MAGNETIC RESONANCE IMAGING MARKERS OF LONG TERM DISABILITY IN RELAPSE-ONSET MULTIPLE SCLEROSIS PATIENTS

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Declaration

I, Leonora Fisniku, confirm that the work presented in this thesis is my own. Where information has been derived from other sources, I confirm that this is indicated in the thesis and acknowledgment.

Leonora Fisniku

Abstract

The aim of this thesis is to assess the ever challenging role of MRI in predicting disability in relapse-onset Multiple Sclerosis (MS) patients. It consists of four parts.

In part one a brief overview of MS is given, looking at the most up-to-date knowledge on aetiology, pathogenesis, most common clinical presentations and the evolution in diagnostic process, prognosis and ever increasing treatment options for MS patients. Then, a brief review of the basic physics concepts and the techniques used to assess disability in MS is given using both conventional and non-conventional MRI.

In part two, the relationship of T_2 white matter lesion volume (T_2WMLV) with long-term disability is assessed in a unique cohort of MS patients seen from the disease onset with a clinically isolated syndrome and followed up with clinical and MRI data every 5 years up to 20 years.

In part three, using cross-sectional data from the same cohort of patients, the role of tissue specific i.e. grey matter and white matter changes in predicting disability at 20 years is assessed, using both atrophy measurements and magnetisation transfer ratio. Comparisons between sub-group of MS patients and controls are also assessed. Furthermore, the relationship of longitudinal T_2WMLV changes with atrophy measurements at 20 years is also explored.

In the fourth and final part of this thesis, a summary of the main findings of this work is given and there is discussion on what the future holds for the role of imaging in predicting disability in MS.

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LIST OF ABBREVIATIONS

2D = Two-dimensional

3D = Three-dimensional

9HPT = 9 Hole peg test

BS = Brainstem syndrome

BMS = Benign Multiple Sclerosis

CIS = Clinically isolated syndrome

CDMS = Clinically definite MS

CNR = Contrast-to-noise-ratio

CPMS = Clinically probable MS

CSF = Cerebrospinal fluid

DMDs = Disease modifying drugs

DIS = Dissemination in space

DIT = Dissemination in time

EBV = Epstein Barr virus

EDSS = Expanded Disability Status Scale

FFT = Fast Fourier Transform

FLAIR = Fast fluid attenuated inversion recovery

FOV = Field of view

FS = Functional system

FSE = Fast spin-echo

FSPGR = Fast spoiled gradient recall

HHV-6 = Human Herpes virus-6

HLA = Human leukocyte antigen

IL = Interleukin

ITW = Inverted timed walk

Gd = Gadolinium

GE = Gradient echo

GMF = Grey matter fraction

LSDMS = Laboratory-supported definite MS

LSPMS = Laboratory-supported probable MS

MHC = Major histocompatibility complex

MRI = Magnetic resonance imaging

MS = Multiple sclerosis

MSFC = Multiple Sclerosis Functional Composite Score

MT = Magnetization transfer

MTR = Magnetization transfer ratio

Nabs = Neutralizing antibodies

NAWM = Normal appearing white matter

NGMV = Normalized grey matter volume

NMR = Nuclear magnetic resonance

NWMV = Normalized white matter volume

ON = Optic neuritis

PASAT = Paced Auditory Serial Addition Test

PD = Proton density

PH = Peak height

PL = Peak location

pu = percentage unit

PPMS = Primary progressive MS

RA = Receptor alpha chain

RF = Radiofrequency

r_s = Spearman Rank correlation coefficient

RRMS = Relapsing-remitting MS

SC = Spinal cord

SD = Standard deviation

SE = Spin echo

SIENAx = Structural Image Evaluation, using Normalization, of Atrophy for cross-sectional measurement

SNR = Signal to noise ratio

SPM = Statistical Parametric Mapping

SPMS = Secondary progressive MS

SPSS = Statistical Package for the Social Sciences

T = Tesla

TE = Echo time

T_2LL = T_2 lesion load

T_2LV = T_2 lesion volume

T_2WMLV = T_2 white matter lesion load

TR = Repetition time

VEP = Visual-evoked potential

WMF = White matter fraction

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14. **Figure 6.2** Predicted rate of lesion growth by 20 year GMF centiles – MS patients only (lesion growth expressed in cm^3)

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INTRODUCTION AND AIMS

Multiple sclerosis (MS) is the commonest cause of neurological deficit in young adults. Clinically isolated syndromes (CIS) such as optic neuritis, brainstem or spinal cord syndromes are frequently the first clinical presentation of MS. However, not all CIS patients convert to MS and for those who do, disability is highly variable. Numerous studies have been done to identify the predictors of conversion to MS, as well as disability. Whilst the former issue has been well addressed in a lot of studies, the second issue has been a rather more challenging one. Whilst the core of diagnosis of MS has been proving dissemination in time and space, and clinical information is still as important as it has ever been, the inclusion of MRI criteria has lead to an earlier diagnosis of MS. With regard to disability, initial hopes for a valuable role of MRI in predicting disability have diminished because of the paradox between MRI and clinical disability. Or is this a real paradox? Are we giving up on the MRI too early? Perhaps yes. *Firstly*, disability in MS accrues slowly; hence longitudinal studies are required to fully assess the predictive value of the MRI. Most of the studies so far have been of a rather short to medium length. Extended longitudinal studies, challenging though they might be, are crucial not only for assessing the predictive value of MRI but also for assessment of the true efficacy of disease modifying therapy. *Secondly*, for a long-time MS was seen as a white matter (WM) inflammatory-demyelinating disease and as of today, still remains classified as a WM disease. Along with this, it was felt that inflammation and demyelination in WM lesions are the most important pathological processes in MS and neurodegeneration is a very late process in MS. However, the ever increasing evidence,

both from pathological and quantitive MRI studies, suggest that neither of these concepts holds true any longer. Not only is MS a more global disease of the CNS - with MS plaques in brain WM tissue being one component - where both the macroscopically normal appearing WM and the grey matter (GM) in addition to the spinal cord are all affected. Also, it is evident that neurodegeneration is a process that occurs early in disease course. The relationship between neurodegeneration and inflammation/demyelination is still unclear. However, evidence has emerged indicating that neurodegeneration is responsible for the irreversible disability in MS but also that it occurs in the GM as well as WM, where it may have an important role in long-term disability. *Finally,* the current available disability scales in MS are heavily waited towards physical disability and do not take into account the full spectrum of MS effects including cognitive dysfunction.

With this in mind, after an overview of the MS and MRI in MS, the work in this thesis looks at the role of longitudinal changes in WM lesions in predicting long-term disability in a rather unique cohort of patients – the longest MRI study of the CIS patients to-date - followed up from the disease onset every five years up to 20 years. It then further explores the role of the cross-sectional tissue specific abnormalities as measured by GM and WM volume and GM and WM magnetisation transfer ratio in addition to the WM lesion volume at 20 years in predicting long-term disability. Finally, it assesses the relationship between the longitudinal changes of WM lesions with tissues specific brain volumes at 20 years.

PART I

INTRODUCTION

Chapter I

Multiple Sclerosis

1.1 Introduction

Multiple sclerosis (MS), the commonest cause of non-traumatic neurological disability in young adults in Europe and North America, is a chronic autoimmune disease of the central nervous system which affects both the white and grey matter (WM and GM). Worldwide it affects nearly 2.5 million individuals (Noseworthy et al., 2000; Weinshenker, 1996). In United Kingdom about 100-200/100,000 people have MS with an incidence of about 7/100,000 of new cases diagnosed with MS each year and a lifetime prevalence of 2/1000 (MacDonald et al., 2000). Worldwide, however, there is a marked geographical variation in prevalence (Compston, 1999) with the incidence of MS reported to be increasing (Gray et al., 2008; Orton et al., 2006). MS usually starts between 20 - 40 years of age and onset is rare after 60 years of age. Although MS occurs in childhood accounting for less than 5% of all MS cases, it is rare before 10-years of age (Renoux et al., 2008). Like other autoimmune diseases, MS has a female predominance with a sex ratio (female/male) of 2:1 although, recently in some regions increase in the incidence of MS in women is shown, to a ratio of more than 3:1 (Orton et al., 2006). In progressive forms of MS the clinical disease onset occurs later than in relapsing-remitting forms and the incidence is similar in males and females (Cottrell et al., 1999; Thompson et al., 1997).

1.2 Aetiology

Currently there is little evidence for a single cause of MS, although the evidence so far supports the hypothesis that a combination of infectious and environmental causes on a background of a genetic susceptibility are relevant aetiological factors.

Genetics

It is evident that MS is not a "pure" genetic disease i.e. not transmitted by Mendelian inheritance. Nevertheless, there is evidence from several sources that there is a genetic predisposition to developing MS. It is believed that MS is triggered by environmental factors (see later) in individuals with rather complex genetic-risk profile (Compston & Coles, 2008).

MS is more common in white people especially those of Northern European descent than in non-white ethnic groups and is very rare in some regions. Also the differences in the distribution of MS within the same region, such as United Kingdom for example, where the frequency of MS is higher in the northern than southern part, (highest in Scotland), may reflect differences in the genetic characteristics of the population at risk (Compston A, 2005). Furthermore, studies of twins and siblings suggest that the genetic factors influence the susceptibility to MS with evidence suggesting that multiple genes, each exerting only a modest effect, probably play a role (Hafler et al., 2007; Islam et al., 2006).

Several candidate genes have been identified as genetic risk factors for susceptibility to MS. Variation in major histocompatibility complex (MHC) on chromosome 6p21 is known to influence susceptibility to MS with the strongest genetic effect coming from

one of the most validated ones, human leukocyte antigen (HLA) DRB1*1501 haplotype (DR15) in the class 2 region (Lincoln et al., 2005; Yeo et al., 2007). But non-HLA genes have also been identified, more recent ones being interleukin 7 and 2 receptor alpha chain (IL7RA and IL2RA) (Hafler et al., 2007; Lundmark et al., 2007; Weber et al., 2008).

But despite the strong genetic link and clustering of MS in families the concordance rate between monozygotic twins is below 30%. While MS has a familial recurrence rate of about 20%, (siblings 5%; parents 2% and children 2%) the reduction in risk changes from 3% in first degree relatives to 1% in second and third degree relatives (Compston & Coles, 2008).

Although there appears to be a genetic influence in predisposing one to develop MS, some believe that the disease manifests only when environmental factors are triggered. The studies done on immigrants support this argument. Overall the migration studies emphasize MS as an exogenous disorder, acquired some years before clinical expression and probably in childhood, whereas studies of indigenous peoples provide more compelling evidence for genetic effects on disease frequency and distribution (Compston A, 2005). Migration studies have shown alteration in the risk of MS - depending on age of migration – of people moving from areas of high risk to those of low risk and vice-versa, supporting the hypothesis of environmental factors modifying the inherent risks of MS dependant on ethnicity, although it becomes important to establish when these influences occur (Compston A, 2005; Dean & Elian, 1997; Elian & Dean, 1987).

Environmental causes

Although genetic susceptibility explains the clustering of MS cases within families and the sharp decline in risk with increasing genetic distance, it can not fully explain the geographic variations in MS frequency and the changes in risk that occur with migration which can be explained perhaps by the influence of environmental factors (Ascherio & Munger, 2007b).

a) Infectious

Many viruses and bacteria have been suspected of causing MS. So far, there is no human study that will definitely establish or dismiss the role of infections in MS. One of the infectious causes which have been thought to cause MS is Epstein Barr virus (EBV) - which also causes infectious mononucleosis - and more recently human herpes virus-6 (HHV-6) (Moore & Wolfson, 2002). Some studies have suggested that developing the infection at a critical period of exposure may lead to conditions conducive to the development of MS a decade later. Although EBV is a ubiquitous B-lymphotropic herpes virus that infects around 90% of the adult population worldwide, studies have shown that the risk of developing MS is higher in those with previous history of infectious mononucleosis and it is extremely low in those who are EBV seronegative (Ascherio & Munger, 2007a; Thacker et al., 2006). A recent study looking at the post-mortem brain specimen of people with MS has shown that nearly 100% of them (21 of 22) had been infected with EBV (Serafini et al., 2007). Whilst more and bigger studies are needed to replicate these results, this recent study has re-opened the enthusiasm that the early studies showed on being close on identifying the cause of MS.

Some believe that the "epidemics" of MS, such as the ones in the Faroe Islands further support the role of environment in the pathogenesis of MS although others believe that this is more likely representative of the increasing recognition of MS (Benedikz et al., 1994). Despite EBV standing out as perhaps the only infectious agent that can explain many of the key features of MS epidemiology, the link between EBV and MS cannot explain the decline in risk among migrants from high to low MS prevalence areas (Ascherio & Munger, 2007a).

b) Non-infectious

Findings from studies on seasonality in MS patients'birth, disease onset and exacerbations, as well as apparent temporal trends in incidence and gender ratio support an influential effect of viruses, metabolic and lifestyle factors on MS risk, vitamin D status, and smoking, in addition to EBV, as factors that may partly explain these epidemiological patterns (Pugliatti et al., 2008).

There is a geographical variation in the incidence of MS in the world. MS is more prevalent in countries with temperate climate including Northern Europe, Southern Canada, Northern United States, South-eastern Australia and New Zealand. It is this striking prevalence of MS that increases with latitude which has made vitamin D deficiency a possible pathogenic co-factor in MS (Handunnetthi et al., 2010; Lucas et al., 2011). A recent study has shown that high levels of 25-hydroxyvitamin D was associated with lower incidence of MS and MS-related disability only in women (Kragt et al., 2009). Whilst the evidence of involvement of vitamin D in pathogenesis of MS can not be ignored, it is far from clear.

Smoking has emerged as a potential factor in the pathogenesis of MS, and although the studies so far do not prove a cause-and effect relation, some suggest that smoking is not only associated with an increase risk of MS (Hernan et al., 2001), but it may also be a risk factor for transforming a relapsing-remitting clinical course to secondary - progressive phase (Hernan et al., 2005). There is suggestion that the differences in smoking habits across the populations could perhaps explain some of the variation in MS incidence, such as the increase in female/male sex ratio in Canada over the past 50 years (Ascherio & Munger, 2007b; Orton et al., 2006). The role of other environmental factors as either protective or risk factors in MS is less well established (Ascherio & Munger, 2007b).

1.3 Pathogenesis

The pathology of MS was originally defined as an inflammatory process, associated with focal plaques of primary demyelination in the WM of the brain and spinal cord (Charcot 1880). Since that first description a lot has changed in our understanding of the pathology in MS. We now know that MS is not exclusively a WM disease and that there is more to MS than just inflammation and demyelination; the pathological spectrum is much wider. Axonal loss and damage are important processes in the pathogenesis of MS and seem to appear more clinically relevant. Whilst there is generally an agreement on the pathological processes occurring in MS i.e. inflammation, demyelination, remyelination and neurodegeneration, there is less agreement on the timing and the order of events and the mechanisms leading to these pathological processes.

On one hand, there are those who believe that MS remains primarily a chronic inflammatory demyelinating disease of the central nervous system involving both brain and the spinal cord in which focal lymphocytic infiltration leads to damage of myelin and axons. Initially this process is transitory leading to remyelination which clinically is manifested by the recovery of neurological dysfunction. Over time the pathological changes become dominated by widespread microglial activation associated with chronic neurodegeneration and clinically manifested by the progressive accumulation of the disability (Compston & Coles, 2008). And whilst there is extensive injury of the so-called normal-appearing WM (NAWM) tissue and GM involving both the cortex and the deep GM, with extensive demyelination and axonal damage, it is believed that this neurodegenerative component does not develop independently of the inflammation and

the inflammation is the driving force of both demyelination and neurodegeneration in MS (Lassmann et al., 2007).

On the other hand there are those who challenge this concept. There is increasing evidence from pathological and Magnetic Resonance Imaging (MRI) studies that the axonal pathology such as axonal transections or acute axonal damage occurs early not only in MS lesions, but also in NAWM. Furthermore the cortical pathology - demyelination and neuroaxonal damage - also appears to be an early event in lesion pathogenesis and contribute to permanent clinical disability. All these findings show not only neurodegeneration being an important component of the pathology of MS but also some believe, it provides evidence for a primary neurodegenerative event in the pathogenesis of MS lesions. The presence of diffuse NAWM injury, marked GM involvement and significant cortical functional reorganizational as well as neurodegeneration - which is only partially linked to demyelination, from the early onset of the disease, has laid to the concept of MS as a "two-stage" disease (i.e. early inflammation followed by late neurodegeneration) to be replaced by the concept of "simultaneous two-component" disease where both processes occur simultaneously (Charil & Filippi, 2007; Hauser & Oksenberg, 2006). Some have even raised questions whether MS could still be considered an autoimmune disease or is indeed a primarily neurodegenerative disease with secondary inflammatory demyelination (Trapp & Nave, 2008).

Immunology

Although the immunological processes in MS are complex and not fully understood, the mainstream view is that MS is caused by an autoimmune attack of the central nervous system (CNS) myelin by myelin-specific CD4 T cells (McLaughlin & Wucherpfennig, 2008). There is considerable evidence that auto-reactive T cells proliferate, cross the blood-brain barrier and enter the CNS under the influence of cellular adhesion molecules and pro-inflammatory cytokines. However other cell populations of the immune system are also involved in the complex series of events leading to the development of MS. B cells contribute to the pathogenesis of MS, through their role as antigen-presenting cells to T cells with matching antigen specificity, the production of pro-inflammatory cytokines and chemokines, and the secretion of auto-antibodies that target structures on the myelin sheath and the axon (McLaughlin & Wucherpfennig, 2008).

The genetic linkage with HLA class II alleles (DRB1*1501, DRB1*0101 and DQB1*0602) and specific alleles of the IL2 and IL7 receptors (also implicated in the pathogenesis of other autoimmune diseases such as type 1 diabetes and Grave's disease) and involvement of auto-reactive T and B cells support the auto-immune hypothesis. These results add to the pathological and immunological studies in suggesting that MS is an autoimmune inflammatory disorder (Compston & Coles, 2008).

WM lesions

The pathological hallmark of MS is the formation of the sclerotic plaques which represents the end stage of a process involving inflammation, demyelination, remyelination, oligodendrocyte depletion and astrocytosis and neuronal and axon

degeneration. Inflammatory infiltrates are typified by the presence of both CD4+ (helper/inducer) and CD8+ (cytotoxic/suppressor) cells but lower numbers of other mononuclear cells (B cells and macrophages/microglial cells) are also consistently found. Lesions are typically sharply demarcated, usually oval or round in shape, often centred around venules [one or several medium sized blood vessels (Lassmann et al., 1998)]. They vary in appearance, size (from less than one millimetre to several centimetres), age (acute or chronic) and although they can be distributed throughout the CNS, their typical sites include: optic nerve, corpus callosum, infratentorial, periventricular and spinal cord – mainly cervical region, as well as GM (see later).

Several attempts have been made to classify the lesions (Lassmann et al., 1998; van, V & De Groot, 2000). More recently Lucchinetti et al have proposed a classification of active lesions in four patterns based on the distribution of myelin loss, the plaque geography and extension, the pattern of oligodendrocyte destruction, and immunoglobulin and complement deposition (Lucchinetti et al., 2000). The authors conclude that this proves the heterogeneity of the MS lesions and further suggest that any single patient has only one of these four patterns. It is important to mention that in this study, much of the material was somewhat unusual – a high percentage of biopsy material, hence not a typical MS presentation and many of the lesions were acute, hence not covering the full spectrum of lesion formation/development. Whilst we still wait for other studies to confirm these findings, they have been challenged by a more recent pathological study performed in a relapsing-remitting MS (RRMS) patient who died either during or shortly after an acute relapse, which showed that in the very early stages of lesion formation, extensive oligodendrocyte apoptosis and microglial activation in myelinated tissue

occurred at sites devoid of T-cell or B-cell infiltrates, although there was inflammation in the regions adjacent to the active lesion (Barnett & Prineas, 2004). These findings and the spectrum of pathological changes in nearby or distant phagocytic plaques suggest, the authors conclude, that the pathological heterogeneity in MS is largely due to evolution of lesional pathology, rather than true pathogenic heterogeneity (Barnett et al., 2006).

It is well established that the axonal loss and injury occurs at an early stage in WM lesions, it is related to the degree of inflammation within lesions and it is considered to be a correlate of the irreversible disability in MS (Trapp et al., 1998).

Normal Appearing White Matter

Neuropathological studies of the macroscopically NAWM has revealed clear abnormalities: diffuse tissue injury such as astrogliosis, microglial activation, patchy demyelination and peri-vascular inflammation as well as evidence of axonal loss which seem to be, at least partially, independent of focal inflammatory demyelinating plaques (DeLuca et al., 2006; Evangelou et al., 2000). Whilst changes in the NAWM are seen at all stages of MS, these are more severe in more disabled patients (Kutzelnigg et al., 2005). They vary from diffuse inflammation (Kutzelnigg et al., 2005) to progressive axonal loss (Bjartmar et al., 2001; Evangelou et al., 2000). However, the axonal loss is an early event not only in lesion formation but also in NAWM (Bjartmar et al., 2001; Evangelou et al., 2000). Whilst the mechanisms of injury in NAWM remains still unclear the knowledge gained from the MRI studies suggest the perhaps that there is a component

due to Wallerian degeneration in NAWM secondary to the axonal transections associated with the inflammatory demyelination within lesions (Narayanan et al., 2006).

Grey Matter

There is now mounting evidence of GM involvement in MS. But despite the knowledge of the presence of GM lesions since early on (Brownell & Hughes, 1962), the assessment of GM pathology and its role in disability in MS has been rather slow until quite recently. This is perhaps due, at least partially, to the difficulties of visualising the GM lesions with current histo-chemical staining for myelin as well as with conventional MRI; the histology still revealing more cortical plaques than MRI (Geurts et al., 2008; Geurts & Barkhof, 2008; Kidd et al., 1999).

Cortical lesions are common in MS and are observed in about 90% of autopsy cases with chronic MS (subpial > leucocortical [i.e. involving cortex as well as juxtacortical WM] > purely intracortical) (Wegner et al., 2006). Cortical demyelination is more extensive in patients with progressive disease and can occur in the near absence of focal WM lesions (Bo et al., 2007; Kutzelnigg et al., 2005; Kutzelnigg et al., 2007). GM lesions differ from WM lesions: they are less inflammatory and rather more destructive (Bo et al., 2003; Bo et al., 2007; Peterson et al., 2001; Trapp & Nave, 2008). Axonal transections and neuronal apoptosis in cortical lesions (Peterson et al., 2001), reduction of glia, neurons and synapses in leucocortical lesions (Wegner et al., 2006), decrease of Purkinje cells in cerebellar cortical lesions (Kutzelnigg et al., 2007) and extensive involvement of the GM in brain and spinal cord (Gilmore et al., 2008), all provide evidence of extensive GM lesions with features of neurodegeneration. Whilst inflammation can lead to axonal loss

(Trapp et al., 1998), the axonal damage of the GM seems to be largely independent of WM lesions. The mechanisms of GM damage are still not fully understood but several mediators of axonal damage have been identified such as CD8 cytotoxic T-cells, macrophages/microglia, cytokines (nitric oxide) and glutamate.

Whilst not much data is available on the pathology, if any, of the non-lesional GM it is believed that the main contribution of the GM pathology to disease progression is through GM lesions. In addition, cortical reorganization may be important in the limitation of functional impairment. Recent functional MRI studies suggest that cortical adaptation in MS patients may help to limit functional impairment (Rocca et al., 2005; Rocca et al., 2010). It is plausible that extensive cortical disease especially in secondary-progressive MS (SPMS) leads to limited cortical plasticity with severe functional consequences .

1.4 Clinical manifestations and natural history

In about 85% of cases the disease starts with an acute episode of neurological deficit known as clinically isolated syndrome (CIS). The most common sites affected are optic nerve, brainstem or spinal cord although other presentations in other locations (and some times multifocal) are recognised. MS affects the whole CNS and other presentations, as well as the above mentioned, such as cognitive and psychiatric symptoms, are also recognised. Whilst most of the above mentioned are not specific to MS, Lhermitte's symptom – paraesthesia of the trunk and limbs on neck flexion and Utoff's phenomenon – blurred vision with increasing body temperature such as after a hot bath or exercise, are relatively specific to MS.

Whilst most of those who present with a CIS convert to MS [as high as 80% (Eriksson et al., 2003)], not all of them do (Brex et al., 2002; O'Riordan et al., 1998c). Optic neuritis (ON) is the most studied CIS perhaps due to the fact that it is the most distinctive and homogenous of the CIS syndromes and because its clinical presentation is more specific for demyelination than symptoms of brainstem or spinal cord that are more heterogeneous and have a rather wider differential diagnosis. Of those who present with a CIS, bilateral simultaneous ON and complete transverse myelitis (Lipton & Teasdall, 1973) have a much lower rate of converting to MS. Whilst the incidence of ON is fairly constant, its association with MS varies [frequent in United Kingdom (Swanton et al., 2006) and infrequent in Japan]. Those studies that have included brainstem and spinal cord syndrome patients in addition to ON patients have reported a similar conversion rate to MS amongst all syndrome presentations (Barkhof et al., 1997; Lee et al., 1991;

O'Riordan et al., 1998c; Tintore et al., 2000). Although a more recent study has shown that the conversion rate of ON versus non-ON CIS, is lower (Tintore et al., 2005), the conversion rate was similar across different CIS presentations when only patients presenting with ON and with an abnormal baseline brain MRI were included. (The role of MRI in predicting conversion to MS will be discussed in Chapter 3).

Most CIS patients who convert to MS will enter a relapsing-remitting course and the majority of them will then progress to the secondary-progressive stage, but the time and the course of this progression varies widely. A minority of CIS patients who convert to MS, can enter the progressive stage after a single relapse. An International panel defined the MS course in 1996 (Lublin & Reingold, 1996) (Table 1.1). Relapses are defined as the first occurrence, recurrence or worsening of symptoms of neurological dysfunction marked by sub-acute onset and a period of stability followed by either partial or complete recovery – the whole process lasting more than 24 hours. Progression is defined as steady worsening of at least more than 6 months.

In ~15% of cases, clinical progression with or without superimposed relapses [primary progressive MS (PPMS)] is seen from the disease onset (Cottrell et al., 1999; Thompson et al., 1997). Whilst there is sometimes discussion whether PPMS and relapse-onset MS are two different entities, most believe that they represent a spectrum of the same disorder.

The level of disability and the disease progression in MS is most often assessed by Expanded Disability Status Scale (EDSS) (Kurtzke, 1983) consist of scoring eight functional systems namely: visual, brainstem, pyramidal, cerebellar, sensory, bowel and

bladder and cerebral function. It ranges from 0 to 10 with 0 meaning no evidence of disability and 10 meaning death due to MS (Table 1.3). The EDSS scores above 4 are mainly driven by ambulation. In clinical trials other disability measurement such as MS Functional Composite Score (MSFC), which takes into account the upper limb function and cognitive impairment of the MS patients, has also been widely used (Cutter et al., 1999; Fischer et al., 1999).

