Understanding long-term disability in multiple sclerosis:

A clinical, MRI and genetic study in patients with clinically isolated syndrome

Wallace J Brownlee BHB MBChB FRACP

Queen Square MS Centre

Department of Neuroinflammation

UCL Institute of Neurology

London WC1N 3BG

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Declaration

I, Wallace J Brownlee 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.

Dr Dan Altmann assisted with the statistical analysis presented in Chapters 4-7 and Dr Carmen Tur assisted with statistical analysis for Chapter 8. Dr Katherine Miszkiel contributed to image-analysis used in Chapters 4-8 and Prof Frederik Barkhof with the image-analysis for chapter 7. Dr Ferran Prados, Dr Arman Eshaghi and Mr Jon Stutters assisted with the brain atrophy measures used in analysis presented in Chapters 6-8. Ms Andreea Manole completed the genotyping for analysis presented in Chapter 8.
Abstract

This thesis concerns a 15 year follow-up study of a cohort of people with clinically isolated syndromes (CIS) suggestive of multiple sclerosis (MS). I investigate (1) how MRI can be used to improve the diagnosis of MS around the time of CIS; (2) early MRI predictors of long-term disease course in people with CIS; and (3) mechanisms responsible for long-term disability and disease progression in relapse-onset MS.

There has been significant evolution of the diagnostic criteria for MS in recent years. I examined the influence of changing diagnostic criteria for MS by retrospectively applying the McDonald criteria in the CIS cohort. I found that MS can be diagnosed significantly earlier in CIS patients using the McDonald criteria. I investigated two possible modifications to dissemination in space (DIS) criteria: firstly whether lesions in the symptomatic region should be included in DIS; and secondly whether the number of periventricular lesions in DIS criteria should be increased from 1 to 3. Including lesions in the symptomatic region improved the performance of MRI criteria but increasing the number of periventricular lesions in DIS did not improve diagnostic accuracy or specificity. These findings will inform future revisions to the diagnostic criteria for MS.

MS-related disability is frequently referable to the spinal cord. I investigated early brain and spinal cord MRI abnormalities in the CIS cohort and found that spinal cord measures explained more of the disability after 5 years than brain MRI measures. Asymptomatic spinal cord lesions at the time of presentation in patients with a non-spinal CIS were the strongest early MRI predictor of disability after 5 years. These findings were then confirmed with long-term follow-up: spinal cord lesions at the time of presentation with CIS and new spinal cord lesions after 1 year and 3 years were
associated with both physical disability and secondary progressive disease course after 15 years. These findings suggest that spinal cord MRI may provide important prognostic information in people with CIS and early MS. Early spinal cord damage may be an important mechanism contributing to long-term disability and disease progression in relapse-onset MS.

Disease course heterogeneity in MS remains poorly explained. I investigated the influence of HLA-DRB1*15:01 on disease course and MRI measures of inflammation and neurodegeneration in the CIS cohort. Carriage of the HLA-DRB1*15:01 allele was associated with a faster accrual of disability, greater inflammatory disease burden and a faster rate of brain atrophy over the 15 year follow-up period. The HLA-DRB1*15:01 allele may not only influence the susceptibility to MS but also the disease phenotype and long-term clinical course.

Neuroaxonal energy failure is thought to be central to disease progression in MS. I applied the novel metabolic imaging method sodium ($^{23}$Na) MRI in patients followed up after 15 years to investigate the relationship of brain sodium accumulation in vivo with long-term disease course and disability. I found evidence of sodium accumulation in grey matter, in normal-appearing white matter and in lesions in people who developed MS. Cortical grey matter sodium concentration was associated with physical disability and cognitive performance at 15 years, even after adjusting for brain atrophy. $^{23}$Na-MRI should be investigated further as a possible outcome measure in future neuroprotection trials.
Acknowledgements

This research would not have been possible without the financial support of the Neurological Foundation of New Zealand and the United Kingdom Multiple Sclerosis Society who jointly funded the 15 year follow-up study.

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Dr Katherine Miszkiel and Prof Frederick Barkhof, also provided their expert input into the image-analysis. I am indebted to Dr Dan Altmann and Dr Carmen Tur who undertook the more complex statistical analysis but also took the time to teach me statistical methods and answer my many questions. I’m thankful for the support and friendship of Dr Rhian Raftopoulos, Dr Hugh Kearney, Dr Varun Sethi, Dr Becky Samson, Dr Niamh Cawley, Dr Will Brown, Dr Karen Chung and Dr Yael Hacohen.

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<tr>
<td>9HPT</td>
<td>9 Hole peg test</td>
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<tr>
<td>ADEM</td>
<td>Acute disseminated encephalomyelitis</td>
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<tr>
<td>BET</td>
<td>Brain extraction tool</td>
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<td>BPF</td>
<td>Brain parenchymal fraction</td>
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<td>CDMS</td>
<td>Clinically-definite multiple sclerosis</td>
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<tr>
<td>CI</td>
<td>Confidence interval</td>
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<td>CIS</td>
<td>Clinically isolated syndrome</td>
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<tr>
<td>CNS</td>
<td>Central nervous system</td>
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<td>CSF</td>
<td>Cerebrospinal fluid</td>
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<td>DIS</td>
<td>Dissemination in space</td>
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<td>DIT</td>
<td>Dissemination in time</td>
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<tr>
<td>DMT</td>
<td>Disease-modifying treatment</td>
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<td>DWI</td>
<td>Diffusion-weighted imaging</td>
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<tr>
<td>DTI</td>
<td>Diffusion tensor imaging</td>
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<td>EBV</td>
<td>Epstein-Bar virus</td>
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<td>EDSS</td>
<td>Expanded Disability Status Scale</td>
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<tr>
<td>FLAIR</td>
<td>Fluid-attenuated inversion recovery</td>
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<td>FOV</td>
<td>Field of view</td>
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<td>FS</td>
<td>Functional system</td>
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<td>FSPGR</td>
<td>Fast spoiled gradient echo</td>
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<tr>
<td>GdE</td>
<td>Gadolinium-enhancing</td>
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<td>GMF</td>
<td>Grey matter fraction</td>
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<tr>
<td>HLA</td>
<td>Human leukocyte antigen</td>
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<td>IQR</td>
<td>Interquartile range</td>
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<tr>
<td>IRR</td>
<td>Incident rate ratio</td>
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<td>Abbreviation</td>
<td>Term</td>
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<tr>
<td>MAGNIMS</td>
<td>Magnetic Resonance Imaging in Multiple Sclerosis Study Group</td>
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<tr>
<td>MS</td>
<td>Multiple sclerosis</td>
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<tr>
<td>MRI</td>
<td>Magnetic resonance imaging</td>
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<td>MSFC</td>
<td>Multiple sclerosis functional composite</td>
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<tr>
<td>MTI</td>
<td>Magnetization transfer imaging</td>
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<tr>
<td>MTR</td>
<td>Magnetization transfer ratio</td>
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<tr>
<td>NAA</td>
<td>N-acetylaspartate</td>
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<td>NAWM</td>
<td>Normal-appearing white matter</td>
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<td>NMOSD</td>
<td>Neuromyelitis optica spectrum disorder</td>
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<tr>
<td>OCBs</td>
<td>Oligoclonal bands</td>
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<tr>
<td>OCT</td>
<td>Optical coherence tomography</td>
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<tr>
<td>PASAT</td>
<td>Paced auditory serial addition test</td>
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<tr>
<td>PBVC</td>
<td>Percentage brain volume change</td>
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<td>PD</td>
<td>Proton density</td>
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<td>PPMS</td>
<td>Primary progressive multiple sclerosis</td>
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<td>PPV</td>
<td>Positive predictive value</td>
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<td>PSIR</td>
<td>Phase-sensitive inversion recovery</td>
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<td>RF</td>
<td>Radiofrequency</td>
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<td>RRMS</td>
<td>Relapsing-remitting multiple sclerosis</td>
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<td>SD</td>
<td>Standard deviation</td>
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<td>SDMT</td>
<td>Symbol digit modalities test</td>
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<tr>
<td>SIENA</td>
<td>Structural Image Evaluation using Normalization of Atrophy</td>
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<td>SPMS</td>
<td>Secondary progressive multiple sclerosis</td>
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<tr>
<td>T1LV</td>
<td>T1-hypointense lesion volume</td>
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<tr>
<td>T2LV</td>
<td>T2-hyperintense lesion volume</td>
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<tr>
<td>TE</td>
<td>Echo time</td>
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<td>Abbreviation</td>
<td>Description</td>
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<tr>
<td>TFE</td>
<td>Turbo field echo</td>
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<tr>
<td>TI</td>
<td>Inversion time</td>
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<tr>
<td>TR</td>
<td>Repetition time</td>
</tr>
<tr>
<td>TWT</td>
<td>Timed 25-foot walk test</td>
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<tr>
<td>UCCA</td>
<td>Upper cervical cord cross-sectional area</td>
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<td>WMF</td>
<td>White matter fraction</td>
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Chapter 1

Introduction to multiple sclerosis and the clinically isolated syndrome

1.1 Introduction

This thesis concerns a 15 year clinical, imaging and genetic study of people with clinically isolated syndromes (CIS) suggestive of multiple sclerosis (MS). This chapter provides background to MS, a common disabling neurological disorder affecting young adults. A CIS is the first clinical manifestation of relapse-onset MS. The clinical features of CIS are reviewed along with risk factors for the development of MS and long-term neurological disability in CIS patients.

1.2 Multiple sclerosis

MS is a chronic, immune-mediated disorder of the central nervous system (CNS) affecting over 2 million people worldwide. In 80-85% of people, the initial course is characterised by relapses and remissions (relapsing-remitting MS, RRMS). In the longer term many people with RRMS will develop neurologic disability either because of poor recovery from relapses or because of a change to a progressive disease course with a gradual increase in disability over time, with or without superimposed relapses (secondary progressive MS, SPMS). In 10-15% the initial course is progressive from the outset with an insidious onset of symptoms and a slow increase in disability over time (primary progressive MS, PPMS).

In this section the epidemiology, aetiology, pathology, diagnosis, natural history and treatment of MS are reviewed with an emphasis on relapse-onset MS.
1.2.1 Epidemiology

MS is a disorder of young adults (peak age at onset 20 – 40 years) and is more common in females than males (Weinshenker et al., 1989). For reasons that are not well understood, the sex ratio in MS is increasing, driven principally by an increase in the incidence of RRMS in young women (Orton et al., 2006). Although MS occurs throughout the world, there are striking geographical differences in prevalence, thought to be due to differences in genetic and environmental risk factors. Epidemiological studies from Europe, North America and Australasia have demonstrated a significant latitudinal gradient in the incidence of MS (more common at higher latitudes), with some notable exceptions such as Sardinia which is an area of unexpectedly high prevalence (Simpson et al., 2011).

1.2.2 Aetiology

The cause of MS is unknown. The current widely held view is that MS develops in genetically susceptible individuals after exposure to one or more environmental triggers (Ascherio et al., 2012). Recently completed genome-wide association studies have found that the majority of genetic loci that influence susceptibility to MS are related to immune system function and there is significant genetic overlap with other autoimmune disorders (International Multiple Sclerosis Genetics Consortium et al., 2011; International Multiple Sclerosis Genetics Consortium et al., 2013). These findings support the concept that MS is primarily an immune-mediated illness.

Genetic Factors

An association between MS and class II human leukocyte antigen (HLA) alleles was first identified more than 40 years ago (Sawcer et al., 2014). Modern genetic
techniques have established HLA-DRB1*1501 as the most important genetic risk factor for MS. In Northern European populations HLA-DRB1*1501 is associated with a three-fold increase in the risk of developing MS, with the risk increased in homozygotes (Moutsianas et al., 2015). A number of other HLA loci have been identified that influence susceptibility to MS; some increase the risk of MS and others exert a protective effect, for example, HLA-A*02:01 (Moutsianas et al., 2015).

Outside the HLA system >100 genetic variants have been identified that also influence susceptibility to MS, although the individual effect sizes are small (International Multiple Sclerosis Genetics Consortium et al., 2013). Many of the loci identified to date are implicated in immune system processes, although how they contribute to the development of MS is poorly understood (Sawcer et al., 2014). Although genetic factors are important in the development of MS the contribution appears to be much smaller than that of environmental factors.

**Environmental factors**

The most compelling evidence for a role of environmental factors in the development of MS comes from migration studies (Ascherio et al., 2012). In a seminal study from South Africa the prevalence of MS was highest in European immigrants compared with South African English and Afrikaners (Dean, 1967). Europeans who immigrated to South Africa as adults had a similar risk of MS to their country of origin, whereas children moving to South Africa before the age of 15 year had a risk similar to those born in South Africa (Dean, 1967). The converse is true for people moving from an area of low-risk to an area of high-risk. For example, MS is uncommon in the West Indies and among immigrants to the United Kingdom from this region in the 1960s
the risk of MS was low. However, in their children the risk of MS was comparable to the general British population (Elian and Dean, 1987).

Collectively these results suggest a role for environmental factors operating in childhood that subsequently lead to MS. A number of environmental risk factors have been proposed including vitamin D insufficiency, viral infections, cigarette smoking and obesity (Ascherio et al., 2012).

**Vitamin D insufficiency**

Vitamin D is a fat-soluble vitamin that has a range of biologic effects. Ultraviolet B radiation in sunlight converts vitamin D precursors in the skin into vitamin D3 (cholecalciferol). In order to become biologically active vitamin D needs to undergo two hydroxylations, the first in the liver to form 25-hydroxyvitamin D3 and the second in the kidneys and other tissues to form 1, 25-hydroxyvitamin D (calcitriol). 1, 25-hydroxyvitamin D3 binds to the vitamin D receptor and acts as a transcription factor for more than 500 genes with effects that extend beyond its well-known role in calcium and bone metabolism. Vitamin D has multiple immunomodulatory actions potentially relevant to the pathogenesis of MS, including down-regulation of antigen-presenting cells, an increase in the number of regulatory T cells, a shift in T helper cell responses away from Th1 / Th17 and decreased production of pro-inflammatory cytokines by activated monocytes (Koch et al., 2013). Vitamin D insufficiency is associated with a number of autoimmune conditions including type 1 diabetes, rheumatoid arthritis and inflammatory bowel disease (Ascherio et al., 2010).
In a longitudinal study in US military recruits in which serum samples were collected prior to disease onset, lower levels of vitamin D were associated with an increased risk of developing MS (Munger et al., 2006). A strong association between low levels of vitamin D and risk of MS was also confirmed in a Mendelian randomisation analysis that investigated three single nucleotide polymorphisms that influence serum vitamin D concentrations (Rhead et al., 2016).

There is some evidence that vitamin D insufficiency may influence the course of MS (Ascherio et al., 2014). This is discussed in section 1.3.2.

*Epstein-Barr virus infection*

Epstein-Barr virus (EBV) is a herpes virus that commonly causes an asymptomatic infection in early childhood and infectious mononucleosis in older children and young adults. Following primary infection with EBV, the virus becomes latent in memory B cells and a complex system of immune surveillance keeps the number of infected B cells at a stable number (Lucas et al., 2011). A history of infectious mononucleosis (by self-report or hospital admission data) and serological evidence of EBV infection is more common in people with MS than in the general population (Goldacre et al., 2004). In a longitudinal study of United States military personnel with serial stored serum samples, 5% of participants were EBV negative at baseline (Levin et al., 2010). During follow-up MS did not develop in any of the participants who remained EBV negative. Ten people who were EBV negative at baseline developed MS, all of whom seroconverted to EBV positive prior to onset of symptoms. These findings support a role for EBV in disease initiation. The mechanism by which EBV influences susceptibility to MS is uncertain (Ascherio et al., 2012).
Cigarette smoking

The risk of MS has been reported to be increased in current smokers, with the risk increasing based on the number pack years (Hernán et al., 2001). Passive smoking may also increase the risk of MS. In a case-control study from France parental smoking was a risk factor for paediatric-onset MS (Mikaeloff et al., 2007).

Obesity

Increased BMI (by self-report) in the teenage years is associated with a two-fold increase in the risk of MS (Munger et al., 2009). These findings have been confirmed in a prospective study of Danish school children aged 7–13 years with BMI measurements available. For every one unit increase in BMI z scores there was a 15-20% increase in the risk of MS (Munger et al., 2013).

1.2.3 Pathology

MS is characterised pathologically by inflammation, demyelination, remyelination, astrogliosis and neuroaxonal loss (Popescu and Lucchinetti, 2012). The major pathological changes can be grouped anatomically into those affecting the white matter, grey matter, meninges and spinal cord.

White matter pathology

A plaque is the pathological description for the multifocal areas of inflammatory demyelination that occur throughout the white matter in people with MS, but with a predilection for the periventricular, juxtacortical and cerebellar white matter of the brain, the optic nerves and the spinal cord (Popescu and Lucchinetti, 2012). Plaques
evolve from acute active lesions to chronic lesions with variable degrees of remyelination.

In the acute active plaque there is breakdown of the blood brain barrier resulting in trafficking of immune cells into the brain. The acute active plaque is hypercellular with infiltration of macrophages and T cells plus proliferation of astrocytes. Although pathologists use relative axonal preservation to help differentiate inflammatory demyelination and infarction (Popescu and Lucchinetti, 2012), considerable axonal transection occurs within acute MS lesions (Trapp et al., 1998). Permanent axonal injury and loss is thought to be the pathological basis for incomplete recovery following relapses in RRMS.

Chronic plaques have a sharply demarcated border with demyelination, loss of axons and oligodendrocytes, a minor inflammatory cell infiltrate and areas of gliosis (Popescu and Lucchinetti, 2012). Half of chronic plaques remain active with a rim of activated microglia, sometimes referred to as slowly-expanding lesions. Ongoing axonal loss occurs within chronic lesions and may be one of the substrates for disability progression (Trapp et al., 1998).

Widespread, diffuse changes occur in the brain within macroscopically normal-appearing white matter (NAWM). These include glial cell proliferation (astrogliosis) and patchy inflammatory cell infiltrates, predominantly centred around small veins and venules (Kutzelnigg et al., 2005; Popescu and Lucchinetti, 2012).
Evidence of axonal injury can be seen pathologically in NAWM (Trapp et al., 1998; Kutzelnigg et al., 2005) and this is thought to contribute to diffuse neuroaxonal loss. The mechanisms of axonal loss within the NAWM are unresolved. In part, it can be explained by the secondary effects of Wallerian degeneration due to axonal transection in remote focal lesions; however, activated microglia (which are increased in number in the NAWM) produce nitric oxide and other free radicals resulting in mitochondrial dysfunction, energy failure and axonal loss (Friese et al., 2014).

**Grey matter pathology**

In recent years there has been increasing recognition of the importance of grey matter pathology in MS including demyelination, neuronal and synaptic loss, and grey matter atrophy (Kutzelnigg et al., 2005; Lucchinetti et al., 2011; Popescu and Lucchinetti, 2012).

Within the cerebral cortex widespread demyelination occurs particularly within the cingulate gyrus, frontal and temporal lobes and cerebellum. Demyelination can be extensive, affecting up to two thirds of the cerebral cortex (Kutzelnigg et al., 2005). Three types of cortical demyelinating lesions can be categorised based on their location (Popescu and Lucchinetti, 2012). The most abundant are subpial lesions extending from the pial surface through the cortical layers, sometimes extending through the full thickness of the cerebral cortex and across multiple gyri. Intracortical lesions form around blood vessels and do not extend to the pial surface or grey-white junction. Finally, leukocortical lesions involve the deeper layers of the cerebral cortex and adjacent white matter.
While grey matter pathology is prominent pathologically in progressive forms of MS (Kutzelnigg et al., 2005), it has also been demonstrated in early RRMS. In a series of patients who underwent brain biopsy for tumefactive demyelination, more than a third were found to have cortical demyelinating lesions (Lucchinetti et al., 2011).

**Meningeal pathology**

Most patients with progressive MS have evidence of a low-grade meningeal inflammatory infiltrate, with or without discrete follicles containing B cells and plasmablasts that resemble secondary lymphoid organs (Magliozzi et al., 2007). Clinically the presence of meningeal lymphoid follicles is associated with an earlier age at disease onset and a more aggressive disease course (Magliozzi et al., 2007). Meningeal pathology has also been documented in early MS and is said to correlate with the extent of cortical demyelination (Lucchinetti et al., 2011).

**Spinal cord pathology**

Demyelinating plaques similar to those found in the brain also occur in the spinal cord in MS with the cervical cord affected more frequently than the thoracic or lumbar cord (Oppenheimer, 1978). Like in the brain, there is considerable neuroaxonal loss within lesions, NAWM and grey matter resulting in spinal cord atrophy (Ganter et al., 1999; Bot et al., 2004b).

1.2.4 **Immunopathogenesis**

Focal areas of demyelination and evidence of neurodegeneration are the major pathological hallmarks of MS (Popescu and Lucchinetti, 2012). Although there is debate as to whether MS is primarily an immune-mediated or neurodegenerative
disorder, inflammation appears to be important both in the development of focal
demyelinating lesions and more diffuse neurodegeneration (Dendrou et al., 2015).

T cells are the major immune cell present in the acute MS lesion. Autoreactive T cells
cross the blood brain barrier using adhesion molecules such as vascular cell adhesion
molecule-1 (VCAM-1) to traffic into the CNS. This interaction is targeted by the
monoclonal antibody natalizumab which blocks very-late antigen-4 (VLA-4), the T cell
ligand for VCAM-1 (Wingerchuk and Weinshenker, 2016). CD4+ T cells become
activated by antigen presenting cells through interactions with MHC class II molecules
with a predominant T_{H1} and T_{H17} phenotype. This leads to production of cytokines
and chemokines that attract circulating monocytes, CD8+ T cells and B cells resulting
in demyelination and injury to axons and oligodendrocytes (Dendrou et al., 2015).
Because of the breakdown of myelin in acute MS lesions it is assumed that the
antigenic target of T cells is a myelin antigen, but this is yet to be identified (Dendrou
et al., 2015).

There are differences in the immunophenotype of relapsing and progressive MS. This
is reflected in their differing responses to the currently available immunomodulatory
treatments (Wingerchuk and Weinshenker, 2016). Relapsing MS is thought to be
mainly due to autoreactive peripheral immune cells crossing the blood brain barrier
and causing inflammation in the brain and spinal cord, whereas progressive MS may
be due to the effects of compartmentalized inflammation driven by immune cells
already present in the CNS (Friese et al., 2014; Dendrou et al., 2015). Widespread
microglial activation occurs resulting in chronic inflammation and production of
inflammatory mediators such as reactive oxygen species that damage mitochondria
(Campbell et al., 2011). The maladaptive reorganisation of ion channels to maintain
ionic homeostasis coupled with the effects of glutamate excitotoxicity leads to irreversible damage to axons and neurodegeneration (Friese et al., 2014). Peripheral plasma cells, B cells and T cells in the CNS can form tertiary lymphoid follicles within the meninges and produce soluble neurotoxic factors that promote cortical demyelination and neuroaxonal injury (Magliozzi et al., 2007; Howell et al., 2011). Rather than occurring in separate phases it is likely these processes occur in parallel, even from the earliest stages of the illness (Friese et al., 2014). Using positron emission tomography, increased binding of PK11195 (a microglial marker) has been reported in patients with CIS (Giannetti et al., 2015).

1.2.5 Clinical features

MS is classified based on the initial disease course as either relapsing or progressive onset. In RRMS the initial course is characterised by relapses, defined as episodes of neurological dysfunction that evolve subacutely over hours or days in the absence of fever or infection (Lublin et al., 2014). Relapses typically last for several weeks and by definition must last at least 24 hours. Relapses usually improve spontaneously with or without steroid treatment with a variable degree of recovery. Relapses commonly result in persistent symptoms and/or abnormal neurological signs (e.g. optic disc pallor, pathologically brisk reflexes). Relapses in RRMS are punctuated by periods of remission in which patients may have ongoing symptoms related to previous relapses or other chronic neurological symptoms (e.g. fatigue, Uhthoff’s phenomenon), but there is no progression of disability between attacks.

The term CIS describes a first episode of neurological symptoms suggestive of MS without clinical or MRI findings sufficient to make a diagnosis (Polman et al., 2011; Lublin et al., 2014). CIS is discussed further in section 1.3.
After a variable latency (usually 10 – 15 years or more), some patients with RRMS have a change in disease course with a steady increase in disability over time, referred to as secondary progression (Scalfari et al., 2014). SPMS typically takes the form of a progressive myelopathy. SPMS is usually a retrospective diagnosis in the context of increasing disability not attributable to relapses over more than 12 months (Lublin et al., 2014). The transition between RRMS and SPMS is subtle and a significant period of diagnostic uncertainty is common (Katz Sand et al., 2014).

In patients with PPMS the disease course is progressive from onset. Patients with PPMS are usually older than patients with RRMS (mean age at onset 40 years) and there is no gender bias (Miller and Leary, 2007). PPMS typically presents with a progressive asymmetric spastic paraparesis or less commonly progressive hemiparesis, cerebellar ataxia, visual failure or dementia (Miller and Leary, 2007). Once established the course of PPMS and SPMS is indistinguishable (Kremenchutzky et al., 2006). Superimposed relapses can occur in both forms of progressive MS, but less often in PPMS than SPMS (Paz Soldan et al., 2015).

Disease course definitions for MS have been recently updated, including the addition of new disease modifiers to describe the clinical phenotype of MS (Lublin et al., 2014). Patients are classified as relapsing or progressive MS with an additional descriptor of whether the disease is active or not (Figure 1.1). Disease activity can be assessed clinically based on the presence of relapses or with MRI showing new T2-hyperintense and/or gadolinium-enhancing lesions. Patients with progressive MS are further classified based on whether the disease is progressing or not progressing. Disease progression is defined as an objective increase in neurological disability, confirmed after 6-12 months.
MS is classified as being relapsing or progressive and patients are further classified as being active or non-active (based on the presence of relapses or MRI disease activity) and with or without progression (assessed clinically).

1.2.6 Diagnosis and differential diagnosis

The core feature of MS is the presence of lesions in the CNS that are disseminated in both time and space. There is no definitive clinical sign or diagnostic test for MS and care is needed to exclude other inflammatory and non-inflammatory CNS disorders.

In the last 30 years MRI has emerged as the key investigation in making a diagnosis of MS. Conventional T2-weighted MRI sequences are highly sensitive to the detection of focal demyelinating lesions in the cerebral white matter. Abnormal brain MRI is seen in almost all patients with established MS and in up to 80% of CIS patients who develop MS (Fisniku et al., 2008a). MRI is also helpful in excluding other pathologies that might mimic MS (e.g. structural or vascular lesions), and identifying abnormalities.
that suggest an alternative diagnosis (e.g. leptomeningeal contrast enhancement, longitudinally-extensive spinal cord lesions).

Other tests that may be helpful in establishing a diagnosis of MS include lumbar puncture and evoked potentials. CSF findings in people with MS include a normal or mildly raised white cell count (<25 cells/cm³, predominantly lymphocytes), normal or mildly raised protein, raised IgG index and IgG oligoclonal bands (OCBs) not present in serum (Freedman et al., 2005). OCBs are found in up to 90% of people with established MS but can also occur in other disorders (Dobson et al., 2013). Evoked potential testing to look for evidence of demyelination in the anterior visual pathways (or less commonly the auditory or sensory pathways) may provide additional evidence for dissemination in space (DIS) in patients with suspected MS, but is less sensitive than MRI or CSF examination (Pelayo et al., 2010).

**Diagnostic criteria for MS**

The first widely accepted diagnostic criteria for MS were proposed by Schumacher in 1965. The Schumacher criteria required objective evidence of two lesions involving different parts of the CNS and at least two or more attacks in a person aged 10 – 50 years with no alternative explanation for the symptoms (Schumacher et al., 1965). Changes were recommended to the diagnostic criteria by an international panel in 1983 (Poser et al., 1983). The Poser criteria introduced the concept of clinically-definite MS (CDMS) requiring two attacks with evidence of two separate lesions. The Poser criteria recognised that para-clinical investigations could also provide alternative evidence for DIS and that the certainty of diagnosis could be increased if unmatched IgG OCBs were found in the CSF (laboratory-supported MS). The Poser criteria have been used widely in clinical settings, research studies and clinical trials.
They remain the gold-standard in evaluating new diagnostic criteria for MS (Swanton et al., 2006; Rovira et al., 2009).

In the years following the publication of the Poser criteria MRI became a widely available tool for diagnosing MS. On this background changes to the diagnostic criteria for MS were proposed in 2001 (McDonald et al., 2001). The original McDonald criteria introduced MRI criteria for DIS and dissemination in time (DIT) that could be used as a substitute for relapses and objective examination findings, including in patients with a CIS. The McDonald 2001 criteria also set out diagnostic criteria for PPMS, which had not been given special consideration by the Poser criteria.

The original McDonald criteria were revised in 2005 (Polman et al., 2005) and 2010 (Polman et al., 2011) with modification of the requirements for DIS and DIT (Table 1.1). In the McDonald 2010 criteria (Table 1.2) DIS requires one or more T2 lesions in at least two regions of the CNS commonly affected in MS – periventricular, juxtacortical, infratentorial regions and the spinal cord (Figure 1.2). If the symptomatic lesion is referable to the spinal cord or brainstem then lesions in this site are excluded from DIS. DIT requires the presence of simultaneous asymptomatic gadolinium enhancing and non-enhancing lesions on a single MRI scan or a new lesion on a follow up scan done at any time point (Figure 1.3). Using the McDonald 2010 criteria a diagnosis of MS can be made in patients at the time of first clinical event (i.e. a CIS) when there is MRI evidence of DIS and DIT.
<table>
<thead>
<tr>
<th>Table 1.1</th>
<th>Comparison of MRI criteria for dissemination in space and time in McDonald 2001, 2005 and 2010 criteria</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>Dissemination in space</td>
</tr>
<tr>
<td>McDonald 2001 criteria (McDonald et al., 2001)</td>
<td>Three of the following:</td>
</tr>
<tr>
<td></td>
<td>- At least one GdE lesion or nine T2 lesions</td>
</tr>
<tr>
<td></td>
<td>- At least one infratentorial lesion</td>
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<tr>
<td></td>
<td>- At least one juxtacortical lesion</td>
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<tr>
<td></td>
<td>- At least three periventricular lesions</td>
</tr>
<tr>
<td></td>
<td>One spinal cord lesion can be substituted for one brain lesion</td>
</tr>
<tr>
<td>McDonald 2005 criteria (Polman et al., 2005)</td>
<td>Three of the following:</td>
</tr>
<tr>
<td></td>
<td>- At least one GdE lesion or nine T2 brain and/or spinal cord lesions</td>
</tr>
<tr>
<td></td>
<td>- At least one infratentorial or spinal cord lesion</td>
</tr>
<tr>
<td></td>
<td>- At least one juxtacortical lesion</td>
</tr>
<tr>
<td></td>
<td>- At least three periventricular lesions</td>
</tr>
<tr>
<td>McDonald 2010 criteria (Polman et al., 2011)</td>
<td>One or more T2 lesion in two of four sites typically affected in demyelination*:</td>
</tr>
<tr>
<td></td>
<td>- Periventricular</td>
</tr>
<tr>
<td></td>
<td>- Juxtacortical</td>
</tr>
<tr>
<td></td>
<td>- Infratentorial</td>
</tr>
<tr>
<td></td>
<td>- Spinal cord</td>
</tr>
</tbody>
</table>

* Lesions in the symptomatic region excluded
<table>
<thead>
<tr>
<th>Presentation</th>
<th>Additional data needed for diagnosis of MS</th>
</tr>
</thead>
<tbody>
<tr>
<td>≥2 attacks; objective clinical evidence of ≥2 lesions or objective clinical evidence of 1 lesion with reasonable historical evidence of a prior attack</td>
<td>None</td>
</tr>
</tbody>
</table>
| ≥2 attacks; objective clinical evidence of 1 lesion | Dissemination in space, demonstrated by either:  
- ≥1 T2 lesion in at least 2 of 4 MS-typical regions of the CNS*  
- Further clinical attack at a different site |
| 1 attack; objective clinical evidence of ≥2 lesions | Dissemination in time, demonstrated by either:  
- Asymptomatic gadolinium-enhancing and nonenhancing lesions  
- A new T2 and/or gadolinium-enhancing lesion on follow-up MRI  
- Second clinical attack |
| 1 attack; objective clinical evidence of 1 lesion | Dissemination in space, demonstrated by either:  
- ≥1 T2 lesion in at least 2 of 4 MS-typical regions of the CNS*  
- Second clinical attack |
| Dissemination in time, demonstrated by either:  
- Asymptomatic gadolinium-enhancing and nonenhancing lesions  
- A new T2 and/or gadolinium-enhancing lesion(s) on follow-up MRI  
- Second clinical attack |
| Insidious neurologic progression suggestive of MS | One year of disease progression plus 2 of 3 of the following:  
- ≥1 T2 lesions in MS-typical regions in the brain*  
- Evidence for DIS in the spinal cord based on ≥2 T2 lesions in the cord  
- Positive CSF |

* MS-typical regions = periventricular, juxtacortical, infratentorial and spinal cord
One or more T2-hyperintense lesion (arrows) must be present at least two of four CNS locations typically affected in demyelination: (A) periventricular, (B) juxtacortical, (C) infratentorial and (D) spinal cord.
Figure 1.3  McDonald 2010 MRI criteria for dissemination in time

Dissemination in time can be demonstrated by either the simultaneous presence of asymptomatic enhancing and non-enhancing lesions on a single scan (figures A and B) or a new T2-hyperintense and/or gadolinium-enhancing lesion on a follow up MRI scan (figures C and D).
**Differential Diagnosis**

The differential diagnosis depends on the clinical setting with different considerations in a patient with a CIS, a relapsing-remitting illness or a slowly progressive neurological deficit (Miller et al., 2008). Many neurologists use an approach of identifying “red flag” clinical (e.g. complete transverse myelopathy, encephalopathy), imaging (e.g. meningeal contrast enhancement, normal brain MRI) or laboratory (e.g. marked CSF pleocytosis, absence of oligoclonal bands) features when evaluating patients with suspected MS (Miller et al., 2008).

Because of the overlapping clinical, MRI and CSF findings two special considerations in the differential diagnosis of MS are acute disseminated encephalomyelitis (ADEM) and neuromyelitis optica spectrum disorder (NMOSD).

**Acute disseminated encephalomyelitis**

ADEM is predominantly a disease in childhood but can occur in adults. The typical presentation is with multifocal neurological deficits and encephalopathy, sometimes following an infection or vaccination. MRI typically shows bilateral, symmetrical lesions in the cerebral white matter, sometimes also involving the deep grey matter (Miller et al., 2008). The CSF typically show a lymphocytic pleocytosis and OCBs may be present, often transiently. Diagnostic criteria for require the presence of encephalopathy in order to differentiate ADEM from MS (Krupp et al., 2013). However, MS can also rarely present with an encephalopathic ADEM-like illness (Schwarz et al., 2001).
Neuromyelitis optica spectrum disorder

NMOSD is an idiopathic inflammatory astrocytopathy of the CNS. Historically, NMOSD was misdiagnosed as MS but the discovery of a pathogenic IgG antibody directed against the aquaporin-4 (AQP4-IgG) water channel has established NMOSD as a specific disease entity (Wingerchuk et al., 2015). The differentiation of MS and NMOSD can be difficult, but accurate diagnosis is essential because of differences in the disease course and response to treatment (Zekeridou and Lennon, 2015).

NMOSD shows a stronger female predominance than MS (female: male ratio 9:1), tends to present in an older age group (mean age at onset 39 years) and is relatively more common in non-white populations. (Pandit et al., 2015) The core clinical features include optic neuritis (often bilateral and/or associated with poor visual recovery), longitudinally-extensive transverse myelitis (with lesions extending over >3 vertebral segments on MRI) and intractable nausea, vomiting and hiccups due to lesions in the area postrema (Kim et al., 2015; Wingerchuk et al., 2015). CSF findings include a lymphocytic pleocytosis, sometimes marked (>50 cells/cm³) and with neutrophils or eosinophils. A minority of patients have unmatched OCBs (Jarius et al., 2011).

A first attack of NMOSD can sometimes mimic a CIS with features suggestive of MS e.g. acute unilateral optic neuritis or partial transverse myelitis (Flanagan et al., 2015). Some patients with NMOSD have brain MRI abnormalities (Matthews et al., 2013) or CSF findings (Jarius et al., 2011) suggestive of MS and a subgroup of patients are AQP4-IgG seronegative. An accurate diagnosis of NMOSD is essential because accrual of disability is relapse-related. Prompt treatment of acute attacks and immunosuppression improves outcomes, whereas MS-specific therapies are not effective and may exacerbate relapses in NMOSD (Zekeridou and Lennon, 2015).
**Misdiagnosis of MS**

Rates of misdiagnosis of MS may be as high as 5-10% (Solomon and Weinshenker, 2013). In a recent survey of MS specialist neurologists in the United States over 95% reported seeing one or more patients in the previous year misdiagnosed with MS. Many of these patients were receiving inappropriate treatments. The common disorders misdiagnosed as MS include small-vessel cerebrovascular disease, migraine, functional neurological disorders and fibromyalgia (Solomon et al., 2012). Potential causes of MS misdiagnosis include the misinterpretation of clinical findings (symptoms not typical of demyelination, absence of objective neurological signs) and application of MRI criteria to diagnose MS in inappropriate clinical settings (Solomon and Weinshenker, 2013).

### 1.2.7 Prognosis

MS is frequently a disabling illness and in most developed countries is the commonest cause of neurologic disability in young adults. On average life expectancy is reduced by 5-7 years with half of deaths attributable to complications of severe MS (Sadovnick et al., 1991).

While many people develop irreversible disability in the long term, others have a much milder disease course with periods of sustained remission and little physical disability. This was highlighted in a prospective, longitudinal study of patients with CIS (Fisniku et al., 2008a). After 20 years, approximately 40% developed SPMS with severe disability. However, another 40% of patients had retained a relapsing course and only had mild disability. The factors that underpin the development of long term disability and those that are protective resulting in a more favourable disease course are poorly understood.
Measuring disability in MS

There is a need to monitor disability in people with MS over time in clinical and research settings. The Kurtzke Expanded Disability Status Scale (EDSS) is the most widely used measure of disability utilised by clinicians, researchers and regulatory authorities (Kurtzke, 1983). The EDSS score is calculated by rating the degree of neurologic disability across seven functional systems (FS) evaluated in the neurologic exam (visual, brainstem, pyramidal, cerebellar, sensory, sphincter and cerebral). The results are combined with the measured walking distance and independence with activities of daily living. A final score of 0 – 10 is given with higher scores indicating more severe disability (Table 1.3).

