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**Title: Diagnostic and Prognostic Magnetic Resonance
Studies in Patients with Clinically Isolated Syndromes**

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Submitted for: Doctor of Medicine.

Date of Submission: August 2004.

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Acknowledgements

There are a number of people I would like to thank as I submit this thesis. First and foremost I wish to thank my supervisors Professors David Miller and Alan Thompson for their unfailing support over the four half years. I am indebted to Dr Katherine Miszkief for guiding me through the marking of over a thousand MRI scans from the Natalizumab and the Clinically Isolated Syndromes studies. My sincere thanks to Dr Gordon Plant who taught me and helped me recruit patients at Moorfields Eye Hospital. I would also like to thank Élan for funding my position marking MRI scans in a pivotal trial of Antegren (Natalizumab) for treatment of MS. Also, I would also like to thank the MS society for funding the NMR unit scanner which was used for patients involved in the Clinically Isolated Syndromes study.

When I started research at the Institute of Neurology, Peter Brex explained the protocol for the CIS study. During the past three years I am grateful to have had the opportunity to collaborate with Riain Gordon, Bill Crum and Nick Fox from the Dementia Research Group on the use of MIDAS for measurement of ventricular volumes. Declan Chard gave much needed help and instruction on the use of SPM99 for segmentation of the brain into grey matter, white matter and CSF. Gerard Davies validated the technique using dummy lesions in 3mm thickness MRI scans. Chris Webb has provided me with much needed computer support and practical advice on image analysis. Hilary Watt and Dan Altmann have helped me with statistical input. The secretarial skills of Tina Holmes, Catherine Noctor are gratefully acknowledged. Claire Middleditch very kindly and carefully proof read my papers. Claudia Wheeler-Kingshott kindly reviewed the physics in chapter 2. Finally I would like to thank all the patients who were involved in the Clinically Isolated Syndromes study.

Publications and presentations arising from this thesis

Dalton CM, Brex PA, Jenkins R, Fox NC, Miszkiet KA, Crum WR, O'Riordan JI, Plant GT, Thompson AJ, Miller DH. Progressive ventricular enlargement in patients with clinically isolated syndromes is associated with the early development of multiple sclerosis. *J Neurol Neurosurg Psychiatry*. 2002 Aug; 73(2):141-7.

Dalton CM, Brex PA, Miszkiet KA, Hickman SJ, MacManus DG, Plant GT, Thompson AJ, Miller DH. Application of the new McDonald criteria to patients with clinically isolated syndromes suggestive of multiple sclerosis. *Ann Neurol*. 2002 Jul;52(1):47-53.

Dalton CM, Brex PA, Miszkiet KA, Fernando K, MacManus DG, Plant GT, Thompson AJ, Miller DH. New T2 lesions enable an earlier diagnosis of multiple sclerosis in clinically isolated syndromes. *Ann Neurol*. 2003 May;53(5):673-6.

Dalton CM, Brex PA, Miszkiet KA, Fernando K, MacManus DG, Plant GT, Thompson AJ, Miller DH. Spinal cord MRI in clinically isolated optic neuritis. *J Neurol Neurosurg Psychiatry*. 2003 Nov; 74(11): 1577-80.

Dalton CM, Chard DT, Davies GR, Miszkiet KA, Altmann D, Fernando K, Plant GT, Thompson AJ, Miller DH. Early development of multiple sclerosis is associated with progressive grey matter atrophy in patients presenting with clinically isolated syndromes *Brain*. 2004 May;127(Pt 5):1101-7.

Presentations

Brex PA Dalton CM. International Workshop on the Measurement of Atrophy in MS. Institute of Neurology, London, 08/12/00. The Measurement of Atrophy in Clinically Isolated Syndromes Suggestive of MS.

CM Dalton, PA Brex, R Gordon, NC Fox, KA Miszkiel, WR Crum, JI. O'Riordan, GT Plant, AJ Thompson, DH Miller. European Neurological Society, Paris, 23/04/01. Progressive ventricular enlargement in patients with Clinically Isolated Syndromes is associated with the development of Multiple Sclerosis. (J Neurol. 2001 Apr;248 Suppl 2:II/26).

CM Dalton, PA Brex, KA Miszkiel, JI O'Riordan, GT Plant, SJ Hickman, AJ Thompson, DH Miller. Awarded Best Platform Presentation ECTRIMS, Dublin, 15/09/01. Diagnosis of Multiple Sclerosis (MS) in patients with Clinically Isolated Syndromes (CIS) using the new McDonald MRI criteria. (Mult Scler. 2001 Sep;7 Suppl 1:S1-137).

CM Dalton, PA Brex, KA Miszkiel, GT Plant, SJ Hickman, DG MacManus, AJ Thompson, D H Miller. Association of British Neurologists. Platform Presentation 04/04/02. Application of the McDonald criteria in patients with Clinically Isolated Syndromes Suggestive of Multiple Sclerosis. (J Neurol Neurosurg Psychiatry. 2002 Aug;73(2):213-36).

CM Dalton, PA Brex, KA Miszkiel, GT Plant, AJ Thompson, DH Miller. American Academy of Neurology Denver, Platform Presentation 16/04/02. Validation of the

McDonald Criteria in Patients with Clinically Isolated Syndromes Suggestive of Multiple Sclerosis. *Neurology*. 2002 Apr 9;58(7 Suppl 3):A1-593.

Dalton C, Brex P, Miszkiel K, Fernando K, Plant G, Thompson A, Miller D. European Neurological Society Berlin, Platform Presentation 25/06/02. Clinically isolated syndromes (CIS) and new T2 lesions on early MRI follow up: a better predictor for clinically definite MS (CDMS) than the new diagnostic (McDonald) criteria? (*J Neurol*. 2002 Jun;249 Suppl 1:I/38).

Dalton CM, Chard DT, Brex PA, Miszkiel KA, Altmann D, Fernando K, Plant GT, Thompson AJ, Miller DH. Progressive grey matter atrophy occurs in the earliest clinical stages of relapse onset Multiple Sclerosis. *Ann Neurol*. 2003;54 (suppl 7):S62
American Neurological Association 128th Annual Meeting San Francisco. Poster 20/10/03.

Dalton CM. Brain and Spinal Cord Lesions in Clinically Isolated Syndromes. Brain and Spinal Cord Atrophy in Clinically Isolated Syndromes. Clinically Isolated Syndromes Workshop 22/01/04-23/01/04.

Abstract

Multiple Sclerosis (MS) is an acquired demyelinating disease of the central nervous system (CNS) which usually presents as a Clinically Isolated Syndrome (CIS). Longitudinal clinical and MRI studies of patients with CIS give insight into the clinical prognostic factors for the development of Relapsing Remitting (RR) and Secondary Progressive (SP) MS.

In this MD thesis, I have discussed the clinical and MRI correlations in a group of 65 patients recruited between 1995 and 1999 and who have been followed for approximately 3 years. In Chapters 3.2 and 3.3, I evaluated baseline MRI brain and spinal cord findings as predictive tests for MS at 3 years.

In 2001, the International Panel on MS diagnosis published revised criteria on the diagnosis of MS. For the first time a diagnosis of MS could be made in patients with CIS suggestive of MS using MRI for evidence of Dissemination in Space (DIS) and Dissemination in Time (DIT). The accuracy of both the new MRI and clinical criteria was evaluated at one and 3 years, in Chapter 4.1. Although the new diagnostic criteria were found to specific for MS, their sensitivity was lower.

While, high specificity was achieved by the requirement of new Gadolinium enhancing lesions at a 3 month follow up scan in patients imaged initially within 3 months of the onset of symptoms, the same requirement reduced sensitivity. New T2 lesions are seen more often on the 3 month follow up scan than new Gadolinium enhancing lesions. Our next project in chapter 4.2, was to evaluate inclusion of new T2 lesions as a predictive test for MS at 3 years. Interestingly, new T2 lesions used as

evidence for DIT were sensitive for a diagnosis of MS at 3 years without a loss in specificity. Finally, as an exploratory exercise in this section, we evaluated new T2 lesions, regardless of evidence of MRI DIS. New T2 lesions were both sensitive and specific for a diagnosis of MS at 3 years. Further evaluation of a new T2 lesion at 3 months together with optimum MRI evidence for DIS is therefore warranted.

Brain atrophy has been evaluated as a surrogate marker in MS. The cause and timing of atrophy and its association with inflammatory MRI lesions are not clear. In chapter 5.1, Ventricular Volume (VV) was analyzed as an atrophy marker in a cohort of 55 patients followed for one year. In Chapter 5.2, Grey Matter (GM), White Matter (WM), Brain Parenchymal Fractions (BPF) and (VV) were analyzed as atrophy markers in 58 patients followed for 3 years. Significant GM atrophy and an increase in VV were seen in those who developed MS. There were moderate correlations between lesions and increase in VV and reduction in GMF and BPF.

In conclusion, although imaging of the brain is extremely helpful in patients with optic neuritis in order to assign risk of MS, imaging of the spinal cord is less useful. The new diagnostic criteria for the diagnosis of MS in patients with CIS are specific. Sensitivity of the diagnostic criteria may be improved by the inclusion of new T2 lesions after 3 months as evidence of DIT. Regional atrophy affecting both GM and VV size was noted in patients with CIS, who went on to develop MS.

Pathogenic process including lesions and atrophy occur in the earliest clinical stages of MS and are only partially related. It is appropriate to measure both processes in future disease modifying treatment trials in patients with CIS or early MS.

Thesis Description

This thesis is divided into two parts and six chapters. In Part 1, Chapters 1 and 2 are introductory in nature. Chapter 1 is a background chapter on Multiple Sclerosis (MS) with a focus on patients presenting with Clinically Isolated Syndromes (CIS). Chapter 2 gives the principles of Magnetic Resonance Imaging (MRI). Conventional and more modern MRI techniques and their application to patients with CIS and MS are discussed.

Patient recruitment, clinical and MRI sequences that form the basis of the studies in Chapters 3 - 5 are explained in the introduction to Part 2. Chapters 3.2 and 3.3, evaluate baseline MRI findings in patients with CIS as predictive tests for MS. The McDonald criteria are applied in chapter 4.1, using MRI evidence of Dissemination in Space (DIS) and Time (DIT). New T2 lesions are also evaluated for evidence of DIT in Chapter 4.2. Chapter 5 evaluates Ventricular Volumes (VV), Grey Matter (GM) White Matter (WM) and Brain Parenchymal fraction (BPF) as markers of atrophy in patients presenting with CIS followed for 1 and 3 years.

Chapter 6 is a summary of the aims of the thesis which are the evaluation of MRI as a predictor for CDMS and testing atrophy as a surrogate marker. T2 lesions on the baseline MRI are sensitive indicators for CDMS at 3 years, but lack the specificity of the McDonald Criteria. The McDonald Criteria are more specific if a new T2 lesion is used as evidence of DIT. GMF and BPF atrophy together with increasing VV are sensitive markers for tissue loss in the first 3 years in patients who develop MS. The relatively modest correlations between lesion load and atrophy suggest that the processes are partly independent and that both will required monitoring as surrogate markers of disability and when evaluating new therapeutic agents for MS.

List of Abbreviations:

Brain Parenchymal Fraction	BPF
Central Nervous System	CNS
Cerebrospinal Fluid	CSF
Clinically Definite Multiple Sclerosis	CDMS
Clinically Probable Multiple Sclerosis	CPMS
Clinically Isolated Syndromes	CIS
Dissemination in Space, Time	DIS, DIT
Grey Matter Fraction	GM GMF
Interferon beta	IFNbeta
Laboratory supported Definite Multiple Sclerosis	LSDMS
Multiple Sclerosis	MS
McDonald Multiple Sclerosis	McDMS
Normal Normal Appearing White Matter	NAWM
Nuclear Magnetic Resonance	NMR
Oligoclonal Bands	OB
Patient, Patients	Pt, Pts
Primary Progressive Multiple Sclerosis	PPMS
Relapsing Remitting Multiple Sclerosis	RRMS
Secondary Progressive Multiple Sclerosis	SPMS
Spin quantum number	S
Statistical Parametric Mapping 1999	SPM99
Ventricular Enlargement, Ventricular Volume	VE, VV
Visual Evoked Potential	VEP
White Matter, White Matter Fraction	WM, WMF

Statement of Conjoint Work

Patient Recruitment (Baseline Data)

The research I have been involved in has been part of a prospective study of patients with Clinically Isolated Syndromes suggestive of MS which began prior to my arrival in 1995. I wish to acknowledge the work of my predecessors and their involvement in this thesis. Between 1995 and 2000 Jonathon O' Riordan and Peter Brex recruited 87/119 patients from the clinical of the National Hospital for Neurology and Neurosurgery and the Neuro-ophthalmology Clinic at Moorfields Eye Hospital. Since January 2000, I have recruited a further 59 patients to this ongoing prospective study.

One Year Follow-up Data used in this thesis

Fifty-three of the 55 patients discussed in the ventricular analysis section of Chapter 5.1 had their follow at one year performed by Peter Brex- the remaining two were seen by me. I performed all the ventricular analyses.

Three Year Follow-up Data

A total of 63 patients have been imaged at three years. Forty-nine were seen by me at their three year follow up scan, the remainder were seen by Peter Brex. I performed all the MRI lesion, brain volume and ventricular volume analyses.

Novel Techniques

During this research, I had the opportunity to use two techniques for atrophy measurement. The MIDAS technique in Chapter 5.1 was developed by Bill Crum (Dementia Research Group, Institute of Neurology). The SPM99 brain segmentation technique used in Chapter 5.2 was developed by Declan Chard and Geoff Parker.

Chapter 1

1. Multiple Sclerosis and Clinically Isolated Syndromes

1.1. Multiple Sclerosis

1.1.1. Introduction

Multiple Sclerosis (MS) is a chronic, inflammatory, demyelinating, autoimmune disorder of the central nervous system (CNS) affecting approximately 1 million people globally and is the commonest cause of neurological impairment in young people.[Williams *et al.* 1995] Usually, the disease is characterised by episodic neurological events disseminated in time and space with increasing disability over many years. The first neurological episode generally occurs between the ages of 20 and 40.[Weinshenker *et al.* 1989][Confavreux *et al.* 1980] Predominately females are affected in a 2:1 ratio.[Paty 1997]

Historically, the first descriptions of MS date back to “The Case of Augustus D’Este” in 1822, where he described his first episode of Optic Neuritis.[Firth 1948] Jean Martin Charcot was the first to study the disease at the Salpetriere, Paris in the latter decades of the 19th century. There has been much progress in the understanding and treatment of MS over the past 20 years. The expanded disability scale and more recently the MS Functional Composite Score are being used as clinical measures for monitoring disability.[Kurtzke 1983][Cutter *et al.* 1999] MRI has emerged as surrogate marker for disease progression with a high sensitivity for detecting MS lesions.[Noseworthy *et al.* 1984] The introduction of clinical measures of disability and MRI surrogate markers of disease progression has facilitated the conduct of large clinical trials, assessing disease modifying therapies.

Symptoms and signs can occur in any part of the CNS. The symptoms and signs seen in 50% of patients at any time include cognitive changes, euphoria, depression, fatigue, optic neuritis, optic atrophy, retinal nerve fibre loss, nystagmus, vertigo, dysarthria, limb ataxia, ataxia of the gait and trunk, sensory loss, increased deep tendon reflexes, weakness in the legs, spasticity, extensor or flexor spasms, bladder disturbance and sexual dysfunction.[Paty 1997]

1.1.2. Aetiology

Data on the geographic distribution of MS, suggest that both genetic and environmental factors influence aetiology. The most widely held hypothesis is that MS is a genetically determined disease with onset triggered by an environmental factor. Epidemiological studies suggest that MS is a predominantly European disease with increased prevalence to areas where Europeans have migrated to. Using the founder father theory genetic studies (1.14) continue to search for susceptibility genes. A summary of recent theories on an environmental trigger is also listed (1.15).