Prognosis

As mentioned earlier, whilst most (~65%) RRMS patients go on to develop SPMS, the rate of progression varies widely. The progressive stage typically starts for both forms (SPMS and PPMS) around 40 years of age (Confavreux & Vukusic, 2006). From natural history studies, several factors have been identified as good prognostic factors in relapse onset MS (first presentation with ON, mono-focal presentation or sensory symptoms; being female; younger age of disease onset; lengthy time between the first to the next episode; low relapse rate on the first 2 years; minimal disability after 5 years) (Confavreux et al., 2003; Eriksson et al., 2003; Kurtzke et al., 1977; Miller et al., 1992; Nilsson et al., 2005) and several others as bad prognostic factors (being male; older age of disease onset; multifocal disease onset; high relapse rate in the first 2-5 years; substantial disability after 5 years) (Eriksson et al., 2003; Miller et al., 2005; Scalfari et al., 2010; Weinshenker et al., 1989). The role of MRI prognostic factors will be discussed in detail in Chapter 3.

The reported time to EDSS 6 (i.e. requiring assistance to walk) varies from 15 years (Canadian and Swedish series) (Kremenchutzky et al., 2006; Weinshenker et al., 1989) to 23 years (French series) (Confavreux et al., 2000). It might be possible that the outcome in MS differ with geography (Ebers & Daumer, 2008). Not all patients develop severe disability and some with little disability after many years are known as benign MS (BMS).

The definition of BMS is based on disease duration of at least 15 years, and an EDSS ≤ 3 (Lublin & Reingold, 1996). Although in some population based studies, a high percentage of people with BMS have been identified who have remained stable with follow-up (Pittock et al., 2004), other studies have shown that the term BMS is rather temporary, as many of these patients go on to develop more disability if they are followed on for long enough (Hawkins & McDonnell, 1999; Hirst et al., 2008; Sayao et al., 2007). New definitions of BMS have been introduced such as lowering of the EDSS score to <=2 although some other authors suggest that it would be best to use an index of progression rather than an arbitrary disease duration and EDSS cut-off (Weinshenker et al., 1989).

Death is attributed to MS in two-third of cases and to increased risk and complications of infections such as skin, pulmonary and urinary infections. The median time to death is 30 years from the disease onset, representing a reduction in life expectancy of 5-10 years.

Table 1.1 Lublin & Reingold (1996) definitions of clinical course of multiple sclerosis (Lublin & Reingold, 1996)

Clinical course	**Definition**
RRMS	Clearly defined disease relapses with full recovery or with sequelae and residual deficit upon recovery; periods between disease relapses characterised by a lack of disease progression.
SPMS	Initially RR disease course followed by progression with or without occasional relapses, minor remissions, and plateaus.
PPMS	Disease progression from onset with occasional plateaus and temporary minor improvements allowed.
PRMS	Progressive disease onset, with clear acute relapses, with or without full recovery; periods between relapses characterised by continuing progression

RRMS = relapsing-remitting multiple sclerosis; SPMS = secondary progressive multiple sclerosis; PPMS = primary progressive multiple sclerosis; PRMS = progressive-relapsing multiple sclerosis

Table 1.2 Kurtzke's Expanded Disability Status Scale (EDSS) (Kurtzke, 1983).

EDSS score	Definition
0	Normal neurological examination (all FS normal, mild cerebellar sign acceptable
1.0	No disability, minimal signs in one FS
1.5	No disability, minimal signs in more than one FS
2.0	Minimal disability in one FS
2.5	Minimal disability in two FS
3.0	Moderate disability in one or mild disability in up to four FS though fully ambulatory
3.5	Fully ambulatory but with a moderate disability in one FS and mild disability in one or two others; or moderate disability in two FS; or mild disability in five FS
4.0	Fully ambulatory without aid; self-sufficient; up and about some 12 hours a day despite relatively severe disability in one FS or combinations of exceeding the limits of previous step. Able to walk without aid or rest for 500m.
4.5	Fully ambulatory without aid; up and about much of the day; may otherwise have some limitation of full activity or require minimal assistance. Able to walk without aid or rest for 300m.
5.0	Ambulatory without aid or rest for 200m; disability severe enough to impair full daily activities.
5.5	Ambulatory without aid or rest for 100m; disability severe enough to impair full daily activities.
6.0	Intermittent or unilateral constant assistance required to walk 100m.
6.5	Constant bilateral assistance to walk 20m without rest.
7.0	Unable to walk 5m even with aid. Essentially restricted to a wheelchair. Transfers alone.
7.5	Unable to walk more than few steps. May need aid with transfers.
8.0	Restricted to bed or chair or perambulated in wheelchair. Generally has effective use of arms. Out of bed for much of the day
8.5	Essentially restricted to bed for much of the day. Retains some self-care functions
9.0	Helpless bed patient; can communicate and eat
9.5	Totally helpless, unable to communicate effectively or eat/swallow
10.0	Death due to MS

FS = Functional system

1.5 Diagnosis

To make a diagnosis of MS, dissemination in time and space is required. And whilst over the years several diagnostic criteria have been designed, the most commonly used are the Poser criteria (Poser et al., 1983) (Table 1.3) and the most recent criteria of the International Panel on the Diagnosis of Multiple Sclerosis (McDonald et al., 2001; Polman et al., 2005) (Polman et al., 2011) (Table 1.4 and 1.5). These sets of criteria emphasise the importance of history taking and clinical examination in establishing the dissemination in space and time (DIS and DIT) which remains the core of the diagnosis of MS. The emergence of new disease modifying drugs (DMDs) and their role in delaying the conversion to MS has lead to a need for a quicker and safer diagnosis of MS. Another reason for updating the criteria is the fact that MRI often shows silent lesions characteristic for MS in people with CIS and that such lesions influence the risk for MS. Whilst one can not make the diagnosis of MS on MRI findings only, the inclusion of the MRI criteria has proved to be the most sensitive paraclinical tool in establishing DIS and DIT at an earlier stage in the clinically manifested disease. MRI is also important in exclusion of conditions that are clinically similar to MS (Charil et al., 2006; Miller et al., 2008).

Other important paraclinical tools used are visual evoked potentials, and oligoclonal bands (IgG) in the CSF, the local synthesis of which can be detected in about 95% of MS patients (Andersson et al., 1994).

In the work presented in this thesis, clinical Poser criteria only, were used to define clinically definite MS: i.e. a second clinical episode with objective new neurological signs was used as a clear evidence for DIS and DIT (CDMS A1).

Table 1.3 Poser's MS diagnostic criteria (Poser et al., 1983)

Category	Attacks	Clinical evidence		Para-clinical evidence	CSF (oligoclonal band or increased IgG index)
A Clinically definite MS (CDMS)					
CDMS A1	2	2			
CDMS A2	2	1	and	1	
B Laboratory-supported definite MS (LSDMS)					
LSDMS B1	2	1	or	1	+
LSDMS B2	1	2			+
LSDMS B3	1	1	and	1	+
C Clinically probable MS (CPMS)					
CPMS C1	2	1			
CPMS C2	1	2			
CPMS C3	1	1	and	1	
D Laboratory-supported probable MS (LSPMS)					
LSPMS	2				+

Table 1.4 International Panel on the Diagnosis of MS criteria (Polman et al., 2005)

Clinical presentation	**Additional data needed for MS diagnosis**
Two or more attacks; objective clinical evidence of two or more lesions	None
Two or more attacks; objective clinical evidence of one lesion	DIS demonstrated by: 1. MRI[a] ***or*** 2. Two or more MRI-detected lesions consistent with MS plus +ve CSF[b] ***or*** 3. Await further clinical attack implicating a different site
One attack; objective clinical evidence of two or more lesions	DIT demonstrated by: 1. MRI[c] ***or*** 2. Second clinical attack
One attack; objective clinical evidence of one lesion (mono-symptomatic presentation; CIS)	DIS demonstrated by: 1. MRI[a] ***or*** 2. Two or more MRI-detected lesions consistent with MS plus +ve CSF ***and*** DIT demonstrated by: 1. MRI[c] ***or*** 2. Second clinical attack

Insidious neurological progression suggestive of MS	One year of disease progression (retrospectively or prospectively) ***and*** Two of the following: a) Positive brain MRI (nine T_2 lesions or four or more T_2 lesions with +ve VEP) b) Positive spinal cord MRI (two focal T_2 lesions) c) +ve CSF

DIS = Dissemination in space; DIT = Dissemination in time; CIS = Clinically isolated syndrome; CSF = Cerbrospinal fluid; VEP = Visual-evoked potential

[a]MRI DIS must fulfil the following criteria: ≥ 3 of the following:

9 T_2 lesions or 1 Gd-enhancing lesion;

≥ 3 periventricular lesions;

≥ 1 juxtacortical lesion

≥ 1 infratentorial lesion

A spinal cord lesion can replace an infra-tentorial lesion

Any number of a spinal cord lesion can be included in total lesion count

[b]Positive CSF determined by iso-electric focusing evidence of oligoclonal IgG bands not present in serum *or* increased IgG index *or* both

[c]MRI DIT must fulfil the following criteria:

A Gd-enhancing lesion ≥ 3 months after CIS onset ***or*** a new T_2 lesion with reference to a baseline scan obtained ≥ 30 days after CIS onset

Table 1.5 The 2010 McDonal Criteria for Diagnosis of MS (Polman et al., 2011).

Clinical presentation	**Additional data needed for MS diagnosis**
≥ 2 attacks; objective clinical evidence of ≥ 2 lesions ***or*** objective clinical evidence of 1 lesion with reasonable historical evidence of a prior attack	None
≥ 2 attacks; objective clinical evidence of 1 lesion	DIS demonstrated by: ≥1 T_2 lesion in at least 2 of 4 MS-typical regions of CNS (PV; JC; IT or SC) ***or*** await a further attack implicating a different CNS site.
1 attack; objective clinical evidence of ≥ 2 lesions	DIT demonstrated by: Simultanious presence of asymptomatic Gd-enhancing and non-enhancing lesions at any time; ***or*** a new T_2 and/or Gd-enhancing lesion (s) on follow up MRI, irrespective of its timing with reference to a baseline scan; ***or*** await a second clinical attack.
1 attack; objective clinical evidence of one lesion (CIS)	DIS demonstrated by: ≥1 T_2 lesion in at least 2 of 4 MS-typical regions of the CNS (PV; JC; IT or SC) ***or*** await a further attack implicating a different CNS site; ***and***

	DIT demonstrated by: Simultanious presence of asymptomatic Gd-enhancing and non-enhancing lesions at any time; ***or*** a new T_2 and/or Gd-enhancing lesion(s) on follow-up MRI, irrespective of its timing with reference to a baseline scan; ***or*** await a second clinical attack.
Insidious neurological progression suggestive of MS (PPMS)	1 year of disease progression (retrospectively or prospectively determined) ***plus*** 2 of 3 of the following criteria: 1. Evidence for DIS in the brain based on ≥1 T_2 lesions in the MS-characteristic (PV, JC or IT) regions. 2. Evidence for DIS in the SC based on ≥2 T_2 lesions in the cord. 3. Positive CSF (isoelectric focusing evidence of oligoclonal bands ***and/or*** elevated IgG index)

MS = multiple sclerosis; CIS = clinically isolated syndrome; DIS = dissemination in space; DIT = Dissemination in time; PV = periventricular; JC = Juxtacortical; IT = infratentorial; SC = spinal cord

1.6 Treatment and management

Despite the emergence of new disease modifying drugs (DMDs), there is no cure for MS as yet. The use of disease modifying treatment in high risk CIS patients have shown that the conversion to MS can be delayed, but not prevented (Beck et al., 2002; Comi et al., 2001; Comi et al., 2009; Kappos et al., 2007). The licensed DMDs, for use in RRMS, such as β-interferon (β-INF) component (1a – Avonex; Rebif and 1b - Betaferon) and Glatimer acetate (Copaxone), have a role in reducing the number of relapses and their severity by a third, but they have either little or no effect on the disease progression (Comi et al., 2001; Comi et al., 2009; Ebers et al., 2010; Jacobs et al., 2000a; Kappos et al., 2007; Kappos et al., 2006b). There are other approved DMDs such as Natalizumab (Miller et al., 2003; Polman et al., 2006; Rudick et al., 2006b) and Mitoxantrone (Edan et al., 1997) which are only used for patients with very active relapsing disease. Mitoxantrone, an unlicensed immunosuppressive treatment, is shown to reduce the rate of relapses and slow the progression of disability in active RRMS patients (Bastianello et al., 1994; Edan et al., 1997; Millefiorini et al., 1997) compared to those who received placebo or methylprednisolone. Natalizumab, a more recently licensed drug on treatment of MS, has shown to significantly reduce the relapse rate by two-third and progression of sustained disability by ~ 40% in RRMS patients when compared to placebo (Miller et al., 2007; Polman et al., 2006).

Fingolimod, the first oral therapy licenced for treatment in RRMS, is without a doubt an exiting news for the MS community. Fingolimod is the first of a new class of drugs that inhibits immune cell migration by integreating with sphingosine-1-phosphate receptor. When compared to placebo it showed significant reduction of relapses as well as of

disease progression (Kappos et al., 2010) and it has shown superiority when compared to β-INF-1a (Cohen et al., 2010).

A lot of research is still on-going to find new DMDs. More recently a randomised blinded trial comparing Alemtuzumab, also known as Campath-1H, a humanized monoclonal antibody which target CD52 lymphocytes and monocytes, with β-INF, Rebif, reduced the disease activity and the rate of relapses by 74% and more importantly reduced the risk of sustained accumulation of disability by 71%, efficacy which was maintained at 36 months despite the fact that nearly 72% of Alemtuzumab treated patients did not receive the month 24 cycle of therapy because of safety concerns (Coles et al., 2008). There are also several emerging oral therapies which in preliminary trials have shown to reduce MRI lesion activity +/- relapse rate (Comi et al., 2008; Giovannoni et al., 2010; Kappos et al., 2006b; Kappos et al., 2008; O'Connor et al., 2006).

Unfortunately none of the DMD's is free of adverse effects. Whilst the β-INF group of drugs cause flu-like syndromes, abnormal blood test the most serious adverse side effect is the occurrence of neutralizing antibodies (Nabs) and whilst the debate still continues there is increasing evidence that their occurrence reduces the efficacy of the DMDs. And whilst there is not very much difference amongst the β-INF treatments with regard to their efficacy, people taking Avonex tend to have a lower rate of Nabs than the rest.
The adverse effects with the monoclonal antibodies are much more serious. Natalizumab causes a life threatening illness – progressive multifocal leukoencephalopathy known as PML – in about 1/500-1/1000 (Clifford et al., 2010; Yousry et al., 2006), whilst

Alemtuzumab causes autoimmune complications such as dysthyroid disease and immune thrombocytopenic purpura. Also, Mitroxantrone is known to cause acute leukemia and cardiotoxicity (each ~1%) and hence its use is limited to highly active cases. The most recently oral therapy, Fingolimod, can also cause bradycardia and arterioventricular block as well as herpesvirus infections. So certainly one of the challenging issues for the neurologists treating MS patients is developing tools and strategies to optimise the risk-benefit ratio of ever growing treatment options for RRMS patients.

Whilst the above mentioned medications are used to treat acute relapsing MS, so far, no drugs have been shown to work on the progressive forms of MS. Experimental trials in progressive MS are currently focused mainly in neuroprotective strategies (Kapoor et al., 2010).

The rest of the treatment of MS is rather symptomatic looking at treating symptoms such as spasticity, bladder symptoms and fatigue. There is a role of high dose, short course corticosteroids in MS during an acute relapse, where they can hasten the recovery but do not influence the final outcome (Beck et al., 1992). A multidisciplinary approach with physiotherapy, occupational therapy, speech and language therapy and neuro-rehabilitation, all integrated, is very important in management of MS patients especially when they are accumulating significant disability (Freeman et al., 1997; Thompson et al., 2010).

1.7 Summary

MS is the commonest cause of neurological disability in the young adults. The concept of MS being only an inflammatory demyelinating disease of the WM is seriously being challenged. There is growing evidence that MS has a neurodegenerative component that seems to be more responsible for the disease progression and disability. From pathological studies there is evidence that neurodegeneration is an early event in the course of disease and appears not only in the WM lesions but also in the so-called NAWM as well as in GM. This concept has been supported by the findings from the MRI studies and MR spectroscopy. The questions of which come first, inflammation or neurodegeneration, and their relationship with each-other are still unclear.

Chapter II

Magnetic Resonance Imaging

2.1 MRI and physics

2.1.1 Basic principles.

Magnetic Resonance Imaging (MRI) is based on the principles of nuclear magnetic resonance (NMR). MRI was not called nuclear MRI because of the negative connotations associated with the word nuclear in the late 1970's. Felix Bloch (Bloch 1946), and Edward Purcell, both of whom were awarded the Nobel Prize in 1952, discovered the magnetic resonance phenomenon independently in 1946, but it took many years later before Lauterbur (1974) and Mansfield (1976) would independently develop the technique for medical imaging. The particle of interest in the work presented is the hydrogen nucleus ^{1}H predominantly in the form of water (which makes up about 75% of the human body) or lipids, although other nuclei with uneven number of protons and neutrons such as ^{19}F, ^{23}Na, and ^{31}P can also be studied with MRI, although they are less abundant in the biological tissues.

^{1}H nuclei contain a single positively charged proton. The nuclei have three intrinsic physical properties which are mass, charge and spin. Hence the ^{1}H nucleus is a continuously rotating positive charge. The combination of spin and charge produces magnetism (Hashemi RH & Bradley WG, 1997).

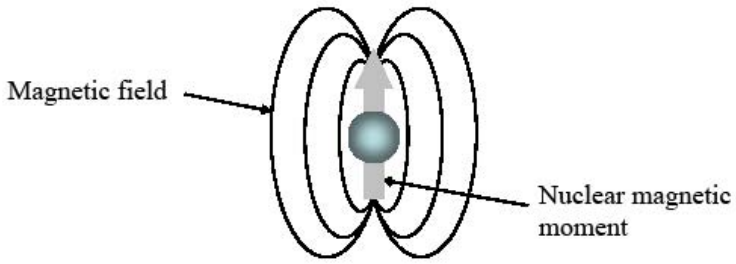
Magnetism and spin are related by the gyromagnetic ratio γ:

Magnetic moment = gyromagnetic ratio x spin

The gyromagnetic ratio depends on the nucleus, for (^{1}H): γ = 42.6 MHz / tesla. The magnetic nuclear moment can be considered as a tiny bar magnet (Figure 2.1). The sum of all these tiny magnet fields is called a net magnetization.

In the absence of a magnet field the protons are aligned randomly so the net magnetization is zero. The NMR phenomenon occurs when these protons are under the influence of an external magnetic field (usually called B_0) such as the one produced by the main coils of the scanner, which by convention is designated as the z-axis.

Figure 2.1: Vector representation of a nuclear magnetic moment



This NMR phenomenon can be described in two ways - classical and quantum mechanics. While the quantum mechanics deals with the individual protons, the classical theory deals with the average, net magnetization vector. Using the quantum mechanics description, in the presence of an external magnetic field (B_0) the protons will try to align themselves along the external field in two ways, based on their energy: either spin-up or parallel (lower energy state) or spin-down or anti-parallel (higher energy state) to B_0. Transitions from the parallel to the anti-parallel state can be induced in the sample under the influence of the electromagnetic radiation of energy. This phenomenon is the resonance feature of NMR.

http://en.wikibooks.org/wiki/Basic_Physics_of_Nuclear_Medicine/MRI_&_Nuclear_Medicine).

Within the magnetic field (B_0) there are more protons aligned parallel (i.e. low energy state) than anti-parallel (i.e. high energy state). Changes back to the parallel state occur spontaneously over a time period which is characteristic of individual tissues and their various pathological conditions.

In addition to the alignment, these protons will also precess (analogous to a gyroscope spinning around in the earths gravitational field) about B_0 at an angular frequency (ω), which is proportional to the main magnetic field strength and the gyromagnetic ratio for the ^1H (γ), given by the Larmor equation:

$$\boldsymbol{\omega_0 = \gamma B_0}.$$

The magnetic vector can be broken down in two components: a longitudinal or z-component (Mz) and the transverse component (Mxy). Longitudinal magnetization is due to a difference in the number of protons in parallel and anti-parallel states. Due to the

slight excess of parallel aligned protons (low energy) the net magnetization (macroscopic magnetization) has a longitudinal component aligned along the z-axis; the same as B_0. Longitudinal magnetization (Mz) is established by placing the patient in the magnet of an MRI scanner only.

To then generate an MRI signal, a radiofrequency pulse (RF) (short electromagnetic signal), usually a 90º or 180º pulse - which generates the B_1 magnetic field - is applied in transverse (x-y) plane at a frequency that matches ω (excitation phase). Only protons that spin with the same frequency as the electromagnetic RF pulse will respond to the RF pulse. During the absorption of the energy two processes occur simultaneously: a) some of the protons resonate and move to the anti-parallel state resulting in the reduction of the longitudinal magnetization (Mz) (Figure 2.2); and b) the protons line up with each-other in phase producing a magnetic sum vector on the XY plane called transverse magnetization (Mxy), a voltage which is detected by the receiver coil placed in the transverse plane. It is what happens between Mz and Mxy that is of importance in MRI.

2.1.2 T_1 and T_2 relaxation times

Switching off of the RF pulse leads to two processes. Firstly, the excited protons progressively return to their lower energy level, causing re-establishment of the longitudinal magnetization vector Mz. The recovery of longitudinal magnetization to the equilibrium state (by giving back the energy they obtained from the RF pulse to their immediate environment - the 'lattice') is known as the T_1 (longitudinal) relaxation time (Figure 2.2). Secondly, the transverse magnetization rapidly decreases (relaxation phase),

this is known as the T_2 relaxation time (Figure 2.3). The transverse relaxation time constant T_2 characterises the decay of magnetisation in the x-y plane to equilibrium.

Although in theory the decay of transverse magnetisation should be characterised by a constant time T2, in reality decay is faster and is characterised by a time constant known as T2*. This is due to the fact that in addition to the interactions with the other magnetic particles, protons are subject to the inhomogeneities in the external magnetic field. The heterogeneous fields arise due to imperfections in the magnetic susceptibility between the adjacent regions in the magnetic field. To ensure that the signal differences are only due to spin-spin interactions and not to additional differences in the external magnetic field, an additional 180° pulse could be applied to reverse dephasing due to field homogeneities.

Figure 2.2 T_1 (longitudinal) relaxation time

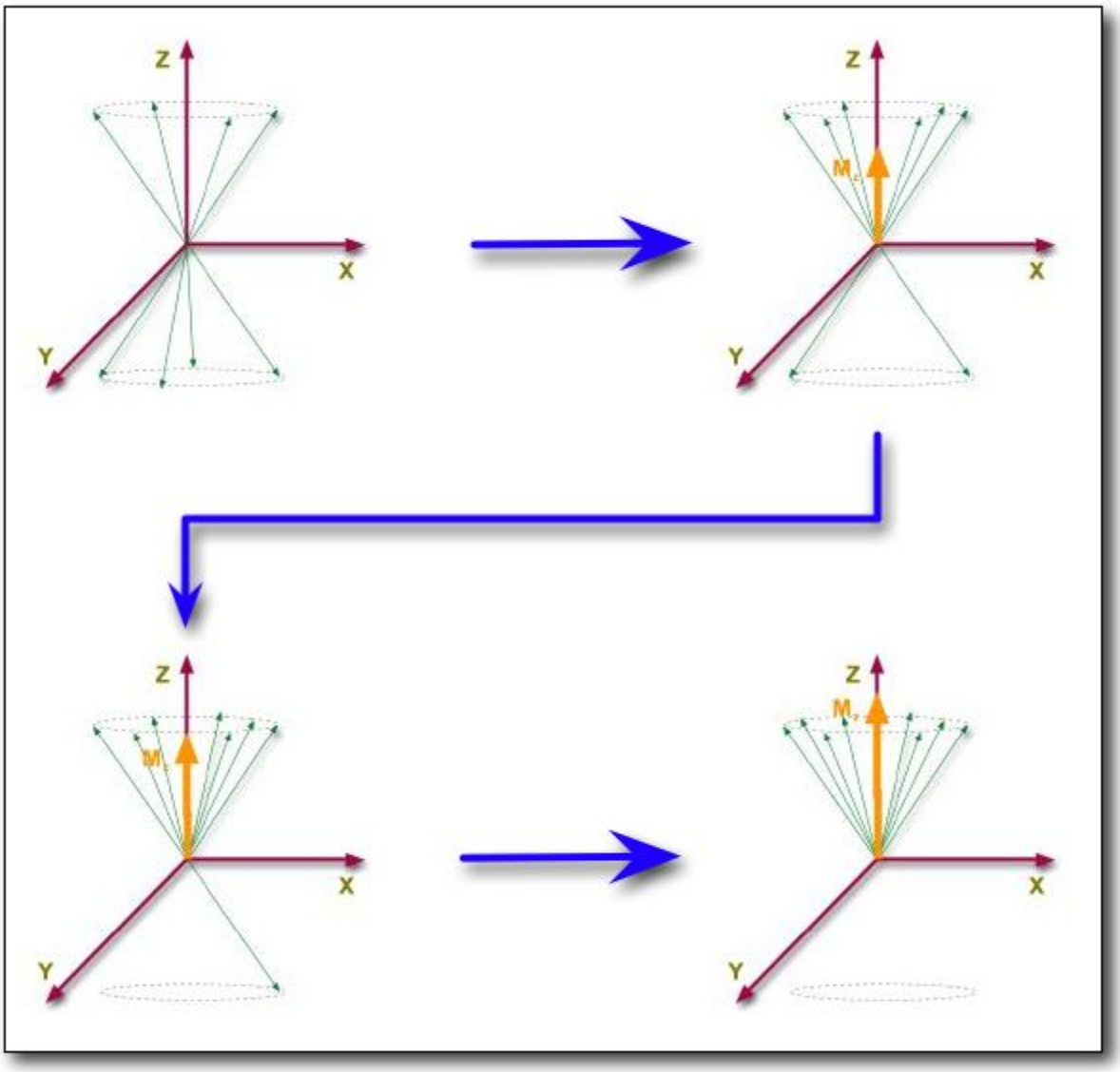
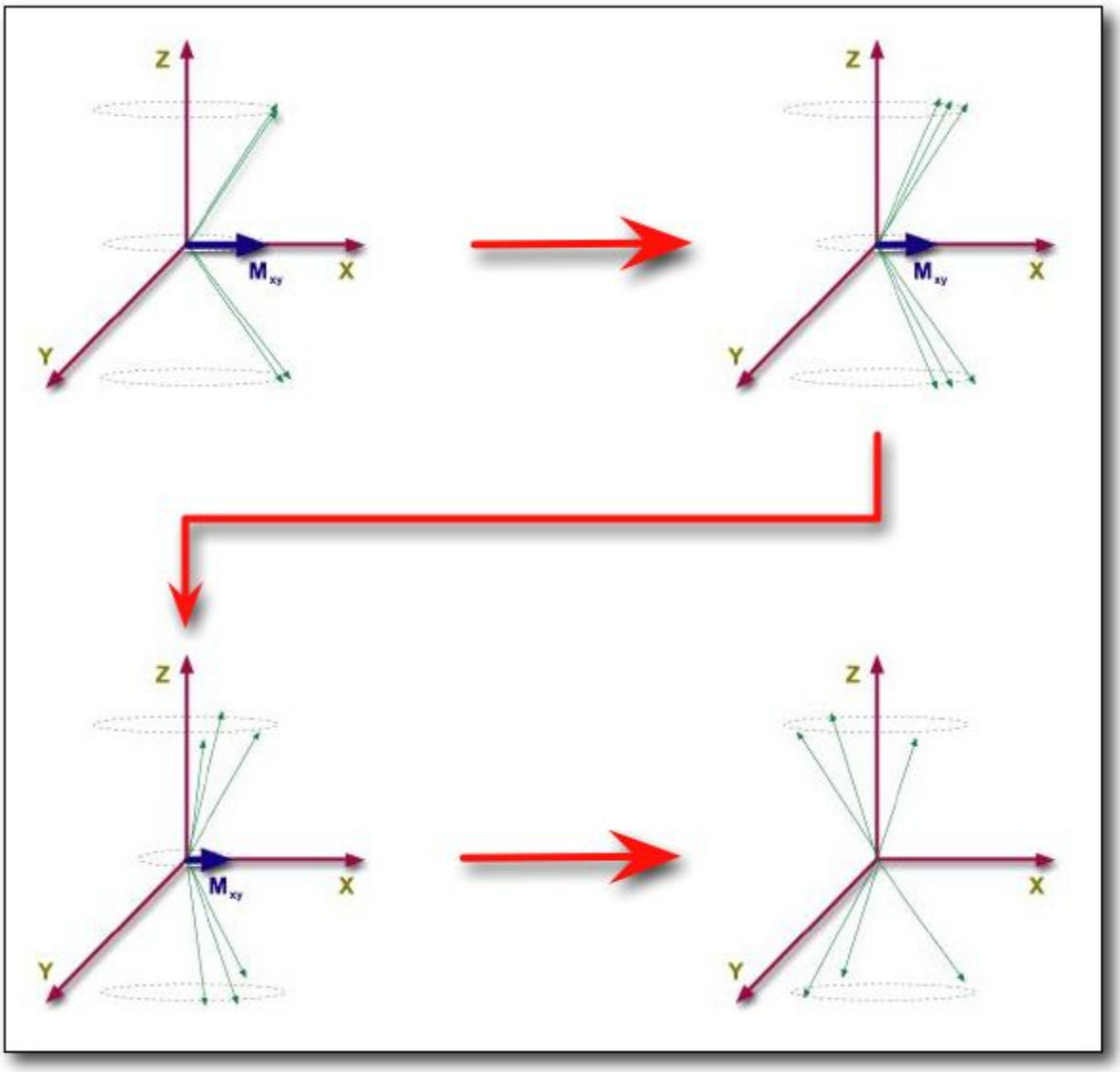


Figure 2.3 T_2 (transverse) relaxation time



T_1 and T_2 relaxation times are inherent properties of the tissue being examined which vary between tissues. T_1 and T_2 relaxation times in addition to the proton density (PD) which is the concentration of the protons in a given tissue, form the basis for much of the contrast observed in MRI sequences. Liquids have long T_1 and T_2 and solids have shorter values as shown in table 2.1. In brain, CSF contains H_2O hence it has a long T_1 and T_2. White matter (WM) is highly structured and hence has a shorter T_1 and T_2. Grey matter (GM) contains more macromolecules than CSF hence exhibits intermediate T_1 and T_2 relaxation times. MS lesions may have a decrease in structure making them detectable on MR images.

