial learning effect due to the PASAT3 and 9HPT. The MSFC is moderately inversely correlated with the EDSS (rho=-0.47 to -0.8 $p<0.001$) mainly due to the ambulatory T25FW measure, with faster 9HPT and higher PASAT3 scores showing strong correlations with lower EDSS scores. The MSFC correlated better (R^2=0.3122) with MRI brain atrophy at 8 years than EDSS (R^2=0.0057) (Rudick *et al.*, 2002). The MSFC was used as the primary trial outcome in the phase 3 trial of interferon beta-1b in SPMS, but this was famously rejected by the Food and Drug Administration (FDA) (Cohen *et al.*, 2001, 2002). The MSFC has been evaluated as a marker of disease progression in MS. MSFC progression was defined as a worsening of 15% or 20% in any of the component tests; i.e. PASAT3, 9HPT, T25FW, sustained for three or more months.

Patient reported outcome measures (PROMS) have an important role in trials and specifically detect subjective measures of physical and psychological function (Doward *et al.*, 2015). The Multiple Sclerosis Impact Scale (MSIS 29v2) has 20 measures of the physical, and 9 measures of the psychological impact of MS on an individual. MSIS 29v2 has high test-retest reliability and good responsiveness with effect sizes of 0.82 for the physical and 0.66 for the psychological items (Hobart, 2001). The (MSQoL-54) has good validity and has shown significant associations with MS related measures of ambulation, employment and depression (Vickrey *et al.*, 1995). The Hospital Anxiety and Depression Scale (HADS) is a brief 14 item self-assessment scale for capturing depression and anxiety states (Zigmond and Snaith, 1983; Breeman *et al.*, 2015). It is

useful to ascertain the prevalence of these states as they can impact on motor and cognitive function in MS (Siepman *et al.*, 2008).

Cognitive measures of disability are described in **section 2.5**.

1.3.8 Management of MS

There are three main categories of treatments for MS; symptomatic therapies, management of the acute relapse, and disease modifying therapies (DMTs) (Perry *et al.*, 2014; Scolding *et al.*, 2015; Doshi and Chataway, 2016, De Angelis *et al.*, 2018*a*; Montalban *et al.*, 2018; Rae-Grant *et al.*, 2018).

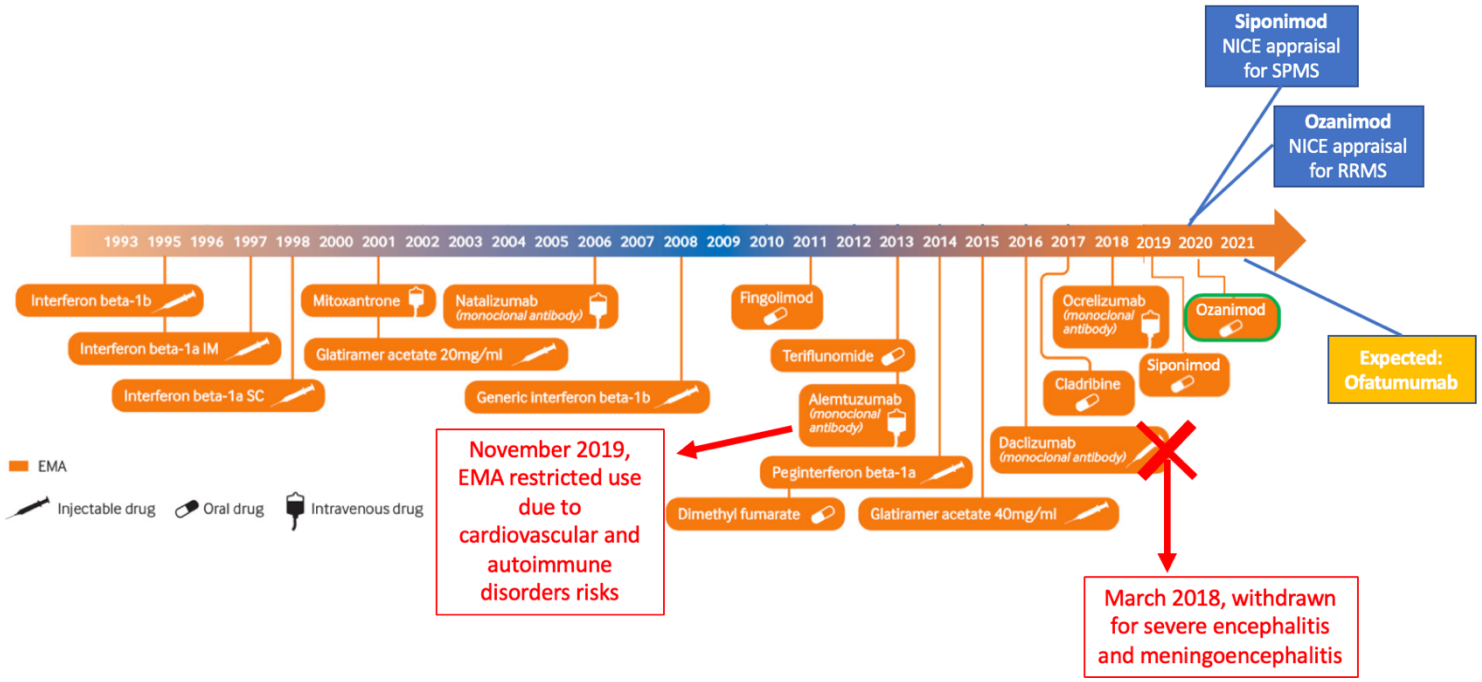
Managing a relapse can be difficult as it is hard to ascertain whether the episode is a true relapse or whether it is a fluctuation of established symptoms. The priority is to rule out an infection, and if the symptoms are of moderate severity patients should be treated with either oral or intravenous high dose methylprednisolone to shorten the duration of the episode (Perry *et al.*, 2014). The choice of route does not alter efficacy as highlighted by the COPOUSEP trial (NCT00984984, ClinicalTrials.gov) (Le Page *et al.*, 2015).

Symptoms experienced in MS are heterogeneous due to the underlying pathology. The main symptoms experienced are pain, bladder issues, fatigue, spasticity and cognitive difficulties. The key to effective symptom management is to remove any co-existent factors; e.g. poor sleep, infection or anaemia. Effective therapy options are both pharmaceutical and non-pharmaceutical and can be applied in a step wise fashion to obtain relief. The management of cognitive impairment in MS is described in **section 2.7**.

Over the last 25 years there has been the development of disease modifying therapies (DMTs) in MS. Currently there are 17 licensed DMTs in the UK for RRMS and DMTs have additional roles for CIS and PPMS, but not SPMS(Perry *et al.*, 2014; Scolding *et al.*, 2015; Montalban *et al.*, 2018;

Rae-Grant *et al.*, 2018). The ultimate aim of DMT is the integration of clinical (no MS relapses or disability progression) and MRI (no new T2 lesions or atrophy) parameters, condensed as 'no evidence of disease activity' (NEDA) (Perry *et al.*, 2014; Scolding *et al.*, 2015). The current licensed DMTs are summarised in **figure 1.7**.

Figure 1.7. Current licensed RRMS disease-modifying therapies (DMTs).



Developed from; (Scolding et al., 2015; Doshi and Chataway, 2016, De Angelis et al., 2018a; Montalban et al., 2018; Rae-Grant et al., 2018). EMA=European Medicines agency.

1.4 Secondary Progressive Multiple Sclerosis

SPMS is defined as the progressive accumulation of disability after an initial relapsing course. The age of secondary progression is usually around 40 years or older, and the speed of disease progression is fairly stable in the same individual (Lorscheider *et al.*, 2016; Plantone *et al.*, 2016; Inojosa *et al.*, 2019). Previously, rates of conversion to SPMS were thought to be up to 80%, however an international cohort study has suggested that this is now around 30% when not previously treated with DMTs (Brown *et al.*, 2019).

1.4.1 Definition of SPMS

As described, the 2013 Lublin classification modifications advised that the diagnosis of SPMS should be retrospective with a history of gradual worsening following a relapsing onset, with MRI, immunological or pathological support required to mark the transition (Lublin, 2014).

Detecting and determining the transition to SPMS from RRMS can be difficult due to subtle changes in symptoms and signs. It is also possible to have transient clinical worsening due to superimposed relapses, i.e. active progression, although this is less likely to be associated with contrast enhancing lesions then in RRMS. There is not a definitive test to determine when exactly secondary progression occurs, and so information gathered from clinical history or outcome measures is supportive and clinical judgement is key. Therefore, the diagnosis is often retrospective with EDSS changes guiding the definition (Ontaneda *et al.*, 2015; Lorscheider *et al.*, 2016; Plantone *et al.*, 2016; Inojosa *et al.*, 2019).

One attempt to derive a consensus for the definition of SPMS comes from work from Lorscheider et al. who developed an objective definition reflecting long-term disability outcomes in SPMS (Lorscheider *et al.*, 2016). Three expert neurologists evaluated 576 data-derived onset definitions for SPMS on the MSBase prospective cohort. The conclusion was that a diagnosis of SPMS was best defined in the absence of relapses, confirmed after 3 months in the main functional system

of the EDSS, with an EDSS ≥4, and pyramidal score ≥2 reaching an accuracy of 87% compared to the consensus diagnosis (Lorscheider *et al.*, 2016). This best definition included a 3-strata progression definition approach whereby there was a worsening of EDSS by 1.5 points if the last EDSS before conversion to SPMS was 0, an increase by 1 point if the EDSS was between 1.0 and 5.5, or an increase by 0.5 points if the EDSS was above 5.5 (Lorscheider *et al.*, 2016). However, this is of course just one approach used in a spectrum of definitions and, therefore, phase 2 and 3 trials must be interpreted accordingly (**table 1.3**) .

1.4.2 Pathology

The transition phase to secondary progression represents a shift from the predominantly inflammatory pathology of relapsing disease, to the axonal injury of progressive MS as described in **section 1.2.3**. Post-mortem studies have shown compartmentalised inflammation in lymphoid follicle-like structures in the brains of 40% of SPMS subjects related to increased meningeal inflammation (Serafini *et al.*, 2004; Magliozzi *et al.*, 2006, 2010; Reynolds *et al.*, 2011; Ciccarelli *et al.*, 2014; Lassmann, 2018). Mitochondrial damage leads to axonal loss and progression in MS. This is related to nitric oxide and glutamate related alterations of ATP synthesis, pore permeability, apoptotic factor release, and electron transport chain dysfunction. When axons are demyelinated sodium channels are upregulated and redistribute increasing energy requirements leading to failure of sodium potassium ATPase pumps. An additional component to pathology in progressive disease is the accumulation of intracellular calcium in axons leading to apoptosis (Lassmann *et al.*, 2012; Dendrou *et al.*, 2015; Mahad *et al.*, 2015). Just as the pathological changes from relapsing to progressive disease are dynamic and not an acute change, the clinical transition to SPMS can be difficult to pinpoint.

1.4.3 Risk factors for conversion to SPMS

The Lyon MS dataset suggested that, despite a low hazard ratio, age at onset was a significant risk factor for conversion to SPMS (hazard ratio 1.03 (95% CI 1.02 to 1.04)) (Vukusic and

Confavreux, 2003). This is however complicated by recent MRI studies showing that although MS brain atrophy decreased by 0.1% every 10 years, whole brain atrophy increases by 0.11% every year of age (Azevedo *et al.*, 2019). Additionally, using the brain age paradigm in machine learning, the brain age gap between estimated and chronological brain age was 20 years in SPMS, larger than any other phenotype (Cole *et al.*, 2020).

A large cohort study showed that there is a higher proportion of females in RRMS than in SPMS (68% versus 61%; p=0.006) (Confavreux and Vukusic, 2006*a*). Additionally, disease duration was twice as long in the SPMS than in the RRMS group (mean $\pm$ SD: 17.6 $\pm$ 9.6 versus 8.7 $\pm$ 8.6 years; p<0.001) (Confavreux and Vukusic, 2006*b*). Initial visual and sensory symptoms delay the onset of progression, and spinal cord presentations decrease the time to secondary progression (Rovaris *et al.*, 2006). Pre- and post- progression relapses both reduce the time to EDSS 6.0 (Soldán *et al.*, 2015).

Smoking has been shown to independently increase secondary progression conversion rates from RRMS in an Iranian cohort. Smoking more than 10 cigarettes per day increased the development of SPMS hazard ratio 2.43 (95% CI 1.28–4.6) p=0.007) (Roudbari *et al.*, 2013). There may also be accumulation of vascular risk factors secondary to increased age, less physical activity and higher homocysteine levels in SPMS which could affect the onset of progressive disease (D'haeseleer *et al.*, 2011). This may explain some of the fibrinogen cortical deposition in terms of pathological changes in SPMS (Yates *et al.*, 2017). Type 3 WM lesions, which are present more in progressive disease, have loss of myelin-associated glycoprotein (MAG) and loss of oligodendrocytes and are similar to acute WM stroke lesions. This, therefore, suggests a vascular aetiology (Aboul-Enein *et al.*, 2003).

1.4.4 Management of secondary progressive MS

Disease modifying therapies and SPMS

There has not been adequate DMT provision for SPMS. However, there is a research drive within Europe and the US to target more progressive MS phenotypes and pathology. Siponimod has recently been licensed by the FDA in the US as a disease modifying therapy for SPMS, and is still under review by the National Institute for Clinical Excellence (NICE) in the UK due to cost-reasons. Siponimod, a selective sphingosine 1-phosphate (S1P) receptor1,5 modulator, was trialled in SPMS in the phase 3 Exploring the Efficacy and Safety of Siponimod in People with SPMS (EXPAND) trial. There was a 21% relative risk reduction of 3 month confirmed disability progression (CDP) in the siponimod arm versus placebo (Kappos *et al.*, 2018).

Phase 2 and 3 trials in SPMS

There are two main approaches to drug trials in SPMS immune modulation and neuroprotection. The completed studies from 2015-2020 and recruiting studies are summarised in **table 1.3**.

In terms of immune modulation, The IMPACT (International MS Secondary Progressive Avonex Controlled Trial) trial was the preliminary trial to use change of MSFC as a primary end point. Overall, at 24 months intramuscular interferon beta-1a was shown to have positive effects on reducing MSFC z-score change, mainly driven by the 9HPT and PASAT3 (Cohen *et al.*, 2001, 2002). Alemtuzumab was trialled in a small study of 36 subjects with SPMS but led to continued disability progression in SPMS (Coles, 2013). The phase 2 ASCEND trial reported no treatment effect of natalizumab on the primary outcome which was a cumulative EDSS, 9HPT, and T25FW score, although did show significant reductions in 9HPT scores in SPMS (Kapoor *et al.*, 2018*b*). Although mitoxantrone was licensed for SPMS in 2000, it was removed from guidance due to safety issues. The phase I randomised controlled trial of Rituximab by Intravenous and Intrathecal Injection Versus Placebo in Patients with Low-Inflammatory Secondary Progressive Multiple Sclerosis (RIVITaLISe) study was terminated early due to a lack of efficacy in SPMS (Komori *et al.*, 2016*a*). Negative or inconclusive results have been found in trials of immunosuppressants;

azathioprine, ciclosporin, cyclophosphamide, and methotrexate (Ontaneda *et al.*, 2015, De Angelis *et al.*, 2018*b*). Autologous haemopoietic stem cell transplantation (AHSCT) had 73% (95% CI 57–88%) progression-free survival at 5 years post-AHSCT in a relapsing MS subgroup. In patients with SPMS, overall 33% (95% CI 24–42%) remained free from EDSS worsening (Muraro *et al.*, 2017). However, at higher EDSS levels there has been shown to be a higher mortality by a meta-analysis (Sormani *et al.*, 2017).

Targeting neuroprotection is a useful drug strategy in SPMS as it targets neuroaxonal loss. The current primary outcome measure of neuroprotection is change in whole brain volume over time, or whole brain atrophy (**section 2.4.2**). Lamotrigine was trialled in SPMS but was found not to lead to an overall difference in the rates of brain atrophy versus placebo (Kapoor *et al.*, 2010). The association of vascular risk factors with disease progression was investigated by the MS-STAT1 trial of 80mg simvastatin. Statins protect the endothelium of vessels and reduce free radical production and glutamate-mediated toxicity. The primary end point was annualised rate of whole brain atrophy and this was significantly slower in the simvastatin versus the placebo arm with a 43% reduction in the annualised rate of atrophy (Chataway *et al.*, 2014). The phase 2 SPRINT-MS trial of ibudilast in both PPMS and SPMS used the parenchymal brain fraction as the primary endpoint. Ibudilast slowed the rate of brain atrophy versus placebo, with 2.5ml less brain-tissue loss over 96 weeks (Fox *et al.*, 2018).

Trial design and drug discovery in SPMS is however difficult. Studies fail due to a lack of change in a relatively short timeframe in terms of the underlying pathology of SPMS. Additionally, there are high levels of disability and more co-morbid factors, this means that outcome measures may not be as accurate. In the SPMS population subject retention and recruitment are more difficult due to disability. This drives the need for alternative trial designs. Most current trial designs in progressive MS are head-to-head or 1:1 trials. This takes time and, as recruitment to SPMS trials can be limited by disability, may prevent best use of the available resources. Multi-arm trial designs, such as MS-SMART described in **section 1.5**, or adaptive designs where there is seamless transition from a phase 2 to phase 3 trial may enhance this in the future (Ontaneda et

al., 2015). These ideas have been adopted and adapted from the multi-arm multi-stage (MAMS) design used in the STAMPEDE trial for prostate cancer (MRC PR08, ISRCTN78818544, NCT00268476) (Sydes *et al.*, 2009). The MAMS design allows stage 1 with placebo and multiple trial drug arms, after which an interim analysis occurs. Stage 2 follows with only those arms which have been successful, or new arms, are added in. The MAMS trial design has recently been proposed for progressive MS trial design by an expert consensus panel.

Table 1.3. Summary of completed and active trials of therapy for progressive multiple sclerosis correct to 2nd January 2020.

1.3 a) Summary of completed Phase 2 and 3 trials in progressive MS between 2015-2020.

Drug(s)	Study title	Phase	Study design (comparator)	Subjects	Primary endpoint(s)	Time frame	Study completion	Results
Andrographolides (Ciampi *et al.*, 2020)	Efficacy, safety and tolerability of andrographolides versus placebo in patients with progressive forms of MS	1/2	RCT (placebo)	44 not active PMS	MRI-BVC	2 y	2020	Positive
Amiloride Fluoxetine Riluzole (Chataway *et al.*, 2020)	MS secondary progressive multi arm randomisation trial (MS-SMART)	2	Multi-arm RCT (placebo)	445 SPMS	MRI-BVC	96 wk	2018	Negative
Biotin (MD1003) (Tourbah *et al.*, 2016, 2019)	Effect of MD1003 in Spinal Progressive MS (MS-SPI). Effect of MD1003 in PMS (SPI2)	3	RCT (placebo)	99 SPMS 55 PPMS 600 PMS	EDSS T25FW	2 y 66m	2016 2021	Negative (phase 3) Negative 2019
Fingolimod (FTY720) (Lublin *et al.*, 2016)	FTY720 in PwPPMS (INFORMS)	3	RCT (placebo)	969 PPMS	EDSS T25FW 9HPT	3 y	2015	Negative
Fluoxetine (Marta, 2019)	Fluoxetine in Progressive Multiple Sclerosis (FLUOX-PMS)	2	RCT (placebo)	72 SPMS 55 PPMS	T25FW 9HPT	2 y	2016	Negative
Ibudilast (MN-166) (Fox *et al.*, 2018)	Safety, Tolerability and Activity Study of Ibudilast in PwPMS (SPRINT-MS)*	2	RCT (placebo)	121 SPMS 134 PPMS	MRI-BVC	3 y	2017	Positive
Idebenone (National Multiple Sclerosis Society, n.d.)	Double Blind Placebo-Controlled Phase I/2 Clinical Trial of Idebenone in Patients with PPMS (IPPoMS)*	1/2	RCT (placebo)	85 PPMS	CombiWISE (Kosa *et al.*, 2016)	2 y	2018	Negative

Drug(s)	Study title	Phase	Study design (comparator)	Subjects	Primary endpoint(s)	Time frame	Study completion	Results
Laquinimod (Active Biotech, n.d.)	A Phase 2 Clinical Study in PwPPMS to Assess the Efficacy, Safety and Tolerability of Two Oral Doses of Laquinimod Either of 0.6 mg/Day or 1.5mg/Day as Compared to Placebo (ARPEGGIO)*	2	RCT (placebo)	374 PPMS	MRI-BVC	48 wk	2017	Negative
Lipoic acid (Spain *et al.*, 2017)	Lipoic Acid for SPMS	2/3	RCT (placebo)	54 SPMS	MRI-BVC	2 y	2015	Positive
Lithium (Rinker, 2016)	A Pilot Trial of Lithium in PMS*	1/2	Crossover RCT (placebo)	20 PMS	MRI-BVC	2 y	2015	Negative
MIS416	A Phase 2B Randomised, Double-Blind, Placebo-Controlled Trial of the Efficacy and Safety of MIS416 in the Treatment of PwSPMS*	2	RCT (placebo)	93 SPMS	Neuromuscular function	1 y	2017	Negative
Natalizumab (Kapoor *et al.*, 2018*a*)	A Clinical Study of the Efficacy of Natalizumab on Reducing Disability Progression in PwSPMS (ASCEND)	3	RCT (placebo)	889 SPMS	EDSS T25FW 9HPT	96 wk	2016	Negative
Ocrelizumab (Montalban *et al.*, 2017)	A Study of Ocrelizumab in PwPPMS (ORATORIO)	3	RCT (placebo)	732 PPMS	EDSS	120 wk	2015	Positive
Oxcarbazepine (Marta, 2019)	Protective role of oxcarbazepine in MS (PROXIMUS)	2	RCT (placebo)	30 SPMS/PRRMS	NFL light chain	48 wk	2018	Negative
Rituximab (IT) (Komori *et al.*, 2016*b*)	Double Blind Combination of Rituximab by Intravenous and Intrathecal Injection Versus Placebo in Patients with Low-Inflammatory Secondary Progressive Multiple Sclerosis (RIVITaLISe)	1/2	RCT (placebo)	44 li-SPMS	CSF CXCL13 CSF BAFF	3 m	2015	Terminated (lack of efficacy)

Drug(s)	Study title	Phase	Study design (comparator)	Subjects	Primary endpoint(s)	Time frame	Study completion	Results
Siponimod (Kappos *et al*., 2018) (BAF312)	Exploring the Efficacy and Safety of Siponimod in PwSPMS (EXPAND)	3	RCT (placebo) [blind phase]	1651 SPMS	EDSS	3 y	2017	Positive
Sunphenon EGCG (epigallocatechin-gallat, EGCG)	Sunphenon in Progressive Forms of MS (SUPREMES)	2/3	RCT (placebo)	60 PMS	MRI-BVC	3 y	2016	Completed Results not reported
Tcelna (imilecleucel-T) (Loftus *et al*., 2009)	Study of Tcelna (Imilecleucel-T) in SPMS (Abili-T)	2	RCT (placebo)	183 SPMS	MRI-BVC	2 y	2016	Negative

Adapted from individual trial papers and the following reviews; (Ontaneda et al., 2015, De Angelis et al., 2018b). NCT for clinical trials have been searched on www.clinicaltrials.gov.uk. RCT=randomised controlled trial, IV=intravenous MRI-BVC=MRI brain volume change, EDSS=Expanded Disability Status Scale, T25FW=Timed 25-foot walk, 9HPT=9 hole peg test, MGD=maximum gait distance, TMT-B=trail-making test-B, CombiWISE=Combinatorial Weight-Adjusted Disability Score, osteopontin=secreted phosphoprotein 1 glycoprotein, NFL light chain=Neurofilament light chain, MRS-NAA=magnetic resonance spectroscopy N-acetylaspartate, CSF=cerebrospinal fluid, CXCL13=chemokine CXCL13, BAFF=B-cell activating factor of the tumour necrosis factor family, GEP=Global evoked potential, ORS=Overall Response Score.

b) Summary of Phase 2 and 3 ongoing treatment trials in progressive MS, as of 2nd January 2020.

*According to putatative pharmaco-mechanistic potential; immunomodulatory, neuroprotective, and regenerative. Drugs marked with § may have additional neuroprotective properties. Drugs marked with * may have additional immune-modulation properties.*

IMMUNOMODULATORY									
Drug(s)	**Study Title**	**Phase**	**Study design (comparator)**	**Subjects (estimated)**	**Primary endpoint(s)**	**Time frame**	**Study completion**	**Status**	**NCT**
Dimethyl fumarate §	Dimethyl Fumarate Treatment of PPMS (FUMAPMS)	2	RCT (placebo)	90 PPMS	NFL light chain	48 wk	2019	Active, not recruiting	02959658
Glatiramer acetate depot	Safety and Efficacy of Monthly Long-acting IM Injection of 40 mg GA Depot in Subjects With PPMS	2	Open label	24 PPMS	Safety	56 wk	2019	Recruiting	03362294
Hydroxychloroquine	Hydroxychloroquine in PPMS	2	Open label	35 PPMS	T25FW	18 m	2020	Recruiting	02913157
Masitinib	A Phase 3 Study to Compare Efficacy and Safety of Masitinib to Placebo in the Treatment of Patients with Primary Progressive or Relapse-free Secondary Progressive Multiple Sclerosis	2/3	RCT (placebo)	450 PPMS or active-SPMS	EDSS	96 wk	2019	Recruiting	01433497
NeuroVax™	A Study of NeuroVax™, a Novel Therapeutic TCR Peptide Vaccine for SPMS	2	RCT (IFA)	150 SPMS	EDSS	48 wk	2019	Not recruiting yet	02149706
Ocrelizumab	A Study of Ocrelizumab in Participants with Primary Progressive Multiple Sclerosis	3	Open label (extension phase)	Variable number PPMS		4 y (max)	2021	Active not recruiting	01194570
Ocrelizumab	A study to evaluate the efficacy and safety of ocrelizumab in adults with PPMS (O'HAND)	3b	Placebo	1000	Time to upper limb disability progression. 9HPT	5.5 y	2028	Recruiting	04035005
Rituximab	Intrathecal Rituximab in PMS (EFFRITE)	2	Open label	12 PMS	Osteopontin	180 d	2019	Unknown	02545959

Siponimod (BAF312)	Exploring the Efficacy and Safety of Siponimod in PwSPMS (EXPAND)	3	Open label (extension phase)	Variable number SPMS	EDSS	3 y	2023	Active not recruiting	01665144
Dimethyl fumarate §	Dimethyl Fumarate Treatment of PPMS (FUMAPMS)	2	RCT (placebo)	90 PPMS	NFL light chain	48 wk	2019	Active, not recruiting	02959658
Glatiramer acetate depot	Safety and Efficacy of Monthly Long-acting IM Injection of 40 mg GA Depot in Subjects With PPMS	2	Open label	24 PPMS	Safety	56 wk	2019	Recruiting	03362294
NEUROPROTECTIVE									
Lipoic acid	Lipoic acid for progressive MS	2	RCT (placebo)	118 PMS	T25FW	2 y	2021	Recruiting	NCT03161028
Melatonin	Safety and efficacy of melatonin in patients with PPMS	2	Placebo	50	Rates of neurological impairment	2 y	2020	Not yet recruiting	NCT03540485
Simvastatin	Multiple sclerosis-Simvastatin trial 2 (MS-STAT12)	3	RCT (placebo)	1180 SPMS	EDSS	182 wk	2023	Recruiting	NCT03387670
REGENERATIVE									
ACTH *	ACTH in progressive forms of MS	2	RCT (placebo)	100 PMS	T25FW	3 y	2022	Active, not recruiting	NCT01950234
ABMT (IV) *	Assessment of bone marrow-derived cellular therapy in PMS (ACTiMuS)	2	Crossover RCT (placebo)	60 SPMS 20 PPMS	GEP	2 y	2018	Recruiting	NCT01815632
Bone marrow-derived cellular therapy	Assessment of Bone Marrow-derived Cellular therapy in PMS (ACTiMuS)	2	Randomised, Crossover assignment	80	GEP	2 y	2019	Recruiting	NCT01915632
Domperidone	Domperidone in SPMS	2	Open label	62 SPMS	T25FW	1 y	2020	Recruiting	NCT02308137

Elezanumab	Study to assess the safety and efficacy of elezanumab when added to standard care in PMS	2	RCT (Placebo)	90 PMS	ORS	52 wk	2021	Recruiting	NCT03737812
Mesenchymal stem cells	Optimal administration mode of autologous mesenchymal bone marrow stem cells in active and progressive MS	2	Randomised (different administration)	36 PMS	MRI-BVC Immunological response	1 y	2018	Recruiting	NCT02166021
Mesenchymal stem cell-derived neural progenitors	Intrathecal administration of autologous mesenchymal stem cell-derived neural progenitors (MSC-NP) in progressive MS	2	Crossover RCT (placebo)	50 PMS	EDSS-Plus	27 m	2023	Recruiting	NCT03355365
autologous Mesenchymal Stromal Stem Cells Secreting Neurotrophic Factors (MSC-NTF cells)	A Phase 2 Open-label Multicentre Study to Evaluate the Safety and Efficacy of Repeated Administration of NurOwn® [Autologous Mesenchymal Stem Cells Secreting Neurotrophic Factors (NTF), MSC-NTF] Cells in Participants with Progressive MS	2	Open label	20	Safety, T25FW, 9HPT	28 m	2020	Recruiting	NCT03799718

1.5 The MS-SMART trial

The recently reported Multiple Sclerosis Secondary Progressive Multi-Arm Randomisation Trial [ClinicalTrials.gov, NCT01910259] (MS-SMART) is a phase 2b multi-arm randomised placebo-controlled trial (Connick *et al.*, 2018; Chataway *et al.*, 2020). The primary outcome was the percentage brain volume change (PBVC) on MRI. The drugs chosen for the MS-SMART trial had different purported neuroprotective mechanisms; fluoxetine, riluzole, and amiloride. These were trialled in a 1:1:1:1 fashion simultaneously against placebo using a multi-arm design. This thesis encompassed SPMS subjects from the MS-SMART trial into a nested, UCL site only, cohort study. This allowed for co-recruitment, and shared outcome measures in addition to a further cognitive battery and rs-fMRI protocol.

1.5.1 Drug selection

The MS-SMART trial design incorporated two elements. Firstly, repurposing of drugs, or drug repositioning, or reprofiling is when already approved drugs are applied to new diseases. This reduces the drug development times and costs (De Angelis *et al.*, 2018*b*). Secondly, by using an adaptive, multi-arm design, the drug arms can be trialled in tandem versus placebo or added to standard care (James *et al.*, 2012).

Vesterinen and colleagues developed a framework to identify oral repurposed drugs targeting neuroprotection in SPMS. They looked at preclinical studies of demyelination, inflammation, axonal loss, and neuro-behavioural changes in experimental autoimmune encephalitis (EAE) models. Phase 2a evidence from other patho-physiologically similar neurodegenerative disorders was also evaluated. From the 120 identified drugs, seven potential drugs; ibudilast, oxcarbazepine, pirfenidone, polyunsaturated fatty acids (including lipoic acid), amiloride, fluoxetine, and riluzole were shortlisted. Amiloride, fluoxetine, and riluzole were trialled in MS-SMART (Vesterinen *et al.*, 2015). Lipoic acid and ibudilast have been trialled separately and have shown positive end-points (De Angelis *et al.*, 2018*b*; Fox *et al.*, 2018).

Riluzole is licensed for use in Motor Neurone disease (MND). It inhibits voltage-dependent sodium channels and modulates glutamate receptors to reduce glutamate release. A study in PPMS showed a reduction in cervical cord atrophy and the number of new T1 hypointense lesions (Kalkers *et al.*, 2002). Riluzole has been shown to lead to persistent sodium channel block and modulate glutamate release in rat studies (Lamanauskas and Nistri, 2008) and in isolated myelinated nerve fibres of frogs (Benoit and Escande, 1991). The action on glutamatergic transmission may be via modulation of receptors (Mizoule *et al.*, 1985; Martin *et al.*, 1993) or inhibition of release (Jehle *et al.*, 2000), or actions on NMDA neurotoxicity (Estevez *et al.*, 1995) in rat models. A phase 2 trial of 16 progressive MS subjects and riluzole found a reduction from -2% (year 1) to -0.2% (year 2) of spinal cord atrophy, reduction in new T1 hypointense brain lesions from 15% in year 1 to 6% in year 2, and reduction in atrophy of the whole brain from -1.0% (year 1) to -0.7% (year 2) (Killestein *et al.*, 2005).

Amiloride is an ASIC-1 antagonist used as a clinical diuretic. ASIC-1 are present in inflammatory lesions and in acidic conditions, they activate and lead to sodium and calcium overload resulting in axonal degeneration via apoptosis (Vergo *et al.*, 2011). A pilot study of 14 PPMS subjects showed reduced rates of brain atrophy with amiloride compared to the pre-treatment phase ($p<0.05$). There were no beneficial effects on acute optic neuritis (Arun *et al.*, 2013).

Fluoxetine is a selective serotonin reuptake inhibitor used clinically to treat depression. It stimulates glycogenolysis and improves mitochondrial energy metabolism leading to neuroprotective mechanisms (Zeis *et al.*, 2015). An exploratory study of non-depressed RRMS and SPMS subjects receiving 20mg fluoxetine or placebo for 24 weeks showed a reduction of gadolinium enhancing lesions in the treated arm at 24 weeks, but this finding was most significant in the last 16 weeks indicating a lag phenomenon of benefit (Mostert *et al.*, 2008). A phase 2 2-year study of a mixed cohort of 42 SPMS (69%) and PPMS subjects randomised to 40mg fluoxetine versus placebo showed no evidence of significant benefit with fluoxetine therapy on measures of disability progression; the EDSS, ambulatory index, or 20% change in the 9HPT but

suggested better grey matter and white matter volume in the fluoxetine group. They suggested a larger sample size and this led to the phase 2 randomised controlled trial of fluoxetine, FLUOX-PMS. 127 subjects with progressive MS (57% SPMS) were assessed for the primary outcomes; time to 12-week confirmed 20% increase in the T25FW or 9HPT. The placebo group showed an unexpectedly low rate of disease progression reducing the statistical power of the study, and overall fluoxetine showed no treatment effect versus placebo on measures of disability (Cambron *et al.*, 2019).

1.5.2 Methods

MS-SMART is an investigator-led, multi-arm, parallel group, double-blind, randomised placebo-controlled trial which was undertaken at 13 neuroscience centres in the UK. Patients were screened for enrolment and met the inclusion and exclusion criteria described in **table 1.4**. The main inclusion criteria included; age 25–65 years, with a confirmed diagnosis of SPMS, EDSS score between 4·0 and 6·5, evidence of steady disability progression in the preceding 2 years (with either an increase of at least 1 point in EDSS score or a clinically documented increase in disability), Beck's Depression Index II score <19, absence of relapse in the 3 months pre-screening, and no concurrent use of DMTs. All subjects gave written, informed consent for the study (Connick *et al.*, 2018; Chataway *et al.*, 2020).

See **section 3.2** for a summary of the test protocol and assessments. After the baseline visit, patients were seen and undertook safety bloods at weeks 4, 8, 12, 24, 36, 48, 72, and 96, with a final safety telephone call at week 100. MRI brain was performed at screening, week 24 and week 96.

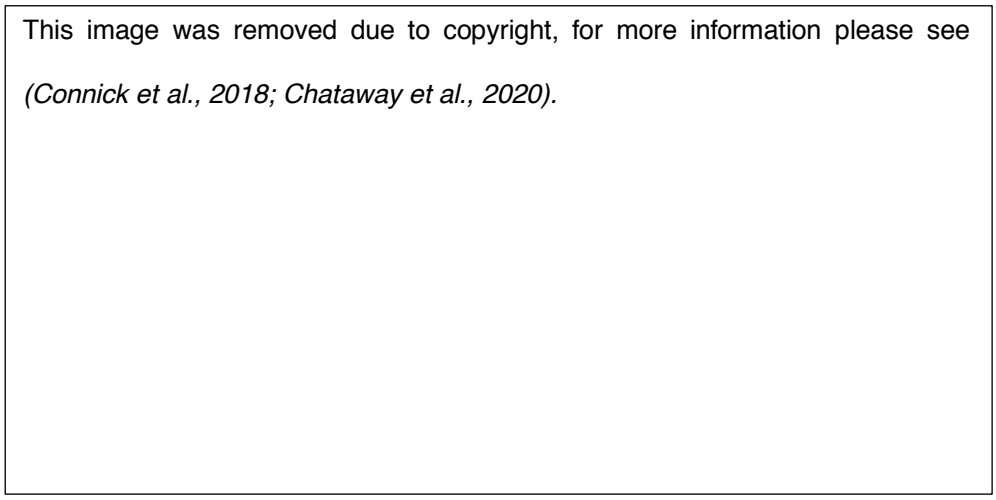
The MRI core protocol included volumetric T1, fluid attenuated inversion recovery (FLAIR), T2, and PD weighted images. To measure the primary endpoint; PBVC, the Structural Image Evaluation using Normalization of Atrophy (SIENA) method was used (see **section 2.4.3**). SIENA is automated and calculates the integral of the edge motion per voxel from the follow-up to

baseline scan. MRI secondary endpoints were counts of new or enlarging T2 lesions at 96 weeks and PBVC at 24 weeks.

Clinical secondary end points were changes from baseline to weeks 48 and 96 in EDSS score, the T25FW, the 9HPT, the PASAT3, the MSFC score, the Symbol Digit Modalities Test (SDMT), high contrast (100%) visual acuity, and Sloan low contrast visual acuity (contrast 5%, 2·5%, and 1·25%).

Sample size calculation for the trial was based on a study from Altmann et al. (Altmann *et al.*, 2009). To allow for a power of 90% statistical significance, the aim was to recruit 110 subjects per arm to detect a change in PBVC of 40% allowing 10% drop-out and 10% discontinuation (Furby *et al.*, 2010). A centralised web-based system randomly assigned patients at baseline (within 30 days of screening) to twice-daily (once daily for the initial 4 weeks) oral treatment of either amiloride 5 mg, fluoxetine 20 mg, riluzole 50 mg, or placebo for 96 weeks. Capsules were identical in appearance. Randomisation included minimisation based on sex, age, EDSS score at randomisation, and trial site.

Table 1.4 The Multiple Sclerosis Secondary Progressive Multi-arm Randomisation Trial (MS-SMART trial) eligibility criteria.



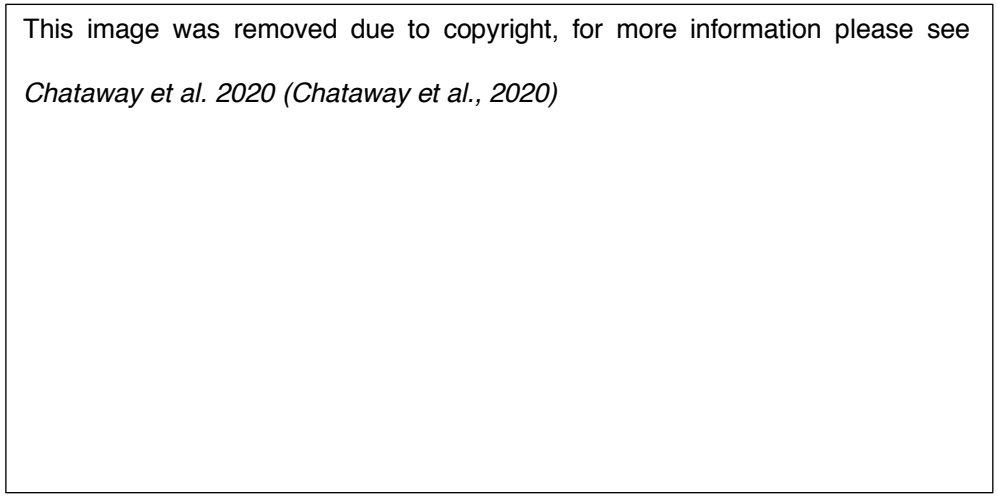
(Connick et al., 2018; Chataway et al., 2020). LFT=liver function tests, ALT=alanine aminotransferase, AST=aspartate aminotransferase, GGT=gamma-glutamyl transferase, WBC=White blood cell count, SSRI=selective serotonin reuptake inhibitor.

1.5.3 Results

Between January 29, 2015, and June 22, 2016, 445 patients were randomly equally allocated to amiloride (n=111), fluoxetine (n=111), riluzole (n=111), or placebo (n=112). Percentage loss to follow-up was 5-9% overall. The primary analysis was a complete-case analysis based on the intention-to-treat population (the 393 patients with data at week 96). These were allocated amiloride (n=99), fluoxetine (n=96), riluzole (n=99), and placebo (n=99). Serious adverse events were minimal, 42 subjects in total across all arms and were not emergent. The three reported trial deaths were unrelated to study treatment; metastatic lung cancer, ischemic heart disease, and sudden death (**figure 1.8)** (Chataway *et al.*, 2020).

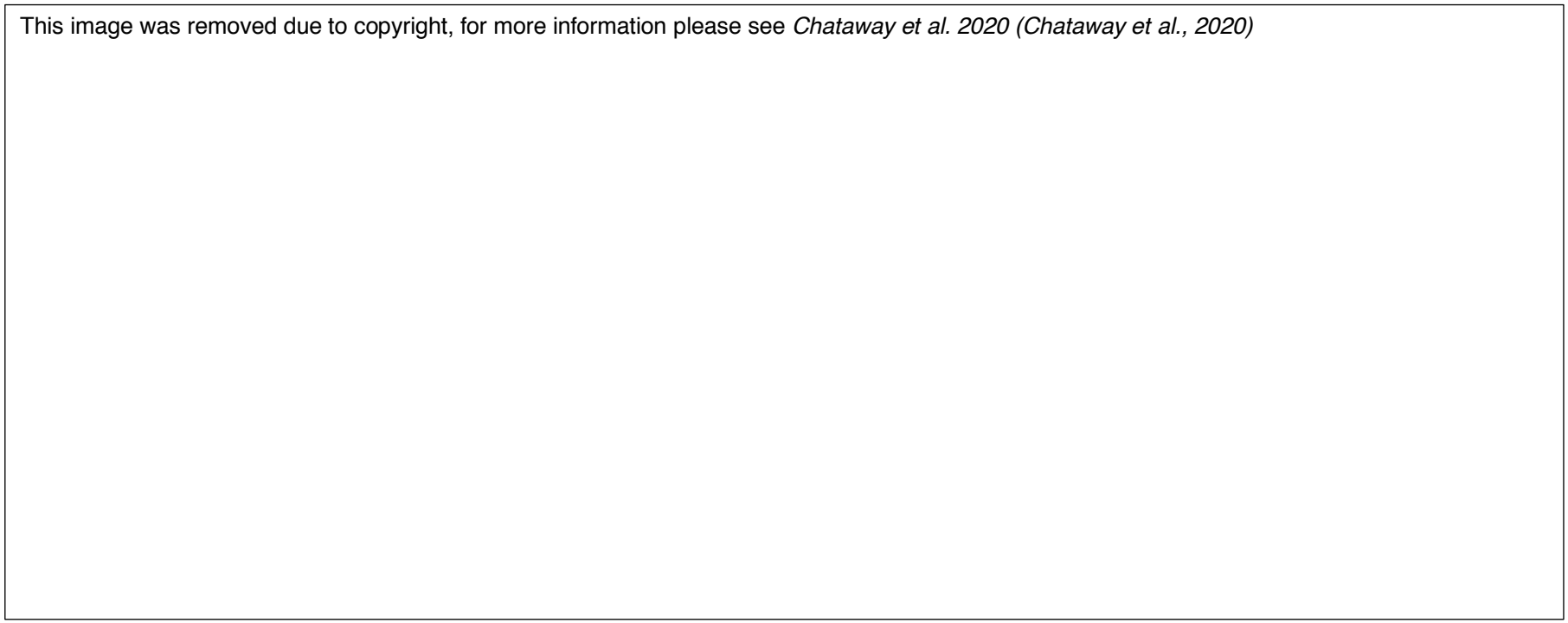
Although not known at the start of this thesis, the MS-SMART trial results indicate that there were no significant differences between group demographic characteristics, and additionally MRI whole brain volume and T2 lesion volume at baseline between drug arms. Mean age was 55.5 years old. Median disease duration was of 21 years (interquartile range 15–29 years), median EDSS was 6 (5.5-6.5), with a median secondary progression of 6 years (interquartile range 3–10 years). **Figure 1.9** shows that the primary outcome measure, PBVC at 96 weeks, showed no difference (amiloride versus placebo, 0·0% (95% CI –0·4 to 0·5; p=0·99); fluoxetine versus placebo –0·1% (–0·5 to 0·3; p=0·86); or riluzole versus placebo –0·1% (95% CI –0·6 to 0·3; p=0·77)). In the placebo arm there were significant changes from baseline to 96 weeks in terms of EDSS scores, the T25FW, and Sloan low contrast visual acuity (contrast 1·25%). The majority of subjects showed no change in baseline EDSS (**Figure 1.10**) (Chataway *et al.*, 2020).

Figure 1.8. The MS-SMART trial profile. 445 subjects were randomised into the trial.



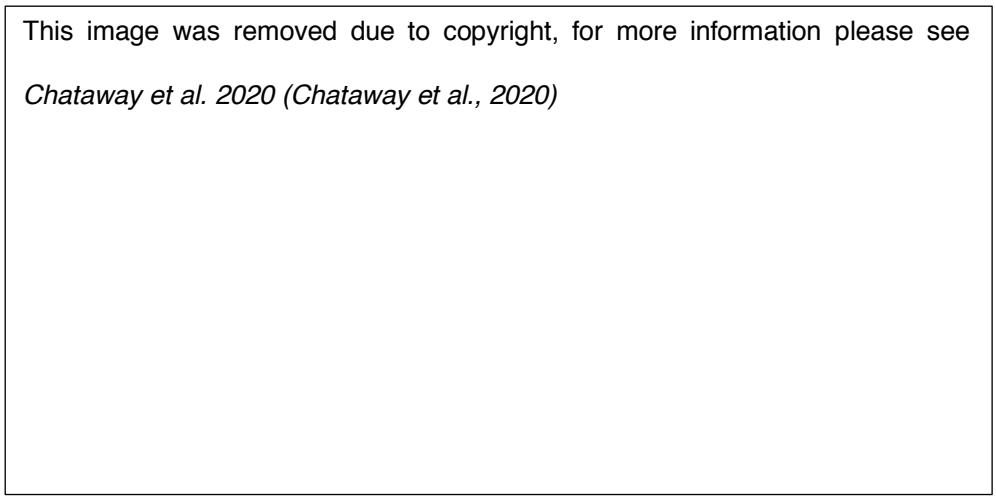
**All patients lost to follow-up at any time during active follow-up (i.e., up to and including the 100-week telephone call). Two patients withdrew after the 96-week MRI scan but before the end of the study (one allocated riluzole and one allocated placebo) and were included in the primary analysis. Two patients allocated riluzole also received fluoxetine prescribed by their family doctor towards the end of the trial. One patient allocated riluzole was withdrawn by a clinician: all other withdrawals were the patient's decision. Adapted from Chataway et al. 2020 (Chataway et al., 2020).*

Figure 1.9. Boxplots of percentage brain volume change (PBVC) by drug arm in MS-SMART. At (A) 24 weeks and (B) 96 weeks.



Horizontal lines are median and IQR; whiskers extend to the minimum and maximum within 1·5 times the IQR; outliers are shown as individual points. Mean PBVC by study group (C), for patients with PBVC data at both 24 and 96 weeks (n=374); whiskers are SD. PBVC=percentage brain volume change. Used with permission from Chataway et al. 2020. (Chataway et al., 2020).

Figure 1.10. Stacked bar charts for change in EDSS in the MS-SMART trial by drug arm.



Therefore, only targeting neuroaxonal damage and loss in SPMS is likely insufficient to slow or stop axonal neurodegeneration. This indicates that different or simultaneous targeting of pathophysiological pathways is required. The MS-SMART trial has indicated the positivity of combination trials which efficiently speed up drug trials and research and has set a precedent in MS drug trial design (Chataway *et al.*, 2020). In terms of this thesis, this trial design may be useful to accelerate our management of cognitive deficits in MS in the future.

2 Cognition and Multiple Sclerosis

2.1 Introduction

Cognitive deficits are common, and untreatable in MS, and affect up to 70% of patients with progressive MS (Langdon, 2011; Connick *et al.*, 2013; Strober *et al.*, 2014) and is evident in around 50% of early MS patients when there is little motor disability (Migliore *et al.*, 2017). This leads to serious socioeconomic effects in this young adult population. Cognitive impairment in MS is not a new concept. In 1877 Charcot described cognitive efficiency and working memory difficulties in his MS patients (Charcot, 1885, 1888). However, it was really around thirty years ago that Rao and colleagues highlighted the importance of cognitive deficits in MS (Rao *et al.*, 1991). Processing speed and episodic memory deficits have been confirmed as the most commonly impaired cognitive domains in MS (Rocca *et al.*, 2015*a*).

A consensus group have highlighted that there are several areas of cognition in MS that require focus and future development (Sumowski *et al.*, 2018). Firstly, cognitive assessment and the measurement of deficits requires integration into clinical trials and standard MS care to recognise this prevalent and debilitating symptom. There are very few longitudinal studies of cognitive change over time or cognitive decline. Further development and a better interpretation of changes in outcome measures over time is required. This will allow the integration and monitoring of cognitive metrics in MS clinical trials (Benedict and Walton, 2012).

As discussed in **chapter 3**, neuroimaging is allowing an insight into the underlying mechanisms of MS cognitive deficits. Cognitive deficits in MS are more due to underlying GM dysfunction than lesional disease alone, supporting a disconnection theory of underlying diffuse anatomical abnormalities (Dineen *et al.*, 2009; Riccitelli *et al.*, 2011, Rocca *et al.*, 2015*b*). Structural damage alone does not fully account for cognitive performance, and so an understanding of the role of functional connectivity (FC) changes is developing (Damoiseaux and Greicius, 2009; Meijer *et*

al., 2018). There is also the suggestion in other neurodegenerative disease such as Alzheimer's disease that functional MRI changes may precede clinical symptoms and structural MRI changes (Corriveau-Lecavalier *et al.*, 2020).

Finally there are currently no effective drug interventions to prevent and treat MS cognitive impairment (Mitolo *et al.*, 2015).

This thesis focuses on cognitive function in SPMS. There is a clear need for knowledge of this phenotypic cognitive profile in the literature (Sumowski *et al.*, 2018). SPMS CI appears to include executive dysfunction in addition to the known working memory and information processing deficits of MS (Connick *et al.*, 2013; Chan *et al.*, 2017). Perhaps this is due to the a less inflammatory trajectory with more neuronal loss in SPMS, and the involvement of deep brain and more extensive grey matter areas (Lassmann, 2018). Therefore, current strategies suggested for the measurement of CI in MS may not be suitable (Benedict *et al.*, 2020).

2.2 Cognitive deficits in MS

Studies have shown that information processing speed is slowed in about 50% of MS patients (Muhlert *et al.*, 2015), and visual learning and memory are affected in a similar proportion (Muhlert *et al.*, 2014). Other cognitive domains affected include attention, and executive function (Muhlert *et al.*, 2013; Preston *et al.*, 2013). Many cognitive domains seem resilient to MS including simple attention, naming and comprehension abilities (Rocca *et al.*, 2015*a*; Sumowski *et al.*, 2018). The interplay between the different cognitive domains is not fully understood. It may be that there are dependent or independent correlations between domains, e.g. processing speed and delayed learning, however it is not certain that these are causal, or that treating one might improve the other (Sumowski *et al.*, 2018). People with MS usually report that they have difficulties with multitasking and word finding, which are not well investigated in the literature. Ideally dual cognitive-cognitive or cognitive-motor tasks may establish a better model of these scenarios in

the research setting (Sumowski *et al.*, 2018). Despite cognitive impairment being present in all types of MS, the individual phenotype of MS affects the pattern of cognitive impairment.

In CIS cognitive function seems to be independent of structural MS damage. Cognitive performance was not significantly correlated with neuroimaging markers of MS, i.e. NAWM, NAGM, T2 lesion load (Glanz *et al.*, 2007). However, FC changes are present compared with controls, indicating more diffuse underlying pathology and this worsens with cognitive performance (Roosendaal *et al.*, 2010*b*). The cognitive impairment in MS study (COGIMUS) was a study of 550 subjects who underwent cognitive and MRI testing and who showed correlation between these markers, particularly with T2 lesion volume. Overall 20% of this early MS group were cognitively impaired(Patti *et al.*, 2010).

Longitudinal studies have shown that in early relapsing MS there are impairments in verbal memory recall and acquisition, and reasoning. After 4.5 years there were additional deficits in verbal fluency and comprehension. At 10 years as disease became more progressive, verbal fluency and comprehension impairments occurred (Amato *et al.*, 2001, 2008*b*).

Despite improved general phenotyping of cognitive deficits in MS, the specific changes in SPMS, and the mechanisms which underlie these deficits, are not clearly understood (Huijbregts *et al.*, 2004, Rocca *et al.*, 2015*a*; Sumowski *et al.*, 2018). People with SPMS performed worse when tasks involved higher order working memory than patients with RRMS and PPMS (Huijbregts *et al.*, 2004). In a comparative study of SPMS and PPMS, fronto-executive dysfunction has been detected only in SPMS using the Addenbrooke's Cognitive Examination-Revised (ACE-R). Visuospatial disturbance was found in overall in progressive MS (Connick *et al.*, 2013). MS-STAT1 had a tandem cognitive sub-study which recruited 140 SPMS subjects (Chan *et al.*, 2017). Neuropsychological evaluation occurred at 0, 12, and 24 months. Cognitive domains tested include; premorbid IQ; general intellectual functioning; verbal and nonverbal memory; semantic memory; visual perceptual function; attention, speed of information processing, and working memory (PASAT3); frontal lobe function (frontal assessment battery, (FAB)). Neuropsychiatric

symptoms were assessed using the Hamilton Depression Scale and the Neuropsychiatric Inventory Questionnaire. Linear mixed models evaluated change at 0,12, and 24 months and the effect of simvastatin at 0 and 24 months. At baseline 45% had abnormalities of frontal lobe function, there was also a significant number of patients (up to 33%) with impairment on tests of verbal and nonverbal memory. Over the entire trial, the whole cohort declined on tests of verbal and non-verbal memory (t score 5.7 points). At 24 months, there was a significant difference in FAB scores between the two treatment groups, with a 0.24-point increase in the mean FAB score observed in the active arm, compared with a decline of 0.92 points in the placebo group: a difference of 1.08 (95% CI 0.09 to 2.14). No treatment effect was observed on any other cognitive or neuropsychiatric measure. At 24 months the primary cognitive decline was in working memory function (Chan *et al.*, 2017).

Cognitive deficits present early in the MS course progress and become be more apparent in SPMS (Huijbregts *et al.*, 2004, Rocca *et al.*, 2015*a*; Sumowski *et al.*, 2018). In PPMS there is dysfunction in all domains of cognition, but this is felt to be less than in SPMS (Amato *et al.*, 2008*b*; Islas and Ciampi, 2019). A comparative study found that RRMS subjects displayed deficits only in working memory and processing speed, as opposed to all cognitive domains in primary progressive disease despite correcting for disability using the EDSS (Ruet *et al.*, 2013*a*). A meta-analysis of 47 studies found more severe cognitive impairment in each cognitive domain in PPMS than RRMS despite matched levels of fatigue and depression which were not due to other heterogeneity (Johnen *et al.*, 2017). Longitudinal studies of PPMS cognitive change are lacking, but overall there may be up to 40% cognitive decline over a two year period (Amato *et al.*, 2008*b*).

2.3 The impact of cognitive impairment in MS

Cognitive impairment is a key cause of unemployment independent of motor disability levels in MS (McCrone *et al.*, 2008). Cognitive function is imperative to aspects of daily life, and are impacted by and impact on MS. Ruet et al undertook a 7-year longitudinal follow-up study of 65 newly diagnosed MS subjects. At baseline 82% of subjects were working, but this reduced to 54%

at 7 years. 73% of those not working at 7 years were cognitively impaired, compared to 45% being cognitively impaired at baseline. Worsening of both motor function (EDSS) and information processing speed were significantly associated with vocational status at 7 years (Ruet *et al.*, 2013*b*).

Cognitive impairment affects many aspects of Health Related Quality of Life (HRQoL) in all MS subtypes (Langdon, 2011). A third of patients have been found to have a major decline in their standard of living after their diagnosis and half of all patients are unable to fulfil employment responsibilities within 10 years of onset (Mitchell *et al.*, 2005; Glanz *et al.*, 2010). Motor weakness and MRI lesions have been shown to correlate less with HRQoL of patients than psychosocial factors including; coping, mood, and perceived support (Mitchell *et al.*, 2005). Despite high levels of motor disability, studies of progressive MS have shown associations only with measures of cognitive function; the SDMT, PASAT3, and the trail making test B (TMT- B)) and overall quality of life, short form 36 (SF- 36) questionnaire (Højsgaard Chow *et al.*, 2018). Quality of life is also related to the ability of a patient to cope with their MS diagnosis and the uncertain disease pathway which is affected by measures of depression, divided attention and verbal intelligence measures. Baseline depression and memory z-scores were shown to be independent predictors for HRQoL at 7 years (Ruet *et al.*, 2013*b*). Work capacity is associated with quality of life in MS, and both are significantly affected by fatigue, cognition and emotional distress (Flensner *et al.*, 2013). Interestingly, the coping ability of the support network, i.e. offspring and partners, was not shown to be affected by patient cognitive or mood factors (Ehrensperger *et al.*, 2008).

In terms of the management of MS itself, cognitive deficiency affects day-to-day functions such as medication adherence (Treadaway *et al.*, 2009; Langdon, 2011; Petracca *et al.*, 2018). In addition there is an impact of cognitive performance on rehabilitation efficacy and performance with therapies (Thompson *et al.*, 1997; Fox *et al.*, 2012; Sumowski *et al.*, 2018).

2.4 Factors impacting cognitive performance in MS

There are several factors which compound the phenotypic variations of MS cognition. Cognitive reserve refers to the mismatch of disability and cognitive performance in neurological diseases. This is partly accounted for by intellectual enrichment, which is determined by education and vocabulary attainment and may increase the threshold before which cognitive impairment occurs. Intelligence quotient (IQ) and years of education account for education attainment and should be considered when reviewing cognition (Sumowski *et al.*, 2018). The cognitive reserve hypothesis may explain some of the differences between MS phenotypes. Task fMRI has investigated this using a working memory task and showed strong correlations between intellectual enrichment, as measured by education and vocabulary, and default mode network FC, and increased prefrontal resting state activity when enrichment was low. Cognitive performance using the SDMT was explained by the level of enrichment, and this was protective against cognitive impairment (Sumowski *et al.*, 2010*a*). In more progressive phenotypes intellectual enrichment seems to remain protective against cognitive impairment. 25 SPMS subjects were investigated with information processing speed and memory tasks, and if the level of intellectual enrichment was high enough, memory deficiency was absent versus healthy controls. Therefore, higher cognitive reserve in the SPMS group cancelled out the disease effects of SPMS on overall cognitive status (Sumowski *et al.*, 2012). In MS, occupational attainment has additionally been shown as an independent predictor of information processing speed, memory, and executive domains after including measures of brain atrophy and IQ in the model (Ghaffar *et al.*, 2012).

As well as cognitive reserve, brain reserve has an impact on cognitive status as explored in **chapter 3**. Brain reserve is hypothesised to be the maximal lifetime brain volume, which is purportedly determined by genes, and corrected by intracranial volume, T2 lesion load, and atrophy. A study of mixed MS phenotype showed that brain and cognitive reserve had separate effects on overall cognitive performance. Cognitive status positively associated with intracranial volume (R^2 0.066, p=0.017). Controlling for brain reserve, higher education (R^2 0.047, p=0.030) and leisure (R^2 0.090, p=0.001) predicted better cognition. At the domain level, brain reserve

supported efficient information processing speed, whereas cognitive reserve prevented memory deficits more (Sumowski *et al.*, 2013). Therefore, as well as intrinsic genetic and disease processes, there are separate factors related to individual environment, i.e. enrichment, which impact overall cognitive performance in MS.

Part of the difficulty understanding cognition in MS is the awareness of a mismatch between classical markers of MS disease progression, and cognitive impairment, i.e. there can be minimal motor disability and MRI structural damage when there is significant impairment. This is something that has been noted historically (Jennekens-Schinkel and Sanders, 1986). The idea of "cognitive relapses" of MS without sensorimotor symptoms may be the only underlying symptom or sign of MS disease activity and therefore effective cognitive monitoring tools are key to detect this (Benedict et al., 2014). The location of MS lesions also plays a role in the type and frequency of cognitive impairment in MS. Lower SDMT scores correlated with more lesions present in the posterior corona radiata, particularly the forceps major (Rossi et al., 2012). It is likely that GM pathology underlies cognitive impairment more than white matter disease in MS, and this is explored in **section 2.4** (Rocca et al., 2015a).

Anxiety has been shown to be associated with overall MS cognitive functioning and lower scores on the SDMT in a cohort of 111 MS subjects (Marrie *et al.*, 2019). Apathy is another factor to be considered in MS cognitive performance. Cognitive function as measured by the BRNB (Brief Repeatable Neuropsychological Battery) was shown to be significantly correlated to depression and apathy scoring systems in a cohort controlled study of RRMS subjects (Niino *et al.*, 2014). Personality has been shown to have an impact on MS cognitive performance. In a study of 80 people with MS, openness was linked to better memory performance, and neuroticism and conscientiousness to worse memory function (Leavitt *et al.*, 2017).

Fatigue is known to compound cognitive function in MS. Current management of MS fatigue highlights that there is currently no significantly effective drug therapy. In a double-blind placebo controlled trial, amantadine hydrochloride showed some significant subjective improvements on

daily function, but not on objective neuropsychological performance (Cohen and Fisher, 1989). Therapy for fatigue is focused on occupational therapy and physiotherapy with graded exercise regimes and review of daily activities. When considering the effect of fatigue on cognitive performance in MS it is useful to consider the different modalities of fatigue. Motor fatigue is due to motor weakness impacting tasks, cognitive fatigue is due to difficulties sustaining attention, and lassitude is a subjective assessment of reduced energy. All of these modalities impact cognitive attainment in MS (Schwid *et al.*, 2002). MS subjects with fatigue have higher levels of proinflammatory cytokines than controls (Heesen *et al.*, 2006).

2.5 Measurement of cognitive deficits in MS

Cognitive assessment is vital to understand the phenotype, course, and prevalence of cognitive impairment and decline in MS (Hobart *et al.*, 2004; Julian, 2011; Cohen *et al.*, 2012; Sumowski *et al.*, 2018). Currently, these are not standard practice in the clinical setting. The benefit of cognitive outcomes in MS are that they allow the screening and early detection of CI, allowing earlier use of interventions and delaying potential decline. However, as described in chapter 1 section 1.3.5, cognitive symptoms due not form part of the current diagnostic criteria for MS. If outcome measures were in routine clinical use, they may allow for better monitoring of clinical function, treatments, and trial outcomes (Benedict *et al.*, 2020).

Cognitive outcome measures encompass neuropsychological tasks, and PROMS. The main cognitive domains affected in MS, and key assessment tools are described below (**table 2.1**).

Table 2.1. Summary of cognitive outcome measures in MS by cognitive domain.

Domain	Test
Learning and memory (verbal)	Selective Reminding Test (SRT)
	California Verbal Learning Test version 2 (CVLT-II)
Learning and memory (visual)	Spatial Recall Test (SPART)
	Brief Visuospatial Memory Test- Revised (BVMT-R)
Visuospatial function	Rey-Osterrieth Complex Figure (ROCF) test–recall task
Information Processing Speed	Paced Auditory Serial Additions Test (PASAT3)
	Symbol digit modalities test (SDMT)
	Letter digit substitution test (LDST)
	Spatial recall test (SPART)
	Controlled Oral Words Association Test (COWAT)
Executive	Word list generation (WLG)
	Verbal Fluency Test (VFT)
	Word learning test (WLT)
	Wisconsin card sorting test (WCST)
	Frontal Assessment Battery (FAB)
	Hayling and Brixton Tests
	Benton Judgement of Line Orientation Test (BJLO)
	Short story test (SST)
	Delis-Kaplan Executive Function System Sorting Test (DKEFS)

Learning and memory (episodic memory)

Episodic memory is the amount of information learnt and recalled; the autobiographical report of spatio-temporal events. In MS the main difficulty is with memory acquisition. However, there are difficulties with encoding, storing, and retrieving long term memories. Episodic memory is the most reliably affected domain in MS (Islas and Ciampi, 2019). It is mediated by the hippocampus and default mode network structures (see **table 3.2 section 3.3.2**) *(Biswal et al., 1995; Beckmann et al., 2005; Smith et al., 2009; Power et al., 2011; Lee et al., 2013).*

The California Verbal Learning Test-II (CVLT-II) is a verbal learning task. It has high sensitivity and good age- sex- normative data. There is an alternative form available increasing the validity for longitudinal use (Delis *et al.*, 2000). The Selective Reminding Test (SRT) is another verbal episodic memory task with high sensitivity and alternate forms are available. However, it relies on recall from long-term memory and not episodic working memory. Normative data is also lacking (Strauss *et al.*, 2006). Both the SRT and CVLT-II rely on verbal recall and so it would be difficult to translate these into app-based tests.

The Brief Visuospatial Memory Test-Revised (BVMT-R) has very high sensitivity to visuospatial memory function. There is good reliability and validity and it is briefer to complete than some other tests available. There are 6 validated versions and good normative age-sex- validated data. However, it is impacted by upper limb motor and sensory disability (Strauss *et al.*, 2006).

Processing speed and attention (working memory)

Processing speed is defined as the amount of work undertaken in a defined time; short term memory span. Information processing speed is strongly affected by attention. In MS, the main impairment is the ability to maintain and manipulate information short term (working memory) and to process this quickly (information processing) (Islas and Ciampi, 2019). Working memory is mediated by fronto-parietal network structures (see **table 3.2 section 3.3.2**) *(Biswal et al., 1995; Beckmann et al., 2005; Smith et al., 2009; Power et al., 2011; Lee et al., 2013)*.

The PASAT3 is a measure of mental flexibility, calculation ability, and has moderate sensitivity for auditory information processing speed. Stimuli are auditory and therefore affected by hearing. Additionally, there are strong practice effects and ceiling effects. Patients tolerate the task poorly and it can be affected by arithmetic acumen and anxiety (Gronwall DM., 1977). PASAT3 is not a sensitive marker of disease progression in MS, therefore the clinical utility is becoming limited (Rudick *et al.*, 2009*a*; Cohen *et al.*, 2012).

The SDMT relies on processing speed, working memory and vision. It was initially developed to test processing speed in the army, but then developed validation in MS. Inter-rater reliability is good to excellent and it is brief to administer. It has international validation and no floor or ceiling effects. It can be influenced by vision (Smith, 1982). It is the most sensitive test of MS cognitive function with a sensitivity of 82% and specificity of 60% (Sumowski *et al.*, 2018). An 18 year follow-up study of RRMS subjects from the intramuscular interferon trial showed that the SDMT had the greatest group decline and may serve as a cognitive performance outcome measure (Strober *et al.*, 2014). An overall expert consensus panel has suggested that a responder definition of change of 4 points or 10% in the baseline SDMT score is meaningful (Benedict *et al.*, 2017).

Executive function

Executive function is procedural memory of learnt motor and cognitive routines. This includes planning, decision-making, response to feedback, inhibition, and flexibility. Executive function requires intact information processing speed and so is affected by this domain (Islas and Ciampi, 2019). Main tests of MS executive function are listed in **table 2.1**. There is considerable variability in the prevalence rates of executive function in MS, overall it is likely a mild deficit, but there is reliability on other cognitive domains (Drew et al., 2008). The Delis-Kaplan Executive Function System Sorting Test (DKEFS) has an alternative form available with moderate sensitivity overall. The Benton Judgement of Line Orientation Test (BJLO) has good reliability and validity as a visuospatial and executive task, but lower sensitivity overall (Sumowski et al., 2018). This is mediated by structures including the cerebellum (see **table 3.2 section 3.3.2**) *(Biswal et al., 1995; Beckmann et al., 2005; Smith et al., 2009; Power et al., 2011; Lee et al., 2013)*.

Language

Language dysfunction is tested by tasks such as object naming, word finding, verbal fluency, grammar, and receptive language (Islas and Ciampi, 2019). The Controlled Oral Words Association Test (COWAT), Word list generation (WLG), Verbal Fluency Test (VFT), and Word learning test (WLT), measure language function but are impacted by attention and executive

function (Strauss et al., 2006). The COWAT has moderate sensitivity for MS cognition and has a validated alternative form (Sumowski et al., 2018).

The effect sizes of the neuropsychological tests in use suggests a hierarchy in MS of the SDMT, BVMT-R, CVLT-II, SRT, COWAT, and then the PASAT3 (Benedict *et al.*, 2017). **Table 2.1** summarises the main cognitive assessments and the domains that they relate to.

Cognitive batteries in multiple sclerosis

Many neuropsychological assessments form part of a composite test battery (**table 2.2**). This allows for testing of more than one cognitive domain. The Neuropsychological Screening Battery for MS (NSBMS) was developed by the National MS Society in the United States. The NSBMS included measures of processing speed, working memory, verbal fluency, and episodic visual and verbal memory. The SDMT outperformed the NSBMS in assessing cognitive impairment in MS, and therefore was suggested as a single sentinel test in MS (Van Schependom *et al.*, 2014). From the NSBMS the BRNB was developed to allow repeat testing. The BRNB consists of tests of processing speed, episodic memory, and language (Rao *et al.*, 1991; Boringa *et al.*, 2001). The BRNB is validated in several countries and has a method for detecting meaningful change over time, the reliable change index (RCI), making it useful in longitudinal study designs (Bever *et al.*, 1995).

Table 2.2 Cognitive test batteries in MS. Summary of the inclusive tests and domains in the main cognitive batteries used in MS.

Cognitive domain	BICAMS Brief International Cognitive Assessment for Multiple Sclerosis	MACFIMS Minimal Assessment of Cognitive Function in MS	BRNB Brief Repeatable Neuropsychological Battery
Processing Speed	SDMT	SDMT PASAT3	SDMT PASAT3
Learning and Memory	CVLT-II BVMT-R	CVLT-II BVMT-R	SRT SPART
Visuospatial function		BJLO	
Executive		DKEFS	
Language		COWAT	COWAT

In 2001 a panel consensus of psychologists and neuropsychologists developed the Minimal Assessment of Cognitive Function in MS (MACFIMS). MACFIMS had good validity and reliability and takes only 90 minutes to complete (Benedict *et al.*, 2002). The 7 component tests evaluate 5 cognitive domains; processing speed, working memory, learning and memory, executive function, visuospatial processing and word retrieval. Regression based normative data account for demographic variables including age, sex, and education, and cross-cultural differences (Benedict *et al.*, 2006; Dusankova *et al.*, 2012). The MACFIMS and the BRNB appear to have comparable sensitivities. The SDMT was the most sensitive individual neuropsychological outcome test in both batteries for cognitive impaired status (Strober *et al.*, 2009).

The Brief International Cognitive Assessment for Multiple Sclerosis (BICAMS) includes the SDMT, the first 5 trials of the CVLT-II, and immediate forms of the BVMT-R. It was recommended as a short clinical cognitive measurement that can be used in practice internationally (Benedict *et al.*, 2012; Langdon *et al.*, 2012). There is good international validity with normative data sets available (Sandi *et al.*, 2015; Ozakbas *et al.*, 2017; Sousa *et al.*, 2018). BICAMS has shown good reliability when compared with other batteries such as the BRNB. If one of the tests contributing to impairment on the BRNB and BICAMs was the SDMT then agreement between the batteries was moderate at 0.46 (Cohen's K statistic), without the SDMT it was poor at 0.3 (Cohen's K statistic) (Niccolai *et al.*, 2015). This further supports evidence for the accuracy of the SDMT for overall MS cognitive impairment.

The MSFC is used as a clinical trial outcome measure of general disability in MS (Rudick *et al.*, 2002). As described in **section 1.2.7**, the MSFC consists of a cognitive measure; PASAT3, ambulatory measure; T25FW, and the 9HPT to assess upper limb function. Results from these three domains are then transformed into z-scores based on a reference population and then averaged to form the composite score. The PASAT3 is a 3 second modification of the original PASAT task and evaluates processing speed and working memory (Strauss *et al.*, 2006). Overall, the MSFC has good inter-rater reliability and predictive value with the EDSS. The MSFC is not used primarily as a trial outcome measure, however a change of 15-20% of the baseline in the individual tests at certain time points; e.g. 3 or 6 months can be. Suggestions of the SDMT replacing PASAT3 in the MSFC have arisen due to longitudinal assessments of changes and correlations overtime. In a 5-year study there were no changes in MSFC over time, whereas EDSS deteriorated. However, if the SDMT was substituted there were correlations at all timepoints with the EDSS, which were not present with PASAT3. However overall these were not significant, and so it is advisable that perhaps the SDMT should be used as an adjunctive test to the MSFC (Brochet *et al.*, 2008).

Cognitive assessment can be considered time consuming in the clinical setting. The development of app-based assessments, e.g. the Processing Speed Test (PST), based on the SDMT, which

is part of the MS Performance Test, a tablet based MSFC, could enhance their use and make them standard care and a possible monitoring tool.

Other paraclinical markers are associated with cognitive function in MS. Coric et al highlighted an example of this with correlation of the thickness of the peripapillary retinal nerve fibre layer (pRNFL) measured by optical coherence tomography (OCT) with cognitive impairment in MS (Coric *et al.*, 2018). sNfL has been found in greater levels in cognitively impaired versus preserved MS subjects; 27.2 versus 20.6iU respectively (Jakimovski *et al.*, 2019). Neuroimaging has revolutionised our understanding of cognition in MS (Rocca *et al.*, 2015*a*), and is discussed in **chapter 3**.

2.6 Definitions of cognitive impairment in MS

The classification of cognitive impairment in MS is not uniform and this leads to difficulty in eliciting reliable comparisons between studies (Sumowski *et al.*, 2018) (**figure 2.1**). A systematic review found 70 methods of classification that can be grouped into 20 approaches, and 3 main classification strategies, all with moderate inter-rater reliability (Fischer *et al.*, 2014).

Figure 2.1 Example of the classification of cognitive impairment.

This image was removed due to copyright, for more information please see Sumowski et al., 2018. (Sumowski et al., 2018).

The bell curve highlights cognitive performance in the population of interest. The red arrows show that there can be a change from baseline with or without meeting the dashed line cut-off for cognitive impairment despite there being an underlying change in function. Adapted from Sumowski et al., 2018. (Sumowski et al., 2018).

Criteria cited were; a critical number of abnormal parameters (n=59), composite indices (n=8), or combinations (n=3). The stringency of the criteria anticorrelated to the prevalence of cognitive impairment, but positively correlated with disease duration. Applying these classification strategies led to a range of prevalence rates of 0 to 68% in early and 4 to 81% in late MS (Fischer *et al.*, 2014). Each individual test can be underpinned by more than one cognitive domain, meaning that there is a lower specificity. It is however useful when creating trial subgroups and to investigate other measures of cognitive dysfunction, e.g. neuroimaging. Grouping patients like this also leads to a heterogenous group who might have differing individual cognitive deficits. This might make it more difficult to understand the underlying mechanisms by advanced MRI than if the research was based on more specific phenotypes, for example working memory impairment or executive dysfunction (Sumowski *et al.*, 2018). The three main classification strategies are explained below.

1) Firstly, a critical number of abnormal parameters. This means performance cut-offs of 1-2 SDs below normal values on a specified number or percentage of tests. This was the most commonly cited strategy in the literature, cognitive impairment was defined as performance 1.5 SD or 2 SD below the normative mean in 18-30% of the individual tests (Fischer *et al.*, 2014; Sumowski *et al.*, 2018).

2) Secondly, classification by composite scoring. Individual test scores are averaged to form an overall average or index of general cognitive function. The tests may have unequal contributions to the overall test score (Fischer *et al.*, 2014; Sumowski *et al.*, 2018).

3) Finally, a combination of the first two strategies. These criteria involved SD thresholds and averaging only those parameters felt to be most sensitive to cognitive impairment or summing the abnormality on parameters (Fischer *et al.*, 2014; Sumowski *et al.*, 2018).

2.7 Cognitive decline in MS

Cognitive decline encompasses a change in a cognitive function from a previous level (**figure 2.1)**. This change in function may mean that the individual is still classed as cognitively preserved even if there is a decline overall. This may be missed by the current grouped qualitative cognitive classification, i.e. impaired and preserved. The majority of cognitive research is cross-sectional and in order to use cognitive measures to monitor and evaluate treatments of cognitive progression in MS, longitudinal studies are required (Sumowski *et al.*, 2018).

Currently, there is no validated clinically meaningful change for cognitive outcome measures in MS. Using measures of physical outcome test changes, certain values have been suggested for use in research (Sumowski *et al.*, 2018). Retrospectively evaluating the tests making up the MSFC and BICAMS using employment as the outcome anchor, there were statistically significant changes in all outcome measures dependent on work status. The T25FW was the best predictor of vocational status, and this was followed by the SDMT (Benedict *et al.*, 2016). Other studies have suggested that cognitive outcome measures are better at predicting change in employment over time, but they have used EDSS rather than pure ambulation assessments. A longitudinal study of employment status found that the SDMT and CVLT-II distinguished those employed from those with employment disability best. Additionally, a change in the CVLT-II total learning score of 2 points gave an odds of employment deterioration of 3.7, versus a change in SDMT score of 4 points giving an odds of 4.2 (Morrow *et al.*, 2010). Consensus from outcome measure experts suggests that research studies should employ co-primary outcomes including a cognitive outcome test and a clinical observation of change. Secondly, this would allow statistically significant determinants of change in test scores correlated with disability or MRI metrics. Finally, using PROMs as anchors is not valid, and other demographics such as employment should be used (Benedict and Walton, 2012).

Longitudinal neuroimaging studies have identified MRI correlates of cognitive decline; atrophy measures, T2 lesion volume, microstructural damage, and cortical lesions (Rocca *et al.*, 2015*a*;

Sumowski *et al.*, 2018). Eijlers et al, undertook a 5-year follow-up study and showed an overall decline in MS cognitive function of 28%. Higher rates were present in progressive MS; 55% in SPMS and 53% in PPMS, versus RRMS patients, 21%. In this study, only measures of cortical grey matter, regional anterior thalamic and temporal atrophy, as well as longitudinal fasciculus lesions, predicted cognitive decline (Eijlers *et al.*, 2018). MRI protocols may provide a suitable metric for cognitive decline, however currently these do not exist in the clinical setting (Rocca *et al.*, 2015*a*; Grzegorski and Losy, 2017; Sumowski *et al.*, 2018).

The best measures of cognitive decline encompass both clinically meaningful change on psychological tests of cognitive function and reliable MRI measures of change over time. A study of 44 people with early MS trialled this over 7 years. From baseline, 50% deteriorated on the memory cognitive domain and 22.7% on processing speed function. Over the 7 year period change in memory function was correlated with baseline magnetisation transfer ratio (MTR) diffuse brain changes, and processing speed with whole and central brain atrophy (Deloire *et al.*, 2011).

2.8 Management of cognitive dysfunction in MS

Current cognitive rehabilitation and treatment are likely to have low efficacy in MS (Amato *et al.*, 2013; Miller *et al.*, 2018; Sumowski *et al.*, 2018), and this has been highlighted by a Cochrane review of 20 randomised trials (Rosti-Otajärvi and Hämäläinen, 2014). Mitolo et al. provide a systematic review of 33 interventional cognitive studies which were felt to have inconclusive effectiveness overall (Mitolo *et al.*, 2015).

There are no current licensed drug therapies specifically for cognitive deficits in MS, however cognitive outcomes have been investigated with some of the DMTs (Amato *et al.*, 2013; Miller *et al.*, 2018; NICE, 2018; Sumowski *et al.*, 2018). Interferon β-1a (avonex) was shown to have significant improvement on information processing speed and learning function in the phase 3 trial in RRMS (Fischer *et al.*, 2000). A further longitudinal follow-up study, COGIMUS (COGnitive

Impairment in MUltiple Sclero-Sis), reviewed the role of subcutaneous interferon β-1a (rebif) on cognition and decline in cognitive performance. At 3 year follow-up there was a 32% lower risk of cognitive impairment with 44 mcg versus 22 mcg rebif (Patti *et al.*, 2010). At 5 years, the dose of rebif continued to have an effect on cognitive impairment, as did gender, with a significantly higher input of male sex on cognitive impairment (Kappos *et al.*, 2009; Patti *et al.*, 2013). In SPMS the role of interferon beta on cognition is not as certain. Avonex was found to have significant effects on MSFC improvement in the IMPACT randomised controlled trial, however this was mainly driven by the 9HPT with some input of the PASAT3 (Cohen *et al.*, 2002). A 2 year follow-up study showed no effect of glatiramer acetate (Copaxone) on cognitive signs in RRMS (Weinstein *et al.*, 1999). However, a multicentre study of 161 RRMS subjects on interferon beta-1a, -1b, or glatiramer acetate showed positive cognitive changes at 12 months. BICAMs was used as the assessment tool, and both first-line DMTs reduced the proportion of cognitive impairment as measured by the individual BICAMs tests (Cinar *et al.*, 2017; Ozakbas *et al.*, 2017).

A 2-year follow-up study of natalizumab found significant reductions in rates of cognitive impairment and fatigue at 1 year and this was sustained (Iaffaldano *et al.*, 2012). A 2 year follow-up study of natalizumab in 51 patients with RRMS showed significant improvements in depression measures and tests of attention at 2 years, especially in the SDMT. There was little change on measures of fatigue (Kunkel *et al.*, 2015). A 3 year follow-up study of 24 RRMS subjects showed significant improvement in tests of memory, attention, and executive function associated with an increased parahimpocampal GM density (Mattioli *et al.*, 2015). These findings suggest that DMTs may decrease cognitive decline by preventing lesion acquisition over time, and so decreasing underlying lesional and non-lesional disease progression (Amato *et al.*, 2006*a*). Siponimod may also improve cognition by reducing whole brain, cortical grey matter, and thalamic atrophy and improving between group differences in SDMT scores (Benedict *et al.*, 2021).

A recent meta-analysis has shown that evidence for the use of DMTs for cognitive symptoms in RRMS is still limited, but that they are effective in improving cognitive test performance (Benedict *et al.*, 2020; Landmeyer *et al.*, 2020). DMT escalation however, is not supported by the current

evidence. There is clearly a need for more cognitive assessments in trials to ascertain DMT applications with a subsequent metanalysis and systematic review (Landmeyer *et al.*, 2020). There has been a recent call to action from stakeholders in the field to escalate this (Benedict *et al.*, 2020).

Other non-DMT, symptomatic agents, have been studied. A small pilot study showed some improvements in verbal memory function with dietary lecithin and physostigmine (Leo and Rao, 1988). The potassium channel blocker, 4-aminopyridine, showed some improvement on MS cognitive function, but this was not significant overall (Smits *et al.*, 1994). Dalfampridine is a symptomatic therapy for MS spasticity, and was shown to have significant effects on PASAT3 score and depression metrics not related to walking improvement (Korsen *et al.*, 2017).

In those with established memory impairment, the stimulant L-amphetamine may be helpful (Sumowski *et al.*, 2011). This work was supported by the immediate improvement in attention, based on the PASAT3 score with methylphenidate (Harel *et al.*, 2009). In a placebo controlled parallel group study, the stimulants amantadine and pemoline did not show significant effects on tests of attention and verbal memory (Geisler *et al.*, 1996). Modafinil targets excessive somnolence and has been studied in MS with conflicting results. However a non-placebo controlled study did show some improvement of several cognitive domains with treatment (Wilken *et al.*, 2008).

Acetylcholinesterase inhibitors are used for memory impairment in Alzheimer's disease and have been studied in MS. Although limited by sample size, a study of the acetylcholinesterase inhibitor, donepezil, showed significant improvements in learning memory with the SRT, and subjective and objective improvements in cognitive functioning, but a larger sample size study negated these results (Krupp *et al.*, 2004, 2011; Amato, 2005; Christodoulou *et al.*, 2006). Another acetylcholinesterase inhibitor, Rivastigmine, did not show a treatment effect on general memory functioning in a 3 month follow-up study (Shaygannejad *et al.*, 2008). The one study of memantine and MS cognition was negative (Lovera *et al.*, 2010).

Non-drug therapies may be helpful in MS cognitive dysfunction. Neuropsychological rehabilitation can involve several different formats and increases a patient's awareness of cognitive impairments. The underlying proposed mechanism is improving neuroplasticity (Benedict and Zivadinov, 2011; Amato *et al.*, 2013; Miller *et al.*, 2018). As neuropsychological therapy is individualised it is difficult to interpret the true impact. Overall, the Cochrane review on the impact of psychological therapies in MS summated that published literature provided only low evidence for improvement (Rosti-Otajärvi and Hämäläinen, 2014). A single randomised controlled trial (RCT) of cognitive rehabilitation, has shown persistent improvement on executive function at 7 months in 60 treated MS subjects (Hanssen *et al.*, 2016). Rehabilitation of cognitive domains can include computer-based therapies which target a wider scope of cognitive domains. A systematic review of rehabilitation techniques over the last ten years has revealed that these not only target memory and that there is expansion into other domains and other modalities including app-based techniques. Cognitive rehabilitation may improve memory function (Goverover *et al.*, 2018). Specific memory rehabilitation may be more useful in targeting MS memory dysfunction, however the evidence for this is sparse. There is evidence of significant improvement in objective memory assessments, but no differences between groups in terms of: subjective memory, mood, activities of daily living (Nair *et al.*, 2016). The Cognitive Rehabilitation for Attention and Memory in people with Multiple Sclerosis (CRAMMS) study is an RCT which will publish final results this year. Preliminary findings suggest a reduction in everyday memory problems and an improvement in mood at both 6 months and 12 months (Lincoln *et al.*, 2015). This finding has led to a summary update as a research recommendation that for memory function in MS, rehabilitation may be useful (NICE, 2018).

Physical activity may improvement subjective measures (Okuda, 2014) of MS fatigue, sleep, cognition, and depression (Bahmani *et al.*, 2019; Mayo *et al.*, 2019). Physical exercise has also been showed to improve measures of cognition, depression and fatigue in progressive MS (Briken *et al.*, 2014). There have been three main reviews of studies of exercise on MS and cognition. Sandroff and colleagues conducted a systematic review of 26 studies (including 6 RCTs)

assessing the impact of exercise and physical activity on cognition in people with MS. Overall exercise is likely to have positive effects on cognitive, pain, depression, fatigue, relapse rate as well as aerobic capacity and cardiovascular risk, but whether this is causal is difficult to confirm (Sandroff *et al.*, 2016). A systematic review included 19 studies (non-randomised) investigating the relationship between physical activity and cognitive performance in people with MS. The authors reported that 10 studies were on physical activity interventions; these reported mixed results on the effectiveness of physical activity in improving cognitive function in people with MS (Morrison and Mayer, 2017). A systematic review included 13 RCTs on the effects of exercise therapy on the quality of life of people with MS in Iran. A meta-analysis of the pooled data revealed significant, large effect sizes for exercise therapy on the mental, physical, and overall quality of life for Iranian people with MS. Due to the heterogeneous nature of the interventions in the included studies, it is however not clear what the content of an effective exercise therapy program should be (Afkar *et al.*, 2017). The evidence for the effects of exercise on cognition in MS is therefore conflicting. Results seem to be positive, but currently, there is insufficient well-designed research to definitively conclude that exercise improves or benefits MS cognition. Currently recruiting studies are trialling blinded non-exercise arms, which are difficult to consider (Motl *et al.*, 2013, 2017). One example in progressive MS is the Improving Cognition in People With Progressive Multiple Sclerosis Using Aerobic Exercise and Cognitive Rehabilitation study (COGEx NCT03679468). This is a novel randomised, multi-arm sham-controlled study of the effects of exercise and cognitive rehabilitation intervention on cognitive function in progressive MS. The study is currently actively recruiting internationally (Feinstein *et al.*, 2019).

Prevention of cognitive decline over time also involves the adaptation of lifestyle factors. These include factors to improve brain reserve; physical exercise (Briken *et al.*, 2014) and mental activity (Sumowski *et al.*, 2012). The two-year MS-STAT1 trial showed a treatment effect of 80mg simvastatin with a significant increase in the frontal assessment battery score of 1.2 points at 2-year follow-up. There were additional improvements in subjective measures of physical functioning and quality of life. These may be related to the cholesterol lowering vascular effects of simvastatin (Chan *et al.*, 2017).

Therefore, when treatments for cognitive impairment are approached, a metacognitive approach to intervention and prevention (Amato *et al.*, 2013; Mitolo *et al.*, 2015) through education of patients is likely to enhance the effects. However, this requires formal linkage study design in the future.

