

The investigation of early MRI in diagnosis and prognosis in patients presenting with a clinically isolated syndrome characteristic of demyelination.

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Declaration

I, Josephine Swanton, confirm that the work presented in this thesis is my own. Where information has been derived from other sources, I confirm that this has been indicated in the thesis. Patient recruitment and examination for the studies detailed in Chapters 5 to 9 were carried out in the NMR Unit, Institute of Neurology, London, UK by Drs Peter Brex, Catherine Dalton, Kryshani Fernando and myself. In the multicentre study of Chapter 7, in addition to the London CIS cohort, each centre (Barcelona, Milan and Amsterdam) was asked to identify CIS patients recruited into prospective MRI and clinical follow-up studies under the supervision of Dr Alex Rovira, Dr Mar Tintore, Professor Xavier Montalban, Dr Frederik Barkhof, Professor Massimo Filippi, Professor Christopher Polman and Dr Marco Rovaris.

All conventional MRI images were reviewed by Dr Katherine Miszkiel (Consultant Neuroradiologist, National Hospital for Neurology and Neurosurgery, London, UK). Statistical analysis for all studies included in this thesis was provided by Dr Dan R. Altmann.

Abstract

This thesis explores the use of early MRI in prognosis and diagnosis in patients presenting with a clinically isolated syndrome (CIS) characteristic of demyelination. This has been investigated in a cohort recruited within 3 months of CIS onset between 1995 and 2004 and followed up clinically and with MRI (planned at 3 months, 1,3 and 5 years).

Current MRI criteria are highly specific for the development of clinically definite multiple sclerosis (CDMS) but have limited sensitivity and are complex. Presented is the evaluation of simplified MRI criteria in my London CIS cohort and in a multicentre CIS cohort. Results from the presented studies show that the MRI criteria can be simplified (dissemination in space: 2 or more lesions in separate but characteristic locations, dissemination in time: an early new T2 lesion) and still maintain high specificity, with improved sensitivity and accuracy.

The prognostic role of early MRI was investigated in the optic neuritis (ON) subgroup, as 80% of my cohort presented with ON and some studies have suggested that such a presentation is associated with more benign disease. Whereas baseline lesion number significantly predicted conversion to CDMS and increased disability at 5 years, other MRI parameters, namely baseline lesion location (periventricular lesions increasing the hazard of CDMS and spinal cord and infratentorial lesions increasing the odds of greater disability at 5 years) and lesion activity (new T2 lesion at 3 month follow-up), were stronger predictors. No non-conventional MRI parameters (spectroscopy, magnetisation transfer ratio or atrophy measures) had a significant prognostic role.

Overall early MRI findings can aid diagnosis and help identify the CIS patients at greatest risk of conversion to CDMS and subsequent disability, which in turn can help direct treatment and clinical follow-up in specialist MS clinics.

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Publications associated with this thesis

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Swanton, J.K., et al., *MRI criteria for multiple sclerosis in patients presenting with clinically isolated syndromes: a multicentre retrospective study*. Lancet Neurol, 2007. **6**(8): p. 677-86.

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Audoin, B., et al., *Selective magnetization transfer ratio decrease in the visual cortex following optic neuritis*. Brain, 2006. **129**(Pt 4): p. 1031-9.

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ABBREVIATIONS

AAN	American Academy of Neurology
ABN	Association of British Neurologists
ADEM	Acute disseminated encephalomyelitis
AEP	Auditory evoked potentials
B ₀	External magnetic field
BBB	Blood brain barrier
BPF	Brain parenchymal fraction
BPV	Brain parenchymal volume
CDMS	Clinically definite multiple sclerosis
CHAMPS	Controlled high risk Avonex multiple sclerosis prevention study
Cho	Choline
CIS	Clinically isolated syndrome
CNS	Central nervous system
CoV	Coefficients of variation
Cr	Creatine
CSF	Cerebrospinal fluid
DIR	Double inversion recovery
DIS	Dissemination in space
DIT	Dissemination in time
DMT	Disease modifying therapy
DSS	Disability status scale
EBV	Epstein Barr virus
EDSS	Expanded disability status scale
ESR	Erythrocyte sedimentation rate
ETOMS	Early treatment of multiple sclerosis study
FLAIR	Fluid attenuated inversion recovery
FOV	Field of view
Glx	Glutamate and glutamine
GMF	Grey matter fraction
GMV	Grey matter volume
Ins	Myo-inositol
MRI	Magnetic resonance imaging

MS	Multiple sclerosis
MSFC	Multiple sclerosis functional composite score
MTI	Magnetisation transfer imaging
MTR	Magnetisation transfer ratio
NAA	N-acetyl aspartate
NABT	Normal appearing brain tissue
NAWM	Normal appearing white matter
NICE	National institute of clinical excellence
NMO	Neuromyelitis optica
OCB	Oligoclonal bands
ON	Optic neuritis
ONTT	Optic neuritis treatment trial
PASAT	Paced auditory serial addition test
PD	Proton density
PML	Progressive multifocal leukoencephalopathy
PPMS	Primary progressive multiple sclerosis
pu	percent units
RF	Radiofrequency
ROI	Region of interest
RRMS	Relapsing remitting multiple sclerosis
SPM	Statistical parametric mapping
SPMS	Secondary progressive MS
SSEP	Somatosensory evoked potentials
TE	Echo time
TIV	Total intracranial volume
TR	Repetition time
VEP	Visual evoked potentials
WMF	White matter fraction
WMV	White matter volume

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Chapter 1

1. Introduction

Multiple sclerosis is an inflammatory, autoimmune disorder of the CNS causing disability in young adults. It is thought to be a T cell-mediated autoimmune disease precipitated by as yet unidentified environmental triggers in a genetically susceptible person. Macrophages, B cells and autoantibodies to myelin antigens [1] some of which have high affinity, are also involved but whether their presence is a result of the destruction or causal is not clear. Previously thought to be a disease of white matter with loss of myelin only, recent work has shown extensive grey matter involvement and loss of axons.

1.1 Aetiology of multiple sclerosis

1.1.1 Genetics

The role of genetic susceptibility is apparent from work such as the Canadian Collaborative Study. Results have shown that family members of MS patients have greater disease risk than the general population: monozygotic twins have about 30% concordance compared with about 5% in dizygotic twins. The risk of MS in offspring of two parents with the disease is significantly higher than for offspring with only one parent (30.5% vs 2.5%) [2]. Full siblings have roughly twice the risk of disease than half siblings (3.5% vs 1.9%) and adopted individuals the same risk as the general population. In families with two or more first degree relatives with MS the disease subtype shows concordance between siblings (but not parent-child) but clinical severity was not concordant [3]. These studies indicate that familial aggregation of MS is due to sharing of genes rather than environment.

MS susceptibility is polygenic and associated with normal polymorphisms rather than genetic mutations. As in other autoimmune diseases, MHC shows the strongest association, and varies with different populations. The extended HLA-DR15 haplotype, particularly the HLA DRB1*1501 allele, has been closely associated with MS in Caucasian patients of northern European heritage [4-6], whereas HLA DPB1*0501 has been associated with the optical-spinal variant in Japan [6]. However the strong linkage disequilibrium occurring in the MHC region makes it

hard to determine whether these alleles or others linked to them are the major candidates.

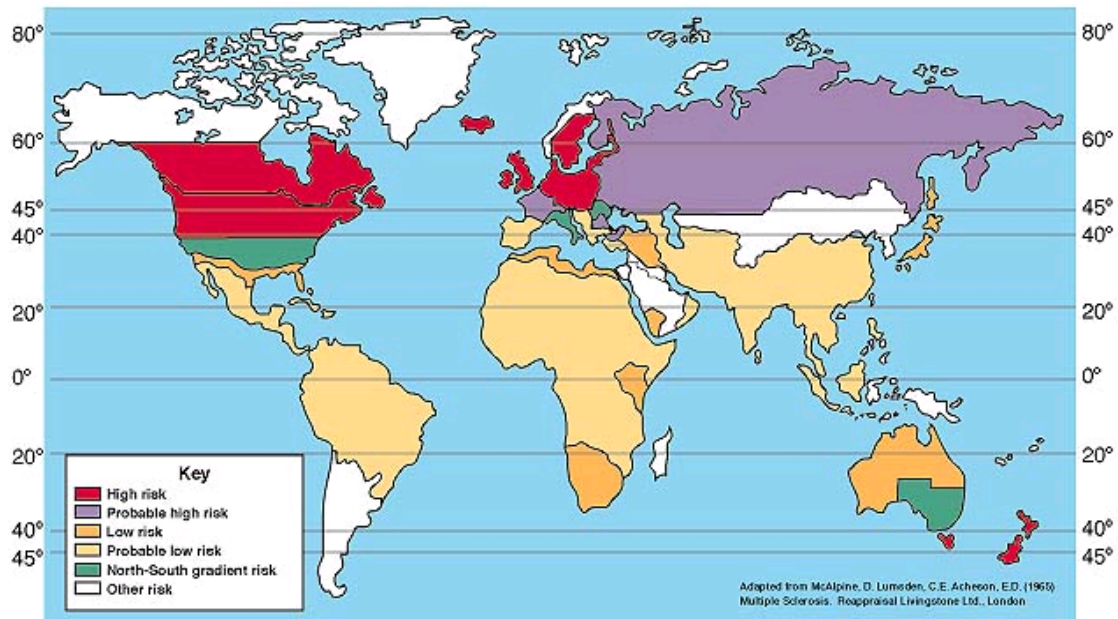
Apart from being associated with a younger age of onset [7, 8], the DR15 haplotype has not been shown to affect phenotype, and no association has been found with disease course, type or outcome.

1.1.2 Epidemiology

MS affects twice as many females as it does males, and over time this difference appears to be expanding with a disproportionate increase in incidence in women over the last hundred years [9]. The reason for female predominance in autoimmune disease is not understood. Androgens tend to be immunosuppressive [10] whereas oestradiol has been shown to enhance the secretion of γ IFN from CD4⁺ T cells *in vitro* [11], thus sex hormones may influence the susceptibility to autoreactivity. However no overwhelming evidence has been provided to support this.

Disease risk varies geographically. The comparison of prevalence studies from different areas is limited by variation in population demographics, regional medical services and survey diagnostic criteria. A geographical relation of the disease was proposed by Kurtzke who observed a variation in disease frequency with latitude and described high risk countries with frequency greater than 80/100,000 [12] (northern Europe, northern United States, southern Canada, New Zealand and southern Australia), medium risk with frequency 30-80/100,000 (southern Europe, South America and the rest of Australia) and low risk with frequency less than 30/100,000 (Africa and Asia) [13], see Figure 1.1.

Figure 1.1 World distribution of multiple sclerosis



The risk of MS is low in distinct ethnic and racial groups such as African blacks, New Zealand Maoris, Chinese, Japanese and Amerindians. Thus European colonisation may partly explain these high risk areas with white Caucasians at greater risk than ethnic and racial groups sharing the same environment but different gene pool. Sicily and Sardinia have high incidences that do not match regions of similar latitude [14] which may be due to unusually high frequency of rare HLA alleles. Thus the geographic distribution of MS may reflect the migration of genetically susceptible alleles.

However a clear relationship has been observed between increasing latitude and increasing prevalence in Australia, with prevalence increasing from 12 per 100,000 in Northern Queenstown to 76 per 100,000 in Hobart, Tasmania [15] with no substantial differences in the distribution of individual countries of origin of the migrant population. This evidence, along with immigration studies, supports an environmental role. Although immigrants from low to high risk areas retain the low risk of their country of origin [16], their children have similar risk to children born in that country [17]. Migration within the USA from high to low risk areas gave migrants an intermediate risk [18]. Such rapid changes in risk over a single generation implicate environmental factors in MS aetiology. The age of migration was important. Younger children migrating developed the high risk of the destination country

whereas older teenagers retained the low risk of the country of origin indicating that risk of disease is established in the first two decades in life [19].

There is likely to be a long latency between exposure to environmental agents and symptom onset making determination of causal factors difficult. Possible factors in the aetiology of MS include exposure to sunlight, and studies suggest an inverse correlation between sunlight exposure and disease risk [20]. More recently mortality from MS was negatively associated with exposure to sunlight [21], and greater levels of actinic skin damage were associated with decreased risk of MS [22]. Vitamin D receptors are present on activated T cells and the most potent vitamin D metabolite inhibits IL2, thus interfering with T cell expansion and potentially autoreactivity [23]. Vitamin D enters cell nuclei bound to the receptor and acts on the promoter region of certain genes via a vitamin D response element (VDRE), of which there are several providing a spectrum of binding affinities for vitamin D. A functioning VDRE has recently been identified next to the HLA DRB1 gene, and has been shown to be highly conserved in the HLA DRB1*1501 haplotype but not so among non-MS associated haplotypes. This study implies direct interactions between vitamin D (a strong candidate in determining MS risk) and HLA DRB1 (the main susceptibility locus for MS) [24].

Other putative causal agents include infections or vaccinations, however no consistent association has been found for any virus or vaccination in MS aetiology. Epstein Barr virus (EBV) has long been touted as a causative agent, and a recent meta-analysis of 14 studies confirmed it as a risk factor for MS [25]. About 90% of healthy adults have evidence of persistent EBV infection compared with nearly 100% of MS adult patients. T cells from MS patients show a stronger immune response to EBV nuclear antigen compared to seropositive controls [26]. The virus, mostly quiescent in B cells, sheds periodically and could explain the relapsing-remitting pattern of the disease. EBV contains a pentapeptide sequence homologous to myelin basic protein, providing a substrate for molecular mimicry (although this is not unique to EBV). *Chlamydia pneumoniae* [27] and human herpes virus 6 [28] are recent yet unsubstantiated candidates.

The evidence is confusing, but it is clear that MS aetiology is multifactorial with environmental factor(s) acting at a population level and genes determining relative familial risk.

1.2 Pathology

MS is a disease of myelin and axonal loss in the central nervous system. It is most conspicuous in white matter but grey matter involvement has recently become evident [29]. There is little information on the pathological changes that occur early in the disease, apart from aggressive variants of MS, or on the events preceding the destructive inflammatory infiltration.

1.2.1 White matter

The hallmark of MS pathology is the white matter plaque. Acute white matter lesions, found during the relapsing-remitting phase of the disease, tend to be centred around a venule and contain blood-borne inflammatory cells: T cells, predominantly CD8⁺, and activated macrophages (see Figure 1.2) [30]. There is evidence of breakdown of the blood brain barrier within acute lesions, believed to be the primary and obligatory event in lesion pathogenesis [31]. Inflammatory cells and mediators target myelin-maintaining, mature oligodendrocytes resulting in complete demyelination. Other cells are caught ‘in the cross-fire’ and a variable degree of axonal transection and loss is observed. Macrophages phagocytose myelin debris (appearing laden with lipid on histology) and remyelination may then follow.

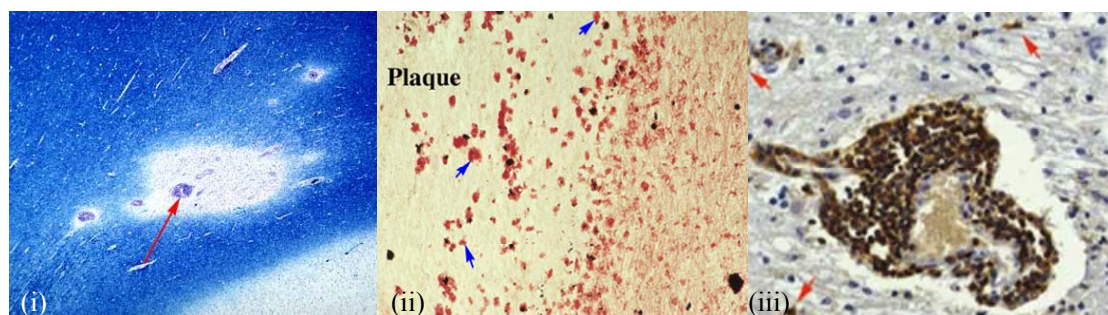


Figure 1.2 Microscopic appearance of an acute lesion, (i) centred around a venule (red arrow), (ii) with lipid laden macrophages in the lesion and at the interface (blue arrows) and (iii) numerous perivenous and parenchymal (red arrows) inflammatory cells. Images from University of California, San Francisco, MS microscopic pathology.

Remyelination is patient-dependent. Whereas in most patients it is limited to the edge of lesions, in a small percentage it occurs extensively [32]. It is also stage-dependent, occurring more frequently early in the disease [33]. Remyelination requires recruitment, differentiation and maturation of multipotent progenitor cells, which rely on intrinsic factors, intact signalling systems between axons and progenitor cells (which in turn require axonal integrity) and a suitable microenvironment. A fault in any area would prevent remyelination [33]. For example, myelin debris inhibits differentiation and maturation of oligodendrocyte progenitor cells [34], explaining the observation that remyelination is more frequent in lesions rich in macrophages [35]. Remyelinated plaques have lower myelin density than normal white matter due to their disproportionately thin sheaths, explaining their ‘shadow plaque’ appearance (see Figure 1.3).

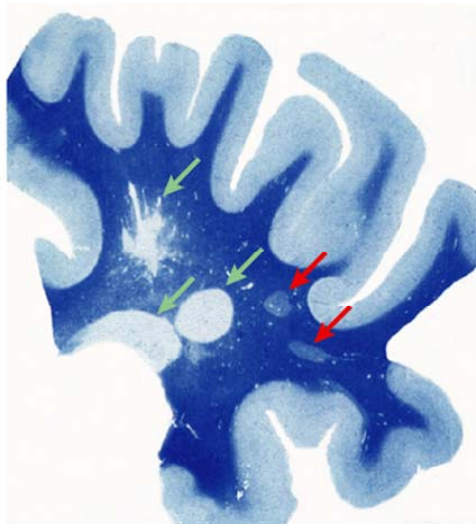


Figure 1.3 Shadow plaques. Section of brain stained with luxol fast blue showing areas of demyelination (green arrows) and areas of remyelination (red arrows) so called shadow plaques. Image from Nature Reviews Neurosciences, Multiple Sclerosis Pathology

Recent work looking at a large number of brain biopsies and autopsies from patients in the early stage of the disease concluded that there are 4 patterns of acute active demyelination:

- pattern I: T cell and macrophage dominant
- pattern II: as above but with antibody and complement (most frequent)
- pattern III: less inflammation but distal oligodendrocyte pathology and apoptosis
- pattern IV: rare and seen exclusively in primary progressive MS, again suggestive of oligodendrocyte pathology

The study concluded that MS was a pathologically heterogeneous condition, with individual patients showing only one of the 4 patterns of acute, active plaques throughout their disease course [36]. Contrary to this however, Barnett *et al* concluded that the pathology is stage-dependent and that the primary event is oligodendrocyte apoptosis with microglial activation and T cell infiltration occurring secondary to this [37], however use of steroids for treatment of the acute lesion in question may have affected the findings.

In the progressive phase, acute demyelinating lesions are rare. Most lesions are inactive, with astrocytosis and glial scars (see Figure 1.4). Some pre-existing plaques remain chronically active, with a less inflammatory infiltrate forming a ‘smouldering rim’ of T cells and activated (resident) microglia. Active demyelination in chronic active plaques is less than in acute plaques with only a few microglia containing myelin degradation products.

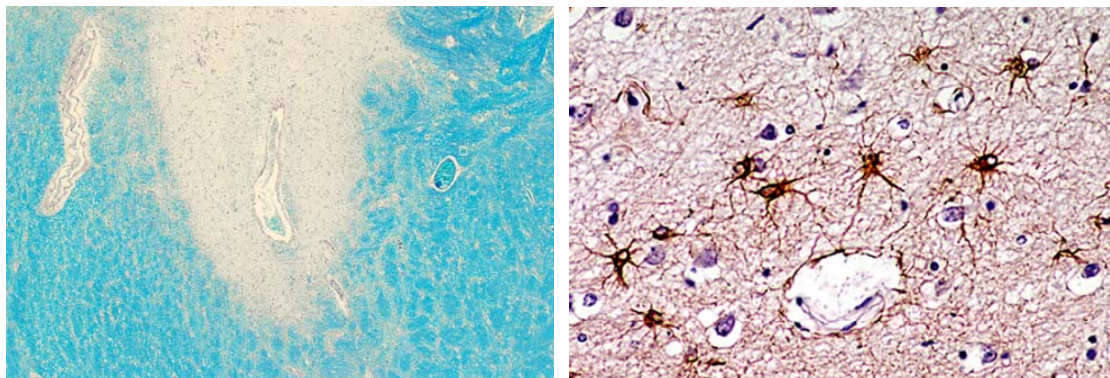


Figure 1.4 Microscopic appearance of chronic demyelinated lesions (i) with very little perivenous inflammation (ii) and astrocytes stained for GFAP (glial fibrillary acidic protein). Images from University of California, San Francisco, MS microscopic pathology.

Non lesion white matter, or normal appearing white matter (NAWM), previously thought to escape the disease process, has been shown to be highly abnormal in autopsy studies [38]. Autopsy studies reveal diffuse inflammation throughout the tissue, predominantly $CD8^+$ T cells and activated microglia, and diffuse axonal injury [39]. This pronounced inflammation is not proportional to lesion load, and processes other than Wallerian degeneration may be implicated.

1.2.2 Grey matter

Grey matter involvement, including cortex, deep grey matter and spinal cord, has become apparent with more sophisticated immunohistochemical staining techniques.

Its involvement is likely to play a role in cognitive function and disability. Kidd *et al* described 3 main types of cortical lesions [40]:

Type I: juxtacortical involving both grey and white matter (most common type)

Type II: transcortical confined to the cortex

Type III: large superficial, subpial lesions

Cortical lesions tend to be sharply demarcated with less lymphocytic infiltration and axonal damage than in white matter plaques [40-42]. Some inflammation is observed however, notably T cells, plasma cells and microglial activation, as well as transected axons and dendrites [41]. Apoptotic loss of neurons are also observed in demyelinated cortex [41]. A particular loss of synapses (by nearly 50%) has been reported and this loss of dendritic arborisation has been implicated as an important feature of MS cortical pathology [43]. Compared to white matter lesions where only about 40% of lesions show remyelination, 95% of cortical lesions appear remyelinated, and investigation of the normal appearing grey matter revealed evidence of remyelination [44], suggesting that cortical lesions are still underestimated.

Grey matter involvement was seen in 88% of chronic MS cases in one study, involving on average 16% of the cortex [44]. Cortical lesions are not restricted to the progressive phase of the disease and are also observed in early MS, with 37% of 262 early MS biopsies showing cortical demyelination [42].

1.2.3 Axonal loss

Axonal loss has only recently been acknowledged and is thought to contribute to the persistent neurological deficits acquired during the progressive phase of the disease. Axonal damage has been demonstrated in acute lesions [45], and to a lesser degree in the margin and centre of chronic active lesions [46]. Smaller diameter axons seem to be more sensitive to damage. Although axonal transection is not observed in normal appearing white matter there is substantial loss of axons in normal appearing white matter tracts. An average axonal loss of 53% in normal appearing corpus callosum [47], and reductions in axonal density of 19-42% in lateral corticospinal tracts [48, 49] was demonstrated in post mortem specimens from MS patients with various degrees of functional impairment. Axonal loss occurs early in the disease, and one

study demonstrated that axons positive for amyloid precursor protein (APP, a marker of acute axonal damage) were five times denser in acute plaques isolated from patients within a year of disease onset than those with disease duration greater than 10yrs [50]. In a post-mortem study of a patient with a 9 month history of MS, 22% axonal loss in descending tracts distal to a terminal brainstem lesion was reported, with preservation of other tracts [51]. In this and other studies, myelin staining was grossly normal since empty tubes of myelin can persist for some time following axonal degeneration, and the white matter can appear normal both pathologically and with conventional MRI.

The observation of axonal transection in active lesions points to involvement of inflammatory mediators such as proteolytic enzymes, excitotoxins, cytokines and free radicals in the destructive process. For example, nitric oxide (NO) and inducible NO synthase are found in high levels in acute lesions [52]. NO impairs mitochondrial function leading to cellular energy depletion [53]. Another mechanism of axonal injury involves the redistribution (across a longer length of axolemma rather than just at the nodes of Ranvier) and change in subtype of sodium channels on demyelinated axons, demonstrated in acute lesions [54]. The diffuse distribution of channels can still support action potential conduction but generates greater energy requirements and causes considerably more sodium influx per impulse than in the myelinated state. Such persistent sodium influx may then drive reverse $\text{Na}^+/\text{Ca}^{2+}$ exchange culminating in the accumulation of calcium and cell death.

As axonal loss is observed in chronic lesions and in patients on immune suppression with cessation of clinical and radiological relapses [55], mechanisms independent of inflammation may be responsible for ongoing axonal loss. Animal studies have shown that oligodendrocytes and some myelin-associated proteins contribute to long-term axonal viability thus demyelination may compromise axons [56, 57]. Axonal death may lead to degeneration of proximal and distal neurons, with the loss of pre- and post-synaptic trophic signals. Mitochondrial DNA damage acquired in the acute inflammatory phase [58] may lead to their production of reactive oxygen species [59] creating a cycle of decline in energy metabolism resulting in axonal degeneration delayed from the acute inflammatory event.

Thus axonal loss occurs early in the disease course and accumulates with disease progression. To overcome axonal loss in the early phase of the disease there is evidence of compensatory cortical adaptation with reorganisation of functional pathways [60]. Permanent disability of the progressive phase may develop when a threshold of axonal loss is reached and the CNS compensatory resources are exhausted.

1.3 Clinical course and diagnosis

1.3.1 Presentation

Eighty five percent of MS patients present with an acute or subacute episode of neurological disturbance characteristic of demyelination, known as a clinically isolated syndrome (CIS). This completely or partially resolves, and is followed months or years later by a second episode, and a diagnosis of relapsing remitting (RR) MS. In the remaining 15%, rather than recovering from the first presentation, the clinical picture is one of slow progression, so called primary progressive (PP)MS. PPMS usually presents as a progressive spinal cord syndrome, but progressive cerebellar syndromes, visual loss or dementia are occasionally seen.

In some patients presenting with a CIS, it will remain a monophasic illness, but 50 to 68% convert to CDMS by 20 years [61-63].

Peak age of symptom onset in MS is 30 years, typically between 15 and 50. Onset after 60 is unusual. Patients with PPMS tend to be older. Twice as many females are affected than males, with a higher ratio of males in PPMS. Lesions occur anywhere in the CNS, but certain 'clinically eloquent' locations, such as the optic nerve, brainstem and spinal cord, give rise to symptoms. The most frequent monofocal presentation is with long-tract symptoms and signs (46%), followed by optic neuritis (21%) and brainstem syndrome (10%) such as internuclear ophthalmoplegia. Twenty three percent present with multifocal abnormalities [64].

PPMS and RRMS differ in their age of onset, gender and clinical course. MRI findings, which will be discussed later, are also remarkably different, with PPMS

patients having significantly fewer lesions. This thesis will concentrate on CIS patients.

1.3.2 Clinical course

In the early phase of RRMS, relapses are followed by remission, with complete or partial recovery of symptoms. Common relapses include sensory symptoms, limb weakness, sphincter dysfunction and balance disturbance, with symptoms resulting from conduction delay along demyelinated axons. Relapse frequency is difficult to estimate as sometimes it can be difficult to distinguish relapses from fluctuations in existing disability. For example, heat further slows conduction, exacerbating symptoms (Uthoff's phenomenon). Also relapse frequency appears to decrease during the clinical course [65]. Hence studies report huge variability in relapse frequency from 0.1 to >1 per year [65] but about 0.5 relapses per year is felt to be a reasonable estimate in a standard RRMS population.

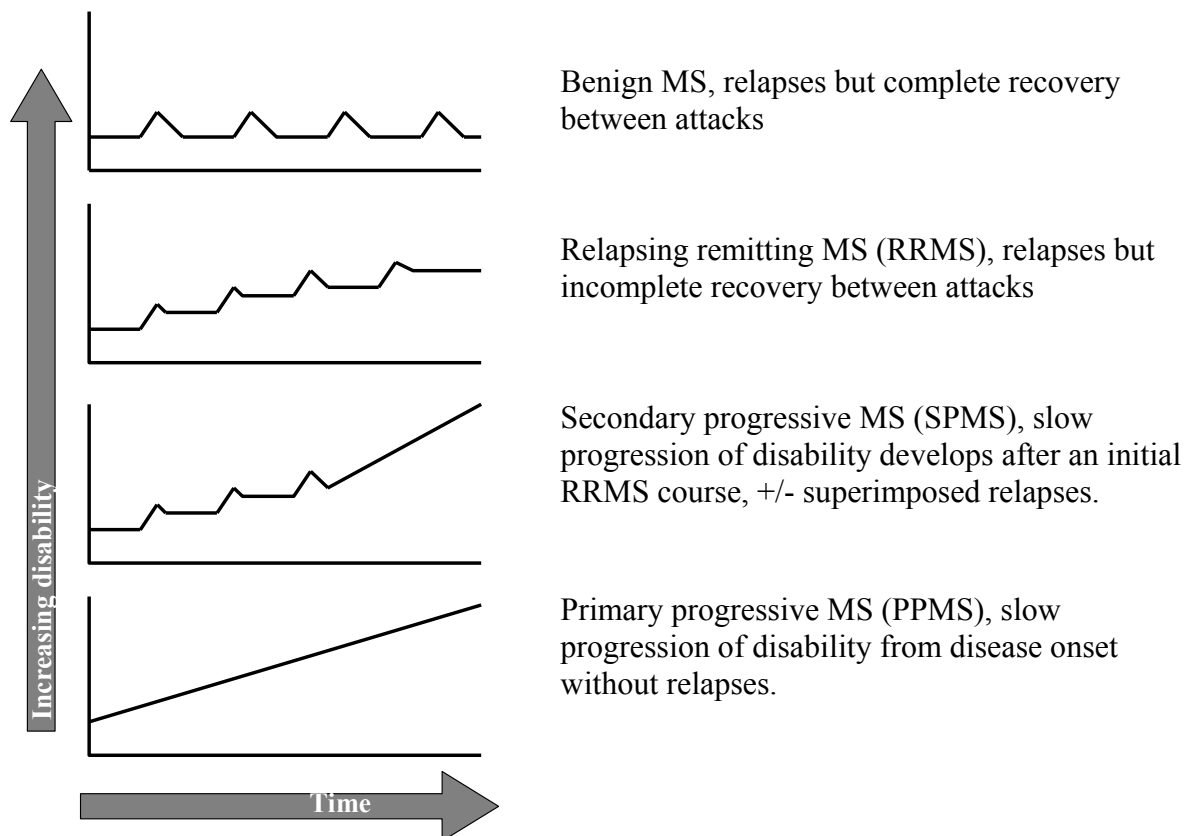
Another phenomenon of demyelination is hyperexcitability with spontaneous discharge giving rise to brief, frequent, paroxysmal, stereotypical symptoms often triggered by movement such as Lhermitte's phenomenon (electric shock sensation radiating down the spine or limbs on neck flexion), trigeminal neuralgia, tonic spasms and phosphenes (flashes of light visualized on eye movement). Throughout the clinical course, patients often complain of fatigue which compounds their physical disability.

Approximately two thirds of RRMS patients develop a secondary progressive course within 25 years of symptom onset [66] and fifty percent of patients with RRMS are unable to walk unaided 15 years after disease onset [65]. During the progressive stage, exercise tolerance slowly declines, and increasing support is required from one to 2 sticks (EDSS 6), a wheelchair (EDSS 7), and assistance with transferring. The progressive phase may be exacerbated by relapses, leading to a sharp decline in function with partial recovery. The pyramidal pattern of weakness in secondary progression is usually accompanied by spasticity, spasms and clonus which can be painful and disturb sleep. Gait disturbance may be caused or exacerbated by cerebellar dysfunction. Upper limb weakness is much less common than paraparesis,

but function may become limited by ataxia. Sphincter dysfunction often occurs in parallel with spastic paraparesis, and symptoms typically include urgency, frequency, incontinence, erectile dysfunction, and constipation. Incomplete voiding may lead to recurrent urinary tract infections which can exacerbate symptoms. Although frank dementia is rare in MS, patients often complain of poor memory and attention. Sensory symptoms are also common, ranging from numbness, tightness, paraesthesia, and can be very troublesome.

On average the life expectancy of people with MS is lower than that of the general population, by about 10 years, as disability predisposes patients to bladder and chest infections [65].

Figure 1.5 Graphical description of the MS subtypes



1.3.3 MS variants

Other variants of MS have been described. Balo's concentric sclerosis is a rare variant which is particularly prominent in the Philippines. The essential features are large plaques consisting of concentric rings of demyelination alternating with

myelinated white matter [67]. It is a histopathology diagnosis, but similar appearance can be seen on MRI (see Figure 1.3).

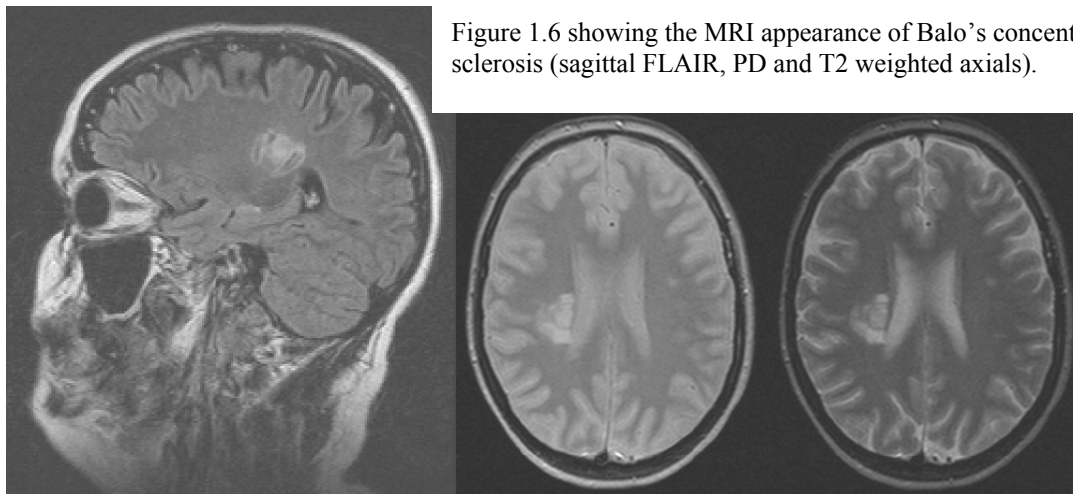


Figure 1.6 showing the MRI appearance of Baló's concentric sclerosis (sagittal FLAIR, PD and T2 weighted axials).

Marburg variant is an aggressive form of MS, with rapid progression and widespread inflammatory lesions, typically of the same age, in the characteristic locations for demyelination; optic nerves, spinal cord, periventricular white matter and brainstem. Marburg variant usually leads to death within months or a very few years from onset due to the severe neurological debilitation. The lesions are generally destructive with tissue necrosis [68]. Inflammatory demyelination can also affect the peripheral nerves in this condition.

Neuromyelitis optica (NMO), or Devic's syndrome, is a variant with optic neuritis and/or myelitis, but no signs or lesions beyond the cord or optic nerves [69]. It is a relapsing disorder in the majority of patients [70], with early residual disability. MS limited to the optic nerves and spinal cord (optico-spinal MS) can be differentiated from NMO by disease severity, MRI appearances, and cerebrospinal fluid (CSF) findings. In NMO the ON is severe with poor visual recovery, and the MRI appearance of transverse myelitis show extensive, sometimes necrotic, spinal cord lesions extending across several vertebrae. CSF may have a pleocytosis (including polymorphonuclear cells not seen in MS) but oligoclonal bands are usually absent. Brain MRI at presentation is often normal or may show changes uncharacteristic of MS, such as hyperintense signal in periependymal regions including the

hypothalamus and periaqueductal brainstem. Ninety percent of NMO patients are female, compared to 70% of MS patients. There is a strong association with autoimmune diseases such as thyroiditis, lupus and Sjögrens syndrome; ANA is positive in 50% of NMO patients [69]. Recently an NMO IgG antibody has been discovered directed against aquaporin 4, but its pathogenicity and the mechanism of demyelination remain uncertain. Aquaporin 4 is not an oligodendrocyte protein, but is found on astrocytes, and is highly expressed in NMO sites. Anti-aquaporin 4 is present in 9% of optico-spinal MS patients, and 73% of NMO patients. CIS patients presenting with idiopathic transverse myelitis and positive anti-aquaporin 4 antibody (present in 38%) are at high risk of recurrence of transverse myelitis or ON. Twenty percent of patients with recurrent ON are anti-aquaporin 4 positive, and their visual outcome is significantly worse than the patients negative for the antibody [69]. NMO diagnostic criteria require ON, acute myelitis and 2 out of the following 3 characteristics: disease onset brain MRI that is not diagnostic for MS, contiguous spinal cord MRI lesion extending over 3 or more vertebral segments, and NMO-IgG seropositive status [71].

1.4 Prognostic markers in CIS/early MS

1.4.1 Conversion to CDMS

Therapeutic studies have given reliable indication of the rate of conversion to CDMS in the 2 years after CIS onset in patients considered high risk, i.e. with lesions on baseline MRI. There have been two therapeutic studies; Early Treatment of Multiple Sclerosis Study (ETOMS) [72], and Controlled High Risk Avonex Multiple Sclerosis Prevention Study (CHAMPS) [73]. The MRI inclusion criteria differed between the two studies. Whereas CHAMPS required two or more clinically silent lesions at least 3mm (at least one lesion must be periventricular or ovoid), ETOMS required more active disease with at least four T2 lesions or three T2 lesions plus one gadolinium-enhancing lesion. By 2 years, 38% and 45% of the placebo arms converted to CDMS in CHAMPS, and ETOMS respectively.

Natural history studies provide long-term data on rates of conversion and prognostic factors in patients with both normal and abnormal MRI at presentation. The Optic Neuritis Treatment Trial [74] followed up patients 10 or more years from onset. The risk of MS was similar in the 3 original treatment groups (intravenous or oral steroids

or placebo); 38% at 10 years and 40% at 12 years, with the median time to diagnosis being 3 years. The 15 year follow-up data has recently been published, and at this point 50% of patients had converted to CDMS [63]. The risk of converting to CDMS decreases with time, but never goes completely. Whereas between baseline and 5 years, 29% of the CIS patients converted, 17% of the remaining CIS patients converted over the next 5 years, and 14% between 10 and 15 years [63]. An Italian study of 10 year follow-up of isolated optic neuritis patients reported a similar result to the 10 year ONTT data, with 42% risk of MS [75].

Follow-up of a mixed CIS cohort (presenting with optic neuritis, brainstem or spinal cord syndromes), found a higher conversion rate compared to the ONTT, with 59% developing CDMS at 10 years and 63% at 20 years [61, 62].

Whether different CIS presentations – ON, brainstem, spinal cord, multifocal – confer different risk of conversion to CDMS has not been established. Eriksson and colleagues found that patients with efferent lesions had twice the risk of a later diagnosis of MS than patients with afferent lesions (optic neuritis or purely sensory symptoms) [76] which could explain the higher rate of conversion in the mixed CIS cohort than the optic neuritis cohorts. Tintore *et al* found that patients presenting with optic neuritis carried a lower risk of conversion to MS, but if only patients with abnormal MRI were compared the risk was not significantly different between the CIS presentations [77]. However in Brex's study, there was no significant difference in risk between the different CIS presentations, irrespective of MRI abnormalities [61]. The discrepancy may be due to the difference in proportion of ON patients presenting with abnormalities on MRI with 77% in England and 51% in Spain.

Therefore there is some evidence to suggest that the type of CIS confers risk of further neurological events with optic neuritis having a lower risk of disease progression.