1.1.3. Epidemiology of Multiple Sclerosis

In the United Kingdom, the age and sex adjusted incidence rate of MS is 7/100,000 per year.[MacDonald *et al.* 2000] The prevalence of MS is 1-2/1000.[MacDonald *et al.* 2000] The lifetime risk is one in 400.[Compston and Coles 2002] The systematic study of MS in populations began in 1929 by Sydney Allison.[Allison 1931] Since 1975 the global distribution of MS has been classified into high (>30/100,000), medium (5-29/100,000) and low (<5/100,000) prevalence bands.[Kurtzke 1975] High prevalence bands now include most of Europe, Israel, Canada, northern US, south-eastern Australia, New Zealand, and easternmost Russia. Medium prevalence areas

include southern US, most of Australia, South Africa, the southern Mediterranean basin, Russia into Siberia, the Ukraine and parts of Latin America. Low prevalence areas include the rest of Asia, Africa and northern South America.[Kurtzke 2000]

Age at migration appears to have an effect on MS risk. Migrants travelling from a high to a lower risk area may retain the MS risk of their birth place, if they are at least age 15 at migration. Migrants aged 11-45 years travelling from a low to high risk area may increase their risk beyond that of the resident population.[Kurtzke 2000]

The recently reported prevalence of MS for Scotland of 184/100,000 is the highest described anywhere in the world for large populations.[Forbes *et al.* 1999] A “North South latitudinal gradient” for MS has been described with notable exceptions including Sardinia which has a prevalence of 152/100 000 compared with 35-90 in the rest of Italy. This may be due to genetic drift in a smaller and more isolated Sardinian population.[Granieri *et al.* 2000]

MS prevalence could in part vary due to variations in ascertainment. A capture recapture method has been used to take into account the number of unobserved cases, which showed latitudinal differences of 180 cases per 100,000 persons in the northern part of the United Kingdom compared with 160 cases per 100,000 in the southern part of the United Kingdom.[Forbes and Swingler 1999] The prevalence of MS in the Republic of Ireland was reported to be 66/100,000,[Hutchinson 1976] compared with a prevalence of 168 estimated in Northern Ireland.[McDonnell and Hawkins 1998][McDonnell and Hawkins 2000] The number of localized population based surveys on MS in the US is small compared with Western Europe and Canada. The association between latitude and risk of MS in the US was corroborated in a study to

examine incidence of MS and its relation to latitude in two ongoing studies of US women in the Nurses' Health Study (NHS), which took place between 1976 and 1994, and in the Nurses' Health Study II (NHS II), which took place between 1989 and 1995.[Hernan *et al.* 1999]

1.1.4. Genetics

An increased risk recurrence in families suggests that genetic predisposition may influence susceptibility to MS. An environmental agent may trigger the disease process in a genetically susceptible population. Associations have been made with Class II major histocompatibility complex (MHC) in particular the DRB1*1501-DQB1*0602 haplotype. Further genetic knowledge has the potential for insight into pathogenesis and novel treatments.

Two systematic screening approaches are being applied in the "GAMES" (The Genetic Analysis of Multiple Sclerosis in Europeans) project – linkage and linkage disequilibrium. The hypothesis used for the research is based on the fact that the characteristic temperate global distribution of MS follows the migration pattern of Northern Europeans and the sharing of a common ancestor. Sawcer's studies to date have yielded 10 markers of greatest interest for association with MS:

1. Three in the HLA region on chromosome 6p (D6S1615, D6S2444 and TNF α)
2. Four regions previously identified by linkage analysis in UK multiplex families (two mapping to chromosome 17q GCT6E11 and D17S1535; one to chromosome 1p GGAA30B06 and one to 19q D19S585)
3. Three novel linkage sites (D1S1590 at 1q; D2S2739 at 2p; D4S416 at 4q).

[Sawcer *et al.* 2002]

1.1.5. Environmental factors

Infection has been implicated as the environmental trigger. Evidence in favour of this hypothesis includes study of transmission and the nature of MS in the Faroe Islands. It has been proposed that three separate “epidemics” of MS occurred beginning 1943 and ending in 1973, which were introduced by asymptomatic British Troops between 1941 and 1942 and that later “epidemics” resulted from transmission by affected but asymptomatic Faroese.[Kurtzke and Hyllested 1986]

A possible role of Chlamydia Pneumonia has been suggested.[Sriram *et al.* 1998][Sriram *et al.* 1999] However, when this possible association was explored by other laboratories, Chlamydia Pneumonia specific DNA could not be detected in the Cerebrospinal Fluid (CSF) of patients with MS. [Boman *et al.* 2000][Saiz *et al.* 2001]

1.1.6. Pathology

Demyelination

Oligodendrocytes are responsible for synthesis and maintenance of the myelin sheath in the CNS. Immune mediated apoptotic death of the oligodendrocytes appears to be crucial in the pathogenesis of demyelination [Rodriguez and Lucchinetti 1999].

Demyelinating lesions

The inflammatory, potentially disabling, MS lesion is the hallmark of demyelination. MS plaques can be subdivided into active and inactive plaques. Myelin degradation products in plaques are a helpful means of determining activity. Remyelination appears to depend on several processes including myelinating oligodendrocytes and

their interaction with axons.[Bruck *et al.* 2003] Four different patterns of plaque formation have been described.

1. Patterns I and II are similar to T-cell-mediated or T-cell plus antibody-mediated autoimmune encephalomyelitis.
2. Patterns III and IV suggest a primary oligodendrocyte dystrophy induced by a virus or toxin.[Lucchinetti *et al.* 2000]

Axonal loss

Early pathological descriptions of MS were characterized by multifocal demyelination with preservation of axons. Recently, there has been a focus on axonal damage which is a key predictor of outcome in MS. Focal patches of axonal injury are quantified using intensely immunoreactive β -APP (β -amyloid precursor protein). Axonal transection has been seen in active MS lesions.[Trapp *et al.* 1998] Reduced axonal density has more recently been quantified in the Normal Appearing White Matter (NAWM) of both brain and spinal cord.[Evangelou *et al.* 2000][Lovas *et al.* 2000]. Axonal loss and gliosis are often marked in chronic lesions of patients with long disease durations and progressive MS.

1.1.7. Pathophysiology of demyelination

Demyelinated axons conduct more slowly than myelinated axons. Delays in visual, brain stem and somatosensory evoked potential conduction are used as diagnostic tools in electrophysiological laboratories. Conduction block may be temperature dependent and one such clinical manifestation is Uhthoff's phenomenon (increased symptoms and signs induced by heat).

1.1.8. Immunology

Autoimmune diseases are more common in the first degree relatives of patients with MS, suggesting that common genetic susceptibility factors for autoimmunity co-exist.[Broadley *et al.* 2000] Deregulation of the immune system in MS may be due to molecular mimicry by T-cells autoreactive for myelin components. Autoantigens implicated include myelin basic protein (MBP), proteolipid protein (PLP), myelin associated glycoprotein and myelin oligodendrocyte glycoprotein (MOG). MS is often considered to be a T-Helper Type 1 cell-mediated disease with proliferation and activation of CD4+ T-cells and secondary macrophage recruitment. Other cells implicated in the disease process include B-cells (plasma cells), CD8+ T-cells and reduced functional activity of natural killer cells. Inflammatory MS lesions are associated with up-regulation of cytokines including IL-2, γ -IFN and TNF- α . IL-12 is produced by activation of chemokines including CCR5 and CXCR3. There is activation of class MHC II molecules on antigen-presenting microglia and macrophages, astrocytes and endothelial cells. Astrocytes and microglia release cytokines and growth promoting factors including insulin derived growth factor, which have potential to mediate tissue damage, repair and remyelination. Increased levels of adhesion molecules including (VCAM-1) have been found in the CSF and serum of MS patients and such upregulation may facilitate recruitment of circulating leukocytes. Selective adhesion molecule inhibition may therefore prevent the formation of new inflammatory lesions.

1.1.9. Diagnosis

Charcot developed diagnostic criteria for MS in 1868. He described a triad of: nystagmus, intention tremor and scanning speech.

1.1.9.1. Schumacher criteria (Table 1.1)

In 1965, George Schumacher lead a panel of clinical neurologists and published the criteria listed in Table 1.1 [Schumacher *et al.* 1965]:

Table 1.1 The Schumacher criteria for the diagnosis of MS

Objective neurological examination abnormalities attributable to CNS dysfunction History or Neurological examination indicating involvement of ≥ 2 parts of the CNS.
Objective neurological evidence of CNS disease predominately reflecting white matter involvement (fiber tract damage).
Involvement of the neuraxis must follow one of the following patterns: ≥ 2 episodes of worsening, each lasting at least 24 hours and ≥ 1 month apart or slow or stepwise progression of signs and symptoms over ≥ 6 months.
The age onset of the disease must be 10 - 50 years and the signs and symptoms cannot be better explained by another disease process. (This decision must be made by a physician competent in clinical neurology)

1.1.9.2. The Poser diagnostic criteria for MS

In 1982, American, Canadian and British MS experts produced revised criteria adding laboratory, neuroimaging, and neurophysiology results to the clinical evaluation. [Poser *et al.* 1983]

Table 1.2 The Poser criteria for the diagnosis of MS

Category	Attacks	Clinical Evidence	Paraclinical Evidence	OB/IgG *
Clinically Definite				
CDMS A1	2	2		
CDMS A2	2	1 and	1	
Laboratory-Supported Definite				
LSDMS B1	2	1 or	1	+
LSDMS B2	1	2		+
LSDMS B3	1	1 and	1	+
Clinically Probable				
CPMS C1	2	1		
CPMS C2	1	2		
CPMS C3	1	1 and	1	
Laboratory-Supported Probable				
LSPMS	2			+

***OB/IgG:** CSF oligoclonal bands and increased production of immunoglobulin G.

1.1.9.3. The McDonald criteria for the diagnosis of MS

In July 2001 revised criteria were published which incorporated patients with CIS and an abnormal MRI (Table 1.3 and applied in Chapter 4). Failure to satisfy the criteria results in possible MS, pending analysis, or not MS. [McDonald *et al.* 2001]

Table 1.3 The McDonald criteria for the diagnosis of MS

Presentation	Attacks	Clinical Lesions	MRI Lesions	OB	VEP	Clinical Data
	≥2	≥2				
	≥2	1	DIS or ≥2	+		
	1	≥2	DIT Or			Second Attack
CIS	1	1	DIS + DIT or ≥2 + DIT	+		Second Attack
PPMS: Insidious progression suggestive of MS			≥9 Brain or ≥2 Cord or 4-8 Brain+1 Cord or 4-8 Brain or	+	+	Progression 1 year

OB = Positive CSF Oligoclonal Bands, VEP (+) = Abnormal visual evoked potential of the type seen in MS (Delayed with preserved waveform)

MRI Dissemination in Space (DIS)

Three of four of the following:	1 gadolinium enhancing lesion or 9 T2 Lesions
	≥ 1 infratentorial lesion
	≥ 1 juxtacortical lesion
	≥ 3 periventricular lesions

One spinal cord lesion can be substituted for one brain lesion.[Tintore *et al* .2001; Barkhof *et al*. 1997].

MRI Dissemination in Time (DIT)

Time of first MRI scan Number	MRI criteria for evidence of DIT
≥3 months after CIS onset	A gadolinium-enhancing lesion. If there is no enhancing lesion at this time, a further scan, 3 months later, is required. A new T2- or gadolinium-enhancing lesion is then sufficient.
<3 months after CIS onset	A second scan done ≥3 months after the clinical event showing a new gadolinium-enhancing lesion or a new T2 lesion on a further scan not < 3 months after the first scan.

1.1.10. Investigations used in the diagnosis of MS

Theoretically, using the new diagnostic criteria, MS may be diagnosed by one clinical episode and MRI evidence of DIS and DIT or two clinical neurological episodes disseminated in time and space. Paraclinical laboratory investigations and MRI are often used in the diagnostic protocol in order to exclude conditions that can mimic MS such as hereditary spastic paraplegias, ataxias, leucodystrophies, vasculopathies, CADASIL, granulomatous and vasculitic diseases. MRI brain and spinal cord are used to observe evidence of lesions disseminated in time and space. Oligoclonal bands present in the CSF but not in the serum are specific for intrathecal inflammation. Delayed visual evoked potentials are further evidence for conduction delay due to demyelination.

1.1.11. Different disease courses in MS

The clinical course of disease progression is heterogeneous. Approximately 85 - 90% of patients present with an acute CIS [Compston *et al.* 1998], and subsequently evolve to a relapsing remitting MS (RRMS). A relapse is defined as a subjective report or objective observation of neurological impairment lasting at least 24 hours. A clinical assessment excludes a pseudoattack, such as change in neurological status due to change in core body temperature or infection. Single paroxysmal episodes including tonic spasms do not constitute a relapse, but multiple episodes over 24 hours do.

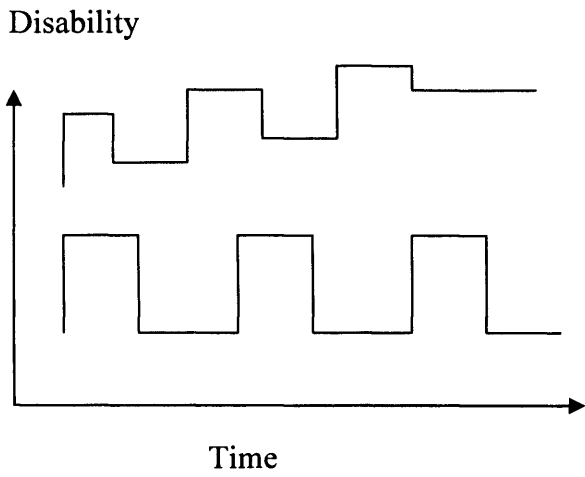
Approximately 70 - 90% of patients initially have RRMS and later convert to Secondary Progressive MS (SPMS), with increasing disability and a paucity of relapses. About 10 - 15% of patients follow a progressive course from the onset of the disease: Primary Progressive MS (PPMS).[Thompson *et al.* 1997][Thompson *et al.*

2000] Some patients with primarily progressive MS may have relapses: Relapsing Progressive Multiple Sclerosis. In large populations, 20% - 40% have benign disease, defined as having less than moderate disability after 15 years.[Lublin and Reingold 1996] Female sex, younger age of onset, and presentation with optic neuritis and sensory symptoms are more likely to have a benign course, although many exceptions occur. The label Benign MS is potentially misleading because many of these patients subsequently become disabled [Hawkins and McDonnell 1999]

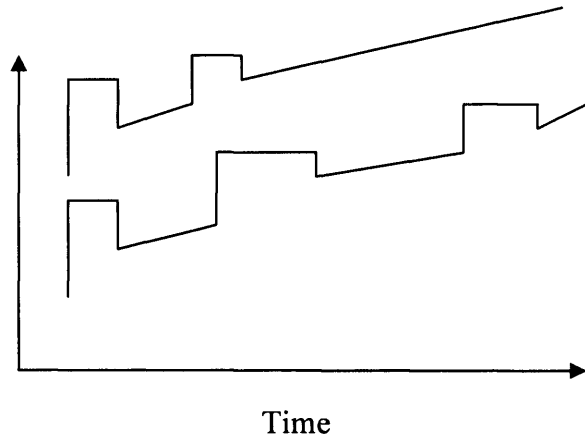
Patients with the greatest risk of disability are those with PPMS and RRMS patients who are older at onset, have pyramidal or cerebellar involvement at onset, and who have frequent or prolonged attacks with incomplete recovery.[Weinshenker 1995] The relative risk for reaching Expanded Disability Status Score (EDSS) 6 (walking 100 m with aids), 8 (wheelchair), and 10 (death from MS) has been calculated using a multivariate analysis for selected predictors of outcome in the London, Ontario cohort.[Ebers 2001] The greatest single predictor is progressive course (relative risk = 6), followed by relapse rate in year 1 and year 2 (relative risk = 3). Determining the "year of progression" seems to be significant for the prognosis.[Minderhoud *et al.* 1988] The time between the first and second attack, polysymptomatic onset and time from onset to EDSS 3 all carry similar risk (relative risk = 2). When patients reach a score of 4 on the EDSS, the progression of disability is not affected by relapses suggesting a dissociation between recurrent acute focal inflammation and progressive degeneration [Confavreux *et al.* 2000; Confavreux *et al.* 2003]. The relapse rate may have an effect when the EDSS is less than 4.