While the EDSS is easy to apply in research and clinical settings it has a number of disadvantages. Firstly, because scores are based on the results of the neurological examination the assessment is inherently subjective and vague definitions of what qualifies as mild, moderate or severe disability have contributed to high intra and inter-observer variability. Secondly, the EDSS is heavily weighted towards ambulatory disability. Between EDSS 4.0 and 7.0 the score is based entirely on the measured walking distance and the need for assistive devices. Although loss of ambulation is an important feature of MS-related disability the EDSS does not give equal representation to other disabiting aspects of the illness, such as cognitive impairment and fatigue.
Table 1.3  Expanded Disability Status Scale (EDSS) score (Kurtzke, 1983)

<table>
<thead>
<tr>
<th>Score</th>
<th>Description</th>
</tr>
</thead>
<tbody>
<tr>
<td>0</td>
<td>Normal neurological examination</td>
</tr>
<tr>
<td>1.0</td>
<td>No disability, minimal signs in one functional system (FS)</td>
</tr>
<tr>
<td>1.5</td>
<td>No disability, minimal signs on two or more FS</td>
</tr>
<tr>
<td>2.0</td>
<td>Minimal disability in one FS</td>
</tr>
<tr>
<td>2.5</td>
<td>Minimal disability in two FS</td>
</tr>
<tr>
<td>3.0</td>
<td>Moderate disability in one FS or mild disability in three or four FS</td>
</tr>
<tr>
<td>3.5</td>
<td>Moderate disability in two FS or mild disability in five FS</td>
</tr>
<tr>
<td>4.0</td>
<td>Able to walk ≥ 500 m but not unrestricted; severe disability in one FS or combinations of less severe disability in other FS</td>
</tr>
<tr>
<td>4.5</td>
<td>Able to walk ≥ 300 m unaided</td>
</tr>
<tr>
<td>5.0</td>
<td>Able to walk ≥ 200 m unaided</td>
</tr>
<tr>
<td>5.5</td>
<td>Able to walk ≥ 100 m unaided</td>
</tr>
<tr>
<td>6.0</td>
<td>Able to walk ≥ 100 m with unilateral assistance</td>
</tr>
<tr>
<td>6.5</td>
<td>Able to walk ≥ 20 m with bilateral assistance</td>
</tr>
<tr>
<td>7.0</td>
<td>Unable to walk 5 m with an aid, essentially restricted to a wheelchair but independent</td>
</tr>
<tr>
<td>7.5</td>
<td>Unable to take more than a few steps, restricted to a wheelchair</td>
</tr>
<tr>
<td>8.0</td>
<td>Restricted to wheelchair with effective use of the arms and retains many self-care functions</td>
</tr>
<tr>
<td>8.5</td>
<td>Restricted to bed much of the day, some use of the arms</td>
</tr>
<tr>
<td>9.0</td>
<td>Bedbound, able to communicate and eat</td>
</tr>
<tr>
<td>9.5</td>
<td>Bedbound, unable to communicate or swallow</td>
</tr>
<tr>
<td>10</td>
<td>Death due to MS</td>
</tr>
</tbody>
</table>

The Multiple Sclerosis Functional Composite (MSFC) was developed to address some of the limitations of the EDSS (Cutter et al., 1999). The MSFC incorporates three objective measures of neurologic function – the timed 25-foot walk (TWT), the 9-hole peg test (9HPT) and the paced auditory serial addition test (PASAT). Although the MSFC addresses some of the issues with EDSS there are a number of disadvantages. The test is time-consuming taking 10 – 15 minutes to complete,
depending on patient cooperation and level of disability. Although the MSFC incorporates a cognitive measure there is no assessment of visual function and so additional tests such as the Sloan low-contrast visual acuity are sometimes added (Balcer et al., 2003). Finally, the PASAT is unpopular with patients but may be substituted for the symbol digit modalities test (SDMT), an alternative test of information processing speed (Smith, 1982). Despite some limitations the MSFC is well-validated and correlates well with disease stage, EDSS score, MRI metrics, health-related quality of life and employment (Cohen et al., 2012).

**Contributions to disability in relapse-onset MS**

Two separate processes drive the accumulation of irreversible disability in relapse-onset MS. The first is relapses, where inflammatory demyelination results in permanent axonal injury with residual neurologic impairment. The second and more significant contribution is the onset of SPMS.

**Relapses**

Among patients with RRMS, relapses occur unpredictably but on average one relapse occurs every two years. Age is an important predictor of relapse rates with higher relapse rates reported in children with MS and young adults compared with older adults (Tremlett et al., 2008b; Gorman et al., 2009). Hormonal factors, particularly around the time of pregnancy (decreased) and the puerperium (increased) exert an effect on relapses (Confavreux et al., 1998). The frequency of relapses tends to reduce with time and relapses are much less common in patients with established SPMS (Paz Soldan et al., 2015).
The impact of relapses on accrual of disability was evaluated in a group of patients with established RRMS (mean baseline EDSS 2.5) enrolled in the placebo arms of a number of randomised control trials (Lublin et al., 2003). EDSS measurements were available before, during and after relapses. 42% of patients had a change of ≥0.5 and 28% had a change of ≥1.0 on the EDSS scale following a relapse (Lublin et al., 2003).

A clinic-based study from Italy aimed to identify predictors of poor recovery following a relapse (Leone et al., 2008). 34% of patients had a change in functional system score of ≥1 and 13% had a change of ≥2 points (severe sequelae) after a relapse. Predictors of severe sequelae included the relapse severity, the duration of the relapse, older age and a greater number of functional systems involved. The number of previous relapses was not predictive of relapse severity. In another clinic-based study, poor recovery from the first relapse predicted poor recovery from subsequent relapses suggesting that in part the variability in recovery from relapses is determined by unknown patient and disease-specific factors (Mowry et al., 2009a).

Recovery following a relapse is thought to be related to the degree of axonal loss within the symptomatic lesion (Trapp et al., 1998). This has been demonstrated in vivo using optical coherence tomography (OCT) in patients with acute optic neuritis where the degree of retinal nerve fibre layer thinning correlates with visual outcome (Henderson et al., 2010). Persistent conduction block in demyelinated axons and failure of remyelination are other possible mechanisms leading to poor recovery. 

Secondary progression

The change in disease course from relapsing to progressive MS is the major determinant of long term disability in relapse-onset MS. In natural history studies the median time to development of SPMS is 15 – 19 years (Confavreux et al., 2003;
Tremlett et al., 2008a; Scalfari et al., 2010; Scalfari et al., 2014). In the London-Ontario natural history cohort two thirds of patients developed SPMS over a mean follow-up period of 28 years (Scalfari et al., 2010; Scalfari et al., 2014).

Patients with SPMS progress through a series of disability milestones that result in the need for assistance to walk, loss of ambulation, impairment of upper limb and sometimes bulbar function, and ultimately death. In the London-Ontario cohort the median time to EDSS 6, 8 and 10 from the onset of the progressive phase was 3, 12 and 30 years respectively (Scalfari et al., 2010). There is much inter-individual variation in the rate of progression in SPMS with periods of stability (plateaus) and periods of increased progression. Superimposed relapses can occur in patients with SPMS, especially soon after onset of progression and in younger patients (Paz Soldan et al., 2015). Age and gender do not appear to affect the course of established SPMS (Confavreux et al., 2003). The number of early relapses (first 2-5 years of the illness) are associated with a shorter latency to the onset of SPMS but do not influence the rate of progression (Confavreux et al., 2000; Scalfari et al., 2014). Studies investigating whether superimposed relapses in patients with SPMS influence disability progression have produced mixed results (Confavreux et al., 2000; Paz Soldan et al., 2015).

**Benign MS**

A subgroup of people with relapse-onset MS have a much milder disease course with periods of sustained remission and little physical disability. This has been termed “benign MS”, a retrospective diagnosis made 15 years after disease onset in patients with an EDSS score of ≤3 (Lublin et al., 1996). The concept of benign MS is controversial. Firstly, the definition is heavily weighted towards ambulatory disability
(as measured by the EDSS), which fails to capture cognitive impairment, fatigue and other important sources of disability in people with MS. In one hospital-based study half of patients meeting criteria for benign MS had significant cognitive impairment, depression and fatigue (Amato et al., 2006). Secondly, the latency to onset of SPMS is highly variable and some evidence suggests that the risk increases with time. In the London-Ontario natural history cohort the risk of developing SPMS increased with time from 53% at 15 years to 69% at 25 years (Scalfari et al., 2014). Another natural history study from Gothenburg found that some patients were still developing SPMS more than 40 years after disease onset (Skoog et al., 2012).

Against this backdrop a number of lines of evidence do support the concept that benign forms of MS exists. Firstly, autopsy studies have found evidence of asymptomatic demyelination in patients without known neurologic symptoms during life. In autopsy series an estimated 20 – 25% of patients with pathologically confirmed demyelination died without a diagnosis of MS (Gilbert and Sadler, 1983). Secondly, an increasing number of people are being identified with the radiologically isolated syndrome with evidence of demyelinating lesions on MRI suggestive of MS in the absence of clinical symptoms (Okuda et al., 2014). These patients have a relatively favourable prognosis with less than half experiencing symptoms in the first 5 years of follow-up (Okuda et al., 2014). Finally, one third of patients with a CIS do not have any further symptoms, even with long term follow up. Up to half of these patients have MRI abnormalities that satisfy the McDonald 2005 criteria without a second clinical attack over follow-up periods of up to 20 years (Chard et al., 2011).
Comorbidities

Comorbid health problems are common in people with MS including affective disorders, hypertension, dyslipidaemia, vascular disease and chronic pulmonary disease (Marrie et al., 2015a). Comorbidities may influence the course of the illness. More rapid progression of disability has been reported in people with MS who have vascular comorbidities (Marrie et al., 2010). Comorbidities also increase the risk of hospitalization and death in people with MS (Marrie et al., 2015b; Marrie et al., 2015c).

1.2.8 Treatment

Disease-modifying treatment (DMT) for RRMS has been available for more than 20 years. There are currently 15 agents approved by the United States Food and Drug Administration and the European Medicines Agency (Wingerchuk and Weinshenker, 2016). Collectively, these treatments have been shown to reduce relapses and the number of new MRI lesions in patients with active RRMS, based on the presence of relapses or MRI evidence of disease activity in the 12-24 months prior to starting treatment (Wingerchuk and Weinshenker, 2016). The available agents differ in their route and frequency of administration, mechanism of action, efficacy and side-effect profile (Wingerchuk and Weinshenker, 2016).

While the currently available treatments reduce relapses and MRI disease activity the effect on disability is less clear. A number of these agents have been shown to reduce confirmed disability progression over follow up periods of 12 – 36 months. However, this largely reflects a reduction in disability attributable to the effects of relapses. In the longer term most disability is due to the development of SPMS. Because the course of MS unfolds over several decades it is not possible to address this issue with a randomised-controlled trial, however, a number of retrospective and
prospective observational studies suggest a benefit of DMT on long-term disability. A 21 year follow up of patients enrolled in the pivotal interferon-β1b (Betaseron) trial showed a survival advantage in patients randomised to early treatment with interferon (Goodin et al., 2012). Injectable treatments (either interferon-β or glatiramer acetate) were found to slow progression of disability compared with historical controls over a 6 year period in a prospective observational study from the United Kingdom (Palace et al., 2015).

Treating patients with MS requires a balance of risks and benefits. While the established MS therapies (interferon-β, glatiramer acetate) are known to have excellent long-term safety, a number of the newer treatments (natalizumab, fingolimod, alemtuzumab), although more effective, are associated with significant toxicity including opportunistic infections, cardiac arrhythmias and secondary autoimmunity. This is relevant when management decisions are being made in young adults with a chronic illness with an unpredictable clinical course and uncertainties about long-term benefits of treatment.

In order to select treatments for individual patients some neurologists use conventional brain MRI findings (T2 lesion load, gadolinium-enhancing lesions) to help guide initial selection and escalation of therapy (Wingerchuk and Weinshenker, 2016). However, the relationship of these measures to long-term disability is limited (Kappos et al., 1999; Fisniku et al., 2008a). There is a need for a better understanding of what drives long-term disability in MS in order to treat patients with MS more effectively with the currently available disease-modifying treatments.
1.3 Clinically isolated syndromes and the relationship to multiple sclerosis

A CIS is the first clinical manifestation of relapse-onset MS. A CIS is defined as a first episode of neurological dysfunction suggestive of demyelination in the absence of fever or infection. There has been evolution in the definition of CIS over the last 15 years as diagnostic criteria for MS have evolved (McDonald et al., 2001; Polman et al., 2005; Polman et al., 2011).

1.3.1 Presentation

The demographic profile of patients with CIS mirrors that of MS. The peak age of onset is between 20 and 40 years (mean age approximately 30 years), and CIS is more common in females than males (Tintore et al., 2015). The symptoms usually evolve subacutely over hours to days and reach their peak within 2 – 3 weeks of onset. A characteristic feature is remission with or without treatment with corticosteroids. Although a CIS can affect any part of the CNS typical presentations include optic neuritis, a brainstem/cerebellar syndrome and partial myelopathy.

**Optic neuritis**

Optic neuritis is the first symptom of MS in approximately 20-30% of patients (Toosy et al., 2014). Patients typically present with blurring of vision in one eye that may progress over hours to days. Pain is common, especially pain on eye movement. The examination shows a reduction in visual acuity that is usually mild to moderate (with median visual acuity 6/24), impaired colour vision and a relative afferent papillary defect. Although a central scotoma is most commonly seen, any type of visual field defect can occur. The optic disc may appear swollen or normal in the acute stage.
MRI of the optic nerve may show T2 hyperintensity, swelling and enhancement after the administration of gadolinium (Toosy et al., 2014).

The “typical” optic neuritis seen in patients with CIS and MS, needs to be differentiated from other causes of an acute optic neuropathy such as anterior ischaemic optic neuropathy, Leber’s hereditary optic neuropathy, NMOSD or neurosarcoidosis plus ophthalmic causes of acute unilateral visual loss. (Toosy et al., 2014). Atypical features that suggest an alternative aetiology include severe visual impairment, absence of pain or very severe pain, a “macular star” or intra-ocular inflammation and bilateral involvement (Miller et al., 2008; Toosy et al., 2014). MRI can assist in the differential diagnosis; longitudinally-extensive optic nerve lesions or involvement of the optic chiasm is suggestive of NMOSD (Kim et al., 2015) and prominent optic nerve sheath enhancement is suggestive of neurosarcoidosis (Toosy et al., 2014).

**Brainstem/cerebellar syndromes**

Brainstem/cerebellar syndromes can take a variety of forms depending on the site of the lesion (Miller et al., 2008). Double vision is the most common presentation due to an internuclear ophthalmoplegia (often bilateral) or a sixth nerve palsy (Miller et al., 2008). Other brainstem/cerebellar manifestations include facial sensory loss, facial weakness, cerebellar ataxia, and vertigo (Miller et al., 2008). Common sites for infratentorial lesions on MRI include the dorsal and ventral surfaces of the pons (in contrast to small vessel cerebrovascular disease which typically involves the belly of the pons), the middle cerebellar peduncle and the cerebellar hemispheres.
**Spinal cord syndromes**

An episode of myelitis presenting with a partial spinal cord syndrome is a common CIS presentation. The symptoms are usually sensory in nature and may start in one limb and ascend to a level involving the trunk or spread to the contralateral side. Lhermitte’s symptom may be present when the lesion involves the cervical cord and mild sphincter disturbance is common. A complete transverse myelitis with bilateral motor and sensory involvement with a clearly defined sensory level, is not typical of CIS/MS and more often occurs in idiopathic transverse myelitis or NMOSD.

In patients with a partial myelitis MRI typically shows a wedge-shaped area of T2-hyperintensity, often placed eccentrically in the cord and/or abutting the pial surface on axial images. Lesions typically extend over 1-2 vertebral bodies, in contrast to NMOSD (Kim et al., 2015).

**Hemispheric syndromes**

Rarely CIS patients can present with cerebral symptoms such as hemiparesis or a visual field defect, sometimes mimicking an acute stroke (Pelayo et al., 2007).

### 1.3.2 Clinical course

Although a CIS can be an isolated, monophasic event it is frequently the first manifestation of MS. This was highlighted in studies done in the pre-MRI era (predominantly in patients with acute optic neuritis) that found that CDMS developed in 30 – 75% of patients with CIS with a higher risk reported in studies from the United Kingdom (Francis et al., 1987) compared with North America (Rizzo and Lessell, 1988) or Scandinavia (Sandberg-Wollheim et al., 1990).
In evaluating patients with CIS two major clinical questions arise. The first is the risk of further clinical episodes and new MRI disease activity (i.e. MS). The second is the risk of long-term neurological disability. A number of natural history studies, observational clinical-MRI studies and clinical trials have provided important insights into the prognosis of patients with CIS. In this section the demographic, genetic and environmental, clinical, MRI and body fluid biomarkers that help predict the development of CDMS and long-term disability in CIS patients are reviewed.

**Risk factors for the development of clinically-definite multiple sclerosis**

**Demographic factors**

Younger age is associated with an increased risk of a second clinical attack and development of CDMS (Polman *et al.*, 2008; Mowry *et al.*, 2009b; Kuhle *et al.*, 2015; Tintore *et al.*, 2015). Younger patients with MS, including children, have a greater burden of radiological disease activity (as measured by increase in T2 lesion load over time and number of gadolinium-enhancing lesions) potentially explaining this finding (Filippi *et al.*, 2001b; Li *et al.*, 2006; Waubant *et al.*, 2009). However, the underlying biological mechanisms responsible for this are unclear.

CIS is more common in women than men (Dobson *et al.*, 2012). While some studies have reported that female sex is a risk factor for CDMS in CIS patients (Rizzo and Lessell, 1988; Optic Neuritis Study Group, 2008; Swanton *et al.*, 2010) others have not (Kuhle *et al.*, 2015; Tintore *et al.*, 2015). A recent meta-analysis of nine studies found no influence of sex on the rate of conversion to CDMS after a CIS (Dobson *et al.*, 2012).
There has been relatively little investigation of the role of ethnicity. In a study from San Francisco, CIS patients with non-white ethnicity (African American, Hispanic and Asian) were at higher risk of CDMS than white patients living in the same geographical area (Mowry et al., 2009b).

**Genetic and environmental factors**

Major genetic and environmental risk factors for MS including HLA-DRB1*1501 (Kelly et al., 1993; Morrissey et al., 1993; Hauser et al., 2000a; Horakova et al., 2011), vitamin D insufficiency (Martinelli et al., 2014; Ascherio et al., 2014) and cigarette smoking (Di Pauli et al., 2008) have been associated with an increased risk of CDMS in CIS patients. The role of EBV infection is uncertain; some studies have reported that higher EBV-IgG titres are a risk factor for CDMS (Lunemann et al., 2010), but others have not (Kuhle et al., 2015; Munger et al., 2015).

**Clinical features**

The risk of CDMS is similar across different CIS subtypes (Morrissey et al., 1993; O’Riordan et al., 1998b; Jacobs et al., 2000; Comi et al., 2001; Brex et al., 2002; Fisniku et al., 2008a; Polman et al., 2008; Mowry et al., 2009b; Tintore et al., 2010; Kuhle et al., 2015; Tintore et al., 2015). Early follow-up of the Barcelona CIS cohort suggested that optic neuritis may be associated with a lower risk of developing CDMS (Tintore et al., 2005). However, patients with optic neuritis in that study had a low rate of asymptomatic T2-hyperintense brain lesions at presentation. After adjustment for MRI findings the risk of MS was similar in patients with optic neuritis and other CIS presentations (Tintore et al., 2005). These findings were confirmed in a much larger cohort of over 1000 CIS patients studied by the same group (Tintore et al., 2015).
Some specific clinical features are associated with a low risk of subsequent MS in patients with CIS. These include papillitis, painless visual loss and presence of retinal haemorrhages / exudates in patients with optic neuritis (Beck et al., 2003), isolated facial sensory loss in brainstem CIS patients (Sastre-Garriga et al., 2010) and a complete transverse myelopathy in patients with acute spinal cord syndromes, which is atypical and associated with a very low risk of MS (Lipton and Teasdall, 1973).

Cognitive impairment (Zipoli et al., 2010) and fatigue (Runia et al., 2015) in patients with CIS may be risk factors for the development of CDMS.

**MRI abnormalities**

The presence of asymptomatic T2-hyperintense brain lesions on MRI is the most important risk factor for CDMS in patients with CIS (Lee et al., 1991; Morrissey et al., 1993; O’Riordan et al., 1998b; Tintore et al., 2001; Brex et al., 2002; Minneboo et al., 2004; Tintore et al., 2006; Fisniku et al., 2008a; Optic Neuritis Study Group, 2008; Tintore et al., 2015). Approximately two-thirds of patients have brain MRI abnormalities at the time of presentation with CIS (Fisniku et al., 2008a; Optic Neuritis Study Group, 2008; Tintore et al., 2015).

Table 1.4 summarises the results of prospective longitudinal observational studies investigating the prognostic significance of asymptomatic T2-hyperintense brain lesions on the development of MS over follow-up periods of up to 20 years.
<table>
<thead>
<tr>
<th>Study</th>
<th>Year</th>
<th>CIS type</th>
<th>Number</th>
<th>Follow up</th>
<th>CDMS (%)</th>
<th>Abnormal MRI</th>
<th>Normal MRI</th>
<th>Total</th>
</tr>
</thead>
<tbody>
<tr>
<td>Tintore et al</td>
<td>2015</td>
<td>Mixed</td>
<td>1015</td>
<td>7 years</td>
<td>56%</td>
<td>7%</td>
<td>39%</td>
<td></td>
</tr>
<tr>
<td>Optic Neuritis Study Group</td>
<td>2008</td>
<td>Optic neuritis</td>
<td>389</td>
<td>15 years</td>
<td>72%</td>
<td>25%</td>
<td>50%</td>
<td></td>
</tr>
<tr>
<td>Fisniku et al</td>
<td>2008</td>
<td>Mixed</td>
<td>107</td>
<td>20 years</td>
<td>82%</td>
<td>21%</td>
<td>63%</td>
<td></td>
</tr>
</tbody>
</table>
In CIS patients with an abnormal brain MRI the risk of MS is approximately 60 – 80% compared with approximately 10 – 25% in patients with a normal MRI brain at presentation (Fisniku et al., 2008a; Optic Neuritis Study Group, 2008; Tintore et al., 2015).

A higher number of T2 lesions at baseline is associated with a higher risk of CDMS, at least over the first 5-7 years after CIS (Morrisey et al., 1993; Optic Neuritis Study Group, 1997; O’Riordan et al., 1998b; Beck et al., 2003; Tintoré et al., 2006; Swanton et al., 2010; Tintore et al., 2015). In the Barcelona CIS cohort followed up over a mean of almost 6 years, the hazards ratio for development of CDMS increased from 5.7 (95% CI 3.5 – 9.4) in patients with 1 – 3 T2 lesions, to 8.7 (5.4 – 14.2) with 4 – 9 T2 lesions and 12.7 (9.1 – 19.7) with 10 or more T2 lesions (Tintore et al., 2015). Similar results were found in a cohort of patients with optic neuritis followed up over a period of 6 years, although in that study measures of lesion location and activity were better predictors of CDMS than lesion number in a multivariate analysis (Swanton et al., 2010).

The effect of T2 lesion number may be less important in the longer term. This was illustrated in a cohort followed up at approximately 5 yearly intervals over the first 20 year after CIS. After 5 years the risk of CDMS was higher in patients with four or more T2 brain lesions at baseline (Morrisey et al., 1993). However, at 14 and 20 years the risk of CDMS was similar in patients with 1 – 3, 4 – 10 and more than 10 T2 lesions at presentation (Brex et al., 2002; Fisniku et al., 2008a). Similar findings were reported in the Optic Neuritis Treatment Trial where a higher a number of T2 lesions at baseline was association with a higher risk of CDMS at 5 years (Optic Neuritis Study Group, 1997) but not at 15 years follow-up (Optic Neuritis Study Group, 2008).
The periventricular region is the most common site for T2 brain lesions in CIS patients (Brex et al., 1999) and one or more periventricular lesions was the strongest baseline MRI predictor of CDMS in patients with optic neuritis followed up over 6 years, independent of other measures of lesion load, location and activity (Swanton et al., 2010). Juxtacortical lesions are a relatively specific finding and have also been found to be associated with the development of CDMS (Barkhof et al., 1997). Infratentorial (Tintore et al., 2010) and spinal cord lesions (Sombekke et al., 2013) occur in up to half of patients with CIS and are associated with an increased risk of CDMS. The value of lesion location is reflected in current diagnostic criteria for MS which emphasise the importance of lesion location over lesion load (Polman et al., 2011).

A core feature of MS is that CNS lesions are disseminated in time. The presence of gadolinium-enhancing and non-enhancing lesions and/or a new T2 lesion provides evidence for DIT using MRI (Dalton et al., 2003; Tur et al., 2008; Rovira et al., 2009). These findings are highly predictive of the development of CDMS in CIS patients, independent of other MRI findings (Swanton et al., 2010).

A number of non-conventional MRI measures have been studied in CIS patients. Whole brain and grey matter atrophy, elevated NAWM myo-inositol concentration, reduced grey matter MTR, and cortical lesions have all be associated with the early development of CDMS over the first 2-3 years after a CIS (Dalton et al., 2002a; Dalton et al., 2004; Fernando et al., 2004; Fernando et al., 2005; Filippi et al., 2010; Calabrese et al., 2011; Perez-Miralles et al., 2013). In a multimodal MRI study investigating the predictive value of baseline MRI abnormalities, conventional MRI measures (particularly lesion location and activity) were associated with the development of CDMS after 6 years in a multivariable analysis, whereas the non-
conventional MRI measures (whole brain and tissue-specific atrophy, magnetization transfer ratio and spectroscopy) were not. The presence of one or more intracortical lesions has been shown to predict CDMS, independent of T2 lesion load (Filippi et al., 2010). However, the sensitivity of this finding was low and only one third of CIS patients had one or more intracortical lesions (Filippi et al., 2010).

Cerebrospinal fluid biomarkers

The presence of unmatched IgG OCBs in the CSF is predictive of conversion from CIS to CDMS (Sandberg-Wollheim et al., 1990; Morrissey et al., 1993; Cole et al., 1998; Tintore et al., 2008; Dobson et al., 2013; Kuhle et al., 2015; Tintore et al., 2015). Some studies have found that the prognostic significance of OCBs may be eclipsed by brain MRI findings (Morrissey et al., 1993), although a recent study in over 1000 CIS patients found that presence of OCBs was associated with development of CDMS independent of brain T2 lesion load (Tintore et al., 2015). A normal brain MRI and negative OCBs is associated with a very low-risk of CDMS (Tintore et al., 2008).

CSF biomarkers other than oligoclonal bands are actively being investigated (Comabella and Montalban, 2014). Recent studies have found that higher levels of CSF chitinase 3-like 1, neurofilament light chain and soluble CD27 predict conversion to CDMS in CIS patients (Brettschneider et al., 2010; Comabella et al., 2010; Arrambide et al., 2016).

Neurophysiologic abnormalities

Although abnormalities in evoked potentials are often seen in CIS patients, the prognostic value for development of CDMS is limited (Pelayo et al., 2010).
Risk factors for the development of disability

Demographic factors

Older age at onset has been consistently associated with shorter times to development of significant disability in relapse-onset MS, principally because of a shorter time to onset of SPMS (Eriksson et al., 2003; Koch et al., 2010; Scalfari et al., 2011; Scalfari et al., 2014). Similarly, male sex is associated with a more disabling course (Confavreux et al., 2003), possibly because of the higher proportion of males with PPMS.

Prospective CIS studies have not found age or sex to be predictive of future disability (Swanton et al., 2009; Tintore et al., 2015), although this might reflect the relatively short duration of follow-up compared with natural history studies.

Genetic and environmental factors

Whether genetic factors influence the course of MS is uncertain. A number of cross-sectional studies have not found an association between HLA-DRB1*1501 status and disability when assessed using the EDSS (Masterman et al., 2000; Hensiek et al., 2002; International Multiple Sclerosis Genetics Consortium et al., 2011; Moutsianas et al., 2015). There have been no previous studies investigating the relationship of HLA-DRB1*1501 (or other genetic factors) and long-term disability in CIS patients.

Vitamin D insufficiency may be a risk factor for disability progression. In a clinical trial of high-risk CIS patients vitamin D levels of <50 nmol/L at presentation was associated with higher disability after 5 years and a trend towards a faster rate of brain atrophy (Ascherio et al., 2014).
Cigarette smoking has been reported to be associated with faster progression of disability in people with established MS (Manouchehrinia et al., 2013) but not with MRI measures of disease progression in patients with CIS or early RRMS, at least in the short-term (Munger et al., 2015; Kvistad et al., 2016).

Clinical features

Natural history studies suggest that the long-term course of CIS patients with optic neuritis or sensory symptoms may be more favourable than those with motor symptoms at onset (Confavreux et al., 2003; Eriksson et al., 2003). However, these studies considered patients with relapse and progressive-onset MS together. In studies restricted to patients with relapse-onset MS the initial presenting symptoms appear to have less prognostic value (Tremlett et al., 2008a; Scalfari et al., 2014). In a prospective CIS cohort followed for 20 years disability was similar at follow-up irrespective of CIS topography (Fisniku et al., 2008a).

Incomplete recovery in CIS patients may be a marker of worse prognosis and shorter time to onset of SPMS (Eriksson et al., 2003).

MRI abnormalities

Brain T2 lesion load at the time of CIS is moderately predictive of future disability (O’Riordan et al., 1998b; Brex et al., 2002; Tintoré et al., 2006; Fisniku et al., 2008a; Tintore et al., 2015). In the Barcelona CIS cohort, patients with 10 or more T2 lesions at baseline were three times as likely to reach EDSS 3 over a mean follow-up period of almost 7 years (Tintore et al., 2015). No increase in the risk of disability was seen in patients with 1 – 3 or 4 – 9 T2 lesion at baseline (Tintore et al., 2015). In the London
CIS cohort followed up over 20 years the risk of EDSS 3 or EDSS 6 was higher in patients with a greater number of T2 lesions at baseline. However, while disability was overall more common with an increasing number of lesions a third of patients with 10 or more lesions baseline accrued minimal neurologic disability (EDSS <3) over 20 years, while nearly a quarter of patients with a normal baseline MRI had an EDSS ≥3 (Fisniku et al., 2008a).

The change in T2 lesion load over time may be a better predictor of disability than baseline T2 lesion number. New T2 lesions after 3 months and change in lesion load over the first year after a CIS are associated with disability 6 years later (Swanton et al., 2009; Di Filippo et al., 2010). In the London cohort followed up over 20 years both clinically and with MRI, the change in T2 lesion volume over the first 5 years was significantly greater in patients who developed SPMS (Fisniku et al., 2008a). The correlation coefficient for change in T2 lesion volume over 5 years and disability after 20 years was higher than for baseline T2 lesion load (r 0.61 vs 0.48). This is consistent with the findings of natural history studies that the number of early relapses (the clinical correlate of T2 lesions) is predictive of disability and shorter time to development of SPMS (Confavreux et al., 2003; Scalfari et al., 2014).

Lesion location appears to be a better predictor of disability that measures of lesion load over the first 5-8 years after a CIS (Minneboo et al., 2004; Swanton et al., 2009; Tintore et al., 2010). In a small cohort of CIS patients from Amsterdam the presence of two or more infratentorial lesions on the baseline MRI was associated with disability after 8 years (Minneboo et al., 2004). Similar findings were reported in a larger CIS cohort from Barcelona; one or more baseline infratentorial lesion doubled the risk of reaching EDSS 3 or more after 7 years. This increase in risk was driven by brainstem
rather than cerebellar lesions (Tintore et al., 2010). In a study of patients with optic neuritis with brain and spinal cord MRI at the time of CIS, spinal cord lesions were found to be the best predictor of disability after 6 years (Swanton et al., 2009).

Gadolinium-enhancing lesions are predictive of relapses and development of CDMS (Kappos et al., 1999; Swanton et al., 2009) but not disability, at least in the short to medium-term (Swanton et al., 2009).

In established MS a number of number non-conventional MRI measures have been correlated with disability progression over time including brain and spinal cord atrophy, grey matter MTR and grey matter lesion load (Agosta et al., 2006; Lukas et al., 2010; Calabrese et al., 2012; Lukas et al., 2015). While similar abnormalities have been found in patients with CIS they have not been found to be associated with disability over follow-up periods of 3 – 6 years (Dalton et al., 2004; Fernando et al., 2004; Fernando et al., 2005; Swanton et al., 2009), but longer follow-up is required to determine the prognostic value of these measures.

Cerebrospinal fluid biomarkers

OCBs are associated with an increased risk of disability in the first 6 years after a CIS, independent of brain MRI abnormalities (Tintore et al., 2015).

Neurophysiologic abnormalities

Abnormalities in multimodal evoked potentials are associated with future disability in patients with CIS independent of brain MRI findings (Pelayo et al., 2010).
Summary

Risk factors for the development of CDMS and disability in CIS patients are summarised in Table 1.5.

<table>
<thead>
<tr>
<th>Demographic</th>
<th>Clinically-definite MS</th>
<th>Disability</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>Younger age</td>
<td>Older age</td>
</tr>
<tr>
<td></td>
<td>Female sex</td>
<td>Male sex</td>
</tr>
<tr>
<td>Genetic</td>
<td>HLA DRB1*15:01</td>
<td></td>
</tr>
<tr>
<td>Environmental</td>
<td>Vitamin D insufficiency</td>
<td>Vitamin D insufficiency</td>
</tr>
<tr>
<td></td>
<td>Smoking</td>
<td></td>
</tr>
<tr>
<td>Clinical</td>
<td>Non-optic neuritis CIS</td>
<td>Motor symptoms at onset</td>
</tr>
<tr>
<td></td>
<td>Cognitive impairment</td>
<td>Poor recovery from CIS</td>
</tr>
<tr>
<td></td>
<td>Fatigue</td>
<td>Number of early relapses</td>
</tr>
<tr>
<td>MRI</td>
<td>Number and location of T2 lesions</td>
<td>&gt;10 T2 brain lesions</td>
</tr>
<tr>
<td></td>
<td>Gadolinium-enhancing lesions</td>
<td>Infratentorial and spinal cord lesions</td>
</tr>
<tr>
<td></td>
<td>New T2 lesions over time</td>
<td>Change in T2 lesion load over first 5 years</td>
</tr>
<tr>
<td>Cerebrospinal fluid</td>
<td>IgG oligoclonal bands</td>
<td>IgG oligoclonal bands</td>
</tr>
<tr>
<td></td>
<td>Neurofilament light chain</td>
<td></td>
</tr>
<tr>
<td></td>
<td>CHI3L-1</td>
<td></td>
</tr>
</tbody>
</table>

1.3.4 Treatment

Treatment with high-dose corticosteroids hastens the recovery from an episode of acute optic neuritis but does not influence the degree of recovery or the subsequent risk of developing MS (Optic Neuritis Study Group, 2008). These results have been generalised to other CIS presentations and corticosteroid treatment is usually considered in CIS patients with disabling symptoms.
Disease-modifying therapies used to treat MS have been investigated in high-risk CIS patients with asymptomatic brain MRI abnormalities suggestive of demyelination (Jacobs et al., 2000; Comi et al., 2001; Kappos et al., 2006; Comi et al., 2009; Comi et al., 2012; Miller et al., 2014; Leist et al., 2014). Collectively these studies have shown that treatment delays the time to a second clinical attack and reduces the number of new T2 brain lesions over 2 – 4 years of follow-up (Table 1.6). Whether there is any long-term advantage to early initiation of DMT at the time of presentation with CIS is uncertain. The BENEFIT study randomised 468 patients with high-risk CIS to interferon β-1b 250 mcg alternate days or placebo (Kappos et al., 2006). After 24 months there was a 38% reduction in the risk of CDMS and positive effects on a number of secondary MRI endpoints. Participants had the option to switch to open-label interferon β-1b at the end of the study or after the development of CDMS. At 3 years there was a 40% reduction in confirmed disability progression in patients randomised to early treatment with interferon β-1b (Kappos et al., 2007), however, this was no longer apparent after 5 years (Kappos et al., 2009). In a recently completed 11 year follow-up, disability was similar in patients randomised to early treatment and those who started treatment after 24 months with a median EDSS 1.5 in both groups (Kappos et al., 2016). More patients in the delayed treatment group developed SPMS compared with the early treatment group (8.3% vs 4.5%), although this was not statistically significant. PASAT scores were higher in the early treatment group suggesting a possible benefit on cognitive outcomes.

The findings of observational studies have confirmed the findings of randomised controlled trials. Early treatment with DMT reduces the risk of CDMS and may reduce the risk of reaching EDSS 3 over follow-up periods of up to 6 years (Tintore et al., 2015).
Table 1.6  Randomised controlled trials of disease-modifying therapies in patients with CIS.

<table>
<thead>
<tr>
<th>Study</th>
<th>Intervention</th>
<th>Duration</th>
<th>Number</th>
<th>CDMS Active</th>
<th>CDMS Placebo</th>
<th>RR</th>
<th>OR</th>
</tr>
</thead>
<tbody>
<tr>
<td>CHAMPS (Jacobs et al., 2000)</td>
<td>IFN β-1a 30 mcg weekly vs placebo</td>
<td>36 months</td>
<td>383</td>
<td>35%</td>
<td>50%</td>
<td>RR 0.56 (0.38-0.81)</td>
<td></td>
</tr>
<tr>
<td>ETOMS (Comi et al., 2001)</td>
<td>IFN β-1a 22 mcg weekly vs placebo</td>
<td>24 months</td>
<td>309</td>
<td>34%</td>
<td>45%</td>
<td>OR 0.61 (0.37-0.99)</td>
<td></td>
</tr>
<tr>
<td>BENEFIT (Kappos et al., 2006)</td>
<td>IFN β-1b 250 mcg alternate days vs placebo</td>
<td>24 months</td>
<td>468</td>
<td>28%</td>
<td>45%</td>
<td>HR 0.50 (0.36-0.70)</td>
<td></td>
</tr>
<tr>
<td>REFLEX (Comi et al., 2012)</td>
<td>IFN β-1a 44 mcg TIW vs IFN β-1a 44 mcg weekly vs placebo</td>
<td>24 months</td>
<td>517</td>
<td>22% (TIW)</td>
<td>38%</td>
<td>HR 0.48 (0.31-0.73)</td>
<td></td>
</tr>
<tr>
<td>PreCISe (Comi et al., 2009)</td>
<td>Glatiramer acetate 20 mg daily vs placebo</td>
<td>36 months</td>
<td>461</td>
<td>25%</td>
<td>43%</td>
<td>HR 0.55 (0.40-0.77)</td>
<td></td>
</tr>
<tr>
<td>TOPIC (Miller et al., 2014)</td>
<td>Teriflunomide 14mg vs teriflunomide 7mg daily vs placebo</td>
<td>24 months</td>
<td>618</td>
<td>24% (14mg)</td>
<td>36%</td>
<td>HR 0.57 (0.38-0.87)</td>
<td></td>
</tr>
<tr>
<td>ORACLE (Leist et al., 2014)</td>
<td>Cladribine 5.25 mg/kg vs cladribine 3.5 mg/kg vs placebo</td>
<td>24 months</td>
<td>616</td>
<td>15% (5.25mg/kg)</td>
<td>34%</td>
<td>HR 0.38 (0.25-0.58)</td>
<td></td>
</tr>
</tbody>
</table>

Abbreviations: CDMS = clinically-definite MS, IFN = interferon, HR = hazard ratio, OR = odds ratio, RR = rate ratio, TIW = three times weekly

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1.4 Rationale for this thesis

MS is a common neurological disorder in young adults with a highly variable clinical course. In the longer term many patients with relapse-onset MS will develop significant neurological disability but others have a much milder disease course. The mechanisms responsible for disease course heterogeneity in MS remain poorly understood, especially the factors important in the development of SPMS. Understanding what course a person’s MS is likely to take is a priority around the time of their first symptoms when neurologists are counselling patients about prognosis and making treatment decisions.