1.4.2 Clinical factors related to disease course

There is a high degree of variability in the final outcome between MS patients, and some patients do not appear to develop significant disability. The proportion of MS patients with a benign course varies between studies. The ONTT reported two thirds

of the patients who had converted to CDMS had no significant disability at 10 and 15 years [63, 78] but the respective figure was 39% at 20 years in the mixed CIS cohort [62]. Thirteen year follow-up of early MS patients found only 5% were defined as benign MS [79]. Some patients develop progressive, irreversible decline in mobility, from limitation in ambulation (EDSS 4) to requirement of a unilateral aid for walking (EDSS 6), to becoming wheelchair bound (EDSS 7) over a period of 8, 20 and 30 years respectively in one natural history study [64]. Thirty one to 50% of RRMS patients are unable to walk unaided 15 years from their first clinical event [61, 65, 79].

Development of a progressive course is the most important factor associated with long-term outcome [79, 80]. Approximately two thirds of RRMS patients develop a secondary progressive course within 25 years of symptom onset [66]. The median time from onset of CIS to secondary progression was 19 years and time from clinically definite/probable MS to secondary progression 12 years [76].

At presentation, several studies report CIS topography and degree of recovery as independent risk factors for secondary progression and poor outcome. By 25 years, patients presenting with an efferent lesion have twice the risk of secondary progression and nearly 3 times the risk of being wheelchair-bound than those presenting with purely sensory symptoms or optic neuritis [76]. Concurring with this, pyramidal, sphincter [79, 81], cerebellar [82, 83] and polysymptomatic presentations [66, 80] have been associated with poor outcome compared to ON or sensory presentations [65, 66, 81, 84, 85]. Complete recovery from the initial attack is also important prognostically [66, 76, 84]. Several studies support early age of onset to be associated with better prognosis [65, 81, 84, 85] and patients over 30 at CIS onset have a 1.7 fold increased risk of secondary progression 25 years from diagnosis than younger patients [76, 79]. Gender is consistently linked with prognosis, with females having better prognosis than males [65, 84, 85]. Family history of MS [82] has also been associated with unfavourable outcome.

In early disease, high relapse frequency in the first 5 years [65, 76, 81] and short first remission [76, 81] have been shown to be predictive of secondary progression and disability. By 25 years, patients having 5 or more attacks in the first 5 years have

twice the risk of secondary progression and nearly 3 times the risk of being wheelchair bound than patients with lower relapse frequency [76]. In agreement, a study compared patients with only 1 attack in the first 2 years with those who experienced 5 or more, and found the first group had significantly slower disease progression than the second, taking 20 years to require a walking aid compared to 7 years [80]. Incomplete remission from early relapses nearly treble the risk of secondary progression and need for a wheelchair at 25 years [76].

None of the early predictors for progression were able to predict the rate of subsequent progression [76]. Only late predictors, such as a shorter time from MS onset to secondary progression (1 to 10 years) and higher number of functional systems involved at onset of progression predicted a faster progression rate. Although time to EDSS 4 is variable among patients, the time course between EDSS 4 and 6 is similar [84], and rate of progression is similar in SPMS and PPMS if time from progression onset rather than disease onset is used as the baseline [64, 80].

Table 1.1 summarising prognostic factors at various stages of disease

Factors predicting conversion to CDMS	Factors at CIS onset predicting secondary progression	Factors in early years predicting secondary progression	Factors predicting rate of progression	Factors predicting disability other than secondary progression
Purely afferent symptoms low risk	Age > 30 [76, 79]	High relapse frequency [76, 80]	Shorter interval between MS onset and progression [76]	CIS due to efferent lesion [76, 79, 82] or polysymptomatic onset [80]
Efferent symptoms high risk	CIS due to efferent lesion	Incomplete remission [76]	Higher number of functional systems involved at onset of progression [76]	Gender (males poorer prognosis)
		Cognitive symptoms in the 5 th disease year [76]		Shorter relapse free interval between first 2 attacks [80]
				Short interval from onset to secondary progression [79] [80]
				Higher EDSS at inclusion [79]
				Family history of MS [82]

1.5 Cognitive impairment

Cognition, including memory, attention and language, relies on distributed interconnected neural networks [86]. The multiple, subcortical connections and pathways of these networks are vulnerable to damage by multifocal lesions or diffuse axonal pathology. Cortical involvement, which has recently become evident, is also likely to adversely affect cognitive function. Various cognitive deficits mainly affecting memory, attention and executive functions are seen in up to 43-60% [87, 88] of MS patients depending on the sample and sensitivity and specificity of neuropsychometric battery used. Language and intellectual functions are generally preserved until late in the disease. Subtle cognitive changes such as deficits in attention, generalised slowing in reaction times and information processing may be detectable at initial presentation in CIS patients [89-93].

In patients converting to CDMS, memory deficits can develop [87, 89, 94], and during the relapsing remitting phase can progress [95], but become more severe when the disease enters the progressive phase [89].

Prediction of cognitive impairment from early clinical factors has not been consistently demonstrated. Neither cross sectional nor longitudinal studies have shown a correlation between performance or decline in cognitive testing and demographic or clinical variables such as disease duration, disability level or relapse rate [93, 95, 96].

1.6 Diagnosis

A diagnosis of MS requires objective confirmation of 2 attacks (symptoms of neurological dysfunction characteristic of demyelination, demonstrated on examination, lasting more than 24 hours) separated by time (at least 1 month) and place. Paraclinical investigations show characteristic features of demyelination which can support the diagnosis, such as oligoclonal bands in cerebrospinal fluid, and delayed conduction on evoked potentials.

1.6.1 Cerebrospinal fluid

Two or more oligoclonal IgG bands (OCB) detected by separation of cerebrospinal fluid (CSF) proteins while not demonstrable in the corresponding serum, indicate a

local B cell response accompanying CSF inflammation. With optimised, standardised methodology, OCB are demonstrated in 90-95% of CDMS patients and 46-61% of CIS patients, but may be present in other disorders [97, 98]. Once present, OCB persist in the MS patient irrespective of disease course or treatment. The antigenic target and the relationship to aetiology and pathogenesis remain to be established. OCB in the CSF are highly sensitive (up to 100%) for the development of CDMS with an equal negative predictive value, and are useful in supporting a diagnosis of MS [99]. However they are not specific (42% in one study) being present in other inflammatory CNS disorders. They have been incorporated into MRI diagnostic criteria, reducing the number of lesions required for dissemination in space. Studies evaluating the MRI criteria with and without OCB have produced conflicting results [100-102]. They may be useful with regards to prognosis in patients with normal MRI. Only 4% of patients with normal MRI and negative OCB convert to CDMS compared to 23% with positive OCB [102] and the hazard ratio of conversion to CDMS with positive OCB is 1.7, adjusting for brain lesions on MRI [103]. No association with disability was found in this study.

1.6.2 Evoked potentials

Brainstem auditory evoked potentials (BAEPs), short latency somatosensory evoked potentials (SSEPs) and pattern reversal visual evoked potentials (VEPs) can confirm the presence of lesions in patients with suspected clinical involvement and document the presence of clinically unsuspected lesions, providing evidence for dissemination in space. Although they provide limited information on the number, location or activity of lesions, they are sensitive to early subclinical damage, give functional information and prolonged latency is felt to be relatively specific for demyelination. BAEP or VEP detect subclinical lesions in about 50% of MS patients and when combined in 71%, whereas SSEPs although frequently abnormal, show only a few silent lesions [104]. VEPs are very sensitive in patients with a history of optic neuritis, especially if there are compatible clinical signs. A review of evoked potentials in patients with CIS, possible or probable MS concluded that only VEPs were clinically useful, with sensitivities ranging from 25-83% and abnormality associated with a risk of CDMS 3 to 9 times that of patients with normal VEPs [105]. The association between CDMS and BAEPs or SSEPs was weak or inconclusive. Therefore, evoked potentials give valuable information in the majority of patients with uncertain

diagnosis and may be useful in patients in whom MRI is negative, equivocal or cannot be performed.

1.6.3 Diagnostic criteria

Diagnostic criteria evolved incorporating these paraclinical investigations [106] and in 1983 the Poser criteria were published. Thus without clinical evidence of 2 attacks, a diagnosis could be made if there was paraclinical evidence of dissemination in space (DIS), see Table 1.2.

Table 1.2 summarising the Poser criteria for diagnosis of MS [106]. ‘Attack’ defined as symptoms of neurological dysfunction characteristic of demyelination, demonstrated on examination, lasting more than 24 hours. * Paraclinical evidence of DIS includes neuroimaging (at this time mainly CT), evoked potentials and expert urological assessment. Laboratory support applies to cerebrospinal fluid examination for oligoclonal bands

	No. of attacks	Clinical evidence of :		Paraclinical evidence of Dissemination in space*	Oligoclonal bands
Clinically definite MS	2	DIS			
	2	only 1 lesion	AND	Yes	
Laboratory supported definite MS	2	only 1 lesion	OR	Yes	+ve
	1	DIS			+ve
	1	only 1 lesion	AND	Yes	+ve
Clinically probable MS	2	only 1 lesion			
	1	DIS			
	1	only 1 lesion	AND	Yes	
Lab supported probable MS	2	only 1 lesion			+ve

1.7 Differential diagnosis

Other conditions can give rise to syndromes, such as optic neuropathy, myelitis and cerebellar ataxia, which may be relapsing-remitting in nature and have MRI and CSF findings similar to MS.

1.7.1 Inflammatory causes

Acute disseminated encephalomyelitis (ADEM) is usually a monofocal, post-infectious illness in children. Headache, drowsiness, fever and seizures usually accompany focal neurology (motor, sensory, visual, ataxia, polysymptomatic) and some degree of encephalopathy is required for diagnosis (from irritability or behavioural change to coma). CSF transiently shows OCB, and grey and white matter lesions often resolve and new lesions at 3 months are rare.

Connective tissue diseases, such as lupus, Sjögrens syndrome, and antiphospholipid syndrome can give rise to optic neuropathy and transverse myelitis. They are usually distinguishable from MS by systemic symptoms characteristic of the underlying condition and a raised ESR and autoantibodies can help with the diagnosis. Behçet's disease, usually distinguishable from MS due to a history of orogenital ulceration, rarely resembles MS and can be a cause of spinal cord or brainstem syndrome.

Primary CNS vasculitis can cause fluctuating symptoms which may involve the optic nerve, brainstem or spinal cord, but headache, reduced level of consciousness and seizures and stroke-like episodes are more characteristic. CSF and angiograms can sometimes aid diagnosis, but a diagnostic biopsy is required to enable aggressive treatment.

1.7.2 Infiltrative causes

Lymphoma can present with subacute and progressive hemiparesis, hemianopia, seizures, reduced level of consciousness and myelopathy. The response to steroids can lead to suspicion of MS, but symptoms recur and progress. A biopsy is required for definitive diagnosis. Sarcoidosis can cause a progressive myelopathy and steroid-dependent optic neuritis. MRI may show meningeal enhancement, but a definitive diagnosis requires a Gallium scan and / or biopsy.

1.7.3 Infective and other causes

Infections (chronic borreliosis, syphilis, HIV, JC virus causing progressive multifocal leukoencephalopathy, chronic measles and Whipples), can cause evolving central neurological symptoms, and CSF and serum serology is required to exclude these as the cause. Other non-inflammatory causes include cerebral autosomal dominant arteriopathy with subcortical infarcts and leukoencephalopathy (CADASIL) where a family history of stroke is important, and mitochondrial disease.

1.8 Current management strategies

Currently no study has shown disease modification in progressive, non-inflammatory MS, therefore disease modifying therapy (DMT) is limited to those patients

experiencing relapses or acute deterioration with evidence of inflammatory activity on MRI.

Beta Interferons have many immunomodulatory actions, such as modulation of plasma cell IgG synthesis, inhibition of leukocyte proliferation, reduction in antigen presentation in microglia and reduction of T cell migration into the brain. Glatimer acetate (Copaxone) also modulates the immune system, by interfering with antigen presentation and inducing suppressor cells. Both classes reduce relapse rate by about 30% and reduce MRI signs of disease activity in 30-80% and disability progression by 30% [107]. Three studies of early treatment of CIS patients (CHAMPS [73], ETOMS [72] and BENEFIT [108]) showed Interferon delays conversion to CDMS in CIS.

Table 1.3 summarising results from Interferon (IFN) treatment trials

Trial	Treatment	F/U duration	Treatment arm conversion rate	Placebo arm conversion rate	HR
CHAMPS	30µg IFN/wk	3 yrs	35%	50%	0.56
ETOMS	22µg IFN/wk	2 yrs	34%	45%	0.69
BENEFIT	250µg IFN alt days	2 yrs then open label 3yrs	34% in immediate group	48% in delayed Rx group	0.5

The long-term benefit of these therapies is not known, and the National Institute of Clinical Excellence (NICE) concluded that such DMTs were clinically effective but not cost effective and for this reason the Risk Sharing Scheme was agreed between pharmaceutical industry and the NHS, with cost of therapy adjusted on a sliding scale if patient outcomes differ from the agreed targets on annual monitoring. Currently, mobile patients who have 2 relapses in 2 years are eligible for DMT, but recent recommendations by the Association of British Neurologists stated that patients with a single disabling relapse in the last year or a diagnosis of McDonald MS within a year of CIS onset should also be eligible for DMT.

Mitoxantrone is an anti-neoplastic agent that inhibits both DNA and RNA synthesis, suppressing B and T cell immunity. Results from clinical trials have demonstrated beneficial effect of treatment in RRMS or SPMS whilst in the inflammatory phase, reducing relapse rate, MRI activity and slowing down progression [109, 110].

Toxicity of mitoxantrone limits its use, and this includes dose dependent cardiac toxicity (limiting cumulative dose to less than 140mg/m²), risk of acute leukaemia (about 1:400) and secondary amenorrhoea. It is restricted to patients with rapidly advancing disease (such as decline in EDSS by ≥ 2 point over a year) unresponsive to other therapies and with demonstrable persistent inflammatory activity with normal cardiac function.

Several monoclonal antibodies strategically targeting the immune system have been developed and have either completed or are mid-trial. These include Natalizumab, Alemtuzumab and Rituximab. Natalizumab (Tysabri), a humanised monoclonal antibody that blocks the adhesion molecule $\alpha 4\beta 1$ integrin and impedes transendothelial migration of lymphocytes, reducing lymphocyte entry into and surveillance of the CNS. Phase III studies showed reduction in relapse rate by two thirds and lesion accumulation by 83% [111, 112]. Two patients in the SENTINEL trial [112] (Natalizumab plus Interferon) developed progressive multifocal leucoencephalopathy, one of whom died. After exhaustive review of all patients exposed to Natalizumab, clinical trials were allowed to resume. It is currently recommended by NICE as monotherapy for rapidly evolving, severe RRMS (2 severe disabling relapses in a year with evidence of increasing lesion load/activity on MRI). However in August 2008 two further cases were reported in patients on Natalizumab monotherapy for more than a year, and the American Academy of Neurology recommends Natalizumab is reserved for patients who have failed other therapy (either with continued disease activity or medication intolerance) or who have particularly aggressive initial disease course. How this will affect NICE guidelines and its use in MS in the UK remains to be seen. As there is currently no screening method to identify patients at risk of PML, surveillance remains clinical vigilance.

Alemtuzumab (Campath-H1), or anti-CD52 (present on T cells and monocytes) causes T cell depletion. In a phase II randomised controlled trial (CAMMS223), applied in up to 3 cycles in two years, Campath was shown to have superior efficacy in comparison to Interferon at 24 and 36 months. Cumulative number of relapses was reduced by 72-87% and the risk of sustained disability by 66-88% over a period of 24 months. The 3 year follow-up data was presented at the American Academy of Neurology in 2008 and similar reductions in relapse rate and disability compared to

Interferon were found at this stage. More excitingly, Alemtuzumab resulted in an improvement in EDSS, with a reduction of 0.39 in EDSS from baseline, and a reduction of 0.77 in comparison to the Interferon group. Serious adverse events included idiopathic thrombocytopenic purpura in 6 patients (1 patient died of intracranial haemorrhage), hyperthyroidism and listeria meningitis in a patient who ate unpasteurised cheese.

Rituximab (anti-CD20) present on all B cells from pre-B cells to memory cells but not plasma cells, has been tested in a Phase II trial in RRMS patients. Results at 48 weeks following 2 infusions found, compared to placebo, 91% relative reduction in gadolinium enhancing lesions and 58% relative reduction in the proportion of patients experiencing relapses during follow-up.

Oral agents are also in development, some of which are in the midst of large phase III trials. Potency of such agents varies, and many are being developed as supplemental therapy, whereas others are potentially as potent as monoclonal antibodies, targeting traffic of lymphocytes into the CNS.

1.9 Measurements of disability

Objective measures of disability are important for monitoring disease, particularly in clinical trials. The Disability Status Scale (DSS) was proposed by Kurtzke in 1955, which graded disability in steps from normal function to death as an ordinal scale, with 1-unit increments [113]. Later the DSS was expanded (EDSS) to include functional systems, to complement the DSS, which was weighted towards ambulation status [114]. The functional scores were supposed to pick up impairment in visual, cerebellar, brainstem, sensory, sphincter and mental functions. However EDSS above 4 remain weighted to ambulation status, and impairment in other functional systems is only reflected in the lower EDSS range. Therefore the sensitivity of the EDSS for reflecting change in neurological impairment is greatly reduced above EDSS 4, which limits its capacity to detect treatment effects in clinical trials. The insensitivity of the EDSS to visual and cognitive function has also been criticised. The EDSS ratings also

range from 0-10, but with 0.5 unit increments, and remain an ordinal scale for which the distances between scores are not equal.

Other scales have been proposed to try to overcome the shortfalls of the EDSS. The MS functional composite (MSFC) is often used in conjunction with the EDSS as it measures manual dexterity (timed 9 hole peg test), attention and working memory (3 second paced auditory serial addition test or PASAT) and mobility (timed 25 foot walk) [115]. Low Contrast Sloan Letter Charts may be added in clinical trials to better capture visual dysfunction. Z scores from the 3 or 4 components are acquired, and averaged for the overall score, yielding a continuous scale that is sensitive to small change in function. In an able population however it may be limited by ceiling effects.

Table 1.4 summarising EDSS

EDSS	Mobility	Criteria
1	unimpaired	minimal (min) signs in 1 functional system (FS)
1.5		min signs in >1 FS
2		min disability in 1FS
2.5		min disability in >1FS OR mild disability in 1 FS
3		moderate (mod) disability in 1FS OR mild disability in 3-4 FS
3.5		mod disability in 1FS AND more than minimal disability in several others
4	restricted	able to walk without rest or aid for >500m
4.5		able to walk without rest or aid for 300m
5		able to walk without rest or aid for 200m
5.5		able to walk without rest or aid for 100m
6	aids required	able to walk without rest with 1 cane 100m
6.5		able to walk without rest with 2 canes 100m
7	wheelchair	max 5m with 2 aids, transfers alone, wheels self
7.5		few steps only, +/- help transferring/wheeling
8		restricted to bed, effective use of arms
9	bedbound	confined to bed, can communicate / eat
9.5		unable to communicate/swallow

Chapter 2

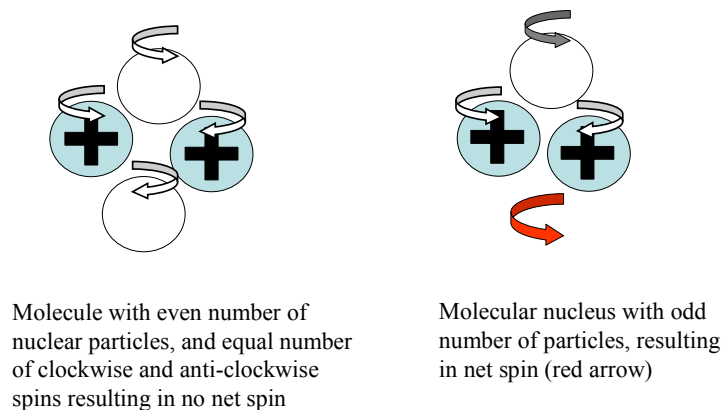
MRI physics

2.1 MRI principles

MRI is a non-invasive technique that can generate high resolution images exploiting the electromagnetic properties of spinning protons (positively charged particles). A full description of MRI physics requires an understanding of quantum mechanics but for the purposes of this thesis a simple model is used.

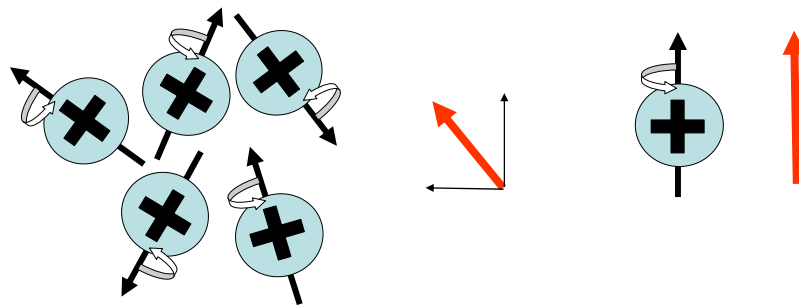
A moving electrical charge, such as a spinning proton, induces a magnetic field, in accordance with Faraday's Law of electromagnetic induction. Atomic nuclei are made up of protons and neutrons (non-charged particles), each spinning about their own axes, either clockwise or anti-clockwise. Nuclei with equal numbers of protons and neutrons have no net spin as the spins cancel each other out. Only atoms with different numbers of the nuclear particles have net spin (see Figure 2.1).

Figure 2.1 Net spin



As protons are positively charged, by spinning they induce magnetic fields around themselves and behave like magnetic dipoles. Magnetic fields have magnitude and direction, denoted by a magnetic moment and the total magnetic moment of the nucleus is the vector sum of the magnetic moments of all the nuclear protons (see Figure 2.2).

Figure 2.2 Magnetic moment



The total magnetic moment of the nucleus (red arrow) is the vector sum of the individual magnetic moments of all the protons in the nucleus (neutrons not shown). Due to differences in alignment, the total magnetic moment is small.

The magnetic moment (red arrow) of a single proton, as in a Hydrogen atom, is relatively large.

Due to the abundance of hydrogen atoms in the body (in the form of water, protein, fats etc), and because the hydrogen atom is a solitary proton giving a large magnetic moment, it is the proton that is imaged in MRI. However, for reasons discussed later, most imaging methods image only those protons in water. MRI works by generating a strong magnetic field which causes the protons to align in an equilibrium position. Energy is briefly put into the system, and absorbed by the protons (also known as spins) altering their energy state. On cessation of energy input, the spins return to the equilibrium position, releasing energy in the process.

2.2 Equilibrium

Exposing spinning protons to an external magnetic field causes the nuclear spins to align (either parallel or anti-parallel) with B_0 . Proportionally more protons align parallel to B_0 than anti-parallel, as this is a lower energy state. At room temperature and 1.5 Tesla, the excess is only 11 in 1 million, but due to the numbers of hydrogen atoms in tissue, this difference is sufficient to produce a measurable net longitudinal magnetisation in the direction of B_0 (see Figure 2.3).

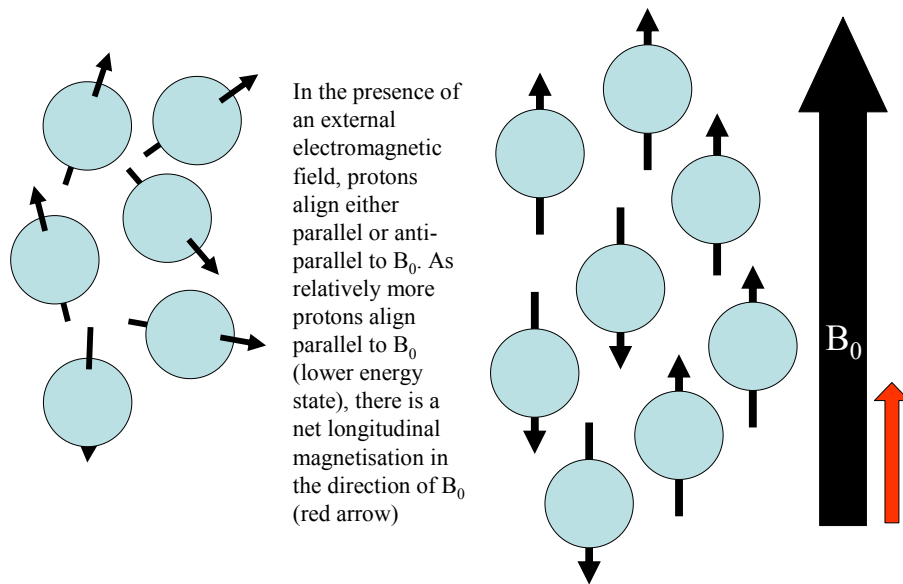


Figure 2.3 Equilibrium: net longitudinal magnetisation

In the presence of an external magnetic field (B_0) protons do not spin perfectly about their axis, but wobble, with gyration of the spinning axis about the axis of the external field B_0 (precession). The precession frequency, also known as the Larmor frequency, is dependent on the gyromagnetic ratio (an intrinsic property of the spin of interest, $\sim 42.6\text{MHz/T}$ for protons in hydrogen atoms), and the strength of the external magnetic field B_0 . Precession generates a rotating transverse magnetic vector in individual spins (see Figure 2.4).

Precession (Larmor) frequency = gyromagnetic ratio \times strength of B_0 ($\nu = \gamma B_0 / 2\pi$).

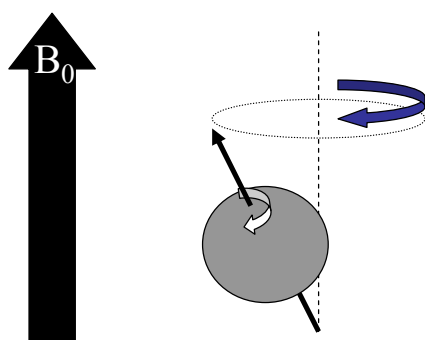


Figure 2.4 Precession
The spinning proton gyrates around the spinning axis (B_0) and generates a rotating transverse magnetic vector (blue arrow)

The protons are aligned with the applied magnetic field, however there is no favoured phase for the precession around B_0 , which means that the protons are evenly spread

out around the magnetic field so there is no net transverse magnetisation (see Figure 2.5).

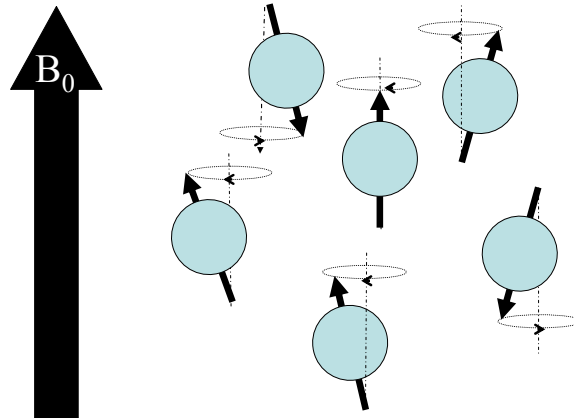


Figure 2.5 Equilibrium: no net transverse magnetisation

Therefore at equilibrium, before excitation pulses are applied, the net magnetisation vector is aligned with the external magnetic field B_0 as there is only longitudinal magnetisation and no transverse component.

2.3 Excitation

By exposing protons to energy, in the form of a radiofrequency (RF) pulse, there is a tendency for them to switch to the anti-parallel state (higher energy state). Energy is most easily absorbed by protons spinning at the same frequency as the RF pulse (resonance) so the RF pulse applied has a frequency equal to the Larmor frequency. These changes result in reduction in net longitudinal magnetisation. The length and amplitude of the RF pulse will determine the extent of the change in the net magnetisation vector. If the proportion of spins anti-parallel to B_0 exceeds that in the parallel state, the net longitudinal magnetisation becomes negative, that is in the opposite direction to B_0 .

The RF pulse also causes phase coherence, and by bringing precessing spins into phase, generates net rotating transverse magnetisation (see Figure 2.6).

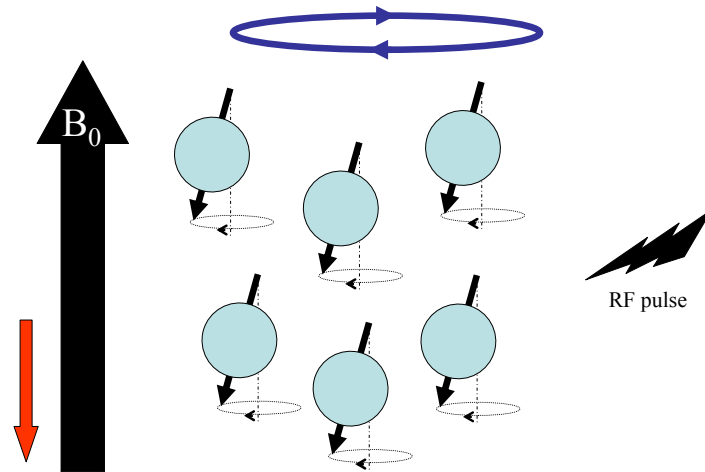


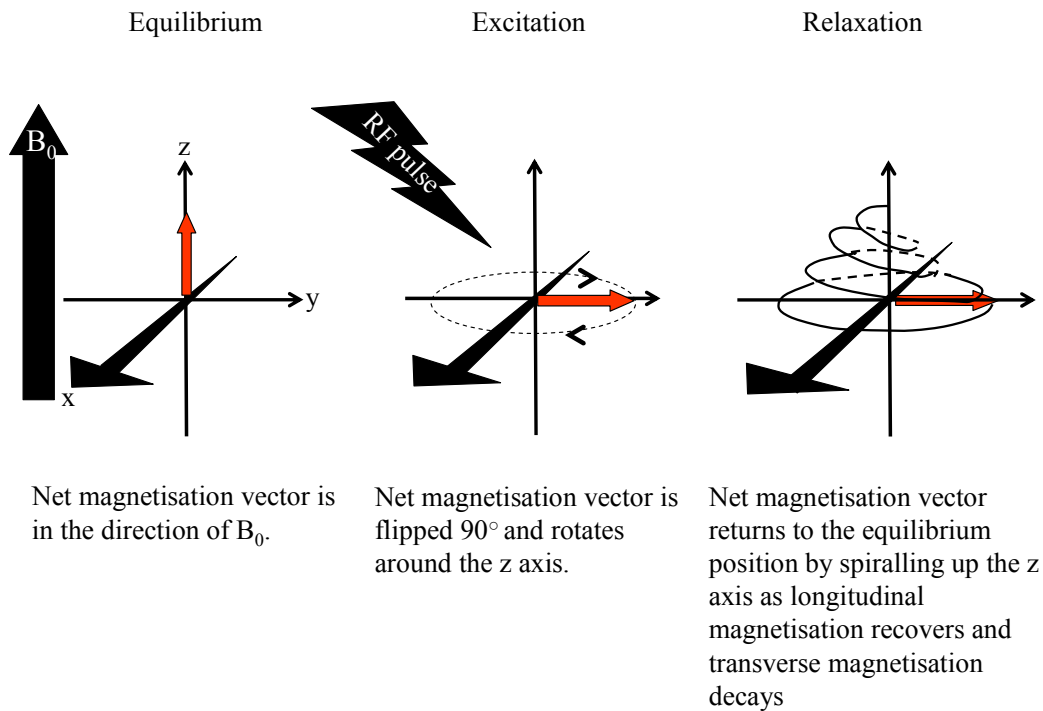
Figure 2.6 Excitation: A greater proportion of spins are in the higher energy state (anti-parallel to B_0), and the net longitudinal magnetisation gets smaller and, in this example, becomes negative (red arrow). The spins precess in phase, generating net transverse magnetisation (blue arrow)

At a quantum level the number of protons switching to the higher energy state, and thus the size and direction of the longitudinal magnetisation, depends on the strength and duration of the RF pulse. Viewing the effects of the RF pulse on the net magnetisation vector, the reduction in longitudinal magnetisation and the rotating transverse component, the net magnetisation vector can be viewed as ‘flipped’ from parallel to B_0 to some angle away from it, rotating around it (see Figure 2.7). The net magnetisation vector is used for explanation of MRI physics as it is simpler to work with than individual spins.

2.4 Relaxation

On cessation of the RF pulse, spinning protons return to equilibrium, with release of energy to their surroundings (spin-lattice interaction) as they tend to return to the lower energy state. The net longitudinal magnetisation returns to the pre-RF pulse position, parallel to B_0 , due to a process known as T1 relaxation or recovery (see Figure 2.7).

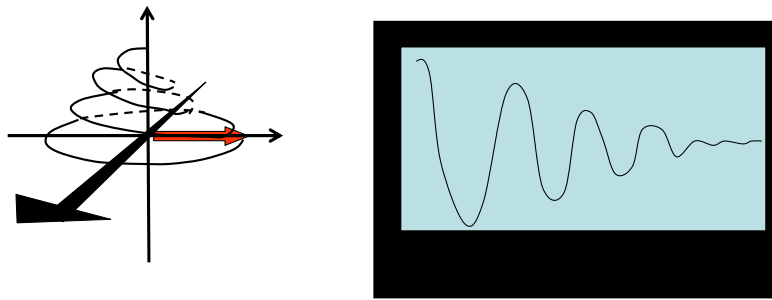
Figure 2.7 Net magnetisation vector during equilibrium, excitation and relaxation



As the longitudinal magnetisation recovers, with release of energy, transverse magnetisation decays, as the spins dephase. This process, known as T2 decay, depends on the interaction of neighbouring spins (spin-spin interactions) and inhomogeneities in the external magnetic field. Unlike T1 relaxation, T2 decay does not involve net energy transfer and is unrelated to field strength.

The recovery of the longitudinal magnetisation and the decay of the transverse magnetisation occur straight after the cessation of the RF pulse. They can be recorded on receiver coils near the sample. Changes in the net longitudinal magnetisation are lost in the overwhelming strength of the external magnetic field. Receiver coils are only sensitive to variations in transverse magnetisation. Faraday's Law of electromagnetic induction explains how the rotating transverse magnetism induces an electric current in the receiver coil, with a voltage oscillating at the Larmor frequency (see Figure 2.8).

Figure 2.8 Induction of electric current in receiver coil



Rotating transverse magnetisation induces an electric current in the receiver coil, with a voltage oscillating at the Larmor frequency.

The time taken for T1 recovery and T2 decay depend on the properties of the tissue in which the proton is spinning (see Figure 2.9). Water molecules are highly mobile with high inherent energy, whereas large molecules such as fat have a slow rate of molecular motion and low inherent energy. Energy transfer (during T1 recovery) is much more efficient, and thus T1 recovery time faster, in fat than water. Spin-spin interactions are greater in molecules with closely bound protons, such as fats, thus these dephase rapidly (short T2) whereas the protons in water molecules have less spin-spin interaction and dephase slowly (long T2) (See Figure 2.10).

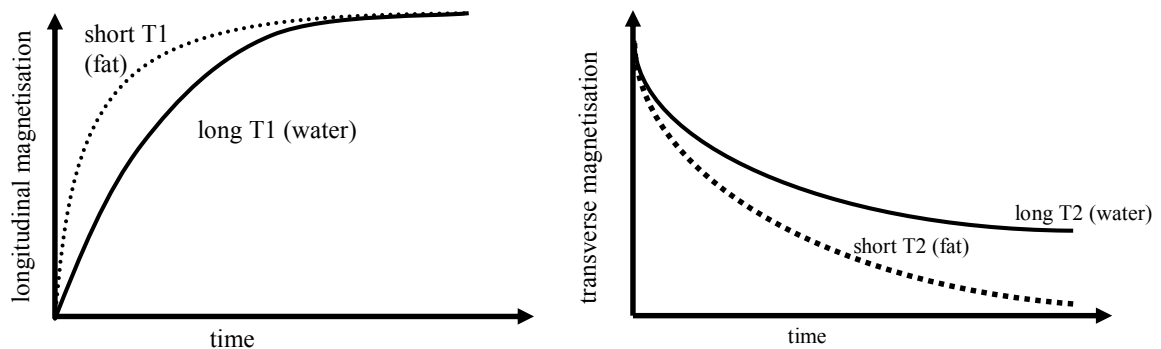
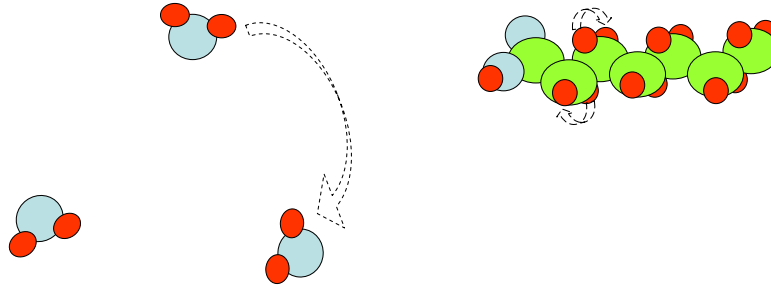


Figure 2.9 Differences in rate of decay in transverse magnetisation and recovery of longitudinal magnetisation in fat and water

Figure 2.10 Spin-spin interactions

Due to high rate of molecular motion and greater distance between molecules, spin-spin interactions are less in water than fat.

Protons in fat are closely bound with slow rate of molecular motion, facilitating spin-spin interactions.

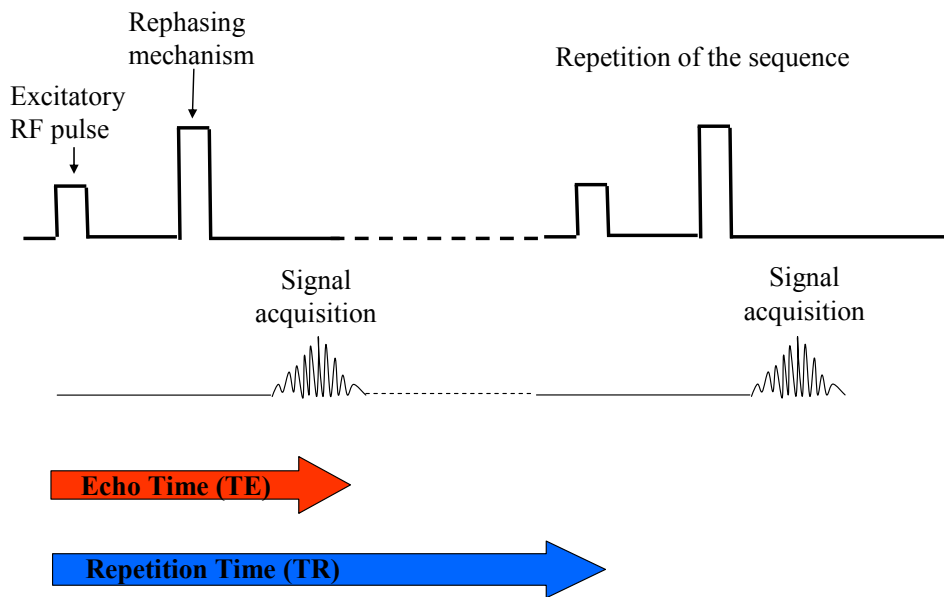


Transverse magnetisation decays faster than it would with pure T2 effects due to inhomogeneities in the external magnetic field. This is known as T2* decay. To ensure the signal differences are only due to spin-spin interactions, rather than additional differences in the external electromagnetic field, the latter must be compensated for. This is achieved with gradients in the electromagnetic field or with 180° pulses to reverse dephasing due to field inhomogeneities.

2.5 Imaging

The above principles can be used to produce 2D or 3D images of an object using the methods described below. One major difference between MRI and the principles described above is that the signal is not measured following the cessation of the RF pulse. This is because it is technically difficult to measure the decay so quickly after the RF pulse. A pulse sequence requires an excitatory RF pulse, and acquisition of the signal at echo time TE from the RF pulse after refocusing the signal by a further RF pulse and application of gradients (see below). The sequence can then be repeated at repetition time TR from the previous RF pulse (see Figure 2.11).