Figure 1.1 Diagrammatic representations of MS

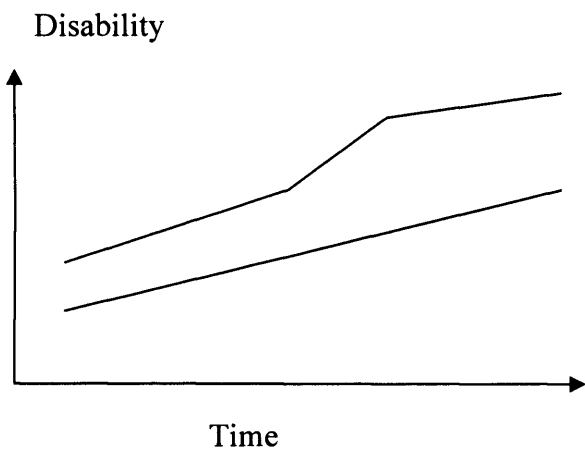
1 Relapsing Remitting



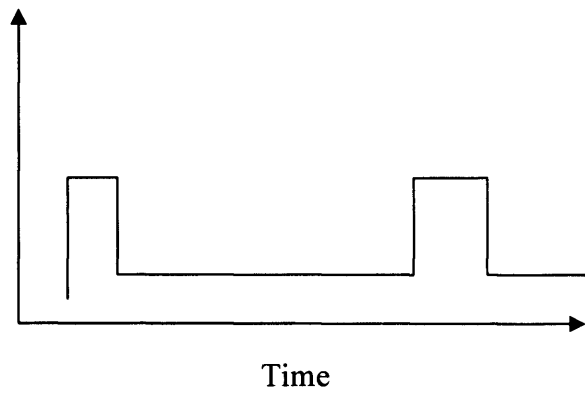
2 Secondary Progressive



3 Primary Progressive



4 Benign



1.1.12. Measurements of impairment and disability

Disability is acquired by incomplete recovery following a relapse and disease progression. In 1955 John Kurtzke described a new scale for evaluating disability in MS, later known as the disability status scale (DSS) which was revised in 1983 John Kurtzke and called Expanded Disability Status Scale (EDSS). The scale was subdivided into steps (1.0, 1.5, 2.0,9.5). The lower portion was divided by Functional Systems (FS) grades namely: Pyramidal, Cerebellar, Brain Stem, Sensory, Bowel, Bladder, Visual, Cerebral, and Other.[Kurtzke1983]

More recently a patient based measure of walking ability (12 Item MS walking scale MSWS-12) called the MS Functional Composite (MSFC) has been developed.[Hobart *et al.* 2003]

The EDSS is used throughout this thesis as a scale for disability. The MSFC has become popular since this project has begun and has been validated concurrently using MRI as a biological disease marker.[Kalkers *et al.* 2001b] The MSFC has not been used in this longitudinal project, because baseline data are not available.

Table 1.4 Kurtzke Expanded Disability Status Scale (EDSS)

0.0 - Normal Neurological Exam

1.0 - No disability, minimal signs on 1 FS

1.5 - No disability minimal signs on 2 of 7 FS

2.0 - Minimal disability in 1 of 7 FS

2.5 - Minimal disability in 2 FS

3.0 - Moderate disability in 1 FS; or mild disability in 3 - 4 FS, though fully ambulatory

3.5 - Fully ambulatory but with moderate disability in 1 FS and mild disability in 1 or 2 FS; or moderate disability in 2 FS; or mild disability in 5 FS

4.0 - Fully ambulatory without aid, up and about 12hrs a day despite relatively severe disability. Able to walk without aid 500 meters

4.5 - Fully ambulatory without aid, up and about much of day, able to work a full day, may otherwise have some limitations of full activity or require minimal assistance. Relatively severe disability. Able to walk without aid 300 meters

5.0 - Ambulatory without aid for about 200 meters. Disability impairs full daily activities

5.5 - Ambulatory for 100 meters, disability precludes full daily activities

6.0 - Intermittent or unilateral constant assistance (cane, crutch or brace) required to walk 100 meters with or without resting

6.5 - Constant bilateral support (cane, crutch or braces) required to walk 20 meters without resting

7.0 - Unable to walk beyond 5 meters even with aid, essentially restricted to wheelchair, wheels self, transfers alone; active in wheelchair about 12 hours a day

7.5 - Unable to take more than a few steps, restricted to wheelchair, may need aid to transfer; wheels self, but may require motorized chair for full day's activities

8.0 - Essentially restricted to bed, chair, or wheelchair, but may be out of bed much of day; retains self care functions, generally effective use of arms

8.5 - Essentially restricted to bed much of day, some effective use of arms, retains some self care functions

9.0 - Helpless bed patient, can communicate and eat

9.5 - Unable to communicate effectively or eat/swallow

10.0 - Death

1.1.13. Treatment of MS

Non pharmacological treatments for MS include physiotherapy, occupational therapy, and speech and language therapy. These services may be available in a multidisciplinary neurorehabilitation clinic. Other non pharmacological treatments, outside the domain of occupational therapy include eye patching and prisms for diplopia. Amantadine or Modafinil may be used for the treatment of fatigue.[Zifko *et al.* 2002][Rammohan *et al.* 2002] Bladder dysfunction may be managed with anticholinergic agents and or intermittent self catheterisation. Trials of intravesical vanilloids and cannabinoids are ongoing. Laxatives and dietetic intervention are used for the treatment of constipation. Sildenafil is being used for male sexual dysfunction and trials are ongoing in female patients.[DasGupta and Fowler 2003] Clonazepam, Isoniazid and beta-blockers can be used for cerebellar tremor. Oral and intrathecal Baclofen, intramuscular Botulinum Toxin and oral Tizanidine are used for treatment of painful spasms associated with spasticity.

Symptomatic Treatments during a Relapse

Over 30 years ago Corticotrophin was shown to hasten recovery from acute relapses.[Rinne *et al.* 1968][Rose *et al.* 1970] In 1983 methylprednisolone administered intravenously was shown to work more rapidly than corticotrophin and has superceded corticotrophin treatment.[Abbruzzese *et al.* 1983][Thompson *et al.* 1989] Short courses of steroids may be used for treatment of relapses such as optic neuritis, diplopia, brainstem vertigo, and sensorymotor dysfunction. Controversy still remains as to the efficacy, route of administration and steroid dosage in MS therapy.[Andersson and Goodkin 1998] At present 1g of intravenous methyprednisolone, administered for three days is a widely used regimen.

Disease modifying agents

The ultimate goal of disease modifying agent drug trials is to reduce the number of relapses and disability. The most recent therapeutic phase II and phase III studies of disease modifying agents have included both clinical (number of relapses) and MRI (new Gadolinium enhancing, T2 and T1 hypointense lesions) parameters as primary and secondary outcome measures.

Evidence from large double blinded placebo controlled clinical trials supports the use of interferon-beta-1a (IFNbeta-1a), interferon-beta-1b (IFNbeta-1b), and glatiramer acetate (GA) for treatment of RRMS.[PRISMS Study Group 1998][PRISMS 2001][Paty and Li 1993;1995][Jacobs *et al.* 1996][Simon *et al.* 1998][Johnson *et al.* 1998][Li and Paty 1999][Comi *et al.* 2001b] In January 2001 the Association of British Neurologists considered the evidence for the effectiveness of these drugs in clinically active RRMS to be conclusive (www.theabn.org/downloads/msdoc.pdf).

Comparison studies between disease modifying agents

In a randomized, controlled, multicenter trial comparing the efficacy and safety of IFNbeta-1a (Rebif) 44 µg Sub-Cutaneously (SC) three times weekly and IFNbeta-1a (Avonex) 30 µg Intra Muscularly (IM) once weekly in 677 patients with RRMS, IFNbeta-1a 44 µg SC was found to be more effective than IFNbeta-1a 30 µg IM on all primary and secondary outcomes investigated after 24 and 48 weeks of treatment.[Panitch *et al.* 2002][Khan *et al.* 2002] In a prospective, non-randomized, open-label treatment trial of immunomodulatory therapy, the effect of IFNbeta-1a (Avonex), IFNbeta-1b (Betaseron) Glatiramer Acetate (GA, Copaxone) and no treatment were compared on the relapse rate in RRMS. One hundred and fifty-six

consecutive patients with clinically definite RRMS with an EDSS score of 4 or less were followed for 18 months. Compared to the untreated group (1.02), the mean annualized number of relapses was significantly reduced in the GA (0.49, $P > 0.0001$) and IFNbeta-1b groups (0.55, $P = 0.001$), in contrast to the IFNbeta-1a treated patients (0.81, $P = 0.106$) who did not show a significant reduction. [Khan *et al.* 2001] The INCOMIN study was a 2-year, prospective, randomised, multicentre study which compared alternate-day IFNbeta-1b 250 µg and once-weekly IFNbeta-1a 30 µg in a cohort of 188 patients with RRMS. The study concluded that alternate day high-dose IFNbeta-1b was more effective than once weekly IFNbeta-1a [Durelli *et al.* 2002].

Serum neutralizing antibodies

Serum neutralizing antibodies to IFNbeta have been found in about 20% of patients on SC IFNbeta-1a preparations and 40% on IFNbeta-1b. These antibodies reduce the clinical efficacy on relapse rate, whether they have an impact long term disability and progression remains unclear. There is a lack of consensus on how these antibodies should be measured. The relative prevalence of antibodies induced by different IFNbeta products seems to be consistent between studies. SC IFNbeta-1b is the most immunogenic, followed by SC IFNbeta-1a, with IM beta-1a being the least immunogenic. Differences between IFNbeta products with regard to their biochemistry, formulation, route of administration, and dose frequency are likely to contribute to these observed differences in immunogenicity [Giovannoni *et al.* 2002].

Myelosuppressive treatment with mitoxantrone

Mitoxantrone has been approved by the Food and Drug Administration (FDA) for the treatment of patients with worsening RRMS or SPMS, following the results of a

multicentre study of Mitoxantrone combined with Methylprednisolone in active disease.[Edan *et al.* 1997] Given the myelosuppressive and cardiotoxic effects of Mitoxantrone, indications for use are limited to patients with RRMS with frequent disabling relapses leading to future permanent severe disability and patients with SPMS whose EDSS increases by ≥ 1 point per year and who do not respond to other current therapies.[Gonsette 2003] The current regime includes an induction phase with a monthly IV dose of 12 mg/m^2 , followed by a maintenance phase with 12 mg/m^2 every 3 months for 2 years (maximum cumulative dose of 140 mg/m^2). The dosage is adapted to the body surface area. Side effects include: amenorrhoea, leukaemia and cardiotoxicity. Cardiotoxicity is reduced by slow infusion over 30 min. Records of three clinical trials including 1,378 patients who received Mitoxantrone revealed an incidence of CHF of $<0.20\%$ in patients with MS who received a mean cumulative dose of 60.5 mg/m^2 Mitoxantrone.[Ghalie *et al.* 2002] A double blind placebo controlled trial of 194 patients with progressive MS treated with Mitoxantrone 12 mg/m^2 , concluded Mitoxantrone was generally well tolerated and reduced disability progression and clinical exacerbations.[Hartung *et al.* 2002]

Prevention of breakdown of the Blood Brain Barrier (BBB)

A novel approach is treatment with Selective Adhesion Molecule Inhibitors (SAMI). Natalizumab is an adhesion molecule inhibitor which prevents trafficking across the Blood Brain Barrier (BBB). In a phase II study, Natalizumab reduced the numbers of new enhancing lesions by 90% and relapses by 50%. Phase III studies are in progress.[Miller *et al.* 2003]

1.1.14. Future possibilities for remyelination

The degree of demyelination in MS may be related to the level of axonal loss. This may be partly due to the loss of oligodendrocytes which are trophic factors for axons. The extent of remyelination correlates with the presence of oligodendrocytes in the lesions.[Lassmann *et al.* 1997] Remyelination may restore function and be neuroprotective. Thus, there is much interest in mechanisms of remyelination in MS.

Although partial remyelination occurs in demyelinating lesions, longlasting myelin repair fails with increasing disability. The proliferative oligodendrocyte progenitor, the most efficient remyelinating cell, has been studied in the rodent and more recently has been identified in human white matter.[Scolding *et al.* 1998]

Oligodendrocyte growth factors have been identified in MS lesions: (IGF1) and (PDGF), which is made by astrocytes. Nine growth factors have been identified in the rodent.[Franklin 2002] Manipulating the Growth factor environment may encourage myelin repair. Early exposure to fibroblast growth factor may be beneficial, whereas late exposure causes further damage. Schwann cells are a realistic candidate for remyelination. Neuronal stem cells may be applicable in the future. Injection of adult neurospheres induced recovery in a chronic MS rodent model.[Pluchino *et al.* 2003] Rat bone marrow stem cells have been shown to convert to neural stem cells and used to repair demyelinated cord lesions. The future of autologous stem cell transplantation is unclear ethically and from a cell type standpoint.

1.2. Clinically Isolated Syndromes CIS

1.2.1. Introduction to CIS and conversion to MS

Approximately 90% of patients with MS present with a CIS.[Compston *et al.* 1998] A CIS as an acute or subacute neurological episode suggestive of demyelination affecting the optic nerves, brainstem or spinal cord, in patients aged between 10 and 50 years. The symptoms and signs may indicate a lesion in the spinal cord (50%), optic nerves (25%) and brain stem (15%).

Optic Neuritis and risk of conversion to MS

Optic neuritis is a common unilateral or bilateral cause of reversible loss of vision in individuals aged 20-49 with an incidence of 1-5 per100, 000 per year.[Jin *et al.* 1998]

It usually presents as a painful subacute loss of vision over a few days or weeks. The degree of visual loss may vary from blurring of vision to no perception of light. The pain is usually present on ocular movement and usually is not severe enough to affect sleep. Other symptoms and signs include deterioration in colour vision, visual field defects, reduced contrast sensitivity, a relative afferent papillary defect in the affected eye and temporary visual deterioration following exercise or heat (Uthoff). On fundus examination, the optic disc appears swollen in 36-58% of cases.[Optic Neuritis Study Group 1991] Signs of improvement were noted in the Optic Neuritis Treatment Trial placebo group within 3 and 5 weeks of onset of symptoms in 79% and 93% of patients respectively.[Optic Neuritis Study Group 1991]

The rate of conversion of a CIS to MS has been studied most in the case of optic neuritis and is approximately 30-70% (Tables 1.5 and 2.1). The risk for developing MS following childhood optic neuritis is considerably lower (Life Table analysis

showed 13% developed MS by 10 years, 19% by 20 years, 22% by 30 years and 26% by 40 years.[Lucchinetti *et al.* 1997] The risk association between optic neuritis and MS varies according to country of origin. The lowest percentage conversion figures were reported from a Chilean retrospective study (4.3%) and a Brazilian prospective study (10.8%).[Alvarez and Cardenas 1989][Lana-Peixoto and Lana-Peixoto 1991] The highest percentage conversion was reported in a prospective Finish study (75%).[Nikoskelainen *et al.* 1981] The low percentages in the Chilean study may be explained by its retrospective nature.[Alvarez and Cardenas1989]

Conversion of a brainstem and spinal cord syndrome to MS

If the brainstem or spinal cord CIS is confirmed to be demyelinating in origin, then MRI is useful in determining the relative risk for future demyelinating episodes. The association between brainstem and spinal cord syndrome with MS is less clear because of the heterogeneity of the syndromes. The MS risk associated with complete spinal cord syndromes was found to be low 1/29 (3%).[Lipton and Teasdall 1973] Incomplete spinal cord syndromes are associated with a much higher risk of MS, 12/15 (80%).[Ford *et al.* 1992] The duration of follow up has important implications on the rate of conversion of brainstem and spinal cord syndromes to MS. A short (3.08 years) study reported 35% conversion.[Sastre-Garriga *et al.* 2003] The longest (14.1 years) clinical and MRI follow up study reported 9/14 (64%) brainstem and 14/21 (67%) spinal cord conversion rates to CDMS. [Brex *et al.* 2002]

1.2.2. Counselling of patients with CIS

In view of the fact that patients with CIS are known to be at risk of MS, we tell all patients with optic neuritis and CIS about the association with MS using both written

and verbal information. In many instances, patients have already learned of the MS association from an information search on the internet. In collaboration with the MS Society of Great Britain and Northern Ireland we have drafted an information sheet regarding optic neuritis and the associated risk of MS. (Appendix 1)

1.2.3. Information and treatment at the time of diagnosis of MS

At the time of diagnosis both written and verbal information should be given regarding treatment and prognosis. Discussion with a MS specialist nurse is also helpful. In the longer term the patient may benefit from discussions with and information from the MS society. Informal lectures and group discussions regarding issues pertaining to newly diagnosed patients are also helpful. Some patients with CIS may request not to have information regarding MS, fearing it may affect mortgages, life insurances etc., and it is essential their wishes are respected.