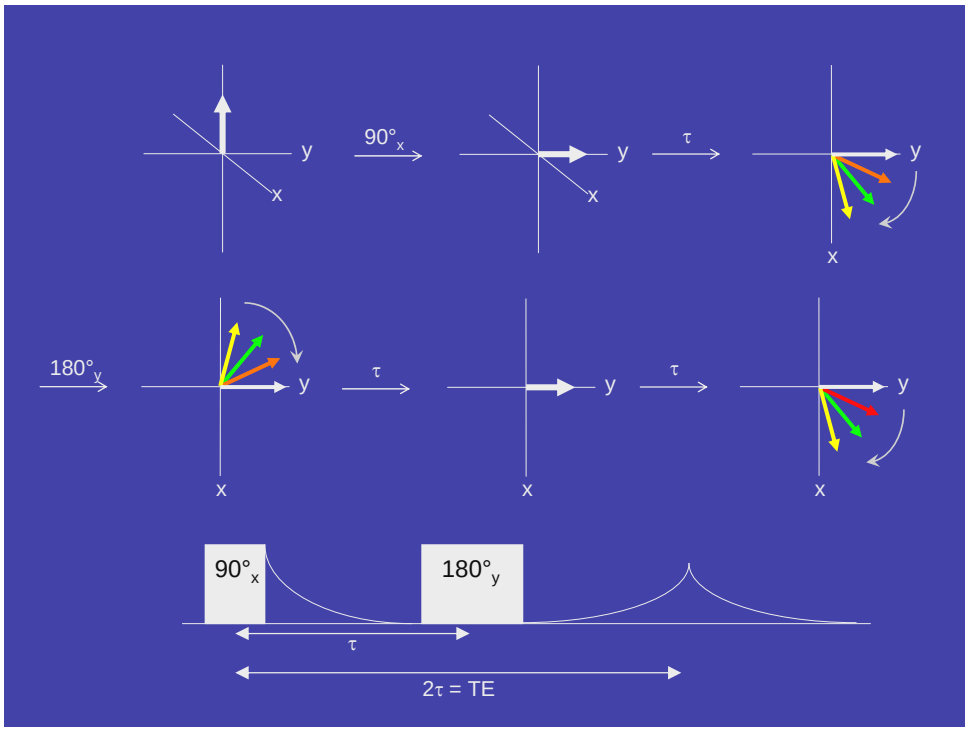
Table 2.1 T_1 and T_2 relaxation times (ms) of different tissues

Tissue	**T_1-relaxation time**	**T_2-relaxation time**
Fluids	1500 – 2000 (long)	700 – 1200 (long)
Water based tissues	400 – 1200 (midrange)	40 – 200 (midrange)
Fat based tissues	100 – 150 (short)	10 – 100 (short)

2.1.3 Imaging sequences

Spin echo (SE) sequence is routinely used in MRI. In order to obtain a SE sequence an initial excitatory 90° RF pulse is given, followed by a second 180° refocusing pulse.

Figure 2.4: Spin Echo sequence



The 90° pulse applied along the x-axis produces transverse magnetisation along the y-axis (T_2 relaxation time). The transverse magnetisation dephases (T_2 decay) due to time-invariant local inhomogeneities. A 180° pulse given at a time τ from the 90° pulse along the x-axis flips the spins from close to the y-axis to –y axis. The spins continue to precess in the same direction and at the same speed as before the 180° was applied, leading to the rephasing of the transverse magnetisation. The spins totally rephase along the –y-axis, to

give a spin echo signal which can be acquired (echo time TE). The time between two excitation 90° pulses represents the repetition time (TR).

TE and TR are important scan acquisition parameters. By varying these values we are able to weight images based on the T_1 and T_2 characteristics of the material imaged. These two parameters (TE and TR) determine the appearance of a spin echo image; different combinations of TE and TR will produce different degrees of contrast (intensity difference) between tissues. Changing TR will change the contrast between tissues with different T_1 relaxation times, changing TE will change the contrast between tissues with different T_2s. TR is almost always longer than TE.

The signal is always proportional to PD. To produce a PD-weighted image, a short TE (avoids T_2-weighting) and a long TR (avoids T_1-weighting) are acquired. To produce a T_1-weighted image in which the intensity contrast is due mainly to the T_1 relaxation properties of the tissue a short TE and TR are acquired. A long TE and TR will produce a T_2-weighted image in which the intensity contrast is due mainly to the T_2 properties of the tissue.

2.1.4 Gradient and image formation

The gradients are magnetic fields that change from point to point, usually in a linear manner. During the acquisition, while the magnetic field B_0 is constantly present, the gradient pulses are applied in a controlled fashion to form an MR pulse sequence (MRI from picture to proton). The gradients used in MRI allow for an image to be constructed. Three orthogonal gradient coils (slice-select, phase encoding and frequency [read or

readout] encoding gradient) are used to allow three-dimensional spatial encoding in the x, y and z directions.

The slice-selective gradient, turned on during the RF pulse, allows excitation of a slice of tissue corresponding to the bandwidth of the slice-selective RF pulse, exciting those protons at the Larmor frequency of the RF pulse. Doing this sequentially with successive excitations selects slices in a predetermined manner to cover the tissue under examination. Slice thickness is dependent on the RF pulse bandwidth and the amplitude of the slice-select gradient.

The frequency-encoding gradient is applied after the slice-select gradient when the echo is being received. This causes differing precession frequencies along the axis such that each part of the sample has a different frequency. It is only applied when the signal is measured, and hence the signal strength at each frequency can be measured.

The phase encoding gradient is applied after the RF pulse excitation and before the frequency-encoding gradient and provides spatial information about the orthogonal direction by causing a phase shift of the protons, with protons at different positions in the gradient precessing at different frequencies. The process is repeated with phase-encoding gradients of increasing amplitude, with a separate TR necessary for each encoding step, except for single shot techniques. The matrix of an image describes the number of frequency encodes x the number of phase encodes (most commonly 256 x 256).

The vector of application of the three gradients dictates the plane of imaging undertaken (axial, sagittal, coronal or oblique). For two-dimensional (2D) imaging the slice-select gradient only determines the plane whilst the other gradients determine the in-plane orientation. For 2D imaging we usually use frequency-encoding for one direction and phase-encoding in the other whilst for three-dimensional (3D) images we use frequency-encoding in one direction and phase-encoding in the remaining two.

For each TR, a line of data is stored in a computer as a series of numbers. This is repeated till all the data set is built up. This data does not correspond to the image and in order for this data to be transformed into an image it must be converted from time-domain to the frequency-domain using a mathematical process known as Fast Fourier Transform (FFT). The image consists of a matrix (number of pixels) with a field of view (FOV) that defines the dimensions of the area studied. FOV = number of pixels * voxel size.

2.1.5 Signal to noise ratio

Signal to noise ratio (SNR) is a measure of signal strength relative to background noise. SNR can be measured from the ratio of the mean voxel signal (from a homogeneous region with high signal intensity within the object of interest) divided by the SD of the background signal (measured from several regions outside the object). Noise is the undesired signal resulting from the MR system, the environment and the patient. It occurs at all frequencies and randomly in time. To increase the SNR usually requires increasing the signal relative to the noise. Parameters that affect the SNR are: the field strength,

proton density, coil type and position, TR, TE, flip angle, number of signal averages and receive bandwidth.

2.1.6 Contrast to noise ratio

Contrast-to-noise-ratio (CNR) is the ratio of the difference in signal intensity between regions with different tissues, and the background noise. CNR is the most important aspect of image quality.

It is controlled by the same factors that affect SNR. CNR is probably the most important image quality factor as the objective of any examination is to produce an image where pathology is clearly seen relative to normal anatomy. Visualization of a lesion increases if the CNR between it and surrounding normal tissue is high. CNR can be increased by the T_1 or T_2-weighting, administration of a contrast agent, magnetization transfer contrast and chemical suppression techniques.

2.2 Conventional MRI techniques in MS

2.2.1 T_2-weighted images

Whilst T_1-weighted images (see 2.2.2) were the first application of the MRI in MS, (followed by T_2 and PD-weighted SE images), T_2-weighted images are now the gold standard sequences for depicting the WM lesions. The images with very little T_1 or T_2-weighting are often called PD-weighted images although all the images have intrinsic PD-weighting properties (Tofts P, 2003). On the T_2 and PD-weighted images, MS lesions appeared as hyperintense areas called T_2 lesions (Figure 2.5) (Bailes et al., 1982).

The T_2 contrast can be achieved using SE, fast spin-echo (FSE) or fast fluid attenuated inversion recovery (FLAIR). Acquisition of a FSE requires the utilization of multiple 180° refocusing pulses, which allows filling of multiple lines of data in one shot; typically between 2-16 lines of data can be filled per TR. The scanning time is a function of TR, number of excitation and phase encodings. By performing the same number of phase encodings but with more than one phase encoding per TR, the acquisition time in FSE is reduced with no reduction in SNR compared to the conventional SE. The increased speed also allows high resolution imaging not achievable with conventional SE.

Clinically silent T_2 WM hyperintense lesions are present in 50-70 % of people who present with a clinically isolated syndrome (CIS) typical of MS such as optic neuritis (ON), brainstem syndrome (BS) and spinal cord syndrome (SC) (Jacobs et al., 1986; Miller et al., 1987; O'Riordan et al., 1998b; Ormerod et al., 1986a) and their presence indicates a higher likelihood of developing clinically definite MS (Brex et al., 2002;

Jacobs et al., 1997; Minneboo et al., 2004; Morrissey et al., 1993; O'Riordan et al., 1998b; Soderstrom et al., 1994; Swanton et al., 2007).

MRI remains the most important paraclinical tool in diagnosing MS. Different MRI criteria have been designed to assess the likelihood of the visible MRI lesions being as a result of MS (Barkhof et al., 1997; Fazekas et al., 1988; Paty et al., 1988). More recently the McDonald Criteria (McDonald et al., 2001) (which were later on revised (Polman et al., 2005; Polman et al., 2011)) have been developed presenting a scheme to better and more reliably diagnose MS. These criteria formally incorporated the MRI features in the established diagnostic workup which includes the neurological history and examination and the use of paraclinical laboratory examination in order to establish the dissemination in time and space which are crucial for the diagnosis of MS.

But, while these T_2 lesions predict conversion to clinically definite MS (Swanton et al., 2007), they only predict subsequent disability to a limited degree (Brex et al., 2002; O'Riordan et al., 1998c). Whilst pathological studies have confirmed that these T_2 lesions correspond with the MS plaques, (Ormerod et al., 1987), these lesions lack pathological specificity being driven by inflammation, demyelination, oedema as well as axonal loss and remyelination (Barkhof et al., 2003). These could explain at least partially the modest correlation of these lesions with disability. Also, most of the studies have been short to medium term and as disability in MS accrues slowly, longitudinal studies are needed to fully assess the potential of MRI in predicting disability.

WM T_2 lesion load has frequently been included in treatment trials to assess the response to the disease modifying treatment and assess disease progression (Comi et al., 2001; Jacobs et al., 2000a; Kappos et al., 2006b).

Whilst the presence of demyelinating lesions in the cortex has been known for a long time (Brownell & Hughes, 1962), their significance has been largely ignored. This is due, at least partially, to the fact that on the conventional MRI most of lesions in the GM are not visible (Geurts et al., 2005) and despite improved sequences they remain largely undetected (Geurts et al., 2005; Geurts et al., 2008). Certainly, other factors such as poor visualization of these lesions with the conventional histological staining methods has played a role on disregarding these lesions for a long time (Geurts & Barkhof, 2008).

2.2.2 Spin-Echo (SE) and T_1-weighted images

T_1-weighted inversion recovery images, which identify the MS lesions as areas of hypointensity compared to the normal white matter, were the first application of MRI in MS patients (Young et al., 1981).

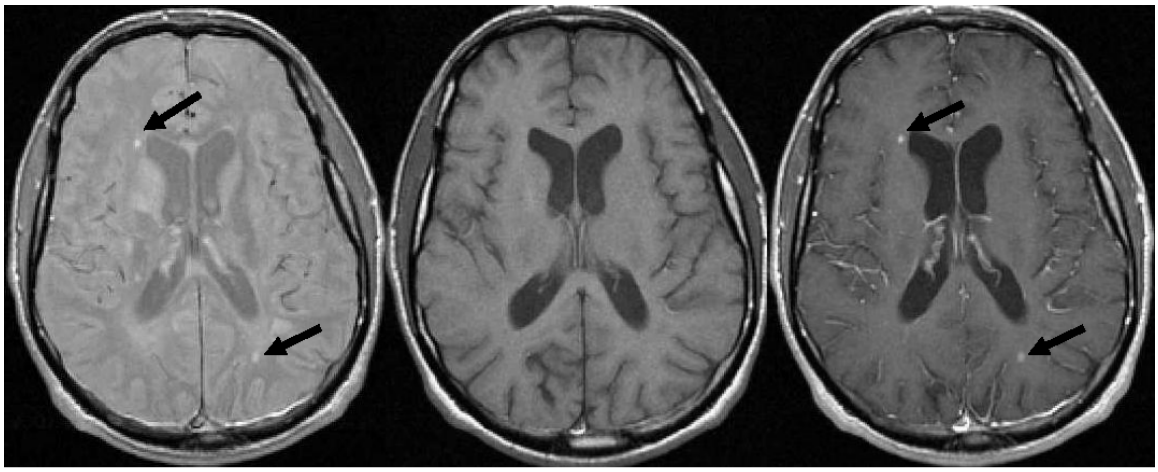
The persistent hypointense lesions seen on the now widely used T_1-weighted SE sequence seem to represent areas of greater tissue matrix damage including axonal loss (Bruck et al., 1997) (van Waesberghe et al., 1999) and in some studies (but not in all of them (O'Riordan et al., 1998a)) have shown better correlation with disability than T_2 lesions (Truyen et al., 1996).

2.2.3 Enhanced images

Gadolinium (Gd) a paramagnetic element, (which has seven unpaired electrons in its electronic structure) that when chelated to, for example, diethylene-triamine-pentacetic-acid (DTPA; one of a number of chelates currently used) to eliminate the toxic effects of free Gd, is used as contrast agent in MRI. Chelated Gd is given intravenously; despite being too large a molecule to cross the blood-brain barrier quickly, it can slowly leak out into brain tissues but rapidly accumulates in lesions where the blood-brain barrier is disrupted resulting in "enhancement" of the active MS lesions (Fig 2.5) (Bruck et al., 1997). The effects of the paramagnetic Gd, is to decrease the T_2 and T_1 relaxation times of the protons in the immediate vicinity of the molecule. Due to the protons exchange with other protons there is an overall reduction of T_1 and T_2. At usual doses (0.1mmolGd/kg) the major effect of the Gd is the reduction of T_1 in tissues where it accumulates, so on post-contrast T_1-weighted images the lesions will have enhanced signals, (i.e. appear hyperintense).

Gd enhanced lesions are used in MS treatment trials to monitor the disease activity. Gd-enhancement has been associated with histopathological evidence of active inflammation and demyelination (Bruck et al., 1997; Katz et al., 1993) and is a consistent feature of new lesions (lasting typically for several weeks) in relapsing forms of MS (Kermode et al., 1990; Miller et al., 1988b; Thorpe et al., 1996).

Figure 2.5 PD-weighted image (left) and SE T_1-weghted images pre (central image) and 20 minutes post intravenous contrast (right) showing two Gd-enhancing lesion (left image).



NB: The same lesions are marked with an arrow on the corresponding PD-weighted image where they appear as hyperintense. Note that no hypointense lesions are seen in the non-contrast SE image.

2.3 Quantitative Imaging Techniques

2.3.1 Atrophy

Brain atrophy is well recognized not only in established MS but also in CIS patients who convert to MS (Dalton et al., 2004). Although atrophy is apparent at earlier stages of relapse-onset MS, is maybe more evident at later stages (Dalton et al., 2006). Whole brain atrophy, as well as tissue specific (i.e. grey matter (GM) and white matter (WM)) and regional atrophy has been observed at different stages of MS (Bakshi et al., 2001; Carone et al., 2006; Chard et al., 2004; Dalton et al., 2004; Fisher et al., 2008; Sastre-Garriga et al., 2005; Zivadinov et al., 2004). Some studies have shown the rate of atrophy been the same across the different subtypes of MS (Kalkers et al., 2002) although other studies have shown more atrophy in the progressive forms (Dalton et al., 2006; Fisher et al., 2008).

Although MS has classically been seen as a WM disease, there is growing evidence that there is an extensive involvement of the GM in MS, (as well as normal appearing WM (NAWM)), and also that the GM pathology is more clinically relevant. GM atrophy in particular seems to be a better predictor of disability than WM atrophy and T_2 lesions (Pirko et al., 2007; Sanfilipo et al., 2005). While brain atrophy may be mainly driven by neurodegeneration, brain volume is also affected by other factors such as oedema, inflammation and demyelination, although to a lesser degree. Brain atrophy has been suggested as a potential surrogate marker of disability progression in MS and increasingly has been included in treatment trials in MS especially of the progressive forms (Altmann et al., 2009; Furby et al., 2008; Kapoor et al., 2010).

The spinal cord is frequently involved in MS patients and is an important element of the acquired disability. Cervical cord atrophy is observed in MS. Sequences such as fast-spoiled gradient echo images have been used to assess the size of the cord and reproducible techniques such as Losseff technique (Losseff et al., 1996) have shown that the cervical cord atrophy was strongly correlated with disability.

2.3.2 Magnetization transfer imaging

Traditionally MRI uses the T_1 and T_2 relaxation times and proton density (PD) of tissue water (hydrogen protons) to generate contrast. Magnetization transfer (MT) imaging is an additional way of obtaining tissue contrast based on the fact that tissues contain two or more separate populations of hydrogen protons: the free highly mobile (water) hydrogen pool, and the immobile restricted hydrogen pool, such as those protons bound to large macromolecular proteins and lipids (such as myelin), found in cellular membranes. The macromolecular pool has very short relaxation times (a very short T_2) and is therefore invisible to conventional imaging. It can be measured indirectly by the use of an off-resonance radiofrequency pulse capable of selectively saturating the macromolecular proton pool. Magnetization transfer (MT) contrast uses additional RF pulses (these pulses are either applied at a frequency away from the Larmor frequency where they are known as off-resonant, or nearer to the centre frequency where they are known as on-resonant) to suppress hydrogen protons that are not free but bound to macromolecules and cell membranes. As a result of the application of these pulses, reduced magnetization is transferred to the MR visible free protons (via exchange with the macromolecular pool)

suppressing the signal in certain types of tissues. The difference (percentage reduction) in the observed signal intensity (of an identical image) before and after the saturation pulse (with and without the saturation) is the magnetization transfer ratio (MTR), which reflects, at least partially, the density of the macromolecular protons in a given tissue.

Although MTR is the result of the combination of several physical quantities and it is highly dependent on the acquisition sequence parameters, it has nevertheless proved to be a sensitive marker of pathological change in the sense that it decreases with increasing histopathological changes (Schmierer et al., 2004; van Waesberghe et al., 1999). A reduction in a tissue's macromolecular content will result in a lower MTR and, consistent with this, a low MTR is associated with demyelination and a reduction in axonal density (Mottershead et al., 2003; van Waesberghe et al., 1999) and to a lesser extent inflammation (Schmierer et al., 2007).

The normalized histogram - a frequency distribution showing the number of voxels with a particular range of MTR parameters values - is a widely used technique to depict brain MTR. Whole brain histogram analysis (any information on the location of abnormalities is lost in this case) and GM, WM and lesion histograms can be acquired. MTR changes are observed at all stages of MS: CIS patients at high risk of converting to MS (Fernando et al., 2005), early relapsing-remitting MS (Davies et al., 2004) and progressive stages. GM (as well as in deep GM (Audoin et al., 2007)) and NAWM reduction in MTR are observed at all stages of MS and seem to reflect the disease phenotype: the histogram metrics are more severely affected the more patient is disabled i.e. abnormalities in

SPMS>RRMS>CIS (Cercignani et al., 2001; Ge et al., 2002; Traboulsee et al., 2002). MTR changes especially of the GM seem to correlate better with disability (Ge et al., 2001a; Khaleeli et al., 2007b).

MTR changes are also observed in MS lesions and these seem to correlate to some extent with disability (Gass et al., 1994).

Other techniques apart from histograms, such as the use of principal component analysis – which maximizes the variability between histograms – have been reported to improve the correlation between whole brain MTR and disability measures (Dehmeshki et al., 2001).

PART II

RELATIONSHIP BETWEEN LONGITUDINALCHANGES IN WHITE MATTER LESION LOAD AND DISABILITY AT 20 YEARS

Chapter III

Disability and T_2 MRI lesions: a 20 year follow up of patients with relapse-onset of MS.

Introduction

As discussed in the previous chapter (Chapter 1) clinically isolated syndromes (CIS), such as optic neuritis, brainstem or spinal cord syndromes are frequently the first clinical presentation of MS. However, not all CIS patients convert to MS and in those who do, disability is highly variable (Boiko et al., 2002; Confavreux & Vukusic, 2006; Kremenchutzky et al., 2006; Nilsson et al., 2005; Runmarker & Andersen, 1993; Weinshenker et al., 1989) and limited prognostic information is provided by early clinical features such as the type of CIS, frequency of early relapses and disability status 5 years after onset.

Numerous studies have investigated the prognostic role of MRI in CIS patients, most with follow up of a few years only. Clinically silent T_2-weighted brain white matter lesions are present in 50-70% of CIS patients (Jacobs et al., 1986; Miller et al., 1987; Ormerod et al., 1986a) and their presence indicates a higher likelihood of developing clinically definite MS (Beck et al., 2003; Brex et al., 2002; Jacobs et al., 1997; Minneboo et al., 2004; Morrissey et al., 1993; Soderstrom et al., 1994).

To assess the potential of MRI to predict disability due to MS requires prolonged follow up, since on average disability accrues only slowly in MS. Tintore et al (2006) followed up 156 patients with CIS after a mean of 7 years and they found that baseline brain MRI

findings helped to differentiate patients with low, medium and high risk for conversion to MS. The number of brain lesions best predicted 5 year disability (Tintore et al., 2006). Minneboo et al reported on 42 CIS patients followed up 8.7 years later and showed that two or more infra-tentorial lesions best predicted long-term disability (Minneboo et al., 2004). Another long term follow up study of 30 patients with relapsing remitting MS reported that T_2 lesion volume correlated strongly with brain tissue loss and clinical disease severity 13 years later (Rudick et al., 2006a)

In a previous report based on data from 14 year follow up of a prospectively recruited CIS cohort, EDSS score at 14 years correlated moderately with lesion volume in MRI at 5 years ($r_s = 0.60$) and with increase of lesion volume over the first 5 years ($r_s = 0.61$) (Brex et al., 2002). I followed up this same CIS cohort and I report here the findings after 20 years. The overall aim was to investigate whether the longitudinal relationships between the MRI and clinical course were maintained over a uniquely long period of 20 years and to assess how lesions evolve in relation to natural history. This investigation includes a comparison of the rate of lesion volume change over time in three distinct subgroups classified according to their clinical status at last follow up: CIS, RRMS or SPMS.

Methods

Subjects

One hundred and forty CIS patients were recruited between May 1984 and July 1987 and had a clinical assessment and brain MRI performed (Miller et al., 1987; Ormerod et al., 1986b; Ormerod et al., 1986a; Ormerod et al., 1987). A follow up was performed approximately one year later in 109 patients (53 optic neuritis; 23 brainstem syndrome and 33 spinal cord syndrome), who had a clinical examination and brain MRI scan repeated at this time (Miller et al., 1988a; Miller et al., 1989). Subsequent follow ups were performed after 5 (89 patients) (Morrissey et al., 1993), 10 (81 patients) (O'Riordan et al., 1998c) and 14 years (71 patients) (Brex et al., 2002).