Fig.2.11 diagrammatic representation of a simple pulse sequence



2.6 Localisation

Spatial localisation requires manipulation of the static magnetic field B_0 to produce gradients in the magnetic field, with linear variation in magnetisation from the centre of the magnet. Magnetic field gradients are applied at a specific time and in a specific plane. Firstly a slice selection gradient is applied at the time of the RF excitation pulse. This is applied perpendicular to the desired slice plane and reduces the precessional frequency of spins at the low end of the gradient and increases the frequency at the high end. The desired slice precesses at the same frequency as the RF pulse (Larmor frequency) and thus only this slice will be excited by it. The bandwidth of the pulse and the strength of the gradient determine the slice thickness.

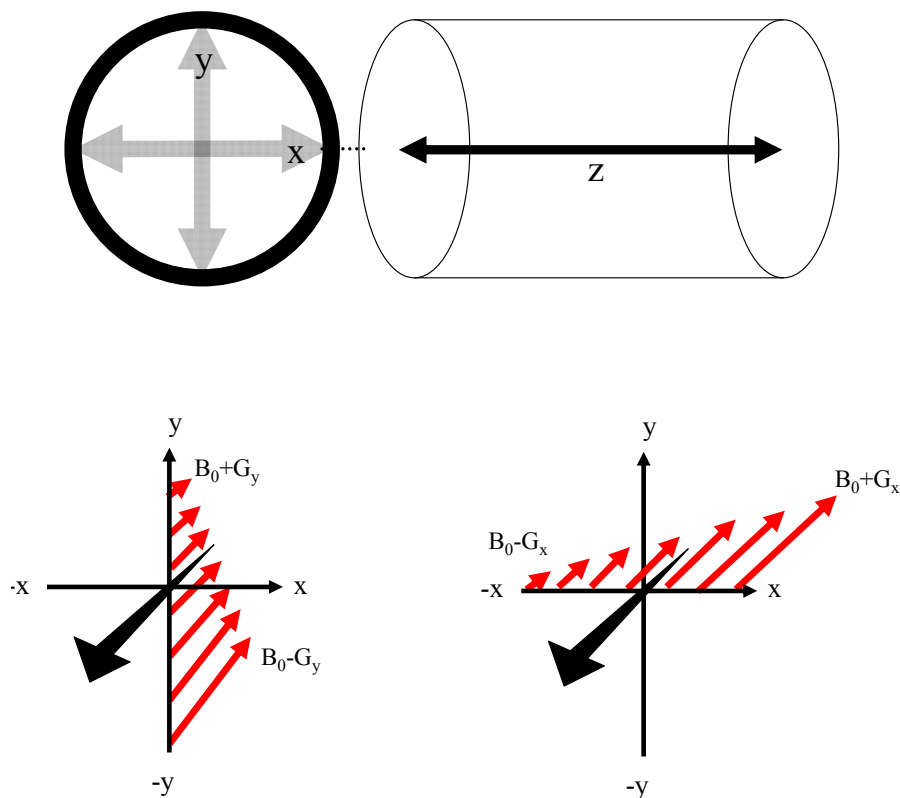
To localise in the image plane, two further magnetic field gradients are applied which spatially encode the spins in the two in-plane directions (see Figure 2.12). The first is brief, and applied after the excitation pulse and is removed before signal acquisition. This is the phase encoding gradient which causes the spins in the direction of the gradient to get out of phase with each other. The gradient causes the spins to precess at different rates during its application, thus they lose phase coherence, when the gradient is removed the neighbouring spins are out of phase but are precessing at the same frequency. The change in phase is proportional to the distance from the centre of the gradient. Multiple phase-encoding gradients are applied at incremental

strengths (one before each acquisition) with stronger gradients resulting in greater phase-shifts between two points. This causes improved differentiation between two adjacent points and better spatial resolution. Shallow phase encoding gradients cause small phase-shifts, and thus poor spatial resolution but they generate high amplitude signal.

Lastly the frequency encoding gradient is applied at the time of signal acquisition, perpendicular to the phase-encoding gradient, and this affects the precessional frequency, speeding up where the gradient is higher and slowing down where it is lower.

To create 3D images a much larger slab of tissue is excited with the 'slice' select gradient and a second phase encoding gradient applied in the same direction, thus encoding the spins in this direction as well.

Figure 2.12 showing axes relative to the magnet and the field gradients on the image plane



2.7 K space

The MR signal is a mixture of amplitudes and frequency- and phase-shifts. The signal is digitised and raw data are written into a data matrix called K space. K space is a spatial frequency domain with 2 (or 3) axes; phase encoded data are stored on the vertical axis and frequency encoded data on the horizontal axis. Every combination of frequency and phase encoding represents a point in K space. Data with high spatial resolution, but low signal amplitude (from steep phase encoding gradients) fill the outer lines of K space and data with low spatial resolution and high signal amplitude fill the central portion of K space. To translate this digitised data into an image requires a 2D (or 3D) inverse Fourier Transform, a complex mathematical procedure which is beyond the scope of this thesis.

2.8 Contrast

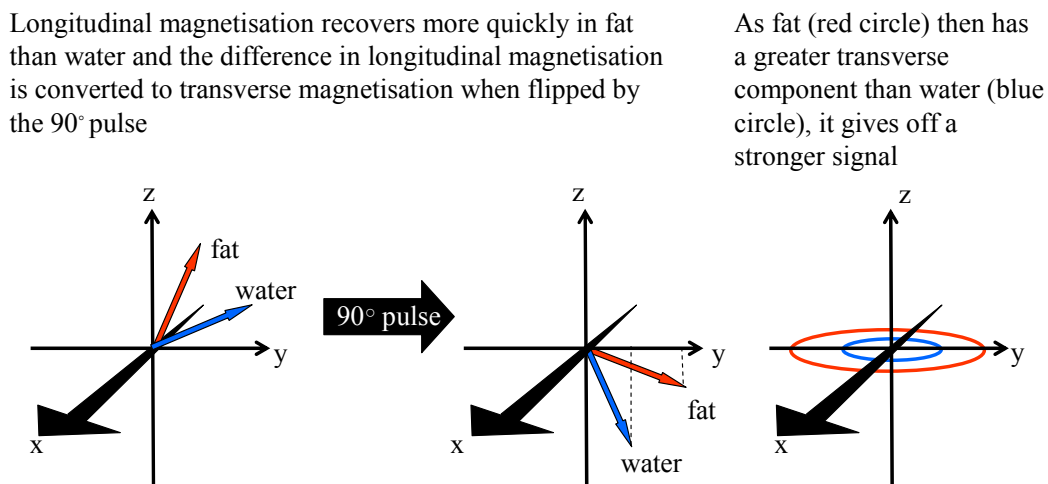
High signal is seen when a tissue has a large transverse component of magnetisation at acquisition and vice versa. Contrast will be achieved when the different tissues in the image give off a range of MR signals, due to differences in their T1 and T2 relaxation times. A third intrinsic contrast mechanism is proton density, which is proportional to the number of protons in each tissue. For example, gases inherently have a low density and will always give a low signal. To produce images where the contrast is predictable, the images are 'weighted' towards one of the 3 contrast mechanisms, depending on the imaging objectives (e.g. anatomy, oedema, tissue-characterisation). There are 3 parameters which are commonly altered to vary the signal contrast between tissues; the time between excitatory pulses (repetition time, TR), the time between excitatory pulse and echo acquisition (echo time, TE) and the initial flip angle induced by the excitatory pulse (gradient echo sequences only).

2.8.1 T1 weighting

The longer the TR, the greater the recovery of longitudinal magnetisation and any differences in signal due to T1 times of the different tissues are lost. To exploit T1 differences in tissues the TR must be short enough to ensure that the longitudinal magnetisation in fat and water have not relaxed back to B_0 . Differences in recovery of longitudinal magnetisation are converted to differences in transverse magnetisation by a 90° pulse, and the more the longitudinal magnetisation has recovered, the greater the transverse component of the net magnetisation vector when it is flipped by 90° (see

Figure 2.12). Therefore in T1 weighted images, tissues with short relaxation times (such as fat) give off high signal and appear bright compared to tissues with longer relaxation times (such as water) which give low signal (see Figure 2.14). A typical T1-weighted sequence has a TR of 300-600ms and a TE of 10-30ms. TE must also be short to diminish the T2 weighting described below. In gradient echo sequences the flip angle can also be used to vary the T1 weighting. A small flip angle will mean that the longitudinal magnetisation recovers very quickly, so minimising T1 weighting, hence a large flip angle should be used.

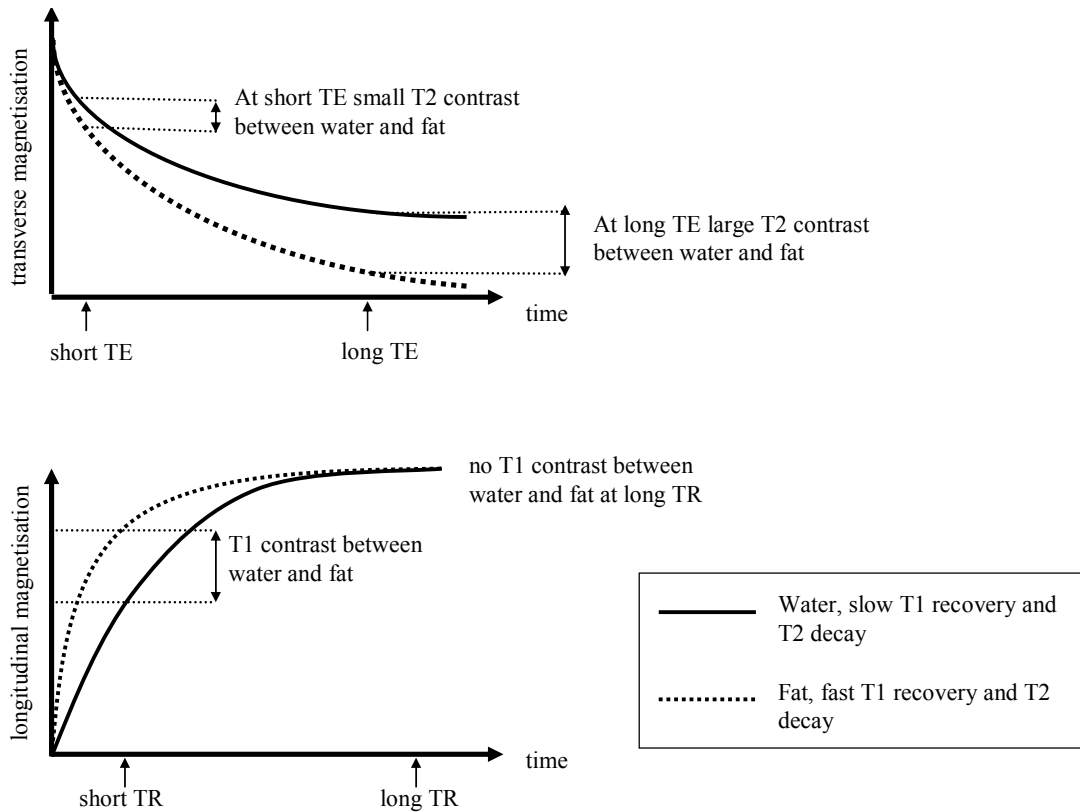
Figure 2.12 T1 weighting



2.8.2 T2 weighting

Sequences with long TRs remove the effects of differences in tissues' T1 properties by allowing full recovery of longitudinal magnetisation in all tissues between acquisitions. Delaying signal acquisition creates contrast between tissues with different T2 decay times. Tissues with slow T2 decay have greater transverse magnetisation at the time of signal acquisition than tissues with fast T2 decay, and thus produce a greater signal (see Figure 2.13). Small flip angles are used in T2 weighted gradient echo sequences to minimise T1 weighting effects. In T2 weighted images fat appears dark and water bright (see Figure 2.14). A T2 weighted sequence has a TR of 2000ms or more and a TE of 70ms or more.

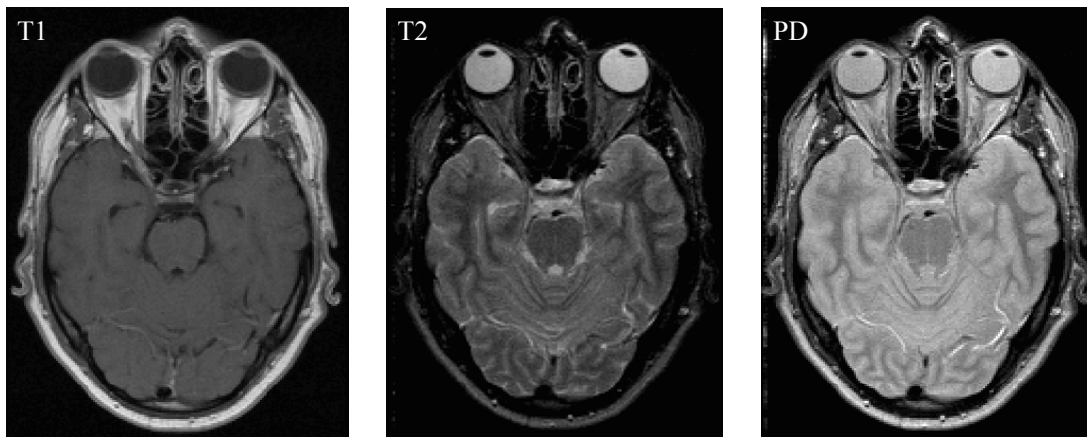
Figure.2.13 T2 weighting. At long TR, T1 weighting is minimised and at long TE T2 weighting is maximised.



2.8.3 PD weighting

PD weighted images minimise both T1 and T2 weighting effects by using a long TR and short TE (and small flip angle). Short TEs do not give enough time for signal decay in tissues with fast T2 times, reducing the tissue contrast due to T2 effects. Signal intensities vary in such sequences due to variation in proton density where tissues with tightly packed protons (such as fat) produce a stronger signal than those with less dense protons (such as water) (see Figure 2.14). A PD weighted sequence has a TR of 2000ms or more and a TE of 10 to 30ms.

Figure 2.14 T1, T2 and PD weighting in the same subject



T1, T2 and PD weighted images showing differences in contrast. In T1 image water is dark, (vitreous humour and CSF in the temporal horns) and orbital fat is bright. Water is bright in T2 weighted images (vitreous humour and temporal horn) but lower signal from orbital fat. Orbital fat and vitreous humour of more similar signal intensity on PD weighted images and right temporal horn not visible on PD weighted image due to lack of contrast with white matter.

2.8.4 Contrast media

Enhancement agents are used in MRI to increase the contrast between pathology and normal tissue. Gadolinium is a paramagnetic agent which produces local magnetic fields and accelerates T1 relaxation time of nearby water protons, resulting in high signal intensity on T1 weighted images. Gadolinium is a rare earth metal that cannot be excreted by the body and is therefore chelated to enable excretion. It can only enter the central nervous system where there is a breach in the blood brain barrier (BBB) and is therefore a marker of BBB breakdown (see Figure 2.15).

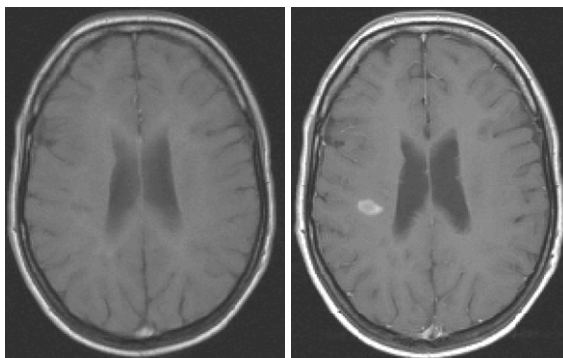


Figure 2.15 Gadolinium enhancing lesion

2.9 Pulse sequences

In designing pulse sequences a balance between image quality and acquisition time has to be achieved. Image quality is determined by spatial resolution and signal to

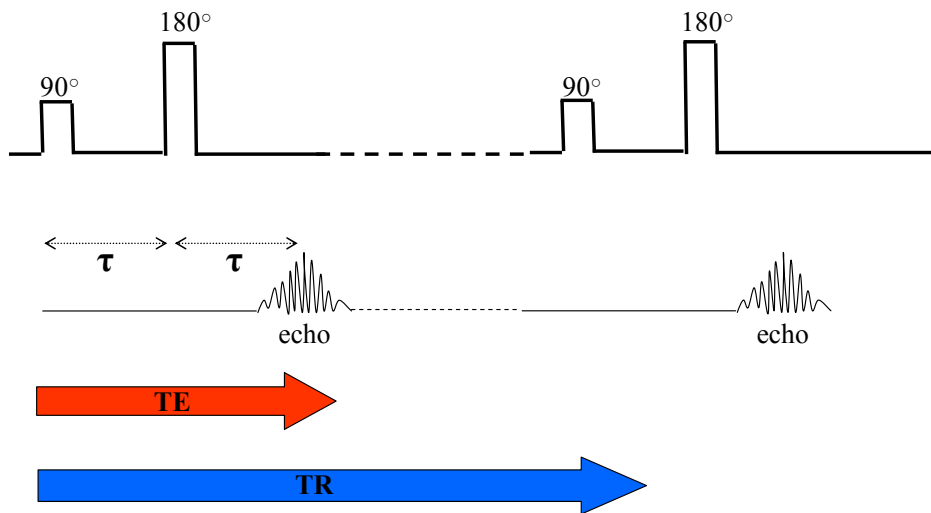
noise ratio. Spatial resolution increases with reduction in voxel volume, which can be achieved by reducing the slice thickness or field of view or increasing the matrix size. Noise is random variation in signal intensity affecting the transverse magnetisation signal and comes from sources such as the patient's body and the MR machinery. Signal to noise ratio is increased at higher field strengths (as the excess in number of spins in the parallel state increases with field strength, thus the number of spins generating the MR signal is greater), with increased voxel size (as there are more spins per voxel), the number of excitations (or averages) per image and the receiver bandwidth (narrow bandwidth records less noise than wide bandwidths, but results in slow sampling and low intensity readout). Acquisition time depends on TR, number of excitations and number of phase encoding steps (increasing phase encoding gradients increases resolution). Sequences have been developed to reduce acquisition time, such as fast spin echo, and ultrafast gradient echo sequences.

2.9.1 Spin Echo

The spin echo sequence is a common imaging sequence. This involves an excitatory 90° RF pulse which causes i) loss of longitudinal magnetisation and ii) maximised transverse magnetisation with phase coherence. Following the excitatory pulse, the longitudinal magnetisation recovers as energy is released to the lattice (T1 recovery) and the spins dephase (T2 decay). To remove the T2* effects, a 180° pulse is given (at a time τ from the 90° pulse). After a 180° pulse, spins that are precessing faster than others (due to stronger electromagnetic field in that part of the magnet) 'go to the back', and slower precessing spins 'get into the lead'. As they continue precessing at their pre- 180° pulse rate, the faster spins will catch up with the slower spins and by time τ from the 180° pulse, the spins will be rephased. The oscillating signal of the precessing spins is maximal at this point and the echo is acquired (echo time TE). The 180° pulse only reverses signal loss due to field inhomogeneities, and differences due to interactions of neighbouring spins (i.e. due to T2 tissue specific differences) remain. Further rephasing 180° pulses can be given (and signal acquired) but due to T2 decay, the signal diminishes from subsequent echoes, thus requiring repetition of an excitatory 90° pulse. The time between two 90° pulses is the repetition time (TR) (see Figure 2.16).

Spin echo sequences can provide good image quality, and are versatile, however they take a long time.

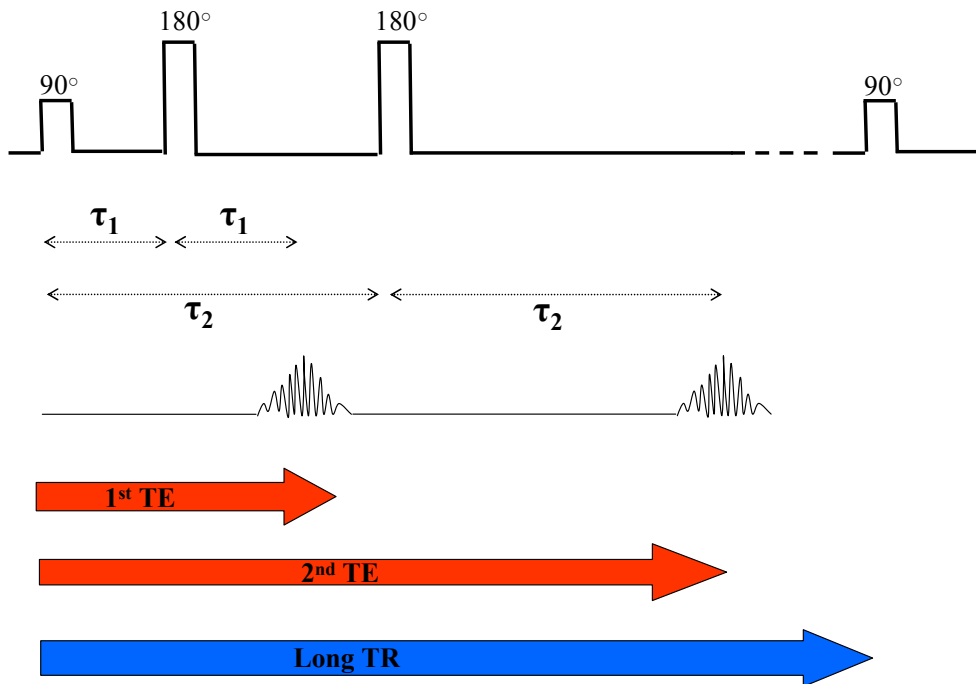
Fig.2.16 diagrammatic representation of a spin echo sequence



2.9.2 Dual echo

Similar to the spin echo sequence, but dual spin echoes allow two images to be acquired with the same (long) TR per slice location. For every 90° pulse, two 180° pulses are given, thus one image is acquired with a short TE (generally proton density weighted) and the second acquired with a long TE (true T2 weighted), see Figure 2.17.

Fig.2.17 Diagrammatic representation of dual echo sequence



2.9.3 Fast spin echo

In fast spin echo for every excitatory 90° pulse, multiple 180° rephasing pulses are applied and spin echoes subsequently acquired, generating an echo train. A phase encoding step is applied with every 180° pulse and data is collected in K space. Several lines of K space are filled for every TR (instead of one line in conventional spin echo) filling K space quickly.

Since every echo in the echo train has a different TE the contrast will be different. Those with longer TEs are T2 weighted and those with shorter TEs PD weighted. When the data is collected in K space, it is ordered so that the strongest signal (highest amplitude produced by the shallowest phase encoding gradient) are used on the echoes produced from the 180° pulses nearest the effective TE (the desired TE), therefore the bulk of the signal has the desired weighting. Steeper phase encoding gradients are performed with 180° pulses at other TEs, generating lower amplitude signal and these provide the spatial resolution.

The advantage of fast spin echo is their speed, being able to acquire 256 phase encodings in as few as 16 TRs, using an echo train length of 16. Because the scan

time is reduced matrix size can be increased to improve spatial resolution. The disadvantage is the signal decays with echo train length, reducing signal to noise. Also with echo train length increase the difference in TEs can affect image quality.

2.9.4 Gradient echo

The sequences described above use 180° RF pulses to rephase spins. This can also be achieved by changes in the magnetic field gradient. After the excitatory RF pulse, the spins start to dephase. A negative gradient is first applied, further slowing down the slow spins and speeding up the faster spins, thus dephasing is accelerated. This is followed by a positive gradient which has the opposite effect, speeding up the slow and slowing down the fast spins, rephasing the signal. This method is less efficient than RF rephasing, as it does not reverse all the dephased spins. Also the rate of transverse decay will be faster as there will be $T2^*$ effects such as B_0 inhomogeneities in the absence of the 180° pulse. The advantages of gradient echo are that it is faster and any flip angle can be used (unlike spin echo which requires an initial 90° pulse) allowing another parameter that can be altered to give the desired weighting. Using a large flip angle gives the images T1 weighting, as the majority of the net magnetisation vector is in the transverse plane. The larger the flip angle, the longer the longitudinal magnetisation recovery takes. Small flip angles reduce T1 weighting and can be quicker due to the reduced TR required for full longitudinal relaxation.

2.10 Non-conventional MRI

Changes observed in the grey matter and normal appearing white matter (NAWM) of biopsies and autopsy studies are not visible on conventional MRI. One reason for this is that conventional MRI visualises protons in association with water, and is unable to visualise protons in macromolecules, such as myelin and cell membranes, due to their very short relaxation times. It is not possible on conventional MRI to separate out the signal from different macromolecular structures and subtle changes in this signal cannot be detected. Magnetisation transfer imaging indirectly views the historically invisible macromolecules.

2.10.1 Magnetisation transfer imaging

In conventional MRI the signal comes from freely mobile protons, such as in water, as these have long T2 relaxation times. The protons in macromolecules and phospholipid bilayers are semi-solid and their motion is restricted compared to water. Due to the close proximity of neighbouring protons, they have strong spin-spin interactions resulting in rapid T2 relaxation times. The signal from this so called 'bound pool' decays too quickly to be visible on conventional MRI however the protons from the bound pool can influence the signal coming from the mobile pool. Protons in the bound pool precess at a broad range of frequencies (tens of kHz), due to variation in macromolecular structures and spin-spin interactions within the individual molecular structures. By contrast the protons in water precess at a narrow range of frequencies (a few Hz). The RF pulses in conventional MRI are at the Larmor frequency of protons in water. In magnetisation transfer imaging, an additional off-resonance pulse is applied, affecting spins in macromolecules precessing at that frequency without exciting water protons. The off-resonance pulse is applied in such a way as to saturate a portion of the macromolecular protons, a dynamic process in which the spins are flipping between parallel and anti-parallel states at such a rate to have a net magnetisation of zero with no transverse component. Magnetisation is then exchanged between the mobile proton pool and bound proton pool causing a reduction in the magnetic resonance visible signal from the water protons. This exchange of nuclear magnetisation occurs both through dipole-dipole interaction and through chemical exchange in which loosely bound protons are exchanged between the two pools. The exchange results in reduction of the free-water transverse magnetisation and a reduction in signal intensity for any given voxel. The difference in signal from two acquisitions with and without the off-resonance pulse can be quantified as a magnetisation transfer ratio. Breakdown of macromolecules, such as myelin and cell membranes, causes a reduction in MTR compared to normal tissue, thus providing a quantitative measure of structural integrity. MTR can be measured in percent units and is calculated using the following formula:

$$\{(M_0 - M_S) / M_0\} \times 100$$

where M_0 and M_S represent the signal intensities without and with the saturation pulse respectively.

The absolute MTR values will depend on the off-resonance pulse used and other scanner specific details, making comparisons between sites difficult. However results are consistent if the imaging is performed with the same sequence on the same imager, with one study finding MTR ranged between 37.6 and 40.6 pu. Age related differences are found however [116].

MTR can be averaged over a region of interest (ROI) to investigate focal disease, but to investigate diffuse changes it is desirable to analyse larger regions. The distribution of values from all pixels in the whole or segmented brain can be displayed in a histogram. Generation of histograms requires data acquisition, definition of the tissue area studied and selection of an interval (bin width) for display of the frequency distribution. As brain volumes differ between subjects, histograms are normalised by dividing all values by the total number of pixels and a frequency distribution is then obtained which represents a fraction of total brain volume in one interval. Histograms can be described by parameters, namely mean MTR, peak height, peak location and percentiles and has allowed detection of subtle diffuse abnormalities which can be missed on ROI analysis [117, 118]. The mean MTR is most commonly referred to in MTR studies which have been shown to vary between patient subgroups. Other histogram parameters give an indication of the focality of the disease process. Reduction in peak location (without loss of peak height) results from a generalised reduction in MTR, suggesting a diffuse disease process with all voxels having reduced MTR values. Peak height reflects the amount of residual normal tissue. Focal changes in the normal appearing tissue are thought to reduce the peak height by increasing the number of voxels with low MTR values. Thus a proportion of voxels have low MTR, but the remainder of the brain tissue is unaffected and has normal MTR values (that is at a normal peak location). The more severe the disease process in these focal abnormalities, the greater the relative increase in the histogram at low MTR, and the histogram peak broadens at lower MTR values. Voxel based analysis compares large regions, and identifies focal MTR differences that are consistent between two groups.

2.10.2 Magnetic Resonance Spectroscopy

As discussed above, in conventional MRI, protons in water molecules are targeted with excitatory RF pulses, which have a narrow range of precessing frequencies. The resulting signal is located using phase and frequency encoding gradients. In spectroscopy, signal location is sacrificed to quantify the protons in different molecules, and hence the molecules themselves. This is possible because protons in different molecules precess at different frequencies due to chemical properties (particularly the electron density associated with the molecule). MRS can look at ^{31}P , ^{19}F and ^1H atoms, (MR active nuclei), but since ^1H is at higher concentrations and existing ^1H imaging hardware can be used, ^1H spectra are most commonly acquired.

A nucleus is surrounded by electrons which also have magnetic susceptibility and become slightly magnetized in the external magnetic field. Their field minimally counteracts B_0 , thus the effective external magnetic field acting on the proton is lower than B_0 , reducing its precession frequency. Hence protons in different chemical environments precess at different frequencies, and the amount of signal at each frequency is detected on the spectrum. The position of the peak on the x axis is measured relative to a reference peak, and the distance between the peak of interest and reference peak is the chemical shift (as it is the shift in the proton's precessional frequency due to the chemical environment). The height of the peak on the y axis is proportional to the number of ^1H nuclei in the molecule with the same chemical shift in the sensitive volume. In this way molecules with known chemical shifts can be quantified.

Chemical shift = nuclear shielding/applied magnetic field

To obtain a spectrum from a region of interest (ROI), an image is first acquired and ROI marked as a cube (the sensitive volume). This is a large voxel compared to conventional MRI as the metabolites of interest are at low concentrations (volumes range typically from 1 to 8mls), see Figure 2.18.

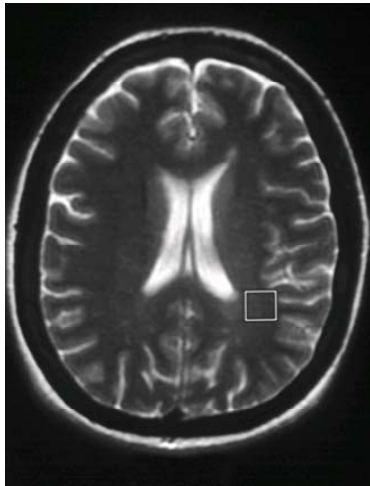


Figure 2.18 showing positioning of a voxel (the sensitive volume) for single voxel spectroscopy

Just as in conventional MRI, following an excitatory pulse, B_0 inhomogeneities must be corrected over the sensitive volume before the resultant signal is acquired. As the voxel is larger than in conventional MRI and the compounds have frequency separations of a few parts per million, correcting the inhomogeneity in the voxel is even more important to prevent the frequencies associated with the same metabolite in different parts of the voxel resulting in multiple peaks. This is done with multiple shim coils generating small magnetic fields to correct B_0 inhomogeneity. Since the bulk of the signal from water and lipid totally dominates the signal from the metabolites, these are suppressed so as to quantify the metabolites at much smaller concentrations.

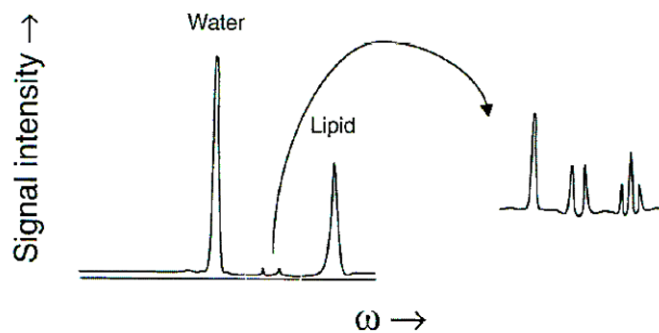


Figure 2.19 Water suppression in spectroscopy. Demonstrating the dominant peaks from water and lipid, and the need to suppress these for investigation of metabolites at lower concentrations

The time of the acquisition depends on the metabolites of interest. At longer echo times, N acetyl containing compounds, creatine and phosphocreatine, and choline containing compounds may be quantified (all of which have T_2 's of about 250ms),

while at shorter echo times additional metabolite peaks may be seen from myo-inositol (Ins), glutamate and glutamine, and mobile lipids, however peak overlap can be a problem. The strength of the magnetic field also affects the spectra, with higher fields generating increased signal intensity and improved spectral separation.

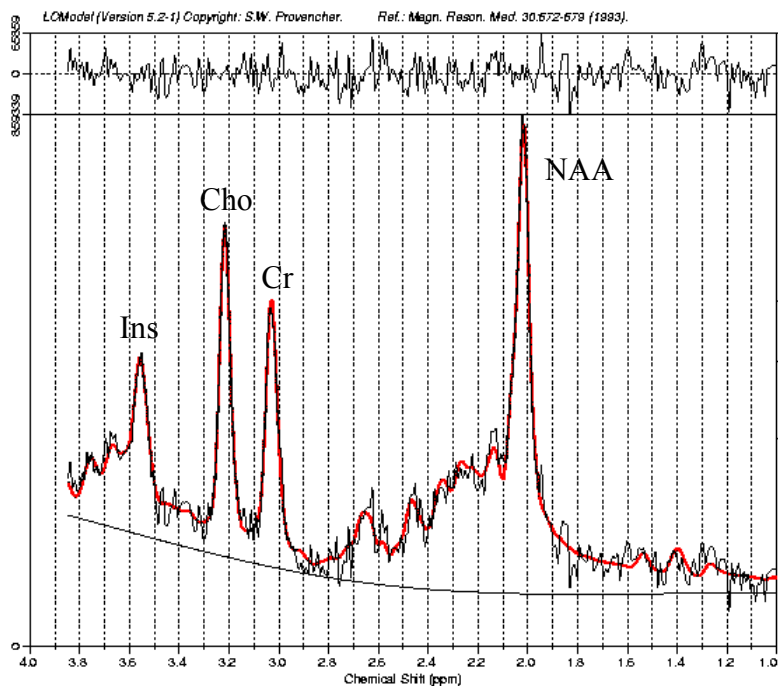


Figure 2.20 spectrum acquired at short echo time from a single voxel in normal appearing white matter in a CIS patient, and metabolite peaks observed.

Deriving reliable data from spectroscopy is limited by several issues, such as variation in tissue composition of voxels, particularly partial-volume effects due to the presence of CSF within the voxel and the position of the voxel in relation to the inhomogenous radio-frequency of the coil. The data must be standardised to enable comparison. A common internal standard is expressing the metabolite of interest relative to a metabolite that is not affected by the disease process. Cr has been used as an internal standard, but studies have shown this is not always stable, and is particularly affected in acute, gadolinium enhancing lesions [119, 120] and correlated with age in healthy adults [121]. Another internal standard used is tissue water or CSF but neither of these are reliable (MRI is very sensitive to changes in tissue water and this is therefore not a stable reference and measurement of CSF signal intensity is error-prone due to the

pulsatile nature of CSF flow). An external standard is often used, with regular measurement of a phantom containing a known concentration of NAA (or other molecule of interest) to provide a calibration factor for quantification of absolute values of metabolites [122].

2.10.3 Measurements of brain atrophy

Several methods have been developed to analyse brain volume or atrophy. Regarding brain volume, the simplest methods are linear measurements of brain or ventricular width, and have also been used to measure corpus callosal width. As well as being time-consuming, these techniques rely on careful positioning, the observer's expertise and results in considerable inter-observer variability. Semi- and fully automated segmentation methods have been developed to reduce the inter-observer variability. These allow quantification of brain parenchymal and/or CSF volumes, driven by the difference in their signal intensities.

MIDAS is a semiautomated technique to measure lateral ventricular volume. The first step involves segmentation of the brain from extracranial tissue (and setting the inferior cut-off as the lowest point of the cerebellum). Brain segmentation requires selection of a signal intensity threshold for the boundary between CSF and brain and is set at 60% of mean brain intensity. Thresholding weakens the links between brain and adjacent structures and the image is eroded to leave only brain tissue. The outer voxels of the brain eroded in the previous step are replaced with a dilation step. Mean whole brain signal intensity is re-calculated and the ventricular-brain boundary is again set at 60% of this value. The lateral ventricles (including the temporal horns but excluding the third and fourth ventricles) are outlined with a seed placing technique and the program automatically outlines the ventricles, recursive 2D growth beyond the ventricles being constrained by the intensity threshold range. High signal structures in the ventricles (such as the choroid plexus) are excluded. Volume is calculated by multiplying the outlined area by the slice thickness. This technique has intra-observer coefficients of variation (CoV) ranging from 0.02% to 0.89%, with inter-observer CoV of 0.32% [123].

Fully automated techniques such as SPM have been developed to reduce inter-observer variance, and allow segmentation of grey and white matter whose signal intensities are variable within the brain and less dissimilar than brain/CSF intensities. After masking to remove extracranial tissue and correcting for intensity inhomogeneity, SPM assigns a probability of a voxel belonging to a tissue based on signal intensity and location. This is assisted by registration of the study image to prior belonging probability maps (generated from segmented images of 151 healthy

subjects). Voxels with greater than 75% probability of belonging to a certain tissue class are thus assigned and mutually exclusive masks are generated. The presence of MS lesions can result in misclassification as grey matter or CSF due to their different signal intensities from surrounding white matter. Masking of lesions prior to segmentation aims to prevent this. Further problems include partial volume effects blurring the boundaries between tissue types impeding their differentiation, and mis-registration due to variability in anatomical landmarks. Intra- and inter-observer CoVs of 0.09% and 0.19% respectively have been reported [123].

As there is a wide range of brain volumes in healthy populations, studies usually try to correct for head size, adjusting the raw inter-subject differences in regional or whole brain measurements. There are two techniques that correct for intracranial volume (ICV), an estimate of head size: proportion and residual methods. The proportion method corrects for head size by dividing the brain tissue volume by the total ICV generating brain fractions. This corrects for head size on an individual basis and is free of any requirement for group based corrections. However errors from numerator and denominator are combined and sources of error are masked in the final outcome ratio. The residual method requires group data and uses the correlation between (raw) absolute parenchymal volumes (APV) and ICV to calculate the regression line for predicted parenchymal volumes (PPV) taking into account ICV. A scatterplot of ICV against APV for each group is fitted with the (least-squares derived) regression line which determines the PPV for a given ICV. It is based on a least-squares solution which by definition minimises the error in derived predicted values. The latter method has been shown to be more robust to errors in brain volume analysis and therefore may provide advantages over the proportion method [124].

The techniques described generate area or volumetric measures, and atrophy can be calculated from serial measurements. However small changes in volume may be missed by these methods due to dependence on reproducibility. Registration based methods have been recently developed to enable direct quantification of brain volume change by spatially registering serial images and subtracting the volume and these techniques are more sensitive to change [125].

3 The use of MRI in the study of multiple sclerosis

3.1 Conventional MRI

3.1.1 Lesion appearance

MRI is very sensitive for detecting inflammatory activity in the white matter of the central nervous system in MS. Typical findings are multiple hyperintense lesions in the white matter on T2 and PD weighted imaging. Over time individual T2 hyperintensities may remain static or increase in size, and occasionally recede or disappear. They tend to accumulate with disease duration, increasing at a rate of 5-10% per year. They can represent pathologically diverse processes, including active demyelination, remyelination and glial scarring [126]. T1 weighted imaging, particularly with contrast can provide some additional information regarding plaque age and degree of axonal and myelin loss.