1.2.4. Diagnosis of MS in patients with CIS

In the past, a definitive diagnosis of MS could not be made at presentation on patients with a CIS. The publication of the McDonald criteria, has for the first time allowed a diagnosis of MS in patients with CIS and an abnormal MRI.[McDonald *et al.* 2001]

1.2.5. Oligoclonal Bands

The presence of oligoclonal bands in the CSF, demonstrated by means of isoelectrophoresis, has been associated with a higher risk of MS.[Miller *et al.* 1989] In one study, 7/43 with optic neuritis developed MS. Although, all had an abnormal MRI, 6/7 had oligoclonal bands, suggesting bands are less sensitive.[Tintore *et al.* 2001] In another study abnormal IgG levels in the CSF correlated more strongly than

abnormal MRIs with the subsequent development of CDMS [Jacobs *et al.* 1997]. More recently the Barkhof MRI criteria for were found to have a better specificity than oligoclonal bands for the development of CDMS (70% versus 43%) [Tintore *et al.* 2001]. The presence of at least 2 lesions and oligoclonal bands resulted in an increased sensitivity but reduced specificity compared with the Barkhof criteria alone for the diagnosis of CDMS at three years. [Tintore *et al.* 2003]

1.2.6. Neurophysiology

Neurophysiology investigations, in particular Visual Evoked Potentials (VEP) have a role in determining dissemination in space, as may Somato Sensory (SSEP), Auditory and Brain Stem Evoked Potentials. However, they have a lower sensitivity compared with MRI. In a study of 51 patients with brain stem CIS, no statistically significant differences were found in neurophysiology parameters between patients who converted to CDMS compared to those who did not.[Sastre-Garriga *et al.* 2003]

1.2.7. McDonald criteria in patients with CIS

In patients with one attack, objective clinical evidence of one lesion (monosymptomatic presentation or CIS), there must be DIS, demonstrated by MRI *or* two or more MRI lesions consistent with MS plus positive CSF oligoclonal bands and DIT, demonstrated by MRI *or* a second clinical attack [McDonald *et al.* 2001].

1.2.8. Treatment of CIS patients with steroids and disease modifying agents

The effect of steroids on optic neuritis and the subsequent development of MS was assessed in 389 patients with acute optic neuritis randomized to receive IV methylprednisolone for 3 days (1 mg per kg) followed by oral prednisolone, (1 mg per

kg) or placebo. Neurological status was assessed for 2 to 4 years. Post hoc analysis found IV methylprednisolone reduced the development of MS over a two-year period. The 5-year cumulative probability of CDMS was 30% and did not differ by treatment group. Steroids have been used for the treatment of optic neuritis, however there are side effects. The more serious side effects reported in the optic neuritis treatment trial include psychotic depression and pancreatitis.[Optic Neuritis Study Group 1997]

The CHAMPS study was the first study to evaluate the use of Interferon in patients after a first demyelinating event.[Jacobs *et al.* 2000] 383 patients were recruited following their first clinical demyelinating event. Patients with optic neuritis, incomplete transverse myelitis, brain-stem and cerebellar syndromes, were included. The trial concluded that initiating treatment with IFNbeta-1a at the time of a first demyelinating event was beneficial for patients with MRI brain lesions. The response to IFNbeta-1a has been assessed using the Barkhof criteria and the treatment effect was more evident as the number of positive criteria increased. The number of patients needed to avoid one patient converting to CDMS decreased from 50 in patients with 1 or 2 positive criteria to 5.6 in patients with 4 positive criteria.[Barkhof *et al.* 2003] In the Etoms study 309 patients with CIS (98 optic neuritis) were randomized to receive IFNbeta-1a 22µg once weekly or placebo.[Comi *et al.* 2001a] MRI requirements included 4 asymptomatic brain lesions (or 3 lesions if one was Gadolinium enhancing). After 2 years the odds ratio for conversion to CDMS was 0.61 in the treatment group compared with placebo (p<0.001).

Table 1.5 Longitudinal studies of optic neuritis and the risk of CDMS (1970 – Present, MRI studies excluded)

Year	Author (Country Retrospective R, Prospective P)[Reference Recent, Past]	Pt	F/U Mean /Med	MS	(%)
2000	Frith (Australia P) [Frith <i>et al.</i> 2000][Hely <i>et al.</i> 1986]	71	13.25 Y	33	46%
1999	Druschky (Germany P)[Druschky <i>et al.</i> 1999]	26	8 Y	14	54%
1999	Landy (Australia P)[Landy 1999; 1983]	134	0.5 – 15		61%
	Landy (Australia R)[Landy and Ohlrich 1970]	51	0.5 – 15		39%
1997	Corona-Vazquez (Mexico R)[Corona-Vazquez <i>et al.</i> 1997]	110	2 Y	13	12%
1995	Rodriguez (USA R) [Rodriguez <i>et al.</i> 1995]	156	13.2 Y		39%
1993	Congia (Italy R)[Congia <i>et al.</i> 1993]	69	4 – 13		53.6%
1991	Mapelli (Italy 1985 P) [Mapelli <i>et al.</i> 1991; 1985]	40	12	10	25%
1991	Scholl (USA P)[Scholl <i>et al.</i> 1991]	81	3.5 Y	35	43%
1991	Lana-Peixoto (Brasil P)[Lana-Peixoto and Lana-Peixoto 1991]	88	4.6 Y	9	10.8%
1990	Sandberg-Wollheim (Sweden P)[Sandberg-Wollheim 1990; 1975]	86	12.1 Y		38.3%
1987	Francis (Compston UK P)[Compston <i>et al.</i> 1978][Francis <i>et al.</i> 1987]	101	11.6 Y	51	51 88%
1989	Gronning (Norway R)[Gronning <i>et al.</i> 1989]	34	12 Y		42%

1989	Alvarez (Chile R)[Alvarez and Cardenas1989]	23	9.7 Y	1	4.3%
1989	Anmarkrud (Norway R) [Anmarkrud and Slettnes 1989]	30	2-11 Y		57%
1988	Rizzo (USA P) [Rizzo, III and Lessell 1988][Cohen <i>et al.</i> 1979]	60	14.9 Y		74%F,34% M
1983	Kinnunen (Finland) [Kinnunen 1983]	296	5.1 Y		19%
1983	Stendahl-Brodin (Sweden P) [Stendahl-Brodin and Link 1983]	30	5 Y	10	33.3%
1981	Nikoskelainen (Finland P)[Nikoskelainen <i>et al.</i> 1981b]	48	7 – 10 Y	36	75%
1974	Nikoskelainen (Finland R)[Nikoskelainen and Riekkinen 1974]	117	10.2 Y	69/109	63.3%
1979	Perkin (UK P) [Perkin 1979; Rose 1970]	78	0.5 – 5.5 Y	45	58%
1976	Hutchinson (UK R) [Hutchinson1976]	132	1-15 14 Y	67	78%
1976	Kahana (Israel R) [Kahana <i>et al.</i> 1976]	105	9.5(3.3–15.6) Y	27	28%
1973	Alter (Hawaii R) [Alter <i>et al.</i> 1973]	28	10 Y		29%-39%
1972	Sandberg (Norway R) [Sandberg 1972]	51	3-7 Y		47%
1972	Percy (Rochester R) [Percy <i>et al.</i> 1972]	24	18 Y		17%

Table 1.6 Longitudinal studies of spinal cord and brainstem syndromes and the risk of CDMS

Year	Author [reference recent, past]	No.	Years f/u	Syndrome	MS Number %
2003	Sastre Garriga [Sastre-Garriga <i>et al.</i> 2003][Tintore <i>et al.</i> 2000]	51	3.08	BS	18/51 (35%)
2002	Brex [Brex <i>et al.</i> 2002][O'Riordan <i>et al.</i> 1999][Morrissey <i>et al.</i> 1993][Miller <i>et al.</i> 1987]	71	14.1	ON, BS, SC	ON 25/36 69%, BS 9/14 64%, SC 14/21 67%
1997	Barkhof [Barkhof <i>et al.</i> 1997][Tas <i>et al.</i> 1995]	74	3.25	ON,SC,SS, M,BS	33/74 45%
1996	Paolino [Paolino <i>et al.</i> 1996]	44	2.17	BS 22 SC 22	30/44 68%
1995	Campi [Campi <i>et al.</i> 1995]	30	1.5	SC	8/30 27%
1994	Filippini [Filippini <i>et al.</i> 1994]	82	2.9	SC BS C	28/82 34%
1992	Ford [Ford <i>et al.</i> 1992]	38	15	SC (I)	12/15 80%
1991	Sharief [Sharief and Thompson 1991]	45	1.5	BS SC	13/45 29%
1973	Lipton [Lipton and Teasdall 1973]	5 Y	2.42	SC (Complete)	1/29 3%

BS = Brain Stem, SC = Spinal Cord, ON = Optic Neuritis, SS = Somatosensory, M = Motor, C = Cerebrum, I = Incomplete

Chapter 2

2. MRI and MS and CIS

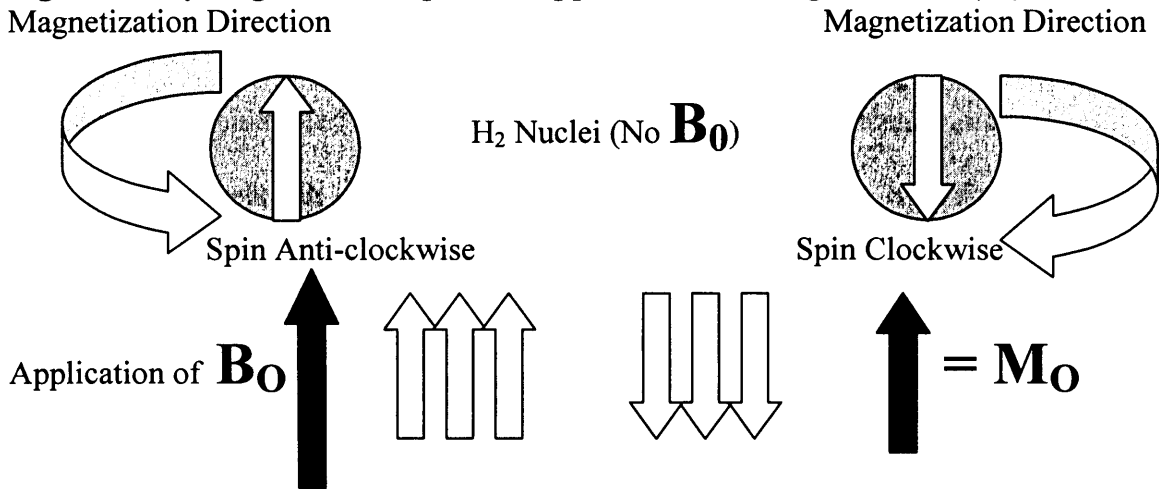
2.1. Basic principles of MRI

In 1946 Bloch and Purcell independently developed theories on Nuclear Magnetic Resonance (NMR) for which they won the Nobel Prize in 1952. The basis of their theory was that a spinning charged hydrogen nucleus creates an electromagnetic field. Between 1950 and 1970 MRI was used for chemical and physical molecular analysis. Raymond Damadian, Lawrence Minkoff, and Michael Goldsmith performed the first live human MRI scan on the 3rd of July 1977. In 1981 Young scanned 10 patients with MS using an inversion recovery sequence. Conventional MRI was shown to be highly sensitive in detecting lesions.[Young *et al.* 1981] MRI has become an essential tool in the diagnosis and management of MS and the evaluation of therapeutic agents for treatment of MS.

2.1.1. Hydrogen protons and spin

MRI is based on the nuclei with odd numbers of protons. The simple hydrogen nucleus contains a positively charged proton which spins creating a small magnetic field called a magnetic dipole moment (MDM). In the absence of any external magnetic field (B_0), the axes of the MDMs cancel one another out. If B_0 is applied, the axes of the MDMs will align themselves like bar magnets, some parallel and some antiparallel with B_0 . One in a million extra spins is parallel to make the net magnetization (M_0) point in the direction of B_0 . This happens at a specific frequency (ω_L), the Larmor frequency, which is proportional to the B_0 .

Figure 2.1 Hydrogen nuclei, spin and application of a magnetic field (B_0)



2.1.2. Magnetic fields and magnetic susceptibility

B_0 is the static magnetic field. The variable magnetic field applied at the Larmor frequency is referred to as the radiofrequency pulse (RF). M_0 is the induced magnetization of intensity (M). The magnetic permeability (μ) or ability of a substance to concentrate magnetic fields is B divided by the acquired magnetic field (H). Substances magnetize when placed in a magnetic field according to their magnetic susceptibility (χ), which is the ratio of the M to H. Magnetic permeability is 1 plus χ . In MRI, B_0 in the order of one Tesla (1T) is 20,000 times the earth's magnetic field. The field is not uniform and shim coils are required to reduce this problem.

Diamagnetic substances have no unpaired electrons and induce a weak magnetic field (M) when placed in B_0 , which reduces the effective magnetic field. The majority of tissues in the body are diamagnetic. Paramagnetic substances have unpaired electrons and become magnetized when placed in B_0 and demagnetize when the field is turned off. Their M is in the same direction as B_0 . Gadolinium, the element on the periodic table with the greatest number of unpaired electrons, is a strongly paramagnetic

substance. Haemosiderin and other iron containing substances have magnetic susceptibilities 100 to 1000 times stronger than paramagnetic substances and are superparamagnetic. Ferromagnetic substances (Iron (Fe), Cobalt (Co) and Nickel (Ni)) are strongly attracted by a magnetic field and become permanently magnetized. The type of magnets can be classified according to strength. Ultra high field magnets (>3.0 T) are mainly for MR Spectroscopy (MRS). High field magnets range from (1.0 to 2.0T). Low field magnets range from (0.1 to 0.2 T) and ultra low field magnets are less than 0.1 T. Magnets may also be classified according to their design as permanent, resistive or superconducting. A superconducting 1.5 T Signa General Electric magnet at the NMR unit, was used in this study.

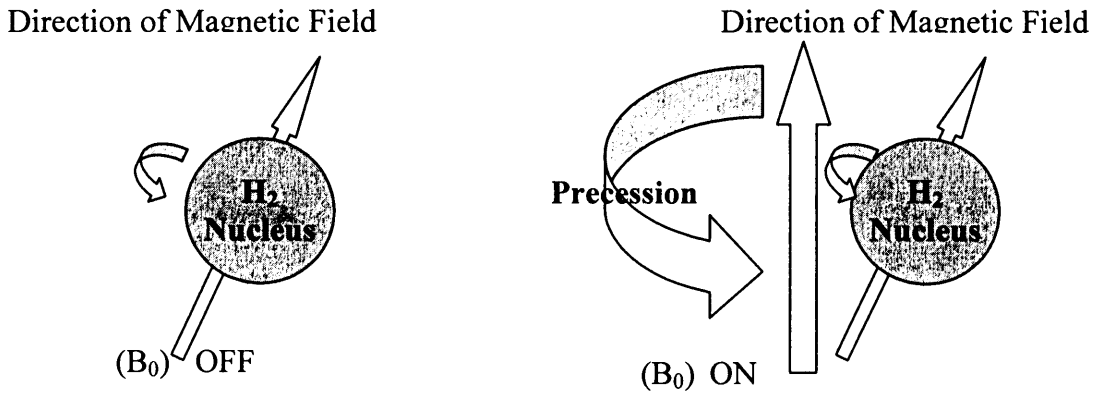
2.1.3. Performing MRI imaging: RF and MR signal

Spinning unpaired electrons in an external magnetic field align with the field, generating M_0 which is parallel to B_0 . In order to measure M_0 , the RF pulse (an electromagnetic wave of frequency ω_L), is applied to a patient and causes some of the spins to change their alignment as a result of a resonance phenomenon. After the RF pulse is turned off, they generate the MR signal as they return to their original alignment.