3 Magnetic Resonance Imaging

3.1 Introduction

MRI has revolutionised our understanding and management of MS over the last 30 years (Miller *et al.*, 1998). As described in **chapter 1**, MRI is a major component of the 2017 revisions to the McDonald diagnostic criteria for MS in terms of providing supportive clinical evidence for dissemination in space and time (Filippi *et al.*, 2016).

Conventional T2-weighted and T1-weighted MRI techniques such as lesion quantification and further characterisation with contrast enhancement indicated the use of MRI as a potential biomarker for MS management in terms of prognostication, follow-up and treatment initiation (Petkau *et al.*, 2008). This has also highlighted the role of MRI as a critical objective trial outcome measure (Daumer *et al.*, 2009). MRI outcomes form part of the delineation of MS activity which is used in current guidance for DMT decision making (Perry *et al.*, 2014; Scolding *et al.*, 2015; Rae-Grant *et al.*, 2018).

Non-conventional MRI metrics have allowed more solid understanding of the underlying pathology of MS. The discovery of diffuse damage to both white matter (WM) and grey matter (GM), not fully detectable on conventional MRI (Chard and Miller, 2009), highlighted the additional cortical pathology of MS. It is the focal lesional and diffuse pathological changes which lead to MS clinical symptomology being highly heterogeneous. Grey matter disease in MS was further clarified with studies of the change in volume, or atrophy, over time and how these occur in different MS subtypes (Eshaghi *et al.*, 2018*b*). This in particular, is of interest in the study of cognition which seemingly associates more with GM pathology (Rocca *et al.*, 2015*a*). It is not only MRI of the brain which is useful for understanding the course of MS. Cervical cord atrophy has an independent association with MS physical disability, as measured by the EDSS (Kearney *et al.*, 2014). Our understanding of the diffuse role of white matter in MS pathology has also expanded with the definition of NAWM and NAGM in MS showing the importance of non-lesional processes in MS (Miller *et al.*, 2003). Proton Magnetic Resonance Spectroscopy (H-MRS) and sodium imaging has allowed the study of metabolic markers for pathology in MS and furthered

our understanding of the underlying changes in MS (Ciccarelli *et al.*, 2014; Petracca *et al.*, 2015). Functional MRI metrics have led to the evaluation of network and global connectivity changes associated with low frequency signals which are key to our understanding of diffuse cognitive changes in MS (Filippi and Rocca, 2013).

This chapter will review the basic principles of MRI, MRI techniques applied in this thesis, and specifically the role they play in our understanding of cognitive changes in MS.

3.2 Basic Principles

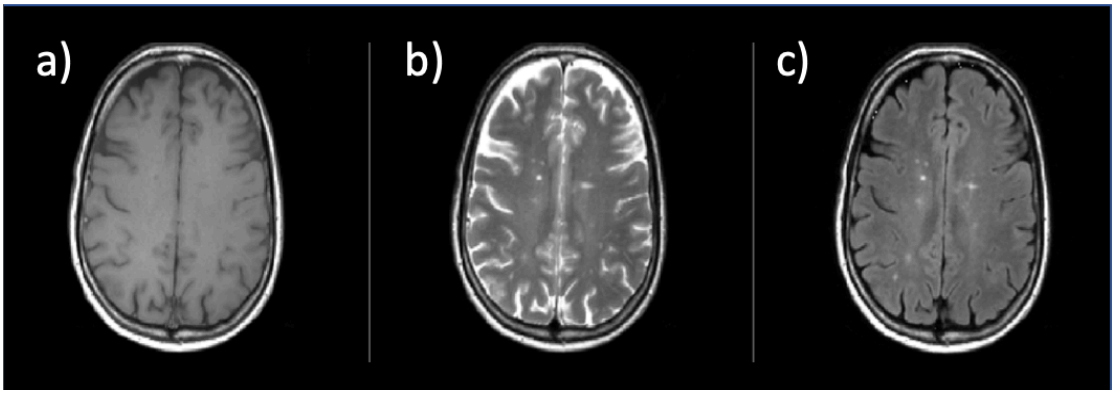
MRI or Nuclear Magnetic Resonance (NMR) research took off in the 1920s. There was interest in understanding why some particles appeared to have a charge, or magnetic field. However, it was in 1946 that Bloch's theory, the underlying principle of MRI, was published for which he was awarded the Nobel Prize in 1952 (Bloch, 1946). A simple explanation is that a spinning charged nucleus has an individual electromagnetic field which is affected or precessed by an external magnetic field. Many charged nuclei together give rise to a grouped or net magnetisation (Chappell *et al.*, 2017).

MRI uses hydrogen nuclei which are abundant in water molecules. The single proton hydrogen nuclei act as individual magnets, but are orientated in different directions at rest. The Larmor equation describes the torque force of an external magnetic field on the net magnetisation, and the rate at which it is precessed. The external magnetic field, the B_0 field, aligns the hydrogen nuclei in the same direction, but at an angle due to the torque force. The B_0 magnetic field relates to the overall strength of the magnetic coil and is reported as tesla (T) (Hashemi and Bradley, 1997; Chappell *et al.*, 2017; Jenkinson and Chappell, 2017).

In the scanner, nuclei will align themselves parallel (longitudinal) or anti-parallel (transverse) to the horizontally orientated B_0 field. The small magnetic field corresponding to the difference between nuclei aligning either way is known as the M_0, and describes the original magnetisation

size. In order to generate a signal which allows image formation the overall magnetic field has to deviate from the central B_0 field or longitudinal axis. This process is known as excitation. Information is collected spatially in three orthogonal axes x, y, and z derived from gradient coils in a coordinate three-dimensional (3D) system. This 3D system generates gradients of magnetic fields produced by each coil; slice select gradient, phase-encoding gradient, frequency encoding (readout) gradient and respond to G_z, G_y, and G_x for axial images respectively. Radiofrequency (RF) signal introduced into the magnetic field flips magnetisation into the x-y plane from the z axis. The size of this RF pulse is determined by the flip angle or α, based on the amplitude and duration of the RF pulse. The RF flip angle determines the contrast of the different tissue compartments (Rocca *et al.*, 2017). The bandwidth, or signal frequency, and slice-select gradient determine slice thickness. The x-y magnetisation vector produced precesses at the same frequency as the nuclei and leads to a voltage or MR output signal in a receiver coil placed in the transverse plane. The RF signal emitted by the sample is collected and transformed into digital data which leads to MRI images (Hashemi and Bradley, 1997; Chappell *et al.*, 2017; Jenkinson and Chappell, 2017).

Figure 3.1. Example of a)T1, b)T2, and c)FLAIR MRI brain images in a subject with SPMS reconstructed from data from my thesis.



Relaxation describes the position of protons at rest in the external magnetic field. T1 and T2 are relaxation time constants relating to regrowth of longitudinal components from thermal agitation, and the decay of transverse components from internuclear interactions back to zero respectively. T1 relaxation time describes the time taken for protons to return to equilibrium by returning energy obtained from the RF pulse to the local environment (lattice). T2 does not require energy transfer, and reflects the rate of transverse magnetisation decay. The T1 and T2 relaxation times are independent of each other and depend on tissue composition. T2 transverse proton decay is affected by inhomogeneities in the B_0 field, and if not compensated for, increases the decay rate and is known as T2* (T2 star). T1 and T2 properties vary by tissue type. Structures containing a lot of water molecules, e.g. CSF, have a long T2 relaxation time due to less tightly bound protons, whereas the opposite is true of tightly bound protons making up lipid dense tissue. The properties of different relaxation times are used to visualise structures variably with MRI (Hashemi and Bradley, 1997; Chappell *et al.*, 2017; Jenkinson and Chappell, 2017).

Different tissues have different contrasts as described, but these can be manipulated in different ways to allow different views. To correct phase incoherence due to T2* a second RF signal can be introduced resulting in the detection of a signal, which is known as echo. TE (variable echo delay time) manipulates the T2 spin delay by using refocusing RF pulses at 90^{O}. TR (repetition time) is the time between pulses. Short TR increases the difference between tissues with fast T1 recovery (e.g. lipids) and long T1 recovery (e.g. water). Short T1 recovery tissues appear bright, and long T1 recovery are dark. The difference in the amount of dephasing by different tissues is increased (based on the T2 relaxation times) if the TE is long. These high T2 weighted images lead to short T2 tissues (proteins and lipids) looking dark, and long T2 tissues (i.e. those with high water content) appearing bright. Proton density (PD) weighted spin echo sequences have long TR and short TE resulting in an image which relies on the density of free tissue protons. TE and TR variations therefore alter tissue properties and are manipulated for MRI imaging as shown in **figure 3.1** and **table 3.1** below (Hashemi and Bradley, 1997; Chappell *et al.*, 2017; Jenkinson and Chappell, 2017).

Table 3.1. TR and TE variations and tissue properties with different weightings.

Weighting	TR	TE	Tissue appearance
T1 weighted	short	short	Short T1, e.g. white matter=bright Long T1, e.g. CSF=dark
T2 weighted	long	long	Short T2, e.g. white matter=dark Long T2, e.g. CSF=bright
Proton Density (PD)	long	short	

(Hashemi and Bradley, 1997; Chappell et al., 2017; Jenkinson and Chappell, 2017)

3.3 Functional MRI

3.3.1 Introduction

Functional MRI (fMRI) detects signal related to brain tissue repair, structural damage, and reorganisation. fMRI gives information about dynamic brain changes which correlate to underlying neuronal activity between functionally connected regions (Jenkinson and Chappell, 2017). The changes in functional connectivity (FC) may be considered a correlate for plasticity of the brain and therefore gives clinical utility above quantitative measures (Guerra-Carrillo *et al.*, 2014, Rocca *et al.*, 2016*a*). fMRI has the possibility of identifying targets for the adaptive capability of the brain and this is therefore an area of research interest (Rocca *et al.*, 2016*a*).

fMRI is sensitive to the oxygenation of haemoglobin in blood which correlates with neuronal firing. Oxygenated haemoglobin (oxyhaemoglobin) and deoxygenated haemoglobin (deoxyhaemoglobin) interact differently with the magnetic field, causing variations in the local magnetic field resulting in detectable MRI signals. These changes are mainly dependent on the concentration of deoxygenated haemoglobin. This is the blood oxygen level dependent (BOLD) effect. A small rise in the cerebral metabolic rate of oxygen consumption ($CMRO_2$) due to increased neuronal activity causes excess increases in the cerebral blood flow (CBF) and cerebral blood volume (CBV). This results in deoxyhaemoglobin variations detected by fMRI. FC is temporal correlation of the BOLD signal from different brain areas (Lowe *et al.*, 2000; Bijsterbosch *et al.*, 2017). BOLD effects can be detected by T2 echo-planar imaging and have better signal to noise ratios (SNR) then other measures of functional changes, e.g. arterial spin labelling (Jenkinson and Chappell, 2017).

fMRI investigates task-related activity, task fMRI, or FC in the absence of cognitive or physical demands, resting state fMRI (rs-fMRI). Both forms of fMRI use the same MRI acquisition, but are differentiated by the presence or absence of a task (Bijsterbosch *et al.*, 2017; Jenkinson and Chappell, 2017). Task fMRI involves a subject undertaking a task whilst in the scanner which may

be passive visual or auditory stimulation, simple motor tasks such as finger tapping, or cognitive tasks such as reading. Using the general linear model (GLM), statistical analysis of changes in the MRI signal in time creates a map of brain activity (Jenkinson *et al.*, 2017). Therefore, task fMRI is useful where the location of brain activity related to a task is important to assess hypothesis-driven functions of working brain networks. This can be useful as a biomarker for clinical change in a disease state (Bijsterbosch *et al.*, 2017; Jenkinson and Chappell, 2017). The focus of this thesis is rs-fMRI and therefore the remainder of this section will focus on this modality.

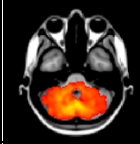
Rs-fMRI studies the low frequency fluctuations of the BOLD signal in the absence of a task. As rs-fMRI is not affected by any task performance it is useful where this is not possible or restricted by disability as in MS (Lee *et al.*, 2013; Barkhof *et al.*, 2014, Sbardella *et al.*, 2015*a*, Castellazzi *et al.*, 2018*a*). An introduction to rs-fMRI, analysis techniques, applications in MS and cognition are described below and has relevance for **chapters 5 and 6** of this thesis.

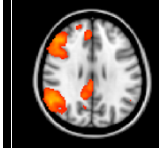
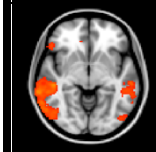
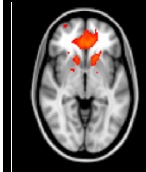
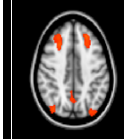
3.3.2 Resting state functional MRI (rs-fMRI)

In 1995, Biswal et al first described the implications and significance of the low frequency (<0.1 Hz) fluctuations in BOLD signal using 512 echo-planar images (EPI) obtained every 250ms in the resting brain. BOLD signal may be affected by cerebral blood flow, and blood oxygenation. However, these low frequency fluctuations were in addition to the peaks related to physiological fluctuations associated with respiration and heart rate at rest. They used a traditional block design fMRI to identify a left somatosensory cortex seed region. During this study, as well as investigating BOLD changes during bilateral finger tapping, the authors asked subjects to refrain from any activity. They noted highly synchronous correlations of the seed region low frequency BOLD fluctuations with the contralateral somatosensory cortex and areas in the same hemisphere at rest (Biswal *et al.*, 1995).

Synchronous low frequency fluctuations in BOLD signal in spatially distinct regions share cortical connections (Lowe *et al.*, 2000). These connections are investigated by rs-fMRI and those areas sharing low frequency BOLD fluctuations are known as resting state networks (RSNs) (Lowe *et al.*, 2000; Gusnard and Raichle, 2001; De Luca *et al.*, 2006). RSNs are identified by their spatial patterns which are consistent in the literature (Damoiseaux *et al.*, 2006). The Default Mode Network (DMN) is the most infamous, and was initially identified by a positron emission tomography (PET)-imaging study whereby the inclusive DMN regions had reduced activity during a cognitive task, but were more active at rest (Raichle *et al.*, 2001). The DMN anatomically comprises the precuneus and posterior cingulate of the medial parietal lobe as the main hubs. The overall functional systems of the brain at rest may be opposing with the DMN composing one, and attention, visual, and somatosensory RSNs making up the other, i.e. task-positive and task-negative systems respectively, relating to underlying cognitive versus non-cognitive function (De Luca *et al.*, 2006; Power *et al.*, 2011). The DMN has been shown to be a cognitive RSN in other diseases such as early Alzheimer's disease (van den Heuvel and Hulshoff Pol, 2010; Barkhof *et al.*, 2014; Gour *et al.*, 2014). Further studies have used techniques such as independent component analysis (ICA), which is described in more detail below, to identify and classify other RSNs (Beckmann *et al.*, 2005). The following table (**table 3.2**) summarises the major RSNs and the underlying anatomical regions that they represent (Smith *et al.*, 2009).

Table 3.2. The major resting state networks (RSNs). Summary of the main RSNs, their underlying anatomical correlations and related functions.

	Resting State Network (RSN)	Anatomical correlates	Functions/domains
	**Default Mode Network (DMN)**	medial parietal lobe; precuneus and posterior cingulate, bilateral infero-lateral parietal lobe, ventromedial frontal cortex	Cognition
	**Somatosensory/sensorimotor network (SMN)** ***first RSN identified. (Biswal *et al.*, 1995)**	Supplementary motor area, sensorimotor cortex, supplementary somatosensory cortex	Action-execution Perception-sensation
	**Auditory network (AN)**	Superior temporal gyrus, Heschl's gyrus, posterior insular	Action-execution-speech Cognition-language-speech Perception-audition
	**Cerebellar (CBLN)**	Cerebellum	Action-execution Perception- sensation - pain
	**Visual (medial/lateral/occipital)** **(MVN, OVN, LVN respectively)**	Occipital pole	Cognition-language-orthography (occipital) Cognition-space (lateral)
	**Executive (ECN)**	Anterior cingulate, paracingulate (medial frontal lobe)	Cognition Action-inhibition Emotion Perception- sensation - pain

	**Frontoparietal – lateralised networks.** **Language network**	Frontoparietal areas, insular, Broca's, Wernicke's areas	Cognition Language Perception-sensation - pain
 	**Right ventral attention network (RVAN)** **Left ventral attention network (LVAN)**	Temporoparietal junction and ventral frontal cortex	Salience Attention
	**Dorsal attention network (DAN)**	Intraparietal sulcus, frontal eye fields	Executive control-attention
	**Frontoparietal control network (FPCN)**	Lateral prefrontal cortex, inferior parietal lobe	Decision-making
	**Salience network (SN)**	Medial superior frontal cortex, anterior insula, anterior prefrontal cortex, anterior cingulate	Goal-directed task performance

*I have derived images of the RSNs in this table from subject independent component analysis data from **section 5.2.3**. (Biswal et al., 1995; Beckmann et al., 2005; Smith et al., 2009; Power et al., 2011; Lee et al., 2013).*

3.3.3 Techniques for analysing rs-fMRI

Gradient-echo EPI sequences are used for rs-fMRI acquisition as it is sensitive to the BOLD effect, and allows for fast image acquisition; 0.5-3 seconds for the whole brain. The TR of rs-fMRI is the time for acquiring one volume. The TE is similar to the T2* of grey matter to allow detection of T2* changes reflecting the BOLD effect. fMRI images are very sensitive to BOLD signal, but are low resolution (voxels 2-3mm), have distortion of their geometry, signal loss, and low tissue contrast. The acquisition of the low frequency fluctuations in the BOLD signal for rs-fMRI requires subjects to be in a resting state, but not asleep. Therefore, it is useful to ask subjects to have their eyes open for the sequence (Bijsterbosch *et al.*, 2017).

Several methods are utilised to analyse rs-fMRI (Azeez and Biswal, 2017), but all require pre-processing. Pre-processing of the BOLD signal includes time shift and intensity correction. To retain the low frequency signals <0.1 Hz and to improve the SNR, spatial smoothing and low pass filtering are used. Head motion correction is an important part of the pre-processing stage as it prevents false signal correlations. Regressing out the average time course of the whole brain is controversial, as although it may reduce intracranial pressure fluctuations due to carbon dioxide, it may affect the physiological basis for BOLD contrast and low frequency fluctuations (Lee *et al.*, 2013; Bijsterbosch *et al.*, 2017). Pre-processing is undertaken by software including the FMRB Software Library (FSL) (FMRIB, 2000). Images are realigned to a slice, co-registered anatomically, and normalised into standard space, e.g. Montreal Neurologic Institute (MNI) space. Finally gaussian spatial smoothing is applied, followed by regressing out motion and temporal filtering (Azeez and Biswal, 2017; Bijsterbosch *et al.*, 2017).

Seed-based correlation analysis is the first voxel-based connectivity analysis to have been used in rs-fMRI. Seed-based analysis requires the preselection of regions of interest (ROIs). The BOLD time course of voxels within the ROIs are correlated with other ROIs' voxels and non-ROI brain voxels. Significance is determined by a specific voxel cut-off. This method is useful for looking at

specific ROIs, but not for understanding whole brain low frequency BOLD fluctuations (Azeez and Biswal, 2017; Bijsterbosch *et al.*, 2017).

Independent component analysis (ICA) is another voxel-based connectivity analysis, but uses algorithms to define statistically independent components. ICA requires more manual input than seed-based analyses as the independent components identified need to be defined as RSNs or artefact e.g. due to noise, cerebrospinal fluid, or white matter. ICA does not require predetermined ROIs and can allow for whole brain analysis (Beckmann *et al.*, 2005; Azeez and Biswal, 2017; Bijsterbosch *et al.*, 2017). ICA and seed-based methods do show similar results in healthy subjects and so can be compared effectively (Rosazza *et al.*, 2012).

To compare rs-fMRI connectivity between groups, which may be the case in a research study, group level analyses are required. Regression analyses use the GLM to attempt to explain the rs-fMRI data (Y) with predictors (X) and the estimate for the predictor (β) and explains noise (ε).

$$Y=\beta X+\varepsilon$$

Several regressors can be put into the GLM to provide an accurate linear model (Jenkinson *et al.*, 2017).

Dual regression analysis is a multi-subject ICA which allows the comparison of group spatial maps. The group maps are inserted as regressors in the GLM. Subject specific differences are derived when the normal time courses are regressed out (Beckmann *et al.*, 2009; Azeez and Biswal, 2017; Bijsterbosch *et al.*, 2017).

Graph theory reviews whole brain RSNs as a series of nodes and connected edges in one model. Computational models look at measures of connectedness. The output of graph theory is a connectivity matrix which summarises the connectivity between regions (Zalesky *et al.*, 2010).

The path length, i.e. the shortest connections between node pairs is a measure of global connectedness. The clustering coefficient relates to neighbouring node connectedness. Understanding these, allows a better understanding of local and whole brain RSNs. Graph theory allows an evaluation of the efficiency of connectivity within the whole brain and can be useful for using rs-fMRI to understand pathology (Lee *et al.*, 2013; Azeez and Biswal, 2017; Bijsterbosch *et al.*, 2017).

3.3.4 Rs-fMRI, disability, and cognition in MS

There are resting state FC changes in all phenotypes of MS, especially in the cognitive RSNs including the DMN hubs. Additionally, compared to healthy controls, there are more FC changes in MS in the cognitive RSNs. Loitfelder et al. 2012 undertook a seed-based connectivity analysis of the anterior cingulate cortex (ACC), a representation of the DMN, in 31 MS subjects (CIS (10), RRMS (16), SPMS (5), HC (31)). People with MS had increased FC between the ACC and the; left angular gyrus, left posterior cingulate cortex (PCC), and the right postcentral gyrus indicating adaptive changes in a sustained attention network (Loitfelder *et al.*, 2012).

Studies have additionally aimed to classify specific RSN abnormalities in the different MS phenotypes. Roosendaal et al undertook a cross-sectional ICA study of FC changes in CIS versus RRMS to evaluate changes as patients progressed to clinically definite MS. Six out of eight RSNs, including the DMN showed increased FC in the CIS group versus controls and RRMS. These changes were lost in RRMS where there was cognitive impairment, grey matter atrophy and DTI white matter changes. This indicates that there is compensatory functional recruitment of these networks secondary to structural loss in CIS which acts as a reserve (Roosendaal *et al.*, 2010*b*). Whether these FC changes are a risk factor for RRMS conversion is not clear but has been purported (Liu *et al.*, 2016).

Rocca at al reviewed FC changes of the DMN in a progressive MS cohort of 33 SPMS and 24 PPMS subjects using ICA analysis. Compared to healthy controls, both SPMS and PPMS

subjects had reduced FC in the ACC but additionally in the medial prefrontal cortex and precentral gyrus. Only ACC FC significantly differed between SPMS and PPMS, lower and greater FC respectively. Anterior DMN FC involving the ACC was reduced more in cognitively impaired versus cognitively preserved progressive MS groups supporting the importance of the anterior DMN in MS cognitive impairment. This FC reduction was correlated to underlying white matter tract damage shown by DTI (Rocca *et al.*, 2010*b*). Therefore there is functional reorganisation of FC within MS phenotypes which changes with disease progression (Janssen *et al.*, 2013; Sacco *et al.*, 2013, Sbardella *et al.*, 2015*a*). Similar changes of reduced ACC resting FC have been shown in cognitively impaired RRMS subjects versus healthy controls indicating that the anterior DMN is important for MS cognitive reserve (Bonavita *et al.*, 2011).

Given that these anterior DMN regions had reduced resting FC in more progressive forms of MS, this could illustrate loss of functional reserve or compensation with disability progression and worsening cognitive impairment (Rocca *et al.*, 2010*b*; Cruz-Gómez *et al.*, 2014; Schoonheim *et al.*, 2017). These findings are supported by the finding of loss of thalamic and left frontal hubs in cognitively impaired MS subjects using graph theory analysis of connectedness (Rocca *et al.*, 2016*b*). Additionally, frontal and parietal regions, making up part of the DMN, have shown increased recruitment in task fMRI of RRMS subjects (Mainero *et al.*, 2006). Therefore, how critical hubs such as the frontoparietal regions hold functional integrity over time is likely key to cognitive function (Rocca *et al.*, 2016*a*). Studies of centrality (graph theory) have shown the importance of the DMN within the reorganisation of RSNs in cognitive impairment in MS (Eijlers *et al.*, 2017).

Studies have also shown contradictory increased FC in RSNs with worsening cognitive function or disability in MS. Thalamic FC, especially in the frontoparietal and occipital regions, was shown to be increased with worse cognitive function in a mixed MS cohort (Schoonheim *et al.*, 2015*a*). These findings were supported by another study of the thalamo-cortical RSN with paradoxical FC increases and cognitive deficits (Tona *et al.*, 2014). Graph theory analysis using eigenvector centrality mapping (ECM), showed increased connectivity of the thalami, and reduced ECM

changes in the ventral stream relating to lower cognitive performance, and decreased ECM in the sensorimotor area inversely correlating to EDSS (Schoonheim *et al.*, 2014). Therefore, converse resting FC changes in the thalami are associated with MS cognitive impairment. Recent studies have indicated a link between increased thalamic and fronto-parietal FC and fatigue in RRMS suggesting that increased FC is perhaps a maladaptive process (Stefancin *et al.*, 2019). Increased connectivity within the frontoparietal and insular networks correlated with lower total MSFC scores in a RRMS cross-sectional study (Faivre *et al.*, 2012). Multimodal studies of the DMN have looked at underlying white matter tract integrity using DTI. A cross-sectional study in a RRMS population showed correlated reduced anatomical connectivity and impaired cognitive function, but that this is anti-correlated to FC, i.e. increased FC with lower cognitive impairment (Hawellek *et al.*, 2011). Increased FC is not only present in RRMS, Basile et al investigated DMN and SMN FC in 14 SPMS and 34 RRMS subjects. Both the SPMS and RRMS groups showed overall increased SMN and DMN FC with positive correlations to the PASAT3, however the SPMS group had decreased anterior SMN FC and increased posterior DMN FC (Basile *et al.*, 2014). A further study of overall FC in RRMS showed both reduced FC in the DMN, working memory, executive SMN, and visual networks and increased FC in the ECN and auditory networks which correlated with higher T2LL and EDSS (Rocca *et al.*, 2012).

These cumulative changes of increased FC go against a compensatory hypothesis, and suggest maladaptation. Perhaps increased and decreased FC represent distributed changes in MS related to structural damage, but also abnormal changes between individual RSNs (Rocca *et al.*, 2016*a*). It could be that there is network collapse as cognitive impairment, disability, and structural damage accumulate above a threshold for network efficiency (Schoonheim *et al.*, 2015*b*). Another hypothesis is that as MS progresses there is an impairment in effective network recruitment, and so there is loss of functional reserve as well as cognitive and physical reserve. This idea was investigated using task fMRI where SPMS patients showed most abnormal network recruitment whilst undertaking a go/no-go task (Loitfelder *et al.*, 2011). However, it is likely that both compensatory and maladaptive changes are represented by FC, and what underlies FC in the resting brain and the BOLD effect is currently not fully understood (Schoonheim *et al.*, 2017).

Other brain RSNs play a key role in cognition and show altered FC in MS cognitive impairment. The hippocampus is part of the DMN and has shown reduced resting FC with other areas using seed-based analysis in relation to structural damage and normal visuospatial memory (Roosendaal *et al.*, 2010*b*). Hippocampal resting FC has been highly correlated to T2 lesion load, and this therefore could account for alterations in connectivity (Rocca *et al.*, 2015*b*). Looking at the cerebellum and seed based analysis of connectivity in progressive MS versus controls, there is independent FC alteration separate to structural changes which correlate inversely to BVMT-R scores (r=-0.393) of visuospatial memory function (Cocozza *et al.*, 2018). RSNs are also altered in other functional systems in MS. Visual RSNs have shown reduced FC following MS optic neuritis and that of the right inferior peristriate cortex correlated with the number of previous episodes of optic neuritis (Gallo *et al.*, 2012).

In addition to cross-sectional analyses of resting FC there are few longitudinal studies (Enzinger *et al.*, 2016). Droby et al performed a longitudinal rs-fMRI study of the effect of lesions on resting FC. Repeat imaging over a 2 month period showed that the presence of a lesion led to increased FC in the contralateral cuneus, contralateral and ipsilateral precuneus indicating functional reorganisation and recruitment in response to lesional tissue damage (Droby *et al.*, 2015). A longitudinal 2-year study of 38 RRMS used graph theory to look at changes of overall brain FC. Over the 2 years worsening of EDSS and the MSFC correlated with decreased brain FC enhancement, as did disease course (Faivre *et al.*, 2016). Two further longitudinal studies of FC reorganisation following cognitive rehabilitation are highlighted below (Parisi *et al.*, 2014; Bonavita *et al.*, 2015).

There are certain factors specific to MS that can affect FC (Filippi *et al.*, 2013*a*; Janssen *et al.*, 2013, Sbardella *et al.*, 2015*a*; Bijsterbosch *et al.*, 2017). Some of the FC variation in MS may be accounted for by gender due to the strong female gender bias in MS (McAlpine *et al.*, 1955). A study of FC using synchronisation likelihood between ROIs, and graph theory showed worse visuospatial memory task performance in male MS subjects which was correlated with reduced

FC and network efficiency (Schoonheim *et al.*, 2012*a*). Therefore, there may be an effect of gender on resting FC in MS and this should be considered in studies (Bijsterbosch *et al.*, 2017). Disease duration has been shown to affect MS RSN recruitment with sensory networks showing increased FC in RRMS with shorter disease duration and cognitive networks showing increased FC in longer disease duration (Castellazzi *et al.*, 2018*b*). A final aspect specific to MS rs-fMRI is the presence of lesions. The presence of high lesion load alters the pattern of FC and may not always be related to disease severity on clinical rating scales. Therefore, there has to be caution when interpreting FC changes (Rocca *et al.*, 2012, Castellazzi *et al.*, 2018*b*). Computational modelling has been used to simulate the effect of lesional and non-lesional GM and WM damage on connectivity in MS. Overall cortical and deep GM damage is related to increased global connectivity, whereas WM damage leads to an initial rise in local connectivity and then global connectivity collapse. This may explain some of the fluctuations in FC of rs-fMRI in MS (Tewarie *et al.*, 2018).

Correlation of resting FC to clinical test outcomes in MS allows better understanding of their underlying mechanism. Rocca et al 2018 showed that reduced thalamic resting FC correlated with better neuropsychological test scores, and reduced global FC with overall T2 lesion load (Rocca *et al.*, 2018). Castellazzi et al. used a different technique, voxel wise correlation, to look at the direct effects of FC on a severity rating scale for MS (Castellazzi *et al.*, 2018*b*). Meijer at al explored whether resting FC or WM and GM volumes affected information processing speed in MS. Both structural and functional metrics predicted information processing speed best in the regression models (Damoiseaux and Greicius, 2009; Meijer *et al.*, 2018). Understanding how RSN changes relate to T25FW scores could be developed as a monitoring tool in trials (Bollaert *et al.*, 2018).

Rs-fMRI could be utilised as a diagnostic tool in MS, but only has limited impact on clinical practice currently. Early work by Bill Seeley and colleagues suggested network imaging could offer a better understanding of disease. They found that patients with neurodegenerative disease and similar symptoms had lesions in different grey matter areas, but that these mapped onto the same

functional networks (Seeley *et al.*, 2009). This understanding has led to a number of studies looking at grey matter lesions in disease and the relationship with functional network imaging (Fox, 2018). Studies of brain stimulation have suggested possible clinical applications of FC, but the reliability should be interpreted with caution (Ozdemir *et al.*, 2020). In a multimodal MRI computational model, rs-fMRI changes along with NAWM DTI, and white matter lesion load together had an accuracy of 88% for differentiating neuromyelitis optica (NMO) from MS (Eshaghi *et al.*, 2015). Rs-fMRI has been used to understand the persistence of cognitive rehabilitation effects in cognitively impaired MS subjects and showed that there is altered FC not only for the trained cognitive domains, but also others (Parisi *et al.*, 2014; Bonavita *et al.*, 2015). The following table summarises key studies of rs-fMRI and cognition in MS **(table 3.3)**. As can be seen the largest SPMS cohort evaluated was 41 subjects cross-sectionally (Rocca *et al.*, 2018). However, there are no specific longitudinal cognitive-rs-fMRI studies of SPMS published to my knowledge, and this is a key area in need of development and evaluation that my thesis aims to target (Enzinger *et al.*, 2016).

Table 3.3. Key studies of rs-fMRI and cognition in MS. *SPMS is in bold font.*

Study	Cohort (n)	Design	Cognitive measures	Outcome
Roosendaal et al. 2010 (Roosendaal *et al.*, 2010*b*)	CIS (14) RRMS (31) HC (41)	Cross-sectional ICA	Stroop, LLT, LDST	In the RRMS group there is loss of RSN changes that were seen in CIS with cognitive impairment, DTI changes, and GM atrophy.
Rocca et al. 2010 (Rocca *et al.*, 2010*b*)	**SPMS (33)** PPMS (24) HC (24)	Cross-sectional ICA	PASAT3, trail making test, SST, WLT, RCFT, VFT	Reduced ACC RS activity within the DMN in cognitively impaired MS subjects & this correlated with PASAT3, WLT, corpus callosum and cingulum DTI changes.
Roosendaal et al. 2010 (Roosendaal *et al.*, 2010*a*)	MS (25) HC (30)	Cross-sectional Seed	LLT	Multimodal (+hippocampal volume +T2 lesions +DTI) Decreased hippocampal FC driven by T2 lesion load, atrophy, but not LLT performance.
Bonavita et al. 2011 (Bonavita *et al.*, 2011)	RRMS (18CI 18CP) HC (18)	Cross-sectional ICA	SRT, SPART, SDMT, PASAT3, WLG	Reduced ACC FC in cognitively impaired RRMS.
Hawellak et al 2011 (Hawellek *et al.*, 2011)	MS (16) HC (16)	Cross-sectional ICA	Trail-making, PASAT3, SDMT, VFT, SPART, Digit span	Multimodal (+DTI) Enhanced DMN connectivity, especially in the posterior cingulate, medial prefrontal cortex, and inferior parietal cortices with CI.
Loitfelder et al. 2012 (Loitfelder *et al.*, 2012)	CIS (10) RRMS (16) **SPMS (5)** HC (31)	Cross-sectional Seed	BRNB, WCST,	Better cognitive performance related to increased FC between the ACC and the; cerebellum, middle temporal gyrus, occipital pole, and the angular gyrus (attentional network).

Faivre et al. 2012 (Faivre *et al.*, 2012).	RRMS (13) HC (14)	Cross-sectional ICA	MSFC	Dorsal and right ventral FPN FC inversely associated with PASAT3, left dorsal FPN FC inversely associated with 9-HPT.
Schoonheim et al. 2012 (Schoonheim *et al.*, 2012*a*)	MS (60) (30F 30M)	Cross-sectional SL GT	LLT, LDST	Reduced FC and network efficiency in male MS and this correlated with visuospatial memory.
Janssen et al. 2013 (Janssen *et al.*, 2013)	RRMS (28) HC (28)	Cross-sectional ICA	PASAT3, letter comparison and pattern comparison	Increased severity of RRMS had reduced motor and executive network, and increased medial visual network FC. No change in DMN between HC and RRMS.
Cruz-Gomez et al. 2014 (Cruz-Gómez *et al.*, 2014)	MS (60) HC (18)	Cross-sectional ICA	BRNB	Lower FC in all RSNs in cognitively impaired MS.
Basile et al. 2014 (Basile *et al.*, 2014).	**SPMS (14)** RRMS (34) HC (25)	Cross-sectional ICA	PASAT3, SDMT	Increased SMN and DMN FC with positive correlations to the PASAT3, the SPMS group had decreased anterior SMN FC and increased posterior DMN FC.
Tona et al. 2014 (Tona *et al.*, 2014)	RRMS (48) HC (24)	Cross-sectional Seed	PASAT3	Inverse correlation of thalamo-cortical resting state functional connections with PASAT3 score.
Schoonheim et al. 2014 (Schoonheim *et al.*, 2014)	MS (128) HC (50)	Cross-sectional GT	BRNB, CST, Stroop, MCT	ECM increased in the bilateral thalamus and PCC, decreased in the sensorimotor areas inversely related to EDSS, and decreased ventral stream related to poor cognition

Parisi et al. 2014 (Parisi *et al.*, 2014)	MS (18 CI)	Longitudinal (6 month)	Cognitive rehabilitation, BRNB, SDMT, SRT, SPART, PASAT3, COWAT, WCST, TEA	Cognitive rehabilitation (12 weeks) lead to persistant RS FC changes at 6 months in the training domain and others.
Schoonheim et al. 2015 (Schoonheim *et al.*, 2015*a*)	MS (157) HC (47)	Cross-sectional	7 domains; BRNB, Stroop, CST, MCT	Multimodal (+DTI+GM atrophy). Increased thalamic FC in CI.
Bonavita et al. 2015 (Bonavita *et al.*, 2015)	MS (18 CI)	Longitudinal	Cognitive rehabilitation, BRNB, SDMT, SRT, SPART, PASAT3, WLG, Stroop	Increased RS FC of the DMN; PCC, inferior parietal cortices. PCC FC negatively correlated to Stroop.
Rocca et al. 2016 (Rocca *et al.*, 2016*b*)	MS (246) HC (55)	Cross-sectional GT	PASAT3	Cognitively impaired RRMs loss thalamic and left frontal lobe hubs.
Eijlers et al. 2017 (Eijlers *et al.*, 2017)	MS (332) HC)96)	Cross-sectional GT	BRNB, SRT, WLG, SDMT, Stroop, MCT	Widespread increased centrality in CI group especially in the DMN.
Van Geest et al. 2018 (van Geest *et al.*, 2018)	MS (29) HC (19)	Cross-sectional ICA	LDST, SDMT, Stroop	Dynamic FC from rs-fMRI to task state is associated with information processing speed changes in MS.

Rocca et al. 2018 (Rocca *et al.*, 2018)	PPMS (13) **SPMS (41)** BMS (29) RRMS (119) CIS (13) HC (98)	Cross-sectional ICA Correlation of RS FC	BRNB	Reduced DMN FC in MS. Reduced FC correlated to T2LL.
Meijer et al. 2018 (Meijer *et al.*, 2018)	MS (330) HC (96)	Cross-sectional ICA	SDMT	Multimodal (+DTI+ atrophy) Worse IPS correlated with less deep GM volume, lower WM integrity, increased FC.

FC=functional connectivity, ICA=independent component analysis, Seed=seed based, GT=graph theory, ECM=Eigenvector Centrality Measure, SL=synchronisation likelihood, ACC=anterior cingulate cortex, PCC=posterior cingulate cortex, FPN=frontoparietal network, DTI=diffusion tensor imaging, GM=grey matter, LDST=letter digit substitution test, SDMT=symbol digit modalities test, PASAT3=Paced Auditory Serial Additions Test, VFT=Verbal Fluency Test, SPART=spatial recall test, WCST=Wisconsin card sorting test, BRNB=Brief Repeatable Battery, Stroop=Stroop Interference Test, LLT=Location Learning Test, TMT-trail making test, SST=short story test, WLT=word learning test, RCFT=Rey-Osterrieth Complex Figure Test, WLG=word list generation, TEA=Test of Everyday Attention, SRT=Selective Reminding Test , COWAT=Controlled Oral Words Association Test, BMS=benign MS, F=female, M=male, LL=lesion load, CI=cognitive impairment, IPS=information processing speed.

3.4 Quantitative MRI

3.4.1 Introduction

Structural MRI is used to determine the shape or size of brain structures. This is usually based on identifying boundaries using contrast variations for delineation. The method for determining this is segmentation and segmentation varies according to whether differentiation is required at the tissue compartment level, or within tissue type, e.g. GM structures. Structural MRI also allows the determination of change of a structure over time. This may be via whole brain metrics, or looking at differences in individual tissue type in a region (Miller *et al.*, 2002; Jenkinson and Chappell, 2017). A famous example of this is the use of voxel based morphometry (VBM) by Maguire and colleagues who showed increases in hippocampal GM over time in taxi drivers (Maguire *et al.*, 2000). This section focuses on MRI atrophy measures which have relevance for **chapter 6**.

3.4.2 Atrophy

Atrophy relates to neuronal loss and is a useful tool in MS as a correlate of the underlying pathological processes and as a reflection of clinical and cognitive disability (Rocca *et al.*, 2017). Brain atrophy is part of normal ageing, but has been shown to be accelerated in MS (Enzinger *et al.*, 2005). Widening of the sulci, enlargement of the ventricles, and brain parenchymal loss can all be visualised on routine MRI imaging. However, quantifying these changes using MRI requires the measurement of brain volumes and brain volume change. This is advantageous in longitudinal study designs, especially when scans are undertaken on the same scanner to prevent bias and to retain precision. MRI brain atrophy has been used as a biomarker for disease state when compared with normal ageing controls and as a measure of cognitive decline in other conditions such as Alzheimer's disease and diabetes mellitus (Fox and Schott, 2004; Van Elderen *et al.*, 2010; Moran *et al.*, 2019). As well as whole brain volumes, regional grey matter volume measurements can useful for understanding different tissue compartments and functionally

significant brain areas, e.g. the thalamus and cerebellum (Sarica *et al.*, 2015, Schoonheim *et al.*, 2015*a*).

3.4.3 Measuring atrophy with MRI

Currently, MRI atrophy measures are used in the research and clinical trial setting for MS, but do not have routine clinical use. This is partly due to a lack of direct comparative studies of different MRI techniques for atrophy, but also due to a lack of data on normative values for volumes and accurate cut offs. Currently, a change in brain volume of -0.4%/year has been shown to be clinically meaningful in terms of disability worsening as measured by the EDSS with a specificity of 80% and sensitivity of 65% (De Stefano *et al.*, 2016). However, there is an error rate of 0.15% in longitudinal brain atrophy validation studies (Smith *et al.*, 2002), and 2.5-3% variation in cortical thickness regional volumes (Tustison *et al.*, 2014). MRI provides a useful measure of atrophy, especially if there is a high contrast between tissues to prevent classification errors, as is the case with T1 weighted scans (Hashemi and Bradley, 1997). To visualise the tissue compartments accurately, ideally high resolution 3D T1-weighted images with near isometric voxel sizes $1mm^3$ should be used (Miller *et al.*, 2002; Honce, 2013; Rocca *et al.*, 2017). Brain volumes require scaling or normalisation to correct for factors e.g. between-subject brain volume variability. Another issue specific to MS is the presence of WM lesions which influence WM and GM brain compartment volumes by diluting the contrast between them. Ideally lesions should therefore be filled (Vrenken *et al.*, 2013; Popescu *et al.*, 2014). Patient factors can also impact atrophy. Hydration, alcohol use, and cardiovascular disease can all effect atrophy rates leading to errors in estimation accuracy. Cohort studies tend to be undertaken for atrophy measures as any errors are averaged out, however this currently limits the use at the individual level for monitoring change over time (Honce, 2013; Rocca *et al.*, 2017).

Over the last twenty years there has been the development of a number of techniques for measuring brain volumes and change over time in MS. Manual segmentation is limited by time

availability and the need for trained experts. Currently, semi-automatic and automatic techniques are more widely used (Honce, 2013; De Stefano *et al.*, 2014; Rocca *et al.*, 2017).

Registration based techniques measure total brain volume change as a combination of WM and GM atrophy. Registration techniques segment the brain, and then using image subtraction, to evaluate brain volume changes over time points (Jenkinson and Chappell, 2017). Structural image evaluation using normalisation of atrophy (SIENA) (Smith *et al.*, 2001) and the brain boundary shift integral (BBSI) (Fox *et al.*, 2000) have been used to measure atrophy or volume change in trials.

SIENA uses FSL software (FMRIB, 2000; Jenkinson *et al.*, 2012) to estimate percentage brain volume change (PBVC) over two time points. Paired T1-weighted scans are first pre-processed using BET (brain extraction tool) to remove non-brain tissues, e.g. skull and orbits. The segmented whole brain includes the external skull which is used to register the two time point T1-weighted scans. This is useful as the skull size does not change in adults and can allow for good spatial orientation. The change in the edge points at the interface between the brain and the cerebrospinal fluid estimates the PBVC over time (Smith *et al.*, 2001, 2002). SIENA can be applied to 2D or 3D T1-weighted pre- or post-contrast MRI images, and slice thickness can be varied (Smith *et al.*, 2002; De Stefano *et al.*, 2014).

BBSI uses digital subtraction to look for differences in the lateral edges of brain tissue and the ventricles between registered interval MRI imaging. The differences are assumed to be due to changes secondary to atrophy (Fox *et al.*, 2000). The boundary shift integral has been modified to allow use with smaller sample sizes (Prados *et al.*, 2015).

Segmentation techniques quantify regional brain areas, e.g. regional GM volumes; total GM volume, cortical grey matter (CGM), and deep grey matter (DGM) (Jenkinson and Chappell, 2017). The cross-sectional version of SIENA is SIENAX. Following brain extraction as described above, the image is registered to a standard brain template normalised for head size by using the

external skull (Smith *et al.*, 2002). This process estimates normalised brain volume (NBV), and after further segmentation, normalised GM and WM volumes are provided. Voxel-based morphometry (VBM) uses statistical parametric mapping (SPM) software to measure GM volume (Ashburner and Friston, 2000). Freesurfer measures the volumes of deep GM structures, and can also measure cortical thickness (Fischl *et al.*, 2004). The advanced normalisation tool (ANTs) software library has tools that can measure GM and WM compartments as well as cortical thickness (Das *et al.*, 2009; Avants *et al.*, 2011; Tustison *et al.*, 2014). Geodesic Information Flow (GIF) uses a graph framework to segment tissues and parcellate tissues to derive volume measures (Cardoso *et al.*, 2015)

Techniques vary in terms of their clinical utility; sample sizes to measure a treatment effect have been evaluated in SPMS and are dependent on the technique used. Overall, SIENA performed better than SIENAX to detect a 50% effect at the 80% power level (Altmann *et al.*, 2009). The Jacobian Integration method (JIM) further improves the ability to evaluate GM atrophy and assess neuroprotection in trials by 4-5 times that of SIENAX (Nakamura *et al.*, 2014).

3.4.4 Atrophy and MS

Atrophy in MS affects both WM and GM and relates to the underlying pathological processes (Siffrin *et al.*, 2010). The pathological basis for WM atrophy is neurodegeneration of axons, myelin loss, and oligodendrocyte loss within lesions. Within NAWM there is axonal damage and loss as well as myelin loss. GM pathology is widespread in cortical lesions, especially type 3 and type IV subpial lesions, the cortex, and also GM parenchymal structures such as the cerebellum and thalamus (**figure 3.2**). GM atrophy is predominantly a sequela of axonal loss and is less due to demyelination. NAGM also relates to cognitive impairment in MS (Bjartmar *et al.*, 2003; Miller *et al.*, 2003; Honce, 2013). Post-mortem studies show significant differences between histological measures of cortical thickness and MRI measures, highlighting the need for more robust analysis techniques (Popescu *et al.*, 2016).

In untreated progressive MS subjects the rate of brain atrophy change is -0.5 to -1% per year (Furby *et al.*, 2010; Chataway *et al.*, 2014, 2020) versus -0.1 to -0.3% per year for healthy controls (Bermel and Bakshi, 2006) **(Figure 3.3)**. Brain atrophy is even present early in MS, i.e. CIS (Chard and Miller, 2009). Brain atrophy occurs faster as disease duration increases, and is greatest overall in SPMS in terms of phenotype **(Figure 3.2).**

Figure 3.2. MRI scan showing brain atrophy changes in relapsing-remitting and secondary progressive multiple sclerosis in a single subject over 13 years.

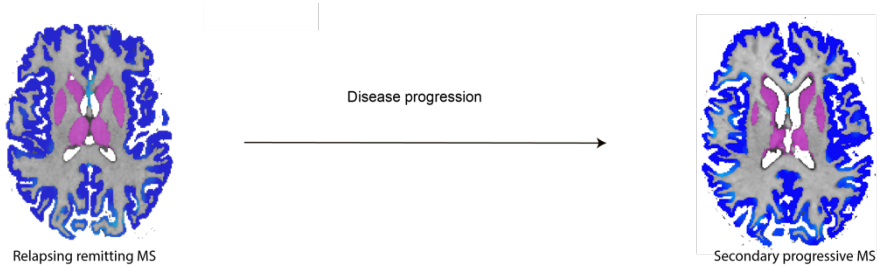
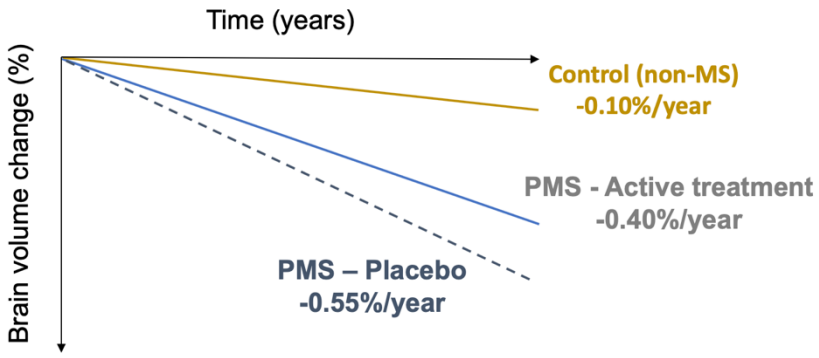


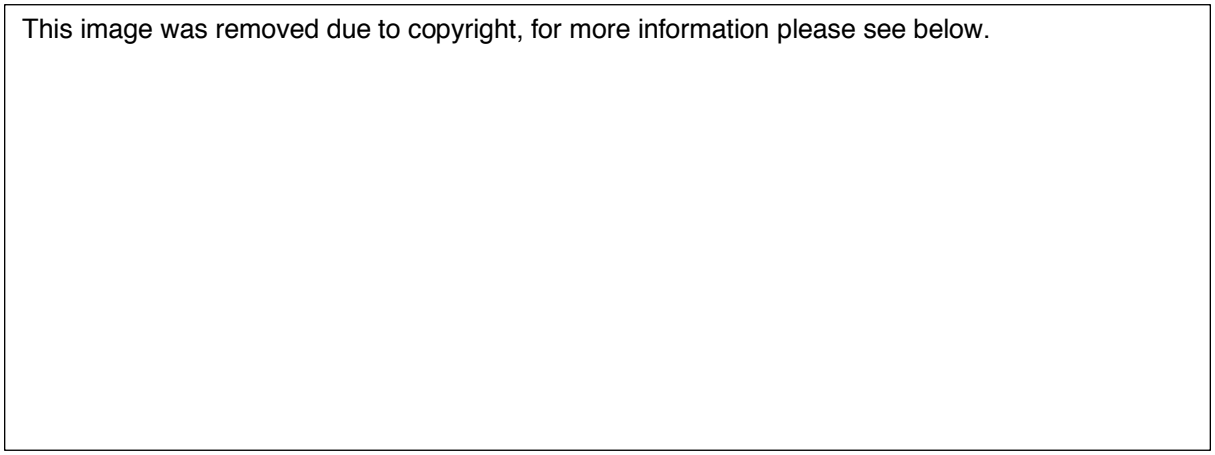
Figure highlights MRI-derived brain atrophy in a patient with relapsing-remitting MS in the scan on the left and MRI after 13 years after which secondary progressive MS had developed. Cortical grey matter is in blue and deep grey matter in purple. Real case image kindly provided courtesy of Arman Eshaghi.

Figure 3.3 Schematic indicating average % whole brain volume differences in controls, those with progressive MS (PMS) with effective treatment, and those not treated with progressive MS across studies.



Brain atrophy can be a marker of MS disease progression and is higher in those with progressive MS than those who are stable (Ingle *et al.*, 2003). Brain volume change is estimated as -0.6%/year in SPMS versus -0.2%/year in age-matched controls (Fox *et al.*, 2000; Chataway *et al.*, 2014) see **Figure 3.4** for a summary. Furby et al undertook a 2-year longitudinal study of atrophy in SPMS and found mean annual whole brain atrophy rate was -0.59% per year. Within this the annual rate of GM atrophy was greater at -1.18% compared with -0.12% for WM (Furby *et al.*, 2010). A further cross-sectional study of 177 SPMS subjects showed significant prediction of the MSFC by the normalised brain volume (Furby *et al.*, 2008). Brain atrophy is highly correlated to T2 lesion load, and a 14 year study showed that this relationship depended most on early T2 lesion accumulation (Chard *et al.*, 2003). New T2 or contrast-enhancing lesions transiently increase brain volume which reverses with steroid therapy (Rocca *et al.*, 2017).

Figure 3.4. The effects of treatment effect on disability progression (hazard ratio) and whole brain atrophy (% change difference) in published progressive MS trials.



With thanks to Jennifer Nicholas for the development of this figure adapted from the Medical Research Councils trials methodology conference 6th-9th October 2019, Brighton, UK.

Grey matter and subcortical atrophy are common in MS and occur in all phenotypes (Miller *et al.*, 2003; Fisher *et al.*, 2008; Honce, 2013; Rocca *et al.*, 2017). Longitudinal studies have shown that the rate of GM atrophy is greater than WM atrophy in MS, and the rate of GM loss increases as MS progresses, but WM atrophy rate remained static (Fisher *et al.*, 2008, Fisniku *et al.*, 2008*b*). As with whole brain atrophy, studies have shown that there is a significant anti-correlation of T2 lesion load and GM volume (Vrenken *et al.*, 2013). However, Steenwijk et al evaluated the role of WM tract abnormality with diffusion weighted MRI, on deep and cortical GM atrophy and found that in SPMS cortical GM atrophy could not be explained by WM disease alone (Steenwijk *et al.*, 2015). GM atrophy was 12.4 times greater in SPMS than in healthy controls in a 4 year follow-up study (Fisher *et al.*, 2008), with the lowest GM and DGM volumes (Eshaghi *et al.*, 2018*b*). PPMS has a predilection for cingulate atrophy (Eshaghi *et al.*, 2014). Overall GM loss correlates more with clinical and cognitive outcome measures than WM loss (Zivadinov *et al.*, 2008; Honce, 2013; De Stefano *et al.*, 2014). GM volume significantly impacts the development of clinically definite MS from CIS in a 3 year cohort study (Dalton *et al.*, 2004). Eshaghi et al showed that only baseline DGM volume predicted time to EDSS progression. There was also the fastest rate of atrophy in the DGM in all MS phenotypes overall, but fastest in the SPMS cohort (Eshaghi *et al.*, 2018*b*).

Regional atrophy appears to occur in different patterns in MS phenotypes and spreads dependent on disease duration and disability level in relapse-onset MS (Eshaghi *et al.*, 2018*a*). GM atrophy is therefore a useful clinical correlate in MS. Reductions in GM volume correlated with cognitive function and overall disability worsening in MS (Amato *et al.*, 2008*a*). The MSFC correlated with GM atrophy more than EDSS in a longitudinal study, perhaps relating to the cognitive input from the PASAT3 in the MSFC (Rudick *et al.*, 2009*b*). Zivadinov et al. undertook a 2 year longitudinal study of 136 RRMS subjects and found that early cortical atrophy predicts disability progression (Zivadinov *et al.*, 2013).

3.4.5 Atrophy and cognition in MS

There are key GM brain areas that have high correlation to cognitive impairment and therefore clinical utility, e.g. the thalamus and cerebellum. Regional thalamic volume may be a good general marker of cognitive impairment, but may not provide a specific domain correlation, e.g. memory, due to the underlying neural basis of neurotransmitters and connections between areas (Schoonheim and Ciccarelli, 2018). Schoonheim et al studied the thalamus with multi-modal MRI looking at FC, diffusivity, and volume. 157 MS patients were graded by cognitive status, and overall all MS patients had lower thalamic volume compared to controls. Thalamic volume, decreased FC, and diffusivity were correlated with and predicted cognitive status (Schoonheim *et al.*, 2015*a*). Thalamic volume correlated with, and predicted, psychomotor speed which correlated with activities of daily living in RRMS (Papathanasiou *et al.*, 2015). Therefore, the thalamus is useful for understanding both day-to-day motor and cognitive function.

The cerebellum, caudate and putamen show early atrophy in RRMS, and this progresses in SPMS, compared to late occurrence in PPMS (Eshaghi *et al.*, 2018*a*). However, the rate of loss in lobar GM regions appeared to be the same in all MS phenotypes (Eshaghi *et al.*, 2018*b*). Most studies of cognitive impairment and the cerebellum look at effects of lesion load (Sarica *et al.*, 2015). Tobyne et al showed that lesions of the middle cerebellar peduncle associated most with cognitive impairment in RRMS (Tobyne *et al.*, 2018). However, a study of regional distribution of

GM atrophy showed that in cognitively impaired SPMS subjects, there was considerably more atrophy of fronto-temporal regions, left hypothalamus, and thalami then in PPMS, but this did not differ in RRMS cognitive impaired subjects (Riccitelli et al., 2011). Riccitelli also showed that those with SPMS who were cognitively impaired had more posterior GM atrophy in the orbital regions, right middle frontal gyrus, middle occipital gyrus, hippocampal, insula, and superior temporal gyrus loss than those with SPMS and no cognitive impairment (Riccitelli et al., 2011).

Benedict et al looked at the role of lobar atrophy on cognitive functioning in MS. Regression models showed that auditory and verbal memory were most predicted by left temporal atrophy. Visuospatial function was most predicted by right and left sided temporal atrophy. Processing speed was, however, most predicted by general whole brain atrophy measures (Benedict *et al.*, 2005).

Given that cognition is strongly correlated to GM and brain atrophy, key atrophy studies with an emphasis on GM and cognition are summarised in the following table (**table 3.4**). As shown, in keeping with the rs-fMRI studies of MS and cognition, there are only 3 longitudinal studies of cognition and GM atrophy including SPMS subjects within the study cohort. Cross-sectional studies focus more on information processing speed and working memory function, and therefore may miss out on the executive dysfunction of SPMS.

Table 3.4. Key studies of regional grey matter atrophy and cognition in MS. *SPMS is in bold font.*

Study	Cohort	Design	Cognitive measures	Outcome
Benedict et al. 2005 (Benedict *et al.*, 2005)	MS (31)	Cross-sectional	PASAT3, SDMT, CVLT, BVMT-R,	Regional atrophy correlated to specific cognitive domains. See main text.
Morgen et al. 2006 (Morgen *et al.*, 2006)	RRMS (19) HC (19)	Cross-sectional	PASAT3, digit span backwards, verbal memory	Greater frontal, parietal, temporal cortical volume loss in RRMS with cognitive impairment.
Houtchens et al. 2007 (Houtchens *et al.*, 2007)	RRMS (26) **SPMS (5)**	Cross-sectional	All domains	Thalamic volume positive correlation with cognitive tests and negative with EDSS.
Glanz et al. 2007 (Glanz *et al.*, 2007)	CIS/MS (92)	Cross-sectional	SRT, SPART, SDMT, PASAT3, COWAT,	49% CI. No correlation with T2LL, NAWM, NAGM, GM, BPF
Fisniku et al 2008 (Fisniku *et al.*, 2008*b*)	CIS (29) RRMS (33) **SPMS (11)** HC (25)	Cross-sectional	MSFC - PASAT3	GM not WM fraction correlated with EDSS and MSFC.
Fisher et al. 2008 (Fisher *et al.*, 2008)	CIS (7) RRMS (36) **SPMS (27)** HC (17)	Longitudinal (4yrs)	MSFC - PASAT3	GM fraction correlation with EDSS and MSFC. Increased contribution of GM atrophy to brain atrophy with increased progression.
Amato et al. 2008 (Amato *et al.*, 2008*a*)	MS (47) EDSS<3.0	Cross-sectional	BRNB Stroop	CI group associated with higher T2LL and neocortical volume.

Audoin et al. 2010 (Audoin *et al.*, 2010)	CIS (62) HC (37)	Cross-sectional	All domains; SRT, PASAT3, SDMT, Visuospatial memory, word list generation	No correlation of cognitive outcomes with deep grey matter structures. EDSS correlates with right cerebellum.
Calabrese et al. 2010 (Calabrese *et al.*, 2010)	RRMS (100) HC (42)	Cross-sectional	BRNB	Cortical thinning predicts cognitive impairment.
Roosendaal et al. 2011 (Roosendaal *et al.*, 2011)	CIS (95) RRMS (657) **SPMS (125)** PPMS (50)	Cross-sectional	PASAT3	Normalised GM volume correlated with cognitive impairment.
Riccitelli et al. 2011 (Riccitelli *et al.*, 2011)	RRMS (22) **SPMS (29)** PPMS (22) HC (39)	Cross-sectional	PASAT3, VFT, RCFT, SST, WLT	Frontal, parietal, and temporal GM atrophy pattern characterises CI (39) vs CP. RRMS and SPMS CI had correlated atrophy to LL. SPMS CI had more frontotemporal, thalamic, and left hypothalamus GM loss. SPMS CI had more orbital, right middle frontal gyrus, middle occipital gyrus, hippocampal, insula, and superior temporal gyrus loss than CP.
Amato et al. 2012 (Amato *et al.*, 2012)	RIS (29) RRMS (26) HC (21)	Cross-sectional	BRNB Stroop test	Lower normalised cortical volume and high LL correlates with cognitive performance in RIS.
Batista et al. 2012 (Batista *et al.*, 2012)	RRMS (59) **SPMS (27)**	Cross-sectional	PASAT3 SDMT	Thalamic atrophy relates to information processing speed.

Schoonheim et al. 2012 (Schoonheim *et al.*, 2012*b*)	MS (80Female 40Male) HC (30 Female 20 Male)	Cross-sectional	7 domains; BRNB, Stroop, CST, MCT	Deep GM reduced in MS (M -11% > F -6.3%) Cognition predicted by thalamic volume, sex, education.
Benedict et al. (2013) (Benedict *et al.*, 2013)	MS (75) HC (18)	Cross-sectional	CVLT, BVMT-R, SDMT, PASAT3, DKEFS	Thalamic volume predicted all cognitive outcomes.
Filippi et al. 2013 (Filippi *et al.*, 2013*b*)	CIS (20) RRMS (34) **SPMS (19)**	Longitudinal (13yrs)	BRNB	GM associated with motor disability worsening in 66% and cognitive decline in 37%.
Tewarie et al. 2013 (Tewarie *et al.*, 2013)	MS (21) HC (17)	Cross-sectional	BRNB, SRT, SPART, SDMT, WLG, CST, MCT	Multimodal (+MEG). MS had lower thalamic volumes, GM volume, and NBV.
Nocentini et al. 2014 (Nocentini *et al.*, 2014)	RRMS (13) **SPMS (5)**	Cross-sectional	All domains; PASAT3, SDMT, CVLT, RCFT, BJLOT, COWAT	SDMT, Delayed- recall CVLT correlated with regional prefrontal, temporal, parietal, and insular atrophy
Schoonheim et al. 2015 (Schoonheim *et al.*, 2015*a*)	MS (157) HC (47)	Cross-sectional	7 domains; BRNB, Stroop, CST, MCT	Multimodal (+DTI+rs-fMRI) Lowest thalamic volume in severe CI.

Modica et al. (2016) (Modica *et al.*, 2016)	MS (71) HC (23)	Longitudinal (3yrs)	BICAMS	Subcortical GM volumes correlate with BICAMS scores. Years of education (cognitive reserve) effected decline in cognitive processing speed.
Bergsland et al. 2016 (Bergsland *et al.*, 2016)	RRMS (44) **SPMS (20)** HC (22)	Longitudinal (3yrs)	BICAMS	Cognitive outcomes related to DGM volume and shape. Atrophy of the anterior and superior left thalamus related to processing speed.
Meijer et al. 2018 (Meijer *et al.*, 2018)	MS (330) HC (96)	Cross-sectional	SDMT	Multimodal (+rs-fMRI). Impaired IPS associated with lower DGM volumes.
Eijlers et al. 2018 (Eijlers *et al.*, 2018)	MS (234) HC (60)	Longitudinal (5yrs)	BRNB, CST, SRT, WLG, Stroop, MCT, RCI	Multimodal (+DTI). Cortical GM and temporal atrophy only were significant for cognitive decline.
Eijlers et al. 2019 (Eijlers *et al.*, 2019)	RRMS (179) PMS (51) HC (59)	Longitudinal (5yrs)	Expanded BRNB – 7 domains	Cortical atrophy PMS>RRMS GM atrophy PMS=RRMS Cognitive decline PMS>RRMS.

RCFT=Rey-Osterrieth Complex Figure Test, SDMT=symbol digit modalities test, MSFC=Multiple Sclerosis Functional Composite, PASAT3=Paced Auditory Serial Additions Test, CVLT=California Verbal Learning Test, SRT=Serial Reading Test , BRNB=Brief Repeatable Battery, BJLOT=Benton Judgement of Line Orientation Test, COWAT=Controlled Oral Words Association Test, VFT=Verbal Fluency Test, SST=short story test, WLT=word learning test, DKEFS= Delis-Kaplan Executive Function System Sorting Test, BICAMS =Brief International Cognitive Assessment for MS, WLG=word list generation, SPART=spatial recall test, RCI=reliable change index, T2LL=lesion load, BPF=brain parenchymal fraction, CI=cognitive impairment, IPS=information processing speed, DGM=deep grey matter, NBV=normalised brain volume, DTI=diffusion tensor imaging, rs-fMRI=resting state function MRI, PMS=progressive MS, RRMS=relapsing remitting MS, MEG=magnetoencephalography, GM=grey matter, NAWM=normal appearing white matter, NAGM=normal appearing grey matter.

4 Cognitive performance and impairment in SPMS

4.1 Introduction

Deficits of working memory and information processing speed affect all phenotypes of MS. As progressive disease develops there is greater dysfunction of other cognitive domains with a disproportionate input of higher order working memory and executive dysfunction in SPMS in literature cohort studies (Connick *et al.*, 2013; Chan *et al.*, 2017). An understanding of the general profile of cognitive function in SPMS is an ideal basis from which to explore cognitive performance.

Large pure SPMS cohorts for furthering our understanding of cognition in SPMS over time are rare. Those which exist suggest that cognitive impairment progresses over time and is associated with worsening on tests known to be the most sensitive and specific for cognitive function in MS, i.e. the SDMT (Strober *et al.*, 2014). Additionally, there is worsening of executive function, and this appeared to show targeted improvement secondary to a putative neuroprotective drug, simvastatin, over time (Chan *et al.*, 2017). This chapter will look at between group changes over 96 weeks in the SPMS and healthy control groups to review if changes might relate to normal ageing processes. Additionally, I evaluate predictors of deterioration on individual tests over time in SPMS.

How we define and determine cognitive status in MS is not uniform in the literature (**section 1.5**) (Fischer *et al.*, 2014). This creates difficulty when interpreting prevalence rates of cognitive impairment, and also in creating suitable outcome measures for trials and research. Given that cognitive symptoms are commonly reported by those with MS, having clear and consistent metrics for cognition is critical to progress our understanding of the underlying mechanistic processes (Sumowski *et al.*, 2018). The experiments in **section 3.5** of this chapter explore strategies for how best to do this in SPMS. I also look at predictive factors of cognitive impairment at follow-up visit from a preserved state.

My chapter aims are to investigate the following aspects of cognitive function in SPMS;

1) Between group differences in the cognitive profile of SPMS versus controls, and which factors predict cognitive performance in SPMS at baseline (**section 4.3**).
2) Within group changes in cognitive performance over time in SPMS versus controls and the factors that predict worsening SPMS cognition over time (**section 3.4**).
3) The effects of using different definitions of cognitive impairment in SPMS, by comparing two thresholds for defining cognitive impairment and how these definitions affect the predictive value of other outcome measures at baseline. Additionally, I look at predictors of SPMS cognitive impairment at follow-up from a preserved state. (**section 3.5**).

4.2 Study Design

This and subsequent chapters of this thesis concern a cohort of patients with SPMS who were co-recruited with the MS-SMART study (Connick *et al.*, 2018) at the University College London site. The rationale and background for the MS-SMART trial have been discussed in **section 1.4**. Embedded clinical assessments were undertaken at baseline alongside MS-SMART trial visits, and at longitudinal, 96-week, follow-up. This study design reduced the visit burden for SPMS subjects, due to some shared assessments for both studies. Having a single assessing rater per visit also enhanced overall efficiency. The additional test time for those undertaking MS-SMART was 30 minutes of further cognitive testing (the total time for all clinical measures is 90 minutes) and 6 more minutes of MRI (rs-fMRI) at 0 and 96 weeks.

4.2.1 Subject Recruitment

Subject recruitment began on the 23th September 2015 and ended on the 8th September 2016. From the 23th September 2015 to the 9th June 2016, I recruited 70 subjects who met the MS-SMART eligibility criteria in **table 1.4**. They were identified at MS-SMART screening visit and provided with patient information leaflets prior to consent. Although 46 were assessed at 0-week MS-SMART visit, prior to commencement of study drug or placebo, 24 subjects were assessed at their 24-week MS-SMART visit. As can be seen in the subsequent sections of this chapter and following chapters, group comparisons of the clinical assessments was made to ensure there were no significant unaccounted-for differences between the 0-week and 24-week SPMS groups before combination for overall SPMS cohort analyses.

16 age and gender matched healthy controls were locally recruited, from the 9th June 2016 to the 8th September 2016. Healthy controls were friends and relatives of SPMS participants or were UCL colleagues. The primary aim was to determine a suitable control baseline for the rs-fMRI data.

4.2.2 Inclusion and exclusion criteria

SPMS subjects met the MS-SMART inclusion and exclusion criteria summarised in **section 1.3**, **table 1.4.** In addition, subjects had to be willing and able to tolerate MRI scanning without contraindication for up to 90 minutes including the full MS-SMART protocol and rs-fMRI protocol.

Healthy controls did not have a history of current or previous neurological disease, or active medical conditions which would contraindicate their participation in the study, and no contraindication to MRI scanning following the MRI protocol described in **chapter 5** (**table 5.1**). Healthy controls were recruited retrospectively, to allow accurate age and gender matching between healthy control and SPMS groups. Inclusion age was 47-61 years of age (matching the mean +/- one SD of the SPMS cohort), and male:female gender ratio; 4:12 (male:female; 17:53 in the SPMS cohort).

4.2.3 Follow-up

Longitudinal assessment was completed between the 11th July 2017 to the 21st May 2018. In the SPMS cohort 59 completed the follow-up visit, alongside 13 healthy controls (**figure 4.1**). I decided that follow-up and quality control would be best done on a single scanner, and therefore all visits were completed on the same scanner pre-scanner upgrade. Time interval between visits was used as a covariate in follow-up analyses looking at between-visit variables.

A summary of the overall cohort partaking in the study in terms of clinical and MRI outcome measures at baseline and follow-up timepoints is provided in **figure 4.2**.

Figure 4.1 Timeline schematic of the study.

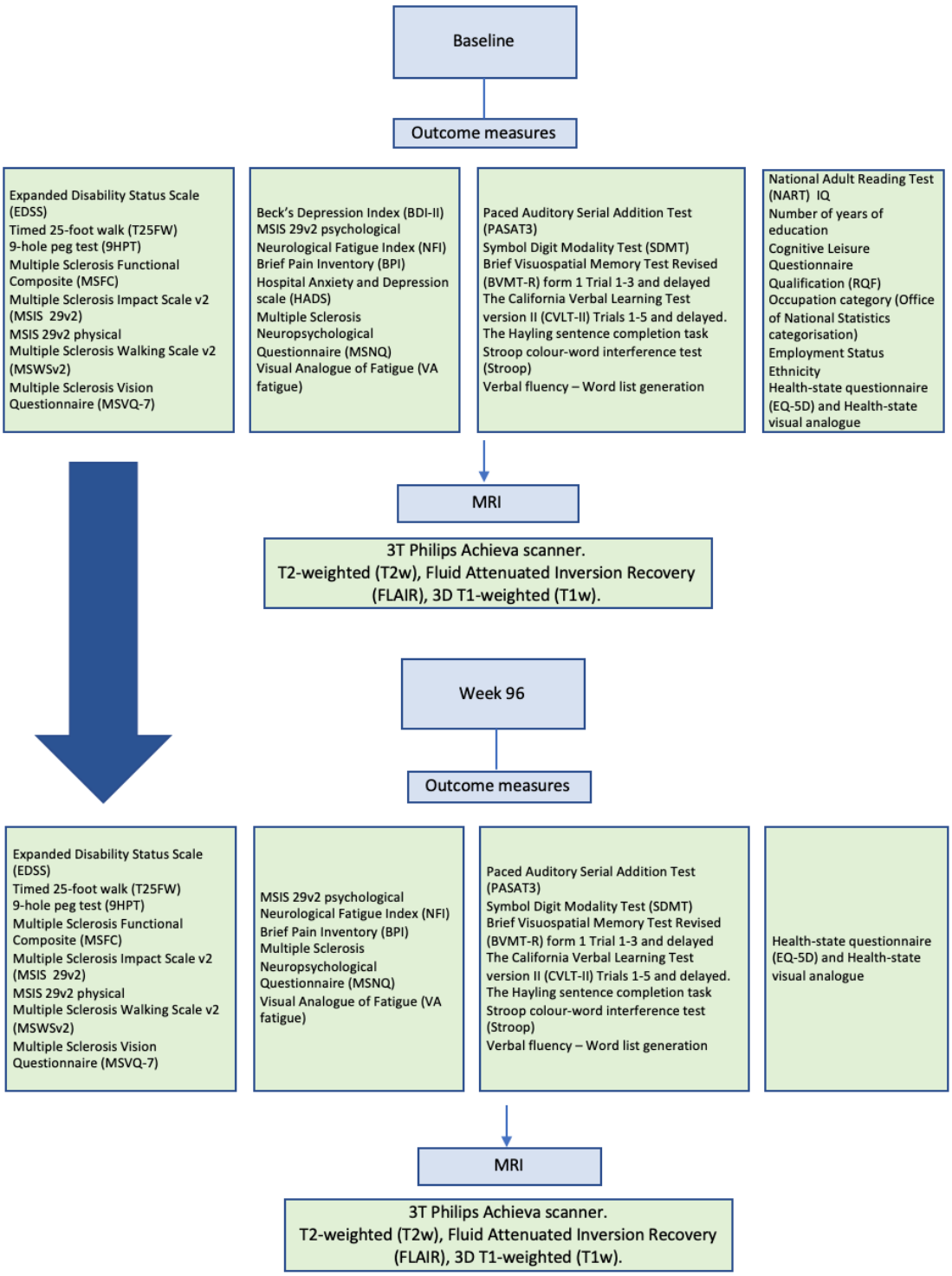
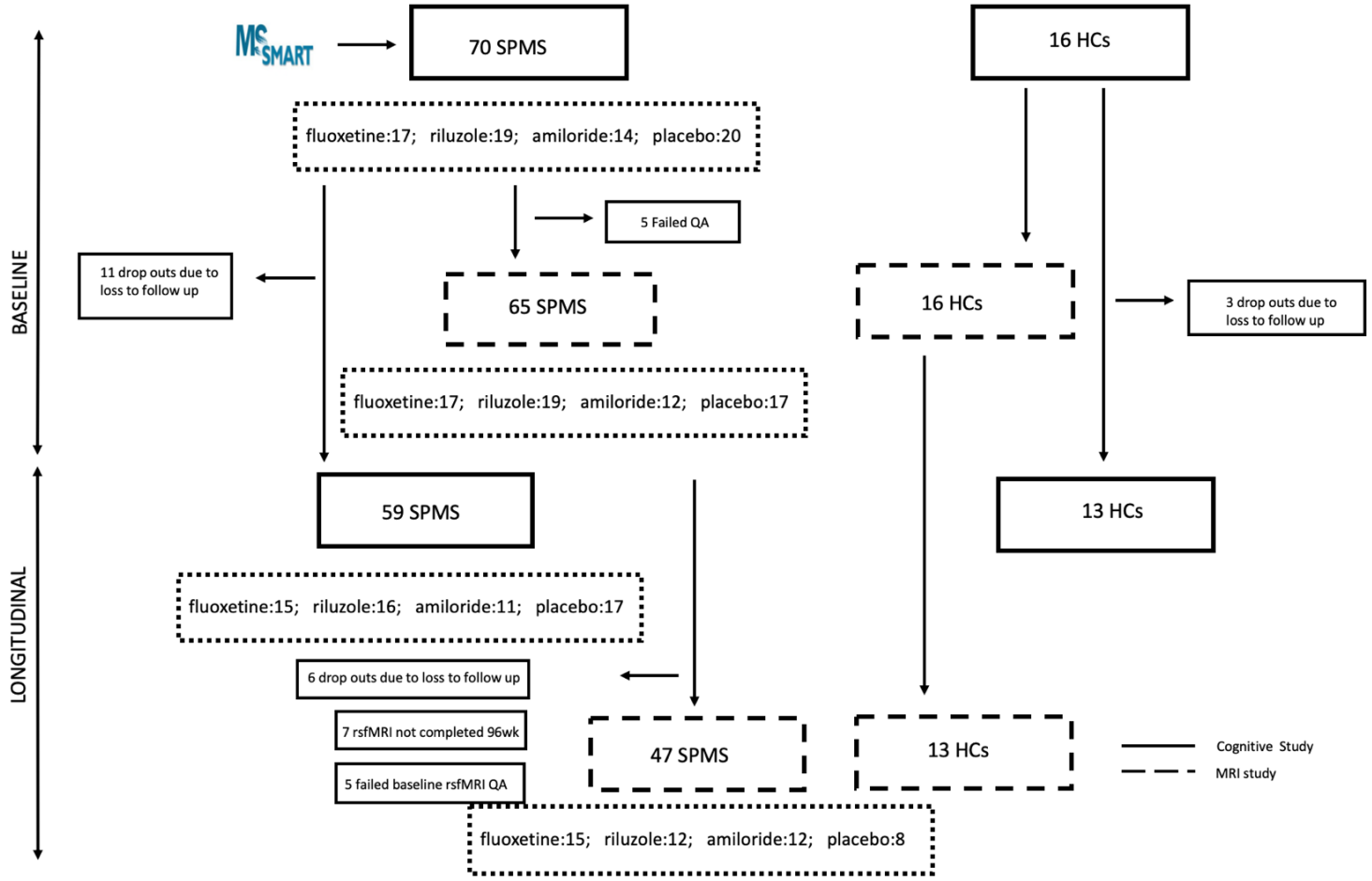


Figure 4.2 Study profile.



Numbers represent the sample size per cohort. Solid boxes show those in the cognitive study. Dotted boxes show drug arm distribution. Dashed boxes show those taking part in the rs-fMRI and GM atrophy MRI study. QA = quality assessment, , HC = healthy controls, SPMS = secondary progressive MS.

4.2.4 Ethical approval and informed consent

Research ethics committee (REC) approval has been gained for this project (REC: London – Queen Square. Study Reference: 09/H0716/77(09/0386-GML02) and appropriate documents for informed consent generated and completed as per UCL policy. MS-SMART had separate REC approval; REC 13/SS/0007, and consent forms which were completed.

4.2.5 Neurological and neuropsychological test protocol

Subjects with SPMS underwent the full MS-SMART battery of assessments as well as further PROMS and cognitive assessments. The choice of additional measures was based on evidence from the literature of what is likely to, and what may affect cognitive function in SPMS. In particular I added weight to the demographic, and non-physical variables from the MS-SMART study which may affect cognitive status in SPMS. A summary of assessments from MS-SMART and additional outcome measures of relevance to this project are in **tables 3.2** and **3.3** below.

Table 4.1. Study assessment battery.

Category	MS-SMART	Additional measures
Demographic	Age at visit Gender Disease duration from first symptom Years of progression Health-state questionnaire (EQ-5D) and Health-state visual analogue	Handedness National Adult Reading Test (NART) for IQ Number of years of education Cognitive Leisure Questionnaire (CLQ) Qualification (RQF) Occupation category (Office of National Statistics categorisation) Employment Status Ethnicity
Physical	Expanded Disability Status Scale (EDSS) Timed 25-foot walk (T25FW) 9-hole peg test (9HPT) Multiple Sclerosis Functional Composite (MSFC) Multiple Sclerosis Impact Scale v2 (MSIS 29v2) MSIS 29v2 physical Multiple Sclerosis Walking Scale v2 (MSWSv2)	Multiple Sclerosis Vision Questionnaire (MSVQ-7)
Non-physical	Beck's Depression Index (BDI-II) MSIS 29v2 psychological Neurological Fatigue Index (NFI) Brief Pain Inventory (BPI)	Hospital Anxiety and Depression scale (HADS) Multiple Sclerosis Neuropsychological Questionnaire (MSNQ) Visual Analogue of Fatigue (VAF)
Cognitive	**Auditory Information Processing Speed** Paced Auditory Serial Addition Test- 3 seconds (PASAT3)	**Visual memory** Brief Visuospatial Memory Test Revised (BVMT-R) form 1 Trial 1-3 and delayed
	Visual Information Processing Speed Symbol Digit Modality Test (SDMT)	**Verbal memory** The California Verbal Learning Test version II (CVLT-II) Trials 1-5 and delayed.
		Executive Function The Hayling sentence completion task Stroop Colour-Word interference task (Stroop) Verbal fluency – Word list generation

Subjects underwent assessment of; demographic, physical and non-physical and cognitive categories. Assessments chosen were objectively and subjectively measured, and had validation for assessment by a medically trained physician.

The following assessments were made at the baseline visit; handedness, estimated premorbid IQ with the National Adult Reading Test (NART) (Nelson and Willison, 1982), years of education Cognitive Leisure Questionnaire (CLQ), qualification (RQF), occupation category (Office of National Statistics categorisation), employment Status, ethnicity as stated by the subject, and subjective neuropsychological status with the Hospital Anxiety and Depression scale (HADS) (Zigmond and Snaith, 1983).

Additionally, those with SPMS underwent Beck's Depression Index (BDI-II), and assessment of motor function using the EDSS (Kurtzke JF., 1983), composite MSFC, T25FW and 9HPT (Rudick *et al.*, 2002). The following cognitive domains were assessed: visual memory (BVMT-R immediate and delayed components) (Benedict, 1997); verbal memory (CVLT-II Trials 1-5 and delayed) (Delis *et al.*, 2000); auditory information processing speed and working memory (PASAT3) (Gronwall DM., 1977; Rudick *et al.*, 2002) and visual information processing speed and working memory (SDMT) (Benedict *et al.*, 2012); Executive function with the Hayling sentence completion task (Burgess and Shallice, 1997), Stroop colour-word interference task (Stroop, 1935), and verbal fluency word list generation (Borkowski JG, Benton AL, 1967)). See **table 4.2** for more information.

Table 4.2. Assessment information.

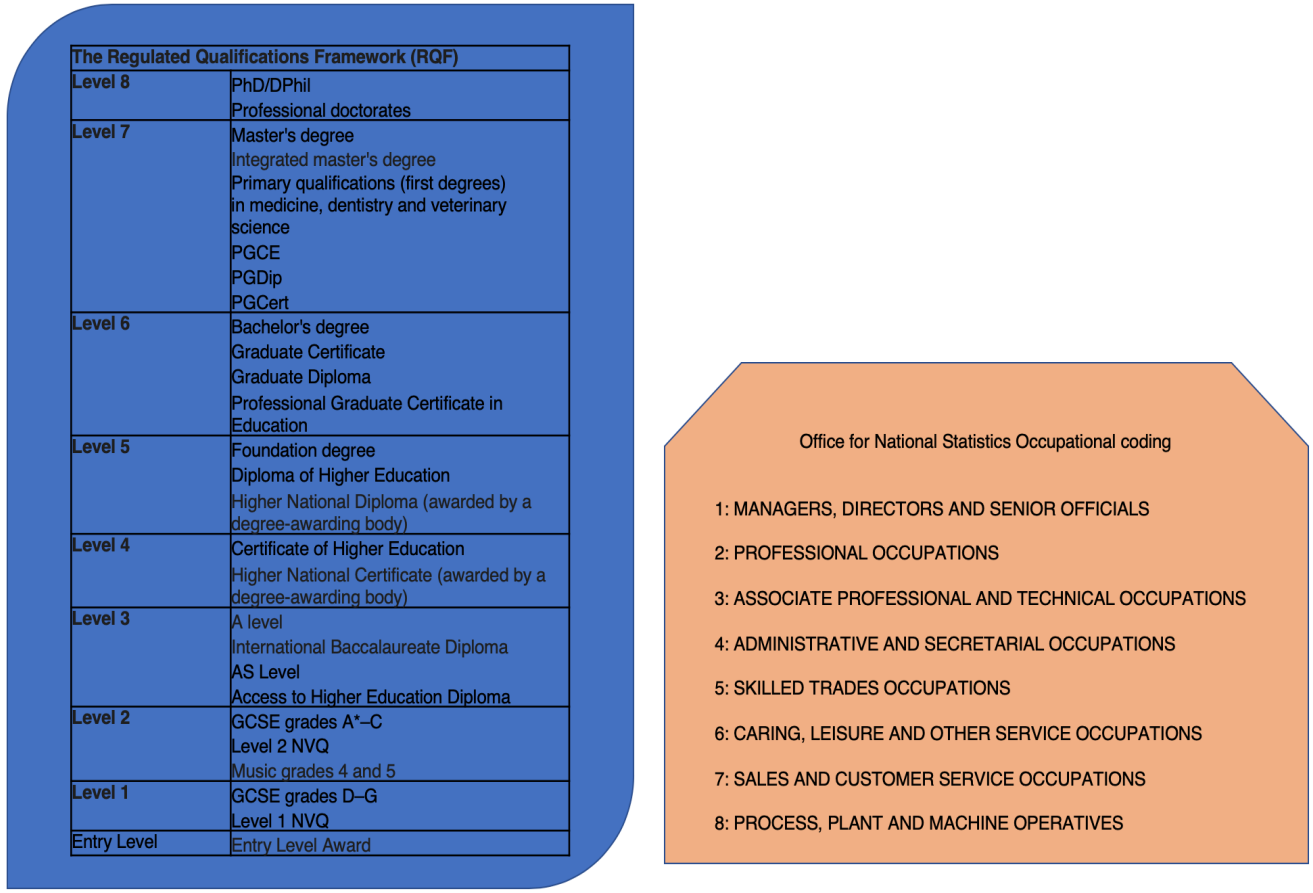
Assessment	Information regarding the assessment
Demographic	
National Adult Reading Test (NART) for IQ	British NART version as an estimate of pre-morbid IQ (Nelson and Willison, 1982). *Baseline only.*
Cognitive Leisure Questionnaire (CLQ)	Likert scale-based questionnaire with 21 questions based on hobbies and interests prior to MS diagnosis. *Baseline only.*
Qualification (RQF)	Based on the UK Regulated Qualifications Framework (RQF) categorisation Entry Level-Level 8 (Ofqual, 2015). *Baseline only.*
Occupation category	Based on the UK Office of National Statistics categorisation 1-7 determined by career classification (Office of National Statistics, 2018). *Baseline only.*
Employment Status	Classified as Full-time/Part-time/Retired as determined by the subject at baseline visit. *Baseline only.*
Health-state questionnaire (EQ-5D) and Health-state visual analogue	The EQ-5D comprises health state classification followed by a health evaluation via a visual analogue scale (VAS) (Herdman *et al.*, 2011).
Ethnicity	Classified subjectively according to the subject's perceived ethnic background. *Baseline only.*
Physical	
Expanded Disability Status Scale (EDSS)	Scaled 0-10 from 0 for no disability versus 10 for death. Functional scores below 4.0 are based on non—ambulatory categories, whereas 4.0 and above are ambulation based functional impairments. Used in MS clinical practice. (Kurtzke, John, 1983). *Repeated at baseline and 96 weeks.*
Timed 25-foot walk (T25FW)	This was directly observed and recorded. An average result from both trials was derived. *Repeated at baseline and 96 weeks.*
9-hole peg test (9HPT)	Gold standard for recording arm and hand function in trials. This was directly observed and recorded. Average results from both trials of both hands (dominant and non-dominant) was derived. *Repeated at baseline and 96 weeks.*

Multiple Sclerosis Functional Composite (MSFC)	The standardised MSFC score was formed of the following tests performed in this order; firstly, the T25FW, 9HPT, and then PASAT3. Scoring represents the average z-score change in the three tasks by formulating a composite (Cutter *et al.*, 1999; Fischer *et al.*, 2001). *Repeated at baseline and 96 weeks.*
Multiple Sclerosis Impact Scale v2 (MSIS 29v2) physical	20 physical and 9 psychological item outcome scale of the impact of MS on daily functions (Hobart, 2001). *Repeated at baseline and 96 weeks.*
Multiple Sclerosis Vision Questionnaire (MSVQ-7)	25 item functional visual symptom questionnaire (Ma *et al.*, 2002). *Repeated at baseline and 96 weeks.*
Multiple Sclerosis Walking Scale v2 (MSWSv2)	12 questions concerning MS related walking limitations during the past 2 weeks. The Likert scale 1-5, is transformed to a 0-100 score (Hobart *et al.*, 2003). *Repeated at baseline and 96 weeks.*
Non-Physical	
Beck's Depression Index (BDI-II)	BDI-II was used as a screening tool for MS-SMART. Score > 18 was an exclusion criterion (Beck, A.T., Steer, R.A., & Brown *et al.*, 1996). *Baseline only.*
Hospital Anxiety and Depression scale (HADS)	14 item ordinal scale; 7 items for anxiety and 7 items for depression. Each item scored 0-3. Score of >8/21 is sensitive and significant for anxiety and depression individually (Zigmond and Snaith, 1983). *Baseline only.*
MSIS 29v2 psychological	20 physical and 9 psychological item outcome scale of the impact of MS on daily functions (Hobart, 2001). *Repeated at baseline and 96 weeks.*
Multiple Sclerosis Neuropsychological Questionnaire (MSNQ)	15 item self-administered questionnaire of recent neuropsychological function (Benedict *et al.*, 2003). *Repeated at baseline and 96 weeks.*
Neurological Fatigue Index (NFI)	10 item validated questionnaire summing the effect of fatigue on daily function (Mills *et al.*, 2010). *Repeated at baseline and 96 weeks.*
Visual Analogue of Fatigue (VAF)	Horizontal centimetre gradated 10cm scale with 0 meaning 'lively and alert', and 10 'absolutely no energy to complete anything at all'. Performed at the beginning and end of the assessment battery. Final scores were subtracted from initial scores to create a summative value. *Repeated at baseline and 96 weeks.*

Brief Pain Inventory (BPI)	Measures the sensory dimension (pain intensity) and reactive dimension (interference of life secondary to pain) (Cleeland and Ryan, 1994). *Repeated at baseline and 96 weeks.*
Cognitive	
Auditory Information Processing Speed **Paced Auditory Serial Addition Test- 3 seconds (PASAT3)**	Practice trials were undertaken as per instructions, and individuals provided answers verbally. Scored out of 60. Performed after the T25FW and 9HPT as per the MSFC. *Repeated at baseline and 96 weeks.*
Visual Information Processing Speed **Symbol Digit Modality Test (SDMT)**	Oral SDMT performed as per MS-SMART trial protocol. *Repeated at baseline and 96 weeks.*
Visual memory Brief Visuospatial Memory Test Revised (BVMT-R) form 1 Trial 1-3 and delayed	Subject used their dominant hand to undertaken the task and wore visual aids as needed. *Repeated at baseline and 96 weeks.*
Verbal memory The California Verbal Learning Test version II (CVLT-II) Trials 1-5 and delayed.	Verbal recall of lists. Recorded forms of the list performed by myself were used for assessments to ensure consistency of administration. *Repeated at baseline and 96 weeks.*
Executive Function The Hayling sentence completion task	Instructions and sentences read and scored as per manual (Burgess and Shallice, 1997). *Repeated at baseline and 96 weeks.*
Stroop Colour-Word interference task (Stroop)	Only Colour-word is stated as a measure for below analyses and not the word list reading task (Stroop, 1935). *Repeated at baseline and 96 weeks.*
Verbal fluency – Word list generation	F, A, and S letter trials performed as per validation studies (Borkowski *et al.*, 1967; Tombaugh *et al.*, 1999; Chapados and Petrides, 2013; Kopp *et al.*, 2013). *Repeated at baseline and 96 weeks.*

Assessments undertaken in this project with specific caveats regarding background, practical undertaking, and scoring. Items in bold were undertaken as part of the MS-SMART protocol. Italics state whether the assessment was at baseline, longitudinal visit or both.