I wanted to follow up - after 20 years - those patients in whom the baseline scans and adequate clinical information of the first clinical episode were still available. The study was approved by the National Hospital for Neurology and Neurosurgery and Institute of Neurology Joint Research Ethics Committee. The NHS Strategic Tracing System, which is a database of people, places and organizations in England and Wales, was used in those cases where the contact details were missing or found to be incorrect. Approval for access to this database for research purposes was obtained from the Security Confidentiality Advisory Group of the NHS Information Authority (the Research Ethics Committee also approved the use of the NHS Strategic Tracing System for research purposes).

Special precaution was needed when approaching those who had been seen at baseline and year 1 and 5 but not at years 10 or 14. During the period covering the earliest time-points of this follow up (i.e., the baseline, year 1 and year 5 assessments that were performed from the mid 1980s to the early 1990s), it was not routine practice to provide patients with information about the link between CIS and MS, nor was there a standard patient information sheet for recruitment which contained this information. Some participants in the study might therefore still have been unaware of the association of their symptoms with MS. It was also uncertain whether subjects would expect, or be sympathetic to, contact from us some 15 or more years after the last time they had been seen. Thus with the Research Ethics Committee approval, the initial contact with such individuals was by a letter which did not provide full details of the project, or mention MS, but simply asked them to get in touch if they were interested in being followed up. Those people who replied positively to this initial approach were invited for clinical examination and brain MRI at which point further information was provided, including the relevance of the study to MS. With those patients who did not reply either positively or negatively to two letters of invitation, I made an attempt to contact them either by phone or through their general practitioner.

Of the 140 subjects who were identified and had been initially recruited with a CIS for a baseline MRI study, 8 were excluded because they had either at baseline or developed subsequently another non-MS diagnosis: 3 at baseline - pontine arteriovenous malformation, pontine haematoma and Leigh's disease; 3 at 5 years - myasthenia gravis, cerebrovascular disease and HIV related complications; one at 10 years - systemic lupus

erythematosus and one at 14 years - cerebrovascular disease. Six other patients had died from non-MS related disease (3 between years 1 and 5 and 3 between years 14 and 20) and there was insufficient information to determine their prior neurological status. Of the remaining 126 subjects, 3 were abroad and could not be traced; 2 did not reply to a second letter of invitation and no contact number was available; one was contacted but did not wish to be followed up; the contact details were unobtainable in 9; and either the baseline scan could not be located or adequate clinical information on the first presentation was not available in 4 subjects.

The remaining 107 patients were included in the study: 104 who were living at the time of the 20 year assessment, and 3 who were known to have died from the complications of severe MS at an earlier timepoint. Of these 107 subjects, the first follow up was after 1 year for 92, 5 years for 7, 10 years for 3 and 20 years for 5 subjects. The disease duration (from CIS onset) at the 20 year follow up was a mean of 20.2 years (range 18 to 27.7 years).

Clinically definite MS was diagnosed by using Poser criteria and was based solely on clinical evidence (Poser et al., 1983). This required a second clinical episode with objective new neurological signs i.e. clear evidence for dissemination in time and space. Patients who did not develop clinically definite MS were classified as remaining CIS at the 20 year follow up. Because the primary focus was to explore the relationship of MRI findings with the clinical course and natural history, MRI evidence only for dissemination in space and time (Polman et al., 2005) was not used to diagnose MS. In

those who developed MS, the clinical course, either relapsing remitting or secondary progressive, was determined by Lublin and Reingold criteria (Lublin & Reingold, 1996). Disability status was determined for all subjects by using EDSS (Kurtzke, 1983) and MSFC (Fischer et al., 1999). RRMS with an EDSS ≤ 3 was defined as benign MS.

MRI Protocol

Baseline and 5 year MRI scans were obtained on a 0.5 Tesla (T) scanner (Picker, Cleveland) and on a 1.5 T GE Signa Echospeed scanner (General Electric Signa, Milwaukee) at 10, 14 and 20 years (Table 3.1). Contiguous, axial slices covering the whole brain were obtained at all visits. In a minority of early baseline scans (1984/1985) slice thickness was 10 mm; it was 5 mm for all subsequent scans. At 20 years T_2-weighted, dual-echo fast spin-echo sequences (FSE) of the brain were obtained on 75 patients (TR 2000ms; TE 17/102ms; 28 x 5 mm [matrix 256 x 256, FOV 24 x 18 cm, 1 NEX]). Of the remaining 32 subjects included in the 20 year clinical follow up, 3 had died of severe MS; one had become claustrophobic and the remainder could not be scanned either because of severe disability (8 subjects) or because lived too far away (20 subjects). In these patients phone EDSS was obtained. Identification of the lesions on the hard copies was performed by me and it was checked for consistency by an experienced neuroradiologist who was blinded to the clinical details. Marking of the lesions was done on the short echo image with the long echo image used as a reference, by myself. Baseline MRI was classified as abnormal if it displayed one or more clinically silent lesions compatible with demyelination. A normal scan might include the CIS symptomatic lesion only. T_2 lesion volume was measured from electronic images using a

semi-automated contouring technique to outline lesions, previously described (O'Riordan et al., 1998a; Sailer et al., 1999). The 20 year T_2 lesion volume was not measured in one patient in whom cerebrovascular disease developed as a second pathology.

Table 3.1 T_2-weighted images and their parameters at all follow up time-points

Time-point	Scanner strength (Tesla)	Sequence	TR (ms)	TE (ms)	Slice thickness (mm)
Baseline May 1984 - Aug 1987	0.5T (Picker Cleveland)	SE	2000	60	5 or 10
1 year follow-up Nov 1984 - Dec 1987	0.5T (Picker Cleveland)	SE	2000	60	5
5 year follow-up Jan 1990 - Jul 1991	0.5T (Picker Cleveland)	SE	2000	60	5
10 year follow-up Jun 1995 - Nov 1996	1.5T (General Electrical Signa, Milwaukee)	FSE	2000	30/90	5
14 year follow-up Jan 1999 - Jul 2000	1.5T (General Electrical Signa, Milwaukee)	FSE	2000	19/95	5
20 year follow-up Nov 2004 - Apr 2007	1.5T (General Electrical Signa, Milwaukee)	FSE	2000	17/102	5

Statistical Analysis

The data was analyzed using the standard statistical software package (SPSS 12, Chicago, IL) and Stata 9.2 (Stata Corporation, College Station, Texas, USA). Spearman rank-correlation was used to evaluate the correlation of the lesion volume or change of lesion volume, on brain MRI, with the EDSS and MSFC score. Because of the skewed (non-normal) nature of T_2 lesion volume data, a non parametric test, Cuzick's test, was used to look for a linear trend across the 3 groups of patients (defined as CIS, RRMS or SPMS at 20 years), to assess whether the lesion variable is associated with these different clinical courses; and the Wilcoxon rank-sum test was used to compare the rate of lesion volume increase between these groups. Longitudinal linear mixed models were used to compare rates of change in lesion volume, with T_2 lesion volume as response variable and time and patient group terms as covariate, using all available data-points for the T_2 lesion volume. A quadratic term was fitted in addition to the linear term in the model for the T_2 lesion volume over time to assess if there was non-linearity in lesion growth. Bootstrap confidence intervals were obtained where residuals showed signs of non-normality. Bootstrap derived p-values are given as ranges, or to fewer decimal places, due to the computer-intensive nature of the method.

Results

The cohort followed up at 20 years was similar to that seen at baseline in terms of mean age of disease onset, gender, type of syndrome and frequency of abnormalities on MRI at presentation (Table 3.2). Disability was assessed by examination on 77 patients. EDSS only was assessed by telephone on 27 patients who were unable to attend the hospital for a clinical examination (Lechner-Scott et al., 2003). Three who had already died from severe complications of MS (Brex et al., 2002) were assigned an EDSS of 10. Three patients were on disease modifying treatment (2 had RRMS and one had SPMS). Another patient had stopped medication three years before the time of examination, after entering secondary progressive stage. The 75 patients who had MRI at 20 years were compared with the 29 who were not scanned at 20 years in terms of the baseline demographic features: the groups were similar with regard to age, gender and frequency of baseline MRI abnormalities, while the scanned group had a high percentage with optic neuritis (55% vs. 41%) and a lower percentage with brainstem presentations (19% vs. 31%). The median EDSS at year 20 was 2.5 in the scanned group and 5.5 in the non-scanned group, higher disability in the latter group reflecting the fact that disability precluded scanning a number of patients.

Table 3.2 Characteristics of the cohort at each follow up time-point

Characteristic	**Baseline**	**1 Year**	**5.3 Year**	**9.7 Year**	**14.1 Year**	**20.2 Year**
Patients who underwent clinical assessment - no.	140	109	89	81	71	107
Exclusion due to alternative diagnosis - no	3	0	3	1	1	0
Deaths not related to MS - no	0	0	3	0	0	3
Female sex - no. (%)	80 (60) [+]	68 (62)	53 (60)	53 (65)	49 (69)	71 (66)
Mean age at presentation.	32[+]	32	31	32	32	32
Range	13-49	13 - 49	13 - 49	17 - 49	19 - 49	13-49
Optic neuritis - no. (%)	69 (50)	53 (49)	44 (49)	42 (52)	36 (51)	54 (51)
Brain stem syndrome - no. (%)	33 (24)§	23 (21)	17 (19)	16 (20)	14 (20)	25 (23)
Spinal cord syndrome - no. (%)	38 (28)	33 (30)	28 (31)	23 (28)	21 (30)	28 (26)
MRI examinations - no.	140	109	89	64	55	75
Electronic data for lesion volume quantification - no	74	---*	69	63	55	75
Abnormal results-baseline MRI-no. (%)	85 (64) [+]	69 (63)	57 (64)	54 (67)	50 (70)	73 (68)
Optic neuritis - no. (%)	42 (64)					36 (67)
Brain stem syndrome - no. (%)	23 (77)					20 (80)
Spinal cord syndrome - no. (%)	20 (54)					17 (61)

Conversion to clinically definite MS - all patients - no. (%)	--	21 (19)	38 (43)	48 (59)	48 (68)	67 (63)
Abnormal scan at baseline	--	21 (30)	37 (65)	45 (83)	44 (88)	60 (82)
Normal scan at baseline	--	0	1 (3)	3 (11)	4 (19)	7 (21)
Lesion volume on MRI - cm3						
Median	0.43	----	1.9	4.3	6.01	7.2
Range	0-55		0-114.3	0-88.6	0-70.2	0-60.8
Median EDSS score						
All patients	0#	-	1.5	2	2	3
Clinically definite MS	-	-	2.5	3.25	3.25	4.0

* Lesion volume measures not available at year 1

+ Based on 133 patients (Apart from CIS type, demographic data was no longer available in 5 patients who were excluded at an earlier time point due to an alternative non-MS diagnosis and 2 who died of non-MS cause before 5 years follow-up)

§ Includes 3 patients excluded after baseline MRI revealed a non-MS diagnosis

As EDSS was not measured during the CIS presentation, 0 represents the presumed EDSS immediately prior to CIS. This value was used to evaluate change of EDSS from baseline to year 5

Conversion to CDMS and disability status

After 20 years, clinically definite MS developed in 67/107 patients (63%). The remaining 40 patients were classified as still CIS – this included 5 with "clinically probable" MS by the Poser criteria that had experienced new neurological symptoms but with no new signs or disability. Clinically definite MS developed in 35/54 (65%) who initially presented with optic neuritis, 15/25 (60%) with a brainstem syndrome and 17/28 (61%) with a spinal cord syndrome. The median EDSS score of the MS group was 4.0 (range 0-10): 39 (58%) had RRMS and 28 (42%) had SPMS. Twenty six/67 (39%) of patients with clinically definite MS had an EDSS score of 6 or more and 26/67 (39%) had an EDSS score of 3 or less. The median EDSS score of the SPMS group was 6.5 (this includes three patients who died of severe complications of disease in whom an EDSS of 10 was assigned). The median EDSS of the optic neuritis onset MS group was 4.0 and in the non–optic neuritis MS group was 5.0 ($p = 0.28$, Wilcoxon rank-sum test).

Normal Brain MRI scans at baseline

Clinically definite MS developed in 7/34 (21%) patients with normal brain MRI scan at baseline, (4 presented with optic neuritis and 3 with spinal cord syndrome). The median EDSS of this MS group was 3.5 (range 1.5 to 7.5) and the median time to converting to MS was 6.0 years (range 1 to 11).

Abnormal Brain MRI scans at baseline

Clinically definite MS developed in 60/73 (82%) patients with an abnormal brain MRI scan. Their median EDSS was 4.25 (range 0 to 10) and median time to converting to MS was 2 years (range 0.5 to 14 years).

Baseline MRI lesion number and clinical outcome

Table 3.3 shows the relationship between baseline lesion number (grouped as 0, 1-3, 4-9 and ≥10) and clinical outcome at 20 years (CIS or clinically definite MS; those with EDSS > 3 or ≥6).

Table 3.3 Baseline MRI lesion number and clinical status at 20 years

	No. of Brain T2 lesions at baseline			
	0 N=34	1-3 N=22	4-9 N=20	10+ N=31
No. CIS at 20 years	27 (79%)	4 (18%)	3 (15%)	6 (19%)
No. clinically definite MS at 20 years	7 (21%)	18 (82%)	17 (85%)	25 (81%)
No. with EDSS >3 at 20 years	9 (26%)	8 36%)	10 (50%)	20 (65%)
No. with EDSS ≥ 6 at 20years	2 (6%)	4 (18%)	7 (35%)	14 (45%)

Longitudinal changes

Patients who developed SPMS tended to have larger baseline T_2 lesion volumes and a greater increase of lesion volume especially over the first 5 years - this was even more evident for those patients who died of complications of MS (Table 3.4 & Figure 3.1). Observing the pattern of median T_2 lesion volume change over 20 years showed that although at baseline there was some overlap, from 5 years onwards the groups of patients (CIS, RRMS, SPMS) were well distinguished. The SPMS group showed a steeper rate of lesion volume increase than RRMS. The increase in SPMS was higher than in RRMS over the first 5 years of the disease ($p = 0.008$, Wilcoxon rank-sum test).

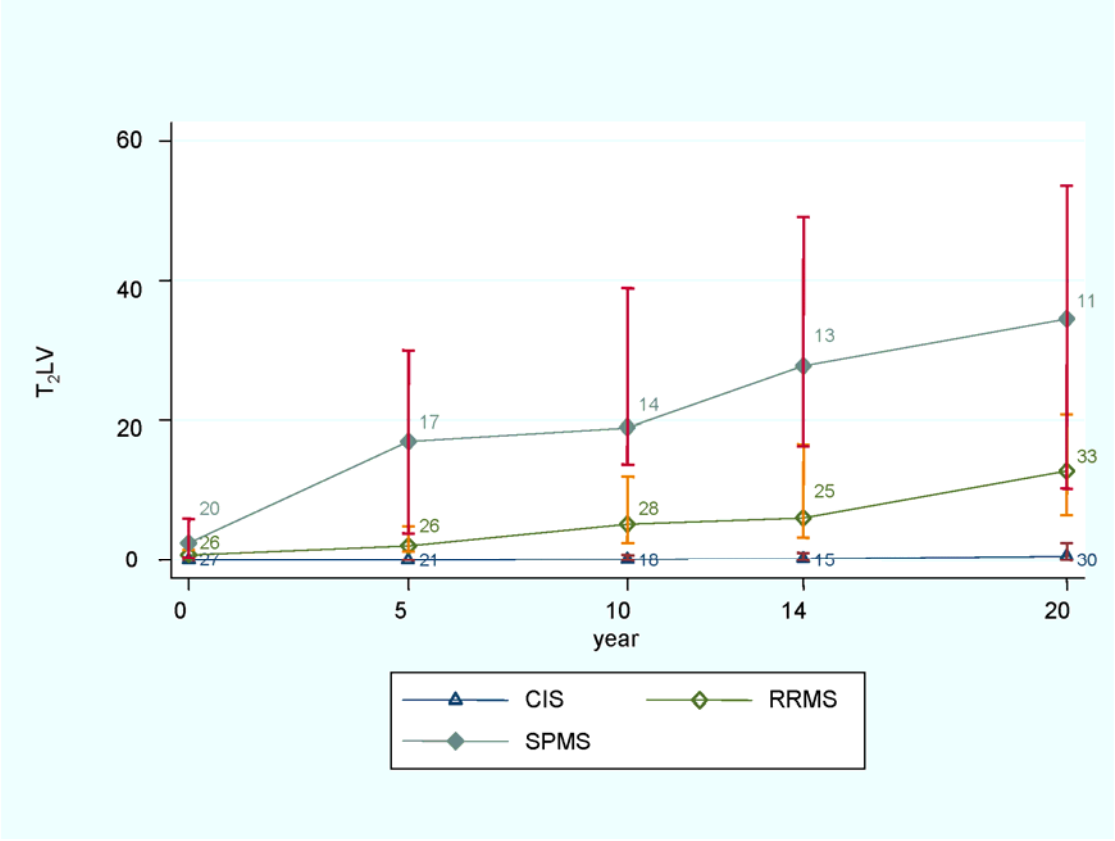
Table 3.4 MRI lesion volumes at each study time-point displayed according to clinical subgroup classification at 20 year follow up

Clinical outcome	**Baseline**	**5 years**	**10 years**	**14 years**	**20 years**
CIS - No.	27	21	18	15	30
Median volume (cm3)	0	0	0.07	0.18	0.5
Range (cm3)	0-2.2	0-10.8	0-16.3	0-15.1	0-13.8
RRMS (All) - No.	26	26	28	25	33
Median volume (cm3)	0.7	2.06	5.1	6.09	12.7
Range (cm3)	0-13.7	0.1-36.5	0.55-40.6	0.97-37.8	0.2-60.8
RRMS (EDSS≤3) - No.	18	18	20	17	22
Median volume (cm3)	0.7	1.8	4.5	5.8	8.7
Range (cm3)	0-13.7	0.5-36.5	0.5-40.6	0.9-37.8	0.2-60.8
RRMS (EDSS>3) - No.	8	8	8	8	11
Median volume (cm3)	0.5	2.7	6.2	9.7	17.5
Range (cm3)	0-4.07	0.1-12.4	0.5-19.9	1.5-23.1	4.7-47.6
SPMS - No.	20*	17*	14§	13	11
Median volume (cm3)	2.5	17	18.9	27.7	34.4
Range (cm3)	0-55	0-114	0.4-88.6	0.5-70.2	0.5-56.4

***** Includes three patients who died of MS

§ Included one patient who died of MS

Figure 3.1 Median T_2 lesion volume (T_2LV) (cm^3) over time for patients groups



NB: Bars show Inter-quartile range; Numbers of patients shown

Cuzick's test was used to look for a linear trend across the three MS subgroups - benign and non-benign RRMS and SPMS - for those patients in whom the baseline and 20 years T_2 lesion volume was available. It showed an increase in lesion growth from the benign group, through the non-benign RRMS group (EDSS > 3), to the SPMS group ($p = 0.08$). Lesion growth over 20 years showed a trend to be higher in SP than RRMS (for the latter, benign and non-benign subgroups were combined; $p = 0.07$, Wilcoxon rank-sum test) and in benign versus non-benign RRMS ($p = 0.08$).

A longitudinal analysis of the gradients, using all available data points, estimated the rate of growth in the RRMS group as 0.80cm³ per year [bootstrap 95% CI: 0.63, 0.99; P<0.001] while in the SPMS group it was estimated as 2.89 cm³ per year [bootstrap 95% CI: 1.78, 4.01; P<0.001]. This is a difference of 2.09 cm³ per year [95% CI: 0.77, 2.96; P<0.001]. Comparing non-benign versus benign RRMS, the rate of lesion growth per year were 1.08 and 0.66 cm³ respectively, a difference of 0.42 cm³ per year [bootstrap 95% CI: - 0.01, 0.90; 0.05 < P < 0.07].

To assess if there was a levelling off or alternatively acceleration in lesion growth, a quadratic term in time was added to the longitudinal models to test for curvature: significant negative or positive quadratic terms indicate, respectively, reducing or accelerating rates of growth. However there is less power in any one group to detect curvature than to detect a difference in curvature between groups. In the whole group there was no evidence of a curved trajectory (bootstrap P > 0.9); nor was there evidence at 5% level for curvature in any of the three groups, though the quadratic terms for both

the SPMS (with bootstrap $0.1 < P < 0.2$) and CIS (bootstrap $0.2 < P < 0.3$) subgroups were negative, consistent with a levelling off, and positive for RRMS (bootstrap $0.1< P < 0.2$), consistent with accelerated growth. There was a borderline significant decelerating curvature in both CIS (bootstrap $P = 0.08$) and SPMS (bootstrap $P = 0.09$) compared to RRMS.

T_2 lesion volumes at all time points were strongly correlated with T_2 lesion volume at 20 years: T_2 lesion volume at baseline vs. 20 years ($n = 55$, $r_s = 0.76$, $P<0.001$); 5 year vs. 20 years ($n = 52$, $r_s = 0.83$, $P<0.001$); 10 years vs. 20 years ($n = 53$, $r_s = 0.89$, $P<0.001$); 14 years vs. 20 years ($n = 47$, $r_s = 0.94$, $P<0.001$).

For the whole cohort, T_2 lesion volume at all time points (0, 5, 10, 14 and 20 years) was significantly correlated with 20 year EDSS (r_s values 0.48 to 0.67, $P < 0.001$) and MSFC z-scores (r_s values [- 0.50] to [-0.61]; $P<0.001$) (Table 3.5). There were similar correlations in those patients who developed clinically definite MS only (Table 3.6).

Table 3.5 Correlation of MRI lesion volumes and changes in lesion volumes at various time points during the study with disability measured at the 20 year follow up for the whole cohort

EDSS				MSFC		
P value	**r_s (95% CI)**	**Patients Number**	**Time point**	**Patients Number**	**r_s (95% CI)**	**P value**
< 0.001	0.48(0.28-0.64)	73	0 yr	49	-0.49(-0.68 – [-0.24])	0.0003
< 0.001	0.62(0.45-0.75)	64	5 yr	48	-0.61(-0.76 – [-0.40])	< 0.001
< 0.001	0.61(0.43-0.74)	64	0-5 yr	48	-0.56(-0.73 – [-0.33])	< 0.001
< 0.001	0.55(0.35-0.71)	60	10 yr	50	-0.50(-0.68 – [-0.25])	0.0002
0.001	0.45(0.20-0.64)	53	5-10 yr	45	-0.49(-0.68 – [-0.23])	0.0005
< 0.001	0.67(0.49-0.80)	53	14 yr	44	-0.59(-0.75 – [-0.36])	< 0.001
0.004	0.40(0.13-0.61)	48	10-14 yr	42	-0.42(-0.64 – [-0.13])	0.005
< 0.001	0.50(0.30-0.65)	74	20 yr	62	-0.53(-0.68 – [-0.33])	< 0.001
< 0.001	0.49(0.24-0.68)	47	14-20 yr	44	-0.51(-0.70 – [-0.25])	0.0004

Table 3.6 Correlation of MRI lesion volumes and changes in lesion volumes at various time points during the study with disability measured at the 20 year follow up for the clinically definite MS cohort

EDSS				MSFC		
P value	**r_s (95% CI)**	**Patients Number**	**Time point**	**Patients Number**	**r_s (95% CI)**	**P value**
0.036	0.31 (0.02-0.55)	46	0 yr	30	-0.41(-0.67 – [-0.06])	0.024
<0.001	0.53 (0.27-0.71)	43	5 yr	30	-0.51(-0.73 – [-0.18])	0.003
<0.001	0.53 (0.28-0.72)	43	0-5 yr	30	-0.45(-0.70 – [-0.11])	0.011
0.005	0.42 (0.13-0.64)	42	10 yr	34	-0.38(-0.63 – (-0.05])	0.025
0.026	0.37 (0.05-0.62)	36	5-10 yr	29	-0.39(-0.66 – [-0.03])	0.033
<0.001	0.57 (0.30-0.75)	38	14 yr	30	-0.50(-0.73 – [-0.17])	0.004
0.020	0.22 (-0.12-0.52)	34	10-14 yr	29	-0.42(-0.68 – [-0.07])	0.020
0.009	0.38 (0.10-0.61)	44	20 yr	39	-0.47(-0.69 – [-0.19])	0.002
0.003	0.50 (0.18-0.72)	32	14-20 yr	30	-0.54(-0.75 – [-0.22])	0.002

NB: the larger patient cohort with EDSS measures includes those who had a telephone EDSS only

For the whole cohort, a significant correlation was found between the change in T_2 lesion volume over the first 5 years and concurrent change of EDSS (r_s = 0.59 [95% CI 0.41-0.72]; P<0.001). There were significant but weaker concurrent correlations for years 5-10 and 10-14 and no significant correlation for years 14-20. These correlations were similar for those who developed clinically definite MS only (Table 3.7).