2.1.4. Precession

In the absence of B_0 , a proton rotates on its axis and creates a magnetic field. If an external magnetic field is then applied, the proton not only rotates about its own axis but also around the axis of B_0 , in a process called precession.

Figure 2.2: Precession of Hydrogen nuclei in a Magnetic Field



2.1.5. The Larmor equation

The rate at which the proton precesses around the external magnetic field (B_0) is expressed using the Larmor equation: $\omega = \gamma B_0$,

ω = Angular precessional frequency of Hydrogen

γ = Gyromagnetic ratio

B_0 = External magnetic field.

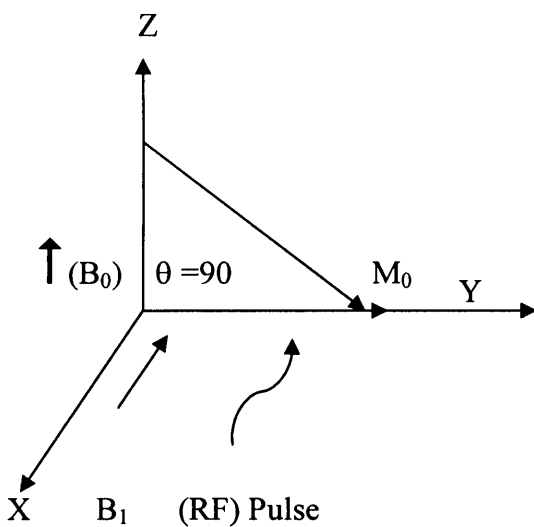
2.1.6. Phase

Phase is the position of each magnetic moment on the precession path around B_0 . Magnetic moments that are in phase are in the same place in the precession path around B_0 . Out of phase magnetic moments are not in the same position on the precession path.

2.1.7. Resonance and flip angle

Following application of the RF pulse, resonance occurs when magnetic moments move to the same position on the precession pathway, resulting in the RF pulse adding energy to the protons. The magnetization is flipped towards the x-y plane forming an angle θ with the z axis. The 90° RF pulse applied in the x plane results in transverse magnetization vector flip into the x-y (M_{xy}) plane.

Figure 2.3 Flip Angle

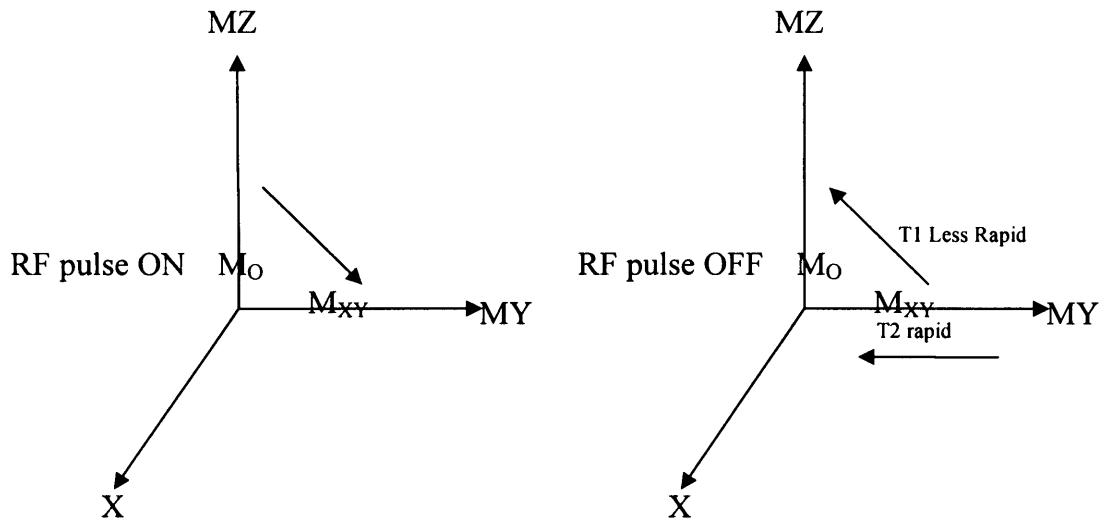


2.1.8. T1 Relaxation Time (Longitudinal Magnetization)

At time $t = 0$, M_0 is aligned with B_0 , hence we measure no signal. Following the induction of a magnetic field B_1 , or RF pulse, spins are excited creating a net magnetization in the orthogonal plane to B_0 (often referred to as the xy plane of a system where z is parallel to B_0). There is a time constant (T_1) depending on the tissue type and strength of magnet, which determines how the spins return to be aligned with B_0 following an exponential curve. T_1 longitudinal relaxation time is recovery of magnetization along the axis of the B_0 field after the RF pulse is turned off. This refers to the time for the spins to return to the longitudinal (z) axis. The spins relax

back to their lowest energy state. Water and fluids have a very long T1 compared with solids which have a long T1 and fat which has a short T1.

Figure 2.4 Radiofrequency Pulse (RF), T1 Longitudinal Magnetization and T2 Transverse Magnetization



2.1.9. T2 Relaxation Time (Transverse Magnetization)

T₂ relaxation time is rapid decay of the M_{xy} component after the RF pulse is turned off and occurs more rapidly than T₁ recovery. T₂ characteristics of a tissue are determined by how fast protons in a tissue dephase. A rapid dephase results in a short T₂ and a slow dephase results in a longer T₂. T₂ transverse relaxation is shorter than T₁ longitudinal relaxation. Water and fluids have a long T₂, solids have a short T₂ and fat has an intermediate T₂.

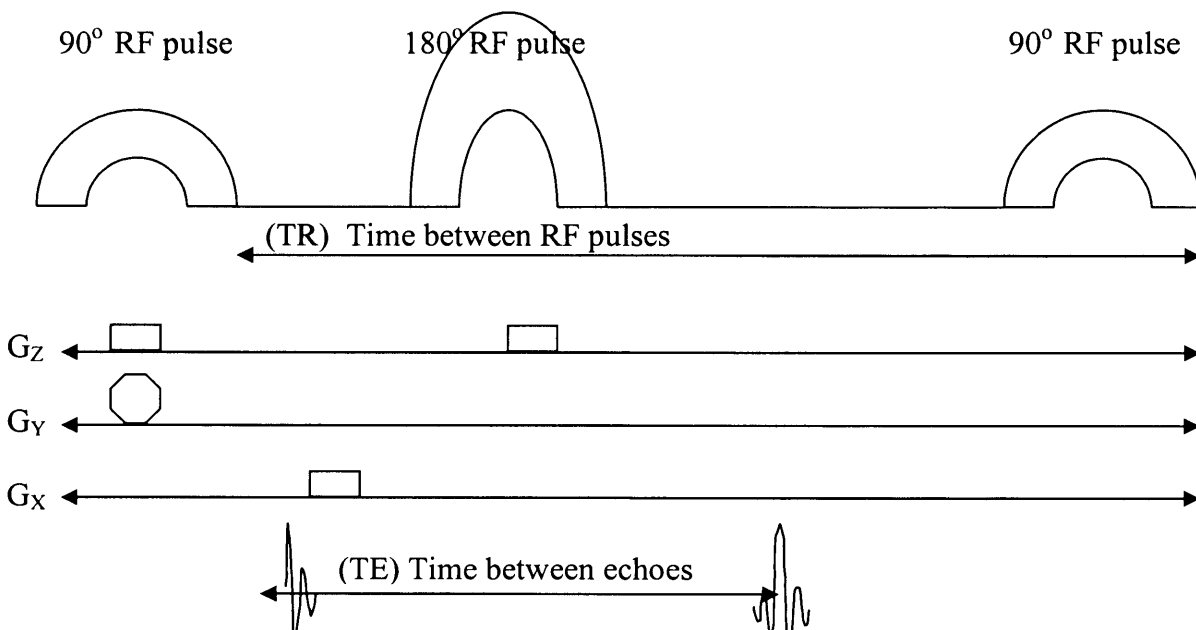
2.1.10. TR (Repetition Time) and TE (Echo Time)

The time between two successive 90° RF pulses is TR. Long TR eliminates T1 effect. TE is the time delay after the RF pulse at which signal measurement is taken. A $2 \times$ TE forms a DSE sequence.

2.1.11. Image construction

Gradients give information on specific origin of each component of the signal. The gradient required in the z gradient is the slice select gradient. The range of frequencies sampled during acquisition determines the slice thickness which can be reduced by decreasing the bandwidth of the RF pulse or by increasing the slice select gradient. The slice select gradient (G_z) is turned on during the 90° pulse and then turned off. A 180° pulse is then applied with (G_z) on. An echo is received during the readout period after time TR. A frequency encoding gradient (G_x) is applied in the x-direction. A phase encoding gradient is applied in the y direction after the RF pulse usually between and the 90° - 180° pulses or between the 180° pulse and the echo.

Figure 2.5 Spin echo pulse sequence diagram



2.1.12. K space

K space is the time-domain data, which shows the signal evolution during acquisition. A Fourier transformation converts the signal from the time domain to the frequency domain so that the Fourier transformation of K-space is the image. The centre of K-space contains the phase encoding step with the weakest gradient (most signal and overall form of the image). The periphery of K-space contains phase encoding steps with the largest gradients (least signal but most detail). The axes on a plot of raw data are K_x , K_y and K_z . [Hashemi and Bradley 1997]

2.2. Conventional MRI techniques to monitor MS

Conventional MRI is an important tool in the diagnosis of MS, monitoring disease progression and evaluation of new disease modifying agents. Conventional MRI may be subdivided into: Dual Echo T2-weighted and post contrast T1-weighted images.

2.2.1. T2 weighted sequences

Three types of T2 weighted sequences may be used: conventional spin echo (CSE), fast spin echo (FSE) and fast FLAIR. The CSE and FSE are equally good at showing MS lesions. FSE is quicker than CSE.

2.2.2. Conventional Spin Echo (CSE) sequence

Following a 90° pulse, the magnetization vector is flipped into the xy plane creating M_{xy} . Following the 90° pulse, there are dephasing effects due to external magnetic field inhomogeneities and spin-spin interactions. At a certain time, $t=TE/2$, after the 90° pulse, the spins are out of phase. A 180° rephasing pulse is then applied. Spins flip into the xy plane and precess in the opposite direction. After another $TE/2$ time, at

$t=TE$, the spins are back in phase and the spin echo is formed. This is sampled to create the image.

2.2.3. Fast image acquisition sequences FSE and RARE

Using spin echo imaging, it is possible to collect echoes at several different echo times within a single TR period. If N is the number of echoes, N lines of K-space can be encoded in a single TR, giving an N-fold reduction in scan time. This gives rise to the RARE (Rapid Acquisition with Relaxation Enhancement) or FSE.[Hennig *et al.* 1986] The Echo Train Length (ETL) refers to the number of echoes used in FSE and it is usually set to an even number (typically 8 or 16).

2.2.4. FLAIR

Fast- Fluid Attenuated Inversion Recovery (FLAIR) detects more lesions in MS than conventional spin echo sequences. Difficulties standardizing fast FLAIR acquisition parameters have limited its use in clinical trials. Other problems include high interscanner variability of MS lesion volumes and poor sensitivity for detecting lesions in the posterior fossa and spinal cord.[Gawne-Cain *et al.* 1997][Filippi *et al.* 1996][Stevenson *et al.* 1997]

2.2.5. Conventional MRI and the diagnosis of MS

Various criteria have been introduced to improve the accuracy of MS diagnosis using MRI. The criteria of Paty and Fazekas have focused on the number size and location of T2 weighted lesions.[Paty *et al.* 1994][Fazekas *et al.* 1988] The Barkhof MRI criteria depend on the fulfilment of three of the four Dissemination in Space (DIS) criteria listed below and include Gadolinium enhancing lesions.[Barkhof *et al.* 1997]

Most recently the McDonald criteria use MRI for evidence of Dissemination in Time (DIT) and DIS. DIS is based on the Barkhof criteria modified to allow the replacement of a brain lesion by a cord lesion.[Tintore *et al.* 2000] One or more Gadolinium enhancing lesions in patients with CIS imaged within three months of the onset of symptoms and again three months later is enough evidence for DIT or a new T2 weighted lesion on a scan performed thereafter.

Paty	Four lesions Three lesions including 1 periventricular lesion [Paty <i>et al.</i> 1994]
Fazekas	Three Lesions including two of the following characteristics: Infratentorial, Periventricular, Lesion > 6 mm [Fazekas <i>et al.</i> 1988]
Barkhof	One Gadolinium or 9 T2 weighted lesions One Infratentorial, One Juxtacortical, Three Periventricular Lesions [Barkhof <i>et al.</i> 1997]

2.2.6 MRI characterization of clinical subtype in MS

RRMS is characterized by the presence of Gadolinium-DTPA enhancing lesions.[Thorpe *et al.* 1996a] SPMS is associated with large confluent T2-weighted hyperintense brain lesions.[Thompson *et al.* 1991] This may be due to the natural history of SPMS which is inherently associated with more severe abnormalities than RRMS.[Nijeholt and Barkhof 2003] PPMS patients may have relatively few brain lesions, which may be small, despite frequent severe disability.[Thompson *et al.* 1991]

Primary and Secondary progressive MS differ with regard to MRI findings. In a 5 year longitudinal study of 11 patients with SPMS and 10 patients with PPPMS, a significant relationship was noted between enhancing lesions and clinical relapses during the initial 6 months and increase in disability 5 years later.[Losseff *et al.*

1996a] A recent 5 year prospective follow up study of 41 patients with PPMS showed significant deterioration in all clinical and MRI measures ($P < 0.01$, $P < 0.001$ respectively). The rates of change of MR measures were more consistent within rather than between patients.[Ingle *et al.* 2003] In patients with PPMS, spinal cord MRI parameters correlate well with spinal cord symptoms. This illustrates the importance of spinal cord involvement in this subgroup of patients.[Nijeholt and Barkhof 2003]

2.2.7 Monitoring the treatment of MS

There have been major difficulties in measuring clinical endpoints in MS treatment trials with much enthusiasm for the use of MRI as surrogate marker. Surrogacy is defined as an outcome that can reliably predict clinical outcome. Unfortunately, conventional MRI scans have a limited correlation with disability so the primary outcome in definitive phase III treatment trials should be clinical. A task force of the US National MS Society was convened to provide international consensus guidelines on the use of MRI in MS clinical trials and their findings were published in 1996.[Miller 1996] In summary their guidelines were:

- 1) Monthly MRI T2-weighted and Gadolinium-enhanced MRI are useful primary endpoints for short term exploratory trials of new agents.
- 2) The primary end point of a definitive trial is clinical. Serial MRI scans at 6- to 12-month intervals are useful secondary end points providing an index of pathological progression.
- 3) In trials of patients presenting with CIS, MRI findings can be used in the entry criteria, and as a secondary outcome measure, but conversion to CDMS should be the primary outcome.

2.2.8 Gadolinium enhancement

Post contrast Gadolinium T1 weighted scans allow discrimination between active and inactive lesions. Gadolinium enhancement occurs as a result of BBB breakdown. Serial gadolinium enhanced imaging has demonstrated disease activity in the absence of relapses with new Gadolinium enhancing lesions occurring 10 times more frequently than relapses.[Barkhof *et al.* 1992][Capra *et al.* 1992][McFarland *et al.* 1992][Stone *et al.* 1995] Clinical worsening of MS has previously been associated with increased enhancement. [Smith *et al.* 1993]

2.2.9 Limitations of conventional MRI imaging

Conventional MRI is unable to distinguish between the different types of pathology occurring in MS namely inflammation, oedema, demyelination, remyelination, axonal loss and gliosis. Also, the relationship between conventional MRI measures of lesion load and clinical measures of disease progression are weak. The discrepancy between conventional MRI lesion load measures and clinical outcome means it would be unwise to rely solely on such MRI measures as primary efficacy variables in MS treatment trials. Newer non invasive MRI techniques are being evaluated as potential surrogate markers for disease progression in MS.