Figure 4.3. Summary of the Regulated Qualifications Framework and the National Office of National Statistics Occupational coding 2010.



The Regulated Qualifications Framework (RQF)	
Level 8	PhD/DPhil Professional doctorates
Level 7	Master's degree Integrated master's degree Primary qualifications (first degrees) in medicine, dentistry and veterinary science PGCE PGDip PGCert
Level 6	Bachelor's degree Graduate Certificate Graduate Diploma Professional Graduate Certificate in Education
Level 5	Foundation degree Diploma of Higher Education Higher National Diploma (awarded by a degree-awarding body)
Level 4	Certificate of Higher Education Higher National Certificate (awarded by a degree-awarding body)
Level 3	A level International Baccalaureate Diploma AS Level Access to Higher Education Diploma
Level 2	GCSE grades A*–C Level 2 NVQ Music grades 4 and 5
Level 1	GCSE grades D–G Level 1 NVQ
Entry Level	Entry Level Award

(Ofqual, 2015; Office of National Statistics, 2018).

4.3 Cognitive performance in SPMS: a cross-sectional analysis

4.3.1 Introduction

As described earlier, in **section 1.5**, SPMS, cognitive performance can be limited more by higher order working memory and executive function than in other phenotypes. This section defines the cognitive profile of this study's SPMS cohort versus healthy controls cross-sectionally, and looks at the associations of this to demographic and clinical outcomes.

The aims of this section are:

1) To define between group differences in the cognitive profile of SPMS subjects versus controls at baseline (**section 4.3.3.2**).
2) To investigate determinants and associations of cognitive performance with outcome measures in SPMS at baseline (**section 4.3.3.3**).

4.3.2 Methods

For this experiment subjects were recruited as per the study design, **section 4.2**. Summaries of the cohorts included in the following analyses are in **figure 4.2**. I reviewed the following SPMS cohorts at baseline:

1) SPMS recruited at 0-week and 24-week MS-SMART visit.
2) Healthy controls versus the overall SPMS.

4.3.2.1 Neurological and neuropsychological test protocol

Subjects completed the assessment batteries stated in **table 4.1,** described further in **table 4.2** in **section 4.2.6.** Previous research carried out at the National Hospital for Neurology and Neurosurgery, University College London, has shown a higher premorbid NART IQ in this MS population (Nelson and Willison, 1982). Based on this evidence, I used age- and gender-matched

healthy control data locally collected during this study to provide the comparative normative dataset.

Raw cognitive outcome test scores for the SPMS groups were converted to z-scores using the normative healthy control data.

$$z=(x-\mu) / \sigma$$

Where x=raw score, μ=normative mean, σ=normative standard deviation.

4.3.2.2 Statistical analysis

I performed statistical analyses using Stata (SE 15.1 for Mac. StataCorp, College Station, TX 77845, USA) (STATACorp LLC, 2017).

Descriptive evaluation and differences between variables by group

Frequency, mean, and SD are presented for the following independent variables (see **table 4.1 and 3.3** for expanded abbreviations);

- Age
- Disease duration from first symptom (years)
- Years of Progression
- 9HPT
- T25FW
- MSFC
- MSIS 29v2
- MSIS 29v2 physical sub-score
- MSWSv2
- MSVQ-7
- Years of education
- NART predicted IQ
- HADS depression score
- HADS anxiety score
- BDI-II
- MSNQ
- MSIS 29v2 psychological sub-score
- Visual Analogue fatigue
- NFI
- BPI
- Cognitive Leisure
- EQ-5D
- Health
- BVMT-R
- BVMT-R retained
- CVLT-II
- CVLT-II delayed
- SDMT
- PASAT3
- Verbal Fluency
- Stroop
- Hayling task

Frequency, median, and range are presented for EDSS. Categorical variables are presented as frequency and frequency ratios according to group and these are; gender, MS-SMART drug arm, qualification, occupation, employment status, and ethnicity as reported by the subject. Frequency of the main categorical variables are plotted as clustered bar charts or pie charts.

Assessment of between group differences was dependent on variable type (numerical, categorical, binary, normal distribution). T-tests were used to test differences in age. The non-parametric Wilcoxon-Mann-Whitney test was used to determine the significance of differences between mean values of at least ordinal variables without assuming normal distribution. Dummy variables were created to determine differences between categorical variables if more than 2 categories. Categorical variable differences were tested with Chi^2 tests if less then 2 variables, i.e. gender. The non-parametric Kruskal-Wallis test was used to test the significance of group differences for categorical independent variables with two or more groups; i.e. drug arm, qualification, employment, occupation, ethnicity, and drug arm.

Results were considered significant if they met a statistical threshold of p-value or chi-squared probability ≤ 0.05. Results are presented as frequency tables with analyses of differences between groups. Variables are divided in the tables according to the division in **table 4.3** into the following groups; covariate, physical disability, non-physical, and demographic. Scatterplots were used to look for non-linearities and normality of the variables. I have plotted boxplots, one-way dot plots with overlying boxplots, or scatterplots of those variables felt to be significantly different between cohorts.

Correlations with cognitive outcome scores

Pearson's pairwise correlations of independent variables were performed according to cohort between all variables and cognitive outcome measures. Results were Bonferroni corrected for multiple comparisons and a statistical threshold of $p \leq 0.05$ was considered significant. These are presented as correlation matrices and scatterplots with 95% confidence intervals (Cis).

Multivariate linear regression models

For the regression models, I divided all of the independent variables into variables likely to affect cognitive scores as suggested by background review of the literature, named covariate predictors, or independent predictors; divided by type into physical disability predictors, non-physical predictors, and demographic predictors (**table 4.3**). A priori multivariable linear regression models were used to find independent predictors of the top four cognitive test scores showing the most significant between group differences in the SPMS versus healthy control groups overall.

To create the models, I undertook manual backwards stepwise elimination of covariate variables to eliminate variables with a significance level of $p>0.05$. This continued until the adjusted R-squared (R^2) value dropped at which point no further covariates were removed. To this covariate model, independent predictors were added singly into the linear model to test individual predictions of the raw cognitive outcome scores. I did not add multiple independent variables to the model to allow a clear evaluation of individual predictors on the response or dependent variable. Categorical variables with greater than two categories, i.e. qualification, occupation category, employment, and ethnicity, were evaluated using multiple categorical groupings with dummy variables. The reference variable was level 1 of the variable, e.g. Entry level on the RQF scale for qualification, and other levels were evaluated as differences from this base level.

To assess for normality, normal quantile plot graphs were generated to compare the quantiles of the variables to the quantiles of a normal gaussian distribution. Where quantiles were skewed from the normal quantile distribution, I undertook log transformation of the variable and normality was re-tested. The predictive performance of each model was evaluated using the adjusted R^2 statistic. Statistical significance of the independent predictors was confirmed if $p\leq0.05$.

To display the results, I created tables of Beta-coefficient (β), p-value, 95% CI, and adjusted R^2. I generated scatterplots of outcome versus predictor variables corrected for relevant covariates to show the fitted values from the model with 95% CIs, when variables were shown to significantly

affect the dependent variable as predicted by the regression model, i.e. had $p \leq 0.05$, to look at the visual linear predictions.

Table 4.3. Predictors that should (covariate) and could (independent predictors) affect cognitive outcome scores for multivariate linear and logistic regression models of cognitive outcome measures.

Covariate predictors	Independent predictors
Age at baseline/96 weeks	**Physical Disability predictors**
Gender	EDSS
Disease duration from first symptom	9HPT
Years of education	T25FW
NART predicted IQ	MSFC
HADS depression score	MSIS 29v2 physical
HADS anxiety score	MSIS 29v2
BDI-II	MSWSv2
	MSVQ-7
	Non-physical predictors
	MSNQ
	MSIS 29v2 psychological
	VAF
	NFI
	BPI
	Demographic predictors
	Drug arm
	CLQ
	RQF Qualification
	Occupation
	Employment Status
	EQ-5D
	Health
	Ethnicity

4.3.3 Results

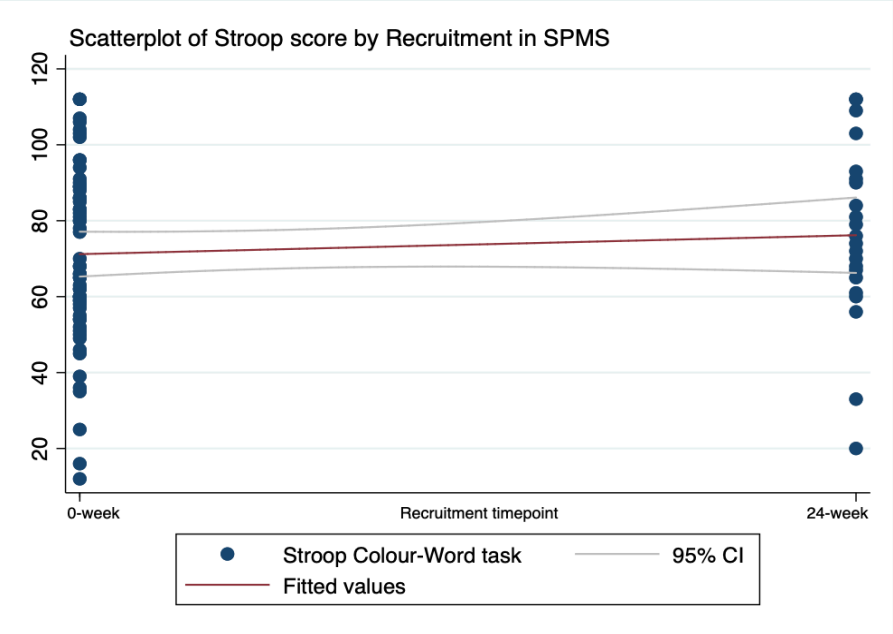
This results section summarises the analyses and associations of cognitive performance of SPMS groups cross-sectionally at baseline visit.

4.3.3.1 0-week versus 24-week starter SPMS between group comparison.

To investigate whether 0-week and 24-week starter SPMS groups could be considered as one SPMS cohort, I determined whether there were any significant differences of variables between the two MS-SMART start date groups. Cross-sectionally, there were 46 SPMS subjects in the 0-week group, and 24 subjects who were recruited to this study 24-weeks into their MS-SMART trial timeline. Review of the general demographics (**table 4.4)** revealed no significant differences between variables of both groups; 0 week (mean age 54.2 ± 7.1 years, gender ratio M:F 10:36, 15.9 ± 2.9 years of education), 24 week (mean age 56.2 ± 6.7 years, gender ratio M:F 7:17, 14.7 ± 2.5 years of education). Regarding MS descriptors; disease duration, years of progression, and EDSS the groups were similar. Although not significant, there was a higher HADS anxiety score in the 24-week group versus the 0-week (6.7 ± 4.4 versus 5.2 ± 3.9 respectively, p=0.17). Depression scores were matched. The only significant difference between both groups was between Stroop test scores (65.6 ± 22.1 (0-week) versus 76.2 ± 23.3 (24-week) p=0.05 (**table 4.5**). However, this is shown to be due to an outlying low value score in the 24-week SPMS cohort which results in more negative skew of the overall mean as shown by the scatter plot (**figure 4.4**).

Given the demographic and clinical similarities of the groups, both 0- and 24-week SPMS groups are considered as a combined SPMS group in the subsequent analyses described in this thesis.

Figure 4.4. Scatterplot of Stroop variation in SPMS by week of recruitment.



Shows the distribution of Stroop scores with 95% confidence intervals.

Table 4.4. Demographic and clinical characteristics of 0-week SPMS versus 24-week SPMS groups. Significant results are in bold font if $p \leq 0.05$. na =not applicable.

BASELINE	0-week SPMS						24-week SPMS						
	n	Mean	SD	Median	Min	Max	n	Mean	SD	Median	Min	Max	p
Age (years)	46	54.17	7.05	55.64	37.83	65.20	24	56.15	6.69	56.51	42.17	65.74	0.26
Gender	46	M:F 10:36					24	M:F 7:17					0.49
Years of education	46	15.85	2.91	16.50	11.00	28.00	24	14.71	2.54	14.00	11.00	20.00	0.13
Drug arm	46	Drug:(n); fluoxetine:11; riluzole:12; amiloride:9; placebo:14					24	Drug:(n); fluoxetine:6; riluzole:7; amiloride:5; placebo:6					0.97
NART IQ	46	114.15	8.31	116.00	77.00	126.00	24	114.21	8.43	116.00	88.00	125.00	1.00
HADS depression	46	5.02	3.42	5.00	0.00	12.00	24	5.25	2.75	5.00	1.00	14.00	0.73
HADS anxiety	46	5.15	3.92	4.00	0.00	18.00	24	6.67	4.39	5.50	1.00	16.00	0.17
BDI-II	46	6.72	5.07	7.00	0.00	17.00	24	7.83	5.10	7.50	0.00	18.00	0.40
Disease duration from first symptom	46	21.11	8.57	20.00	6.00	44.00	24	23.83	9.90	22.00	10.00	46.00	0.30
Duration of progression	46	7.74	4.64	7.00	2.00	22.00	24	8.17	5.95	6.00	2.00	21.00	0.75

BASELINE	0-week SPMS						24-week SPMS						
	n	Mean	SD	Median	Min	Max	n	Mean	SD	Median	Min	Max	p
EDSS	46	5.68	0.85	6.0	4.0	6.5	24	5.79	0.78	6.0	4.0	6.5	0.84
9HPT (sec)	46	34.03	16.94	29.49	20.08	108.73	24	39.18	16.49	34.34	21.50	76.53	0.12
T25FW (sec)	45	17.81	15.97	10.95	4.50	79.30	24	20.79	26.72	13.58	5.05	138.80	0.58
MSFC score	46	-0.16	0.93	-0.06	-4.94	1.00	24	0.00	1.12	0.17	-4.95	0.91	0.13
MSIS 29v2 physical	46	47.96	10.36	48.00	23.00	67.00	24	49.52	14.21	51.00	25.26	75.00	0.72
MSIS 29v2	46	65.24	14.10	67.00	32.00	92.00	24	68.25	19.67	64.50	38.76	106.00	0.78
MSWS v2	46	40.32	10.89	42.00	14.00	54.00	24	41.79	10.05	43.00	20.00	54.00	0.21
MSVQ-7	46	21.34	28.65	10.98	4.50	180.00	24	21.65	30.71	13.58	5.05	159.40	0.69
MSNQ	46	22.48	11.81	20.00	2.00	53.00	24	21.58	14.18	19.00	0.00	55.00	0.66
MSIS 29v2 psychological	46	17.28	5.33	17.00	9.00	27.00	24	18.74	6.77	16.50	9.00	33.00	0.50
VAF	45	1.17	1.33	0.90	-2.60	4.60	18	0.65	1.53	0.70	-2.10	4.90	0.10
NFI	46	17.23	3.87	17.24	9.42	27.42	24	17.99	5.25	18.05	9.42	30.00	0.72
BPI	46	2.78	2.24	2.21	0.00	7.43	22	3.78	2.39	3.29	0.00	7.86	0.10
CLQ	46	19.26	4.43	20.00	11.00	31.00	23	20.74	5.06	21.00	14.00	33.00	0.28
Qualification by RQF	46	RQF:(n); Entry level:1; Level 1:16; Level 2:2; Level 3:4; Level 5:1; Level 6:18; Level 7:3; Level 8:1					24	RQF:(n); Entry level:0; Level 1:6; Level 2:1; Level 3:7; Level 5:1; Level 6:8; Level 7:1; Level 8:0					0.51
Occupation	46	Occupation Category:(n); 1:6; 2:16; 3:3; 4:12; 5:4; 6:4; 7:1; 8:0					24	Occupation Category:(n); 1:2; 2:9; 3:3; 4:6; 5:1; 6:2; 7:1; 8:0					0.95
Employment Status	46	Employment Status:(n); Full-time:6; Part-time:17; Retired:23					24	Employment Status:(n); Full-time:5; Part-time:10; Retired:9					0.54
EQ-5D	46	0.69	0.18	0.72	0.35	1.00	24	0.65	0.18	0.66	0.25	0.89	0.35
Health-state analogue	46	65.22	18.41	64.50	0.95	100.00	24	63.17	23.45	69.00	5.00	100.00	0.95
Ethnicity	46	Ethnicity:(n); Belgian:1; Cingalese:1; English:43; German:1; Italian:0; Urdu:0; Welsh:0					24	Ethnicity:(n); Belgian:0; Cingalese:0; English:24; German:0; Italian:0; Urdu:0; Welsh:0					0.65

Table 4.5. Cognitive characteristics of 0-week SPMS versus 24-week SPMS. Significant results are in bold font if p≤0.05. na =not applicable.

BASELINE	0-week SPMS						24-week SPMS						
	n	Mean	SD	Median	Min	Max	n	Mean	SD	Median	Min	Max	p
BVMT-R trials 1-3	46	12.37	6.57	12.00	2.00	26.00	24	12.67	6.43	11.00	3.00	27.00	0.85
BVMT-R trials 1-3 z-score	46	-0.50	1.06	-0.56	-2.17	1.69	24	-0.46	1.04	-0.72	-2.01	1.85	0.85
BVMT-R retained	46	91.37	35.29	100.00	0.00	175.00	24	89.91	33.22	100.00	0.00	150.00	0.88
BVMT-R retained z-score	46	-0.20	0.89	0.01	-2.52	1.91	24	-0.24	0.84	0.01	-2.52	1.28	0.88
CVLT-II trials 1-5	46	43.91	12.05	43.50	15.00	66.00	24	45.75	9.68	46.00	27.00	66.00	0.57
CVLT-II trials 1-5 z-score	46	-1.15	1.20	-1.13	-4.05	1.03	24	-0.88	0.91	-0.85	-2.64	1.03	0.41
CVLT-II delayed	46	9.41	4.19	9.00	0.00	16.00	24	9.88	3.59	10.00	2.00	16.00	0.58
CVLT-II delayed z-score	46	-1.54	1.83	-1.72	-5.64	1.34	24	-1.34	1.57	-1.28	-4.77	1.34	0.57
PASAT3	46	39.91	11.26	41.00	14.00	58.00	24	42.04	12.52	44.00	17.00	60.00	0.36
PASAT3 z-score	46	-0.42	0.93	-0.33	-2.57	1.07	24	-0.25	1.04	-0.09	-2.32	1.24	0.36
SDMT	46	46.15	13.15	49.00	17.00	74.00	24	49.38	6.90	48.00	39.00	62.00	0.60
SDMT z-score	46	-1.47	1.41	-1.17	-4.59	1.50	24	-1.13	0.74	-1.28	-2.24	0.22	0.60
Hayling test	46	6.24	1.40	6.00	2.00	10.00	24	6.21	1.77	6.00	3.00	10.00	0.37
Hayling test z-score	46	-0.38	1.37	-0.61	-4.51	3.29	24	-0.41	1.73	-0.61	-3.54	3.29	0.37
Verbal fluency	46	14.08	4.12	13.33	7.33	24.67	22	15.82	5.67	15.33	4.67	27.67	0.16
Verbal fluency z-score	46	-0.77	0.86	-0.93	-2.19	1.44	22	-0.41	1.19	-0.51	-2.74	2.07	0.16
Stroop	46	65.59	22.07	65.00	12.00	106.00	22	76.18	23.30	75.00	20.00	112.00	**0.05**

4.3.3.2 Healthy controls versus SPMS group comparison.

Between group comparison of healthy control (n=16) and the combined SPMS (n=70) groups (**table 4.6**) at baseline revealed good demographic matching with no significant differences; 55.8±4.7 years M:F 4:12 in the healthy control, and 54.9±6.9 years M:F 17:53 in the SPMS group. The MS-specific clinical outcomes were; median EDSS of 6.0 (range 4.0-6.5), mean disease duration of 22±9.1 years, mean years of progression of 7.9±5.1 years, mean 9HPT of 35.8±16.9 seconds, and mean T25FW of 18.9±20.2 seconds. Distribution of SPMS subjects into the different MS-SMART drug arms was not uniform with more subjects in the riluzole and placebo groups overall; Drug:(number of subjects); fluoxetine:17; riluzole:19; amiloride:14; placebo:20. This is due to the random recruitment of UCL site only SPMS subjects from MS-SMART. **Figure 4.5** shows that significant differences were driven by outliers in both healthy control and SPMS groups. In particular there is negative skew of median years of education in the SPMS group.

The clustered bar charts in **figure 4.6** highlight categorical variables. Review of qualification status indicates that the qualification attainment in the SPMS group was skewed and peaked at both the GCSE RQF level 1 and the undergraduate degree RQF level 6, whereas in the control group there was greater higher degree level qualification ($p \leq 0.01$). As expected, in terms of employment status, the majority of SPMS subjects were retired, compared to full-time employment prevailing in the control group ($p \leq 0.01$). There were no significant between group differences in type of employment.

Figure 4.5. Boxplot of significant differences between healthy controls and SPMS at baseline.

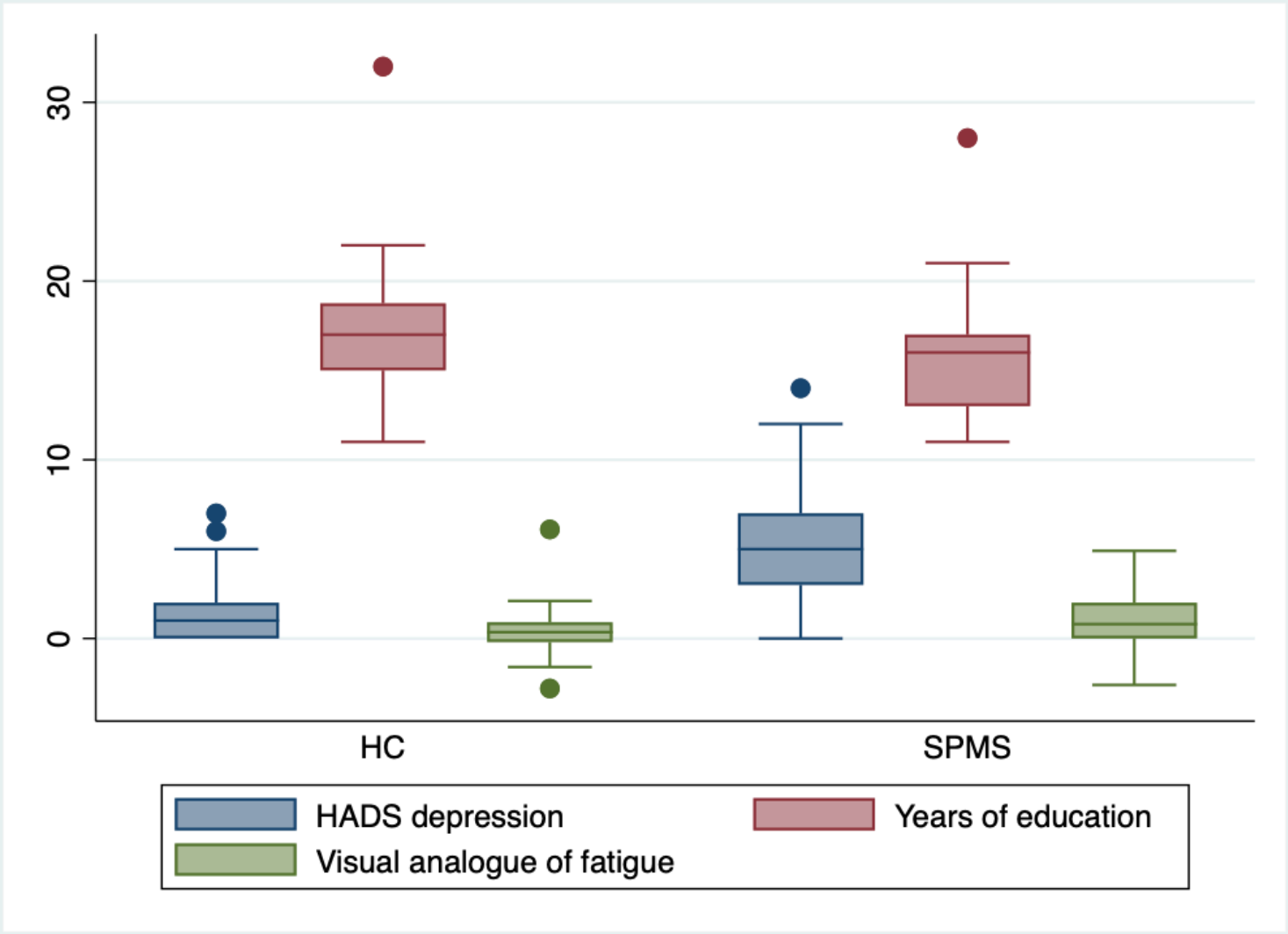


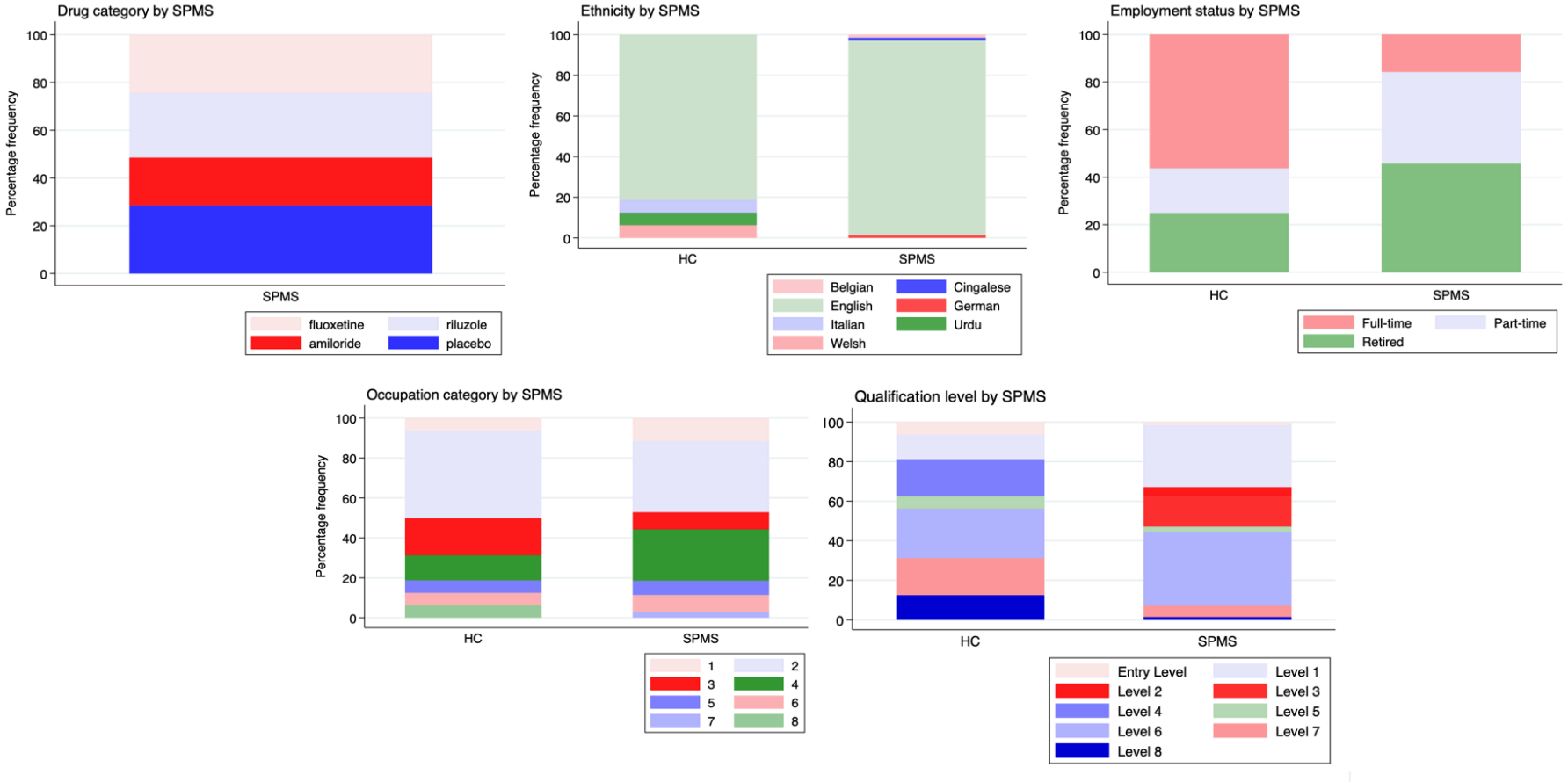
Table 4.6. Demographic and clinical characteristics of healthy controls versus SPMS at baseline. Significant results are in bold font if $p \leq 0.05$. na =not applicable.

BASELINE	Healthy Controls						SPMS						
	n	Mean	SD	Median	Min	Max	n	Mean	SD	Median	Min	Max	p
Age (years)	16	55.75	4.72	57.21	47.22	61.65	70	54.85	6.94	55.64	37.8	65.7	0.62
Gender	16		M:F	4:12			70		M:F	17:53			0.95
Years of education	16	17.56	4.92	17.00	11.00	32.00	70	15.46	2.82	16	11	28	**0.01**
Drug arm	0			na			70	Drug:(n); fluoxetine:17; riluzole:19; amiloride:14; placebo:20					na
NART IQ	16	114.88	7.65	116.00	99.00	126.00	70	114.17	8.29	116	77	126	0.74
HADS depression	16	1.81	2.26	1.00	0.00	7.00	70	5.10	3.2	5	0	14	**≤0.01**
HADS anxiety	16	6.44	3.37	6.00	1.00	14.00	70	5.67	4.12	5	0	18	0.30
BDI-II	0			na			70	7.10	5.07	7.00	0.00	18.00	na
Disease duration from first symptom	0			na			70	22.04	9.07	20.50	6.00	46.00	na
Duration of progression	0			na			70	7.89	5.09	7.00	2.00	22.00	na

BASELINE	Healthy Controls	SPMS	

	n	Mean	SD	Median	Min	Max	n	Mean	SD	Median	Min	Max	p
EDSS	0			na			70	5.72	0.82	6.0	4.0	6.5	na
9HPT (sec)	0			na			70	35.80	16.85	31.18	20.08	108.73	na
T25FW (sec)	0			na			69	18.85	20.21	12.65	4.50	138.80	na
MSFC score	0			na			70	-0.10	0.99	0.10	-4.95	1.00	na
MSIS 29v2 physical	0			na			70	48.49	11.74	48.50	23.00	75.00	na
MSIS 29v2	0			na			70	66.27	16.14	66.00	32.00	106.00	na
MSWS v2	0			na			70	40.83	10.56	42.00	14.00	54.00	na
MSVQ-7	0			na			70	21.45	29.15	12.75	4.50	180.00	na
MSNQ	16	16.19	6.57	18.00	4.00	25.00	70	22.17	12.58	20.00	0.00	55.00	0.12
MSIS 29v2 psychological	0			na			70	17.78	5.85	17.00	9.00	33.00	na
VAF	16	0.55	1.89	0.35	-2.80	6.10	63	1.02	1.40	0.80	-2.60	4.90	**0.04**
NFI	0			na			70	17.49	4.37	17.64	9.42	30.00	na
BPI	0			na			68	3.10	2.32	2.71	0.00	7.86	na
CLQ	16	20.88	4.65	22.00	13.00	27.00	69	19.75	4.66	20.00	11.00	33.00	0.27
Qualification	16	RQF:(n); Entry level:1; Level 1:2; Level 2:0; Level 3:0; Level 4:3; Level 5:1; Level 6:4; Level 7:3; Level 8:2					70	RQF:(n); Entry level:1; Level 1:22; Level 2:3; Level 3:11; Level 4:3; Level 5:2; Level 6:26; Level 7:4; Level 8:1					**≤0.01**
Occupation	16	Occupation Category:(n); 1:1; 2:7; 3:3; 4:2; 5:1; 6:1; 7:0					70	Occupation Category:(n); 1:8; 2:25; 3:6; 4:18; 5:5; 6:6; 7:2; 8:0					0.35
Employment Status	16	Employment Status:(n); Full-time:9; Part-time:3; Retired:4					70	Employment Status:(n); Full-time:11; Part-time:27; Retired:32					**≤0.01**
EQ-5D	0			na			70	0.67	0.18	0.70	0.17	1.00	na
Health-state analogue	0			na			70	64.51	20.13	65.00	5.00	100.00	na
Ethnicity	16	Ethnicity:(n); Belgian:0; Cingalese:0; English:13; German:0; Italian:1; Urdu:1; Welsh:1					70	Ethnicity:(n); Belgian:1; Cingalese:1; English:67; German:1; Italian:0; Urdu:0; Welsh:0					**≤0.01**

Figure 4.6. Summary of categorical variables at baseline in the SPMS versus healthy control groups at baseline. *Stacked clustered bar charts of the percentage frequencies of the categorical variables; drug category, qualification level, occupation category, ethnicity, and employment status by SPMS.*



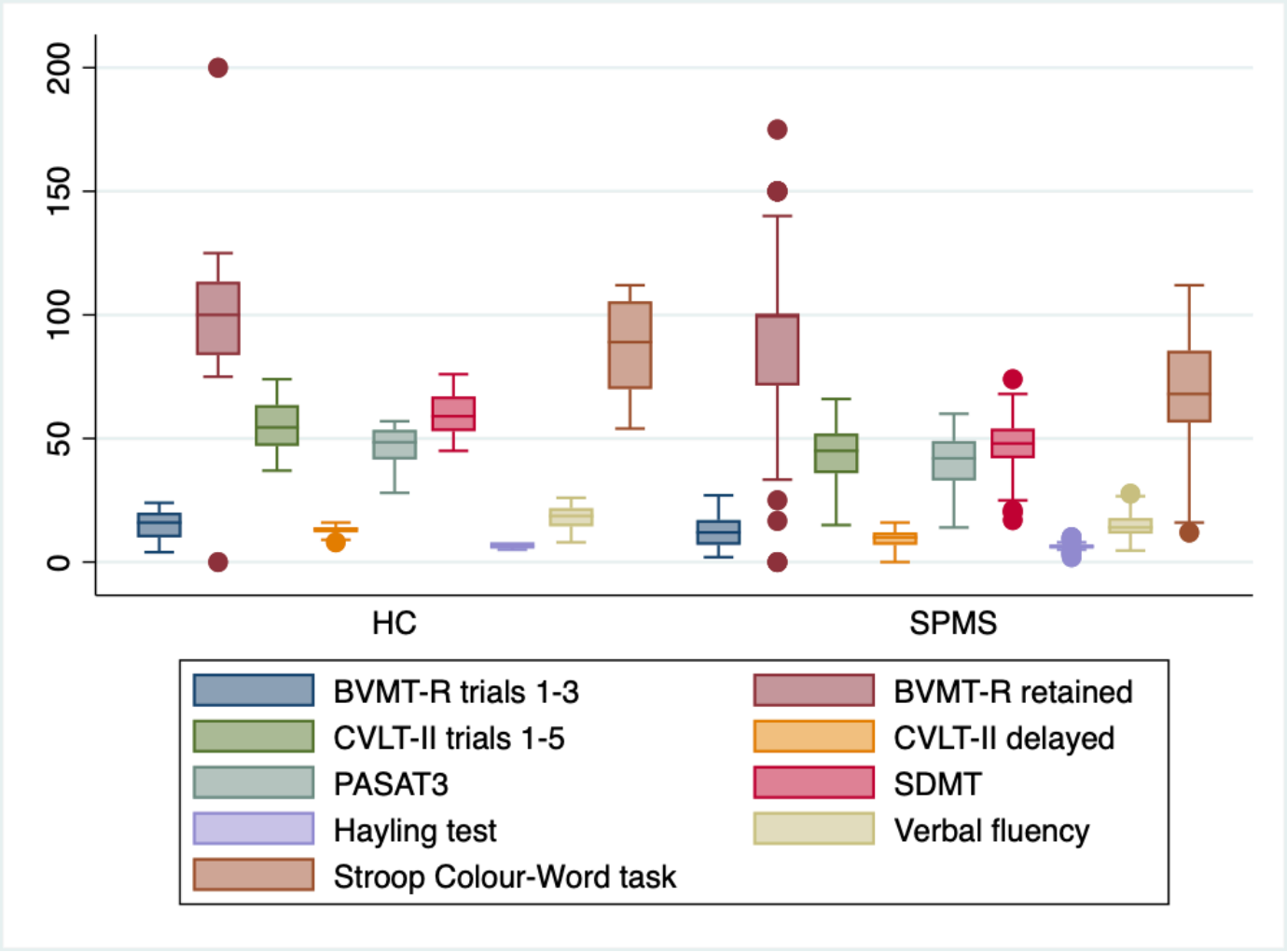
As shown in **figure 4.2,** there are variations in both control and SPMS group sizes at baseline and at follow-up visit as well as by clinical versus MRI study. This has led to subsequent non-significant differences in the proportions of SPMS subjects per MS-SMART drug arm at each study time-point. Review of the study variables between SPMS drug arm groups cross-sectionally at baseline visit showed no significant differences (data not shown).

Raw cognitive test scores show that the primary differences between SPMS and healthy control groups were within tests of information processing speed and retained verbal memory (**table 4.7**) at baseline visit. The most significant between group difference was for the SDMT; mean score of 59.9±9.4 for controls, and 47.3±11.4 for SPMS, $p \leq 0.01$. Both the immediate and delayed components of the CVLT-II test of verbal memory had significant group differences with $p \leq 0.01$ between SPMS and control groups, and the delayed component more so, indicating difficulty recalling information. Thereafter, the Stroop task differentiated healthy controls and SPMS best, with scores of 87.3±20 and 69±22.9 respectively ($p \leq 0.01$). Further executive deficits were shown by worse verbal fluency scores in the SPMS cohort. There were no significant differences between both groups in terms of the BVMT-R and Hayling task despite overlapping domains with other outcomes. **Figure 4.7** summarises the cognitive performance of the groups and shows that the median, 25% and 75% quartiles of the BVMT-R were skewed significantly by outliers in both groups.

Table 4.7. Cognitive characteristics of healthy controls versus SPMS at baseline. Significant results are in bold font if p≤0.05. na =not applicable.

BASELINE	Healthy Controls						SPMS						
	n	Mean	SD	Median	Min	Max	n	Mean	SD	Median	Min	Max	p
BVMT-R trials 1-3	16	15.50	6.21	16.00	4.00	24.00	70	12.47	6.48	12.00	2.00	27.00	0.10
BVMT-R trials 1-3 z-score	16			na			70	-0.49	1.04	-0.56	-2.17	1.85	0.10
BVMT-R retained	16	99.43	39.47	100.00	0.00	200.00	70	90.87	34.35	100.00	0.00	175.00	0.39
BVMT-R retained z-score	16			na			70	-0.22	0.87	0.01	-2.52	1.91	0.39
CVLT-II trials 1-5	16	55.06	10.63	54.50	37.00	74.00	70	44.54	11.26	45.00	15.00	66.00	**≤0.01**
CVLT-II trials 1-5 z-score	16			na			70	-1.05	1.11	-0.95	-4.05	1.03	**≤0.01**
CVLT-II delayed	16	12.94	2.29	13.50	8.00	16.00	70	9.57	3.98	10.00	0.00	16.00	**≤0.01**
CVLT-II delayed z-score	16			na			70	-1.47	1.73	-1.28	-5.64	1.34	**≤0.01**
PASAT3	16	46.50	8.82	48.50	28.00	57.00	70	40.64	11.66	42.00	14.00	60.00	0.06
PASAT3 z-score	16			na			70	-0.36	0.97	-0.25	-2.57	1.24	0.06
SDMT	16	59.94	9.35	59.00	45.00	76.00	70	47.26	11.44	48.00	17.00	74.00	**≤0.01**
SDMT z-score	16			na			70	-1.36	1.22	-1.28	-4.59	1.50	**≤0.01**
Hayling test	16	6.63	1.02	6.00	5.00	8.00	70	6.23	1.52	6.00	2.00	10.00	0.31
Hayling test z-score	16			na			70	-0.39	1.49	-0.61	-4.51	3.29	0.31
Verbal fluency	16	17.77	4.92	18.67	8.00	26.00	68	14.64	4.71	14.00	4.67	27.67	**0.01**
Verbal fluency z-score	16			na			68	-0.66	0.99	-0.79	-2.74	2.07	**0.01**
Stroop	16	87.31	20.04	89.00	54.00	112.00	68	69.01	22.86	68.00	12.00	112.00	**≤0.01**
Stroop z-score	16			na			68	-0.91	1.14	-0.96	-3.76	1.23	**≤0.01**

Figure 4.7. Boxplot of raw cognitive test scores in the SPMS and healthy control cohorts at baseline.



4.3.3.3 Associations of cognitive outcome scores and independent variables: cross-sectional analysis at the baseline visit.

To review the associations between cognitive assessments and other baseline variables I used Pearson's pairwise correlations and multiple linear regression models for the SPMS group.

Pearson's pairwise correlations: cross-sectional analysis at the baseline visit.

Considering associations of cognitive outcomes with demographic variables (**figure 4.8**); female gender significantly associated with verbal memory and information processing speed; the CVLT-II immediate (Rho=0.29 p=0.01), CVLT-II delayed (Rho=0.26 p=0.03) and SDMT (Rho=0.26 p≤0.03). IQ was significantly associated with age (Rho=0.35 p≤0.01), and additionally the PASAT3 (Rho=0.36 p≤0.01), verbal fluency (Rho=0.43 p≤0.01), and Stroop tasks (Rho=0.31 p=0.01). Duration of disease and progression in years did not associate with the cognitive outcome measures. Occupation did not associate with cognitive outcomes, but negatively associated with qualification level (Rho=-0.4 p≤0.01), and positively with retirement (Rho=0.27 p=0.02) in the SPMS group. This means that interestingly, those with higher qualification levels, were more likely to be in managerial or professional roles, and those in more physical and administrative roles were more likely to be retired. Consideration must be made for the fact that occupational levels are inverse to service and educational attainment. Those who were retired had significantly lower scores on the SDMT (Rho=-0.26 p=0.03), Hayling (Rho=-0.43 p≤0.01), and Stroop (Rho=-0.52 p≤0.01). Health economic variables and ethnicity did not show significant associations with cognitive scores (**figure 4.8**).

Reviewing correlations of cognitive assessments and physical variables, greater EDSS disability associated with a lower SDMT score (Rho=-0.25 p=0.04). There were no associations of significance between the 9HPT or T25FW with cognitive outcome scores. A better SDMT score correlated with a lower MSWS subjective score, and higher SDMT and PASAT3 scores associated with lower MSIS29v2 overall (SDMT Rho=-0.4 and p≤0.01, PASAT3 Rho=-0.27 and p=0.02) and lower physical scores (SDMT Rho=-0.35 and p≤0.01, PASAT3 Rho=-0.30 and

p=0.01). Positive associations of the MSFC were present with the BVMT-R, PASAT3, and SDMT (**figure 4.8**).

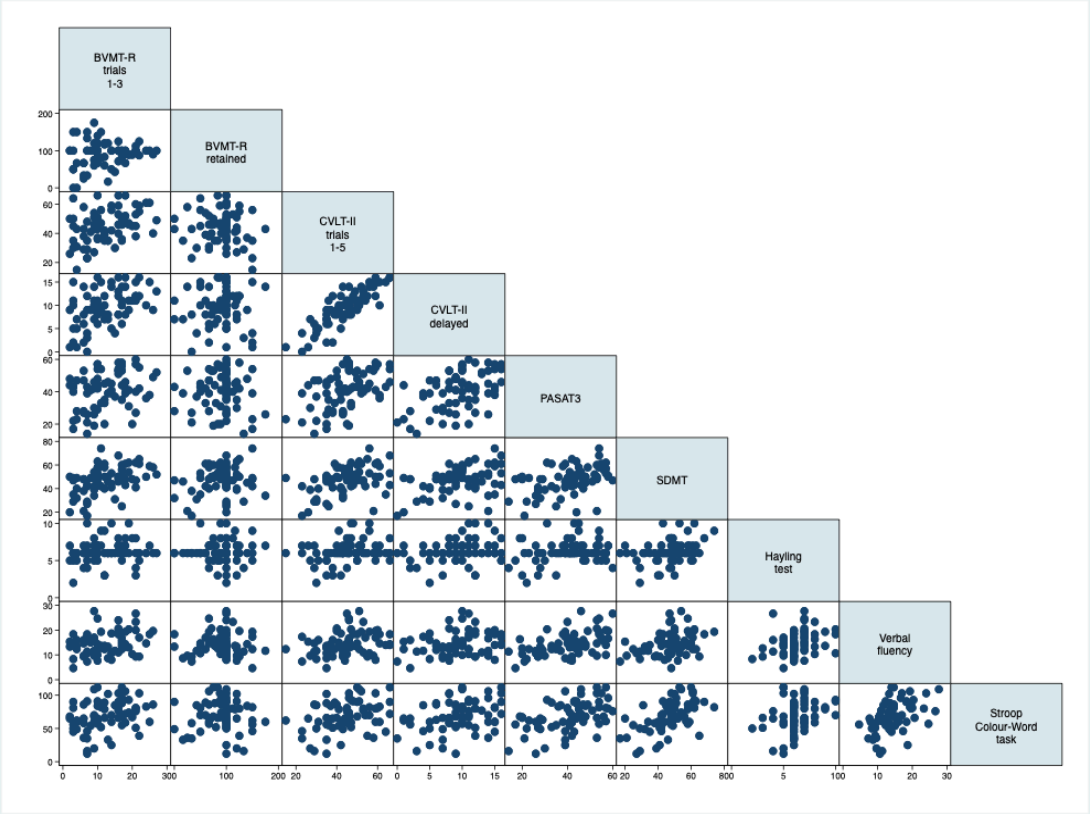
Psychological variables showed associations with cognitive outcome measures (**figure 4.8**). HADS anxiety had negative correlations with; the delayed component of the CVLT-II (Rho=-0.24 p=0.04), PASAT3 (Rho=-0.37 p≤0.01), SDMT (Rho=-0.25 p=0.04), and the Stroop task (Rho=-0.26 p=0.03). The MSNQ showed significant negative associations (p≤0.01) with the CVLT-II immediate (Rho=-0.29), CVLT-II delayed (Rho=-0.27), SDMT (Rho=-0.28), Hayling (Rho=-0.24), and the Stroop task (-0.25). A higher level of fatigue on the VAF associated with a worse outcome on the Hayling task (Rho=-0.29, p=0.02), and the NFI was also associated with executive dysfunction using the verbal fluency (Rho=-0.29, p=0.02) and Stroop tasks (Rho=-0.26, p=0.03). The NFI had additional positive associations with the MSIS29v2 psychological subscore (Rho=0.62, p≤0.01).

Between the cognitive outcome measures (**figure 4.8**), there were significant associations between the SDMT and all cognitive outcome measures (p≤0.05) apart from the BVMT-R retained. The most significant associations were between the Stroop and SDMT (Rho=0.57 p≤0.01), and the delayed CVLT-II and the SDMT (Rho=0.86 p≤0.01). There were significant associations between visual and verbal components of working memory; the BVMT-R trials 1-3, the CVLT-II trials 1-5 (Rho=0.4 p≤0.01), and CVLT-II delayed component (Rho=0.44 p≤0.01). The Hayling test showed the least association with other measures of executive function; verbal fluency (Rho=0.29 p=0.02), and the Stroop (Rho=0.28 p=0.02), and only otherwise significantly associated with the CVLT-II immediate (Rho=0.27 p=0.02) delayed (Rho=0.26 p=0.03), and the SDMT (Rho=0.32 p=0.01).

Figure 4.8 Table and graph matrix of the Pearson's pairwise correlations between the individual cognitive outcome measures. Cognitive tasks are labelled by number on the horizontal title field. Rho values are presented with p-values in brackets.

Variables	(1)	(2)	(3)	(4)	(5)	(6)	(7)	(8)	(9)
(1) BVMT-R trials 1-3	1.00								
(2) BVMT-R retained	0.15	1.00							
	(0.21)								
(3) CVLT-II trials 1-5	0.40*	-0.10	1.00						
	(0.00)	(0.43)							
(4) CVLT-II delayed	0.46*	0.03	0.86*	1.00					
	(0.00)	(0.81)	(0.00)						
(5) PASAT3	0.31*	-0.02	0.50*	0.51*	1.00				
	(0.01)	(0.85)	(0.00)	(0.00)					
(6) SDMT	0.45*	0.21	0.44*	0.42*	0.43*	1.00			
	(0.00)	(0.09)	(0.00)	(0.00)	(0.00)				
(7) Hayling test	0.13	0.03	0.27*	0.26*	0.22	0.32*	1.00		
	(0.27)	(0.81)	(0.02)	(0.03)	(0.06)	(0.01)			
(8) Verbal fluency	0.28*	0.02	0.28*	0.24*	0.49*	0.36*	0.29*	1.00	
	(0.02)	(0.85)	(0.02)	(0.05)	(0.00)	(0.00)	(0.02)		
(9) Stroop Colour-Word	0.30*	0.00	0.36*	0.36*	0.49*	0.57*	0.28*	0.47*	1.00
	(0.01)	(0.98)	(0.00)	(0.00)	(0.00)	(0.00)	(0.02)	(0.00)	

Significant pairwise correlation with Rho ($p<0.05$)*



Multivariate linear regression models: cross-sectional analysis.

Between group differences for healthy controls and SPMS (**section 4.3.3.1**) at the baseline visit showed that the greatest differences in cognitive outcome scores were for the SDMT, CVLT-II delayed, CVLT-II trials 1-5, and the Stroop task. I created a priori linear regression models of these dependent predictor variables (**section 4.3.2.2**). Significant models are shown as scatterplots with fitted lines of linear prediction from the model and 95% CIs.

Reviewing determinants of the baseline SDMT (**table 4.8**), manual backwards stepwise elimination showed that HADS anxiety was the most significant covariate predictor for the model (β=-1.07, $p \leq 0.01$, 95% CI -1.77 to -0.38). Additional covariate independent variables were gender (β=7.45, p=0.02, 95% CI 1.51 to 13.39), BDI-II (β=0.60, p=0.04, 95% CI 0.04 to 1.16), age (p=0.14, 95% CI -0.71 to 0.10), and IQ (p=0.17, 95% CI -0.10 to 0.57). The covariate independent variable model predicted SDMT with an adjusted R^2 of 0.15. In terms of additional independent variables, the MSIS29v2 physical ($p \leq 0.01$, 95% CI -0.62 to -0.14, adjusted R^2=0.25) predicted SDMT best. Other physical variables significantly improved the covariate model predicting SDMT

including the; EDSS (p ≤0.01, 95% CI -7.91 to -1.56, adjusted R^2=0.24), MSFC (p=0.02, 95% CI 0.43 to 5.56, adjusted R^2=0.2), MSIS29v2 overall (p ≤0.01, 95% CI 15.2 to 91.92, adjusted R^2=0.23), and the MSWS (p=0.01, 95% CI -0.53 to -0.07, adjusted R^2=0.22). The only other significant variables were sub-categories of qualification (overall adjusted R^2=0.20) including differences from; entry level to level 1 (GCSE grades D-G) (p=0.03 95% CI -53.69 to -2.69), entry level to level 2 (GCSE grades A*-D) (p=0.03 95% CI -58.51 to -3.92), entry level to level 3 (A Level) (p=0.02 95% CI -55.66 to -5.52), and entry level to level 6 (Batchelor degree) (p=0.01 95% CI -60.65 to -8.47).

For the CVLT-II trials 1-5 (**table 4.9**) manual stepwise elimination revealed associations with variables for the base covariate model (adjusted R^2=0.14); which were negative for greater age (β=-0.38, p=0.08, 95% CI -0.81 to 0.04) and increased HADS anxiety score (β=-0.56, p=0.09, 95% CI -1.19 to 0.08), and positive for higher IQ (β=0.35, p=0.04, 95% CI 0.02 to 0.67), female gender (β=8.23, p=≤0.01, 95% CI 2.35 to 14.11), and disease duration (β=0.14, p=0.37, 95% CI -0.16 to 0.44). Adding to this model, the MSNQ significantly negatively associated with CVLT-II trials 1-5 (β=-0.26, p=0.03, 95% CI 0.04 to 0.68). Other significant predictors were; qualification (p=0.16, adjusted R^2=0.19), and more significantly occupation (p=0.03, adjusted R^2=0.25), particularly the difference between category 3 (associate professionals) and 1 (managers) (p≤0.01, 95% CI 4.83 to 26.26). The delayed component of the CVLT-II (**table 4.10**) had age (p=0.25), HADS anxiety (p=0.04), gender (p=0.03), IQ (p=0.04), education (p=0.42), and disease duration (p=0.44) as significant covariates of interest in the model (adjusted R^2=0.12) after manual backwards stepwise elimination. The model was, as with the immediate CVLT-II, most significantly enhanced by the difference between managerial and associate professional occupations (overall: p=0.02, adjusted R^2=0.25, level 3-1: β=5.36, p ≤0.01, 95% CI 1.56 to 9.17).

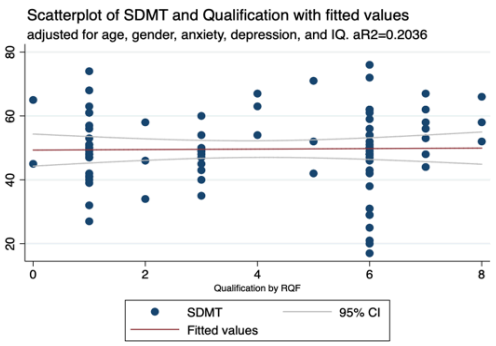
Modelling the Stroop test (**table 4.11**); the significant covariates in the base model (adjusted R^2=0.19) were; age (β=-0.79, p=0.07), HADS anxiety (β=-0.96, p=0.28), IQ (β=1.06, p ≤0.01), HADS depression (β=-1.15, p=0.31), and disease duration (β=-0.41, p=0.20). The only significant predictor was the difference between full-time and retired employment status (β=-23.65, p≤0.01,

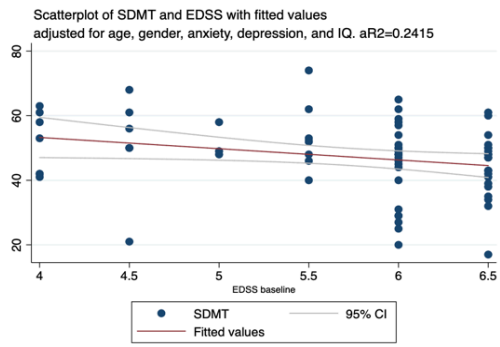
95% CI -39.46 to -7.83), with employment status overall giving a significant prediction of Stroop ($p \leq 0.01$, adjusted $R^2=0.34$).

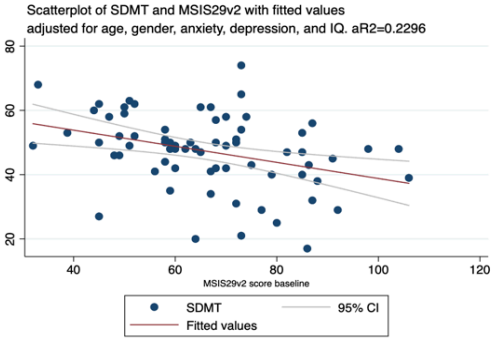
Table 4.8. Multivariate linear regression models and scatterplots of significant a) disability, b) neuropsychological, c) demographic predictors for baseline SDMT score.

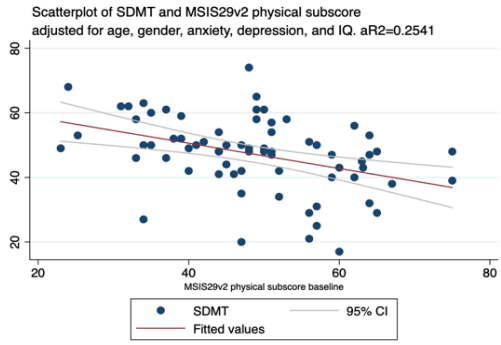
SDMT Predictors	n	β-Coefficient	p-value	95% CI		R^2	Adjusted R^2
Co-variate model							
HADS anxiety	70	-1.07	≤**0.01**	-1.77	-0.38	0.21	0.15
Gender		7.45	**0.02**	1.51	13.39		
BDI-II		0.60	**0.04**	0.04	1.16		
Age		-0.30	0.14	-0.71	0.10		
NART IQ		0.23	0.17	-0.10	0.57		
Physical disability predictors							
EDSS	70	-4.74	≤**0.01**	-7.91	-1.56	0.31	0.24
9HPT	70	0.10	0.19	-0.05	0.26	0.23	0.16
T25FW	69	-0.07	0.29	-0.20	0.06	0.22	0.15
MSFC	70	3.00	**0.02**	0.43	5.56	0.27	0.20
MSIS 29v2 physical	70	-0.38	≤**0.01**	-0.62	-0.14	0.32	0.25
MSIS 29v2	70	-0.27	**0.01**	15.20	91.92	0.30	0.23
MSWSv2	70	-0.30	**0.01**	-0.53	-0.07	0.28	0.22
MSVQ-7	70	-0.04	0.43	-0.12	0.05	0.22	0.14
Non-physical predictors							
MSNQ	70	-0.15	0.28	-0.41	0.12	0.22	0.15
MSIS 29v2 psychological	70	-0.28	0.41	-0.96	0.39	0.22	0.14
VAF	63	-1.06	0.34	-3.29	1.16	0.21	0.13
NFI	70	-0.15	0.66	-0.83	0.53	0.21	0.14
BPI	68	-0.31	0.67	-1.74	1.12	0.21	0.14
Demographic predictors							
CLQ	69	-0.14	0.65	-0.73	0.46	0.22	0.14
RQF	70		0.14			0.34	0.20
-level1 – entry level			**0.03**	-53.69	-2.67		
-level2 – entry level			**0.03**	-58.51	-3.92		
-level3 – entry level			**0.02**	-55.66	-5.52		
-level6 – entry level			**0.01**	-60.65	-8.47		
Occupation	70		0.40			0.29	0.15
Employment Status	70		0.09			0.27	0.19
EQ-5D	70	6.07	0.45	-9.73	21.86	0.22	0.14
Health	70	0.00	1.00	-0.14	0.14	0.21	0.13
Ethnicity	70		0.19			0.27	0.17

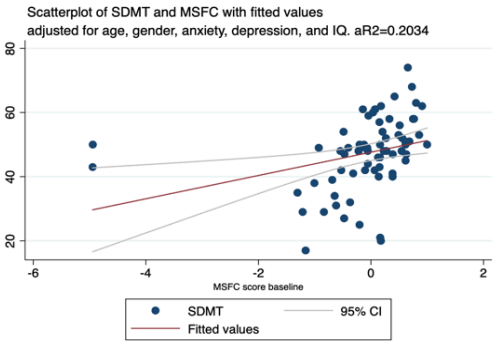
*All models include age at baseline, gender, hospital anxiety and depression scale anxiety subscore, NART IQ, and baseline BDI-II score which were shown to be the most significant independent covariate predictors for SDMT score at baseline using stepwise linear regression of covariates that should affect cognitive function (**table 4.3**). Significant results are in bold font if $p \leq 0.05$. na =not applicable. Scatterplots of significant predictors are below with fitted linear predictions.*


Scatterplot of SDMT and Qualification with fitted values
adjusted for age, gender, anxiety, depression, and IQ. aR2=0.2036
Qualification by RQF
SDMT
95% CI
Fitted values


Scatterplot of SDMT and EDSS with fitted values
adjusted for age, gender, anxiety, depression, and IQ. aR2=0.2415
EDSS baseline
SDMT
95% CI
Fitted values


Scatterplot of SDMT and MSIS29v2 with fitted values
adjusted for age, gender, anxiety, depression, and IQ. aR2=0.2296
MSIS29v2 score baseline
SDMT
95% CI
Fitted values


Scatterplot of SDMT and MSIS29v2 physical subscore
adjusted for age, gender, anxiety, depression, and IQ. aR2=0.2541
MSIS29v2 physical subscore baseline
SDMT
95% CI
Fitted values


Scatterplot of SDMT and MSFC with fitted values
adjusted for age, gender, anxiety, depression, and IQ. aR2=0.2034
MSFC score baseline
SDMT
95% CI
Fitted values

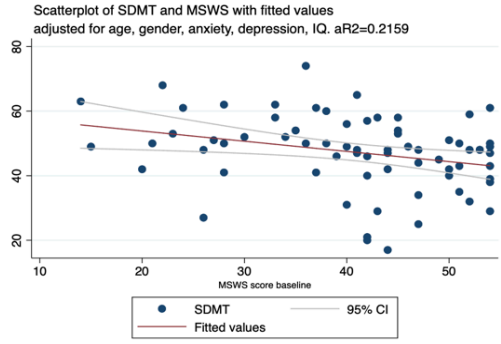
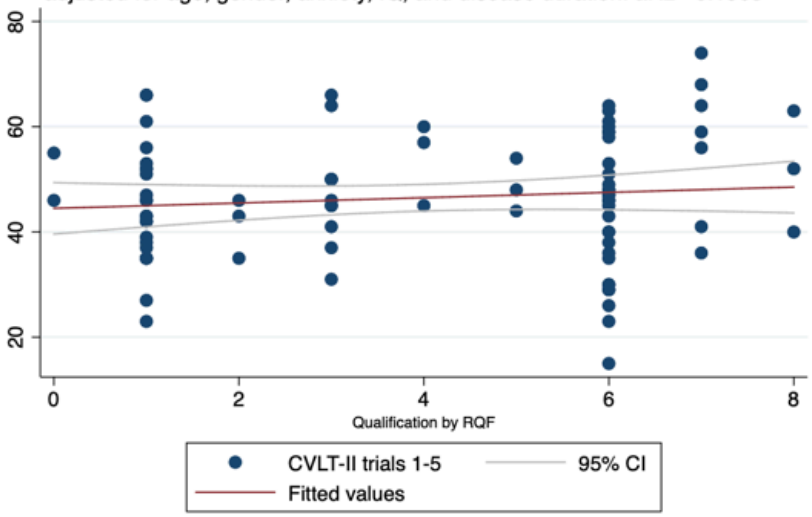

Scatterplot of SDMT and MSWS with fitted values
adjusted for age, gender, anxiety, depression, IQ. aR2=0.2159
MSWS score baseline
SDMT
95% CI
Fitted values

Table 4.9. Multivariate linear regression models of and scatterplots of significant a) disability, b) neuropsychological, c) demographic predictors for baseline CVLT-II trials 1-5 score.

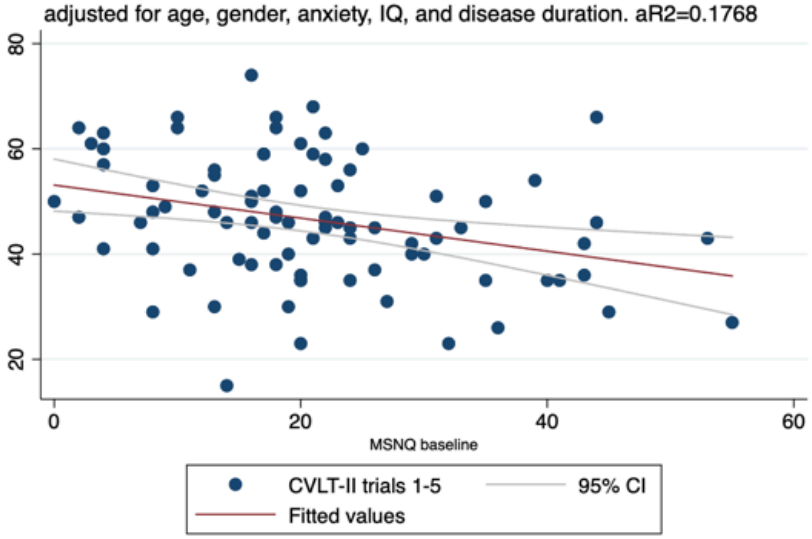
CVLT-II 1-5 Predictors	n	β-Coefficient	p-value	95% CI		R^2	Adjusted R^2
Co-variate model							
Age	70	-0.38	0.08	-0.81	0.04	0.20	0.14
HADSa		-0.56	0.09	-1.19	0.08		
Gender		8.23	**0.01**	2.35	14.11		
NART IQ		0.35	**0.04**	0.02	0.67		
Disease Duration		0.14	0.37	-0.16	0.44		
Physical disability predictors							
EDSS	70	-1.50	0.37	-4.84	1.84	0.21	0.14
9HPT	70	0.09	0.23	-0.06	0.24	0.22	0.14
T25FW	69	0.02	0.74	-0.11	0.15	0.20	0.12
MSFC	70	1.28	0.32	-1.29	3.85	0.21	0.14
MSIS 29v2 physical	70	-0.09	0.51	-0.34	0.17	0.21	0.13
MSIS 29v2	70	-0.07	0.53	-0.28	0.14	0.21	0.13
MSWSv2	70	-0.02	0.84	-0.27	0.22	0.20	0.12
MSVQ-7	70	0.01	0.29	-17.70	57.55	0.20	0.12
Non-physical predictors							
MSNQ		-0.26	**0.03**	0.04	0.68	0.25	0.18
MSIS 29v2 psychological	70	-0.11	0.75	-0.81	0.58	0.20	0.13
VAF	63	-1.20	0.28	-3.37	0.98	0.20	0.11
NFI	70	0.10	0.78	-0.60	0.79	0.20	0.13
BPI	68	-0.54	0.43	-1.91	0.83	0.20	0.12
Demographic predictors							
CLQ	69	-0.10	0.75	-0.68	0.49	0.20	0.13
RQF	70		0.16			0.33	0.19
-level1 – entry level			**0.03**	-54.34	-2.79		
-level2 – entry level			**0.04**	-56.43	-1.93		
-level6 – entry level			**0.02**	-57.67	-5.73		
-level8 – entry level			**0.05**	-66.93	-0.37		
Occupation	70		**0.03**			0.37	0.25
-category3 - 1			**0.01**	4.83	26.26		
Employment Status	70		0.16			0.25	0.16
EQ-5D	70	1.19	0.88	-14.52	16.91	0.20	0.12
Health	70	-0.08	0.27	-0.21	0.06	0.22	0.14
Ethnicity	70		0.71			0.22	0.12

*All models include age at baseline, gender, hospital anxiety and depression scale anxiety subscore, NART IQ, and disease duration from first symptom which were shown to be the most significant independent covariate predictors for CVLT-II trials 1-5 score at baseline using stepwise linear regression of covariates that should affect cognitive function (**table 4.3**). Significant results are in bold font if $p \leq 0.05$. na =not applicable. Scatterplots of significant predictors are below with fitted linear predictions.*

Scatterplot of CVLT-II and Qualification with fitted values
adjusted for age, gender, anxiety, IQ, and disease duration. aR2= 0.1905



Scatterplot of CVLT-II and MSNQ with fitted values
adjusted for age, gender, anxiety, IQ, and disease duration. aR2=0.1768



Scatterplot of CVLT-II and Occupation category with fitted values
adjusted for age, gender, anxiety, IQ, and disease duration. aR2=0.2452

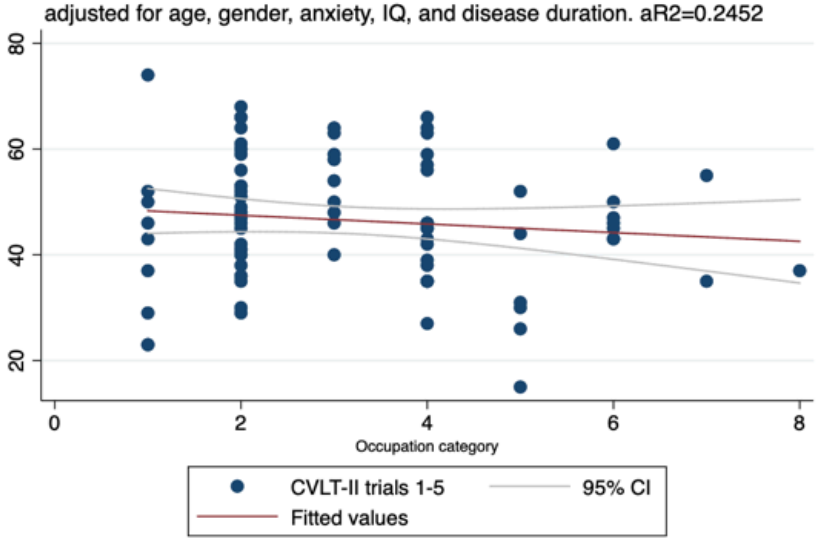


Table 4.10. Multivariate linear regression models of and scatterplots of significant a) disability, b) neuropsychological, c) demographic predictors for baseline CVLT-II retained score.

CVLT-retained Predictors	n	β-Coefficient	p-value	95% CI		R^2	Adjusted R^2
Co-variate model							
Age	70	-0.09	0.25	-0.24	0.06	0.19	0.12
HADSa		-0.24	**0.04**	-0.47	-0.01		
Gender		2.43	**0.03**	0.30	4.55		
NART IQ		0.12	**0.04**	0.00	0.24		
Education		-0.14	0.42	-0.47	0.20		
Disease Duration		0.04	0.44	-0.07	0.15		
Physical disability predictors							
EDSS	70	-0.57	0.35	-1.78	0.63	0.22	0.12
9HPT	70	0.03	0.33	-0.03	0.08	0.21	0.12
T25FW	69	0.01	0.64	-0.03	0.06	0.21	0.11
MSFC	70	0.52	0.26	-0.40	1.43	0.21	0.12
MSIS 29v2 physical	70	-0.01	0.87	-0.10	0.08	0.19	0.10
MSIS 29v2	70	-0.01	0.87	-0.08	0.07	0.19	0.10
MSWSv2	70	0.00	0.98	-0.09	0.09	0.19	0.10
MSVQ-7	70	0.00	0.89	-0.03	0.03	0.19	0.10
Non-physical predictors							
MSNQ	70	-0.06	0.18	-0.16	0.03	0.22	0.13
MSIS 29v2 psychological	70	-0.01	0.91	-0.26	0.24	0.19	0.10
VAF	63	-0.45	0.26	-1.25	0.34	0.22	0.12
NFI	70	0.06	0.63	-0.19	0.31	0.20	0.11
BPI	68	0.11	0.66	-0.39	0.60	0.20	0.11
Demographic predictors							
CLQ	69	-0.15	0.15	-0.36	0.06	0.23	0.14
RQF	70		0.45			0.28	0.12
Occupation	70		**0.02**			0.38	0.25
-category 3 - 1		5.36	**0.01**	1.56	9.17		
Employment Status	70		0.17			0.24	0.14
EQ-5D	70	-0.62	0.83	-6.31	5.07	0.19	0.10
Health	70	-0.04	0.10	-0.09	0.01	0.23	0.14
Ethnicity	70		0.83			0.21	0.09

*All models include age at baseline, gender, hospital anxiety and depression scale anxiety subscore, NART IQ, and years of education which were shown to be the most significant independent covariate predictors for CVLT-II retained score at baseline using stepwise linear regression of covariates that should affect cognitive function (**table 4.3**). Significant results are in bold font if $p \leq 0.05$. na =not applicable. Scatterplots of significant predictors are below with fitted linear predictions.*

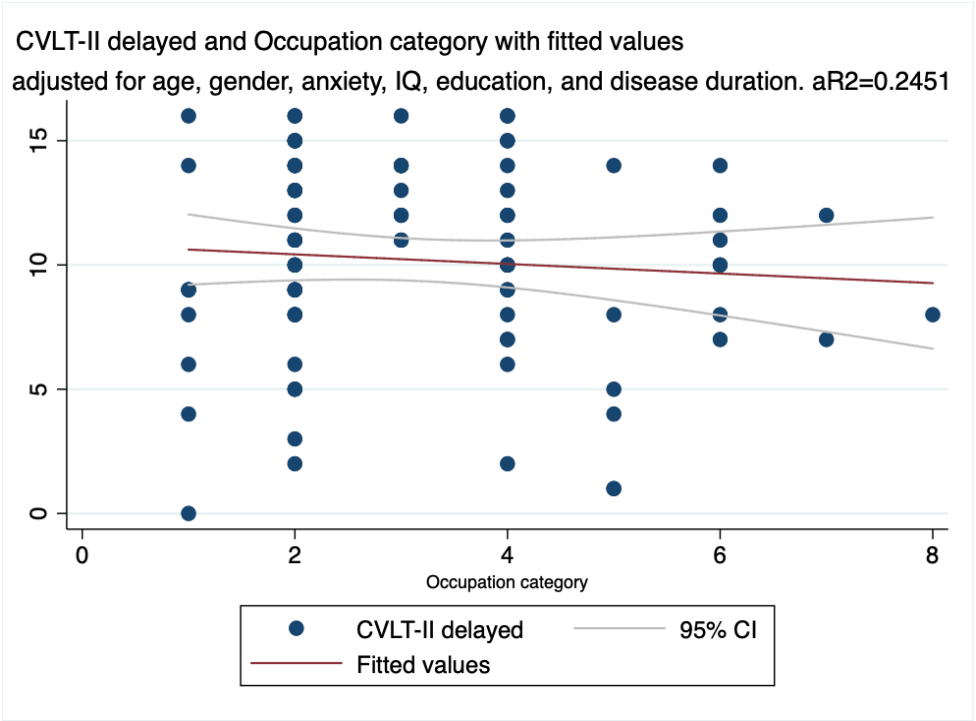
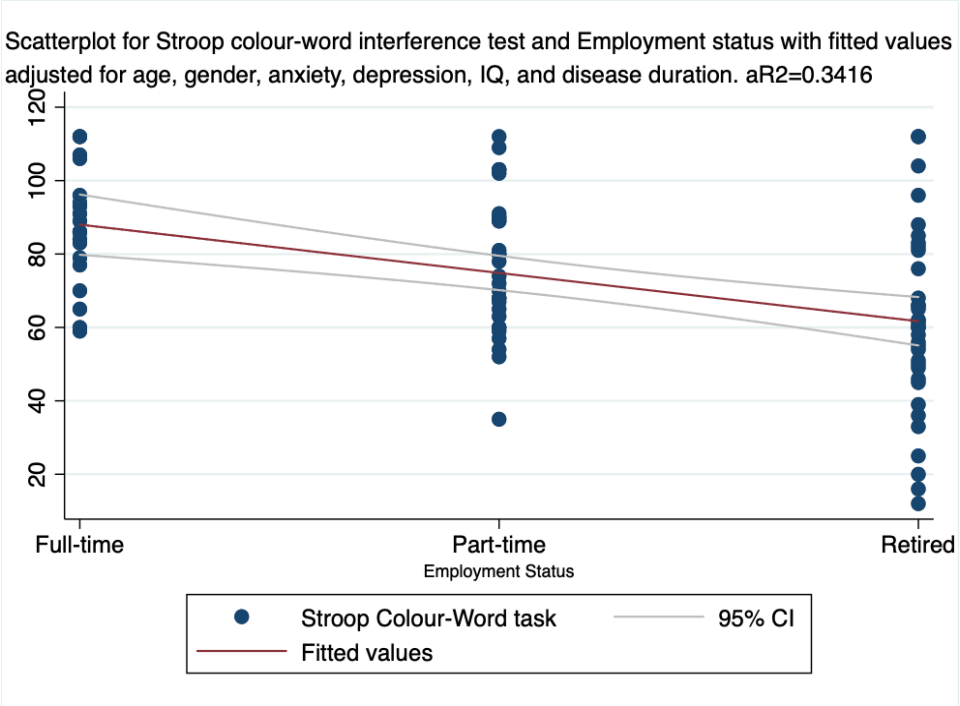

CVLT-II delayed and Occupation category with fitted values
adjusted for age, gender, anxiety, IQ, education, and disease duration. aR2=0.2451
0
5
10
15
0
2
4
6
8
Occupation category
CVLT-II delayed
95% CI
Fitted values

Table 4.11. Multivariate linear regression models of and scatterplots of significant a) disability, b) neuropsychological, c) demographic predictors for baseline Stroop score.

Stroop Predictors	n	β-Coefficient	p-value	95% CI		R^2	Adjusted R^2
Co-variate model							
Age	70	-0.79	0.07	-1.64	0.05	0.25	0.19
HADSa		-0.96	0.28	-2.74	0.81		
NART IQ		1.06	**<0.01**	0.41	1.71		
HADSd		-1.15	0.31	-3.41	1.11		
Disease Duration		-0.41	0.20	-1.03	0.22		
Physical disability predictors							
EDSS	68	-1.06	0.74	-7.41	5.29	0.26	0.18
9HPT	68	0.03	0.84	-0.27	0.33	0.25	0.18
T25FW	67	0.03	0.79	-0.22	0.28	0.26	0.19
MSFC	68	3.94	0.12	-1.07	8.94	0.28	0.21
MSIS 29v2 physical	68	-0.12	0.65	-0.64	0.40	0.26	0.18
MSIS 29v2	68	-0.05	0.82	-0.47	0.38	0.25	0.18
MSWSv2	68	-0.29	0.25	-0.78	0.21	0.27	0.20
MSVQ-7	68	-0.01	0.89	-0.19	0.16	0.25	0.18
Non-physical predictors							
MSNQ	68	-0.26	0.33	-0.79	0.27	0.27	0.19
MSIS 29v2 psychological	68	0.31	0.65	-1.04	1.65	0.26	0.18
VAF	63	-1.44	0.50	-5.67	2.79	0.23	0.14
NFI	68	-0.39	0.59	-1.85	1.06	0.26	0.18
BPI	66	0.41	0.78	-2.53	3.36	0.24	0.16
Demographic predictors							
CLQ	67	-0.27	0.64	-1.45	0.90	0.24	0.17
RQF	68		0.94			0.28	0.13
Occupation	68		0.96			0.27	0.13
Employment Status	68		**0.00**			0.41	0.34
-full-time - retired	68	-23.65	**0.00**	-39.46	-7.83		
EQ-5D	68	3.06	0.84	-27.97	34.10	0.25	0.18
Health	68	-0.11	0.46	-0.39	0.18	0.26	0.19
Ethnicity	68		0.99			0.26	0.15

*All models include age at baseline, hospital anxiety and depression scale anxiety subscore, hospital anxiety and depression scale depression subscore, NART IQ, and disease duration from first symptom which were shown to be the most significant independent covariate predictors for Stroop interference score at baseline using stepwise linear regression of covariates that should affect cognitive function (**table 4.3**). Significant results are in bold font if $p \leq 0.05$. na =not applicable. Scatterplots of significant predictors are below with fitted linear predictions.*


Scatterplot for Stroop colour-word interference test and Employment status with fitted values adjusted for age, gender, anxiety, depression, IQ, and disease duration. aR2=0.3416
120
100
80
60
40
20
Full-time
Part-time
Retired
Employment Status
Stroop Colour-Word task
95% CI
Fitted values

4.3.4 Discussion

The main findings of this section support additional fronto-executive dysfunction in SPMS to the working memory and information processing speed deficits present in other MS phenotypes. Therefore, I have shown that a "BICAMS-plus" protocol including tests of the fronto-executive domain in addition to those within the BICAMS protocol is needed in SPMS (Langdon *et al.*, 2012). Additionally, I show the importance of occupation type and employment status in determining performance on individual cognitive tasks. Finally, the nested design allied to the MS-SMART trial allowed for novel co-recruitment, but also sharing of outcome measures and metrics.

70 subjects with SPMS and 16 healthy controls were evaluated cross-sectionally at baseline. The demographic features of the SPMS cohort were in keeping with a more advanced SPMS cohort (**table 4.6**); age 54.9±6.9 years, M:F 17:53, median EDSS of 6.0 (range 4.0-6.5), mean disease duration of 22±9 years, and mean years of progression of 7.9±5.1 years, matching the core characteristics of the MS-SMART cohort (445 SPMS) (Chataway *et al.*, 2020) and similar to that in the MS-STAT1 study (140 SPMS) (Chataway *et al.*, 2014). There were no differences with the healthy controls in terms of baseline demographics of age, and gender (55.8±4.7 years and M:F 4:12), and premorbid intelligence (**table 4.6**).

In keeping with the literature (Huijbregts *et al.*, 2004; Muhlert *et al.*, 2015; Sumowski *et al.*, 2018), working memory and information processing speed were most significantly impaired in this SPMS cohort (p=<0.001) as shown by group differences in the SDMT (**table 4.7**). This was followed by verbal learning memory (CVLT-II) and fronto-executive (Stroop) domains. SPMS cohorts have been shown to have a greater extent of executive dysfunction than in PPMS (Huijbregts *et al.*, 2004; Achiron *et al.*, 2013; Connick *et al.*, 2013; Chan *et al.*, 2017) in the majority, but not all studies (Ruano *et al.*, 2017). Auditory information processing speed (PASAT3) and word list generation (Verbal Fluency) were less impaired but are perhaps more reliant on attention and executive function (Muhlert *et al.*, 2013; Preston *et al.*, 2013) than working memory. Visuospatial working memory was retained in this cohort. RRMS studies suggest strong correlation between

PASAT3 and SDMT with similar sensitivity and specificity between the two tests (López-Góngora *et al.*, 2015), however the SDMT is more reliant on vision for visual working memory and this is more impacted in SPMS (Smith, 1982; Connick *et al.*, 2013; Van Schependom *et al.*, 2014, 2015). The SDMT correlated most with tests of executive function, the Stroop, and working memory, the CVLT-II. This suggests and supports that changes in working memory, information processing speed, attention and executive domains may be particularly prominent in SPMS and so may offer a sensitive marker of cognitive dysfunction in this group (Connick *et al.*, 2013; Sumowski *et al.*, 2018).

There are several independent factors which compound the phenotypic variations of cognition. As with the literature, I have shown that lower limb disability markers, i.e. EDSS and T25FW, associate with cognitive function in MS (Huijbregts *et al.*, 2004; Achiron *et al.*, 2013; Migliore *et al.*, 2017; Ruano *et al.*, 2017). I show that worse EDSS significantly associated with the SDMT task in terms of Pearson's pairwise correlations and also positively with the T25FW (Rho=0.48 $p \leq 0.01$). This indicates a lower limb disability predominance of associations for visual information processing speed and working memory. The multivariate linear regression model for SDMT supported this, whereby mainly physical disability predictors enhanced the model significantly above the covariates; EDSS, MSIS29v2 (total and physical subscale), and the MSFC. This may encompass cognitive reserve in terms of disability and cognitive performance over and above IQ and years of education (Sumowski *et al.*, 2010*a*, 2018). This was not the case for other cognitive outcome assessments, supporting the SDMT as a sentinel marker of cognitive function and clinical disability prediction in MS (Van Schependom *et al.*, 2014; Benedict *et al.*, 2015). This also supports the use of the SDMT as an adjunctive cognitive measure of disability in composite clinical trial outcome markers (Benedict *et al.*, 2017; Goldman *et al.*, 2019).

Despite recruiting a cohort with a restricted BDI-II and not meeting criteria for clinical depression or anxiety, in the SPMS group HADS anxiety correlated negatively with the SDMT, Stroop, and CVLT-II as did the MSNQ. In the multivariate regression models, HADS anxiety was the most significant covariate overall for all of the outcome measures. Anxiety has been shown to be

associated with overall cognitive functioning and lower scores on the SDMT in a cohort of 111 MS subjects (Marrie *et al.*, 2019). HADS depression scores indicate that the SPMS group expectedly had significantly higher depression indices (Grech *et al.*, 2019) (**figure 4.5**) despite not reaching clinically diagnostic significance, than the controls (5.1±3.2, versus 1.8±2.3 respectively, $p<0.001$). However, this was not a significant predictor of cognitive performance.

Subjective neuropsychological wellbeing, MSNQ, was significantly ($p<0.001$) negatively correlated with the CVLT-II immediate (Rho=-0.29), CVLT-II delayed (Rho=-0.27), SDMT (Rho=-0.28), Hayling (Rho=-0.24), and the Stroop (-0.25). Additionally, the MSNQ significantly negatively associated with CVLT-II trials 1-5 (β=-0.26, p=0.027, 95% CI 0.04 to 0.68, adjusted R^2=0.18) in the multivariate regression model. This supports the use of PROMS as a useful tool for evaluating cognitive function in SPMS, focusing on the key cognitive domains affected (Cohen *et al.*, 2012; Doward *et al.*, 2015). SPMS subjects reported higher levels of fatigue than controls (p=0.004), although this had no impact on cognitive performance, and therefore possibly cognitive fatigue (Schwid *et al.*, 2002).

45% of those with SPMS were retired, versus 25% in the control group. This is in keeping with rates of early retirement due to MS in Europe, but importantly also correlates significantly with care costs of 36,500 Euros for moderate disease (EDSS 4.0– 6.5) (Kobelt *et al.*, 2006). Cadden et al. suggested that employment status was significantly predicted only by a cognitive composite score and not by fatigue or depression markers (Cadden *et al.*, 2014). Although theirs' was a mixed MS phenotype, in this study I found supportive evidence with positive associations with retirement and significantly lower scores on the SDMT (Rho=-0.26 p=0.03), Hayling (Rho=-0.43 $p\leq0.01$), and Stroop (Rho=-0.52 $p\leq0.01$) only in SPMS. This was supported by the multivariate linear regression model for the Stroop task which only significantly associated with the difference between full-time and retired employment status (β=-23.65, p=0.004, 95% CI -39.46 to -7.83), overall giving a significant prediction of Stroop (p=0.001, adjusted R^2=0.34).