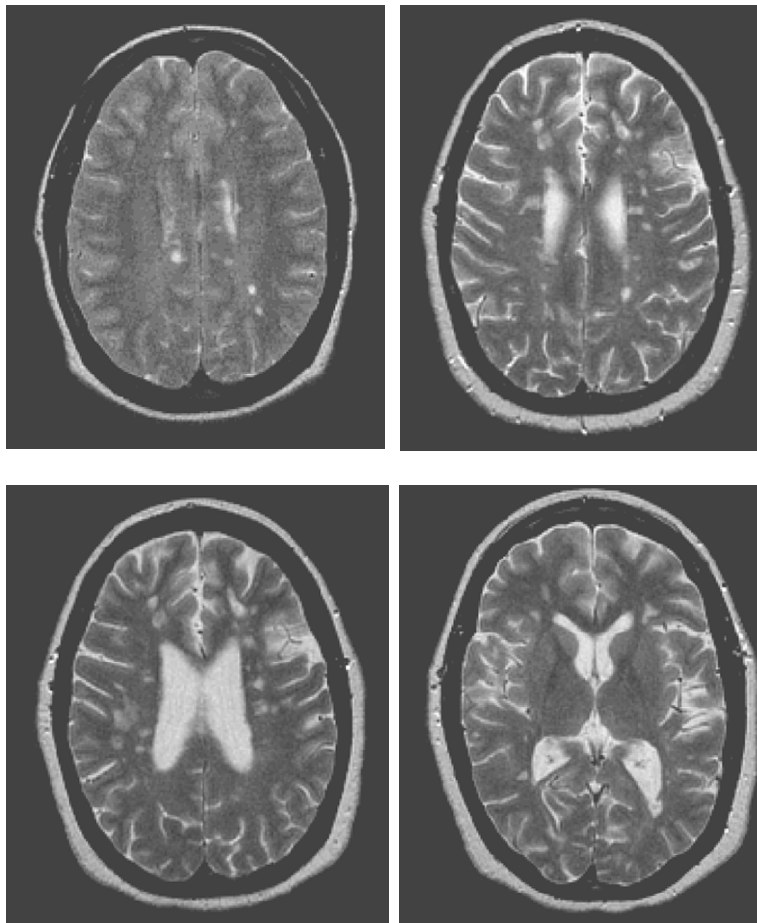


Figure 3.1 Typical MRI findings on T2 weighted images in CIS and early MS, demonstrating multiple, focal hyperintensities in a periventricular distribution.

Acutely, due to breakdown in the blood brain barrier, lesions enhance with gadolinium, a finding which has been shown to correlate with active demyelination on histology [127]. Enhancement may precede new T2 lesions by hours or days, and persist for 2 to 8 weeks so providing a marker of recent disease activity. On corresponding T2-weighted scans, acute lesions may appear target-like, with surrounding oedema which later resolves (see Figure 3.2). Being a marker of acute inflammation, gadolinium enhancing lesions are more frequently seen in relapses than remissions [128], and in relapsing-remitting more than secondary progressive patients [129, 130]. Scanned monthly for 10 months, 90% of untreated RRMS patients would have enhancing lesion(s) [131]. The number of enhancing lesions is low, with a median of 0 to 1 [132, 133], and declines with disease duration [134]. Like T2 lesions, the majority are asymptomatic.

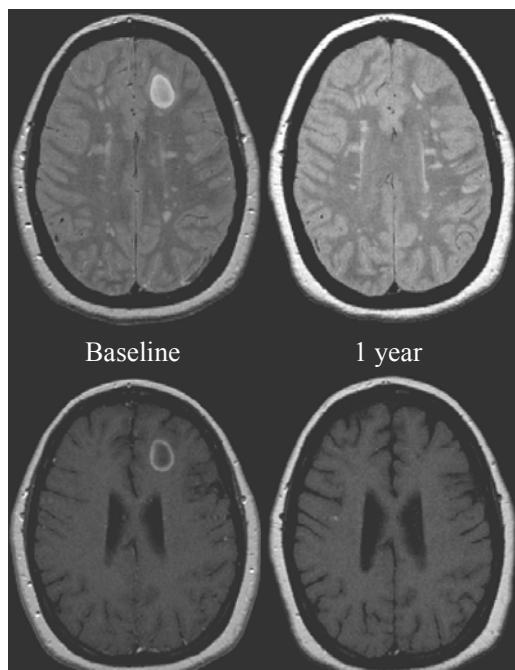


Figure 3.2 Acute lesion on PD- and T1 weighted images at baseline, demonstrating surrounding oedema on PD weighted MRI, with resolution of oedema and accrual of new lesions at 1 year. The corresponding T1 weighted image shows acute Gd enhancement and subsequent development of a black hole.

Most gadolinium-enhancing lesions appear hypointense on T1-weighted scans (see Figure 3.3), but only 40% remain hypointense 6 months later, the remainder recovering to some degree back to isointense with the normal appearing white matter [135]. Histopathology studies have shown that the degree of hypointensity correlates with axonal loss [136], thus persistent T1 hypointensities represent irreversible structural damage and axonal loss.

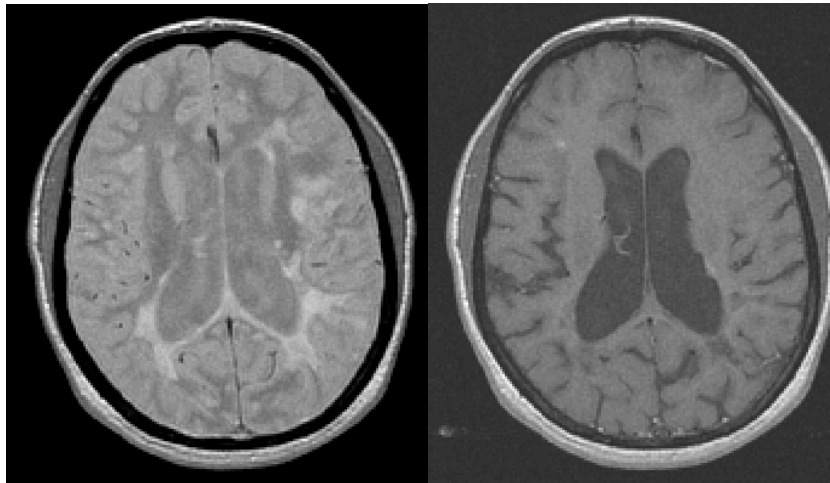
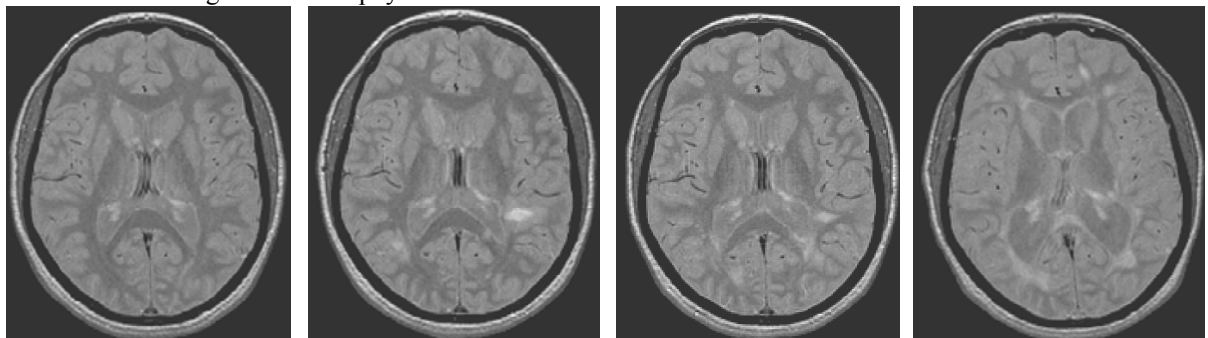


Figure 3.3 T1 hypointensities, demonstrating some PD-weighted lesions appear hypointense on corresponding T1 image

Figure 3.4 Change in lesion appearance during follow-up. Demonstrating a new lesion at 3 months with surrounding oedema, corresponding to Gd enhancing lesion on T1 post contrast. At subsequent 1 year scan, oedema has resolved but lesion appears hypointense on corresponding T1 MRI. MRI at 5 years shows accumulation of lesions, with further T1 hypointensity development. Ventricular enlargement and sulcal widening indicate atrophy.

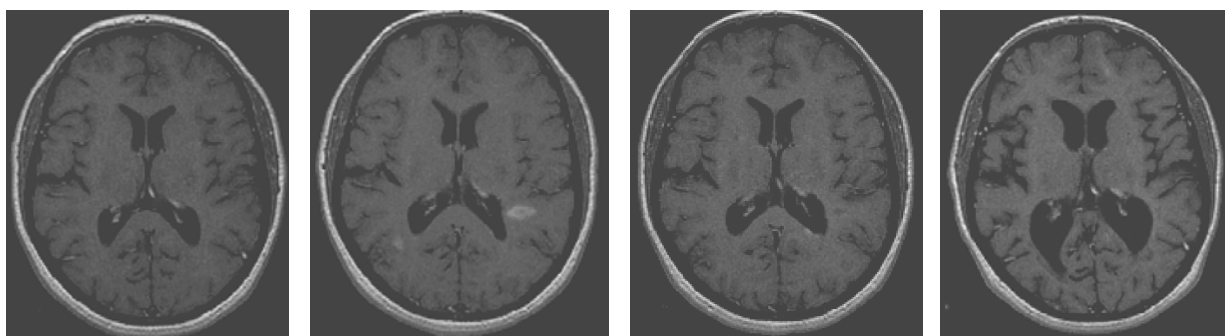


Baseline

3 months

1 year

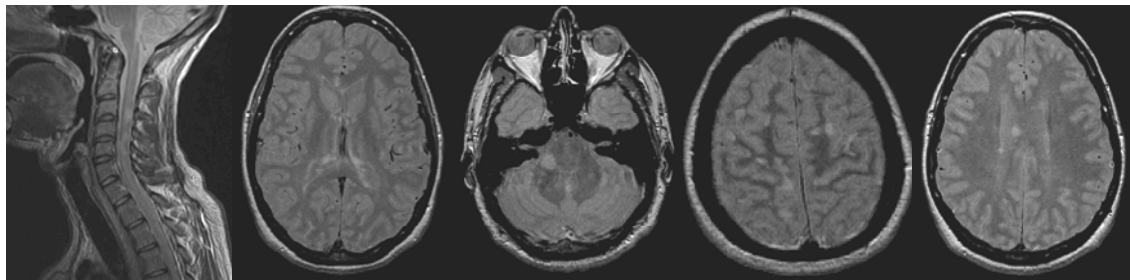
5 years



3.1.2 Location of lesions

CNS locations considered characteristic of demyelination are periventricular, corpus callosum, juxtacortical (this direct subcortical zone is typically spared in hypoxic/ischaemic disease), infratentorial (other diseases tend to be confined to the supratentorial compartment) and spinal cord (see Figure 3.5). Lesions are characteristically ovoid, representing breakdown in the blood brain barrier of venules with inflammation extending into adjacent white matter.

Figure 3.5 Characteristic locations for demyelination, showing (from left) spinal cord, callosal, infratentorial, juxtacortical and periventricular lesions



At first presentation with a clinically isolated syndrome (CIS), asymptomatic brain lesions are seen on T2 weighted MRI in 50-74% of patients [99, 137-140], and in 95% of patients with CDMS. Asymptomatic spinal cord lesions are seen in 27-42% of CIS patients [140-142], and are usually accompanied by brain lesions. Spinal cord lesions in patients with CDMS are most frequent in the cervical cord, tend to be focal, rarely cause cord expansion (3%) or appear hypointense on T1 weighted images (<0.1%) [143].

MRI activity tends to be greater than clinical activity in RRMS and for every clinical relapse there may be 5 to 10 new T2 lesions. Similarly, with monthly gadolinium enhanced T1 weighted imaging, about 10 gadolinium enhancing lesions will be observed for every clinical relapse [131, 144].

3.1.3 Conventional MRI features associated with prognosis

MRI is the most sensitive paraclinical test in MS diagnosis and a normal MRI at presentation carries a negative predictive value of early conversion of 97% [99, 145]. The presence of one or more MRI lesions at CIS presentation increases the risk of CDMS, with 82% of patients with abnormal baseline MRI converting to CDMS by 20

years compared to 21% of patients with normal imaging [61, 62]. Other studies have reported similar results (see Table 3.1).

Table 3.1 comparing conversion rates in CIS cohorts with normal and abnormal baseline MRI (*symptomatic lesion excluded)

CIS cohorts	% developing CDMS		Duration of follow-up
	with normal MRI	with abnormal MRI	
ON only [63, 74]	22%	56%	10 years
	25%	72%	15 years
Mixed CIS cohort [61, 62, 145, 146]	6%	72%	5 years
	11%	83%	10 years
	19%	88%	14 years
	21%	82%	20 years
ON only [75]	0%	52%	6.3 years
Mixed CIS cohort [147]	8%	60%	7 years

Short follow-up studies suggested that the number or grade of MRI abnormalities influences the risk of developing CDMS. Morrissey *et al* showed at 5 years the risk of progression to MS in a mixed CIS cohort was 54% in patients with 1-3 lesions and 85% in patients with 4 or more [145]. Similar findings were reported from the two year follow-up of the North American optic neuritis treatment trial (ONTT), in which 17% of patients with grade 2 MRI abnormalities (one periventricular or ovoid lesion >3mm in size) developed CDMS compared to 36% of patients with grade 3 or 4 MRI abnormalities (2 or more periventricular or ovoid lesion >3mm in size) [148]. Recently Tintore *et al* demonstrated risk of conversion in a mixed CIS cohort by 7 years was clearly stratified by baseline lesion number [147]. However longer follow-up of both the ONTT and early mixed CIS cohort found the risk of CDMS at 14-15 years was not significantly different in patients with 1 or more lesions (ranging from 81-85% in the mixed CIS cohort [61, 62] and 60-78% in the ONTT depending on lesion number (see Table 3.2)) [63, 74]. Together these results suggest that baseline lesion number may predict early conversion. It should be noted however that the early follow-up studies are based on MRIs acquired 15 to 20 years ago, and were of lower resolution than those acquired more recently. It is likely that the number of lesions was underestimated by today's standards. Longer follow-up of more recent cohorts

are required to determine the risk of baseline lesion number visible at higher resolution.

Table 3.2 showing percentages of patients converting to CDMS with 7-20 year follow-up depending on number of brain lesions at baseline. Note that the MRIs in the studies marked with an * were acquired in the 1980's.

	Percentage of patients converting to CDMS by number of lesions											F/U
	0	1	2	3	4	5	6	7	8	9	≥10	
Mixed CIS cohort [147]	8%	30%			50%						73%	7y
Mixed CIS cohort* [61, 62, 145, 146]	6%	17%	67%		92%						80%	5y
		54%										
	11%	33%	87%		87%						85%	
		79%										
	19%	89%			87%						88%	
21%	82%			85%						81%	20y	
ON only* [63, 78]	21%	51%	50%			70%			57%			10y
	25%	60%	68%	78%								15y
		65%										

Evidence of MRI dissemination in time is strongly predictive of conversion to CDMS. A new lesion at 1 year was identified in 68% of CIS patients with abnormal baseline MRI, of whom 81% developed CDMS after 5 years compared to 22% of patients not acquiring new lesions [145]. In patients with normal baseline imaging, only 31% acquired new lesion(s), of whom 20% developed CDMS by 5 years compared to no patients with persistently normal imaging. Both the appearance of new T2 lesions and new gadolinium lesions at 3 months follow-up were highly predictive of CDMS at 12 to 19 months [140] (specificity 81% and 89%, sensitivity 92% and 54% respectively). Using only the baseline scan, the presence of a gadolinium enhancing lesion was found to be the most useful imaging correlate for predicting CDMS by 1 year (specificity 80%, sensitivity 61%) [149]. Therefore the identification of T2 and gadolinium lesions at baseline and new lesions at follow-up are helpful in predicting the risk of conversion to CDMS.

The prediction of long-term disability is more uncertain and perhaps more important. The ONTT found no correlation between baseline MRI findings and disability at 10 and 15 years [63, 74], whereas a consistent association between baseline lesion number and EDSS at 10, 14 and 20 years was found in the mixed CIS cohort (correlation coefficients of 0.45 - 0.48) [61, 62, 146]. None of the patients in this mixed CIS cohort with normal baseline MRI had developed clinically significant disability at 14 years, whereas over a third of patients with abnormal baseline MRI were unable to walk unaided [61]. By 20 years 6% of patients with normal baseline MRI were unable to walk unaided compared to 25% of patients with abnormal imaging [62]. In a recent study 10 or more lesions increased the hazard of significant disability at 7 years more than three fold [147], concurring with findings from the earlier CIS cohort followed up at 10 years in which three quarters of patients with less than 10 lesions at baseline had little or no disability ($EDSS \leq 3$) and three quarters of patients with more than 10 lesions having significant disability ($EDSS > 3$) [146].

The chronological relationship of EDSS and lesion load has been investigated, and early change in lesion volume found to correlate more strongly with later rather than concurrent disability [61, 112, 150]. These findings led to an ‘MRI-clinical disconnect theory’, that the CNS is able to compensate for MS-related pathology up until a certain threshold, beyond which functional deterioration and clinical progression become manifest. This is supported by the finding in over a thousand MS patients that T2 lesion load correlated with disability until EDSS 4.5, following which the relationship plateaued [151]. These data suggest that pathogenic processes additional to T2-visible lesions might become more important over time.

The relationship with disability and lesion activity has also been investigated in CDMS patients. Gadolinium enhancement is a marker of disease activity, and predicts the occurrence of relapses [152-154] but their significance regarding disability is not clear. Whereas a cross sectional study found a correlation between EDSS and number of enhancing lesions [155], longitudinal studies have failed to show their ability to predict later disability [150, 152, 153, 156]. Location of lesions for prognosis has been proposed and one study found the presence of two or more infratentorial lesions to be a strong predictor of disability [156].

As well as physical disability, cognitive impairment is an important aspect of MS. Although correlations between total lesion load and cognitive impairment in established disease are recognised [157, 158], the relationship between neuropsychology scores and lesion load in patients with early disease has been weak [159] or absent [93, 95, 96].

The correlations of early conventional MRI markers and later disability have been moderate at best. Therefore it seems that in the early years, relapse rate and lesion load has an influence on time to develop moderate disability, (EDSS 4) but not the subsequent evolution to more severe disability. The histological observation of cortical lesions and changes in the normal appearing white matter (NAWM) ‘invisible’ on conventional MRI may add important prognostic information and explain the missing variation in disability.

3.1.4 MRI in diagnosis

With the recognition of the sensitivity of MRI to MS pathology and its ability to predict CDMS in CIS patients, came its integration into diagnostic criteria as paraclinical evidence of dissemination in space (DIS). Such criteria were designed to be used only in CIS cases considered absolutely characteristic of demyelination, such as unilateral optic neuritis, bilateral intranuclear ophthalmoplegia and partial transverse myelitis. Early criteria involved number of lesions on a single scan [160, 161] but when tested on cohorts of CIS patients were found to have low specificity. The relative importance of individual MRI parameters at presentation was subsequently evaluated to predict conversion to CDMS using logistic regression [162]. The presence of a gadolinium enhancing lesion and a juxtacortical lesion were the most relevant, followed by an infratentorial lesion and three periventricular lesions. The presence of three out of four of the above was more specific (78% compared to 54%) and accurate than the earlier criteria (80% compared with 69%). These were later modified and 9 or more T2 lesions were allowed in place of a gadolinium enhancing lesion (since accuracy was found to be optimal at a minimum of 9 lesions) [163].

Table 3.3 summarising early MRI diagnostic criteria

	Required MRI findings
Paty's criteria [160]	4 lesions or 3 lesions one of which is periventricular
Fazekas' criteria [161]	3 lesions, including 2 of the following characteristics: infratentorial location, periventricular location, lesion >6mm
Barkhof's criteria[162]	3 of the following: ≥ 9 T2 lesions or ≥ 1 Gd enhancing lesion ≥ 3 periventricular lesions ≥ 1 juxtacortical lesion ≥ 1 infratentorial lesion

The early MRI criteria appeared to support evidence of DIS but failed to address the need for dissemination in time (DIT), fundamental to a diagnosis of MS. Brex *et al* following up a CIS cohort at 1 year found that the presence of new T2 lesions at follow-up and enhancing lesions at baseline provided the best combination of sensitivity and specificity for CDMS [140]. In 2001 the McDonald criteria were published which included MRI evidence for DIT to improve the specificity of MRI criteria. The modified Barkhof criteria were retained as evidence of DIS and a gadolinium lesion more than 3 months from symptom onset confirmed DIT (DIT could also be fulfilled with a new T2 lesion with reference to a baseline scan more than 3 month from symptom onset). DIS is also fulfilled with 2 T2 lesions and oligoclonal bands in the CSF. The criteria are highly specific for the development of CDMS at 3 years, (greater than 90%), but they have limited sensitivity (59%) [164]. Sensitivity increases to 76-83% if the 1 year scan is used for evidence of DIS and DIT (when a new T2 lesion subsequent to a scan at least 3 months after the attack could count for DIT), but specificity drops to 83-86% [164, 165]. In response to demonstration that a new T2 lesion on 3 month follow-up scan as evidence of DIT could be used to increase sensitivity from 58% to 84% without compromising specificity, [166] the McDonald criteria were recently modified, with a new T2 lesion (with reference to a baseline scan more than a month from CIS onset) acting as evidence of DIT [167]. The use of the McDonald criteria improves the rate of diagnosis, with the McDonald 2001 criteria more than doubling diagnosis of MS within a year of presentation with a clinically isolated syndrome [164]. This is potentially important as clinical trials have shown delay to CDMS with early Interferon treatment and the Association of British Neurologists (ABN) guidelines

have recently recommended disease modifying therapy for patients with McDonald MS.

3.2 Non-conventional MRI

An autopsy study suggested that T2 weighted MRI may detect only about 65% of plaques in the white matter identified grossly on pathological inspection [168]. Therefore conventional MRI fails to detect a substantial proportion of MS lesions, particularly in the grey matter where lesions are rarely seen on MRI but frequently evident at post mortem. Conventional MRI also lacks pathological specificity. Following the acute active demyelinating stage, signal intensity and borders of lesions appear the same on T2 weighted images, irrespective of the wide variability seen on histopathology. T2 weighted imaging does not allow distinction between remyelinated and fully demyelinated, gliotic lesions or assessment of the degree of axonal loss, differentiation of which could have prognostic implications. Also, MRI reveals no abnormality in non lesion white matter, although it can appear highly abnormal in autopsy studies [38]. Non-conventional MRI techniques, such as magnetisation transfer imaging, spectroscopy and diffusion, offer a new perspective on MRI imaging, quantifying abnormalities in what appears normal appearing brain tissue on conventional imaging.

3.2.1 Double Inversion Recovery

Cortical lesions are poorly visualised on T2 weighted MRI [40] due to poor contrast resolution between grey matter and lesions in cortex compared to lesions in the white matter. Grey matter lesions tend to be less inflammatory, and therefore have shorter relaxation times than white matter lesions. FLAIR acquisition identifies a few more cortical lesions than PD or T2 weighted images, but a newer technique, double inversion recovery (DIR) identifies considerably more, with 538% gain compared with conventional spin echo, and 152% gain compared to FLAIR. It still detects much lower numbers of intracortical lesions compared with histopathology studies [168].

In a large study of CIS, relapsing-remitting and secondary progressive MS patients, DIR identified cortical lesions in 58% of patients, including 36% of CIS patients. The mean number of cortical lesions was significantly higher in secondary progressive MS patients compared to CIS patients. Thus cortical lesions seem to increase with

disease activity and their presence at CIS onset may have important prognostic implications, but their ability to predict disability or CDMS has not been evaluated. Three types of intracortical lesions were identified by DIR: 60% small round/ovoid lesions, 31% worm shaped lesions which followed the profile of gyri with extension into the grey matter, and the rest were wedge shaped with subpial base and apex towards the white matter [169].

3.2.2 Magnetisation Transfer Imaging

Magnetisation transfer imaging (MTI) and ratio (MTR) offer improved pathological specificity, correlating with axonal density and myelin in lesions in autopsy studies [136]. However animal models of experimental allergic encephalitis have shown that MTR is also reduced in inflammation, in the absence of demyelination and axonal loss [170, 171] and may indicate changes in myelin content or structural changes in response to inflammation, not necessarily a loss of myelin. Therefore MTR changes are not specific for changes in myelin content but represent changes in myelin pathology including oedema, axonal loss and more general tissue injury.

There is wide variation in lesion MTR which may reflect different stages of plaque development and tissue loss. Post mortem studies have shown that compared with mildly hypointense T1-weighted lesions, severely hypointense lesions have the lowest MTR and myelin content [126], whereas remyelinated lesions have higher MTR than demyelinated lesions [172]. Voxel-based analysis of a gadolinium-enhancing lesion observed over 6 months *in vivo* and subsequently biopsied, showed MTR was low and stable in the lesion core. On subsequent histopathology this corresponded to the demyelinated centre, with diffuse presence of macrophages and marked loss of oligodendrocytes. Over follow-up, increase in MTR was noted in lesion voxels bordering the normal appearing white matter (NAWM), which corresponded on histopathology to the partially remyelinated lesion border [173]. MTR has also been found to correlate with myelin and axonal density in NAWM [136].

MTR reduction in lesions relative to NAWM has been demonstrated *in vivo* and studies have shown that mean lesion MTR significantly declines with plaque age [174], with detectable change over a year [175]. Progressive reduction in MTR in

pre-lesion white matter has been demonstrated up to 2 years prior to lesion detection on conventional MRI [174, 176]. These findings suggest myelin pathology precedes lesion visualisation and progresses following its appearance on conventional MRI.

Autopsy studies have shown the disease process in MS is not restricted to lesions, and MTR can detect abnormalities in NAWM *in vivo* [177]. Compared to controls, reduction in MTR in NAWM has been reported in patients with minimal disability and disease burden [178, 179] and therefore pathology in normal appearing brain tissue (NABT) is not restricted to the progressive phase of the disease. Some studies have shown reduction in NAWM MTR of CIS patients compared to controls [180-182], although this was not found in smaller studies [183, 184], and may be greater in patients with abnormal imaging. In the same way that lesions accrue, changes in NAWM progress. Significant decline in NAWM MTR over a year, not found in controls, has been observed in CIS, RRMS and SPMS patients [185] resulting in SPMS patients having the lowest values of MTR [186]. This possible evidence for myelin pathology is potentially irreversible, as CIS patients who have not had a further event 14 years later still had detectable MTR reduction in NAWM when compared to controls [181].

MTR reduction has also been detected in grey matter, in patients at all stages of the disease [178, 179] including CIS patients [182]. Again, change in MTR over a year in grey matter is detectable in CIS [187], RRMS [187, 188] and SPMS patients [187], with MTR decreasing at a greater rate in grey matter than NAWM [188]. Back extrapolation of the data in this latter study suggested that changes in the NAWM began prior to symptom onset, and in the grey matter soon after the first clinical event. MTR reduction in grey matter may reflect cortical lesions not visible on conventional MRI. However voxel based analysis of the occipital cortex in CIS optic neuritis patients showed selective reduction in MTR in the visual cortex compared to controls [189]. The selective location of MTR reduction argues against this being due to cortical lesions, as the reduction would be more global, and suggests a more specific link with the symptomatic inflammatory demyelinating optic nerve lesion, via a trans-synaptic pathogenic mechanism.

Studies have shown an association between MTR, particularly in the grey matter, and disability. Cross sectional studies in CDMS patients have shown moderate correlation of MSFC [190] or EDSS and grey matter MTR parameters; mean [179], peak height [190, 191] and peak location [190]. Whereas a PPMS study found a correlation with regional NAWM MTR and EDSS [192], other studies have not found an association between histogram NAWM parameters and disability [179, 191]. An association between MTR and cognitive impairment has also been reported in a mixed MS cohort [193], and in both early [159] and later disease [193].

Medium and longer follow-up of a mixed cohort of CIS, RRMS and SPMS have shown that certain MTR parameters may predict worsening of disability. Worsening of EDSS at 4.5 years was predicted by baseline T2 lesion volume and percentage change in whole brain MTR over 1 year [175] but grey matter peak height and average lesion MTR percentage change over 1 year predicted longer term disability [187]. The prognostic power of MTR has not been fully explored in CIS patients, but studies suggest that early MTR changes do not predict CDMS independently of T2 lesions [182, 184, 189, 194] although one study has shown baseline NABT MTR independently predicts CDMS [180].

3.2.3 Magnetic Resonance Spectroscopy

Magnetic resonance spectroscopy (MRS) offers further insight into MS pathology by quantifying certain metabolites which have been shown to correlate with cell types or disease processes. Water-suppressed proton MR spectra detect protons associated with N-acetyl aspartate (NAA), choline (Cho), creatine (Cr) and phosphocreatine, myo-inositol (Ins), glutamate and glutamine (Glx) and free lipids. NAA has been shown *in vitro* cell culture analysis to be a neuronal marker [195], and correlates with axonal loss in lesion biopsies [196], although it is also present in oligodendrocyte precursor cells. Ins is most raised in chronic lesions with predominant astrocytosis and is therefore a marker of gliosis [195], but is also raised in acute lesions and is a marker of active myelin breakdown [196]. Cho is thought to represent inflammation [197] and membrane turnover [196]. Cr is found at high concentration in glial cells (highest in oligodendrocytes and astrocytes [195]), and is a putative marker of gliosis. As creatine phosphate serves as a phosphate reserve and buffer in ADP/ATP metabolism in neurons, reduction in Cr has been described as indicating metabolic

dysfunction [198]. It was thought to be a stable metabolite [199], changing little throughout the brain in MS, and therefore used in ratios to express relative concentrations of other metabolites. However studies have suggested it may not be as stable as first thought. Cr is raised in acute, gadolinium enhancing lesions [119, 120], and found to correlate with age in healthy adults [121]. Glutamate is a neurotransmitter which is metabolised and released by neurons. It is cleared from the synaptic cleft by astrocytes and converted to glutamine which is re-taken up by neurons and regenerated into glutamate. An excess of glutamate has been implicated in the neuroexcitotoxicity cascade, and may be involved in neuronal loss.

Deriving reliable data from spectroscopy is limited by several issues, such as variation in tissue composition of voxels, particularly partial-volume effects due to the presence of cerebrospinal fluid within the voxel and the position of the voxel in relation to the inhomogeneous radio-frequency of the coil. The data must be standardised to enable comparison. A common internal standard is expressing the metabolite of interest relative to a metabolite that is not affected by the disease process. Cr has been used as an internal standard, but studies have shown this is not always stable, as described above. Another internal standard used is tissue water or cerebrospinal fluid but neither of these are reliable (MRI is very sensitive to changes in tissue water and this is therefore not a stable reference and measurement of cerebrospinal fluid signal intensity is error-prone due to the pulsatile nature of cerebrospinal fluid flow). An external standard is often used, with regular measurement of a phantom containing a known concentration of NAA (or other molecule of interest) to provide a calibration factor for quantification of absolute values of metabolites [122].

In acute lesions, transient changes in metabolites have been demonstrated. Reduction in NAA [140, 199-203] and raised Cho [119, 140, 201, 204-207], Cr [119], free lipid peaks [199, 205, 206] and glutamate [120] have been detected, which partially recover over follow-up [119, 199, 203, 205]. Permanent changes in metabolites are observed in chronic lesions, particularly those that appear hypointense on T1 images. These include reduced NAA [120, 205, 206, 208] and raised Ins [120, 207, 208]. These findings suggest inflammation, membrane turnover and transient axonal dysfunction in acute lesions, with more permanent damage in chronic lesions.

Changes including raised Cho [204, 205] and free lipid peaks [205] have been detected in pre-lesion normal appearing white matter (NAWM). These findings are consistent with MTR changes in NAWM prior to lesion development and suggest inflammation and/or membrane turnover is occurring for some time prior to lesion visualisation on conventional MRI.

Cross sectional studies have shown reduction in NAA in NAWM of patients at all stages of CDMS [192, 202, 209, 210] including early relapsing-remitting MS [178, 210] but reduction is greatest in secondary progressive MS patients [200]. Although NAA was significantly lower in secondary progressive MS than relapsing-remitting MS patients, further decline in NAA over follow-up was only detected in the relapsing-remitting MS patients [200, 211, 212], suggesting progressive axonal loss in the relapsing remitting but not progressive phase. The changes in NAWM NAA ratios over time appeared to be independent of lesion volumes or clinical activity, suggesting that axonal damage is partly dissociated from MRI detectable or clinical disease activity [213]. Other metabolite changes in the NAWM of MS patients include elevated Ins [210, 214], Cho [120, 201, 215], Cr [201, 215], and Glutamate [120] compatible with the autopsy finding of increased inflammation and glial cells in NAWM.

Reduction in NAA has also been reported in the grey matter of CDMS patients [207, 216] including early RRMS patients [210]. Cho [210] and Glx [210, 214] were also reduced in the grey matter.

CIS cohorts have been studied to investigate how early in the disease course changes in the NAWM can be detected. Studies of whole brain or white matter have found reduction in NAA [217, 218] and increased Cho [218, 219] in CIS patients compared to controls; however these have included lesions which consistently show metabolite derangement. One longitudinal study of a patient with a single lesion adjacent to the corpus callosum showed transient reduction in NAA in the homologous site in the contralateral hemisphere (with highest density of axonal projections to or from the lesion) that was not detected elsewhere in the NAWM [203]. Recently a high field single voxel study demonstrated reduction in NAA in CIS patients [220] not detected at lower field strength [221, 222]. The same group found NAA was only significantly

lower in the subgroup of patients who converted to definite MS suggesting a prognostic role [223]. Fernando *et al* demonstrated raised Ins compared to controls at lower field strength, which was not significant in the smaller high field study [221]. Together these findings suggest that there is evidence of increased inflammation and axonal injury at the onset of the disease.

Some cross sectional and longitudinal studies have shown correlation of various metabolite measures with EDSS, MSFC and/or cognitive function in CDMS patients. Correlation of white matter (i.e. including lesions) NAA:Cr with EDSS has been demonstrated in patients with early or mild disease, [224] with decrease over 30 months correlating with increase in EDSS in RRMS but not SPMS patients [211]. This may reflect changes in lesion NAA as studies of NAA in NAWM (i.e. excluding lesions) have not correlated with outcome [207, 209, 210, 214]. Studies have shown a correlation with NAWM Ins and EDSS [207, 214] and MSFC [210, 214] suggesting a role for gliosis rather than axonal loss in disability progression. Cortical grey matter Glx [210] and NAA [214] have been shown to correlate with EDSS and MSFC in some but not all [225] studies.

3.2.4 Atrophy

Brain lesion volume measurement is sensitive for monitoring disease evolution but is only modestly correlated with clinical disability. Measurements of brain atrophy are thought to reflect the neurodegenerative components of the condition, namely axonal loss and demyelination, and therefore better reflect the irreversible pathology occurring in MS, and may be better surrogate markers of disability or predictors of outcome than T2 lesion volume. Other variables affect brain volume measurement such as hydration status and oedema.

MS patients have reduced brain volume compared to healthy age- and gender-matched controls [226-228], with one study showing 3.5% brain parenchymal fraction reduction [226]. With annual atrophy of 0.5 to 0.7% [229, 230], volume change can be detected over as little as 3 months [229, 231].

Brain segmentation, and more recently programs that measure mean and regional cortical thickness have shown that atrophy occurs in both grey [232, 233] and white

matter [226] in MS patients. Most cortical areas appear to be involved [233], with frontal regions being particularly affected [233-235] and pre-central gyri (primary motor cortex) involvement in patients with severe disability.

Atrophy rate appears to be similar across all MS subtypes [227, 230, 232, 236] and occurs early in the disease [226, 237]. Atrophy is even detectable in CIS patients within a year of symptom onset [233, 238, 239], however this is mainly significant in those CIS patients who have a high risk of conversion to CDMS [233, 238-241]. Volume loss in CIS patients appears to be mainly due to grey matter rather than white matter atrophy [233, 241] which may be due to increasing lesion load and oedema during the early stage masking white matter atrophy.

Cross sectional atrophy measures brain parenchymal fraction [227, 229], ventricular fraction [227] and cortical measures (thickness [235, 242] and volume [232]) have been found to correlate with disability. In regression analysis of one study, every 0.08mm loss of cortex caused a unit rise in EDSS [242]. Another study found atrophy in areas corresponding to clinical presentation and correlation with individual functional system scores of the EDSS [233]. Longitudinal atrophy studies have found concurrent correlation with disability [229, 237, 243] and importantly the ability to predict disability status at 8 year follow-up [229].

Cognitive impairment has been associated with volume loss in MS, and cognitive function shown to correlate with cortical atrophy, brain parenchymal volume loss and increased ventricular width [95, 234, 244].

Cross sectional measures of brain atrophy have been related to MRI measures of axonal damage [232] and lesion burden [226, 232, 239, 245], but this relationship does not fully explain the extent of acquired brain atrophy with only 33 to 50% of atrophy measures explained by variation in T2 lesion volume [226, 242, 245]. Some studies have shown a delayed relationship with lesions [246, 247], but the results suggest that atrophy is due to more diffuse processes occurring in normal appearing tissue.

In summary histopathology studies have revealed changes beyond white matter demyelination, including axonal transection and loss, cortical demyelination, and inflammation in normal appearing tissue. Compatible changes have been detected *in vivo* using newer quantitative MRI

3.3 Aims

The aims of the studies in this thesis are to investigate diagnosis and prognosis in CIS patients using MRI parameters obtained at baseline (within 3 months of CIS onset) and early follow-up (3 months from baseline). Previous imaging studies of CIS cohorts have provided great insight into the prognostic power of MRI in MS; however images from these studies were acquired in the 1980's at low resolution. Since this time, MRI techniques have improved and quantitative techniques developed. Between 1996 and 2004, patients within 3 months of CIS onset were recruited into an MRI and clinical follow-up study at the NMR research unit at the Institute of Neurology. This allows investigation of conventional and non-conventional MRI measures acquired at higher resolution and their association with, and prediction of, clinical outcome.

3.3.1. Investigation of the global frequency of MRI abnormalities in isolated optic neuritis

The geographical distribution of MS is well known although poorly understood. It is also clear that the presence of lesions on MRI in CIS patients increases the risk of CDMS. The frequency of such abnormalities varies between cohorts reported from different regions of the world. The first aim of this thesis is to investigate whether this geographical difference in frequency of MRI abnormalities and development of CDMS are related. This is potentially important for interpretation of results from MRI studies and evaluation of diagnostic criteria as they may not apply to patients from other prevalence regions.

3.3.2. Modification of MRI criteria for the diagnosis of MS in CIS patients

Improving diagnosis of MS is important for follow-up in specialist clinics and potentially for the initiation of disease modifying treatment. The recognition of the sensitivity of MRI to MS pathology has led to its incorporation into diagnostic criteria. The McDonald criteria were published in 2001 and allow MS to be diagnosed in a CIS patient where there is MRI evidence of dissemination in space and time (DIS and DIT). These criteria are highly specific for the development of CDMS but have limited sensitivity. They were modified in 2005, with a major modification being the allowance of an early new T2 lesion to count as DIT. The 2005 McDonald criteria have not yet been evaluated but are potentially more sensitive than the 2001 criteria.

However they remain complex and require administration of gadolinium. The second aim of this thesis is to simplify the MRI diagnostic criteria for MS to improve sensitivity and accuracy, without compromising specificity and remove the need for a contrast agent. These simplified criteria along with the McDonald criteria are evaluated with the Poser criteria as the 'gold standard' in my cohort.