2.3. Non conventional MRI techniques

2.3.1. T1 hypointense lesion analysis

T1 hypointense lesions are defined as lesions that are hypointense on moderately T1-weighted conventional spin-echo sequences.[Barkhof *et al.* 2000] T1-weighted

hypointense lesions in MS patients correspond to areas of axonal loss. T1 – hypointense lesions are associated with:

- 1) Low magnetization transfer ratios (postmortem) [van Waesberghe *et al.* 1999].
- 2) Low N-acetylaspartate concentrations [van Walderveen *et al.* 1999]
- 3) Decreased histological axonal density (Postmortem unfixed whole brains) [van Walderveen *et al.* 1998]

2.3.2. Magnetization Transfer

Magnetization Transfer (MT) is a continuous measure based on interactions between protons in a relatively free environment (tissue water) and where motion is restricted (macromolecules of myelin). Off-resonance irradiation is applied, which saturates the magnetization of less mobile protons. As free and bound protons are exchanged, the MRI signal intensity is reduced as a consequence of the protons' transfer. MTR imaging provides a non-specific assessment of structural integrity as it is affected by changes of macromolecules, such as myelin.

2.3.3. Protein Magnetic Resonance Spectroscopy (MRS) in MS

Protein Magnetic Resonance Spectroscopy (MRS) is used to investigate metabolic changes in the brain. The primary peak is produced by N-acetyl aspartate (NAA) at 2.0 ppm with secondary peaks at 3.0 ppm (creatine/phosphocreatine (Cr)), 3.2 ppm (choline-containing compounds) and 3.54 ppm (myo-inositol). Changes in the signal intensity of N-acetylaspartate (NAA) which is localized in the neurons and neuronal processes enable assessment of axonal loss or dysfunction.[Provencher 1993][Arnold *et al.* 1990][Davie *et al.* 1994] Spectroscopy studies have shown a reduced N-acetyl-aspartate in lesions suggesting axonal damage. Reductions in NAA have also been

shown in normal appearing white matter in MS.[Davie *et al.* 1997][Fu *et al.* 1998] MRS reduced NAA has been shown to correlate with disability suggesting a strong correlation between axonal loss and disability.[Matthews *et al.* 1998][Grimaud *et al.* 1999][De Stefano *et al.* 1998] More recently spectroscopic data acquired using point resolved spectroscopic localization (PRESS) and processed using LC Model to estimate metabolite concentrations in mmol per litre has revealed changes in cortical grey matter (GM) and normal appearing white matter (NAWM).[Chard *et al.* 2002a]

2.3.4. Spinal cord imaging in MS

T2 weighted spinal cord lesions can be detected in 47-90% of patients with MS. In a study of 485 lesions collected from a cohort of 10 patients with relapsing remitting MS, 42 or (9%) were in the spinal cord.[Thorpe *et al.* 1996b] Thus if MRI brain alone is used as a marker of disease activity approximately 10 lesions will be missed. Spinal cord lesions often cause more symptoms than MRI brain lesions, hence the rationale for imaging of the spinal cord.[Kidd *et al.* 1996] In one large study with 91 MS patients (28 RR, 32 SP and 31 PP), there was correlation between the number of focal brain T1 lesions and the number of focal spinal T2 lesions ($r=0.22$; $p<0.05$).[Nijeholt *et al.* 1998] Although the presence of cord lesions can increase the confidence in making a diagnosis of MS, this does not appear the case with CIS presenting with optic neuritis (Chapter 3.3).

Cord atrophy has been used as a potential marker of axonal loss in longitudinal studies of patients with CIS, RRMS, PPMS and SPMS using an automated technique.[Losseff *et al.* 1996c] In one longitudinal study disability inversely correlated at entry and follow-up with the cord area and changes and disability

correlated inversely with changes in cross-sectional area ($r = -0.4$, $p = 0.04$) suggesting cord area be a useful marker of disease evolution.[Filippi *et al.* 1997b]

2.3.5. Atrophy

Atrophy of both the brain and spinal cord (2.3.4) due to irreversible tissue loss has been described in all stages MS. Atrophy may develop following the appearance of inflammatory lesions. Significant correlations have been found between brain volume and MR neuronal markers indicating axonal loss. The pathological basis of atrophy in MS is due to loss of myelin and a marked degree of axonal loss.[Lassmann 2002] As a consequence of axonal loss in a lesion, there is Wallerian degeneration along the fibre pathways that traverse the lesion. A proportionately greater loss of small axons with relative preservation of larger axons has been noted in both the brain and spinal cord.[Ganter *et al.* 1999][Evangelou *et al.* 2001] Cerebral atrophy correlates with proton MR spectroscopic NAA/Cr ($r = 0.67$, $p < 0.001$), suggesting damage to the normal-appearing tissue rather than the extent and intrinsic pathology of macroscopic lesions seems to be important in the destructive process.[De Stefano *et al.* 2002] Significant correlations have also been found between T1 hypointense lesion load and atrophy suggesting a direct link between hypointense abnormalities detected in T1-weighted brain scans and cerebral atrophy.[Sailer *et al.* 2001]

The methodological requirements for global and regional brain volume measurements and spinal cord areas are reproducibility, sensitivity to change, stability over time, accuracy and practicality to implement.[Miller *et al.* 2002] Both global brain and upper cervical cord cross sectional areas are measured using highly reproducible techniques and sensitive to change within 6-12 months.[Losseff *et al.* 1996b][Losseff

et al. 1996c] Regional measurements of atrophy including ventricular volumes are also sensitive to tissue loss.[Fox *et al.* 2000][Redmond *et al.* 2000] The optic nerve is frequently involved in MS, however the small dimensions and artefact make measurement difficult. In a one year study of optic neuritis patients, atrophy of the affected optic nerve has been demonstrated.[Hickman *et al.* 2002] Brain and cord atrophy have roles as markers of disease progression. [Simon 2001].

Segmentation techniques are used to calculate:

- Normalized CSF volume[Wolinsky *et al.* 2001]
- Whole Brain Ratios (WBR) (Whole Brain – CSF / Intradural Volume)
- Brain Intracranial Capacity Ratio (BICCR) = $100 \times (\text{GM} + \text{WM} + \text{lesions}) / (\text{GM} + \text{WM} + \text{lesions} + \text{CSF})$ [Collins *et al.* 2001]
- Fuzzy Connectedness Segmentation FCS[Ge *et al.* 2000]
- SPM-based segmentation[Chard *et al.* 2002c]
- Template driven segmentation of the brain[Guttmann *et al.* 2000]
- Sienax.[Zhang *et al.* 2001] and brain surface modelling[Smith *et al.* 2001]
-

Registration based techniques include:

- MIDAS[Freeborough *et al.* 1997]
- Voxel Based Morphometry VBM[Ashburner and Friston 2000]

2.3.6. Gradient echo sequences

Gradient echo also called gradient recalled echo (GRE) and partial flip angle techniques significantly reduce scanning time by using small flip angles and allowing very short TR values. An RF pulse yielding a smaller flip angle α is used instead of

the usual 90° pulse. An important application of the GRE is the ability to employ three dimensional (3D) imaging using high speed GRE due to very short TRs. GRE sequences are acquired one slice at a time (Sequential Scanning). The shorter scan time is determined by reducing the TR, using the following equation: Scan Time = TR x Ny x NEX, where TR = Repetition Time, Ny = Number of Phase Encoding Steps and NEX = Number of Excitations.

2.3.7. Diffusion sequences

Diffusion Tensor Imaging (DTI) has been used to investigate brain tissue microstructure in vivo. Reduced fractional anisotropy (FA) and increased mean diffusivity (MD) have been found in the genu, body and splenium of the corpus callosum in MS patients compared with controls which correlated with lesion load measures.[Ciccarelli *et al.* 2003b]

2.3.8. Tractography

Diffusion tractography has been used to determine paths of anatomical connection using diffusion tensor information. Fast marching tractography was used to generate maps of connectivity. Regions of voxels with highest connectivity to an anatomically defined starting point were identified for each tract investigated. Reproducibility of the technique using coefficient of variation was 1.2 - 18.6%, emphasising the importance of assessing normal controls before investigating white matter pathology.[Ciccarelli *et al.* 2003a][Parker *et al.* 2002].

2.3.9. Functional Magnetic Resonance Imaging (fMRI)

Functional MRI is used to measure changes in signal that occur on brain activation due to the changes in concentration of deoxygenated haemoglobin. fMRI has been

used to localize specific motor or sensory functions in the cortex or to monitor altered or adaptive responses following injury and/or during recovery. Changes have been shown in patients who have recovered from optic neuritis [Werring *et al.* 2000].

2.4. MRI in patients with CIS suggestive of MS

Multi-parametric MRI has been applied to patients with CIS in order to determine the value of MRI, in predicting early development of Poser CDMS. MRI studies have focused on the lesion load seen on T2 images. Longitudinal studies have assessed the predictive value of T2 abnormalities for the development of CDMS. Multifocal cerebral white matter lesions are present in 50-70% of patients presenting with a CIS.

Numerous studies have shown that the presence of cerebral white matter lesions in patients with CIS at the time of presentation is associated with an increased risk for the development of CDMS. Approximately 30% of patients with an abnormal MRI will develop MS after one year,[Brex *et al.*19991] approximately 50% after 5 years [Morrissey *et al.* 1993], 80% after 10 years [O'Riordan *et al.* 1998b] and 88% after a mean of 14.1 years [Brex *et al.* 2002].

Longer term study of patients with CIS suggestive of MS followed for a mean of 14.1 years has revealed that MRI lesion volume in the first 5 years of the disease is of prognostic value in assessing future disability.[Brex *et al.* 2002] The correlations between conventional MRI lesion load measures and disability were only moderate suggesting better surrogate markers are required. The longitudinal MRI studies of patients with CIS and the subsequent risk for the development of MS are presented in Table 2.1.

Table 2.1 Longitudinal MRI studies of patients with CIS and the subsequent risk for the development of MS

Yr	Author	Syndrome	Patient	Mean/Med f/u	MS/MRI+MSMRI-
2003	ONSG [Beck and Cleary 1997][Beck et al. 1993]	ON	388	10 Y	86/160 38/191
2003	Tintore [Tintore et al. 2000][Rio et al. 1997]	ON, BS, SC, PS	139	1 Y	36/101 2/38
2002	Brex [Brex et al. 2002][O'Riordan et al. 1998b][Morrissey et al. 1993][Miller et al. 1988, 1989]	ON, BS, SC	71	14.1 Y	44/50 4/21
2000	Ghezzi[Ghezzi et al. 2000, 1999, 1996]	ON	102	6.5 Y	42/71 0/31
1998	Söderström[Soderstrom et al.1998, 1995, 1994]	ON	147	6 Y	42/75 5/41
1998	Deya [Deya et al. 1998]	ON	28	4.5 Y	2/10 0/15
1997	Barkhof [Barkhof et al.1997][Tas et al.1995]	ON,SC,SS,MS,BS	74	3.25 Y	32/61 1/13
1997	Jacobs [Jacobs et al. 1991][Jacobs et al. 1997]	ON	74	5.6 Y	16/42 5/32
1996	Paolino [Paolino et al.1996]	BS SC	44	2.17 Y	18/22 12/22
1996	Frederiksen [Frederiksen et al. 1996][Frederiksen et al. 1991]	ON	50	0.92 Y	7/31 0/29
1995	Campi [Campi et al. 1995]	SC	30	1.5 Y	8/11 0/19
1994	Filippini [Filippini et al..1994]	Suspected MS	82	2.9 Y	19/36 1/25
1992	Ford [Ford et al. 1992]	SC	15	3.25 Y	11/12 1/3
1991	Lee [Paty et al. 1988][Lee et al. 1991]	ON SC	91	2 Y	52/118 3/66

Part 2

Chapter 3

3. Introduction, baseline MRI brain and spinal cord studies in CIS

3.1 Introduction to MRI studies in patients with CIS (Chapters 3, 4 and 5)

In 1995 a new prospective follow-up study of patients presenting with CIS was commenced. The study received ethical approval from the medical ethics committees of The National Hospital for Neurology and Neurosurgery and Moorfields Eye Hospital. Written, informed consent was obtained from patients before entry into the study and participation was voluntary.

3.1.1 Methods: Patients

Patients between 16 and 50 years of age with CIS suggestive of MS were recruited within 12 weeks of the onset of symptoms. A CIS is defined as an acute isolated event affecting one region of the central nervous system that is presumed to be demyelinating, with no previous history of possible demyelinating events. The development of maximal symptoms and signs must be evident within 14 days of symptom onset, and appropriate investigations carried out to exclude alternative diagnoses. Inclusion in the study is based solely on clinical features and is not influenced by MRI. Baseline assessments, both clinical and MRI, were performed less than three months from clinical onset

Careful history taking and examination excluded patients with a previous history of neurological symptoms which may have been suggestive of MS. Prior to entering the study each patient received a Patient Information Sheet. History and examination

were performed at each research visit, in order to ascertain clinical relapses consistent with Clinically Probable and CDMS. Patients with a diagnosis of MS were referred to an appropriate follow up neurology clinic.

3.1.2 Methods: MRI

All MRI brain and spinal cord Images were acquired using a 1.5-Tesla Signa (General Electric, Milwaukee, WI, USA) imager at the NMR Unit, Institute of Neurology, Queen Square, London WC1N 3BG. Imaging was performed at baseline (within three months of the onset of symptoms). Imaging was performed again three months later, one and three years later. Repositioning for all follow up MRI scans was achieved by using a protocol based on identification of standardized anatomical landmarks.[Gallagher *et al.* 1997] The sequences are listed in the order in which they were done. The sequences performed but not used in this thesis are also outlined.

3.1.2.1 Brain volume sequence (baseline, one and three years)

Patients initially had a 3D inversion-prepared fast spoiled gradient recall (3d FSPGR) sequence (TR=16 ms; TE=4.2 ms; TI=450 ms; matrix=256x160; FOV=300x225 mm; interpolated during reconstruction to a final in-plane resolution 1.2x1.2 mm); NEX=1, with 124x1.5 mm slices covering the whole brain. This brain volume measurement sequence was not used in this thesis.

3.1.2.2 Spectroscopy (three months, one year and three years)

Single voxel spectroscopy was performed at three months, one and three years using a fast spin echo (FSE) axial localising scan (TR 3000 ms, TE 84 ms, Matrix 256x192, 5 mm thickness, 1.5 mm gap). Then a single voxel was manually placed in the posterior

parietal and centrum semi-ovale NAWM. The MRS acquisition was a PRESS sequence (TR=3000 ms, TE=30 ms; 192 averages). Absolute in vivo metabolic concentrations were estimated using the Linear Combination Model (LCModel) [Provencher 1993] and were not evaluated in this thesis.

3.1.2.3 Magnetization Transfer Ratio (MTR) (three months)

A dual-echo spin-echo sequence was performed with and without presaturation pulses using an interleaved sequence producing 28 inherently coregistered 5-mm-thick axial PD (TR=1720 ms, TE=30 ms; 0.75 NEX) and T2-weighted images (TR=1720 ms, TE=80 ms; 0.75 NEX). Calculated MTR images were produced. This was not evaluated in this thesis [Brex *et al.* 2001b].

3.1.2.4 Gadolinium (baseline, three months one year and three years)

Each patient was then given 0.1 mmol/kg of gadolinium-DTPA intravenously. A T1-weighted spin-echo brain image (TR=600ms TE=14 ms) was acquired 15 minutes after the injection. For each sequence, 46 x 3 mm contiguous axial slices were acquired with a field of view=24 cm, matrix=256 x 256 and number of excitations=1.

3.1.2.5 PD/T2 weighted brain imaging: baseline, three months one & three years

Proton density /T2 images were acquired using a dual echo fast spin echo (FSE) sequence with a repetition time (TR) 3200 ms, and effective time (TE) 15/90 ms.

3.1.2.6 MRI of the spinal cord (baseline, one year and three years)

Nine contiguous, 3mm thick, sagittal slices were obtained through the spinal cord using PD- and T2-weighted fast spin echo (FSE) images (TR 2500 ms, TE 56/98 ms)

and a T1-weighted spin-echo sequence (TR 500ms TE 19 ms), using phased array coils and a 48 cm field of view, with a Matrix 512x512. Sixty 1mm sagittal slices were acquired using an inversion prepared fast spoiled gradient echo (FSPGR) for cord volume analysis (TR 15.6 ms, TE 4.2 ms, TI 450 ms, flip angle 20, matrix 256x256).