Table 3.7 Correlations between concurrent changes in MRI lesion volumes and changes in EDSS score

All patients				Clinically definite MS only		
P value	**r_s (95% CI)**	**Number of patients**	**Interval**	**Number of patients**	**r_s (95% CI)**	**P value**
< 0.001	0.59 (0.41-0.72)	62	0-5	43	0.69 (0.49-0.82)	< 0.001
0.002	0.40 (0.15-0.60)	53	5-10	36	0.43 (0.12-0.66)	0.008
0.002	0.41(0.15-0.62)	48	10-14	34	0.46 (0.14-0.69)	0.006
0.165	0.20 (-0.08-0.46)	47	14-20	32	-0.03 (-0.37-0.31)	0.853

Discussion

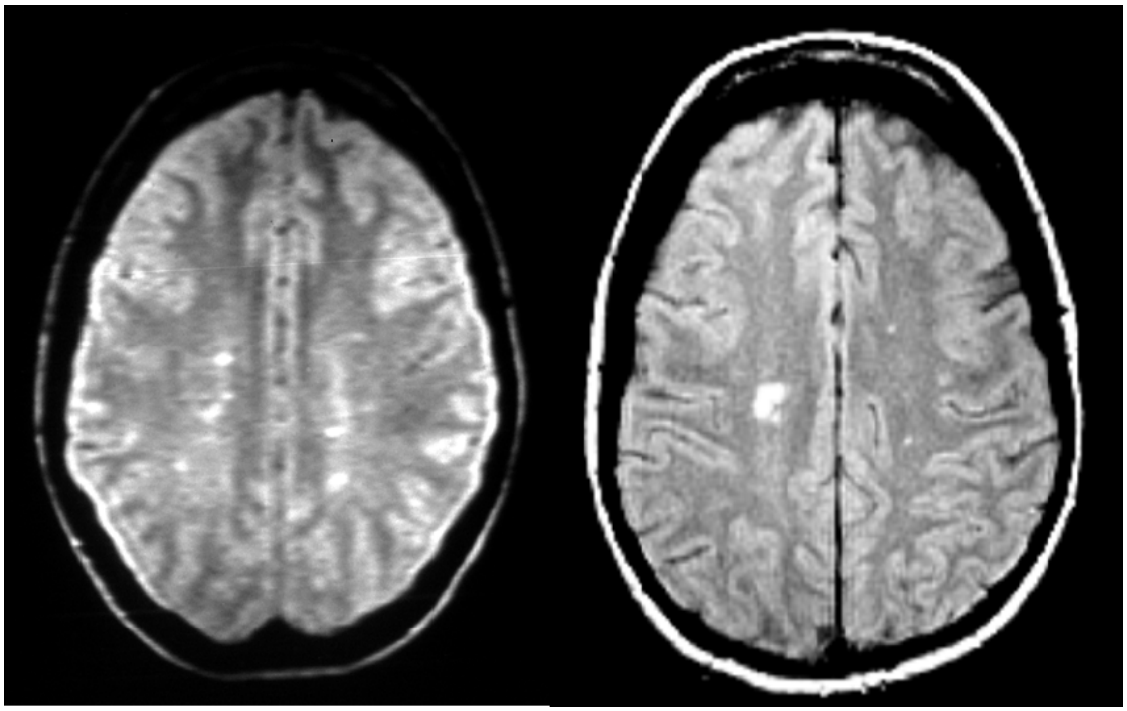
This report provides prospectively acquired, longitudinal MRI and clinical data over a period averaging 20 years from CIS onset. By comparison, long term follow up of MRI findings and clinical course in other CIS cohorts has ranged from 7 to 10 years (Beck et al., 2004; Minneboo et al., 2004; Tintore et al., 2006). The cohort at 20 years has similar demographic features to the original cohort in terms of mean age, gender, clinical presentation and frequency of baseline MRI abnormalities (Table 3.2). The number of the cases ascertained at 20 year follow up was about 50% higher when compared with the 14 year follow up, and provides a robust long-term assessment of the MRI–clinical relationship in relapse-onset MS. The 20 year follow up with clinical and MRI data is the longest study of the CIS patients. It also reflects the unmodified natural history of MRI lesion load in CIS and MS: only 4/107 patients were treated with disease modifying therapies; all 4 after developing clinically definite MS. Other long-term studies have often included substantial proportions of patients who received disease modifying therapies, which may modify the relationships between MRI and disability (Rudick et al., 2006a).

Baseline MRI and long-term clinical outcome

This data confirms and extends the findings of the previous follow up studies of this cohort (Brex et al., 2002; Morrissey et al., 1993; O'Riordan et al., 1998c). It shows that after 20 years, CIS patients with an abnormal MRI scan at presentation are far more likely to convert to clinically definite MS than those with a normal scan (82% versus

21%; Table 3.3). It is however noteworthy that 18% of those with an abnormal scan at presentation did *not* develop clinically definite MS. This proportion is higher than that reported at 14 year follow up (12%), which likely reflects more complete ascertainment of cases in this latest follow up. The detection of such "non-converting" cases after 20 years (Figure 3.2) supports the approach of the new MS diagnostic criteria that do not allow a diagnosis of MS in CIS patients with a single abnormal MRI scan without evidence for dissemination in time (Polman et al., 2005). On the other hand it appears that the higher risk for MS that is associated with an abnormal scan is evident whether there are few or many lesions.

Figure 3.2 T_2-weighted images of a 49 year old female who had an abnormal baseline brain MRI and had remained CIS 20 years later



NB: The baseline brain MRI (image on the left) of a patient seen at baseline after CIS presentation with optic neuritis showing several hyperintense lesions. The image on the right was acquired 20 years later. The scan acquired at 20 years had 12 new hyperintense lesions. Despite an abnormal scan this patient did not convert to MS.

Brain T_2 lesion detection, as several studies have shown, is resolution and slice thickness dependent (Bradley & Glenn, 1987; Nielsen et al., 2006; Sicotte et al., 2003). Whilst it is possible that with contemporary MR imaging acquiring thinner slices on higher field strength magnets, the proportion of people with normal baseline MRI scan converting to MS might be lower than that reported in the present study (21%), recent CIS studies also report conversion to MS of patients with a normal brain MRI (Swanton et al., 2007).

The clinically definite MS group in this study exhibited a wide spectrum of disability at 20 years. Whilst 42% had developed secondary progression, 39% had a benign course with minimal disability (EDSS≤3). The percentage of people with benign MS in this cohort is higher than that reported on other cohorts of patients (Eriksson et al., 2003; Nilsson et al., 2005). Optic neuritis presentation has been associated with better outcome in several reports (Beck et al., 2003; Beck et al., 2004; Confavreux et al., 2003; Nilsson et al., 2005) and nearly half of this cohort presented with optic neuritis. However, the rate of conversion to clinically definite MS was high (67%) and the 20 year disability status of the optic neuritis group did not differ significantly from the non-optic neuritis group, so this does not appear to explain the high proportion of minimally disabled MS patients in this cohort. However, patients in this cohort were followed up prospectively from their first episode, before a diagnosis of MS was established, and efforts were made to follow up all patients, whether or not they had developed MS at an earlier time point. Some of those with mild/benign MS may not be followed up at a routine MS or general neurology clinics and may not be detected in prevalence studies.

Patients with a higher number of lesions at baseline were somewhat more likely to be disabled after 20 years: an EDSS of 6 or more, indicating that assistance is required for walking, was seen in 45% with ≥10 lesions, 35% with 4-9 lesions, 18% with 1-3 lesions and 6% with no lesions suggesting that lesion number has some predictive effect for disability (Table 3.3). Conversely, about one third (35%) of patients with ≥10 lesions had minimal disability after 20 years (EDSS≤3) and 18% had not developed clinically definite MS. Such a wide spectrum of outcomes indicates that MRI lesion number at CIS presentation provides limited prediction for long term disability.

MRI lesion volume evolution over 20 years

This study sheds new light on lesion load evolution and the natural history of CIS and MS. The rate of lesion volume growth over 20 years was clearly higher in those developing SPMS than in those who retained a relapsing remitting course, and this difference became clearly evident 5 years after the first CIS presentation (Figure 1). These findings should be investigated in other cohorts of patients with more frequent follow ups to determine whether an earlier than 5 year timepoint might show these differences (lesion volume measures were not available at the one year follow up in the present cohort). Reliable early identification of patients at high risk for secondary progression is important: for such individuals there is a high priority to develop disease modifying treatments that delay or prevent secondary progression.

Immunomodulatory treatment with beta interferon in CIS patients with abnormal MRI for up to over 3 years decreases the number of new MRI lesions, delays development of

clinically definite MS (Comi et al., 2001; Jacobs et al., 2000b; Kappos et al., 2006a), and possibly slows development of early disability (Kappos et al., 2007). Whilst this natural history cohort data suggests that slowing of lesion volume increase might delay or prevent secondary progression, an alternative consideration is that the white matter T_2 lesion volumes seen in this study may have increased in parallel with anatomically and mechanistically separate pathological changes that are more directly responsible for long term disability e.g. pathology in normal appearing brain white and grey matter or spinal cord disease. It is plausible that if a therapy prevents new T_2 lesions but not abnormalities in normal appearing white and grey matter, secondary progression and disability will still evolve. There is a need to investigate the long term relationship of other MRI measures that are abnormal in CIS and early relapsing remitting MS (e.g. brain volume and quantitative measures of normal appearing white and grey matter (Dalton et al., 2004; Fernando et al., 2004; Fernando et al., 2005)) with the long term clinical course both in untreated patients and those on disease modifying treatment.

Despite the loss of some subjects to clinical and/or MRI examination and changes in MRI technology a robust long term relationship of lesion volume and EDSS was observed, albeit of a moderate strength only. The lesion volumes at all time points were correlated with the 20 year EDSS suggesting that the moderate predictive value of T_2 lesion volume appears early in the disease.

Several reasons might explain why brain lesion volume and disability are only moderately – rather than strongly – correlated. *First*, not all patients were followed up

with MRI. *Secondly*, the detection of the T_2 hyperintense lesions and lesion load is resolution dependent (Erskine et al., 2005; Filippi et al., 1995) and the use of 5mm thick slices (or 10mm in few scans at baseline) will have limited detection of smaller lesions. In addition changes in scanner hardware and software (Table 3.1), during the follow up period could have had some effect on a lesion measurement precision (earlier measurements less sensitive than the later ones). However, it should not have affected the relative ranking of patients with regard to lesion volumes, and hence the strength of clinical correlations observed. Furthermore there was no evidence of a step increase in T_2 lesion volume between year 5 and 10 when the change in scanner and field strength (from 0.5 to 1.5 Tesla) occurred. *Thirdly,* with current MRI techniques, intra-cortical lesions remain largely undetected (Geurts et al., 2005; Kidd et al., 1999). *Fourthly*, demyelinating lesions also occur in the spinal cord in CIS and MS patients (O'Riordan et al., 1998b). It is possible that some patients in this cohort had more severe spinal disease with less abnormality in the brain. MS cord pathology may be independent of concomitant brain changes, develop at different rates according to the disease phenotypes and be associated to medium-term disability accrual (Agosta et al., 2007). *Fifthly*, despite a high sensitivity to global tissue damage, the T_2 lesion volume measure lacks pathological specificity – it is collectively sensitive to inflammation, oedema, demyelination and axonal loss as well as remyelination and is therefore not specific for the irreversible damage to myelin and axons that underpin irreversible disability in MS (Guttmann et al., 1995). Furthermore, lesions occur in semi-random locations which may or may not be clinically eloquent. *Sixthly*, abnormalities in normal appearing white and grey matter are also detected early on the disease (Audoin et al., 2006; Fernando et al.,

2004; Fernando et al., 2005) and have the potential to influence the clinical course. The concurrent correlation between T_2 lesion volume and EDSS change was most apparent in the first quinquennium and was not present in the fourth and final follow up interval (years 14-20) (Table 3.6). This suggests that the mechanisms of disability progression may change over time. Disability may be more dependent on lesion volume changes earlier on and less dependent later on (Li et al., 2006). The investigation of other MRI measures - atrophy, normal appearing white and grey matter and the spinal cord abnormality - could explore the hypothesis that they may be more closely related to disability changes at a later stage.

Finally, when looking at the associations between lesion volume and disability it is important to take into account the limitations of EDSS scale – e.g. intraobserver variability, being heavily weighted towards physical disability with limited assessment of key clinical features such as cognitive and sphincter dysfunction. In addition, interpreting the association between a succession of changes and a later outcome can be difficult for a number of reasons. Subjects with large changes during one period often have smaller changes over the next period, due to a phenomenon similar to regression to the mean. There also may be "ceiling effects", where subjects with large early changes have less room for later changes. The rate of EDSS change in MS is not constant over time, with typically longer periods stationed at certain levels (e.g. 3 and 6) than others. Furthermore, there were some differences in the patient cohorts studied at the various study time-points - it is possible that this has influenced the results.

Is the MRI lesion volume increase linear or non-linear?

This analysis suggests a relatively linear – or constant – increase in lesion volume in MS over the 20 years of follow up, whether patients retain a relapsing remitting course or develop secondary progression. Further analysis investigating for non-linear changes revealed a non-significant trend in secondary progressive patients – who had an overall 3-fold greater increase in volumes than those who were still relapsing remitting – for the rate of lesion volume increase to slow over time. However, unlike the findings in a recent cross-sectional study (Li et al., 2006), a definite or complete plateauing of lesion volume with the higher levels of disability associated with secondary progression could not be detected , at least within the 20 year time frame of the study. Because of their disability, proportionately more of the secondary progressive MS patients were not able to undergo MRI scans at later time-points and the imaging data at these times reflects a group of slightly less disabled patients – whether this contributes to the slight non-linearity is uncertain. Future longitudinal studies of larger cohorts may provide clarification.

In summary this long-term follow up study demonstrates that T_2 brain lesions have a moderate correlation with disability and their predictive value appears early on the disease. Lesion load continues to increase for at least 20 years in relapse-onset MS patients and the rate of lesion growth in those who develop SPMS is higher than those who retain a relapsing-remitting course.

PART III

TISSUE SPECIFIC ABNORMALITIES AND THEIR RELATIONSHIP WITH DISABILITY IN RELAPSE-ONSET MULTIPLE SCLEROSIS

Chapter IV

Grey matter atrophy is related to long-term disability in multiple sclerosis

Introduction

As emphasised in earlier chapters, MRI detectable WM lesions are usually seen early in relapse onset MS, and in people who develop a CIS suggestive of MS they are associated with conversion to clinically definite MS (Polman et al., 2005; Swanton et al., 2007), although only predict subsequent disability to a limited degree (Brex et al., 2002). Work presented in Chapter 3 also confirmed these findings.

Brain atrophy is also seen from clinical disease onset in MS (Brex et al., 2000); it is prominent in the later stages of the disease, and is more marked in SP compared with RR phenotypes of MS (Dalton et al., 2006; Miller et al., 2002), although the relative influence of disease phenotype and disease duration on such atrophy is uncertain.

From pathological studies extensive cortical damage has been observed predominantly in progressive forms of MS, suggesting that GM pathology may be an important determinant of irreversible disability (Pirko et al., 2007). Whilst whole brain atrophy has been well explored, the advent of new MRI acquisition and analysis tools now makes it possible to determine the relative extent of both GM and WM atrophy. Recent work investigating the progression of tissue specific atrophy - measured using methods based on Statistical Parametric Mapping (SPM) segmentations - after first presentation with a CIS, showed significantly greater GM compared with WM atrophy in those subjects who developed clinically definite MS within three years (Dalton et al., 2004). Furthermore, in

people with early RRMS, GM atrophy over 2-years was more rapidly progressive than WM atrophy (Tiberio et al., 2005). These studies suggest that progressive GM atrophy occurs early in the clinical course of MS, and in the case of CIS, is of direct and immediate clinical relevance. Whilst some studies have detected predominantly GM atrophy (Chard et al., 2002; Dalton et al., 2004; De et al., 2003; Quarantelli et al., 2003; Sastre-Garriga et al., 2004) not all have: indeed some observed mostly WM atrophy (Ge et al., 2001b) and it remains to be definitively determined which tissue is most affected at any given stage of the disease, particularly in the longer term.

The relationship between WM lesions and brain atrophy also remains unclear, with current evidence suggesting a partial discordance between these pathological manifestations of MS (Anderson et al., 2006) both in cross-sectional and longitudinal studies (Chard et al., 2003; Chard et al., 2002; Dalton et al., 2004; De et al., 2003; Ge et al., 2000; Paolillo et al., 2000; Rudick et al., 2006a; Sailer et al., 2003). The suggestion that there is at least a partial discordance between T_2 lesion load (T_2LL) and atrophy measures during the evolution of MS is supported by the observation that whilst disease-modifying therapies such as beta interferon are relatively effective in preventing new WM lesion formation, their effect in reducing atrophy has been modest (Filippi et al., 2004; Zivadinov et al., 2007), and in some studies not evident at all (Kappos et al., 2006b; Molyneux et al., 2000).

With this context, the *primary objective* of this study was to estimate GM and WM volumes in the same cohort of CIS patients followed up 20 years from clinical disease

onset (See Chapter 3), and to assess the relationship between these measures of tissue specific atrophy, clinical course, and disability, in particular investigating the hypothesis that GM atrophy will correlate better with clinical disease severity. *Secondary objectives* were to: (i) evaluate the relationship of GM and WM volumes with T_2LL, and (ii) investigate the relative contributions of GM and WM volumes and T_2LL to disability.

Methods

Subjects

This report is based on 20 year follow up data of a cohort who had clinical and MRI assessments at approximately 5-yearly intervals after presenting with a CIS suggestive of MS (Brex et al., 2002) (See Chapter 3). Clinical status was documented at the 20 year follow up in 107 patients (Chapter 3), of whom 75 had an MRI examination, with data from two patients excluded (one who developed cerebrovascular disease and one who did not complete the scanning protocol). The remaining 73 patients are the subject of this report.

Clinically definite MS was diagnosed on clinical grounds alone (Poser et al., 1983). Disability was assessed using the expanded disability status scale (EDSS) (Kurtzke, 1983) and MS functional composite scores (MSFC) (Fischer et al., 1999). The clinical course of MS (RRMS or SPMS) was defined by Lublin and Reingold criteria (Lublin & Reingold, 1996). Those clinically definite MS patients with an EDSS ≤ 3 were defined as benign MS. Patients were studied a mean (SD) of 20 (1.5) years, (range 18-27) following

the CIS [49 women and 24 men; mean age 51.4 (7.2) years]; 29 were still classified as CIS [mean disease duration 20.4 (2.06); mean age 51.5 (8.4) years], 33 had developed RRMS [mean disease duration 19.7 (1.1); mean age 51 (6.1) years] and 11 SPMS [mean disease duration 19.8 (0.68); mean age 52 (7.3)]. The median EDSS was 2.5 [range 0-8] for all patients and 3.25 [range 1-8] for MS patients only. Three patients were on disease modifying treatments. MRI was also performed in 25 healthy controls [14 women and 11 men; mean age 41.7 (7.7) years].

The study was approved by the National Hospital for Neurology and Neurosurgery and Institute of Neurology Joint Research Ethics Committee. All study participants gave written informed consent.

Image acquisitions and processing

Whole brain MRI was performed on a 1.5 Tesla GE Signa scanner (General Electric, Milwaukee, WI) as follows: (1) 2D dual-echo proton density (PD) (TE 17ms) and T_2 (TE 102ms) weighted fast spin-echo (FSE) [repetition time (TR) 2000 ms; 28 × 5 mm slices; field of view 24 × 18 cm; in-plane resolution of 1.1 mm]; (2) 3D axial T_1-weighted inversion-prepared fast spoiled gradient recall [TR 10.9 ms; TE 4.2 ms; inversion time 450 ms; 124 × 1.5 mm slices; imaging matrix 256 × 160, interpolated to a final in-plane resolution of 1.1 mm]. The identification of lesions on hard copies of the PD-weighted images, with reference to the T_2-weighted images was performed by myself and checked for consistency by an experienced neuroradiologist blinded to the clinical details. This was then used as a reference for contouring of the lesions on the PD-weighted digital

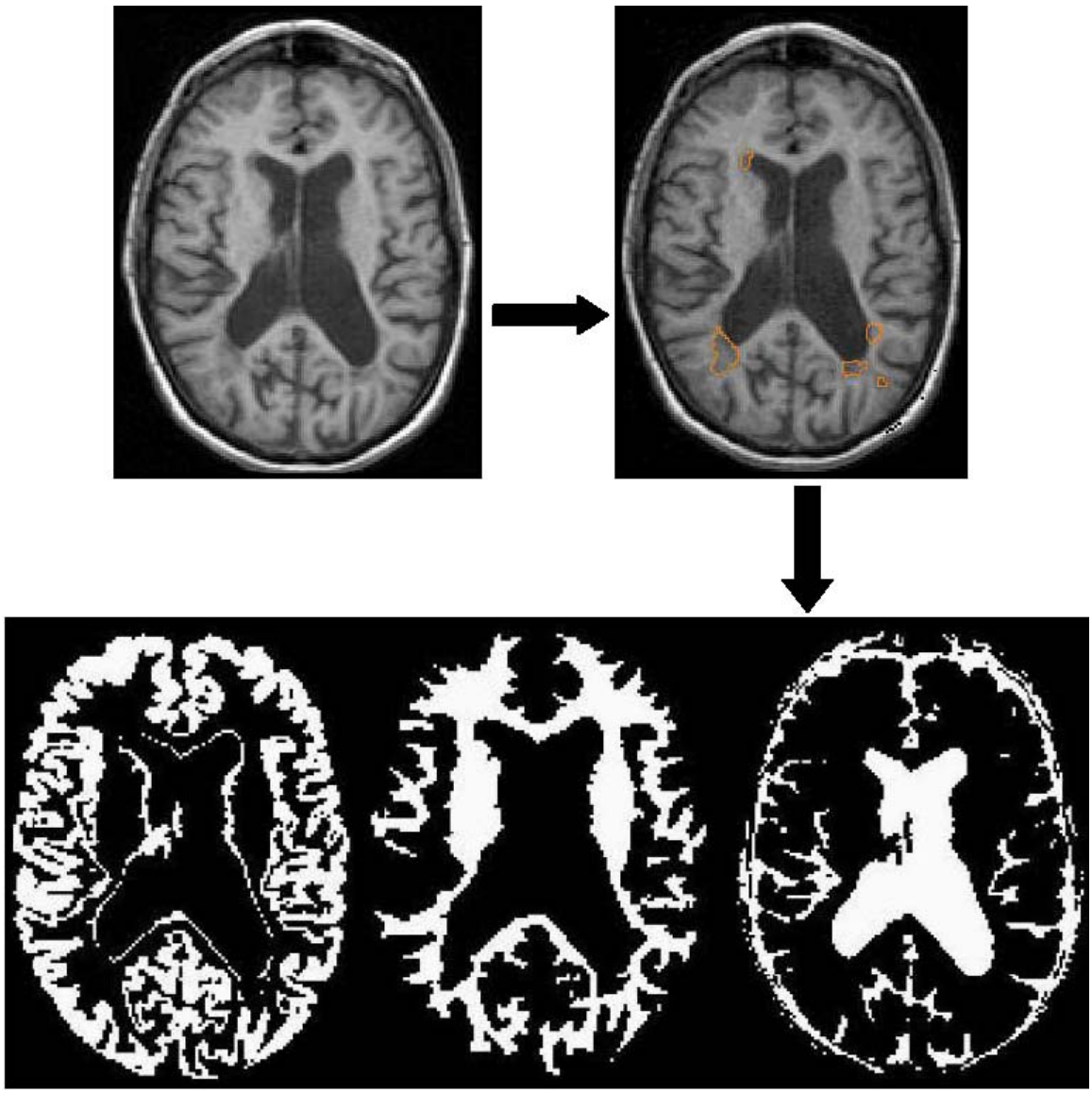
images, using a semi-automated local thresholding technique implemented in the image display package DispImage (Plummer, Department of Medical Physics and Bioengineering, University College London) (Sailer et al., 1999) (Fig 4.1). Then, a computer program summed all the individual lesion volumes (calculated as surface area of each lesion multiplied by slice thickness) and T_2LLs were generated.

Segmentation of the axial 3D T_1-weighted images into WM, GM and cerebrospinal fluid (CSF) was performed using SPM2 (Statistical Parametric Mapping; Wellcome Department of Cognitive Neurology, Institute of Neurology, Queen Square, London), following a previously described method (Chard et al., 2002) (software available free to the research community at www.nmrgroup.ion.ucl.ac.uk/atrophy). The processing parameters for SPM2 were set to 0.01 for the bias correction, and 30 for the bias cutoff. Briefly, lesion masks, derived from the 3D T_1-weighed lesion contours, were subtracted from GM, WM and CSF masks using in house software; this yielded 4 mutually exclusive tissue masks. From these masks the GM, WM, T_1 lesion and CSF volume were calculated. WM and GM fraction volumes (WMF and GMF) relative to total intracranial volume] were derived, corrected for lesion misclassification as GM (Figure 4.1) (Chard et al., 2002). All the tissue masks were inspected, and no significant segmentation errors were detected.

To assess the robustness of results obtained using SPM2, I re-processed the data using SIENAx (Structural Image Evaluation, using Normalization, of Atrophy for cross-sectional measurement), a fully automated technique, to obtain the normalized GM and

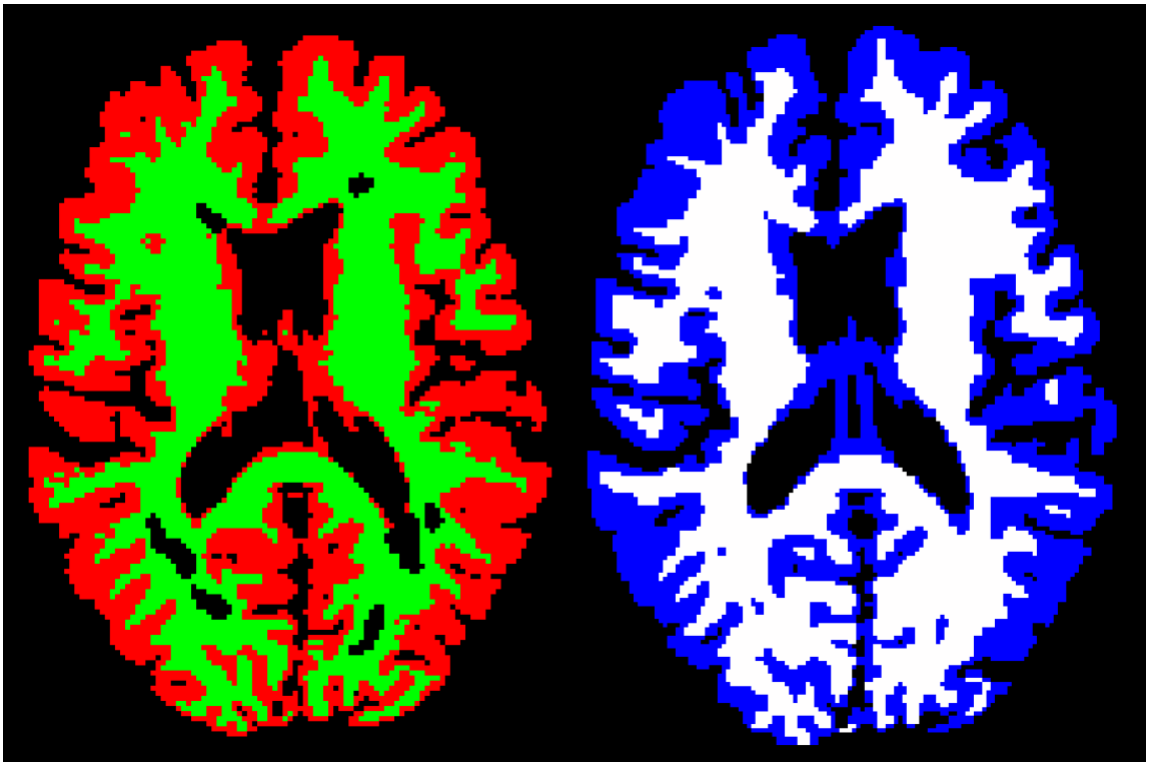
WM volumes (NGMV and NWMV) (Figure 4.2) (Smith et al., 2002). Briefly, SIENAx first extracts brain and skull voxels from the input MR data, using the Brain Extraction Tool (www.fmrib.ox.ac.uk/fsl). The brain image is then affine-registered to standard space brain and skull images, derived from the MNI152 standard space reference set, with the skull registration used to determine the head size normalization factor. Next, tissue type segmentation, with partial volume estimation, is carried out in order to calculate the total volume of brain tissues, including separate estimates of volumes of GM, WM and ventricular CSF. The estimated volumes for a subject are then multiplied by the normalization factor to obtain NGMV and NWMV (normalized CSF and whole brain volumes were also obtained - data not presented here).

Figure 4.1 Segmentation of a 3D T_1-weighted FSPGR scan into GM, WM and CSF mask



Top left – original 3D FSPGR scan; top right – contouring of the hypointense lesions; bottom raw – (from left to right) GM, WM and CSF mask. Lesion contouring (top right) was done so to correct for lesion misclassification as GM.