Against this background the major aims of this thesis are: (1) to improve the diagnosis of MS using MRI to facilitate an early, accurate diagnosis; (2) to identify early MRI predictors of long-term disability in relapse-onset MS; and (3) to identify the pathological changes that underpin disease course heterogeneity in relapse-onset MS using conventional and novel MRI techniques. To address these aims I conducted a clinical, MRI and genetic study in a group of people with CIS who have been followed up prospectively over the last 15 years. The patients were all recruited within 3 months of CIS and seen for scheduled follow-up irrespective of clinical status over the first 5 years after disease onset. I conducted a long-term follow-up of this cohort approximately 15 years after presentation with CIS. I analysed data collected over a 15 year period with the major results presented in this thesis.

This introductory chapter provides background to MS and particularly the difficulties in predicting long-term disease at the time of onset. Chapter 2 provides an overview of the use of MRI to understand the pathological basis of MS. In Chapter 3 the characteristics of the CIS cohort I studied are described with the major results of the
follow-up study presented in subsequent chapters. Chapters 4 and 5 concern the diagnosis of MS in CIS patients, including the influence of changing diagnostic criteria for MS and possible changes to diagnostic criteria to improve the diagnosis of MS in people with CIS. Chapters 6 and 7 investigate early MRI abnormalities and how they relate to the development of neurological disability with a focus on the prognostic value of spinal cord MRI. In Chapter 8, I investigate the influence of *HLA-DRB1*15:01 (the most important genetic risk factor for MS) for the development of long-term disability and MRI-detected brain tissue damage. Finally, in Chapter 9 I apply sodium MRI, a novel metabolic imaging technique to investigate disease mechanisms responsible for long-term disability and secondary progression in relapse-onset MS.
Chapter Two

Use of magnetic resonance imaging to understand the pathological basis of multiple sclerosis

2.1 Introduction

In the last 30 years MRI has emerged as an important tool for accurately and reproducibly imaging key pathological changes in the brain, spinal cord and optic nerve in people with MS *in vivo* (Filippi et al., 2012). MRI is now the central investigation for making the diagnosis of MS (Polman et al., 2011; Rovira et al., 2015) and in monitoring the course of the disease (Wattjes et al., 2015). MRI has facilitated the development of disease-modifying treatments for relapsing forms of MS and provided insights into the pathological changes relevant to disease progression and disability.

The major pathological changes in MS include focal areas of demyelination in white and grey matter, diffuse injury within normal-appearing tissues and widespread neuroaxonal loss (Popescu and Lucchinetti, 2012). A number of conventional and non-conventional MRI measures are sensitive to the detection of these pathological abnormalities. These are reviewed in this chapter with emphasis on techniques applied in this thesis.

2.2 White matter lesions

White matter lesions on MRI appear hyperintense on proton density (PD) and T2-weighted sequences (Figure 2.1). MS lesions are typically small (<1cm in diameter) and may appear round or ovoid (Fazekas et al., 1999). Brain lesions in MS have a
predilection for certain anatomical sites including the periventricular white matter, the corpus callosum, the subcortical U-fibres abutting the cerebral cortex (juxtacortex) and the brainstem/cerebellum (Fazekas et al., 1999). Focal white matter lesions seen on MRI are the radiological correlate of plaques seen pathologically in patients with MS (Filippi et al., 2012). White matter lesions visible on conventional MRI are not pathologically specific and reflect varying degrees of inflammation, demyelination, astrogliosis and axonal loss within focal lesions (Filippi et al., 2012).

Figure 2.1 White matter lesions in multiple sclerosis.

T2-hyperintense white matter lesions in MS on (A) proton density-weighted, (B) T2-weighted and (C) FLAIR sequences.

FLAIR improves the sensitivity of MRI for the detection of supratentorial white matter lesions, particularly around the lateral ventricles and juxtacortical white matter close to the brain-CSF interface (Stevenson et al., 1997). In a combined MRI-pathological study, FLAIR detected 71% of pathologically confirmed white matter lesions compared with 63% for T2-weighted spin-echo sequences at 1.5T (Geurts et al., 2005a). 2D-FLAIR may be less sensitive than PD/T2-weighted sequences for detection of infratentorial lesions (Stevenson et al., 1997), potentially because FLAIR is more prone to artefacts within the posterior fossa (Rovira et al., 2015). Recently,
the added utility of a 3D FLAIR with higher resolution and decreased slice thickness compared with standard 2D FLAIR and 2D PD/T2-weighted sequences has been demonstrated (Gramsch et al., 2015). 3D FLAIR may improve the detection of juxtacortical and infratentorial lesions compared with standard 2D sequences (Gramsch et al., 2015).

Up to 30% of white matter lesions in MS appear hypointense compared with the surrounding NAWM on T1-weighted spin echo sequences (Figure 2.2). These lesions are also known as “black holes”. Acute MS lesions may show T1-hypointensity due to the effects of oedema but only half of these lesions evolve into a persistent black hole over time (Bagnato et al., 2003). Persistent black holes represent areas of significant axonal loss pathologically (van Waesberghe et al., 1999)

![Figure 2.2](image)

Figure 2.2  T1-hypointense and T1-isointense white matter lesions.

White matter lesions in MS on (A) T2-weighted and (B) T1-weighted scans. A subset of T2-hyperintense lesions appear hypointense on the T1-weighted scan.
T2-weighted scans are unable to differentiate acute active plaques from chronic lesions. A contrast-enhanced T1-weighted scan obtained after the administration of a gadolinium contrast agent can be helpful in identifying acute active MS lesions. The presence of contrast enhancement indicates breakdown of the blood brain barrier within acute lesions (Cotton et al., 2003; Filippi et al., 2012). Lesions may show nodular, ring or open-ring patterns of enhancement and the pattern may change as the lesion evolves. In a study of patients studied with weekly MRI scanning, the mean duration of enhancement was three weeks and so the presence of contrast enhancement is helpful in identifying new or acute lesions (Cotton et al., 2003).

A number of technical factors influence the detection of contrast-enhancing lesions including the dose of gadolinium given and the timing of the post-contrast scan (Silver et al., 1997).

The major safety concern with gadolinium-based contrast agents is nephrogenic systemic fibrosis, a rare multisystem fibrosing disorder affecting skin, soft tissues and sometimes other organs seen in patients with moderate to severe renal dysfunction with a glomerular filtration rate of <30ml/minute (Thomsen, 2016). More recently, a number of cases have been reported of gadolinium deposition in the dentate nuclei, pons and basal ganglia in patients with repeated exposure to gadolinium-based contrast agents to monitor brain tumours and normal renal function (McDonald et al., 2015). These abnormalities were noted in vivo with hyperintensity on T1-weighted images in these structures and confirmed pathologically in a subset of patients (McDonald et al., 2015). The clinical significance of gadolinium deposition in these structures is uncertain and is under review by the United States Food and Drug Administration (United States Food and Drug Administration, 2015).
2.3 Grey matter lesions

Involvement of grey matter is a major finding pathologically in MS (Kutzelnigg et al., 2005; Lucchinetti et al., 2011; Popescu and Lucchinetti, 2012). Compared with lesions in the white matter, grey matter lesions are typically smaller, have poor tissue contrast with normal-appearing grey matter and may be obscured by partial volume effects of CSF. T2-weighted and FLAIR sequences detect 3% and 5% respectively of pathologically-confirmed intracortical lesions, although the sensitivity is higher for leukocortical lesions involving both grey and white matter (Geurts et al., 2005a).

Double-inversion recovery (DIR) is a non-conventional MRI technique using inversion times that suppress the signal from both CSF and white matter, increasing the contrast between grey and white matter (Geurts et al., 2005b). On DIR cortical lesions appear hyperintense compared with the normal appearing grey matter. DIR detects 500% more cortical lesions than T2 spin-echo and 150% more than FLAIR sequences (Geurts et al., 2005b). However, the overall sensitivity is low, with less than 20% of pathologically-confirmed cortical grey matter lesions visible on DIR (Seewann et al., 2012). The sensitivity of DIR is significantly higher for leukocortical lesions, compared with intracortical or subpial lesions (Seewann et al., 2012).

Another approach to detection of grey matter lesions is phase-sensitive inversion recovery (PSIR), a T1-weighted sequence that uses a phase-sensitive reconstruction to provide greater contrast between grey and white matter (Figure 2.3). PSIR is more sensitive than DIR with a three-fold increase in the detection of cortical grey matter lesions (Sethi et al., 2012). However, PSIR remains insensitive to the detection of subpial lesions (Sethi et al., 2012).
2.3 Cortical grey matter lesions in multiple sclerosis

Phase-sensitive inversion recovery scan showing multiple leukocortical and intracortical grey matter lesions (arrow heads) at the level of the insula (A) and at the cerebral convexity (B) in a person with multiple sclerosis.

2.4 Spinal cord lesions

MS commonly affects the spinal cord. An episode of partial myelitis is a common first symptom of MS and progressive MS frequently takes the form of a spastic paraparesis. Spinal cord MRI is technically more challenging than brain MRI. The spinal cord is a much smaller structure than the brain and spinal cord MRI is more prone to artefacts due to patient motion, surrounding CSF and the effects of respiration and the cardiac cycle (Kearney et al., 2015b).

Spinal cord lesions are found in up to 90% of patients with established MS (Kidd et al., 1993; Nijeholt et al., 1998; Lukas et al., 2013). Spinal cord lesions are more commonly found in the cervical than thoracic or lumbar spine (Kidd et al., 1993). Lesions typically extend over 1 or sometimes 2 segments and are peripherally located.
in the cord abutting the subpial surface. Like in the brain, spinal cord lesions often involve both white and grey matter structures (Kearney et al., 2015c). Spinal cord lesions can be detected on PD or T2-weighted images (Figure 2.4). STIR sequences are more sensitive to the detection of spinal cord lesions (Thorpe et al., 1994; Rocca et al., 1999; Bot et al., 2000), but also more prone to artefacts (Rovira et al., 2015). Acquisition of images with at least two types of tissue contrast (PD, T2 or STIR) and in two planes (sagittal and axial) is recommended to improve lesion detection and accuracy in the spinal cord in MS (Rovira et al., 2015).

Post-contrast T1-weighted spinal cord scans can be helpful in demonstrating acute active lesions (Figure 2.4). The number of new, enhancing spinal cord lesions over time is much less than in the brain (Thorpe et al., 1996).

(A) T2-weighted sagittal scan of the spinal cord showing two T2-hyperintense lesions at C2 and C2/C3 (arrows) and (B) post-contrast T1-weighted scans obtained 15 minutes after the administration of gadolinium showing contrast enhancement in the lesions seen on the T2-weighted scan (arrows).
Diffuse spinal cord abnormalities are sometimes seen, particularly on PD-weighted scans (Lukas et al., 2013). These are more common in patients with a progressive disease course and significant disability (Lukas et al., 2013).

2.5 Brain atrophy

Brain atrophy is a major feature of MS pathologically and is thought to principally reflect neuroaxonal loss within lesions and normal-appearing tissue, although other processes such as demyelination and the extent of inflammation also influence brain volume in MS (Miller et al., 2002; Rocca et al., 2017). MRI is sensitive to the detection of brain tissue loss and this may be visible qualitatively once established with gross brain parenchymal atrophy, ventricular enlargement and widening of the cerebral sulci (Figure 2.5).

![Figure 2.5 Brain atrophy in multiple sclerosis. Post-contrast T1-weighted scans in a patient with multiple sclerosis at (A) baseline, (B) 1 year, (C) 3 years and (D) 8 years showing brain tissue loss with progressive enlargement of the lateral ventricles and sulcal widening. Gadolinium-enhancing lesions are also visible.](image-url)
Brain atrophy has been shown to begin early in the course of MS and occurs at a similar rate throughout the course of the disease and across different MS subtypes (Chard et al., 2002b; Dalton et al., 2004; De Stefano et al., 2007; De Stefano et al., 2010). Multiple studies have reported a consistent rate of whole brain atrophy in MS of 0.5-1%/year compared with 0.1-0.3%/year in healthy controls (De Stefano et al., 2007). Studies investigating tissue-specific brain atrophy in MS have found that grey matter atrophy occurs at a faster rate than white matter atrophy (Chard et al., 2004). Grey matter atrophy is more closely linked to disease progression, physical disability and cognitive impairment than white matter atrophy (Dalton et al., 2004; Fisher et al., 2008; Fisniku et al., 2008b; Roosendaal et al., 2011; Filippi et al., 2013). Grey matter atrophy has a predilection for certain brain regions. Thalamic and deep grey matter atrophy begins early in the course of MS (Henry et al., 2008; Azevedo et al., 2015) and occurs at a faster rate than cortical grey matter atrophy in established MS (Eshaghi et al., 2016). Within cortical grey matter regions atrophy may progress at different rates and the distribution of atrophy may be influenced by the disease phenotype (Ceccarelli et al., 2008; Eshaghi et al., 2014).

There are a number of manual, semi-automated and fully automated methods for measuring brain volumes and change in volumes over time in MS (Miller et al., 2002; Pelletier et al., 2004; Rocca et al., 2017). For accurate assessment of brain volumes image contrast is important and T1-weighted scans that provide excellent tissue contrast between the brain and the CSF are typically used. Although brain atrophy can be measured using standard 2D T1-weighted scans, a high-resolution 3D (volumetric) acquisition with near isotropic voxel sizes of ~1mm³ is preferred (Miller et al., 2002; Rocca et al., 2017).
Registration-based techniques including Structural Image Evaluation using Normalization of Atrophy (SIENA) and Brain Boundary Shift Integral (BBSI) have been widely used in observational studies and also in clinical trials to measure brain atrophy in MS. SIENA estimates the percentage brain volume change (PBVC) over two time points using paired T1-weighted MRI scans (Smith et al., 2002). The first step uses a brain extraction tool (BET) to remove the skull and other non-brain tissues resulting in a segmented whole brain image (including the cerebellum and upper brainstem) and the external skull. The skull (which remains a constant size in adults) is used to register two T1-weighted scans in order to normalize for differences in scan geometry. In order to estimate the change in brain volume over time SIENA locates the edge points of the brain at the brain-CSF interface, including the ventricular system. The change in brain edge points between the two scans is used to estimate the PBVC. SIENA has been used widely in research studies and also in clinical trials to measure brain atrophy over time.

SIENAX is a cross-sectional form of SIENA and involves a similar set of steps. Following BET the extracted brain is registered to a standard brain with the external skull used to normalize for head size (Smith et al., 2002). SIENAX estimates the normalised brain volume (NBV) and through an additional segmentation step the normalised grey matter and white matter volume can also be estimated. SIENA and SIENAX can be applied to 2D or 3D T1-weighted scans obtained pre or post-contrast and with varying slice thickness (Smith et al., 2002; De Stefano et al., 2010). SIENA and SIENAX are used in image-analysis presented in Chapters 6, 7 and 8.

BBSI is another registration-based technique used to estimate brain volume changes over time in MS (Freeborough and Fox, 1997). The baseline and follow-up images
are registered and digital subtraction is used to detect differences in the lateral edges of the brain parenchyma and ventricles that are assumed to be the result of changes in tissue boundaries due to atrophy (Freeborough and Fox, 1997). Modifications to BBSI may improve the detection of brain volume changes with reductions in the sample sizes required to test putative disease-modifying drugs in neurodegenerative conditions, including MS (Prados et al., 2015).

2.6 Spinal cord atrophy

Progressive spinal cord atrophy occurs in people with MS and is thought to reflect neuroaxonal loss involving white matter tracts and spinal cord grey matter (Evangelou et al., 2005). Spinal cord atrophy, usually measured as the upper cervical cord cross-sectional area (UCCA) on MRI, is associated with disability and is more marked in progressive forms of MS (Losseff et al., 1996; Rocca et al., 2011; Lukas et al., 2013; Kearney et al., 2014a; Lukas et al., 2015).

As in the brain, spinal cord atrophy is typically measured using T1-weighted 3D volumetric scans with an inversion recovery pulse to suppress the signal from CSF and improve the delineation of the spinal cord from surrounding structures (Miller et al., 2002). Spinal cord atrophy is usually measured in the upper cervical segments (C2/C3) where spondylotic changes are uncommon and the CSF spaces are wide, improving spinal cord-CSF contrast (Losseff et al., 1996).

The first reproducible method for determining spinal cord atrophy was described by Losseff and colleagues. Using this technique sagittally-acquired volumetric scans of the cervical cord are reformatted in the axial plane to obtain five contiguous slices at
the level of C2/C3. A region of interest is then drawn manually around the edge of the spinal cord and a further region drawn at the outer CSF space in the spinal canal. By calculating the mean signal intensity of the spinal cord and CSF the edge of the spinal cord can be accurately identified and the mean UCCA over five slices estimated. Using this method, progressive spinal cord atrophy has been observed in patients with RRMS and progressive MS over follow-up periods of 1-3 years (Stevenson et al., 1998; Rashid et al., 2006). The rate of spinal cord atrophy is approximately 1%/year in established MS with little change in UCCA observed in healthy controls (Rashid et al., 2006).

The Losseff method has been superseded by another semi-automated technique using an active surface model (Horsfield et al., 2010). Either high-resolution T1-weighted images acquired in the axial plane or reformatted sagittal images at the level of C2/C3 can be used. Using this technique the spinal cord is modelled as a cylinder. An operator places a region of interest in the centre of the spinal cord. The active surface model algorithm is used to automatically identify cord outline from a radius generator. The UCCA is then calculated as a mean over five slices. This approach has been found to be more accurate and more reproducible than previous methods and has been applied in multicentre studies (Rocca et al., 2011; Kearney et al., 2014a) and in a recent clinical trial in PPMS (Yaldizli et al., 2015). The sequence of steps used in image-analysis for calculating the UCCA using the active surface model are shown in Figure 2.6. This technique is applied in analyses presented in Chapters 6, 7 and 8 of this thesis.
Step 1
Sagittally-acquired images are reformatted in the axial plane to create five axial slices at the level of C2/C3.

Step 2
A region of interest is placed in the centre of the spinal cord on each axial slice.

Step 3
The active surface model algorithm identifies the edge of the spinal cord and the UCCA is calculated as a mean over five slices.

Figure 2.6 Estimating the upper cervical cord cross-sectional area using the active surface model.
Spinal cord grey matter damage is prominent in MS and more marked in people with progressive MS (Kearney et al., 2015c; Kearney et al., 2015d). Grey matter atrophy is more marked than atrophy in spinal cord white matter tracts and correlates more strongly with disability than the UCCA, cord white matter area or grey matter atrophy in the brain (Schlaeger et al., 2014). Fully automated approaches to segmentation of spinal cord grey and white matter have now been described that are likely to improve the accuracy and reproducibility of this technique (Prados et al., 2016).

2.7 Normal-appearing white and grey matter

Important pathological changes occur in MS outside MRI-visible focal lesions. These include neuroaxonal dysfunction and loss, microglial activation and astrogliosis (Popescu and Lucchinetti, 2012). A number of non-conventional MRI measures including magnetization transfer imaging (MTI), diffusion MRI and magnetic resonance spectroscopy (MRS) are sensitive to these changes.

**Magnetization transfer imaging**

Protons exist in two distinct pools – in free water and bound to large macromolecules, for example, myelin. The bound protons within macromolecules do not contribute to the MR signal in conventional MRI due to faster spin-spin interactions and very short relaxation times (Ropele and Fazekas, 2009). However, because they are bound to proteins and lipids this pool of protons can provide important information on tissue microstructure and pathological abnormalities such as demyelination and axonal loss. MTI exploits the transfer of protons between the larger pool in free water and the smaller number of bound proteins following a selective radiofrequency saturation pulse (Ropele and Fazekas, 2009). The difference between the magnetisation transfer before and after a selective radiofrequency saturation pulse is expressed as
the magnetization transfer ratio (MTR). Histopathological studies suggest that MTR correlates with myelin and axon counts (Schmierer et al., 2004).

MTI is sensitive to the detection of tissue-damage within NAWM and normal-appearing grey matter (NAGM), with reductions in MTR in people with MS compared with healthy controls (Filippi et al., 1999; Agosta et al., 2006). These abnormalities begin early in the course of the disease (Davies et al., 2004; Fernando et al., 2005) and are associated with progression of physical disability and cognitive impairment over time (Agosta et al., 2006; Hayton et al., 2009; Tur et al., 2011a; Tur et al., 2011b).

Recent work using MTR has found evidence of a gradient of tissue damage in white matter and grey matter with more marked MTR abnormalities closer to the brain-CSF interface. In periventricular white matter, MTR is reduced compared with healthy controls but this difference is most marked in the white matter closest to the lateral ventricles (Liu et al., 2015). Similarly, in cortical grey matter reduced MTR is seen in the inner and outer cortex compared with healthy controls, but the difference is more marked in the outer than the inner cortex (Samson et al., 2014). A preferential reduction in outer cortical MTR is consistent with subpial demyelination and neuronal loss found post-mortem in people with SPMS (Kutzelnigg et al., 2005; Magliozzi et al., 2010). Subsequent work has investigated MTR abnormalities across the whole brain, including in the brainstem/cerebellum and the deep grey matter, with similar findings of a gradient of MTR abnormality that is maximal closest to the ventricles (Pardini et al., 2016). The pathological basis for these observations in unclear but the findings could suggest a susceptibility of the tissues at the brain-CSF interface to demyelination/neurodegeneration or the presence of pathogenic mediators within the CSF that diffuse into the brain parenchyma resulting in tissue damage.
**Diffusion MRI**

Diffusion MRI is a tool for investigating tissue microstructure *in vivo* by measuring water motion within tissues. Free water diffuses equally in all directions and is said to be isotropic. In most biological tissues the presence of cell membranes and other structures means that water movement is hindered or anisotropic. The diffusion properties of a given tissue are therefore largely determined by the tissue’s microstructure. For example, in tightly compacted tissues such as white matter, water motion is significantly more anisotropic than in less compact structures like the cerebral cortex. Pathological processes that disrupt tissue microstructure can influence water motion and be detected using diffusion MRI.

The diffusion model most frequently applied in MS is diffusion tensor imaging (DTI) in which water is assumed to diffuse through tissues in a Gaussian (normal) distribution that can be modelled as a tensor. Based on the directions of water movement (the diffusion tensor’s eigenvectors), DTI metrics are calculated that describe tissue microstructure, including the fractional anisotropy (FA), mean diffusivity (MD), axial diffusivity (AD) and radial diffusivity (RD). A number of studies have reported decreases in FA and increases in mean diffusivity MD within NAWM in people with MS compared with healthy controls (Werring *et al.*, 2000; Filippi *et al.*, 2001a; Rovaris *et al.*, 2002; Ciccarelli *et al.*, 2003). These abnormalities occur early in the course of MS, including in patients with CIS (Gallo *et al.*, 2005). More marked diffusion abnormalities in the NAWM occur in patients with SPMS compared with patients with RRMS (Preziosa *et al.*, 2011). Combined imaging and histopathological studies have found correlations of DTI metrics with myelin content and axon counts (Mottershead *et al.*, 2003; Schmierer *et al.*, 2007), confirming that DTI abnormalities reflect pathological changes relevant to MS.
Diffusion MRI data can also be used to investigate damage to specific white matter fibre tracts (diffusion tractography) relevant to disability in MS. Damage to the corpus callosum in particular has been associated with progression of physical disability and cognitive impairment over time (Dineen et al., 2009; Bodini et al., 2013)

**Magnetic resonance spectroscopy**

Unlike the other MRI techniques described so far, $^1$H-MRS provides metabolic rather than structural information. Changes in the levels of N-acetylaspartate (NAA, a neuroaxonal marker), choline (a marker of cell turnover) and myo-inositol (an astroglial marker) reflect pathological processes within a given voxel or voxels. Within acute demyelinating lesions there is evidence of a reduction in NAA due to axonal injury, increases in choline due to inflammation and/or breakdown of myelin and increased lactate due to inflammation (Rovira and Alonso, 2013). MRS is sometimes helpful in clinical settings to differentiate tumefactive demyelinating lesions from neoplasms (Rovira and Alonso, 2013).

Within NAWM, $^1$H-MRS displays metabolic changes including a decrease in NAA and increase in myo-inositol (Fu et al., 1998; Sarchielli et al., 1999; Chard et al., 2002a; Rovira and Alonso, 2013). These abnormalities are thought to reflect neuroaxonal loss and astrogliosis respectively, prominent pathological changes seen in NAWM in MS (Lucchinetti et al., 2011). Spectroscopic abnormalities begin early in the course of MS (Chard et al., 2002a; Sastre-Garriga et al., 2005), including in patients with CIS. Increases in myo-inositol (Fernando et al., 2004; Wattjes et al., 2008) and decreases in NAA (Wattjes et al., 2008) in NAWM in CIS patients are associated with the development of MS. In a large cohort of patients with predominantly RRMS the ratio of myo-inositol to NAA in NAWM at baseline was associated with the annualised
PBVC and progression over disability measured using the MSFC over 4 years of follow-up (Llufriu et al., 2014). These findings suggest that metabolic abnormalities in NAWM detected using $^1$H-MRS reflect pathological changes relevant to disease progression in MS.

Metabolic abnormalities in cortical and deep grey matter are also detectable using $^1$H-MRS. Reductions in cortical grey matter NAA are most marked in people with progressive MS (Adalsteinsson et al., 2003; Sastre-Garriga et al., 2005), while spectroscopic abnormalities are found in the deep grey matter even in early RRMS (Inglese et al., 2004).

### 2.8 Sodium MRI

Sodium ($^{23}$Na) MRI is a novel metabolic imaging technique applied in this thesis (Chapter 9). Sodium is found in all biological tissues and has an essential role in tissue homeostasis and maintaining the integrity of cell membranes. In the nervous system and the heart, changes in intracellular sodium concentration mediated by voltage-gated sodium channels, are responsible for the generation and propagation of action potentials. In the brain (and other biological tissues) tissue sodium comprises two distinct pools: a larger intracellular compartment (80-85%) with a sodium concentration of approximately 10-15mmol and an extracellular compartment (15-20%) with a much higher sodium concentration of approximately 140mmol. This concentration gradient of intracellular and extracellular sodium is maintained by the action of the energy dependent $\text{Na}^+$/K$^+$-ATPase pump. The $\text{Na}^+$/K$^+$-ATPase pump is present in almost all eukaryotic cells and exchanges three intracellular Na$^+$ ions for two extracellular K$^+$ ions. Because this action is against the electrochemical gradient of sodium and potassium the action of the $\text{Na}^+$/K$^+$-ATPase pump requires energy in the
form of adenosine triphosphate (ATP). Tissue sodium concentrations may rise when there is expansion of the extracellular space (e.g. oedema, tissue destruction) or an increase in intracellular sodium because of failure of the \( \text{N}^+/\text{K}^+\)-ATPase pump. Increases in tissue sodium concentration have been found in a number of neurological diseases including stroke, glioma and neurodegenerative diseases, as well as MS (Madelin and Regatte, 2013), reflecting either an increase in extracellular volume, an increase in intracellular sodium concentration, or both.

Like hydrogen, sodium \((^{23}\text{Na})\) had an odd number of protons resulting in a net nuclear spin. When exposed to an external magnetic field \(^{23}\text{Na}\) will generate radiofrequency energy and is MRI visible. The concentration of sodium in biological tissues is many times less than hydrogen ions resulting in a significantly lower signal-to-noise ratio with longer acquisition times and the need for large voxel sizes than with \(^1\text{H}\) MRI (Petracca et al., 2016a). Sodium displays a bi-exponential relaxation time with the major component of the T2 signal very difficult to detect because of a short TE. These technical challenges have been overcome with the use of higher field strengths (≥3T) and sequences utilising ultra-short TE facilitating signal detection.

\(^1\text{H}\) MRI provides information on tissue structure whereas \(^{23}\text{Na}\) MRI quantifies tissue sodium concentrations providing information on the functional and metabolic state of the tissue being investigated. The tissue sodium concentration is usually quantified with the use of phantoms containing agar gels with a known concentration of sodium (Figure 2.7). The phantoms are placed within the field of view and tissue sodium concentration is estimated using the phantom signal.
Figure 2.7  Sodium MRI.

Axial image at the level of the lateral ventricles obtained using a sodium coil. Two phantoms are visible at the lateral edges of the field of view with differing sodium concentrations indicated by differences in the signal intensity.

$^{23}$Na MRI measures total brain sodium and reflects a composite of both extracellular and intracellular sodium. The intracellular sodium concentration is of greater biological interest since this is the major determinant of cellular function and integrity. A number of different approaches have been used to try and suppress the signal from extracellular sodium and accurately quantify intracellular sodium concentration (Petracca et al., 2016a). Shift reagents do not cross the cell membrane and have been used to quantify intracellular sodium in ex vivo and animal studies. These substances are toxic and cannot be used in humans. A number of MRI techniques have been used, including the use of multiple quantum filters, inversion recovery pulses and diffusion-based techniques that exploit differences in the magnetic resonance properties of intracellular and extracellular sodium ions.
A small number of studies have investigated sodium MRI in MS (Table 2.1). The first application of $^{23}$Na MRI was in 17 patients with early RRMS (mean disease duration 5.8 years) and mild disability (median EDSS 2) who were compared with a group of age and sex-matched healthy control subjects (Inglese et al., 2010). A region of interest analysis was used to compare total sodium concentrations in grey matter, NAWM and lesions. Compared with healthy controls the MS patients showed a higher total sodium concentration in grey matter and NAWM. Within white matter lesions the total sodium concentration was higher in gadolinium-enhancing and non-enhancing lesions compared with NAWM.

Zaaroui et al studied a small group of patients (n=23) with very early RRMS with a disease duration of <5years and established RRMS with a disease duration of >5years (Zaaroui et al., 2012). Increased total sodium concentration was found in white matter lesions in all patients. Patients with established RRMS, but not patients with very early RRMS, had increased total sodium concentration in NAWM and grey matter compared with healthy control subjects. Grey matter sodium concentration was found to correlate EDSS. These findings suggest that early in the course of MS increases in sodium concentration are confined to areas of focal damage but over time more widespread changes occur in NAWM and grey matter. This is supported by a study in 20 patients with CIS (mainly unilateral optic neuritis) where evidence of increased total sodium concentration was found in asymptomatic T2 brain lesions but not in NAWM or grey matter (Cawley et al., 2016).

Two studies have extended these findings in patients with progressive forms of MS (Paling et al., 2013; Maarouf et al., 2014). Paling and colleagues studied 70 patients with RRMS, SPMS and PPMS who underwent $^{23}$Na MRI and conventional $^1$H MRI at
In keeping with previous studies, MS patients displayed higher total sodium concentrations in cortical and deep grey matter and NAWM compared with healthy controls. Tissue sodium concentrations were higher in all tissue compartments in SPMS patients compared with RRMS patients, although findings were similar in PPMS and RRMS patients. Significant correlations were found between tissue sodium concentrations and measures of physical disability and cognitive impairment. In a multivariable linear regression analysis deep grey matter sodium concentration was independently associated with EDSS and walking speed while T1-hypointense lesion sodium concentration was associated with performance on the 9HPT and the PASAT. In a smaller study involving 20 patients with progressive MS, Maarouf and colleagues also found increased grey matter total sodium concentrations in patients compared with healthy controls (Maarouf et al., 2014). No differences were found in NAWM and no significant correlations were found between tissue sodium concentration and disability measures using the EDSS and MSFC.

Finally, Maarouf and colleagues also studied 58 patients with early RRMS who underwent detailed neuropsychological testing (Maarouf et al., 2017). 21 patients were found to be cognitively impaired and 37 were cognitively preserved. The cognitively impaired patients showed higher total sodium concentration in grey matter and NAWM compared with patients who were cognitively preserved. In a multivariable model, grey matter sodium concentration explained more of the variability in cognition that grey matter atrophy. These findings suggest that metabolic abnormalities in cortical grey matter are relevant to the development cognitive impairment in MS and may precede tissue loss.
Table 2.1  Application of sodium MRI in multiple sclerosis: Summary of studies to date

<table>
<thead>
<tr>
<th>Study</th>
<th>Patient group</th>
<th>Sample size</th>
<th>Major findings</th>
</tr>
</thead>
<tbody>
<tr>
<td>Inglese et al</td>
<td>RRMS</td>
<td>n=17</td>
<td>Increased TSC in lesions, NAWM and grey matter</td>
</tr>
<tr>
<td>Zaaroui et al</td>
<td>RRMS</td>
<td>n=23</td>
<td>Increased TSC in NAWM and grey matter</td>
</tr>
<tr>
<td>Paling et al</td>
<td>RR/SP/PPMS</td>
<td>n=70</td>
<td>TSC progressive MS &gt; RRMS</td>
</tr>
<tr>
<td></td>
<td></td>
<td></td>
<td>Associations found between deep grey matter and T1 lesions TSC and disability measures</td>
</tr>
<tr>
<td>Maarouf et al</td>
<td>SP/PPMS</td>
<td>n=20</td>
<td>Higher grey matter TSC in progressive MS</td>
</tr>
<tr>
<td>Eisele et al</td>
<td>RRMS</td>
<td>n=65</td>
<td>TSC Gd+ lesions &gt; T1-hypointense&gt;T1-isointense lesions</td>
</tr>
<tr>
<td>Cawley et al</td>
<td>CIS</td>
<td>n=20</td>
<td>TSC increased in asymptomatic T2 brain lesions.</td>
</tr>
<tr>
<td>Maroouf et al</td>
<td>RRMS</td>
<td>n=58</td>
<td>TSC higher in grey matter and NAWM of cognitively impaired compared with cognitively preserved RRMS patients</td>
</tr>
</tbody>
</table>
2.5 Conclusions

MRI is an established research tool used to understand the pathological basis of MS. Conventional MRI techniques including T2-weighted and post-contrast T1 weighted readily detect focal lesions in MS. A number of non-conventional techniques are sensitive to the detection of brain and spinal cord atrophy, grey matter pathology and abnormalities in normal-appearing tissues that are relevant to the development of disability and disease progression.

A number of the MRI techniques described are applied in this thesis to improve our understanding of the prognosis of people with CIS and investigate mechanisms of long-term disability in MS. Chapter 3 describes the CIS cohort studied and the MRI acquisition protocol used to address the main research questions in this thesis.
Chapter 3

Clinically isolated syndromes study design

3.1 Introduction

Subsequent chapters of this thesis concern follow-up of a cohort of patients with CIS who have been followed as part of a longitudinal clinical-MRI study since 1995. This chapter outlines the study design including patient recruitment, follow-up and assessments over a mean time of 15 years since presentation with CIS.

3.2 Study design

3.2.1 Recruitment, inclusion and exclusion criteria

Between 1995 and 2004 CIS patients seen at Moorfields Eye Hospital and the National Hospital for Neurology and Neurosurgery were invited to take part in a longitudinal clinical-MRI study. Patients aged 16 – 50 years with a typical CIS presentation suggestive of MS were included in the study. Investigations including routine blood tests, serological testing, visual evoked potentials and lumbar puncture were done as clinically indicated. All of the patients were assessed clinically and with MRI within 3 months of CIS onset (“baseline”).

Patients were excluded if there was a history of any previous neurological symptoms or if there was a contraindication to MRI scanning.
3.2.2 Follow-up

The patients were invited to return for scheduled clinical and MRI follow-up after 3 months, 1 year and 3 years irrespective of clinical status. Further follow-ups were conducted after approximately 5 years (range 4.0 – 11.8 years) and 15 years (range 10.9 – 19.7 years). During the course of the study patients who developed a neurological disorder other than MS were excluded.

An overview of the study, including the number of patients who were assessed at each time point clinically and with MRI is shown in Figure 3.1.

3.2.3 Ethical approval and patient consent

The study was approved by the institutional ethics committees at the National Hospital for Neurology and Neurosurgery and Moorfields Eye Hospital. The 15 year follow-up study was approved by the National Research Ethics Service (NRES) Committee London (City Rd and Hampstead). Written informed consent was obtained from study participants at the time of study entry and at each subsequent follow-up visit.

3.2.3 Clinical assessment

Patients were assessed by a clinical research fellow around the time of CIS (baseline) and at each follow-up visit. Dr Jonathan O’Riordan, Dr Peter Brex, Dr Catherine Dalton, Dr Kryshani Fernando and Dr Josephine Swanton recruited patients into the study and/or conducted the clinical assessments up to 5 years. I re-recruited patients at the 15 year follow-up, obtained consent for continued participation in the study and conducted all of the clinical assessments.
Figure 3.1  Clinically isolated syndromes study overview.

The CIS patients were seen at baseline (within 3 months of symptom onset) and then for scheduled follow up after approximately 3 months, 1 year, 3 years, 5 years and 15 years. The number of patients who were assessed clinically and with MRI at each time point is shown.

Number of patients

<table>
<thead>
<tr>
<th>Clinical assessment</th>
<th>Baseline</th>
<th>3 m</th>
<th>1 year</th>
<th>3 years</th>
<th>~5 years</th>
<th>~15 years</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>178</td>
<td>155</td>
<td>145</td>
<td>123</td>
<td>139</td>
<td>166</td>
</tr>
<tr>
<td>MRI scanning</td>
<td>178</td>
<td>155</td>
<td>145</td>
<td>123</td>
<td>111</td>
<td>106</td>
</tr>
</tbody>
</table>
At each study visit the patients were asked about new or progressive neurological symptoms and were examined neurologically. CDMS was diagnosed prospectively when there was a second clinical attack and objective evidence of two separate CNS lesions (Poser et al., 1983). At the 15 year follow-up MS was diagnosed using the McDonald 2010 criteria, either on clinical grounds or when there was MRI evidence of DIS and DIT, or both (Polman et al., 2011).

In patients with MS the disease course was classified at each visit as either RRMS or SPMS using the Lublin and Reingold 1996 disease course definitions (Lublin et al., 1996).