3.1.2.7 MRI hard copy analysis

All MRI brain and cord scans were reviewed by a consultant neuroradiologist (Dr IF Moseley 1995 – 2000, Dr KA Miszkiel 2000 – 2003). The baseline and follow up lesions loads were marked by the Neuroradiologist, who marked all T1 weighted Gd enhancing, T2 hyperintense and T1 hypointense lesions at baseline. In parallel processes, the month 3 studies were then compared side by side with the baseline scans in order to identify all new Gadolinium enhancing, T2 hyperintense and new T1 hypointense lesions. The process was repeated at one and three years.

3.1.2.8 Electronic image analysis: T2, T1 lesion volume analysis

All lesions marked on the hard copy were outlined on the on a Sun workstation (Sun Microsystems Inc, Mountain View, CA) using a semiautomated local thresholding technique, or manually [Plummer 1992] and from this lesion volumes were generated.

3.1.2.9 Electronic image analysis of atrophy: ventricular volumes and SPM99

The ventricles were measured on baseline, one and three years follow up T1-weighted scans using the MIDAS interactive image analysis package and a semi-automated seed placing technique.[Freeborough *et al.* 1997] The baseline and 3-year follow-up T2-weighted images were segmented using a fully automated technique into Grey

Matter (GM), White Matter (WM), CSF and other tissues using SPM99 [Chard *et al.* 2002c].

3.1.3. Statistical Tests used to Evaluate MRI Criteria

Conventional definitions have been used to evaluate the accuracy of MRI criteria as a test for Poser CDMS at 3 years. Based on the new MRI lesions after three months and one year as the test, and CDMS at 3 years as the disease, the number of True Positives (TP: new MRI criteria positive, CDMS positive), True Negatives (TN: new MRI criteria negative, CDMS negative), False Positives (FP: new MRI criteria positive, CDMS negative) and False Negatives (FN: new MRI criteria negative, CDMS positive) were calculated and used to determine:

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

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

Positive predictive value: Percentage people with a positive test who have the disease ($TP/TP+FP$)

Negative predictive value: Percentage people with a negative test who do not have disease ($TN/TN+FN$)

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

3.2. Baseline MRI brain predictors of early conversion to CDMS in patients with CIS suggestive of MS

3.2.1 Aims

The aim of this chapter was to assess the relevance baseline MRI brain lesion load as a predictive test for the development of MS at three years using sensitivity, specificity, positive, negative predictive value and accuracy of baseline lesions for diagnosis of MS at three years.

3.2.2 Methods

Patients

Since 1995, patients between 16 and 50 years of age with CIS suggestive of MS have been recruited consecutively and imaged within three months of the onset of symptoms. This study was conducted at the National Hospital for Neurology and Neurosurgery and Moorfields Eye Hospital. Local ethics approval was obtained and written informed consent was obtained from each patient, prior to scanning. Most patients were recruited from the Optic Neuritis Clinic at Moorfields Eye Hospital, so there is a bias towards optic neuritis in the study sample. A CIS is defined as an acute isolated event affecting any part of the central nervous system that is presumed to be demyelinating, with no previous history of demyelinating events. Patients were assessed clinically and with brain MRI at baseline, three months, one year and three years. Those with further relapses were diagnosed as having CDMS according to the Poser criteria.[Poser *et al.*, 1983]

MRI brain

Proton density/T2-weighted images fast spin echo and T1-weighted spin-echo images were acquired on a 1.5T Signa scanner following intravenous injection of 0.1 mmol/kg of Gadolinium. An experienced neuroradiologist, who was blinded to the clinical diagnosis, reviewed all the images. The number and location of high signal T2/proton density lesions was noted and number gadolinium enhancing and T1 hypointense lesions counted on both the MRI brain.

3.2.3 Results

Baseline clinical and MRI data

This study is based on the 63 patients who have been studied up to and including year three. Forty-four patients presented with optic neuritis, fourteen with a brain stem syndrome, four with a spinal cord syndrome and one patient presented with an MRI demyelinating lesion of the optic tract. The median age of onset of symptoms was 32 years (range 17-50). There were 35 females and 28 males. Twenty-one of the 44 patients (48%) with an abnormal and one out of 19 (5%) with a normal MRI brain scan developed CDMS at three years.

Baseline MRI lesion type as a predictor of CDMS at three years (Table 3.1)

One or more T2 lesions on baseline MRI brain were the most sensitive indicators of CDMS at 3 years (95%), but with low specificity 56%. One or more T1 hypointense or Gadolinium enhancing lesion although less sensitive (68% and 59% respectively) were more specific for CDMS at three years (75% and 85% respectively). The most sensitive lesion position was periventricular with a sensitivity of 91%, compared with cerebellar 45%, subcortical 73% and spinal cord 55%. Of these the spinal cord lesion

was the most specific with a specificity of 76%. Increasing the number of periventricular lesions to three or more reduced sensitivity to 77% and increased specificity to 83%.

Increasing the baseline lesion number caused a progressive decrease in sensitivity, (three or more lesions: 86% and nine or more lesions: 59%). Specificity of three or more lesions was 68% and of nine or more lesions 83%. The specificity of nine or more gadolinium enhancing lesions was 100% however the sensitivity was only 14%.

Baseline Barkhof criteria as a predictor of CDMS at three years (Table 3.2)

The sensitivity of the Barkhof criteria [Barkhof et al 1997] including the spinal cord was 77% and the specificity was 80%.

3.2.4 Discussion

MRI scanning at baseline within three months of the onset of symptoms is a helpful indicator of patients at risk for the development of further episodes. Fulfilling the criteria for dissemination in time and space helps to delineate those at higher risk. Various stricter criteria such as selecting patients with more than two Gadolinium enhancing lesions indicate those patients with blood brain barrier (BBB) breakdown and ongoing inflammation. These patients are at most risk of developing symptoms; however the number of patients fulfilling this test is small. This results in high specificity and false negative rate so using this test is of limited value. The added value of follow-up MRI findings in improving sensitivity and specificity is assessed in Chapter 4.

Table 3.1 Baseline MRI findings as predictors of CDMS at 3 years

Criterion	T2 brain	Cord	T1 lesions	Gd	Cerebellar	Ventricular (≥ 3)	Subcortical	All locations
TP	21	12	15	13	10	20 (17)	16	5
FN	1	10	7	9	12	2 (5)	6	2
TN	23	31	30	35	30	27 (34)	24	39
FP	18	10	10	6	11	14 (7)	17	17
Sens	95%	55%	68%	59%	45%	91% (77%)	73%	71%
Spec	56%	76%	75%	85%	73%	66% (83%)	59%	70%
Ppv	54%	55%	60%	68%	48%	59% (71%)	48%	23%
Npv	96%	76%	81%	80%	71%	93% (87%)	80%	95%
Accuracy	70%	68%	71%	76%	63%	75% (81%)	63%	70%

Table 3.2 Combination of baseline MRI criteria as predictors of CDMS at 3 years

Criterion	≥ 3 lesions	≥ 4 lesions	≥ 9 lesions	≥ 3 Gd lesions	≥ 4 Gd lesions	≥ 9 Gd lesions	Barkhof cord
TP	19	18	13	10	6	3	17
FN	3	4	9	12	16	19	5
TN	28	29	34	38	39	41	33
FP	13	12	7	3	2	0	8
Sens	86%	82%	59%	45%	27%	14%	77%
Spec	68%	71%	83%	93%	95%	100%	80%
Ppv	59%	60%	65%	7%	75%	100%	68%
Npv	90%	88%	79%	76%	71%	68%	87%
Accuracy	59%	48%	32%	21%	71%	70%	79%

3.3 Spinal cord MRI in clinically isolated optic neuritis

3.3.1 Aims

The purpose of this study is to document the frequency of MRI abnormalities in both the brain and spinal cord and to determine the potential of spinal cord lesions to enable a diagnosis of MS and to predict the future clinical course.

3.3.2 Introduction

Using the new diagnostic criteria formulated by an international panel of experts, both brain and spinal cord MRI lesions may be used to diagnose MS in patients with clinically isolated optic neuritis [McDonald *et al.* 2001]. While spinal cord imaging is essential when the CIS involves the spinal cord to exclude other diagnoses, its value in diagnosis of patients with optic neuritis has not been systematically investigated. In recent years we have been prospectively recruiting patients with isolated optic neuritis for investigation with brain and spinal cord MRI. The purpose of this study is to document the frequency of MRI abnormalities in both regions and to determine their potential to enable a diagnosis of MS and to predict the future clinical course. We now report the findings in a cohort of 115 patients with optic neuritis who have had both brain and spinal cord imaging at presentation, and have been followed up for periods ranging from 0 to 72 months (mean 18 months).[Dalton *et al.* 2003]

3.3.3 Methods

Patients

Since 1995, patients between 16 and 50 years of age with optic neuritis have been recruited from the physicians' clinic at Moorfields Eye Hospital. Approval was

received from the medical ethics committee. Consent was obtained from all patients before entry into the study. Inclusion in the study was based solely on clinical features and was not influenced by MRI. Patients with a previous history of possible demyelinating events were excluded. Baseline clinical and MRI assessments were performed less than three months from clinical onset. Appropriate investigations were carried out to exclude alternative diagnoses.

MRI brain was performed at baseline and again after three months, one year and three years. Images were acquired using a 1.5-Tesla Signa (General Electric, Milwaukee, WI, USA) imager. Each patient was given 0.1 mmol/kg of gadolinium-DTPA prior to imaging. Proton density (PD)- and T2-weighted images were acquired using a dual echo fast spin echo (FSE) sequence with a repetition time (TR) 3200 ms, and effective time (TE) 15/90 ms. A T1-weighted spin-echo image (TR=600ms TE=14 ms) of the brain was acquired 15 minutes after the injection of the gadolinium-DTPA. For each sequence, 46 x 3 mm contiguous axial slices were acquired. The field of view=24 cm, matrix=256 x 256 and number of excitations=1.

MRI of the spinal cord was performed at baseline, one year and three years. Nine contiguous, 3mm thick, sagittal slices were obtained through the whole of the spinal cord with PD- and T2-weighted fast spin echo (FSE; TR 2500 ms, TE 56/98 ms), and T1-weighted spin-echo (TR 500ms TE 19 ms) sequences following administration of gadolinium-DTPA. Both sequences employed phased array coils and a 48 cm field of view, with a 512x512 matrix.

Clinical assessment

Patients were assessed clinically at baseline, three months, one year and three years. Those who experienced further relapses with new signs were classified as having clinically definite MS (CDMS) according to the criteria of Poser.[Poser *et al.* 1983] MS was diagnosed using the new McDonald criteria using clinical and/or MRI evidence of dissemination in time and space. Disability was measured using EDSS [Kurtzke1983].

Image analysis

The images were reviewed by an experienced neuroradiologist, who was blinded to the clinical diagnosis. The number and location of high signal PD/T2 lesions and gadolinium enhancing lesions was noted. The baseline, three month, one year and three year PD/T2-weighted and gadolinium enhanced T1-weighted scans were analyzed for evidence of Dissemination in Space (DIS) and Time (DIT) using the McDonald criteria. DIS, based on the criteria of Barkhof and Tintore [Barkhof *et al.* 1997][Tintore *et al.* 2000] consisted of three out of four of the following:

1. At least one gadolinium enhancing or nine T2 lesions
2. At least one infratentorial lesion
3. At least one juxtacortical lesion
4. At least three periventricular lesions

The criteria allowed the replacement of a brain lesion with a cord lesion in any of the above categories. One or more gadolinium enhancing lesions at three months (brain only) was used as evidence of Dissemination in Time (DIT) or a new T2-weighted lesion on the one or three year follow up scans (brain and spinal cord). Patients were also divided into those with no brain lesions, 1-8 brain lesions

inclusive and 9 or more brain lesions and these subgroups were subsequently classified for MRI evidence of DIS.

3.3.4 Results

Baseline clinical data

One hundred and fifteen patients with optic neuritis had imaging of the brain and spinal cord within three months of the onset of symptoms: 113 had unilateral and 2 bilateral optic neuritis, age (mean, median, range) (32, 31, 16-49), gender (76 female, 39 male) and disease duration in weeks (6, 5, 1-12).

MRI DIS: baseline data (Table 3.3)

Eighty-one (70%) patients had one or more brain lesions and 31 (27%) had one or more cord lesions. Forty one/115 (36%) had DIS using brain scanning alone (44/115 (38%) using brain and spinal cord). Patients were subdivided into those with a normal brain MRI (no lesions), 1-8 brain lesions and greater than 9 lesions.

Normal brain MRI

Of the 34 patients with a normal brain MRI scan, 4 (12%) had spinal cord lesions. Using the present diagnostic criteria, patients with cord lesions but without MRI brain lesions can not be included as having MRI evidence of DIS.

1-8 brain lesions

Of the 39 patients with between 1 and 8 lesions, 8 (21%) had spinal cord lesions. The number of patients with DIS on the baseline scan was increased by the

inclusion of spinal cord lesions from 2 (5%) to 5 (13%). Lesion location in the 3 patients requiring cord lesions for dissemination in space was:

- 1 Periventricular=3, gadolinium enhancing=1 and cord=1.
- 2 Infratentorial=1, juxtacortical=2, periventricular=2 and cord=3.
- 3 Infratentorial=2, periventricular=3 and cord=2.

Lesion location in the 34/39 patients with between 1 and 8 lesions who did not have DIS despite inclusion of the spinal cord was: cord lesions (4), infratentorial (5), juxtacortical (15), periventricular (19), periventricular ≥ 3 (6) discrete cerebral white matter neither periventricular nor juxtacortical (14) and gadolinium enhancing (6).

Nine or more brain lesions

In the 42 patients with 9 or more brain lesions, 19 (45%) had spinal cord lesions. On brain MRI alone 39/42 (93%) had DIS and this number was unchanged when cord MRI findings were incorporated. When multiple cord lesions were allowed to substitute for an identical number of brain lesions, there was no alteration in the number of patients who had DIS. Only two patients with more than one cord lesion lacked DIS at baseline.

Follow up: MRI DIT and DIS and diagnosis of MS (Table 3.3)

The follow up study is ongoing and the present report includes all those currently followed-up at each time point: 94 have been studied at 3 months, 64 at one year (one of whom did not have cord MRI) and 44 at 3 years (3 of who did not have cord MRI). Although the new diagnostic criteria incorporating brain MRI findings markedly increase the number of patients with a diagnosis of MS at 3 months, one and three years, when compared with the number developing CDMS, imaging of the spinal cord had almost no additional impact on the diagnosis. As spinal cord

imaging was not performed at three months, the impact of new spinal cord lesions on DIT could only be evaluated at one and three years. Overall, addition of cord MRI findings to brain MRI and clinical findings enabled a diagnosis of MS using the McDonald criteria in only one/64 (1.6%) and 2/44 (4.5%) additional patients who were followed up at one and 3 years respectively. These individuals all had between 1 and 8 brain lesions at baseline.

At one year, only one/11 patients with new spinal cord lesions did not have clinical (new relapse) or brain MRI (new brain lesion) evidence of DIT – this single patient had a normal brain scan at baseline and one year. One further patient with a new brain lesion at one year and cord lesion at baseline had MRI evidence of DIS according to the McDonald criteria only if the spinal cord lesion was included. (4 periventricular, 2 juxtacortical, 1 discrete and 1 cord lesion).

At three years, 2/10 patients with new spinal cord lesions did not have MRI evidence of DIS although both had new brain lesions and no further symptoms. In patients with an abnormal baseline scan, the occurrence of new T2 brain lesions as evidence of DIT at 3 months or 1 year had a sensitivity of 85% and specificity of 79% for CDMS at 3 years. Adding new cord lesions at one year resulted in no increase in sensitivity and specificity dropped from 79% to 75% (Table 3.4).

3.3.5 Discussion

Patients with clinically isolated optic neuritis and asymptomatic brain lesions have an increased risk for the development of MS [Brex *et al.* 2002]. Although spinal

cord imaging is mandatory in isolated spinal cord syndrome patients in order to detect treatable alternative disorders such as compressive lesions, its role is unclear in patients with optic neuritis. The key question is whether cord lesions increase the frequency of DIS and DIT and thereby enable a more frequent and earlier diagnosis of MS.