Figure 4.2 Overlaid GM (red and blue) and WM (green and white) masks using SPM2 (left) and SIENAx (right)



NB: Lesion mask was used for the SPM2 segmentation (left) to remove lesions – shown as black areas within the green WM mask.

Statistical Analyses

Group comparisons of the brain tissue volumes were performed using linear regression with group indicator and age and gender covariates. To assess the associations between the brain volume measurements, T_2LL, and disability (EDSS and MSFC and its components), Spearman rank correlation was used.

To assess the relative contribution of the WM and GM volume loss and T_2LL to accrued disability, ordinal logistic regression (for EDSS) and linear regression (for MSFC) were used. The predictive value to disability of tissue specific volume measurements obtained was assessed separately for each technique (i.e. SPM and SIENAx). Both EDSS (categorised as follows: ≤ 1.5; >1.5 and ≤ 3; >3 and ≤ 6; >6) and MSFC (as a continuous variable) were modelled as response variables, with tissue volumes, lesion load, age and gender as covariate predictors. Lesion load was log transformed to improve Normality before inclusion in the regression models; where the T_2LL was zero (10 subjects), the log volume was given a value of 0.01 to include these subjects. Changing the EDSS category intervals, or the small value given for the log volume where the T_2LL was zero, did not materially change the results.

MRI covariates were entered together and removed singly by manual backwards stepwise exclusion until all model predictors were significant at $p<0.1$. Age and gender were added to the final models but omitted if the adjusted coefficients were both non-significant and not materially different from unadjusted coefficients. Models were applied to the whole cohort of patients and the MS subgroup separately.

The data were analyzed using SPSS 11 (SPSS Inc, Chicago, IL, USA) and Stata 9.2 (Stata Corporation, College Station, TX, USA). Statistical significance was taken at $p<0.05$.

Results

Tissue specific volumes and clinical subgroups: Tables 4.1 & 4.2 for SPM measurements & Tables 4.3 & 4.4 for SIENAx measurements.

Using SPM2, tissue specific volumes were significantly lower in MS patients and MS subgroups (RRMS and SPMS) versus controls. Significant GM atrophy and WM atrophy was seen in MS patients compared with controls. There was significantly more GM atrophy, but not WM atrophy, in SPMS versus RRMS and RRMS versus CIS. There was significantly greater GM atrophy but, not WM atrophy, in those (non-benign) MS patients with an EDSS > 3 (22 patients) compared with those (benign) MS patients with an EDSS ≤ 3 (22 patients). There were no significant differences for any of the volume measurements between the controls and those remaining classified as a CIS after first presentation.

SIENAx yielded similar results consistent with the GMF regarding GM volume measurements. For the WM volume measurements, there were no significant differences in any group comparison.

Table 4.1 Mean and Median (SD) of brain volume measurements using SPM2

Groups (Number of patients)	**GMF** Mean; Median (SD)	**WMF** Mean; Median (SD)
Controls (25)	0.51; 0.52 (0.01)	0.29; 0.29 (0.01)
All patients (73)	0.49; 0.49 (0.03)	0.28; 0.28 (0.01)
CIS (29)	0.50; 0.50 (0.02)	0.28; 0.28 (0.01)
MS (44)	0.47; 0.48 (0.03)	0.28; 0.28 (0.01)
RRMS (33)	0.48; 0.49 (0.02)	0.28; 0.28 (0.01)
Benign MS (22)	0.49; 0.49 (0.02)	0.28; 0.28 (0.01)
Non-benign MS (22)	0.46; 0.46 (0.03)	0.28; 0.27 (0.01)
SPMS (11)	0.45; 0.45 (0.03)	0.27; 0.27 (0.01)

CIS = Clinically isolated syndrome; MS = Multiple sclerosis (RRMS and SPMS); RRMS = Relapsing-remitting MS; SPMS = Secondary progressive MS; GMF = Grey matter fraction; WMF = White matter fraction; Benign MS = EDSS $\leq$ 3; Non-benign MS = EDSS > 3

Table 4.2 Age and gender-adjusted mean difference between patient subgroups and controls

Group comparisons	**GMF**		**WMF**	
	Adjusted mean difference 95% CI	p-value	Adjusted mean difference 95% CI	p-value
MS-Controls	**-0.027 [-0.041- (-0.014)]**	**<0.001**	**-0.009 [-0.017 – (-0.001)]**	**0.017**
MS-CIS	**-0.028 [-0.039 – (-0.017)]**	**<0.001**	-0.003 [-0.010 – 0.003]	0.318
SPMS-Controls	**-0.046 [-0.063 – (-0.028)]**	**<0.001**	**-0.013 [-0.024 – (-0.002)]**	**0.018**
RRMS-Controls	**-0.021 [-0.035 – (-0.008)]**	**0.002**	**-0.008 [-0.017 – (-0.001)]**	**0.042**
SPMS-CIS	**-0.046 [-0.062 – (-0.030)]**	**0.001**	-0.006 [-0.016 – 0.003]	0.179
RRMS-CIS	**-0.022 [-0.033 – (-0.010)]**	**<0.001**	-0.002 [-0.009 – 0.004]	0.540
SPMS-RRMS	**-0.024 [-0.040 – (-0.008)]**	**0.003**	-0.004 [-0.014 – 0.005]	0.361
Benign-Non-benign MS	**0.022 [0.004 – 0.040]**	**0.01**	0.004 [-0.004 – 0.013]	0.328
CIS-Controls	0.001 [-0.013 – 0.015]	0.089	-0.006 [-0.015 – 0.002]	0.142

NB: Bold - significant at $p < 0.05$; CIS = Clinically isolated syndrome; MS = Multiple sclerosis (RRMS and SPMS); RRMS = Relapsing-remitting MS; SPMS = Secondary progressive MS; GMF = Grey matter fraction; WMF = White matter fraction; Benign MS = EDSS ≤ 3; Non benign MS = EDSS > 3

Table 4.3 Normalized brain volume measurements in controls and clinical subgroups

Groups (Number of patients)	**NGMV** Mean (cc); Median (SD)	**NWMV** Mean (cc); Median (SD)
Controls (25)	910; 916 (40.4)	663; 669 (33.0)
CIS (29)	885; 888 (40.8)	658; 657 (36.0)
All patients (73)	858; 870 (60.7)	653; 652 (35.4)
CDMS (44)	840; 853 (65.4)	650; 649 (35.0)
RRMS (22)	856; 865 (60.4)	649; 649 (33.7)
Benign MS (22)	874; 885 (61.8)	659; 660 (31.0)
Non-benign MS (22)	806; 798 (50.3)	640; 643 (36.8)
SPMS (11)	795; 793 (61.3)	652; 646 (40.6)

CIS = Clinically isolated syndrome; CDMS = Clinically definite multiple sclerosis; RRMS = Relapsing-remitting MS; SPMS = Secondary progressive MS; NGMV = Normalized gray matter volume; NWMV = Normalized white matter volume; Benign MS = EDSS $\leq$ 3; Non-benign MS = EDSS > 3

Table 4.4 Adjusted mean difference between patient subgroups & controls using SIENAx

Group comparisons	**NGMV**		**NWMV**	
	Adjusted mean difference (cc) 95% CI	p-value	Adjusted mean difference (cc) 95% CI	p-value
MS-Controls	**-56.52 [-85.05 - (-27.99)]**	**<0.001**	-14.11 [-33.99 - 5.76]	0.162
MS-CIS	**-47.60 [-71.64 – (-23.57)]**	**<0.001**	-8.62 [-25.37 - 8.11]	0.309
SPMS-Controls	**-96.63 [-133.91 – (-59.35)]**	**<0.001**	-11.12 [-38.43 - 16.17]	0.420
RRMS-Controls	**-43.49 [-71.98 – (-15.00)]**	**0.003**	-15.08 [35.95 - 5.78]	0.155
SPMS-CIS	**-87.06 [-121.02 – (-53.11)]**	**<0.001**	-5.68 [-30.55 - 19.18]	0.651
RRMS-CIS	**-33.92 [-58.48 – (-9.37)]**	**0.007**	-9.64 [27.63 - 8.33]	0.290
SPMS-RRMS	**-53.14 [-86.08 – (-19.47)]**	**0.002**	3.95 [-20.70 - 28.62]	0.751
Benign-Non benign MS	**65.25 [30.05 – 100.44]**	**0.001**	17.69 [-3.30 – 38.70]	0.09
CIS-Controls	-8.91 [-39.51 - 21.68]	0.564	-5.48 [-26.80 - 15.82]	0.610

NB: Bold - significant at $p < 0.05$; MS = Multiple sclerosis (all cases); CIS = Clinically isolated syndrome; RRMS = Relapsing-remitting MS; SPMS = Secondary progressive

MS; NGMV = Normalized gray matter volume; NWMV = Normalized white matter volume; Benign MS = EDSS ≤ 3; Non benign MS = EDSS > 3

MRI measures and disability Table 4.5

GMF correlated significantly with EDSS and MSFC for all patients and for the MS subgroup alone. WMF showed no such correlations. T_2LL also correlated with EDSS (r_s = 0.49; p<0.001) and MSFC (r_s = -0.53; p<0.001) for all patients as well as in the MS subgroup: (r_s = 0.38; p = 0.009) and (r_s = -0.43; p=0.005) respectively. Correlations of SIENAx measurements with clinical features are presented in Table 4.6.

Table 4.5 Correlations of brain volume measurements with clinical features

	EDSS N=73* (44§)	**MSFC** N=67* (41§)	**Z-PEG** N=70* (42§)	**Z-WALK** N=68* (40§)	**Z-PASAT** N=68* (42§)
	r_s (p-value)				
GMF*	**-0.48 (<0.001)**	**0.56 (<0.001)**	**0.59 (<0.001)**	**-0.40 (0.001)**	**0.27 (0.026)**
GMF§	**-0.41 (0.005)**	**0.55 (<0.001)**	**0.44 (0.003)**	**-0.49 (0.001)**	**0.32 (0.038)**
WMF*	-0.20 (0.086)	0.03 (0.784)	0.16 (0.176)	-0.11 (0.337)	-0.07 (0.537)
WMF§	-0.11 (0.443)	0.10 (0.526)	0.28 (0.071)	-0.09 (0.560)	-0.04 (0.761)

* All patients

§ MS subgroup only

Bold: significant at $p < 0.05$; r_s = Spearman Rank correlation coefficient; GMF = Grey matter fraction; WMF = White matter fraction; EDSS = Expanded disability status scale; MSFC = Multiple sclerosis functional composite score

Table 4.6 Correlations of SIENAx brain volume measurements with clinical features

	EDSS N=*73(§44)	**MSFC** N=*62(§39)	**Z-PEG** N=*62(§39)	**Z-WALK** N=*62(§39)	**Z-PASAT** N=*62(§39)
	r_s (p-value)				
*NGMV	**-0.62 (<0.001)**	**0.49 (<0.001)**	**0.62 (<0.001)**	**-0.34 (0.006)**	0.19 (0.12)
§NGMV	**-0.53 (<0.001)**	**0.39 (0.01)**	**0.54 (<0.001)**	**-0.45 (0.005)**	0.14 (0.38)
*NWMV	**-0.31 (0.006)**	0.15 (0.24)	0.12 (0.33)	**-0.29 (0.02)**	0.18 (0.15)
§NWMV	**-0.28 (0.05)**	0.14 (0.39)	0.24 (0.12)	-0.19 (0.24)	0.21 (0.19)

* All patients

§ CDMS patients only

Bold: significant at $p < 0.05$; r_s = Spearman Rank correlation coefficient; CIS = Clinically isolated syndrome; CDMS = Clinically definite multiple sclerosis (all cases); RRMS = Relapsing-remitting MS; SPMS = Secondary progressive MS; NGMV = Normalized grey matter volume; NWMV = Normalized white matter volume

Correlations of lesions with grey and white matter volumes

T_2LL correlated significantly with GMF (r_s = -0.63; p<0.001) but not with WMF (r_s = -0.15; p = 0.19) for the whole cohort of patients and for the MS subgroup only: (r_s = -0.66; p<0.001) and (r_s = -0.18; p = 0.22) respectively.

T_2LL correlated significantly with NGMV (r_s = -0.57; p<0.001) but not with NWMV (r_s = -0.14; p = 0.22) for all patients and for the MS subgroup only: (r_s = -0.67; p<0.001) and (r_s = -0.10; p = 0.50) respectively.

Predicting Disability

For the whole cohort of patients, only GMF predicted EDSS category: there was an estimated 68% (p<0.001) reduction in the odds of having greater disability per 1 SD higher GMF.

GMF only also, independently predicted disability as measured by MSFC scores; there was an estimated 0.62 increase (p<0.001) in MSFC per 1 SD higher GMF.

Restricting regression models to the MS subgroup of patients, only GMF independently predicted disability, whether EDSS or MSFC: there was a 59% (p = 0.007) reduction in the odds of being in more severe EDSS category per 1 SD higher GMF; and there was a 0.67 increase (p = 0.001) in MSFC per 1 SD higher GMF.

On the regression models using SIENAx measurements, only NGMV independently predicted disability whether EDSS or MSFC, on the whole cohort of patients and on the

MS subgroup only. For the whole cohort of patients there was a 79% ($p<0.001$) reduction in odds of being in a more severe EDSS category per 1 SD higher NGMV; and there was a 0.54 increase ($p<0.001$) in MSFC per 1 SD higher NGMV.

In the MS subgroup, there was a 71% ($p = 0.001$) reduction in odds of being in a more severe EDSS category per 1 SD higher NGMV; and there was a 0.52 increase ($p = 0.010$) in MSFC per 1 SD higher NGMV.

The independent MRI predictors of the MSFC components for the SPM2 and SIENAx measurements separately, for all patients and MS group only are presented in Table 4.7.

Table 4.7 Independent MRI predictors of MSFC components in regression models

	SPM2 (p-value)		**SIENAx (p-value)**	
	All patients	*CDMS only*	*All patients*	*CDMS only*
9HPT (N=70/42)	GMF (<0.001)	T_2LL (<0.001) Age (0.006) Gender (0.056)	NGMV (<0.001) T_2LL (0.029) Age (0.031)	NGMV (0.014) T_2LL (0.013) Age (0.006)
ITW (N=68/40)	GMF (<0.001) Age (0.002)	GMF (<0.001) Age (0.032)	NGMV (<0.001) Age (0.019) Gender (0.001)	NGMV (<0.001) Age (0.003) Gender (0.023)
PASAT (N=68/42)	GMF (0.024)	None	None	None

N = number of patients shown – all patients/CDMS

MSFC = Multiple Sclerosis Functional Score; CDMS = Clinically definitive MS; SPM2 = Statistical parametric mapping; SIENAx = Structural Image Evaluation, using Normalization, of Atrophy for cross-sectional measurement; GMF = Grey matter fraction WMF = White matter fraction; NGMV = Normalised GM volume; NWMV = Normalized WM volume; 9HPT = 9 Hole peg test; PASAT = Paced auditory serial additional test; T_2LL = T2-weighted lesion load; ITW = Inverted timed walk

Discussion

This study builds on previous work (Carone et al., 2006; Chard et al., 2003; Sanfilipo et al., 2005), characterising tissue specific brain atrophy in a group of people with MS or CIS who have a uniquely long and homogeneous disease duration (~20 years). It has allowed an exploration of the associations and role as predictors of MRI measures - tissue specific (GM and WM) atrophy and (WM) lesion load - with clinical phenotype and disability, relatively free of confounding by variability in disease duration.

In this cohort of patients both GM and WM atrophy was seen in MS patients compared with controls, and the extent of GM atrophy was greater than that of WM atrophy in keeping with some previous studies (Chard et al., 2002; Dalton et al., 2004; De et al., 2003; Quarantelli et al., 2003; Sastre-Garriga et al., 2004). Furthermore, there was significantly more GM, but not WM, atrophy in SPMS versus RRMS, and RRMS versus those remaining CIS patients. It should be noted that GM atrophy has not been a universal finding in MS, and that a definitive consensus on the location and timing of brain atrophy has yet to be reached; however a significant number of recent studies suggest that GM atrophy is a consistent finding throughout the clinical course of MS, seemingly mirroring clinical status (Dalton et al., 2004; Sanfilipo et al., 2005; Sastre-Garriga et al., 2005; Tiberio et al., 2005). The apparent discrepancy in some previous studies may represent a combination of cohort related and technical factors. Although there is no universally accepted gold-standard method for measuring GM and WM volumes, the SPM-based approach has provided consistent findings in several previous studies (Chard et al., 2002; Dalton et al., 2004; Sanfilipo et al., 2005; Tiberio et al.,

2005), and in the present study, the robustness of the results obtained using SPM-based methods have been consolidated by very similar findings with another widely used segmentation method - SIENAx.

Given the relatively homogeneous disease duration and age distribution of the clinical subgroups included in the present work, the association of GM atrophy with clinical status is not explained by these factors; rather, the findings suggest a direct link between GM atrophy and clinical disease severity.

Differential tissue specific atrophy in MS may be partially explained by variable degrees of inflammatory activity in WM and GM (Bo et al., 2003; Peterson et al., 2001), with relatively greater compensation of cell loss by inflammatory infiltrates and oedema in WM compared with GM. Differential inflammatory noise in the volumetric measures may also lead to greater attenuation of WM compared with GM associations with clinical parameters. However, it may be expected that eventually atrophy, if progressive, would reach a magnitude where it would no longer be disguised by inflammatory interference; given this, my observations in MS patients with relatively long disease duration suggest that WM atrophy is truly less progressive than that of GM, and not simply the result of compensation by, and short-term fluctuations associated with, inflammation. In addition, whilst no clear evidence of an association between WM atrophy and disability was detected, 50% of MS patients in the present cohort had a benign clinical course, and it is conceivable that larger cohort with more severe disability (e.g. EDSS 7 and higher) might exhibit more WM atrophy; further work is required to

explore this possibility. Considered overall, these findings suggest that measures of GM atrophy will be more useful than WM volume in natural history studies or treatment trials, for example in a study of potentially neuroprotective agents, although serial studies should further investigate the relationship between longitudinal GM volume and clinical changes. A recent longitudinal study of MS patients by Fisher et al, showed that the GM atrophy rate, but not WM atrophy rate – which was constant across MS subtypes, increased with disease severity: from 3.4 fold normal in CIS patients converting to RRMS to 14 fold normal in SPMS (Fisher et al., 2008).

GM, but not WM volume measurements, correlated with clinical disability (EDSS, MSFC and its components). Although T_2LL correlated significantly with disability, GMF was a better predictor of disability when included in the regression models. Whilst noting the caveats about inflammatory noise discussed previously, these data suggest that GM atrophy has more clinical relevance in the long term than either lesion load or WM atrophy in people with MS, being more closely related to long term disability and clinical course. The present study findings consolidate and extend the observation made in several previous studies that MRI markers of GM involvement correlate more strongly with measures of physical disability than WM lesion load (Carone et al., 2006; Fisher et al., 2008; Pirko et al., 2007; Sanfilipo et al., 2005; Sanfilipo et al., 2006) .

The amount of tissue loss in MS probably represents a balance between several pathological processes: irreversible neuronal and axonal loss, myelin loss, and reversible neuro-axonal atrophy, on the one hand; with partial compensation by inflammation associated cellular infiltrates, and cellular [including axonal (Fisher et al., 2007)] and

interstitial oedema on the other. With regard to the mechanisms of brain atrophy, there may be: (i) antegrade and retrograde neuroaxonal tract degeneration associated with focal WM inflammatory lesions (Trapp et al., 1998), with a potentially significant delay between axonal demyelination and subsequent neuroaxonal degeneration, and (ii) a more widespread process directly targeting neurons, myelin [including cortical demyelination (Bo et al., 2003; Peterson et al., 2001)] and glia.

GM (but not WM) volume measurements correlated with WM lesion load, which is in keeping with other studies (Chard et al., 2002; Dalton et al., 2004; Sanfilipo et al., 2005; Tiberio et al., 2005). This correlation might reflect secondary degeneration from WM lesions to GM. That the degree of correlation is only moderate suggests that processes independent of WM lesions are also contributing to GM atrophy in MS. One such explanation might be GM demyelinating lesions that - although not visible on conventional MRI - are commonly found at autopsy (Bo et al., 2003; Peterson et al., 2001). Our findings emphasise that further research to elucidate pathogenic mechanisms in MS should focus on GM as well as WM pathology.

In this study SIENAx and SPM-based methods generally provided similar results when investigating the relationship of GM and WM volumes with clinical course, clinical features and T_2LL. However, WM segmentation obtained by SIENAx appeared to be less accurate compared with SPM2, as deep GM was not as clearly segmented, and misclassification of WM lesions was not adjusted for, perhaps explaining why there were no differences between the NWMV (derived from SIENAx) between MS patients and

controls, while differences were seen in WMF (derived from SPM2). Nevertheless, taken the findings reinforced the conclusion that GM pathology is a significant contributing factor to the clinical course in relapse onset MS.

When considering the significance of the findings observed in this study it is important to take into account a few limitations. *Firstly*, neither WM lesion volume nor tissue specific brain atrophy measurement is pathologically specific. WM lesions on T_2-weighted MRI may contain variable amounts of inflammation, demyelination, oedema and axonal loss. The brain volume measurements, while affected by the same factors, are thought to be more specifically weighted towards neurodegeneration. *Secondly*, the brain volume measurement data is cross-sectional, and does not provide any direct information on the temporal evolution of atrophy, information which can only be gathered using serial MRI data. Hence, one cannot determine whether the atrophy observed in this study occurred immediately before, or many years prior to, this study. Although the patients were scanned at earlier time-points (Brex et al., 2002), there has been a major scanner hardware upgrade since then, rendering it difficult to directly compare measurements from earlier scanning with that obtained at 20-years. *Thirdly*, some of the more disabled patients were not able to be scanned, so this data is relatively biased towards a less disabled subset of the patients presented in chapter 3. *Fourthly*, spinal cord involvement makes an important contribution to locomotor disability in MS and was not included in this investigation. *Finally*, with the SPM based methods, misclassification of lesions or non brain tissue as GM may lead to a relative underestimation of the apparent magnitude of GM disease effects; however, correction for lesion misclassification was performed

and quality assurance review of the scans found no additional significant segmentation errors – thus, there should not have been significant misclassification effects.

Notwithstanding these caveats, the study clearly found that in MS patients with a relatively long and homogeneous disease duration (~20 years), GM atrophy is greater than WM atrophy, and reflects disease subtype and disability. It also helps to understand why a limited relationship between (WM) lesions and disability in MS has been evident in many previous MRI-clinical studies of both natural history and therapeutic intervention, and highlights a need to better understand and monitor GM pathology in MS.

Chapter V

Magnetisation transfer ratio abnormalities reflect clinically relevant grey matter damage in multiple sclerosis

Introduction

In multiple sclerosis (MS), the relationship between the white matter (WM) lesion load, as assessed using conventional MRI, and disability is only moderate (Brex et al., 2002)(see chapter 3). This has prompted a search for other MRI markers of disease progression that may either better explain clinical outcomes, or provide additional clinically relevant information. A variety of MRI techniques have been developed and, of these, brain atrophy measures and magnetization transfer ratio (MTR) have shown particular promise in MS, in particular tissue specific estimates of the grey matter (GM) damage. GM atrophy, more so than WM lesion accrual or WM atrophy, maybe associated with long term disability (Fisher et al., 2008; Pirko et al., 2007). These findings were also confirmed in the study presented in Chapter 4. MTR changes in the GM especially in early relapsing-remitting MS (RRMS) and primary progressive MS, have shown promise as predictors of future disability (Agosta et al., 2006; Khaleeli et al., 2007a).

Whilst recent histopathological studies in MS have reported extensive cortical demyelination (Bo et al., 2003; Gilmore et al., 2008; Kutzelnigg et al., 2005; Moll et al., 2008) (including deep GM nuclei (Cifelli et al., 2002; Gilmore et al., 2008; Huitinga et al., 2004; Vercellino et al., 2005)), a study in an experimental model of MS found no clear association between regional cortical demyelination and atrophy (Pomeroy et al.,

2008). This suggests that some elements of intrinsic cortical damage in MS may be at least partly independent of tissue atrophy, or may proceed it (Khaleeli et al., 2007a), and that assessment of both may provide complementary information. It is this that is explored in this work, using tissue specific MTR as a measure of intrinsic tissue damage (Mottershead et al., 2003; Schmierer et al., 2004; van Waesberghe et al., 1999), alongside tissue specific volumetric measures to assess atrophy (see Chapter 4). MTR imaging has proven a sensitive marker of tissue damage in MS and, at least in the WM, appears to be mainly influenced by demyelination and axonal loss (Dousset et al., 1992; Schmierer et al., 2004) and to a lesser degree by inflammation (Dousset et al., 1992; Schmierer et al., 2007), although pathological features other than demyelination may also be important in the determinants of the MTR (Gareau et al., 2000). MTR abnormalities within normal-appearing (NA)WM and GM have been detected from the earliest clinical stages of MS (Audoin et al., 2004; Davies et al., 2004; Fernando et al., 2005), and may increase over time (Fisher et al., 2008; Rovaris et al., 2003). Several studies have reported that MTR changes in NAWM and GM may differ between clinical subgroups and are correlated with disability (Agosta et al., 2006; Ge et al., 2001a; Khaleeli et al., 2007b; Ramio-Torrenta et al., 2006; Rovaris et al., 2003; Santos et al., 2002; Zivadinov et al., 2001).

By studying a cohort of patients with a long and homogenous disease duration since their first presentation with a clinically isolated syndrome (CIS) suggestive of MS, this work aimed to:

i. Assess how GM and NAWM MTR metrics at 20 years reflect disease subtype (CIS, RRMS or secondary progressive MS (SPMS)) and their level of disability;

ii. Evaluate the relative clinical relevance of the MTR histogram metrics, tissue specific brain atrophy estimates, and WM lesion load measures.

Methods

Subjects

The patients included in this study are part of a previously described cohort of CIS patients followed up approximately every five years for 20 years since their first presentation with a CIS suggestive of MS (Chapter 3 & 4). In seventy-five out of 107 patients seen at 20 years clinical and MRI data were acquired. Six patients were excluded (one patient developed cerebro-vascular disease in addition to MS; 2 patients did not complete the full scanning protocol and the GM-WM segmentation failed in 3 patients). The remaining 69 patients were included in this study [45 female and 24 male; mean age 51.4 years, range (39.4 - 67.5)]. Twenty eight subjects had remained CIS and 41 had converted to clinically definite MS (Poser et al., 1983) (31 had RRMS and 10 had evolved to SPMS)(Lublin & Reingold, 1996). Patients were followed up a mean of 20.0 years (range 18 – 27) from their first episode of CIS suggestive of MS. Clinical measures included Expanded Disability Status Scale score (EDSS) (Kurtzke, 1983) and MS functional composite scores (MSFC) (Fischer et al., 1999). Only two patients were on disease modifying treatment and both had RRMS. MRI data was also obtained in 19 healthy controls [11 females and 8 male; mean age 39.91 (6.53) years, range (30.57 – 57.30)].