Disability was rated at each visit using the EDSS (Kurtzke, 1983). At the 5 year and 15 year follow-up the patients were also assessed with the three components of the MSFC, including tests of walking speed (TWT), upper limb function (9HPT) and information processing speed (PASAT). Additional cognitive testing was done at the 15 year follow-up including the SDMT (Smith, 1982), tests of visual and verbal memory from the Adult Memory and Information Processing Battery (Coughlan and Hollows, 1985) and two tests of executive function, the Brixton and Hayling Tests (Burgess and Shallice, 1997). The National Adult Reading Test (NART) was used to estimate premorbid intelligence (Nelson, 1982).

Patients who were unable or unwilling to attend in person after 5 and 15 years were invited to take part in a structured telephone interview to establish disease course (CIS, RRMS, SPMS) and disability was measured using the telephone EDSS (Lechner-Scott et al., 2003). The telephone EDSS has been validated previously for use in clinical trials (Lechner-Scott et al., 2003) and has also been used in previous
observational studies (Fisniku et al., 2008a). Although the telephone EDSS shows a robust correlation with examined EDSS score at all levels the correlations are particularly strong in patients with significant ambulatory problems (EDSS >4).

3.3 MRI acquisition protocol

3.3.1 Baseline to 5 years

All MRI scans obtained over the first 5 years of the study were done on the same 1.5T Signa scanner (General Electric, Wisconsin, USA). There was a major hardware and software upgrade in April 2004, after the last patient was recruited into the study. The scanner upgrade affected follow-up MRI scans done after April 2004.

Details of the MRI protocol over the first 5 years of the study are shown in Table 3.1. Patients were given 0.1mmol/kg gadolinium-diethylenetriamine pentaacetic acid (DTPA) and axial proton-density (PD)/T2-weighted and post-contrast T1-weighted fast-spin echo scans of the brain were acquired. Spinal cord MRI included sagittal T2-weighted and post-contrast T1-weighted scans of the whole spine and a volume acquired inversion prepared fast spoiled gradient echo (FSPGR) scan of the cervical cord. Brain and spinal cord MRI were obtained at all time points except at the 3 month follow-up when brain MRI alone was acquired.

Volumetric 3D-T1-weighted brain MRI, 1H spectroscopy and magnetization transfer imaging were added to the study protocol in 1997-1998. These were available in a subset of patients but were not analysed as part of this thesis.
### Table 3.1 MRI acquisition parameters: baseline to 5 years.

<table>
<thead>
<tr>
<th>Orientation</th>
<th>Slice thickness, mm</th>
<th>TR, ms</th>
<th>TE, ms</th>
<th>Matrix</th>
</tr>
</thead>
<tbody>
<tr>
<td><strong>Brain</strong></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>T2/PD weighted</td>
<td>Axial</td>
<td>46 x 3</td>
<td>3200</td>
<td>15/90</td>
</tr>
<tr>
<td>Post-contrast T1-</td>
<td>Axial</td>
<td>46 x 3</td>
<td>600</td>
<td>17</td>
</tr>
<tr>
<td>weighted</td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td><strong>Spinal cord</strong></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>T2/PD-weighted (whole cord)</td>
<td>Sagittal</td>
<td>9 x 3</td>
<td>2500</td>
<td>56/92</td>
</tr>
<tr>
<td>FSPGR (cervical cord)</td>
<td>Sagittal</td>
<td>60 x 1</td>
<td>15.6</td>
<td>4.2</td>
</tr>
</tbody>
</table>
3.3.2 15 years

The MRI scans at 15 years were all done on the same 3T Achieva TX scanner (Philips Healthcare, Best, The Netherlands) between January 2014 and December 2015. There was no major hardware or software upgrade during the study period. Only brain MRI was obtained at 15 years and contrast was not administered. In addition to standard PD/T2-weighted, FLAIR and T1-weighted 2D spin echo sequences for detection of white matter lesions, an inversion-prepared 3D T1-weighted turbo field echo (TFE) scan was also obtained to measure brain atrophy and for tissue segmentation. Three novel MRI measures were also obtained: PSIR, DWI and sodium MRI. Analysis of the sodium MRI data is presented in Chapter 9.

Details of the MRI protocol at 15 years are presented in Table 3.2.
Table 3.2 MRI acquisition parameters: 15 years

<table>
<thead>
<tr>
<th>MRI Type</th>
<th>Orientation</th>
<th>Slice thickness, mm</th>
<th>TR, ms</th>
<th>TE, ms</th>
<th>TI, ms</th>
<th>Matrix</th>
</tr>
</thead>
<tbody>
<tr>
<td><strong>1H MRI</strong></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>T2/PD weighted</td>
<td>Axial</td>
<td>50 x 3</td>
<td>3500</td>
<td>19/85</td>
<td></td>
<td>240x180</td>
</tr>
<tr>
<td>FLAIR</td>
<td>Axial</td>
<td>50 x 3</td>
<td>8000</td>
<td>125</td>
<td>2400</td>
<td>240x180</td>
</tr>
<tr>
<td>2D T1-weighted spin echo</td>
<td>Axial</td>
<td>50 x 3</td>
<td>625</td>
<td>10</td>
<td></td>
<td>240x180</td>
</tr>
<tr>
<td>3D T1-weighted TFE</td>
<td>Sagittal</td>
<td>180 x 1</td>
<td>6.9</td>
<td>3.1</td>
<td>824</td>
<td>256x256</td>
</tr>
<tr>
<td>PSIR</td>
<td>Axial</td>
<td>75 x 2</td>
<td>7306</td>
<td>13</td>
<td>400</td>
<td>256x256</td>
</tr>
<tr>
<td>DWI</td>
<td>Axial</td>
<td>60 x 2.5</td>
<td>13846</td>
<td>82</td>
<td></td>
<td>76x87</td>
</tr>
<tr>
<td><strong>23Na MRI</strong></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Sodium scan</td>
<td>Axial</td>
<td>80 x 3</td>
<td>120</td>
<td>2.9</td>
<td></td>
<td>80x80</td>
</tr>
<tr>
<td>PD/T2-weighted</td>
<td>Axial</td>
<td>50 x 3</td>
<td>3875</td>
<td>19/85</td>
<td></td>
<td>200x200</td>
</tr>
</tbody>
</table>
3.4 Image-analysis

3.4.1 Lesion number, location and activity

Scans obtained from baseline to 5 years were reviewed by two neuroradiologists. Dr Katherine Miszkiel reviewed all the scans prospectively and counted the number of T2-hyperintense brain and spinal cord lesions on PD/T2-weighted scans. The number of T2-hyperintense lesions in four mutually-exclusive locations – the periventricular, juxtacortical and infratentorial regions in the brain, plus the spinal cord, was recorded. Post-contrast T1-weighted scans were used to identify the number of T1-hypointense lesions in the brain and the number of GdE lesions in the brain and spinal cord.

Scans obtained at baseline, 1 year and 3 years were re-reviewed by Prof Frederik Barkhof as part of analyses presented in Chapter 7. He counted the number of new supratentorial and infratentorial lesions seen at the 1 year and 3 year follow up.

3.4.2 Lesion volume

I identified T2-hyperintense brain lesions on PD-weighted scans obtained at all time points in conjunction with T2-weighted and FLAIR scans (available in all patients at 15 years) to aid in lesion detection. I used a semi-automated edge-finding tool (JIM6.0, Xinapse Systems, Aldwincle, UK) to draw around the edge of lesions and the T2 lesion volume (T2LV) was calculated by multiplying lesion area by slice thickness. The process was repeated using 2D T1-weighted spin echo scans to identify T1-hypointense lesions and calculate the T1 lesion volume (T1LV).
3.4.3 Brain atrophy

The 2D T1-weighted fast-spin echo scans were used for the volumetric brain measures. T1-hypointense lesion masks were used to fill lesions in order to improve segmentation accuracy (Prados et al., 2014). Using the baseline MRI scan, the NBV was calculated using SIENAX and follow-up scans were registered with the baseline MRI scan to calculate the PBVC over time using SIENA (Smith et al., 2002).

Dr Arman Eshaghi, Dr Ferran Prados and Mr John Stutters assisted with the SIENAX and SIENA analysis.

3.4.4 Spinal cord atrophy

Dedicated volumetric spinal cord imaging was available at baseline, 1, 3 and 5 years. I calculated the UCCA using the active surface model as described in Chapter 2. Firstly, the sagittally-acquired FSPGR scans of the cervical cord were reformatted in the axial plane at the level of C2/C3. A region of interest marker was placed in the centre of the spinal cord and the active surface model used to calculate the UCCA (in mm²) as a mean over five slices. Analyses were done using JIM6 (Xinapse Systems, Aldwincle, UK).

3.4.4 Reproducibility

The image-analysis used in this thesis involved well-established techniques (lesion counts, lesion volume, brain and spinal cord atrophy measures) and intra-rater reproducibility was not measured.
3.5 Characteristics of the CIS cohort

A total of 178 patients were included in the study. The baseline characteristics of the study patients are shown in Table 3.3.

Table 3.3 Baseline demographic and clinical characteristics of the cohort

<table>
<thead>
<tr>
<th></th>
<th>Values</th>
</tr>
</thead>
<tbody>
<tr>
<td>Age, mean (SD)</td>
<td>32.3 (7.4)</td>
</tr>
<tr>
<td>Female, n (%)</td>
<td>114 (64)</td>
</tr>
<tr>
<td>White ethnicity, n (%)</td>
<td>145 (81)</td>
</tr>
<tr>
<td>CIS topography, n (%)</td>
<td></td>
</tr>
<tr>
<td>Optic neuritis</td>
<td>145 (81.5)</td>
</tr>
<tr>
<td>Brainstem syndrome</td>
<td>21 (12)</td>
</tr>
<tr>
<td>Spinal cord syndrome</td>
<td>11 (6)</td>
</tr>
<tr>
<td>Hemispheric syndrome</td>
<td>1 (0.5)</td>
</tr>
<tr>
<td>Baseline EDSS, median (IQR)</td>
<td>1 (1)</td>
</tr>
</tbody>
</table>

The demographic profile of study patients was typical of people with CIS with a mean age of approximately 32 years and two-thirds female. There was an over-representation of optic neuritis in the cohort; 82% patients in this study compared with 30 – 40% in other large prospective CIS series (Eriksson et al., 2003; Tintore et al., 2015). From the total cohort of 178 patients, differing numbers of patients were included in various analyses presented in this thesis depending on the availability of clinical and MRI data. The inclusion and exclusion criteria for each analysis is described in detail in subsequent chapters.
Chapter 4

Earlier and more frequent diagnosis of multiple sclerosis in CIS patients using the McDonald criteria

4.1 Introduction

The McDonald criteria allow MS to be diagnosed in CIS patients when there is MRI evidence of dissemination in DIS and DIT (McDonald et al., 2001; Polman et al., 2005; Polman et al., 2011). The original criteria published in 2001 were modified in 2005 to increase the importance of spinal cord lesions in DIS and simplify the requirements for DIT (Polman et al., 2005). There was a further revision in 2010 with a major simplification of the requirements for both DIS and DIT, as summarised in Table 1.1 (Polman et al., 2011).

When applied to patients with CIS the McDonald criteria have been shown to have a high sensitivity and specificity for the development of CDMS over follow-up periods of 24-36 months (Dalton et al., 2002b; Swanton et al., 2007; Montalban et al., 2010; Runia et al., 2013). The application of the McDonald criteria to patients with a CIS should facilitate an earlier diagnosis of MS by using MRI to provide evidence for DIS and DIT, rather than waiting for a second clinical attack to occur (Poser et al., 1983). However, few previous studies have quantified how much sooner MS can be diagnosed using the McDonald criteria (Thouvenot et al., 2012; Runia et al., 2013).
I investigated the time to diagnosis of MS using the various iterations of the McDonald criteria in the CIS cohort over the first ~5 years of follow up. I also investigated the frequency with which MS could be diagnosed using MRI criteria in patients who had no further clinical events over this time (i.e. MRI-only MS).

4.2 Methods

Patients

This investigation is based on follow-up of the CIS cohort up to approximately 5 years. I included all patients from the cohort with at least one follow-up MRI scan at any time point (for demonstration of DIT) and at least 36 months of clinical follow-up.

Clinical assessment

Patients with CDMS were identified prospectively during scheduled follow-up of the cohort. CDMS was diagnosed according to the Poser criteria, requiring a second clinical attack with objective evidence of two separate lesions (Poser et al., 1983).

MRI acquisition and image-analysis

PD/T2-weighted and post-contrast T1-weighted scans done at the time of presentation and at follow-up were analysed. The number, location and activity (i.e. presence of gadolinium-enhancement) of brain and spinal cord lesions was reported by Dr Katherine Miszkiel, Consultant Neuroradiologist.
**Application of MRI criteria to diagnose MS**

I retrospectively assigned a diagnosis of McDonald MS using the 2001, 2005 or 2010 criteria using clinical and MRI data obtained over ~5 years of follow-up (McDonald et al., 2001; Polman et al., 2005; Polman et al., 2011). MS was diagnosed when there was MRI evidence of DIS and DIT or on clinical grounds (i.e. at the time of a second clinical attack), whichever occurred first.

**Statistical analysis**

The time to diagnosis of MS using the four different diagnostic criteria (Poser criteria and McDonald 2001, 2005 and 2010 criteria) was compared using a Kaplan-Meier survival analysis. Times to diagnosis of MS were skewed. Differences in means and proportions of patients were compared using the Wilcoxon signed-rank test and McNemar’s test respectively with p values <0.05 considered significant.

4.3 Results

From the 178 patients included in the study, 157 (88%) had sufficient clinical and MRI follow up to be included in this analysis. The reasons for exclusion were <36 months clinical follow-up (n=11), no follow-up MRI scan (n=8) and a disorder other than MS (n=2).

The baseline clinical and MRI characteristics of the patients included in this analysis grouped by diagnosis at follow-up is shown in Table 4.1. 129 (82%) patients presented with optic neuritis and 28 (18%) patients had other presentations...
(brainstem/cerebellar, spinal cord or hemispheric syndromes). The mean follow-up was 5.8 years (range 3.0 – 11.9 years).

Table 4.1  Baseline clinical and MRI characteristics of patients stratified by diagnosis at follow up after ~5 years using the Poser criteria

<table>
<thead>
<tr>
<th></th>
<th>CDMS</th>
<th>CIS</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>(n=71)</td>
<td>(n=86)</td>
</tr>
<tr>
<td>Age, mean (SD)</td>
<td>32.3 (7.9)</td>
<td>32.8 (7.2)</td>
</tr>
<tr>
<td>Female, n (%)</td>
<td>53 (75)</td>
<td>47 (55)</td>
</tr>
<tr>
<td>Optic neuritis, n (%)</td>
<td>56 (79)</td>
<td>73 (85)</td>
</tr>
<tr>
<td>Baseline MRI findings, median (range)</td>
<td></td>
<td></td>
</tr>
<tr>
<td>Brain T2 lesion number</td>
<td>11 (0 - 142)</td>
<td>0 (0 - 57)</td>
</tr>
<tr>
<td>GdE lesion number</td>
<td>0 (0 - 21)</td>
<td>0 (0 - 13)</td>
</tr>
<tr>
<td>Spinal cord lesion number</td>
<td>1 (0 -11)</td>
<td>0 (0 - 5)</td>
</tr>
</tbody>
</table>

Only one patient received DMT prior to the development of CDMS. The patient started glatiramer acetate 7 months after presenting with optic neuritis. Five months later she relapsed with a spinal cord syndrome and a diagnosis of CDMS was made.

**Patients who developed CDMS**

71 (45%) patients had a second clinical attack over the ~5 year follow-up period and were classified as CDMS. Figure 4.1 shows a Kaplan-Meier survival curve for the time to diagnosis of MS using the Poser and McDonald criteria in patients who had a second clinical attack. The mean time to development of CDMS was 23.1 months.
compared with 12.0, 10.5 and 6.2 months using the 2001, 2005 and 2010 McDonald criteria respectively (p<0.001 for all comparisons with time to CDMS).

Using the McDonald criteria 36 (51%), 40 (56%) and 54 (76%) of patients who developed CDMS could be diagnosed with MS using the 2001, 2005 and 2010 MRI criteria within three months of CIS onset.

32 (45%) patients who developed CDMS had asymptomatic gadolinium-enhancing and non-enhancing lesions on the first MRI scan and could be diagnosed with MS using the McDonald 2010 criteria at the time of presentation with CIS.
Figure 4.1  Kaplan-Meier survival curve showing time to diagnosis of MS using Poser and McDonald criteria
Patients who remained CIS

86 (55%) patients remained CIS (i.e. did not have a second clinical attack over the ~5 years of follow-up). 24 (28%), 26 (30%) and 36 (42%) CIS patients satisfied 2001, 2005 and 2010 MRI criteria for McDonald MS (MRI-only MS) but did not have a second clinical episode. A significantly greater number of CIS patients had MRI-only conversion to MS when the 2010 criteria were applied compared with the 2001 (p<0.001) and 2005 criteria (p = 0.001).

The majority of patients with MRI-only MS satisfied the McDonald within 12 months of symptom onset; 18 (75%), 18 (69%) and 26 (72%) patients using the 2001, 2005 and 2010 criteria respectively.

4.4 Discussion

In patients with CIS a diagnosis of MS can be made significantly earlier using the McDonald MRI criteria to provide evidence for DIS and DIT rather than waiting for a second clinical attack. However, a third of CIS patients who had no further clinical attacks over a mean follow up period of ~5 years also satisfied MRI criteria for a diagnosis of MS.

I found that with each revision of the McDonald criteria, a diagnosis of MS can be made at a significantly earlier time point. Among patients who ultimately developed CDMS, the mean time to diagnosis of MS halved from 12.0 months with the 2001 criteria to 6.2 months with the 2010 criteria. This reflects a time gain of 16.9 months when compared with the mean time to a second clinical attack and a diagnosis of CDMS. Although a number of studies have investigated the sensitivity, specificity and
accuracy of the McDonald criteria in different CIS cohorts, few have quantified how much sooner a diagnosis of MS can be made (Thouvenot et al., 2012; Runia et al., 2013). In a clinic-based series of CIS patients from The Netherlands, the mean time to diagnosis of 2010 and 2005 McDonald MS was 23.6 and 26.1 months compared with 27.9 months for CDMS (Runia et al., 2013). However, spinal cord imaging was not done which is important for determining DIS (particularly when applying the McDonald 2010 criteria), and not all patients had follow up MRI scans to demonstrate DIT. In a small cohort of 49 CIS patients from France, reported in abstract form at the ECTRIMS 2012 meeting, the mean time to diagnosis of MS was 151 and 321 days for the 2010 and 2005 criteria respectively, compared with 500 days for CDMS, which is similar to my findings (Thouvenot et al., 2012).

The time gain with each revision of the McDonald criteria reflects the less stringent requirements for DIS and DIT as MRI criteria have evolved (McDonald et al., 2001; Polman et al., 2005; Polman et al., 2011). The Magnetic Resonance Imaging in Multiple Sclerosis (MAGNIMS) study group evaluated the performance of the 2001 and 2005 criteria in a large multi-centre European CIS cohort that included a subset of patients reported here (Swanton et al., 2007). Patients had a baseline MRI scan (within 3 months of CIS onset) and a follow up MRI after approximately 3 months. They found a sensitivity of 47.1% and 60.0% for the 2001 and 2005 criteria respectively for the development of CDMS at three years. The changes to the requirements for DIS and DIT that were incorporated into the 2010 criteria have been shown to be more sensitive than earlier iterations of the McDonald criteria for a diagnosis of CDMS over follow-up periods of up to 36 months (Swanton et al., 2007; Rovira et al., 2009; Montalban et al., 2010). I found that the successive iterations of the McDonald criteria have allowed an earlier diagnosis of MS. Importantly, 45% of
patients who ultimately developed CDMS in this cohort could be diagnosed with MS using the 2010 McDonald criteria with a single MRI scan at the time of presentation.

Establishing a diagnosis of MS at an earlier time point is desirable for patients and treating neurologists. Natural history studies suggest that events early in the course of MS, including the time to the second clinical attack and the number of relapses in the first few years of the illness, are important predictors of long-term disability (Confavreux et al., 2003; Scalfari et al., 2010). An early and accurate diagnosis of MS may also facilitate early initiation of treatments that reduce relapses rates and accrual of disability (Wingerchuk and Weinshenker, 2016).

A significant number of CIS patients satisfy MRI criteria for a diagnosis of MS in the absence of further neurological symptoms, at least of the first 5 years or so after presentation. Long-term follow-up studies have shown that 18 – 28% of CIS patients with MRI abnormalities at baseline do not develop CDMS (Fisniku et al., 2008; Optic Neuritis Study Group, 2008). It is therefore not surprising that when applying the McDonald criteria that a proportion of patients with CIS have MRI-only conversion to MS, even with follow up of up to 20 years (Chard et al., 2011). The number of CIS patients with MRI-only conversion to MS has increased with each revision of the McDonald criteria. I found that 42% of CIS patients who did not have a second clinical attack satisfied McDonald 2010 criteria during follow up, usually within 12 months of CIS onset. Whether these patients have a mild form of MS that remains largely subclinical is unknown. They may still be at risk of long term disability from late relapses or the development of SPMS, the so called “single-attack progressive MS” phenotype (Kremenchutzky et al., 2006). These patients may also be at risk of cognitive impairment, fatigue and affective disorders that can occur in people with
benign MS and in the radiologically isolated syndrome, even in the absence of significant physical disability (Amato et al., 2006; Amato et al., 2012).

Recent studies have suggested that the prognosis of relapse-onset MS in contemporary patient cohorts may be rather better than in natural history studies conducted in the pre-MRI era (Confavreux et al., 2003; Scalfari et al., 2010). In a population-based study from France, 244 patients with relapse-onset MS were followed up prospectively over 10 years after CIS and only 8% reached EDSS 6 (Kerbrat et al., 2015). Similarly, Capra and colleagues studied the accrual of disability in relapse-onset MS patients seen at a single Italian MS centre since 1980 (Capra et al.). The patients were grouped into epochs of 5 years from 1980 to 2016 and the age and rate at which patients reached EDSS 6 was noted. Between 1980 and 1995 the age at EDSS 6 was stable. However, since 1996 the age at which EDSS 6 was reached has progressively increased in each 5 yearly epoch. While treatment with DMT is likely to be a major factor contributing to these observations the possibility arises that the McDonald criteria are identifying patients with milder forms of MS. There is the potential for the natural history of relapse-onset MS to be favourably modified by changes to the diagnostic criteria, independent of any effect of DMT, referred to as the Will-Rogers phenomenon (Sormani et al., 2008).

This study has some limitations. Firstly, participants had scheduled MRI follow up over the first ~5 years after CIS onset, irrespective of clinical status. In routine clinical practice CIS patients would usually have a diagnostic MRI at presentation then a follow up MRI if required to demonstrate dissemination in time, rather than repeated MRI scans. However, the majority of CIS patients who satisfied the McDonald MRI criteria (including those with MRI-only MS) did so within 12 months of CIS onset.
suggesting that my findings are not simply a function of the intensity of MRI follow up. Secondly, this cohort is biased towards patients with optic neuritis. It has been suggested that optic neuritis may be more benign prognosis than brainstem or spinal cord syndromes (Tintore et al., 2005; Tintore et al., 2015), potentially accounting for the significant number of patients in this study with MRI-only MS. However, more than 80% of the patients with optic neuritis in the study had abnormal brain MRI at presentation indicating a high-risk group for the development of CDMS. Finally it is possible that with longer follow up the proportion of CDMS patients will increase, although most people with MS experience a second attack in the first 2-3 years after a CIS.

4.5 Conclusions

The McDonald criteria allow MS to be diagnosed sooner and more often in patients with CIS. While the McDonald criteria do facilitate an earlier diagnosis of MS, up to 1 in 3 CIS patients who satisfy the McDonald 2010 MRI criteria for a diagnosis of MS do not have further clinical events, at least in the medium-term. Changes to the diagnostic criteria for MS may be one of the factors contributing to an improved prognosis of MS in the treatment era.

The incorporation of MRI into diagnostic criteria represents a significant advance in the management of people with MS. However, further modifications to MRI criteria for diagnosing MS are likely in the future. In Chapter 5 I investigate two proposed modifications to DIS criteria to improve the diagnosis of MS in people with CIS.
Chapter 5

Modifications to MRI criteria to improve the diagnosis of MS in people with clinically isolated syndromes

5.1 Introduction

There has been significant evolution of the diagnostic criteria for MS over the last 15 years (McDonald et al., 2001; Polman et al., 2005; Polman et al., 2011). As presented in Chapter 4 the McDonald criteria are helpful in establishing a diagnosis of MS at an earlier time point. However, fewer than half of CIS patients who develop MS can be diagnosed at the time of presentation with a single contrast-enhanced MRI scan of the brain and spinal cord using current MRI criteria (Rovira et al., 2009).

Recently, the MAGNIMS group has recommended further changes to MRI criteria to improve the diagnosis of MS in CIS patients (Filippi et al., 2016). These changes include the inclusion of lesions in the symptomatic region, optic nerve and cerebral cortex in DIS, and an increase in the number of lesions required in the periventricular region to confirm DIS from 1 to 3. In this chapter I investigate two of the proposed changes to DIS criteria in the CIS cohort:

1. Should lesions in the symptomatic site should be included in DIS criteria?

2. Does increasing the number of periventricular lesions required for DIS improve the diagnostic accuracy of MRI criteria for a diagnosis of MS?
5.2 Should lesions in the symptomatic region be included in dissemination in space in patients with clinically isolated syndromes?

5.2.1 Introduction

In the 2010 revisions to the McDonald criteria there was a major simplification of the requirements for DIS (Polman et al., 2011). The presence of one or more T2-hyperintense lesions in two of four sites typically affected in demyelination (periventricular, juxtacortical, infratentorial, spinal cord) provides MRI evidence for DIS (Polman et al., 2011). When the patient’s symptoms are referable to the brainstem/cerebellum or spinal cord, then lesions in the symptomatic region are excluded (Polman et al., 2011). The criteria are ambiguous as to whether all lesions in the symptomatic region should be excluded or only the symptomatic lesion (Rovira et al., 2015).

The rationale for exclusion of symptomatic lesions in DIS was to maximise the specificity of MRI criteria by requiring MRI evidence of at least two asymptomatic lesions to establish DIS (Swanton et al., 2006; Swanton et al., 2007). However, in previous diagnostic criteria for MS, whether clinical criteria (Poser et al., 1983) or incorporating MRI (McDonald et al., 2001; Polman et al., 2005), symptomatic lesions have always contributed to DIS. The exclusion of lesions in the symptomatic region may reduce the sensitivity of the McDonald 2010 criteria in patients with brainstem/cerebellar and spinal cord syndromes (Ruet et al., 2011; Kang et al., 2014), potentially leading to delays in the diagnosis and management of MS.
I wanted to investigate whether inclusion of lesions in the symptomatic region improves the performance of DIS criteria for a diagnosis CDMS in patients with clinically isolated brainstem/cerebellar or spinal cord syndromes suggestive of MS.

5.2.2 Methods

Patients

Patients from the CIS cohort who presented with brainstem/cerebellar or spinal syndromes were included in this analysis (n=32). Two patients were excluded; one with myelitis who developed NMOSD and one with a brainstem syndrome who was lost to follow-up.

Clinical assessment

The patients were followed prospectively for the development of CDMS. The date of the second clinical attack was recorded and used to determine the time to diagnosis of CDMS.

MRI acquisition and image-analysis

PD/T2-weighted and post-contrast T1-weighted scans of the brain and spinal cord obtained at baseline and a follow-up PD/T2-weighted and post-contrast T1-weighted brain scan after approximately 3–12 months were analysed. The number and location of T2-hyperintense lesions and the number of contrast-enhancing lesions was recorded by Dr Katherine Miszkiel, Consultant Neuroradiologist.
DIS criteria

I retrospectively applied three DIS criteria to the baseline MRI scans:

1. The McDonald 2010 DIS criteria with lesions in the symptomatic region excluded (Polman et al., 2011)

2. Modified criteria 1: The McDonald 2010 DIS criteria modified to include asymptomatic lesions in the symptomatic region. For example, a cerebellar hemisphere lesion in a patient with bilateral internuclear ophthalmoplegia.

3. Modified criteria 2: The McDonald 2010 DIS criteria modified to include any lesion in the symptomatic region in DIS. For example, a single spinal cord lesion in a patient with myelitis.

DIT criteria

The McDonald 2010 DIT criteria were also applied retrospectively, requiring either the simultaneous presence of gadolinium-enhancing and non-enhancing lesions on the same MRI scan or a gadolinium-enhancing and/or new T2-hyperintense lesion on the follow-up MRI done after 3 or 12 months (Polman et al., 2011).

Statistical analysis

The sensitivity, specificity and accuracy of the three DIS criteria (alone and in combination with DIT criteria) for the development of CDMS and 95% confidence intervals were calculated. I also analysed the time to diagnosis of MS based on clinical
evidence alone (CDMS) or using the different MRI criteria (each requiring both DIS and DIT). The times to diagnosis of MS were not normally distributed and comparison was made using the one-sample Wilcoxon signed-rank test.

### 5.2.3 Results

30 patients were included in this analysis. Table 5.1 shows the baseline demographic, clinical and MRI findings. 23 (77%) patients had one or more T2-hyperintense lesions in the symptomatic region. The symptomatic lesion was able to be localised in 22 (73%) patients based on the neurological examination findings, the presence of contrast-enhancement, or both. 11 (37%) patients had one or more asymptomatic lesion in the symptomatic region.

**Table 5.1 Baseline patient characteristics.**

<table>
<thead>
<tr>
<th></th>
<th>Braintstem/cerebellar syndrome (n=20)</th>
<th>Spinal cord syndrome (n=10)</th>
<th>All patients (n=30)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Age, mean (SD)</td>
<td>30.6 (8.3)</td>
<td>32.6 (9.9)</td>
<td>31.25 (8.8)</td>
</tr>
<tr>
<td>Female, n (%)</td>
<td>14 (70)</td>
<td>4 (40)</td>
<td>18 (60)</td>
</tr>
<tr>
<td>Number of lesions in the symptomatic site</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>0 lesions</td>
<td>5</td>
<td>2</td>
<td>7</td>
</tr>
<tr>
<td>1 lesion</td>
<td>9</td>
<td>3</td>
<td>12</td>
</tr>
<tr>
<td>2 or more lesions</td>
<td>6</td>
<td>5</td>
<td>11</td>
</tr>
<tr>
<td>Baseline EDSS, median</td>
<td>2.25</td>
<td>2</td>
<td>2</td>
</tr>
</tbody>
</table>
During follow-up 15 (50%) patients developed CDMS. None of the patients received disease-modifying treatment prior to developing CDMS.

Figure 5.1 shows a Kaplan-Meier survival curve with the time to diagnosis of CDMS based on the number of lesions in the symptomatic region. CDMS developed in 1/7 (14%) patients with a normal MRI of the symptomatic region, 7/12 (58%) patients with a single lesion in the symptomatic region and 10/11 (91%) patients with two or more lesions.

Figure 5.1  Kaplan-Meier survival curve showing time to diagnosis of clinically-definite MS based on the number of lesions in the symptomatic region.
Table 5.2 shows the performance of the McDonald 2010 DIS criteria and the two modified criteria alone and in combination with DIT criteria for the development of CDMS. All of the DIS criteria had high sensitivity and specificity for the development of CDMS (Table 5.2). However, the two modified criteria were more sensitive and more accurate than the McDonald 2010 DIS criteria. All three criteria had the same specificity. The inclusion of any lesion in the symptomatic region had the highest overall sensitivity and accuracy for the development of CDMS.

Table 5.2 Performance of the McDonald 2010 DIS criteria and the modified DIS criteria for the development of clinically-definite MS.

<table>
<thead>
<tr>
<th></th>
<th>Sensitivity (95%CI)</th>
<th>Specificity (95%CI)</th>
<th>Accuracy (95%CI)</th>
</tr>
</thead>
<tbody>
<tr>
<td><strong>DIS criteria only</strong></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>McDonald 2010 DIS criteria</td>
<td>73% (45-92%)</td>
<td>73% (45-92%)</td>
<td>73% (54-87%)</td>
</tr>
<tr>
<td>Modified criteria 1</td>
<td>80% (51-96%)</td>
<td>73% (45-92%)</td>
<td>77% (57-90%)</td>
</tr>
<tr>
<td>Modified criteria 2</td>
<td>87% (60-98%)</td>
<td>73% (45-92%)</td>
<td>80% (61-92%)</td>
</tr>
<tr>
<td><strong>DIS + DIT criteria combined</strong></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>McDonald 2010 criteria</td>
<td>67% (39-88%)</td>
<td>73% (45-92%)</td>
<td>70% (50-84%)</td>
</tr>
<tr>
<td>Modified criteria 1</td>
<td>73% (45-92%)</td>
<td>73% (45-92%)</td>
<td>73% (54-87%)</td>
</tr>
<tr>
<td>Modified criteria 2</td>
<td>80% (51-96%)</td>
<td>73% (45-92%)</td>
<td>77% (57-90%)</td>
</tr>
</tbody>
</table>
The mean time to diagnosis of MS (requiring both DIS and DIT) was: (i) CDMS: 24.9 months; (ii) McDonald 2010 criteria: 8.9 months, (iii) McDonald 2010 DIS criteria modified to include asymptomatic lesions in the symptomatic region: 6.7 months; and (iv) McDonald 2010 DIS criteria modified to include any lesion in the symptomatic region: 4.8 months. The McDonald criteria and the two modified criteria allowed for an earlier diagnosis of MS compared with the time to development of CDMS (p<0.001 for all comparisons). However, the time to diagnosis was not significantly different when the modified criteria were compared with the McDonald 2010 criteria.

5.2.4 Discussion

I found that inclusion of lesions in the symptomatic region in patients with clinically isolated brainstem/cerebellar and spinal cord syndromes improves the overall performance of DIS criteria for a diagnosis of CDMS, with no drop in specificity.

This study provides evidence to support the recommendation of the MAGNIMS group that lesions in the symptomatic region should contribute to DIS (Filippi et al., 2016). I found that inclusion of both asymptomatic and symptomatic lesions in the symptomatic region increased the sensitivity of DIS criteria for the development of CDMS. However, modified DIS criteria that include any lesion in the symptomatic region may be preferable, firstly because the sensitivity was higher and secondly because the criteria would be easier to apply in a clinical setting. Determining whether a lesion is symptomatic or asymptomatic is sometimes difficult and not always possible on the basis of clinical findings alone.
The findings of this analysis are supported by other studies that have investigated the prognostic significance of symptomatic lesions in patients with CIS (Caucheteux et al., 2015; Tintore et al., 2016). Caucheteux and colleagues studied 146 CIS patients with a well-defined symptomatic lesion on MRI (including patients with acute optic neuritis). Patients in whom a symptomatic lesion could not be identified on MRI were excluded. The presence of a well-defined symptomatic lesion plus at least one other asymptomatic lesion typical of demyelination (or any white matter lesion in combination with positive CSF) had a very high sensitivity for the development of MS (Caucheteux et al., 2015). The prognostic significance of the symptomatic lesion was also highlighted in analyses from the Barcelona CIS cohort. A single symptomatic lesion in patients with brainstem/cerebellar or spinal cord syndromes carried a similar risk of CDMS as an asymptomatic brain lesion in patients with optic neuritis, and the risk was significantly higher than in CIS patients with a normal MRI at presentation (Tintore et al., 2016). Similarly, in my investigation patients with a single lesion in the symptomatic region were at significantly higher risk of CDMS than patients with no lesions, although the risk was lower than in patients with two or more lesions in the symptomatic region.

Some limitations of this study need to be noted. Firstly, the patients studied all had syndromes that were typical of a first demyelinating event suggestive of MS, such as bilateral internuclear ophthalmoplegia and partial myelitis. The McDonald MRI criteria should only be applied to patients with typical CIS syndromes and care should be taken to exclude conditions that might mimic MS. Secondly, I tested the two modified DIS criteria in a relatively small cohort of patients. This is reflected in the large confidence intervals for sensitivity, specificity and accuracy for the McDonald DIS criteria and the modified DIS criteria. However, the results are encouraging and the criteria should be tested in a larger, multi-centre CIS cohort.
5.3 Periventricular lesion number in dissemination in space criteria in patients with clinically isolated syndrome

5.3.1 Introduction

The McDonald 2010 criteria give equal weighting to asymptomatic T2-hyperintense periventricular, juxtacortical, infratentorial regions and spinal cord lesions when determining DIS (Polman et al., 2011). Although commonly seen in people with CIS and MS, periventricular lesions are not a specific finding and can also be seen in other inflammatory disorders, small vessel cerebrovascular disease, migraine and healthy aging (Fazekas et al., 1988; Nielsen et al., 2005; Absinta et al., 2012; Liu et al., 2013).

The use of ≥1 periventricular lesion in DIS criteria has never been formally tested and earlier iterations of the McDonald criteria required ≥3 periventricular lesions (McDonald et al., 2001; Polman et al., 2005). This was based on the work of Barkhof and colleagues who found that ≥3 periventricular lesions provided the optimal cut-off in terms of sensitivity and specificity for development of CDMS in CIS patients using receiver operator curve characteristics (Barkhof et al., 1997). However, in that study the prognostic value of brain MRI abnormalities was considered in isolation, whereas current diagnostic criteria require evidence of DIS in at least two sites plus DIT to make a diagnosis of MS (Polman et al., 2011).

The MAGNIMS group has recently proposed that the number of lesions required to confirm DIS in the periventricular region be increased from ≥1 to ≥3 (Filippi et al., 2016). This recommendation was based on expert opinion that a single periventricular lesion was not sufficiently specific enough to determine whether the lesion was due
to demyelination or another pathology, particularly if both symptomatic and asymptomatic lesions are included in DIS (Filippi et al., 2016). For example, in a middle-aged patient presenting with a brainstem or spinal cord syndrome, a single periventricular brain lesion may be insufficient to say there is evidence of DIS.

I wanted to investigate the role of periventricular lesion number in DIS. I examined the performance of ≥1, ≥2 and ≥3 periventricular lesions in DIS criteria for the diagnosis of CDMS in order to determine whether increasing the number of periventricular lesions improves the specificity or accuracy of MRI criteria for a diagnosis of MS.

5.3.2 Methods

Patients

I included patients from the CIS cohort who had MRI scans at baseline and a follow-up MRI scan within 12 months to demonstrate DIS. All of the patients had at least 36 months of clinical follow-up.

Clinical assessment

The patients were followed up prospectively for the development of CDMS.

MRI acquisition and image-analysis

MRI scans obtained at baseline (brain and spinal cord) and after 3 - 12 months (brain only) were analysed.
**DIS criteria**

I retrospectively applied the McDonald 2010 DIS criteria requiring ≥1 asymptomatic lesion in two or more sites (periventricular, juxtacortical, infratentorial and spinal cord). I then applied two modified DIS criteria requiring ≥2 or ≥3 periventricular lesions to satisfy DIS in the periventricular region. All other requirements of the McDonald 2010 criteria were unchanged.