Within the Magnims European multicentre collaborative research network which studies MRI in MS, a collaborative study was initiated with groups from Barcelona, Milan and Amsterdam who are investigating similar CIS cohorts. This allows evaluation in a larger cohort with a greater number of patients presenting with non-ON CIS. Also as the McDonald criteria are currently accepted globally, European evaluation is important due to the differences in MS prevalence and frequency of MRI abnormalities.

3.3.3 Early MRI in optic neuritis: risk for CDMS and disability

The final aim is to identify prognostic markers from baseline and early follow-up scans which would be potentially useful for patient counselling and improved targeting of disease modifying therapy. It is not clear from earlier MRI studies of CIS patients whether number of lesions at baseline increases the risk of CDMS or disability, and whether location or activity of lesions contributes independently. Also quantitative measures from normal appearing brain tissue have been shown to be abnormal in CIS patients compared to controls, and whether such measures are stronger markers of clinical outcome has not been determined and is investigated in this thesis. The risk of a second clinical event is investigated in all patients followed up at least once, but the risk of disability is limited to the subgroup followed up at 5 years to allow a spectrum of disability to develop. As 80% of my cohort presented with ON and location of lesions is investigated, this part of the thesis is limited to the ON presentations, to remove the potential difficulty in differentiating symptomatic from asymptomatic lesions in brainstem and spinal cord syndromes.

Chapter 4 Methods

4.1 Patients

Patients aged 16 to 50 within 3 months of CIS onset were recruited from the wards and clinics of the National Hospital for Neurology and Neurosurgery and from the Neuroophthalmology clinic at Moorfields Eye Hospital from 1995 to 2004 following approval from the medical ethics committees of both hospitals. Written, informed consent was obtained from all patients prior to study entry and participation was voluntary at all stages. Follow-up visits were planned for 3 months, 1, 3 and 5 years from baseline and involved clinical assessment and MRI. Follow-up of this CIS cohort is on-going, and several other research fellows preceded me in the patients' recruitment and follow-up. All patients bar one had been recruited prior to my starting this project. I assessed 5 patients at 3 months, 14 at 1 year, 29 at 3 years and 128 at 5 years. Some of the data from the early assessments had been analysed by my predecessors and I completed the data set. Although not used in this thesis, I also analysed the 5 year data from the same MRI acquisitions as described in this thesis.

CIS was defined as an acute isolated event affecting one region of the CNS, presumed to be demyelinating in aetiology, with no previous history of possible demyelinating events, and with maximal symptoms and signs by 14 days of onset. Appropriate investigations were conducted to exclude alternative diagnoses. Study inclusion was based on clinical characteristics and was not influenced by MRI findings.

Patients reported any further neurological events and were classified as CDMS where there was symptomatic and examination evidence of a second neurological episode attributable to demyelination and occurring in a CNS location separate from the initial episode, of more than 24 hours duration and more than 4 weeks from the initial attack. When no symptoms were reported, patients were reviewed clinically to confirm clinical status. Disability was recorded at each visit with the Kurtzke expanded disability scale.

The NHS Strategic Tracing System was used in those cases where the contact details were found to be incorrect, following approval for access from the Security Confidential Advisory Group of the NHS Information Authority and local Research Ethics Committee. Patients who had emigrated and left no forwarding address were

not contactable. When patients were unable or unwilling to attend for a follow-up visit, clinical status was assessed by telephone [248].

Different subgroups of patients were used for the analyses in the results chapters. Figure 4.1 shows the number of CIS patients recruited and followed-up at the end of my study, and Figure 4.2 shows the same data for the ON subgroup.

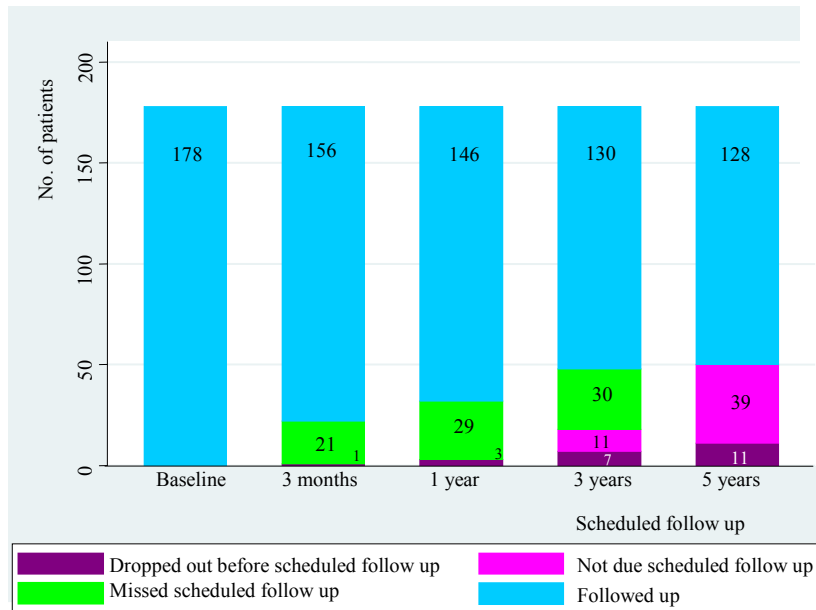


Figure 4.1 showing the numbers of CIS patients who had been followed up at the scheduled time-points by the end of the project

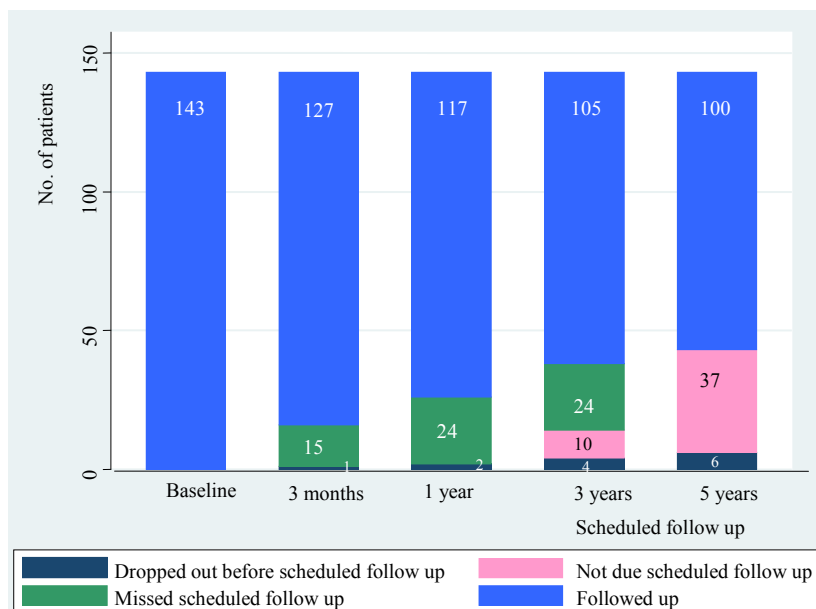


Figure 4.2 showing the numbers of optic neuritis patients who had been followed up at the scheduled time-points by the end of the project

The number of patients analysed in the results chapters depended on how many had been followed up at the time of the analyses, and the type of analysis performed. This is explained below.

Chapter 5: included all optic neuritis patients (ON) initially recruited (n=143) (see Figure 4.2).

Chapter 6: included the 81 CIS patients who had been followed up for 3 or more years at the time of the analysis (2005), along with 9 other CIS patients who had developed CDMS prior to the 3 year scheduled follow up.

Chapter 7: the London cohort of this multicentre study was composed of the 111 CIS patients who had been followed up at 3 years at the time of the analysis (2007).

Chapter 8: included all 142 ON patients who had been followed up at least once (one patient dropped out after the baseline scan) (see Figure 4.2)

Chapter 9: included only the ON patients who had been followed up at 5 years (n=100).

4.2 MRI acquisition protocols

MRI studies were acquired on a 1.5 Tesla GE Signa Echospeed scanner (General Electric Medical Systems, Milwaukee, WI, USA).

4.2.1. Conventional brain MRI

The following brain images were acquired at each visit 5 minutes after administration of 0.1mmol/kg of gadolinium-diethylenetriamine pentaacetic acid (DTPA). For each sequence 46x3mm contiguous slices were acquired in the axial oblique plane (parallel to the anterior/posterior commissural line), with field of view 24cm, matrix 256x256, and 1 excitation:

Proton density and T2-weighted images: dual echo fast spin echo sequence, TR 3200ms and TE 15/90.

T1 weighted images: spin echo sequence TR 600ms TE 14ms

4.2.2. Spinal cord MRI

Spinal cord sequences were performed at baseline, 1, 3 and 5 years after gadolinium administration. For each sequence 9x3mm contiguous sagittal slices were obtained through the whole spinal cord, employing phased array coils, 48cm field of view and 512x512 matrix.

PD and T2 weighted FSE: TR 2500ms TE 56/98

T1 weighted: TR 500ms TE 19ms

4.2.3. Magnetisation transfer imaging

Obtained at 3 month and 5 year visits (from October 1998). A dual echo spin echo sequence (28x5mm contiguous axial oblique slices parallel to the anterior/posterior commissural line) covering the whole brain was performed prior to gadolinium administration using an interleaved sequence; TR 1720ms TE 30/80ms, number of excitations 0.75, matrix 256x128, field of view 24x24cm. Magnetisation and non-magnetisation transfer images were acquired for both TEs; the interleaved nature of the sequence removes the need for coregistration of the images. The sequence was magnetisation weighted by the application of a presaturation pulse (Hamming apodized 3 lobe sinc pulse, duration 64ms, flip angle of 1430 degrees and a peak amplitude of 14.6 μ T giving a normal bandwidth of 62.5 Hz, applied 2Hz from the water resonance). MTR maps were calculated on a pixel by pixel basis using the short echo data because of higher signal to noise compared to the longer echo data.

4.2.4. Spectroscopy

¹H-MRS was acquired prior to gadolinium administration at 3 months, 1, 3 and 5 years from baseline (from August 1997). A fast spin echo axial localising scan (TR 3000ms, TE 14/84ms, matrix 256x192, slice thickness 5mm, interslice gap 1.5mm) was acquired first. Single voxel ¹H-MRS was then acquired from the NAWM in the posterior parietal or centrum semi-ovale regions. The voxel was manually placed so as to maximise the area of NAWM sampled whilst excluding lesions, grey matter and CSF. Either hemisphere could be chosen as studies have shown no evidence for hemispheric differences in NAWM metabolite concentrations [249]. The ¹H-MRS acquisition used a point-resolved spectroscopy (PRESS) sequence with TR 3000ms, TE 30ms and 192 averages. Shimming and water suppression were optimised using a standard automated prescan (GE Medical Systems, Milwaukee, WI, USA), and an ongoing quality assurance programme confirmed that water suppression remained stable throughout the study period.

4.3 Image analysis

4.3.1 Lesions

Hard copies were reviewed by an experienced neuroradiologist (Dr Katherine Miskiel) blinded to the patients' clinical status. Number and location of T2, gadolinium-enhancing and T1 hypointense lesions were marked on baseline images as were new lesions identified at follow-up through close comparison with previous films. All further image analysis was performed on Sun workstations (Sun Microsystems Inc. Palo Alto CA). Dispimage image display software (D.L Plummer University College London Hospitals, London UK), a semi-automated thresholding technique, was used to contour lesions (identified above) on the computer images for calculation of lesion volumes and for lesion masking in subsequent analyses. T2 hyperintense lesions were contoured on the PD-weighted images, using the T2-weighted image for reference. T1 hypointense brain lesions were contoured on the spin echo images. Number of cord lesions identified by the neuroradiologist was also noted. Lesion volumes were calculated by 2 observers (myself n=78 and Kryshani Fernando n=100), and 22 lesion volumes were repeated by both observers to assess agreement. Regression coefficient was 0.99 (95% CI 0.91-1.07 p<0.001, constant -30.42 95% CI -278.79 to 217.95 p=0.801), and 0.98 without the constant (95% CI 0.93-1.04 p<0.001). The mean difference between the raters' lesion volumes was 0.23mls (95% CI 0.01 to 0.64). To test reproducibility, 10 lesion volumes were repeated by the same observer several days apart. Intra-rater regression coefficient was 1.03 (95% CI 1.00-1.06 p<0.001, constant 40.56 95% CI -180.87 to 99.75 p=0.524) and remained 1.03 without the constant (95% CI 1.00-1.05 p<0.001). The mean difference between the 10 lesion volumes was 0.14mls (95% CI 0 to 0.43mls).

4.3.2 Magnetisation transfer imaging

The MTR data was processed by Kryshani Fernando. Lesions were contoured on the non-magnetisation transfer images to create lesion masks which were used to remove the lesions from the images before subsequent analyses. A whole brain mask (excluding CSF and other non-brain parenchyma) was generated in SPM99 (Wellcome Department of cognitive Neurology, Institute of Neurology, Queen Square London UK) and then applied to the (inherently registered) calculated MTR map. The MTR map was then segmented into grey matter and white matter, using a maximum likelihood algorithm with a probability threshold of 75% certainty to allow

separate NAWM and NAGM analyses. Partial volume effects were minimized using a 10 percent units thresholding and outer voxel erosions. NAWM and NAGM histograms, normalized for brain volume, were generated with a bin width of 0.1 percent units and a smoothing window of 0.3 percent units, and the following parameters measured: peak height, peak location, mean MTR. MTR values at the 25th, 50th and 75th percentiles were also obtained.

4.3.3 Spectroscopy

The spectroscopy data was analysed by Kryshani Fernando. Metabolite concentrations (N-acetyl aspartate (NAA), creatine (Cr), choline (Cho), glutamate and glutamine (Glx) and myo-inositol (Ins)) were estimated using the linear combination model (LCModel), a fully automated program requiring input of a calibration factor. The spectra were assessed for quality of acquisition and processing by an experienced observer blinded to the clinical data (Kryshani Fernando) and quality control was achieved by weekly scanning of a phantom solution of 50mM NAA. The calibration factor was adjusted appropriately to compensate for any temporal variations noted in the metabolite phantom measures.

4.3.4 Brain tissue segmentation

2D T2 weighted images were segmented using SPM99, a fully-automated technique, following semi-automated lesion contouring (described above) and masking to prevent misclassification. Images were registered and segmented automatically (correcting for image inhomogeneity) into images representing the probability of any given voxel containing grey matter (GM), white matter (WM), CSF and other tissues based on localisation and signal intensity. A voxel was partitioned to one mask only (the lesion mask overrode all SPM99 tissue classifications). Segmentations were inspected to qualitatively confirm adequate segmentation. GM, WM (segmented WM plus lesions) and brain parenchymal (BP) volumes (GM +WM + lesion volumes) were normalized by dividing by total intracranial volume, generating GM-, WM- and BP fractions (GMF, WMF, BPF). Segmentation was performed by myself (n=66) and Catherine Dalton (n= 58). In 19 cases segmentation could not be performed as the images were not available.

4.3.5 Ventricular volume

A second volumetric measure was obtained from 2D scans using MIDAS interactive algorithm in T1-weighted post-gadolinium brain images. Whole brain was initially segmented using a semi-automated interactive morphologic technique and mean signal intensity over these brain regions was calculated. Ventricular regions were outlined using a semi-automated seed placing thresholding technique with the ventricular-brain boundary set at 60% of whole brain signal intensity. The ventricular region consisted of the lateral ventricles including the temporal horns but excluding the 3rd and 4th ventricles. High signal structures within the ventricles on enhanced images (e.g. blood vessels) were excluded. Ventricular volumes were automatically calculated from the outlined regions by multiplying the total area outlined by the slice thickness. Ventricular volumes were quantified by two separate observers (myself n=66 and Catherine Dalton n=58). In 19 cases electronic images could not be located in the archives. Ten ventricular volumes were measured by both observers to assess agreement and the regression coefficient was 0.97, (95% CI 0.95-0.99 p<0.001, constant -1.88, 95% CI -220.54 to 216.79 p=0.985) and 0.97 on removal of the constant (95% CI 0.96-0.98 p<0.001). The mean difference in observers' ventricular volumes was 0.22mls (95% reference range 0.02 to 0.80). To assess reproducibility, 10 ventricular volumes were repeated by the same observer. Intra-rater regression coefficient was 0.99 (95% CI 0.97 to 1.01 p<0.001, constant 50.89 95% CI -143.25 to 245.04 p=0.562) and 1.00 without the constant (95% CI 0.99 to 1.01 p<0.001). Mean difference in the 10 ventricular volumes by the same rater was 0.07mls (95% reference range 0.03 to 0.37).

Chapter 5

Is the frequency of MRI abnormalities in isolated optic neuritis related to the prevalence of multiple sclerosis? A global comparison

5.1 Introduction

The presence of brain lesions on MRI at presentation with clinically isolated optic neuritis (ON) has been shown to increase the risk of developing clinically definite multiple sclerosis (CDMS) [61, 74, 147] but the proportion of ON patients with MRI abnormalities varies widely between cohorts reported from different regions of the world [77, 148, 250-252]. A question arising is whether the frequency of MRI abnormalities and development of MS in ON patients are related to the prevalence of MS *per se* [253] as this would have potential implications in the use of MRI in diagnosis and prognosis of MS. This was explored by examining the frequency of MRI abnormalities in my London ON cohort and other published studies and the prevalence of MS reported from similar geographical locations.

5.2 Methods

London optic neuritis cohort

143 patients with acute ON were recruited between 1995 and 2004 from Moorfields Eye Hospital in London and underwent brain MRI. All patients were referred by ophthalmologists and reviewed by a single, experienced neuroophthalmologist. Patients with a history of previous neurological events or signs of abnormality outside the optic nerves were excluded. Brain MRI was performed on a 1.5 Tesla scanner within 3 months of symptom onset. The scans were reviewed by an experienced neuroradiologist and the numbers of high signal lesions on both PD- and T2-weighted scans were noted. Scans were reported normal if there were no lesions compatible with demyelination.

Other optic neuritis cohorts

Prospective brain MRI studies of 5 other large ON cohorts from around the world were identified and the frequency of MRI abnormalities noted [77, 148, 250-252]. Other features of the studies also noted included geographical location and year of study, number of subjects, method of referral, recruitment criteria including age range and history of previous or bilateral ON or clinical features such as disc pallor,

duration of symptoms at time of MRI and definition of abnormality on MRI and slice thickness.

Regional MS prevalence estimates

Regional MS prevalence data was acquired from a recent review by Rosati [253] and compared in the countries in which the identified ON studies had been performed: >80/100,000 was defined as high prevalence, 30-80/100,000 medium and <30/100,000 low. As there may be variation in regional MS prevalence within a single country, data from a region close to that of the ON study was used where possible. An exception was the United States, where estimated average prevalence for the country as a whole was used because ON MRI data came from the Optic Neuritis Treatment Trial (ONTT), which was a multicentre trial involving patients from across the country. Using the annually conducted National Health Interview Survey of the United States from 1989 to 1994, the national prevalence of MS was estimated at 85/100,000 [254].

5.3 Results

London cohort

The patients were aged 16-49 years (median 31.9 years), 97 (68%) were female and 46 (32%) male. Two had bilateral sequential ON (2 to 3 weeks interval between episodes) and 141 had acute unilateral ON. Brain MRI performed in all cases within 3 months of symptom onset (median 5 weeks, range 1 to 12). One or more asymptomatic brain lesions compatible with demyelination were present in 112 (78%) patients (median 5 lesions, range 0 to 142).

Other optic neuritis cohorts

Data from other ON cohort studies is summarised in Table 5.1. The frequency of MRI abnormalities ranged from 14% [252] (Japan) to 65% [250] (Sweden).

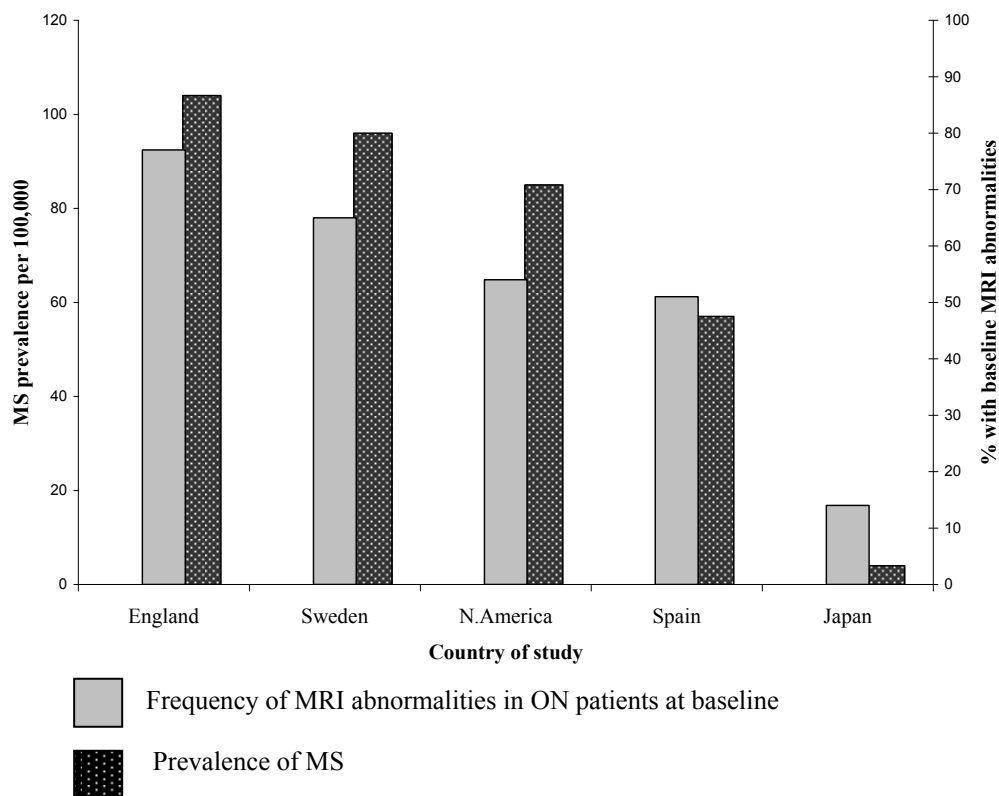
Table 5.1 comparing methodology and percentage of isolated ON patients with baseline MRI abnormalities and nearest regional MS prevalence estimate. *In the Danish study, abnormal MRI was defined as the percentage of patients with at least 2 lesions and data for one or more lesions was not given, therefore this data was excluded from Figure 5.1. **Data represents the 90% ONTT patients in whom MRI findings are reported.

	Years of recruitment	MRI slice thickness	Age range (years)	Maximum time from symptom onset to MRI	% with abnormal MRI	MS prevalence (/100,000) [253]
England, London n=143	1995-2004	3mm	16-49	3 months	78%	104
Sweden, Stockholm n=116 ^[250]	1990-1995	5mm	12-57	24 weeks	65%	96
Denmark, Copenhagen, n=120 ^[251]	1987-1993	4mm	12-59	4 weeks	53%*	112
North America, n=389 ^[148]	1988-1991	5mm	18-46	8 days	54% **	85 ^[254]
Spain, Barcelona, n=123 ^[77]	1995-2001	5mm	14-50	3 months	51%	57
Japan n=70 ^[252]	1991-1996	Not specified	15-55	14 days	14%	1-4

Brain MRI in ON and regional prevalence of MS

Where available, the frequency of MRI abnormalities in ON and a corresponding reported regional prevalence for MS is provided in Figure 5.1.

Figure 5.1 showing the variation in percentage of isolated ON patients with baseline MRI abnormalities and the prevalence of MS in that country



5.4 Discussion

In comparison with rates previously reported in other geographical regions (14-65%) [77, 148, 250-252], a higher proportion (78%) of my cohort of ON patients from southern England exhibited brain MRI abnormalities suggestive of demyelination at presentation. A high prevalence of MS is well documented in southern England [253]. Inspection of the frequency of MRI abnormalities in ON cohorts and corresponding regional MS prevalence suggest that the two are broadly related (Figure 5.1), although the apparent link is not entirely consistent and should be interpreted with caution because of the observational nature of the analysis and multiple methodological differences between reported studies. Such global differences in MRI abnormalities may affect accuracy of diagnostic criteria based on MRI parameters, depending on the strength of the association with MS prevalence. For example in regions with low MS prevalence, diagnostic criteria may be less specific, over-diagnosing patients with abnormalities on MRI at presentation and vice versa, unless the frequency of such abnormalities is closely associated to regional prevalence. Multicentre studies are therefore important in the assessment of MRI criteria. Similarly the prognostic role of MRI may also vary geographically.

It is likely that the Japanese cohort was phenotypically different in some visual features from other cohorts e.g. there was a high frequency of poor visual acuity in the Japanese cohort during the acute phase: 61% had visual acuity worse than 6/60 compared with 36% of the ONTT cohort. MS is much less common in Japan than in Europe or North America. Furthermore, 15-40% of cases of MS in Japan are of the optico-spinal type and 58-63% of this group have been shown to be positive for anti-aquaporin 4 antibody, or NMO-IgG [255, 256], a marker of neuromyelitis optica (NMO) [256]. NMO was previously reported to have fewer lesions on brain MRI than seen in conventional MS [255-257]. However NMO-IgG has brought about change in NMO diagnostic criteria, and up to 60% of patients fulfilling these criteria were found to have brain lesions on MRI, although these were atypical for MS [258]. The overall evidence suggests that ON in Japan is more often associated with NMO or an isolated syndrome with normal MRI and less often due to classical MS.

The ONTT [148] recruited patients from all over the United States limiting comparison of geographical prevalence in studies from smaller, well-defined

catchment areas such as the Scandinavian studies [250, 251]. However the ONTT provided a wealth of clinical and MRI data and, like the London cohort, included a multiethnic and immigrant rich population. By contrast in the Swedish study all patients were Caucasian and only 4% were of non-Swedish origin [250]. Variation in migration patterns and ethnicity may influence MS risk and prevalence and add complexity when comparing studies from different geographical regions.

Considering studies from the last 20 years, the incidence of ON in Japan (1992-1993) of 1.6 to 2/100,000 [259] appears to be similar to incidences reported in Sweden (1990-1995) 1.46/100,000 [260]; Croatia (1985-2001) 1.6/100,000[261], and the UK (1995-1996) 1/100,000 [262]. A higher incidence was reported from Minnesota with age and sex adjusted incidence of 5.1/100,000 [263] (1985 to 1991) although epidemiological ascertainment is likely to be particularly high in this well-defined local region that is served by the Mayo Clinic. Earlier studies report incidences less than 1 per 100,000 and there may have been improvement in case ascertainment or an increase in incidence with time. A study in Hawaii compared the incidence of ON in the Oriental and Caucasian populations and found no significant difference between the two races (0.7 and 1.1/100,000 respectively) [264]. Taken together, these data suggest that the incidence of ON is relatively similar across regions where the prevalence of MS varies markedly: it is therefore plausible that the frequency with which ON is due to MS will also vary. Since the studies reported did not include other known causes (e.g., sarcoidosis, vasculitis, syphilis) it seems likely that in low MS prevalence regions – especially when MRI is normal – ON is often a monophasic inflammatory syndrome. In regions such as Asia with relatively high optico-spinal MS prevalence, neuromyelitis optica may potentially be the underlying cause.

A clinically isolated syndrome study from Barcelona [77] reported that subjects with a brainstem or spinal cord syndrome had a higher frequency of MRI abnormalities at presentation (76%) and a higher conversion rate to clinically definite MS than did those with ON (of whom 51% had abnormal MRI at presentation). The Barcelona study also showed that when only those with abnormalities on MRI were compared, no significant difference in risk for conversion to MS was found between the clinically isolated syndrome subtypes. A medium (~50%) prevalence of MRI

abnormalities in ON patients is concordant with the medium prevalence of MS reported in the same region of Spain [253].

The hypothesis that there is a link between proportions of ON patients with MRI abnormalities and corresponding regional prevalence of MS is not proven by the present work, which is observational in nature. Potential biases exist that limit interpretation of the various ON cohort datasets: these include possible referral biases; different inclusion and exclusion criteria (e.g. age range, unilateral and/or bilateral ON); variations in time from onset of visual symptoms to having MRI; the stringency with which ON patients with other neurological features were excluded; differential proportions of long-term resident versus more frequent migrant populations; use of different MRI scanners, field strengths, acquisition sequences and slice thicknesses; and variable definitions of abnormality on MRI. Furthermore, the cited MS prevalence studies were performed at different time periods and may have had their own biases related to case identification and diagnosis. While the observations support a link between frequency of MRI abnormalities in isolated ON and regional MS prevalence, a prospective epidemiological study design that uses a standardised approach to case identification and diagnosis would be required for definitive clarification.

These caveats aside, this study suggests a link between frequency of MRI abnormalities in ON and MS prevalence. This may have global implications in performance of MRI criteria and prognostic markers for MS.

Chapter 6

Modification of MRI criteria for multiple sclerosis in patients presenting with clinically isolated syndromes.

6.1 Introduction

In 85% of patients with MS the onset is with a clinically isolated syndrome (CIS) defined as an acute clinical event of presumed demyelinating aetiology, such as optic neuritis, bilateral internuclear ophthalmoplegia or partial transverse myelitis, in a patient with no history of neurological symptoms suggestive of demyelination. An essential requirement in making the diagnosis of MS is that there should be objective evidence for central nervous system (CNS) white matter lesions disseminated in space (DIS) and time (DIT). Past criteria relied mainly on clinical evidence for DIS and DIT [106]. Brain MRI criteria for DIS have been developed to improve prediction of clinically definite MS (CDMS) [162, 163, 265]. These criteria have focused on the number, activity (presence of gadolinium enhancement) and location of lesions, and include lesions in three regions that are considered characteristic for demyelination: juxtacortical, infratentorial and periventricular. The McDonald 2001 MRI criteria also require evidence of DIT [266]. While the McDonald criteria have high specificity for subsequent development of CDMS when applied in prospectively followed cohorts with CIS [164, 165], they have several limitations [267, 268]. Notably, the complex MRI DIS criteria [162, 163] (developed for brain MRI by Barkhof *et al* and Tintore *et al*, in addition allowing a spinal cord lesion to substitute for a brain lesion [162, 165]) (see Table 6.1) have been considered too stringent. The DIS criteria also include gadolinium enhancement, which – strictly speaking - is a feature of lesion activity rather than location. In addition the requirement for a gadolinium-enhancing lesion for DIT on a 3-month follow-up scan resulted in low sensitivity at this time point (i.e. many cases who develop CDMS do not fulfil the criteria after 3 months [164]). When a new T2 lesion on follow-up scanning was allowed as evidence for DIT if the reference scan was obtained within 3 months of CIS onset, the criteria became more sensitive and remained highly specific [166].

An early and accurate diagnosis of MS is increasingly important for counselling individual patients and potentially for making decisions on use of disease modifying treatments. This preliminary study explores whether the MRI criteria for DIS and

DIT could be modified to improve the accuracy of early diagnosis. The combination of a less stringent definition for DIS and allowing a new T2 lesion *per se* after 3 months as evidence for DIT are evaluated in my London CIS cohort who have been followed up for 3 years or until the development of CDMS.

6.2 Methods

Rationale for modified criteria

The MRI criteria for DIS were modified with the intention of: (i) retaining the 4 anatomical regions that were included in the McDonald 2001 criteria as they are considered characteristic for demyelination i.e. periventricular, juxtacortical, infratentorial or spinal cord; (ii) reducing to a minimum the number of lesions and regions needed for radiological DIS i.e. there had to be ≥ 1 lesions(s) in ≥ 2 of the 4 regions; (iii) removing the option to include gadolinium enhancement as a feature of DIS (i.e. only T2 lesions and their location are considered). The DIS criteria were evaluated on the 3 month brain scans with and without the inclusion of baseline cord MRI findings. In cases of brainstem and spinal cord syndromes, all lesions within the symptomatic region were excluded.

The rationale for modifying the DIT criteria was that in an earlier study of a subgroup of 56 patients from the currently reported cohort, a new T2 lesion at 3 months follow-up was more sensitive but almost as specific as a gadolinium enhancing lesion (required by the McDonald 2001 criteria) for the development of CDMS [166]. Thus, the modified DIT criteria required ≥ 1 new T2 lesion(s) at 3 months follow-up (a new lesion on the 3 month scan could also contribute to DIS if situated in the regions specified by the criteria).

Patients

This exploratory study was undertaken in 90 prospectively recruited CIS patients (38 males, 52 females, median age 32 years, range 17-50): 67 with optic neuritis (ON; unilateral in 66, bilateral sequential in 1), 15 with a brainstem syndrome, seven with a spinal cord syndrome, and one with an optic tract lesion. Written informed consent was obtained from all patients. Patients were followed clinically until they developed CDMS or for a mean of greater than 3 years if they did not. The study was approved by the Joint Medical Ethics Committee of the Institute of Neurology and National

Hospital for Neurology and Neurosurgery.

MRI

The first MRI was performed within three months of clinical onset (median 5.5 weeks, range 1-12), and consisted of a T2-weighted and gadolinium enhanced T1-weighted brain and spinal cord scan (see Chapter 4 for protocol details [164]). The second MRI was performed approximately 3 months after the first (median 12 weeks, range 9-49) and consisted of a T2-weighted and gadolinium enhanced T1-weighted brain scan.

Statistical analysis

The McDonald 2001 and modified criteria were assessed by comparison with the gold standard for the disease that was defined as CDMS by the Poser criteria [106]. The number of true positives (TP=criteria positive and developing CDMS by 3 years), true negatives (TN=criteria negative and not developing CDMS by 3 years), false positives (FP=criteria positive but not developing CDMS by 3 years) and false negatives (FN=criteria negative and developing CDMS by 3 years) were calculated and used to determine the following:

Sensitivity: the probability of the test finding the disease among those who have the disease ($TP/TP+FN$).

Specificity: the probability of the test finding no disease among those who do not have the disease ($TN/TN+FP$)

Accuracy: the proximity to the true value ($TP+TN/TP+TN+FP+FN$)

6.3 Results

Thirty nine of the 90 patients (43%) developed CDMS as defined by the Poser criteria [106] during follow-up after a median of eight months from clinical onset (mean 14, range 2-48). The 51 (57%) patients who did not develop CDMS were followed up for a median of 39 months (mean 41, range 33-64). Both the McDonald 2001 and modified criteria had a high specificity for development of CDMS but the modified criteria were more sensitive and accurate (Table 6.1).

Both DIT criteria *per se* had high specificity for CDMS but a new T2 lesion was considerably more sensitive than a new enhancing lesion. The DIS criteria *per se*

were less specific than the DIT criteria. The modified criteria for MS had a higher specificity in the ON than non-ON subgroup (95% versus 83%; Table 6.2).

The modified criteria had a high specificity for CDMS in the subgroups of patients whose first scan was performed less than or more than 6 weeks from symptom onset (specificities 93% and 91% respectively; Table 6.3).

Table 6.1 Sensitivity, specificity and accuracy of the McDonald 2001 and modified criteria for CDMS
 TP = true positive (criteria positive & CDMS), FP = false positive (criteria positive & not MS), TN = true negative (criteria negative & not MS) FN = false negative (criteria negative & CDMS)
 Sensitivity = TP / (TP+FN), Specificity = TN / (TN+FP), Accuracy = (TP+TN)/(TP+TN+FP+FN)

	TP	FP	TN	FN	Sensitivity	Specificity	Accuracy
McDonald criteria for MS: brain MRI only	18	3	48	21	46%	94%	73%
McDonald criteria for MS: brain & cord MRI	18	3	48	21	46%	94%	73%
Modified criteria for MS: brain MRI only	29	4	47	10	74%	92%	84%
Modified criteria for MS: brain & cord MRI	30	4	47	9	77%	92%	86%
McDonald criteria for DIS: brain MRI only	30	11	40	9	77%	78%	78%
McDonald criteria for DIS: brain & cord MRI	31	11	40	8	79%	78%	79%
Modified criteria for DIS: brain MRI only	35	13	38	4	90%	75%	81%
Modified criteria for DIS: brain & cord MRI	37	15	36	2	95%	71%	81%
McDonald criteria for DIT	19	5	46	20	49%	90%	72%
Modified criteria for DIT	30	6	45	9	77%	88%	83%

McDonald criteria for DIS on baseline scans:

3 out of: ≥ 9 T2 brain lesion or ≥ 1 Gd lesion
 ≥ 1 infratentorial lesion
 ≥ 3 periventricular lesions
 ≥ 1 juxtacortical lesion
 (1 spinal cord lesion can substitute for 1 brain lesion)

Modified criteria for DIS on baseline scans:

≥ 1 T2 lesion(s) in ≥ 2 of the following regions
 periventricular
 juxtacortical
 infratentorial[#]
 spinal cord*
[#] and * excluded in cases of brainstem and spinal cord syndromes respectively

McDonald criteria for DIT on 3month scans:

≥ 1 new gadolinium enhancing lesion

Modified criteria for DIT on 3month scans:

≥ 1 new T2 lesion

Table 6.2 Performance of the McDonald 2001 and modified criteria* in optic neuritis and non-optic neuritis subgroups

TP = true positive (criteria positive & CDMS), FP = false positive (criteria positive & not MS), TN = true negative (criteria negative & not MS) FN = false negative (criteria negative & CDMS)
Sensitivity = TP / (TP+FN), Specificity = TN / (TN+FP), Accuracy = (TP+TN)/(TP+TN+FP+FN)

		TP	FP	TN	FN	Sensitivity	Specificity
Optic neuritis patients	McDonald criteria for MS	12	2	37	16	43%	95%
	Modified criteria for MS	20	2	37	8	71%	95%
Non-optic neuritis patients	McDonald criteria for MS	6	1	11	5	55%	92%
	Modified criteria for MS	10	2	10	1	91%	83%

Table 6.3 Sensitivity and specificity of the McDonald 2001 and modified criteria* for clinically definite MS in subgroups first scanned less than and more than 6 weeks after CIS onset.

TP = true positive (criteria positive & CDMS), FP = false positive (criteria positive & not MS), TN = true negative (criteria negative & not MS) FN = false negative (criteria negative & CDMS)
Sensitivity = TP / (TP+FN), Specificity = TN / (TN+FP), Accuracy = (TP+TN)/(TP+TN+FP+FN)

		TP	FP	TN	FN	Sensitivity	Specificity
Patients < 6/52 from CIS onset (n=45)	McDonald criteria for MS	8	2	26	9	47%	93%
	Modified criteria for MS	13	2	26	4	76%	93%
Patients > 6/52 from CIS onset (n=45)	McDonald criteria for MS	10	1	22	12	45%	96%
	Modified criteria for MS	17	2	21	5	77%	91%

*Brain and spinal cord MRI findings are included, except that in patients with a brainstem or spinal cord syndrome, lesions in the symptomatic region (i.e. infratentorial and spinal cord respectively) were excluded.

6.4 Discussion

The modified MRI criteria for MS proposed in this study were more accurate than the McDonald 2001 criteria in my London cohort. This was because of an increased sensitivity of both the DIS and DIT components whilst maintaining a high overall specificity. As well as improving the overall accuracy of diagnosing MS in patients with typical CIS, the modified criteria are also less complex than the existing criteria and should be easier to use. In not requiring gadolinium-enhanced MRI, there are potential savings in time and cost.

High specificity is especially important in order to avoid diagnosing a disease when it is not present. It is notable that in both the McDonald 2001 and modified criteria the

DIT component was required to maintain specificity greater than 90%. Both DIS components alone were less specific especially for the modified criteria; this should discourage making a diagnosis of MS in CIS patients based solely on the findings of a single scan.

Although the present study was confined only to patients with a typical CIS, the lower specificity of MRI DIS criteria *per se* – especially the modified criteria - suggest that if MRI is used to establish a diagnosis of MS, the MRI DIT criteria should also be required in patients who have equivocal *clinical* evidence for DIT e.g. a typical CIS plus another vaguely defined neurological episode.