Type of employment was also different with more administrative roles than professional roles in the SPMS group versus controls. This could be explained by the significantly lower duration of education in the SPMS group, 15.5±2.8 years, versus 17.6±4.9 years in the control group (p=0.006), or bias in the recruiting method of the control group. However, occupation type only correlated positively with retirement (Rho=0.27 p=0.02) and negatively associated with qualification level (Rho=-0.4 p≤0.01) not years of education. In the multivariate regression models, the difference between category 3 (associate professionals) and 1 (managers) (adjusted R^2=0.25, p=0.005, 95% CI 4.83 to 26.26) was significant for the CVLT-II and the delayed component (adjusted R^2=0.25, p=0.007, 95% CI 1.56 to 9.17). Additionally, differences between entry level to level 6 (Batchelor degree) (p=0.01 95% CI -60.65 to -8.47) qualification improved the prediction of the SDMT, and the CVLT-II (p=0.156, adjusted R^2=0.19) in the multivariate regression models. These tasks rely on attention, processing speed, and working memory indicating that these are domains key to employment. Occupational attainment was associated with employment status in this SPMS cohort, and has been shown as an independent predictor of information processing speed, memory, and executive domains after including measures of brain atrophy and IQ in the model (Ghaffar *et al.*, 2012).

There are limitations of the design of this study. By co-recruiting with MS-SMART, and meeting the inclusion criteria for this, a critique is of excluding SPMS subjects with depression. Up to 50% of people with MS have been shown to have clinical symptoms of depression, which are more common in a progressive phenotype (Grech *et al.*, 2019). Those meeting a relevant BDI-II score representing moderate-severe depression were excluded in the MS-SMART eligibility criteria (**section 1.3, table 1.4**). This was to avoid potential double-dosing of a selective serotonin reuptake inhibitor (SSRI) for which trial medication would have had to be stopped for initiation. However, whilst recruiting for the MS-SMART trial only 7/90 of the ineligible group met this criterion. The aim of this thesis was not to assess a neuroprotective or treatment effect on cognitive function in SPMS, but to define the performance. I have shown that there were no differences between MS-SMART drug arms in terms of variables in the SPMS group cross-sectionally.

As with other studies based at the National Hospital of Neurology and Neurosurgery (Nelson and Willison, 1982; Muhlert *et al.*, 2015), this SPMS cohort had higher premorbid IQ (reflected by reading ability) (114.2±8.29), and this unintentionally matched the locally recruited healthy controls (114.8±7.65). This is supportive of the data from Rocca et al suggesting generally retained intellectual function in MS (Rocca *et al.*, 2015*a*; Sumowski *et al.*, 2018). In the literature, the age (mean 48±12 years), social class distribution and test results used for NART standardisation predicted a mean premorbid intelligence performance IQ of 109.1±11.5 (Nelson and Willison, 1982). This created a dilemma when deciding on which normative dataset against which to calculate the z-scores for the cognitive outcomes as there was significant variability in the combined demographic information used for each test (Casaletto and Heaton, 2017). As an example, when thinking about the SDMT, CVLT-II, and BVMT-R; the main US dataset utilised by the BICAMS (Parmenter *et al.*, 2010) for normative z-scores standardises for age, sex, and education using a regression-based approach. O'Connell provides a dataset of 100 Irish subjects with normative values more closely matched to the UK population (O'Connell *et al.*, 2015). The Italian dataset (Goretti *et al.*, 2014) provides some re-test evaluation of the BICAMS incorporated tests in a much larger dataset, but with considerably higher normative age-standardised scores. However, these datasets do not provide normative values for the CVLT-II delayed or BVMT-R retained tests. Manualised normative data which mainly rely on US based military and paediatric datasets would be a consideration, however these use variations of combined demographics for standardisation which vary by test (Strauss *et al.*, 2006). I decided, therefore, to use local control data as they were well matched to the SPMS subjects in terms of IQ, age, education and local cultural factors. This also keeps the 'baseline' consistent between tests with the least variability. A limitation is the number of controls available, however given that manualised and literature normative datasets also vary in number, this was felt to be less relevant. This is not uncommon in larger longitudinal (Modica *et al.*, 2016; Eijlers *et al.*, 2018) and cross-sectional (Schoonheim *et al.*, 2015*a*; Meijer *et al.*, 2018) cognitive-MRI studies where the main emphasis is on providing normative data for the MRI metrics. I did compare results with the standardised literature

normative scores where available, and there were no significant differences for z-scores between this method or that in the study (data not shown).

This section shows that the SPMS group show deficits of information processing speed, working memory, and executive function versus the control cohort. These changes are mainly predicted and associated with premorbid factors including employment status, and so indicate the importance of cognitive reserve, and loss of this with pathology, explicitly in SPMS (Sumowski *et al.*, 2018).

4.4 Changes in cognitive performance in SPMS over time: a longitudinal analysis

4.4.1 Introduction

There are very few longitudinal cohorts of cognition and SPMS in the literature (Connick *et al.*, 2013; Chan *et al.*, 2017; Sumowski *et al.*, 2018). This section provided me with the opportunity to review the change in cognitive performance over time.

The aims of this section are:

1) To show how cognitive function, in terms of individual tests, changes in SPMS over time (**section 4.4.3.1**).
2) To investigate what associates with and predicts cognitive worsening at the individual test level in SPMS over time (**section 4.4.3.2**).

4.4.2 Methods

Subjects were recruited as per **section 3.2**, and undertook the neuropsychological and clinical battery described further in **table 4.2** in **section 3.2.7.**

4.4.2.1 Statistical analysis

Descriptive evaluation and differences between variables

This was evaluated by control or SPMS group and were undertaken cross-sectionally at follow-up as described in **section 4.3.2.2**. In addition visit interval in days was calculated. The non-parametric Kruskal-Wallis test was used to test the significance of group differences between both time points for the cohorts. A statistical threshold of $p \leq 0.05$ was considered significant. One-way dot plots with overlying box plots, graphs of mean change over time, and violin plots have been created to show changes over time. Violin plots show boxplots with overlying estimated kernel densities. For the violin plots, the white dot represents the median, thick grey bar represents the interquartile range, and the thin grey line represents the distribution of data. The

rest of the plot represents the kernel density estimation to show the overall distribution shape of the data and the probability of each value.

Multivariable logistic regression models

Multivariable logistic regression models of the binary outcome; worse or not worse for each cognitive outcome measure at follow-up, were developed. This was for the 59 SPMS subjects in the longitudinal clinical analysis (see **figure 4.2**). The independent predictors tested were as for the multivariate linear regression models (**table 4.3**) i.e. divided into the following categories; physical disability predictors, non-physical predictors, and demographic predictors. Covariates chosen for the models were those which were most frequently significant in the healthy control versus SPMS groups, i.e. HADS anxiety in the follow-up cohort, age at follow-up and gender in the follow-up cohort. Models were performed for the following dependent variables;

1) Worsening on the PASAT3 at 96-weeks
2) Worsening on the SDMT at 96-weeks
3) Worsening on the BVMT-R at 96-weeks
4) Worsening on the BVMT-R retained at 96-weeks
5) Worsening on the CVLT-II immediate at 96-weeks
6) Worsening on the CVLT-II delayed at 96-weeks
7) Worsening on the Hayling at 96-weeks
8) Worsening on the Verbal fluency at 96-weeks
9) Worsening on the Stroop at 96-weeks

The longitudinal models included variables at baseline and longitudinal timepoints. Stata reports McFadden's pseudo R-squared as the relative fit of two models, but not the absolute fit of the models. Therefore, likelihood ratio Chi^2 (LR) were gathered per model to show the overall model fit. To review the sensitivity; the probability of the model predicting a positive outcome for a given observation, and the specificity; the probability that the model predicts a negative outcome for an observation, I plotted ROC (receiver operating characteristic) curves. ROC curves plot values of

the sensitivity against 1-specificity. When the model has high sensitivity and specificity the ROC curve hugs the top left corner of the plot. The area under the ROC curve (AUROC) was calculated to define the ability of the model to distinguish between positive and negative outcomes. The AUROC ranges from 0-1, with values closer to 1 indicating that the model is better at correctly classifying the outcome of interest.

Tables of significantly associated factors have been produced. Tables include; OR, p-value, 95% CI, LR, AUROC. ROC curves are shown graphically where relevant.

4.4.3 Results

4.4.3.1 Evaluation of between group differences: longitudinal analysis.

This results section summarises the change in raw scores of cognitive tests in those subjects completing follow-up. Summaries of changes in the all variables from baseline in the healthy control and SPMS groups (visit) and between control and SPMS groups at the follow-up timepoint (cohort) are shown in **table 4.12**.

Cross-sectionally, at the follow-up timepoint, there were no significant differences in demographic variables between the healthy control (n=13) and SPMS (n=59) groups (**table 4.12**). There was a significantly shorter interval between visits in the healthy control versus SPMS cohorts (mean 661.2 and SD 22.3 days versus 672.9 and SD 61.7 days respectively, p=<0.01) due to the MRI scanner upgrade described in **section 3.2**. As with baseline (**table 4.4**), the cross-sectional comparison revealed significant differences between qualification between the groups, with most control subjects attaining bachelor's degrees or higher, and in the SPMS group there was again a dichotomy, as with baseline, with peaks at A-level, or bachelor's degree level (p=0.01). Most in the control group were employed full-time (n=7), whereas in the SPMS group 22 were employed part-time group, and 27 subjects were retired (p=0.02) (**table 4.12**). As with baseline (**table 4.5**) there were significantly lower cognitive scores in the SPMS versus control group, but this was not

sustained for the BVMT-R, PASAT3, or Hayling suggesting less sensitivity and specificity for their domains (**table 4.12**).

Reviewing differences from baseline to follow-up, there was only a significant difference in the healthy control group for the BVMT-R immediate recall with a positive change of 7.8 in mean score, p=0.02, from baseline to follow-up. In the SPMS group there was worsening in the MSISv2 physical component (mean score=54.1, change=6.0, p=0.01), psychological component (mean score=20.4, change=2.9, p=0.01), and MSIS overall (mean score=74.5, change=8.9, p=0.01). This indicates a greater impact of MS-related disability over time in the SPMS group. In terms of other PROMS, there was worsening of the MSWS (mean score=45.3, change=4.5, p=0.02), MSNQ (mean score=29.2, change=7.9, p=<0.01), BPI (mean score=4.5, change=1.4, p=<0.01), and EQ-5D (mean score=0.6, change=-0.1, p=<0.01). In the SPMS group there was also significant improvement in the raw score of the BVMT-R visual memory task (change=6.7, SD=8.4, p<0.01), but this was not sustained in terms of the z-score. This is highlighted by the mean and median change between baseline and longitudinal visits on the strip-plot (boxplot with overlying dot plot) in **figure 4.9**. There was raw score worsening overall in the delayed verbal memory task, the CVLT-II, verbal fluency, and Stroop (**figure 4.9**). Although not significant, there was general worsening of the z-scores of the BVMT-R retained, CVLT-II immediate and delayed components, SDMT, Hayling, verbal fluency FAS, and Stroop task in the SPMS group (**figure 4.10**). The most significant change over time in SPMS is in the verbal memory domain; the delayed and immediate components of the CVLT-II, and Stroop.

I qualitatively reviewed the differences in variables cross-sectionally and over time by MS-SMART drug arm (**table 4.13 and figure 4.11**). As with baseline visit, there were no differences between drug arms in the demographic, clinical, or cognitive variables at follow-up (**table 4.13**). The majority (n=17) of the 59 subjects were in the placebo arm where there was generalised worsening of verbal memory, information processing speed and executive function, but this was only significant for Stroop z-score over time. The violin plot in **figure 4.11** indicates that this might be due to a greater spread of probability of Stroop scores then at baseline. Subjects in the

fluoxetine arm (n=15) showed preservation of demographic, physical, psychological, and cognitive functions over time with no significant differences (**table 4.13**). In the riluzole group (n=16) there was significant worsening of the MSNQ (change -4.9, p=0.02). However, there was significant improvement or lack of worsening in several other PROMS; the MSIS (change 7.1, p=0.05), the MSWS (change 10.8, p=0.05), MSNQ (change -4.9, p=0.02) and EQ-5D (change 0, p=0.05). Additionally, the BVMT-R showed significant improvement overall in this group (change 8.8, p=0.01), but paradoxically the BVMT-R retained z-score significantly worsened (change -0.3, p=0.05) (**table 4.13**). **Figure 4.11** indicates that this difference is related to the greater spread of data and probability of scores in the BVMT-R task, but not BVMT-R retained in the riluzole group. The amiloride group had the smallest proportion of subjects; 11 out of 59. There was significant improvement of the BPI pain score in the amiloride group (change -1.2, p=0.02). As with riluzole, there was also apparently significant improvement of the BVMT-R with amiloride (change 20.2, p=0.03) **figure 4.11**. Due to small group sizes and multiple comparisons this data should be treated qualitatively.

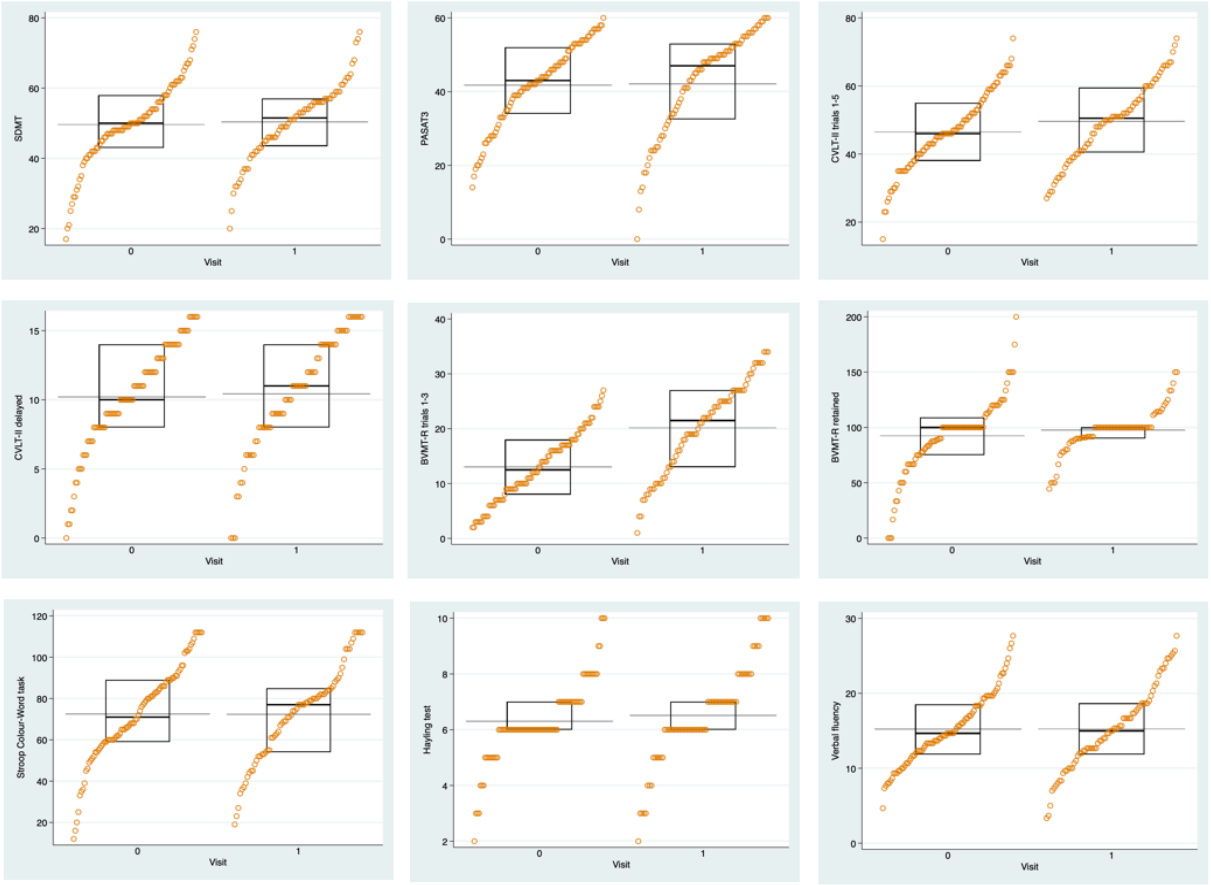
Table 4.12. Demographic, clinical, and cognitive characteristics in healthy control and SPMS groups at the follow-up timepoint and changes over time. *Negative changes over time and p-values≤0.05 are in bold.*

FOLLOW-UP	Healthy controls					SPMS					
	n	Mean/median	SD/range	change	p-value (visit)	n	Mean/median	SD/range	change	p (visit)	chi^2 (cohort)
Age (years)	13	57.8	4.63	1.8	0.16	58	56.7	6.9	1.9	0.14	0.69
Gender	13	M:F 3:10		na	na	59	M:F 13:46		na	na	0.94
Interval	13	661.2	22.3	na	na	58	672.9	61.7	na	na	**<0.01**
EDSS	13	na	na	na	na	53	6.0	2.5-8.0	0.0	0.45	na
9HPT	13	na	na	na	na	54	35.8	14.9	0.3	0.78	na
T25FW	13	na	na	na	na	53	28.5	40.6	8.9	0.35	na
MSFC	13	na	na	na	na	53	-0.3	1.4	**-0.2**	0.77	na
MSISphy	13	na	na	na	na	52	54.1	11.9	6.0	**0.01**	na
MSIS	13	na	na	na	na	52	74.5	15.7	8.9	**0.01**	na
MSWS	13	na	na	na	na	52	45.3	8.9	4.5	**0.02**	na
MSVQ	13	na	na	na	na	53	13.5	4.7	**-9.1**	0.99	na
MSNQ	13	15.8	7.4	0.8	0.76	55	29.2	11.4	7.9	**<0.01**	na
MSISpsy	13	na	na	na	na	52	20.4	5.6	2.9	**0.01**	na
VAF	12	0.4	1.5	-0.7	0.40	57	1.1	1.7	0.1	0.93	0.22
NFI	13	na	na	na	na	52	18.5	4.8	1.3	0.13	na
BPI	13	na	na	na	na	52	4.5	2.6	1.4	**<0.01**	na
Qualification	13	RQF:(n); Level 1:2; Level 4:2; Level 6:4; Level 7:3; Level 8:2				59	RQF:(n); Entry level:1; Level 1:19; Level 2:2; Level 3:10; Level 5:2; Level 6:20; Level 7:4; Level 8:1				**0.01**
Occupation	13	Occupation Category:(n); 1:1; 2:5; 3:3; 4:2; 5:1; 8:1				59	Occupation Category:(n); 1:6; 2:22; 3:5; 4:15; 5:3; 6:6; 7:2				0.25
Employment	13	Employment Status:(n); Full-time:7; Part-time:2; Retired:4				59	Employment Status:(n); Full-time:10; Part-time:22; Retired:27				**0.02**
EQ-5D	13	na	na		na	52	0.6	0.2	**-0.1**	**<0.01**	na
Health	13	na	na		na	52	61.6	20.0	**-4.1**	0.25	na
Ethnicity	13	Ethnicity:(n); English:10; Italian:1; Urdu:1; Welsh:1				59	Ethnicity:(n); Belgian:1; Cingalese:1; English:56; German:1				**0.02**

	n	Mean/median	SD/range	change	p-value (visit)	n	Mean/median	SD/range	change	p (visit)	chi² (cohort)
BVMT-R	13	23.5	8.6	7.8	**0.02**	59	19.4	8.4	6.7	**<0.01**	0.14
BVMT-R z	13	0.0	1.0	0.0	0.86	59	-0.5	1.0	0.0	0.99	0.14
BVMT-R ret	13	104.0	27.8	4.5	0.68	58	96.1	18.1	5.0	0.54	0.22
BVMT-R ret z	13	0.0	1.0	0.0	0.82	58	-0.3	0.7	**-0.1**	0.10	0.22
CVLT-II	13	58.5	10.0	1.5	0.84	59	47.6	11.2	1.7	0.43	**<0.01**
CVLT-II z	13	0.0	1.0	-0.2	0.63	59	-1.1	1.1	**-0.2**	0.24	**<0.01**
CVLT-II d	13	14.0	2.8	0.5	0.17	58	9.6	4.1	**-0.4**	0.61	**<0.01**
CVLT-II d z	13	0.0	1.0	-0.3	0.29	58	-1.6	1.5	**-0.3**	0.19	**<0.01**
PASAT3	13	46.9	12.2	-0.9	0.80	53	41.1	14.0	0.2	0.57	0.10
PASAT3 z	13	0.2	1.0	-0.1	0.80	53	-0.3	1.2	0.0	0.56	0.10
SDMT	13	59.5	8.4	-0.5	0.86	59	48.4	10.6	0.2	1.00	**<0.01**
SDMT z	13	0.0	1.0	0.0	0.94	59	-1.3	1.3	**-0.1**	0.75	**<0.01**
Hayling	13	6.9	1.0	0.2	0.70	57	6.4	1.9	0.1	0.63	0.25
Hayling z	13	0.0	1.0	-0.1	0.42	57	-0.5	2.0	**-0.2**	0.14	0.25
VF	13	18.5	4.8	0.4	0.76	59	14.5	5.5	**-0.2**	0.80	**0.02**
VF z	13	0.0	1.0	-0.1	0.90	59	-0.8	1.1	**-0.2**	0.26	**0.02**
Stroop	13	91.6	20.1	3.2	0.70	58	68.0	21.6	**-2.4**	0.55	**<0.01**
Stroop z	13	0.0	1.0	-0.1	0.94	58	-1.2	1.1	**-0.3**	0.10	**<0.01**

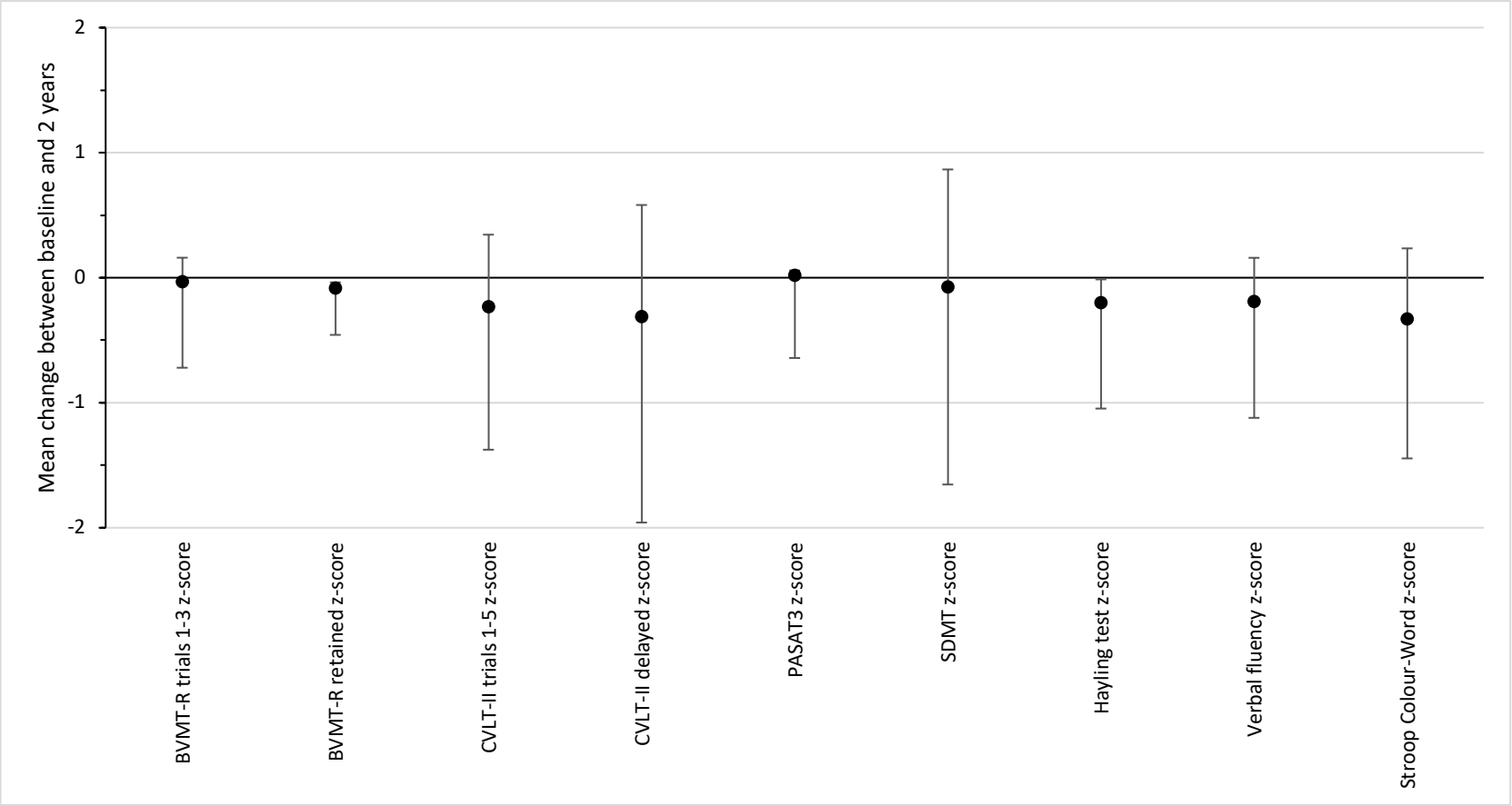
Interval= visit interval in days, 9HPT= Average nine hole peg test in seconds, T25FW= average timed 25 foot walk in seconds, MSISphy = MSIS 29v2 physical score, MSIS= MSIS 29v2 total score, MSWS = MS walking score v2, MSVQ= MS Vision Questionnaire-7, MSNQ= MS neuropsychological questionnaire, MSISpsy= MSIS 29v2 psychological score, VAF= Visual analogue of fatigue, NFI= Neurological fatigue index, BPI= Brief Pain Inventory, Qualification = Qualification by RQF, Occupation = Occupation, Employment = Employment Status, EQ-5D= Health-state questionnaire, Health = Health-state analogue, BVMT-R = BVMT-R trials 1-3, BVMT-R z = BVMT-R trials 1-3 z-score, BVMT-R ret = BVMT-R retained, BVMT-R ret z = BVMT-R retained z-score, CVLT-II = CVLT-II trials 1-5, CVLT-II z = CVLT-II trials 1-5 z-score, CVLT-II d = CVLT-II delayed, CVLT-II d z = CVLT-II delayed z-score, PASAT3 z = PASAT3 z-score, SDMT z = SDMT z-score, Hayling = Hayling test, Hayling z = Hayling test z-score, VF = Verbal fluency, VF z = Verbal fluency z-score, Stroop = Stroop Colour-Word task, Stroop z = Stroop Colour-Word z-score.

Figure 4.9 One-way dot plots with overlying box plots of the cognitive assessment scores at visit 0 (baseline) and visit 1 (follow-up). From top left to bottom right; SDMT, PASAT3, CVLT-II trials 1-5, CVLT-II delayed, BVMT-R trials 1-3, BVMT-R retained, Stroop colour-word task, Hayling test, verbal fluency task.



Grey lines represent mean, black lines within boxes represents median.

Figure 4.10 Plot of mean change between baseline and follow-up assessments with 95% confidence intervals for the cognitive outcome measures in the SPMS group.



Dots represent mean change over time. Whiskers represent 95% confidence intervals.

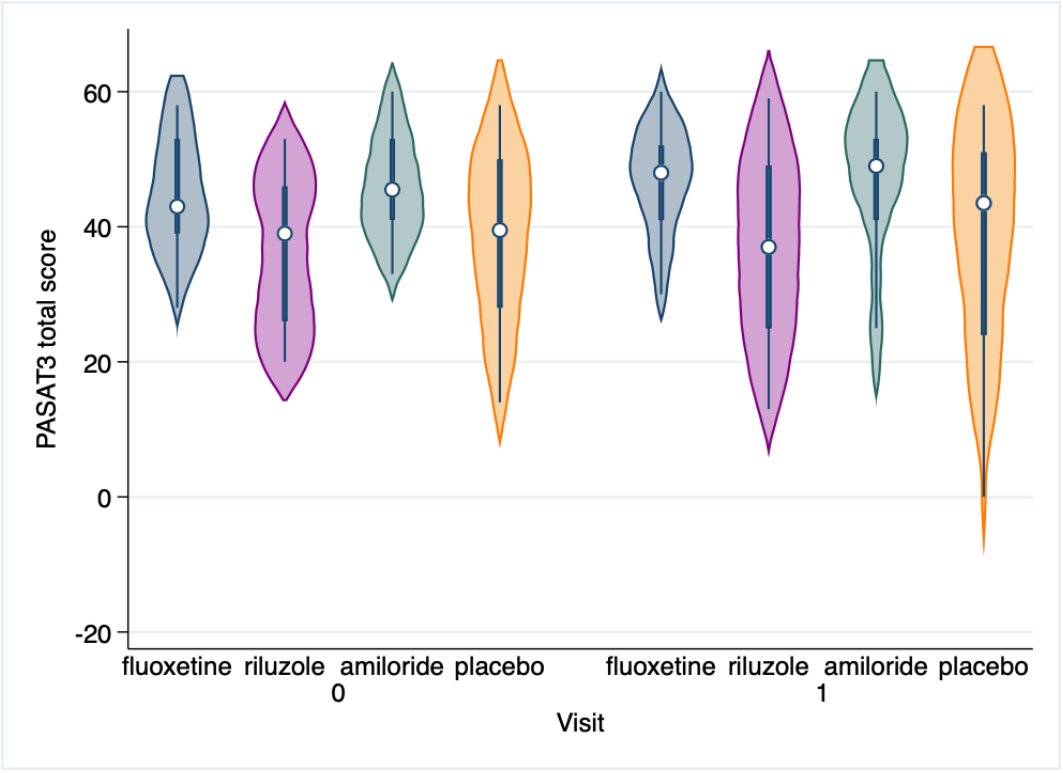
Table 4.13. Characteristics of SPMS groups by drug arm at the follow-up timepoint and changes over time. *Negative changes over time and p-values ≤0.05 are in bold.*

	Placebo					Fluoxetine					Riluzole					Amiloride					
	n	Mean/median	SD/range	change	p (visit)	n	Mean/median	SD/range	change	p (visit)	n	Mean/median	SD/range	change	p (visit)	n	Mean/median	SD/range	change	p (visit)	chi² (cohort)
Age (years)	16	55.2	5.9	1.9	0.25	15	55.6	8.2	1.8	0.55	16	58.7	7.0	1.8	0.43	11	57.6	6.0	1.8	0.34	0.42
Gender	16	M:F 4:13		na	na	15	M:F 1:14		na	na	16	M:F 4:12		na	na	11	M:F 4:7		na	na	0.48
Interval	16	677.6	67.2	na	na	15	668.9	62.3	na	na	16	673.9	48.0	na	na	11	669.9	77.0	na	na	0.39
EDSS	15	6.0	2.5-8	0.0	0.86	13	6.0	3-8	0.0	0.98	15	6.0	2-6.5	0.0	0.58	10	6.0	3-6.5	0.0	0.59	0.90
9HPT	16	32.4	10.9	0.1	0.68	15	35.6	15.8	**-0.1**	0.66	13	37.9	15.2	0.0	0.66	10	38.7	19.7	0.1	0.94	1.00
T25FW	15	37.1	58.7	1.3	0.46	13	36.5	48.5	**-2.3**	0.98	15	20.5	13.9	**-0.7**	0.71	10	17.3	16.3	4.3	0.50	0.93
MSFC	15	-0.6	1.9	15.8	0.87	13	-0.3	1.6	12.4	0.84	15	-0.2	0.7	1.5	0.61	10	0.3	0.4	5.2	0.53	0.78
MSISphy	15	54.1	11.8	**-0.4**	0.23	13	54.6	12.4	0.0	0.13	14	55.7	11.3	**-0.1**	0.08	10	51.0	13.3	**-0.1**	0.48	0.82
MSIS	15	73.9	14.1	4.4	0.18	13	76.5	16.8	8.2	0.10	14	75.8	14.9	7.1	**0.05**	10	71.0	19.4	4.1	0.48	0.88
MSWS	15	44.3	9.4	5.6	0.79	13	44.5	10.5	12.8	0.32	14	47.6	6.6	10.8	**0.05**	10	44.4	9.6	6.3	0.27	0.98
MSVQ	15	14.1	4.3	1.6	0.32	14	13.1	3.1	4.0	0.43	14	14.1	4.8	8.1	0.60	10	12.3	6.8	4.5	0.94	0.43
MSNQ	15	28.3	11.0	**-8.3**	0.11	14	32.1	10.0	**-21.1**	0.12	15	27.3	14.2	**-4.9**	**0.02**	11	29.4	10.4	**-0.1**	0.36	0.56
MSISpsy	15	19.9	3.7	5.5	0.26	13	21.9	6.2	9.0	0.06	14	20.1	5.9	11.0	0.10	10	20.0	7.2	5.6	0.50	0.88
VAF	16	1.3	1.7	1.2	0.98	15	1.4	1.1	4.7	0.22	16	1.3	2.0	3.7	0.84	10	0.1	1.7	2.2	0.18	0.21
NFI	15	19.6	4.2	0.3	0.09	13	18.9	6.4	0.4	0.42	14	17.8	2.5	0.2	0.46	10	17.3	6.0	**-0.9**	0.70	0.59
BPI	15	4.5	2.4	2.7	0.20	13	4.0	3.4	2.2	0.29	14	4.4	2.5	0.8	0.23	10	5.3	2.0	**-1.2**	**0.02**	0.74
EQ-5D	15	0.6	0.2	0.0	0.13	13	0.6	0.2	0.0	0.42	14	0.6	0.2	0.0	**0.05**	10	0.5	0.3	0.0	0.12	0.76
Health	15	61.9	18.9	**-0.1**	0.58	13	62.2	19.7	**-0.1**	0.41	14	64.3	21.0	**-0.1**	0.95	10	56.4	22.6	**-0.2**	0.52	0.27

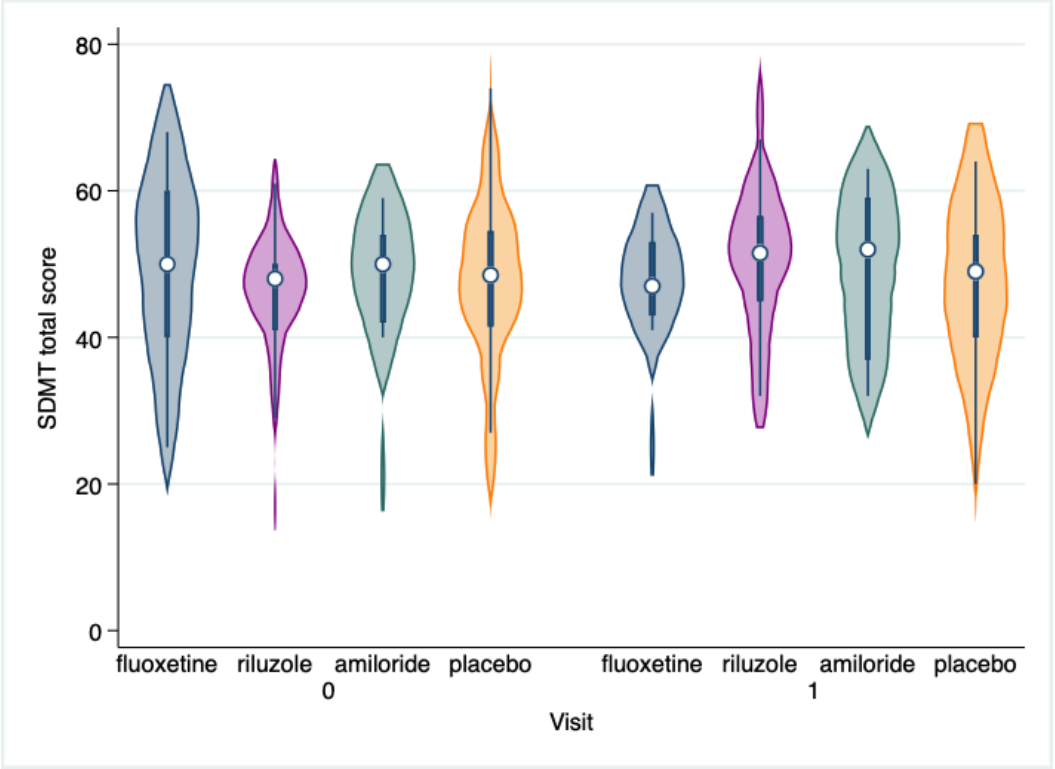
	n	Mean/median	SD/range	change	p (visit)	n	Mean/median	SD/range	change	p (visit)	n	Mean/median	SD/range	change	p (visit)	n	Mean/median	SD/range	change	p (visit)	chi² (cohort)
BVMT-R	17	16.2	8.4	5.0	0.06	15	20.1	8.7	7.8	0.02	16	20.7	9.3	8.8	**0.01**	11	21.6	5.7	4.7	0.12	0.35
BVMT-R z	17	-0.8	1.0	-0.2	0.55	15	-0.4	1.0	0.1	0.76	16	-0.3	1.1	0.3	0.41	11	-0.2	0.7	**-0.4**	0.31	0.35
BVMT-R ret	16	88.6	18.2	-5.5	0.96	15	102.3	15.4	14.3	0.77	16	94.4	22.3	**-3.6**	0.39	11	100.8	10.9	20.2	**0.03**	0.36
BVMT-R ret z	16	-0.6	0.7	-0.4	0.17	15	-0.1	0.6	0.2	0.36	16	-0.4	0.8	**-0.3**	**0.05**	11	-0.1	0.4	0.4	0.28	0.36
CVLT-II	17	42.4	12.1	1.1	0.97	15	49.3	9.3	0.0	1.00	16	49.6	12.5	7.4	0.06	11	50.6	8.3	**-3.3**	0.36	0.14
CVLT-II z	17	-1.6	1.2	**-0.3**	0.22	15	-0.9	0.9	**-0.4**	0.25	16	-0.9	1.3	0.3	0.47	11	-0.8	0.8	**-0.7**	0.09	0.14
CVLT-II d	16	7.9	3.9	**-0.6**	0.61	15	10.6	4.1	**-0.5**	0.87	16	9.5	4.9	0.4	0.62	11	10.9	2.2	**-1.3**	0.24	0.14
CVLT-II d z	16	-2.2	1.4	**-0.3**	0.54	15	-1.2	1.5	**-0.4**	0.47	16	-1.6	1.8	0.1	0.88	11	-1.1	0.8	**-0.8**	0.11	0.14
PASAT3	15	36.3	17.7	**-1.2**	0.88	13	45.2	8.2	1.1	0.71	15	36.9	14.3	**-0.1**	1.00	10	49.1	8.5	2.0	0.52	0.15
PASAT3 z	15	-0.7	1.5	**-0.1**	0.87	13	0.0	0.7	0.1	0.68	15	-0.7	1.2	0.0	0.98	10	0.3	0.7	0.2	0.50	0.15
SDMT	17	46.7	12.3	**-1.1**	0.93	15	47.1	7.9	**-3.1**	0.33	16	51.0	11.4	4.7	0.15	11	48.7	10.6	**-0.1**	1.00	0.73
SDMT z	17	-1.5	1.5	**-0.1**	0.88	15	-1.5	1.0	**-0.5**	0.25	16	-1.0	1.4	0.4	0.19	11	-1.3	1.3	**-0.2**	0.77	0.73
Hayling	15	6.3	2.0	**-0.5**	0.54	15	6.5	1.6	0.5	0.30	16	6.1	2.1	0.1	0.78	11	6.8	2.2	0.6	0.25	0.72
Hayling z	15	-0.6	2.1	**-0.8**	0.17	15	-0.4	1.6	0.1	0.98	16	-0.8	2.2	**-0.2**	0.20	11	-0.1	2.3	0.3	0.82	0.72
VF	17	12.9	5.8	**-2.1**	0.20	15	15.3	6.1	0.1	0.83	16	15.5	5.6	1.9	0.35	11	14.5	4.2	**-0.7**	0.69	0.56
VF z	17	-1.2	1.2	**-0.6**	0.09	15	-0.7	1.3	**-0.1**	0.76	16	-0.6	1.2	0.2	0.66	11	-0.8	0.9	**-0.3**	0.34	0.56
Stroop	16	56.6	25.3	**-13**	0.15	15	74.3	16.2	5.2	0.57	16	71.5	19.4	0.4	0.84	11	71.0	21.7	**-1.3**	0.97	0.23
Stroop z	16	-1.7	1.3	**-0.9**	**0.04**	15	-0.9	0.8	0.1	0.93	16	-1.0	1.0	**-0.2**	0.55	11	-1.0	1.1	**-0.3**	0.49	0.23

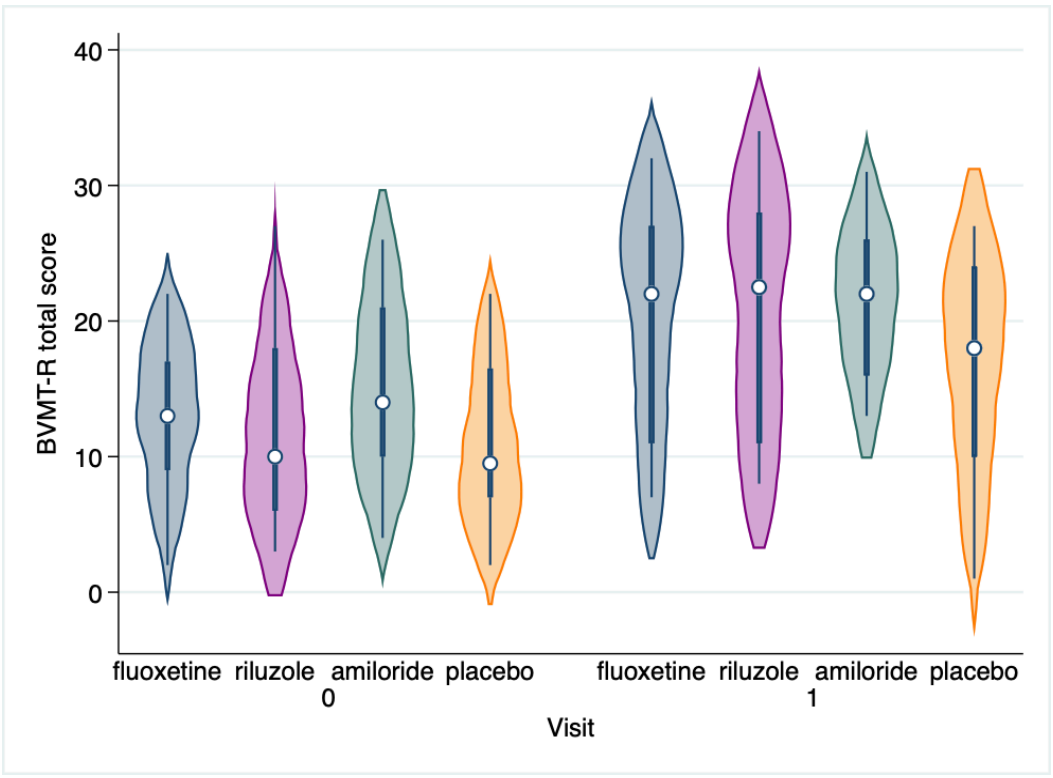
Interval= visit interval in days, 9HPT= Average nine hole peg test in seconds, T25FW= average timed 25 foot walk in seconds, MSISphy = MSIS 29v2 physical score, MSIS= MSIS 29v2 total score, MSWS = MS walking score v2, MSVQ= MS Vision Questionnaire-7, MSNQ= MS neuropsychological questionnaire, MSISpsy= MSIS 29v2 psychological score, VAF= Visual analogue of fatigue, NFI= Neurological fatigue index, BPI= Brief Pain Inventory, EQ-5D= Health-state questionnaire, Health = Health-state analogue, BVMT-R = BVMT-R trials 1-3, BVMT-R z = BVMT-R trials 1-3 z-score, BVMT-R ret = BVMT-R retained, BVMT-R ret z = BVMT-R retained z-score, CVLT-II = CVLT-II trials 1-5, CVLT-II z = CVLT-II trials 1-5 z-score, CVLT-II d = CVLT-II delayed, CVLT-II d z = CVLT-II delayed z-score, PASAT3 z = PASAT3 z-score, SDMT z = SDMT z-score, Hayling = Hayling test, Hayling z = Hayling test z-score, VF = Verbal fluency, VF z = Verbal fluency z-score, Stroop = Stroop Colour-Word task, Stroop z = Stroop Colour-Word z-score.

Figure 4.11. Violin plots of changes in cognitive outcomes by drug arm at visit 0 (baseline) and visit 1 (follow-up) in the SPMS group. a) PASAT3, b) SDMT, c) BVMT-R trials 1-3, d) BVMT-R retained, e) CVLT-II trials 1-5, f) CVLT-II delayed, g) Hayling test, h) Stroop colour-word interference task, i) Verbal fluency task.

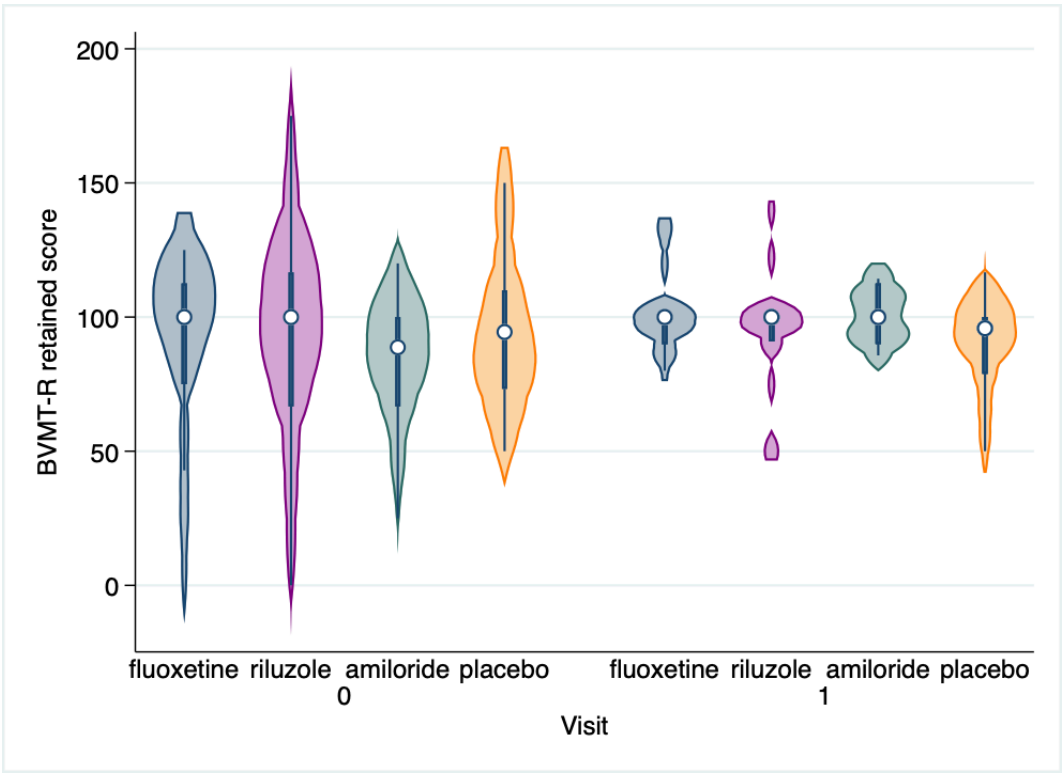


a) b)

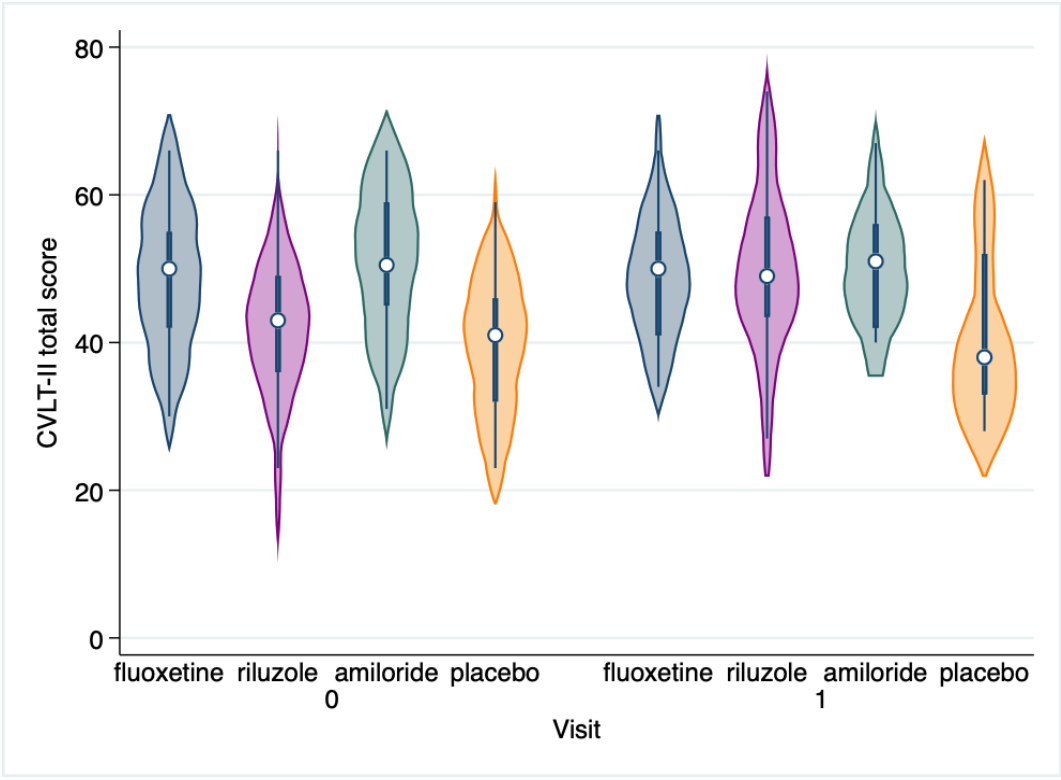



BVMT-R total score
40
30
20
10
0
fluoxetine riluzole amiloride placebo
0
fluoxetine riluzole amiloride placebo
1
Visit

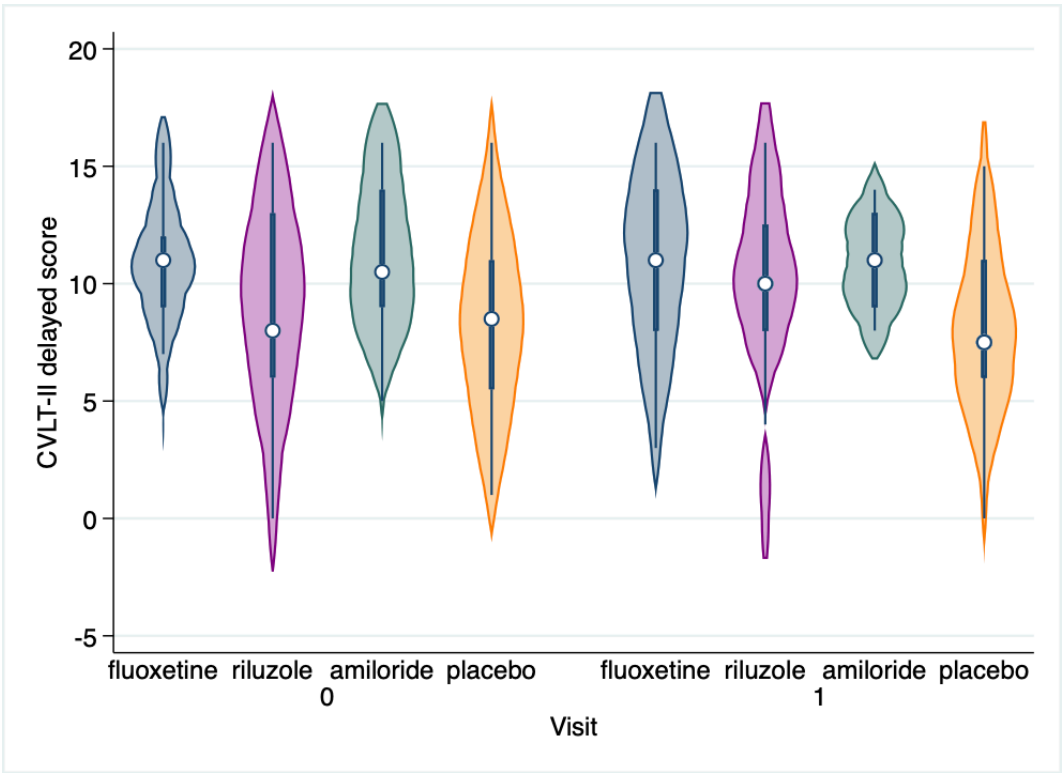
c)


BVMT-R retained score
200
150
100
50
0
fluoxetine riluzole amiloride placebo
0
fluoxetine riluzole amiloride placebo
1
Visit

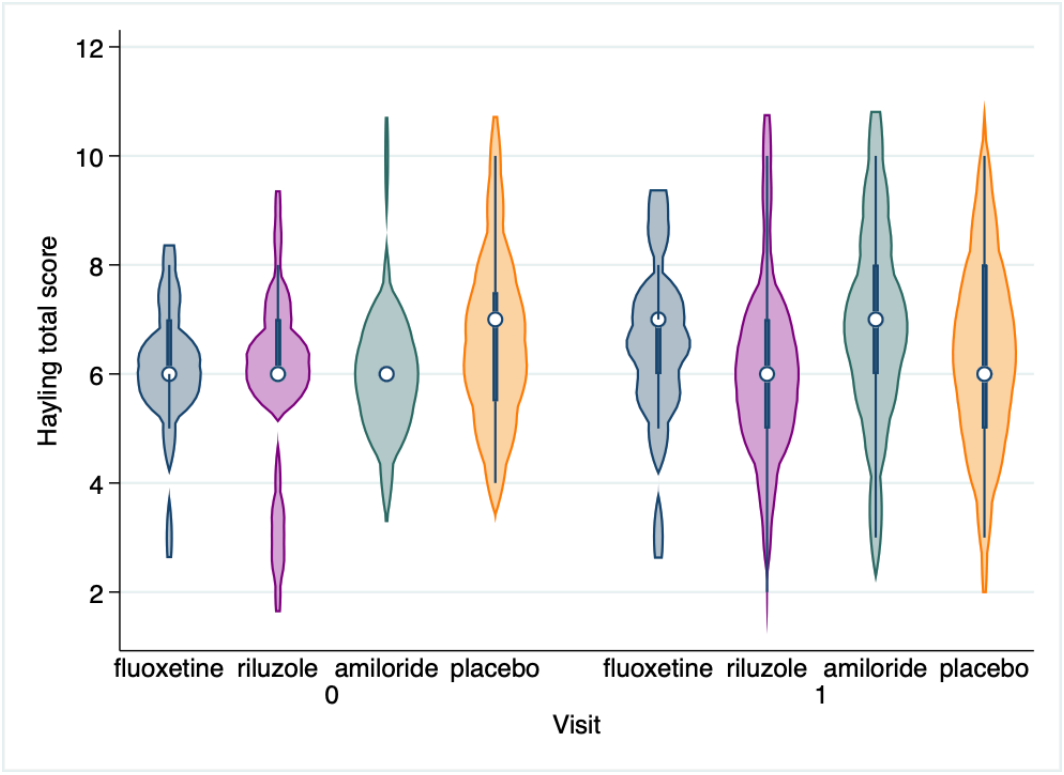
d)


80
60
40
20
0
CVLT-II total score
fluoxetine riluzole amiloride placebo
0
fluoxetine riluzole amiloride placebo
1
Visit

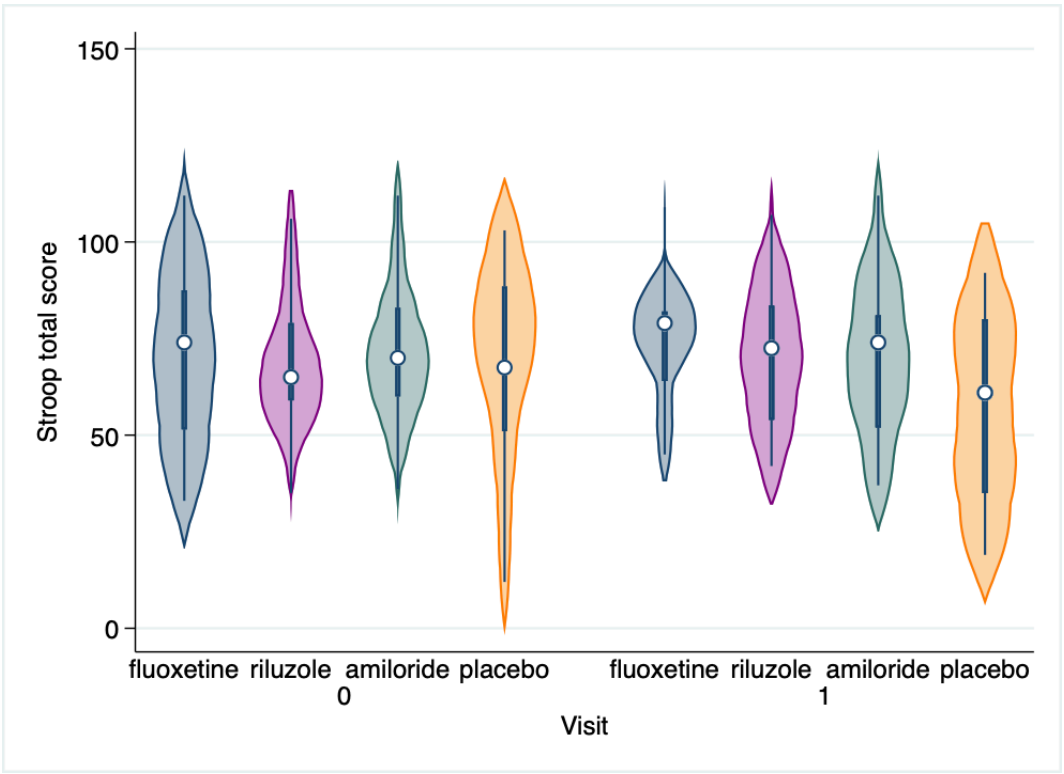
e)


20
15
10
5
0
-5
CVLT-II delayed score
fluoxetine riluzole amiloride placebo
0
fluoxetine riluzole amiloride placebo
1
Visit

f)

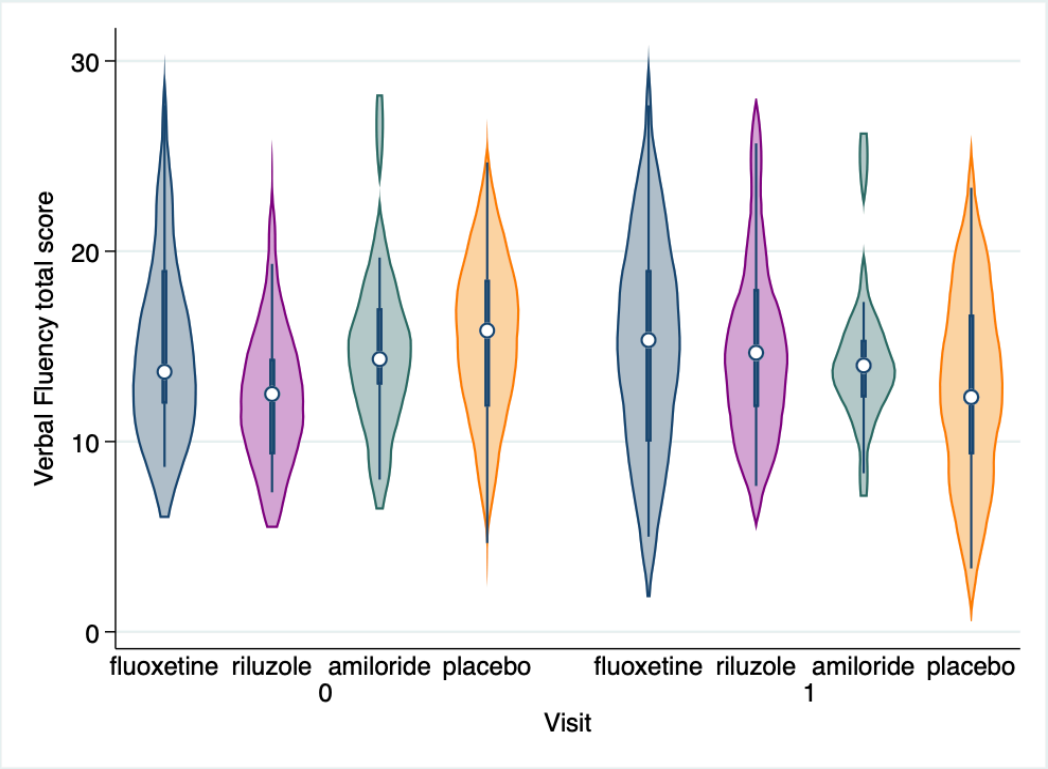

Hayling total score
12
10
8
6
4
2
fluoxetine riluzole amiloride placebo
0
fluoxetine riluzole amiloride placebo
1
Visit

g)


Stroop total score
150
100
50
0
fluoxetine riluzole amiloride placebo
0
fluoxetine riluzole amiloride placebo
1
Visit

h)

i)



The white dot represents the median. The thick grey bar represents the interquartile range. The thin grey line represents the distribution of data. The rest of the plot represents the kernel density estimation to show the overall distribution shape of the data and the probability of each value.

4.4.3.2 Associations of worsening of cognitive function in SPMS over time.

This analysis reviews associations of deterioration of each of the cognitive outcomes from baseline to follow-up visit in the SPMS group. Each analysis has a covariate model including age, HADS anxiety score and gender at follow-up. This is because they were shown to be the most significant variables in the baseline linear regression models (**tables 4.9-4.12**). The greatest proportion of cognitive decline in the 59 SPMS subjects occurred in the verbal fluency (n=54), and BVMT-R tasks (n=42).

25 subjects worsened on the SDMT over time (**table 4.14 a)**). The SDMT worsening covariate model with age, gender, and HADS anxiety as predictors is not significant at the 5% level (LR=1.99). The overall predictive quality of the covariate model is an AUROC of 0.6. There were only cognitive significant predictors of SDMT worsening. The most predictive model is from the addition of the SDMT z-score at follow-up to the covariate model (OR=3.43, p=<0.01, 95% CI 1.65 to 7.13, LR 19.96, AUROC 0.84). For each one point increase in the SDMT at follow-up there is a 1.16 times increased chance of worsening of the SDMT. However, for both SDMT and SDMT-z score at baseline and follow-up there is considerable confounding with LR of above 19. The baseline BVMT-R is overall the most predictive model with the least confounding (OR=0.87, p=0.01, 95% CI 0.79 to 0.96, LR 11.30). A point increase on the BVMT-R at baseline there is a 13% reduction in the chance of worsening on the SDMT (AUROC=0.73).

The SDMT deteriorated by 4 points in 25 subjects over time (**table 4.14 b)**). The covariate model was not significant at the 5% level (LR=2.33) with a predictive probability of 0.61. A greater baseline score on the BVMT-R (OR=0.84, p=<0.01, 95% CI 0.75 to 0.94, LR 14.39, AUROC 0.76) and SDMT (OR=0.85, p=<0.01, 95% CI 0.77 to 0.94, LR 22.44, AUROC 0.83) reduced the risk of 4 point worsening on the SDMT by 16% and 15% respectively. However, the baseline SDMT model understandably showed considerable confounding. At follow-up the SDMT showed significant positive confounding (OR=1.16, p<0.01, LR=19.96, AUROC=0.84). The CVLT-II associated best (AUROC=0.72) at follow-up, (OR=1.07, p=<0.02, 95% CI 1.01 to 1.14, LR 9.23,

AUROC 0.72), with a one point increase in the CVLT-II at follow-up indicating a 7% chance of greater worsening on the SDMT by 4 points.

The covariate model for PASAT worsening (n=35) was not significant in terms of individual predictors, and gave an overall LR of 4.19 AUROC=0.64. (**table 4.14 c)**). This covariate model was significantly improved by the addition of the PASAT3 (OR=1.09, p=0.01, 95% CI 1.03-1.15, LR of 15.12, AUROC=0.79) and PASAT3 z-score (OR=2.76 p=0.01, 95% CI 1.36-5.61, LR of 15.12, AUROC=0.79) at follow-up, for which a higher follow-up score indicated greater worsening of PASAT3 at follow-up, however both show expected confounding. A greater predictive property of the covariate model was provided by the addition of premorbid IQ (p=0.02, 95% CI 1.02 to 1.24, LR 11.54, AUROC 0.76) for which a unit higher score was associated with a 1.13 times likelihood of PASAT3 worsening at follow-up. A higher HADS depression score (p=0.03, 95% CI 1.05 to 2.23, LR 10.44, AUROC 0.71) was associated with a worsening of PASAT3 at follow-up per unit increase by a factor of 1.53. Increases in the number of years of progression (p=0.02, 95% CI 0.73 to 0.97, LR 10.80, AUROC 0.74), and MSNQ (p=0.03, 95% CI 0.88 to 0.99, LR 8.62, AUROC 0.72) were associated with less worsening of PASAT3 at follow-up by 0.84, and 0.94 times respectively.

The BVMT-R immediate (n=42) and delayed (n=22) components were both negatively associated with lower scores in their parent BVMT-R and BVMT-R retained tasks at baseline corrected by age, gender and HADS anxiety, but with considerable confounding (**table 4.14 d) and e)**). Subjects were less likely to worsen on the BVMT-R at follow-up by a factor of 0.91 with a unit lower SDMT score (OR=0.91, p=0.02, 95% CI 0.84-0.98, LR 11.23, AUROC=0.76) and BDI-II score (OR=0.86, p=0.04, 95% CI 0.74-0.99, LR of 8.21, AUROC=0.72) at baseline. There was a positive association of BVMT-R deterioration for every year increase in disease duration (OR=1.10, p=0.03, 95% CI 1.01-1.20, LR 9.56, AUROC=0.72). Decline on the BVMT-R was associated positively with the CVLT-II verbal memory (OR=1.07, p=0.04, 95% CI 1.00-1.13, LR 8.17, AUROC=0.74) and Stroop executive tasks (OR=1.03, p=0.04, 95% CI 1.00-1.06, LR 8.21, AUROC=0.72) at follow-up.

30 subjects deteriorated on the immediate CVLT-II verbal memory task versus 21 for the delayed component (**table 4.14 f) and g)**). A higher baseline CLQ score, indicating less leisure activity interaction, was associated with 16% reduction in the odds of worsening on the CVLT-II per unit increase (OR=0.84, p=0.02, 95% CI 0.73 to 0.97, LR 9.36, AUROC 0.72). An increase in PASAT3 score at follow-up by one point was associated with a lower chance of CVLT-II worsening by 6% (OR=0.94, p=0.02, 95% CI 0.89 to 0.99, LR 8.02, AUROC=0.69). A higher MSVQ-7 score, indicating poor subjective visual function, was associated with a lower odds of CVLT-II delayed component decline, by a factor of 0.84 (OR=0.84, p=0.03, 95% CI 0.72 to 0.98, LR=8.80, AUROC=0.77).

In terms of executive function, as shown by **table 4.14 h),** there were no non-confounding variables associated with worsening on the Hayling task (23 subjects). Of interest, the verbal fluency task (**table 4.14 i)**) deteriorated in more subjects than any other task; 54 subjects. Verbal fluency scores at either timepoint were not significant additions to the covariate model. However, a greater NART IQ at baseline was associated with 31% reduction in the chance of deterioration on the verbal fluency task per unit increase (OR=0.69, p=0.06, 95% CI 0.46 to 1.01, LR=7.67, AUROC=0.89), but this did not quite reach significance. The most predictive model of worsening on the Stroop (25 subjects) included follow-up age, gender, and HADS anxiety as well as employment status at baseline (**table 4.14 j)**). Those who were retired at baseline were positively associated with Stroop decline by a factor of 2.46 (OR=2.46, p=0.04, 95% CI 1.04 to 5.84, LR=4.82, AUROC=0.70).

Tables 3.14 a)-j). Logistic regression models of worsening of cognitive assessments over time and significant associated predictors. a) SDMT, b) worsening of the SDMT by 4 points c) PASAT3, d) BVMT-R trials 1-5, e) BVMT-R retained, f) CVLT-II trials 1-3, g) CVLT-II delayed, h) Hayling test, i) Verbal fluency, j) Stroop colour-word interference task. *Receiver operator curve characteristics are provided to aid interpretation. Only significantly associated or almost-significant variables are shown in the tables below (p= ≤ 0.05 in bold). All variables in* ***table 4.3*** *were tested.*

a) Independent predictors of SDMT Worsening (n=25)						
Predictors	Odds Ratio	p-value	95% Confidence Interval		LR	AUROC
Age	1.02	0.70	0.94	1.10	1.99	0.60
Gender	0.48	0.27	0.13	1.77		
HADS anxiety	1.07	0.39	0.92	1.24		
BASELINE						
SDMT	0.85	**<0.01**	0.77	0.94	22.44	0.83
SDMT z-score	0.22	**<0.01**	0.09	0.54	22.44	0.83
PASAT3	0.94	**0.04**	0.89	1.00	6.47	0.69
PASAT3 z-score	0.50	**0.04**	0.25	0.98	6.49	0.69
BVMT-R	0.87	**0.01**	0.79	0.96	11.30	0.73
BVMTR z-score	0.42	**0.01**	0.22	0.78	11.30	0.73
LONGITUDINAL						
SDMT	1.16	**<0.01**	1.06	1.26	19.96	0.84
SDMT z-score	3.43	**<0.01**	1.65	7.13	19.96	0.84
PASAT3	0.95	**0.02**	0.90	0.99	7.65	0.68
PASAT3 z-score	0.51	**0.02**	0.28	0.91	7.65	0.68
CVLT-II	1.06	**0.03**	1.01	1.12	7.32	0.70
CVLT-II z-score	1.82	**0.03**	1.06	3.14	7.32	0.70
BVMT-R	1.07	**0.04**	1.00	1.15	6.50	0.67
BVMT-R z-score	1.84	**0.04**	1.02	3.32	6.50	0.67

b) Independent predictors of SDMT Worsening by 4 points (n=25)						
Predictors	Odds Ratio	p-value	95% Confidence Interval		LR Chi2	AUROC
Age	1.04	0.40	0.95	1.13	2.33	0.61
Gender	0.46	0.25	0.13	1.70		
HADS anxiety	1.07	0.37	0.92	1.24		
BASELINE						
BVMT-R	0.84	**<0.01**	0.75	0.94	14.39	0.76
BVMT-R z-score	0.34	**<0.01**	0.17	0.69	14.39	0.76
SDMT	0.85	**<0.01**	0.77	0.94	22.44	0.83
SDMT z-score	0.22	**<0.01**	0.09	0.54	22.44	0.83
LONGITUDINAL						
BVMT-R	1.09	**0.03**	1.01	1.17	7.78	0.71
BVMT-R z-score	2.02	**0.03**	1.08	3.76	7.78	0.71
CVLT-II	1.07	**0.02**	1.01	1.14	9.23	0.72
CVLT-II z-score	2.02	**0.02**	1.15	3.57	9.23	0.72
SDMT	1.16	**<0.01**	1.06	1.26	19.96	0.84
SDMT z-score	3.43	**<0.01**	1.65	7.13	19.96	0.84
PASAT3	0.95	**0.03**	0.91	1.00	7.15	0.68
PASAT3 z-score	0.53	**0.03**	0.30	0.95	7.15	0.68

c) Independent predictors of PASAT3 Worsening (n=35)						
Predictors	Odds Ratio	p-value	95% Confidence Interval		LR	AUROC
Age	0.93	0.15	0.85	1.03	4.19	0.64
Gender	0.43	0.26	0.10	1.89		
HADS anxiety	0.95	0.48	0.81	1.10		
BASELINE						
NART IQ	1.13	**0.02**	1.02	1.24	11.54	0.76
HADS depression	1.53	**0.03**	1.05	2.23	10.44	0.71
Years of progression	0.84	**0.02**	0.73	0.97	10.80	0.74
LONGITUDINAL						
PASAT3	1.09	**0.01**	1.03	1.15	15.12	0.79
PASAT3 z-score	2.76	**0.01**	1.36	5.61	15.12	0.79
MSNQ	0.94	**0.03**	0.88	0.99	8.62	0.72

d) Independent predictors of BVMT-R Worsening (n=42)						
Predictors	Odds Ratio	p-value	95% Confidence Interval		LR	AUROC
Age	0.99	0.84	0.90	1.09	3.55	0.65
Gender	0.39	0.27	0.07	2.06		
HADS anxiety	1.12	0.22	0.94	1.33		
BASELINE						
BVMT-R	0.73	**<0.01**	0.62	0.87	27.58	0.90
BVMT-R z-score	0.15	**<0.01**	0.05	0.42	27.58	0.90
BDI-II	0.86	**0.04**	0.74	0.99	8.21	0.72
Disease duration	1.10	**0.03**	1.01	1.20	9.56	0.72
SDMT	0.91	**0.02**	0.84	0.98	11.23	0.76
SDMT z-score	0.45	**0.02**	0.23	0.90	10.07	0.76
LONGITUDINAL						
BVMT-R	1.15	**<0.01**	1.05	1.26	15.49	0.81
BVMT-R z-score	3.28	**<0.01**	1.52	7.04	15.49	0.81
CVLT-II	1.07	**0.04**	1.00	1.13	8.17	0.74
CVLT-II z-score	1.89	**0.04**	1.02	3.51	8.17	0.74
Stroop	1.03	**0.04**	1.00	1.06	8.21	0.72
Stroop z-score	1.88	**0.04**	1.03	3.44	8.21	0.72

e) Independent predictors of BVMT-R retained Worsening (n=22)						
Predictors	Odds Ratio	p-value	95% Confidence Interval		LR	AUROC
Age	0.93	0.12	0.86	1.02	4.36	0.66
Gender	0.52	0.33	0.14	1.91		
HADS anxiety	0.96	0.63	0.82	1.12		
BASELINE						
BVMT-R retained	0.90	**<0.01**	0.84	0.96	39.85	0.91
BVMT-R retained z-score	0.01	**<0.01**	<0.01	0.18	39.85	0.91
LONGITUDINAL						
Nil						

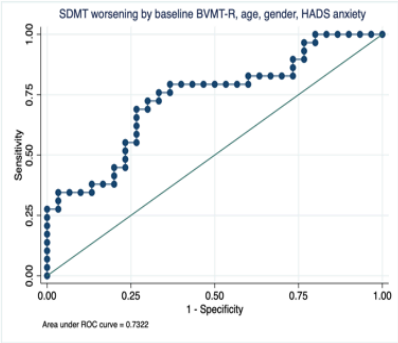
f) Independent predictors of CVLT-II Worsening (n=30)						
Predictors	Odds Ratio	p-value	95% Confidence Interval		LR	AUROC
Age	1.02	0.67	0.94	1.11	2.73	0.59
Gender	0.33	0.11	0.09	1.30		
HADS anxiety	1.01	0.85	0.88	1.17		
BASELINE						
CVLT-II	0.84	**<0.01**	0.76	0.92	24.76	0.84
CVLT-II z-score	0.15	**<0.01**	0.05	0.43	24.76	0.84
Cognitive Leisure	0.84	**0.02**	0.73	0.97	9.36	0.72
CVLT-II delayed	0.80	**0.01**	0.67	0.95	10.26	0.74
CVLT-delayed z-score	0.59	**0.01**	0.39	0.90	10.26	0.74
LONGITUDINAL						
CVLT-II	1.14	**<0.01**	1.05	1.23	19.79	0.81
CVLT-II z-score	3.64	**<0.01**	1.70	7.78	19.79	0.81
CVLT-delayed	1.26	**0.01**	1.07	1.48	12.05	0.74
CVLT-II delayed z-score	1.88	**0.01**	1.19	2.95	12.05	0.74
PASAT3	0.94	**0.02**	0.89	0.99	8.02	0.69
PASAT3 z-score	0.47	**0.02**	0.25	0.89	8.02	0.69

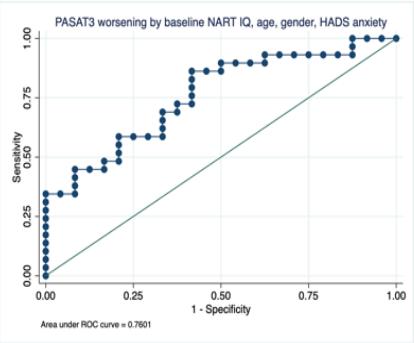
g) Independent predictors of CVLT-II delayed Worsening (n=21)						
Predictors	Odds Ratio	p-value	95% Confidence Interval		LR	AUROC
Age	1.04	0.33	0.96	1.14	2.33	0.62
Gender	1.22	0.78	0.31	4.79		
HADS anxiety	1.10	0.21	0.95	1.28		
BASELINE						
CVLT-delayed	0.75	**0.01**	0.62	0.92	13.10	0.79
CVLT-II delayed z-score	0.52	**0.01**	0.33	0.82	13.10	0.79
CVLT-II	0.88	**<0.01**	0.82	0.96	15.59	0.79
CVLT-II z-score	0.27	**<0.01**	0.12	0.63	15.59	0.79
LONGITUDINAL						
CVLT-delayed	1.51	**<0.01**	1.19	1.92	21.11	0.83
CVLT-II delayed z-score	3.13	**<0.01**	1.61	6.06	21.11	0.83
MSVQ	0.84	**0.03**	0.72	0.98	8.80	0.77
BVMT-R	1.09	**0.03**	1.01	1.17	7.68	0.71
BVMT-R z-score	2.06	**0.03**	1.07	3.95	7.68	0.71
CVLT-II	1.09	**0.01**	1.02	1.16	11.52	0.75
CVLT-II z-score	2.34	**0.01**	1.27	4.30	11.52	0.75

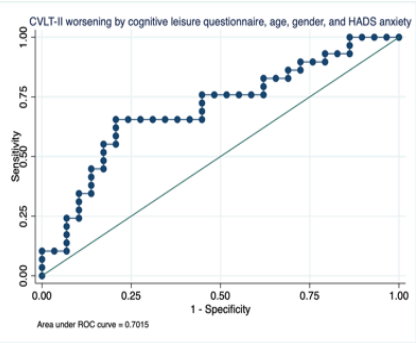
h) Independent predictors of Hayling Worsening (n=23)						
Predictors	Odds Ratio	p-value	95% Confidence Interval		LR	AUROC
Age	1.05	0.29	0.96	1.14	3.32	0.64
Gender	0.39	0.17	0.10	1.49		
HADS anxiety	0.95	0.49	0.81	1.11		
BASELINE						
Hayling	0.39	**<0.01**	0.20	0.76	16.95	0.82
Hayling z-score	0.38	**<0.01**	0.19	0.75	16.95	0.82
LONGITUDINAL						
Hayling	3.28	**<0.01**	1.62	6.64	28.28	0.87
Hayling z-score	3.11	**<0.01**	1.59	6.09	28.28	0.87

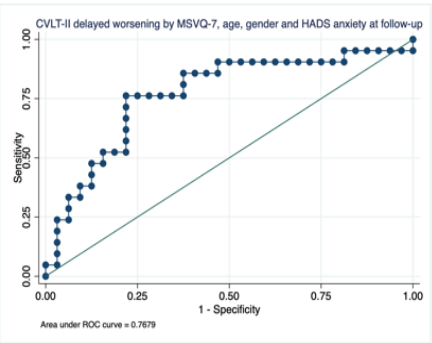
i) Independent predictors of Verbal Fluency Worsening (n=54)						
Predictors	Odds Ratio	p-value	95% Confidence Interval		LR	AUROC
Age	0.91	0.38	0.73	1.13	1.68	0.71
Gender	2.26	0.53	0.17	29.45		
HADS anxiety	1.12	0.59	0.74	1.69		
BASELINE						
NART IQ	0.69	0.06	0.46	1.01	7.67	0.89
LONGITUDINAL						
SDMT	1.15	0.06	1.00	1.34	6.45	0.87
SDMT z-score	3.31	0.06	0.96	11.35	6.45	0.87

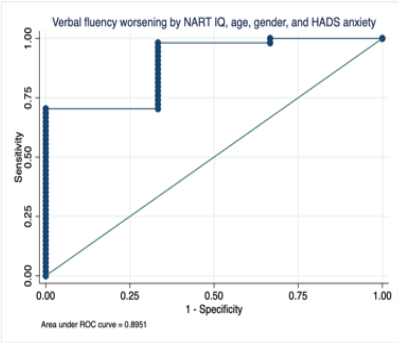
j) Independent predictors of Stroop Worsening (n=25)						
Predictors	Odds Ratio	p-value	95% Confidence Interval		LR	AUROC
Age	1.01	0.80	0.93	1.10	0.29	0.53
Gender	0.90	0.88	0.25	3.24		
HADS anxiety	1.04	0.62	0.90	1.20		
BASELINE						
Stroop	0.92	<0.01	0.87	0.96	23.14	0.84
Stroop z-score	0.18	<0.01	0.07	0.46	23.14	0.84
Employment status	2.46	0.04	1.04	5..84	4.82	0.70
PASAT3	0.95	0.05	0.90	1.00	4.32	0.65
PASAT3 z-score	0.52	0.05	0.27	1.01	4.32	0.65
Verbal fluency	0.84	0.02	0.73	0.97	7.31	0.67
Verbal fluency z-score	0.44	0.02	0.22	0.86	7.31	0.67
LONGITUDINAL						
Stroop	0.18	<0.01	0.07	0.46	23.14	0.84
Stroop z-score	4.40	<0.01	1.85	10.44	19.06	0.80
MSVQ	0.87	0.05	0.75	1.00	4.98	0.67
BVMT-R	1.13	<0.01	1.04	1.22	11.32	0.75
BVMT-R z-score	2.79	<0.01	1.42	5.47	11.32	0.75
CVLT-II	1.05	0.07	1.00	1.10	3.76	0.65
CVLT-II z-score	1.60	0.07	0.96	2.66	3.76	0.65
CVLT-delayed	1.22	0.01	1.04	1.43	7.74	0.71
CVLT-delayed z-score	1.74	0.01	1.12	2.69	7.74	0.71
SDMT	1.10	0.01	1.03	1.17	9.83	0.74
SDMT z-score	2.15	0.01	1.24	3.72	9.83	0.74

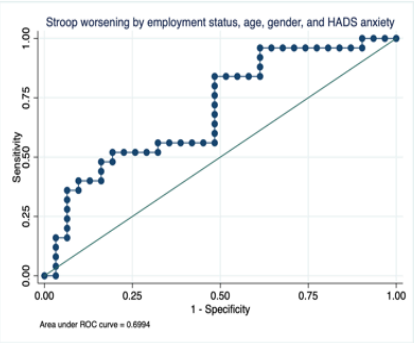

SDMT worsening by baseline BVMT-R, age, gender, HADS anxiety
Sensitivity
1 - Specificity
Area under ROC curve = 0.7322

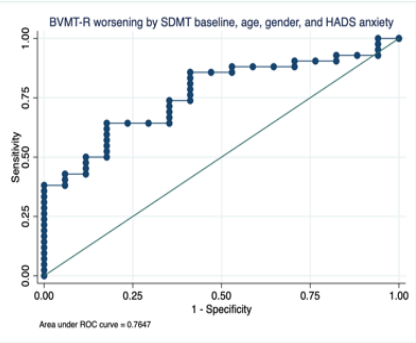

PASAT3 worsening by baseline NART IQ, age, gender, HADS anxiety
Sensitivity
1 - Specificity
Area under ROC curve = 0.7601


CVLT-II worsening by cognitive leisure questionnaire, age, gender, and HADS anxiety
Sensitivity
1 - Specificity
Area under ROC curve = 0.7015


CVLT-II delayed worsening by MSVQ-7, age, gender and HADS anxiety at follow-up
Sensitivity
1 - Specificity
Area under ROC curve = 0.7679


Verbal fluency worsening by NART IQ, age, gender, and HADS anxiety
Sensitivity
1 - Specificity
Area under ROC curve = 0.8951


Stroop worsening by employment status, age, gender, and HADS anxiety
Sensitivity
1 - Specificity
Area under ROC curve = 0.6994


BVMT-R worsening by SDMT baseline, age, gender, and HADS anxiety
Sensitivity
1 - Specificity
Area under ROC curve = 0.7647

4.4.4 Discussion

There are very few longitudinal studies of cognition in SPMS (Connick *et al.*, 2013; Chan *et al.*, 2017; Sumowski *et al.*, 2018). It is important to look at individual cognitive outcome measure scores as there can be a change in baseline without meeting the criteria for impairment and this may be missed by the grouped qualitative cognitive classifications (Sumowski *et al.*, 2018), see **figure 2.1**. I also aimed to determine predictors of changes in the cognitive profile of SPMS between timepoints.

The main findings of this section are, firstly, that in SPMS there is deterioration in information processing speed, working memory, and executive function over time. Secondly, supporting the results of **section 4.3**, the SDMT appears to be a purer test of cognitive decline in SPMS in that it is only associated with cognitive outcome measures. This suggests that raw SDMT change is a robust measure of cognitive differences in SPMS over time, and adds evidence of potential use as a trial outcome measure. Finally, baseline enrichment with higher IQ, employment status, and leisure activities protected against worsening of the executive and verbal memory domains in this SPMS cohort. Social engagement in a study may account for improvements in quality of life measures at follow-up.

Over time, there was significant improvement in the SPMS group in subjective outcomes of physical and neuropsychological function, i.e. PROMS. This may be due to social and psychological benefits of trial participation. Pain measures were worse, but overall quality of life was significantly improved (**table 4.12**) in the SPMS arm. This could be related to visuospatial memory being most intact in this cohort, and therefore less likelihood of deterioration in quality of life measures as per the observations of Ruet et al (Ruet *et al.*, 2013*b*). In the MS-STAT1 study improvement in quality of life measures was stated to be in relation to the cholesterol lowering vascular effects of simvastatin (Chan *et al.*, 2017), although perhaps given the similarities of findings in this cohort, this may be a sequelae of clinical trial participation, with associated impacts on social engagement (Lincoln *et al.*, 2015; Ontaneda *et al.*, 2015). Only cognitive outcome

measures were shown to associate with quality of life measures despite high levels of motor disability in another longitudinal follow-up study (Højsgaard Chow *et al.*, 2018).

Cognitive deterioration in the domains was not significant in the SPMS group, but as with other studies represented overall cognitive decline in tests of information processing speed, attention, working memory, and executive domain (Amato *et al.*, 2006*b*; Bergendal *et al.*, 2007). In this study delayed memory deteriorated by 30% (CVLT-II delayed), once standardised, and information processing speed (SDMT) by 10% (**figure 4.14**). Deloire et al had greater rates of deterioration; from baseline 50% deteriorated on memory and 22.7% on processing speed function, however theirs' was a 7 year duration study of a mixed MS phenotype (Deloire *et al.*, 2011).

Creating logistic regression models of worsening on the individual cognitive tasks, allowed a review of factors that could be targeted against cognitive decline (**table 4.14**). There were intra-test associations of worsening of raw cognitive scores in mainly the information processing speed, working memory, and executive domains, particularly the SDMT, Stroop, and CVLT-II. Decline on the SDMT was only significantly associated with cognitive outcome measures. This suggests a sensitive and specific representation of intrinsic cognitive function and reserve without effects of external factors as it was a pure cognitive representation (Smith, 1982; Parmenter *et al.*, 2007; Sonder *et al.*, 2014; Benedict *et al.*, 2015; Sumowski *et al.*, 2018). This indicates why the SDMT is a suitable sentinel measure of cognitive decline over time in newly diagnosed patients (Hankomaki *et al.*, 2014). As with worsening on the SDMT (**table 4.14 a)**), deterioration of the SDMT by 4 points (**table 4.14 b)**) was significantly associated only with cognitive outcome measures. An expert consensus panel has suggested that a responder definition of change of 4 points or 10% in the baseline SDMT score is a meaningful marker of MS cognitive deterioration (Benedict *et al.*, 2017). The results from this section support the role of the SDMT as a sensitive and specific representation of intrinsic cognitive function and reserve without effects of external factors (Smith, 1982; Parmenter *et al.*, 2007; Sonder *et al.*, 2014; Benedict *et al.*, 2015, 2017; Sumowski *et al.*, 2018). However, SDMT decline by 4 points does not associate with executive

function which was shown to have a significant impact on overall cognitive performance in SPMS in this study and others (Connick *et al.*, 2013). Therefore, I conclude that only raw worsening of the SDMT is interpretive of this.