The three sets of DIS criteria with varying numbers of periventricular lesions were tested separately in the subgroup of patients who had imaging of the symptomatic region (n=31) to investigate the influence of periventricular lesion number when symptomatic lesions are included in DIS. In addition to the 30 patients with brainstem/cerebellar and spinal cord syndromes described in section 5.2, I also included one patient who presented with a visual field defect due to a hemispheric lesion.

**DIT criteria**

The McDonald 2010 DIT criteria were applied retrospectively in combination with the DIS criteria above with varying numbers of periventricular lesions.

**Statistical analysis**

I investigated the frequency of ≥1, ≥2 and ≥3 periventricular lesions on baseline MRI scans and the positive predictive value (PPV) of periventricular lesion number for the development of CDMS. I calculated the sensitivity, specificity and accuracy with 95% confidence intervals of DIS criteria with ≥1, ≥2 and ≥3 periventricular lesions (alone and in combination with DIT) for the development of CDMS.
5.3.3 Results

155 CIS patients (mean age 32.2 years, 66% female) were included, 124 patients with optic neuritis and 31 patients with a non-optic neuritis presentation who had imaging of the symptomatic region (brainstem/cerebellar syndrome \(n=20\), spinal cord syndrome \(n=10\), hemispheric syndrome \(n=1\)).

During follow-up 93 (60%) patients developed CDMS. The positive predictive value (PPV) of periventricular lesion number for the development of CDMS is shown in Table 5.3. A single periventricular lesion had a very high PPV for CDMS, irrespective of other baseline MRI findings.

Table 5.3  Number of periventricular lesions at baseline and positive predictive value (PPV) of periventricular lesions for the development of CDMS.

<table>
<thead>
<tr>
<th></th>
<th>All patients (n=155)</th>
<th>CDMS (n=93)</th>
<th>PPV</th>
</tr>
</thead>
<tbody>
<tr>
<td>0 lesions, n (%)</td>
<td>53 (34)</td>
<td>14 (15)</td>
<td>26%</td>
</tr>
<tr>
<td>1 lesion, n (%)</td>
<td>22 (15)</td>
<td>15 (16)</td>
<td>68%</td>
</tr>
<tr>
<td>2 lesions, n (%)</td>
<td>12 (8)</td>
<td>9 (10)</td>
<td>75%</td>
</tr>
<tr>
<td>≥3 lesions, n (%)</td>
<td>68 (45)</td>
<td>55 (60)</td>
<td>81%</td>
</tr>
</tbody>
</table>
The performance of DIS criteria using ≥1, ≥2 and ≥3 periventricular lesions for development of CDMS is shown in Table 5.4. One or more periventricular lesion had the highest sensitivity and accuracy but the specificity was lower than with DIS criteria requiring ≥2 or ≥3 periventricular lesions. When DIS was combined with DIT criteria, the sensitivity and accuracy of ≥1 periventricular lesion was higher than ≥2 or ≥3 periventricular lesions and the specificity was the same.

Table 5.4 Performance of diagnostic criteria for development of clinically-definite MS with varying numbers of periventricular lesions in all patients (n=155).

<table>
<thead>
<tr>
<th></th>
<th>Sensitivity (95%CI)</th>
<th>Specificity (95%CI)</th>
<th>Accuracy (95%CI)</th>
</tr>
</thead>
<tbody>
<tr>
<td><strong>DIS only</strong></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>≥1 PV lesion</td>
<td>80% (68-91%)</td>
<td>73% (61-81%)</td>
<td>77% (70-84%)</td>
</tr>
<tr>
<td>≥2 PV lesions</td>
<td>72% (59-83%)</td>
<td>76% (63-85%)</td>
<td>74% (66-81%)</td>
</tr>
<tr>
<td>≥3 PV lesions</td>
<td>69% (60-79%)</td>
<td>77% (64-87%)</td>
<td>72% (64-78%)</td>
</tr>
<tr>
<td><strong>DIS + DIT combined</strong></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>≥1 PV lesion</td>
<td>62% (50-71%)</td>
<td>79% (66-89%)</td>
<td>69% (62-77%)</td>
</tr>
<tr>
<td>≥2 PV lesions</td>
<td>56% (45-66%)</td>
<td>79% (66-89%)</td>
<td>65% (57-73%)</td>
</tr>
<tr>
<td>≥3 PV lesions</td>
<td>54% (43-64%)</td>
<td>79% (66-89%)</td>
<td>64% (56-72%)</td>
</tr>
</tbody>
</table>
The results were similar when lesions in the symptomatic region were included in DIS in the subgroup of patients who had imaging of the symptomatic region (n=31); ≥1 periventricular lesion was more sensitive and more accurate than ≥2 or ≥3 periventricular lesions and there was no overall change in specificity when DIS was considered in conjunction with DIT (Table 5.5).

Table 5.5 Performance of diagnostic criteria for development of clinically-definite MS with varying numbers of periventricular lesions and inclusion of lesions in the symptomatic region (n=31).

<table>
<thead>
<tr>
<th></th>
<th>Sensitivity (95%CI)</th>
<th>Specificity (95%CI)</th>
<th>Accuracy (95%CI)</th>
</tr>
</thead>
<tbody>
<tr>
<td><strong>DIS only</strong></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>≥1 PV lesion</td>
<td>87% (57-99%)</td>
<td>69% (41-89%)</td>
<td>71% (49-86%)</td>
</tr>
<tr>
<td>≥2 PV lesions</td>
<td>73% (45-92%)</td>
<td>69% (41-89%)</td>
<td>68% (45-83%)</td>
</tr>
<tr>
<td>≥3 PV lesions</td>
<td>67% (38-88%)</td>
<td>69% (41-89%)</td>
<td>68% (45-83%)</td>
</tr>
<tr>
<td><strong>DIS + DIT combined</strong></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>≥1 PV lesion</td>
<td>80% (52-97%)</td>
<td>75% (48-93%)</td>
<td>77% (57-91%)</td>
</tr>
<tr>
<td>≥2 PV lesions</td>
<td>73% (45-92%)</td>
<td>75% (48-93%)</td>
<td>74% (54-89%)</td>
</tr>
<tr>
<td>≥3 PV lesions</td>
<td>67% (38-88%)</td>
<td>75% (48-93%)</td>
<td>71% (49-86%)</td>
</tr>
</tbody>
</table>
5.3.4 Discussion

Increasing the number of periventricular lesions required for DIS may reduce the sensitivity of the MRI criteria for a diagnosis of CDMS, possibly reflecting the lower lesion load at this earliest clinical stage of MS. Although a high sensitivity facilitates an early diagnosis, a high specificity is also important, in order to avoid misdiagnosis of MS. I found that DIS criteria requiring \( \geq 1 \) or \( \geq 2 \) PV lesions were more sensitive but less specific than DIS criteria requiring \( \geq 3 \) periventricular lesions. However, when DIS criteria was combined with DIT, the specificity of the 2010 McDonald criteria for CDMS was the same whether the minimum number of PV lesions was 1, 2 or 3.

I separately investigated the effect of varying periventricular lesion number when lesions in the symptomatic site were included in DIS in the small subgroup of patients with a non-optic neuritis presentation (n=31). An increase in the number of periventricular lesions required for DIS yielded similar findings to those reported in the whole cohort. A larger cohort should be studied to more definitively investigate the effect of including symptomatic region lesions with varying number of periventricular lesions, ideally in combination with other changes to DIS recommended by MAGNIMS (Filippi et al., 2016).

I studied a group of young adults with well-defined clinical presentations typical of demyelination (mainly acute optic neuritis) in whom the pre-test probability of MS was high. How MRI criteria perform outside this setting, for example, in people with atypical presentations, in young children (<12 years) or in older adults (>50 years) is uncertain. In this setting the likelihood of alternative diagnoses other than MS may be higher. Periventricular lesions occur in many disorders, including migraine (Absinta et al., 2012; Liu et al., 2013) and small vessel cerebrovascular disease (Nielsen et al.,...
2005), and a previous study found that ≥3 periventricular lesions was the best MRI brain measure to differentiate MS from other neurological disorders (Nielsen et al., 2005).

MRI criteria are intended for use in patients in whom a diagnosis of MS is clinically suspected, rather than to differentiate MS from other neurological disorders and should only be applied in settings where MS is strongly suspected, such as a young adult with a typical CIS. In this group of patients I found that increasing the number of periventricular lesions required for DIS may not improve the performance of MRI criteria for MS.

5.4 Conclusions

The McDonald criteria have evolved significantly over the last 15 years and further changes are likely as the evidence base for MRI criteria grows. The findings presented in this chapter support the inclusion of lesions in the symptomatic region in DIS in patients with brainstem/cerebellar and spinal cord syndromes. However, an increase in the number of periventricular lesions required for DIS from ≥1 to ≥3 does not enhance the diagnostic accuracy and may potentially reduce the performance of MRI criteria, at least in young adult patients with typical CIS presentations.

My findings highlight the high prognostic value of conventional MRI abnormalities in predicting the development of CDMS in patients with CIS. In Chapters 6 and 7 I investigate how these early MRI measures used to establish a diagnosis of MS are associated with the development of disability.
Chapter 6

Spinal cord lesions and atrophy are associated with the development of disability over the first 5 years after a clinically isolated syndrome

6.1 Introduction

In people with MS, disability is commonly referable to the spinal cord resulting in weakness, sensory alteration, sphincter problems and neuropathic pain (Swingler and Compston, 1992). Pathological studies investigating spinal cord damage in MS have found evidence of focal lesions involving grey and white matter, neuroaxonal loss and atrophy (Kearney et al., 2015b). MRI often detects spinal cord abnormalities in established MS (Kidd et al., 1993; Kearney et al., 2015b); focal T2-hyperintense lesions are seen in 75% or more of patients, most commonly in the cervical cord (Kidd et al., 1993; Nijeholt et al., 1998; Lukas et al., 2013). Spinal cord atrophy can also be detected in vivo using MRI by measuring the UCCA, and has robust correlations with physical disability (Losseff et al., 1996; Nijeholt et al., 1998; Stevenson et al., 1998; Lin et al., 2003; Rashid et al., 2006; Furby et al., 2008; Bonati et al., 2011; Rocca et al., 2011; Lukas et al., 2013; Kearney et al., 2014a; Lukas et al., 2015).

Spinal cord MRI abnormalities are also seen in people with CIS. Up to half of CIS patients have asymptomatic spinal cord lesions (O’Riordan et al., 1998a; Sombekke et al., 2013) and these can be helpful in establishing a diagnosis of MS (Polman et al., 2011). Spinal cord lesions may have prognostic value and the presence of spinal cord lesions in CIS patients is associated with an increased risk of disability over the first few years after a CIS (Swanton et al., 2009; Arrambide et al., 2017).
Spinal cord atrophy has been previously reported in CIS patients with abnormal brain MRI (Brex et al., 2001; Biberacher et al., 2015), a group known to be at high-risk for MS (Fisniku et al., 2008a; Tintore et al., 2015). Only one previous study has investigated spinal cord atrophy longitudinally in patients with CIS and no change in UCCA was found over a follow-up period of one year (Brex et al., 2001). This contrasts with findings in patients with established MS where progressive spinal cord atrophy is evident over follow-up periods of 12–36 months (Lin et al., 2003; Rashid et al., 2006; Lukas et al., 2015) and may be associated with progression of disability (Lin et al., 2003; Lukas et al., 2015).

I wanted to investigate longitudinal changes in spinal cord lesions and UCCA over the first 5 years after CIS to determine whether spinal cord involvement is one of the mechanisms responsible for disability progression in early relapse-onset MS.

6.2 Methods

Patients

I included patients from the CIS cohort who had brain and spinal cord MRI at the time of presentation and at follow-up after 3-5 years. I excluded patients who presented with a spinal cord CIS in order to investigate the significance of clinically silent, early spinal cord MRI abnormalities present at the time of CIS.

Clinical assessments

MS was diagnosed using the McDonald 2010 criteria (Polman et al., 2011). Disability was assessed at baseline and follow-up in all patients using the EDSS (Kurtzke,
1983). A subgroup of patients were also assessed at follow-up with the TWT and 9HPT, components of the MSFC (Cutter et al., 1999).

**MRI acquisition and image-analysis**

MRI of the brain and spinal cord was obtained at baseline (within 3 month of CIS onset) and at follow-up after 3-5 years. All of the scans were done on the same 1.5 T Signa scanner (General Electric, Wisconsin, USA). There was a major hardware upgrade during the study. Some of the follow-up MRI scans were obtained after the scanner upgrade and this was considered in the statistical analysis (see below).

**Brain MRI measures**

I identified T2-hyperintense brain lesions on PD/T2-weighted scans and T1-hypointense brain lesions on post-contrast T1-weighted scans. A semi-automated edge-finding tool (JIM6, Xinapse systems, Aldwincle, UK) was used to outline lesions and to calculate T2LV and T1LV. The lesion load at baseline and the change in T2LV and T1LV from baseline to follow-up was recorded.

I calculated NBV at baseline using SIENAX and the PBVC between baseline and follow-up using SIENA from T1-weighted fast spin echo scans after lesion filling (Smith et al., 2002; Prados et al., 2014). SIENAX and SIENA analyses were done using FMRIB Software Library (FSL) version 5.0.2 with the assistance of Dr Ferran Prados, Dr Arman Eshaghi and Mr Jon Stutters.
Spinal cord MRI measures

The number of spinal cord lesions was identified from sagittal PD/T2-weighted scans of the whole cord by Dr Katherine Miszkiel. The number of spinal cord lesions at baseline and the change in spinal cord lesion number was recorded (Figure 6.1).

![Spinal cord lesions](image)

Figure 6.1  Spinal cord lesions.

Sagittal T2-weighted scans of the whole spine in a CIS patient. At baseline (A) a single spinal cord lesion is visible at T8/9. At follow-up (B) after 5 years a new lesion is visible at the level of C2.

For spinal cord atrophy, I calculated the UCCA at baseline and follow-up from dedicated volumetric scans of the cervical cord (Figure 6.2). The sagittally-acquired images were reformatted in the axial plane to obtain five contiguous 3 mm slices at
the level of the C2/C3 disc. An active surface model was then used to measure the mean UCCA over the five slices, as described in Chapter 2 (Horsfield et al., 2010; Rocca et al., 2011; Kearney et al., 2014b). The change in UCCA between baseline and follow-up (in mm²) was calculated. The analyses were done using JIM6.

Figure 6.2  Spinal cord atrophy

Reformatted axial images at the level of C2/C3 used to calculate the UCCA over a mean of five slices at baseline (A) and follow-up after 5 years (B).

**Statistical analysis**

I grouped the patients based on their clinical status after 5 years as CIS, MS with EDSS <3 and MS with EDSS ≥3. Univariable comparisons of MRI measures between patient groups (CIS vs MS, MS with EDSS <3 vs MS with EDSS ≥3) were tested using
the non-parametric Wilcoxon rank sum test for lesion and brain atrophy measures, due to their skewed distribution. Linear regression was used for comparisons of UCCA, with adjustment for age and sex.

Multiple linear regression models were used to identify independent MRI predictors of physical disability at follow-up (EDSS, TWT, 9HPT). Separate models were constructed for brain and spinal cord MRI measures in order to compare the proportion of variance explained using the R-square ($R^2$) statistic and to restrict the number of covariates. Only variables with $p<0.08$ were retained in models. The predictor variables from the separate brain and spinal cord models were then combined, and manual backwards stepwise elimination used to find the best overall combined model. The omitted MRI variables were then entered singly and retained if $p<0.08$.

Disease duration was included as a covariate in all models in order to account for differences in the duration of follow-up. The potential effect of a scanner upgrade during the follow-up period was assessed by adjusting for those subjects whose baseline and follow-up straddled the upgrade. Potential confounding by age and sex was examined by entering these into the final models. The results of the final models were confirmed using a non-parametric bias-corrected and accelerated bootstrap with 1000 replicates (Carpenter and Bithell, 2000).
6.3 Results

I included 131 patients from the CIS cohort in this analysis. The reasons for exclusion were spinal cord CIS (n=10), no follow-up MRI scan at 3 or 5 years (n=35) and diagnosis other than MS (n=2). The patients included in this analysis comprised 114 (87%) patients with optic neuritis, 16 (12%) patients with a brainstem/cerebellar syndrome and 1 (1%) patient with a hemispheric syndrome. The mean duration of follow-up was 5.3 years (range 3.0–7.9 years).

93 (71%) patients were diagnosed with MS using the McDonald 2010 criteria. 15 (16%) patients had an EDSS score of ≥3 at follow-up (median EDSS 3.5, range 3–6.5), and were classified as disabled. Four of these patients had developed SPMS. 78 (84%) MS patients had not developed significant disability (median EDSS 1, range 0–2.5). Disability was similar after 5 years in patients with optic neuritis and other CIS presentations (median EDSS 1 in both groups). During follow-up 23 (18%) patients started DMT, either beta interferon or glatiramer acetate.

The baseline characteristics of the patients included in this analysis grouped by clinical status at approximately 5 years is shown in Table 6.1.
Table 6.1  Baseline demographic characteristics and MRI findings at baseline and follow grouped by clinical status after 5 years.

<table>
<thead>
<tr>
<th></th>
<th>All patients (n=131)</th>
<th>CIS at 5 years (n=38)</th>
<th>MS after 5 years</th>
<th>p†</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td></td>
<td></td>
<td>EDSS &lt; 3 (n=78)</td>
<td></td>
</tr>
<tr>
<td></td>
<td></td>
<td></td>
<td>EDSS ≥ 3 (n=15)</td>
<td></td>
</tr>
<tr>
<td>Age, years</td>
<td>32.6 (7.5)</td>
<td>32.5 (6.9)</td>
<td>32.8 (7.9)</td>
<td>31.5 (7.4)</td>
</tr>
<tr>
<td>Female, n (%)</td>
<td>83 (63)</td>
<td>18 (47)</td>
<td>55 (71)</td>
<td>10 (67)</td>
</tr>
<tr>
<td>Optic neuritis, n (%)</td>
<td>114 (87)</td>
<td>34 (89)</td>
<td>69 (88)</td>
<td>11 (73)</td>
</tr>
<tr>
<td>Baseline EDSS, median (IQR)</td>
<td>1 (1)</td>
<td>1 (1)</td>
<td>1 (1)</td>
<td>1 (1.75)</td>
</tr>
</tbody>
</table>

All data presented as mean (SD) unless otherwise stated
† p values for comparisons between MS patients with EDSS <3 and ≥3 at follow-up.
**Brain MRI abnormalities**

Brain MRI findings at baseline and at follow-up are shown in Table 6.2. MS patients classified as disabled at follow-up (EDSS ≥3) had a greater T2LV at baseline (mean 5.12 vs 1.72 ml, p<0.001) and follow-up (18.20 vs 4.89 ml, p<0.001) compared with the MS patients without significant disability. The findings were similar for brain T1LV at baseline (0.65 vs 0.21ml, p=0.009) and at follow-up (5.06 vs 0.64ml, p=0.031).

The MS patients showed significantly greater brain atrophy compared with the patients who remained CIS (annualised PBVC -0.40 vs -0.26%, p=0.009), and there was significantly greater brain atrophy in the MS patients who were disabled at follow-up compared with those without disability (annualised PBVC -0.67 vs -0.35%, p=0.047).

**Spinal cord MRI abnormalities**

Spinal cord MRI measures at baseline and follow-up are shown in Table 6.2.

**Spinal cord lesions**

None of the patients who remained CIS had spinal cord lesions at baseline or follow-up. Among the patients who were diagnosed with MS, the number of spinal cord lesions at baseline (median [range] 2 [0 – 7] vs 0 [0 – 5], p=0.001) and at follow-up (median [range] 5 [1 – 14] vs 1 [0 – 9], p<0.001) was higher in patients with disability compared with those without significant disability.

There was no difference in the number of spinal cord lesions at baseline or follow-up in patients with optic neuritis and a non-optic neuritis CIS.
Spinal cord atrophy

The mean UCCA at baseline and follow-up grouped by clinical status at ~5 years is shown in Figure 6.3.

After adjusting for age and sex there was no significant difference in the UCCA at baseline in patients who developed MS compared with those who remained CIS, (unadjusted means 74.66 vs 78.25 mm², adjusted difference -2.21 mm², p=0.11), or in MS patients with and without disability at follow-up (unadjusted means 76.65 vs 74.27 mm², adjusted difference 2.22 mm², p=0.27). The mean annualised change in UCCA showed significantly more atrophy in patients with MS compared with those who remained CIS (unadjusted means -0.42 vs -0.09 mm²/year, adjusted difference -0.37 mm²/year, p<0.001), and in MS patients with disability compared with those without significant disability (unadjusted means -0.91 vs -0.32 mm²/year, adjusted difference -0.59 mm²/year, p<0.001).
Table 6.2 Baseline demographic characteristics and MRI findings at baseline and follow up grouped by clinical status after 5 years.

<table>
<thead>
<tr>
<th>Baseline MRI measures</th>
<th>All patients (n=131)</th>
<th>CIS at 5 years (n=38)</th>
<th>MS after 5 years</th>
<th>p†</th>
</tr>
</thead>
<tbody>
<tr>
<td>Brain T2LV, ml</td>
<td>1.63 (3.0)</td>
<td>0.06 (0.1)</td>
<td>1.72 (2.9)</td>
<td>5.12 (4.1)</td>
</tr>
<tr>
<td>Brain T1LV, ml</td>
<td>0.20 (0.6)</td>
<td>0 (0)</td>
<td>0.21 (0.6)</td>
<td>0.65 (1.1)</td>
</tr>
<tr>
<td>NBV, cm³</td>
<td>1576 (72)</td>
<td>1580 (66)</td>
<td>1582 (68)</td>
<td>1549 (82)</td>
</tr>
<tr>
<td>SC lesion number, n</td>
<td>0.54 (1.2)</td>
<td>0 (0)</td>
<td>0.50 (0.9)</td>
<td>2.07 (1.9)</td>
</tr>
<tr>
<td>UCCA, mm²</td>
<td>75.70 (7.8)</td>
<td>78.25 (7.2)</td>
<td>74.27 (7.7)</td>
<td>76.65 (7.8)</td>
</tr>
<tr>
<td>Annualised change in MRI measures</td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Δ Brain T2LV, ml</td>
<td>0.56 (1.3)</td>
<td>0 (0)</td>
<td>0.51 (0.8)</td>
<td>2.28 (2.8)</td>
</tr>
<tr>
<td>Δ Brain T1LV, ml</td>
<td>0.12 (0.5)</td>
<td>0 (0)</td>
<td>0.07 (0.1)</td>
<td>0.67 (1.4)</td>
</tr>
<tr>
<td>PBVC, %</td>
<td>-0.37 (0.3)</td>
<td>-0.26 (0.2)</td>
<td>-0.35 (0.3)</td>
<td>-0.67 (0.5)</td>
</tr>
<tr>
<td>Δ SC lesion number, n</td>
<td>0 (0.3)</td>
<td>0 (0)</td>
<td>0 (0.4)</td>
<td>0.83 (1.1)</td>
</tr>
<tr>
<td>Δ UCCA, mm²</td>
<td>-0.32 (0.5)</td>
<td>-0.09 (0.3)</td>
<td>-0.32 (0.4)</td>
<td>-0.91 (0.8)</td>
</tr>
</tbody>
</table>

All data presented as mean (SD)

† p values for comparisons between MS patients with EDSS <3 and ≥3 at follow-up.
Figure 6.3  Change in upper cervical cord area from baseline to 5 years grouped by disability status at follow-up
Independent associations between MRI measures and disability

Multivariable regression models were constructed that included brain MRI measures only, spinal cord MRI measures only and the combination of both in order to determine independent associations between MRI measures and disability at follow-up (Tables 6.3, 6.4 and 6.5). EDSS scores were available for all patients at follow-up. Walking speed and 9HPT scores were available for 101 (77%) patients.

Multivariable regression analysis: EDSS at follow-up

Independent associations between MRI measures and EDSS at follow-up are shown in Table 6.3. In the brain MRI-only model the change in T2LV, the squared change in T2LV and PBVC were independently associated with EDSS at follow-up ($R^2 = 0.39$). Change in T2LV showed evidence of a non-linear association with EDSS, and the squared term was retained in models. The small negative coefficient for the squared change in T2LV suggests a levelling off of the association between T2LV and EDSS as T2LV increases.

In the spinal cord-only model baseline spinal cord lesion number, change in spinal cord lesion number and change in UCCA were independently associated with EDSS at follow-up ($R^2 = 0.53$).

When the brain and spinal cord MRI measures were combined all the MRI measures from the separate models above remained significant. Combining brain and spinal cord MRI measures only modestly increased the predictive power of the model (adjusted $R^2 = 0.61$) compared to spinal cord measures alone (adjusted $R^2 = 0.51$). Bootstrapping confirmed the regression results, except for baseline UCCA in the combined model which became borderline significant (Table 6.3).
Table 6.3  Multiple linear regression models investigating independent associations of MRI measures with EDSS after 5 years (n=131)

<table>
<thead>
<tr>
<th>MRI measure</th>
<th>Coefficient</th>
<th>95% CI</th>
<th>p</th>
<th>R² (Adjusted R²)</th>
</tr>
</thead>
<tbody>
<tr>
<td><strong>Brain MRI measures</strong></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Change in T2LV</td>
<td>0.19</td>
<td>0.12, 0.26</td>
<td>&lt;0.001</td>
<td>0.39 (0.37)</td>
</tr>
<tr>
<td>Squared Change in T2LV</td>
<td>-0.0032</td>
<td>-0.0047, -0.0017</td>
<td>&lt;0.001</td>
<td></td>
</tr>
<tr>
<td>PBVC</td>
<td>-0.14</td>
<td>-0.25, -0.02</td>
<td>0.020</td>
<td></td>
</tr>
<tr>
<td><strong>Spinal cord MRI measures</strong></td>
<td></td>
<td></td>
<td></td>
<td>0.53 (0.51)</td>
</tr>
<tr>
<td>Baseline SC lesion number</td>
<td>0.40</td>
<td>0.26, 0.54</td>
<td>&lt;0.001</td>
<td></td>
</tr>
<tr>
<td>Change in SC lesion number</td>
<td>0.19</td>
<td>0.11, 0.28</td>
<td>&lt;0.001</td>
<td></td>
</tr>
<tr>
<td>Change in UCCA</td>
<td>-0.15</td>
<td>-0.21, -0.09</td>
<td>&lt;0.001</td>
<td></td>
</tr>
<tr>
<td><strong>Brain and spinal cord MRI measures combined</strong></td>
<td>0.64 (0.61)</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Change in T2LV</td>
<td>0.10</td>
<td>0.04, 0.16</td>
<td>0.002</td>
<td></td>
</tr>
<tr>
<td>Squared Change in T2LV</td>
<td>-0.0017</td>
<td>-0.0029, -0.00039</td>
<td>0.011</td>
<td></td>
</tr>
<tr>
<td>PBVC</td>
<td>-0.11</td>
<td>-0.21, -0.02</td>
<td>0.018</td>
<td></td>
</tr>
<tr>
<td>Baseline SC lesion number</td>
<td>0.37</td>
<td>0.24, 0.50</td>
<td>&lt;0.001</td>
<td></td>
</tr>
<tr>
<td>Change in SC lesion number</td>
<td>0.12</td>
<td>0.04, 0.21</td>
<td>0.004</td>
<td></td>
</tr>
<tr>
<td>Baseline UCCA</td>
<td>-0.01</td>
<td>-0.033, 0.0040</td>
<td>0.122</td>
<td></td>
</tr>
<tr>
<td></td>
<td></td>
<td>(-0.028, 0.00036)</td>
<td>(0.053)</td>
<td></td>
</tr>
<tr>
<td>Change in UCCA</td>
<td>-0.11</td>
<td>-0.18, -0.05</td>
<td>&lt;0.001</td>
<td></td>
</tr>
</tbody>
</table>

*Bootstrap confidence interval and p-value.
Spinal cord lesion number was the only baseline MRI measure associated with EDSS at follow-up. Univariably, baseline spinal cord lesion number explained a significant proportion of the variability in EDSS at follow-up (adjusted $R^2=0.24$).

*Multivariable regression analysis: TWT and 9HPT at follow-up*

 Associations between MRI measures and walking speed at 5 years are shown in Table 6.4. In the brain-only model the change in T2LV and with borderline significance baseline NBV were associated with walking speed at follow-up ($R^2=0.13$). Baseline spinal cord lesion number and change in UCCA were associated with walking speed in the spinal cord-only model ($R^2=0.26$). In the combined model only change in T2LV and baseline spinal cord lesion number remained significant ($R^2=0.29$).

 Associations between MRI measures and upper limb dexterity measured using the 9HPT are shown in Table 6.5. In the brain-only model the change in T2LV and baseline NBV were independently associated with 9HPT speed ($R^2=0.27$). In the spinal cord-only model baseline spinal cord lesion number and the change in UCCA were independently associated with 9HPT speed ($R^2=0.34$). In the combined model all of the brain and spinal cord MRI measures from the two separate models remained significant ($R^2=0.41$).

 For both the TWT and 9HPT the findings were similar to EDSS: spinal cord MRI measures explained more of the variation in walking speed and 9HPT performance at 5 years than brain MRI measures, and the combination of both only modestly increased the predictive power of the models over spinal cord MRI measures alone (Table 6.4). Baseline spinal cord lesion number and the change in T2LV were consistently associated with all three measures of physical disability at follow-up.
Table 6.4  Multiple linear regression models investigating independent associations of MRI measures with walking speed at 5 years (n=101)

<table>
<thead>
<tr>
<th>MRI measure</th>
<th>Coefficient</th>
<th>95% CI</th>
<th>p</th>
<th>R² (Adjusted R²)</th>
</tr>
</thead>
<tbody>
<tr>
<td><strong>Brain MRI measures</strong></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Change in T2LV</td>
<td>-0.004</td>
<td>-0.007, -0.004</td>
<td>0.002</td>
<td></td>
</tr>
<tr>
<td>Baseline NBV</td>
<td>1.32⁻⁷</td>
<td>-2.86⁻⁷, 2.68⁻⁷</td>
<td>0.055</td>
<td></td>
</tr>
<tr>
<td><strong>Spinal cord MRI measures</strong></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Baseline SC lesion number</td>
<td>-0.015</td>
<td>-0.023, -0.008</td>
<td>&lt;0.001</td>
<td></td>
</tr>
<tr>
<td>Change in UCCA</td>
<td>0.004</td>
<td>0.007, 0.001</td>
<td>0.004</td>
<td></td>
</tr>
<tr>
<td><strong>Brain and spinal cord MRI measures combined</strong></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Change in T2LV</td>
<td>-0.004</td>
<td>-0.006, -0.002</td>
<td>0.001</td>
<td></td>
</tr>
<tr>
<td>Baseline SC lesion number</td>
<td>-0.019</td>
<td>-0.026, -0.011</td>
<td>&lt;0.001</td>
<td></td>
</tr>
</tbody>
</table>
Table 6.5  Multiple linear regression models investigating independent associations of MRI measures with 9HPT speed at 5 years (n=101)

<table>
<thead>
<tr>
<th>MRI measure</th>
<th>Coefficient</th>
<th>95% CI</th>
<th>p</th>
<th>R² (Adjusted R²)</th>
</tr>
</thead>
<tbody>
<tr>
<td><strong>Brain MRI measures</strong></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Change in T2LV</td>
<td>-0.0004</td>
<td>-0.0006, -0.0002</td>
<td>&lt;0.001</td>
<td></td>
</tr>
<tr>
<td>Baseline NBV</td>
<td>4.78×10⁻⁶</td>
<td>2.51×10⁻⁶, 7.04×10⁻⁶</td>
<td>&lt;0.001</td>
<td></td>
</tr>
<tr>
<td><strong>Spinal cord MRI measures</strong></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Baseline SC lesion number</td>
<td>-0.002</td>
<td>-0.004, -0.001</td>
<td>0.001</td>
<td></td>
</tr>
<tr>
<td>Change in UCCA</td>
<td>0.001</td>
<td>0.001, 0.002</td>
<td>&lt;0.001</td>
<td></td>
</tr>
<tr>
<td><strong>Brain and spinal cord MRI measures combined</strong></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Change in T2LV</td>
<td>-0.0002</td>
<td>-0.0004, -0.0001</td>
<td>0.008</td>
<td></td>
</tr>
<tr>
<td>Baseline NBV</td>
<td>2.83×10⁻⁷</td>
<td>6.29×10⁻⁹, 5.03×10⁻⁸</td>
<td>0.012</td>
<td></td>
</tr>
<tr>
<td>Baseline SC lesion number</td>
<td>-0.002</td>
<td>-0.003, -0.001</td>
<td>0.003</td>
<td></td>
</tr>
<tr>
<td>Change in UCCA</td>
<td>0.0007</td>
<td>0.0002, 0.0012</td>
<td>0.007</td>
<td></td>
</tr>
</tbody>
</table>
Effect of covariates on multivariate linear regression models

Age, sex and scanner upgrade all had a negligible effect on regression coefficients and were not retained in the models. Because of differences in the length of follow-up from presentation with CIS (range 3.0 – 7.9) disease duration was retained as a covariate in the models.

None of the models were adjusted for DMT use. Patients who started DMT had greater disability at follow-up (mean EDSS 2.28 vs 1.21, p<0.001), likely reflecting reverse causality with more severely affected patients receiving treatment. This is consistent with a greater change in T2LV (9.19 vs 2.15ml, p<0.001), PBVC (-3.24 vs -1.82%, p=0.003), baseline spinal cord lesion number (median 2 vs 0, p<0.001), and change in UCCA (-2.88 vs 1.50 mm² p=0.034) in patients who received DMT compared with patients who were not treated. Since these differences also likely reflect reverse causality, adjustment for DMT is not appropriate (Weinberg, 1993), with a DMT covariate tending to “steal” the effect of MRI variables.

6.4 Discussion

I found that spinal cord MRI abnormalities explained more of the variability in physical disability approximately 5 years after CIS than brain MRI measures. These findings suggest that spinal cord abnormalities, detected using MRI, may be important in the evolution of disability in the first few years after a non-spinal CIS.

A number of studies in patients with established MS have found a poor correlation between spinal cord lesion load and disability (Kidd et al., 1993; Nijeholt et al., 1998; Lukas et al., 2013). Previous studies have largely been cross-sectional in nature and
included MS patients with different disease durations and clinical courses, whereas the cohort of patients I studied have been followed longitudinally from the time of first presentation with CIS.

Because of their location close to pathways involved in walking, balance and sensation, spinal cord lesions may be more likely to cause physical impairment than brain lesions. New spinal cord lesions seen on MRI are more likely to be symptomatic than new brain lesions (Thorpe et al., 1996) and an incomplete recovery is more common following a spinal cord relapse (Leone et al., 2008). The spinal cord lesions seen at baseline in this group of patients with a non-spinal CIS were all asymptomatic. How they contribute to future disability is unclear, although one potential explanation is that axonal loss in focal lesions reduces the functional reserve to prevent permanent deficits when new pathological changes develop in the future. Whether the importance of focal spinal cord lesion accumulation lessens over time, particularly after the onset of secondary progression, is uncertain. Previous studies in patients with progressive MS have found no relationship between spinal cord lesions and disability (Nijeholt et al., 1998; Lukas et al., 2013).

The change in UCCA was independently associated with EDSS and 9HPT performance at follow-up. Spinal cord atrophy in MS is thought to reflect neuroaxonal arising from axonal transection and Wallerian degeneration from focal lesions but also diffuse changes in the normal-appearing grey and white matter (Kearney et al., 2015b). Spinal cord atrophy, as measured by the UCCA, has been correlated with disability in established MS (Losseff et al., 1996; Nijeholt et al., 1998; Stevenson et al., 1998; Furby et al., 2008; Bonati et al., 2011; Rocca et al., 2011; Lukas et al., 2013; Kearney et al., 2014a). Previous studies have suggested that spinal cord atrophy
begins early in the course of MS (Brex et al., 2001; Rashid et al., 2006; Biberacher et al., 2015). I found that UCCA at the time of CIS was associated with EDSS at follow-up, although only with borderline significance.

There have been few longitudinal studies of spinal cord atrophy in people with CIS and early RRMS. In a pilot study that included patients from this cohort cohort no change in UCCA was seen over a follow-up period of one year (Brex et al., 2001). In a small study of patients with early RRMS (disease duration <3 years), the rate of spinal cord atrophy was significantly greater than in healthy controls, however, there was no correlation between cord atrophy and change in disability over 3 years (Rashid et al., 2006). This contrasts with my findings that significantly greater spinal cord atrophy occurred in MS patients, and that the change in UCCA was associated with EDSS at follow-up. These differences might be explained by the longer duration of follow-up, the larger sample size or the use of a more robust method for detecting spinal cord atrophy (Losseff et al., 1996; Horsfield et al., 2010; Kearney et al., 2014b).

Spinal cord imaging is more technically challenging than brain MRI and does require additional scan time. In clinical practice spinal cord MRI is not always used in the diagnosis and monitoring of patients with MS. Recent European and North American guidelines recommend spinal cord imaging in CIS patients presenting with a spinal cord syndrome and in patients where brain MRI is not diagnostic of MS (Rovira et al., 2015; Traboulsee et al., 2016). Whether routine spinal cord imaging should be done in patients with a non-spinal CIS is controversial (Hutchinson, 2014). My findings suggest that spinal cord imaging may not only be helpful diagnostically (Sombekke et al., 2013), but may also provide significant prognostic information when counselling patients with a CIS and early RRMS about how their condition might progress.
Overall the spinal cord MRI measures explained more of the variability in physical disability at 5 years than the brain MRI measures. Of the brain MRI measures investigated, the change in T2LV from baseline to follow-up was independently associated with EDSS, walking speed and hand dexterity at follow-up. These findings are in keeping with other studies that have found that change in T2 lesion load over time is associated with later disability in patients with CIS and early MS (Fisniku et al., 2008a; Swanton et al., 2009; Di Filippo et al., 2010).

Some limitations should be noted. Firstly, disability at 5 years was measured using tests that primarily assess walking (EDSS, TWT) and upper limb function (9HPT). These metrics are likely to be more heavily influenced by spinal cord disease, potentially explaining the stronger relationship with spinal cord than brain MRI measures. Secondly, measures of lesion load but not lesion location were investigated in the multivariable models. Infratentorial brain lesions may have greater functional consequences than supratentorial lesions and previous studies have found that infratentorial lesions are associated with the development of disability in patients with CIS (Minneboo et al., 2004; Tintore et al., 2010). Similarly, in the spinal cord the location of lesions (cervical, thoracic or lumbar) may influence the clinical symptoms and the resulting disability. Finally, I excluded people who presented with a spinal cord CIS in order to investigate the prognostic significance of early, asymptomatic spinal cord involvement in CIS patients. There were too few patients with clinically isolated myelitis in the cohort to separately investigate the influence of asymptomatic spinal cord lesions in patients with a spinal CIS.
6.5 Conclusions

I found that spinal cord MRI measures were more strongly associated with physical disability than brain MRI measures in the first 5 years after a non-spinal CIS. These findings suggest that spinal cord lesions and atrophy may be important in the evolution of disability in early relapse-onset MS.