The study was approved by the National Hospital for Neurology and Neurosurgery and Institute of Neurology Joint Research Ethics Committee. All study participants gave written informed consent.

Image Acquisitions and Processing

The following whole brain sequences were acquired in all subjects at the 20 year follow up time-point, using a 1.5 Tesla GE Signa scanner (General Electric, Milwaukee, WI, USA): i) 2D interleaved dual spin echo (SE) [repetition time (TR) 1720 ms; echo times (TE)s 30/80 ms); 28 × 5 mm slices; matrix 256 × 256; field of view 24 × 24 cm^2]. Both echoes were acquired with and without a MT pre-saturation pulse to calculate a MTR map (Barker et al., 1996). The saturating MT pulse was 1 kHz off-water resonance with an on-resonance equivalent flip angle of 1430°; ii) 2D dual-echo proton density (TE 17ms) and T_2 (TE 103ms) weighted fast SE [TR 2000 ms; 28 × 5 mm slices; field of view 24 × 18 cm^2; in-plane resolution of 1.1 mm]; iii) 3D axial T_1-weighted inversion-prepared fast spoiled gradient recall [TR 10.9 ms; TE 4.2 ms; inversion time 450 ms; 124 × 1.5 mm slices; imaging matrix 256 × 160, interpolated to a final in-plane resolution of 1.1 mm; flip angle 20º].

The long TE images of sequences (i) and (ii) were co-registered using FLIRT (www.fmrib.ox.ac.uk/fsl/), and the same transformation was applied to the short echo images. Lesions were outlined using a semi-automated method as previously described (Sailer et al., 1999). T_2 lesion volumes (T_2LL) were obtained using the proton density-weighted scans (short echo) of sequence (ii) and 3D T_1-weighted lesion load was

computed from sequence (iii). SPM2 www.nmrgroup.ion.ucl.ac.uk/atrophy (Ashburner & Friston, 2005) was used to segment the long echo images from sequence (i) into GM, WM (both NAWM and lesions) and CSF. (In the three cases where segmentation failed the T_2LL was very large, which is the reason for the failure). These segmentations were combined to obtain WM, GM, and whole brain masks. Lesions were then removed to obtain NAWM and GM masks which were applied to the calculated MTR maps. A 10 percentage unit (pu) lower threshold was applied as were two successive erosions of WM and a single erosion of the GM to minimise partial volume effects. Brain volume normalized NAWM and GM histograms were generated with a bin width of 0.1 pu and a smoothing window of +/- 0.3pu. The peak height (PH), peak location (PL) and mean MTR were extracted for NAWM and GM.

WM, GM and brain parenchymal fraction (WMF, GMF and BPF) were obtained from sequence (iii) using SPM2 (Wellcome Department of Cognitive Neurology, London, UK), corrected for lesion misclassification as GM (Chard et al., 2002) (see Chapter 4).

Statistical Analyses

Z-scores for MSFC subtests were calculated using the same sample of patients as a reference and used to derive MSFC scores (Cutter et al., 1999).

Cross-sectional group comparisons of the MTR parameters were performed using linear regression with group indicator, and age, gender and BPF as covariates. To assess the associations between the MTR histogram parameters and T_2LL, brain volume

measurements and clinical outcomes, due to non-Normality of the variables as was shown by normal probability plots, Spearman's rank correlation was used.

To assess the relative contribution of the MTR histogram parameters, tissue specific brain volumes and T_2LL to accrued disability: (i) ordinal logistic regression was used for EDSS, categorised as follows: ≤1.5; >1.5 and ≤3; >3 and ≤6; >6, and (ii) linear regression was used for MSFC and its components: 9 hole peg test (9HPT); Paced Auditory Serial Addition test (PASAT) and timed walk (the inverse of timed walk was used as it was more normally distributed). EDSS and MSFC (and its components) were modelled as response variables, with MTR parameters, tissue volumes, lesion load, age and gender as covariate predictors. Lesion load was log transformed to improve normality before inclusion in the regression models. Where the T_2LL was zero (10 subjects), the log volume was given a value of 0.01 to include these subjects.

For each response variable, MRI covariates were entered together and removed singly by manual backwards stepwise exclusion until all model predictors were significant at $p<0.1$ (a less stringent p-value threshold was used to take a more conservative approach in detecting potential confounds). To minimize the number of model covariates, a multi-stage approach was used. First, GM and NAWM MTR histogram parameters were modelled separately and then the best predictors from the two (GM and NAWM) models combined to obtain the best overall MTR model. After establishing the best MTR predictors, T_2LL and tissue volume (GMF and WMF) measurement were added to obtain a final model. Age and gender were added to the final models but omitted if the adjusted

coefficients were both non-significant and not materially different from unadjusted coefficients.

The data were analyzed using Stata 9.2 (Stata Corporation, College Station, TX, USA). Statistical significance was taken at $p<0.05$.

Results

The median EDSS for all patients (MS patients and those who had remained CIS) was 2.5 (range 0-8) and for MS patients only, 3.0 (range 1-8).

Group comparisons

The mean, median and standard deviation (SD) of the GM and NAWM MTR histogram metrics for all patients and healthy subjects are presented in Table 5.1.

Group comparisons are presented in Table 5.2 and Figure 5.1. When age and gender were included as covariates, GM PH was found to be significantly lower in: (i) MS patients (SPMS and RRMS) versus controls; (ii) SPMS and RRMS versus those subjects remaining CIS and (iii) SPMS versus RRMS. In the NAWM, mean MTR and PL were significantly lower in: (i) MS patients (SPMS and RRMS) versus controls; (ii) SPMS and RRMS versus those subjects remaining CIS, but not between (iii) SPMS versus RRMS. WM PH was lower in SPMS compared to all other groups. There were no differences between controls and those remaining CIS in any MTR metrics.

Table 5.1 Mean and median (Standard Deviation) of the MTR histogram metrics

MTR histogram metrics		Mean; Median (SD)				
		Controls N = 19	CIS N = 25	MS (N = 41)		All patients N = 69
				RRMS N = 31	SPMS N = 10	
GM	Mean MTR (pu)	32.65; 32.66 (0.44)	32.41; 32.34 (0.55)	32.30; 32.27 (0.82)	31.83; 31.63 (0.82)	32.27; 32.27 (0.74)
	PL MTR (pu)	33.31; 33.40 (0.37)	33.29; 33.40 (0.30)	33.68; 33.50 (0.99)	33.37; 33.25 (1.00)	33.48; 33.50 (0.80)
	PH % volume/pu	12.10; 12.54 (1.20)	11.27; 11.15 (1.24)	10.64; 10.60 (0.99)	9.24; 8.86 (1.49)	10.69; 10.73 (1.33)
NAWM	Mean MTR (pu)	38.56; 38.52 (0.38)	38.49; 38.56 (0.41)	37.99; 38.24 (0.91)	37.77; 37.90 (0.84)	38.16; 38.35 (0.78)
	PL MTR (pu)	38.71; 38.80 (0.38)	38.66; 38.70 (0.52)	38.25; 38.50 (0.78)	38.00; 38.25 (0.69)	38.38; 38.50 (0.70)
	PH % volume/pu	19.62; 19.86 (2.28)	18.84; 18.85 (1.75)	19.01; 19.30 (1.80)	16.8; 17.87 (3.17)	18.63; 19.18 (2.12)

N = number of subjects shown; CIS = Clinically isolated syndrome; MS = Multiple sclerosis; GM = Grey Matter; NAWM = Normal appearing white matter; MTR = Magnetization transfer ratio; PL = Peak location; PH = Peak height; pu = Percentage unit

Table 5.2 Age- and sex-adjusted mean difference (with p-values in brackets) [95% CI] of the MTR histogram metrics between patient subgroups and control subjects

Subgroups	GM			NAWM		
	Mean [pu]	PL [pu]	PH % volume/pu	Mean [pu]	PL [pu]	PH % volume/pu
MS – Controls	-0.27 (0.205) [-0.71 to 0.15]	0.47 (0.049)* [0.002 to 0.94]	-0.92 (0.009)** [-1.60 to -0.24]	-0.65 (0.005)** [-1.10 to -0.19]	-0.47 (0.027)* [-0.89 to -0.05]	-0.55 (0.350) [-1.73 to 0.62]
CIS – Controls	-0.03 (0.861) [-0.48 to 0.40]	0.16 (0.499) [-0.32 to 0.65]	0.05 (0.876) [-0.64 to 0.75]	-0.09 (0.694) [-0.56 to 0.37]	-0.002 (0.992) [-0.43 to 0.42]	-0.07 (0.896) [-1.29 to 1.13]
MS – CIS	-0.23 (0.141) [-0.55 to 0.08]	0.30 (0.082) [-0.03 to 0.65]	-0.97 (<0.001)** [-1.48 to -0.47]	-0.55 (0.001)** [-0.89 to -0.22]	-0.47 (0.003)** [-0.77 to -0.16]	-0.47 (0.278) [-1.34 to 0.39]
SPMS – Controls	-0.55 (0.054)^ [-1.11 to 0.01]	0.28 (0.369) [-0.33 to 0.90]	-1.79 (<0.001)** [-2.64 to -0.94]	-0.81 (0.008)** [-1.40 to -0.21]	-0.65 (0.019)* [-1.20 to -0.10]	-1.71 (0.026)* [-3.21 to -0.21]
RRMS –Controls	-0.19 (0.389) [-0.63 to 0.25]	0.53 (0.032)* [0.04 to 1.02]	-0.65 (0.056)^ [-1.32 to 0.01]	-0.60 (0.013)* [-1.07 to -0.13]	-0.41 (0.058)^ [-0.84 to 0.01]	-0.19 (0.739) [-1.38 to 0.98]
SPMS – CIS	-0.50 (0.037)* [-0.98 to -0.03]	0.11 (0.654) [-0.40 to 0.64]	-1.83 (<0.001)** [-2.55 to -1.11]	-0.71 (0.006)** [-1.22 to -0.21]	-0.64 (0.007)** [-1.11 to -0.18]	-1.61 (0.013)* [-2.89 to -0.34]
RRMS – CIS	-0.14 (0.383) [-0.48 to 0.18]	0.37 (0.050)* [-0.01 to 0.74]	-0.69 (0.008)** [-1.20 to -0.18]	-0.50 (0.006)** [-0.86 to -0.14]	-0.41 (0.015)* [-0.73 to -0.08]	-0.09 (0.828) [-1.00 to 0.80]
SPMS – RRMS	-0.35 (0.136) [-0.83 to 0.11]	-0.25 (0.337) [-0.77 to 0.26]	-1.14 (0.002)** [-1.85 to -0.42]	-0.20 (0.410) [-0.71 to 0.29]	-0.23 (0.307) [-0.69 to 0.22]	-1.51 (0.019)* [-2.78 to -0.25]

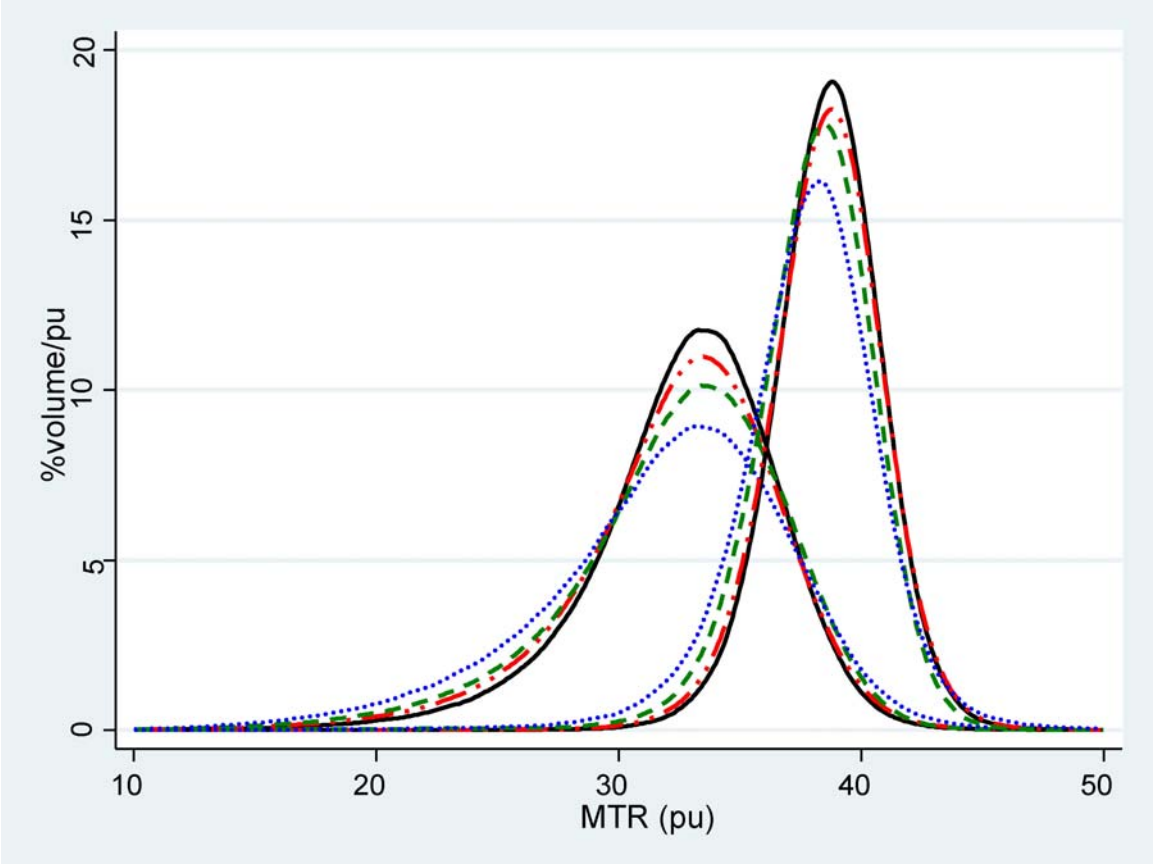
MS = Multiple sclerosis; RRMS = Relapsing-remitting MS; SPMS = Secondary progressive MS; CIS = Clinically isolated syndrome; GM = Grey Matter; NAWM = Normal appearing white matter; MTR = Magnetization transfer ratio; PL = Peak location PH = Peak height; pu = percentage unit;

^ Indicates significance level at $0.05 < p \leq 0.1$

* Indicates significance level at $0.01 < p \leq 0.05$

** Indicates significance level at $p \leq 0.01$

Figure 5.1 MTR histogram profiles for the GM and NAWM



NB: The average histogram profiles for the GM (left) and NAWM (right) for all subgroups: controls (solid line), CIS (dot dash line), RRMS (dashed line) and SPMS (dotted line). The peak location is the modal MTR value in pu; the peak height is the number of normalised brain units at the modal MTR value.

GM = Grey matter; NAWM = Normal appearing white matter; CIS = Clinically isolated syndrome; RRMS = Relapsing remitting multiple sclerosis; SPMS = Secondary progressive MS; pu = percentage unit

When BPF was also included as covariate (along with age and gender), the only significant differences observed were: lower GM PH in SPMS versus (i) CIS [age and gender adjusted mean difference (B) = -0.88; p = 0.011] and (ii) RRMS (B = -0.66; p = 0.036), and lower NAWM PH in SPMS versus RRMS (B = -1.28; p = 0.053).

Univariate correlations of MTR parameters with clinical outcomes and other MRI measures are presented in Table 5.3 and Figure 5.2 & Figure 5.3.

Table 5.3 Correlations of MTR histogram parameters with T_2LL, tissue specific brain volumes and disability (MS group only)

MTR histogram metrics		T_2LL	GMF	WMF	EDSS	MSFC
		r_s (p-value)				
GM	Mean	-0.04 (0.780)	0.12 (0.435)	0.43 (0.005)**	-0.29 (0.060)	0.30 (0.064)
	PL	0.33 (0.035) *	-0.15 (0.350)	0.23 (0.141)	-0.12 (0.428)	0.12 (0.448)
	PH	-0.47 (0.002)**	0.71 (<0.001) **	0.01 (0.921)	-0.49 (0.001)**	0.51 (0.001)**
NAWM	Mean	-0.69 (<0.001)**	0.36 (0.019)*	0.30 (0.051)*	-0.27 (0.079)	0.34 (0.037)*
	PL	-0.64 (<0.001)**	0.35 (0.024)*	0.20 (0.196)	-0.25 (0.107)	0.33 (0.040)*
	PH	-0.31 (0.045)*	0.38 (0.013)*	-0.002(0.992)	-0.25 (0.113)	0.17 (0.287)

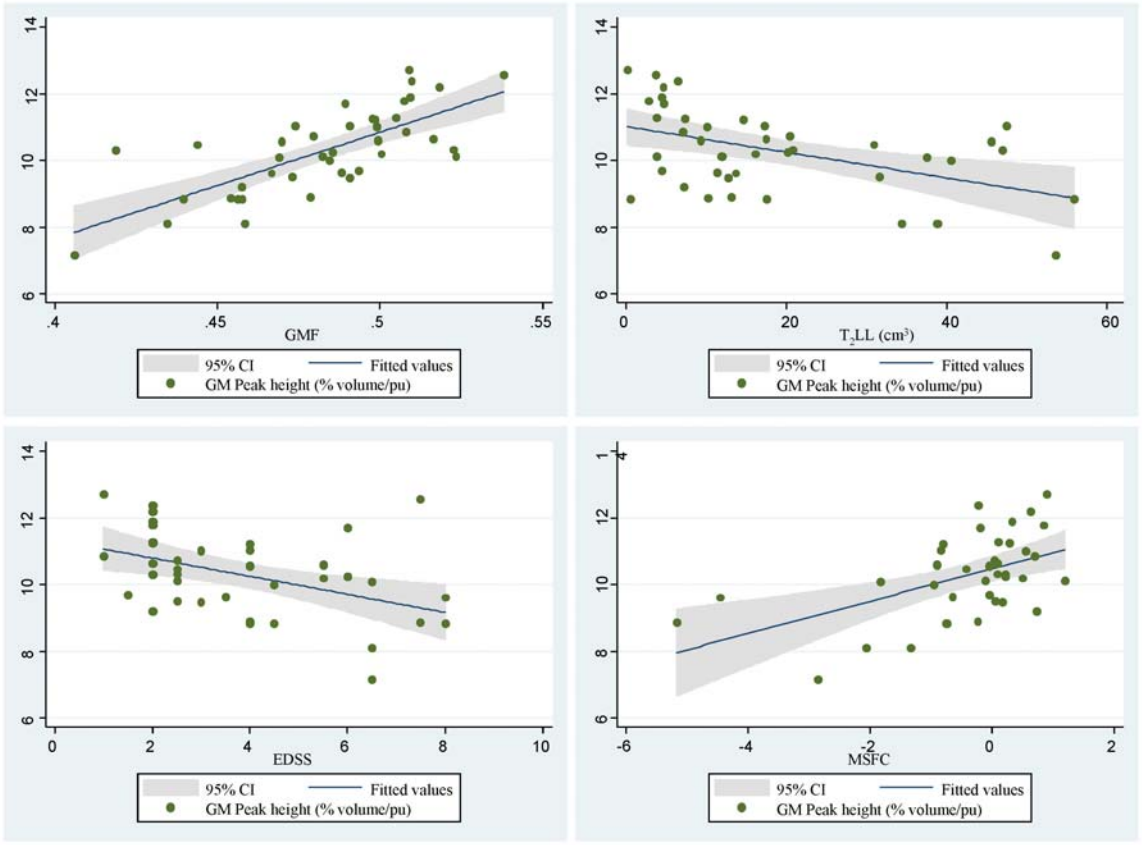
GM = Grey matter; NAWM = Normal appearing white matter; MTR = Magnetization transfer ratio; PL = Peak location; PH = Peak height; T_2LL = T_2 lesion load; WMF = WM fraction; GMF = GM fraction; EDSS = Expanded disability status scale; MSFC = Multiple sclerosis functional score

r_s = Spearman Rank correlation coefficient

* Indicates significant level at $0.01 < p < 0.05$

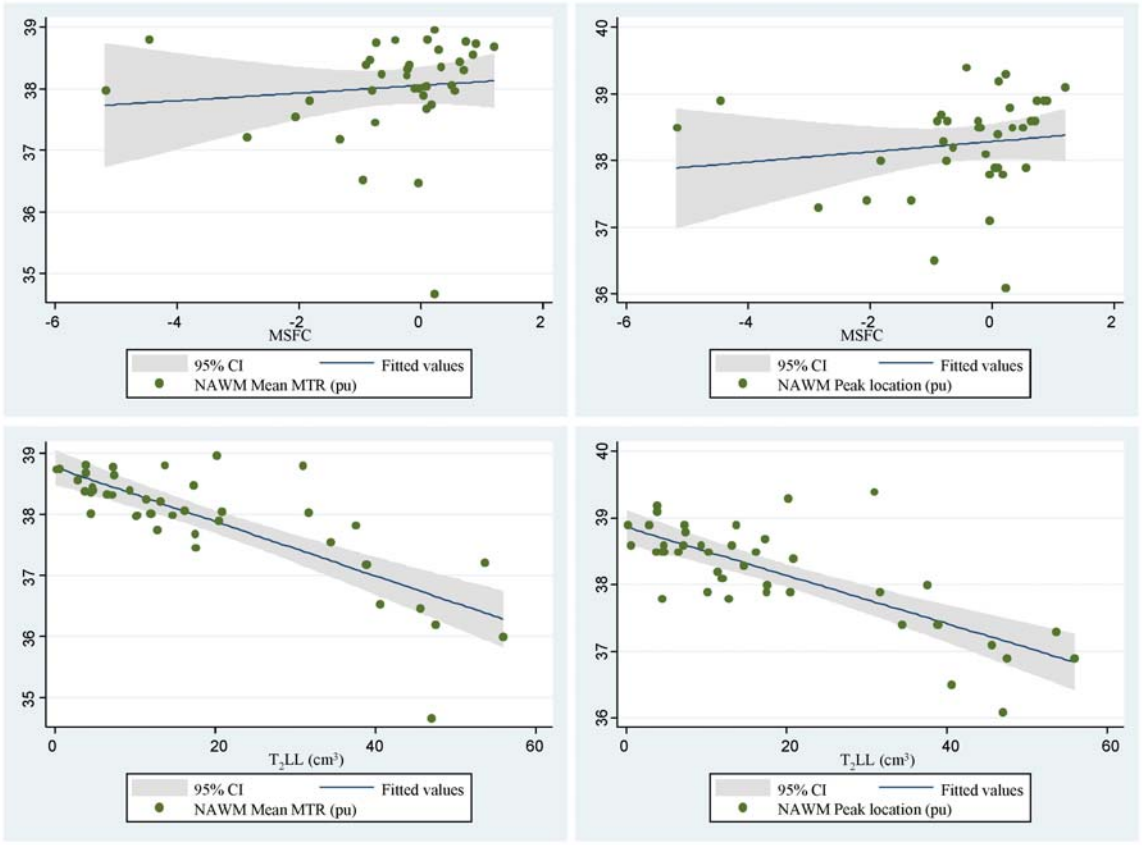
** Indicates significant level at $p < 0.01$

Figure 5.2 GM peak height plotted against GMF (top right), T_2 lesion load (top left), EDSS (bottom right) and MSFC (bottom left)



GM = Grey matter; GMF = Grey matter fraction; EDSS = Expanded disability status scale; MSFC = Multiple sclerosis functional score

Figure 5.3 NAWM mean MTR (left) and peak location (right) plotted against Multiple Sclerosis Functional Score and T_2 lesion load



NAWM = Normal appearing white matter; MTR = Magnetisation transfer ratio

Regression models: Table 5.4

EDSS

Of the MTR parameters, GM PH was the only independent predictor of disability: there was an estimated 76% ($p = 0.001$) reduction in odds of a patient having greater disability per 1SD higher GM PH.

GM PH remained the only independent predictor, even after adding T_2LL and WMF measures to the best MTR predictors. Because of the strong correlation between GM PH and GMF ($r_s = 0.71$; $p < 0.001$) their independent contribution could not be estimated in the same model (inclusion of both GM PH and GMF in the same model rendered them both statistically non-significant); however substituting GM PH with GMF, there was an estimated 66% ($p = 0.005$) reduction in the odds of a patient having higher disability per 1 SD higher GMF.

MSFC

Of the MTR parameters, GM PH was the only independent predictor of disability: there was a 0.86 ($p = 0.001$) increase in z-MSFC per 1SD higher GM PH.

GM PH remained the only independent predictor, even after adding T_2LL and WMF to the best MTR predictors. Because of the strong correlation between GM PH and GMF ($r_s = 0.71$; $p < 0.001$) their independent contribution could not be estimated in the same model; however substituting GM PH with GMF: there was an estimated 0.67 ($p < 0.001$) increase in MSFC per 1SD higher GMF.

Similar observations were evident when investigating 9HPT and inverted timed walk (Table 5.4). None of the MRI parameters predicted PASAT.

Table 5.4 Independent predictors in regression models in MS group only

Variables*	**EDSS N = 41**	**MSFC N = 38**	**9HPT N = 39**	**Inverted timed walk N = 37**	**PASAT N = 39**
MTR metrics only	GM-PH ($p = 0.001$)	GM-PH ($p = 0.001$)	GM-PH ($p = 0.001$)	GM-PH ($p < 0.001$) *and* gender ($p = 0.018$)	None
GM-PH; GMF/WMF; T_2LL	Either, GM-PH ($p = 0.001$) *or* GMF ($p = 0.005$)	Either, GMF ($p < 0.001$) or GM-PH ($p = 0.001$)	Either, GM-PH ($p = 0.001$) *or* GMF ($p = 0.015$) *and* T_2LL ($p = 0.007$)	Either, GMF ($p < 0.001$) *or* GM-PH *and* gender ($p = 0.041$)	None

* Age and gender were used as covariates on all models; (N = number of patients shown); GM = Grey matter; GMF = GM fraction; WMF = White matter fraction; PH = Peak height; MTR = Magnetization transfer ratio; T_2LL = T_2-weighted lesion load; EDSS = Expanded disability status scale; MSFC = Multiple Sclerosis functional composite score 9HPT = 9 Hole peg test; PASAT = Paced Auditory Serial Addition Test

Discussion

This study builds on work presented in Chapter 4 with this cohort in two ways: it characterises intrinsic tissue specific changes, as measured by MTR, in a group of people with long and homogeneous disease duration not markedly confounded by the potential effects of disease modifying treatments, and it explores the relative contribution of GM and WM MTR, and atrophy, to clinical outcomes in MS. The main observations were that: (i) MTR abnormalities can be found in GM and NAWM; (ii) both are associated with clinical outcomes, although GM more so than NAWM; (iii) GM MTR PH and GM atrophy (GMF) measures explain similar degrees of variability in disability scores; and (iv) such measures are more clinically relevant in the longer term than T_2LL estimates.

MTR parameters (especially PH in GM and less so, mean MTR and PL in NAWM) were generally lower the more clinically advanced a patient's MS had become following a consistent stepwise pattern: they were lower in SPMS than in RRMS, and lower in RRMS than in healthy controls and those remaining classified as CIS, but there were no differences between CIS and healthy controls. These results suggest that MTR abnormalities in GM and NAWM – but more so in GM – may mark on-going disease activity, rather than reflecting any residual effects of a CIS.