The greatest proportion of subjects worsened on the verbal fluency (54 out of 59), which is telling as this has been shown to be a measure of executive function relating most to the prefrontal lobe (Delis *et al.*, 2001; Chapados and Petrides, 2013; Preston *et al.*, 2013). More intellectual enrichment, i.e. higher IQ, significantly reduced the chance of worsening by 31%. Education may also play a role in enrichment, but this did not affect the cognitive reserve for verbal fluency. Sumowski et al showed preservation of memory function in 25 SPMS subjects if the intellectual enrichment level was high enough, however this was cross-sectional (Sumowski *et al.*, 2012). Amato and colleagues showed that after 10 years of disease duration, verbal fluency and comprehension impairments occurred, supporting the need for an assessment of executive function in SPMS (Amato *et al.*, 2001, 2008*b*). As with baseline visit, employment status significantly protected against Stroop deterioration over time, with retirement leading to a 2.4 times increased risk. This may be related to factors improving brain reserve and employment, such as more physical exercise (Briken *et al.*, 2014) and mental activity (Sumowski *et al.*, 2012). CVLT-II worsening was lessened by more engagement in cognitive leisure activities at baseline, and this has been shown to improve cognitive reserve but not brain atrophy in relation to the SDMT and the SRT in a smaller cohort of MS subjects (n~30) (Sumowski *et al.*, 2010*b*).

PASAT3 was associated with more worsening if the IQ at baseline was conceptually different (i.e. those with better initial performance had more to lose), and if the HADS depression score was higher. Contradictorily, a higher BDI-II score was associated significantly with less deterioration of the BVMT-R task. These results indicate the heterogeneity and difficulty of evaluating a single cognitive outcome measure to represent a cognitive domain. The PASAT3 has overlap with information processing speed, attention, and visual working memory domains, therefore interpreting these associations is not always clear (Fischer *et al.*, 2014, Rocca *et al.*, 2015*a*; Sumowski *et al.*, 2018).

A limitation of this follow-up study is that the method of recruitment and small sample size did not allow for sufficiently powered treatment effects of MS-SMART to be evaluated. However, as shown, there were no differences in variables cross-sectionally. At an individual drug level, the placebo group showed significant changes only for the Stroop task over time, again suggesting that executive function and visual working memory may be particularly susceptible in SPMS (Huijbregts *et al.*, 2004; Achiron *et al.*, 2013; Chan *et al.*, 2017). Those with higher brain reserve may retain intact cognitive function, however, this cannot be reliably evaluated by this study's design (Grech *et al.*, 2019). There are some positive improvements in the BVMT-R visuospatial working memory task in the riluzole arm over time, however with z-score standardisation this was shown in fact to be worsening. Although the improvement is hypothetically plausible as Riluzole acts on beta-amyloid pathology via glutamate receptor modulation and gene expression in mouse models to improve memory in early Alzheimer's disease (Okamoto *et al.*, 2018). Amiloride appeared to improve long-term working memory over time, i.e. recall after a delay with the BVMT-R. Again hypothetically, this could be via action on brain atrophy in a small PPMS study (n=14) with improvements for cognitive reserve over time (Arun *et al.*, 2013). However, both riluzole and amiloride arms had very small sample sizes in this study, and were not shown to have an impact on brain atrophy rates in the recently reported MS-SMART trial (n=445) (Chataway *et al.*, 2020). These changes are therefore more likely due to multiple comparison testing and therefore not significant or relevant overall. However, they provide a rationale for future longitudinal adequately powered trials of neuroprotection and cognitive function in SPMS (Ontaneda *et al.*, 2015).

I highlight deterioration in SPMS in the information processing speed, working memory, and executive control cognitive domains. Additionally, increased cognitive reserve in terms of higher IQ, employment status, and leisure activities is protective against executive and verbal working memory decline. The SDMT shows further utility as a pure cognitive marker of longitudinal change in SPMS.

4.5 Definitions of cognitive impairment in SPMS: a cross-sectional and longitudinal analysis

4.5.1 Introduction

There are substantial differences in definitions of cognitive impairment in MS (Fischer *et al.*, 2014). Focus may be on impairment at the individual test level, however to evaluate the prevalence of overall cognitive impairment in MS the most common and robust method has been the critical number of abnormal parameters on 18-30% of individual tests (**section 2.6**) (Fischer *et al.*, 2014). This definition also adds to the variability in prevalence of cognitive impairment in terms of the threshold required and the number of tests or domains impaired. These definitions may critically impact upon associations between 'cognitive impairment' and other outcome measures such as vocational status or motor ability (Ruet *et al.*, 2013*b*). There is also an urgency to better understand cognitive reserve or capacity and the impact of this factor on cognitive status in MS (Sumowski *et al.*, 2010*a*, 2012, 2018), as well as anxiety (Marrie *et al.*, 2019) and fatigue (Schwid *et al.*, 2002).

This section also reviews the factors associated with cognitive decline over time, i.e. from a preserved to an impaired state at follow-up. Lifestyle (Sumowski *et al.*, 2012) and simvastatin therapy (Chan *et al.*, 2017) have been considered protective against cognitive decline over time in SPMS, however other factors need evaluation.

The aims of this section are:

1) To define the cognitive status of the SPMS cohort by two critical number of abnormal parameters criteria at baseline visit (**section 3.5.3.1**).
2) To determine cross-sectional associations with cognitive impairment using both criteria at baseline visit (**section 3.5.3.2**).
3) To investigate the phenotype and associations of the development of cognitive impairment at follow-up visit from a preserved state (**section 3.5.3.3**).

4.5.2 Methods

4.5.2.1 Neurological and neuropsychological test protocol

Subjects were recruited as per **section 4.2**, and undertook the neuropsychological and clinical battery described further in **table 4.2** in **section 4.2.7.**

To ascertain impairment at the individual and global test level, SPMS subjects were sub-divided into cognitively impaired (CI) and cognitively preserved (CP) based on the two main critical number of abnormal parameters classifications criteria for determining cognitive impairment in MS.

These are:

1) **Conservative criteria**; cognitive impairment is a z-score of -1.96SD (i.e. p=0.05, two-tailed) or less on two or more individual tests from at least two cognitive domains compared to healthy control data (Strauss *et al.*, 2006; Patti *et al.*, 2009; Fischer *et al.*, 2014).

2) **Lenient criteria**; cognitive impairment is a z-score of -1.5SD (i.e. p=0.05, one tailed) or less on two or more individual tests from at least two cognitive domains compared to healthy control data (Strauss *et al.*, 2006; Patti *et al.*, 2009; Fischer *et al.*, 2014).

4.5.2.2 Statistical analysis

Analyses were performed according to the following group divisions for the SPMS group;

1. ***cognitively impaired*** z-score ≤-1.96SD versus cognitively preserved ≤-1.96SD on ≥2 domains at baseline and follow-up timepoints.
2. ***cognitively impaired*** z-score ≤-1.5SD versus cognitively preserved ≤-1.5SD SPMS on ≥2 domains at baseline and follow-up timepoints.

Descriptive evaluation and differences between variables

Variables were described, and group differences ascertained according to type using the methods described in **sections 4.3.2.2.**

Correlations of impairment of cognitive outcomes

Tetrachoric correlations were used to determine binary-binary variable correlations; i.e. impairment on individual tests and overall cognitive impairment using the 2 criteria.

Variance analysis

I used variance to review the proportional input of each cognitive outcome test score on overall cognitive impairment using the 2 definitions. Results are presented as percentage bar charts.

Multivariable logistic regression models

I developed multivariable logistic regression models of the binary outcome; cognitive impairment and the development of cognitive impairment at follow-up, were developed. The independent predictors tested were as for the multivariate linear regression models (**table 4.3**) i.e. divided into the following categories; physical disability predictors, non-physical predictors, and demographic predictors. Covariates in the model were those which were most frequently significant in the healthy control versus SPMS groups, i.e. HADS anxiety, age and gender. Models were performed at baseline and longitudinally for the following dependent variables;

1. Cognitive impairment at baseline z-score ≤-1.96SD on ≥2 domains.
2. Cognitive impairment at baseline z-score ≤-1.5SD on ≥2 domains.

3. Cognitive impairment at follow-up from cognitively preserved at baseline z-score ≤-1.96SD on ≥2 domains.
4. Cognitive impairment at follow-up from cognitively preserved at baseline z-score ≤-1.5SD on ≥2 domains.

Longitudinal models of change at the follow-up timepoint included variables at both visits. Stata reports McFadden's pseudo R-squared as the relative fit of two models, but not the absolute fit of the models. Therefore, likelihood ratio chi-squared were gathered per model to show the overall model fit. To review the sensitivity; the probability of the model predicting a positive outcome for a given observation, and the specificity; the probability that the model predicts a negative outcome for an observation, AUROC was calculated to define the ability of the model to distinguish between positive and negative outcomes. Tables of tested variables have been produced and include; OR, p-value, 95% confidence intervals, LR, and AUROC.

4.5.3 Results

4.5.3.1 Cognitive impairment in SPMS by criteria; cross-sectional analysis

Evaluation of between group differences: Critical number of abnormal parameters z-score ≤-1.96SD on ≥2 domains.

There were no significant differences in demographic variable for the cognitively preserved (49) (mean age 54.7±7.1 years, M:F 9:40, years of education 15.6±3 years, disease duration from first symptom 22.4±9.5 years, EDSS median=6.0 range 4.0-6.5) and impaired (21) (mean age 55.3±6.7 years, M:F 8:13, years of education 15.1±2.3 years, disease duration from first symptom 21.3±8.3 years, EDSS median=6.0 range 4.0-6.5) SPMS groups (**table 4.15**). As expected, there was a significantly higher IQ in the preserved group (115.2±8.3 versus 111.8±7.9). Additionally, there were significantly worse physical, neuropsychological and fatigue measures in SPMS groups with cognitive impairment versus without (MSFC-0.32±0.56 versus -0.01±1.12 p=0.002, MSIS 29v2 73.1±16.43 versus 63.35±15.27 p=0.03, MSIS 29v2 physical score 53.6±11.1 versus 46.3±11.4 p=0.02, MSNQ 28.19±13.9 versus 19.6±11.16 p=0.02, VAF 1.68±1.43 versus 0.76±1.31 p=0.03, NFI 19.7±4.7 versus 16.6±3.9 p=0.01). These significant differences are highlighted by boxplots which also show a greater range of spread of MSIS 29v2 results in the preserved group (**figure 4.12**). Cognitively impaired subjects were most likely to be retired, n=18, compared with part-time employment prevailing in the preserved group (p≤0.01) (**figure 4.13**).

Apart from the retained portion of the BVMT-R there are significantly worse results in the working memory, information processing speed, and executive function tasks in those with cognitive impairment compared to those without. These are all significant at p≤0.01 (**table 4.16**). Boxplots in **figure 4.14** suggest that this is due to outliers in the preserved group, and a higher median and interquartile range for the BVMT-R retained in the impaired group. There were no significant differences in drug arm distribution between the groups as shown by the boxplots in **figure 4.15**. However, cognitively impaired subjects appear to have greater variations in scores.

Table 4.15. Demographic and clinical characteristics of SPMS groups with and without cognitive impairment (z-score of ≤-1.96SD on ≥2 domains) at baseline.

na=not applicable. Significant results are in bold font if p≤0.05.

BASELINE	Cognitively Preserved -1.96SD						Cognitively Impaired -1.96SD						
	n	Mean	SD	Median	Min	Max	n	Mean	SD	Median	Min	Max	p
Age (years)	49	54.68	7.1	56.13	39.11	65.74	21	55.25	6.71	55.16	37.83	65.2	0.76
Gender	49			M:F 9:40			21			M:F 8:13			0.08
Years of education	49	15.59	3.03	16	11	28	21	15.14	2.29	16	11	19	0.63
Drug arm	49	Drug:(n); fluoxetine:12; riluzole:12; amiloride:12; placebo:13					21	Drug:(n); fluoxetine:5; riluzole:7; amiloride:2; placebo:7					0.51
NART IQ	49	115.18	8.33	116	77	126	21	111.81	7.89	114	88	123	**0.04**
HADS depression	49	4.59	2.78	4	0	12	21	6.29	3.8	6	0	14	0.09
HADS anxiety	49	5.04	3.47	4	0	14	21	7.14	5.13	6	0	18	0.16
BDI-II	49	6.73	4.75	6	0	18	21	7.95	5.78	7	0	17	0.42
Disease duration from first symptom	49	22.35	9.45	20	6	46	21	21.33	8.29	21	6	36	0.99
Duration of progression	49	7.63	4.88	7	2	21	21	8.48	5.64	8	2	22	0.61

BASELINE	Cognitively Preserved -1.96SD						Cognitively Impaired -1.96SD						
	n	Mean	SD	Median	Min	Max	n	Mean	SD	Median	Min	Max	p
EDSS	49	5.7	0.82	6	4	6.5	21	5.76	0.83	6	4	6.5	0.75
9HPT (sec)	49	35.82	18.48	30.45	20.08	108.73	21	35.75	12.64	32	20.48	68.6	0.39
T25FW (sec)	48	18.95	22.21	12.15	4.5	138.8	21	18.63	15.15	13.4	5.35	52.9	0.83
MSFC score	49	-0.01	1.12	0.16	-4.95	1	21	-0.32	0.56	-0.22	-1.31	0.55	**≤0.01**
MSIS 29v2 physical	49	46.29	11.41	48	23	75	21	53.62	11.14	56	33	75	**0.02**
MSIS 29v2	49	63.35	15.27	62	32	104	21	73.1	16.43	72	45	106	**0.03**
MSWS v2	49	39.88	11.16	42	14	54	21	43.05	8.83	43	26	54	0.30
MSVQ-7	49	22.65	33.48	12.65	4.5	180	21	18.63	15.15	13.4	5.35	52.9	0.94
MSNQ	49	19.59	11.16	18	0	44	21	28.19	13.88	24	2	55	**0.02**
MSIS 29v2 psychological	49	17.05	5.45	16	9	29	21	19.48	6.53	17	10	33	0.17
VAF	45	0.76	1.31	0.5	-2.6	4.9	18	1.68	1.43	1.7	-0.7	4.6	**0.03**
NFI	49	16.56	3.89	16.05	9.42	27.42	21	19.67	4.74	19.29	10.65	30	**0.01**
BPI	48	2.95	2.16	2.79	0	7.43	20	3.46	2.69	2.43	0	7.86	0.64
CLQ	48	19.35	4.61	19	11	33	21	20.67	4.76	21	13	31	0.27
Qualification by RQF	49	RQF:(n); Entry level:1; Level 1:13; Level 2:2; Level 3:9; Level 5:1; Level 6:18; Level 7:4; Level 8:1					21	RQF:(n); Entry level:0; Level 1:9; Level 2:1; Level 3:2; Level 5:1; Level 6:8; Level 7:0; Level 8:0					0.67
Occupation	49	Occupation Category:(n); 1:5; 2:21; 3:5; 4:11; 5:1; 6:5; 7:1					21	Occupation Category:(n); 1:3; 2:4; 3:1; 4:7; 5:4; 6:1; 7:1					0.10
Employment Status	49	Employment Status:(n); Full-time:10; Part-time:25; Retired:14					21	Employment Status:(n); Full-time:1; Part-time:2; Retired:18					**≤0.01**
EQ-5D	49	0.7	21	0.7	0.17	1	21	0.62	0.21	0.68	0.25	0.95	0.25
Health-state analogue	49	65	21.31	68	5	100	21	63.38	17.5	60	30	100	0.55
Ethnicity	49	Ethnicity:(n); Belgian:1; Cingalese:0; English:47; German:1; Italian:0; Urdu:0; Welsh:0					21	Ethnicity:(n); Belgian:0; Cingalese:1; English:20; German:0; Italian:0; Urdu:0; Welsh:0					0.36

Figure 4.12. Boxplots of significant characteristic differences between SPMS groups with and without cognitive impairment (z-score of ≤-1.96SD on ≥2 domains) at baseline.

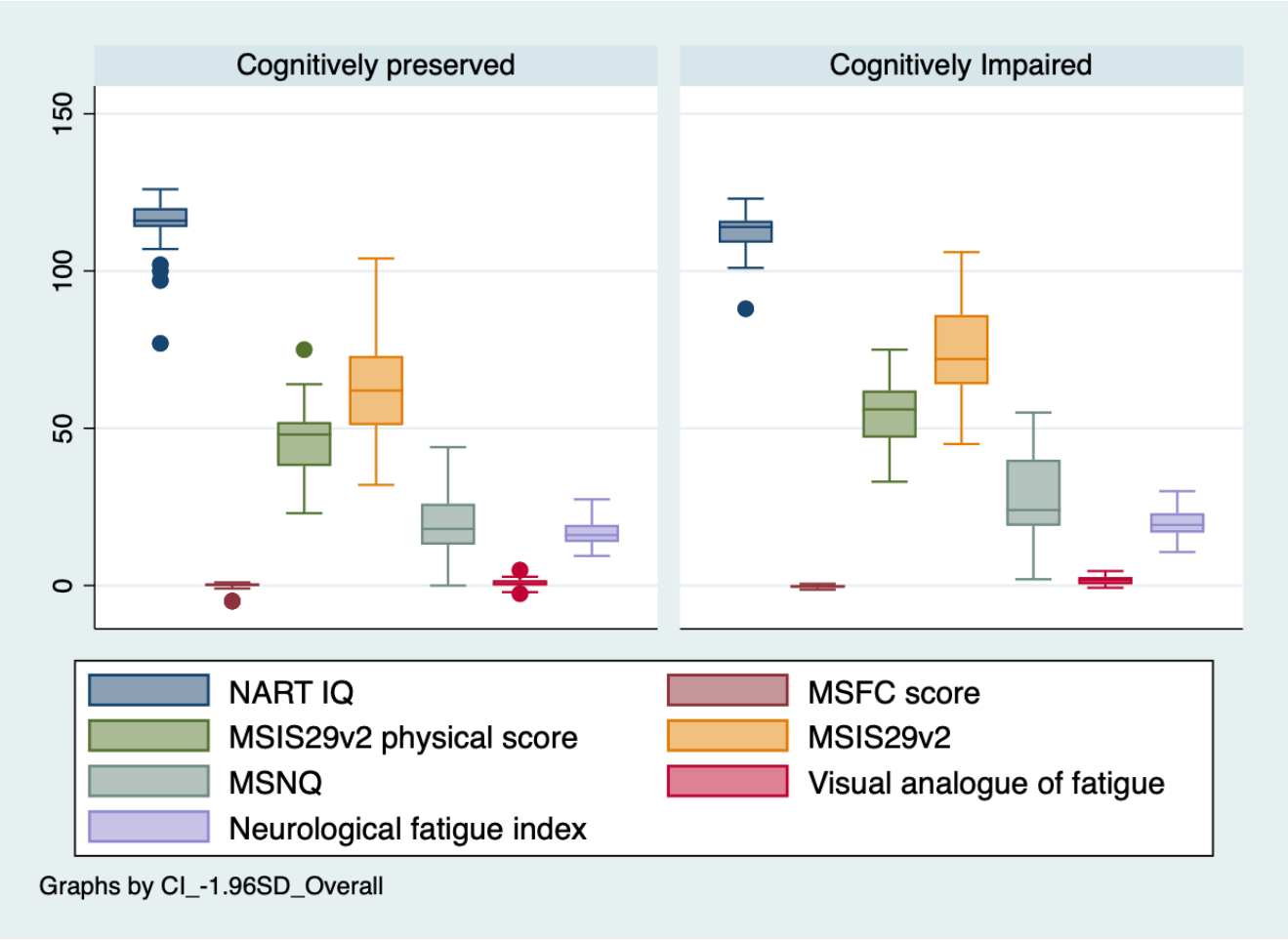
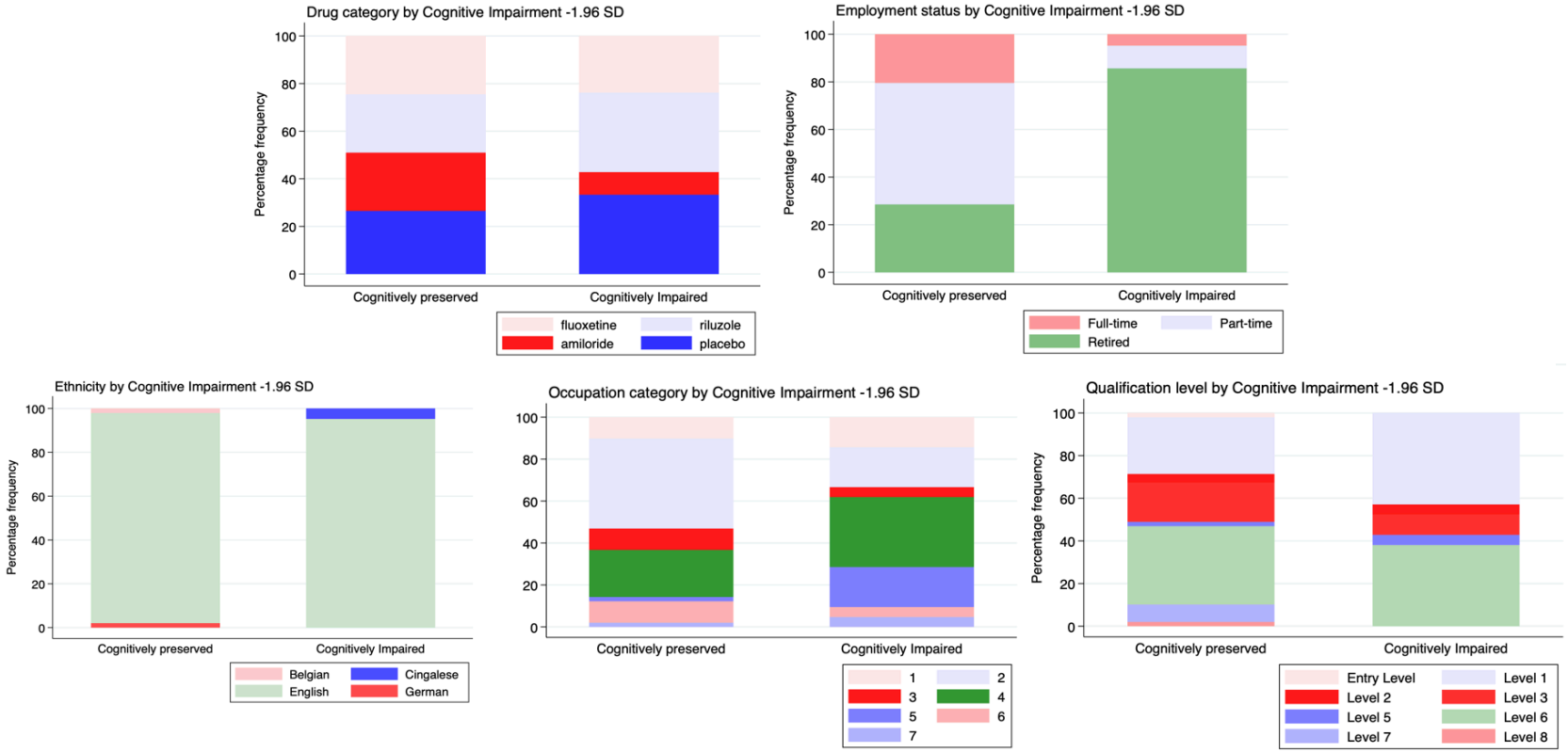


Figure 4.13. Summary of categorical variables at baseline in SPMS groups with and without cognitive impairment (≤-1.96SD on ≥2 domains). Stacked *clustered bar chart summaries of the percentage frequencies of the categorical variables; drug category, qualification level, occupation category, ethnicity, and employment status by Cognitive Impairment at ≤-1.96SD on ≥2 domains versus those without impairment. Pie charts show the differences in employment status in the cognitively impaired and preserved group.*



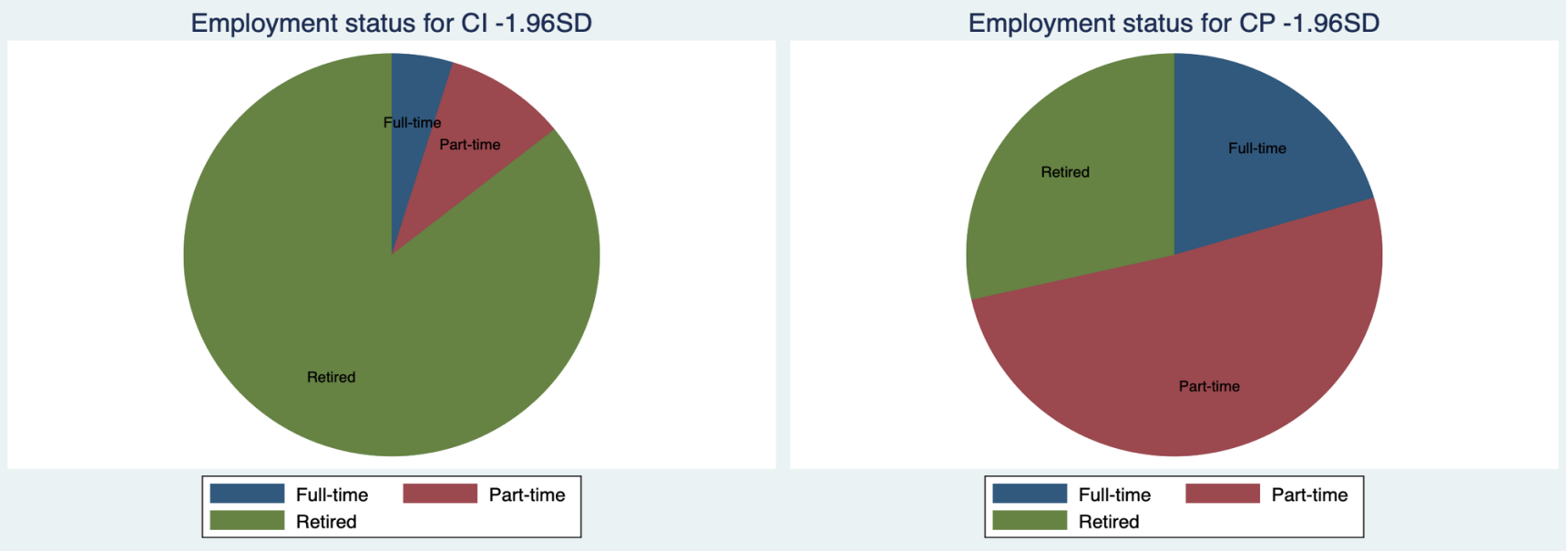

Employment status for CI -1.96SD
Full-time
Part-time
Retired
Full-time
Part-time
Retired
Employment status for CP -1.96SD
Full-time
Retired
Part-time
Full-time
Part-time
Retired

Table 4.16. Cognitive characteristics of SPMS groups with and without cognitive impairment (z-score of ≤-1.96SD on > 2 domains) at baseline. *na=not applicable. Significant results are in bold font if p≤0.05.*

BASELINE	Cognitively Preserved -1.96SD						Cognitively Impaired -1.96SD						
	n	Mean	SD	Median	Min	Max	n	Mean	SD	Median	Min	Max	p
BVMT-R trials 1-3	49	14.33	6.23	16.00	2.00	27.00	21	8.14	4.87	7.00	2.00	21.00	**≤0.01**
BVMT-R trials 1-3 z-score	49	-0.19	1.00	0.08	-2.17	1.85	21	-1.19	0.78	-1.37	-2.17	0.89	**≤0.01**
BVMT-R retained	49	94.89	28.49	100.00	0.00	175.00	21	81.49	44.63	100.00	0.00	150.00	0.37
BVMT-R retained z-score	49	-0.12	0.72	0.01	-2.52	1.91	21	-0.45	1.13	0.01	-2.52	1.28	0.37
CVLT-II trials 1-5	49	47.90	9.93	47.00	15.00	66.00	21	36.71	10.41	35.00	23.00	64.00	**≤0.01**
CVLT-II trials 1-5 z-score	49	-0.76	1.04	-0.76	-4.05	1.03	21	-1.73	0.98	-1.89	-3.02	0.84	**≤0.01**
CVLT-II delayed	49	10.88	3.12	11.00	1.00	16.00	21	6.52	4.15	7.00	0.00	15.00	**≤0.01**
CVLT-II delayed z-score	49	-0.90	1.36	-0.84	-5.20	1.34	21	-2.80	1.81	-2.59	-5.64	0.90	**≤0.01**
PASAT3	49	44.22	10.24	45.00	19.00	60.00	21	32.29	10.60	30.00	14.00	53.00	**≤0.01**
PASAT3 z-score	49	-0.07	0.85	0.00	-2.16	1.24	21	-1.06	0.88	-1.24	-2.57	0.66	**≤0.01**
SDMT	49	51.88	8.57	50.00	25.00	74.00	21	36.48	10.07	39.00	17.00	52.00	**≤0.01**
SDMT z-score	49	-0.86	0.92	-1.06	-3.74	1.50	21	-2.51	1.08	-2.24	-4.59	-0.85	**≤0.01**
Hayling test	49	6.69	1.31	6.00	4.00	10.00	21	5.14	1.46	6.00	2.00	7.00	**≤0.01**
Hayling test z-score	49	0.07	1.28	-0.61	-2.56	3.29	21	-1.45	1.42	-0.61	-4.51	0.37	**≤0.01**
Verbal fluency	48	15.92	4.51	14.67	8.00	27.67	20	11.58	3.74	11.00	4.67	18.67	**≤0.01**
Verbal fluency z-score	48	-0.39	0.94	-0.65	-2.05	2.07	20	-1.30	0.78	-1.42	-2.74	0.19	**≤0.01**
Stroop	48	78.23	17.32	79.50	39.00	112.00	20	46.90	19.25	49.50	12.00	85.00	**≤0.01**
Stroop z-score	48	-0.45	0.86	-0.39	-2.41	1.23	20	-2.02	0.96	-1.89	-3.76	-0.12	**≤0.01**

Figure 4.14 Boxplots of cognitive outcome differences between SPMS groups with and without cognitive impairment (z-score of ≤-1.96SD on ≥2 domains) at baseline.

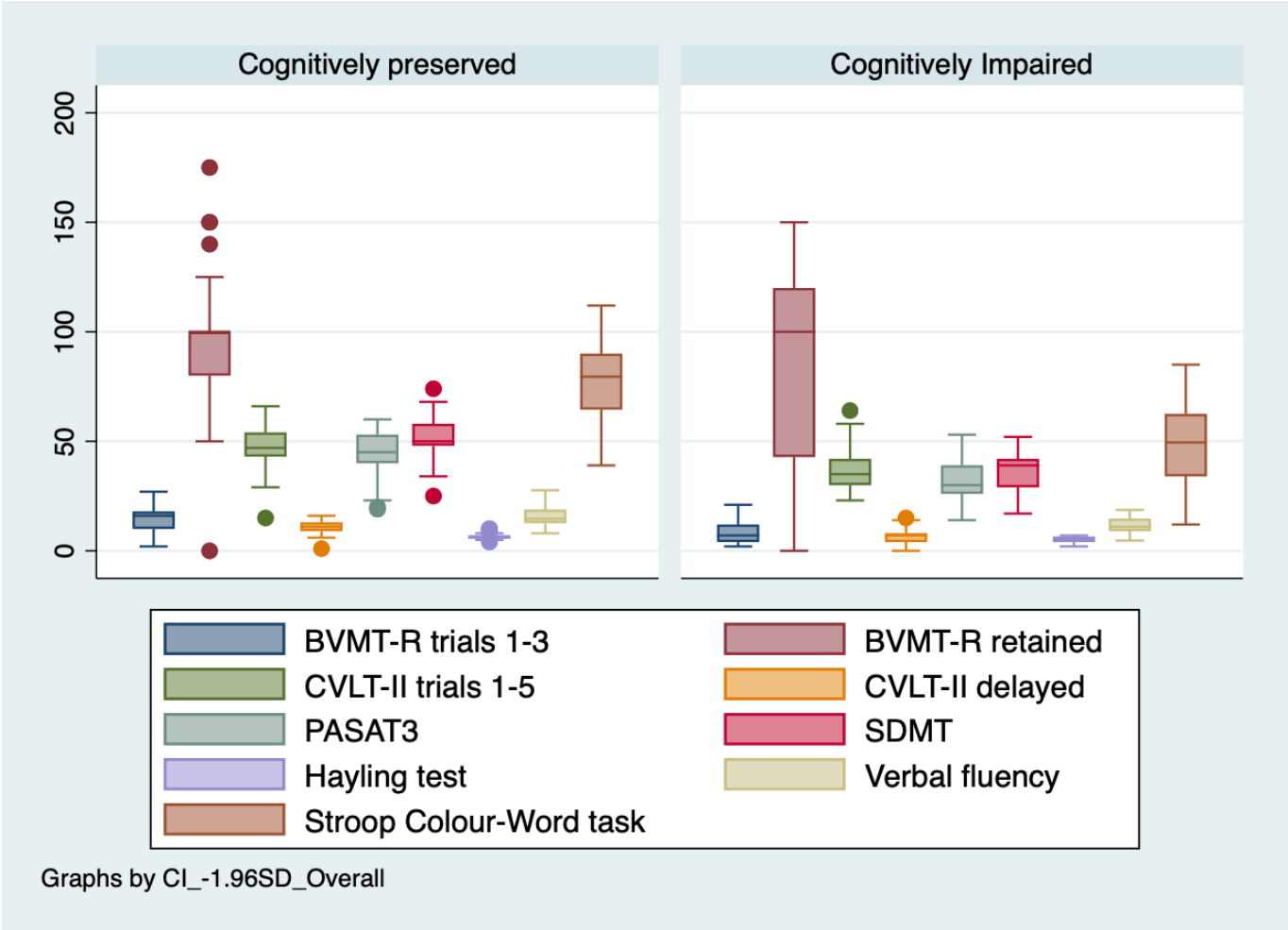
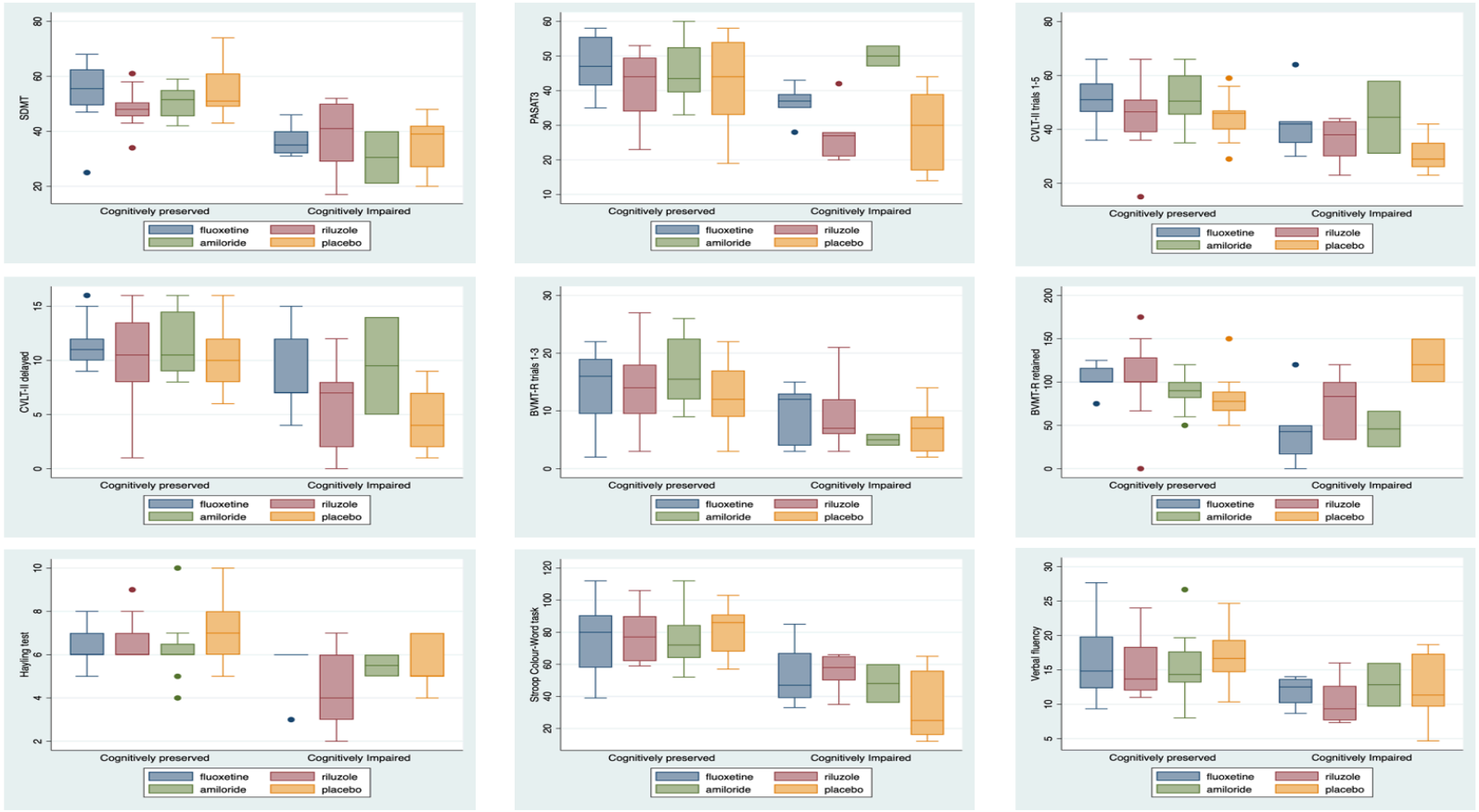


Figure 4.15 Boxplots of cognitive outcome differences between SPMS groups with and without cognitive impairment (z-score of ≤-1.96SD on ≥2 domains) by drug arm at baseline.



Evaluation of between group differences: Critical number of abnormal parameters z-score ≤-1.5SD on ≥2 domains.

As with the conservative criteria, there are similar demographic variables lenient cognitively preserved (n=37) (mean age 55.5±6.8 years, M:F 7:30, years of education 15.7±3.2 years, disease duration from first symptom 21.6±9.6 years, EDSS median=6.0 range 4.0-6.5) and those impaired (n=33) (mean age 54.1±7.1 years, M:F 10:23, years of education 15.2=1±2.4 years, disease duration from first symptom 22.6±8.51 years, EDSS median=6.0 range 4.0-6.5) **(table 4.17)**. There was also significantly higher NART IQ in the preserved cohort (117.11±8.3 versus 110.9±7.9). Physical and pain measures were significantly worse in the SPMS group with cognitive impairment than without respectively (MSFC-0.4±0.97 versus 0.16±0.95 p=≤0.01, MSIS 29v2 71.3±17.2 versus 61.8±13.9 p=0.019, MSIS 29v2 physical score 52.4±11.6 versus 44.98±10.82 p=0.01, BPI 3.84±2.56 versus 2.48±1.92 p=0.039). These significant differences are highlighted by boxplots which also indicate a greater range of spread of MSIS 29v2 results in the impaired versus preserved SPMS group and lower IQ outliers in both groups (**figure 4.16**). There were significant differences in occupation (p=0.006) and employment (p≤0.01) between the groups (**table 4.17** and **figure 4.17**). Those who were cognitively impaired were most likely to be retired, n=24, compared with most who were preserved being in part-time employment, n=21 (**figure 4.17**).

Similar to the ≤-1.96SD on ≥2 domains criteria, apart from the BVMT-R retained there are significantly worse results in the working memory, information processing speed, and executive function tasks in the cognitively impaired group. These are all significant at p≤0.001, excluding the Hayling task where p=0.006 (**table 4.18**). Boxplots in **figure 4.18** again indicate outliers in the preserved group, and higher median and interquartile range for the BVMT-R retained in the impaired group.

Table 4.17. Demographic and clinical characteristics of SPMS groups with and without cognitive impairment (z-score of ≤-1.5SD on ≥2 domains) at baseline.

na=not applicable. Significant results are in bold font if p≤0.05.

BASELINE	Cognitively Preserved -1.5SD						Cognitively Impaired -1.5SD						
	n	Mean	SD	Median	Min	Max	n	Mean	SD	Median	Min	Max	p
Age (years)	37	55.49	6.82	56.54	39.11	65.74	33	54.14	7.12	54.59	37.83	65.20	0.42
Gender	37			M:F 7:30			33			M:F 10:23			0.27
Years of education	37	15.73	3.17	16.00	11.00	28.00	33	15.15	2.39	16.00	11.00	21.00	0.48
Drug arm	37	Drug:(n); fluoxetine:8; riluzole:9; amiloride:10; placebo:10					33	Drug:(n); fluoxetine:9; riluzole:10; amiloride:4; placebo:10					0.48
NART IQ	37	117.11	5.98	117.00	97.00	126.00	33	110.88	9.32	112.00	77.00	123.00	**≤0.01**
HADS depression	37	4.49	2.36	4.00	0.00	9.00	33	5.79	3.84	6.00	0.00	14.00	0.19
HADS anxiety	37	4.89	3.20	5.00	0.00	12.00	33	6.55	4.85	6.00	0.00	18.00	0.27
BDI-II	37	7.03	4.00	7.00	0.00	15.00	33	7.18	6.12	7.00	0.00	18.00	0.79
Disease duration from first symptom	37	21.57	9.63	20.00	6.00	46.00	33	22.58	8.51	21.00	6.00	40.00	0.44
Duration of progression	37	7.70	5.00	6.00	2.00	21.00	33	8.09	5.26	7.00	2.00	22.00	0.78

BASELINE	Cognitively Preserved -1.5SD						Cognitively Impaired -1.5SD						
	n	Mean	SD	Median	Min	Max	n	Mean	SD	Median	Min	Max	p
EDSS	37	5.58	0.89	6	4	6.5	33	5.88	0.72	6	4	**6.5**	0.17
9HPT (sec)	37	37.18	20.37	31.78	20.08	108.73	33	34.24	11.85	30.58	20.48	68.6	0.83
T25FW (sec)	37	18.46	23	10.95	4.5	138.8	32	19.3	16.78	13.65	5.35	79.3	0.32
MSFC score	37	0.16	0.95	0.27	-4.95	1	33	-0.4	0.97	-0.2	-4.94	0.61	**≤0.01**
MSIS 29v2 physical	37	44.98	10.82	44	24	64	33	52.42	11.63	52	23	75	**0.01**
MSIS 29v2	37	61.81	13.94	60	33	87	33	71.27	17.17	70	32	106	**0.02**
MSWS v2	37	37.89	10.8	40	14	54	33	44.12	9.38	47	15	54	**≤0.01**
MSVQ-7	37	19.01	26.04	10.95	4.5	159.4	33	24.17	32.48	13.75	5.35	180	0.23
MSNQ	37	19.89	11.77	19	0	44	33	24.73	13.14	21	2	55	0.2
MSIS 29v2 psychological	37	16.83	4.87	16	9	25	33	18.85	6.71	17	9	33	0.28
VAF	34	0.8	1.3	0.5	-2.1	4.9	29	1.28	1.48	1.4	-2.6	4.6	0.11
NFI	37	16.62	3.93	16.05	9.42	27.42	33	18.48	4.68	18.45	9.42	30	0.08
BPI	37	2.48	1.92	2.14	0	7.43	31	3.84	2.56	3.57	0	7.86	**0.04**
CLQ	36	20.36	4.61	20	13	33	33	19.09	4.71	19	11	31	0.26
Qualification by RQF	37	RQF:(n); Entry level:0; Level 1:12; Level 2:1; Level 3:5; Level 5:1; Level 6:14; Level 7:3; Level 8:1					33	RQF:(n); Entry level:1; Level 1:10; Level 2:2; Level 3:6; Level 5:1; Level 6:12; Level 7:1; Level 8:0					0.83
Occupation	37	Occupation Category:(n); 1:1; 2:17; 3:5; 4:11; 5:0; 6:3; 7:0					33	Occupation Category:(n); 1:7; 2:8; 3:1; 4:7; 5:5; 6:3; 7:2					**≤0.01**
Employment Status	37	Employment Status:(n); Full-time:8; Part-time:21; Retired:8					33	Employment Status:(n); Full-time:3; Part-time:6; Retired:24					**≤0.01**
EQ-5D	37	0.7	0.17	0.74	0.17	1	33	0.65	0.19	0.68	0.25	0.95	0.25
Health-state analogue	37	67.32	19.24	70	5	100	33	61.36	20.93	60	20	100	0.14
Ethnicity	37	Ethnicity:(n); Belgian:1; Cingalese:0; English:35; German:1; Italian:0; Urdu:0; Welsh:0					33	Ethnicity:(n); Belgian:0; Cingalese:1; English:32; German:0; Italian:0; Urdu:0; Welsh:0					0.41

Figure 4.16. Boxplots of cognitive outcome differences between SPMS groups with and without cognitive impairment (z-score of ≤-1.5SD on ≥2 domains) at baseline.

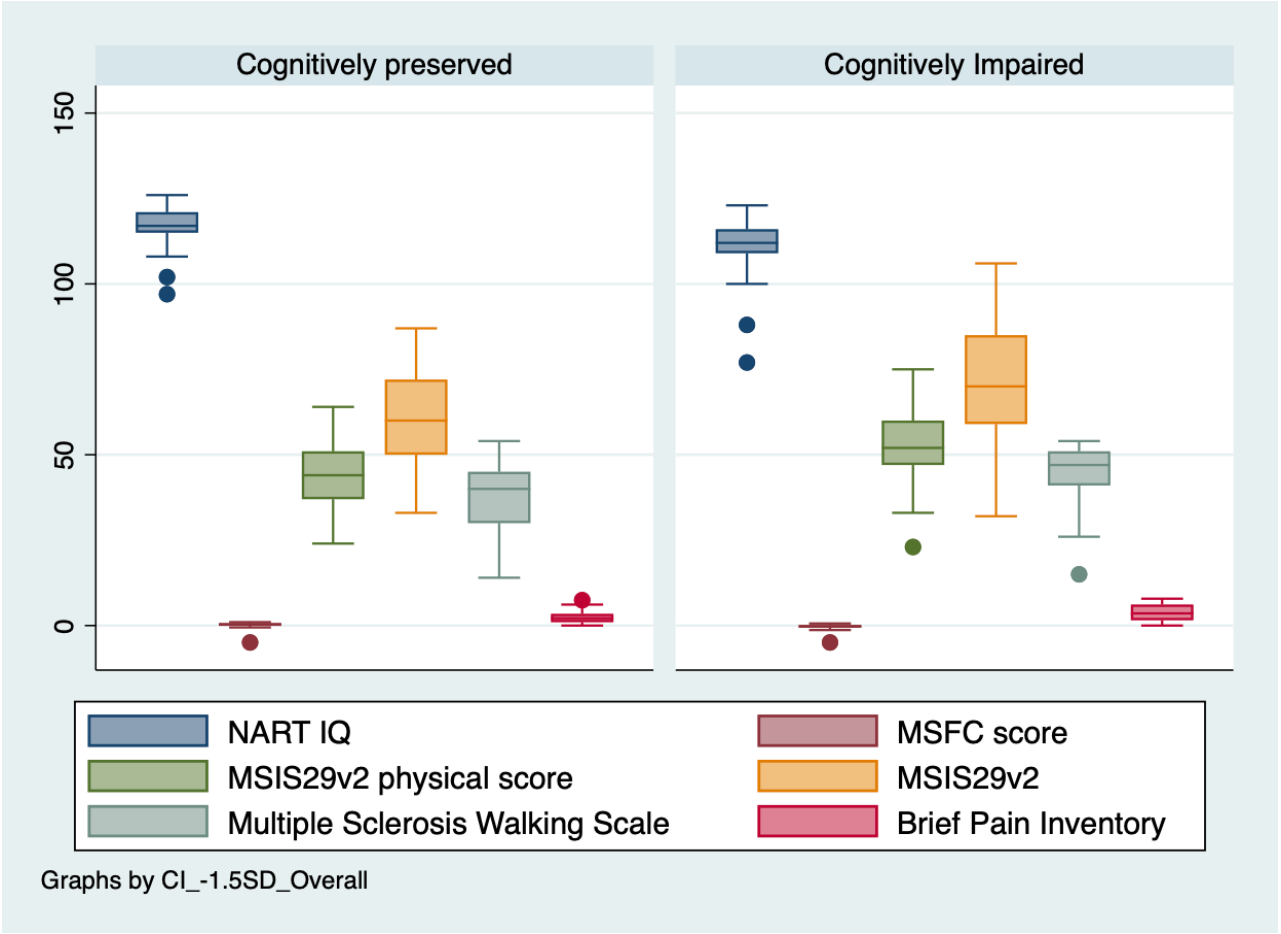
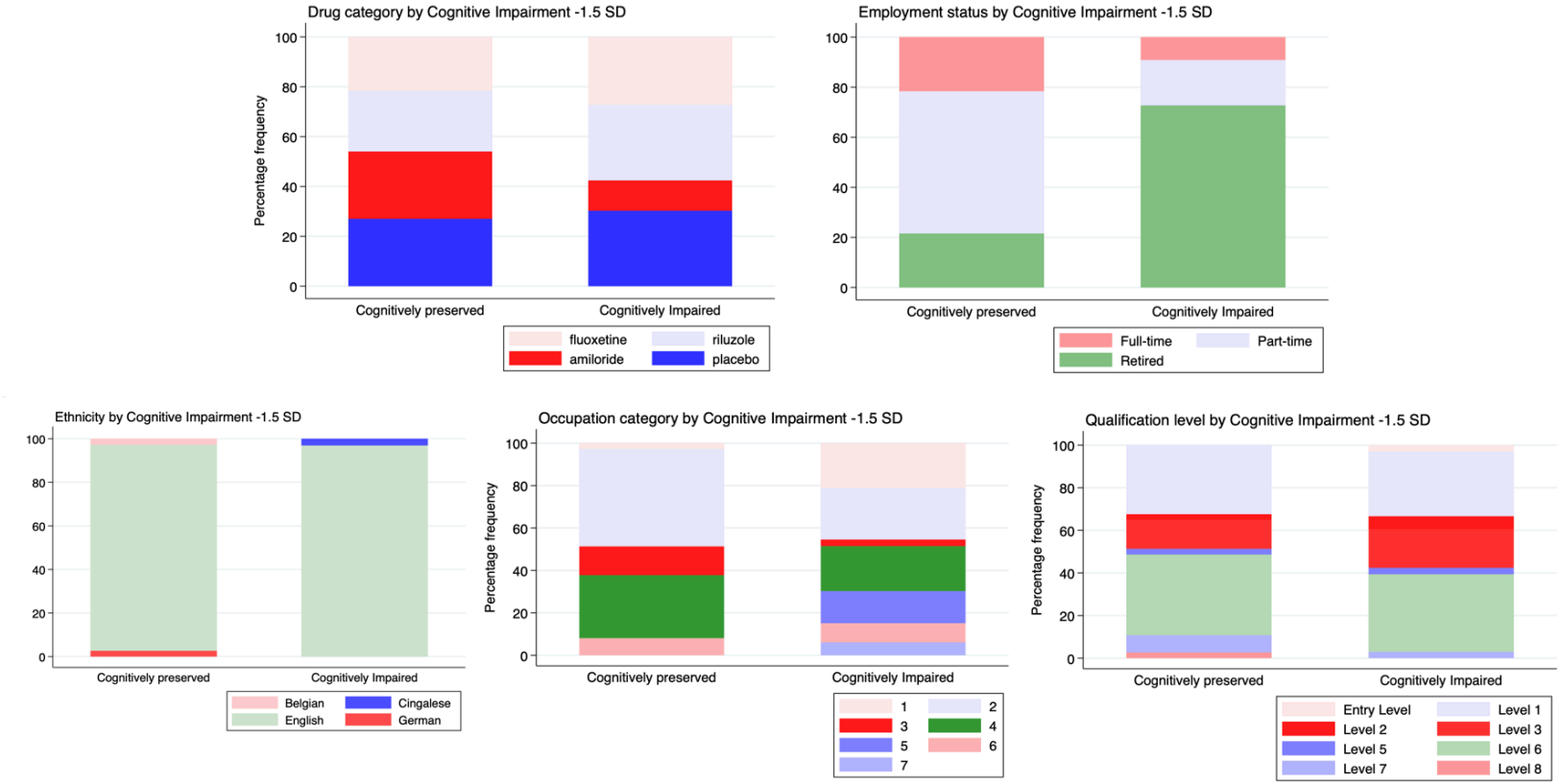


Figure 4.17. Summary of categorical variables at baseline in SPMS groups with and without cognitive impairment (≤-1.5SD on ≥2 domains). *Summaries of the percentage frequencies of the categorical variables; drug category, qualification level, occupation category, ethnicity, and employment status by Cognitive Impairment at ≤-1.5SD on ≥2 domains versus healthy controls. Pie charts show the differences in occupation in the cognitively impaired and preserved group.*



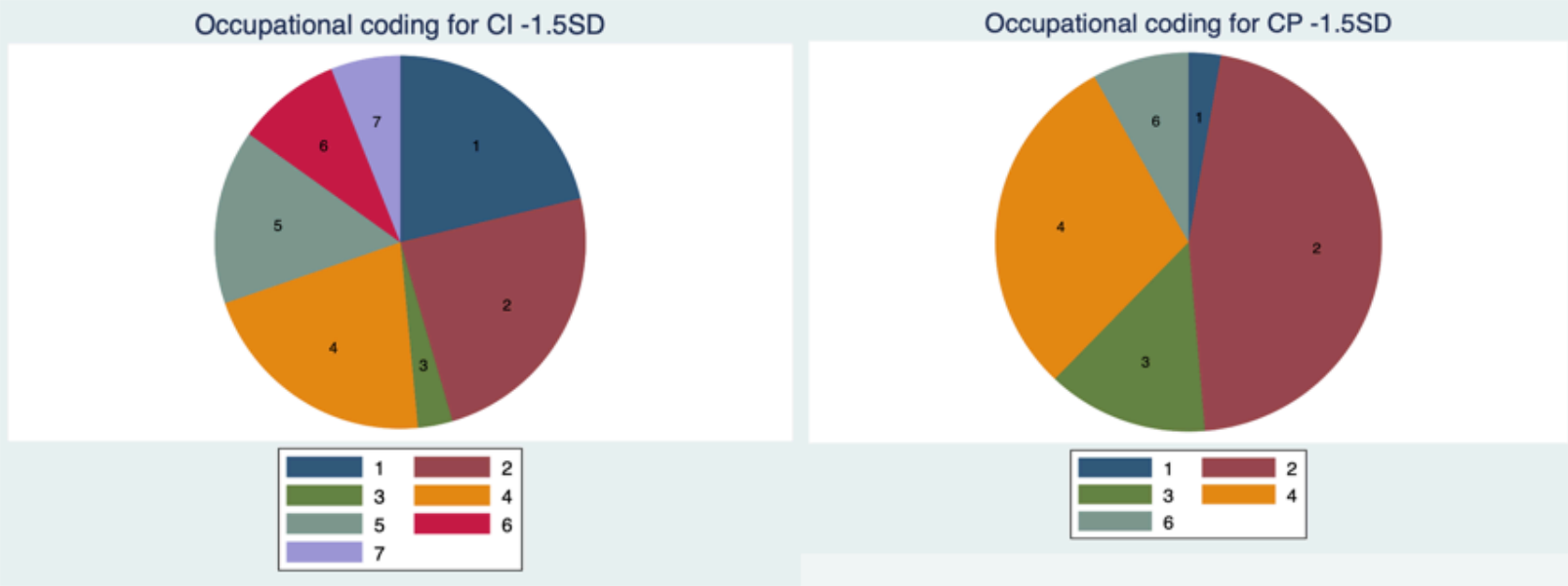
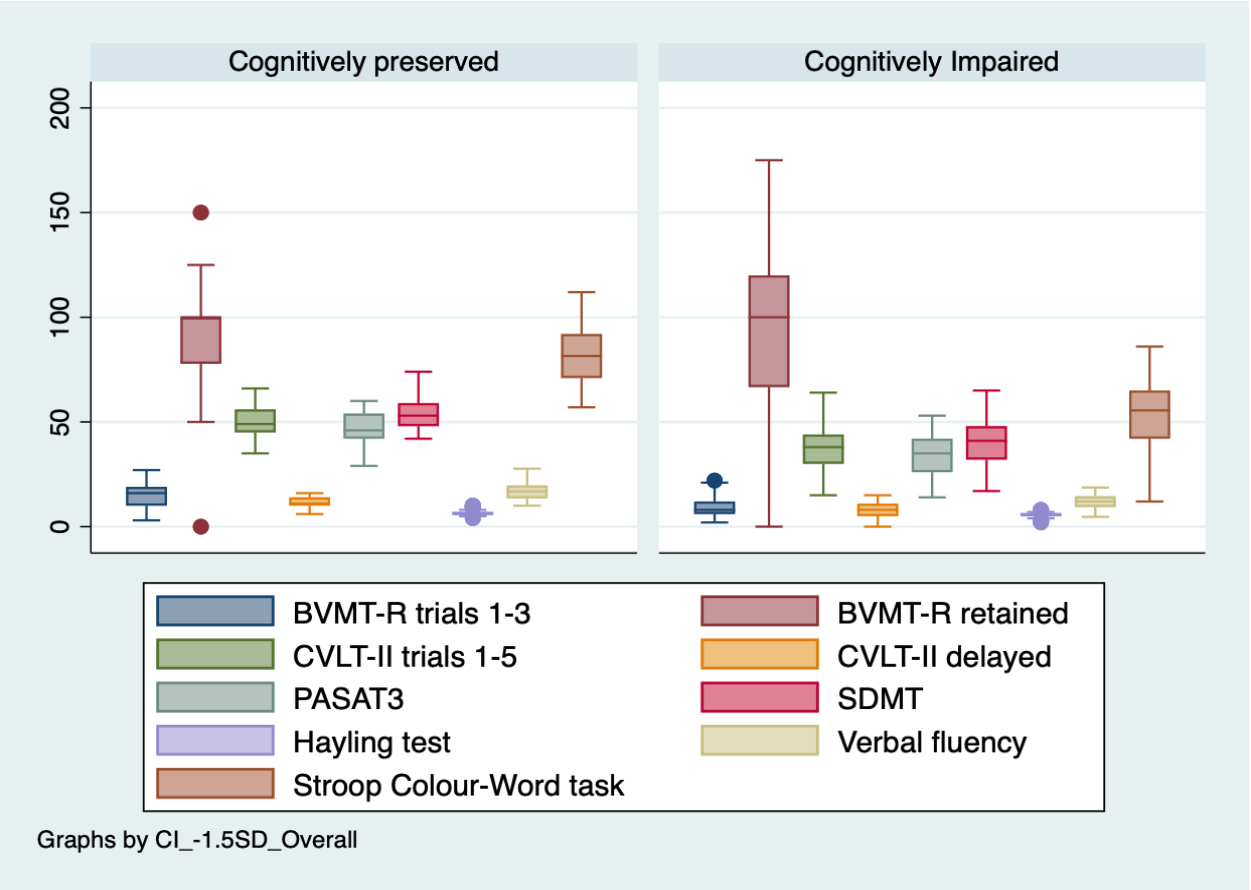

Occupational coding for CI -1.5SD
1
2
3
4
5
6
7
Occupational coding for CP -1.5SD
1
2
3
4
6

Table 4.18. Cognitive characteristics of SPMS groups with and without cognitive impairment (z-score of ≤-1.5SD on ≥2 domains) at baseline.

na=not applicable. Significant results are in bold font if p≤0.05.

BASELINE	Cognitively Preserved -1.5SD						Cognitively Impaired -1.5SD						
	n	Mean	SD	Median	Min	Max	n	Mean	SD	Median	Min	Max	p
BVMT-R trials 1-3	37	15.41	5.93	16.00	3.00	27.00	33	9.18	5.47	8.00	2.00	22.00	**≤0.01**
BVMT-R trials 1-3 z-score	37	-0.02	0.95	0.08	-2.01	1.85	33	-1.02	0.88	-1.21	-2.17	1.05	**≤0.01**
BVMT-R retained	37	90.51	25.98	100.00	0.00	150.00	33	91.27	42.25	100.00	0.00	175.00	0.76
BVMT-R retained z-score	37	-0.23	0.66	0.01	-2.52	1.28	33	-0.21	1.07	0.01	-2.52	1.91	0.76
CVLT-II trials 1-5	37	49.84	8.55	49.00	35.00	66.00	33	38.61	11.05	38.00	15.00	64.00	**≤0.01**
CVLT-II trials 1-5 z-score	37	-0.61	0.98	-0.66	-4.05	1.03	33	-1.55	1.04	-1.61	-3.77	0.84	**≤0.01**
CVLT-II delayed	37	11.30	2.86	11.00	6.00	16.00	33	7.64	4.20	8.00	0.00	15.00	**≤0.01**
CVLT-II delayed z-score	37	-0.72	1.25	-0.84	-3.02	1.34	33	-2.31	1.83	-2.15	-5.64	0.90	**≤0.01**
PASAT3	37	47.14	8.24	46.00	29.00	60.00	33	33.36	10.64	35.00	14.00	53.00	**≤0.01**
PASAT3 z-score	37	0.17	0.68	0.08	-1.33	1.24	33	-0.97	0.88	-0.83	-2.57	0.66	**≤0.01**
SDMT	37	53.89	7.25	53.00	42.00	74.00	33	39.82	10.74	41.00	17.00	65.00	**≤0.01**
SDMT z-score	37	-0.65	0.78	-0.74	-1.92	1.50	33	-2.15	1.15	-2.03	-4.59	0.54	**≤0.01**
Hayling test	37	6.76	1.42	6.00	4.00	10.00	33	5.64	1.43	6.00	2.00	8.00	**≤0.01**
Hayling test z-score	37	0.13	1.39	-0.61	-2.56	3.29	33	-0.96	1.40	-0.61	-4.51	1.34	**≤0.01**
Verbal fluency	36	17.02	4.52	16.83	10.00	27.67	32	11.97	3.31	12.00	4.67	18.67	**≤0.01**
Verbal fluency z-score	36	-0.16	0.95	-0.20	-1.63	2.07	32	-1.21	0.69	-1.21	-2.74	0.19	**≤0.01**
Stroop	36	83.33	15.54	81.50	57.00	112.00	32	52.91	18.72	55.50	12.00	86.00	**≤0.01**
Stroop z-score	36	-0.20	0.78	-0.29	-1.51	1.23	32	-1.72	0.93	-1.59	-3.76	-0.07	**≤0.01**

Figure 4.18. Boxplots of cognitive outcome differences between SPMS groups with and without cognitive impairment (z-score of ≤-1.5SD on ≥2 domains) at baseline.



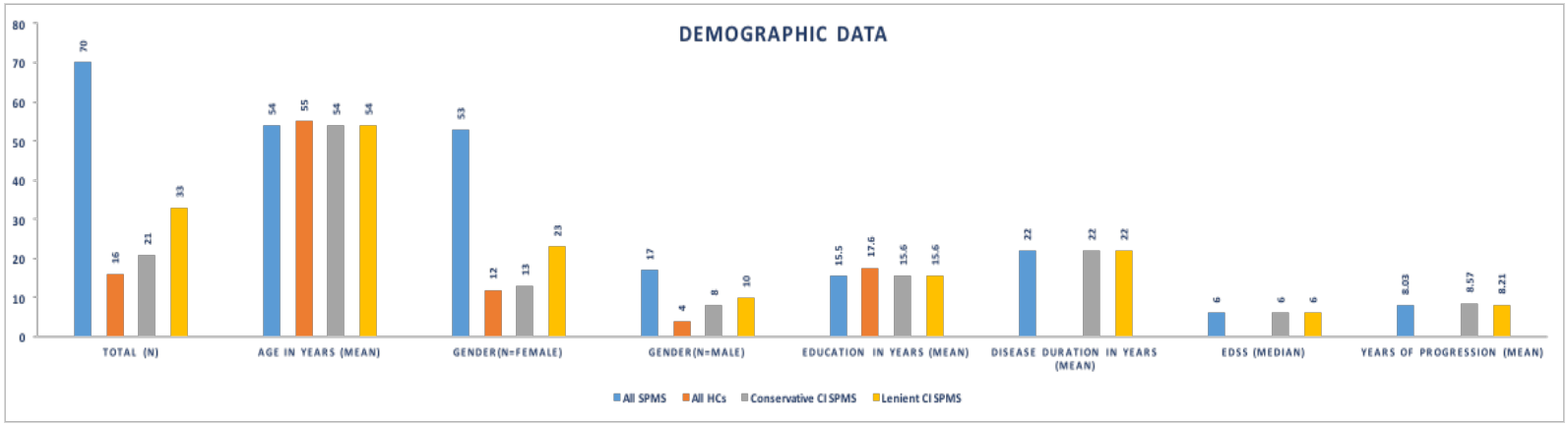
Comparison of the conservative and lenient criteria at baseline.

The bar charts in **Figure 4.19** summarise the similarities in demographic features in terms of age, disease duration, median EDSS, and mean years of progression in the overall SPMS, and cognitively impaired SPMS groups using the 2 criteria. There are differences in gender ratios between groups (SPMS M:F 17:53, z-score ≤-1.96SD on ≥2 domains M:F 8:13, z-score ≤-1.5SD on ≥2 domains M:F 4:12) indicating that the CI ≤-1.96SD on ≥2 domains criteria shows a higher proportion of males versus females.

47.1% of subjects were impaired overall on the more lenient criteria, versus 30% on the conservative criteria (**figure 4.20**). The sequence impairment followed the same pattern regardless of criteria; verbal memory most (40% lenient criteria, versus 25.7% conservative), then visual information processing speed (34.3% lenient criteria, versus 24.3% conservative), executive function (21.9% lenient criteria, versus 10% conservative), auditory information processing speed (14.3% lenient criteria, versus 8.6% conservative), and visual memory (14.3% lenient criteria, versus 7.1% conservative).

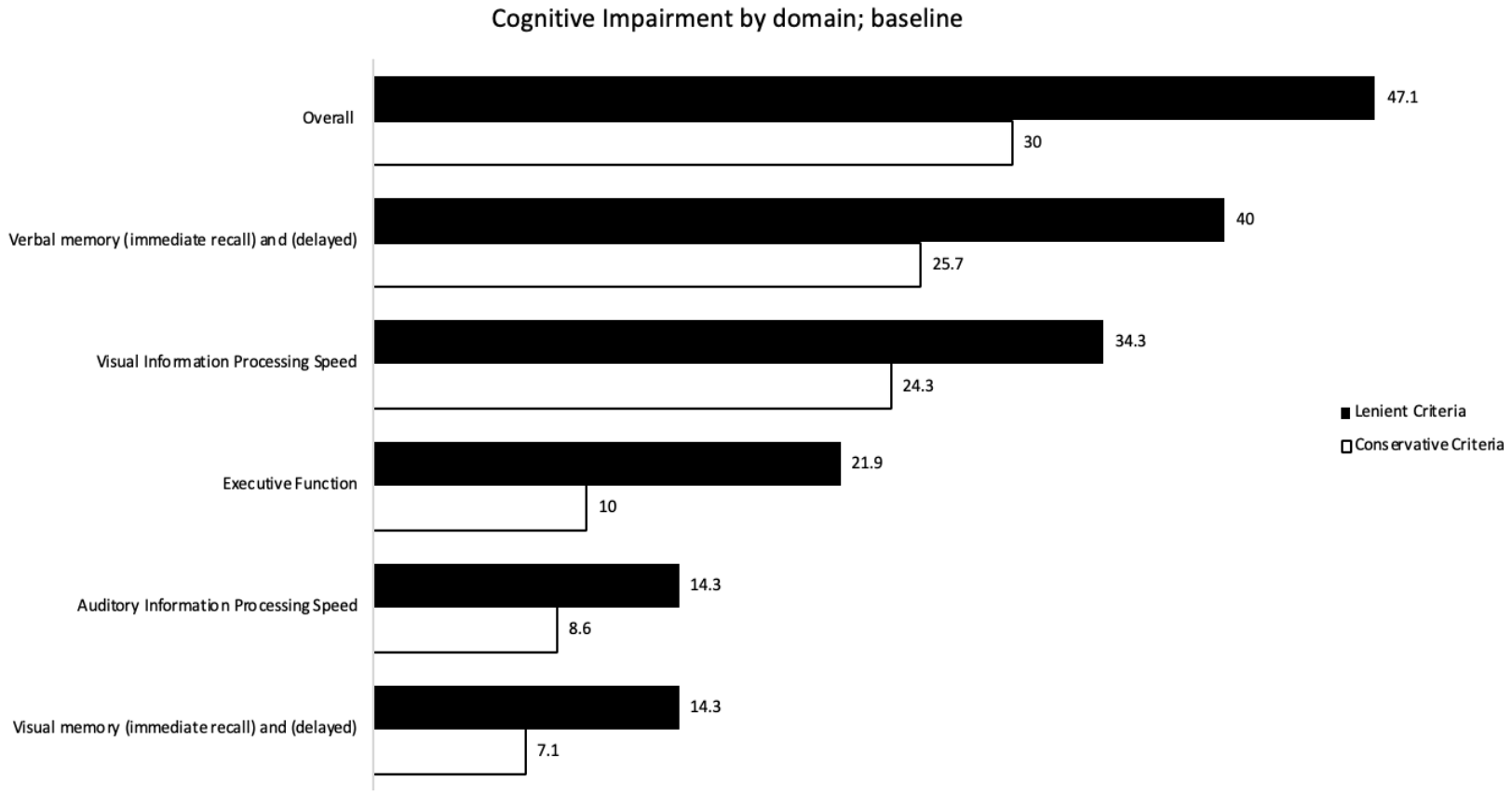
At baseline visit, the main outcome measures driving cognitive impairment in the SPMS ≤-1.96SD on ≥2 domain group were the delayed component of the CVLT-II (76.19%), and the SDMT (71.43%), with the BVMT-R retained score driving impairment least (9.52%) (**figure 4.20***).* Reviewing associations with the SDMT score in the previous section, the immediate CVLT-II task has the most significant pairwise correlation (rho 0.4416, 2-sided exact p=<0.01) (**figure 4.8**). The main drivers of impairment for the SPMS ≤-1.5SD on ≥2 domain group were also the CVLT-retained (54.55%) and SDMT (51.52%), however the lower percentages likely account for the dilution of the population in terms of SD cut-off (**figure 4.20***).*

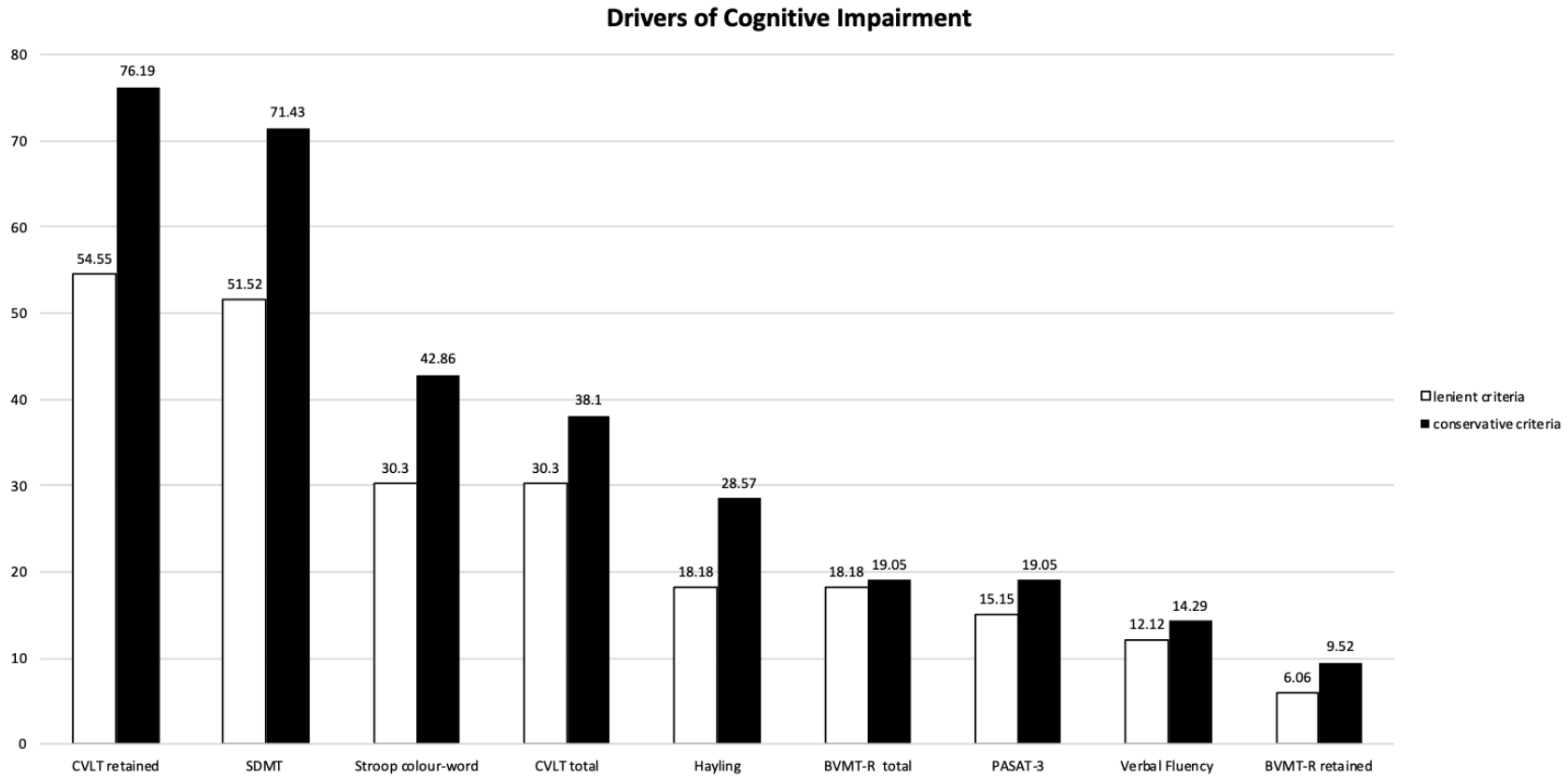
Figure 4.19. Bar chart of demographic variables in the healthy control, SPMS, and cognitively impaired SPMS groups at baseline.



Cognitive impairment; conservative=z-score of ≤-1.96SDs on ≥2 domains and lenient=z-score of ≤-1.5SDs on ≥2 domains.

Figure 4.20. Bar charts of percentage impairment rates by domain and the input of individual cognitive tests to overall cognitive impairment using two different criteria at baseline. a) percentage impairment by individual cognitive domain and overall b) individual test drivers of overall cognitive impairment by percentage input.





Cognitive impairment; lenient=z-score of ≤-1.5SDs on ≥2 domains(black) and conservative=z-score of ≤-1.96SDs on ≥2 domains(white).

4.5.3.2 Associations of cognitive impairment: cross-sectional analysis

I developed logistic regression models to look for associations of the dependent binary outcome measure cognitive impairment on either the ≤-1.96SD on ≥2 domains or ≤-1.5SD on ≥2 domains critical number of abnormal parameters criteria. There were 70 SPMS subjects who were evaluated cross-sectionally. Age, gender, and HADS anxiety were used a priori for the covariate variables (**section 4.3.3.3**). All demographic, physical, and cognitive variables were evaluated individually (**table 4.3**), however only significant associations are presented below.

Critical number of abnormal parameters criteria ≤-1.96SD on ≥2 domains.

There were 21 subjects with cognitive impairment at baseline (**table 4.19**). The covariate model (LR=8.55) was only significant for HADS anxiety (OR=1.17, p=0.03, 95% CI 1.02 to 1.34). The overall predictive quality of the covariate model is an AUROC of 0.72. The most significant non-cognitive association was employment status where there was an 11.33 times higher rate of cognitive impairment if unemployed (OR=11.33, p=<0.01, 95% CI 3.06 to 41.90, LR=29.46) with a predictive probability of 0.88. However, there is considerable confounding of this predictor with the dependent variable indicating additional interactions. As with the group cognitive status differences in **table 4.15** and **figure 4.12**, the VAF (OR=1.79, p=0.02, 95% CI 1.09 to 2.96, LR=13.5, AUROC=0.75) and NFI score (OR=1.2, p=0.02, 95% CI 1.03 to 1.41, LR=14.43, AUROC=0.77) had additional significant associations; 79% and 20% respectively, and improved the predictive probabilities of cognitively impaired status above the covariate model (**table 4.19**).

All of the cognitive tasks, apart from the BVMT-R retained significantly enhanced the probability of the covariate model (**table 4.19**). An increase by one point on the Stroop (OR=0.09, p=<0.01, 95% CI 0.83 to 0.84, LR=39.19, AUROC=0.91) was associated with a reduction of 91% of being cognitively impaired at baseline, compared with a 9% reduction of impairment per point increase in the CVLT-II (OR=0.91, p=0.01, 95% CI 0.85 to 1.01, LR=19.13, AUROC=0.82) at baseline. This indicates that the Stroop is the most discriminatory for cognitive impaired status and the

CVLT-II the least. The SDMT (OR=0.83, p=<0.01, 95% CI 0.74 to 0.95, LR=34.62, AUROC=0.9) gives the second best predictive probability for cognitive impairment at ≤-1.96SD on ≥2 domains, with a single point increase in score associating with a 17% reduction in cognitive impairment.

Table 4.19. Logistic regression models of significant predictors of SPMS cognitive impairment with a z-score of ≤-1.96SD on ≥2 domains. *Significant results are in bold font if p≤0.05.*

Independent predictors of Cognitive Impairment at -1.96SD at baseline						
Predictors	**OR**	**p-value**	**95% CI**		**LR**	**AUROC**
Age	1.05	0.27	0.96	1.14	8.55	0.72
Gender	0.29	0.05	0.08	0.97		
HADS anxiety	1.17	0.03	1.02	1.34		
VAF	1.79	0.02	1.09	2.96	13.50	0.75
Neurological Fatigue Score	1.20	0.02	1.03	1.41	14.43	0.77
Employment Status	11.33	<0.01	3.06	41.90	29.46	0.88
BVMT-R trials 1-3	0.81	<0.01	0.71	0.92	22.90	0.83
CVLT-II trials 1-5	0.91	0.01	0.85	1.01	19.13	0.82
CVLT-II delayed	0.73	<0.01	0.60	0.97	22.26	0.81
PASAT3	0.88	<0.01	0.82	0.89	25.83	0.87
SDMT	0.83	<0.01	0.74	0.95	34.62	0.90
Hayling test	0.33	<0.01	0.17	37.58	28.75	0.87
Verbal fluency	0.75	<0.01	0.62	0.78	21.29	0.81
Stroop	0.09	<0.01	0.83	0.84	39.19	0.91

Critical number of abnormal parameters criteria ≤-1.5SD on ≥2 domains.

Overall, 33 out of 70 SPMS subjects were cognitively impaired using the critical number of abnormal parameters criteria ≤-1.5SD on ≥2 domains. The covariate model (LR=4.38, AUROC=0.64) was not significant for any covariate predictors (**table 4.20**).

Addition of the MSFC (OR=0.37, p=0.04, 95% CI 0.14 to 0.96, LR=11.06) most enhanced the predictive probability of the covariate model (AUROC=0.83) with a single point improvement on the MSFC leading to a 63% reduction in cognitive impairment. Unemployed status was, as with the ≤-1.96SD on ≥2 domains criteria, the most significantly associated variable resulting in a 7.54 times higher chance of being cognitively impaired (OR=7.54, p≤0.01, 95% CI 2.71 to 20.96, LR=25.29) and a predictive probability of 0.82. The EDSS which did not show significant between group differences (**table 4.17**), is significantly associated with a 2.15 times increased chance of cognitive impairment at ≤-1.5SD on ≥2 domains (OR=2.15, p=0.03, 95% CI 1.07 to 4.32, LR=9.51) with a predictive probability of 0.71 overall (**table 4.20**).

As with the conservative criteria, impairment on the lenient criteria is associated with cognitive impairment on all of the cognitive tasks apart from the retained component of the BVMT-R. Impairment on the lenient criteria is associated with and most discriminated by the Stroop with a point increase indicating a 13% lower chance of cognitive impairment (OR=0.87, p=<0.01, 95% CI 0.82 to 0.93, LR=46.5, AUROC=0.92). However, there is the most confounding of this variable and the dependent variable. A single point increase of the SDMT at baseline is associated with a 20% lower risk of cognitive impairment at baseline (OR=0.8, p=<0.01, 95% CI 0.71 to 0.9, LR=37.58) with a predictive probability of 0.88 (**table 4.20**).

Table 4.20. Logistic regression models of significant predictors of SPMS cognitive impairment with a z-score of ≤-1.5SD on ≥2 domains. *Significant results are in bold font if p≤0.05.*

Independent predictors of Cognitive Impairment at -1.5SD at baseline						
Predictors	**OR**	**p-value**	**95% CI**		**LR**	**AUROC**
Age	0.99	0.792	0.92	1.06	4.38	0.64
Gender	0.52	0.257	0.17	1.62		
HADS anxiety	1.11	0.112	0.98	1.26		
NART IQ	0.87	<0.01	0.79	0.96	15.75	0.76
EDSS	2.15	0.03	1.07	4.32	9.51	0.71
MSFC	0.37	0.04	0.14	0.96	11.06	0.83
MSIS29v2	1.05	0.03	1.00	1.09	9.39	0.70
MSIS29v2 physical subscore	1.07	0.02	1.01	1.12	10.73	0.71
MSWS	1.07	0.02	1.01	1.13	11.11	0.74
BPI	1.34	0.03	1.02	1.75	9.03	0.71
Employment Status	7.54	<0.01	2.71	20.96	25.29	0.82
BVMT-R trials 1-3	0.83	<0.01	0.75	0.92	19.93	0.80
CVLT-II trials 1-5	0.89	<0.01	0.83	0.95	21.50	0.81
CVLT-II delayed	0.76	<0.01	0.64	0.90	17.66	0.77
PASAT3	0.85	<0.01	0.78	0.92	33.59	0.86
SDMT	0.80	<0.01	0.71	0.90	37.58	0.88
Hayling test	0.50	<0.01	0.31	0.78	17.18	0.79
Verbal fluency	0.69	<0.01	0.56	0.84	26.93	0.83
Stroop	0.87	<0.01	0.82	0.93	46.50	0.92

4.5.3.3 Cognitive impairment in SPMS over time by criteria; longitudinal analysis

Evaluation of between group differences longitudinally.

Comparisons were made from baseline to follow-up per cohort (p-value by visit), and cross-sectionally between cohorts at the follow-up timepoint (Chi^2 probability) (**table 4.21** and **table 4.22**). I developed violin plots of mean, median, and kernel density probabilities of values for each of the cognitive outcome measures to demonstrate differences both cross-sectionally per visit, and longitudinally (**figure 4.21** and **4.22**).

Critical number of abnormal parameters criteria z-score ≤-1.96SD on ≥2 domains.

In the follow-up group there were 21 subjects cognitively impaired (35.6%) and 38 subjects cognitively preserved (**table 4.21**). Between visits there was only significant improvement in the BVMT-R in the cognitively impaired group (mean 14.57±7.92 SD, change over time 6.77 points p=0.01). There were some non-significant changes of note at follow-up from baseline. At follow-up, there was a lower number of years of education (mean 15.24±3.91 years, change over time -0.16 years, p=0.6), lower disease duration (mean 21.57±10.71 years, change over time -1.03 years, p=0.45), and less years of progression (mean 7.1±5.01 years, change over time -0.9 years, p=0.35). Additionally, there was non-significantly lower HADS anxiety score (mean 5.1±3.18, change over time -2.3, p=0.23), lower HADS depression (mean 4.57±2.31 SD, change over time -1.9, p=0.07) and BDI-II score (mean 8±4.69 SD, change over time -0.6, p=0.78) (**table 4.21**).

In the cognitively preserved group (n=38) there were significantly higher scores in the subjective MSIS 29v2 physical component (mean 52.81±12.36 SD, change over time 5.94, p=0.03) MSIS 29v2 psychological subscale (mean 73.28±16.54 SD, change over time 9.37, p=0.03), and MSIS 29v2 overall (mean 4.57±2.31 SD, change over time 3.43, p=0.03). There was additional worsening of the MSNQ (mean 28.7±11.33 SD, change over time 6.85, p=<0.01), and BPI (mean

4.08±2.76 SD, change over time 1.08, p=0.05). Over time there was significant improvement in the EQ-5D (mean 0.58±0.22 SD, change over time -0.11, p=0.02). As with the cognitively impaired group, there was also significant improvement in the BVMT-R over time (mean 22.11±7.44 SD, change over time 7.73, p=<0.01) (**table 4.21**).

Between groups at the follow-up timepoint, there were significant differences only in the cognitive outcome measures cross-sectionally by cognitive status. This was at a <1% significance level for each task, apart from the BVMT-R retained (p=0.8) and PASAT3 (p=0.78) which did not show discriminatory differences for cognitive impairment at this timepoint (**table 4.21**). The violin plots in **figure 4.21** summarise the cross-sectional and longitudinal changes over time for the SPMS groups by cognitive status. The lack of significant changes in the BVMT-R retained and PASAT3 may be due to the higher kernel density around the median and mean values at baseline and this not persisting at follow-up.