Over the first 5 years of follow-up relatively few patients in the cohort developed significant disability and only four had SPMS. In the next chapter I investigate the prognostic significance of a number of conventional MRI measures (including spinal cord lesion number) and the development of disability and secondary progressive disease course after 15 years in the CIS cohort.
Chapter 7

Early MRI predictors of long-term disability and secondary progressive disease course in patients with clinically isolated syndrome

7.1 Introduction

Natural history studies have identified demographic and clinical factors associated with shorter latency to the onset of SPMS and development of irreversible disability in relapse-onset MS. These include older age, male sex, a motor presentation, poor recovery from the initial attack, a shorter interval between the first and second attack and a higher number of relapses in the first few years after disease onset (Eriksson et al., 2003; Scalfari et al., 2010; Scalfari et al., 2014). These factors are associated with a worse prognosis in large populations of people with established relapse-onset MS, but have limited utility in counselling individual patients about long-term prognosis.

In people with CIS, conventional MRI is helpful in predicting a second clinical attack and MS; up to 80% of CIS patients with asymptomatic T2-hyperintense brain lesions develop CDMS compared with approximately 20% of patients with a normal brain MRI (Fisniku et al., 2008a; Optic Neuritis Study Group, 2008; Tintore et al., 2015). However, the relationship between brain T2 lesion load and long-term disability is less robust. Previous studies with long-term follow up have only found a modest relationship between brain T2 lesion load at the time of presentation with CIS and physical disability 15 – 20 years later (Fisniku et al., 2008a; Optic Neuritis Study Group, 2008). The change in brain T2 lesion load, particularly in the first few years after disease onset, may have a stronger relationship with long-term disease course, albeit still limited (Fisniku et al., 2008a). There is a need for better prognostic markers.
to help predict future disability and personalise treatment plans in people with CIS and early RRMS.

Conventional MRI measures other than T2 lesion load, including lesion location and activity may be better predictors of future disability, at least in the short to medium term. The presence of asymptomatic infratentorial (Minneboo et al., 2004; Swanton et al., 2009; Tintore et al., 2010), spinal cord (Swanton et al., 2009; Arrambide et al., 2017) and gadolinium-enhancing lesions (Swanton et al., 2009; Di Filippo et al., 2010) are associated with the development of disability over the first 3 – 7 years after a CIS. Whether these factors are important in the evolution of disability in the longer term, when MS-related disability is mainly determined by the onset of secondary progression rather than the effects of individual relapses, is uncertain (Scalfari et al., 2014). In addition to the short duration of follow-up, previous studies have been limited to subgroups of CIS patients, for example acute optic neuritis (Swanton et al., 2009); have considered the prognostic significance of individual MRI measures (e.g. infratentorial lesions) in isolation, rather than in combination with other potentially important MRI abnormalities (Minneboo et al., 2004; Tintore et al., 2010); and most have not included spinal cord imaging (Minneboo et al., 2004; Fisniku et al., 2008a; Di Filippo et al., 2010; Tintore et al., 2010; Tintore et al., 2015). In Chapter 6 I found that spinal cord lesions and atrophy were more strongly associated with disability 5 years after a CIS than brain MRI abnormalities.

The aim of this analysis was to identify early MRI predictors of long-term outcomes in relapse-onset MS. I wanted to investigate the relationship of early brain and spinal cord lesions plus atrophy in patients with CIS with disability and secondary progressive disease course in the longer term.
7.2 Methods

Patients

All patients in the CIS cohort who were followed up after approximately 15 years were included in this investigation. Patients who developed a condition other than MS during follow-up were excluded.

Clinical assessment

MS was diagnosed using the McDonald 2010 criteria (Polman et al., 2011). Disease course was classified as RRMS or SPMS using the Lublin and Reingold 1996 disease course definitions (Lublin et al., 1996). Disability was assessed using the EDSS score (Kurtzke, 1983). In patients who were unable to attend in person for a follow-up visit, I assessed clinical status and disability by telephone interview that included the telephone EDSS (Lechner-Scott et al., 2003).

MRI acquisition and image-analysis

MRI scans of the brain and spinal cord obtained at baseline, 1 year and 3 years were included in this analysis. The number of supratentorial, infratentorial and spinal cord T2 lesions at baseline and the number of new lesions at 1 and 3 years were analysed, along with the number of gadolinium-enhancing lesions (brain and spinal cord combined) at each time point (Figure 7.1).
Figure 7.1 Representative images of patients classified as CIS, RRMS and SPMS at 15 years. MRI scans obtained at baseline (the time of CIS), 1 year and 3 years were analysed.
The NBV at baseline was measured using SIENAX and the PBVC from baseline to 1 year and baseline to 3 years using SIENA. The UCCA was measured at baseline, 1 year and 3 years using the active surface model. The absolute change and percentage change in UCCA from baseline to 1 year and baseline to 3 years was calculated. The methods for determining brain and spinal cord atrophy are described in more detail in earlier chapters.

**Statistical analysis**

Univariable linear regression was used to compare differences in MRI measures at baseline, 1 year and 3 years based on disease course at 15 years (CIS, RRMS, SPMS).

Multivariable logistic and linear regression models were used to identify independent MRI predictors of SPMS disease course and disability (measured using the EDSS) at 15 years. For each of the two outcomes three separate models were constructed using MRI data available from each of the three time points:

i. *Baseline model* – predictor variables were supratentorial, infratentorial, spinal cord and gadolinium-enhancing lesion number (investigating both binary [0/1+] and categorical [0/1/2+] groupings); and NBV and UCCA as continuous variables.

ii. *1 year model* – baseline MRI predictors (again using binary and categorical groupings) plus the change in supratentorial, infratentorial and spinal cord lesion number at 1 year, the number of gadolinium-enhancing lesions at 1
year; and as continuous variables the PBVC from baseline to 1 year and the change in UCCA from baseline to 1 year.

iii. 3 year model – baseline and 1 year MRI predictors (again using binary and categorical groupings) plus the change in supratentorial, infratentorial and spinal cord lesion number at 3 years, the number of gadolinium-enhancing lesions at 3 year; and as continuous variables the PBVC from baseline to 3 years and the change in UCCA from baseline to 3 year.

For the multivariable logistic regression of the binary outcome of SPMS at 15 years, models were built using manual backwards stepwise elimination of variables with p>0.08; to the final model each of the omitted variables was added singly, and retained if p<0.08. Additionally, the following potential confounders were tested, and retained only if they materially affected either the MRI coefficients or their p values: age at onset, gender, type of CIS presentation and disease duration (time from CIS onset to 15 year follow-up assessment). These potential confounders all had a negligible impact, so were not included the final reported models. The model C-statistic (range 0.5 [no predictive power] to 1 [perfect prediction]) was used to measure the performance of the models.

The same process was used to build linear regression models for 15-year EDSS, but using the \( R^2 \) statistic to measure predictive performance. Although the linear regression residuals showed no obvious non-normality, as a precaution the EDSS model inferences were checked using a non-parametric bias-corrected and accelerated bootstrap with 2000 replications (Carpenter and Bithell, 2000). Statistical significance is reported as p<0.05.
7.3 Results

178 patients were recruited into the study and 166 (93%) were followed up after a mean of 15.1 years (range 11.2 – 19.7 years). Two patients were diagnosed with NMOSD during follow-up (one each with myelitis and optic neuritis) and were excluded, leaving 164 patients.

The baseline demographic and clinical profile of the patients followed up at 15 years and the patients lost to follow-up is shown in Table 7.1. Among the 12 (7%) patients lost to follow-up, males were over-represented, but other baseline characteristics were similar.

<table>
<thead>
<tr>
<th>Table 7.1</th>
<th>Baseline clinical and demographic characteristics of the CIS cohort</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>15 year follow-up*</td>
</tr>
<tr>
<td></td>
<td>(n=164)</td>
</tr>
<tr>
<td></td>
<td>Lost to follow-up</td>
</tr>
<tr>
<td></td>
<td>(n=12)</td>
</tr>
<tr>
<td>Age, mean (SD)</td>
<td>32.2 (7.6)</td>
</tr>
<tr>
<td>Female, n (%)</td>
<td>109 (66)</td>
</tr>
<tr>
<td>Presentation, n (%)</td>
<td></td>
</tr>
<tr>
<td>Optic neuritis</td>
<td>134 (82)</td>
</tr>
<tr>
<td>Brainstem syndrome</td>
<td>19 (11.5)</td>
</tr>
<tr>
<td>Spinal cord syndrome</td>
<td>10 (6)</td>
</tr>
<tr>
<td>Hemispheric syndrome</td>
<td>1 (0.5)</td>
</tr>
<tr>
<td>Baseline EDSS, median (IQR)</td>
<td>1 (1)</td>
</tr>
<tr>
<td>Abnormal brain MRI, n (%)</td>
<td>125 (76)</td>
</tr>
<tr>
<td>Abnormal spinal MRI, n (%)</td>
<td>58 (35)</td>
</tr>
</tbody>
</table>

* Excluding two patients who developed NMOSD.
Disease course and disability at 15 years was assessed by telephone interview in 47 (29\%) patients. The reasons for telephone interview were patient choice (n=34), living overseas (n=8), severe disability (n=3) and pregnancy (n=2).

**Development of multiple sclerosis**

119 (73\%) patients developed MS and 45 (27\%) patients remained CIS. Among the 119 patients with MS, 101 (85\%) had clinically-definite or clinically-probable MS using the Poser criteria and 18 (15\%) patients satisfied the McDonald 2010 MRI criteria for MS, but did not experience a second clinical attack during follow-up. The number of patients who developed MS was similar in patients who presented with optic neuritis (74\%), brainstem/cerebellar (65\%) and spinal cord (64\%) syndromes. Disease course in the MS patients at 15 years was classified as RRMS in 94 (79\%) patients and SPMS in 25 (21\%) patients.

34 (29\%) MS patients were receiving DMT at the time of follow-up or had been exposed to one or more DMTs in the past, including beta interferon (n=22), glatiramer acetate (n=11), natalizumab (n=5), fingolimod (n=4), dimethylfumarate (n=1), teriflunomide (n=1) and alemtuzumab (n=1).

**Development of disability**

The median EDSS at follow-up was 0 (range 0 – 1) in patients who remained CIS and 2.0 (range 0 – 10) in patients with MS. A wide range of disability encompassing all levels of the EDSS was seen in the patients with MS (Figure 7.2). Three patients had died from complications of severe MS at follow-up and were assigned EDSS 10.
Figure 7.2  EDSS scores after 15 years in the patients who developed multiple sclerosis (n=119)
The median EDSS at follow-up was similar in patients with different CIS topographies: optic neuritis 1.5 (range 0 – 10), brainstem syndrome 1.5 (range 0 – 10) and spinal cord syndrome 2.5 (range 0 – 8.5).

**Baseline MRI findings**

The baseline brain MRI showed one or more asymptomatic T2-hyperintense lesion(s) in 125 (76%) patients. MS developed in 111 of 125 (89%) patients with an abnormal brain MRI at baseline and 8 of 39 (21%) with a normal brain MRI. The median EDSS at 15 years was 2 (range 0 – 10) in patients with an abnormal baseline brain MRI and 0 (range 0 – 4) in patients with a normal brain MRI.

The baseline spinal cord MRI showed one or more asymptomatic T2-hyperintense lesion(s) in 58 (35%) patients. MS developed in all 58 (100%) patients with an abnormal spinal cord MRI at baseline and in 62 of 106 (58%) patients with a normal spinal cord MRI. The median EDSS at 15 years was 2.5 (range 0 – 10) in patients with an abnormal baseline spinal cord MRI and 1.5 (range 0 – 9) in patients with a normal spinal cord MRI.

The baseline MRI findings grouped by disease course at 15 years are shown in Table 7.2. Compared with the patients with RRMS at follow-up, the SPMS patients showed a greater number of spinal cord lesions (1.60 vs 1.00, p=0.007) and a trend towards a higher number of gadolinium-enhancing lesions (2.72 vs 1.29, p=0.084) at baseline. There were no differences in the brain or spinal cord volumetric measures at baseline.
Table 7.2  Baseline MRI findings grouped by clinical status at 15 years.

<table>
<thead>
<tr>
<th></th>
<th>CIS at 15 years (n=45)</th>
<th>RRMS (n=94)</th>
<th>SPMS (n=25)</th>
<th>p*</th>
<th>All patients (n=164)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Supratentorial lesions, n</td>
<td>0.58 (1.3)</td>
<td>16.72 (23.0)</td>
<td>18.33 (19.3)</td>
<td>0.630</td>
<td>12.53 (20.3)</td>
</tr>
<tr>
<td>Infratentorial lesions, n</td>
<td>0 (0)</td>
<td>0.98 (1.3)</td>
<td>2.32 (3.8)</td>
<td>0.133</td>
<td>0.94 (1.9)</td>
</tr>
<tr>
<td>Spinal cord lesions, n</td>
<td>0 (0)</td>
<td>1 (1.8)</td>
<td>1.60 (1.7)</td>
<td>0.007</td>
<td>0.82 (1.6)</td>
</tr>
<tr>
<td>GdE lesions, n</td>
<td>0 (0)</td>
<td>1.29 (3.1)</td>
<td>2.72 (4.9)</td>
<td>0.084</td>
<td>1.15 (3.1)</td>
</tr>
<tr>
<td>NBV, cm³</td>
<td>1589 (76)</td>
<td>1589 (69)</td>
<td>1562 (84)</td>
<td>0.723</td>
<td>1585 (74)</td>
</tr>
<tr>
<td>UCCA, mm²</td>
<td>77.56 (7.2)</td>
<td>74.64 (7.7)</td>
<td>76.35 (6.5)</td>
<td>0.678</td>
<td>75.77 (7.5)</td>
</tr>
</tbody>
</table>

All data presented as mean (SD)

* p values for comparisons between RRMS and SPMS patients
**Follow-up MRI findings**

The MRI findings at 1 year and 3 years are shown in Tables 7.3 and 7.4.

At 1 year the patients who developed SPMS showed a higher number of new supratentorial (16.50 vs 3.92, p<0.001), infratentorial (2.30 vs 0.24, p<0.001) and spinal cord (0.85 vs 0.23, p=0.001) lesions compared with the patients with RRMS after 15 years. The patients with SPMS at follow-up also had a higher number of gadolinium-enhancing lesions at 1 year (3.70 vs 0.85, p=0.005) compared with those with RRMS.

The findings at 3 years were similar: the patients with SPMS had a higher number of new supratentorial, infratentorial and spinal cord lesions compared with those with RRMS (Table 7.4). However, the number of gadolinium-enhancing lesions at 3 years was similar in the two groups.

The rate of brain atrophy was similar at 1 and 3 years in patients with SPMS and RRMS at follow-up (Tables 7.3 and 7.4). A faster rate of spinal cord atrophy from baseline to 3 years was observed in the patients who developed SPMS compared with those with RRMS (-3.10 vs -1.14%, p=0.029).
Table 7.3  Follow-up MRI findings at 1 year grouped by clinical status at 15 years.

<table>
<thead>
<tr>
<th></th>
<th>CIS at 15 years</th>
<th>RRMS (n=79)</th>
<th>SPMS (n=20)</th>
<th>p*</th>
<th>All patients (n=136)</th>
</tr>
</thead>
<tbody>
<tr>
<td>New supratentorial lesions, n</td>
<td>0 (0)</td>
<td>3.92 (5.6)</td>
<td>16.5 (28.1)</td>
<td>&lt;0.001</td>
<td>4.71 (12.5)</td>
</tr>
<tr>
<td>New Infratentorial lesions, n</td>
<td>0 (0)</td>
<td>0.24 (0.7)</td>
<td>2.3 (3.5)</td>
<td>&lt;0.001</td>
<td>0.48 (1.6)</td>
</tr>
<tr>
<td>New Spinal cord lesions, n</td>
<td>0 (0)</td>
<td>0.23 (0.6)</td>
<td>0.85 (0.93)</td>
<td>0.001</td>
<td>0.26 (0.6)</td>
</tr>
<tr>
<td>GdE lesions, n</td>
<td>0 (0)</td>
<td>0.85 (1.8)</td>
<td>3.70 (8.2)</td>
<td>0.005</td>
<td>1.04 (3.6)</td>
</tr>
<tr>
<td>PBVC, %</td>
<td>-0.11 (0.3)</td>
<td>-0.42 (0.5)</td>
<td>-0.46 (0.7)</td>
<td>0.772</td>
<td>-0.34 (0.5)</td>
</tr>
<tr>
<td>Percentage UCCA change, %</td>
<td>-0.03 (1.14)</td>
<td>-0.36 (2.2)</td>
<td>-1.50 (2.8)</td>
<td>0.057</td>
<td>-0.44 (2.1)</td>
</tr>
</tbody>
</table>

All data presented as mean (SD)

*p values for comparisons between RRMS and SPMS patients
Table 7.4 Follow-up MRI findings at 3 years grouped by clinical status at 15 years.

<table>
<thead>
<tr>
<th></th>
<th>CIS at 15 years (n=35)</th>
<th>RRMS (n=69)</th>
<th>SPMS (n=17)</th>
<th>p*</th>
<th>All patients (n=121)</th>
</tr>
</thead>
<tbody>
<tr>
<td>New supratentorial lesions, n</td>
<td>0.06 (0.3)</td>
<td>11.91 (21.7)</td>
<td>31.56 (57.2)</td>
<td>0.026</td>
<td>11.0 (27.7)</td>
</tr>
<tr>
<td>New Infratentorial lesions, n</td>
<td>0 (0)</td>
<td>0.86 (1.9)</td>
<td>3.75 (4.7)</td>
<td>&lt;0.001</td>
<td>0.98 (2.45)</td>
</tr>
<tr>
<td>New Spinal cord lesions, n</td>
<td>0 (0)</td>
<td>0.61 (1.0)</td>
<td>3.19 (2.2)</td>
<td>&lt;0.001</td>
<td>0.77 (1.5)</td>
</tr>
<tr>
<td>GdE lesions, n</td>
<td>0 (0)</td>
<td>0.96 (3.7)</td>
<td>1.75 (2.3)</td>
<td>0.412</td>
<td>0.78 (2.9)</td>
</tr>
<tr>
<td>PBVC, %</td>
<td>-0.42 (0.6)</td>
<td>-1.19 (1.0)</td>
<td>-1.59 (1.9)</td>
<td>0.230</td>
<td>-1.02 (1.1)</td>
</tr>
<tr>
<td>Percentage UCCA change, %</td>
<td>-0.27 (1.3)</td>
<td>-1.14 (2.7)</td>
<td>-3.10 (4.7)</td>
<td>0.029</td>
<td>-1.14 (2.8)</td>
</tr>
</tbody>
</table>

All data presented as mean (SD)

*p values for comparisons between RRMS and SPMS patients
**MRI predictors of SPMS: multivariable logistic regression analysis**

The results of the multivariable logistic regression analysis investigating early MRI predictors of SPMS at 15 years are presented in Table 7.5. In the baseline MRI model gadolinium-enhancing lesions and spinal cord lesions were independently associated with SPMS at 15 years (C-statistic 0.76). In patients with no gadolinium-enhancing lesions and no spinal cord lesions at baseline the estimated risk of SPMS at 15 years was 5.3% (95% CI 1.1 – 9.5%), compared with 45.5% (95% CI 24.7 – 66.4%) in those with at least one spinal cord lesion and two or more gadolinium-enhancing lesions.

In the model incorporating baseline and follow-up MRI data over 1 year, baseline gadolinium-enhancing lesions remained significant and the number of new spinal cord and infratentorial lesions at 1 year were independently associated with SPMS (C-statistic 0.86). In patients with no gadolinium-enhancing lesions at baseline and no new spinal or infratentorial lesions after 1 year the estimated risk of SPMS after 15 years was 3.0% (95% CI 0 – 6.2%), compared with 85.2% (95% CI 67.7 – 100%) in people with two or more gadolinium-enhancing lesions at baseline and new spinal and infratentorial lesions at 1 year.

Finally, in the model incorporating all MRI data from baseline to 3 years, new spinal cord lesions and with borderline significance new infratentorial lesions between baseline and 3 years were associated with SPMS course at 15 years (C-statistic 0.89). In patients with no new spinal or infratentorial lesions over the first 3 years after CIS the estimated risk of SPMS after 15 years was 0.9% (95% CI 0 – 2.7%), compared with 53.1% (31.7 – 74.6%) in patients with new spinal and infratentorial lesions.
Table 7.5  Multivariable logistic regression models investigating early MRI predictors of secondary progressive disease course after 15 years.

<table>
<thead>
<tr>
<th></th>
<th>Odds ratio</th>
<th>95% CI</th>
<th>p</th>
<th>C-statistic</th>
</tr>
</thead>
<tbody>
<tr>
<td><strong>Baseline (n=164)</strong></td>
<td></td>
<td></td>
<td></td>
<td>0.76</td>
</tr>
<tr>
<td>Baseline GdE lesions</td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>1</td>
<td>1.33</td>
<td>0.35, 5.07</td>
<td>0.678</td>
<td></td>
</tr>
<tr>
<td>2+</td>
<td>3.16</td>
<td>1.08, 9.23</td>
<td>0.035</td>
<td></td>
</tr>
<tr>
<td>Baseline spinal cord lesions</td>
<td>4.71</td>
<td>1.72, 12.92</td>
<td>0.003</td>
<td></td>
</tr>
<tr>
<td><strong>Baseline – 1 year (n=136)</strong></td>
<td></td>
<td></td>
<td></td>
<td>0.86</td>
</tr>
<tr>
<td>Baseline GdE lesions</td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>1</td>
<td>2.31</td>
<td>0.47, 11.40</td>
<td>0.306</td>
<td></td>
</tr>
<tr>
<td>2+</td>
<td>4.58</td>
<td>1.19, 17.71</td>
<td>0.027</td>
<td></td>
</tr>
<tr>
<td>New spinal cord lesions</td>
<td>5.72</td>
<td>1.67, 19.56</td>
<td>0.005</td>
<td></td>
</tr>
<tr>
<td>New infratentorial lesions</td>
<td>7.02</td>
<td>2.06, 23.94</td>
<td>0.002</td>
<td></td>
</tr>
<tr>
<td><strong>Baseline – 3 years (n=121)</strong></td>
<td></td>
<td></td>
<td></td>
<td>0.89</td>
</tr>
<tr>
<td>New spinal cord lesions</td>
<td>38.68</td>
<td>4.67, 320.53</td>
<td>0.001</td>
<td></td>
</tr>
<tr>
<td>New infratentorial lesions</td>
<td>3.28</td>
<td>0.87, 12.31</td>
<td>0.079</td>
<td></td>
</tr>
</tbody>
</table>
**MRI predictors of disability: multivariable linear regression analysis**

The results of the multivariable linear regression models investigating independent MRI predictors of disability at follow-up are shown in Table 7.6.

In the model investigating baseline MRI predictors of long-term disability, baseline supatentorial lesions, gadolinium-enhancing lesions and spinal cord lesions were independently associated with EDSS at 15 years (R²=0.31).

In the 1 year model, baseline gadolinium-enhancing lesions and spinal cord lesions remained significant, and in the smaller group of patients with MRI follow-up at 1 year (n=136) the baseline NBV was also borderline significant. New supatentorial, infratentorial and spinal cord lesions at 1 year were also independently associated with disability at 15 years (R²=0.48).

Finally, in the model investigating all time points over the first 3 years after CIS, baseline gadolinium-enhancing lesions and spinal cord lesions remained significant predictors of disability at 15 years, and new spinal cord lesions and the PBVC at 3 years were also associated with EDSS (R²=0.58).
Table 7.6  Multivariable linear regression models investigating MRI predictors of EDSS at 15 years.

<table>
<thead>
<tr>
<th>MRI measure</th>
<th>Coefficient</th>
<th>95% CI</th>
<th>( p )</th>
<th>( R^2 ) (Adjusted ( R^2 ))</th>
</tr>
</thead>
<tbody>
<tr>
<td><strong>Baseline (n=164)</strong></td>
<td></td>
<td></td>
<td></td>
<td>0.31 (0.30)</td>
</tr>
<tr>
<td>Baseline spinal cord lesions</td>
<td>1.53</td>
<td>0.84, 2.23</td>
<td>&lt;0.001</td>
<td></td>
</tr>
<tr>
<td>Baseline GdE lesions</td>
<td>1.32</td>
<td>0.58, 2.06</td>
<td>0.001</td>
<td></td>
</tr>
<tr>
<td>Baseline supratentorial lesions</td>
<td>0.96</td>
<td>0.20, 1.71</td>
<td>0.014</td>
<td></td>
</tr>
<tr>
<td><strong>Baseline – 1 year (n=136)</strong></td>
<td></td>
<td></td>
<td></td>
<td>0.48 (0.45)</td>
</tr>
<tr>
<td>Baseline spinal cord lesions</td>
<td>0.71</td>
<td>0.03, 1.38</td>
<td>0.040</td>
<td></td>
</tr>
<tr>
<td>Baseline GdE lesions</td>
<td>1.27</td>
<td>0.57, 1.96</td>
<td>&lt;0.001</td>
<td></td>
</tr>
<tr>
<td>Baseline NBV</td>
<td>-3.71e-6</td>
<td>-7.60e-6, 1.89e-7</td>
<td>0.062</td>
<td></td>
</tr>
<tr>
<td>New spinal cord lesions 1 year</td>
<td>0.73</td>
<td>-0.08, 1.54</td>
<td>0.058</td>
<td></td>
</tr>
<tr>
<td>New infratentorial lesions 1 year</td>
<td>1.90</td>
<td>1.04, 2.78</td>
<td>&lt;0.001</td>
<td></td>
</tr>
<tr>
<td>New supratentorial lesions 1 year</td>
<td>0.79</td>
<td>0.13, 1.45</td>
<td>0.019</td>
<td></td>
</tr>
<tr>
<td><strong>Baseline – 3 years (n=121)</strong></td>
<td></td>
<td></td>
<td></td>
<td>0.58 (0.56)</td>
</tr>
<tr>
<td>Baseline spinal cord lesions</td>
<td>0.88</td>
<td>0.21, 1.54</td>
<td>0.011</td>
<td></td>
</tr>
<tr>
<td>Baseline GdE lesions</td>
<td>1.04</td>
<td>0.36, 1.72</td>
<td>0.003</td>
<td></td>
</tr>
<tr>
<td>New spinal cord lesions 3 years</td>
<td>2.30</td>
<td>1.65, 2.95</td>
<td>&lt;0.001</td>
<td></td>
</tr>
<tr>
<td>PBVC 3 years</td>
<td>-0.53</td>
<td>-0.79, -0.26</td>
<td>&lt;0.001</td>
<td></td>
</tr>
</tbody>
</table>
7.4 Discussion

I investigated early brain and spinal cord abnormalities over the first 3 years after CIS to identify the most robust predictors of long-term disability and secondary progressive disease course at 15 years. The major findings of this analysis include: (1) a consistent association of spinal cord lesions, both at the time of presentation with CIS and new spinal cord lesions over the first 3 years of follow-up, with physical disability and disease course after 15 years; (2) an association of asymptomatic gadolinium-enhancing lesions at the time of CIS with long-term disease outcomes; (3) a stronger association of lesional MRI measures in the earliest stages of relapse-onset MS with later disability and secondary progression compared with measures of brain and spinal cord atrophy.

Previous studies have suggested that lesions in clinically-eloquent sites, such as the brainstem, cerebellum and spinal cord, may be important predictors of later disability in CIS patients, at least in the short to medium term (Minneboo et al., 2004; Swanton et al., 2009; Tintore et al., 2010). I found that asymptomatic spinal cord lesions were the only baseline MRI predictor of disability after ~5 years in patients with a non-spinal CIS (Chapter 6). The importance of spinal cord lesions in the longer term is confirmed in this same cohort of patients with long-term follow-up: spinal cord lesions at baseline were associated with disability and the development of SPMS at 15 years. The importance of spinal cord lesions present at the time of CIS is highlighted in the models incorporating changes in MRI measures over 1 and 3 years after CIS. Baseline spinal cord lesions remained a significant predictor of EDSS at 15 years independent of changes in brain and spinal cord lesions and atrophy at 1 and 3 years. In addition to the spinal cord lesions seen at the time of CIS onset, the number of new spinal cord lesions after 1 year and 3 years was also independently associated with disability and SPMS at 15 years. Collectively these findings suggest that early spinal
cord damage is an important mechanism underlying both development of disability and secondary progression in early relapse-onset MS.

Pathological studies have found evidence of substantial axonal loss within chronic spinal cord lesions in MS (Lovas et al., 2000) and this may result in more widespread neuroaxonal loss at distant sites due to the effects of Wallerian degeneration (Dziedzic et al., 2010). Neuroaxonal loss within spinal cord pathways involved in locomotion, sensation and sphincter function may have important functional consequences, ultimately resulting in physical disability. In a recent study that quantified cervical cord lesion load on axial images with high in-plane resolution, patients with SPMS had a higher spinal cord lesion load compared RRMS patients, even after adjusting for differences in disease duration (Kearney et al., 2015a). These findings support the concept that focal spinal cord damage may be one of the factors involved in the development of secondary progression in relapse-onset MS.

Like the spinal cord, the brainstem and cerebellum contain pathways critical for balance and locomotion. Two previous studies found that infratentorial lesions seen at the time of presentation with CIS are associated with physical disability in the short to medium term (Minneboo et al., 2004; Tintore et al., 2010). Infratentorial lesions seen at baseline were not independently associated with long-term outcomes, possibly because the effect is eclipsed by the impact of spinal cord lesions. However, accrual of new infratentorial lesions over time was associated with disability and secondary progressive disease course at 15 years, confirming the prognostic importance of lesion location.
Gadolinium-enhancing lesions at the time of presentation with CIS were also associated with disability and the development of secondary progressive course after 15 years. Importantly, in the models examining MRI predictors of EDSS at follow-up, the association of gadolinium-enhancing lesions remained even after including changes in MRI measures at 1 and 3 years. Gadolinium-enhancing lesions reflect areas of acute inflammatory activity associated with breakdown of the blood-brain barrier (Filippi et al., 2012). A meta-analysis of observational studies and patients enrolled in the placebo arms of clinical trials found that gadolinium-enhancing lesions are predictive of relapse rates in the short-term (Kappos et al., 1999). The association of early gadolinium-enhancing lesions with long-term disability is consistent with findings from natural history studies showing that the number of early relapses in the first few years after disease onset is associated with long-term disability and development of SPMS in relapse-onset MS (Confavreux et al., 2003; Scalfari et al., 2014).

In this cohort of patients followed prospectively from CIS onset, lesional MRI measures, particularly lesion location (spinal cord, infratentorial) and lesion activity (i.e. gadolinium-enhancement), were more consistently associated with disability and secondary progressive disease course at 15 years than early brain or spinal cord atrophy. A similar rate of whole brain atrophy was observed over the first 3 years after CIS onset in patients who developed MS irrespective of disease course at 15 years. This is consistent with previous studies showing that brain atrophy begins early in the course of MS and progresses at a similar rate throughout the course of the illness, irrespective of disease phenotype or disease duration (De Stefano et al., 2010). The PBVC from baseline to 3 years, however, was independently associated with disability after 15 years, although not with the development of SPMS. Significant spinal cord atrophy was also observed over the first 3 years after CIS, with a faster
rate of spinal cord atrophy in patients with SPMS at 15 years compared with those with RRMS. Early changes in spinal cord atrophy (up to 3 years) were not independently associated with outcomes after 15 years, at least when considered in conjunction with other MRI measures. This contrasts with the findings presented in Chapter 6 that change in UCCA was associated with physical disability, although in that analysis change in UCCA over a longer period (~5 years) was investigated rather than over 1 and 3 years in these models.

A wide range of disability was observed at 15 years reflecting the variable prognosis of relapse-onset MS. Disability was overall low at 15 years: only 1 in 4 patients had an EDSS of 3 or more at follow-up and only 1 in 7 developed SPMS. This contrasts with the much worse prognosis reported in natural history studies done in the 1970s and 1980s (Confavreux et al., 2003; Tremlett et al., 2006; Scalfari et al., 2010; Scalfari et al., 2014). For example, in the London-Ontario cohort, half of patients required unilateral assistance to walk 15 years after disease onset (Scalfari et al., 2014). There are a number of possible explanations for the differences in the rate of disability progression. Firstly, I studied a group of people who were followed prospectively from disease onset at the time of presentation with a CIS. The patients were followed up irrespective of disease status and not through a hospital-based MS clinic, potentially biased towards more severely affected patients. Secondly, patients with optic neuritis are over-represented in this cohort. Optic neuritis usually accounts for 30-40% of people with CIS, compared with 80% in the cohort that I studied (Tintore et al., 2015). Some studies have suggested that optic neuritis may be associated with a better prognosis compared with other CIS presentations (Eriksson et al., 2003; Tintore et al., 2005; Tintore et al., 2015). However, more than three quarters of the patients with optic neuritis in this cohort had an abnormal baseline MRI scan indicating a group at high-risk for the development of MS and future disability. Thirdly, during the course of
the study DMT for RRMS became available in the United Kingdom. These treatments reduce relapse rates and may reduce progression of disability over time, improving the prognosis of people with MS. Less than a third of patients in the study received DMT, and most patients who were treated received first-line injectable therapies (beta interferon or glatiramer acetate) rather than the more potent oral or monoclonal antibody therapies. Like in Chapter 6 no adjustment was made for DMT in the multivariable models because criteria for treating patients in the United Kingdom at the time of the study required two attacks in two years so more severely affected patients received treatment leading to reverse causality. Finally, the diagnosis of MS was made using the McDonald criteria and includes people who in the past would have been labelled as having CIS rather than MS. The so-called Will-Rogers phenomenon describes the apparent improvement in prognosis with changes to diagnostic criteria (Sormani et al., 2008), as discussed in Chapter 4. However, the rate of SPMS was still low at 15 years in patients in whom the diagnosis of MS was clinically-definite (<25%). My findings of a more favourable long-term outcome than would be expected from natural history studies is consistent with other contemporary observational cohort studies (Fisniku et al., 2008a; Kerbrat et al., 2015; Tintore et al., 2015; Cree et al., 2016).

The strengths of this study include the prospective design, the longitudinal MRI acquisition (including both brain and spinal cord), the uniquely long follow-up duration and high rate of follow-up after 15 years. Some limitations also need to be noted. Firstly, the two outcome measures used, the EDSS and secondary progression, are both heavily weighted towards ambulatory disability. Cognitive impairment, fatigue and affective disorders commonly occur in people with MS even in the absence of significant physical disability (Amato et al., 2006). Cognitive testing was done in patients who retuned in person for the 15 year follow-up and analyses are ongoing.
Secondly, clinical status after 15 years was assessed in a significant number of patients by telephone interview because not all patients were able to return for a follow-up visit to be examined in person. The use of telephone EDSS has been validated previously for use in clinical trials (Lechner-Scott et al., 2003) and has also been used in previous longitudinal clinical-MRI studies (Fisniku et al., 2008a). Thirdly, as discussed earlier, the CIS cohort studied is mainly comprised of people with optic neuritis. Validation of the predictive models in other CIS cohorts, particularly cohorts with a more representative number of patients with brainstem/cerebellar and spinal cord syndromes would be of interest. Finally, although the mean duration of follow-up was over 15 years the course of MS often unfolds over much longer and the number of people developing secondary progression and worsening disability in this cohort is likely to increase with time (Skoog et al., 2012; Scalfari et al., 2014). However, the findings do identify early MRI predictors associated with a more aggressive course over the first 15 years after disease onset and may potentially help inform treatment decisions in patients with CIS and early relapse-onset MS.

7.5 Conclusions

MRI abnormalities seen around the time of presentation with CIS and over the first few years after disease onset may be helpful in predicting the development of long-term disability and secondary progression in early relapse-onset MS. Early changes in spinal cord and infratentorial lesion load plus the number of gadolinium-enhancing lesions seen around the time of presentation with CIS displayed the most consistent association with disability and secondary progressive disease course at 15 years. These findings suggest that the accrual of focal lesions in clinically-eloquent sites and the extent of early inflammatory disease activity may be important mechanisms that contribute to long-term disability and secondary progression in relapse-onset MS.
In keeping with previous studies a wide range of clinical outcomes was observed in this cohort of patients followed for approximately 15 years after disease onset. The underlying biological factors responsible for disease course heterogeneity in MS remain poorly understood. In Chapter 8 I investigate how HLA DRB1:15*01, the most important genetic susceptibility factor for MS, influences disease course in relapse-onset MS.
Chapter 8

*HLA-DRB1*1501 is associated with the development of disability and MRI-detected tissue damage over the first 15 years after a clinically isolated syndrome

### 8.1 Introduction

Over 100 genetic loci have been identified that influence susceptibility to MS, many of which are implicated in immune system processes relevant to the pathogenesis of MS (International Multiple Sclerosis Genetics Consortium *et al.*, 2013) The extended *HLA-DRB1*1501 haplotype is the most important genetic risk factor for MS (Moutsianas *et al.*, 2015). In Northern European populations up to half of people with MS carry at least one *HLA-DRB1*1501 allele (compared with 10-20% of healthy controls), and *HLA-DRB1*1501 positivity is associated with an approximately threefold increase in the risk of developing MS (Moutsianas *et al.*, 2015).

The course of MS is highly variable with significant inter-individual variation in disease onset, relapse rates, MRI abnormalities and long-term disability. Genetic factors may be one of the mechanisms responsible for these differences (Hensiek *et al.*, 2007). The *HLA-DRB1*1501 allele has consistently been associated with younger age at disease onset (Masterman *et al.*, 2000; Hensiek *et al.*, 2002; International Multiple Sclerosis Genetics Consortium *et al.*, 2011; Moutsianas *et al.*, 2015), with each copy of the *HLA-DRB1*1501 allele decreasing age at onset by approximately 11 months (International Multiple Sclerosis Genetics Consortium *et al.*, 2011). However, a number of studies have found no relationship between *HLA-DRB1*1501 and disease course (relapse or progressive-onset MS) or disease severity measured using the EDSS or the Multiple Sclerosis Severity Score (MSSS) (Masterman *et al.*, 2000;
Hensiek et al., 2002; International Multiple Sclerosis Genetics Consortium et al., 2011; Moutsianas et al., 2015).