High diagnostic specificity was obtained from brain MRI findings alone, and inclusion of spinal cord MRI findings only slightly increased the overall diagnostic accuracy of the modified criteria. Whilst cord MRI is a primary investigation for spinal cord CIS, its role in patients with ON or brainstem syndromes appears more limited; it may be helpful, especially if brain MRI is abnormal but the DIS criteria are not fulfilled. The similar outcomes for the subgroups first scanned more or less than 6 weeks from symptom onset suggests that the exact timing of the first scan (within 3 months of symptom onset) is not crucial to the performance of the diagnostic criteria.

In order for MRI criteria to be applied reliably, several conditions should be met. First, the CIS should be unambiguously typical of those seen in MS e.g. unilateral ON, bilateral internuclear ophthalmoplegia, partial myelopathy. Neither the McDonald 2001 nor these modified criteria have been tested in cohorts with clinically atypical or equivocal syndromes, nor have they been rigorously compared in established MS versus other white matter diseases. Furthermore, the non-ON cohort in this study was small and other studies of larger non-ON CIS cohorts are warranted. In some populations, the frequency of MRI abnormalities may differ between ON and non-ON CIS [77], and this may influence the performance of diagnostic criteria.

Secondly, CIS diagnosis should be made by an experienced clinician, normally a neurologist or (in ON) a neuroophthalmologist. Thirdly, the criteria should only be applied in younger adults (ages 16-50 years): in children, monophasic acute disseminated encephalomyelitis is more commonly seen, and in older adults non-

specific MRI white matter lesions are frequently encountered. Fourthly, the MRI scans should be of high quality, with careful attention to re-positioning and consistency of image acquisition, and they should be interpreted by an experienced neuroradiologist. Finally, cerebrospinal fluid examination for oligoclonal bands may still be a useful investigation especially where clinical features are atypical; their value in combination with MRI criteria warrants further investigation in prospectively followed CIS cohorts.

This preliminary study suggests that modification of the McDonald 2001 criteria can improve their sensitivity whilst maintaining specificity, within the limits described above. While the modifications used in the present study indicate potential areas for revision of the existing criteria in patients with CIS, it would be prudent that their performance is further evaluated in other CIS cohorts, especially non-ON cases. Also as the frequency of MRI abnormalities appears to vary geographically, it would be beneficial to assess the criteria in cohorts from regions of different MS prevalence.

Chapter 7

MRI criteria for multiple sclerosis in patients presenting with clinically isolated syndromes: a multicentre study

7.1 Introduction

By requiring MRI evidence of dissemination in time (DIT) and space (DIS) the McDonald 2001 criteria [266] are highly specific for the development of clinically definite (CD)MS in patients presenting with a clinically isolated syndrome (CIS), however their sensitivity is limited by stringent DIS criteria and gadolinium enhancement for DIT [164, 165]. In 2005, the McDonald criteria were revised [167]. Changes in imaging criteria included: (i) a greater role for spinal cord lesions as evidence for DIS whilst retaining essentially the same Barkhof-Tintore DIS findings for brain abnormalities, and (ii) allowing a new T2 lesion occurring any time after 30 days from CIS onset as evidence of DIT [167]. The performance of these criteria in predicting CDMS has not been reported.

The results from the preliminary study in chapter 6 suggested that the MRI DIS criteria could be simplified (≥ 1 T2 lesion(s) in ≥ 2 of 4 locations considered characteristic for MS in the McDonald criteria: juxtacortical, periventricular, infratentorial and spinal cord), without compromising specificity if evidence of DIT was sought (a new T2 lesion on a follow-up scan regardless of the timing of a baseline scan). As well as being simpler than the McDonald 2001 criteria, they were more sensitive with similar (high) specificity in my London CIS cohort. Also these new criteria are based on the findings of T2-weighted images alone and do not require gadolinium enhancement. It was important to evaluate the new criteria in a larger CIS cohort, ideally with more non-optic neuritis presentations, as the previous study had suggested the criteria performed less well in such cases. My observational study of optic neuritis CIS patients in Chapter 5 suggested variation in frequency of MRI abnormalities in different geographical regions. Therefore it was decided to evaluate the criteria in a multicentre European cohort to test how robust the MRI criteria were in regions of different MS prevalence and frequency of MRI abnormalities.

Within a European multicentre collaborative research network that studies MRI in MS (called Magnims), data was collected on 282 CIS patients from 4 centres -

Barcelona, Amsterdam, Milan and London - who had two MRI scans within 12 months of CIS onset and were followed up clinically. Some of the patients from one centre are included in previous reports evaluating diagnostic criteria [164, 269]. The performance of the two McDonald (2001 & 2005) and new MRI diagnostic criteria with respect to development of CDMS was investigated.

7.2 Patients and Methods

Patient selection

Each centre was asked to identify CIS patients recruited into prospective MRI and clinical follow-up studies who had the following: (i) baseline scan obtained within 3 months of CIS onset; (ii) follow-up scan obtained between 3 and 12 months of CIS onset (timing dependent on individual centre study protocols and some inevitable variation in scheduling appointments); (iii) clinical follow-up for 3 years or until development of CDMS if this was within 3 years; (iv) not on disease modifying treatment before having a second confirmed clinical episode (i.e. developing CDMS). Each of the centres was a secondary or tertiary referral centre, and consecutive patients were included at each centre.

A total of 282 patients were identified who had two MRI scans within one year of CIS onset and for whom clinical status (CDMS or not) at latest follow-up was known. Of these, 63 who had not developed CDMS had been followed up for less than 3 years, 13 had started disease modifying treatment prior to development of CDMS (4 of these had also been followed up for less than 3 years without developing CDMS) and 2 had a baseline scan much longer than 3 months following CIS onset (29 and 34 weeks). That left a core group of 208 patients who fulfilled all 4 of the above features (including 2 whose first scan was a few days more than 3 months). In this core group, the performance of each of the diagnostic criteria (specificity, sensitivity, accuracy, positive predictive value and likelihood ratio) was investigated, with CDMS within 3 years as the outcome measure.

Clinical details

The following clinical information was provided by each centre: age of CIS-onset, gender, topography of CIS (and second event if applicable), disability using the Kurtzke expanded disability status scale (EDSS) [114]; and the dates of symptom

onset, disease-modifying therapy (DMT), MRI scans and last follow-up visit. Patients were classified as CDMS according to the Poser criteria i.e. where there was symptomatic and examination evidence of a second neurological episode attributable to demyelination of more than 24 hours duration, more than 4 weeks from the initial attack [106]. Alternative diagnoses were ruled out by appropriate tests according to local standards. Diagnosis of CDMS was confirmed by an experienced neurologist at each centre based on clinical information only and regardless of MRI findings.

MRI acquisition and analysis

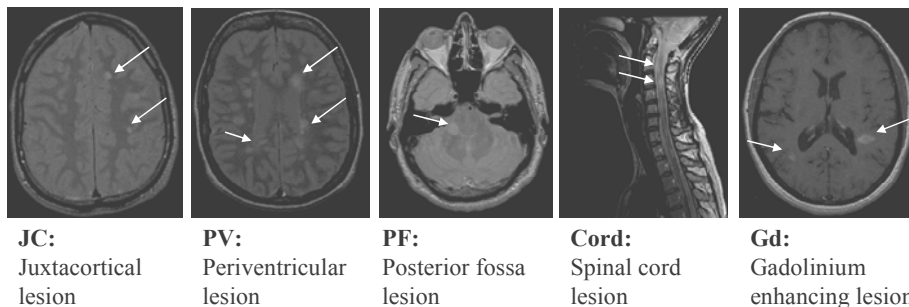
MRI scans were performed on 1.5 Tesla (94%) or 1.0 Tesla (6%) scanners and in 278 cases consisted of axial Proton density (PD) and T2-weighted fast spin echo (FSE) and post contrast T1-weighted spin echo brain MRI (in 4 cases, fast-fluid-attenuated-inversion-recovery (FLAIR) brain images were provided in place of the PD images). T2-weighted sagittal MR images through the spinal cord were also available, on the baseline scan only, in 130 patients; slice thickness varied from 3 to 5mm. For follow-up brain scans, internal landmarks were used for slice positioning identical to baseline.

Areas of increased signal intensity present on both T2 and PD (or FLAIR) images (referred to as T2 lesions) were marked independently by two readers blinded to clinical status. Both readers were trained by experienced neuroradiologists to identify and classify lesions. Only areas of increased signal intensity marked by both readers were counted. Brain lesions were classified as periventricular, juxtacortical, infratentorial or discrete (supratentorial lesions that were neither juxtacortical nor periventricular). Gadolinium enhancing lesions identified on the T1-weighted scans were confirmed on the PD- and T2-weighted scans. Follow-up brain scans were closely compared with the baseline images for new T2 and gadolinium enhancing lesions. The brain and cord images were reported as normal if they had no lesions compatible with demyelination.

Classification of scans using MRI criteria

Table 7.1 Three MRI criteria for Dissemination in Space and Time for MS

	McDonald 2001 ^[266]	McDonald 2005 ^[167]	New criteria ^[269]
Dissemination in space (on either baseline or follow-up MRI)*	3 or more of: 9 T2 lesions or 1 Gd-enhancing lesion 3 or more PV lesions 1 or more JC lesions 1 or more PF lesions 1 cord lesion can replace 1 brain lesion	3 or more of: 9 T2 lesions or 1 Gd lesion 3 or more PV lesions 1 or more JC lesions 1 or more PF lesions or spinal cord lesion Any number of cord lesions can be included in total lesion count	≥1 lesion in each of ≥2 characteristic locations: PV JC PF Cord All lesions in symptomatic region excluded in brainstem and spinal cord syndromes
Dissemination in time	(i) a Gd-enhancing lesion at least 3 months after CIS onset (ii) A new T2 lesion with reference to a prior scan at least 3 months after CIS onset.	(i) A Gd-enhancing lesion at least 3 months after CIS onset (ii) A new T2 lesion with reference to a baseline scan obtained at least 30 days after CIS onset.	A new T2 lesion on follow-up MRI irrespective of timing of baseline scan



* The McDonald 2001 and 2005 DIS criteria also include the presence of two or more T2 lesions plus cerebrospinal fluid (CSF) oligoclonal bands. Because CSF was not examined systematically in the Magnims cohort, only the MRI criteria for DIS are considered in this study

Each scan was classified according to the McDonald 2001 and 2005 and new criteria (Table 7.1), plus their separate components of DIS and DIT. For the new criteria, all lesions within the symptomatic region were excluded in patients presenting with brainstem or spinal cord syndromes (not required for the McDonald criteria).

Statistical analysis

Core cohort

The performance of the MRI criteria was evaluated using the clinical status of the core cohort patients at 3 years as the outcome: CDMS or CIS. The number of true

positives (TP; fulfilling the MRI criteria and developing CDMS), false positives (FP; fulfilling the criteria but not developing CDMS), true negatives (TN; not fulfilling the criteria and not developing CDMS) and false negatives (FN; not fulfilling the criteria but developing CDMS) was determined. From this, the sensitivity, specificity, accuracy, positive predictive value, and both positive and negative likelihood ratios - all with 95% confidence intervals - were calculated:

Sensitivity: the probability of the test finding CDMS among those who have CDMS (TP/TP+FN)

Specificity: the probability of the test finding no disease among those who do not have CDMS (TN/TN+FP)

Accuracy: the proximity to the true value (TP+TN/TP+TN+FP+FN)

Positive predictive value: the proportion of patients with positive test results who are correctly diagnosed (TP/TP+FP)

Positive likelihood ratios: how many times more likely patients with CDMS are to have a positive test result than patients without CDMS (sensitivity/1-specificity)

Negative likelihood ratio: how many times less likely patients without CDMS are to have a positive test result than patients with CDMS (1-sensitivity/specificity)

Survival cohort

The time to CDMS from CIS onset was analysed using Cox proportional hazards regression; patients who did not develop CDMS were censored at the time of last follow-up. The following covariates were entered into all the Cox regressions, in order to adjust the effects of the diagnostic criteria: gender (binary), disease modifying treatment (binary), centre (categorical: Barcelona, Amsterdam, Milan, reference category London) and type of patient (categorical: brainstem, spinal cord, 'other', reference category optic neuritis). Estimates can also be considered age-adjusted, since although age was not included in final models, its inclusion (non-significant) did not materially alter estimates.

In order to assess the effect of the diagnostic predictors, two types of model were used to generate the curves in Figures 7.1 and 7.2 respectively:

i) Figure 7.1 shows predicted survival curves for the four groups 'neither DIS nor DIT', 'DIS only (not DIT)', 'DIT only (not DIS)' and 'both DIS and DIT'. The aim

of the first type of model, used for Figure 7.1, was to estimate the independent effects (as measured by hazard ratios) of DIS and DIT: in these models DIS and DIT were adjusted for each other, so that any estimated effect of, say, DIS, was due to just the patients' DIS status and not also to their DIT status. These models were also used to assess (using an extra DIS+DIT indicator) whether there was any additional effect of being both DIS and DIT over and above the accumulation of the independent DIS and DIT effects. There was no evidence for such an additional effect, and so the combined DIS+DIT term was omitted from these models: this implies that on average the increase in hazard due to a patient being DIS is the same whether that patient is DIT positive or not; and that the most efficient and potentially least biased estimate of the hazard ratios 'DIS only' vs. 'neither', and 'both' vs. 'DIT only', are given by a single weighted average of the two comparisons (and correspondingly for DIT). It should be noted therefore that although, for example, the hazard ratio reported with the 'DIS only' curves in Figure 7.1 are 'DIS only' vs. 'neither', the same quantity also estimates 'both' vs. 'DIT only' (and correspondingly for 'DIT only'). The 'both DIS and DIT' vs. 'neither' hazards ratio was estimated by accumulating the separate DIS and DIT effects.

ii) Figure 7.2 shows predicted survival curves for the four groups 'neither DIS nor DIT', 'DIS only or both DIS and DIT' (i.e. all DIS), 'DIT only or both DIT and DIS' (i.e. all DIT), and 'both DIS and DIT'. The aim of the second type of model, used for Figures 7.2a, b and c, was to estimate the effects of DIS or DIT *per se*, unadjusted for each other, that is, including, in the DIS estimate, any effect due to DIS positive patients tending also to be DIT positive; and *vice versa*. The hazard ratios associated with the two middle curves in Figure 7.2 are thus 'DIS or both' vs. 'neither' and 'DIT or both' vs. 'neither'.

In the type ii) models, the 'both DIS and DIT' vs. 'neither' effect was estimated explicitly using a DIS+DIT term, rather than, as in models in i), by accumulating separate DIS and DIT estimates. As a result the estimated hazards ratio of 'both' vs. 'neither' is very slightly different in i) than in ii), but their great similarity confirms the evidence from the data that the 'both' effect can be considered as the accumulation of the two independent effects. In calculating the plots in Figures 7.1 and 7.2, covariate values were set at the sample mean, thus predicting the survival

curves for varying DIS and DIT status of patients with otherwise ‘average’ covariate values.

To compare statistically the relative performance of the three diagnostic criteria, terms from the three criteria were entered together (along with the covariates) in a Cox regression model. This was repeated for the DIS terms, the DIT terms, and finally the DIS+DIT terms; the latter model is reported in full in Table 7.6.

The natural origin for a survival time scale is CIS onset and all 282 patients were included in a survival analysis from CIS onset, which estimated the proportion who were conversion free (i.e. had not developed CDMS) at last follow-up. However, 29 patients developed CDMS before their second MRI scan. Most of these were both DIS and DIT positive at the follow-up scan (20, 23 or 25 by the McDonald 2001, 2005 or new criteria respectively), but their status prior to conversion cannot be known precisely, in contrast with the remaining 253 patients; as a precaution, a secondary survival analysis was carried out using the second scan as origin for the time scale, thus excluding the 29 early converters and an additional six patients whose conversion time was the same as their second scan. This secondary analysis thus comprised 247 patients. There was no evidence that the proportional hazards assumption of the Cox models was violated. Analyses were carried out in Stata 9.2 (Stata Corporation, College Station, Texas, USA). Statistical significance is taken at the 5% level.

7.3 Results

Clinical and MRI features of the three year “core” cohort (Table 7.2)

The demographic data of the cohort (n=208) followed up for 3 years or until development of CDMS is summarised in Table 7.2. Median age of the cohort was 31 years. One hundred and twenty three (59%) patients presented with optic neuritis, 42 (20%) with a brain stem and 27 (13%) a spinal cord syndrome; most of the remaining cases had an isolated cerebral hemisphere syndrome. Median EDSS at baseline was 2 (range 0-8). Baseline MRI scans were performed a median of 6.2 weeks from CIS onset (range 0-13.6); 72 were performed less than 30 days from CIS onset. Follow-up scans were all performed a median of 4.7 months from CIS onset (range 3.0 to 11.9).

The median interval between baseline and follow-up scans was 3.2 months (range 1.5 to 10.0 months).

Table 7.2 Demographic, clinical and MRI findings in the CIS cohorts followed up for 3 years

Characteristic		3-year core cohort (n=208)	All cases (n=282)
Age of onset (yr)		31.0 yrs (range 14-52)	31.0 (range 14-52)
Gender (M/F)		72:136 (35 % ♂ 65% ♀)	102:180 (36 % ♂ 64% ♀)
Center location	London	109 (52.4%)	111 (39.4%)
	Barcelona	60 (28.9%)	118 (41.8%)
	Amsterdam	13 (6.2%)	23 (8.2%)
	Milan	26 (12.5%)	30 (10.6%)
Presenting clinical syndrome	Optic neuritis	123 (59.0%)	155 (55.0%)
	Brainstem syndrome	42 (20.2%)	57 (20.2%)
	Spinal cord syndrome	27 (13.0%)	45 (16.0%)
	Other	16 (7.7%) (9 hemispheric, 1 polyregional, 6 unknown)	25 (8.8%) (12 hemispheric, 1 polyregional, 12 unknown)
Median EDSS baseline		2 (range 0-8)	2 (range 0-8)
Median time between event onset and baseline MRI		6.2 weeks (range 0-13.6 weeks)	5.1 weeks (range 0-34 weeks)
Median time between baseline and follow-up MRI		3.2 months (range 1.5-10.0 months)	3.2 months (range 1.5-10.0 months)
Median time between event onset and follow-up MRI		4.7 months (range 3.0-11.9 months)	4.6 months (range 2.5-11.9 months)
Median duration of follow-up in non-converters		45.0 months (range 36-119 months)	39.1 months (range 3.8-119 months)
No. with abnormal baseline brain MRI (%)		163 (78.4%)	213 (75.5%)
No. with abnormal follow-up brain MRI (%)		167 (80.3%)	218 (77.3%)
No. with abnormal baseline cord MRI (%)		45/130 (34.6%)	52/147 (35.4%)
No. fulfilling 2005 McDonald criteria for DIS at baseline (%)		95 (46%)	123 (44%)
No. fulfilling 2005 McDonald criteria for DIS at follow-up (%)		102 (49%)	130 (46%)
Median no. of T2 lesions at baseline		6 (range 0-120)	6 (range 0-120)
Median no. of T2 lesions at follow-up		7 (range 0-122)	7 (range 0-122)
No. with new T2 lesion (%)		88 (42.3%)	115 (40.8%)
No. with Gd lesion on follow-up (%)		55 (26.4%)	72 (25.5%)
No. developing CDMS (%)		85 (40.9%)	101 (35.8%)
Median time to CDMS (range)		7.9 months (1-35 months)	12 months (1 to 68 months)

Baseline MRI

Brain MRI was abnormal in 163 (78%) at baseline. Abnormal baseline cord imaging was seen in 45/130 (35%) of patients where cord imaging was available; 5/45 with

abnormal cord imaging had normal brain imaging. The 2001 and 2005 McDonald and new DIS criteria were fulfilled in 95 (46%), 95 (46%) and 117 (56%) respectively

Follow-up MRI

Brain MRI was abnormal in 167 (80%) at follow-up. The McDonald 2001 and 2005 and new DIS criteria were fulfilled in 104 (50%), 102 (49%) & and 123 (59%) respectively. New T2 lesions were seen in 88 (42%) and gadolinium enhancing lesions in 55 (26%) of follow-up scans.

Clinical follow-up

After 2 years 69/208 (33%) and after 3 years 85/208 (41%) had developed CDMS.

Performance of the MRI diagnostic criteria (Table 7.3)

With CDMS after 3 years as the outcome, the performance of the 2001 and 2005 McDonald and new criteria, along with their DIS and DIT components alone, are all presented in Table 7.3. The main findings are: (i) all 3 criteria have high specificity (87-91%) and positive predictive value (77-79%) for CDMS; (ii) the new criteria (72%) are more sensitive than the 2005 McDonald criteria (60%), which in turn are more sensitive than the 2001 McDonald criteria (47%); (iii) for all 3 criteria, having both DIS and DIT gives higher specificities and positive likelihood ratios than either the DIS or DIT components alone; (iv) the DIT component of all 3 criteria is more specific than DIS alone with higher positive likelihood ratios.

A new T2 lesion *per se* had a specificity of 80% when all cases were considered and 81% in those whose baseline scan was performed more than 30 days from CIS onset (n=136); a gadolinium enhancing lesion *per se* on follow-up (all cases) had a specificity of 89%. When the new criteria were applied in the subgroup whose baseline scan was performed more than 30 days from CIS onset (n=136), specificity was 87% and sensitivity 69%.

Table 7.3 Performance of MRI criteria for development of clinically definite MS

OVERALL CRITERIA								
DIS	DIT	No. test positive	Sensitivity (95% C.I)	Specificity (95% C.I)	Accuracy (95% C.I)	PPV (95% C.I)	+ve L.R. (95% C.I)	-ve L.R (95% C.I)
McDonald 2001	McDonald 2001	51	47.1% (36-58%)	91.1% (85-95%)	73.1% (67-79%)	78.4% (65-89%)	5.3 (3-10)	0.6 (0.5-0.7)
McDonald 2005	McDonald 2005	66	60.0% (49-70%)	87.8% (81-93%)	76.4% (70-82%)	77.3% (65-87%)	4.9 (3-8)	0.5 (0.3-0.6)
New criteria	New criteria	77	71.8% (61-81%)	87.0% (80-92%)	80.8% (75-86%)	79.2% (68-88%)	5.5 (3-9)	0.3 (0.2-0.5)
DISSEMINATION IN SPACE CRITERIA								
DIS	DIT	No. test positive	Sensitivity (95% C.I)	Specificity (95% C.I)	Accuracy (95% C.I)	PPV (95% C.I)	+ve L.R. (95% C.I)	-ve L.R (95% C.I)
McDonald 2001	-----	104	76.5% (66-85%)	68.3% (59-76%)	71.6% (65-78%)	62.5% (52-72%)	2.4 (2-3)	0.4 (0.2-0.5)
McDonald 2005	-----	102	75.3% (65-84%)	69.1% (60-77%)	71.6% (65-78%)	62.8% (53-72%)	2.4 (2-3)	0.4 (0.2-0.5)
New criteria	-----	123	85.9% (77-92%)	59.4% (50-68%)	70.2% (63-76%)	59.4% (50-68%)	2.1 (2-3)	0.2 (0.1-0.4)
DISSEMINATION IN TIME CRITERIA								
DIS	DIT	No. test positive	Sensitivity (95% C.I)	Specificity (95% C.I)	Accuracy (95% C.I)	PPV (95% C.I)	+ve L.R. (95% C.I)	-ve L.R (95% C.I)
-----	McDonald 2001	55	51.8% (41-63%)	88.6% (82-94%)	73.6% (67-79%)	75.9% (63-86%)	4.6 (3-8)	0.5 (0.4-0.7)
-----	McDonald 2005	80	68.2% (57-78%)	82.1% (74-88%)	76.4% (70-82%)	72.5% (61-82%)	3.8 (3-6)	0.4 (0.3-0.5)
-----	New criteria	88	74.1% (63-83%)	79.7% (71-86%)	77.4% (71-83%)	71.6% (61-81%)	3.7 (3-5)	0.3 (0.2-0.5)
INDIVIDUAL DISSEMINATION IN TIME FEATURES								
DIT on follow-up scan		No. test positive	Sensitivity (95% C.I)	Specificity (95% C.I)	Accuracy (95% C.I)	PPV (95% C.I)	+ve L.R. (95% C.I)	-ve L.R (95% C.I)
New gadolinium enhancing lesion		55	49.4% (38-60%)	89.4% (83-94%)	73.1% (67-79%)	76.4% (63-87%)	4.7 (3-8)	0.6 (0.5-0.7)
New T2 lesion All cases		88	74.1% (63-83%)	79.7% (71-86%)	77.4% (71-83%)	71.6% (61-81%)	3.7 (3-5)	0.3 (0.2-0.5)
New T2 lesion & baseline scan at least 30 days after CIS onset (n=136)		56	68.9% (56-80%)	81.3% (71-89%)	75.7% (68-83%)	75.0% (62-86%)	3.7 (2-6)	0.4 (0.3-0.6)

Table 7.3 footnotes

DIS = dissemination in space
+ve L.R. = positive likelihood ratio
PPV = positive predictive value

DIT = dissemination in time
-ve L.R. = negative likelihood ratio
95% CI = 95% confidence interval

When the baseline spinal cord MRI findings were excluded, there was slight decrease in specificity (New: 86% vs. 87%, McDonald 2001: 90% vs. 91%, McDonald 2005: 87% vs. 88%) and sensitivity (New: 69% vs. 72%; 2001 McDonald 2001: 46% vs. 47%; 2005 McDonald: 58% vs. 60%) of all 3 criteria.

If the lesions in the symptomatic region were included in brainstem and spinal cord syndromes, the new criteria were slightly more sensitive and less specific (73% and 85% respectively.)

Clinical and MRI features of the survival analysis cohort (Table 7.2)

The clinical characteristics of this group are very similar to the core cohort. There were 180 females (64%) and 102 males; median age at CIS-onset was 31 years (range 14-52), median EDSS was 2 (range 0-8); 155 (55%) presented with optic neuritis, 57 (20%) with a brainstem syndrome, 45 (16%) with a spinal cord syndrome and 25 (9%) with other syndromes. All patients converting to CDMS before the end of their follow-up period were included as CDMS in this analysis (i.e. including those converting after 36 months). Duration of follow-up ranged from 3.3 to 119 months, median 40 months. One hundred and one patients (35.8%) converted to CDMS a median 12 months from CIS onset (range 1 to 68 months). Of the 282 patients included in the survival analysis, 55 had received disease modifying treatment, which was initiated before a second neurological event in 13 patients and after a diagnosis of CDMS in 42 patients. Patients who only received disease modifying treatment after CDMS conversion were included in the 'not receiving treatment' group.

Conversion free survival analyses (Figures 7.1 & 7.2 and Tables 7.4 & 7.5)

Table 7.4: Cox regression survival analysis from time of CIS onset: all hazards ratios are compared with having neither DIS nor DIT (n=282)

Criteria		Hazard ratio	95% C.I.	P
McDonald 2001	DIS only	3.30	2.0 to 5.6	<0.001
	DIT only	2.52	1.6 to 4.1	<0.001
	All DIS	6.34	3.7 to 10.8	<0.001
	All DIT	8.43	4.8 to 14.9	<0.001
	DIS&DIT	9.17	5.2 to 16.3	<0.001
McDonald 2005	DIS only	2.64	1.6 to 2.1	<0.001
	DIT only	3.44	2.1 to 5.6	<0.001
	All DIS	6.72	3.8 to 11.8	<0.001
	All DIT	8.24	4.7 to 14.5	<0.001
	DIS&DIT	9.71	5.4 to 17.3	<0.001
New	DIS only	3.75	2.0 to 6.9	<0.001
	DIT only	3.45	2.1 to 5.7	<0.001
	All DIS	7.76	4.1 to 14.7	<0.001
	All DIT	10.33	5.4 to 19.9	<0.001
	DIS&DIT	12.33	6.4 to 23.9	<0.001

The following covariates had significant (or borderline significant) associations with conversion free survival probability and were therefore retained in the model for all survival analyses: (i) gender (less conversion in males); (ii) study centre (less conversion in Barcelona versus London patients); (iii) CIS location (higher conversion in non optic neuritis than optic neuritis patients); (iv) disease modifying treatment (slightly lower conversion in those receiving treatment). Age was not significant, and made no material difference to estimates when included, and so was omitted from all models.

Table 7.5: Cox regression survival analysis from time of CIS onset: Hazards ratios when all 3 'both DIS and DIT' criteria are entered with covariates (n=282)

		Hazard ratio	95% C.I	p
New criterion		4.46 ^a	2.4-8.4	<0.001*
2001 McDonald criterion		1.29 ^a	0.7-2.5	0.47
2005 McDonald criterion		1.41 ^a	0.6-3.2	0.41
Male vs. female		0.56	0.3-0.9	0.018*
Site: vs. London	Barcelona	0.49	0.3-0.9	0.016*
	Amsterdam	0.64	0.3- 1.5	0.30
	Milan	0.65	0.3-1.3	0.24
CIS type: Vs. Optic neuritis	Brainstem	1.58	0.9-2.8	0.11
	Spinal cord	1.96	1.0-3.9	0.058
	Other	1.86	0.8-4.1	0.13
Treatment		0.27	0.08-0.9	0.04*

^aHazard ratio for 'both DIS and DIT' vs. 'neither or one only'.

In Table 7.4 the ‘DIS only’ and ‘DIT only’ hazard ratios estimate the independent increased conversion risks due to a patient having DIS or DIT, controlling for each other. These hazard ratios were used to construct the corresponding survival curves in Figure 7.1. The ‘DIS&DIT’ survival curve in Figure 7.1 was constructed by accumulating the hazards ratios of these independent DIS and DIT risks, making the assumption that the combined risk to a patient with both is simply the product of the two independent effects; this is the ‘DIS+DIT’ hazard ratio is shown in Figure 7.1.

The ‘All DIS’ and ‘All DIT’ hazard ratios in Table 7.4 reflect the increased hazard ratios without controlling for each other, thus including any effect due to DIS positive patients tending also to be DIT positive and *vice versa*. These hazard ratios were used to construct the corresponding survival curves in Figure 7.2. The ‘DIS&DIT’ hazard ratio in Table 7.4 is estimated directly from an explicit comparison of the ‘both DIS & DIT’ group with patients who are neither DIS nor DIT, without assuming independent effects, and this hazard ratio is used to construct the corresponding survival curves in Figure 7.2. The ‘DIS&DIT’ hazard ratios with and without the independence assumption are quite similar, tending to confirm the validity of the independence assumption.

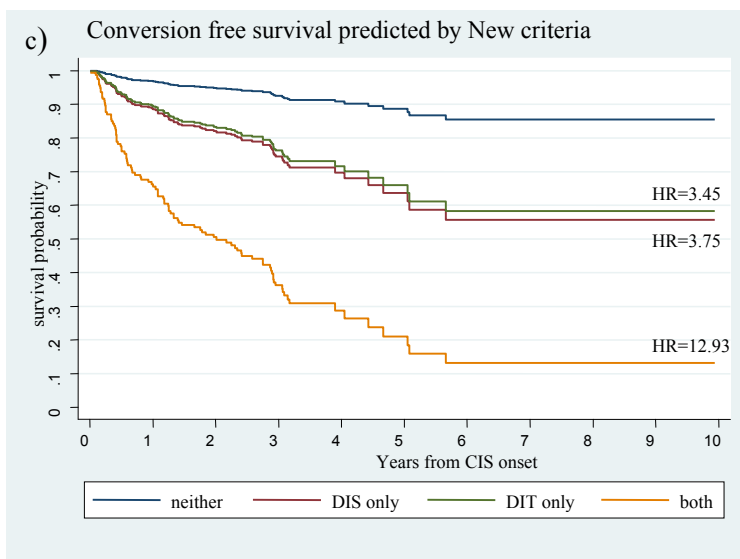
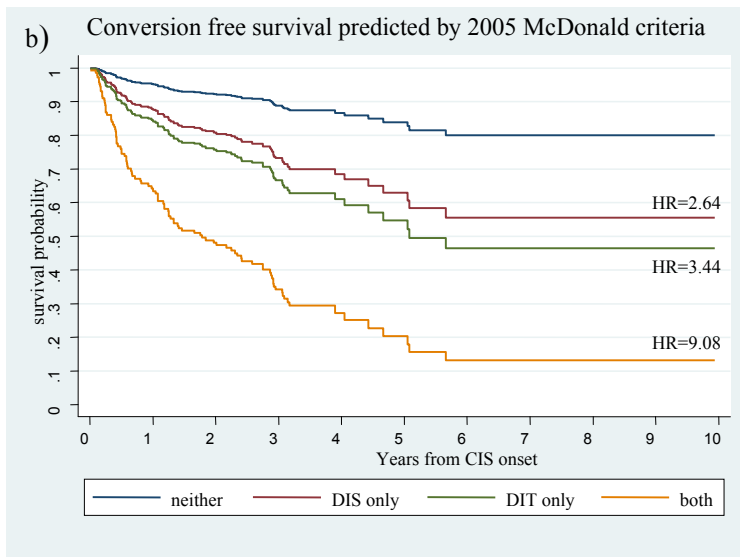
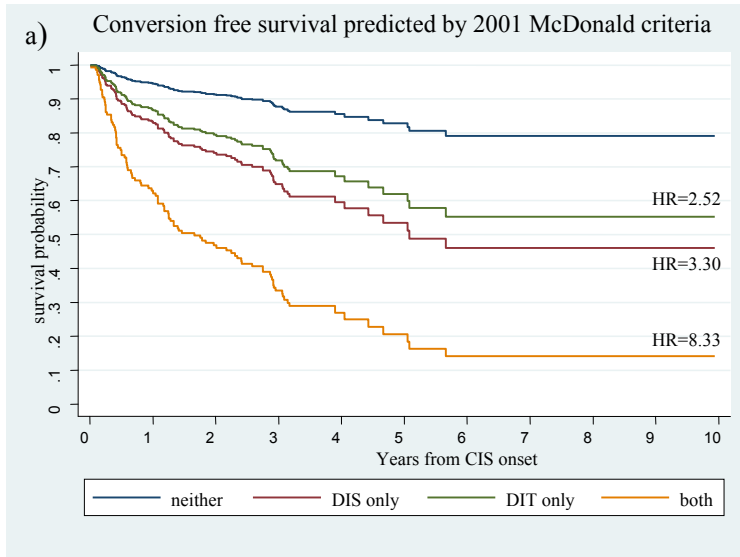


Figure 7.1: Survival probabilities for
 (i) neither DIS nor DIT
 (ii) DIS only (adjusted for DIT)
 (iii) DIT only (adjusted for DIS)
 (iv) both DIS and DIT.
 Figure 7.1a: 2001 McDonald criteria
 Figure 7.1b: 2005 McDonald criteria
 Figure 7.1c: New criteria
 All graphs adjusted for other covariates.

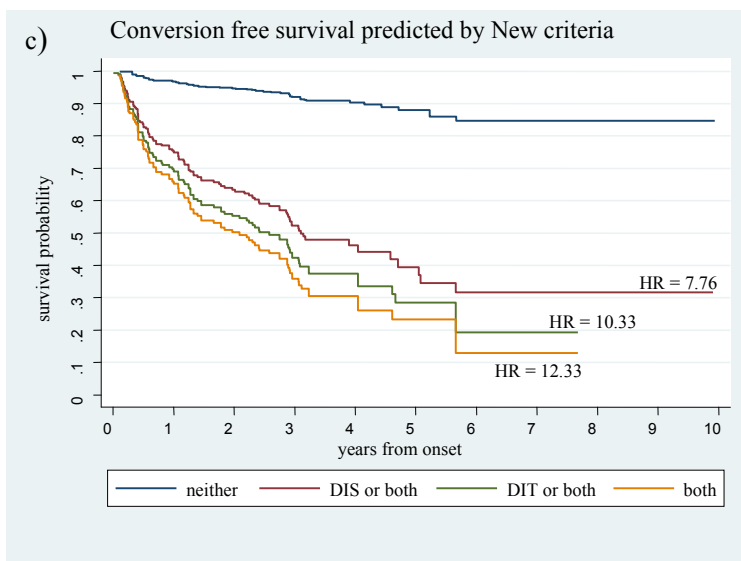
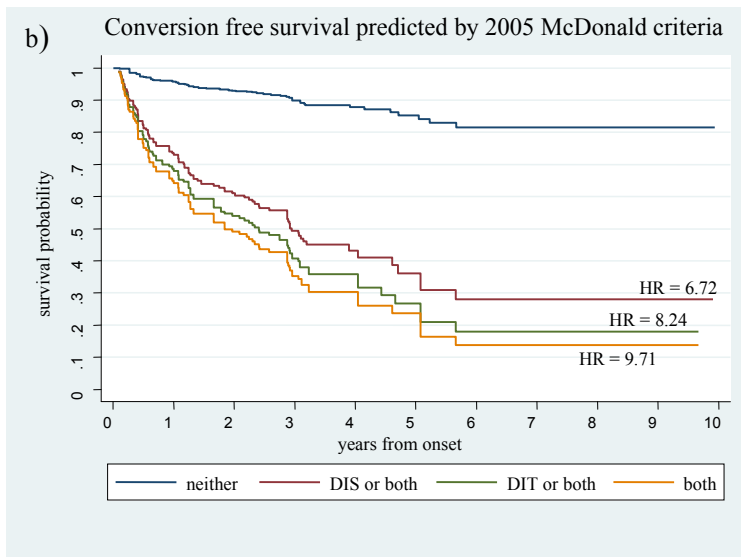
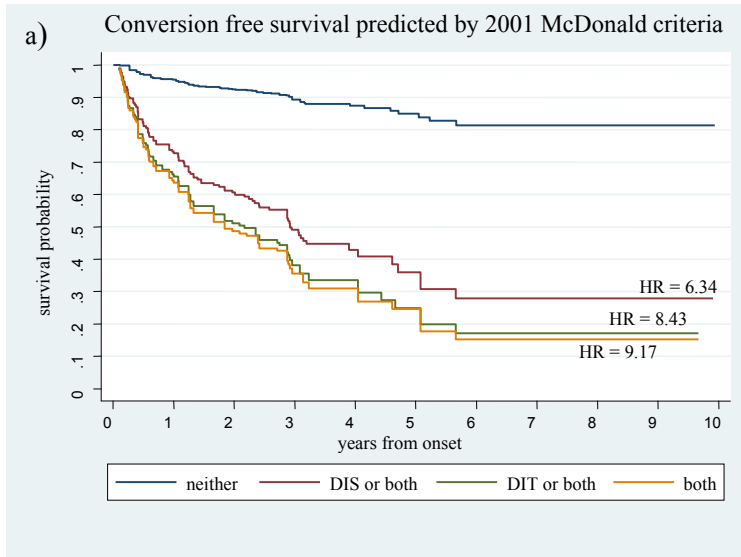


Figure 7.2: Survival probabilities for
 (i) neither DIS nor DIT
 (ii) **all** DIS (i.e. not adjusted for DIT)
 (iii) **all** DIT (i.e. not adjusted for DIS)
 (iv) both DIS and DIT.
 Figure 7.2a: 2001 McDonald criteria
 Figure 7.2b: 2005 McDonald criteria
 Figure 7.2c: New criteria
 All graphs adjusted for other covariates.

For all three sets of criteria, Table 7.4 shows estimated hazard ratios are higher for patients with both DIS and DIT than for those positive on just one criterion alone, and this is reflected in the Fig 7.1 survival curves. For the new and the 2001 McDonald criteria, 'DIS only' has a higher hazard ratio than 'DIT only', but this is reversed for the 2005 McDonald criteria, as reflected in the Fig 7.1 curves. The 'only' hazard ratios are highest for the new criteria.