Table 4.21. Demographic, clinical and cognitive characteristics of SPMS groups with and without cognitive impairment (z-score of ≤-1.96SD on ≥2 domains) at the follow-up timepoint and changes over time. *na=not applicable. Significance levels; bold font p≤ 0.05. Negative change over time; bold font.*

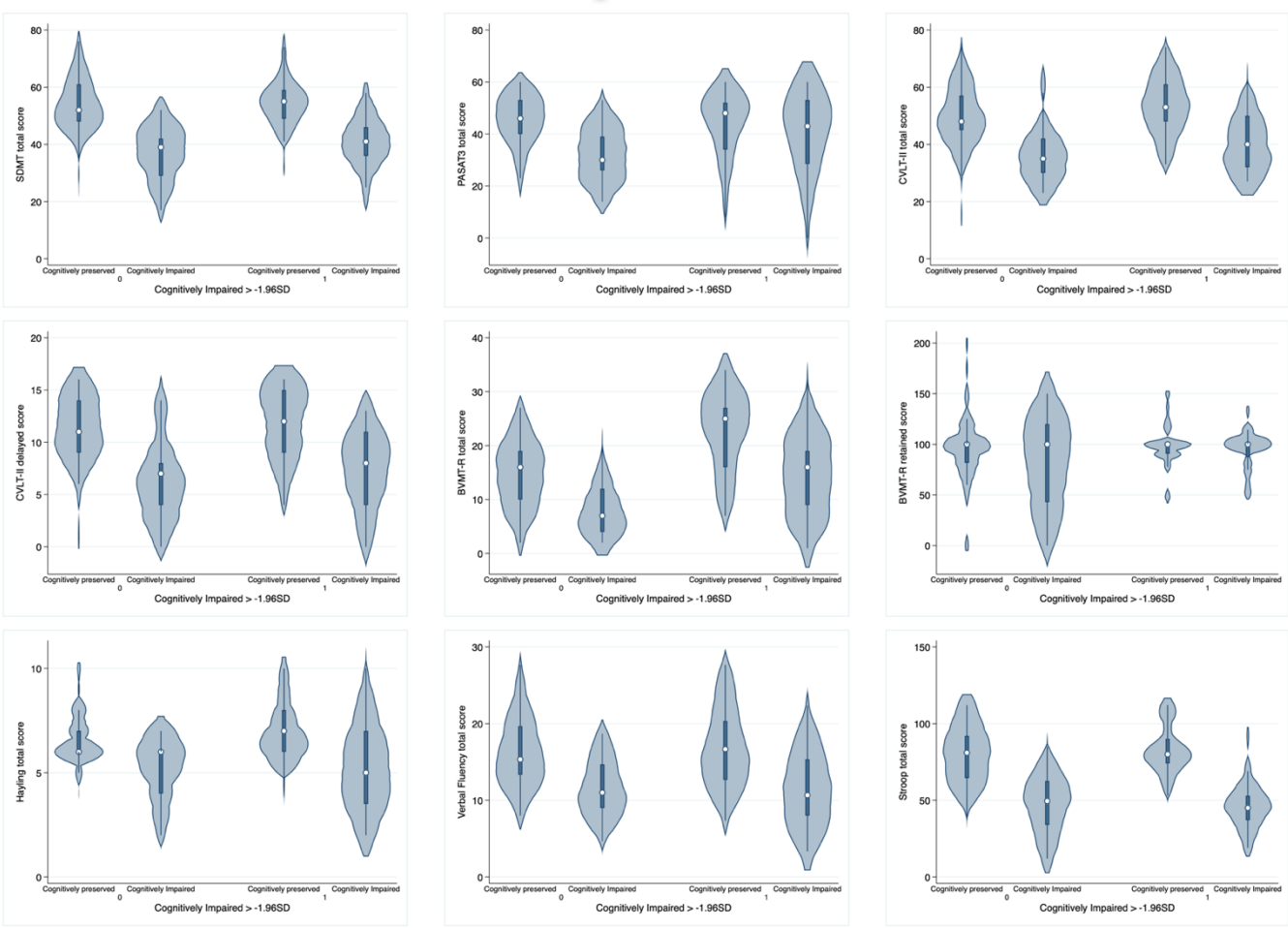
	Cognitively Preserved -1.96SD								Cognitively Impaired -1.96SD								
	n	Mean	SD	Median	Min	Max	Change	p (visit)	n	Mean	SD	Median	Min	Max	Change	P (visit)	Chi-2 (cohort)
Age	37	56.01	6.38	56.82	43	66.79	1.31	0.46	21	57.97	7.62	59.54	39.06	67.56	3.44	0.18	0.14
Gender	38	Female:29, Male:9					**-0.03**	0.73	21	Female: 17, Male:4					0.08	0.59	0.68
Interval	38	673.66	46.65	681	446	751	0.77	na	21	663.71	76.18	684	449	753	1.85	na	0.73
Edu	38	15.55	2.21	16	12	21	0.15	0.58	21	15.24	3.91	14	11	28	**-0.16**	0.6	0.28
Drug arm	38	fluoxetine:12, riluzole:10, amiloride:8, placebo:8					**-0.2**	0.88	21	fluoxetine:3, riluzole:6, amiloride:3, placebo:9					0.39	0.7	0.24
IQ	38	114.89	8.27	116	77	125	**-0.09**	0.85	21	113.86	8.09	115	97	126	0.86	0.71	0.46
HADS d	38	5.21	3.28	5	0	12	0.46	0.32	21	4.57	2.31	4	0	9	**-1.9**	0.07	0.5
HADS a	38	5.95	4.44	5	0	18	0.7	0.51	21	5.1	3.18	5	0	14	**-2.3**	0.23	0.71
BDI-II	38	6.55	5.27	6	0	18	0	0.88	21	8	4.69	8	0	17	**-0.6**	0.78	0.22
Disease duration	38	23.68	7.87	22	12	46	0.63	0.56	21	21.57	10.71	19	6	44	**-1.03**	0.45	0.26
Progression duration	38	8.47	4.76	8	2	20	0.49	0.51	21	7.1	5.01	4	3	21	**-0.9**	0.35	0.14
EDSS	33	5.65	1.28	6	2.5	8	**-0.04**	0.62	20	5.85	1.17	6	2.5	8	0.02	0.66	0.71
9HPT	33	33.39	14.71	29.45	17.92	80.2	**-2.38**	0.59	21	39.49	14.86	34.15	20.88	71.03	4.9	0.33	0.07
T25FW	33	30.7	42.35	13.7	5.2	180	11.03	0.35	20	24.84	38.36	14.93	5.45	180	5.52	0.82	0.84
MSFC	33	-0.28	1.44	0.02	-5.56	0.9	**-0.24**	0.34	20	-0.21	1.22	0.14	-4.64	0.95	0.07	0.3	0.91
MSISphys	32	52.81	12.36	52	30	75	5.94	**0.03**	20	56.05	11	55	32	77	4.52	0.27	0.36
MSIS	32	73.28	16.54	71	46	100	9.37	**0.03**	20	76.45	14.51	73	50	103	6.05	0.3	0.52
MSWS	32	44.66	10.16	49	21	54	4.66	0.06	20	46.25	6.58	46.5	34	54	3.32	0.34	0.96
MSVQ-	36	12.81	4.09	12.5	7	22	**-10.97**	0.84	17	15	5.5	14	7	26	**-4.32**	0.82	0.18
MSNQ	37	28.7	11.33	30	6	49	6.85	**<0.01**	18	30.28	11.88	29	9	55	3.68	0.29	0.62
MSISpsy	32	20.47	6.29	19.5	11	34	3.43	**0.03**	20	20.4	4.47	19	14	29	1.53	0.33	0.92
VAF	36	0.93	1.48	1	-2	7	**-0.12**	0.66	21	1.36	1.97	1	-3	5	**-0.11**	0.71	0.27
NFI	32	17.81	5.08	17.64	7.32	30	1.39	0.19	20	19.62	4.28	18.45	13.86	30	0.3	0.87	0.19
BPI	32	4.08	2.76	4.64	0	8.71	1.08	**0.05**	20	5.2	2.24	5.21	0	8.29	1.72	0.07	0.14
CLQ	37	20.05	4.75	20	13	33	0.51	0.58	21	19.29	4.68	18	13	30	**-1.44**	0.45	0.58
Qualification	38	Entry:1, 1:9, 2:2, 3:8, 5:2, 6:14, 7:2, 8:0					0.06	0.91	21	3.52	1:10, 2:0, 3:2, 5:0, 6:6, 7:2				0.05	0.48	0.28
Occupation	38	1:5, 2:15, 3:4, 4:7, 5:2, 6:3, 7:2					0.01	0.96	21	3.48	1:1, 2:7, 3:1, 4:8, 5:1, 6:3				**-0.19**	0.82	0.5
Employment	38	Full-time:7, Part-time:17, Retired:14					0.04	0.89	21	2.48	Full-time:3, Part-time:5, Retired:13				**-0.32**	0.25	0.17
EQ-5D	32	0.58	0.22	0.63	-0.12	0.94	**-0.11**	**0.02**	20	0.54	0.19	0.58	0.17	0.92	**-0.06**	0.37	0.31
Health	32	60.56	20.72	62.5	30	100	**-5.35**	0.23	20	63.2	19.16	60	30	95	**-1.67**	0.88	0.59
Ethnicity	38	Belgian:0, English:38, German:0					0.04	0.41	21	Belgian:1, Cingalese:1, English:18, German:1					**-0.03**	0.67	0.13

	Cognitively Preserved -1.96SD								Cognitively Impaired -1.96SD								
	n	Mean	SD	Median	Min	Max	Change	p (visit)	n	Mean	SD	Median	Min	Max	Change	p (visit)	Chi-2 (cohort)
BVMT-R	38	22.11	7.44	25	8	34	7.73	**<0.01**	21	14.57	7.92	16	1	32	6.77	**0.01**	**<0.01**
BVMT-R z	38	-0.16	0.87	0.18	-1.81	1.23	0.01	0.99	21	-1.04	0.93	-0.87	-2.62	1	0.2	0.53	**<0.01**
BVMT-R ret	37	96.89	17.36	100	50	140	3.11	0.64	21	94.57	19.68	100	50	133.33	13.57	0.6	0.8
BVMT-R ret z	37	-0.26	0.62	-0.14	-1.94	1.29	**-0.09**	0.11	21	-0.34	0.71	-0.14	-1.94	1.05	0.13	0.53	0.8
CVLT-II z	38	51.74	9.69	51	33	74	3.49	0.1	21	40.19	10	40	27	64	**0.06**	0.99	**<0.01**
CVLT-II z	38	-0.68	0.97	-0.75	-2.55	1.54	**-0.03**	0.78	21	-1.83	1	-1.85	-3.15	0.55	**-0.43**	0.27	**<0.01**
CVLT-II d	37	11.14	3.24	11	4	16	0.35	0.86	21	6.95	4.09	8	0	13	**-0.38**	0.87	**<0.01**
CVLT-II d z	37	-1.03	1.17	-1.08	-3.61	0.72	**-0.15**	0.39	21	-2.55	1.48	-2.17	-5.06	-0.36	**-0.11**	0.89	**<0.01**
PASAT3	33	41.94	12.73	45	8	60	**-1.32**	0.77	20	39.6	16.11	43	0	60	7.33	0.08	0.78
PASAT3 z	33	-0.26	1.05	0	-3.07	1.24	**-0.11**	0.78	20	-0.45	1.33	-0.17	-3.73	1.24	0.61	0.07	0.78
SDMT	38	52.71	8.63	53	32	74	0.49	0.67	21	40.48	9.38	41	20	58	4.01	0.24	**<0.01**
SDMT z	38	-0.81	1.03	-0.78	-3.29	1.73	0.03	0.82	21	-2.27	1.12	-2.21	-4.72	-0.18	0.24	0.53	**<0.01**
Hayling	37	7.03	1.5	7	4	10	0.28	0.31	20	5.3	2.08	5	2	10	0.23	0.91	**<0.01**
Hayling z	37	0.11	1.57	0.08	-3.06	3.23	**-0.01**	0.27	20	-1.7	2.18	-2.02	-5.16	3.23	**-0.18**	0.57	**<0.01**
VF	38	16.24	5.01	15.33	7.33	27.67	0.44	0.86	21	11.41	5.08	10.67	3.33	22.33	**0.2**	0.89	**<0.01**
VF z	38	-0.48	1.03	-0.66	-2.31	1.88	**-0.06**	0.47	21	-1.47	1.05	-1.62	-3.14	0.78	**-0.1**	0.64	**<0.01**
Stroop	37	80.03	13.31	80	54	112	2.02	0.78	21	46.86	16.41	45	19	92	**2.15**	1	**<0.01**
Stroop z	37	-0.58	0.66	-0.58	-1.87	1.01	**-0.12**	0.31	21	-2.22	0.82	-2.32	-3.61	0.02	**-0.09**	0.35	**<0.01**

Change= change over time, chi-2= chi square, p= p-value, age= Age (years), edu= years of education, IQ= NART IQ, HADS d=HADS depression, HADS a= HADS anxiety, BDI-II= Beck's Depression Index, Disease duration = Disease duration from first symptom, Progression duration = Duration of progression, Interval= visit interval in days, 9HPT= Average nine hole peg test in seconds, T25FW= average timed 25 foot walk in seconds, MSISphy = MSIS 29v2 physical score, MSIS= MSIS 29v2 total score, MSWS = MS walking score v2, MSVQ= MS Vision Questionnaire-7, MSNQ= MS neuropsychological questionnaire, MSISpsy= MSIS 29v2 psychological score, VAF= Visual analogue of fatigue, NFI= Neurological fatigue index, BPI= Brief Pain Inventory, CLQ = Cognitive Leisure Questionnaire, Qualification = Qualification by RQF, Occupation = Occupation, Employment = Employment Status, EQ-5D= Health-state questionnaire, Health = Health-state analogue, BVMT-R = BVMT-R trials 1-3, BVMT-R z = BVMT-R trials 1-3 z-score, BVMT-R ret = BVMT-R retained, BVMT-R ret z = BVMT-R retained z-score, CVLT-II = CVLT-II trials 1-5, CVLT-II z = CVLT-II trials 1-5 z-score, CVLT-II d = CVLT-II delayed,

CVLT-II d z = CVLT-II delayed z-score, PASAT3 z = PASAT3 z-score, SDMT z = SDMT z-score, Hayling = Hayling test, Hayling z = Hayling test z-score, VF = Verbal fluency, VF z = Verbal fluency z-score, Stroop = Stroop Colour-Word task, Stroop z = Stroop Colour-Word z-score.

Figure 4.21. Violin plots of cognitive outcomes at baseline and follow-up time points by cognitive impairment (z-score of ≤-1.96SD on ≥2 domains) in SPMS.



Critical number of abnormal parameters criteria z-score ≤-1.5SD on ≥2 domains.

At the follow-up timepoint there were 34 subjects cognitively impaired (57.6%) and 24 subjects cognitively preserved (**table 4.22**). Between visits there was significant worsening in the MSNQ in the cognitively impaired group (mean 29.45±11.8 SD, change over time 5.7 points, p=0.05). There was significantly lower EQ-5D score in this group at follow-up (mean 0.55±0.19, change over time -0.09, p=0.05). As with the conservative criteria, there was non-significant improvement on the HADS anxiety score (mean 4.97±3.02, change over time -1.21, p=0.43), and HADS depression (mean 4.79±2.46 SD, change over time -1, p=0.36), and a shorter disease duration in the cognitive impaired group (mean 22.79±10.1 years, change over time -0.94 years, p=0.46). There was significant improvement in the impaired group over time on the BVMT-R (mean 15.82±7.92 SD, change over time 6.52 points p=<0.01) and PASAT3 (mean 39.5±14.9 SD, change over time 6.26 points p=0.04) (**table 4.22**).

The cognitively preserved group also showed significant improvement in the MSNQ over time (mean 28.92±11.15 SD, change over time 6.03 points p=<0.01) which could be a chance finding. However, there was worsening of the BPI (mean 4.08±2.76 SD, change over time 1.08, p=0.05) and Health state (mean 57.64±19.82 SD, change over time -9.81 points p=0.05). There was significant improvement in the BVMT-R as with the impaired group (mean 24.32±6.33 SD, change over time 9.03 points p=<0.01), but also additionally the z-score of the CVLT-II (mean -0.3±0.94 SD, change over time 4.17 points p=0.01) (**table 4.22**).

Between cognitive status groups at the follow-up timepoint, there are significant differences in the physical component of the MSIS 29v2 (Chi^2=0.05) and the NFI (Chi^2=0.03). There were significant differences in all of the cognitive outcome measures at a <1% significance level apart from the BVMT-R retained (chi2 p=0.13), PASAT3 (chi2 p=0.35), and Hayling (chi2 p=0.02) which did not show discriminatory differences cross-sectionally (**table 4.22**). The violin plots in **figure 4.22** summarise the cross-sectional and longitudinal changes over time by cognitive status. The lack

of significant changes in the BVMT-R retained, PASAT3 and Hayling may be due differences in the kernel density probabilities around the median and mean values.

Table 4.22. Demographic, clinical and cognitive characteristics of SPMS groups with and without cognitive impairment (z-score of ≤-1.5SD on ≥2 domains) at the follow-up timepoint and changes over time. *na=not applicable. Significance levels; bold font p≤ 0.05. Negative change over time; bold font*

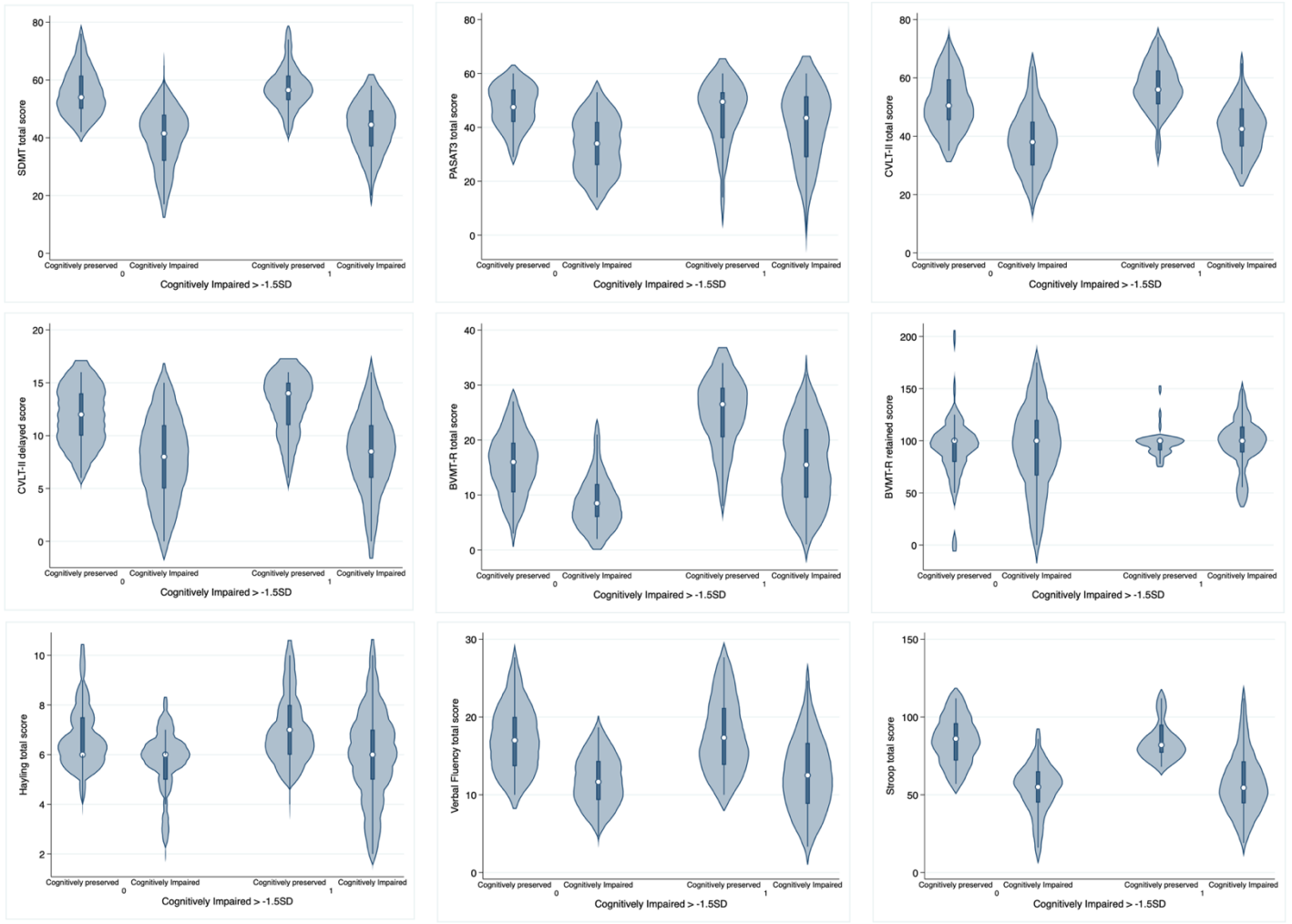
	Cognitively Preserved -1.5SD								Cognitively Impaired -1.5SD								
	n	Mean	SD	Median	Min	Max	Change	p (visit)	n	Mean	SD	Median	Min	Max	Change	p (visit)	Chi-2 (cohort)
Age	24	56.84	6.54	57.15	45.93	66.7	1.53	0.46	34	56.63	7.16	58.09	39.06	67.56	2.72	0.15	0.96
Gender	25	Female:19, Male:6					0	0.8	34	Female:27, Male:7					0.01	0.82	0.76
Interval	25	673.66	46.65	681	446	751	**-0.89**	na	34	671.2	56.6	684	449	709	0.62	na	0.72
Edu	25	15.52	2.45	16	12	21	**-0.08**	0.87	34	15.38	3.23	16	11	28	0.38	0.98	0.6
Drug arm	25	fluoxetine:7, riluzole:6, amiloride:7, placebo:5					**-0.21**	0.9	34	fluoxetine:8, riluzole:10, amiloride:4, placebo:12					0.21	0.89	0.32
IQ	25	115.08	9.57	116	77	125	**-0.94**	0.58	34	114.1	7.07	115	97	126	2.39	0.33	0.23
HADS d	25	5.24	3.59	5	0	12	0.38	0.41	34	4.79	2.46	5	0	11	**-1**	0.36	0.66
HADS a	25	6.56	5.01	6	0	18	0.84	0.3	34	4.97	3.02	4	0	14	**-1.21**	0.43	0.34
BDI-II	25	6.12	4.62	6	0	16	**-0.7**	0.44	34	7.76	5.35	7.5	0	18	0.38	0.65	0.26
Disease duration	25	23.12	7.36	21	12	46	0.82	0.46	34	22.79	10.1	19.5	6	44	**-0.94**	0.46	0.59
Progression duration	25	7.36	3.91	7	2	16	**-0.64**	0.89	34	8.44	5.46	7.5	3	21	0.48	0.99	0.75
EDSS	23	5.41	1.21	6	2.5	6.5	**-0.17**	0.8	30	5.97	1.22	6	2.5	8	0.05	0.3	0.06
9HPT	20	37.62	17.39	30.97	17.92	80.2	0.09	0.81	34	34.67	13.4	31.35	20.05	71.03	1.82	0.85	0.72
T25FW	23	17.62	16.09	10.6	5.35	68.25	**-1.74**	0.86	30	36.82	51	17.25	5.2	180	16.96	0.32	0.12
MSFC	23	0.13	0.49	0.29	-0.8	0.86	0.02	0.48	30	-0.54	1.69	0.03	-5.56	0.95	**-0.16**	0.59	0.22
MSISphys	22	50.45	13.63	49.5	30	75	4.83	0.22	30	56.7	9.76	55.5	32	77	5.55	0.06	**0.05**
MSIS	22	71	18.34	68	46	100	8.42	0.13	30	77.07	13.2	72	50	103	7.72	0.09	0.15
MSWS	22	42.41	10.92	47.5	21	54	4.29	0.2	30	47.37	6.53	49	34	54	3.29	0.22	0.13
MSVQ-	23	12.22	4.17	11	7	20	**-7.77**	0.98	30	14.5	4.83	14	7	26	**-11.52**	0.93	0.08
MSNQ	24	28.92	11.15	29	6	49	6.03	**<0.01**	31	29.45	11.8	30	9	55	5.7	**0.05**	0.82
MSISpsy	22	20.55	6.52	19.5	11	34	3.59	0.85	30	20.37	4.96	19.5	13	30	2.18	0.1	0.96
VAF	23	0.63	1	1	-2	3	**-0.33**	0.85	34	1.4	1.96	1	-3	7	0.07	0.92	0.07
NFI	22	17.03	4.92	16.44	7.32	30	0.45	0.78	30	19.59	4.54	18.87	9.42	30	1.69	0.18	**0.03**
BPI	22	4.55	2.66	5.07	0	8.14	1.97	**<0.01**	30	4.48	2.61	4.79	0	8.71	0.63	0.36	0.79
CLQ	24	20.92	5.16	20.5	13	33	0.39	0.79	34	18.97	4.25	18.5	13	30	0.06	0.89	0.21
Qualification	25	Entry level:1, 1:6, 3:5, 5:2, 6:9, 7:2, 8:0					0.06	0.72	34	Entry level:0, 1:13, 2:2, 3:5, 5:0, 6:11, 7:2, 8:1					0.15	0.74	0.37
Occupation	25	1:1, 2:12, 3:3, 4:5, 5:2, 6:1, 7:1					0.05	0.53	34	1:5, 2:10, 3:2, 4:10, 5:1, 6:5, 7:1					**-0.14**	0.76	0.37
Employment	25	Full-time:4, Part-time:12, Retired:9					0.1	0.61	34	Full-time:6, Part-time:10, Retired:18					**-0.29**	0.28	0.32

EQ-5D	22	0.59	0.24	0.63	-0.12	0.94	**-0.1**	0.08	30	0.55	0.19	0.58	0.17	0.92	**-0.090**	**0.05**	0.29
Health	22	57.64	19.82	57	30	95	**-9.81**	**0.05**	30	64.47	19.9	62.5	30	100	1.120	0.77	0.23
Ethnicity	25	Belgian:0, English:25, German:0					0	0.46	34	Belgian:1, Cingalese:1, English:31, German:1					0.07	0.66	0.51

	Cognitively Preserved -1.5SD								**Cognitively Impaired -1.5SD**								
	n	**Mean**	**SD**	**Median**	**Min**	**Max**	**Change**	**p (visit)**	**n**	**Mean**	**SD**	**Median**	**Min**	**Max**	**Change**	**p (visit)**	**Chi-2 (cohort)**
BVMT-R	25	24.32	6.33	26	8	34	9.03	**<0.01**	34	15.82	7.92	15.5	1	32	6.52	**<0.01**	**<0.01**
BVMT-R z	25	0.1	0.74	0.3	-1.81	1.23	0.12	0.61	34	-0.89	0.93	-0.93	-2.62	1	0.11	0.52	**<0.01**
BVMT-R ret	24	94.38	6.99	100	77.78	100	5.82	0.62	34	97.23	23	100	50	140	3.55	0.96	0.13
BVMT-Rret z	24	-0.35	0.25	-0.14	-0.94	-0.14	**-0.03**	0.06	34	-0.24	0.83	-0.14	-1.94	1.29	**-0.09**	0.31	0.13
CVLT-II z	25	55.56	9.36	55	33	74	4.17	**0.01**	34	41.79	8.61	41.5	27	64	2.31	0.59	**<0.01**
CVLT-II z	25	-0.3	0.94	-0.35	-2.55	1.54	0.06	0.29	34	-1.67	0.86	-1.7	-3.15	0.55	**-0.2**	0.24	**<0.01**
CVLT-II d	24	12.21	2.93	12	6	16	0.73	0.28	34	7.79	3.8	8	0	15	**-0.12**	0.62	**<0.01**
CVLT-II d z	24	-0.65	1.06	-0.72	-2.89	0.72	**-0.04**	0.96	34	-2.24	1.37	-2.17	-5.06	0.36	**-0.08**	0.46	**<0.01**
PASAT3	23	43.09	12.72	48	8	60	**-2.46**	0.41	30	39.5	14.9	43.5	0	60	6.26	**0.04**	0.35
PASAT3 z	23	-0.16	1.05	0.25	-3.07	1.24	**-0.2**	0.42	30	-0.46	1.24	-0.13	-3.73	1.24	0.52	**0.04**	0.35
SDMT	25	55.84	7.44	56	41	74	1.6	0.38	34	42.85	9.18	43.5	20	58	2.78	0.35	**<0.01**
SDMT z	25	-0.44	0.89	-0.42	-2.21	1.73	0.19	0.42	34	-1.99	1.09	-1.91	-4.72	-0.18	0.14	0.65	**<0.01**
Hayling	24	7.12	1.57	7	4	10	0.25	0.36	33	5.91	1.97	6	2	10	0.35	0.71	0.02
Hayling z	24	0.21	1.64	0.08	-3.06	3.23	**-0.04**	0.46	33	-1.06	2.07	-0.97	-5.16	3.23	**-0.02**	0.4	0.02
VF	25	17.24	4.57	15.67	12	27.67	0.41	0.94	34	12.52	5.33	12.17	3.33	24.67	0.97	0.75	**<0.01**
VF z	25	-0.27	0.94	-0.59	-1.35	1.88	**-0.08**	0.4	34	-1.24	1.1	-1.32	-3.14	1.26	0.06	0.75	**<0.01**
Stroop	24	82.88	10.52	80.5	68	112	0.94	0.72	34	57.53	21.3	54.5	19	107	5.76	0.74	**<0.01**
Stroop z	24	-0.43	0.52	-0.55	-1.17	1.01	**-0.16**	0.13	34	-1.69	1.06	-1.84	-3.61	0.76	0.08	0.61	**<0.01**

Change= change over time, chi-2= chi square, p= p-value, age= Age (years), edu= years of education, IQ= NART IQ, HADS d=HADS depression, HADS a= HADS anxiety, BDI-II= Beck's Depression Index, Disease duration = Disease duration from first symptom, Progression duration = Duration of progression, Interval= visit interval in days, 9HPT= Average nine hole peg test in seconds, T25FW= average timed 25 foot walk in seconds, MSISphy = MSIS 29v2 physical score, MSIS= MSIS 29v2 total score, MSWS = MS walking score v2, MSVQ= MS Vision Questionnaire-7, MSNQ= MS neuropsychological questionnaire, MSISpsy= MSIS 29v2 psychological score, VAF= Visual analogue of fatigue, NFI= Neurological fatigue index, BPI= Brief Pain Inventory, CLQ = Cognitive Leisure Questionnaire, Qualification = Qualification by RQF, Occupation = Occupation, Employment = Employment Status, EQ-5D= Health-state questionnaire, Health = Health-state analogue, BVMT-R = BVMT-R trials 1-3, BVMT-R z = BVMT-R trials 1-3 z-score, BVMT-R ret = BVMT-R retained, BVMT-R ret z = BVMT-R retained z-score, CVLT-II = CVLT-II trials 1-5, CVLT-II z = CVLT-II trials 1-5 z-score, CVLT-II d = CVLT-II delayed, CVLT-II d z = CVLT-II delayed z-score, PASAT3 z = PASAT3 z-score, SDMT z = SDMT z-score, Hayling = Hayling test, Hayling z = Hayling test z-score, VF = Verbal fluency, VF z = Verbal fluency z-score, Stroop = Stroop Colour-Word task, Stroop z = Stroop Colour-Word z-score.

Figure 4.22. Violin plots of cognitive outcomes at baseline and follow-up time points by cognitive impairment (z-score of ≤-1.5SD on ≥2 domains) in SPMS.

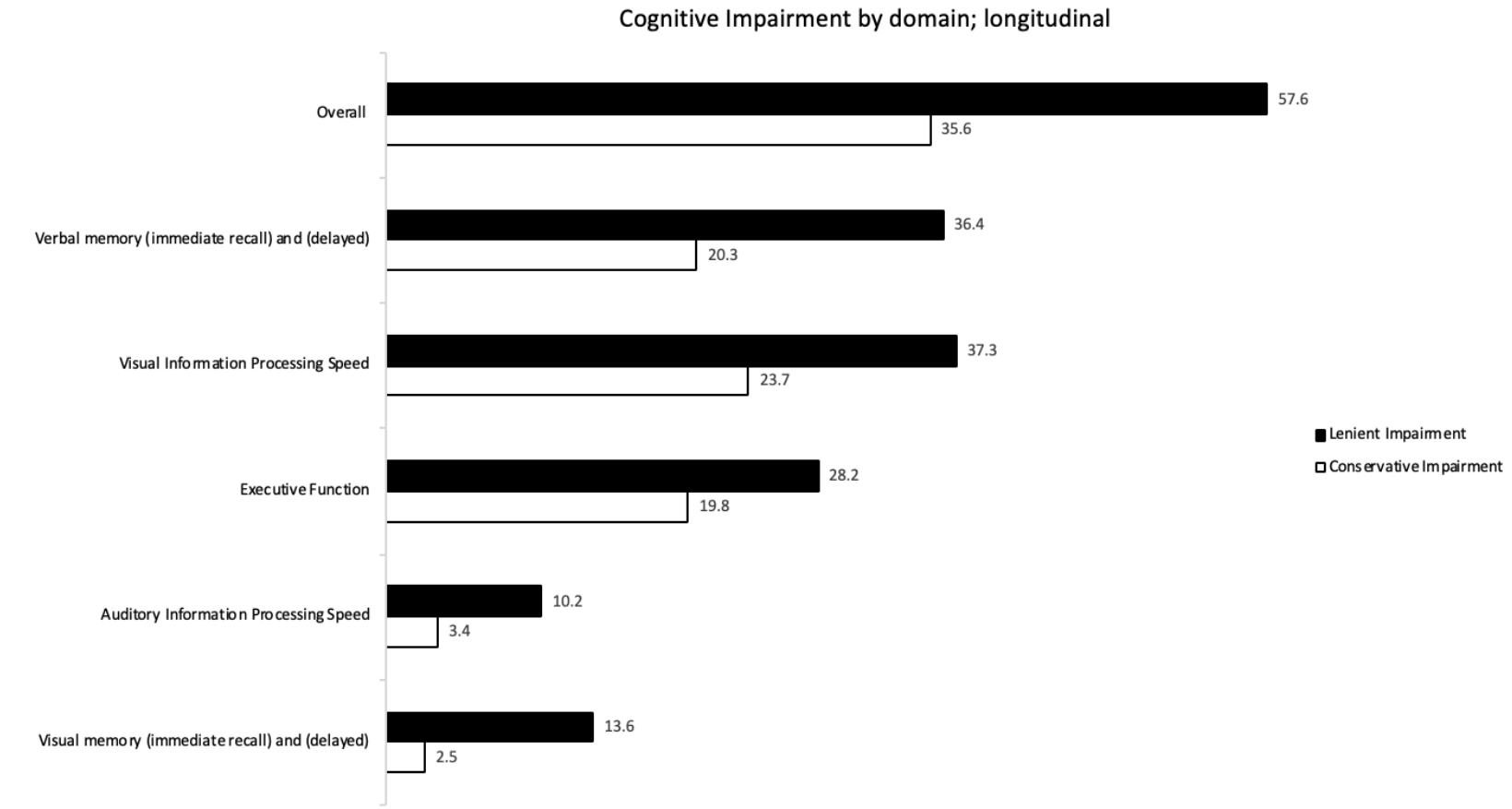


Comparison of the conservative and lenient criteria at follow-up.

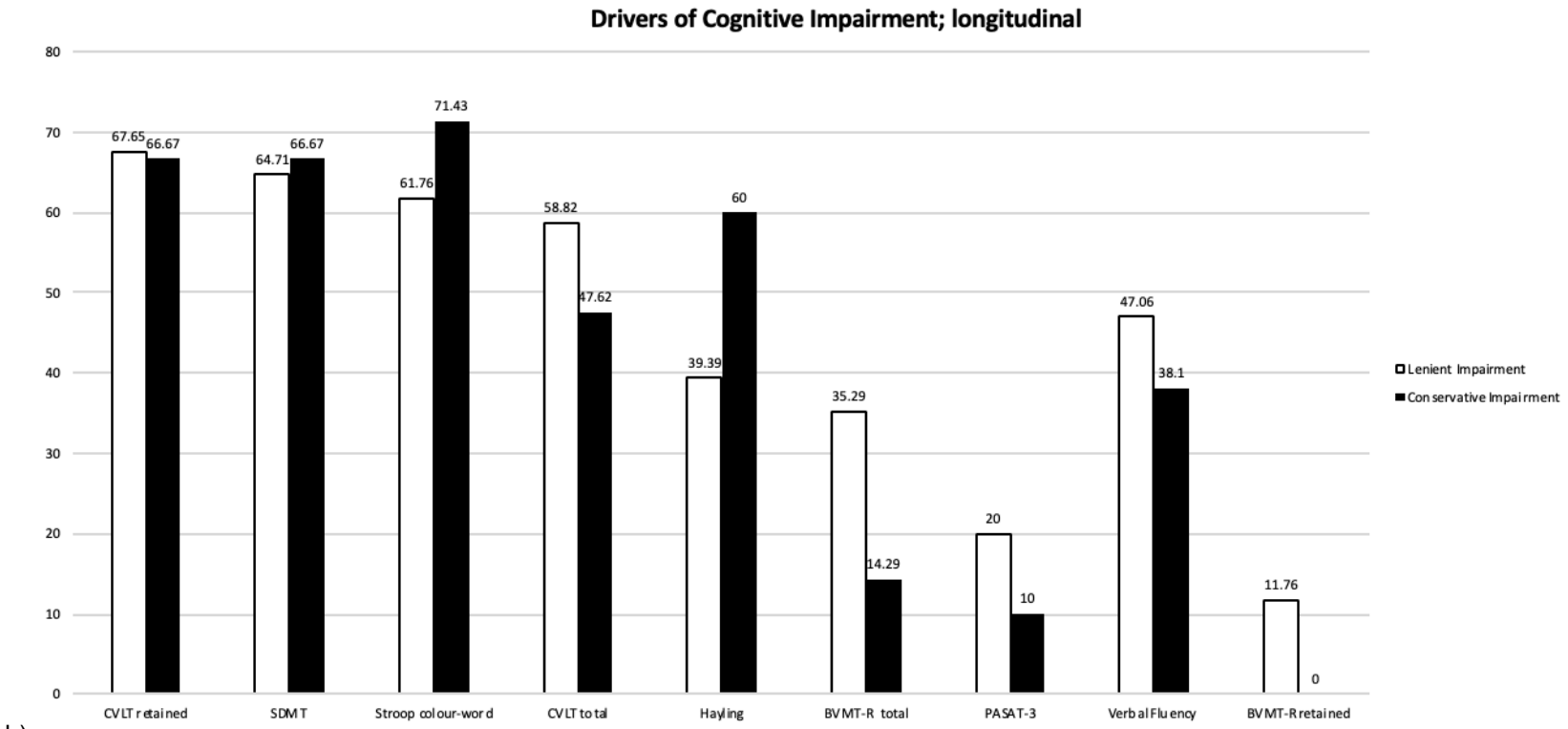
The bar charts in **figure 4.23** summarise the proportions of cognitive impairment by domain using the two different criteria; 57.6% for the lenient criteria (*≤-1.5SD on ≥2 domains) and 35.6% on the conservative criteria (≤-1.96SD on ≥2 domains).* As with baseline (**figure 4.20**); the sequence of impairment followed the same pattern regardless of criteria at the follow-up visit, verbal memory (36.4% lenient criteria, versus 20.3% conservative), then visual information processing speed (37.3% lenient criteria, versus 23.7% conservative), executive function (28.2% lenient criteria, versus 19.8% conservative), auditory information processing speed (10.2% lenient criteria, versus 3.4% conservative), and visual memory (13.6% lenient criteria, versus 2.5% conservative). At follow-up visit, the main outcome measures driving conservative criteria cognitive impairment in SPMS were the Stroop (71.43%), followed jointly by the delayed CVLT-II and the SDMT (66.67%) (**figure 4.23***).* The main drivers of lenient criteria cognitive impairment were the delayed CVLT-II (67.65%) and the SDMT (64.71%). As with the baseline evaluation (**figure 4.20**) the main differences are due to the stringency of the criteria in the same SPMS cohort. **Figure 4.24** highlights the percentages of cognitively impaired and preserved subjects using both z-score ≤-1.96SD and ≤-1.5SD on ≥2 domains criteria if missing subjects from the baseline visit are included. There is much less variation in the number of subjects who are impaired at follow-up from baseline (30% conservative definition, and 48.57% lenient definition at follow-up versus 30% and 47.1% at baseline respectively). The proportion of those cognitively preserved is also reduced. This suggests fewer differences in the cohort if accounting for missing subjects and therefore cross-sectionally the baseline and follow-up cohorts may have had more similarities if there had been no drop-outs (15.71% drop-out).

To summarise, as expected, there were significantly more cognitively impaired SPMS subjects at the follow-up timepoint with either criteria from baseline (≤-1.96SD on ≥2 domains p=0.011, and ≤-1.5SD on ≥2 domains p=0.006). There were, however, no significant differences between visit timepoints within the cognitively impaired or preserved cohorts.

Figure 4.23. Bar charts of percentage impairment rates by domain and the input of individual cognitive tests to overall cognitive impairment using two different criteria at the follow-up timepoint. a) percentage impairment by individual cognitive domains and overall b) individual test drivers of overall cognitive impairment by percentage input.



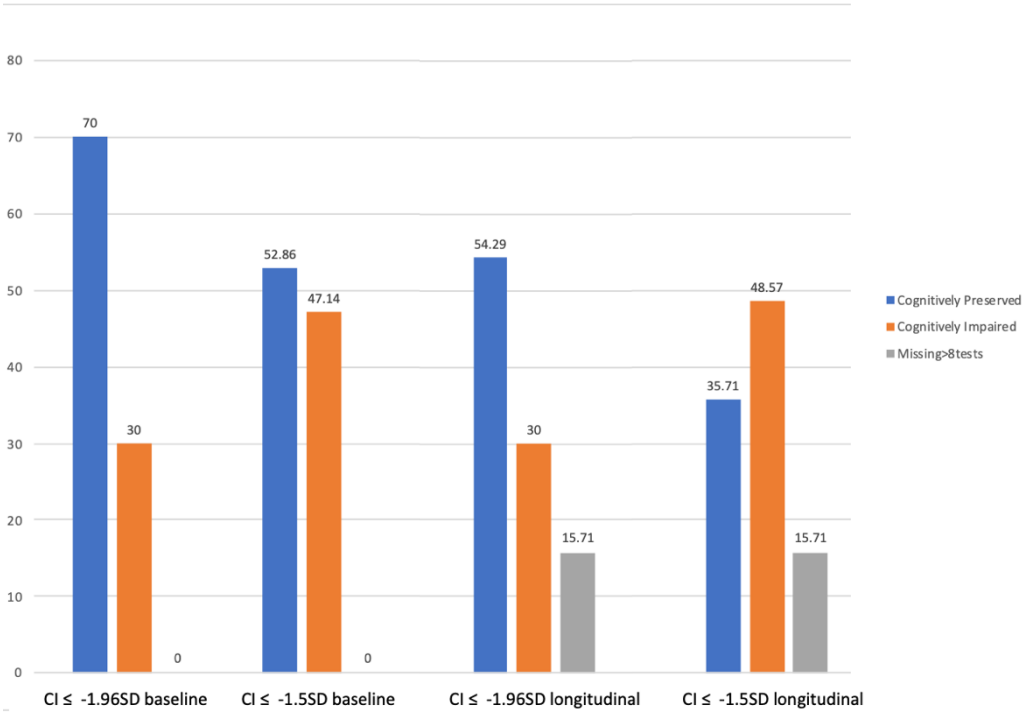
a)



b)

Cognitive impairment; lenient=z-score of ≤-1.5SDs on ≥2 domains(black) and conservative=z-score of ≤-1.96SDs on ≥2 domains(white).

Figure 4.24. Bar chart of percentages of cognitive impaired and preserved SPMS subjects over time including missing subjects.



4.5.3.4 Associations of cognitive decline over time: longitudinal analysis

I developed logistic regression models to look for associations of the dependent binary outcome; the development of cognitive impairment from a preserved state on either the conservative or lenient critical number of abnormal parameters criteria. Age, gender, and HADS anxiety were used a priori for the covariate variables based on the linear cross-sectional regression models per test at baseline (**section 4.3.3.2**). All demographic, physical, and cognitive variables were evaluated individually at baseline and follow-up timepoints (**table 4.3**), however only significant associations are presented.

Conservative critical number of abnormal parameters criteria.

14 subjects developed cognitive impairment from a preserved state using the z-score *≤-1.96SD on ≥2 domains criteria. The covariate model was not significant for age, gender, or HADS anxiety* (LR=1.5) and the overall predictive probability was an AUROC curve of 0.61 (**table 4.23**). Although the study was not powered to show an effect, differences in baseline MS-SMART drug arm significantly associated with cognitive decline over time (OR 2.36, p=0.01, 95% CI 1.20 to 4.62 LR=9.17) with a predictive probability of 0.75 AUROC. The greatest predictive probability was in the amiloride arm AUROC 0.96 (versus fluoxetine as base variable). An increase in the baseline MSFC score resulted in a 6.42 greater risk of cognitive decline from baseline to follow-up (OR 6.42, p=0.03, 95% CI 1.26 to 32.76 LR=8.73, AUROC 0.74). At follow-up a higher, i.e. worse, MSVQ-7 result at follow-up significantly increased the risk of cognitive impairment by 1.2 times (OR 1.2, p=0.04, 95% CI 1.01 to 1.42 LR=5.62 AUROC=0.71). The remainder of the significant follow-up timepoint variables were cognitive outcome measures, with significance at more than the 5% level. The most associated was the Stroop task with a single point increase accounting for a 9% lower chance of cognitive impairment developing from baseline visit (OR 0.91, p=<0.01, 95% CI 0.87 to 0.96, LR=23.05, AUROC=0.88) (**table 4.23**).

Table 4.23. Logistic regression model of cognitive decline in SPMS over time with a z-score of ≤-1.96SD on ≥2 domains. *Significance levels; bold font p≤ 0.05.*

Independent predictors of cognitive impairment at follow-up from cognitively intact -1.96SD (n=14)						
Predictors	**Odds Ratio**	**p-value**	**95% CI**		**LR**	**AUROC**
Age	1.05	0.35	0.95	1.16	1.50	0.61
Gender	0.90	0.89	0.20	4.08		
HADS anxiety	0.95	0.59	0.79	1.14		
BASELINE						
Drug arm	2.36	**0.01**	1.20	4.62	9.17	0.75
Fluoxetine	Omitted					
Riluzole	1.00				1.06	0.67
Amiloride	1.00				7.65	0.96
Placebo	1.00				2.42	0.68
MSFC	6.42	**0.03**	1.26	32.76	8.73	0.74
FOLLOW-UP						
MSVQ	1.20	**0.04**	1.01	1.42	5.62	0.71
CVLT-II	0.89	**<0.01**	0.82	0.96	13.25	0.79
CVLT-II z-score	0.31	**<0.01**	0.14	0.68	13.25	0.79
CVLT-II delayed	0.82	**0.02**	0.69	0.97	7.45	0.73
CVLT-delayed z-score	0.58	**0.02**	0.36	0.93	7.45	0.73
SDMT	0.89	**<0.01**	0.82	0.96	12.92	0.80
SDMT z-score	0.37	**<0.01**	0.19	0.72	12.92	0.80
Hayling	0.53	**0.01**	0.34	0.84	10.91	0.78
Hayling z-score	0.55	**0.01**	0.35	0.85	10.91	0.78
Verbal fluency	0.79	**<0.01**	0.67	0.92	13.84	0.79
Verbal fluency z-score	0.31	**<0.01**	0.14	0.68	13.84	0.79
Stroop	0.91	**<0.01**	0.87	0.96	23.05	0.88
Stroop z-score	0.16	**<0.01**	0.06	0.45	23.05	0.88

Lenient critical number of abnormal parameters criteria.

17 SPMS subjects developed cognitive impairment from a preserved state using the lenient definition threshold from baseline visit. As with the more stringent criteria, the covariate model was not significant (LR=3.07) and had a predictive probability of 0.62 AUROC (**table 4.24**). At baseline visit there were no non-cognitive variables associated with cognitive impairment. The most predictive and significant cognitive test at baseline was the BVMT-R for which an increase in score by one point increased the risk of cognitive decline over time by 16% (OR 1.16 p=0.01, 95% CI 1.04 to 1.29, LR=11.69, AUROC=0.76). At follow-up timepoint worsening by one point on the NFI, i.e. worse, led to a 20% increased chance of cognitive decline (OR 1.20, p=0.04, 95% CI 1.01 to 1.44, LR=9.53, AUROC=0.74). The remainder of the associated variables were cognitive outcome measures, of which the most predictive model (AUROC=0.8) included the CVLT-II (OR 0.9, p=0.01, 95% CI 0.84 to 0.97, LR=13.89) where a single point increase in follow-up score reduced the chance of cognitive impairment by 10% (**table 4.24**).

Table 4.24. Logistic regression model of cognitive decline in SPMS over time with a z-score of ≤-1.5SD on ≥2 domains. *Significance levels; bold font p≤ 0.05.*

Independent predictors of cognitive impairment at follow-up from cognitively intact -1.5SD (n=17)						
Predictors	**Odds Ratio**	**p-value**	**95% CI**		**LR**	**AUROC**
Age	1.04	0.47	0.94	1.14	3.07	0.62
Gender	0.75	0.70	0.18	3.12		
HADS anxiety	0.88	0.18	0.74	1.06		
BASELINE						
BVMT-R	1.16	**0.01**	1.04	1.29	11.69	0.76
BVMT-R z-score	2.48	**0.01**	1.27	4.86	11.69	0.76
SDMT	1.08	**0.03**	1.01	1.16	9.12	0.72
SDMT z-score	1.96	**0.04**	1.02	3.74	8.11	0.71
PASAT3	1.09	**0.02**	1.02	1.18	10.58	0.75
PASAT3 z-score	2.94	**0.02**	1.22	7.05	10.58	0.75
Hayling	1.60	**0.04**	1.02	2.49	7.81	0.72
Hayling z-score	1.61	**0.04**	1.02	2.55	7.81	0.72
Stroop	1.04	**0.04**	1.00	1.07	7.44	0.73
Stroop z-score	2.07	**0.04**	1.05	4.11	7.44	0.73
FOLLOW-UP						
NFI	1.20	**0.04**	1.01	1.44	9.53	0.74
BVMT-R	0.91	**0.02**	0.84	0.99	9.10	0.75
BVMT-R z-score	0.45	**0.02**	0.23	0.89	9.10	0.75
CVLT-II	0.90	**0.01**	0.84	0.97	13.89	0.80
CVLT-II z-score	0.35	**0.01**	0.17	0.73	13.89	0.80
CVLT-II delayed	0.85	**0.04**	0.73	0.99	7.60	0.72
CVLT-delayed z-score	0.64	**0.04**	0.41	0.99	7.60	0.72
SDMT	0.91	**0.01**	0.84	0.97	12.47	0.77
SDMT z-score	0.44	**0.01**	0.24	0.79	12.47	0.77
Verbal fluency	0.85	**0.02**	0.75	0.97	10.13	0.76
Verbal fluency z-score	0.47	**0.02**	0.25	0.86	10.13	0.76
Stroop	0.95	**<0.01**	0.91	0.98	14.10	0.79
Stroop z-score	0.34	**<0.01**	0.17	0.70	14.10	0.79

4.5.4 Discussion

This large SPMS cohort provides an overview of cross-sectional cognitive impairment and decline over time. This study is novel in that it aims to evaluate impairment by two cognitive impairment criteria, and thus allows a direct comparison of the criteria in a single population cross-sectionally and over time. The criteria chosen were felt to be the most robust and most frequently used in the literature (Fischer *et al.*, 2014); critical number of abnormal parameters. I chose a more lenient (≤-1.5SD on two or more domains) (Muhlert *et al.*, 2015), and more stringent (≤-1.96SD on two or more domains) threshold to allow further evaluation from the published review.

The main findings of this section is that a more stringent criteria leads to more sensitive evaluation of cognitive impairment in SPMS. Additionally, the main associations of this status are occupational and employment related factors. Baseline cognitive outcome scores predict the development of cognitive impairment at follow-up most from a preserved state.

Reviewing cognitive impairment in SPMS, regardless, of the criteria used, at baseline (n=70) and follow-up (n=59) visit the patterns of dysfunction in the cognitive domains were the same regardless of which criteria was used: verbal memory, then information processing speed, executive dysfunction, auditory information processing speed, and visual memory. This is in keeping with the literature (Muhlert *et al.*, 2013, 2014, 2015; Sumowski *et al.*, 2018). This confirms the disproportionate effects of pathology in SPMS on working memory and executive function (Huijbregts *et al.*, 2004; Connick *et al.*, 2013), above the general processing speed and episodic memory deficits seen in MS more broadly (Rocca *et al.*, 2015*a*).

Using the lenient criteria, overall there was a greater proportion of cognitive impairment (47.1%) versus 30% with the conservative criteria. This worsened at follow-up with 57.6% versus 35.6% impaired respectively in the two criteria cohorts. The percentage change between visits was not felt to be significant. Although studies have suggested a greater rate of overall cognitive

impairment in SPMS; 55%, there are key differences in demographic characteristics, in cognitive battery, and less stringent criteria may be utilised (Eijlers *et al.*, 2018).

Although not significant, the z-score ≤-1.96SD on ≥2 domains group shows a higher proportion of males versus females 8:13, compared to SPMS overall and the lenient threshold SPMS group, and this has been previously reported (Schoonheim *et al.*, 2012*b*). This supports possible positive hormonal effects of female sex on cognitive function (Amato *et al.*, 2019). IQ was higher in the preserved group overall, indicating the role of intellectual enrichment and cognitive reserve despite no differences in years of education (Sumowski *et al.*, 2010*a*). As in the literature, measures of fatigue were significantly higher in those with SPMS and cognitive impairment suggesting cognitive fatigue (Schwid *et al.*, 2002) regardless of criteria, but only predicted cognitive impairment with the conservative criteria in the logistic regressions model ($p=0.02$, AUROC 0.75 to 0.77). Additionally, there were significantly higher rates of retirement in the cognitively impaired groups ($p<0.05$) which support the role of employment status and cognitive performance which were highlighted by cognitive performance measures in this cohort (**section 4.3.4**) (Cadden *et al.*, 2014). This was supported by the logistic regression models (**tables 4.21 and 4.22**) which showed unemployed status as the most significant predictor of cognitive impairment regardless of criteria used. A longitudinal follow-up study showed that 73% of those not working at 7 years were cognitively impaired, compared to 45% being cognitively impaired at baseline (Ruet *et al.*, 2013*b*). Independent of motor disability, cognitive impairment is key to unemployment in MS (McCrone *et al.*, 2008). Of interest is the greater frequency of physical predictors of cognitive impairment with the more lenient criteria despite being the same SPMS cohort. The EDSS is associated with a 2.15 times higher rate of cognitive impairment per point increase in EDSS, but is not significant for the ≤-1.96SD on ≥2 domains criteria. This provides evidence for the lower sensitivity and specificity of a less stringent criteria, and the pitfalls of interpreting cognitive studies in the literature (Sumowski *et al.*, 2018).

The cross-sectional baseline prevalence of verbal memory impairment was 40% versus 25.7%, visual information processing speed 34.3% versus 24.3%, executive function 21.9% versus 10%,

auditory information processing 14.3% versus 8.6%, and visual memory 14.3% versus 7.1% in the ≤-1.5SD on two or more domains criteria and ≤-1.96SD on two or more domains criteria respectively. Often verbal and visual memory are combined as working memory as are the two components of information processing speed, again introducing more heterogeneity and diversity in prevalence rates in the literature (Fischer *et al.*, 2014; Strober *et al.*, 2014). These rates are in keeping with longitudinal SPMS cohorts (Connick *et al.*, 2013; Strober *et al.*, 2014; Chan *et al.*, 2017), although the FAB impairment in the MS-STAT1 study was significantly higher than that of working memory at 45% and has not been replicated here or elsewhere (Chan *et al.*, 2017). Although treatment effects were not powered for in this study, there were no significant differences in cross-sectional or follow-up rates of cognitive impairment for any of the MS SMART drug arms (Chataway *et al.*, 2020). Looking at associations of cognitive impairment, there were differences cross-sectionally. Overall, the delayed component of the CVLT-II (54.55% versus 76.19%), SDMT (51.52% versus 71.43%), Stroop (30.3% versus 42.86%), and CVLT-II (30.3% versus 38.1%) had the greatest inputs in the classification of cognitive impairment (≤-1.5SD versus ≤-1.96SD on two or more domains criteria) at baseline. This follows the patterns of working memory, information processing speed, and executive dysfunction key to SPMS (Connick *et al.*, 2013; Sumowski *et al.*, 2018). At follow-up visit the Stroop task was the biggest contributor to overall impairment using the conservative criteria. This would have been missed using the lenient criteria, and demonstrates the worsening of executive function in SPMS over time (Amato *et al.*, 2006*b*; Bergendal *et al.*, 2007). Perhaps using a more stringent criteria allows for more precise evaluation of cognitive deterioration over a shorter period of time which would be useful for research trials and studies (Connick *et al.*, 2013).

These differences between criteria not only lead to differences in prevalence rates of cognitive impairment but also considerably alter the pattern of associations with clinical outcomes (Fischer *et al.*, 2014). Therefore, this work highlights the need for consensus and uniform criteria for evaluating cognitive performance and impairment in SPMS and MS more generally. This will ensure the comparability of studies across the literature that evaluate cross-sectional and longitudinal changes in cognition over time and correlations with allied clinical and MRI metrics

(Rocca *et al.*, 2015*a*; Sumowski *et al.*, 2018). I suggest from this study's results using the ≤-1.96SD on two or more domains criteria to account for a purer evaluation of SPMS function and to allow greater sensitivity and specificity of associations. Given the frequency of executive dysfunction in SPMS, a "BICAMS-plus" protocol, as I have developed here, would be most sensitive and specific for this (Connick *et al.*, 2013; Chan *et al.*, 2017; Sumowski *et al.*, 2018).

To determine what predicts and associates with the development of cognitive impairment in SPMS, I designed logistic regression models of impairment at follow-up from a preserved state at baseline visit. 14 subjects developed cognitive impairment at follow-up using the conservative criteria, and 17 with the lenient criteria. Of interest, only the less stringent criteria associated with baseline cognitive outcome measures, and particularly with visuospatial working memory, information processing speed, and executive tasks. The conservative criteria suggested significant input from MS-SMART drug arm to the development of cognitive impairment, and particularly so in the amiloride arm (AUROC 0.96). In the cognitive performance **section 4.3**, Amiloride appeared to improve long-term working memory, i.e. recall after a delay with the BVMT-R. Perhaps, by the action on brain atrophy as shown in a small PPMS study (n=14) (Arun *et al.*, 2013). However, this must be interpreted cautiously due to the study not being powered to look for this effect. These changes are more likely due to multiple comparison testing due to the low numbers of subjects leading to the lack of definite conclusions. Better cognitive scores at follow-up intuitively associated with a lower chance of cognitive decline and more cognitive reserve regardless of criteria in keeping with the work of Sumowski et al (Sumowski *et al.*, 2010*a*, 2013).

A relevant critique is whether any measure of 'generalised' cognitive function leads to a lower specificity of MS cognition. Individual tests can be underpinned by more than one cognitive domain, which creates difficulty in defining the different domains. It is however useful when estimating prevalence, creating trial subgroups and investigating associations with measures of pathology, e.g. from neuroimaging. Grouping patients like this also leads to a heterogenous group who might have differing patterns of cognitive deficits. This may make it more difficult to understand the underlying mechanisms than if the research was based on more specific cognitive

phenotypes, working memory impairment and executive dysfunction for example (Sumowski *et al.*, 2018).

4.6 Conclusions

This chapter suggests and supports that specifically: working memory, information processing speed, attention and executive domains are key cognitive domains affected by SPMS (Connick *et al.*, 2013; Sumowski *et al.*, 2018).

The literature provides evidence that cognitive performance affects occupational and vocational status, and is also associated with depression, anxiety, and fatigue. In this study I show that attention, processing speed, and working memory domains are key to employment, and vice versa, with retirement and lower qualifications predicting lower SDMT scores. Higher IQ, being employed, and undertaking more premorbid leisure activities are associated with less worsening on executive and verbal memory tasks. This supports and furthers the evidence of the importance and interplay of intellectual enrichment and cognition (Sumowski *et al.*, 2010*b*, 2012; Briken *et al.*, 2014).

There is a strong association between physical measures of MS disability and cognitive outcomes (Strober *et al.*, 2014). Lower limb disability associations, i.e. the EDSS and T25FW, show predominance for SDMT. Therefore, the SDMT provides at least an adjunctive measure of clinical disability prediction in studies. Given the additional cognitive associations of the SDMT, I have shown that this a sentinel marker of cognitive function in MS (Van Schependom *et al.*, 2014; Benedict *et al.*, 2015). There were only cognitive significant predictors of SDMT worsening, and therefore the SDMT is a sensitive and specific marker of cognitive performance in SPMS. When reviewing worsening by the agreed consensus of 4 points, the SDMT is not associated with executive function which is a key deficit in SPMS. Therefore this study supports the use of the SDMT as an adjunctive cognitive measure of disability in composite clinical trial outcome markers (Benedict *et al.*, 2017; Goldman *et al.*, 2019), but perhaps a general review of SDMT worsening is required in SPMS. Another longitudinal study has suggested that over 5 years the SDMT associated with the EDSS in MS, but not significantly, and therefore was only suggested as an adjunct to the MSFC as a trial outcome measure (Brochet *et al.*, 2008).

Anxiety, but not depression was a significant independent predictor of tests of information processing speed, verbal working memory, and executive function. However, given that this cohort was selectively recruited not to have depression this interpretation requires some caution.

Overall, a more stringent approach to defining SPMS cognitive impairment is required. This prevents confounding of non-cognitive markers on sensitivity analysis when looking at a more lenient threshold. Information processing speed, verbal memory, and executive function contribute most to overall cognitive impairment, particularly when I used the most stringent z-score of -1.96SD or less on two or more individual tests from at least two cognitive domains. This indicates the requirement of a "BICAMS-plus" protocol to evaluate additional delayed working memory and executive deficits in SPMS. The associations with other metrics were also substantially linked to the definition of cognitive impairment used. In particular the odds of unemployment, a key contributor to lower rates of quality of life, were up to 11 times when patients were classified as cognitively impaired using the most stringent definition. Overall, grouped criteria for cognitive impairment may miss heterogeneous differences within the cohort, but they are vital for estimating prevalence rates, and to define trial cohorts to allow inter-study evaluation.

5 Resting state functional MRI and cognition in SPMS

5.1 Introduction

I have confirmed in **chapter 4** that the cognitive profile of SPMS has more fronto-executive deficits compared to other MS phenotypes (Sumowski *et al.*, 2018). What is less well understood is the underlying dynamic brain changes of SPMS with and without cognitive impairment in terms of functional connectivity (FC) of the brain (Guerra-Carrillo *et al.*, 2014, Rocca *et al.*, 2016*a*) (**section 4.3)**. A benefit of rs-fMRI is that by investigating the brain at rest, task performance is not affected by physical disability, which is prevalent in this study's SPMS cohort with median EDSS of 6.0 (Lee *et al.*, 2013; Barkhof *et al.*, 2014, Sbardella *et al.*, 2015*a*). I designed this advanced rs-fMRI experiment to look at whole brain FC in SPMS overall, and SPMS with and without cognitive impairment.

As described in **section 4.2**, this study cohort is advantageous as it is, to my knowledge, the largest pure SPMS cross-sectional cohort investigated by rs-fMRI in the literature to date and therefore provides a unique exploration of SPMS with and without cognitive impairment (**section 4.3.4 table 4.3)**. Rocca et al. 2018 had a cross-sectional mixed MS group with 41 SPMS subjects (Rocca *et al.*, 2018). There is no other longitudinal pure SPMS cohort investigated by rs-fMRI and therefore this is a novel experimental design (Enzinger *et al.*, 2016) (**section 4.3.4 table 4.3)**. Finally, I used a whole brain voxel-based analysis rather than a seed-based rs-fMRI approach, to evaluate global FC changes allowing for a more total view of FC changes in SPMS with and without cognitive impairment (Bijsterbosch *et al.*, 2017).

This chapter is divided into two main parts: in the first part I investigated rs-fMRI FC changes in SPMS when compared with heathy controls (**section 5.2**), and in the second part I looked at the classification, and development of cognitive impairment in SPMS (**section 5.3**).

By undertaking the following experiments, I aimed to investigate, in a large cohort of people with SPMS, the following objectives:

1. Differences in global rs-fMRI FC between SPMS and healthy controls cross-sectionally at two timepoints and the changes over time (**section 5.2**).
2. Differences in rs-fMRI FC in SPMS with and without cognitive impairment using two different critical number of abnormal parameters classifications of cognitive impairment, and the predictive FC changes of a preserved state at baseline leading to cognitive impairment in SPMS at the follow-up visit (**section 5.3**).

5.2 Between group global rs-fMRI FC changes in SPMS versus healthy controls

5.2.1 Introduction

Chapter 4, indicated key characteristic differences in cognition between SPMS and controls and how these change over time **(section 4.3)**. There is, however, little understanding of the underlying dynamic FC changes in SPMS (**section 3.3.2)**.

Rocca et al. has described specific changes within the hubs of the DMN in more progressive forms of MS. Within the anterior DMN; the anterior cingulate gyrus, medial prefrontal gyrus, and precentral gyrus have shown reduced FC in the SPMS group, with sparing of the anterior cingulate gyrus in PPMS (Rocca *et al.*, 2010*a*). These FC changes hypothetically indicate loss of functional and cognitive reserve as disability progresses (Rocca *et al.*, 2010*b*; Cruz-Gómez *et al.*, 2014; Schoonheim *et al.*, 2017). In a comparative study of RRMS and SPMS, both groups showed positive correlations of FC in the sensorimotor network (SMN) and DMN with the PASAT3 task, although the SPMS group had reduced anterior SMN and increased posterior DMN connectivity overall compared with RRMS (Basile *et al.*, 2014). Therefore, some of the FC changes in SPMS might relate to cognitive performance and reserve. FC reductions in the DMN, working memory network (WMN), SMN, and visual networks correlate with higher T2LL and EDSS, and reductions in the executive networks, and auditory networks in RRMS (Rocca *et al.*, 2012). Hippocampal resting FC also correlates with T2LL on fMRI (Rocca *et al.*, 2015*b*). These findings support

evidence that there are alterations in network FC as MS disability progresses, but are not based on global approaches (Rocca *et al.*, 2012).

What underlies these RSN changes and their meaning is therefore complex to interpret in the context of progressive disability in MS. FC changes in RSNs may represent compensatory or maladaptive mechanisms, however this may be too simplistic an interpretation given the range of FC changes, and therefore cumulative FC changes likely amalgamate with progression over time and lead to lesser network efficiency and collapse (Schoonheim *et al.*, 2015*b*). However, the differences in resting FC patterns with progressive MS phenotypes may indicate inadequate network recruitment as indicated by task based fMRI experiments (Loitfelder *et al.*, 2011). In a multimodal computational study, rs-fMRI changes along with NAWM DTI, and white matter lesion load together had an accuracy of 88% for differentiating neuromyelitis optica (NMO) from MS, and therefore there is an underlying clinical utility of rs-fMRI in MS (Eshaghi *et al.*, 2015).

There are few rs-fMRI longitudinal studies in MS cohorts to allow evaluation of changes in SPMS over time (Enzinger *et al.*, 2016) (**section 3.3.4, table 3.3**). FC has been shown to increase in the precuneus, and cuneus in relation to lesion accumulation in RRMS (Droby *et al.*, 2015), and decreased brain connectivity, using graph theory metrics, correlated with worsening of additional disability markers; the EDSS and MSFC, over time (Faivre *et al.*, 2016).

The hypothesis of this section is to evaluate between group differences of global rs-fMRI FC changes in SPMS versus healthy controls at a global brain level at two separate timepoints and to look at changes over time.

5.2.2 Methods

5.2.2.1 Study design

The design of this study, subject recruitment and clinical assessments have been summarised in **chapter 4**, **section 4.2**.

5.2.2.2 MRI acquisition

Subjects underwent single session MRI using a 3T Philips Achieva scanner (Philips Healthcare, Guildford, UK) with a 32 head-coil. The protocol included fast field echo-echo planar imaging, conventional T2-weighted (T2) and FLAIR images, which were acquired for lesion detection (**table 5.1**).

5.2.2.3 Structural MRI analysis

Lesion load evaluation, lesion filling, and tissue segmentation analysis:

Lesions were semi-automatically outlined using JIM software (JIM 7.0 Xinapse Systems, Leicester, UK) on the T2 images using FLAIR images for reference and were used to determine T2LL. Lesion masks were registered to the 3DT1 scan using the NiftyReg toolbox freely available at http://niftyreg.sf.net (Modat *et al.*, 2014) and filled according to recently developed methods (Prados *et al.*, 2016).

Cross-sectional structural imaging evaluation using normalisation of atrophy (SIENAX) (Smith *et al.*, 2001, 2002) was used to estimate total intracranial volume (TIV), normalised for individual head size. Brain and skull 3DT1 images were registered to the MNI 152 (Montreal Neurological Institute) atlas space to obtain the volumetric scaling factor for correcting for individual volume variations in head size (Smith *et al.*, 2002). The resulting 3DT1 images were segmented into whole brain volume (WBV), grey matter (GM), white matter (WM), and cerebrospinal fluid (CSF), while removing non-brain tissue voxels, using the Geodesic Information Flow (GIF) algorithm

(Cardoso *et al.*, 2015). Normalised grey matter fraction (NGMF) was calculated by dividing normalised grey matter volume by the normalised whole brain volume (NBV).

Table 5.1. MRI acquisition protocol

Acquisition	Fast field echo-echo planar imaging	3D T1-weighted volumetric	Dual echo PD/T2- weighted	T2-weighted FLAIR
Scanner	3T Philips Achieva	3T Philips Achieva	3T Philips Achieva	3T Philips Achieva
Coil	32-head coil	32-head coil	32-head coil	32-head coil
Slice orientation	axial-oblique	sagittal-oblique	axial-oblique	axial-oblique
Voxel size	RL (mm) 3 AP (mm) 3 isotropic	1x1x1 (mm^3)	RL (mm) 3 AP (mm) 3 isotropic	RL (mm) 3 AP (mm) 3 isotropic
Slice thickness (mm)	2.7	1	2.7	2.7
TE (ms)	35	3.1	13	120
TR (ms)	2600	6.9	2900	9500
Flip angle (o)	90	8		
FOV (mm2)	192 x 192	256 x 256	256 x 256	256 x 256
Slices	46	180 sagittal		
Volumes	120	n/a	n/a	n/a

5.2.2.4 Rs-fMRI analysis

Data pre-processing:

Individual subjects' rs-fMRI were pre-processed using FSL (FMRIB, 2000; Jenkinson *et al.*, 2012) tools and included motion correction, brain extraction, spatial smoothing using a Gaussian kernel of full-width-at-half-maximum (FWHM) of 5mm, and high pass temporal filtering equivalent to 120 seconds (0.008Hz) (**figure 5.1)**. Thereafter, manual inspection was undertaken using FSLview to evaluate for further motion or MRI artefacts (FMRIB, 2000; Jenkinson *et al.*, 2012). Individual rs-fMRI volumes were then linearly registered to the corresponding structural three dimensional (3D) T1 scan and subsequently to standard space (MNI152) using the NiftyReg toolbox (Modat *et al.*, 2014).

Resting State Network Identification:

The pre-processed rs-fMRI series, containing 120 time points for each subject, were temporally concatenated across subjects to create a single 4-dimensional dataset. This was then processed using Independent Component Analysis (ICA) with the Multivariate Exploratory Linear Optimized Decomposition into Independent Components (MELODIC) toolbox (Group-ICA) (Beckmann *et al.*, 2005; Damoiseaux *et al.*, 2006; Smith *et al.*, 2009; Cole *et al.*, 2010). ICA decomposed the 4-dimensional dataset into independent components (ICs), which resulted in spatial maps, each with an associated time course, (**figure 5.1**). Some ICs were identified as RSNs based on their power spectra and comparison to previous literature, while others were associated with noise and white matter artefact (**figure 5.2**) (Smith *et al.*, 2009).

Dual regression:

A non-parametric permutation test (dual regression) was then applied to compare the group-specific maps for each IC **figure 5.3**. Cross-sectionally, I tested the between group statistical differences of FC changes in SPMS versus healthy controls using different contrasts (baseline SPMS<baseline HC, baseline SPMS>baseline healthy controls, longitudinal SPMS<longitudinal HC, longitudinal SPMS> longitudinal HC) at baseline and follow-up visits respectively. Changes

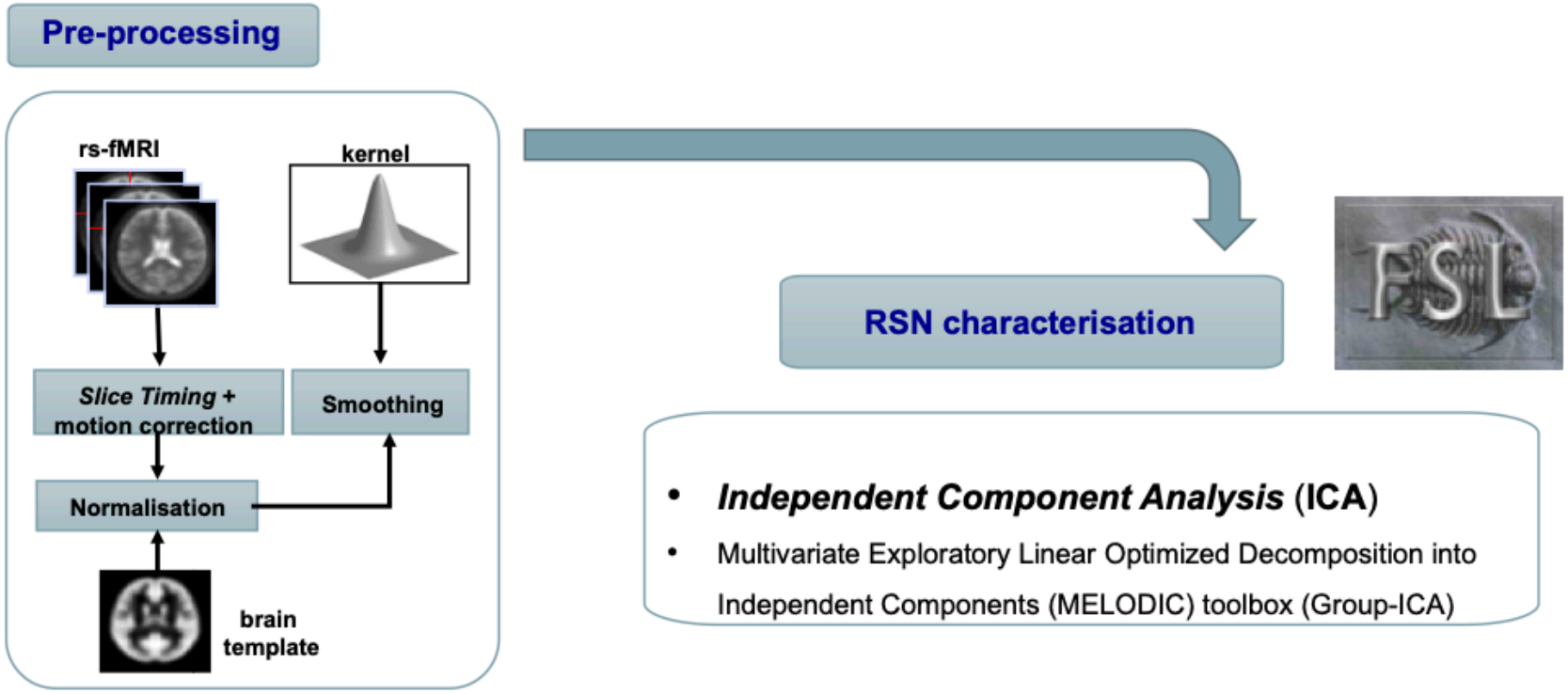
over time were evaluated using the following pairs of contrasts (baseline SPMS>longitudinal SPMS, baseline SPMS<longitudinal SPMS, baseline HC>longitudinal HC, baseline HC<longitudinal HC). I ran the dual regression analyses using age, gender, handedness, pIQ, years of education, MS SMART drug arm, normalised GM fraction, and T2LL as additional covariates in the baseline general linear model, and added interval between scans in days for the baseline-longitudinal comparisons.

The results were threshold-free cluster enhancement (TFCE) corrected. Voxels that survived a statistical threshold of $p \leq 0.05$ were considered significant and were saved as *tstatFC* maps. The 95% threshold is reported as the main finding. In addition, multiple comparison correction, i.e. Bonferroni adjustment of the p value, was trialled to assess its influence on the overall pattern of RSN FC changes. I therefore re-ran the dual regression analyses at 95%, 99%, and (Bonferroni) 99.86% thresholds accordingly, and these Bonferroni-corrected results are shown for comparison (**figure 5.8**).

Global map production:

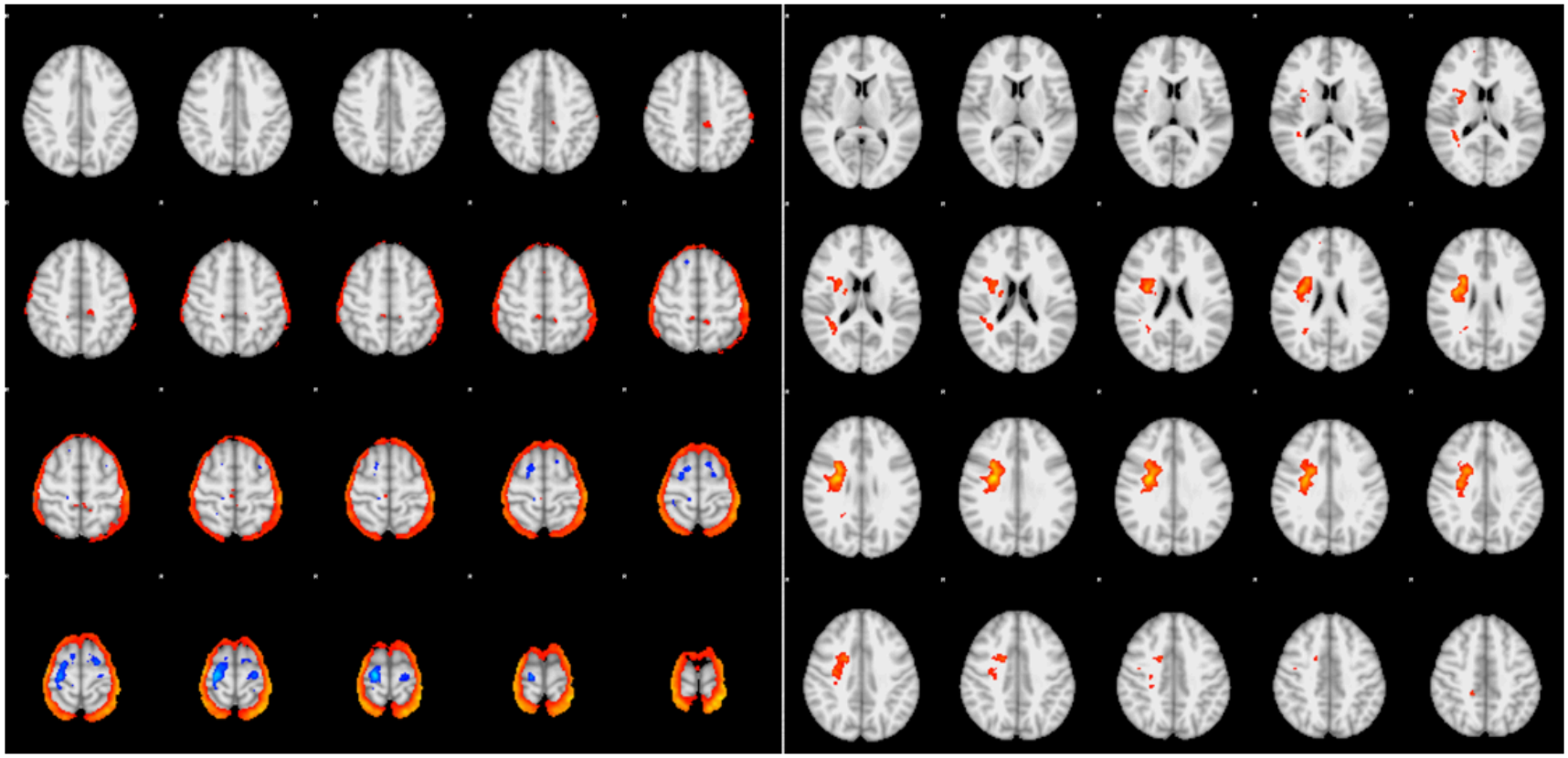
The TFCE corrected *tstatFC* maps were processed further by grey matter correction, for which I used an FSL tool (FMRIB, 2000; Jenkinson *et al.*, 2012), to correct for partial volume effects (**figure 5.4)**. The grey-matter corrected maps were visualised using the SPM xjView toolbox (https://www.alivelearn.net/xjview) to allow for any cluster voxel-thresholding for the finalised image (a threshold of 100 voxels per cluster was used for spatial map images). Additionally, anatomical locations of each cluster in the thresholded maps were reported using the xjView toolbox (https://www.alivelearn.net/xjview). I post-processed the thresholded maps into 3D images using the visualisation tool; BrainNet Viewer (Xia *et al.*, 2013).

Figure 5.1. Rs-fMRI pre-processing and RSN characterisation. Independent Component Analysis using the FSL toolkit was undertaken on the pre-processed images to determine the resting state networks (RSNs).



(Smith et al., 2009; Jenkinson et al., 2012).

Figure 5.2. Examples of artefacts from independent component analysis. Noise artefact (left) and white matter artefact (right).



I derived these images from subject data in this thesis.

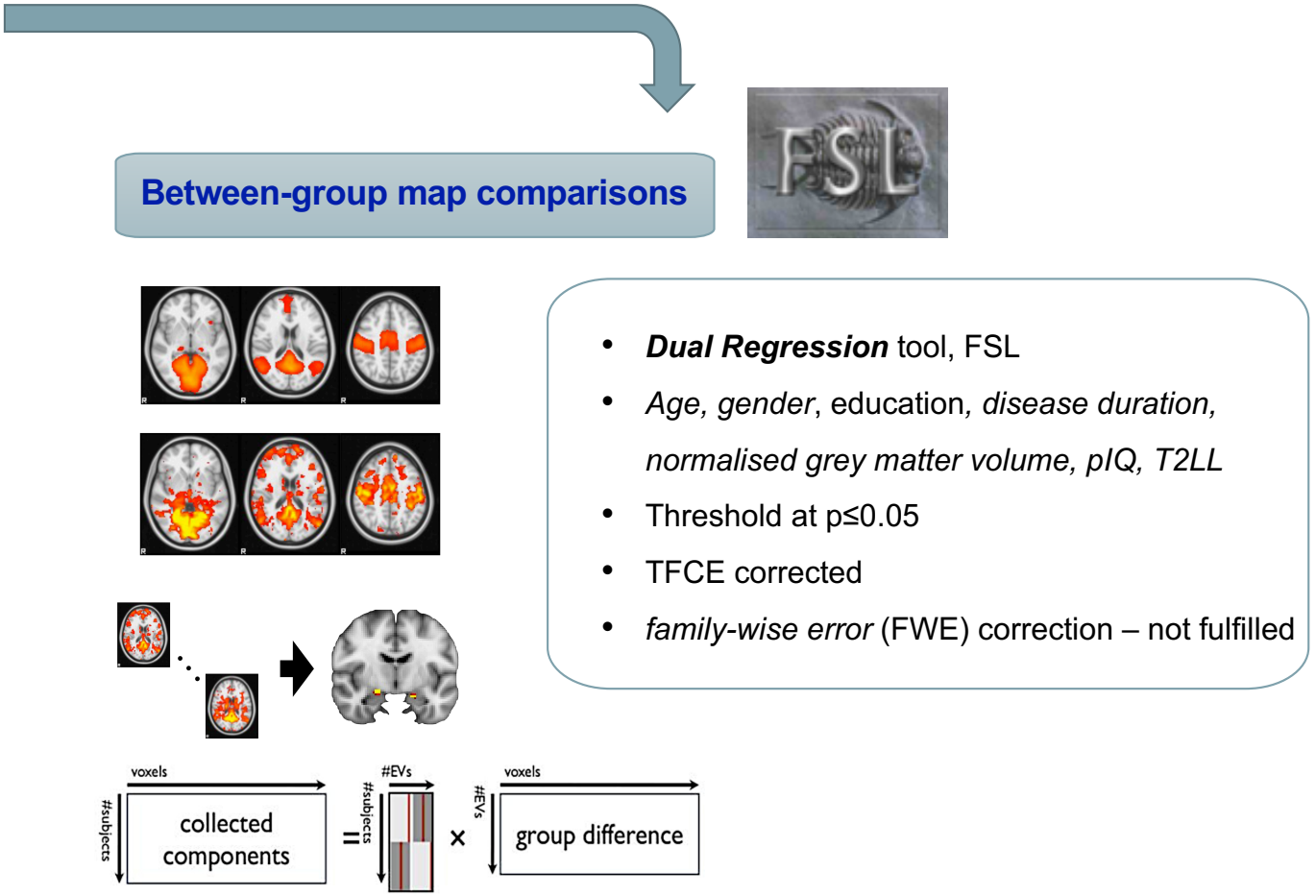
Global network analysis:

In order to study the FC changes within each RSN, and to establish a ranking of the networks, for each considered contrast, I calculated the percentage of altered voxels (% altered voxels) within each RSN. Specifically, the % altered voxels index was calculated as the number of altered voxels in the *tstatFC* map ($N_{tstatFC}$) with respect to the total number of voxels of the RSN map itself (N_{RSN}):

$$\% \text{ altered voxels} = \frac{N_{tstatFC}}{N_{RSN}}$$

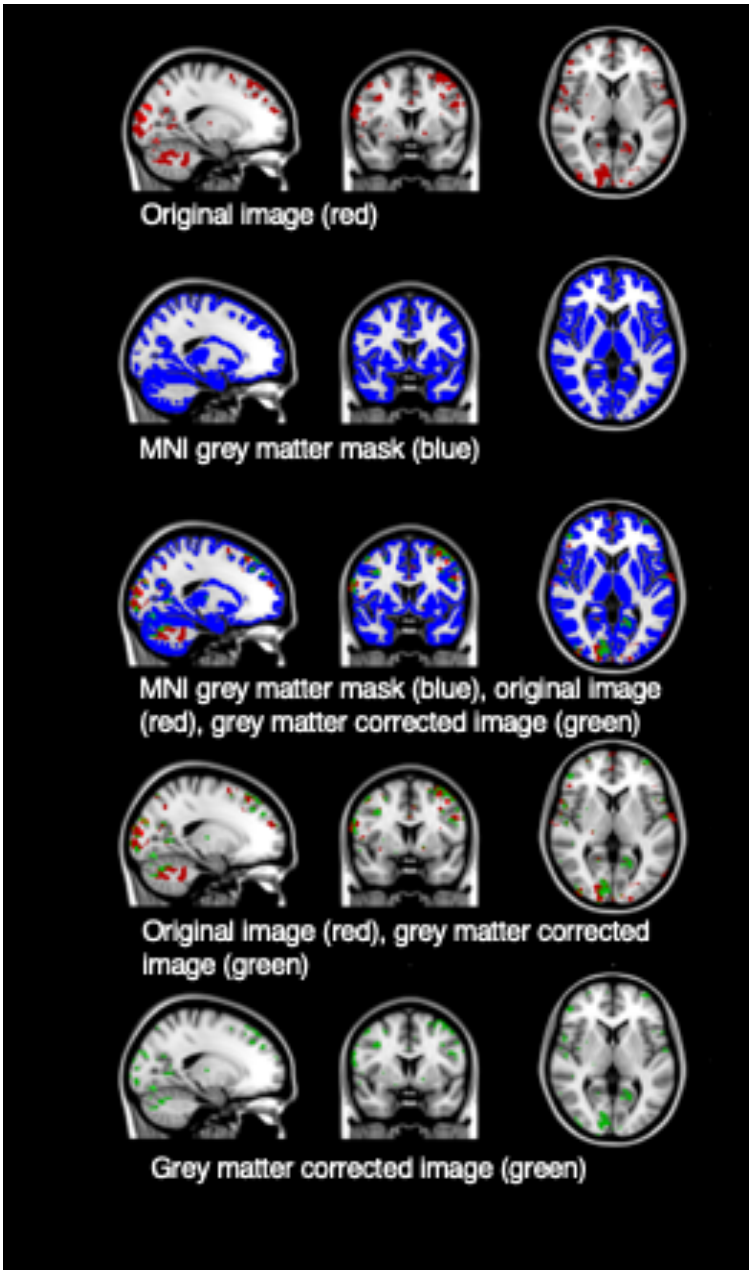
For each contrast, I was therefore able to rank the RSN alterations in terms of the changes in their % altered voxels. I have focused on reporting the RSNs with at least 5% of altered voxels in the global maps as the other changes could be due to noise, and so these are shown in the figures only, but not described in the text.

Figure 5.3. Dual regression analysis. After the creation of a general linear model, the dual regression tool in FSL was used to perform a non-parametric permutation test on the pre-processed rs-fMRI to review between group differences.



(Smith et al., 2009; Jenkinson et al., 2012).

Figure 5.4. Grey matter correction of the global map functional alterations.



I created these images using subject data from this thesis and the FSL tool, FSL Eyes (FMRIB, 2000).

5.2.2.5 Statistical Analysis

As per **section 3.3.2.2,** I performed the statistical analyses using Stata (SE 15.1 for Mac. Stata Corp, College Station, TX 77845, USA). Between group differences in demographic, clinical, and imaging variables were dependent on variable type (numerical, categorical, binary, normal distribution). T-tests were used to test differences in age. Scatterplots were used to look for non-linearities and normality of the variables. Categorical variable differences were tested with chi-squared tests if less then 2 variables, i.e. gender. Non-parametric Mann-Whitney U-tests were applied for group differences in education level, IQ, clinical indices (expanded disability status score, timed 25-foot walk test, and the nine-hole peg test) and covariate imaging metrics (NBV, normalised grey matter fraction, and T2LL). Neuropsychological measures were analysed as raw scores and z-scores versus healthy controls per cohort. Group differences in these neuropsychological tests were assessed at the individual test level by non-parametric Mann-Whitney U-tests following inspection of the residuals of the variables. The Kruskal-Wallis test was used to test for between group differences over time. Results were Bonferroni corrected for multiple comparisons and an exact probability value (p) statistical threshold of $p \leq 0.05$ was considered significant. Results are presented as frequency tables with analyses of differences between groups.

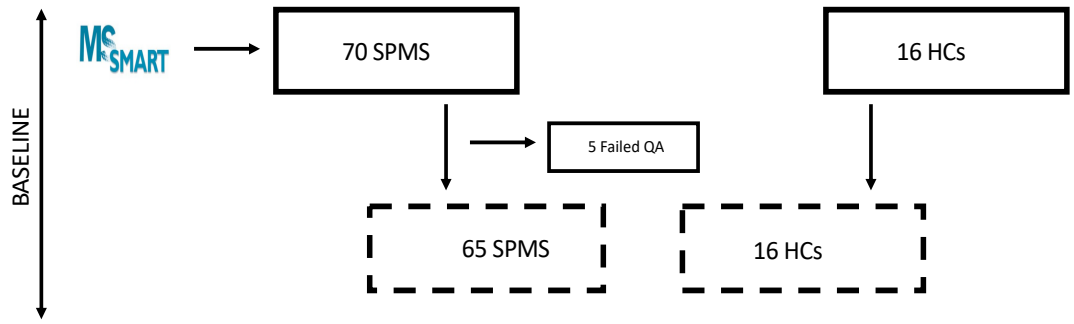
5.2.3 Results

5.2.3.1 Between group global rs-fMRI changes in SPMS versus healthy controls at baseline

The clinical-cognitive profile of the cohort.

The SPMS cohort was similar to that of **section 3.3** cross-sectionally, but 5 SPMS subjects were excluded during manual quality assessment due severe motion artefacts in the images (**figure 5.5**)**.** Of these 65 subjects, 24 subjects were assessed at their 24 week MS-SMART trial visit, instead of at baseline, due to study start date as described in **section 3.2**. The MSFC showed significant between group differences due to apparent improvements of the T25FW in the 24-week starters. Although, this is confounded by worse 9HPT scores in this same group, therefore the MSFC difference alone cannot be accurately interpreted (**table 5.2)**. Differences in the Stroop task between 0- and 24-week SPMS groups were due to an outlier in the 24-week group as shown in the scatterplot (**figure 4.4 section 3.3.3.1)**. Between group analysis of the 0- and 24-week SPMS groups therefore showed no significant differences in measures of interest and, as per **chapter 4**, these were pooled as a single SPMS cohort (N=65) here after.

Figure 5.5. Schematic of subjects in the baseline rs-fMRI analyses.