A number of studies have investigated the influence of the HLA-DRB1*1501 allele on MRI-detected brain and spinal cord pathology in patients with CIS, relapse-onset MS and PPMS, with inconsistent findings (Hauser et al., 2000b; Zivadinov et al., 2003; Zivadinov et al., 2007; Okuda et al., 2009; Sombekke et al., 2009; Horakova et al., 2011; Van der Walt et al., 2011; Tur et al., 2014; Isobe et al., 2016; Yaldizli et al., 2016b). Some studies have found more focal T2-hyperintense lesions (Hauser et al., 2000b; Okuda et al., 2009; Sombekke et al., 2009; Horakova et al., 2011; Tur et al., 2014), brain atrophy (Zivadinov et al., 2007; Okuda et al., 2009; Isobe et al., 2016), and microstructural tissue damage (Okuda et al., 2009; Tur et al., 2014) in HLA-DRB1*1501-positive compared with HLA-DRB1*1501-negative patients, but others have not (Zivadinov et al., 2003; Van der Walt et al., 2011; Yaldizli et al., 2016b).

Previous studies have largely been cross-sectional in nature and included heterogeneous cohorts of MS patients with varying disease courses and disease duration, potentially explaining these conflicting findings.

The aim of this work was to investigate the influence of HLA-DRB1*1501 on the development of disability and MRI measures of inflammation and neurodegeneration. I wanted to investigate whether HLA-DRB1*1501 may be one of the factors responsible for disease course heterogeneity in a well-characterised, prospective recruited CIS cohort who were deeply phenotyped with MRI over the first 15 years after disease onset.
8.2 Methods

Patients

I included patients from the CIS cohort in this analysis who provided a blood sample that was able to be used for genotyping (n=107) as part of the 15 year follow-up. Clinical and MRI data were included from all available time points from baseline to 15 years.

Clinical assessment

MS was diagnosed using the McDonald 2010 criteria (Polman et al., 2011) and disease course was classified using the Lublin and Reingold 1996 criteria (Lublin et al., 1996). Disability was assessed at each time point using the EDSS (Kurtzke, 1983) and at the 15 year follow-up using the TWT, 9HPT, PASAT (Cutter et al., 1999) and the SDMT (Smith, 1982).

MRI acquisition and image-analysis

The patients underwent PD/T2-weighted and post-contrast T1-weighted MRI scans of the brain and whole spinal cord at baseline, 1, 3 and 5 years on the same 1.5T Signa scanner (General Electric, Wisconsin, USA). There was a major hardware and software upgrade during the study period that was considered in statistical analyses (see below). At 15 years the patients underwent MRI brain only with PD/T2-weighted and T1-weighted scans without contrast. The 15 year MRI scans were all done on the same 3T Achieva TX scanner (Philips Healthcare, Best, The Netherlands). Technical details of the MRI protocols are presented in Chapter 3.
MRI measures analysed for this investigation included: brain T2LV, brain T1LV, brain gadolinium-enhancing lesion number, brain atrophy (NBV at baseline and the PBVC over time), spinal cord lesion number and the UCCA.

**Genotyping**

Genomic DNA was extracted from whole blood using standard techniques. Sanger sequencing was used to identify the rs3135388 single nucleotide polymorphism (SNP). The A allele of rs3135388 is strongly associated with HLA-DRB1*1501 and patients heterozygous or homozygous for the A allele are inferred to be HLA-DRB1*1501-positive (Benesova et al., 2013). All genotyping was done by Dr Andreea Manole, Neurogeneticist, who was blinded to the clinical status and MRI findings.

**Statistical analysis**

Mixed-effects models were used to assess differences at baseline and over time in the MRI measures between HLA-DRB1*1501-positive and HLA-DRB1*1501-negative patients, with the MRI measure of interest as the dependent variable. Linear mixed-effects models were used for all MRI measures except for gadolinium-enhancing lesion number, for which a Poisson mixed-effects model was used. The independent variables included time (in years), genotype (HLA-DRB1*1501-positive or negative) and an interaction term ‘time X genotype’. All models were adjusted for age, sex, scanner upgrade and scanner type (1.5 T or 3 T).

The mixed-effects models were used to examine differences at baseline and over time in the MRI measures between HLA-DRB1*1501-positive and HLA-DRB1*1501-negative patients. When the p value for the regression coefficient for genotype was
<0.05, it was assumed that differences in MRI measures at baseline between the *HLA-DRB1*1501-positive and *HLA-DRB1*1501-negative groups were significantly different. When the *p* value for the regression coefficient of the interaction term ‘time X genotype’ was <0.05, it was assumed that there were significant differences in the change in MRI measures over time in the *HLA-DRB1*1501-positive and *HLA-DRB1*1501-negative groups. Bootstrapping with 1000 replicates was applied to confirm confidence intervals and *p* values in the final models.

### 8.3 Results

The cohort of 107 CIS patients comprised 89 (83%) with optic neuritis, 12 (11%) with a brainstem/cerebellar syndrome, 5 (5%) with partial myelitis and 1 (1%) patient with a hemispheric syndrome.

Fifty-two (49%) patients were *HLA-DRB1*1501-positive (49 [94%] heterozygous, 3 [6%] homozygous) and 55 (51%) were *HLA-DRB1*1501-negative. The baseline demographic and clinical profile of the cohort grouped by *HLA-DRB1*1501 status is shown in Table 9.1. The *HLA-DRB1*1501-positive patients were younger than the *HLA-DRB1*1501-negative patients (mean age 31.1 vs 34.4 years, *p*=0.036) at the time of CIS, but other demographic and clinical characteristics were similar.
Table 8.1 Baseline demographic and clinical characteristics of the genotyped cohort grouped by HLA-DRB1*1501 status.

<table>
<thead>
<tr>
<th></th>
<th>HLA-DRB1*1501- positive (n=52)</th>
<th>HLA-DRB1*1501- negative (n=55)</th>
<th>p</th>
</tr>
</thead>
<tbody>
<tr>
<td>Age, years</td>
<td>31.1 (7.0)</td>
<td>34.4 (7.8)</td>
<td>0.036</td>
</tr>
<tr>
<td>Female, n (%)</td>
<td>36 (65)</td>
<td>40 (73)</td>
<td>0.535</td>
</tr>
<tr>
<td>Optic neuritis, n (%)</td>
<td>45 (87)</td>
<td>44 (80)</td>
<td>0.439</td>
</tr>
<tr>
<td>White ethnicity, n (%)</td>
<td>44 (85)</td>
<td>48 (87)</td>
<td>0.692</td>
</tr>
<tr>
<td>Baseline EDSS, median (IQR)</td>
<td>1 (0.63)</td>
<td>1 (0.5)</td>
<td>0.306</td>
</tr>
</tbody>
</table>

**Development of multiple sclerosis**

Figure 8.1 shows the clinical course at 15 years grouped by HLA-DRB1*1501 status. MS developed in 47/52 (90%) HLA-DRB1*1501-positive patients and 41/55 (75%) HLA-DRB1*1501-negative patients (odds ratio=3.21, 95%CI 1.07 – 9.68, p=0.032). SPMS developed in 11/52 (21%) HLA-DRB1*1501-positive patients and 7/55 (13%) HLA-DRB1*1501-negative patients (odds ratio=1.84, 95%CI 0.65 – 5.18, p=0.248).

The mean time to a second clinical attack was 18.7 months in HLA-DRB1*1501-positive patients and 24.2 months in HLA-DRB1*1501-negative patients, but this was not significant (p=0.229).
Figure 8.1 Clinical course at 15 years grouped by *HLA-DRB1*1501 status.
Development of disability

Disability was low in both groups after 15 years; the median EDSS was 2 (range 0 – 10) in the HLA-DRB1*1501-positive group and 1.5 (range 0 – 10) in the HLA-DRB1*1501-negative group. Figure 8.2 shows the rate of change over time in the EDSS in HLA-DRB1*1501-positive and negative patients. The HLA-DRB1*1501-positive patients showed faster progression of disability compared with the HLA-DRB1*1501-negative patients (annualised change in EDSS 0.14/year vs 0.08/year, p<0.025).

![Figure 8.2](image)

Figure 8.2 Change in EDSS over time grouped by HLA-DRB1*1501 status.

At 15 years the HLA-DRB1*1501-positive patients had slower walking speed (mean 0.17 vs 0.20 feet/second, p=0.025) and worse performance on the PASAT (mean Z score -0.73 vs -0.23, p=0.050) and the SDMT (mean Z score -1.01 vs -0.32, p=0.039) compared with HLA-DRB1*1501-negative patients.
Baseline MRI findings

Baseline MRI findings grouped by HLA-DRB1*1501 status are shown in Table 8.2. The HLA-DRB1*1501-positive patients had a higher T2LV (mean 2.50 ml vs 1.14 ml, p<0.01) at baseline and a greater number of GdE lesions (mean 1.90 vs 0.51, p<0.001) compared with the HLA-DRB1*1501-negative patients. These differences remained even after excluding patients who remained CIS after 15 years.

The T1LV, number of spinal cord lesions, NBV and UCCA were similar in both groups at baseline.

Longitudinal changes in MRI measures: All patients

The longitudinal changes in MRI measures in the HLA-DRB1*1501-positive and HLA-DRB1*1501-negative patients is shown in Table 8.3.
<table>
<thead>
<tr>
<th></th>
<th>All patients</th>
<th>Patients with MS at 15 years</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>HLA-DRB1*1501 positive</td>
<td>HLA-DRB1*1501 negative</td>
</tr>
<tr>
<td></td>
<td>(n=52)</td>
<td>(n=55)</td>
</tr>
<tr>
<td>T2LV, ml</td>
<td>2.50 (4.31)</td>
<td>1.15 (2.17)</td>
</tr>
<tr>
<td>T1LV, ml</td>
<td>0.29 (0.71)</td>
<td>0.12 (0.47)</td>
</tr>
<tr>
<td>GdE lesion number, n</td>
<td>1.90 (3.76)</td>
<td>0.51 (1.49)</td>
</tr>
<tr>
<td>NBV, cm³</td>
<td>1579 (69)</td>
<td>1581 (74)</td>
</tr>
<tr>
<td>SC lesion number, n</td>
<td>0.98 (1.41)</td>
<td>0.89 (1.84)</td>
</tr>
<tr>
<td>UCCA, mm²</td>
<td>75.56 (7.97)</td>
<td>75.17 (7.41)</td>
</tr>
</tbody>
</table>

† Approximate p values obtained from bootstrap analysis for all variables except gadolinium-enhancing lesion number and NBV which were examined using univariable linear regression.

NS (non-significant) indicates p>0.05.
Table 8.3  Annualised change in MRI measures in HLA-DRB1*1501-positive and negative patients.

<table>
<thead>
<tr>
<th></th>
<th>HLA-DRB1*1501-positive (n=52)</th>
<th></th>
<th>HLA-DRB1*1501-negative (n=55)</th>
<th></th>
<th>p†</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>Rate of change (95% CI)</td>
<td>p†</td>
<td>Rate of change (95% CI)</td>
<td>p†</td>
<td></td>
</tr>
<tr>
<td>T2LV, ml</td>
<td>0.79 (0.57, 1.07)</td>
<td>&lt; 0.01</td>
<td>0.34 (0.23, 0.68)</td>
<td>&lt;0.01</td>
<td>&lt; 0.025</td>
</tr>
<tr>
<td>T1LV, ml</td>
<td>0.23 (0.18, 0.30)</td>
<td>&lt; 0.01</td>
<td>0.11 (0.08, 0.16)</td>
<td>&lt;0.01</td>
<td>&lt; 0.01</td>
</tr>
<tr>
<td>PBVC, %</td>
<td>-0.43 (-0.50, -0.36)</td>
<td>&lt;0.01</td>
<td>-0.31 (-0.39, -0.25)</td>
<td>&lt;0.01</td>
<td>&lt; 0.05</td>
</tr>
<tr>
<td>SC lesion number, n</td>
<td>0.27 (0.19, 0.39)</td>
<td>&lt;0.01</td>
<td>0.19 (0.11, 0.32)</td>
<td>&lt;0.01</td>
<td>NS</td>
</tr>
<tr>
<td>UCCA, mm²</td>
<td>-0.45 (-0.58, -0.25)</td>
<td>&lt;0.01</td>
<td>-0.23 (-0.43, -0.09)</td>
<td>&lt;0.01</td>
<td>&lt; 0.05</td>
</tr>
</tbody>
</table>

† Approximate p values obtained from bootstrap analysis.

NS (non-significant) indicates p>0.05.
Brain MRI measures

The T2LV increased in both groups over time (Figure 8.3), but the annualised change in T2LV was significantly higher in the HLA-DRB1*1501-positive patients compared with the HLA-DRB1*1501-negative patients (adjusted difference 0.45 ml/year, 95% CI 0.11 to 0.71, p<0.025).

The T1LV also increased in both groups over time (Figure 8.3), but the annualised change in T1LV was significantly higher in HLA-DRB1*1501-positive patients compared with the HLA-DRB1*1501-negative patients (adjusted difference 0.13 ml/year, 95% CI 0.03 to 0.20, p<0.01).
Figure 8.3  Change in brain lesion load over time in HLA-DRB1*1501-positive and HLA-DRB1*1501-negative patients.

Brain lesion load increased in both groups over time, however, the rate of increase in T2LV and T1LV was significantly faster in the HLA-DRB1*1501-positive compared with HLA-DRB1*1501-negative patients.
Figure 8.4 shows the change in GdE lesion number over time by \textit{HLA-DRB1*1501} status over the first ~5 years of follow-up (gadolinium was not given at the 15 year follow-up). In the Poisson model the \textit{HLA-DRB1*1501}-positive patients had a higher number of GdE at all time points compared with those who were \textit{HLA-DRB1*1501}-negative (incident rate ratio [IRR] = 4.45, 95%CI 1.82 to 10.91, \( p=0.001 \)).

![Graph showing change in gadolinium-enhancing lesion number over time in HLA-DRB1*1501-positive and HLA-DRB1*1501-negative patients](image)

Figure 8.4  Change in gadolinium-enhancing lesion number over time in \textit{HLA-DRB1*1501}-positive and \textit{HLA-DRB1*1501}-negative patients

Both the \textit{HLA-DRB1*1501}-positive and \textit{HLA-DRB1*1501}-negative patients had evidence of brain atrophy over time (Figure 8.5) but the annualised PBVC measured using SIENA was significantly greater in \textit{HLA-DRB1*1501}-positive compared with \textit{HLA-DRB1*1501}-negative patients (adjusted difference -0.12%/year, 95%CI -0.19 to -0.01, \( p<0.01 \)).
Figure 8.5  PBVC over time in *HLA-DRB1*1501-positive and *HLA-DRB1*1501-negative patients.

Given the observed differences in T2 lesion load in *HLA-DRB1*1501-positive and *HLA-DRB1*1501-negative patients the brain atrophy models were repeated after adjusting for T2LV. The results were similar: the annualised PBVC was significantly greater in the *HLA-DRB1*1501-positive compared with the *HLA-DRB1*1501-negative patients (adjusted difference -0.12%/year, 95%CI -0.20 to -0.02, p<0.05)

**Spinal cord MRI measures**

The number of spinal cord lesions increased over time in both the *HLA-DRB1*1501-positive and *HLA-DRB1*1501-negative patients over the first ~5 years of follow-up (Figure 8.6). There was a trend towards a greater increase in spinal cord lesion
number over time in the HLA-DRB1*1501-positive patients, but this was not significant (adjusted difference 0.07 ml/year, 95%CI -0.32 to 0.38, p>0.05).

Although the UCCA was similar between the two groups at baseline, the HLA-DRB1*1501-positive group had evidence of greater spinal cord atrophy compared with HLA-DRB1*1501-negative patients (adjusted difference -0.22 mm²/year, 95%CI -0.36 to -0.01, p<0.05) over the first ~5 years of follow-up (Figure 8.6).
Figure 8.6  Change in spinal cord lesion load and atrophy over time in HLA-DRB1*1501-positive and HLA-DRB1*1501-negative patients.
**Longitudinal changes in MRI measures: MS patients only**

Patients who remained CIS were over-represented in the *HLA-DRB1*1501-negative group. All analyses were repeated after excluding patients who remained CIS after 15 years (Table 8.4).

In the subgroup of patients with MS at 15 years the findings were similar to the whole study cohort. The *HLA-DRB1*1501-positive MS patients had a greater annualised change in T2LV (adjusted difference 0.52 ml/year, 95%CI 0.24 to 0.70, p<0.01) and T1LV (adjusted difference 0.17 ml/year, 95%CI 0.11 to 0.24, p<0.01) compared with the *HLA-DRB1*1501-negative patients. The *HLA-DRB1*1501-positive MS patients also had a higher number of GdE lesions at each time point (IRR = 3.20, 95%CI 1.36 to 7.50, p=0.007).

The results of the brain and spinal cord atrophy were also similar in the subgroup of patients with MS after 15 years. The *HLA-DRB1*1501-positive MS patients had a greater annualised change in PBVC (adjusted difference -0.14%/year, 95%CI -0.26 to -0.11, p<0.01) and UCCA (adjusted difference -0.19 mm²/year, 95%CI -0.34 to -0.13, p<0.05) compared with the *HLA-DRB1*1501-negative MS patients.
Table 8.4  Annualised change in MRI measures in *HLA-DRB1*1501*-positive and negative MS patients*

<table>
<thead>
<tr>
<th></th>
<th><em>HLA-DRB1</em>1501-positive (n=47)</th>
<th></th>
<th><em>HLA-DRB1</em>1501-negative (n=41)</th>
<th></th>
<th>p†</th>
</tr>
</thead>
<tbody>
<tr>
<td>Rate of change (95% CI)</td>
<td>0.93 (0.67, 1.18)</td>
<td>p† &lt;0.01</td>
<td>0.41 (0.30, 0.59)</td>
<td>p† &lt;0.01</td>
<td>&lt;0.01</td>
</tr>
<tr>
<td>T2LV, ml</td>
<td>0.20 (0.14, 0.28)</td>
<td>p† &lt;0.01</td>
<td>0.11 (0.08, 0.16)</td>
<td>p† &lt;0.05</td>
<td>&lt;0.01</td>
</tr>
<tr>
<td>PBVC, %</td>
<td>-0.46 (-0.53, -0.39)</td>
<td>p† &lt;0.01</td>
<td>-0.29 (-0.41, -0.19)</td>
<td>p† &lt;0.01</td>
<td>&lt;0.01</td>
</tr>
<tr>
<td>SC lesion number, n</td>
<td>0.30 (0.20, 0.43)</td>
<td>p† &lt;0.01</td>
<td>0.26 (0.15, 0.43)</td>
<td>p† &lt;0.01</td>
<td>NS</td>
</tr>
<tr>
<td>UCCA, mm²</td>
<td>-0.49 (-0.65, -0.26)</td>
<td>p† &lt;0.01</td>
<td>-0.30 (-0.54, -0.13)</td>
<td>p† &lt;0.01</td>
<td>&lt;0.05</td>
</tr>
</tbody>
</table>

† Approximate p values obtained from bootstrap analysis.

NS (non-significant) indicates p>0.05.
8.4 Discussion

The extent to which genetic factors contribute to disease course heterogeneity in MS is unresolved. In this prospective, longitudinal study of CIS patients I found evidence that \textit{HLA-DRB1*1501} allele may influence disease course, including (1) an earlier age at disease onset; (2) an increased risk of conversion to MS; (3) an increased rate of disability progression over time, measured using the EDSS, and worse cognitive performance at 15 years; (4) a higher brain T2 lesion load at the time of presentation with CIS and a faster increase in T2LV over time; (5) a higher number of gadolinium-enhancing lesions at all time points over the first ~5 years of follow-up; and (6) evidence of a faster rate of brain and spinal cord atrophy, even after adjusting for differences in brain T2 lesion load.

I found that brain T2 lesion load was higher in \textit{HLA-DRB1*1501}-positive compared with \textit{HLA-DRB1*1501}-negative patients at the time of CIS. Although brain T2LV increased in both groups over time, the rate of increase was faster in \textit{HLA-DRB1*1501}-positive than in \textit{HLA-DRB1*1501}-negative patients. In keeping with these findings, brain gadolinium-enhancing lesion number was also higher in the \textit{HLA-DRB1*1501}-positive group at the time of presentation and at all subsequent follow-up time points. Importantly, these differences were maintained after excluding people who remained CIS after 15 years, suggesting that the observed differences were not simply due to an over-representation of CIS patients with a monophasic disease course in the \textit{HLA-DRB1*1501}-negative group.

Conventional MRI is highly sensitive to the detection of focal inflammatory pathology in MS (Filippi et al., 2012). T2-hyperintense lesions reflect areas of focal demyelination, astrogliosis and axonal loss, with contrast-enhancement on T1-
weighted images indicating the presence of blood-brain barrier dysfunction in acute demyelinating lesions (Filippi et al., 2012). \( HLA-DRB1^*1501 \) and other class II human leukocyte antigen alleles are involved in immune processes such as antigen presentation and T cell activation that could plausibly influence inflammatory disease activity in MS. The finding of a more inflammatory disease phenotype in \( HLA-DRB1^*1501 \)-positive patients is supported by other radiological, immunological and pathological studies. Previous MRI studies in patients with CIS (Hauser et al., 2000b; Horakova et al., 2011), RRMS (Okuda et al., 2009) and PPMS (Tur et al., 2014) have reported a greater brain T2 lesion load in \( HLA-DRB1^*1501 \)-positive compared with \( HLA-DRB1^*1501 \)-negative patients when assessed cross-sectionally. A single previous longitudinal study found a greater change in brain T2LV in \( HLA-DRB1^*1501 \)-positive compared with \( HLA-DRB1^*1501 \)-negative patients with PPMS over a follow-up period of 5 years, in keeping with the findings presented here in patients with CIS and early RRMS (Tur et al., 2014). A number of studies have found an association between \( HLA-DRB1^*1501 \) and cerebrospinal fluid IgG oligoclonal bands (Masterman et al., 2000; Mero et al., 2013) and this has been confirmed in a recent large study conducted by the International MS Genetics Consortium (Goris et al., 2015). Finally, a recent post-mortem study found that \( HLA-DRB1^*1501 \) carriage was associated with a greater extent of demyelination within focal spinal cord lesions and a greater inflammation in brain lesions and in normal-appearing tissues (DeLuca et al., 2013; Yates et al., 2015).

Although the NBV was similar in \( HLA-DRB1^*1501 \)-positive and negative patients at baseline I found that the \( HLA-DRB1^*1501 \)-positive patients had a faster rate of whole brain atrophy over the first 15 years after CIS. A faster rate of spinal cord atrophy, measured as change in the UCCA, was also observed in \( HLA-DRB1^*1501 \)-positive group over the first ~5 years of follow-up. Previous MRI studies have found evidence
of greater whole brain atrophy (Okuda et al., 2009) and subcortical grey matter atrophy (Isobe et al., 2016) in HLA-DRB1*1501-positive compared with HLA-DRB1*1501-negative patients, plus evidence of more microstructural tissue damage assessed using 'H magnetic resonance spectroscopy and MTI in normal-appearing tissues (Okuda et al., 2009; Tur et al., 2014).

It is uncertain whether the observed differences in brain atrophy and microstructural tissue damage in the HLA-DRB1*1501-positive group is due to a more inflammatory disease phenotype or due to an independent effect on neurodegeneration. Focal demyelinating lesions result in significant neuroaxonal loss (Trapp et al., 1998) and early changes in brain T2 lesion load on MRI are associated with the development of later brain atrophy in MS (Chard et al., 2003). After adjusting for brain T2 lesion load, I found that differences in whole brain and spinal cord atrophy between the HLA-DRB1*1501-positive and negative were maintained. However, conventional MRI is insensitive to the detection of diffuse inflammation within normal-appearing tissues and the meninges that may be relevant to disability progression and tissue damage in MS (Kutzelnigg et al., 2005; Magliozzi et al., 2007; Magliozzi et al., 2010). A previous post-mortem study has suggested a greater extent of diffuse parenchymal and meningeal inflammation in patients who carry the HLA-DRB1*1501 allele (DeLuca et al., 2013) that may partly contribute to the faster rate of brain and spinal cord atrophy observed.

In contrast to brain MRI findings, I found no significant association of HLA-DRB1*1501 status with spinal cord lesion number, although the number of new spinal cord lesions was overall higher in HLA-DRB1*1501-positive compared with HLA-DRB1*1501-negative patients. This contrasts with two previous MRI studies that reported greater
spinal cord involvement in HLA-DRB1*1501-positive compared with HLA-DRB1*1501-negative MS patients with more focal and diffuse spinal cord lesions (Sombekke et al., 2009; Qiu et al., 2011). I counted spinal cord lesion number using sagittal T2-weighted scans only, which is known to under-estimate the number of cord lesions (Kearney et al., 2015b), and the presence of diffuse spinal cord lesions was not recorded, although these are uncommon in CIS and early RRMS (Bot et al., 2004a). In a previous post-mortem study the number of spinal cord lesions was similar in HLA-DRB1*1501-positive and negative patients, however, the extent of lesions measured by the area of demyelinating plaques was significantly greater in patients who carried the HLA-DRB1*1501 allele (DeLuca et al., 2013).

I found that HLA-DRB1*1501 is a risk factor for conversion to MS in patients with CIS, probably reflecting the higher proportion of patients in the HLA-DRB1*1501 group with asymptomatic T2 brain lesions at presentation (Kelly et al., 1993). Although there was a trend towards a shorter time to a second clinical attack in HLA-DRB1*1501-positive compared with HLA-DRB1*1501-negative patients, this was not statistically significant. In keeping with previous retrospective studies I also found that HLA-DRB1*1501 is associated with a younger age at disease onset (Masterman et al., 2000; Hensiek et al., 2002; International Multiple Sclerosis Genetics Consortium et al., 2011). How HLA-DRB1*1501 might influence age of onset in MS is uncertain. Age is strongly linked to inflammatory disease activity in MS; relapse rates and MRI measures of disease activity are greater in children than adults with MS (Gorman et al., 2009) and relapse rates in adults are more strongly linked to age than disease duration (Kalincik et al., 2013; Scalfari et al., 2016). One possibility is that more inflammatory disease activity in HLA-DRB1*1501-positive patients might result in a shorter latency to disease onset, and this is supported by the finding of more inflammatory MRI phenotype in the HLA-DRB1*1501-positive patients in this study.
Other than age at disease onset, previous studies have not found an association of HLA-DRB1*1501 with disease course, at least when assessed cross-sectionally in patients with established MS (Masterman et al., 2000; Hensiek et al., 2002; International Multiple Sclerosis Genetics Consortium et al., 2011; Van der Walt et al., 2011; International Multiple Sclerosis Genetics Consortium et al., 2013; Moutsianas et al., 2015). In contrast to previous studies I found evidence that HLA-DRB1*1501 was associated with a faster rate of disability progression over the 15 year follow-up period measured using EDSS. At 15 years the HLA-DRB1*1501-positive patients also had slower walking speed and worse performance in tests of information processing speed compared with the HLA-DRB1*1501-negative group.

The main limitation of this study is the small sample size. A number of much larger studies have not consistently found an association between HLA-DRB1*1501 status and MS phenotype, other than age at disease onset and presence of CSF oligoclonal bands (International Multiple Sclerosis Genetics Consortium et al., 2011; International Multiple Sclerosis Genetics Consortium et al., 2013; Moutsianas et al., 2015). In contrast to most previous studies I studied a homogeneous cohort of patients, all followed prospectively from the time of presentation with CIS. The patients were deeply phenotyped with MRI (in addition to clinical assessments) including MRI measures of inflammation and neurodegeneration. Importantly, the patients were studied longitudinally over the first 15 years after CIS, unlike previous MRI studies that have largely been cross-sectional in nature (Hauser et al., 2000b; Zivadinov et al., 2003; Okuda et al., 2009; Sombekke et al., 2009; Van der Walt et al., 2011; Yaldizli et al., 2016b). The findings are similar to a previous longitudinal study of patients with early PPMS (disease duration < 5 years) who had several MRI scans over a follow-up period of 5 years (Tur et al., 2014). In that study HLA-DRB1*1501-positive PPMS
patients had a greater T2 lesion load at each time point and faster accumulation of tissue injury in grey matter and NAWM measured using MTR.

A further possible limitation is the over-representation of patients with optic neuritis in this cohort. In some studies patients with optic neuritis have been found to have a more favourable course than patients with brainstem and spinal cord syndromes (Tintore et al., 2005). However, the frequency of HLA-DRB1*1501 positivity is similar in patients with optic neuritis and other CIS presentations (Kelly et al., 1993; Horakova et al., 2011) and the high proportion of patients with optic neuritis in our cohort is unlikely to account for the observed differences between HLA-DRB1*1501-positive and negative patients.

8.5 Conclusions

These findings provide evidence that HLA-DRB1*1501 influences the rate of disability progression, the accumulation of new brain lesions and brain and spinal cord atrophy. HLA-DRB1*1501 may be one of the mechanisms underlying disease course heterogeneity in patients with relapse-onset MS.

In the next chapter I investigate energy failure, another possible mechanism for long-term disability and disease progression in MS using sodium MRI, a novel metabolic imaging technique.
Chapter 9

Grey matter sodium accumulation is associated with long-term disability and cognitive impairment in relapse-onset multiple sclerosis

9.1 Introduction

Energy failure is thought to be central to the pathophysiology of MS and may provide a link between demyelination and neurodegeneration (Lassmann et al., 2012), the two pathological hallmarks of MS. Following inflammatory demyelination, compensatory electrophysiological changes occur in demyelinated axons in order to preserve neuroaxonal function, at least in the short term. There is up-regulation of sodium channels to maintain conduction with redistribution of sodium channels across the axolemma. The high energy requirements of the Na⁺/K⁺-ATPase pump, potentially compounded by primary mitochondrial dysfunction in MS (Lassmann et al., 2012), may overwhelm neuronal ATP production resulting in failure of the Na⁺/K⁺-ATPase pump and intracellular sodium accumulation. Energy failure may give rise to neuroaxonal dysfunction and ultimately cell death, and is thought to be an important mechanism contributing to disability and disease progression in MS (Lassmann et al., 2012).

Sodium (²³Na) MRI is a novel imaging technique able to quantify tissue sodium concentrations in vivo, providing metabolic rather than structural information in tissues. ²³Na MRI has been applied previously in a small number of studies in people with relapsing and progressive forms of MS (Inglese et al., 2010; Zaaraoui et al., 2012; Paling et al., 2013; Maarouf et al., 2014; Maarouf et al., 2017). Collectively these studies have found evidence of brain sodium accumulation within lesions and normal-appearing tissues in MS, most marked in patients with progressive forms of
MS (Paling et al., 2013) and in patients with greater physical disability and cognitive impairment (Zaaraoui et al., 2012; Paling et al., 2013; Maarouf et al., 2017). These findings suggest that metabolic abnormalities detected using $^{23}$Na MRI may be relevant to disability and disease progression.

I applied $^{23}$Na MRI cross-sectionally at 15 years in the CIS cohort using a recently optimised protocol with smaller voxel size (3mm$^3$ compared with 4mm$^3$ in previous studies), and a 3D cones technique that has been shown to improve tissue contrast and signal-to-noise ratio with clinically acceptable scanning times (Riemer et al., 2014). The aims of the study were (1) to investigate the relationship of tissue sodium concentrations in the brain with long-term disease course in a cohort of patients well-matched for disease onset and (2) to investigate the relationship of tissue sodium concentrations with physical disability and cognitive impairment.

9.2 Methods

Patients

Patients who attended the 15 year follow-up were invited to take part in the $^{23}$Na MRI study. I also recruited a group of healthy controls with no known neurological disorder who underwent the same MRI protocol.

Clinical assessment

MS was diagnosed using the McDonald 2010 criteria (Polman et al., 2011). Disability was assessed cross-sectionally at 15 years using the EDSS, TWT and 9HPT as described previously (Kurtzke, 1983; Cutter et al., 1999). The TWT raw scores were transformed into an inverse to determine walking speed (in seconds) and 9HPT times
were transformed into a z score using normative values from the MSFC taskforce database (Cutter et al., 1999).

I assessed cognition using (1) tests of verbal and visual memory from the Adult Memory and Information Processing Battery (Coughlan and Hollows, 1985); (2) tests of information processing speed, the PASAT (Cutter et al., 1999) and the SDMT (Smith, 1982); and (3) tests of executive function, the Hayling and Brixton tests (Burgess and Shallice, 1997). Premorbid intellectual function was estimated using the National Adult Reading Test (NART) (Nelson, 1982). The raw test scores for each of the cognitive tests were transformed into a z score using published age-matched normative data from healthy controls. Patients were classified as being cognitively impaired if performance was abnormal (≥1.5 standard deviations from normative values) in one test in at least two of the cognitive domains assessed (memory, information processing speed, executive function).

Self-reported fatigue was measured using the Fatigue Severity Scale (Krupp, 1989). Responses were converted into a mean value from 0-7, with higher values indicating greater fatigue.

**MRI acquisition**

Details of the full image acquisition protocol are summarised in Chapter 3. Briefly, all of the MRI scans were obtained on the same 3.0 T scanner (Achieva, Phillips Healthcare Systems). Using a 32-channel head coil the following ¹H-MRI scans were obtained: (i) a PD/T2-weighted 2D fast spin multi echo scan for identification of T2-
hyperintense lesions; (ii) a T1-weighted 2D spin echo scan for identification of T1-hypointense lesions; and (iii) a T1-weighted 3D TFE scan for segmentation purposes.

The 32-channel head coil was changed to $^{23}$Na coil (RAPID biomed) and the subject was returned to the scanner with two calibration phantoms containing a solution of NaCl with a sodium concentration of 33 mM and 66 mM respectively. The NaCl concentration in the calibration phantoms was chosen to encompass the range of physiological values expected in the brain. A sodium scan was obtained followed by a $^1$H PD/T2-weighted fast spin multi echo scan obtained using the body coil.

Figure 9.1 shows examples of raw sodium images, sodium concentration maps and standard $^1$H T2-weighted and T1-weighted images obtained.
Figure 9.1 $^{23}$Na and $^1$H scans in a healthy control and subjects with CIS, RRMS and SPMS.
Image analysis

T2-hyperintense and T1-hypointense white matter lesions were manually outlined on PD/T2-weighted and T1-weighted 2D spin echo scans using a semi-automated edge-finding tool (JIM6.0, Xinapse Systems, Aldwincle, UK). The T2LV and T1LV was calculated by multiplying lesion area by slice thickness. The T2-hyperintense lesion masks were co-registered to the 3D T1-weighted TFE scan. White matter lesions were filled using a patch-based lesion filling technique (Prados et al., 2014). Brain extraction and probabilistic tissue segmentation of the lesion-filled 3D T1-weighted TFE scans was done using Geodesic Information Flows (GIF) to create cortical grey matter, deep grey matter and NAWM masks (Cardoso et al., 2015). The brain parenchymal fraction (BPF), grey matter fraction (GMF) and white matter fraction (WMF) were calculated by dividing the tissue volumes by the total intracranial volume.

A fully-automated image analysis pipeline was used to determine the total sodium concentration in tissues. The signal intensity in calibration phantoms was used to quantify the total sodium concentration on a voxel-by-voxel basis. A series of registration steps were then used to quantify total sodium concentration: (1) the sodium scan was registered to the PD/T2-weighted scan obtained using the body coil; (2) the PD/T2-weighted scan obtained using the body coil was registered to the PD/T2-weighted scan obtained using the 32-channel head coil; and (3) the PD/T2-weighted scan obtained using the 32-channel head coil was registered to the 3D T1-weighted TFE. Each of these registration steps were then reversed in order to transform the segmented tissue masks (cortical grey matter, deep grey matter, NAWM, T1-isointense lesions, T1-hypointense lesions) into sodium space and calculate total sodium concentration (in mM).
Partial volume correction was done to remove voxels with contamination of CSF containing unrealistically high sodium concentrations (Paling et al., 2013).

**Statistical analysis**

All clinical, $^1$H-MRI and $^{23}$Na-MRI data are presented as mean (SD) unless otherwise stated.

Differences in clinical measures between groups were compared using univariable linear regression, except for EDSS which was compared using the non-parametric Wilcoxon rank-sum test. Differences in $^1$H-MRI findings between groups were compared using univariable linear regression, with age and sex included as covariates when comparing brain volumetric measures. Finally, differences in total sodium concentration in cortical grey matter, deep grey matter, NAWM and lesions between groups was compared using univariable linear regression using grey matter fraction (GMF) or white matter fraction (WMF) as a covariate depending on the tissue type being examined.

Univariate correlations between tissue-specific total sodium concentrations and scores on tests of physical disability, cognition and fatigue were examined using Spearman’s rank correlation for EDSS and Pearson’s correlation coefficient for other measures.

Multivariable linear regression was used to identify independent associations between tissue-specific total sodium concentrations and clinical measures. Significant univariate predictor variables were entered into the model and removed
by stepwise backward manual elimination of variables with $p>0.08$. Each of the omitted variables was then tested in the final models. Because EDSS is not normally distributed the final regression model was checked using non-parametric bias-corrected and accelerated bootstrap with 1000 replicates to confirm associations. Tissue-specific brain atrophy (GMF, WMF or both) was included as covariates in all models. The estimated premorbid IQ measured using the NART was also included as a covariate in all models examining associations with cognitive measures. Age, sex and disease duration had no material effect on regression coefficients and were not included as covariates.

9.3 Results

A total of 130 subjects were included: 96 patients from the CIS cohort and 34 healthy controls. The patient group comprised 18 patients with CIS, 65 with RRMS and 13 with SPMS after ~15 years. The demographic and clinical characteristics of the different patients groups and healthy controls is shown in Table 9.1. The healthy controls were younger than the patient group (mean age 35.5 vs 47.4 years, $p<0.001$) but the sex ratio was similar.

A summary of the clinical assessments in the patient group is shown in Table 9.2. Compared with the patients who remained CIS at 15 years the MS patients had more physical disability, worse performance on cognitive tests (with the exception of visual memory) and greater self-reported fatigue ($p<0.05$ for all comparisons). The SPMS patients had worse performance on each of the clinical measures compared with the RRMS patients ($p<0.05$ for all comparisons), with the exception of verbal memory, which was similar in patients with SPMS and RRMS.
Table 9.1  Demographic characteristics of healthy controls and patients grouped by clinical status at 15 years.

<table>
<thead>
<tr>
<th></th>
<th>Healthy controls (n=34)</th>
<th>CIS (n=18)</th>
<th>RRMS (n=65)</th>
<th>SPMS (n=13)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Age, years</td>
<td>35.5 (10.1)</td>
<td>49.0 (7.2)</td>
<td>46.8 (7.6)</td>
<td>47.8 (7.8)</td>
</tr>
<tr>
<td>Female, n (%)</td>
<td>23 (66)</td>
<td>12 (67)</td>
<td>49 (77)</td>
<td>9 (69)</td>
</tr>
<tr>
<td>Disease duration, years</td>
<td>-</td>
<td>14.5 (2.1)</td>
<td>14.6 (2.4)</td>
<td>15.0 (2.8)</td>
</tr>
<tr>
<td>DMT, n (%)</td>
<td>-</td>
<td>0 (0)</td>
<td>18 (28)</td>
<td>4 (31)</td>
</tr>
</tbody>
</table>
Table 9.2  Physical disability and cognitive assessments in the patient groups.