Volume normalised MTR histogram measures are principally influenced by three factors: the average MTR values within a tissue, tissue variability, and skew. This means for two tissues with similar mean MTR, an increase in tissue heterogeneity will lead to a reduction in PH. For tissues with different mean MTR values, an increase in tissue

heterogeneity will tend to mask such differences. It is therefore worth considering a given MTR histogram parameter in the context of its standard deviation. The PL relative to the mean MTR provides some insight into the skew of the histogram. A PL lower than the mean MTR, suggests the histogram is skewed towards higher values.

In this study, we see that the main disease effects in GM appear to be an increase in tissue heterogeneity, with greater skew towards lower MTR values (PL significantly increases, while mean MTR marginally reduces) when compared to healthy controls. In the NAWM tissue heterogeneity also increases, but not to a degree that this masks a reduction of the mean MTR values (as well as reduction in concurrent PL). This differential disease effect may in part be due to the much greater ability using current MRI techniques to detect (and therefore exclude) WM lesions. Hence, the GM is more likely to contain demyelinated lesions than the NAWM, and so be more heterogeneous. Further, given its relatively small myelin content (compared to the WM) a larger proportional reduction in GM myelination is probably required to be detected using MTR. In summary, it is plausible that the GM MTR findings reflect a greater mixture of lesional and non-lesional cortex – the later constituting the majority of GM tissue – with the results being a combination of reduced mean MTR and increased PL, whereas NAWM MTR findings reflect a more subtle non-lesional diffuse abnormalities (e.g. a combination of microglial activation (Kutzelnigg et al., 2005), inflammation, demyelination, astrogliosis and axonal loss) with reductions in both the mean and PL.

The study presented in Chapter 4 on a larger subset of the same cohort of patients, showed that GM but not WM atrophy measurements correlated with disability. However, in this study the association between MTR measures and clinical outcomes appears to be less tissue specific than for atrophy. Whilst GM MTR was more closely related to clinical outcomes, NAWM MTR parameters were also correlated with clinical scores: GMF, but not WMF correlated with clinical outcomes. This disparity may in part reflect differences in the nature of GM and WM pathology, in particular noting that WM lesions appear more inflammatory than those in GM (Bo et al., 2003). If this holds true for normal-appearing tissues, we may expect that inflammation associated oedema will tend to compensate for atrophy associated with cell loss more in the WM more than GM, while simultaneously diluting cell densities more in the WM than GM, which in turn may influence the strength of associations between tissue specific MRI measures with clinical outcomes.

A recent histopathological study of an experimental model of MS has suggested that cortical demyelination and atrophy may not be closely linked (Pomeroy et al., 2008). In contrast in this data, it is shown that GMF and GM MTR PH were strongly correlated (r_s = 0.71). Whilst the above discrepancy may represent known difficulties to translating findings from animal models of MS into human disease and vice-versa, if we assume that the substrates of MTR change in the GM are similar to those in the WM (i.e. weighted to a degree towards myelination, but not purely so (Schmierer et al., 2004)), these findings would suggest a link between GM demyelination and atrophy; if not, it would suggest that MTR changes in the GM, compared with the WM, at least in long-term MS, are less

weighted towards demyelination, and perhaps share another common substrate such as neuronal and or axonal loss.

Whilst a correlation of brain atrophy and MTR has been evident in previous studies looking at early RRMS patients (Davies et al., 2005; Phillips et al., 1998), MTR especially of the GM, appeared to be independent of brain atrophy when distinguishing MS patients from controls (Davies et al., 2004; Fernando et al., 2005), this was not the case in this study. Given that disease associated atrophy is generally more marked, and spans a greater range, in the present cohort (disease duration ~20 years) compared with people with shorter disease duration, this observation may be the result of greater power to detect correlations rather than true biological differences. It may also relate to partial volume effects, which tend to increase at tissue volumes decrease. Whilst measures were taken to limit this, we cannot entirely discount this possibility. However, it may also represent a true pathological link between intrinsic tissue damage and atrophy with connected features including demyelinating lesions and neuronal loss.

Correlation between T_2LL and NAWM MTR was stronger than with GM MTR. However, at best such correlations accounted for about 50% of shared variability, suggesting that global WM and more particularly GM MTR changes are at least partly independent of focal WM lesion formation. In addition, GM PH was a better predictor of clinical outcomes than T_2LL, reinforcing the conclusion from previous work presented in Chapter 4 that GM pathology is an important determinant of disability in MS.

When considering these results, in addition to those noted above, several other limitations should be kept in mind. *Firstly*, studies have reported mean GM MTR (Davies et al., 2004) and the others have reported PH (Agosta et al., 2006) as better correlated with disability, but differences in MTR sequences across different scanners and centres (Rovaris et al., 2008), and variation in processing methodologies (Fernando et al., 2005; Filippi, 1999; van Buchem et al., 1999), may influence the relative sensitivity of the various MTR metrics to pathology. *Secondly* the cohort of patients presented here has a bias towards those with lower disability (benign-MS) of all who were seen at 20 years (it was not possible to scan some of the more disabled patients), potentially limiting the scope to fully investigate associations between MRI measures and disability. *Thirdly*, current image techniques have limited ability to detect cortical lesions (Geurts et al., 2005; Geurts et al., 2008); thus there is uncertainty to what extend such lesions account for the GM MTR change seen. Future MR hardware (e.g. high field scanners at 3 Tesla and above) and sequence developments (e.g. double inversion recovery, phase sensitive inversion recovery) should focus on better detection of GM lesions (Geurts et al., 2005; Nelson et al., 2007). *Fourthly*, no assumption can be made about the longitudinal changes in MTR and atrophy in predicting disability. While a subgroup of the patients had similar sequences acquired at 14 year follow up, a major scanner hardware upgrade, rendered it difficult to directly compare measurements from earlier scanning with that obtained at 20 years.

Not withstanding these limitations, this study shows that GM damage – as marked by MTR changes – is related to disability in MS, and is a better predictor of clinical

outcomes than NAWM MTR, WM lesion load or WM atrophy in patients with longstanding MS.

Chapter 6

Early brain white matter lesion formation predicts long-term grey more than white matter atrophy in relapse onset multiple sclerosis

Introduction

Magnetic resonance imaging white matter (WM) lesions (T_2LL) are usually a conspicuous feature early in the clinical course of relapse-onset multiple sclerosis (MS), but whilst they have a role in predicting who will convert to clinically definite MS following a clinically isolated syndrome (CIS) (Polman et al., 2005; Swanton et al., 2007) they predict the long-term disability only modestly (Brex et al., 2002). In contrast, brain atrophy although evident from disease onset (Brex et al., 2000; Dalton et al., 2004) , is more readily apparent in the later clinical stages of MS (Dalton et al., 2006; Fisher et al., 2008). Further, there is growing evidence suggesting that in the long-term in MS there is more GM than WM atrophy, and that GM atrophy is more closely related to disability (Fisher et al., 2008; Pirko et al., 2007).

The long-term relationship between changes in T_2LL with brain atrophy is uncertain. A previous study involving people 14 years after first presentation with a CIS, showed that early rather than later changes in WM T_2LL predicted whole brain atrophy at 14 years, but the tissue specificity of the atrophy measures in this association was not investigated (Chard et al., 2003). Cross-sectional work has hinted at more consistent associations between GM rather than WM atrophy and T_2LL accrual, and so building on this previous study, using recently acquired 20 year follow up data in the same cohort, I sought to

confirm these previous findings, and in addition characterize the relationship between lesion accrual and tissue specific (GM and WM) atrophy.

Methods

Subjects

The patients included in this study are part of a larger cohort of patients seen from their first presentation with a CIS and followed up approximately every 5 years for 20 years described in details in Chapters 3 and 4. Seventy-five out of 107 patients seen at 20 years had the clinical and MRI data acquired. Only 73 were included in this study as one did not complete the full scanning protocol, and the other developed cerebrovascular disease in addition to MS. Forty patients had T_2LL collected at all data time-points available. Twenty-five healthy controls were imaged in parallel with the CIS cohort's 20 year follow up.

The diagnosis of clinically definite MS was made on the clinical grounds (Poser et al., 1983) and the course of MS was based on the Lublin et al criteria (Lublin & Reingold, 1996). Disability in patients was measured by using Expanded Disability Status Scale (EDSS) (Kurtzke, 1983). The study was approved by the National Hospital for Neurology and Neurosurgery and Institute of Neurology Joint Research Ethics Committee. All study participants gave written informed consent.

Image Acquisitions and Processing

Baseline and 5 year MRI scans were obtained on a 0.5 Tesla scanner (Picker, Cleveland) and on a 1.5 Tesla GE Signa Echospeed scanner (General Electric Signa, Milwaukee) at 10, 14 and 20 years. Contiguous, axial slices covering the whole brain were obtained at all visits. In a minority of early baseline scans (1984/1985) slice thickness was 10 mm; it was 5 mm for all subsequent scans. At 20 years, the following sequences were acquired: i) 2D dual-echo proton density (TE 17ms) and T2 (TE 102ms) weighted fast SE [TR 2000 ms; 28 × 5 mm slices; field of view 24 × 18 cm^2; in-plane resolution of 1.1 mm]; ii) 3D axial T_1-weighted inversion-prepared fast spoiled gradient recall [TR 10.9 ms; TE 4.2 ms; inversion time 450 ms; 124 × 1.5 mm slices; imaging matrix 256 × 160, interpolated to a final in-plane resolution of 1.1 mm; flip angle 20º].

Using a previously described technique (Sailer et al., 1999), T_2LL were obtained using the proton density-weighted scans (short echo) of sequence (i) and 3D T_1-weighted lesion load was computed from sequence (ii). WM, GM and brain parenchymal fraction (WMF, GMF and BPF) were obtained from sequence (ii) using SPM2 (Wellcome Department of Cognitive Neurology, London, UK), corrected for lesion misclassification as GM (Chard et al., 2002).

Statistical Analyses

The data were analyzed using Stata 9.2 (Stata Corporation, College Station, TX, USA). Statistical significance was taken at $p<0.05$.

Longitudinal linear mixed models were used to assess the rate of T_2LL accrual, the confounding effect of slice thickness on T_2LL and rate of growth, and the associations of rate of T_2LL growth and tissue specific atrophy. T_2LL was used as a response variable with time, patient group (CIS or MS at 20 year follow up), GMF and WMF as covariates with additional interactions with time where appropriate, using all available data time-points for T_2LL. The models were run on the all patients, and on the subset of patients who had T_2LL data available for all time-points. To assess for non-linearity, in addition to the linear term, a quadratic term was also fitted. GMF and WMF were expressed as standardized values for convenience of interpretation.

Results

The demographics of all the subjects are shown on Table 6.1. The 20 year GM and WM fractional volumes were lower in MS patients compared with controls ($p < 0.001$ and $p = 0.01$ respectively). There was no evidence of non-linear lesion load growth in either group and there was no evidence of difference in linearity between the two groups ($p = 0.352$). Slice thickness did not significantly influence estimated lesion volumes or the gradient of lesion growth.

Lesion volume at *baseline* was lower by 1051.4mm^3 ($p = 0.044$) for every additional 1 SD higher GMF at 20 years for all patients, 26.3mm^3 lower per 1 SD higher GMF ($p = 0.984$) for those patients who did not develop MS, and 1419.1mm^3 ($p = 0.035$) for those who did. However, the difference in association between MS and non-MS was not significant ($p = 0.316$). For every 1SD greater 20 year GMF, the annual *rate* of lesion

growth decreased by 526.5mm^3; ($p < 0.001$) for all patients; 533.2mm^3; ($p < 0.001$) for MS patients only and by 87.3mm^3 ($p = 0.517$) for those who remained CIS (see Figure 6.1 and 6.2).

Lesion volume at *baseline* was lower by 1895.9mm^3; $p < 0.001$ for every 1 SD higher WMF at 20 years. The association was different in those who developed MS compared with those who remained CIS ($p = 0.018$): in the group who remained CIS, the baseline lesion volume was lower by 493.8 mm^3; $p = 0.917$, per additional SD WMF at 20 years, while in the MS group it was lower by 2621.2mm^3; ($p < 0.001$). The overall *rate* of lesion growth was not associated with WMF at 20 years in either group of patients.

Restricting the analysis to the MS patients ($N = 27$) in whom all the T_2LL at all-time-points was available, change in T_2LL over the first 5 years was more closely related to GMF than subsequent T_2LL changes: 1 SD higher T_2LL in years 0-5 predicting 1.3 SD lower GMF ($p < 0.001$) versus 1 SD higher T_2LL in years 5-20 predicting 0.5 SD lower GMF ($p < 0.001$). Only the changes in T_2LL over the first 5 years predicted WMF at 20 years: 1 SD higher lesion growth predicted 1.03 SD lower WMF ($p = 0.01$).

Table 6.1 Demographics of all subjects scanned at 20 years

Demographics	All patients	Patients with all T_2LL available	Controls
Subjects (all)	73*$	40	25
CDMS	67	27	---
RRMS	39	21	---
Female : Male	49 : 24	26 : 14	14 : 11
Mean follow-up (years) (range)	20 (18-27)	19.7 (18-26)	---
Mean age (SD)	51.4 (7.2)	50.8 (6.5)	41.7 (7.7)
Median (range) EDSS			
All patients	2.5 (0-8)	2.5 (0-8)	---
CDMS only	3.25 (1-8)	3.0 (1-8)	
Mean (SD)			
GMF	0.49 (0.03)	0.49 (0.03)	0.51 (0.01)
WMF	0.28 (0.01)	0.28 (0.01)	0.29 (0.01)

MS = Multiple Sclerosis; CDMS = Clinically definite MS; GMF = Grey matter fraction; WMF = White matter fraction; T_2LL = T_2-weighted WM lesion load

* 107 patients were seen at 20 years. The demographics of the whole cohort are reported on chapter 3.

$ 2 other patients were excluded from the analysis – one developed cerebrovascular disease in addition to MS and the other patient did not complete the full MRI scanning protocol

Figure 6.1 Predicted rate of lesion growth by the 20th centile GMF at 20 years – all patients (lesion growth expressed in cm^3)

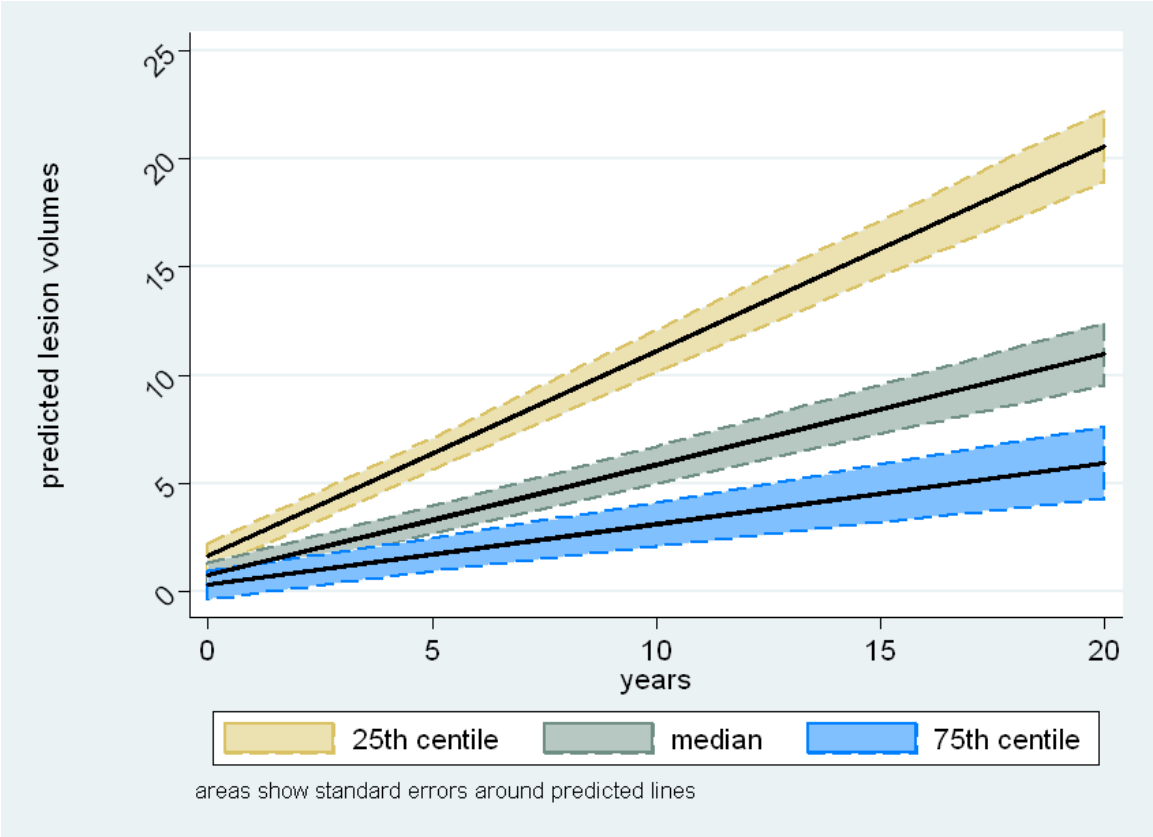
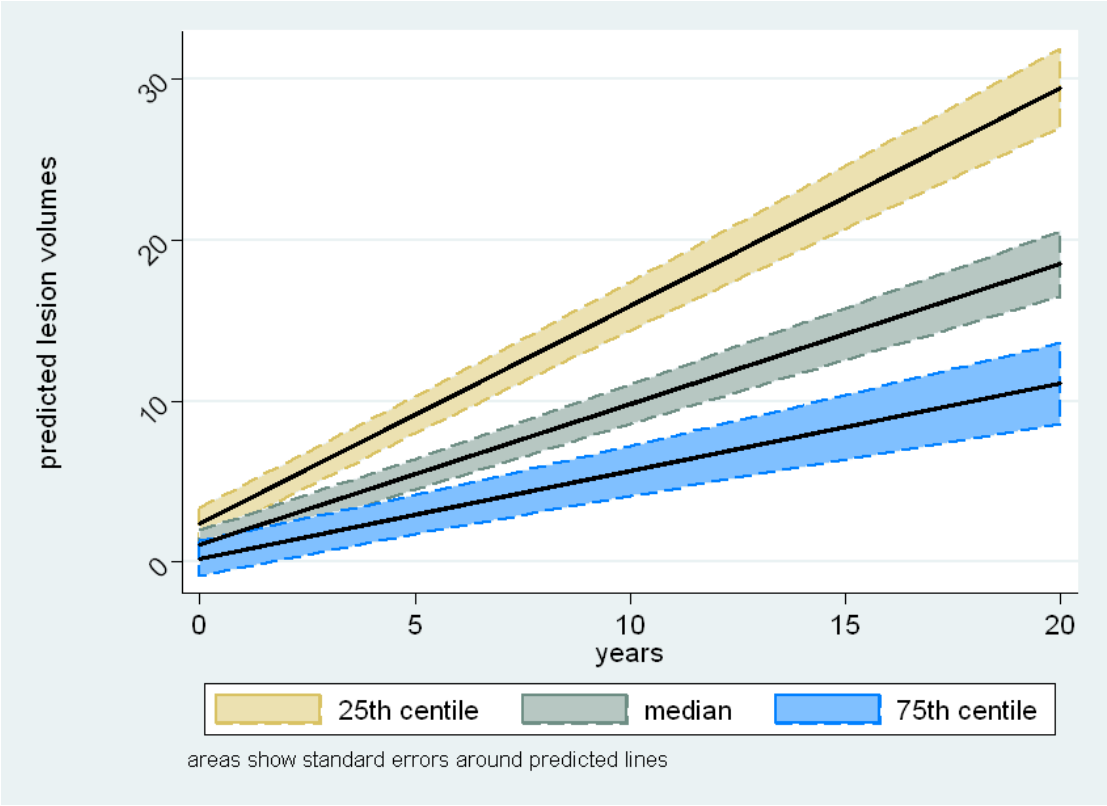


Figure 6.2 Predicted rate of lesion growth by 20 year GMF centiles – MS patients only (lesion growth expressed in cm^3)



Discussion

The main findings of this study were: i) baseline T_2LL predicts long-term WM atrophy more than GM atrophy; iii) subsequent T_2 lesion accrual, particularly in the first 5 years, is a better predictor of long-term GM rather than WM atrophy; iv) the changes in T_2LL only partially explain the brain atrophy at 20 years.

Given that the atrophy was only measured at 20 year follow up time-point, one cannot determine when the observed atrophy occurred, or whether there is a temporal difference in the evolution of lesion load and atrophy. It is known from other studies that early increasing GM atrophy occurs in MS (Chard et al., 2002; Dalton et al., 2004). Nevertheless, there are several possible explanations why there may be a delay between lesion formation and atrophy. *Firstly*, slowly evolving tract-mediated neurodegeneration may occur following the occurrence of an acute focal demyelinating lesion; for example thinning of the retinal nerve fibre layer on optical coherence tomography is not fully evident until ~6 months after an epoisode of optic neuritis (Henderson et al., 2010). *Secondly*, both lesion formation and tissue atrophy may be related to the same underlying process, but occur with different latencies. *Thirdly,* tissue atrophy may occur at the time of lesion formation but be masked by inflammation associated oedema and cellular infiltration, and subsequently only become manifest as this settles.

Why should the longitudinal increase in lesion load relate more to later GM (rather than than WM) atrophy? One possibility is that GM tissue is more vulnerable to inflammatory

lesion-mediated neurodegeneration. Neurons and their projections make up more of the GM than WM (Chard & Miller, 2009), so even if loss of neurons and axons are equally related to lesion load formation in both WM and GM, correlations with WM lesion loads are more likely to be found, or appear to be stronger, with GM rather than WM atrophy. Another possibility is differential inflammatory activity of WM lesions compensating for volume loss in WM more than GM with the GM lesions being less inflammatory (Peterson et al., 2001).

The explanation of a stronger relationship between baseline T_2LL and later WM (rather than GM) atrophy is uncertain. It may be that WM atrophy is a marker of a very early process in the disease, whereas GM atrophy is more influenced by events taking place throughout the disease course. Previous serial studies in early RRMS support this concept in reporting early but stable WM atrophy with increasing GM atrophy (Chard et al., 2004; Tiberio et al., 2005).

In addition to those study limitations noted above, two others are particularly worth mention: we did not find a significant confounding effect of the slice thickness on lesion volumes and rate of lesion growth, but could not assess the reproducibility of early versus later measures, as such may be underestimating the strength of the association between early lesion formation and later atrophy; the segmentation of the brain tissues using SPM (as with other methods) is imperfect, and in the case of SPM tends to misclassify the T_1 hypointense lesions as GM – a lesion mask was used to correct for this, but small residual errors may remain.

In conclusion, in this study we found that in the longer-term following a CIS suggestive of MS, the rate of lesion growth is related more to later GM than WM atrophy and early more so than later accumulation of WM lesions predicts brain atrophy.

PART IV

CONCLUSIONS

Chapter VII

SUMMARY & CONCLUSIONS

The aim of this thesis was to assess the role of MRI as a predictor of disability in relapse-onset MS patients. I was fortunate enough to be able to work with a rather unique cohort of patients who were seen from the disease onset with a clinically isolated syndrome and followed up prospectively with clinical and MRI data every five years up to 20 years. The data gathered is unique not only because it is the longest MRI study of MS patients but also because it has given me a unique cohort of patients with different subtypes of MS who also have a rather homogenous disease duration as well as age of disease onset. Furthermore, this is a drug naive cohort of patients as only very few patients were on disease modifying treatment, hence the observed relationship between the MRI and the clinical disability is representative of the "true natural" relationship of MRI and disability in MS.

So what does this data add to what had already been known about this relationship? Are the findings confirmatory to the overwhelming number of MRI studies done in MS? Does this data accept or reject the concept of the MRI paradox in MS? What are the service and the novelty that this work offers to research in MS and the MS community as a whole and where does the future of imaging lie in MS?

The main findings from this work are:

i. CIS patients with an abnormal MRI scan at presentation are more likely to convert to clinically definite MS than those with a normal MRI scan (82% versus 21%).

ii. MS patients exhibit a wide spectrum disability 20 years after disease onset.

iii. MRI lesions on the baseline scan provide limited prediction for long term disability.

iv. There is moderate correlation of T_2 lesion volume at all time point with disability at 20 years.

v. Concurrent correlation between T_2 lesion volume and EDSS change is mainly in the first 5 years and weak or absent at later intervals.

vi. Lesion load continues to increase for at least 20 years in relapse onset MS patients and it is higher in those who develop SPMS than those who retain a relapsing remitting course.

vii. In MS patient with a long and homogenous disease duration there is more GM damage than WM damage as measured by atrophy and MTR.

viii. GM damage reflects disease subtype and disability more so than WM damage, independent of disease duration or age.

ix. Earlier more so than later lesion accumulation is related to subsequent GM and WM atrophy.

I believe the data from this work has direct implications in the care of MS patients especially as it offers help in being able to counsel the patients regarding their chances of converting to MS after their first presentation with a CIS. The baseline MRI scan offers the strongest predictive value to conversion to MS independent of the clinical presentation. MRI studies had shown that conventional MRI measures such as lesion volumes modestly predict disability in MS on short and medium follow up studies. Data from this work robustly confirms these findings but furthermore, sheds new light in establishing that the lesion volumes continue to increase and are on average three times higher in SPMS than RRMS.

It was this modest predictive value of lesion load for disability in MS that made me look at other MR measures of tissue specific involvement. And although the GM involvement has been known for years, hence the observed differences between MS and controls was not a surprise, this data quite significantly showed that not only there is more GM atrophy than WM atrophy in MS patients with a long disease duration but also that GM atrophy is more clinically relevant to long term disability than WM atrophy or indeed WM lesions. The MTR data also suggest the importance of GM pathology for long term disability.

This work was not devoid of limitations which are discussed in detail in each relevant result chapter. Nevertheless despite these limitations and taking into account the shortcomings of MRI technology, imperfections of the disability scales which are currently available to us and the “hiccups” of the analysis techniques used, I believe that this data has shed some light in our understanding of MS. In particular, it has emphasised

that MS is a complex generalized disease where the WM lesions are only a component – albeit a clinically relevant one - of the pathology and that GM (along with so-called NAWM and the spinal cord) pathology also plays an important role. Overall, my work has illuminated the so-called clinical-MRI paradox in MS by identifying *in vivo*, through long term follow up and use of quantitative and tissue-sepcific measures, key aspects of MS pathology.

In future, further development of pathologically specific MRI hardware (e.g., high field scanners) and quantitative measures including spectroscopy, diffusion tensor imaging, volumetric measurement, magnetization transfer ratio and other techniques for assessment of the GM involvement, along with improvement of the clinical outcome measures and more longitudinal studies, challenging though they might be, will clarify the role of MRI as a predictive tool in MS. This will be extremely important as the emergence of new disease modifying treatment requires robust surrogate markers to assess their efficacy.

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