Solid border=clinico-cognitive cohorts, dashed border=rs-fMRI cohorts. SPMS=secondary progressive MS, HC=healthy controls, QA=quality assurance.

Table 5.2. Clinical, cognitive, and MRI characteristics of 0-week and 24-week SPMS groups at baseline.

		0-week SPMS (n=45)	24-week SPMS (n=20)	p
Gender (M:F)		10:35	5:15	ns
Age (years)		54.1±7.1	56.0±7.3	ns
Education (years)		15.8±2.9	15.0±2.6	ns
Disease duration (years)		21.1±8.7	24.1±10.0	ns
Progression (years)		7.8±4.6	7.7±5.8	ns
EDSS (median/range)		6.0 (4.0-6.5)	6.0 (4.0-6.5)	ns
T25FW (seconds)		17.9±16.1	15.2±8.0	ns
9-HPT (seconds)		34.3±17.0	37.8±15.9	ns
MSFC		-0.17±0.93	0.28±0.37	**≤0.05**
NART pIQ		114.0±8.4	115.4±6.8	ns
Visual memory	**BVMT-R total recalled**	12.3±6.6	13.8±6.5	ns
	BVMT-R retained (%)	92.3±35.1	89.8±35.3	ns
Verbal memory	**CVLT-II total**	43.6±12.1	47.3±9.0	ns
	CVLT-II delayed	9.3±4.2	10.2±3.7	ns
Auditory information processing speed	**PASAT3**	39.8±11.4	43.5±12.3	ns
Visual information processing speed	**SDMT**	46.0±13.3	50.7±6.6	ns
Executive function	**Hayling task**	6.2±1.3	6.1±1.6	ns
	Verbal Fluency	14.2±4.1	16.0±6.1	ns
	Stroop C-W	65.5±22.3	79.8±21.0	**≤0.05**
NBV (ml)		1423.1±82.5	1410.8±56.6	ns
GM volume (ml)		842.0±46.0	836.7±32.5	ns
T2LV (ml)		121.0±113.1	108.0±83.2	ns

Values are presented as ratio for gender, median and range for EDSS, and means ± standard deviation for all other measures. Significant differences are in bold font if p≤0.05, ns = not significant.

In the pooled SPMS versus healthy control between group comparison a total of 65 SPMS and 16 healthy controls were considered (**table 5.3**). Both cohorts were well matched for age, gender, years of education, and predicted IQ. The SPMS group had median EDSS of 6.0 and a long disease duration of 22±9.1 years. As per **section 3.3**, there were significant differences, as expected, in group comparisons for the CVLT-II, SDMT, Verbal fluency, Stroop, NBV, and NGMF with lower values in the SPMS versus control groups. However, PASAT3 and BVMT-R were relatively preserved in the SPMS group, but, as described in **section 3.3,** these outcome measures have been shown to have least impact on the cognitive profile of SPMS in this study. I have summarised key descriptive demographic and clinical scores for the healthy control and SPMS groups in **table 5.3.**

Table 5.3. Clinical, cognitive, and MRI characteristics, and group differences between healthy controls and SPMS.

		HC (n=16)	SPMS (n=65)	p
Gender M:F		4:12	15:50	0.87
Age (years)		55.75±4.72	54.68±7.15	0.69
Years of education		17.56±4.92	15.57±2.84	0.07
NART IQ		114.88±7.65	114.45±7.89	0.78
Disease duration from first symptom		na	22.02±9.14	na
EDSS		na	5.69±0.84	na
9HPT (sec)		na	6.0 (4.0-6.5)	na
T25FW (sec)		na	17.11±14.07	na
MSFC score		na	-0.03±0.82	na
MSIS29v2		na	65.14±15.66	na
Visual Memory	**BVMT-R trials 1-3**	15.5±6.21	12.77±6.57	0.15
	BVMT-R trials 1-3 z-score	na	-0.44±1.06	0.15
	BVMT-R retained	99.43±39.47	91.52±34.9	0.46
	BVMT-R retained z-score	na	-0.2±0.88	0.46
Verbal Memory	**CVLT-II trials 1-5**	55.06±10.63	44.77±11.25	**<0.01**
	CVLT-II trials 1-5 z-score	na	-1.04±1.11	**<0.01**
	CVLT-II delayed	12.94±2.29	9.58±4.03	**<0.01**
	CVLT-II delayed z-score	na	-1.46±1.76	**<0.01**
Information Processing Speed	**PASAT3**	46.5±8.82	40.94±11.68	0.08
	PASAT3 z-score	na	-0.34±0.97	0.08
	SDMT	59.94±9.35	47.45±11.77	**<0.01**
	SDMT z-score	na	-1.34±1.26	**<0.01**
Executive Function	**Hayling test**	6.62±1.02	6.12±1.4	0.24
	Hayling test z-score	na	-0.49±1.36	0.24
	Verbal fluency	17.77±4.92	14.68±4.8	**0.02**
	Verbal fluency z-score	na	-0.65±1	**0.02**
	Stroop	87.31±20.04	69.59±22.72	**0.01**
	Stroop z-score	na	-0.88±1.13	**0.01**
NBV (ml)		1476.81±61.49	1419.33±75.27	**0.01**
GM volume (ml)		843.55±27.16	840.37±42.14	1.00
NGMF		0.57±0.01	0.59±0.01	**<0.01**
T2LV (ml)		na	116.71±104.38	na

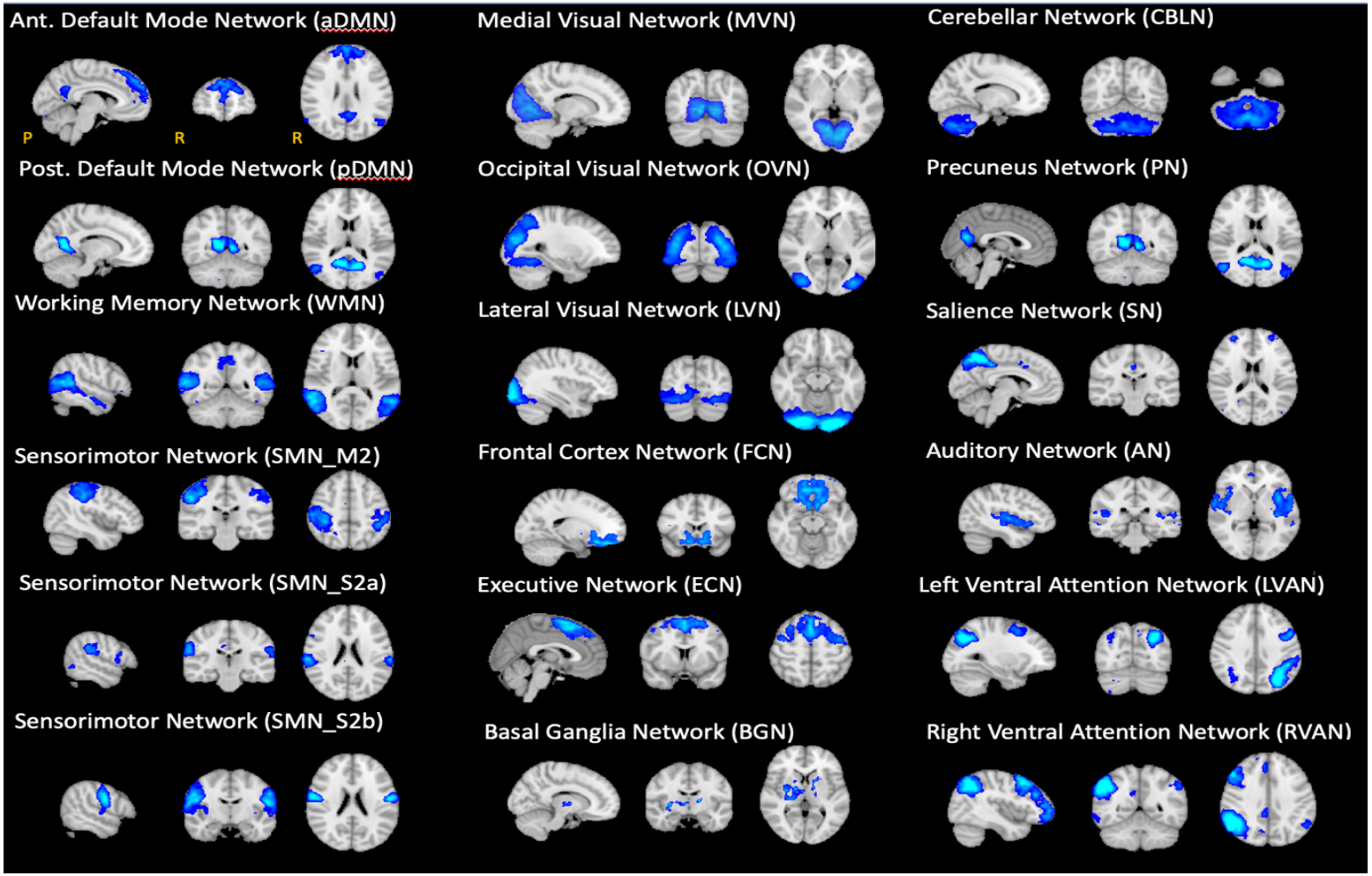
Values are presented as ratio for gender, median and range for EDSS, and means ± standard deviation for all other measures. Significant differences are in bold font if p≤0.05, na=not applicable.

RSN identification.

ICA processing of the rs-fMRI images identified 61 independent components. Of these I manually classed 18 as RSNs; denoted by their spatial pattern and low frequency spectra (Damoiseaux *et al.*, 2006; Beckmann *et al.*, 2009; Cole *et al.*, 2010; Castellazzi *et al.*, 2014). The remaining ICs were due to noise, partial volume effects from CSF, and white matter artefacts (Gour *et al.*, 2014) (**figure 5.2)**.

The 18 RSNs which I identified were the: Medial visual network (MVN), occipital visual network (OVN), lateral visual network (LVN), cerebellar network (CBLN), precuneus network (PN), anterior default mode network (aDMN), posterior default mode network (pDMN), salience network (SN), executive network (EN), sensorimotor network (SMN_S2a), sensorimotor network (SMN_S2b), sensorimotor network (SMN_M2), right ventral attentional network (RVAN), left ventral attentional network (LVAN), frontal cortex network (FCN), auditory network (AN), working memory network (WMN or LNp), and basal ganglia network (BGN) (**figure 5.6**).

Figure 5.6. Resting State Networks identified using independent component analysis.



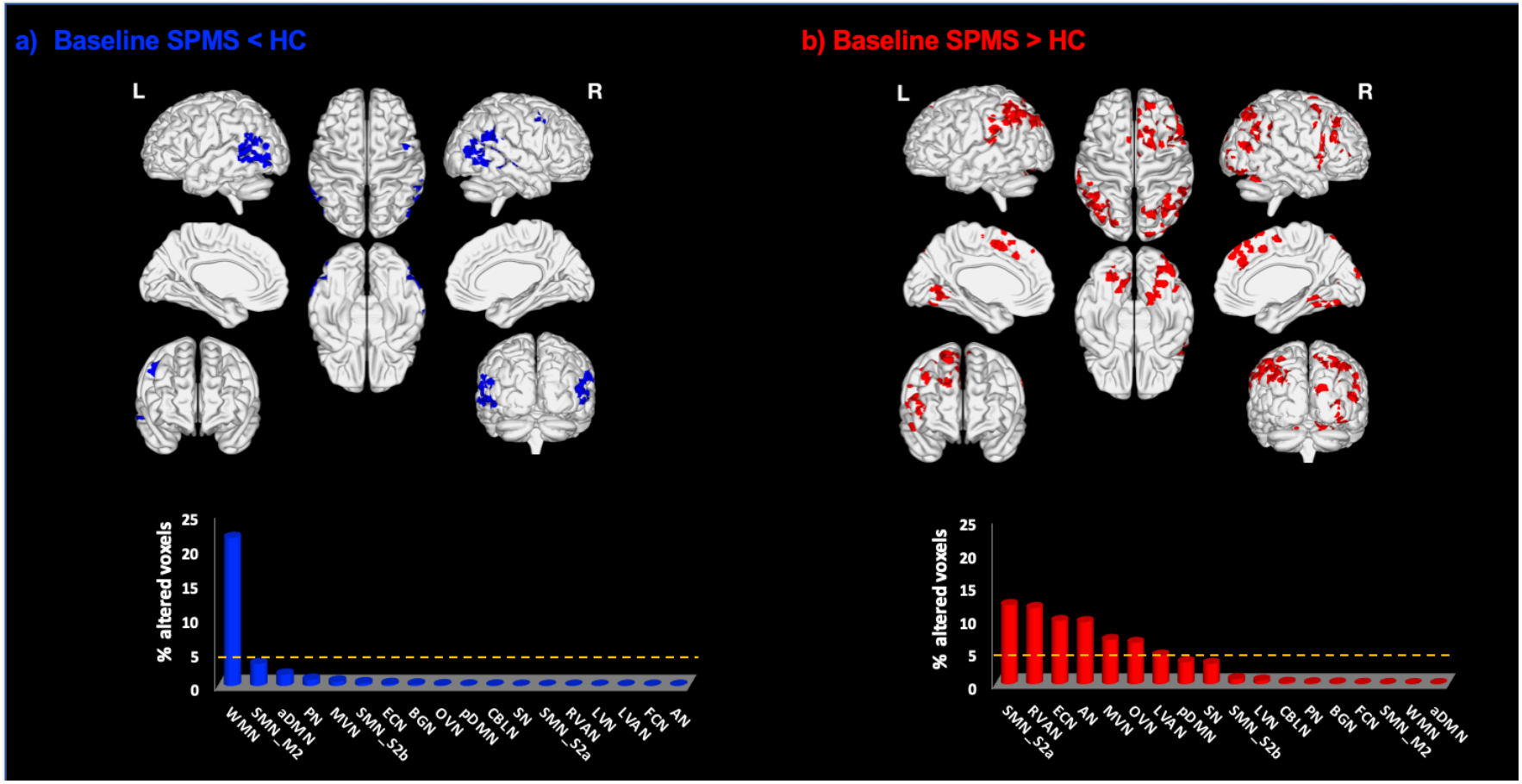
Images are shown according to radiological convention.

Between group FC changes in SPMS versus healthy controls at baseline.

I found both significantly increased and decreased FC ($p \leq 0.05$ TFCE-corrected) within RSNs in the SPMS group when compared to healthy controls **(figure 5.7**). Out of the 18 identified RSNs, 11 showed reduced FC in SPMS when comparing to HCs (SPMS<HC) ($p \leq 0.05$ TFCE-corrected). However, only a cognitive RSN; the working memory network (WMN), showed reduced FC in the SPMS group (SPMS<HC) with more than 5% altered voxels. From an anatomical perspective, these functional alterations largely comprise the middle temporal gyrus, superior temporal and frontoparietal regions (**figure 5.7a**). There were significant FC increases in 16 out of 18 RSNs in the SPMS group versus healthy controls (SPMS>HC) ($p \leq 0.05$ TFCE-corrected). Extensive alterations were detected in attentional and executive cognitive networks (RVAN, ECN, LVAN), sensory (SMN_S2a, AN) and visual networks (MVN, OVN). Anatomically these FC changes relate to the frontal and parietal lobes, and the middle occipital gyrus (**figure 5.7b**). See **table 5.4** for a summary of percentage alterations in voxels per RSN for these between group analyses.

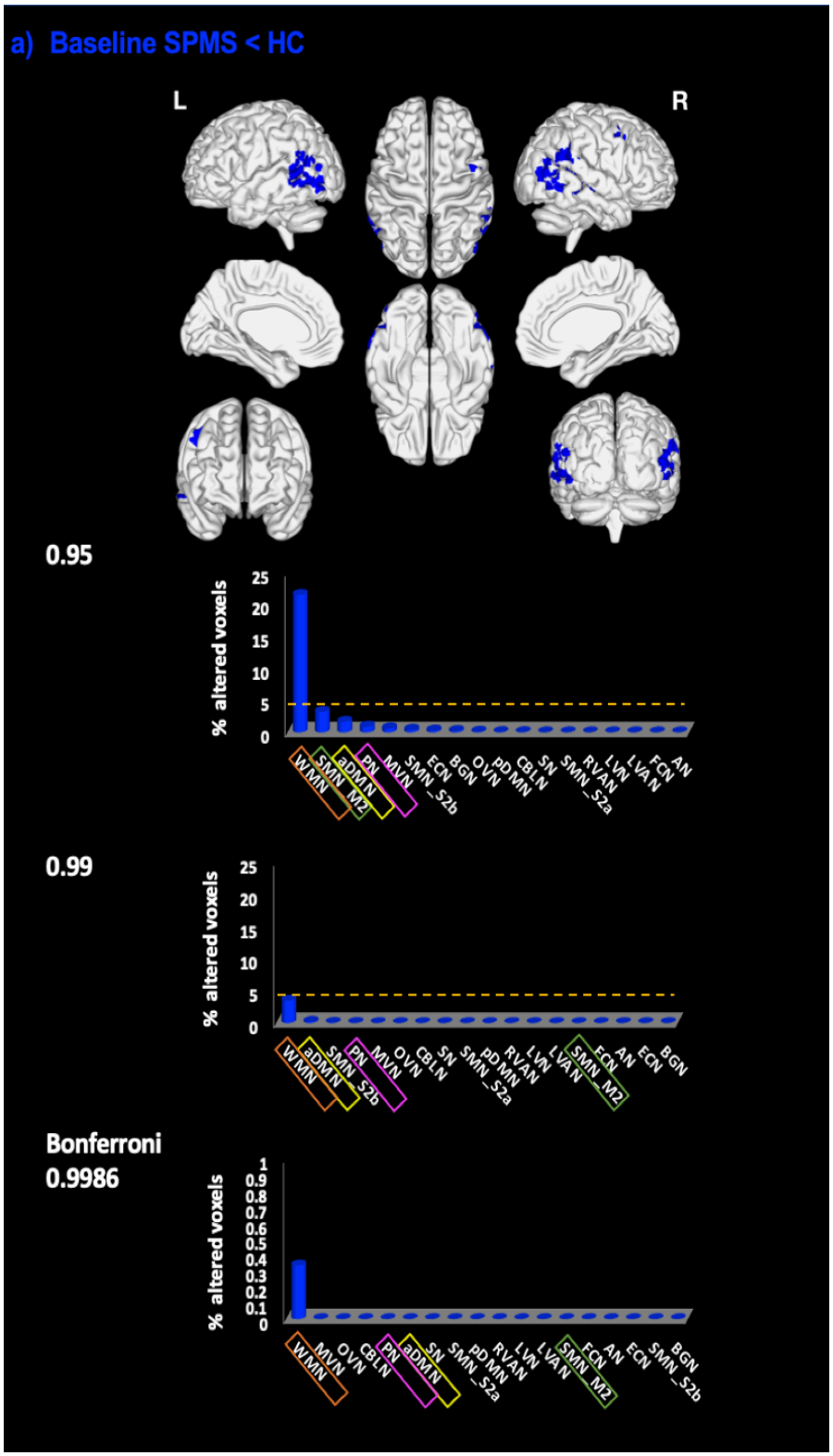
When Bonferroni adjustment of the p value was used, the overall percentages of altered voxels decreased substantially (**figure 5.8).** The WMN showed consistent FC reduction, and the RVAN showed a persistent FC increase in the number of altered voxels in the SPMS group when compared with the control group. Despite having decreased % alterations of the voxels, the overall spatial pattern of FC changes in the key networks was similar. Therefore, for the subsequent rs-fMRI analyses in this section, a threshold of $p \leq 0.05$ TFCE-corrected is used.

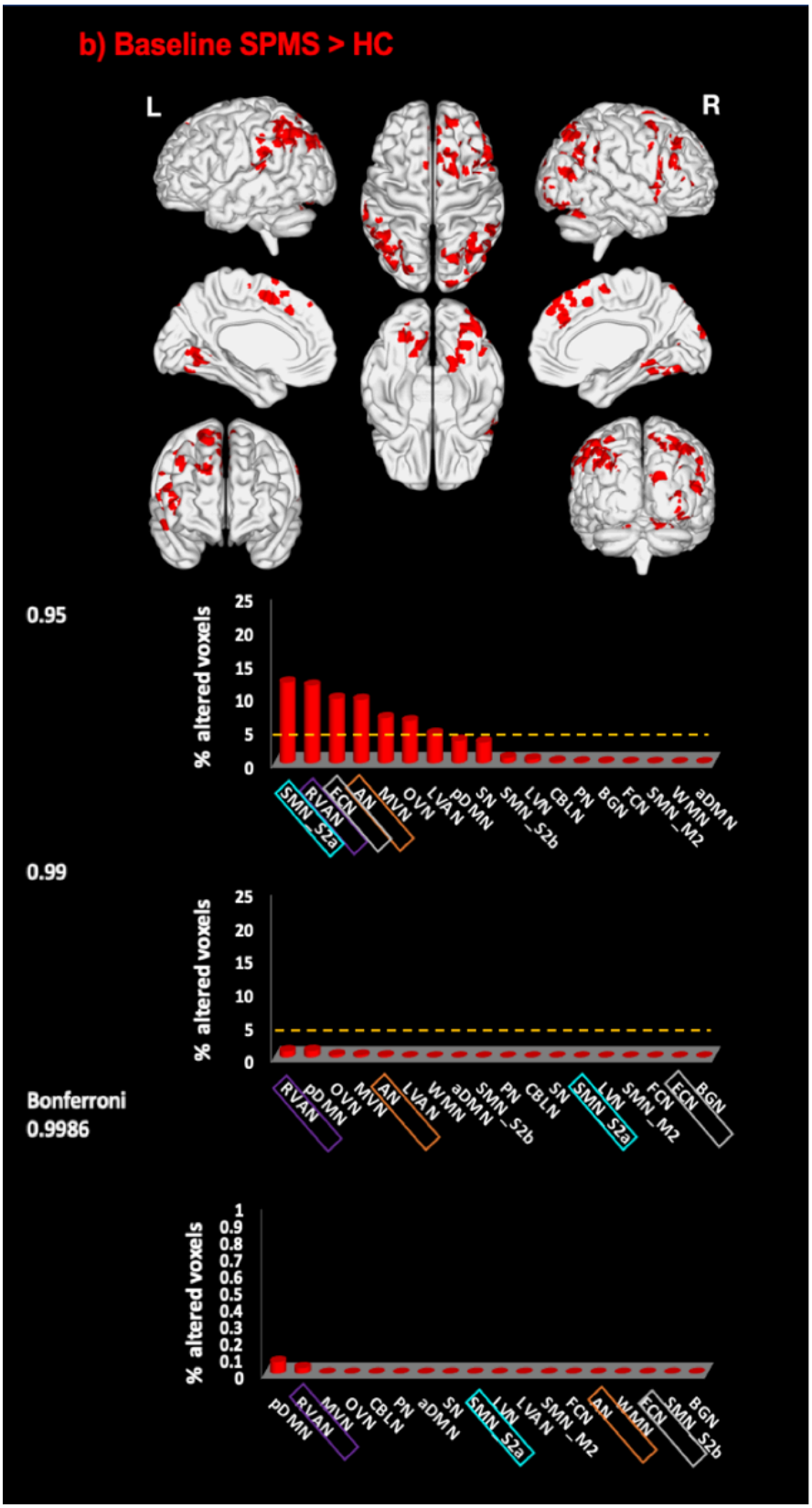
Figure 5.7 Between group FC changes in SPMS versus healthy controls at baseline. a)3D global map of (blue) brain RSNs showing decreased FC in SPMS versus HCs (SPMS<HC). The bar chart of % altered voxels shows that the greatest FC reduction was in the working memory network. b) 3D global map highlights which RSNs (red) had increased FC in SPMS versus HCs (SPMS>HC). The bar plot indicates that the sensorimotor and right ventral attentional networks had the greatest voxel alterations.



Yellow dashed lines represent those changes above and below the 5% level. Images are shown according to radiological convention. Percentage altered voxels=% altered voxels. FC changes are significant at the p≤0.05 TFCE-corrected threshold

Figure 5.8. Multiple comparison correction of FC changes in SPMS versus healthy control groups at baseline. a) Baseline SPMS<HC. b) Baseline SPMS>HC. FC changes for different correction thresholds; 0.95 and 0.99, and bonferroni (0.9986) correction for the 18 RSNs identified. Coloured boxes represent the top 4 altered networks.





Yellow dashed lines represent those changes above and below the 5% level. Images are shown according to radiological convention. Percentage altered voxels=% altered voxels.

Table 5.4. Summary of FC changes at baseline between SPMS versus healthy control groups.

Healthy Controls versus SPMS			
SPMS<HC RSN FC	**% voxel alterations**	**SPMS>HC RSN FC**	**% voxel alterations**
WMN	21.4	**SMN_S2a**	12
SMN_M2	3.2	**RVAN**	11.5
aDMN	1.6	**ECN**	9.6
PN	0.9	**AN**	9.4
MVN	0.6	**MVN**	6.7
SMN_S2b	0.4	**OVN**	6.2
ECN	0.3	**LVAN**	4.3
BGN	0.2	**pDMN**	3.3
OVN	0.1	**SN**	3.1
pDMN	0	**SMN_S2b**	0.8
CBLN	0	**LVN**	0.5
SN	0	**CBLN**	0.2
SMN_S2a	0	**PN**	0.1
RVAN	0	**BGN**	0.1
LVN	0	**FCN**	0
LVAN	0	**SMN_M2**	0
FCN	0	**WMN**	0
AN	0	**aDMN**	0

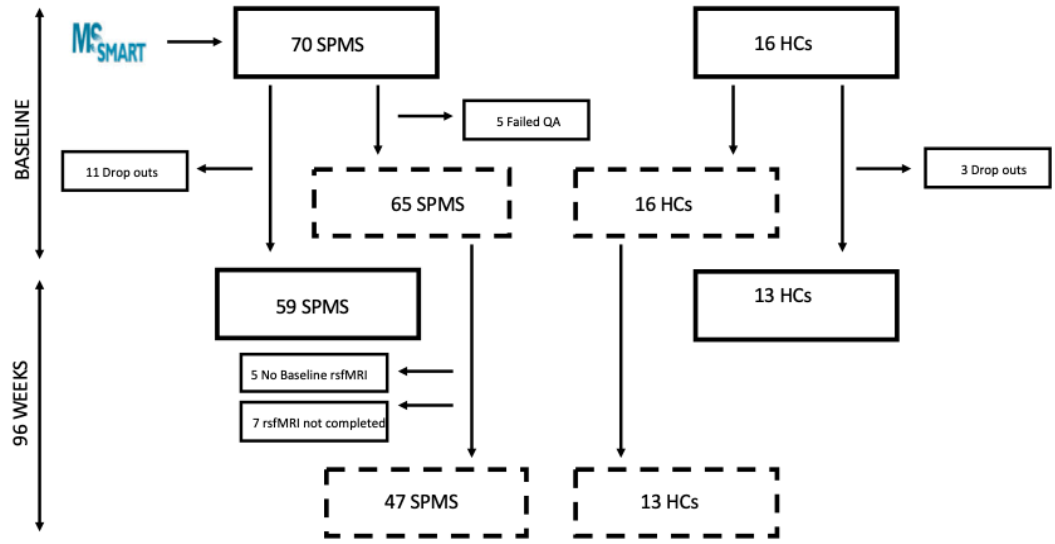
*Percentage alterations of voxels (% altered voxels) are presented as percentages (%) hierarchically. See **figure 5.6** for abbreviations of RSNs.*

5.2.3.2 Between group global rs-fMRI changes in SPMS versus healthy controls at the follow-up visit and within group changes over time

Changes in the clinical-cognitive cohort over time.

From baseline, 27.7% of SPMS and 18.8% of control subjects dropped out over time (**figure 5.9**). Of these drop-outs, 5 SPMS subjects did not complete MRI imaging and 7 SPMS subjects did not complete rs-fMRI scanning at follow-up and therefore were not including in the follow-up cohort. A further SPMS subject was excluded from the rs-fMRI follow-up cohort due to significant motion degradation. The 3 healthy control subjects dropped out as one was unable to undertake the visit in the scheduled time interval due to illness, and two could no longer commit to the study visit due to distance (**chapter 4, figure 4.2**). There were 47 SPMS and 13 healthy controls evaluated at the follow-up timepoint (**figure 5.9**).

Figure 5.9. Schematic of subjects in the rs-fMRI analyses at baseline and follow-up timepoints.



Solid border = clinico-cognitive cohorts, dashed border = rs-fMRI cohorts.

Cross-sectional between group differences at the follow-up visit, indicated in **table 5.5**, parallel those at baseline. The core demographics are well matched in terms of age, gender, education, and IQ. There are key expected differences in working memory, information processing speed, and executive function in SPMS which are not reflected by the BVMT-R, PASAT3, and Hayling task. These tasks have been shown to have least between group differences in SPMS versus controls in **section 3.3**. Controls had a shorter interval between visits in days, by a mean of 5 days, but as explained in **section 3.2,** this was due to the MRI scanner upgrade. NBV and NGMF are again expectedly significantly ($p<0.01$) higher in the control group; NBV 1449.61±61.66ml and 1419.97±63.13 ml, and NGMF 0.58±0.01 and 0.58±0.05 in the control versus SPMS groups respectively. Between visits, there was significant improvement in the BVMT-R in both cohorts. The BVMT-R improved by 7.84 $p=0.02$ in controls, and by 7.08 $p=<0.01$ in SPMS subjects. This likely represents the inflated composite scoring of this task and upper limb reliance as explained and illustrated in **section 3.4**. In the SPMS group subjects provided evidence of increase in subjective physical and psychological well-being by improvements in the MSIS29v2, mean score change of 9.26 $p=0.01$, mirroring those findings in **section 3.4**.

Table 5.5. Clinical, cognitive, and MRI characteristics of healthy control and SPMS groups at the follow-up timepoint and changes over time.

		Healthy controls (N=13)				SPMS (N=47)				
		BASELINE	**FOLLOW-UP**	**change**	**p**	**BASELINE**	**FOLLOW-UP**	**change**	**p**	**chi-2**
Gender M:F		0.77±0.44	0.77±0.44	na	na	10:37	10:37	na	na	0.89
Age (years)		55.99±4.63	57.8±4.63	1.81	0.33	55.13±6.86	56.96±6.92	1.83	0.2	0.88
Interval (days)		661.15±22.34	661.15±22.34	na	na	666.98±66.86	666.98±66.86	na	na	**0.02**
Years of education		18.04±5.13	18.04±5.13	na	na	15.51±3.07	15.51±3.07	na	na	na
NART IQ		115.69±7.85	115.69±7.85	na	na	114.87±4.99	114.87±6.99	na	1	na
Disease duration		na	na	na	na	22.74±9.33	22.74±9.33	na	na	na
EDSS		na	na	na	na	6.0 (4.0-6.5)	6.0 (4.0-6.5)	**-0.02**	0.64	na
9HPT (sec)		na	na	na	na	34.68±15.29	37.04±15.42	2.36	0.46	na
T25FW (sec)		na	na	na	na	16.37±14.84	25.03±36.29	8.66	0.27	na
MSFC score		na	na	na	na	0.1±0.55	-0.16±1.19	**-0.26**	0.55	na
MSIS29v2		na	na	na	na	64.04±16.77	73.3±15.81	9.26	**0.01**	na
Visual Memory	**BVMT-R 1-3**	15.62±6.55	23.46±8.56	7.84	**0.02**	12.6±6.5	19.68±8.52	7.08	**<0.01**	0.19
	BVMT-R 1-3 z	0.02±1.06	0±1	**-0.02**	0.86	-0.47±1.06	-0.44±1	0.03	0.69	0.19
	BVMT-R retained	99.51±43.06	104.03±27.81	4.52	0.68	91.44±35.32	95.64±17.45	4.2	0.66	0.19
	BVMT-R retained z	0±1.09	0±1	0	0.82	-0.2±0.89	-0.3±0.63	**-0.1**	0.08	0.19
Verbal Memory	**CVLT-II 1-5**	57.08±10.84	58.54±10.01	1.46	0.84	45.26±10.5	47.6±11.71	2.34	0.3	**<0.01**
	CVLT-II 1-5 z	0.19±1.02	0±1	**-0.19**	0.63	-0.92±0.99	-1.09±1.71	**-0.17**	0.57	**<0.01**
	CVLT-II delayed	13.54±2.03	14±2.77	0.46	0.17	9.83±3.95	9.57±4.32	**-0.26**	0.89	**<0.01**
	CVLT-II delayed z	0.26±0.88	0±1	**-0.26**	0.29	-1.35±1.72	-1.6±1.56	**-0.25**	0.35	**<0.01**
Information Processing Speed	**PASAT3**	47.77±8.18	46.92±12.22	**-0.85**	0.8	41.28±11.26	41.13±14.34	**-0.15**	0.67	0.12
	PASAT3 z	0.23±0.68	0.16±1.01	**-0.07**	0.8	-0.31±0.93	-0.32±1.19	**-0.01**	0.65	0.12
	SDMT	60±8.93	59.54±8.38	**-0.46**	0.86	48.64±11.25	47.79±11.23	**-0.85**	0.63	**<0.01**
	SDMT z	0.01±0.95	0±1	**-0.01**	0.94	-1.21±1.2	-1.4±1.34	**-0.19**	0.46	**<0.01**
Executive Function	**Hayling test**	6.77±1.09	6.92±0.95	0.15	0.7	6.23±1.4	6.48±1.99	0.25	0.47	0.33
	Hayling test z	0.14±1.07	0±1	**-0.14**	0.42	-0.38±1.37	-0.47±2.08	**-0.09**	0.27	0.33
	Verbal fluency	18.1±4.56	18.54±4.84	0.44	0.76	14.9±4.85	15.33±5.61	0.43	0.72	0.06
	Verbal fluency z	0.07±0.95	0±1	**-0.07**	0.9	-0.6±1.01	-0.66±1.16	**-0.06**	0.69	0.06
	Stroop	88.46±19.38	91.62±20.12	3.16	0.7	71.6±21.94	68.32±23.57	**-3.28**	0.58	**<0.01**
	Stroop z	0.06±0.97	0±1	**-0.06**	0.94	-0.78±1.09	-1.16±1.17	**-0.38**	0.17	**<0.01**
NBV (ml)		1478.51±65.42	1449.61±61.66	**-28.9**	0.19	1419.97±63.13	1403.96±65.79	**-16.01**	0.25	**0.02**
GM volume (ml)		844.99±26.42	827.08±25.13	**-17.91**	0.09	841.8±34.39	829.14±34.75	**-12.66**	0.11	0.86
NGMF		0.57±0.01	0.58±0.01	0.01	0.38	0.59±0.01	0.58±0.05	**-0.01**	0.61	**0.01**
T2LV (ml)		na	na	na	na	110.85±87.72	111.75±88.11	0.9	0.94	na

Values are presented as ratio for gender, median and range for EDSS, and means ± standard deviation for all other measures. Significant differences are in bold font if p (between visits) or chi-squared probability value (between cohorts) ≤0.05, na=not applicable. Values are presented as ratio for gender, median and range for EDSS, and means ± standard deviation for all other measures. z=z-score. Negative changes over time are in bold.

Between group FC changes in SPMS versus healthy controls at the follow-up timepoint and within group FC changes over time.

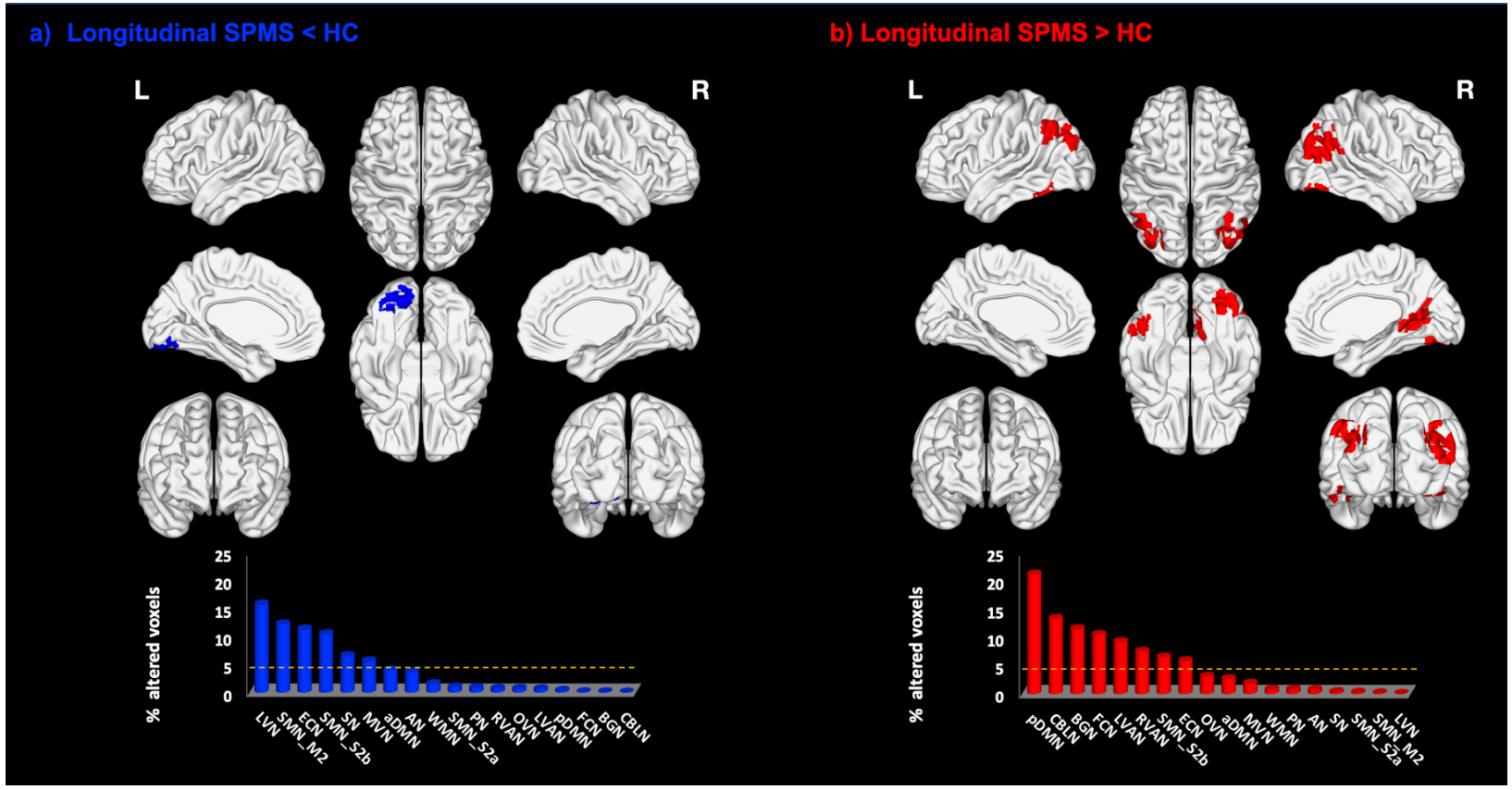
I analysed between group FC changes of SPMS versus healthy controls and found that the main significant reductions in FC in the SPMS group (SPMS<HC) were within the lateral and medial visual networks, SMN, but additionally key cognitive networks: the executive control network (ECN) and salience network (SN) ($p \leq 0.05$ TFCE-corrected) (**figure 5.10 a)**). Anatomically, the main changes are in the left cerebellar region, occipital lobe and medial occipital gyrus which are related to visual processing attention, and visual priming for working memory indicating overlap with the major FC reductions in SPMS in the baseline analysis (**figure 5.7a)**). 17 out of 18 RSNs showed significant FC increases ($p \leq 0.05$ TFCE-corrected) in the SPMS versus control group (SPMS>HC) (**figure 5.10 b)**). These changes were in the key cognitive networks of the posterior DMN, and executive-attentional networks; the CBLN, BGN, FCN, and left and right VAN mirroring the results at the baseline timepoint. Anatomically, these changes were within the cerebellum especially the left and posterior lobe, parietal lobe, occipital lobe, temporal lobe, middle temporal gyrus, and posterior cingulate gyrus. There was additional increased FC in the precuneus which is the main hub of the DMN in the SPMS group at the follow-up visit analysis.

Over time, within group analysis showed that there is increased FC in the SPMS group (baseline SPMS<longitudinal SPMS) in the posterior regions of the DMN and precuneus network, the SMN and occipital and medial visual networks ($p \leq 0.05$ TFCE-corrected) (**figure 5.11a)**). These FC increases relate to the inferior temporal lobe, occipital lobe, parietal lobe, frontal lobe, and precuneus. There were additional increases in FC in the superior parietal lobule relating to visuospatial and working memory function, and the posterior cingulate gyrus implicated in episodic memory retrieval, topographic memory and recall. Reductions in FC over time in the SPMS group (baseline SPMS>longitudinal SPMS) occurred in all of the 18 RSNS, but most in ECN (59% altered voxels), anterior DMN (57.4% altered voxels), and LVAN (48.2% altered voxels) (**table 5.6**) (**figure 5.11b)**). These FC network reductions relate to the cerebellum,

temporal lobe, occipital lobe, frontal lobe, lingual gyrus, middle and inferior temporal gyrus, precuneus, and the inferior parietal lobule relating to working memory function and event retrieval.

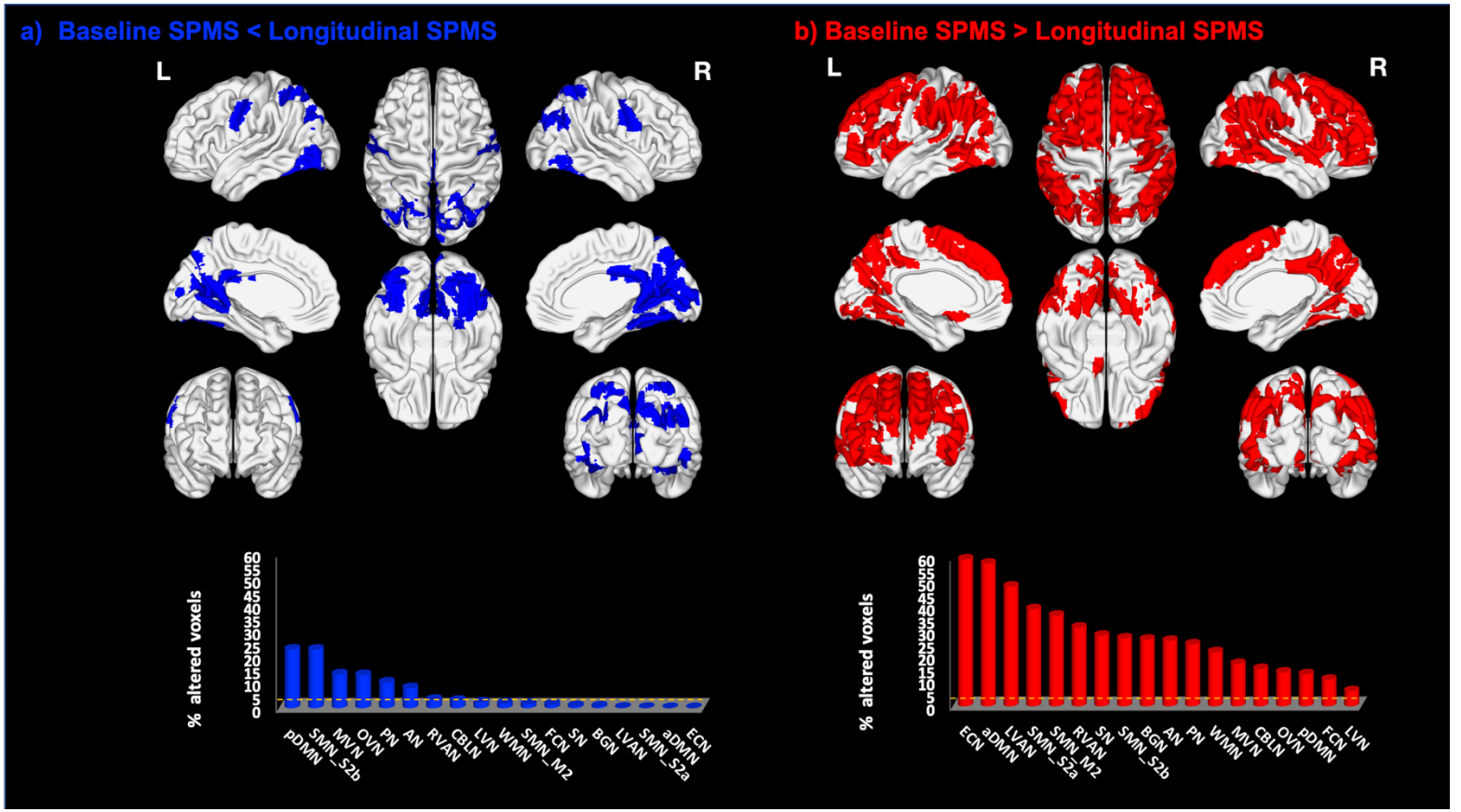
Over time healthy control within group analysis showed significantly increased FC (baseline HC<longitudinal HC) in the MVN, and RVAN at the 5% altered voxel level ($p \leq 0.05$ TFCE-corrected) (**figure 5.12a)**). Anatomically these changes were in the occipital lobe and cuneus. There were FC reductions over time (baseline HC>longitudinal HC) in all of the 18 RSNs, particularly in the deeper cognitive memory and attentional control networks (aDMN, WMN, LVAN, CBLN, and BGN) (**figure 5.12b)**). These changes relate to all cerebellar regions, the middle and superior temporal gyrus, temporal lobe, frontal lobe, inferior frontal gyrus, and precuneus. These areas are associated with working memory and episodic long-term memory, as well as semantic processing and attentional functions.

Figure 5.10. Between group FC changes in SPMS versus healthy controls at the follow-up timepoint. a) 3D global map of (blue) brain RSNs showing decreased FC in SPMS versus HCs (SPMS<HC). The bar chart of (% altered voxel shows that the greatest FC reduction was in the lateral visual network. b) 3D global map highlights which RSNs (red) had increased FC in SPMS versus HCs. The bar plot indicates that the posterior default mode network had the greatest voxel alterations.



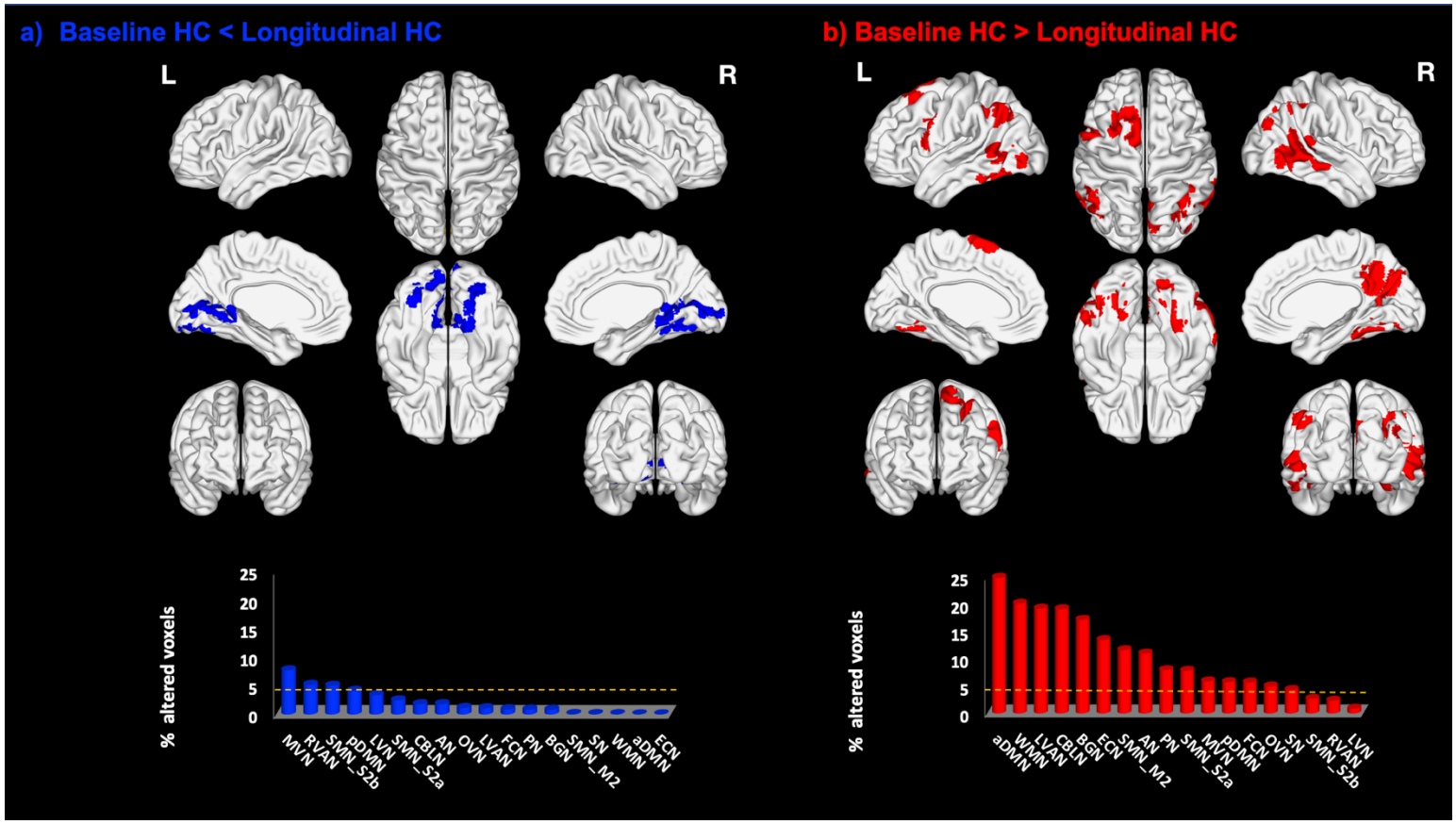
Yellow dashed lines represent those changes above and below the 5% level. Images are shown according to radiological convention. Percentage altered voxels=% altered voxels. FC changes are significant at the p≤0.05 TFCE-corrected threshold.

Figure 5.11. Within group FC changes over time in SPMS. a) 3D global map of (blue) brain RSNs showing increased FC over time in SPMS (baseline SPMS<longitudinal SPMS). The bar chart of % altered voxels shows that the greatest FC increase was in the posterior default mode network. b) 3D global map highlights which RSNs (red) had reduced FC over time in SPMS. The bar plot indicates that the executive control network had the greatest voxel reductions.



Yellow dashed lines represent those changes above and below the 5% level. Images are shown according to radiological convention. Percentage altered voxels=% altered voxels. FC changes are significant at the p≤0.05 TFCE-corrected threshold.

Figure 5.12. Within group FC changes over time in healthy controls. a) 3D global map of (blue) brain RSNs showing increased FC over time in controls (baseline HC<longitudinal HC). The bar chart of altered voxels shows that the greatest FC increase was in the medial visual network. b) 3D global map highlights which RSNs (red) had reduced FC in the controls over time (baseline HC>longitudinal HC). The bar plot indicates that the anterior default mode network had the greatest voxel reductions.



Yellow dashed lines represent those changes above and below the 5% level. Images are shown according to radiological convention. Percentage altered voxels=% altered voxels. FC changes are significant at the p≤0.05 TFCE-corrected threshold.

Table 5.6. Summary of FC changes between the SPMS versus healthy control groups at the follow-up timepoint and changes over time.

Healthy Controls versus SPMS				Baseline versus Longitudinal SPMS				Baseline versus Longitudinal HC			
SPMS<HC RSN FC	% voxel altered	SPMS>HC RSN FC	% voxel altered	baseSPMS<long SPMS RSN FC	% voxel altered	baseSPMS>long SPMS RSN FC	% voxel altered	baseHC<longHC RSN FC	% voxel altered	baseHC>longHC RSN FC	% voxel altered
LVN	15.7	**pDMN**	21.0	**pDMN**	22.6	**ECN**	59.0	**MVN**	7.7	**aDMN**	24.8
SMN_M2	12.1	**CBLN**	13.2	**SMN_S2b**	22.6	**aDMN**	57.4	**RVAN**	5.1	**WMN**	20.0
ECN	11.2	**BGN**	11.4	**MVN**	13.1	**LVAN**	48.2	**SMN_S2b**	5.0	**LVAN**	19.1
SMN_S2b	10.4	**FCN**	10.4	**OVN**	12.8	**SMN_S2a**	39.0	**pDMN**	4.1	**CBLN**	19.0
SN	6.5	**LVAN**	9.1	**PN**	9.9	**SMN_M2**	36.5	**LVN**	3.3	**BGN**	17.1
MVN	5.6	**RVAN**	7.5	**AN**	7.7	**RVAN**	31.6	**SMN_S2a**	2.4	**ECN**	13.4
aDMN	4.0	**SMN_S2b**	6.4	**RVAN**	3.2	**SN**	28.4	**CBLN**	1.8	**SMN_M2**	11.6
AN	3.6	**ECN**	5.8	**CBLN**	2.8	**SMN_S2b**	27.3	**AN**	1.8	**AN**	10.9
WMN	1.5	**OVN**	3.1	**LVN**	2.2	**BGN**	26.8	**OVN**	1.1	**PN**	7.9
SMN_S2a	0.9	**aDMN**	2.6	**WMN**	1.8	**AN**	26.3	**LVAN**	1.1	**SMN_S2a**	7.8
PN	0.8	**MVN**	1.8	**SMN_M2**	1.6	**PN**	24.9	**FCN**	0.9	**MVN**	5.8
RVAN	0.7	**WMN**	0.7	**FCN**	1.4	**WMN**	21.7	**PN**	0.8	**pDMN**	5.7
OVN	0.6	**PN**	0.7	**SN**	0.7	**MVN**	17.0	**BGN**	0.8	**FCN**	5.7
LVAN	0.6	**AN**	0.7	**BGN**	0.7	**CBLN**	15.0	**SMN_M2**	0.1	**OVN**	4.9
pDMN	0.3	**SN**	0.3	**LVAN**	0.2	**OVN**	13.6	**SN**	0.0	**SN**	4.3
FCN	0.0	**SMN_S2a**	0.2	**SMN_S2a**	0.1	**pDMN**	12.8	**WMN**	0.0	**SMN_S2b**	2.7
BGN	0.0	**SMN_M2**	0.1	**aDMN**	0.0	**FCN**	10.7	**aDMN**	0.0	**RVAN**	2.3
CBLN	0.0	**LVN**	0.0	**ECN**	0.0	**LVN**	6.1	**ECN**	0.0	**LVN**	0.9

*Percentage alterations of voxels (% altered voxels) are presented as percentages (%) hierarchically. See **figure 5.6** for abbreviations.*

5.2.4 Discussion

The main findings of this section are that in SPMS there is reduced FC in key cognitive and sensory processing domains relating to temporoparietal regions. Over time in SPMS these reductions in FC involve more posterior brain networks, including the posterior DMN hubs and cerebellum. Increases in FC were more fronto-parietal in SPMS and increased within group over time. This suggests that these areas of FC changes are key underlying dynamic changes in SPMS. I am now going to discuss in turn the results at the individual visit timepoints and the FC changes over time.

Overall, SPMS (n=65 at baseline, 47 at follow-up) and controls (n=16 at baseline, and 13 at follow-up) were matched for age, gender, years of education, and predicted NART IQ. The pattern of cognitive impairment in SPMS is in line with the literature, with impairments in working memory, long-term memory, information processing speed, and executive function (Connick *et al.*, 2013, Rocca *et al.*, 2015*a*; Sumowski *et al.*, 2018). This SPMS MRI cohort has a high physical disability level, with a median EDSS of 6.0, and long disease duration.

Between group rs-fMRI analyses of SPMS versus healthy controls at baseline and follow-up timepoints shows that there are both increases and reductions of FC in RSNs involving cognitive and sensory domains. When compared to controls, SPMS subjects have the greatest FC reductions in key cognitive processing (WMN and DMN) and sensory (SMN) networks, which have been shown to have impairment throughout the spectrum of MS phenotypes (Rocca *et al.*, 2010*a*; Batlle *et al.*, 2017). Anatomically, the reductions of % altered voxels were mainly in temporoparietal and frontal areas known to underlie working memory, attentional, language, and visual function (Steenwijk *et al.*, 2015). Reductions in the FC of sensorimotor, attentional, and executive networks have been highlighted in literature multimodal imaging studies and indicate the importance of the cingulate, precuneus and thalamus in SPMS (Steenwijk *et al.*, 2014; Tewarie *et al.*, 2014, Schoonheim *et al.*, 2015*b*). The greatest FC increases in SPMS were in the networks comprising the frontoparietal lobes; the attentional networks, SMN, and ECN, when

compared to controls at the baseline timepoint. There were further FC increases in posterior and deep brain regions; pDMN, CBLN, and BGN, at the follow-up visit, although these differences may represent differences in cohort size at this timepoint. These RSNs underlie specific cognitive functions, particularly processing speed; for instance the anterior cingulate cortex (ACC) has been consistently associated with executive function, and both changes in anterior cingulate structure and executive dysfunction are common in SPMS, more so than other MS phenotypes (Amato *et al.*, 2008*b*; Connick *et al.*, 2013; Chan *et al.*, 2017). The increased FC in the posterior DMN hubs in the SPMS group may indicate lesional tissue damage as has been highlighted in a RRMS study (Droby *et al.*, 2015). Only the ACC showed reduced FC in SPMS which differentiated it from PPMS in a cross-sectional ICA study (Rocca *et al.*, 2010*b*). The increased FC in the deeper networks, the BGN, in the follow-up visit SPMS versus control between group analysis may represent maladaptive responses due to worsening disability over time or associations with other disease components (Stefancin *et al.*, 2019) (**section 3.4**). The increases in FC of the pDMN in the SPMS group versus controls have been shown in comparison studies of SPMS and RRMS (Basile *et al.*, 2014). This may indicate increased disability, and in this SPMS cohort, worsening of cognitive and physical disability over time, even if not significantly so, at the descriptive characteristic level.

Within group changes over time show increased FC in the SPMS follow-up group in more posterior brain areas; the pDMN, PN, and visual networks, than at baseline. There is also reduced FC at follow-up in the more frontoparietal networks; the ECN, aDMN, and LVAN. The increased FC in more posterior DMN hubs and brain regions over time may indicate functional reorganisation and recruitment in response to lesional tissue damage (Droby *et al.*, 2015). However, the reductions in FC in the executive control, working memory and attentional networks over time in the SPMS group show maladaptive changes in the cognitive regions indicating reductions in network efficiency (Schoonheim *et al.*, 2015*b*). These changes also mimic those seen in this SPMS cohort's cognitive outcome measures over time (**section 3.3.3.3)**. The underlying reductions in cerebellar FC in SPMS over time have been shown to indicate worse visuospatial memory function in progressive MS (Cocozza *et al.*, 2018). In the healthy control

group both increases and decreases in FC over time related to more anterior brain areas and networks. There were reductions in attentional, working memory, and anterior DMN areas which were in keeping with the non-significant changes in these cognitive domains between visits.

5.3 Global rs-fMRI changes relating to cognitive status in SPMS

5.3.1 Introduction

Chapter 3 (**section 3.5**) showed key quantitative changes in cognitively impaired versus preserved SPMS subjects, and the variations in prevalence and characteristics by the definition used. However, what is not so well understood is the underlying dynamic changes in terms of brain connectivity with cognitive status in SPMS.

The anterior DMN, and particularly the ACC, has been shown to have reduced FC in cognitively impaired progressive MS in a seed-based approach (Rocca *et al.*, 2010*b*). The anterior DMN has been highlighted as a key region for preservation of cognitive reserve with reductions seen in cognitive impairment (Bonavita *et al.*, 2011). Additionally, there appears to be loss of key frontoparietal hubs which form part of the DMN in cognitively impaired MS subjects (Rocca *et al.*, 2016*b*), which are retained in task based fMRI studies in RRMS (Mainero *et al.*, 2006). Further studies of centrality have supported the importance of the DMN in MS cognitive impairment (Eijlers *et al.*, 2017). Additional areas showing FC changes relating to MS cognitive impairment include altered FC in the thalamus (Schoonheim *et al.*, 2014, 2015*a*; Tona *et al.*, 2014). In RRMS, however, these paradoxical changes do not occur with increased thalamic and DMN connectivity in the cognitively preserved state (Hawellek *et al.*, 2011). Perhaps this is in relation to inverse correlations of lesion load and global FC in MS (Rocca *et al.*, 2018). Reductions in cerebellar FC have been shown to correlate with improvements in MS visuospatial memory function (Cocozza *et al.*, 2018).

There are few rs-fMRI longitudinal studies in MS cohorts to allow evaluation of changes over time (Enzinger *et al.*, 2016) and no longitudinal studies have looked specifically at cognitive impairment in MS.

The aims of this section are to evaluate:

a. How resting FC changes with the definition of cognitive impairment in SPMS using two criteria (**section 4.3.3.1**).
b. Between group FC changes at the follow-up timepoint in SPMS with and without cognitive impairment (**section 4.3.3.2**).
c. The predictive FC changes for the development of SPMS cognitive impairment over time from a preserved state (**section 4.3.3.3**).

5.3.2 Methods

The methods and subjects used for the analyses were the same as **section 4.2.** However, I investigated the presence of FC changes within the RSNs between cognitive impaired (CI) and cognitively preserved (CP) SPMS groups using additional contrasts at both timepoints (baseline CI>baseline CP, baseline CI<baseline CP, longitudinal CI>longitudinal CP, longitudinal CI<longitudinal CP), and changes over time (baseline CP>longitudinal CI, baseline CP<longitudinal CI) in the general linear model. CI and CP was classified using the two critical number of abnormal parameter criteria:

1) Conservative criteria, overall cognitive impairment is a z-score of -1.96SD on two or more individual tests from at least two cognitive domains compared to healthy control data (Strauss *et al.*, 2006; Patti *et al.*, 2009; Fischer *et al.*, 2014).

2) Lenient criteria, overall cognitive impairment is a z-score of -1.5SD on two or more individual tests from at least two cognitive domains compared to healthy control data (Strauss *et al.*, 2006; Patti *et al.*, 2009; Fischer *et al.*, 2014).

5.3.3 Results

5.3.3.1 Between group global rs-fMRI FC changes in SPMS with and without cognitive impairment using two different classifications

The clinical-cognitive profile of the cohort.

29% of the SPMS group were classified as cognitively impaired defined by a z-score of ≤-1.96SDs on ≥2 individual from ≥2 cognitive domains (conservative), compared to 46% of those classified as impaired defined by a z-score of ≤-1.5SDs on ≥2 individual from ≥2 cognitive domains (lenient).

Using the z-score of ≤-1.96SDs on ≥2 domains criteria, the cognitively impaired (19 subjects) and cognitively preserved (46 subjects) SPMS groups were well matched in terms of age, gender, years of education, and IQ (**table 5.7**). However, there were significantly better MSFC scores ($p<0.01$) in the preserved versus impaired group, which were not driven by the T25FW or 9HPT. Overall, cognitive outcome scores were expectedly significantly worse ($p<0.01$) in the cognitively impaired versus cognitively preserved group. As in **section 3.5**, the delayed component of the BVMT-R was not significantly impaired highlighting its poor discriminatory power in this study (**table 5.7**). As shown in **section 3.3**, in SPMS cognitive performance; working memory and information processing speed domains were most affected, with additional executive dysfunction. NBV and NGMF were significantly higher in the cognitively preserved group. With the z-score ≤-1.5SDs on ≥2 domains criteria, 30 SPMS subjects were impaired and 35 preserved. Those impaired using this criteria had lower IQ 111.47±8.72 versus 117±6.13 in the preserved group respectively $p<0.01$. There was also significantly better subjective disability and psychological function in the preserved group (MSIS29v2 score 60.77±13.55 versus 70.23±16.62 $p=0.02$) (**table 5.7**). These findings are in keeping with **section 3.5**.

Table 5.7. Clinical, cognitive, and MRI characteristics of SPMS groups with and without cognitive impairment using two different criteria at baseline.

		CI ≤-1.96SDs on ≥2 domains			CI ≤-1.5SDs on ≥2 domains		
		CP (46)	CI (19)	p	CP (35)	CI (30)	p
Gender M:F		8:38	7:12	0.09	6:9	9:21	0.22
Age (years)		54.54±7.29	55±6.96	0.82	55.44±6.99	53.79±7.34	0.36
Years of education		15.63±3.08	15.42±2.22	0.94	15.69±3.25	15.43±2.31	0.90
NART IQ		114.98±8.56	113.16±5.97	0.14	117±6.13	111.47±8.72	**<0.01**
Disease duration from first symptom		22.02±9.55	22±8.29	0.44	21.46±9.88	22.67±8.31	0.36
Duration of progression		7.5±4.74	8.47±5.54	0.55	7.54±4.77	8.07±5.25	0.74
EDSS		6.0 (4.0-6.5)	6.0 (4.0-6.5)	0.90	6.0 (4.0-6.5)	6.0 (4.0-6.5)	0.20
9HPT (sec)		35.95±18.64	33.92±10.68	0.70	38.01±20.65	32.26±9.69	0.59
T25FW (sec)		16.7±13.82	18.07±14.97	0.84	15.24±11.03	19.36±16.97	0.22
MSFC score		0.09±0.89	-0.33±0.57	**<0.01**	0.3±0.42	-0.42±1.01	**<0.01**
MSIS29v2		62.7±15.32	71.05±15.27	0.06	60.77±13.55	70.23±16.62	**0.02**
Visual Memory	**BVMT-R 1-3**	14.61±6.28	8.32±5.03	**<0.01**	15.69±5.91	9.37±5.66	**<0.01**
	BVMT-R 1-3 z	-0.14±1.01	-1.16±0.81	**<0.01**	0.03±0.95	-0.99±0.91	**<0.01**
	BVMT-R retained	96.18±28.46	80.24±46.01	0.30	91.4±25.75	91.67±43.72	0.74
	BVMT-R retained z	-0.08±0.72	-0.49±1.17	0.30	-0.2±0.65	-0.2±1.11	0.74
Verbal Memory	**CVLT-II 1-5**	47.76±10.18	37.53±10.62	**<0.01**	49.71±8.73	39±11.23	**<0.01**
	CVLT-II 1-5 z	-0.78±1.07	-1.65±1	**<0.01**	-0.63±1	-1.51±1.06	**<0.01**
	CVLT-II delayed	10.83±3.17	6.58±4.36	**<0.01**	11.2±2.9	7.7±4.37	**<0.01**
	CVLT-II delayed z	-0.92±1.38	-2.77±1.9	**<0.01**	-0.76±1.26	-2.28±1.91	**<0.01**
Information Processing Speed	**PASAT3**	44.78±9.87	31.63±10.58	**<0.01**	47.31±8.44	33.5±10.54	**<0.01**
	PASAT3 z	-0.02±0.82	-1.11±0.88	**<0.01**	0.19±0.7	-0.95±0.87	**<0.01**
	SDMT	52.11±8.73	36.16±10.56	**<0.01**	54.23±7.21	39.53±11.15	**<0.01**
	SDMT z	-0.84±0.93	-2.54±1.13	**<0.01**	-0.61±0.77	-2.18±1.19	**<0.01**
Executive Function	**Hayling**	6.52±1.13	5.16±1.54	**<0.01**	6.57±1.22	5.6±1.43	**0.02**
	Hayling z	-0.1±1.1	-1.43±1.5	**<0.01**	-0.05±1.19	-1±1.39	**0.02**
	Verbal fluency	15.97±4.58	11.44±3.77	**<0.01**	17.17±4.51	11.76±3.26	**<0.01**
	Verbal fluency z	-0.38±0.96	-1.32±0.79	**<0.01**	-0.13±0.94	-1.26±0.68	**<0.01**
	Stroop	78.36±17.69	47.67±19	**<0.01**	83.5±15.78	53.28±18.42	**<0.01**
	Stroop z	-0.45±0.88	-1.98±0.95	**<0.01**	-0.19±0.79	-1.7±0.92	**<0.01**
NBV (ml)		1437.18±69.1	1376.11±73.6	**0.01**	1440.86±69.39	1394.21±75.13	**0.01**
GM volume (ml)		850.24±34.3	816.48±50.2	**0.01**	851.84±32.94	826.99±47.98	**0.02**
NGMF		0.59±0.01	0.59±0.01	0.31	0.59±0.01	0.59±0.01	0.65
T2LV (ml)		79.84±71.11	205.97±119.21	**<0.01**	65.93±60.3	175.95±114.18	**<0.01**

CI= cognitively impaired. CP = cognitively preserved. Values are presented as ratio for gender, median and range for EDSS, and means ± standard deviation for all other measures. Significant differences are in bold font if p (between visits) or chi-squared probability value (between cohorts) ≤0.05, na=not applicable. Values are presented as ratio for gender, median and range for EDSS, and means ± standard deviation for all other measures. z=z-score.

Between group FC changes in SPMS with and without cognitive impairment at baseline using two different classification criteria.

Comparison of RSNs between the cognitively impaired ≤-1.96SDs on ≥2 domains and cognitively preserved SPMS cohorts showed significantly reduced FC (CI<CP) in cognitive (posterior DMN, and Right Ventral Attentional Network (RVAN)), sensory SMN_S2b, visual (Lateral Visual Network (LVN)), and cerebellar RSNs above the 5% altered voxel level (p≤0.05 TFCE corrected) (**figure 5.13a)**). These RSNs associate with more posterior brain regions: the cerebellum, occipital lobe, and inferior parietal lobe. The greatest RSN FC increases (CI>CP) in the cognitively impaired SPMS group occurred in sensory (AN), cognitive (SN), and deep (BGN) RSNs (p≤0.05 TFCE corrected) (**figure 5.13b)).** Anatomically these changes related to the precuneus, parietal lobe and the temporal lobe. See **table 5.8** for a summary of percentage alteration in voxels per RSN.

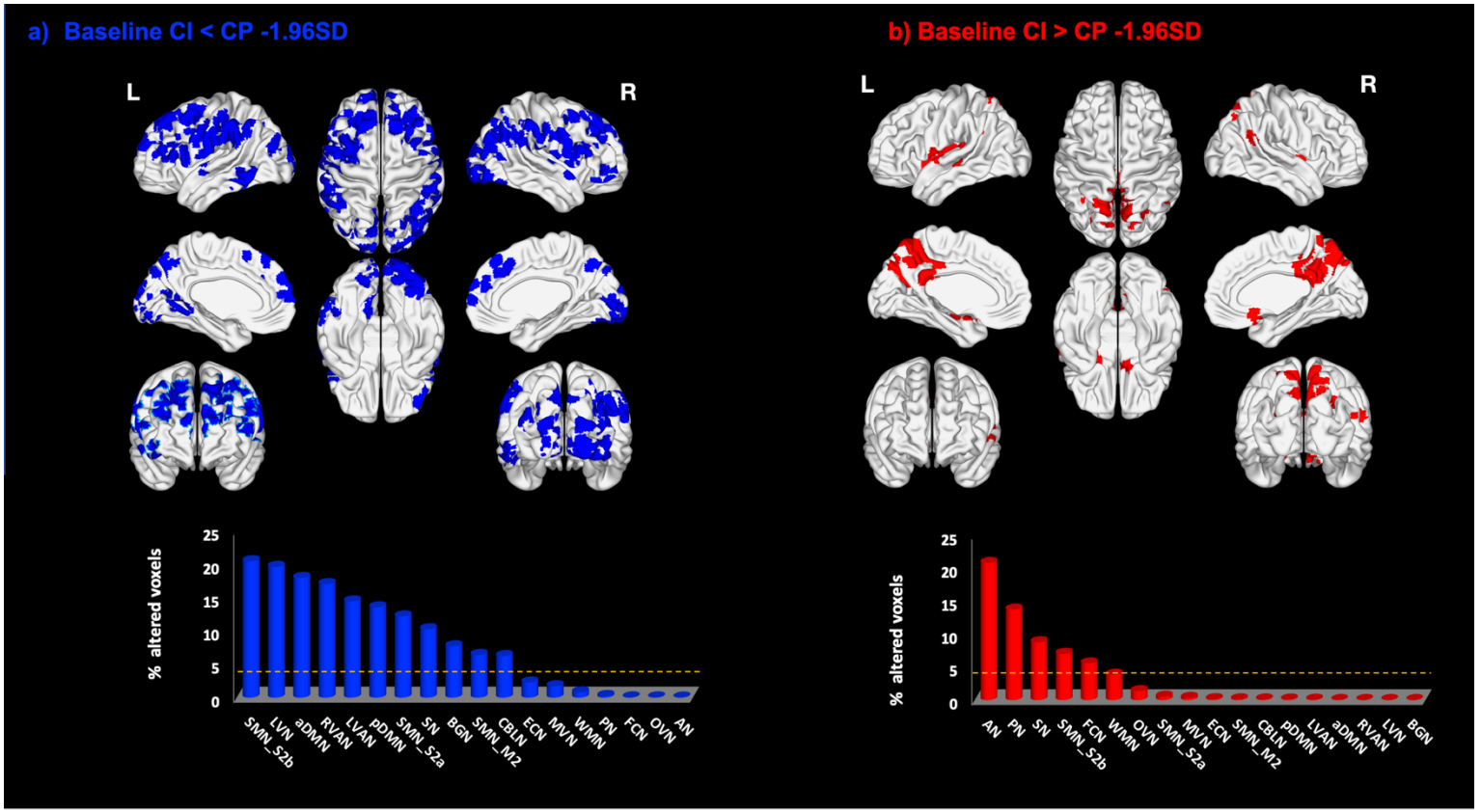
Multiple comparison correction, i.e. Bonferroni adjustment of the p-value, showed persistent FC decreases (CI<CP)in the; SMN, LVN, and RVAN in the cognitively impaired group with z-score ≤-1.96SDs on ≥2 domains. The precuneus and salience networks show increases in FC (CI>CP) overall throughout the correction thresholds (**figure 5.14**). Therefore, the patterns of spatial changes are the same when thresholds are altered and therefore, as with the SPMS versus control analyses (**figure 5.8**), Bonferroni adjustment does not add to the final outcome, and so a 5% significance level has been adopted for the rs-fMRI analyses here in.

Those cognitively impaired SPMS subjects with z-score ≤-1.5SDs on ≥2 domains versus those cognitively preserved showed similar FC reductions (CI<CP) as the more stringent classification, with reduced FC in the posterior DMN, LVN, RVAN, and CBLN (p≤0.05 TFCE corrected) (**Figure 5.15a)**). These FC changes associate with more posterior brain regions; the cerebellum, occipital lobe, and the middle occipital gyrus associated with word recognition. In the cognitively impaired SPMS group, FC was increased (CI>CP) in the auditory network, salience network, and BGN above 5% altered voxels (p≤0.05 TFCE corrected) (**Figure 5.15b)**). These FC increases are

anatomically in the visuospatial, working memory and executive regions; the frontal lobe, inferior frontal gyrus, occipital lobe, middle temporal gyrus, and temporo-parietal lobes.

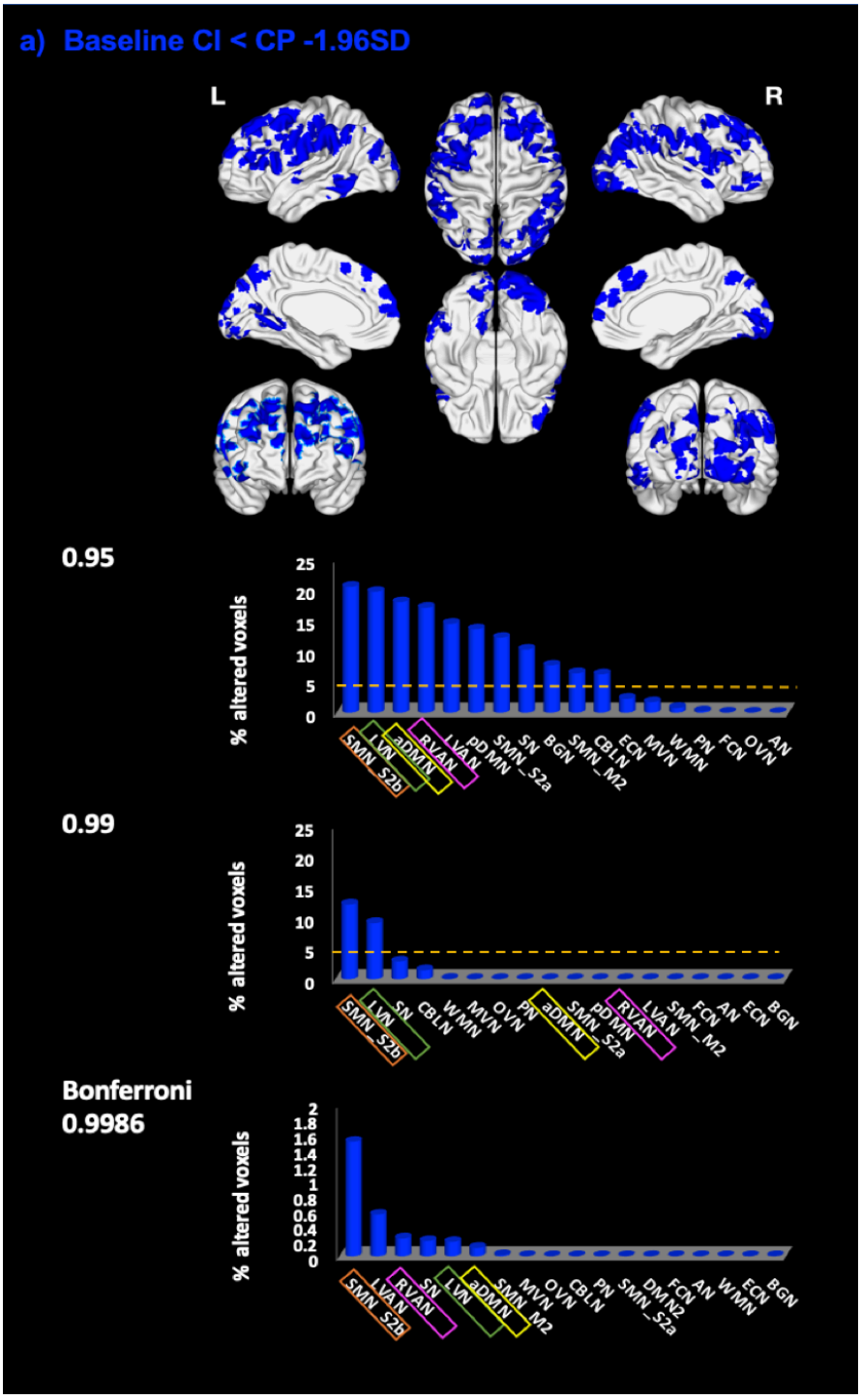
Therefore, both criteria are similar in terms of FC changes, but a more stringent criteria gives more dynamic FC alterations that may be otherwise missed.

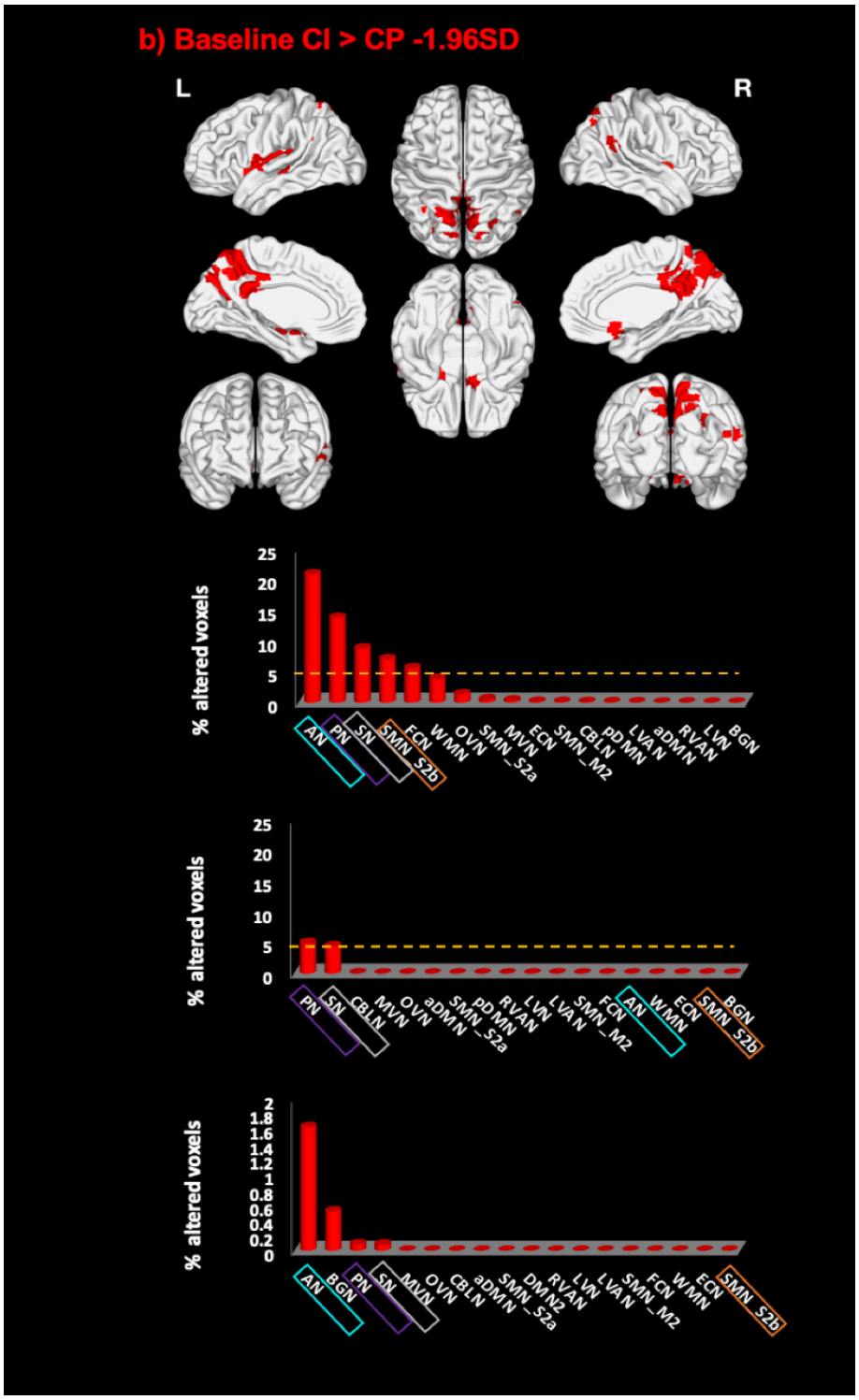
Figure 5.13. Between group FC changes in SPMS groups with and without cognitive impairment (z-score of ≤-1.96SD on ≥2 domains) at baseline. **a)** 3D global map of (blue) RSNs showing decreased FC on the global map in SPMS CI group (CI<CP). The bar chart of % altered voxels shows that the greatest FC reduction was in the sensorimotor network (SMN_S2b), lateral visual network (LVN), and default mode network (DMN). **b)** 3D global map of (red) RSNs with increased FC (p≤0.05 TFCE-corrected) in SPMS CI (CI>CP). The bar plot indicates that the auditory (AN) and cognitive precuneus networks (PN) had the greatest % altered voxels.



CI=cognitively impaired. CP=cognitively preserved. Yellow dashed lines represent those changes above and below the 5% level. Images are shown according to radiological convention. Percentage altered voxels=% altered voxels. FC changes are significant at the p≤0.05 TFCE-corrected threshold.

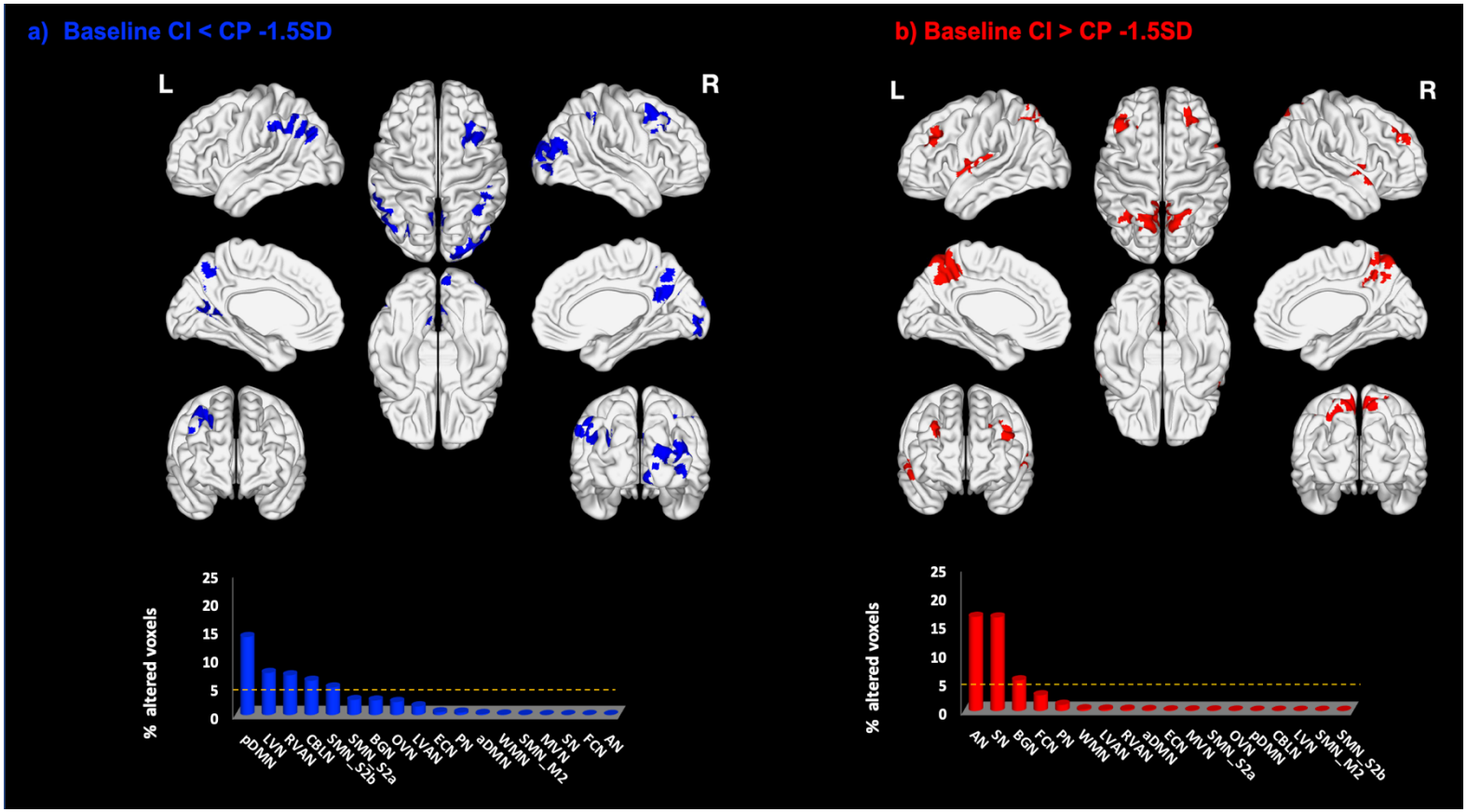
Figure 5.14. Multiple comparison correction of FC changes in SPMS groups with and without cognitive impairment (z-score of ≤-1.96SD on ≥2 domains) at baseline. Shown as % altered voxels for different correction thresholds; 0.95 and 0.99, and Bonferroni (0.9986) correction for the 18 RSNs identified. Boxes represent the top 4 altered networks.





CI=cognitively impaired. CP=cognitively preserved. Yellow dashed lines represent those changes above and below the 5% level. Images are shown according to radiological convention. Percentage altered voxels=% altered voxels.

Figure 5.15. Between group FC changes in SPMS groups with and without cognitive impairment (z-score of ≤-1.5SD on ≥2 domains) at baseline. **a)** 3D global map of (blue) RSNs showing decreased FC on the global map in SPMS CI (CI<CP). The bar chart of % altered voxels shows that the greatest FC reduction was in the posterior default mode network (pDMN). **b)** 3D global map of (red) RSNs with increased FC in SPMS CI (CI>CP). The bar plot indicates that the auditory network (AN) had the greatest % altered voxels.



CI= cognitively impaired. CP = cognitively preserved. Yellow dashed lines represent those changes above and below the 5% level. Images are shown according to radiological convention. Percentage altered voxels=% altered voxels. FC changes are significant at the p≤0.05 TFCE-corrected threshold.

Table 5.8. Summary of FC changes at baseline between SPMS groups with and without cognitive impairment using two different criteria.