As would be expected, Table 7.4 shows the 'All DIS' and 'All DIT' hazard ratios are closer to the 'DIS+DIT' hazard ratios, since they include the effect of patients with both; and this is reflected in the proximity of the survival curves for these groups in Figure 7.2. For all three sets of criteria, the 'All DIT' hazards ratios are higher than for 'All DIS', again shown in Figure 7.2. Table 7.4 shows the 'All DIS' and 'All DIT' hazards ratios are highest for the new criteria; this is reflected in Figure 7.2c, by the wider gap between the 'neither' curve and the other groups: the 'neither' New group has higher conversion free survival than for the other criteria, with rather similar curves for the other groups.

When the three indicator terms for being DIS positive (by the three criteria) were entered together into a model (along with the covariates), the hazards ratios for the two McDonald criteria were substantially reduced and lost statistical significance, suggesting no evidence of further predictive contribution once the new criteria was in the model. When the corresponding new criteria term was entered into the model, similar reductions in hazard ratios and loss of significance for the two McDonald criteria occurred for the DIT positive criterion, and for the 'both DIS and DIT' criterion. The details of the latter model are given in Table 7.5. This shows that the hazard ratio for being both new DIS and DIT positive, vs. neither or one only, is 4.46, and still highly significant, whereas the corresponding hazard ratios for the McDonald 2001 and 2005 criteria are respectively 1.29 and 1.41, and no longer significant (when entered singly into the model the new and the 2001 and 2005 McDonald criteria gave highly significant hazard ratios of respectively 6.54, 4.21 and 5.12).

The secondary analysis using time from second MRI scan, and excluding patients who converted before the second scan, gave very similar comparative results between the three sets of criteria, but with hazard ratios reduced throughout by around 15%.

7.4 Discussion

Because all 3 criteria have a high specificity for CDMS, they provide a reliable diagnosis of MS within the first year after CIS onset. The similar positive predictive values (77-79%) indicate that most patients who fulfil any of the 3 criteria will develop CDMS within 3 years and further support their reliability as diagnostic tools.

The 2001 McDonald criteria are less sensitive in detecting cases of MS in the first year of CIS follow-up. This relates to the requirement for a gadolinium enhancing lesion as evidence for DIT on follow-up when the baseline scan is performed less than 3 months from CIS onset (as usually occurs in practice). The McDonald 2005 criteria allows a new T2 lesion after 30 days from CIS onset and this is the main reason for increased diagnostic sensitivity compared with the 2001 criteria (60% vs. 47%). The new criteria allow a new T2 lesion seen at any time on a follow-up scan, which further increases sensitivity compared to the 2005 McDonald criteria (72% vs. 60%), although the more liberal new DIS criteria also contributes.

For all 3 diagnostic criteria, the DIS component *per se* was less specific for CDMS than the DIT criteria *per se*. Consistent with this observation; the survival curves show a higher HR for all DIT cases versus all DIS cases (Figure 7.2). The greater conversion to CDMS when there is DIT is consistent with studies in established MS, which show that relapses are more likely to occur in patients who develop new MRI lesions [153]. The lower specificity and positive predictive value of all the DIS criteria *per se* caution against making a diagnosis of MS in CIS patients until there is also evidence of DIT. The new DIS criteria were least specific (59%), and suggest that two lesions in two MS-characteristic locations are not sufficient to diagnose MS in CIS patients.

A recent study from the same Magnims collaboration assessed the Barkhof-Tintore (i.e. 2001 McDonald) brain DIS criteria alone in a cohort of 349 patients followed up for at least 2 years, and found a higher specificity (79% vs. 68%) and lower sensitivity (49% vs. 75%) than the same criteria applied in my study cohort followed for 2 years [265]. A contributory cause for this difference is that there was a second

follow-up scan in the present study that identified additional cases of DIS. Another potential reason is that in the previous study, lesions were defined as areas of increased signal intensity greater than 3mm, whereas no such rule was applied in the present study and small white matter abnormalities that were considered to be definite were called lesions. While requiring a minimum lesion size may increase the specificity of DIS criteria, they will also become less sensitive, i.e. more cases of MS do not fulfil the criteria. In the North American Optic Neuritis Treatment Trial, lesions had to be >3mm in size for a scan at baseline to be considered abnormal and this may have contributed to the unusually low frequency of patients in whom any MRI abnormality was reported (46%) [74]. Deciding lesion size during reporting is problematic since actual measurement is impractical. It is also noteworthy that the specificity of both DIT and the overall diagnostic criteria were high in my study without requiring a minimum lesion size. It is doubtful whether a minimum lesion size is necessary, especially in the brain and when imaging is of high quality and has the slice thickness of this study (3-5mm).

The high overall specificity of the new criteria (DIS + DIT both present) suggests that as long as DIT is present, the simpler new DIS criteria may be sufficient for diagnosis. The new criteria had the highest positive likelihood ratio and lowest negative likelihood ratio of the criteria tested, and were therefore the strongest at ruling in and out the disease respectively. Whereas sensitivity and specificity evaluate how the outcome predicts a particular test result, likelihood ratios do the reverse, i.e. evaluate how a particular test result predicts the risk of the outcome [270]. The simplified new criteria were also the only criteria that remained significant in the survival curve analysis when all 3 criteria were added to the model. Compared to the McDonald DIS criteria, the new criteria should be easier to remember and apply in practice; furthermore, they do not require gadolinium enhanced imaging, which provides time and cost savings that could be helpful in some health delivery settings and avoid rare adverse effects. Not including gadolinium enhancement in DIS criteria may seem logical since it is a feature of lesion state or activity, not location *per se*. The advantage of not administering gadolinium (time, costs & safety) most likely will counter-balance the slight loss of differential diagnostic information (e.g. leptomeningeal enhancement in sarcoidosis). A limitation of the new criteria is that they require two scans in order to show both DIS and DIT, and it is noted that when

the first scan is obtained at least 3 months after CIS onset, it is possible – using the McDonald criteria - for both DIS and DIT to be fulfilled on the single scan that includes gadolinium enhanced images.

Spinal cord MRI was available in over a half of the patients at baseline, although inclusion of cord lesions as allowed by each of the three diagnostic criteria only slightly improved their specificity and sensitivity (increases ranging from 1-3%). Similar experience has been reported in previous analyses of a subgroup of CIS patients from one of the centres, where most patients had isolated optic neuritis [141, 166], but not in a study of patients recently diagnosed with MS, which found a larger increase of sensitivity for the McDonald 2001 criteria [143]. Cord images were not available at follow-up to determine their impact on DIT. However, in established relapsing remitting MS, serial MRI shows considerably more new lesions occurring in the brain than in the cord [271] and it seems improbable that the situation is different in CIS patients. Spinal cord imaging will be required when there is a spinal cord CIS, especially if there is clinical concern that it is not due to inflammatory demyelination; it is unlikely to alter diagnostic classification in most patients with a non-spinal cord CIS, although detection of characteristic cord lesions may increase confidence that the syndrome is due to demyelination [272].

In the 2005 McDonald DIT criteria, a new T2 lesion on follow-up scanning must be identified with reference to a scan obtained at least 30 days from CIS onset - this is intended to ensure that new T2 lesions are not attributable to the presenting event (monophasic disease) [167]. However, in the new criteria, a new T2 lesion on follow-up scan is counted as DIT irrespective of timing of the baseline scan. Since many patients have their first scan within 30 days of CIS onset, it is advantageous to allow this scan as the baseline for determining subsequent DIT. The new criteria were equally specific (87%), whether applied to all cases or to the subgroup of patients with baseline scans after >30 days.

In addition to the MRI criteria, several covariates had a significant effect on risk of conversion to CDMS. Females had a higher risk than males and this finding is consistent with some previous reports of cohorts with optic neuritis [273, 274]. Baseline brain MRI was more often abnormal in females than males (78% vs. 72%)

and this may have contributed to their higher risk of conversion. Compared with the London cohort, conversion to CDMS was less common in Barcelona patients. Baseline brain MRI abnormalities were less common in the Barcelona cohort (65% vs., 76%) and the prevalence of MS is probably lower in Spain than in the United Kingdom, as discussed in chapter 5 [77, 275]. These factors may have influenced the risk for developing CDMS. In chapter 6 concern was raised that the modified criteria were less specific in the non optic neuritis cohorts than the optic neuritis cohort. In this multicentre study with greater numbers of non optic neuritis patients, there was a trend for higher conversion rates in the non optic neuritis versus optic neuritis cohorts. This may also be related to the higher frequency of brain MRI abnormalities seen at presentation in the former groups (84% of non optic neuritis and 68% of optic neuritis) [77]. The lower rate of conversion to CDMS in the small group of 13 patients who received disease modifying treatment before having a second clinical episode is consistent with the known effect of such treatment in delaying the time to convert from CIS to CDMS [72, 276, 277]. Importantly, the influence of all these covariates was taken into account in the survival model and the clear effect of the MRI diagnostic criteria on risk for CDMS was retained.

There are several limitations to the present study. First, scanning was limited to the first year of follow-up; it does not evaluate the imaging criteria at longer intervals following CIS, although since CDMS will develop in more patients over time, their main use is to make an early and accurate diagnosis. Secondly, clinical follow-up in the core cohort was limited to 3 years. However, many who develop MS will do so within 3 years of a CIS, and the survival analysis captured additional subjects with later conversion to CDMS. There is also some evidence that a short interval to the first relapse has a poorer long-term prognosis [80, 81, 84], which gives added relevance to criteria that predict early conversion. Furthermore, a recently reported Kaplan Meier analysis of a CIS cohort followed up for a median of 7 years showed that the presence on baseline MRI of DIS using the Barkhof-Tintore criteria was a risk factor for both conversion to CDMS and disability [147]. Further investigation is warranted to evaluate the criteria after 5 or more years when more patients would have converted to CDMS. It may be that with longer follow-up, the DIS criteria alone prove to be more reliable in predicting long-term risk of MS as is suggested by the

survival curves. Longer follow-up would also clarify the long-term outcome following an early diagnosis of MS based on both MRI DIS and DIT.

Thirdly, in all patients, scanning was performed on 1.5 Tesla or lower field strength scanners; 3 Tesla scanners are now entering practice and seem to impact at least the McDonald criteria [278]. Fourthly, all but 4 patients in this study had dual echo T2-weighted (fast) spin echo rather than FLAIR imaging; the latter is more sensitive to juxtacortical and less sensitive to infratentorial lesions. While FLAIR imaging is useful as an adjunct to conventional T2-weighted scanning, the diagnostic criteria should be evaluated on the latter scans. Fifthly, disease modifying treatment was given before a second clinical event to 13 patients in the survival analysis cohort. Of these patients the majority did not go on to have a second event thus explaining the significance of disease modifying treatment in the survival analysis. However, these patients were excluded from the analysis of the diagnostic criteria in the core cohort. Sixthly, because many patients did not have a lumbar puncture, cerebrospinal fluid findings were not included in the evaluation; however, cerebrospinal fluid oligoclonal bands are included in the McDonald DIS criteria. Seventhly, the cohort remained weighted to ON presentation, (55% of the overall cohort), and spinal cord and brainstem presentations were under-represented. As non-ON presentations were significantly associated with increased conversion to CDMS in the Cox regression analysis, it would be helpful to have a more representative spread of CIS topographies for evaluation of the criteria in the different subgroups.

Finally, the criteria were applied retrospectively to cohorts that were recruited in centres with an interest in MS and neuroimaging, and only cases considered to have a clinical syndrome characteristic for demyelination were included. The performance of the criteria in CIS patients with clinical features considered atypical for MS and in patients with other multifocal diseases of CNS white matter has not been addressed, nor have the criteria been assessed in a new, prospectively recruited cohort. Nevertheless, the patients in this study reflect the spectrum of CIS encountered at presentation in patients who go on to develop MS and, unlike the placebo arm of cohorts studied in trials of beta Interferon in CIS who were selected for the presence of MRI abnormalities, the present study includes patients with normal baseline MRI;

inclusion of such cases is necessary to properly evaluate diagnostic criteria. Overall, this study supports the use of MRI diagnostic criteria for MS in CIS patients.

Chapter 8

Early MRI in optic neuritis. I: the risk for clinically definite MS

8.1 Introduction

As discussed in the previous chapters, white matter lesions characteristic of demyelination are seen on MRI in 50-74% of CIS patients at presentation [99, 137, 138, 222, 279], and their shape, size, activity and location aid diagnosis. Whether such MRI characteristics have an additional prognostic role is of equal interest, and their ability to predict outcome is investigated in the following two chapters.

Thirty eight to 78% of patients presenting with a CIS converted to clinically definite (CD)MS in studies of up to 20 years follow-up [61, 74, 147, 156, 280-282]. MRI studies have shown that patients with no lesions at presentation have a 15-22% risk of CDMS compared to 56-88% of patients with 1 or more lesions [61, 74, 147]. While the simple presence of lesions on MRI increases the likelihood of developing CDMS, the predictive effect of more detailed early MRI measures – including lesion number and volume, location and activity – has been less studied.

Whereas long-term follow-up of an ON cohort and another mixed CIS cohort recruited in the 1980s showed no obvious effect of lesion number on the risk for CDMS [61, 74, 280], follow-up of a later CIS cohort (recruited in the 1990s) showed that risk of CDMS was stratified by baseline lesion number [147]. MRI contrast agents were not used in the early studies, and gadolinium-enhancing lesions (which are typically present for 2 to 8 weeks during the initial evolution of a new lesion and correspond to active inflammation on histology [127]) have since been associated with conversion to CDMS [149, 162, 283, 284]. New T2 lesions on follow-up scans have also been reported to predict a subsequent clinical episode [140, 145, 149]. Certain locations of white matter lesions, namely periventricular, corpus callosum, juxtacortical, infratentorial and spinal cord are considered characteristic of demyelination and their relative contribution in predicting CDMS has been investigated at 0.5 or 0.6T [162]. Higher field strengths are now routinely acquired and may preferentially influence detection of lesions in certain locations. The spinal cord was not imaged in the early CIS cohort studies and asymptomatic cord lesions are found in 27%-42% of CIS patients at presentation [140-142]. These may add

independent prognostic information in patients with brain lesions [143, 285]. Lastly, changes in normal appearing brain tissue detected by non-conventional, quantitative MR techniques may help differentiate CIS patients at greatest risk of conversion to CDMS [180, 241, 286].

It is potentially important to identify patients who are at high risk of early conversion to CDMS, as such patients may be at greater risk of developing significant disability [81].

I therefore have now investigated in the ON subgroup of a CIS cohort who have been followed in the NMR Research Unit over the last decade [140-142, 164, 166, 241, 287] whether brain lesion number at presentation demonstrated at 1.5T increased the hazard of a second clinical episode after an average follow-up of ~5 years. I also investigated whether location of lesions in the brain and spinal cord, or new brain lesion activity on an early follow-up scan or non-conventional MRI parameters adds prognostic information independent of total lesion number and volume.

I have limited this follow-up report to the 80% of my CIS cohort who presented with ON due to limited statistical power of the small non-ON groups and to ensure all brain and cord lesions were asymptomatic.

8.2 Methods

Patients

Patients aged between 16 and 50 within 3 months of ON onset with no previous neurological history suggestive of demyelination or evidence of CNS abnormality outside the optic nerves, were consecutively recruited from a Neuroophthalmology clinic at Moorfields Eye Hospital between 1995 and 2004 as part of a prospective follow-up study. All patients were reviewed by a single neuroophthalmologist and blood tests were performed as required to exclude other causes. Local ethics committee approval was obtained prior to starting the study, and informed consent was obtained from patients at each visit. Follow-up assessments were planned at 3 months, 1, 3 and 5 years from baseline and included MRI, history of further events, clinical examination and documentation of EDSS. Patients were classified as CDMS where there was symptomatic and examination evidence of a second neurological

episode attributable to demyelination and occurring in a CNS location separate from the initial episode, of more than 24 hours duration and more than 4 weeks from the initial attack. Duration of follow-up was calculated as the time from ON onset to the last follow-up visit. When patients were unwilling or unable to attend for follow-up, but were happy to be contacted, history was obtained over the telephone. As it was not possible remotely to confirm a second clinical episode, only patients who had been given a diagnosis of CDMS by a neurologist locally were classified as such for the purposes of this study.

MRI acquisition protocols

All images were acquired on a 1.5 Tesla GE scanner and have been described in detail in chapter 4 and summarised in Table 8.1. In this report, MRI findings at baseline and 3 month follow-up only are considered as the focus is on *early* imaging predictors.

Table 8.1 summarising MRI acquisition protocols and image analysis, FOV: field of view, MTI: magnetisation transfer imaging, MRS: magnetic resonance spectroscopy GMF: grey matter fraction, WMF: white matter fraction, BPF: brain parenchymal fraction, TIV: total intracranial volume, NAWM normal appearing white matter, GM: grey matter

	Slice thickness	Orientation	Matrix	FOV	TR/TE (comments)	Image analysis
Brain post contrast, baseline and 3 months	46x3mm	contiguous axial slices	256x256	24cm	3200/15ms 3200/90ms 600/14ms	<i>Number and location of T2, Gd-enhancing, T1-hypointense lesions</i> identified by neuroradiologist and contoured using semi-automated thresholding technique to calculate volume [288]. <i>Ventricular volume</i> measured on T1 weighted scans at baseline using MIDAS [239, 289]. <i>GMF, WMF and BPF</i> calculated from SPM99 segmented T2-weighted images following lesion masking [241]: GMF=GM/TIV, WMF=WM+lesion volume/TIV, BPF=GM+WM+lesion volume/TIV
Cord post contrast, baseline	9x3mm	contiguous sagittal slices	512x512	48cm	2500/56ms 2500/98ms 500/19ms	Number of T2, Gd-enhancing lesions identified by neuroradiologist
MTI [182, 290] pre contrast, 3 months	28x5mm	contiguous axial oblique slices	256x128	24cm	1720/30ms 1720/80ms (MT weighted with application of presaturation pulse.)	Lesion masks created on non-MT images, whole brain mask generated and segmented into NAWM and GM in SPM99 and applied to calculated MTR map determining NAWM and GM pixels. Normalised NAWM and GM histograms generated.
¹H-MRS [221] pre contrast, 3 months		single voxel manually placed in NAWM			3000/30ms (PRESS sequence with 192 averages with optimised shimming and water suppression)	Metabolite concentrations estimated using LCModel and spectra assessed for quality of acquisition and processing by observer blinded to the clinical data

Statistical analysis

Cox proportional hazard regression was used to assess the association between early MRI parameters and time to CDMS in all patients followed up at least once from baseline scan (n=142). In patients not converting to CDMS, duration of follow-up was used as the censoring time.

Cox proportional hazard regression analysis was performed in 4 stages.

Stage 1: the strongest independent predictors of CDMS on the baseline scan were determined from multivariate analysis from within each of 4 baseline scan variable types: (i) *lesion load* (T2 lesion number, T1-hypointense and T2 lesion volume); (ii) *T2 lesion location* (periventricular, juxtacortical, discrete cerebral, infratentorial and spinal cord); (iii) *lesion activity* (gadolinium enhancing lesions) and (iv) *tissue volumetric measures* (grey and white matter fractions (GMF, WMF), brain parenchymal fraction (BPF) and ventricular volume).

Stage 2: the significant predictors from analysis within the 4 baseline scan variable types were entered together to identify the strongest overall independent predictors on the baseline scan.

Stage 3: the 3 month scan was analysed in the same way as Steps 1 and 2 for the following variables: (i) *new lesion activity* (new T2 lesions, gadolinium enhancing lesions), (ii) $^1\text{H-MRS}$ measures (N-Acetyl aspartate (NAA), and myo-inositol (Ins) in normal appearing white matter (NAWM)), and (iii) *MTR measures* (NAWM and grey matter peak height, peak location and mean).

Stage 4: the significant baseline and 3 month variables were entered together to identify the strongest overall independent predictors from both scans.

Number of lesions were categorised into groups for statistical analysis. If there was a linear trend across the ordinal categories, and no evidence of non-linearity, the ordinal score was entered into the models. For analysis of baseline brain T2 lesion number,

patients were grouped by percentiles to ensure similar sized groups (0, 1-4, 5-14 and 15 or more brain lesions). The number of lesions at each given location and the number of active lesions (gadolinium enhancing or new T2 lesions) were analysed as (i) binary variable (i.e. presence of 1 or more lesions) and (ii) ordinal variable (grouped into 0,1 and 2 or more lesions). The baseline measures location and activity were chosen as they are useful in the differential diagnosis of white matter lesions [162, 266, 267] but it is less clear whether they affect prognosis.

Volumetric measures (including lesion volumes), MTR and MRS variables were analysed as continuous variables.

Age at onset and gender were entered and all models can be considered adjusted for these covariates. Where significant their respective hazard ratios (HR) are quoted. Disease modifying therapy was not initiated prior to developing CDMS and was not adjusted for.

All statistical analysis was performed on Stata 9.2 statistical software (Stata Corporation, College Station, Texas, USA).

8.3 Results

Of 143 ON patients recruited, one was lost to follow-up after the baseline visit (a further patient was lost after the 3 month visit and two after both 1 and 3 year visits). Therefore 142 ON patients reviewed at least once from baseline were included in the Cox regression analysis. Demographic details and MRI findings are summarised in table 8.2.

Table 8.2: summarising demographic details of the cohort and MRI abnormalities at baseline and 3 month follow-up and number of patients converting to CDMS, ON: optic neuritis, CDMS: clinically definite MS

Age at ON onset	32			
Gender	46 (32%) male 96 (68%) female			
Median EDSS at baseline	1 (IQR 1-1)			
Median delay from ON onset to baseline MRI	5 weeks (IQR 3-8)			
Median delay from ON onset to 3 month MRI	20 weeks (IQR 13-29)			
Number attending 3 month follow-up	127 (1 patient did not receive contrast)			
Number converting to CDMS by final follow-up	57 (47%)			
Number converting to CDMS before 3 month follow-up	8 (6%)			
Median time to CDMS	16 months (IQR 6-36)			
Duration of follow-up	non-converters 62 months (IQR 37-90) converters 61 months (57-80)			
MRI findings	No. of patients with:		% converting to CDMS with:	
	no lesions	≥ 1 lesion	no lesions	≥ 1 lesion
Brain at baseline	31 (22%)	111 (78%)	10%	49%
Periventricular at baseline	51 (36%)	91 (64%)	18%	53%
Juxtacortical at baseline	59 (42%)	83 (58%)	27%	49%
Discrete at baseline	65 (46%)	77 (54%)	23%	55%
Infratentorial at baseline	97 (68%)	45 (32%)	34%	53%
Spinal cord	104 (74%)	37 (26%)	34%	57%
Brain and/or cord at baseline	28 (20%)	114 (80%)	7%	48%
Gd enhancing at baseline	105 (74%)	37 (26%)	30%	68%
New T2 at 3 months	81 (64%)	46 (36%)	22%	74%
Gd enhancing at 3 months	102 (81%)	24 (19%)	31%	79%

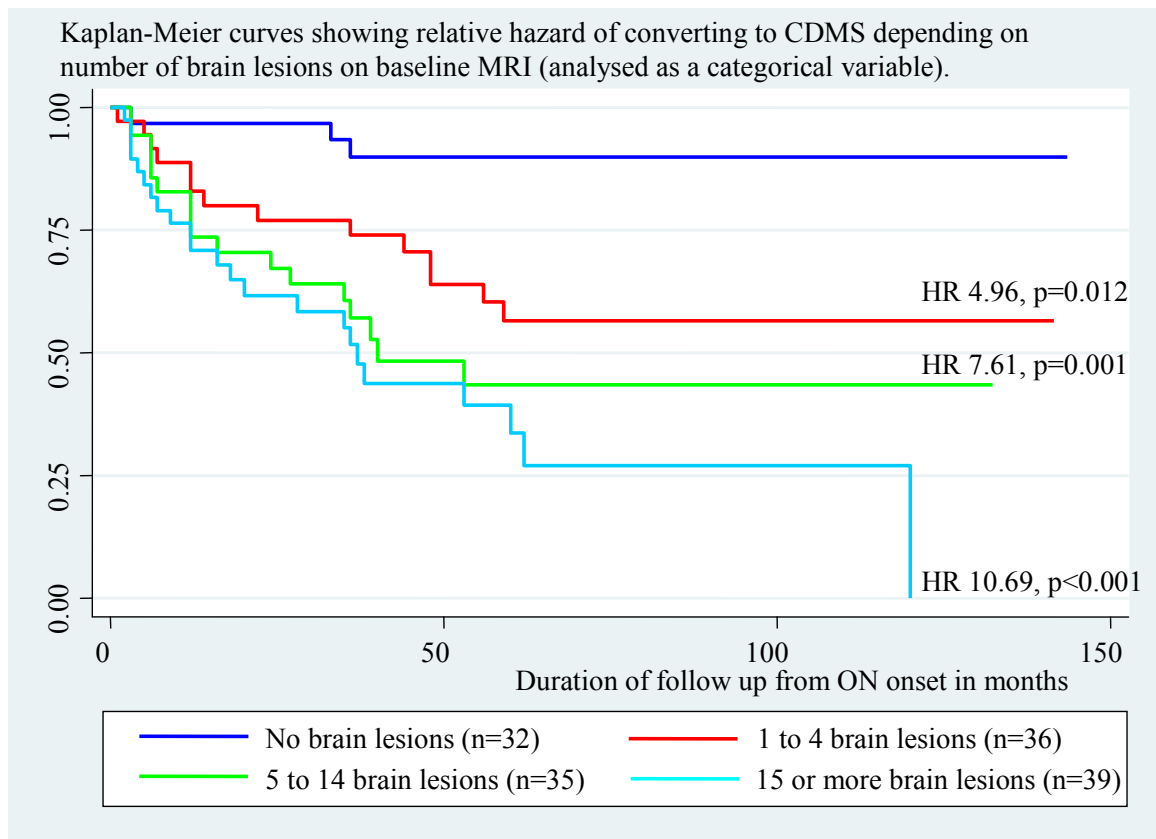
Baseline scan measures

(i) Brain lesion number

Abnormal baseline brain MRI *per se* was associated with a 7 fold increase in hazard of CDMS compared to normal brain MRI (HR 7.04 95% CI 2.19-22.56, $p < 0.001$).

Higher numbers of baseline brain T2 lesions were associated with increased conversion hazard: HR 1.81 (95% CI 1.40-2.34 $p < 0.001$) was estimated per lesion group increase (female gender also associated with a significant increased hazard of conversion in this model: HR 2.17 95% CI 1.12-4.21 $p = 0.043$). Linearity of the grouping variable was confirmed. For comparison with other studies, Figure 8.1 depicts the hazard of conversion per baseline T2 lesion grouping category with respective hazard ratios (i.e. analysed as categorical rather than ordinal variable).

Figure 8.1 Kaplan Meier curve showing probability of converting to CDMS in ON patients and how this probability is affected by number of brain lesions on baseline MRI



Neither lesion volumetric measures nor the binary variable, abnormal brain MRI, contributed further to hazard of CDMS.

(ii) Lesion location (Table 8.3)

The number of lesions observed separately at each of the 5 possible, mutually exclusive locations (periventricular, juxtacortical, discrete cerebral, infratentorial and spinal cord) were entered together, while adjusting for total lesion volume, age and gender. All 5 locations were significant in univariate analysis.

Periventricular location was significant in the ordinal model (HR 1.83 95% CI 1.21-2.78 p=0.004), and approached significance in the binary model (HR 2.37 95% CI 0.99-5.70 p=0.053). After stepwise removal of variables which were contributing least to the models, only periventricular location, T2 lesion volume and gender remained significant.

Table 8.3 showing binary and ordinal models analysing lesion location adjusted for total lesion volume after stepwise removal of variables contributing least to the model.

	Hazard ratio	p	95% Confidence interval
Binary model			
Presence of periventricular lesion(s)	3.56	0.001	1.71-7.44
T2 lesion volume	1.09	0.003	1.02-1.16
Female gender	2.29	0.014	1.18-4.45
Ordinal model			
Periventricular lesion no. (0,1,≥ 2)	1.91	<0.001	1.34-2.73
T2 lesion volume	1.08	0.011	1.01-1.15
Female gender	2.31	0.013	1.19-4.47

(iii) Baseline lesion activity (gadolinium enhancement)

Gadolinium enhancing lesions were associated with a significant increase in hazard of conversion to CDMS whether analysed as binary (HR 3.19, 95% CI 1.88-5.40, $p < 0.001$) or ordinal variables (HR 1.94, 95% CI 1.45-2.60, $p < 0.001$) and on adjustment for T2 lesion volume, (HR 2.64, 95% CI 1.62-4.29 $p < 0.001$ and 1.82, 95% CI 1.37-2.41 $p < 0.001$) respectively. Age and gender were not significant in analysis of baseline lesion activity.

(iv) Tissue volumetric measures

No volumetric measures (GMF, WMF, BPF or ventricular volume) were significant in Cox proportional hazards regression, even in univariate analysis.

Multivariate analysis of baseline scan measures

The significant variables from the baseline MRI parameters (gender, brain T2, gadolinium enhancing and periventricular lesions) were entered together to determine the significant independent predictors of CDMS at this time point.

In the ordinal model, the contribution from brain T2 lesion number was no longer significant (HR 1.21 95% CI 0.81-1.82 $p = 0.350$). In the binary model, abnormal brain MRI *per se* was not significant (HR 2.30, 95% CI 0.65-10.74 $p = 0.172$), and periventricular location approached significance (HR 2.30 95% CI 0.97-5.41 $p = 0.057$). The ordinal variable, brain T2 lesion number, was not significant in the binary model if it replaced abnormal brain MRI, (HR 1.28 95% CI 0.88-1.85 $p = 0.201$), (see Table 8.4).

Table 8.4 showing the models with baseline MRI parameters after removal of non-contributing brain T2 lesion number.

	Hazard ratio	p	95% Confidence interval
Binary			
Presence of periventricular lesion(s)	3.50	0.001	1.67-7.33
Presence of Gd enhancing lesion(s)	2.09	0.008	1.21-3.61
Female gender	2.00	0.041	1.03-3.89
Ordinal			
Periventricular lesion no. (0,1,≥ 2)	1.92	<0.001	1.35-2.74
Gd enhancing lesion no. (0,1,≥ 2)	1.52	0.008	1.12-2.08
Female gender	2.02	0.039	1.03-3.93

3 month scan measures

In analysis of gadolinium enhancing and new T2 lesions at 3 months, only the latter were significant in binary (HR 6.78 95% CI 3.71-12.39 p<0.001) or ordinal models (HR 3.21 95% CI 2.26-4.54 p<0.001) and on exclusion of early converters (HR 6.31 95% CI 3.34-11.96 p<0.001 and HR 3.09 95% CI 2.11-4.52 p<0.001 respectively).

No MTR measures or metabolite concentrations were significant in univariate analysis.

Combining baseline and 3 month scan measures (Table 8.5)

When the significant variables from the baseline (gender, periventricular and gadolinium enhancing lesions) and 3 month analyses (new T2 lesions) were combined, baseline gadolinium enhancing lesions did not remain significant. Higher HRs were observed for new T2 than for periventricular lesions. The number of baseline brain lesions when added later did not contribute to prognosis independently of new T2 and periventricular lesions. Gender remained significant only in binary models.

Table 8.5 showing the final model with the strongest independent predictors of CDMS in ON patients from baseline and 3 month follow-up scans, including and excluding the early converters (patients converting before the follow-up scan)

Excluding early converters	Hazard ratio	p	95% Confidence interval
Binary			
Presence of baseline periventricular lesion(s)	2.62	0.029	1.10-6.22
Presence of new T2 lesion(s) at 3/12	4.52	<0.001	2.29-8.97
Female gender	2.43	0.026	1.11-5.31
Ordinal			
Baseline periventricular lesion no. (0,1,≥ 2)	1.58	0.028	1.05-2.39
No. of new T2 at 3/12 (0,1,≥ 2)	2.56	<0.001	1.71-3.84

Including early converters	Hazard ratio	p	95% Confidence interval
Binary			
Presence of baseline periventricular lesion(s)	2.41	0.033	1.07-5.42
Presence of new T2 lesion(s) at 3/12	4.93	<0.001	2.58-9.41
Female gender	2.10	0.039	1.04-4.26
Ordinal			
Baseline periventricular lesion no. (0,1,≥ 2)	1.51	0.037	1.02-2.22
No. of new T2 at 3/12 (0,1,≥ 2)	2.71	<0.001	1.86-3.93

8.4 Discussion

Effect of lesion number

In contrast to results from the earliest CIS cohorts, the results from this study show that there is an increasing risk of conversion to CDMS with increasing number of lesions at baseline. This supports work by Tintore *et al* [147] who found that the number of lesions at presentation differentiated patients' risk of CDMS. The optic neuritis treatment trial (ONTT) [74] found no significant difference in hazard of conversion to CDMS at 10 years in groups with 1 brain lesion compared to more than 1 lesion. A Kaplan-Meier curve suggested differentiation between patients in the lesion groups although these appeared to converge over time. Similar findings have been reported in the 20 year follow-up from my centre of a mixed CIS cohort with the same risk of CDMS in patients with few or many lesions [280].

There are two possible explanations for the different effect of lesion number between the earliest and more recent studies. Firstly the earliest studies used less sensitive methodology: the 20 year CIS follow-up cohort was scanned in the 1980s at lower field strength, and both the CIS and ONTT cohorts scans were acquired without contrast at greater slice thickness with spacing. It is possible that the findings of

recent studies reflect more sensitive lesion detection because scanning was performed at a higher field strength and image resolution. Secondly patients from lower lesion load groups may convert to CDMS with longer follow-up: the ONTT follow-up study was reported after 10 years and the early CIS cohort has been followed for 20 years. Tintore's and this follow-up study suggest lesion load predicts conversion within a range of 5 to 7 years. However time to CDMS may be important as early conversion is reported as a risk factor for disability in some studies [76, 80, 81, 84] and may be relevant in considering disease modifying treatments, which delay conversion to CDMS [72, 73, 108] and possibly disability [108]. However, the present study also showed that lesion location and activity measures were stronger predictors of CDMS than lesion load *per se*.

Effect of lesion location

Although location is helpful in the differential diagnosis of white matter lesions, it has been less clear whether lesion location in CIS patients affects prognosis. This study showed, adjusting for total lesion volume, that periventricular lesions increased the risk of conversion to CDMS and this variable remained consistently significant in subsequent models. This supports the inclusion of periventricular location in several diagnostic criteria [160-162, 266]. A reason why periventricular lesions may be more predictive of CDMS than other locations is that they occur more frequently (Table 8.2) and with fewer patients converting in the absence of lesions in this compared to other locations. Periventricular lesion number correlated strongly with total T2 brain lesion number (Spearman's $r=0.88$). If the ordinal variable of brain lesion number and periventricular lesions were analysed together, neither remained significant due to this strong correlation, and because of this total lesion volume was adjusted for in the analysis of lesion location. However in the multivariate model of the baseline measures, gadolinium enhancing lesions and periventricular lesions remained significant but brain lesion number did not, suggesting that the former two measures provide sufficient information, one reflecting baseline lesion load and the other lesion activity. In effect periventricular lesions may be the best independent marker for lesion load. It should be noted that although total lesion volume was accounted for, regional volume of lesions was not. Further analysis could investigate regional lesion volume and risk of conversion to CDMS. However, the identification of periventricular lesions *per se* as a predictor for CDMS has practical value as they are

readily observed on reading scans in routine clinical practice, whereas measures of lesion volume are not.

In some studies cord lesions aid sensitivity of MRI diagnostic criteria [143, 285], but these were in cohorts of patients with clinically probable or definite MS many of whom had clinical evidence of spinal cord involvement. In this study asymptomatic spinal cord lesions, not investigated in the earlier CIS cohorts, were not significant in analysis with other baseline location variables, and therefore did not contribute to prediction independent of brain lesion measures.

However, in the next chapter, I report an effect of cord lesions on the prognosis for disability.

Effect of lesion activity

When only one baseline scan was considered, gadolinium enhancing lesion(s) were an independent predictor of CDMS. However this effect was lost when follow-up scans were considered and new T2 lesions emerged as the strongest overall independent predictor of CDMS. New T2 lesion(s) are usually permanent, whereas gadolinium enhancing lesions are transient, lasting for several weeks on average. The former provides a more sensitive measure of interval disease activity when the follow-up scan is performed several months or longer after the baseline study. This greater sensitivity probably explains the stronger predictive effect of new T2 lesions. The result suggests that there is a prognostic role for repeat imaging in the assessment of CIS patients and that non-contrast imaging may suffice at follow-up.

Non-lesion MRI measures

Perhaps disappointingly, none of the non-lesion measures obtained at a single early time point – grey matter and white matter tissue volumes, MTR measures and NAWM metabolite measures – were predictive of future development of CDMS in the present optic neuritis cohort. Abnormalities in the NAWM and grey matter have been reported in CIS cohorts including the present cohort [180, 182, 184, 217, 219-221, 223]. A possible explanation for the lack of predictive effect is that the abnormalities in NAWM and grey matter at this very early stage of disease evolution

are mild and of themselves unlikely to cause significant clinical manifestations. There is evidence from serial studies in MS cohorts that abnormalities in the NAWM and grey matter increase over time [175, 187, 188, 193, 200, 211, 212, 218] and may also correlate with measures of clinical impairment [175, 187, 211, 218]. There is a previous report of increasing grey matter atrophy and ventricular enlargement over a period of 1 to 3 years in a subgroup of the present CIS cohort that was associated with the development of CDMS [239, 241]. Longitudinal investigation is now required of the evolution of non-lesion MR measures of NAWM and grey matter over a longer period of time and their relationship to the clinical course.

Age was not significant in any of the analysis whereas the female gender was associated with 2 fold increase in hazard of conversion to CDMS in many models. Baseline brain MRI was more frequently abnormal in women than men (81% vs 72%) and this may have contributed to the higher conversion. This is consistent with previous reports in patients with ON [273, 274].

Study limitations

A limitation of this study was that not all patients had a 3 month follow-up scan and therefore the numbers of patients analysed at baseline and 3 months was different (142 and 127). Also only a subgroup of patients had MTR (n=89) and spectroscopy (n=85) as these were only acquired from 1998 onwards, after a proportion of patients had already been recruited and scanned. The smaller numbers may have affected the results.

By only including the ON patients, conclusions cannot be drawn about predictors of conversion in other CIS patients and further investigation is needed for these other presentations. Some studies report lower rate of MRI abnormality and conversion to MS in ON compared to non-ON CIS [65, 66, 76, 77, 81, 84, 85]. However, similar rates of MRI abnormality in ON and non-ON CIS cohorts have been seen in Southeast England cohorts (including this one) followed in the NMR Unit [275, 280].

8.5 Conclusion

When two early scans obtained 3 months apart in ON patients were analysed, only baseline periventricular lesions and new T2 lesions at follow-up were independent

predictors of CDMS. A new T2 lesion on an early follow-up scan was the strongest predictor of CDMS.

Chapter 9

Early MRI in optic neuritis. II: the risk for disability

9.1 Introduction

In the previous chapter I explored the association of characteristic MRI findings in optic neuritis (ON) clinically isolated syndrome (CIS) patients and conversion to clinically definite (CD)MS and reported the features of lesion location and activity independently predicting conversion. In this chapter I investigate how the same MRI parameters predict disability at ~6 years.

Of the 38-78% CIS patients converting to clinically definite MS (CDMS) [61, 78, 147, 156] the subsequent clinical course is very variable. Up to two thirds of patients have minimal disability after 10 years [78, 291] whereas up to a half require aid to walk after 15 years [61, 292, 293].

Whereas the optic neuritis treatment trial (ONTT) found no correlation between baseline MRI findings and disability at 10 years [78], other studies of mixed CIS cohorts have found a significant, although modest, association between baseline lesion load and disability at up to 20 year follow-up [61, 62, 146, 147].