<table>
<thead>
<tr>
<th></th>
<th>CIS patients (n=18)</th>
<th>All MS (n=78)</th>
<th>RRMS (n=65)</th>
<th>SPMS (n=13)</th>
</tr>
</thead>
<tbody>
<tr>
<td>EDSS, median (range)</td>
<td>0 (0-1)</td>
<td>2 (0-7)**</td>
<td>2 (0-6.5)</td>
<td>6 (3.5-7)##</td>
</tr>
<tr>
<td>1/TWT, seconds</td>
<td>0.24 (0.03)</td>
<td>0.18 (0.06)**</td>
<td>0.20 (0.04)</td>
<td>0.09 (0.05)##</td>
</tr>
<tr>
<td>9HPT, z score</td>
<td>0.78 (0.70)</td>
<td>0.23 (0.91)*</td>
<td>0.50 (0.61)</td>
<td>-1.16 (0.82)##</td>
</tr>
<tr>
<td>Verbal memory, z score</td>
<td>0.21 (0.60)</td>
<td>-0.32 (0.58)**</td>
<td>-0.27 (0.54)</td>
<td>-0.59 (0.74)</td>
</tr>
<tr>
<td>Visual memory, z score</td>
<td>0.24 (0.49)</td>
<td>-0.06 (0.65)</td>
<td>-0.01 (0.63)</td>
<td>-0.35 (0.76)##</td>
</tr>
<tr>
<td>PASAT, z score</td>
<td>0.21 (0.68)</td>
<td>-0.48 (1.09)*</td>
<td>-0.32 (0.99)</td>
<td>-1.32 (1.25)##</td>
</tr>
<tr>
<td>SDMT, z score</td>
<td>0.31 (0.90)</td>
<td>-0.65 (1.38)**</td>
<td>-0.34 (1.18)</td>
<td>-2.12 (1.38)##</td>
</tr>
<tr>
<td>Brixton test, z score</td>
<td>-0.19 (0.98)</td>
<td>-1.06 (1.18)*</td>
<td>-0.93 (1.09)</td>
<td>-1.74 (1.44)##</td>
</tr>
<tr>
<td>Hayling test, z score</td>
<td>0.20 (0.86)</td>
<td>-0.63 (1.04)**</td>
<td>-0.56 (1.01)</td>
<td>-0.97 (1.15)*</td>
</tr>
<tr>
<td>Fatigue severity score</td>
<td>2.4 (1.3)</td>
<td>4.3 (3.80)**</td>
<td>3.5 (1.8)</td>
<td>5.6 (1.5)##</td>
</tr>
</tbody>
</table>

All data presented as mean (SD) unless otherwise indicated

*p<0.05 compared with CIS, **p<0.01 compared with CIS, #p<0.05 compared with RRMS, ##p<0.05 compared with RRMS.


$^{1}$H-MRI findings

The structural $^{1}$H-MRI findings in patients and healthy controls are shown in Table 9.3. The GMF (adjusted difference -0.003, p=0.281) and WMF (adjusted difference -0.001, p=0.788) were similar in patients who remained CIS at 15 years and healthy controls. The MS patients had a lower GMF (adjusted difference -0.011, p<0.001) and lower WMF (adjusted difference -0.011, p=0.002) compared with the healthy controls. The MS patients also had lower GMF (adjusted difference -0.009, p=0.002) and lower WMF (adjusted difference -0.011, p=0.004) compared with the patients who remained CIS. The SPMS had a lower GMF (adjusted difference -0.008, p=0.020) and lower WMF (adjusted difference -0.011, p=0.017) compared with the RRMS patients.

The patients with SPMS had a higher T2LV (17.97 vs 9.81 ml, p=0.026) and higher T1LV (5.87 vs 1.90 ml, p<0.001) compared with the patients with RRMS.
<table>
<thead>
<tr>
<th></th>
<th>Healthy controls (n=34)</th>
<th>CIS patients (n=18)</th>
<th>All MS (n=78)</th>
<th>RRMS (n=65)</th>
<th>SPMS (n=13)</th>
</tr>
</thead>
<tbody>
<tr>
<td>BPF</td>
<td>0.768 (0.009)</td>
<td>0.767 (0.008)</td>
<td>0.747**</td>
<td>0.751**</td>
<td>0.731**.##</td>
</tr>
<tr>
<td>GMF</td>
<td>0.458 (0.009)</td>
<td>0.453 (0.006)</td>
<td>0.445**</td>
<td>0.447</td>
<td>0.438**.##</td>
</tr>
<tr>
<td>WMF</td>
<td>0.310 (0.011)</td>
<td>0.314 (0.010)</td>
<td>0.302**</td>
<td>0.303**</td>
<td>0.293**.##</td>
</tr>
<tr>
<td>T2LV, ml</td>
<td>0.12 (0.30)</td>
<td>11.08 (12.00)</td>
<td>9.81 (11.60)</td>
<td>17.97##</td>
<td></td>
</tr>
<tr>
<td>T1LV, ml</td>
<td>0 (0)</td>
<td>2.54 (3.52)</td>
<td>1.90 (2.91)</td>
<td>5.87##</td>
<td></td>
</tr>
</tbody>
</table>

**p<0.05 compared with healthy controls. ##p<0.05 compared with RRMS.
**23Na-MRI findings: healthy controls**

Tissue sodium concentrations in healthy controls are shown in Table 9.4. In healthy control subjects the total sodium concentration was higher in cortical grey matter than deep grey matter. The TSC was also higher in cortical grey matter and deep grey matter compared with white matter.

No correlation was found between total sodium concentration in cortical grey matter, deep grey matter or white matter with age or sex in the healthy controls.

**23Na-MRI findings: patients**

The total sodium concentrations in cortical grey matter, deep grey matter, NAWM and lesions are shown in Table 9.4.

In the patients who remained CIS at 15 years, no significant differences were observed in the total sodium concentrations in cortical grey matter (adjusted difference 0.023 mM, p=0.971), deep grey matter (adjusted difference 1.28 mM, p=0.087) and NAWM (adjusted difference 1.16 mM, p=0.203) compared with healthy controls.

In MS patients, the total sodium concentration in cortical grey matter (adjusted difference 2.12 mM, p=0.005), deep grey matter (adjusted difference 1.90 mM, p=0.018) and NAWM (adjusted difference 1.99 mM, p=0.012) was higher than that in healthy controls.
Compared with the patients with RRMS, the SPMS patients had higher total sodium concentration in cortical grey matter (adjusted difference 1.88 mM, p=0.040) and deep grey matter (adjusted difference 2.10 mM, p=0.037). The total sodium concentration in NAWM was similar in patients with SPMS and RRMS (adjusted difference 1.58 mM, p=0.181).

In the MS patients, the total sodium concentration in white matter lesions was significantly higher than in NAWM (adjusted difference 10.73 mM, p<0.001). The total sodium concentration was significantly higher in T1-hypointense compared with T1-isointense white matter lesions (adjusted difference 8.14 mM, p<0.001). The overall total sodium concentration was higher in white matter lesions in SPMS patients compared with those with RRMS (adjusted difference 7.73 mM, p=0.002). The SPMS patients also showed higher total sodium concentration in T1-isointense lesions (adjusted difference 6.48 mM, p=0.003) and a trend towards higher total sodium concentration in T1-hypointense lesions (adjusted difference 6.55 mM, p=0.054) compared with the RRMS patients.
Table 9.4  Total sodium concentrations in healthy controls and subjects grouped by clinical status at 15 years.

<table>
<thead>
<tr>
<th></th>
<th>Healthy controls</th>
<th>CIS patients</th>
<th>MS patients</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>(n=34)</td>
<td>(n=18)</td>
<td>All MS</td>
</tr>
<tr>
<td>Cortical grey matter</td>
<td>40.63 mM (2.33)</td>
<td>40.68 mM (1.74)</td>
<td>42.82 mM** (3.53)</td>
</tr>
<tr>
<td>Deep grey matter</td>
<td>34.83 mM (2.79)</td>
<td>36.13 mM (1.69)</td>
<td>36.69 mM* (3.74)</td>
</tr>
<tr>
<td>NAWM</td>
<td>32.03 mM (3.44)</td>
<td>32.92 mM (2.43)</td>
<td>34.85 mM* (4.05)</td>
</tr>
<tr>
<td>White matter lesions</td>
<td></td>
<td></td>
<td>All lesions</td>
</tr>
<tr>
<td></td>
<td></td>
<td></td>
<td>45.58 mM (8.53)</td>
</tr>
<tr>
<td>T1-isointense lesions</td>
<td></td>
<td></td>
<td>43.93 mM (5.83)</td>
</tr>
<tr>
<td>T1-hypointense lesions</td>
<td></td>
<td></td>
<td>52.08 mM (7.43)</td>
</tr>
</tbody>
</table>

All data presented as mean (SD).

*p<0.05 compared with healthy controls , **p<0.01 compared with healthy controls. *p<0.05 compared with RRMS. **p<0.01 compared with RRMS.
Correlations between tissue sodium concentrations and disability

Univariate correlations between tissue sodium concentrations and measures of physical disability, cognitive performance and fatigue in all subjects are shown in Table 9.5.

The total sodium concentration in cortical grey matter, deep grey matter and NAWM total sodium concentration was positively correlated with EDSS and negatively correlated with walking speed, upper limb dexterity, speed of information processing, visual memory, executive function and fatigue. No correlation was seen between total sodium in any of the tissue compartments and verbal memory.

The total sodium concentration in T1-hypointense lesions and T1-isointense lesions was positively correlated with EDSS and negatively correlated with walking speed, upper limb dexterity, SDMT and the Brixton test. No correlation was observed between the total sodium concentration in T1-hypointense lesions or T1-isointense lesions with memory, PASAT, the Hayling test and fatigue.
Table 9.5. Univariate correlations between tissue sodium concentrations and clinical measures

<table>
<thead>
<tr>
<th></th>
<th>Cortical GM</th>
<th>Deep GM</th>
<th>NAWM</th>
<th>All lesions</th>
<th>T1-hypointense lesions</th>
<th>T1-isointense lesions</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>$r$</td>
<td>$p$</td>
<td>$r$</td>
<td>$p$</td>
<td>$r$</td>
<td>$p$</td>
</tr>
<tr>
<td>EDSS</td>
<td>0.453</td>
<td>&lt;0.001</td>
<td>0.290</td>
<td>0.004</td>
<td>0.377</td>
<td>&lt;0.001</td>
</tr>
<tr>
<td>Walking speed</td>
<td>-0.446</td>
<td>&lt;0.001</td>
<td>-0.345</td>
<td>0.001</td>
<td>-0.399</td>
<td>&lt;0.001</td>
</tr>
<tr>
<td>z 9HPT</td>
<td>-0.462</td>
<td>&lt;0.001</td>
<td>-0.409</td>
<td>&lt;0.001</td>
<td>-0.385</td>
<td>&lt;0.001</td>
</tr>
<tr>
<td>z Verbal memory</td>
<td>-0.195</td>
<td>0.062</td>
<td>-0.135</td>
<td>0.198</td>
<td>-0.164</td>
<td>0.199</td>
</tr>
<tr>
<td>z Visual memory</td>
<td>-0.272</td>
<td>0.009</td>
<td>-0.320</td>
<td>0.002</td>
<td>-0.272</td>
<td>0.009</td>
</tr>
<tr>
<td>z PASAT</td>
<td>-0.350</td>
<td>0.001</td>
<td>-0.307</td>
<td>0.003</td>
<td>-0.344</td>
<td>0.001</td>
</tr>
<tr>
<td>z SDMT</td>
<td>-0.483</td>
<td>&lt;0.001</td>
<td>-0.410</td>
<td>&lt;0.001</td>
<td>-0.455</td>
<td>&lt;0.001</td>
</tr>
<tr>
<td>z Brixton test</td>
<td>-0.385</td>
<td>&lt;0.001</td>
<td>-0.341</td>
<td>0.001</td>
<td>-0.346</td>
<td>0.001</td>
</tr>
<tr>
<td>z Hayling test</td>
<td>-0.296</td>
<td>0.004</td>
<td>-0.277</td>
<td>0.001</td>
<td>-0.324</td>
<td>0.002</td>
</tr>
<tr>
<td>Fatigue severity score</td>
<td>0.240</td>
<td>0.024</td>
<td>0.271</td>
<td>0.010</td>
<td>0.239</td>
<td>0.024</td>
</tr>
</tbody>
</table>
Associations between tissue sodium concentrations and disability: All patients

Results of the multivariable linear regression models investigating independent associations of tissue-specific total sodium concentration and clinical measures are shown in Table 9.6.

Cortical grey matter sodium concentration was independently associated with measures of physical disability including EDSS ($R^2=0.26$), walking speed ($R^2=0.23$) and upper limb dexterity ($R^2=0.23$), measured using the 9HPT. Cortical grey matter sodium concentration was also independently associated with speed of information processing measured using the PASAT ($R^2=0.29$) and SDMT ($R^2=0.31$), and with performance on the Brixton test ($R^2=0.36$), a measure of executive function. Deep grey matter sodium concentration was associated with visual memory with borderline significance ($R^2=0.13$). No association was found between tissue sodium concentrations with verbal memory, the Hayling test or the Fatigue Severity Scale.

All of the multivariable linear regression models were adjusted for brain atrophy. The NART score was also included as a covariate in the models examining cognition.
Table 9.6.  Associations between tissue-specific total sodium concentrations and clinical measures in all patients (n=96)

<table>
<thead>
<tr>
<th>Clinical measure</th>
<th>Tissue sodium concentration</th>
<th>Coefficient</th>
<th>95% CI</th>
<th>p</th>
<th>$R^2$</th>
</tr>
</thead>
<tbody>
<tr>
<td>EDSS</td>
<td>Cortical grey matter</td>
<td>0.174</td>
<td>0.061, 0.287</td>
<td>0.003</td>
<td>0.26</td>
</tr>
<tr>
<td>Walking speed</td>
<td>Cortical grey matter</td>
<td>-0.006</td>
<td>-0.010, -0.002</td>
<td>0.003</td>
<td>0.23</td>
</tr>
<tr>
<td>9HPT</td>
<td>Cortical grey matter</td>
<td>-0.096</td>
<td>-0.148, -0.051</td>
<td>0.001</td>
<td>0.23</td>
</tr>
<tr>
<td>PASAT</td>
<td>Cortical grey matter</td>
<td>-0.105</td>
<td>-0.177, -0.032</td>
<td>0.005</td>
<td>0.29</td>
</tr>
<tr>
<td>SDMT</td>
<td>Cortical grey matter</td>
<td>-0.156</td>
<td>-0.246, -0.066</td>
<td>0.001</td>
<td>0.31</td>
</tr>
<tr>
<td>Visual memory</td>
<td>Deep grey matter</td>
<td>-0.054</td>
<td>-0.107, -0.000</td>
<td>0.052</td>
<td>0.13</td>
</tr>
<tr>
<td>Brixton test</td>
<td>Cortical grey matter</td>
<td>-0.067</td>
<td>-0.120, -0.014</td>
<td>0.014</td>
<td>0.36</td>
</tr>
</tbody>
</table>
Associations between tissue sodium concentrations and disability: MS only

The multivariable linear regression analysis was repeated after excluding patients who remained CIS (n=18), firstly to confirm findings in the MS-only group and secondly to examine associations between total sodium concentration in white matter lesions with disability measures (none of the CIS patients at 15 years had typical demyelinating white matter lesions). The results are presented in Table 9.7.

In the MS-only model (n=78), both cortical grey matter and T1-hypointense lesion sodium concentration were associated with EDSS ($R^2=0.25$), walking speed ($R^2=0.24$) and upper limb dexterity ($R^2=0.32$). The associations with the cognitive tests was similar in the patients with MS to the whole cohort: cortical grey matter total sodium concentration was associated with PASAT ($R^2=0.29$), SDMT ($R^2=0.31$) and the Brixton test ($R^2=0.38$), and deep grey matter total sodium concentration was associated with visual memory ($R^2=0.12$), again with borderline significance.
Table 9.7. Associations between tissue-specific total sodium concentrations and clinical measures in the MS patients (n=78)

<table>
<thead>
<tr>
<th>Clinical measure</th>
<th>Tissue sodium concentration</th>
<th>Coefficient</th>
<th>95% CI</th>
<th>p</th>
<th>$R^2$</th>
</tr>
</thead>
<tbody>
<tr>
<td>EDSS</td>
<td>Cortical grey matter</td>
<td>0.128</td>
<td>0.010, 0.246</td>
<td>0.035</td>
<td>0.25</td>
</tr>
<tr>
<td></td>
<td>T1-hypointense lesions</td>
<td>0.020</td>
<td>0.000, 0.041</td>
<td>0.055</td>
<td></td>
</tr>
<tr>
<td></td>
<td></td>
<td></td>
<td>(0.002, 0.037*)</td>
<td>(0.023*)</td>
<td></td>
</tr>
<tr>
<td>Walking speed</td>
<td>Cortical grey matter</td>
<td>-0.005</td>
<td>-0.009, -0.001</td>
<td>0.023</td>
<td>0.24</td>
</tr>
<tr>
<td></td>
<td>T1-hypointense lesions</td>
<td>-0.001</td>
<td>-0.002, -0.0005</td>
<td>0.032</td>
<td></td>
</tr>
<tr>
<td>z 9HPT</td>
<td>Cortical grey matter</td>
<td>-0.091</td>
<td>-0.152, -0.030</td>
<td>0.004</td>
<td>0.32</td>
</tr>
<tr>
<td></td>
<td>T1-hypointense lesions</td>
<td>-0.014</td>
<td>-0.025, -0.004</td>
<td>0.008</td>
<td></td>
</tr>
<tr>
<td>z PASAT</td>
<td>Cortical grey matter</td>
<td>-0.101</td>
<td>-0.180, -0.023</td>
<td>0.012</td>
<td>0.29</td>
</tr>
<tr>
<td>z SDMT</td>
<td>Cortical grey matter</td>
<td>-0.143</td>
<td>-0.240, -0.046</td>
<td>0.005</td>
<td>0.31</td>
</tr>
<tr>
<td>z Visual memory</td>
<td>Deep grey matter</td>
<td>-0.060</td>
<td>-0.119, 0.000</td>
<td>0.051</td>
<td>0.12</td>
</tr>
<tr>
<td>z Brixton test</td>
<td>Cortical grey matter</td>
<td>-0.066</td>
<td>-0.118, -0.015</td>
<td>0.013</td>
<td>0.38</td>
</tr>
</tbody>
</table>

*Bootstrap confidence interval and p-value.
9.4 Discussion

I investigated the relationship of total sodium concentration in grey matter, NAWM and lesions with long-term physical disability and cognitive impairment in a large cohort of patients, who were well-matched for disease onset and followed prospectively from presentation with CIS. The major findings of this work include: (1) higher total sodium concentration in normal-appearing tissues and white matter lesions in people with MS compared with healthy controls; (2) higher total sodium concentrations in cortical and deep grey matter, plus white matter lesions, in people with SPMS compared with those with RRMS; (3) a consistent association of cortical grey matter total sodium concentration with clinical measures of physical disability and cognitive performance. These findings suggest that $^{23}$Na-MRI detects metabolic abnormalities in the brain that are relevant to disease course and disability in relapse-onset MS.

In line with previous studies I found evidence of a widespread increase in total brain sodium in people with MS, not just confined to lesional tissue, but also normal-appearing white matter and grey matter (Inglese et al., 2010; Zaaraoui et al., 2012; Paling et al., 2013; Maarouf et al., 2014; Maarouf et al., 2017). Higher total sodium concentration was observed in both cortical and deep grey matter in people with MS compared with healthy controls. In white matter, a gradient of total sodium concentration was seen with the highest values measured in T1-hypointense lesions, followed by T1-isointense lesions and then NAWM in people with MS. Total sodium concentrations in grey matter and white matter lesions were higher in people with SPMS compared with those with RRMS, suggesting a greater degree of neuroaxonal metabolic dysfunction and neuroaxonal loss in SPMS. These findings are consistent with a greater extent of tissue damage detected using $^1$H-MRI in patients with SPMS compared with patients with RRMS (Filippi et al., 1999; Fisniku et al., 2008b; Fisniku
et al., 2009). Microstructural tissue damage and tissue loss detectable using $^1$H-MRI may result in metabolic changes in tissues detectable using $^{23}$Na-MRI.

No increase in total sodium concentration in grey or white matter was seen in the patients who remained CIS after 15 years. The structural $^1$H-MRI findings also showed no difference in brain volume measurements in the patients with CIS at 15 years compared with healthy controls. These findings are supported by previous $^1$H-MRI studies showing that patients who remain CIS in the longer term do not have detectable structural (Fisniku et al., 2008b; Fisniku et al., 2009) or metabolic (Kapeller et al., 2002) abnormalities of the sort seen in people with MS, and MRI findings are similar to healthy control subjects.

Independent associations were observed between $^{23}$Na-MRI abnormalities and measures of clinical impairment. Total sodium concentration in cortical grey matter was independently associated with EDSS, walking speed and upper limb dexterity both in the whole cohort and in the subgroup of patients with MS after 15 years. Cortical grey matter total sodium concentration was also associated with performance on tests of information processing speed and executive function. Grey matter pathology is recognised as being a major substrate for clinical impairment in MS (Geurts and Barkhof, 2008). Cortical grey matter pathology detected using $^1$H-MRI, including grey matter lesions (Calabrese et al., 2012), atrophy (Fisniku et al., 2008b) and microstructural damage (Fisniku et al., 2009; Yaldizli et al., 2016a), is associated with both physical disability and cognitive impairment in MS. Established metabolic imaging methods such as $^1$H-MRS have also found an association between cortical grey matter abnormalities and physical disability in MS (Sastre-Garriga et al., 2005).
Previous $^{23}$Na-MRI studies have also reported an association of grey matter total sodium concentration with measures of clinical impairment. In a mixed cohort of patients with RRMS, SPMS and PPMS univariate correlations were found between both cortical and deep grey matter total sodium concentration and measures of physical disability (Paling et al., 2013). In multivariable models, deep grey matter total sodium concentration was independently associated with EDSS and walking speed. In a cohort of early RRMS patients with little physical disability (mean disease duration 3.1 years, median EDSS 1), grey matter total sodium concentration was independently associated with cognitive impairment (Maarouf et al., 2017). Although the authors did not investigate cortical and deep grey matter separately, a voxel-wise analysis was done to investigate the topography of grey matter sodium accumulation. Widespread increases in total sodium concentration were seen in cognitively impaired patients, mainly in neocortical areas (Maarouf et al., 2017).

In the group of patients with MS, total sodium concentration in T1-hypointense lesions was also independently associated with measures of physical disability including EDSS, TWT and 9HPT. These findings are in keeping with the extent of pathological changes observed in MS with the gradient of demyelination and neuroaxonal injury in white matter, being most severe in T1-hypointense lesions and least severe in NAWM (van Waesberghe et al., 1999; Schmierer et al., 2004).

Some limitations should be noted, both to the work presented here and more generally to $^{23}$Na-MRI. Firstly, the healthy control group were significantly younger than the patients with CIS and MS. However, I found no relationship between age and total sodium concentrations in tissue-compartments, in keeping with previous studies (Paling et al., 2013). It is unlikely that the increase in total sodium
concentration found in people with MS is due to older age, particularly since there was no difference between healthy controls and patients who remained CIS. Secondly, although I studied a large number of patients (this study is largest application of $^{23}$Na-MRI in MS to date), the number of patients with SPMS who were scanned as part of the 15 year follow-up was small ($n=13$). Despite the small sample size clear differences were seen, particularly in cortical and deep grey matter, between the SPMS and RRMS group, findings that are consistent with a previous study with a larger sample of patients with SPMS (Paling et al., 2013). Thirdly, only a selective set of cognitive tests were administered rather than a comprehensive neuropsychological evaluation such as the Brief Repeatable Battery or MACFIMS. However, the tests chosen cover the three most commonly affected cognitive domains in MS, and included the SDMT plus tests of verbal and visual memory. An expert panel has recently highlighted that these tests should be the highest priority in brief cognitive assessments in people with MS (Langdon et al., 2012). Finally, this is an exploratory study and in the statistical analyses presented no correction was made for multiple comparisons.

Although $^{23}$Na-MRI is a promising new imaging technique with the potential to provide tissue-specific metabolic information in vivo there are a number of technical challenges and limitations that need to be taken into consideration. The concentration of $^{23}$Na in tissues is significantly lower than $^1$H with a much lower signal-to-noise ratio. Consequently the voxel size required is significantly larger, typically 4x4x4 mm in previous studies (Inglese et al., 2010; Zaaraoui et al., 2012; Paling et al., 2013; Maarouf et al., 2014; Maarouf et al., 2017). A larger voxel size increases the risk of partial volume effects, especially from CSF which contains a much higher sodium concentration than tissues (~150 mM). A number of steps were taken to reduce the effects of partial volume in this study. Firstly, the scans were acquired using an
optimised $^{23}$Na-MRI protocol that included a higher resolution (3x3x3 mm) and more efficient sampling of $k$-space using a 3D cones technique (Riemer et al., 2014). These changes have been shown to improve tissue contrast and increase signal-to-noise ratio (Riemer et al., 2014). Secondly, the image-analysis pipeline included a partial volume correction to identify voxels with CSF contamination (Soret et al., 2007). Thirdly, as an additional precaution all of the analyses were adjusted for tissue-specific atrophy (GMF, WMF or both), which has not been done in previous studies.

The total sodium concentration in tissues represents a composite of intracellular and extracellular sodium. The increased total sodium concentration observed in grey matter, NAWM and lesions in people with MS could be due to an increase in intracellular sodium due to axonal metabolic dysfunction, an increase in extracellular sodium due to expansion of the extracellular space from neuroaxonal loss, or both. A number of approaches have been described for measuring intracellular sodium concentration in vivo (Petracca et al., 2016a). A recent study at 7 T used triple quantum filtering to exploit the differences in the relaxation properties between intracellular and extracellular sodium (Fleysher et al., 2013; Petracca et al., 2016b). This technique allows quantification of total sodium concentration, intracellular sodium concentration and the intracellular sodium volume fraction (a surrogate measure of the extracellular sodium). This approach has been applied in a study of 19 patients with RRMS. Compared with healthy controls, the RRMS patients showed a significant increase in intracellular sodium concentration and decrease in intracellular sodium volume fraction (Petracca et al., 2016b). These findings suggest that both an increase in intracellular sodium concentration and expansion of the extracellular space contribute to the increase in total sodium concentration in MS.
Sodium channel blockade has been proposed as a neuroprotective strategy in MS (Raftopoulos and Kapoor, 2013) based on results of animal studies showing that sodium channel blocking agents ameliorate axonal loss in EAE models (Bechtold et al., 2004). These observations are supported by a recent phase II that demonstrated a neuroprotective effect of phenytoin on retinal nerve fibre layer thinning in patients with acute optic neuritis (Raftopoulos et al., 2016). Phase II studies of putative neuroprotective agents in MS typically use brain atrophy as the primary outcome measure. $^{23}$Na-MRI is potentially a novel MRI endpoint for future neuroprotection studies, particularly of agents targeting ion homeostasis or mitochondrial function that might influence intracellular sodium concentration. Longitudinal studies are required to determine whether $^{23}$Na-MRI is sensitive to changes in total sodium concentration over time and to undertake sample size calculations for future clinical trials.

### 9.5 Conclusions

$^{23}$Na-MRI detects pathological abnormalities that are relevant to long-term disease progression, physical disability and cognitive dysfunction in relapse-onset MS. These associations remain even after adjusting for brain atrophy suggesting that $^{23}$Na-MRI may detect neuroaxonal metabolic abnormalities. Metabolic as well as structural abnormalities in cortical grey matter may be an important mechanism contributing to disability and disease progression in relapse-onset MS.
Chapter 10

Conclusions and future directions

10.1 Introduction

I completed a 15 year follow-up study of people with CIS with three major objectives: (1) to improve the diagnosis MS in people with CIS using MRI; (2) to investigate the prognostic value of early MRI abnormalities in people with CIS and early MS; and (3) to investigate the pathological mechanisms that underpin the development of secondary progression and long-term disability in relapse-onset MS. The major findings presented in this thesis relating to each of these objectives is summarised here along with future directions.

10.2 Improving the diagnosis of multiple sclerosis in people with CIS

The McDonald criteria allow the use of MRI to provide evidence of dissemination in time and space, rather than waiting for a second clinical attack. I applied the McDonald 2001, 2005 and 2010 criteria retrospectively in the CIS cohort and found that each revision of the criteria has allowed for an earlier diagnosis of MS. Using the current McDonald 2010 criteria the mean time to diagnosis of MS was 6.2 months compared with 23.1 months using the Poser criteria, and almost half of the CIS patients could be diagnosed with MS using a single contrast-enhanced MRI scan. In clinical settings the application of the McDonald 2010 criteria in patients with typical CIS presentations should allow for earlier diagnosis and treatment of MS.

I found that when applying the McDonald criteria, MS is diagnosed sooner but also more often in CIS patients. With each revision of the McDonald 2010 the number of
CIS patients with MRI-only conversion to MS has increased. Patients with milder forms of MS may be identified when the diagnosis is made using MRI criteria, rather than on the basis of clinical features alone. Changing diagnostic criteria for MS may influence the natural history of MS, independent of the effects of disease-modifying treatments. Clinicians should be mindful of this when counselling patients with CIS and early MS about how their condition might progress in the future.

The MAGNIMS group has recently proposed changes to the DIS criteria. I investigated two of these recommendations to provide evidence to support future revisions to the diagnostic criteria for MS. I found that inclusion of lesions in the symptomatic region improved performance of MRI criteria for a diagnosis of MS with higher sensitivity/accuracy and the same specificity as the McDonald 2010 criteria. There was a trend towards earlier diagnosis with DIS criteria that included symptomatic lesions, but this was not significant in the relatively small cohort studied. I also investigated the influence of periventricular lesion number on the performance of MRI criteria. The MAGNIMS group has proposed increasing the number of periventricular lesions required for DIS from 1 to 3. This recommendation was based on expert consensus that a single periventricular lesion may be insufficient to say there is DIS. I found that increasing periventricular lesion number from 1 to 3 resulted in a reduction in sensitivity and accuracy with no overall improvement in specificity.

Misdiagnosis of MS is an important contemporary clinical issue. Misapplication of the McDonald criteria in patients with presentations not typical of demyelination or those without objective neurological signs is a major contributor to misdiagnosis. My findings confirm previous studies that when the McDonald criteria are applied in young adults with symptoms typical of MS (e.g. unilateral optic neuritis) the criteria
have a very high sensitivity, specificity and accuracy. An important finding is that DIT significantly enhances the specificity of MRI criteria for diagnosis of MS. Demonstration of both DIS and DIT remains essential in making a diagnosis of MS and should be retained in future diagnostic criteria for MS.

10.3 Prognostic value of early MRI abnormalities in people with CIS and early multiple sclerosis

I investigated early MRI abnormalities seen at the time of presentation with CIS and over the first 3 years of follow-up in order to identify the most robust predictors of disease course at 15 years. The presence of asymptomatic spinal cord lesions at the time of CIS was independently associated with secondary progressive disease course and physical disability at 15 years. Spinal cord MRI is not done routinely as part of the diagnostic process in people with suspected MS. Although spinal cord imaging may be helpful diagnostically, my findings suggest that it might also provide important prognostic information in people with CIS and early MS. The number of new spinal cord lesions after 1 year and 3 years was also associated with SPMS and disability at 15 years suggesting that spinal cord MRI may also have a role in monitoring the course of MS.

A higher number of asymptomatic gadolinium-enhancing lesions at the time of presentation with CIS was also associated with secondary progressive disease course and physical disability after 15 years. This association remained significant even when changes in MRI measures over the first 3 years of follow-up were included in models. As well as providing evidence of DIT, gadolinium-enhancing lesions at the time of presentation with CIS may also provide important prognostic information.
Established MRI techniques already in clinical use might provide useful prognostic information in patients with CIS and early MS. Obtaining spinal cord and post-contrast T1-weighted scans in addition to standard PD/T2-weighted or FLAIR scans of the brain may not only assist in the diagnosis and differential diagnosis of suspected MS, but also provide information helpful in counselling patients about prognosis and personalising treatment plans. It will be important to confirm these findings in larger CIS cohorts, particularly cohorts with a more representative number of patients with brainstem/cerebellar and spinal cord syndromes.

10.4 New insights into the mechanisms of disease progression in relapse-onset multiple sclerosis

The mechanisms responsible for disease course heterogeneity in MS, particularly those underlying the development of secondary progression, remain poorly understood. My findings identify a number of mechanisms that may potentially influence the onset of secondary progression and irreversible disability in relapse-onset MS: (1) early inflammatory disease activity, particularly the accrual of lesions in clinically-eloquent sites; (2) the HLA-DRB1*1501 allele which was associated with a greater extent of inflammatory disease activity, neurodegeneration and disability progression; and (3) energy failure detectable in vivo as an increase in total sodium concentration in brain tissues, particularly cortical grey matter.

I found that gadolinium-enhancing lesion load at the time of presentation with CIS was independently associated with disability and the development of SPMS at 15 years. This is consistent with previous observations in natural history studies that the extent of early inflammatory activity (measured clinically in the form of relapses) is associated with shorter latency to the onset of SPMS. Focal lesions in clinically-
elocent sites may be of particular importance in disease progression in relapse-onset MS. Asymptomatic spinal cord lesions seen at the time of CIS and new spinal cord lesions over time was associated with disability at 5 years and with both disability and secondary progressive disease course at 15 years. Similarly, accrual of new spinal and infratentorial lesions over the first 3 years after presentation with CIS was also associated with disability and secondary progression at 15 years. Collectively, these observations point to a link between early inflammatory disease, particularly at clinically-eloquent sites, and later disease progression in relapse-onset MS. My findings also support the concept that early initiation of disease-modifying treatment may prevent or delay the onset of SPMS.

The course of relapse-onset MS is highly variable. At 15 years there was a wide range of outcomes in the CIS cohort with all levels of disability observed. It has been suggested that genetic factors may be one of the mechanisms responsible for disease course heterogeneity in MS. However, studies to date have produced conflicting results. I investigated whether the effect of the HLA-DRB1*1501 haplotype on MRI measures of inflammation and neurodegeneration plus disability progression over the first 15 years after CIS. I found that HLA-DRB1*1501-positive patients had a greater extent of inflammatory disease activity (higher number of gadolinium-enhancing lesions and faster rate of change in T2LV) and also neurodegeneration (faster rate of brain atrophy, spinal cord atrophy and change in T1LV) compared with the HLA-DRB1*1501-negative patients. The HLA-DRB1*1501-positive patients also displayed a faster rate of disability progression measured using the EDSS and had worse performance on cognitive testing at 15 years. My findings suggest that HLA-DRB1*1501 does exert an effect on the course of relapse-onset MS.
Energy failure within demyelinated axons is thought to be a major factor contributing to chronic neuroaxonal loss and disease progression in MS. I applied the novel metabolic imaging technique $^{23}$Na-MRI in the CIS cohort at 15 years to investigate energy failure in vivo. I found evidence of widespread increases in total sodium concentration in white matter lesions, NAWM and grey matter in people with MS. The total sodium concentration in cortical grey matter was consistently associated with measures of physical disability and cognitive impairment. Metabolic abnormalities in cortical grey matter may be an important contributor to disease progression and disability in relapse-onset MS and $^{23}$Na-MRI might be a future outcome measure in clinical trials of neuroprotective agents that target energy failure.

10.5 Future directions

Further revisions to the diagnostic criteria for MS are anticipated following a meeting of an international panel in 2016. The findings presented in this thesis highlight both the importance of symptomatic lesions in DIS and the high sensitivity/specificity of a single periventricular lesion in MRI criteria. I was not able to test other recently recommended changes to dissemination in space criteria such as inclusion of optic nerve or cortical grey matter lesions because this data was not collected in the CIS cohort. These and other possible changes to the diagnostic criteria for MS should ideally be confirmed in a larger, multicentre study. Data collected as part of this thesis has been included in a multicentre study involving seven other European centres organised by the MAGNIMS group. The findings from this thesis and other studies will inform revisions to the diagnostic criteria for MS.

I found that conventional MRI abnormalities including spinal cord lesions and gadolinium-enhancing lesions may be helpful in predicting long-term disease course
in patients with CIS and early MS. The cohort studied is predominantly untreated and the extent to which disease-modifying treatments attenuate these associations is uncertain. Prospective studies in treated cohorts of patients with CIS and early MS will be required to determine the prognostic significance for long-term disability and whether they predict a differential response to disease-modifying therapies.

My analyses focussed on secondary progressive disease course and EDSS at 15 years, two outcome measures heavily weighted towards ambulatory disability. I also assessed cognition, fatigue and mood in the CIS cohort at the 15 year follow-up. Analyses are ongoing to determine whether the MRI variables associated with long-term physical disability are the same as those associated with cognitive impairment, fatigue and affective disorders.

Much of the variability in the early course of MS, including relapse rates, accrual of new focal inflammatory lesions, and ultimately neurodegeneration measured in the form of brain and spinal cord atrophy, is poorly understood. My findings suggest a possible role for \textit{HLA-DRB1*1501} in disease heterogeneity in MS. The effect of \textit{HLA-DRB1*1501} may be influenced by interactions with other HLA and non-HLA genes, and this warrants further investigation. Future genotype-phenotype studies in MS should focus on patient cohorts that have been deeply phenotyped both clinically and with MRI or other biomarkers.

Neuroprotection to slow or prevent progressive neuroaxonal loss in MS is an area of unmet need. Novel imaging methods are required to facilitate the discovery of neuroprotective agents. $^{23}$Na-MRI is a promising new metabolic imaging technique to provide information about energy failure \textit{in vivo}. Longitudinal studies of $^{23}$Na-MRI are
required to assess the sensitivity of this technique to change over time and to provide sample size calculations for future phase II trials. A limitation of currently available $^{23}$Na-MRI techniques at 3T is the inability to separately quantify intracellular sodium (reflecting neuroaxonal metabolic dysfunction) and extracellular sodium concentration (reflecting expansion of the extracellular space and neuroaxonal loss). This is an area of active interest and a number of approaches are being explored.

10.6 Conclusions

In this 15 year follow-up study of people with CIS I found that use of MRI allows for an earlier diagnosis, and potentially earlier treatment, of RRMS. Evidence is presented to help refine the diagnostic criteria for MS further. Early MRI abnormalities in CIS patients may provide important prognostic as well as diagnostic information. Asymptomatic spinal cord and gadolinium-enhancing lesions at presentation with CIS are associated with disability and SPMS course 15 years later. Early inflammatory disease activity (potentially modified by carriage of the $HLA-DRB1*1501$ allele), especially accrual of focal lesions at clinically-eloquent sites, in early relapse-onset MS may be important mechanisms underlying later disease progression. Metabolic abnormalities, particularly in cortical grey matter, may contribute to physical disability and cognitive impairment in relapse-onset MS and may be a putative target for neuroprotection strategies.
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