CI versus CP SPMS (≤-1.96 SDs on ≥2 domains)				CI versus CP SPMS (≤-1.5 SDs on ≥2 domains)			
CI<CP RSN FC	% voxel alterations	CI>CP RSN FC	% voxel alterations	CI<CP RSN FC	% voxel alterations	CI>CP RSN FC	% voxel alterations
SMN_S2b	20.4	**AN**	20.8	**pDMN**	13.8	**AN**	16.4
LVN	19.6	**PN**	13.7	**LVN**	7.4	**SN**	16.3
aDMN	17.9	**SN**	8.8	**RVAN**	7.0	**BGN**	5.3
RVAN	17	**SMN_S2b**	7	**CBLN**	6.1	**FCN**	2.7
LVAN	14.4	**FCN**	5.6	**SMN_S2b**	4.9	**PN**	1.1
pDMN	13.5	**WMN**	3.8	**SMN_S2a**	2.7	**WMN**	0.3
SMN_S2a	12.2	**OVN**	1.4	**BGN**	2.6	**LVAN**	0.2
SN	10.2	**SMN_S2a**	0.5	**OVN**	2.3	**RVAN**	0.2
BGN	7.7	**MVN**	0.3	**LVAN**	1.5	**aDMN**	0.1
SMN_M2	6.4	**ECN**	0.1	**ECN**	0.5	**ECN**	0.1
CBLN	6.2	**SMN_M2**	0.1	**PN**	0.4	**MVN**	0.1
ECN	2.3	**CBLN**	0.1	**aDMN**	0.2	**SMN_S2a**	0.1
MVN	1.7	**pDMN**	0.1	**WMN**	0.1	**OVN**	0.0
WMN	0.8	**LVAN**	0	**SMN_M2**	0.0	**pDMN**	0.0
PN	0.2	**aDMN**	0	**MVN**	0.0	**CBLN**	0.0
FCN	0	**RVAN**	0	**SN**	0.0	**LVN**	0.0
OVN	0	**LVN**	0	**FCN**	0.0	**SMN_M2**	0.0
AN	0	**BGN**	0	**AN**	0.0	**SMN_S2b**	0.0

*CI=cognitively impaired. CP=cognitively preserved. Percentage alterations of voxels (% altered voxels) are presented as percentages (%) hierarchically. See **figure 5.6** for abbreviations of RSNs.*

5.3.3.2 Between group global rs-fMRI changes in SPMS with and without cognitive impairment at the follow-up timepoint

The results in the last **section 4.3.3.1** show that the most sensitive criteria for showing the dynamic changes of cognitive impairment in SPMS was the conservative criteria, z-score ≤-1.96 SDs on ≥2 domains. Therefore, this criteria is used a priori for further analyses in this section.

Changes in the clinical-cognitive cohort over time.

Using the ≤-1.96SDs on ≥2 domains criteria, more subjects were cognitively impaired at follow-up; 19 SPMS subjects were impaired and 28 preserved, versus 12 impaired and 35 preserved at baseline visit. At the follow-up visit those subjects cognitively impaired and preserved were well matched in terms of age, gender, education, visit interval and IQ. There were no significant differences in the main physical outcome measures of disability; the EDSS, 9HPT, T25FW, MSFC, and MSIS29v2. As, expected from the baseline visit, there were significant differences cross-sectionally between the preserved and impaired groups for all cognitive tasks, but not the PASAT3, and BVMT-R retained which had poor discriminatory power. GM volume was again significantly lower in the cognitively impaired versus preserved group; 817.45±36.83ml versus 843.61±26.14ml p=0.01, respectively (**table 5.9**). Additionally, over time, within the cognitively preserved group, there was improvement in the MSIS29v2, BVMT-R, and CVLT-II verbal learning task (**table 5.9**).

Between group FC changes in SPMS with and without cognitive impairment at the follow-up timepoint.

At the follow-up visit, the main FC reductions in the cognitively impaired group (CI<CP) were in the ECN with 11.1% voxel alterations, the remainder of FC reductions were less than 5% and so likely noise, but included cognitive (WMN), sensory (AN), and attentional networks (RVAN, and LVAN) (p≤0.05 TFCE corrected) (**figure 5.16a)**) (**table 5.10**). This is similar to the baseline results

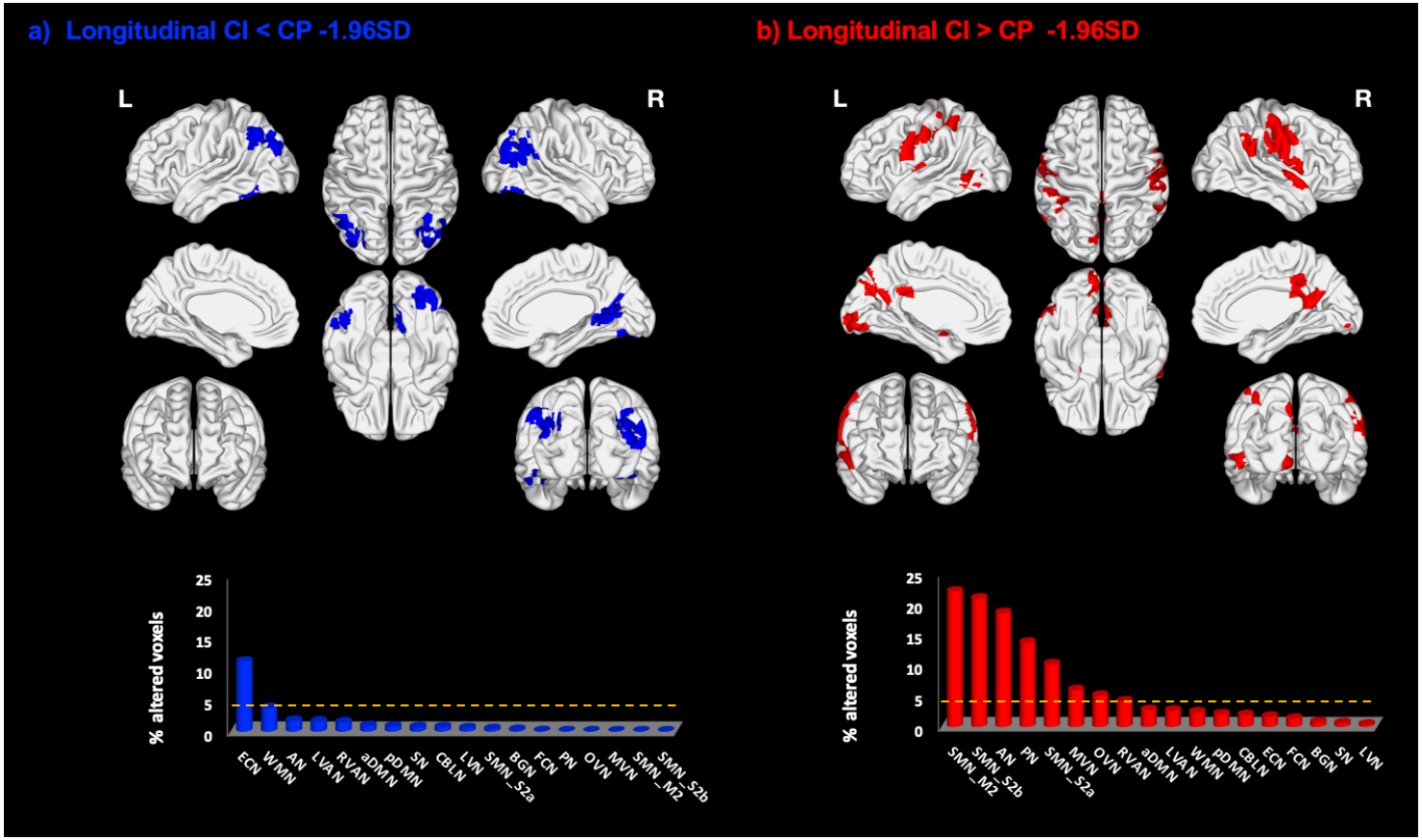
(**figure 5.13a)**), apart from the additional reduction in the ECN. Anatomically, these changes relate to the cerebellum, occipital lobe, posterior cingulate, limbic lobe, temporal lobe and middle temporal gyrus, and the precuneus. This relates to semantic processing, word generation, topographic and episodic working memory, and include the main hub of the DMN. The main FC increases in the impaired group (CI>CP) ($p \leq 0.05$ TFCE corrected) (**figure 5.16b)**) (**table 5.10**) mirrored those at the baseline timepoint (**figure 5.13b)**); including all sensorimotor networks, the sensory AN, and the main hub of the DMN, the precuneus network. These changes relate to the occipital lobe, lingual gyrus, temporal lobe and superior temporal gyrus, posterior cingulate, frontal and parietal lobes.

Table 5.9. Clinical, cognitive, and MRI characteristics of SPMS groups with and without cognitive impairment at the follow-up timepoint and changes over time.

		Baseline CCP (35)	Long CCP (28)	change	p	Baseline CCI (12)	Long CCI(19)	change	p	chi-2
Gender M:F		7:28	6:22	**-0.01**	0.90	3:9	4:15	0.04	0.80	0.98
Age (years)		55.56±6.67	56.62±6.4	1.06	0.52	53.87±7.54	57.45±7.77	3.58	0.16	0.50
Interval (days)		671.34±62.41	671.46±57.96	0.12	0.84	654.25±77.65	660.37±79.43	6.12	0.82	0.96
Years of education		15.54±3.3	15.46±2.36	**-0.08**	0.77	15.42±2.39	15.58±3.96	0.16	0.90	0.69
NART IQ		115.77±6.85	115.68±5.79	**-0.09**	0.88	112.25±7.02	113.68±8.5	1.43	0.60	0.50
Disease duration from first symptom		22.91±9.93	23.82±8.27	0.91	0.53	22.25±7.69	21.16±10.75	**-1.09**	0.46	0.21
Duration of progression		7.51±4.82	8.07±4.73	0.56	0.52	7.92±5.42	6.95±5.25	**-0.97**	0.47	0.20
EDSS		6.0 (4.0-6.5)	5.5 (4.0-6.5)	**-0.08**	0.88	6.0 (4.0-6.5)	6.0 (4.0-6.5)	**-0.06**	0.85	0.37
9HPT (sec)		35±16.43	36.29±16.26	1.29	0.86	33.75±11.94	37.94±14.72	4.19	0.44	0.49
T25FW (sec)		15.09±13.76	25.25±34.76	10.16	0.31	20.09±17.74	24.72±39.41	4.63	0.75	0.94
MSFC score		0.22±0.46	-0.14±1.17	**-0.36**	0.21	-0.27±0.64	-0.18±1.25	0.09	0.29	0.91
MSIS29v2		62.14±16.44	71.7±16.83	9.56	**0.04**	69.58±17.22	75.58±14.37	6.00	0.35	0.41
Visual Memory	**BVMT-R trials 1-3**	14.54±6.09	23.14±6.93	8.6	**<0.01**	6.92±4.29	14.58±8.21	7.66	**0.01**	**<0.01**
	BVMT-R trials 1-3 z	-0.15±0.98	-0.04±0.81	0.11	0.57	-1.38±0.69	-1.04±0.96	0.34	0.33	**<0.01**
	BVMT-R retained	95.46±30.59	96.75±15.21	1.29	0.60	79.72±46.09	94±20.65	14.28	0.56	0.79
	BVMT-R retained z	-0.1±0.77	-0.26±0.55	**-0.16**	0.10	-0.5±1.17	-0.36±0.74	0.14	0.65	0.79
Verbal Memory	**CVLT-II trials 1-5**	47.14±10.28	52.82±9.68	5.68	**0.03**	39.75±9.5	39.89±10.24	0.14	0.90	**<0.01**
	CVLT-II trials 1-5 z	-0.75±0.97	-0.57±0.97	0.18	0.54	-1.44±0.89	-1.86±1.02	**-0.42**	0.27	**<0.01**
	CVLT-II delayed	10.89±3.33	11.5±3.37	0.61	0.23	6.75±4.14	6.74±4.04	**-0.01**	0.85	**<0.01**
	CVLT-II delayed z	-0.89±1.45	-0.9±1.22	**-0.01**	0.53	-2.7±1.8	-2.62±1.46	0.08	0.87	**<0.01**
Information Processing Speed	**PASAT3**	44.29±10.44	41.61±13.3	**-2.68**	0.72	32.5±8.98	40.42±16.11	7.92	**0.05**	1.00
	PASAT3 z	-0.06±0.86	-0.28±1.1	**-0.22**	0.73	-1.04±0.74	-0.38±1.33	0.66	**0.05**	1.00
	SDMT	52.29±9.31	53.61±8.69	1.32	0.54	38±9.74	39.21±8.87	1.21	0.76	**<0.01**
	SDMT z	-0.82±1.0	-0.71±1.04	0.11	0.60	-2.35±1.04	-2.42±1.06	**-0.07**	0.81	**<0.01**
Executive Function	**Hayling test**	6.57±1.2	7.18±1.52	0.61	0.11	5.25±1.54	5.39±2.17	0.14	0.97	**0.01**
	Hayling test z	-0.05±1.17	0.27±1.59	0.32	0.41	-1.34±1.51	-1.61±2.28	**-0.27**	0.55	**0.01**
	Verbal fluency	16±4.56	17.6±4.95	1.6	0.22	11.52±4.25	12±4.91	0.48	0.91	**<0.01**
	Verbal fluency z	-0.37±0.95	-0.19±1.02	0.18	0.70	-1.31±0.89	-1.35±1.01	**-0.04**	0.81	**<0.01**
	Stroop	79.38±17.12	83.89±12.07	4.51	0.29	47.55±17.59	45.37±16.36	**-2.18**	0.86	**<0.01**
	Stroop z	-0.4±0.85	-0.38±0.6	0.02	0.98	-1.98±0.88	-2.3±0.81	**-0.32**	1.49	**<0.01**
NBV (ml)		1428.91±60.52	1410.36±67.18	**-18.55**	0.24	1393.9±65.94	1394.54±64.3	0.64	0.12	0.40
GM volume (ml)		845.08±31.68	837.2±33.11	**-7.88**	0.12	832.22±41.33	817.26±34.52	**-14.96**	0.31	**0.04**
NGMF		0.59±0.01	0.58±0.05	**-0.01**	0.85	0.6±0.01	0.57±0.06	**-0.03**	0.60	0.90
T2LV (ml)		86.85±76.22	127.24±97.64	40.39	0.10	180.85±83.91	88.93±67.95	**-91.92**	0.00	0.23

CCI=cognitively impaired z-score ≤-1.96SDs on ≥2 domains. CCP=cognitively preserved z-score ≥-1.96SDs on ≥2 domains. Values are presented as ratio for gender, median and range for EDSS, and means ± standard deviation for all other measures. Significant differences are in bold font if p (between visits) or chi-squared probability value (between cohorts) ≤0.05, na=not applicable. z=z-score. Negative changes over time are in bold.

Figure 5.16. Between group FC changes in SPMS groups with and without cognitive impairment (z-score of ≤-1.96SD on ≥2 domains) at the follow-up timepoint. a) 3D global map of (blue) brain RSNs showing decreased FC in CI SPMS (CI<CP). The bar chart of % altered voxels below shows that the greatest FC reduction was in the executive control network (ECN). b) 3D global map highlights which RSNs (red) had increased FC in CI SPMS (CI<CP). The bar plot indicates that the sensori-motor network (SMN-M2) had the greatest voxel alterations.



CI= cognitively impaired. CP = cognitively preserved. Yellow dashed lines represent those changes above and below the 5% level. Images are shown according to radiological convention. Percentage altered voxels=% altered voxels. FC changes are significant at the p≤0.05 TFCE-corrected threshold.

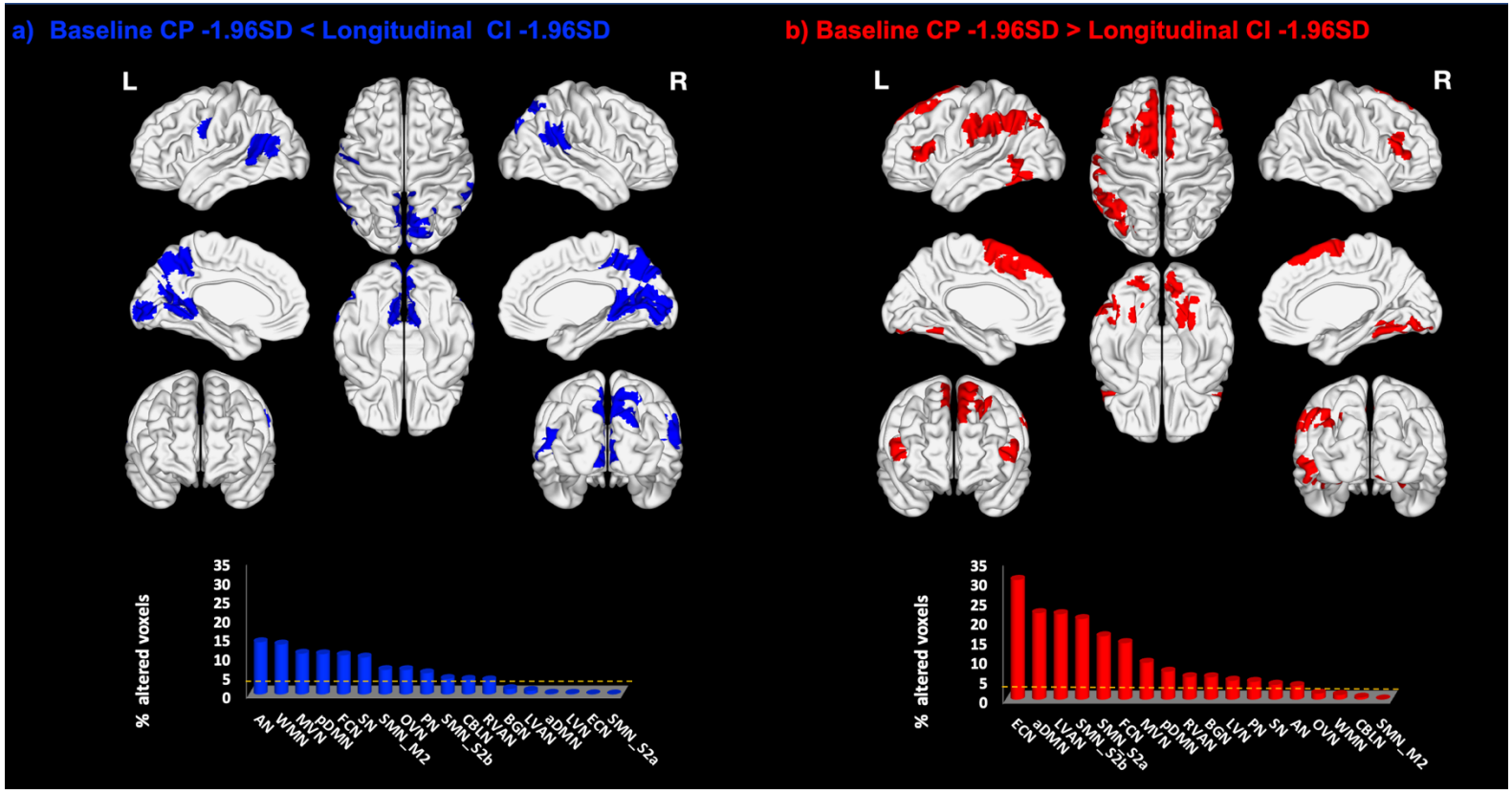
The predictive FC changes for the development of SPMS cognitive impairment over time from a preserved state.

13 SPMS subjects developed new cognitive impairment at the follow-up visit from a preserved state at baseline using the z-score ≤-1.96SDs on ≥2 domains criteria.

Over time, the greatest FC reductions in the baseline preserved group compared to the longitudinal impaired group (baseline CP<longitudinal CI) were in the sensory (AN), cognitive (WMN, pDMN, PN), executive (FCN or FPCN), and visual networks (MVN, OVN) (**figure 5.17a)**, **table 5.10**). These changes relate anatomically to the occipital lobe, frontal lobe, cuneus, precuneus, lingual gyrus, posterior cingulate, temporal lobe and superior temporal gyrus. These areas relate to language and speech attention, topographic and episodic memory, and visuospatial memory and processing.

The greatest FC increases in the baseline preserved group (baseline CP>longitudinal CI) were in the executive networks (ECN and FCN), attentional networks (LVAN and RVAN), sensorimotor networks, anterior and posterior DMN, deep BGN, and MVN (**figure 5.17b)**, **table 5.10**). Anatomically these increases in FC correlate to the cerebellum, occipital lobe, temporal lobe, frontal lobe, and inferior parietal and inferior frontal gyri. These areas relate to working memory function and language, attentional, as well as visual and executive control.

Figure 5.17. Between group FC changes in SPMS subjects developing cognitive impairment (z-score of ≤-1.96SD on ≥2 domains) at follow-up from a preserved state. a) 3D global map of (blue) brain RSNs showing decreased FC in baseline CP (baseline CP<longitudinal CI). The bar chart of % altered voxels below shows that the greatest FC reduction was in the auditory network (AN) b) 3D global map highlights which RSNs (red) had increased FC in baseline CP (baseline CP>longitudinal CI). The bar plot indicates that the executive control network (ECN) had the greatest % altered voxels.



CI=cognitively impaired. CP=cognitively preserved. Yellow dashed lines represent those changes above and below the 5% level. Images are shown according to radiological convention. Percentage altered voxels=% altered voxels. FC changes are significant at the p≤0.05 TFCE-corrected threshold.

Table 5.10. Summary of FC changes of SPMS groups with and without cognitive impairment at the follow-up timepoint and changes over time.

CI versus CP SPMS (≤-1.96 SDs on ≥2 domains)				**baseline CP versus longitudinal CI SPMS (≤ 1.96 SDs on ≥2 domains)**			
CI<CP RSN FC	% voxel alterations	CI>CP RSN FC	% voxel alterations	baseCP<longCI RSN FC	% voxel alterations	baseCP>longCI RSN FC	% voxel alterations
ECN	11.1	**SMN_M2**	21.9	**AN**	13.7	**ECN**	30.4
WMN	3.7	**SMN_S2b**	20.8	**WMN**	13.1	**aDMN**	22.0
AN	1.7	**AN**	18.4	**MVN**	10.7	**LVAN**	21.7
LVAN	1.6	**PN**	13.6	**pDMN**	10.6	**SMN_S2b**	20.5
RVAN	1.4	**SMN_S2a**	10.1	**FCN**	10.3	**SMN_S2a**	16.1
aDMN	0.9	**MVN**	5.9	**SN**	9.7	**FCN**	14.4
pDMN	0.8	**OVN**	5.0	**SMN_M2**	6.4	**MVN**	9.4
SN	0.7	**RVAN**	4.1	**OVN**	6.4	**pDMN**	7.1
CBLN	0.7	**aDMN**	2.7	**PN**	5.6	**RVAN**	5.7
LVN	0.5	**LVAN**	2.6	**SMN_S2b**	4.1	**BGN**	5.7
SMN_S2a	0.4	**WMN**	2.3	**CBLN**	3.8	**LVN**	4.8
BGN	0.2	**pDMN**	2.0	**RVAN**	3.7	**PN**	4.5
FCN	0.0	**CBLN**	1.9	**BGN**	1.4	**SN**	3.8
PN	0.0	**ECN**	1.6	**LVAN**	0.9	**AN**	3.4
OVN	0.0	**FCN**	1.3	**aDMN**	0.2	**OVN**	1.5
MVN	0.0	**BGN**	0.7	**LVN**	0.2	**WMN**	1.2
SMN_M2	0.0	**SN**	0.6	**ECN**	0.0	**CBLN**	0.5
SMN_S2b	0.0	**LVN**	0.3	**SMN_S2a**	0.0	**SMN_M2**	0.0

CI=cognitively impaired. CP=cognitively preserved. Summary of RSN alterations by group according to increases and decreases in FC in the CI versus CP dual regression analyses using the ≤-1.96 standard deviations on ≥2 domains criteria at follow-up cross-sectionally, and additionally for the group baseline CP to CI conversion at follow-up.

5.3.4 Discussion

The role of this experiment (**section 4.3)** was to review differences in cognitively impaired and preserved SPMS subjects, and to review changes by choice of classification of impairment used. **Section 3.4** indicated that less stringent criteria lead to an overestimation of cognitive impairment prevalence in SPMS. Whether this impacts on dynamic FC changes is unknown in the literature. I further evaluated changes in SPMS with and without cognitive impairment using the most robust criteria at the follow-up timepoint. Given the longitudinal nature of this study, I was able to directly compare FC changes in those who were preserved at baseline and developed cognitive impairment over time. This is a novel approach, not in the literature, to my knowledge.

The main findings of this section are that a more stringent criteria of cognitive impairment (z-score ≤-1.96 SD on ≥2 domains) leads to better representation of the dynamic FC rs-fMRI changes in SPMS and suggests that this should be used. In terms of development of cognitive impairment over time, the baseline preserved group show reduced FC in more posterior cognitive RSNs and increased FC in the executive RSNs, suggesting that these are key resting FC changes predisposing to SPMS cognitive impairment.

Regardless of classification, the cognitively impaired and preserved SPMS groups were matched in terms of age, gender, years of education, and IQ. There were significantly better results for the MSFC composite score ($p<0.01$) in the preserved versus impaired groups which were not driven by the T25FW or 9HPT. Overall, cognitive outcome scores were significantly worse ($p<0.01$) in the impaired versus preserved group as expected, however the delayed component of the BVMT-R was not significantly impaired. Caution is required when interpreting this, as the choice of test and reliance on upper limb motor function can impact prevalence of impairment in SPMS given the level of physical disability, EDSS=6.0. This may have led to the BVMT-R delayed component, which relies on both working memory and upper limb motor function, not having significant group differences between the cognitively impaired and cognitively preserved cohorts as explained further in **section 3.5** (Fischer *et al.*, 2014; Sumowski *et al.*, 2018). There were, however, key

differences in prevalence rates, with 29.3% impaired using the ≤-1.96 SD on ≥2 domains criteria, versus 46.2% using the ≤-1.5 SD on ≥2 domains criteria at baseline. Over time the CVLT-II, MSIS29v2, and BVMT-R showed significant improvements from baseline in the preserved group using the conservative criteria. This perhaps indicates better subjective and clinical working memory measures in this group. These changes are keeping with findings of **chapter 4** of this thesis (**section 4.5**) and other SPMS cognitive follow-up studies and differences in prevalence rates by definition used (Fischer *et al.*, 2014; Muhlert *et al.*, 2015; Chan *et al.*, 2017).

Cross-sectional comparisons of cognitively impaired versus preserved SPMS subjects at both timepoints revealed similar patterns of FC changes. By comparing global FC in the cognitively impaired versus cognitively preserved SPMS groups, choice of criteria was shown to alter the number of RSNs showing % altered voxels, however the patterns of spatial FC changes were the same. This is a key point, which has not been investigated in MS cognitive impairment previously in the literature. By using a more lenient criteria, i.e. ≤-1.5 SD on ≥2 cognitive domains, RSN alterations were missed. Therefore, these analyses suggest using a more stringent classification to understand underlying resting dynamic brain MRI changes.

As expected from other rs-f MRI studies of MS cognition (Rocca *et al.*, 2010*a*, 2014), changes in the RSNs, especially the reduction of FC in the DMN, in the cognitively impaired SPMS groups indicates involvement of widespread cognitive brain regions, which form part of more than a single RSN. Given that the attentional networks; the RVAN, LVAN, and ECN, showed increased FC (SPMS>HC) in the SPMS group overall, it appears that the reduction in FC in these network areas in the cognitively impaired state (CI<CP) may be the key component of the mechanism for cognitive impairment in SPMS subjects. Changes in processing networks and areas including the DMN and cerebellum in the cognitively impaired groups are in line with recent reports showing the contribution of cerebellar structural connectivity to the DMN to explain SDMT scores (Savini *et al.*, 2019). Furthermore, the DMN and cerebellar networks have been shown to be dysregulated in other neurological diseases (Castellazzi *et al.*, 2014, 2018*a*; Guell *et al.*, 2018; Savini *et al.*, 2019). ACC RSNs showed reduced FC overall in SPMS, but this was further reduced in the

cognitively impaired versus preserved groups indicative of the development of more cognitive impairment in SPMS (Rocca *et al.*, 2010*b*). Reductions in cerebellar FC are illustrative of worse executive and visuospatial function and have been shown to anti-correlate to the BVMT-R, which might indicate why the BVMT-R showed no significant discriminatory power between the cognitively impaired and preserved groups cross-sectionally given that the impaired groups showed lower cerebellar FC (Cocozza *et al.*, 2018). At the follow-up timepoint spatial FC changes were replicated using the more stringent criteria. There was further FC reduction in the ECN in the cognitively impaired SPMS group suggesting worsening in this domain since the baseline visit in this group.

Increased FC in the cognitively impaired groups versus preserved groups (CI>CP) cross-sectionally occurred in the sensory (AN), precuneus, and sensorimotor networks. Such widespread global brain FC increases indicate maladaptive FC alterations. These FC changes might relate to structural white and grey matter damage in MS, however this does not occur in the SPMS cohort alone. This may indicate abnormal network recruitment, as cognitive impairment is established (Loitfelder *et al.*, 2011). Alternatively, this could indicate a collapse of the networks due to accumulation of physical and cognitive disability and structural damage (Schoonheim *et al.*, 2015*b*).

Exploring the development of cognitive impairment from a preserved state over time indicated that 13 SPMS subjects developed cognitive impairment from baseline visit. Using the ≤ -1.96 SD on ≥2 domains criteria, those preserved at baseline had reduced FC (baseline CP<longitudinal CI) in more posterior brain networks; the pDMN, PN, and visual networks, as well as the working memory and auditory network. This indicates less connectivity in key visuospatial memory, working memory, and processing speed RSNs in this preserved group at baseline. There was increased FC at baseline (baseline CP>longitudinal CI) in the ECN, anterior DMN, SMN, and LVAN. This suggests more resting FC in the working memory, language, visual, and executive RSNs. From the global FC changes indicated, it cannot simply be stated that FC alterations in these RSNs in the baseline preserved subjects led to cognitive impairment at follow-up. However,

lower FC was seen in these executive and attentional areas in the baseline cognitive impaired groups, whereas there is increased FC in those who transitioned. Therefore, there is anti-correlation between the established cognitively impaired groups and the transitioning preserved group at baseline indicating that these networks play a key role in the development of cognitive impairment in SPMS over time and FC and efficiency falters here (Schoonheim *et al.*, 2017). Increased FC in the transitioned cognitively impaired groups (baseline CP<longitudinal CI) is present in the posterior DMN and has been shown to be present in literature cohorts of cognitively impaired SPMS (Basile *et al.*, 2014).

5.4 Conclusions

Studies have focussed on individual DMN regions and individual structural alterations in progressive MS (Rocca *et al.*, 2010, 2014). It is understood, however, that cognitive impairment results from both white and grey matter dysfunction in MS (Rocca *et al.*, 2015*a*). A more global assessment of FC of the whole brain, as I have used, allows for a better evaluation of alternative biomarkers of disability in MS.

The cross-sectional studies at the two timepoints in **sections 5.2**, and **5.3** investigate global RSN FC in SPMS not only to highlight changes compared to healthy subjects, but also to understand possible differences between SPMS with and without cognitive impairment. First, the group comparison of SPMS versus healthy controls shows that there are both increases and reductions of FC in RSNs involving cognitive and sensory domains. Posterior and deep brain networks and areas show increased FC in the SPMS group, and this has been shown in the literature compared to RRMS subjects (Basile *et al.*, 2014). By comparing global FC in cognitively impaired and preserved SPMS groups defined by the two different classifications, I show that there are important changes in the attentional networks of SPMS patients, not sustained in the cognitively impaired state. Additionally, more stringent cognitive impairment criteria are required to show the greatest FC alterations in networks. Reduced FC in the medial prefrontal cortex and precentral gyrus, and increased FC in the ACC, corresponding with DMN areas, correlate with cognitive impairment in terms of information processing speed and disease progression in SPMS (Rocca *et al.*, 2010*b*, 2018; Basile *et al.*, 2014). It is likely, however, that both compensatory and maladaptive changes are represented by FC, and what underlies FC in the resting brain and the BOLD effect is currently not fully understood (Schoonheim *et al.*, 2017).

Cognitive deficits and the role of underlying FC changes is currently not established at the voxel level (Filippi and Rocca, 2013, Sbardella *et al.*, 2015*b*; Rocca *et al.*, 2018). Studies have focussed on individual DMN regions and individual structural alterations in progressive MS (Rocca *et al.*, 2010, 2014) which lead to difficulty when interpreting and comparing with more global changes.

Therefore, I have shown that when studying MS cognition using rs-fMRI, classifying cohorts as binary impaired versus preserved groups may be a useful approach.

There are key increases in FC in posterior and attentional brain networks in SPMS versus controls, which are inverse when cognitive impairment is established, i.e. reductions of FC in the LVAN and RVAN compared to increases within SPMS overall. Although the literature has evidence of ACC increased FC in SPMS with cognitive impairment (Basile *et al.*, 2014), associations between changes in executive and attentional function and FC reductions in SPMS have not been documented. This may be due to the targeted nature of seed-based approaches, or more global graph based metrics. However, these alterations in executive function in the SPMS cognitive profile are supported by this studies results (**section 3.3 and 3.4**) and others (Connick *et al.*, 2013; Chan *et al.*, 2017).

The development of cognitive impairment from a preserved state appears to relate to reduced FC in the working memory, posterior DMN, and visual networks and increased FC in the executive control, and more anterior DMN hubs at baseline. This interplay and global regional FC alteration indicates differences in functional recruitment and network efficiency (Loitfelder *et al.*, 2011, Schoonheim *et al.*, 2015*b*).

Some considerations need to be taken when interpreting this study. Firstly, the pre-processing methodology involved the exclusion of five test subjects due to motion. There is also a mismatch in terms of healthy control and SPMS groups in terms of number which may impact on the degree of % altered voxels in terms of RSNs for this comparison, but this is common in such studies (Rocca *et al.*, 2016*a*, 2018). However, basic RSN results are in line with previous studies (Meijer *et al.*, 2018). Also, healthy control and SPMS cohorts were well matched in terms of age, gender, years of education, and IQ. IQ was additionally added as a co-variate to the GLM for dual regression in addition to MRI brain volume measures which can affect cognitive function (Sumowski *et al.*, 2018). Multiple comparison correction with Bonferroni were performed. As can

be seen, although there is a reduction in overall % altered voxels, the pattern of resting network FC changes was the same, and therefore a threshold of $p \leq 0.05$ TFCE corrected was used.

It is likely that there is underlying functional reorganisation that results in the FC changes seen in MS. One can hypothesise that such reorganisation is the source of variability in previously reported FC changes used to describe cognitive deficits in MS where both increases and decreases in the same RSNs associate with cognitive impairment (Tewarie *et al.*, 2018). Cognitive performance and FC metrics are indeed affected by the interplay between the degree of impairment and preserved network efficiency (Schoonheim *et al.*, 2015). Therefore, it is likely that compensatory and maladaptive FC changes occur in tandem in MS cognitive dysfunction (Penner and Aktas, 2017; Rocca and Filippi, 2017; Schoonheim *et al.*, 2017, Castellazzi *et al.*, 2018*b*). However, as stated above, the current methodology used in studies is too variable to allow for definite conclusions about which are compensatory or maladaptive. Additionally, given the bidirectional FC changes in this large SPMS cohort with cognitive impairment it is likely to represent both mechanisms related to the underlying non-uniform nature of MS pathology with inflammation and neurodegeneration present (Lassmann, 2018). Longitudinal studies, like this study, should contribute further to the understanding of such mechanisms and potentially establish a conversion threshold for associations of FC and cognitive status (Schoonheim *et al.*, 2015*b*; Tewarie *et al.*, 2018). MRI protocols may, therefore, provide a suitable metric for cognitive decline, however currently these do not exist in the clinical setting (Rocca *et al.*, 2015*a*; Grzegorski and Losy, 2017; Sumowski *et al.*, 2018).

6 Measures of regional grey matter atrophy and cognition in SPMS

6.1 Introduction

In the previous **chapters 4 and 5**, I have summarised changes and associations of cognitive performance and impairment in this SPMS cohort. A distinguishing feature of SPMS which I have highlighted is the high proportion of executive dysfunction in addition to the working memory and information processing speed deficits clinically. Relapse-onset MS has been shown to have earlier atrophy involving GM in the cerebellum, caudate, and putamen than in PPMS; which may relate to this executive predominance (Eshaghi *et al.*, 2018*a*). Of interest, looking at the resting functional changes, I emphasised loss of FC in the cognitive networks in **chapter 5**, particularly the DMN, in SPMS overall. With cognitive impairment there is further loss of FC in the attentional networks. Anatomically, these FC changes in SPMS, and more so with cognitive impairment, occur mostly in frontotemporal and posterior brain regions; the occipital lobe and cerebellum (**section 5.2.4**). Studies have emphasised the importance of GM atrophy in these regions (**section 3.4.5**) (Riccitelli *et al.*, 2011). The cerebellum is a predilection site in MS both for motor and cognitive deficits in RRMS and PMS with 49% of patients with clinically definite MS having cerebellar WM lesions with additional cortical GM cerebellar lesions (Mormina *et al.*, 2017). Topographically, the posterior cerebellum has more cognitive associations and the anterior cerebellum is involved in motor tasks (Buckner, 2013; Koziol *et al.*, 2014). Information processing speed impairment was associated with atrophy of the posterior cerebellar lobules, particularly vermis VI level (Damasceno *et al.*, 2014). The cerebellum is functionally part of several behavioural and cognitive circuits, including the SMN and DMN, which project throughout the cerebrum (**section 3.3.4**). Increased connectivity between the ACC and the cerebellum associated with higher PASAT score in CIS, RRMS, and SPMS subjects versus controls suggesting an adaptive process (Loitfelder *et al.*, 2012). However, a seed-based study correcting for structural damage showed increased FC between cerebellar lobule VIIb and the right precentral gyrus which inversely correlated with visuospatial scores from the BVMT-R in PMS which suggests a maladaptive mechanism of FC increase (Cocozza *et al.*, 2018).

In this chapter, I look at the differences in whole brain, deep, cortical, and regional GM volumes in SPMS with and without cognitive impairment to see if the spatial pattern in the literature is replicated. Additionally, given the posterior FC changes with cognitive impairment in the cerebellum described in **chapter 5**, and the fact this is a key target site for MS pathology, I look more closely at the posterior cerebellar changes in terms of regional GM volume and FC in SPMS cognitive impairment. Therefore, the aims of this chapter are:

1. To look at differences in overall, deep, cortical, and regional GM volumes in SPMS with and without cognitive impairment, and the associations of these to cognitive impairment in SPMS (**sections 6.3.1 and 6.3.2**).
2. To review cerebellar changes in volume and FC at the follow-up timepoint in the cognitively impaired SPMS group (**section 6.3.3**).

6.2 Methods

6.2.1 Study design

The design of this study and subject recruitment and clinical assessment has been summarised in **section 4.2**.

The SPMS cohorts considered for this analysis were; all SPMS, cognitively impaired SPMS (a z-score of -1.96SD or less on two or more individual tests from at least two cognitive domains), and cognitively preserved SPMS. This cognitive impairment classification was shown to be most sensitive and specific to cognitive function in **chapter 4**, **section 4.5** and additionally showed the greatest amount of dynamic FC change in **chapter 5, section 5.3.**

6.2.2 MRI acquisition and protocol

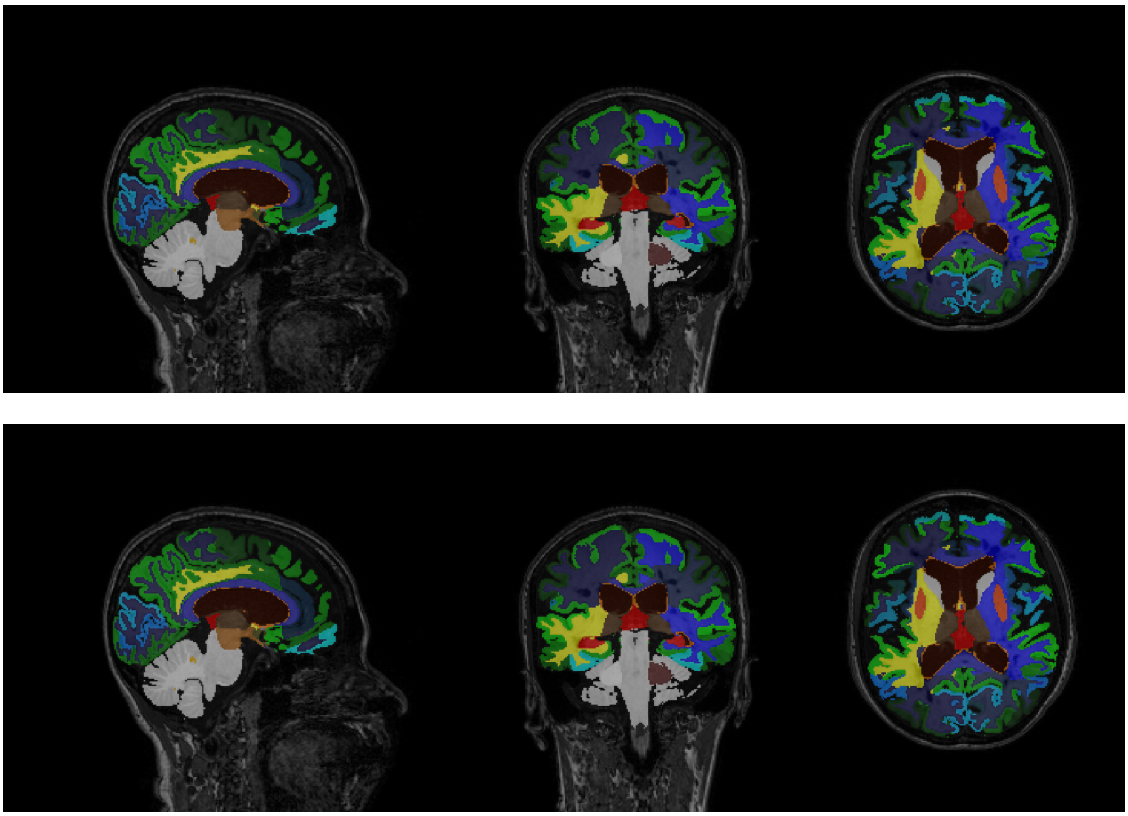
MRI acquisition and protocol have been summarised in **section 5.2.2.2** (**table 5.1**).

6.2.3 Structural MRI analysis

Section 5.2.2.3 summarises the methods for T2 lesion load evaluation, lesion filling, and tissue segmentation analysis for normalised whole brain volume (NBV), grey matter (GM), white matter (WM) volumes using the Geodesic Information Flow (GIF) algorithm (Cardoso *et al.*, 2015). Subsequent read outs of regional volumes from GIF was undertaken. The following brain parcellations were obtained and normalised using the scaling factor for correcting for individual volume variations in head size (Smith *et al.*, 2002) as described in **section 5.2.2.3**, and grouped as below:

- Hippocampus:
    - Left hippocampus
    - Right hippocampus
- Thalamus:
    - Left Thalamus
    - Right Thalamus
- Caudate:
    - Left caudate
    - Right caudate
- Cerebellum:
    - Cerebellar Vermal Lobules I-V
    - Cerebellar Vermal Lobules V3-X
    - Cerebellar Vermal Lobules VI-VII
    - Left Cerebellum Exterior
    - Left Cerebellum White Matter
    - Right Cerebellum Exterior
    - Right Cerebellum White Matter

Figure 6.1. Examples of regional brain parcellations from the Geodesic Information Flow (GIF) algorithm.



I derived these images from subject-data in this thesis. GIF parcellation images (Cardoso et al., 2015) were overlaid onto the subject specific 3D T1-weighted structural MRI scan and captured using FSLview (FMRIB, 2000).

To look at change over time, I calculated atrophy from the difference between baseline and longitudinal volumes at 96 weeks, and then derived percentage change. To calculate percentage annualised change in volumes, I divided this by the interval in days between visits and multiplied by 365.

The percentage brain volume change (PBVC) was quantified using the SIENA method as previously described in **chapter 3** (Smith *et al.*, 2002, 2004). This method was used to calculate the PBVC at 96 weeks from baseline. As for the cross-sectional analysis of normalised whole brain volume, 3D-T1 baseline and follow-up scans (i.e. 96-week scans) were reoriented, bias-field corrected and the 3D-T1 scans were lesion filled. The scans were then analysed with GIF (Cardoso *et al.*, 2015), which extracted the brain and gave additional measures of total intracranial volume (TIV), GM (deep grey matter (DGM) plus cortical grey matter (CGM)), WM, and CSF

volumes for the two timepoint scans. The resulting brain volumes between the two timepoints were co-registered and the SIENA algorithm was applied to calculate the PBVC. Unlike the SIENAX normalised brain volume pipeline which is across subjects, the registration is done between scans at different timepoints within subjects by registering a single timepoint scan to a standard common template (Smith *et al.*, 2002, 2004).

6.2.4 Rs-fMRI analysis

Rs-fMRI was handled as **per section 5.2.2.4**.

After pre-processing, RSN identification, and dual regression analysis at the follow-up timepoint as described, I utilised the SPM xjView toolbox (https://www.alivelearn.net/xjview) to derive anatomical locations of each cluster in the thresholded maps. This was to calculate proportions of increased or decreased cerebellar FC alteration for the overall SPMS and cognitively impaired SPMS cohort in voxels. This is to allow some comparison with regional cerebellar volume changes at the follow-up visit.

The output of the SPM visualiser toolbox is regional voxels with FC alteration as per a combined Automatic Anatomical Labelling (AAL) and Talairach atlas. This provides a group constant value for FC voxels up or down in the cerebellar regions for the cognitively impaired group, which I summed into a whole cerebellar region. Dividing this value by the total number of altered voxels in the FC up or down analyses this provided percentage alterations in the cerebellum for the cognitively impaired group at follow-up.

The output of GIF in terms of regional volumes in voxels uses the GIF atlas. Once again, as per the structural MRI analysis, I summed the cerebellar regions to make a total cerebellar volume for the cognitive impaired cohort. I calculated the proportion of the cerebellar volume (%) out of whole brain volume at follow-up for comparison.

Associations between the constant group FC changes and variable subject-level structural volumes of the cerebellum are not statistically possible. Therefore, voxels of cerebellar volume, atrophy, proportion, FC altered voxels, and FC alteration (%) have been tabulated and provided as a column graph for visual comparison.

6.2.5 Statistical analysis

As with the other chapters, statistical analyses were undertaken using Stata (SE 15.1 for Mac. Stata Corp, College Station, TX 77845, USA).

Between group differences for gender, age, and MRI volume (longitudinal timepoint) and atrophy variables were tested dependent on variable type (numerical, categorical, binary, normal distribution). Scatterplots were used to look for non-linearities and normality of the variables. T-tests were used to test differences in age. Categorical variable differences were tested with chi-squared tests if less then 2 variables, i.e. gender. Non-parametric Mann-Whitney U-tests were applied for group differences in the for longitudinal MRI volumes and atrophy measures. Results were Bonferroni corrected for multiple comparisons and an exact probability value (p) statistical threshold of $p \leq 0.05$ was considered significant. Results are presented as frequency tables with analyses of differences between groups; healthy controls versus SPMS, cognitively impaired (a z-score of -1.96SD or less on two or more individual tests from at least two cognitive domains) versus cognitively preserved.

Multivariable logistic regression models of cognitively impairment (a z-score of -1.96SD or less on two or more individual tests from at least two cognitive domains) and the associations of regional MRI volume at follow-up, and atrophy were developed. This was for the 47 SPMS subjects in the longitudinal MRI cohort (see **figure 4.2**). The independent predictors tested were the regional volumes and regional atrophy measures at follow-up, including T2 lesion load. Covariates chosen for the models were those which were most frequently significant in the SPMS group linear regressions for cognitive performance (**section 3.3.3.2**), i.e. HADS anxiety, age and

gender in the follow-up cohort. Stata reports McFadden's pseudo R-squared as the relative fit of two models, but not the absolute fit of the models. LR were gathered per model to show the overall model fit. To review the sensitivity; the probability of the model predicting a positive outcome for a given observation, and the specificity; the probability that the model predicts a negative outcome for an observation, the AUROC was calculated to define the ability of the model to distinguish between positive and negative outcomes. Tables of independent models have been produced. Tables include; OR, p-value, 95% confidence interval, LR, and AUROC.

6.3 Results

This study looks at the SPMS cohort and then division into cognitively impaired and preserved. As with the longitudinal rs-fMRI study in **chapter 5**; 47 SPMS were included in this analysis. Of the SPMS group, 19 were cognitively impaired using the ≤-1.96SD on two or more domains criteria, 28 were cognitively preserved. This criteria has been shown in previous chapters (**4 and 5**) to be the most robust based on cognitive performance and rs-fMRI changes.

6.3.1 Regional MRI volumes, and regional grey matter atrophy in the SPMS cohorts at follow-up

Demographic and clinical-cognitive characteristics of the longitudinal SPMS MRI cohorts are summarised In **chapter 5** (**tables 5.5 and 5.9),** and are therefore not repeated. **Table 6.1** summarises the parcellated regional brain volumes (mls) from the GIF read-out, PBVC for NBV, and percentage annualised change for other volumes.

As described, the complete SPMS group showed significant deficits of working memory, information processing speed, and attention not reflected by the PASAT3, BVMT-R, and Hayling task (**table 5.5**). The SPMS group has expectedly lower NBV, WM, and GM volume then healthy control data available in the literature (Eshaghi *et al.*, 2018*b*). PBVC in the SPMS cohort logically matched that of the placebo arm of the MS-SMART study (Chataway *et al.*, 2020). Deep GM annualised atrophy was lower than in the literature cohort at -0.6% versus -1.45%, as was cortical GM atrophy at -0.83% versus -1.11%, but may represent differences in sample size (Eshaghi *et al.*, 2018*b*). Cerebellar GM annualised atrophy was similar in this study to the literature cohort at -0.9% versus -0.98% (Eshaghi *et al.*, 2018*b*).

Males were non-significantly over-represented in the cognitively impaired SPMS group overall. Preserved and impaired groups were well matched in terms of age, gender, education, visit

interval and IQ, but showed expectedly lower cognitive scores in all domains for the cognitively impaired group except for the PASAT3, and BVMT-R (**table 5.9**) although in **chapter 3 section 3.5,** these tests were shown to have least input to SPMS cognitive impaired status. As expected, T2LL was higher in the cognitively impaired group, although not significantly so (**table 6.2**). There was significantly higher PBVC (p=0.03) in the cognitively impaired versus preserved group, and this was higher than in the SPMS group overall. There is significantly lower overall GM volume in the cognitively impaired group (p=0.04) with non-significantly greater % annualised GM atrophy (-0.83% versus -0.82%). This difference is driven by lower cortical GM volume in the impaired group (775.61±40.08 ml versus 786.85±30.29ml respectively).

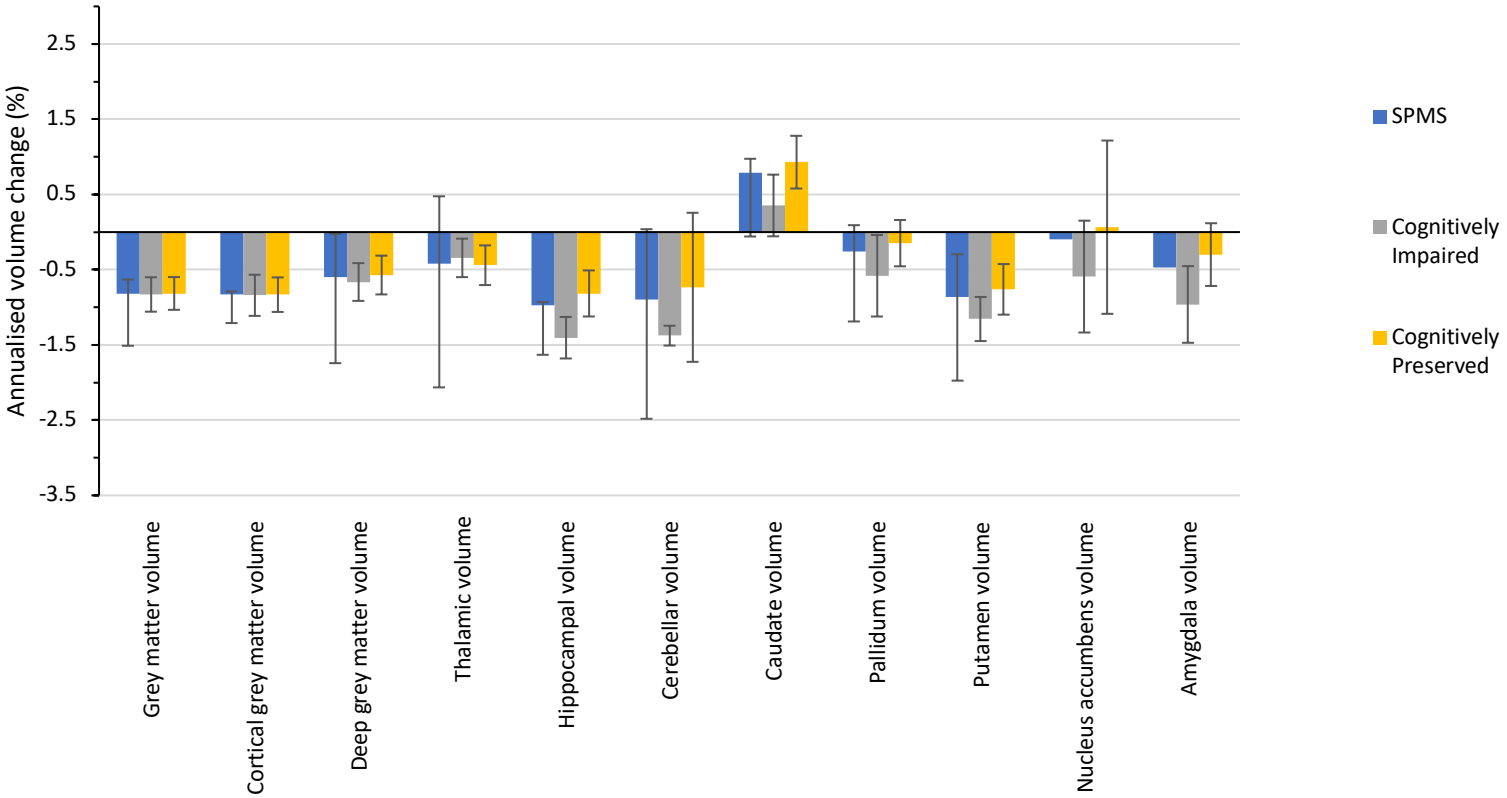
As shown in **figure 5.2**, annualised % change in regional brain volumes by cohort. Although not significant, hippocampal, cerebellar and putamen volumes have the greatest % annualised atrophy in the cognitively impaired SPMS group. There is relative preservation and seemingly an increase in caudate volume in all of the SPMS cohorts, but these are least in the cognitively impaired group.

Table 6.1. Summary of MRI volume metrics for the SPMS follow-up cohorts.

	Overall SPMS (n=47)		Cognitively Impaired SPMS (n=19)		Cognitively Preserved SPMS (n=28)		long	change
	Mean ± SD	% change	Mean ± SD	% change	Mean ± SD	% change	p	p
Gender M:F	10:37		4:15		6:22		0.98	
Age (years)	56.96±6.92		55.66±7.7		57.4±6.69		0.5	0.96
Normalised brain volume (ml)	1403.96±65.79	-1.41* (-0.79)	1376.60±67.90	-1.75* (-0.95)	1413.34±63.33	-1.15* (-0.62)	0.4	**0.03**
White matter volume (ml)	574.82±38.23	-0.33	556.89±36.11	-0.49	580.97±37.46	-0.28	0.7	0.34
Grey matter volume (ml)	829.14±34.75	-0.82	819.71±41.32	-0.83	832.37±32.23	-0.82	**0.04**	0.1
Cortical grey matter volume (ml)	783.98±32.96	-0.83	775.61±40.08	-0.84	786.85±30.29	-0.83	**0.05**	0.1
Deep grey matter volume (ml)	45.15±3.3	-0.6	44.11±3.05	-0.66	45.52±3.39	-0.57	0.57	0.07
T2LV (ml)	11.18±8.81	0.72	18.15±8.42	0.19	8.78±7.68	0.89	0.23	0.26
Thalamic volume(ml)	14.04±1.29	-0.42	13.50±1.39	-0.34	14.23±1.22	-0.44	0.95	0.13
Hippocampal volume (ml)	12.00±0.84	-0.97	12.11±0.59	-1.4	11.97±0.91	-0.82	0.46	0.17
Cerebellar volume (ml)	153.11±14.73	-0.9	150.57±18.57	-1.38	153.98±13.38	-0.73	0.18	0.41
Caudate volume (ml)	8.47±1.12	0.79	8.23±0.90	0.35	8.55±1.18	0.93	0.56	0.17
Pallidum volume (ml)	2.39±0.20	-0.26	2.44±0.15	-0.58	2.376±0.21	-0.15	0.29	**0.04**
Putamen volume (ml)	12.68±1.38	-0.86	12.11±1.07	-1.16	12.87±1.43	-0.76	0.18	**0.02**
Nucleus accumbens volume (ml)	1.70±0.15	-0.1	1.73±0.18	-0.59	1.70±0.15	0.07	0.78	0.79
Amygdala volume (ml)	2.69±0.18	-0.47	2.73±0.16	-0.96	2.68±0.19	-0.3	0.86	0.47

*Results are presented as normalised mean and SD, atrophy, and percentage annualised change (% change). * Percentage Brain Volume Change (PVBC), brackets contain the annualised PBVC. Group differences are between cohort MRI volumes, and between cohort MRI atrophy measures. A significant result is $p \leq 0.05$, and is in bold. Na= not applicable. LONG = significance between longitudinal values of the cognitively impaired and preserved groups, CHANGE = significance between change over time of the cognitively impaired and preserved groups.*

Figure 6.2. Graph of percentage annualised grey matter volume change in SPMS; overall, with, and without cognitive impairment (z-score of ≤ -1.96SD on > 2 domains).



Column graph with error bars of annualised regional normalised volume change (%) for; whole brain, white matter, grey matter, cortical grey matter, deep grey matter, T2 lesion, thalamic, hippocampal, cerebellar, caudate, pallidum, putamen, nucleus accumbens, and amygdala volume. Summarised for overall SPMS, cognitively impaired (z-score ≤-1.96SD on two or more domains) SPMS, and cognitively preserved SPMS cohorts.

6.3.2 Regional grey matter MRI predictors of cognitive impairment in SPMS

The results of the multivariable logistic regression analysis investigating regional volume and atrophy predictors of cognitive impairment in SPMS using the ≤-1.96SD on two or more domains criteria is shown in **table 6.2**. For all of the individual models, the covariate predictors; age, gender, and HADS anxiety were not significant.

Looking at the individual regional volumes, as with the group differences between the cognitively preserved and impaired SPMS, only total GM and CGM volume are significant independent predictors of impairment (GM; OR=1.00, p=0.05, LR=4.34, AUROC=0.67. CGM; OR=1.00, p=0.04, LR=5.02, AUROC=0.68). In terms of atrophy, however, it is deep GM atrophy which predicts SPMS cognitive impairment (DGM atrophy; OR=1.00, p=0.04, LR=5.75, AUROC=0.68). Atrophy of deep pallidal and putaminal regions are significant drivers of SPMS cognitive impairment with AUROC of 0.69 and 0.71 respectively (**table 6.2**). Caution should be taken when interpreting the multivariable logistic models, as overall ORs are 1.0 for all MRI metrics, indicating little overall effect in terms of increased risk or chance of SPMS cognitive impairment. I also looked at the associations of SPMS cognitive impairment and NBV, WMV, and T2LL at follow-up and changes from baseline over time including PBVC. There were not significant associations with the dependent variable, however the AUROC for PBVC was 0.79 (results not shown).

Table 6.2. Independent regional grey matter predictors of cognitive impairment (z-score of ≤-1.96SD on ≥2 domains).

Independent MRI predictors of cognitive impairment ≤-1.96SD on two or more domains						
Predictors	**OR**	**p**	**95% CI**		**LR**	**AUROC**
Age	1.02	0.74	0.93	1.11	0.21	0.56
Gender	0.97	0.97	0.23	4.15		
HADS anxiety	0.98	0.83	0.84	1.15		
LONGITUDINAL						
Grey matter volume (ml)	1.00	**0.05**	1.00	1.00	4.34	0.67
Cortical grey matter volume (ml)	1.00	**0.04**	1.00	1.00	5.02	0.68
Deep grey matter volume (ml)	1.00	0.63	1.00	1.00	0.45	0.59
Thalamic volume (ml)	1.00	0.88	1.00	1.00	0.24	0.56
Hippocampal volume (ml)	1.00	0.77	1.00	1.00	0.30	0.57
Cerebellar volume (ml)	1.00	0.24	1.00	1.00	1.63	0.64
Caudate volume (ml)	1.00	0.72	1.00	1.00	0.34	0.57
Pallidum volume (ml)	1.00	0.16	1.00	1.01	2.37	0.61
Putamen volume (ml)	1.00	0.15	1.00	1.00	2.44	0.63
Nucleus accumbens volume (ml)	1.00	0.84	1.00	1.00	0.25	0.55
Amygdala volume (ml)	1.00	0.96	1.00	1.00	0.22	0.55
Grey matter atrophy (ml)	1.00	0.12	1.00	1.00	3.09	0.67
Cortical grey matter atrophy (ml)	1.00	0.14	1.00	1.00	2.74	0.67
Deep grey matter atrophy (ml)	1.00	**0.04**	1.00	1.00	5.75	0.68
Thalamic atrophy (ml)	1.00	0.06	0.99	1.00	4.44	0.67
Hippocampal atrophy (ml)	1.00	0.12	0.99	1.00	2.79	0.62
Cerebellar atrophy (ml)	1.00	0.37	1.00	1.00	1.02	0.58
Caudate atrophy (ml)	1.00	0.22	1.00	1.00	2.82	0.62
Pallidum atrophy (ml)	0.99	**0.05**	0.97	1.00	4.62	0.69
Putamen atrophy (ml)	0.99	**0.02**	0.99	1.00	8.94	0.71
Nucleus accumbens atrophy (ml)	0.99	0.51	0.98	1.01	0.65	0.54
Amygdala atrophy (ml)	1.00	0.69	0.99	1.01	0.37	0.55

LR= likelihood ratio, AUROC = area under the receiver operator curve. Significant results are in bold if p≤0.05.

6.3.3 Comparisons of structural and functional measures of the cerebellum.

This analysis builds on the findings of more posterior FC changes including the cerebellum from the rs-fMRI SPMS versus controls and SPMS with cognitive impairment results at follow-up in **chapter 5** (**sections 5.2 and 5.3**). Due to the nature of the general linear model for dual regression, only the cognitively impaired SPMS group are shown, as the FC changes would be the inverse for the preserved group. The cerebellum was chosen a priori as it is quantifiable using both the SPM (rs-fMRI) and GIF (structural) atlases.

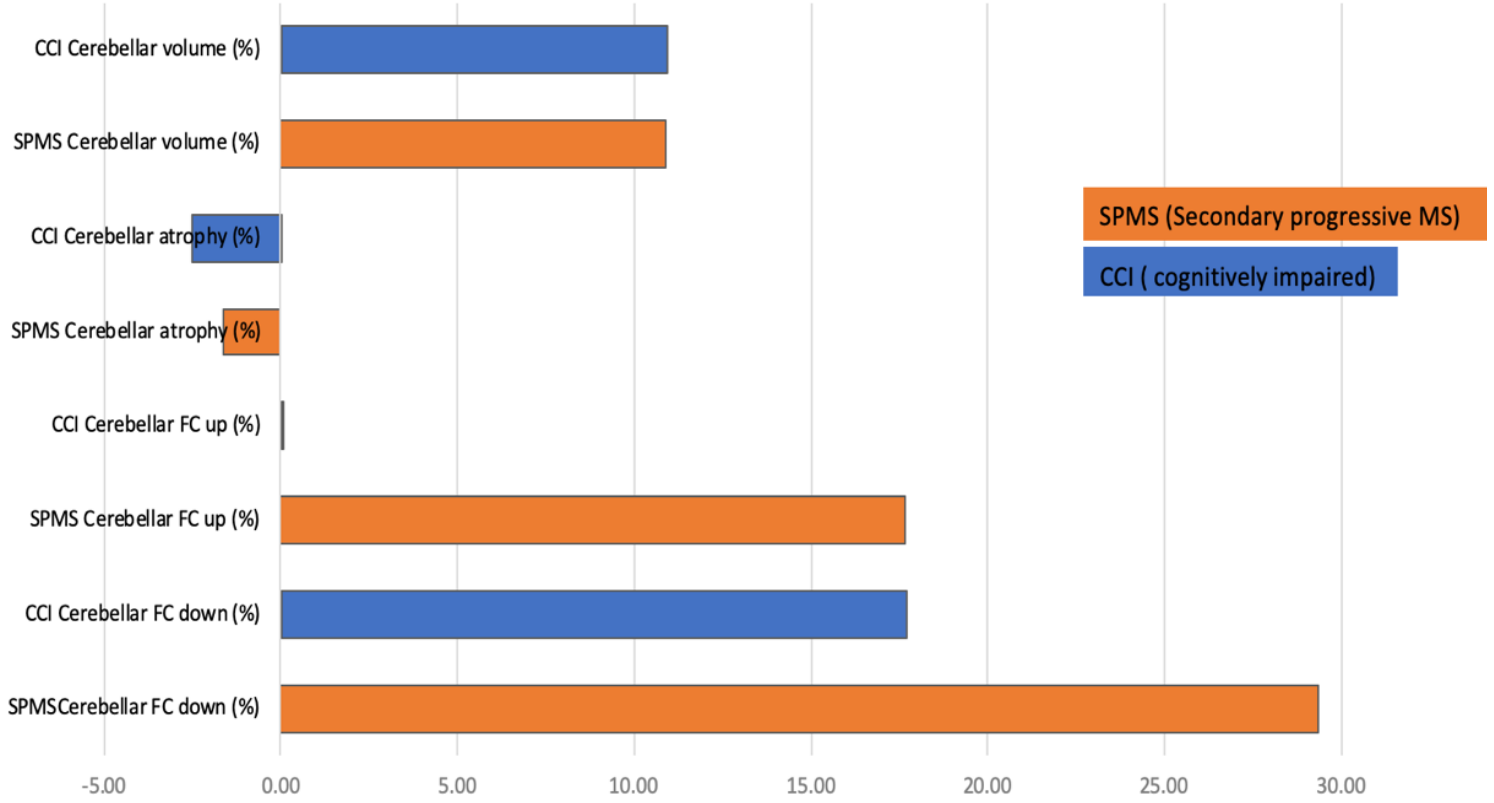
Group constants for FC and mean for subject-variable cerebellar volumes and atrophy are shown in **table 6.3**. As expected, the cognitively impaired SPMS subjects have lower cerebellar volume, and greater change in volume and percentage over time then the SPMS group overall. In the cognitively impaired SPMS group there was greater reduction in the number of voxels with FC reduction, but proportionally this was lower than in the SPMS group overall (17.7% versus 29.4%). However, looking at where FC is up in the cerebellar region, this is much lower, 0.1% versus 17.65% in the cognitively impaired versus SPMS cohort as a whole. These changes are shown graphically as percentage proportions for ease of comparison in **figure 6.3**. Therefore, despite being almost matched in terms of % cerebellar volume overall, there is greater cerebellar atrophy in the cognitively impaired SPMS group, with less cerebellar FC increases and reductions than in SPMS overall indicating alterations in connectivity when cognitive impairment is defined.

Table 6.3. Summary of cerebellar FC, volume, and atrophy measures in the SPMS and cognitively impaired SPMS cohorts.

	Total number of voxels (mm3)	Total number of voxels (ml)	% proportion/ change
SPMS Cerebellar FC decreased	370.00	0.37	29.37
Cognitively impaired SPMS Cerebellar FC decreased	1598.00	1.60	17.71
SPMS Cerebellar FC increased	1592.00	1.60	17.65
CI SPMS Cerebellar FC increased	9.00	0.009	0.10
SPMS Cerebellar atrophy	-3023.64	-3.02	-1.64
CI SPMS Cerebellar atrophy	-3784.19	-3.78	-2.49
SPMS Cerebellar volume	158844.39	158.84	10.90
CI SPMS Cerebellar volume	150566.9	150.57	10.92

FC = functional connectivity, CI=cognitively impaired.

Figure 5.3. Column graph of cerebellar rs-fMRI FC changes, volume, and atrophy measurements in SPMS and SPMS with cognitive impairment (z-score of ≤-1.96SD on ≥2 domains).



CCI = SPMS with cognitive impairment (z-score of ≤ -1.96SD on > 2 domains). FC = functional connectivity.

6.4 Discussion

I investigated a priori, whole, regional and deep regional GM MRI volumes and atrophy to identify changes in SPMS and cognitive impairment in SPMS.

The main findings of this analysis were that in the cognitively impaired group there was a significantly greater rate of annualised GM atrophy and lower GM and CGM volumes at follow-up. The main predictors of cognitive impairment in SPMS were GM and CGM volume, also DGM atrophy and deep GM regional atrophy within the pallidum and putamen. Finally, proportionally there were less FC increases in the cerebellum in the cognitively impaired SPMS group, and this was related to lower cerebellar volume and greater cerebellar atrophy over time than in SPMS overall.

Brain atrophy measures reflect neurodegeneration of both WM and GM in MS (Siffrin *et al.*, 2010; De Stefano *et al.*, 2016; Rocca *et al.*, 2017). Previous studies have suggested annualised PBVC rates of -0.5 to -1%/year in SPMS (Fox *et al.*, 2000; Furby *et al.*, 2010; Chataway *et al.*, 2014, 2020) (**chapter 3, figure 3.3**). Therefore the SPMS cohort in this study is in keeping with this at -0.79%/year annualised PBVC (overall PBVC -1.45%) (Chataway *et al.*, 2020). There was also significantly greater annualised PBVC as expected in those SPMS subjects with cognitive impairment (-0.98%/year) (Rocca *et al.*, 2015*a*).

Both lesional and parenchymal, e.g. cerebellar and thalamic, GM atrophy in MS are an outcome of axonal loss more than demyelination (Bjartmar *et al.*, 2003; Miller *et al.*, 2003; Honce, 2013). GM volume is also known to be lower in SPMS cohorts overall (Roosendaal *et al.*, 2011, Eshaghi *et al.*, 2018*b*). Annualised GM atrophy in this SPMS cohort was -0.82 to -0.83%/year with and without cognitive impairment respectively, and therefore, less than in other progressive MS non-cognitive cohort studies where this was -1.18%/year (Furby *et al.*, 2010). Cortical GM annualised atrophy rates were high at -0.84%/year in the impaired versus -0.83%/year in the preserved group, indicating that this is not only due to WM disease alone (Steenwijk *et al.*, 2015). Deep GM

atrophy rates were lower than in longer duration cohort studies at -0.6%/year versus -1.45%/year (Eshaghi *et al.*, 2018*b*). The SPMS group had expectedly lower DGM volumes than literature controls (Eshaghi *et al.*, 2018*b*), and overall the cognitively impaired group had a greater rate of DGM atrophy, -0.66%/year versus -0.57%/year in the cognitively preserved cohort. Despite not increasing the risk of cognitive impairment significantly per percent change, DGM atrophy was a significant predictor of cognitive impairment in this cohort. This is in keeping with other studies of deep GM atrophy and cognition (Bergsland *et al.*, 2016; Modica *et al.*, 2016).

Thalamic annualised atrophy was -0.42%/year in the SPMS group overall, however, this was lower than expected at -0.34%/year in the cognitively impaired group despite a lower thalamic volume. This is likely due to the lower change in volume over time in the cohort sample. The greatest annualised percentage change in regional GM volume with cognitive impairment versus cognitively intact SPMS was within the cerebellum (-1.38%/year versus -0.73%/year) and hippocampi (-1.40%/year versus -0.82%/year). Hippocampal volume is known to reflect working memory function and has been shown to be lower in progressive MS (Roosendaal *et al.*, 2010*a*; Damjanovic *et al.*, 2017). In the logistic regression model of cognitive impairment, it was deeper GM nuclei atrophy that were significant predictors; the putamen and pallidum. This supports the correlations of deep grey matter structures and cognitive function in SPMS (Rocca *et al.*, 2015*a*, 2017; Sumowski *et al.*, 2018). In relapse-onset MS the cerebellum and putamen show early atrophy and this progresses in SPMS, compared to later occurrence in PPMS (Eshaghi *et al.*, 2018*a*). The posterior changes of atrophy in the cognitively impaired SPMS group are supported by findings from Riccitelli et al who also showed that those with SPMS who were cognitively impaired had more posterior and deep GM atrophy (Riccitelli et al., 2011).

In **chapter 5**, in addition to temporoparietal FC reductions in the SPMS and cognitively impaired cohorts, there were changes in the cerebellar regions anatomically. Given that these were large enough to be captured as voxel FC changes, and the high annualised % atrophy rate within the cerebellum of cognitively impaired subjects, I derived a proportion of FC alterations; either up or down for comparison with the regional cerebellar changes. Rs-fMRI studies have shown disrupted

cerebellar FC within the cerebellar network (Dogonowski *et al.*, 2014; Rocca *et al.*, 2018), but also attentional and sensorimotor networks which include the cerebellum (Loitfelder *et al.*, 2012; Sbardella *et al.*, 2017). As expected, the cognitively impaired SPMS subjects have a lower cerebellar volume, and greater change in volume and percentage over time as described above, relating to attention, working memory, and verbal fluency in cohort studies (Sarica *et al.*, 2015). The cognitively impaired SPMS group showed greater % reduction in FC in the cerebellum compared proportionally to FC increases. Looking at the SPMS group overall, it is clear that this may represent functional maladaptation in cognitive impairment (Tewarie *et al.*, 2014, Schoonheim *et al.*, 2015*b*; Cocozza *et al.*, 2018). However, in the literature both increases and decreases in FC have been related to the cerebellum (Mormina *et al.*, 2017). The results here suggest that functional changes are proportionately greater by voxel % than structural atrophy in the cerebellum as a whole. Perhaps this supports functional changes preceding structural atrophy in SPMS with cognitive impairment (Corriveau-Lecavalier *et al.*, 2020). Caution must be taken in conclusions made on the cerebellum as a whole entity given the topographical anatomical functions (Buckner, 2013; Koziol *et al.*, 2014). Additionally studies used non-uniform methodology and definitions which makes direct comparisons difficult.

There were some limitations to this study. The sample size is small and the variations in atrophy rates from the literature may also be effected by skew as shown by the standard deviations limiting the averaging out of errors in the cohort (Honce, 2013; Rocca *et al.*, 2017). Secondly, although the derivation of proportion FC and volume changes allows for some comparison of cerebellar functional and structural function in cognitive impairment, it is limited. Methodologically, the atlases from which the regions are derived vary and segment the cerebellum into different regions, therefore limiting the analysis to the whole cerebellum, and not allowing for sub-regional analyses. Rs-fMRI dual regression analysis provides a summed FC value for FC for the cohort, and not at the subject-level as per structural MRI analysis. Therefore, statistically this limits any further descriptions other than quantitative variations in terms of voxels. Conclusions made are therefore speculative, but do suggest that functional connectivity changes may supersede or even precede atrophy in the cerebellum as a whole. Further seed based and graph theory based

approaches with correction for volume metrics may be a solution, but are also dependent on having a consensus on definitions of cognitive impairment and the atlas used.

6.5 Conclusions

This chapter adds to the literature for SPMS PBVC rates over time. Cognitive impairment is shown to be determined independently by GM volume, CGM volume, DGM atrophy with further deep GM associations. The greatest annualised regional GM atrophy occurs within the hippocampi and cerebellum, known to have key roles in working memory and working memory, attention, and executive functions respectively. Looking at an a priori measure of cerebellar rs-fMRI FC, I have highlighted that there is disruption of FC increases in the cerebellum, more than decreases in the cognitively impaired SPMS group overall. This likely represents functional maladaptation and network inefficiency.

7 Conclusions and future directions

I undertook a 2-year follow-up study of cognition in SPMS using clinical, functional MRI, and structural MRI measures. The major objectives of my study were; firstly, to better define the cognitive profile of SPMS in a large cohort, and to look at relevant associations. Secondly, to provide a sensitivity analysis of the main criteria for defining cognitive impairment in SPMS, and to look at associations for impairment and developing cognitive impairment. Thirdly, to look at underlying dynamic rs-fMRI changes of SPMS with and without cognitive impairment and changes over time. Finally, I looked at structural regional GM volume measures in SPMS with and without cognitive impairment. I will look at these themes in turn below.

7.1 Insights into cognitive performance in SPMS

This study supports the literature in that specifically: working memory, information processing speed, attention and executive domains are sensitive to pathology in SPMS (Connick *et al.*, 2013; Sumowski *et al.*, 2018). Therefore, current strategies which do not include tests of executive function will miss specific SPMS determinants and potentially weaken any use of these tests as prognostic markers. In terms of executive function, verbal fluency and the Stroop task were most insightful, in keeping with other studies showing their use for assessing more fronto-executive function (Chapados and Petrides, 2013). My work suggests the use of a "BICAMS-plus" protocol with added higher working memory and executive domain coverage.

The SDMT was shown to be the most sensitive outcome of cognitive performance and decline over time in terms of associations. This supports its role as a good clinical correlate of cognitive performance in MS with the benefit of short duration (Van Schependom *et al.*, 2014). However, for a longitudinal clinical trial I support that the SDMT may not be sufficient as a sole progression marker, and requires a physical and executive composite (Benedict *et al.*, 2017; Goldman *et al.*, 2019). The SDMT, CVLT-II, and Stroop tasks differentiated SPMS from healthy controls most. A key component of these tasks is visual function and perhaps this links the impairments in these outcomes due to visual symptoms. However, there were no associations of these tasks and the MSVQ-7 in the SPMS. Although vision was not further assessed in my thesis and may require

the use of OCT measures of retinal nerve fibre layers which are shown to correlate with progression in MS (Gordon-Lipkin *et al.*, 2007). This is a suggested future linked project, as OCT was an exploratory MS-SMART outcome, and so I am collaborating with another colleague who collected this data at UCL.

I have shown that employment status was predicted by information processing speed, attention, and working memory tasks and vice versa. Higher IQ, being employed, and undertaking more premorbid leisure activities are associated with less decline on executive and verbal memory tasks. This provides further evidence of the importance and interplay of intellectual enrichment on cognitive reserve in MS, particularly SPMS (Sumowski *et al.*, 2010*b*, 2012; Briken *et al.*, 2014).

7.2 Improving the definition of cognitive impairment in SPMS

I have shown that the criteria used to define cognitive impairment in SPMS impacts on the cognitive profile of impaired tests and associations with outcomes. In particular, information processing speed, verbal memory, and executive function were the greatest contributors to overall cognitive impairment in SPMS when using the z-score of -1.96SD or less on two or more individual domain tests. I show that there are differences in prevalence rates of cognitive impairment using both tested definitions, but that overall a more stringent criteria allows for more clear and pure evaluation of SPMS cognition. Employment is shown to be a vital association with cognitive impairment, with unemployment increased the odds of cognitive impairment by 11 fold. This shows that employment status should be noted during any study of MS cognitive impairment.

7.3 The dynamic rs-fMRI changes of SPMS and the development of cognitive impairment over time

This is the largest pure SPMS longitudinal whole brain rs-fMRI study of cognition in the literature, and is the first to look at the classification of cognitive impairment with rs-fMRI.

I highlight more frontoparietal and posterior RSN changes, particularly in the cognitive RSNs in SPMS which further the literature in terms of the development of these changes over time in MS (Rocca *et al.*, 2016*a*), and versus RRMS (Basile *et al.*, 2014). Over time, there are key increases in FC in posterior and attentional brain networks in SPMS, which are opposite when cognitive impairment is established.

I have shown that rs-fMRI shows key FC reductions in more posterior, attentional and cognitive RSNs with the development of cognitive impairment. Additionally, reductions of FC in these areas increase the tendency of developing impairment over time from a preserved state. It is likely, that both compensatory and maladaptive changes are represented by FC, and what underlies FC in the resting brain and the BOLD effect needs more evaluation (Schoonheim *et al.*, 2017).

If a more stringent pooled cognitive criteria is used then there is a greater overview of FC alterations which are otherwise missed. This is a novel finding and has not been shown in the literature to date. A multimodal approach is likely needed to understand this better (Schoonheim *et al.*, 2015*a*; Meijer *et al.*, 2018; Savini *et al.*, 2019). My results further the literature by establishing some factors affecting the reliability of rs-fMRI and clinical applications in studying cognition in SPMS (Fox, 2018).

7.4 Regional grey matter changes and cognitive impairment in SPMS

I show that cognitive impairment is determined independently by GM volume, CGM volume, and DGM atrophy, with further deep GM atrophy associations. Additionally, looking at the annualised regional GM atrophy rates, this is greatest in areas allied to working memory, attention, and

executive functions; i.e. hippocampi and cerebellum. I developed an a priori measure of proportion of FC and volume alteration of the cerebellum. There is greater functional then structural disruption in cognitively impaired SPMS versus SPMS overall in the cerebellum suggesting that dynamic changes may have a greater input then structural. This was a very limited sample size, and therefore is a future direction, but suggests that functional connectivity changes are greater than structural atrophy in SPMS cognitive impairment. Future approaches should include seed-based rs-fMRI analyses and graph matrix connectivity with topographic regional cerebellar volumes for direct correlations.

7.5 Limitations

There are limitations to the clinical and imaging methodologies used to test the hypotheses in this thesis, as highlighted at the end of each experimental chapter.

Firstly, **chapter 2** summarises the challenges in researching and defining cognitive symptoms in MS. With no current consensus on the definition and measurement of cognitive impairment in MS developing this project was clearly imperative (Sumowski *et al.*, 2018; Benedict *et al.*, 2020). The experimental **chapter 4** firstly aimed to confirm the prevalence and pattern of cognitive deficits in SPMS specifically, and then explored the definitions. I utilised the BICAMS given the current recommendations (Langdon *et al.*, 2012), but also tested delayed components of working memory and executive function given the known prevalence in SPMS (Connick *et al.*, 2013; Chan *et al.*, 2017). I chose an a priori approach to the definitions which I tested based on the systematic review of what are the most robust currently used criteria (Fischer *et al.*, 2014). Therefore, a key limitation is that this work aims to state a potential base from which future cognitive studies of SPMS can learn, but is not powered to look at the strength of the battery of tests or definitions used.

The 'clinico-radiological' paradox highlights the mismatch of MS cognitive symptoms and conventional MRI measures, such as lesion volume (Rocca *et al.*, 2015*a*). Therefore, it is key to

develop more advanced neuroimaging techniques to gain an understanding of the underlying pathophysiology of cognitive dysfunction essential for diagnosing, monitoring and developing specific treatments. A major aim for the fMRI aspect of this thesis was to develop the current understanding of underlying FC changes in SPMS with and without cognitive impairment in a much larger cohort than in the previous studies (see **table 3.3**) to allow a benchmark from which the field can further progress. **Section 3.3** shows that posterior network changes predominate in more progressive forms of MS, however the meaning of the direction of FC change is less clear.

In the rsfMRI field there is heterogeneity in methodological aspects of studies and a lack of standardised protocols which could influence the direction of findings (Hohenfeld *et al.*, 2018; Tewarie *et al.*, 2018). However, given the division of those cognitively impaired or not, this project's strength is that is provides a picture of the dynamic whole brain FC changes in cognitive impairment which may be altered by disease duration, atrophy, or white matter lesion volume too. This whole brain analysis provides an honest and thorough baseline interpretation of overall FC changes in multiple networks.

The results suggest that both increased and decreased FC occur when a threshold of cognitive impairment is reached suggesting and confirming a degree of compensatory functional reorganisation and network collapse (Schoonheim *et al.*, 2015*b*, 2017). This is a clear limitation of this project in terms of the interpretation of whether changes are maladaptive or compensatory. This supports more of a network efficiency theory in MS rather than simply increased or decreased FC being meaningful. However, decreased FC in the posterior and attentional networks do support literature findings of the DMN too. This is not only the case in MS, in other neurodegenerative conditions such as Alzheimer's disease, rs-fMRI suggested consistent FC decreases in the DMN (Badhwar *et al.*, 2017), yet a recent review of rs-fMRI studies in several neurodegenerative diseases, including Alzheimer's, argued that the evidence for meaningful interpretation of directional FC change is not yet suitable for use as an imaging biomarker (Hohenfeld *et al.*, 2018).

The data generated was large and so I had to create an a priori cut off of 5% change to allow key FC changes to be noted. This is similar to what is done in DTI and has been replicated by Castellazzi (Castellazzi *et al.*, 2018*b*) but a critique may be that smaller voxel changes may actually be key.

Recent work in Alzheimer's disease has supported functional MRI changes occurring before structural volume loss (Corriveau-Lecavalier *et al.*, 2020). This has been hypothesised in MS, and so to test this in this dataset I derived the proportional total cerebellar change as this is a predilection site for MS. As stated in **section 6.4** the findings are limited as they interpret the cerebellum as a whole despite the topographical divisions, however correcting for this and atlas differences the proportion of functional voxel change is greater than cerebellar atrophy. This supports the need for multimodal studies going forward(Schoonheim *et al.*, 2015*a*; Tewarie *et al.*, 2018).

7.6 Future directions

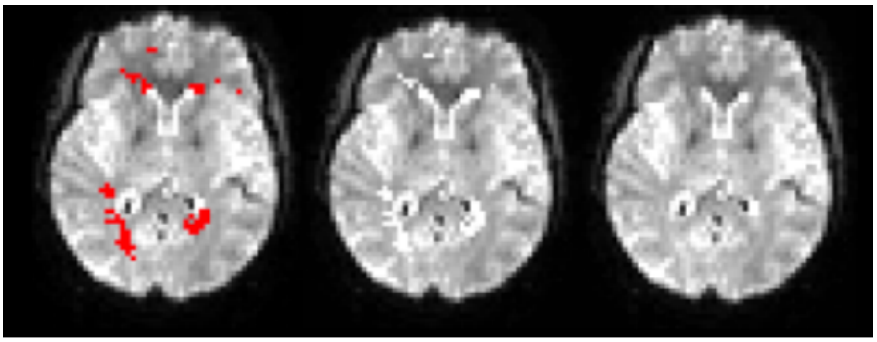
To expand the work in this thesis I have developed a series of future work. Firstly, the rs-fMRI FC literature on cognition in MS has not yet been subject to systematic review, and so the specificity and reliability of FC as a marker of cognitive dysfunction has not been established. My first task is to publish an ongoing systematic review of rs-fMRI changes and cognitive in impairment in MS which I have been undertaking since December 2019 to outline the state of the field and provide a critical analysis of findings to date.

Secondly, the experimental cross-sectional and longitudinal data in **chapters 4, 5 and 6** are in the drafting stages of publication. Work from chapters 4 and 5 has been presented as poster and oral presentations at local and international meetings to date.

The key utility of this work is to support the need for a consensus for defining cognitive impairment in the MS field and the alterations in fMRI findings dependent on this. To further our understanding of the underlying MS biology this must be uniform in the field.

An essential point when investigating MS subjects is the role of MS lesions. This is not currently considered routine practice for functional MRI. A specific caveat for pre-processing is the need to consider correction for brain lesions, and as with atrophy analyses, lesion filling of the rs-fMRI may increase the accuracy of results (Garrison *et al.*, 2015; Castellazzi *et al.*, 2019). I show preliminary work which I undertook from this thesis with my colleagues Dr Castellazzi and Dr Prados below. The figure on the left shows the lesion mask overlaid on the rs-fMRI native space. In the centre image lesions are visualised on the rs-fMRI, and on the right rs-fMRI with lesions filled using a multi-time-point modality agnostic lesion filling algorithm (Prados *et al.*, 2016).

Figure 7.1. The role of lesion filling of rs-fMRI.



Rs-fMRI images with filled lesions. Figure developed from subjects in this study with Gloria Castellazzi.

To elucidate direct rs-fMRI voxel correlations with the SDMT I have developed a two-stage dual regression project, the results of which are drafted for publication. This novel approach allows correction for network changes directly.

Finally, to further the findings of this study in terms of cognitive profile and impairment classification a larger multimodal MRI longitudinal cohort-control study of SPMS and cognition is

required to look at the effects and associations of outcome measures. This will allow the evaluation of potential therapies such as proposed rehabilitation, exercise, and computer-assisted programs. Seed-based fMRI and task-based fMRI will be approaches suitable to develop this further.

To summarise, in this thesis I highlight that executive dysfunction is a major contributor to cognitive impairment in SPMS and must be measured. I show that a stringent pooled criteria is superior, and is required to create a more pure cognitive cohort, adding power to studies of cognition. With the development of impairment, there is greater loss of executive and information processing speed which relate most to measures of employment and fatigue. I demonstrate that the rs-fMRI changes of cognitive impairment are more posterior FC reductions. I also show that deeper GM changes associate most with cognitive impairment in SPMS. I finally introduce the idea that overall, FC alterations in the cerebellum may have more input then structural volume changes indicating more widespread dynamic network dysfunction.

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