The relationship of disability with gadolinium enhancement, a marker of disease activity, has not been consistently demonstrated in patients with CDMS [150, 152, 153, 156] and has not been investigated in unselected CIS cohorts [61, 78, 147]. Lesion location may also be important for prognosis, with one study concluding two or more infratentorial lesions were a significant predictor of disability [156].

Post mortem MS studies have shown abnormalities in the normal appearing white matter (NAWM) [38, 39] and cortex [40] and compatible changes [136, 172, 196] have been demonstrated *in vivo* less than a year from CIS onset using non-conventional MRI [181, 182, 187, 220, 221]. The potential of these non-lesion measures to identify CIS patients at greater risk of disability has not yet been explored through long-term clinical follow-up.

About 80% of the CIS cohort I have been investigating in the NMR Unit presented with ON, and some natural history studies report that ON presentation is associated with a more benign disease course than other CIS presentations [76, 77, 82] (although this was not found in all follow-up studies [61, 72, 276]). As in the previous chapter, I limited this follow-up report to ON only, in view of these observations, the small size of the non-ON CIS groups in the cohort and the potential difficulty in differentiating symptomatic from asymptomatic lesions in brainstem or spinal cord CIS. The aim of this study was to identify independent, early MRI predictors of subsequent disability after ~6 years in patients who present with isolated ON. Only scans obtained at baseline and 3 months later were used in this analysis.

9.2 Methods

Patients

Patients aged 16-50 within 3 months of ON onset with no previous neurological history suggestive of demyelination or evidence of CNS abnormality outside the optic nerves, were consecutively recruited from a Neuroophthalmology clinic at Moorfields Eye Hospital between 1995 and 2004 as part of a prospective follow-up study. All patients were reviewed by a single neuroophthalmologist and blood tests were performed as required to exclude other causes. Local ethics committee approval was obtained prior to starting the study, and informed consent was obtained from patients at baseline and each subsequent visit (planned at 3 months, 1, 3 and 5 years from baseline). Only patients who had reached the scheduled 5 year time point were included in this analysis. Follow-up visits included MRI, history of further events, clinical examination and documentation of EDSS. All examinations and EDSS scores at 5 years were performed by a single physician. Patients were defined as CDMS using the Poser criteria [106]. (The clinical criteria defined by Poser for CDMS, i.e. requiring clinical evidence of dissemination in space and time, were used as the aim was to investigate early MRI parameters predicting clinical outcome).

MRI acquisition protocols and image analysis

All images were acquired on a 1.5 Tesla GE scanner and have been described in detail in Chapter 4 and where referenced. In this report, MRI findings at baseline and 3 month follow-up only are considered as the focus is on *early* imaging predictors. MRI acquisition protocols and image analysis are summarized in Table 9.1.

Table 9.1 summarising MRI acquisition protocols and image analysis, FOV: field of view, MTI: magnetisation transfer imaging, MRS: magnetic resonance spectroscopy, GMF: grey matter fraction, WMF: white matter fraction, BPF: brain parenchymal fraction, TIV: total intracranial volume, NAWM normal appearing white matter, GM: grey matter

	Slice thickness	Orientation	Matrix	FOV	TR/TE (comments)	Image analysis
Brain post contrast, baseline and 3 months	46x3mm	contiguous axial slices	256x256	24cm	3200/15ms 3200/90ms 600/14ms	<i>Number and location of T2, Gd-enhancing, T1-hypointense lesions identified by neuroradiologist (KAM) and contoured using semi-automated thresholding technique to calculate volume [288]. Ventricular volume measured on T1 weighted scans at baseline using MIDAS [239, 289]. GMF, WMF and BPF calculated from SPM99 segmented T2-weighted images following lesion masking [241]: GMF=GM/TIV, WMF=WM+lesion volume/TIV, BPF=GM+WM+lesion volume/TIV</i>
Cord post contrast, baseline	9x3mm	contiguous sagittal slices	512x512	48cm	2500/56ms 2500/98ms 500/19ms	Number of T2, Gd-enhancing lesions identified by neuroradiologist (KAM)
MTI [182, 290] pre contrast, 3 months	28x5mm	contiguous axial oblique slices	256x128	24cm	1720/30ms 1720/80ms (MT weighted with application of presaturation pulse.)	Lesion masks created on non-MT images, whole brain mask generated and segmented into NAWM and GM in SPM99 and applied to calculated MTR map determining NAWM and GM pixels. Normalised NAWM and GM histograms generated.
¹H-MRS [221] pre contrast, 3 months		single voxel manually placed in NAWM			3000/30ms (PRESS sequence with 192 averages with optimised shimming and water suppression)	Metabolite concentrations estimated using LCModel and spectra assessed for quality of acquisition and processing by observer blinded to the clinical data (KTF)

Statistical analysis

Spearman correlation identified associations with MRI parameters and 5 year EDSS. Ordinal logistic regression was performed, with ranked follow-up EDSS as the dependent variable (cut-offs chosen to make approximately equal groups: EDSS 0, 1, 1.5-2, ≥ 2.5) and MRI parameters as the independent variables. Univariate regression analysis identified significant predictors from the parameter types: baseline lesion load (T2 lesion number, T2 and T1-hypointense lesion volume), location (periventricular, juxtacortical, discrete cerebral, infratentorial and spinal cord), lesion activity (gadolinium enhancing lesions), volumetric measures (grey matter, white matter and brain parenchymal fractions (GMF, WMF, BPF), ventricular volume); 3 month follow-up new lesion activity (gadolinium enhancing and new T2 lesions)

spectroscopy (N-Acetyl aspartate (NAA), myo-Inositol (Ins)) and MTR (mean, peak height, peak location in NAWM and NAGM) measures.

Multiple regression was performed in three stages.

Stage 1: multiple regression identified the strongest of the significant predictors within each variable type, (lesion number, location, volumetric measures, lesion activity, spectroscopy and MTR) for each of the 2 time points separately.

Stage 2: the significant predictors of different variable type from stage 1 were entered together to identify the strongest independent predictors for each time point scan.

Stage 3: the variables from both time point scans were entered together, to identify the strongest overall independent predictors.

Stages 1 to 3 were repeated, firstly excluding the patients whose EDSS had been obtained by telephone, and secondly in the subgroup of patients who had converted to CDMS by final follow-up.

When variables did not remain significant in multivariate analysis, they were dropped stepwise (manually) from the model, and their contribution tested with the likelihood ratio (LR) test. When the LR test was borderline significant ($p < 0.1$), the variable was maintained in the model, except in the final model where only variables $p < 0.05$ were reported.

Number of lesions were categorised into groups for statistical analysis. For analysis of baseline brain lesion number, patients were grouped by lesion number according to percentiles to ensure similar sized groups (0, 1-4, 5-14 and ≥ 15 lesions). Other lesion measures (location, gadolinium enhancing, new lesions) were grouped into binary (0, ≥ 1) and ordinal (0, 1, 2 and ≥ 3 lesions) variables. If there was no evidence of non-linearity, the ordinal score was entered into the model. Volumetric measures and non-conventional MRI parameters were analysed as continuous variables.

Other covariates (age, gender, baseline EDSS, disease modifying treatment and duration of follow-up) were added to the model at the end.

Statistical analysis was performed using Stata 9.2 statistical software (Stata Corporation, College Station, Texas, USA).

9.3 Results

Of 143 ON patients recruited, 106 were recruited at least 5 years earlier, and of these 100 were successfully followed up at the “5 year” time point (median duration of follow-up was actually 6.2 years), and phone EDSS was obtained for 25 patients unable to attend for clinical follow-up [248]. Demographic and clinical details are summarised in Table 9.2.

Table 9.2 summarising demographic data of the 100 ON patients followed up at the scheduled 5 year time point (‘5 years’), median follow-up actually 6 years.

Median age at CIS onset (IQR)		31.9 (28.2-37.9)
Gender		33 (33%) ♂: 67 (67) ♀
Median time from CIS onset to baseline MRI (IQR)		5 weeks (4-8)
Median time from CIS onset to follow-up MRI (IQR)		20 weeks (18-23)
Median duration of follow-up (IQR)		6.1 years (5.1 to 7.2)
Clinical vs phone [248] EDSS		75:25
Median EDSS in all patients at ‘5 years’ (range)		1 (0-8.5)
Median EDSS in patients remaining CIS at ‘5 years’ (IQR)		1 (0-1)
Median EDSS in patients CDMS at ‘5 years’ (IQR)		2 (1-3)
EDSS at ‘5 years’	0	21 (21%)
	1	40 (40%)
	1.5-2	23 (23%)
	≥2.5	16 (16%)
CIS RRMS SPMS	} at ‘5 years’	52 (52%)
		43 (43%)
		5 (5%)
MS by McDonald criteria at 5 years		66 (66%)
Median time to CDMS (IQR)		17 months (6 to 36)

Baseline and follow-up MRI findings are shown in Table 9.3 and results of univariate correlation and regression analysis are shown in Table 9.4. Lesion volume measures were not significant in univariate regression, however on exclusion of a single outlier both became significant (T2 and T1-hypointense lesion volumes ORs 2.13, p=0.034 and 1.26 p=0.011 respectively). No brain tissue volume measures were significant.

Table 9.3 summarising MRI findings at baseline and 3 months in all patients and in the 5 year EDSS subgroups MTR magnetisation transfer ratio, NAWM normal appearing white matter, NAGM normal appearing grey matter.

	All patients	EDSS 0	EDSS 1	EDSS 1.5-2	EDSS≥2.5
Baseline: Median (Interquartile range)					
Brain T2 lesion number	5 (1-16)	0 (0-4)	3 (0-16)	6 (3-16)	15 (6-29)
Gd lesion number	0 (0-1)	0 (0-0)	0 (0-0)	0 (0-1)	2 (0-6)
T2 lesion volume, mls	0.46 (0.05-1.81)	0 (0-0.34)	0.19 (0-1.67)	0.79 (0.23-1.81)	2.08 (0.48-4.13)
T1 hypointense lesion volume, mls	0 (0-0.15)	0 (0-0)	0 (0-0.15)	0.03 (0-0.25)	0.09 (0-0.54)
Cord T2 lesion number	0 (0-1)	0 (0-0)	0 (0-0)	0 (0-1)	1 (0-2)
Number of infratentorial lesions	0 (0-1)	0 (0-0)	0 (0-0)	0 (0-2)	1 (0-2)
Number of periventricular lesions	2 (0-7)	0 (0-1)	1 (0-7)	3 (1-8)	7 (2-12)
Number of juxtacortical lesions	1 (0-4)	0 (0-1)	1 (0-3)	1 (0-5)	4 (1-9)
Number of discrete cerebral lesions (IQR)	1 (0-43)	0 (0-0)	0 (0-4)	1 (0-3)	2 (0-5)
Ventricular volume	6.41 (4.02-10.13)	6.27 (4.00-8.40)	5.97 (3.27-10.71)	6.86 (4.57-10.76)	5.98 (4.07-8.75)
Grey matter fraction	0.49 (0.47-0.51)	0.50 (0.49-0.51)	0.49 (0.47-0.51)	0.48 (0.48-0.51)	0.48 (0.46-0.50)
White matter fraction	0.37 (0.36-0.39)	0.37 (0.36-0.38)	0.37 (0.36-0.39)	0.37 (0.36-0.38)	0.38 (0.36-0.40)
Brain parenchymal fraction	0.86 (0.85-0.87)	0.87 (0.85-0.88)	0.86 (0.85-0.88)	0.86 (0.85-0.87)	0.85 (0.84 – 0.87)
3 month follow-up: Median (Interquartile range)					
Number of new T2 lesions	0 (0-1)	0 (0-0)	0 (0-0)	1 (0-1)	2 (1-8)
Number of Gd lesions	0 (0-0)	0 (0-0)	0 (0-0)	0 (0-1)	1 (0-6)
N-Acetyl Aspartate	8.16 (7.61-8.84)	7.75 (7.37-9.36)	8.14 (7.54-8.73)	8.56 (8.12-9.42)	8.14 (7.26-8.84)
Myo-inositol	3.82 (3.06-4.43)	4.00 (3.38-4.64)	3.82 (2.78-4.15)	4.12 (3.31-4.65)	3.29 (2.74-5.01)
MTR NAWM peak height,	20.88 (19.80-21.87)	19.76 (18.92-21.62)	20.63 (19.41-21.41)	20.78 (19.71-22.26)	21.11 (19.87-22.12)
MTR NAWM peak location,	38.4 (38.0-38.7)	38.4 (38.2-38.6)	38.4 (38.0-38.8)	38.4 (38.0-38.7)	38.2 (37.7-38.5)
MTR NAWM mean	38.14 (37.84-38.42)	38.16 (37.89-38.28)	38.14 (37.86-38.55)	38.21 (37.89-38.47)	38.11 (37.54-38.39)
MTR NAGM peak height,	13.05 (12.33-13.73)	12.78 (12.46-13.68)	13.07 (12.33-13.72)	12.52 (11.83-13.11)	13.20 (12.12-14.04)
MTR NAGM peak location,	33.2 (32.9-33.5)	33.1 (32.7-33.3)	33.1 (32.9-33.5)	33.1 (32.8-33.6)	33.4 (32.8-33.6)
MTR NAGM mean	32.32 (32.05-32.61)	32.05 (31.98-32.41)	32.33 (32.11-32.55)	31.89 (32.46-32.63)	32.26 (32.08-32.71)

Table 9.4 Univariate analysis with spearman rank correlation coefficients and ordinal logistic regression derived odds ratios for baseline and follow-up MRI parameters (NB for regression analysis, lesion number variables were analysed as score variables * grouped 0,1,2,≥3 lesions or # 0, 1-4, 5-14, ≥15 lesions). MTR: magnetisation transfer ratio, NAWM: normal appearing white matter, NAGM: normal appearing grey matter

	No. of patients	Spearman correlation coefficient	p value	Odds ratio	p value
BASELINE					
T2 brain lesion number #	100	0.425	<0.001	2.068	<0.001
T2 brain lesion volume	100	0.422	<0.001	1.104	0.108
T1 hypointense brain lesion volume	100	0.313	0.002	1.575	0.092
T2 cord number *	100	0.367	<0.001	2.067	0.001
Infratentorial lesion number*	100	0.372	<0.001	2.500	<0.001
Periventricular lesion number*	100	0.387	<0.001	1.809	<0.001
Juxtacortical lesion number*	100	0.333	<0.001	1.556	0.002
Gd lesion number*	100	0.428	<0.001	2.072	<0.001
Grey matter fraction	100	-0.222	0.027	0.894	0.088
White matter fraction	100	0.077	0.448	1.040	0.687
Brain parenchymal fraction	100	-0.178	0.077	0.852	0.058
Ventricular volume (mls)	100	0.084	0.433	1.027	0.450
3 MONTH FOLLOW-UP					
Number of new T2 lesions *	87	0.510	<0.001	2.470	<0.001
Number of Gd lesions *	87	0.383	<0.001	2.514	0.001
N-Acetyl aspartate	74	0.180	0.427	1.246	0.389
Myo-inositol	74	-0.049	0.097	0.932	0.743
MTR NAWM peak height	72	0.163	0.202	1.248	0.167
MTR NAWM peak location	72	-0.089	0.486	0.848	0.735
MTR NAWM mean	72	-0.079	0.537	0.883	0.811
MTR NAGM peak height	72	-0.052	0.684	0.934	0.740
MTR NAGM peak location	72	0.158	0.216	2.030	0.212
MTR NAGM mean	72	0.102	0.426	1.493	0.494

Logistic regression: Multivariate analysis of relationship between early MRI measures and EDSS at follow-up

1.) Baseline scan measures

i. Lesion load The ordinal brain lesion variable (subsequently referred to as brain lesion number) was the strongest of the lesion load variables and a two-fold increase in risk of greater disability was estimated per lesion group increase (HR 2.07 95% CI 1.47-2.91 p<0.001). T2 and T1-hypointense lesion volumetric measures did not contribute independently even on exclusion of the outlier (ORs for brain lesion number in analysis with T2 and T1-hypointense lesion volumes 1.78 (95% CI 1.14-2.80) and 2.00 (95% CI 1.37-2.90, both p<0.001) respectively).

ii. Lesion location All 5 possible, mutually exclusive locations were analysed together (periventricular, juxtacortical, infratentorial, discrete cerebral, spinal cord), adjusting for brain lesion number. In the binary model, only spinal cord lesions remained significant, and infratentorial lesions approached significance. In the ordinal model, infratentorial and spinal cord lesions were both significant. After stepwise removal of non-contributing variables, only infratentorial, spinal cord and brain lesion number remained. In the binary model, the contribution from infratentorial lesions borderline significant ($p=0.064$) and was therefore retained. See Models 5a in Table 9.5.

iii. Lesion activity When baseline gadolinium lesion(s) were added to Models 5a, brain lesion number no longer remained significant and was therefore dropped. In addition, in the binary model, spinal cord lesions did not remain significant, but removal showed their contribution to that model was borderline ($p=0.087$), and was therefore retained (see Models 5b in Table 9.5).

iv. Tissue volumetric measures No volumetric measures were significant when added to the multivariate Models 5b.

2.) 3 month follow-up scan measures

i. New lesion activity In analysis of gadolinium enhancing and new T2 lesions at 3 months, gadolinium enhancing lesions became insignificant leaving new T2 lesion(s) with a binary OR of 11.51 (95% CI 3.14-42.21 $p<0.001$) and ordinal OR of 2.42 (95% CI 1.23-4.78, $p=0.005$).

ii. Other variables No spectroscopy or MTR measure at 3 months were significant in univariate analysis and were therefore not further investigated.

3.) Multivariate analysis combining baseline and 3 month follow-up scan measures

Combining the significant baseline (spinal cord, infratentorial locations and gadolinium enhancing lesions) and 3 month parameters (new T2 lesions), gadolinium enhancing lesions became borderline significant in the ordinal model ($p=0.054$). Infratentorial lesions did not remain significant in the binary analysis. These variables were therefore dropped from the final model, see Model 5c in Table 9.5.

Table 9.5 showing Models 5a (analysis of 5 possible baseline lesion locations, adjusting for baseline lesion number, after removal of variables not contributing significantly to the models), Models 5b (analysis of all significant baseline lesion variables, baseline lesion load, location and activity measures, after serial removal of variables not contributing to the model) and Models 5c (showing the final model in all ON patients with significant lesion variables from baseline and 3 month follow-up MRI after serial removal of variables not contributing to the models).

BINARY MODEL 5a (grouped 0, ≥1) n=100			
Location	Odds ratio	95% CI	p
Infratentorial	2.36	0.95-5.86	0.065
Spinal cord	2.51	1.02-6.15	0.044
Grouped brain lesions	1.66	1.15-2.40	0.007
ORDINAL MODEL 5a (grouped 0, 1, 2, ≥3) n=100			
Location	Odds ratio	95% CI	p
Infratentorial	1.80	1.08-3.03	0.025
Spinal cord	1.62	1.04-2.51	0.031
Grouped brain lesions	1.87	1.08-2.29	0.019
BINARY MODEL 5b (grouped 0, ≥1) n=100			
Location /enhancement	Odds ratio	95% CI	p
Infratentorial	3.37	1.37-8.29	0.008
Spinal cord	2.18	0.89-5.33	0.088
Gd enhancing	4.93	2.21-10.98	<0.001
ORDINAL MODEL 5b (grouped 0, 1,2, ≥3) n=100			
Location /enhancement	Odds ratio	95% CI	p
Infratentorial	2.22	1.34-3.70	0.002
Spinal cord	1.65	1.07-2.55	0.023
Gd enhancing	1.84	1.25-2.70	0.002
BINARY MODEL 5c (grouped 0, ≥1) n=87			
Parameter	Odds ratio	95% CI	p
Baseline			
Spinal cord	3.30	1.26-8.68	0.015
Gd enhancing	2.78	1.02-7.59	0.045
3 months			
New T2 3months	7.12	2.64-19.18	<0.001
ORDINAL MODEL 5c (grouped 0,1,2, ≥3) n=87			
Parameter	Odds ratio	95% CI	p
Baseline			
Infratentorial	1.82	1.06-3.11	0.030
Spinal cord	1.94	1.23-3.06	0.004
3 months			
New T2 3months	2.06	1.34-3.18	0.001

Other covariates

Age, gender, baseline EDSS, disease modifying therapy and time between symptom onset and 5 year follow-up inserted into the models individually were not significant.

Excluding phone-EDSS patients

Similar results were obtained at all 3 stages if we repeated the analysis excluding patients whose EDSS had been obtained over the phone rather than in person (n=75 at baseline and 69 at 3 months follow-up). The final binary and ordinal models contained only baseline spinal cord (OR's 4.41, 95% CI 1.47-13.24, p=0.008, and OR 2.35, 95% CI 1.35-4.07, p=0.002) and new T2 lesions at 3 months (OR's 6.89, 95% CI 2.44-19.47, p<0.001 and 1.87, 95% CI 1.19-2.93, p=0.007).

CDMS patients only

If only those ON patients converting to CDMS by 6 years are investigated (n=48), no measures of baseline brain lesion load, location or activity were significant in univariate analysis. Only baseline cord lesions, GMF and BPF were significant in univariate analysis, however the volumetric measures did not remain significant after adjusting for age, leaving just cord lesions (ordinal OR 1.92, 95% CI 1.14-3.26, p=0.015, binary OR 5.07, 95% CI 1.60-16.11, p=0.006). No variable from the 3 month follow-up visit remained significant in the patients who had converted to CDMS at 6 years.

No other covariates were significant in the analysis of CDMS patients.

9.4 Discussion

In the previous chapter, the strongest independent predictor of conversion to CDMS was new T2 lesion(s), with periventricular lesions also being significant predictors, potentially acting as a surrogate marker of baseline lesion load. Brain lesion number was not significant in analysis with these other MRI measures. Similarly, in this study, early independent MRI predictors of disability in ON patients were measures of lesion location (spinal cord and infratentorial lesions) and activity (baseline gadolinium-enhancement and new T2 lesions at follow-up) rather than lesion load.

Effect of baseline lesion load

In this ON cohort, lesion number was a significant predictor of the 6 year EDSS in univariate analysis in agreement with the recently reported mixed CIS cohorts followed up at 7 and 20 years [61, 62, 146, 147]. However an important new finding

in the present cohort is that baseline lesion number was no longer significant in analysis including other MRI lesion location and activity parameters, which were not investigated in these other studies. Analysis of the NMR Unit's earlier mixed CIS cohort also found brain lesion load at 5 years, cross-sectional and increase from baseline, correlated more strongly with EDSS at 14 and 20 years than did baseline measures [61, 62]. It may be that lesion increase over time is more predictive than one baseline measure and the significant effect of new T2 lesions at 3 months in the present study supports this idea: investigation of longer term changes in T2 number and volume in the present cohort will be of interest.

Due to the presence of a single outlier, the continuous variable lesion volume was significant in spearman correlation (sensitive to values' rank) but not in logistic regression (sensitive to values' magnitude) where lesion number, analysed as an ordinal variable, was significant.

The significant association between baseline brain lesion number and 6 year disability did not remain so in analysis of the patients converting to CDMS. Although still significant in the Unit's earlier CIS cohort, the correlation between baseline T2 lesion volume and 20 year EDSS was weaker in the CDMS subgroup [62]. The Barcelona CIS study does not report this subgroup analysis [147]. The overall findings – in my and the Unit's earlier CIS cohorts – suggest that baseline brain lesion load *per se* is a better predictor of conversion to CDMS than disability. That brain lesion load did not remain significant in the analyses including measures of lesion location and activity suggests that the latter lesion measures are more important with respect to disability than overall lesion load *per se*.

Lesion location and the effect of spinal cord and infratentorial lesions

The spinal cord is considered a characteristic location for demyelination. Asymptomatic cord lesions, were seen in 26% of my ON cohort, and were reported at an earlier stage of study recruitment in 27% and 42% of CIS patients from the same cohort at presentation [141, 142]. Whether cord lesions add independent prognostic information in patients with brain lesions has not been clear and the previous chapter did not reveal any independent influence of cord lesions on the risk for CDMS. However this study shows the presence of asymptomatic cord lesion(s) at ON

presentation significantly increased the odds of higher disability at 6 years and in this effect was independent of and stronger than brain lesion load; there may therefore be a role for cord MRI in ON patients in considering the risk for future disability.

Infratentorial lesions were significant in ordinal but not binary analysis, suggesting the number of infratentorial lesions influences the risk for disability. This study is concordant with the findings from a Dutch CIS cohort that 2 or more baseline infratentorial lesions in CIS patients were significant predictors of time to EDSS \geq 3 [156]. Asymptomatic infratentorial lesions are found in CIS patients on MRI. An early study found brainstem and cerebellar lesions in 14% and 11% of ON CIS patients respectively [138]. Brainstem or cerebellar lesions were found in 31% of my ON cohort, and the increase in detection may be due to improvement in imaging technology. A role for infratentorial lesions in disability was supported by a study in which patients with progressive MS had similar numbers of supratentorial lesions but higher numbers of infratentorial lesions than age-, sex- and disease duration-matched patients with benign MS [294].

Lesions in the spinal cord or brainstem are more likely to affect a clinically strategic pathway than supratentorial lesions. Although not always clinically eloquent at presentation, they may result in delayed symptoms through secondary degeneration or when subject to further episodes of inflammatory demyelination. The presence of such lesions at presentation may indicate a propensity for further lesions in the same region which themselves cause symptoms and subsequent sustained disability. In the supratentorial compartment there is potential for compensatory mechanisms and reorganisation of functional pathways; this potential may be less in the spinal cord and infratentorial regions where the threshold of axonal damage needed before clinical deficits develops may be lower.

The lesion locations investigated were relatively large areas of the CNS rather than specific pathways that have immediate functional relevance. For example, lesions in specific tracts such as the internal capsule may have significant effects on later disability, and were not investigated in this study.

Effect of lesion activity measures

MRI evidence of lesion activity added significant prognostic information with regards to disability in the whole CIS cohort, with both gadolinium-enhancing lesions at baseline and (more consistently) new T2 lesions on the follow-up scan being independent predictors of disability in the final model (Models 5c). However, neither of these remained significant predictors of disability when analysis was confined to the subgroup who converted to CDMS. In the previous chapter, new T2 lesions were strongly significant in the final model predicting CDMS *per se*. These findings suggest that the lesion activity variables may be predicting conversion to CDMS rather than disability *per se*. This idea is supported by other studies which have found gadolinium lesions predict relapse occurrence but not later disability [152, 153].

Non-lesion measures

In the previous chapter, MTR and MRS parameters obtained 20 weeks from CIS onset were not significantly associated with conversion to CDMS in the ON cohort. Similarly there was no association with disability at 6 years. Although changes in MTR and MRS have been detected in CIS patients compared to controls, including this cohort, [181, 182, 187, 221], it is possible that the abnormalities in the NAWM and grey matter are mild at such an early stage of disease and unlikely to manifest clinically. Longitudinal studies of CDMS patients have demonstrated changes in MTR [187] and MRS [211] over time, some of which correlated with clinical impairment [187, 211]. Further longitudinal investigation of CIS cohorts is required of the evolution of non-lesion MR measures and their relationship to the clinical course.

In this study, baseline GMF was an independent significant predictor of disability in the subgroup of patients who converted to CDMS, but was confounded by age. Longitudinal atrophy measures were associated with the concurrent development of CDMS in this CIS cohort [239, 241]. Future analysis should investigate the relationship between longitudinal atrophy with later disability.

Study limitations

The study has several limitations. Firstly, tissue volumetric measures were generated from 2D acquisitions as 3D acquisitions were not available in the patients recruited before 1998.

Secondly, only about three-quarters of patients had MRS or MTR due to quality of acquisition or processing and lack of availability before 1998. Nevertheless, it seems likely that had there been a clear predictive effect of these MR measures an effect should have been apparent.

Thirdly, this study was limited to the ON subgroup and is not necessarily applicable to non-ON presentations; some [76, 77, 82] but not all [61, 72, 276] studies have suggested that ON has a better long-term prognosis than non-ON presentations. When investigating the predictive role of lesion location in patients with brainstem and spinal cord CIS, special care will be needed to identify clinically silent infratentorial and spinal cord lesions.

Fourthly, EDSS has been criticised for its emphasis on ambulation, which may explain the association with spinal cord lesions. MSFC was not used due to ceiling effects in a relatively unimpaired cohort. Also EDSS was obtained over the phone in 25% of my cohort. Although clinical examination would have been preferable, in these patients it was impractical. Reassuringly similar results were obtained on their exclusion.

Finally, only 12% of my cohort had $EDSS \geq 3$ at ~6 years and the median EDSS of the CDMS patients was 2. In the Unit's earlier CIS cohort the median EDSS of the CDMS group was 3.25 and 4.0 after 14 and 20 years respectively [61, 62]. More prolonged follow-up is needed to determine whether the early predictors identified in this study are still relevant in the cohort as more disability emerges.

9.5 Conclusion

Several MRI lesion measures on scans obtained within 6 months of ON onset - number of baseline spinal cord and infratentorial lesions, baseline gadolinium lesions

and new T2 lesions after 3 months - predicted disability after 6 years; only spinal cord lesions were predictive of disability in those developing CDMS.

Chapter 10 Summary

This thesis explores the diagnostic and prognostic roles of MRI at the earliest clinically manifest stage of relapse-onset MS, the clinically isolated syndrome (CIS). To be able to distinguish the patients at greatest risk of developing CDMS and, possibly more importantly, disability would help counsel patients and direct current and emerging disease modifying therapies.

MRI has aided diagnosis of MS by detecting characteristic lesions on brain and cord images which help distinguish demyelination from other conditions. This has allowed incorporation of MRI findings into diagnostic criteria [161, 162, 167, 266, 267], with MRI evidence of dissemination in time and space replacing the need for clinical evidence, thus leading to earlier diagnosis, and potentially to therapeutic intervention. Trials have shown early treatment with disease modifying drugs delay subsequent clinical episodes and disability [72, 276] although the long-term benefit of these agents is still under investigation. In addition to the therapeutic benefits of early diagnosis, access to MS specialist clinics and personnel is equally important.

MRI findings however are not the same around the world, with frequency of abnormalities at CIS presentation varying in a similar way to the variation in MS prevalence (Chapter 5). This has implications on the use of MRI in diagnosis and prognosis globally, as criteria evaluated in one region may not be as accurate in regions with different frequencies of MRI abnormalities.

10.1 Diagnosis

The results from Chapters 6 and 7 of this thesis show that sensitivity and accuracy of diagnostic criteria can be improved whilst maintaining high specificity. The criteria proposed in this thesis are also simpler than the current McDonald criteria [167, 266], requiring just 2 lesions in separate but characteristic locations for DIS and a new T2 lesion as evidence of DIT, irrespective of timing of the baseline scan. In the preliminary study where the criteria were tested in my London CIS cohort, the sensitivity increased to 77% compared to 46% for the McDonald criteria, with specificity high at 92% (vs 94% for the McDonald criteria [266]). It was noted however that the criteria performed less well in the (small) non-ON subgroup.

Further evaluation of the MRI criteria was performed in a multicentre European CIS cohort, important in light of the observation that the frequency of MRI abnormalities in CIS patients varies in reports of other CIS and ON cohorts. It was also hoped to increase the proportion of non-ON presentations for better evaluation in that subgroup.

Several points have come to light from these studies. Firstly the proposed simplified criteria performed well against the McDonald 2001 [266] and recently modified McDonald 2005 criteria [167]. The simplified criteria were the most sensitive at detecting cases of MS in the first year of CIS follow-up (72% vs. 47% and 60% for the 2001 and 2005 McDonald criteria respectively) whilst maintaining high specificity (87% for the simplified criteria, 91% and 88% for the 2001 and 2005 McDonald criteria), thus being effective in excluding patients who do not develop CDMS. Secondly all DIT criteria were more specific than DIS criteria supporting a role for repeat imaging. Thirdly, spinal cord imaging only marginally increased the specificity and sensitivity of the criteria, suggesting it is of limited value in non-spinal cord syndromes. Fourthly, not all CIS presentations carried the same risk of CDMS. As highlighted in the preliminary study, in which the criteria performed less well in the non-ON subgroup, Cox regression analysis showed that CIS presentation was an independent risk factor for CDMS, with non-ON presentations having increased risk of conversion compared to ON patients. Finally conversion was lower in Barcelona than the other centres, where MS prevalence was also lowest [253]. However even on adjustment for these covariates in the survival analysis, the clear effect of the MRI diagnostic criteria on risk for CDMS was retained, and most strongly for the simplified criteria.

Concern has been raised as to the timing of the baseline scan. To be confident that subsequent new lesions were disseminated in time from the initial clinical episode, the McDonald 2005 criteria stipulated that the baseline scan must be more than 30 days from CIS onset, comparable to the clinical requirement for DIT. My results show that in these characteristic CIS presentations, (95% unifocal disease in the age range 16-50) the criteria were equally specific whether applied to cases with baseline imaging performed less or more than 30 days from CIS onset.

Therefore the proposed MRI criteria are more sensitive, accurate and simpler than the current McDonald 2005 criteria, whilst maintaining high specificity.

10.2 Prognosis

Whether MRI findings considered characteristic for demyelination affect prognosis was explored in the ON subgroup of my CIS cohort in Chapters 8 and 9. This was divided into risk of converting to CDMS and risk of disability. Analysis was restricted to the MRI parameters acquired at baseline and 3 months follow-up as the focus was on *early* predictors of clinical outcome.

Our investigation of MRI parameters estimated hazard of conversion to CDMS and disability doubled with each increase in lesion group, supporting the findings by Tintore *et al*, where risk of conversion and disability increased with lesion number [147]. Long-term follow-up of two other CIS cohorts at 10 and 20 years found baseline lesion load did not predict conversion to CDMS [78, 280], as patients with a single lesion and multiple lesions had the same risk of CDMS. A modest correlation between baseline lesion load and disability at 20 years was found in the mixed CIS cohort [280]. The discrepancy between earlier and more recent studies may be due to differences in scan quality, with the recent studies being acquired at higher field strengths and/or resolution. Also it is likely that patients with lower lesion loads convert to CDMS with longer follow-up, and this is supported by the apparent convergence of curves in the Cox regression analysis in the ONTT by 10 years [78]. However as early conversion is reported as a risk factor for disability in some studies [80, 81, 84], distinguishing such patients may help with counselling and potentially directing disease modifying therapy. Therefore these results suggest number of lesions detected on scans of today's image resolution and field strength influence risk of conversion to CDMS and development of disability.

However, the prognostic studies in this thesis suggest that MRI lesion characteristics *other* than the total number, not investigated in the earlier studies, are better independent predictors in ON CIS. Although baseline brain lesion number was significant in univariate analysis predicting conversion to CDMS or disability at 5 years, it did not remain so in analysis with MRI parameters of lesion location and activity.

Lesion activity was a significant risk factor, and new T2 lesions were the strongest predictors of CDMS and disability. For the most part gadolinium-enhancing lesions did not contribute independently of new T2 lesions, although were strong predictors if only baseline imaging was considered. Gadolinium enhancing lesions are transient whereas new T2 lesions are usually permanent and are therefore a more sensitive measure of new disease activity. As neither new T2 lesions nor gadolinium enhancing lesions remained significant in analysis predicting disability in the subgroup who converted to CDMS, the lesion activity variables may be predicting conversion to CDMS rather than disability *per se* as subsequent clinical episodes are required to develop disability. Just as we concluded in the evaluation of MRI diagnostic criteria, repeat imaging is helpful in the assessment of CIS patients. Although risk of CDMS increases with increasing baseline brain lesion load, this becomes insignificant in analysis with new T2 lesions. Thus specificities are improved with addition of DIT criteria.

Investigation of lesion location in the ON CIS group revealed that independent of total lesion load periventricular location was consistently associated with an increased risk of conversion to CDMS and seemed to act as a surrogate measure of baseline lesion load. Just as spinal cord lesions added little to the performance of MRI criteria, they did not independently predict conversion to CDMS. However, spinal cord lesions were consistently associated with increased disability, and in the CDMS subgroup were the only predictor. Therefore there is a potential role for spinal imaging in non-spinal cord presentations.

Disappointingly none of the non-lesion measures were significant in predicting CDMS or disability. Although MTR and spectroscopy data was not available for all patients, it was for the majority and if they had had a clear predictive effect this should have been apparent. Changes have been detected in NAWM and grey matter in CIS patients compared to controls [180, 182, 184, 217, 219-221, 223], but this study suggests they are too mild at this early stage to cause clinical manifestations. Just as lesions accumulate over time, so too do changes in non-lesion brain tissue [175, 187, 188, 200, 211, 218] which have been shown to correlate with measures of

clinical impairment [175, 187, 211, 218]. Longitudinal studies are required to assess their relationship with outcome.

10.3 Future questions

Our observational study of global MS prevalence suggested an association with MRI abnormalities in ON patients at presentation. It would be useful to take this hypothesis further with a prospective study as the accuracy of MRI in diagnosis and prognosis will vary globally depending on the strength of this association.

Regarding diagnosis, the studies in this thesis were performed retrospectively on cohorts of patients presenting with a clinical syndrome characteristic of demyelination on scans acquired at 1.5T. Future work should evaluate the diagnostic criteria in a new, prospectively recruited CIS cohort, ideally in another multicentre study. This could be an opportunity to compare the accuracy of the criteria at 1.5T and higher field strengths. As these are becoming increasingly available their increased sensitivity to white matter lesions will require re-evaluation of the criteria [295, 296]. The non-ON CIS subgroup remained a small proportion of the multicentre analysis of the diagnostic criteria, and ideally future prospective cohorts would have a better balance of CIS topography. Also it is important to assess how the criteria perform in a clinically mixed cohort, and whether they can distinguish demyelination from other multifocal white matter conditions. Longer follow-up will be important to assess whether the specificity of the criteria falls as patients with lower lesion loads and slower lesion accrual convert with time increasing the number of false negatives. As newer imaging techniques become available, such as double inversion recovery which has higher sensitivity to cortical lesions [168], the incorporation of such findings into diagnostic criteria will be of interest. Lastly, investigating whether the presence of oligoclonal bands in the CSF allows reduction of the DIS or DIT criteria to maintain accuracy would be important.

Regarding the role of MRI in prognosis, both studies were limited to ON presentations and may not extrapolate to non-ON CIS. Further investigation of a mixed CIS cohort would be of interest. It would be interesting to investigate whether the clinically silent spinal cord and infratentorial lesions predict disability through secondary degeneration or by representing a predisposition to inflammation in these

locations giving rise to brainstem or spinal cord syndromes. That is, can sustained disability arise from initially clinically silent lesions or must they manifest clinically? In light of recent work on the association between neuromyelitis optica and anti-aquaporin 4 antibody [297], it would be interesting to investigate the antibody's presence in the sera of the ON cohort and determine whether it is associated with those presenting with spinal cord lesions and/or developing disability.

Longer follow-up would help determine whether the MRI parameters highlighted in this thesis as being important for predicting conversion to CDMS continue to be so as more patients with lower lesion loads convert. Also only 12% of my cohort had $EDSS \geq 3$ at ~6 years. With time a more disabled cohort with potentially greater spread of disability may emerge, and it is important to investigate whether the significant early MRI predictors of disability remain so. This thesis concentrated on early MRI parameters, only looking at the scans obtained at baseline and 3 month follow-up. The timing of the second scan was not investigated and as new T2 lesions were the strongest predictor of CDMS it would be useful to know the optimum time for a repeat scan. It seems likely that earlier repeat imaging lowers the sensitivity but increases the specificity and vice versa. Early non-lesion measures did not contribute to prognosis in this study, although differences have been detected in CIS patients at this early stage. Longitudinal study of these quantitative variables will be important to elicit the stage when they become important for prognosis, which may be as the association between T2 visible lesions and disability plateaus. The disadvantage of longitudinal quantitative measures is the inherent delay in obtaining them and sensitivity to change in hardware.

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