Overall, 27% of our patients had MRI evidence of spinal cord lesions at presentation. However, they contributed little to the diagnosis. Although there were 4 patients with cord lesions but no brain lesions, these subjects do not have DIS according to current definitions and therefore a diagnosis of MS cannot be made using existing MRI criteria. Two of the 4 have been followed for three years and have had no further symptoms.

In patients with between one and eight brain lesions inclusive, cord imaging modestly increased the number of patients with DIS at baseline by 8%; however, at three months (the earliest time point at which a diagnosis of MS can be made) no additional patients were diagnosed with MS (although follow up MRI was confined to the brain at this time point). In those patients with 9 or more brain lesions, cord lesions were much more common, being found in almost one half of subjects, but their presence did not increase the number who fulfilled the MRI criteria for DIS or who developed MS at each follow up time point.

The present study is limited by the fact that cord imaging was not performed at three months. Nevertheless, brain MRI, on average, detects many more lesions than

cord MRI and the latter is most often abnormal only when there are already at least 9 brain lesions present and when the new criteria for DIS are very often already fulfilled by brain findings alone. In established MS, new cord lesions occur less often on follow up than new brain lesions [Thorpe et al. 1996b][Silver *et al.* 2001] and usually when there are concurrent new brain lesions. Notably, the new spinal cord lesions seen at three years when compared with one year did not increase the number of patients exhibiting DIT over that period, and increased the number of patients with a diagnosis of MS at three years by only 2 out of a total of 44 (4.5%) (Table 3.3). We doubt therefore that additional cord imaging at month three would have affected the rate of early MS diagnosis.

In summary, using existing criteria, imaging of the spinal cord is of limited diagnostic value in patients with optic neuritis with between 1 and 8 MRI brain lesions. If in future the diagnostic criteria were revised to allow the presence of any cord lesions as evidence of DIS and DIT, then cord imaging would have a diagnostic role in more patients, but overall it appears that this will be a relatively small subgroup. Any such extension of the diagnostic criteria should first be validated by confirming its accuracy for predicting CDMS.

Table 3.3 Impact of spinal cord lesions on the diagnosis of MS

	Time	Pts	0 brain lesions	1 - 8 brain lesions	≥9 brain lesions
Number pt brain lesions (c)	Baseline	115	34 (4)	39 (8)	42 (19)
% patients with cord lesions		115	12%	21%	45%
Mean, (Range) cord lesions		115	0.1 (0-1)	0.4 (0-4)	1.2 (0-11)
DIS (b)		115	0	2(5%),	39(93%),
DIS (b + c)				5(13%)	39(93%)
New brain lesions	3 Months	94	1/28 (4%)	8/32 (25%)	23/34 (68%)
McDonald MS (b)		94	0/28	1/32 (3%)	17/34(50%)
McDonald MS (b+ base c)		94	0/28	1/32 (3%)	17/34(50%)
CDMS		94	0/28	0/32	9/34 (26%)
New brain lesions	1 year	64	0/17	11/22 (50%)	18/25 (72%)
New cord lesions		63	2/17	3/22 (14%)	6/24 (25%)
New brain or cord lesions		64	2/17 (12%)	11/22 (50%)	19/25 (76%)
McDonald MS (b)		64	1/17(6%)	7/22 (32%)	22/25 (88%)
McDonald MS (b & c)		64	1/17(6%)	8/22 (36%)	22/25 (88%)
CDMS		64	1/17(6%)	1/22 (5%)	8/25 (32%)
New brain lesions	3 years	44	3/15 (20%)	11/15 (73%)	12/14 (86%)
New cord lesions		41	1/15 (7%)	4/14 (29%)	5/12 (42%)
New brain or cord lesions		44	3/15 (20%)	11/15 (73%)	13/14 (93%)
McDonald MS (b)		44	1/15(7%)	5/15 (33%)	14/14 (100%)
McDonald MS (b+c)		44	1/15(7%)	7/15 (47%)	14/14 (100%)
CDMS 3 years		44	1/15(7%)	4/15 (27%)	9/14 (64%)
(brain = b, cord = c)					

Table 3.4: Relationship of new lesions after one year with CDMS after 3 years in patients with an abnormal baseline MRI brain (41 patients)

	New Brain Lesions*	New Brain & Cord lesions*
CIS +	6	7
MS +	11	11
CIS -	22	21
MS -	2	2
Sensitivity (95% Confidence Interval)	85% (55% 98%)	85 % (55% 98%)
Specificity (95% Confidence Interval)	79% (59% 92%)	75 % (55% 89%)
PPV (95% Confidence Interval)	65% (38% 86%)	61% (36% 83%)
NPV (95% Confidence Interval)	92% (73% 99%)	91% (72% 99%)
Accuracy (95% Confidence Interval)	80% (65% 91%)	78% (62% 89%)

CIS+ = New lesions after one year and CIS after 3 years

MS+ = New lesions after one year and CDMS after 3 years

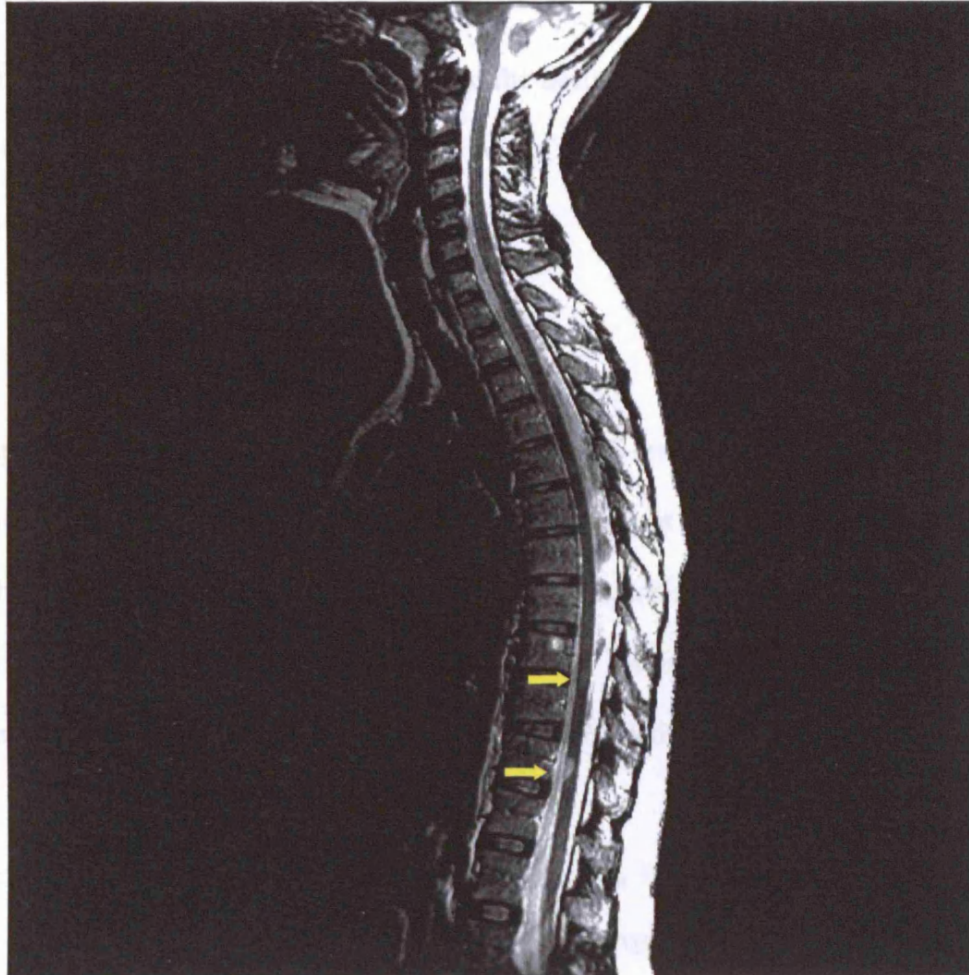
CIS- = No new lesions after one year and CIS after 3 years

MS- = No new lesions after one year and MS after 3 years

*Brain MRI findings only were available at 3 months; brain and cord at one year

Footnote: Based on the new MRI lesions after one year as the test, and CDMS at 3 years as the disease, the number of True Positives (TP: new MRI lesions positive, CDMS positive), True Negatives (TN: new MRI lesions negative, CDMS negative), False Positives (FP: new MRI lesions positive, CDMS negative) and False Negatives (FN: new MRI lesions negative, CDMS positive) were calculated and used to determine: sensitivity, specificity, positive predictive value, negative predictive value and accuracy.

Figure 3.1 T2-weighted sagittal MRI through the spinal cord in a 30-year old female with isolated optic neuritis. Two intrinsic lesions are seen in the thoracic cord (arrowed).



Chapter 4

4. Application of the McDonald criteria and new T2 lesions

4.1. Application of the McDonald criteria to patients with CIS suggestive of MS

4.1.1. Aims

The aim of this study was to determine the accuracy of the new McDonald MRI and clinical criteria in a cohort of patients with CIS who have been followed for one and three years, respectively.

4.1.2. Introduction

Approximately 90% of patients with MS initially present with a clinically isolated inflammatory, demyelinating syndrome of acute onset in the central nervous system. The most common types of CIS are optic neuritis, brainstem and spinal cord syndromes in an individual without a previous history suggestive of demyelination and in whom appropriate investigations have excluded an alternative diagnosis.

Long-term follow-up studies indicate that between 30-80% [Hely *et al.* 1986][Francis *et al.* 1987][Rizzo, III and Lessell 1988][Sandberg-Wollheim *et al.* 1990][Rodriguez *et al.* 1995][Jacobs *et al.* 1997][O'Riordan *et al.* 1998b][Barkhof *et al.* 1997][Soderstrom *et al.* 1998][Ghezzi *et al.* 1999][Ghezzi *et al.* 2000][Morrissey *et al.* 1993] of patients with CIS will develop clinical evidence for Dissemination in Time (DIT) and Space (DIS) thereby fulfilling the traditional criteria for a diagnosis of CDMS.[Poser *et al.* 1983][Schumacher *et al.* 1965] About 50-70% of patients with CIS have disseminated lesions on brain MRI characteristic of MS at first presentation, and the presence of such lesions increases the likelihood of developing CDMS.[Jacobs *et*

al. 1997][O'Riordan *et al.* 1998b][Barkhof *et al.* 1997][Soderstrom *et al.* 1998][Ghezzi *et al.* 1999][Ghezzi *et al.* 2000][Morrissey *et al.* 1993][Tintore *et al.* 2000][Brex *et al.* 2002][Brex *et al.* 2001c][Brex *et al.* 1999b] One year follow-up of patients with CIS frequently reveals new, clinically silent MRI lesions that also appear to increase the chances of developing CDMS.[Brex *et al.* 1999b][Brex *et al.* 2001c] Diagnosis of MS in patients with CIS may be beneficial since the early course of MS with axonal loss [Trapp *et al.* 1998] may determine the relative risk of progression.[Weinshenker *et al.* 1989]

Recently, the International Panel of McDonald and colleagues has prepared new guidelines for the diagnosis of MS.[McDonald *et al.* 2001] These rely, as have the previous clinically based Poser criteria,[Poser *et al.* 1983] on objective evidence of Dissemination in Time (DIT) and Space (DIS). However, the revised criteria have included MRI evidence of DIS and DIT with potential to enable an earlier diagnosis, especially in patients with CIS.[McDonald *et al.* 2001]

At the time this study was performed, the McDonald criteria had not yet been evaluated in a population of patients with CIS. Assessment of the relevance of the new criteria in this subgroup should include an evaluation of an early MRI-supported diagnosis of MS in terms of specificity, positive predictive value and accuracy for the development of CDMS. An accurate diagnosis is essential since prognosis, counselling and advice regarding treatment is likely to be given to patients once a diagnosis of MS has been established.

In a prospective follow up of a CIS cohort first seen between 1984 and 1987, it was found, by the early 1990s, that baseline brain T2 lesion load and number seen on a 0.5 Tesla scanner were predictive of clinical outcome after 5 years.[Morrissey et al. 1993][Filippi *et al.* 1994]. However, since these early studies began, there were significant developments in MR technology, including the widespread use of higher field scanners (1.5 Tesla), gadolinium enhancement, routine acquisition of thinner slices, spinal cord imaging and measurements of atrophy. Therefore, in 1995, a new, prospective follow up study from first presentation of a separate cohort of patients with a CIS began. The purpose of this ongoing study is to obtain multiple MR measurements using a 1.5 Tesla scanner, and to determine, by serial clinical and MRI follow up at regular, pre-specified intervals, the prognostic significance of the early MR findings. Initial findings, with up to one year of follow up, have already been reported [Brex *et al.* 2001a][Brex, et al. 2001b][Brex, et al 2001c][Brex *et al.* 2000][Brex *et al.* 1999a][O'Riordan *et al.* 1998a]. The present study analyses serial clinical and MRI data that was prospectively acquired for up to three years from this cohort of patients. By retrospective application of the new McDonald criteria to prospectively acquired data, it addresses the value of the new diagnostic criteria as follows:

1. Determining the frequency with which patients with CIS fulfil the new criteria for MS at three months, one year and three years, and comparing that with the frequency of Poser CDMS at each time point.
2. Evaluating the relationship between the new criteria for MS and the traditional criteria for Poser CDMS in terms of sensitivity, specificity, positive and negative predictive value and accuracy of the former for the latter.

4.1.3. Methods

Patients

Since 1995, patients have been recruited from the wards and clinics of the National Hospital for Neurology and Neurosurgery and from the Neuro-Ophthalmology clinic at Moorfields Eye Hospital, London, UK. Patients between 16 and 50 years of age have been included. The study has received approval from the medical ethics committees of both hospitals. Written, informed consent is obtained from all patients before entry into the study and participation is voluntary at all stages. A CIS is defined as an acute isolated event affecting one region of the central nervous system that is presumed to be demyelinating, with no previous history of possible demyelinating events. The development of maximal symptoms and signs must be evident within 14 days of symptom onset, and appropriate investigations carried out to exclude alternative diagnoses. Inclusion in the study is based solely on clinical features and is not influenced by MRI. Baseline assessments, both clinical and MRI, must be performed less than three months from clinical onset

MRI acquisition protocols

MRI brain is performed at baseline (within three months of the onset of symptoms) and again after three months, one year and three years. Images are acquired using a 1.5-Tesla Signa (General Electric, Milwaukee, WI, USA) imager. Each patient is given 0.1 mmol/kg of gadolinium-DTPA prior to imaging. Proton density (PD)- and T2-weighted images are acquired using a dual echo fast spin echo (FSE) sequence with a repetition time (TR) 3200 ms, and effective time (TE) 15/90 ms. A T1-weighted spin-echo image (TR=600ms TE=14 ms) of the brain is acquired 15 minutes

after the injection of the gadolinium-DTPA. For each sequence, 46 x 3 mm contiguous axial slices are acquired. The field of view=24 cm, matrix=256 x 256 and number of excitations=1.

MRI of the spinal cord is performed at baseline, one year and three years using PD- and T2-weighted fast spin echo (FSE) images (TR 2500 ms, TE 56/98 ms). A T1-weighted spin-echo sequence (TR 500ms TE 19 ms) is also performed following administration of gadolinium-DTPA. Additional sequences for MR spectroscopy and magnetization transfer imaging are obtained at the month 3 visit and a 3D cord acquisition is obtained at baseline and one year to investigate cord atrophy.[Brex *et al* 1999a][Brex *et al*. 2001a]

Clinical assessment

All patients are assessed clinically at baseline, three months, one year and three years and those with further relapses with new signs disseminated in space and time (at least one month after the CIS) are classified as having CDMS according to the criteria of Poser.[Poser *et al*. 1983] Disability is recorded at each visit using the Kurtzke EDSS.[Kurtzke1983].

The clinical diagnosis of MS had in all instances made prior to application of the McDonald MRI criteria (see below), and although the clinician making the diagnosis had seen the scans and been aware of the presence or absence of MR abnormalities these had not been quantified. MS was diagnosed using the new McDonald criteria of clinical and/or MRI evidence of Dissemination in Time (DIT) and Space (DIS). The

McDonald clinical criteria for DIS and DIT are those for CDMS; the MRI criteria are outlined in the next section.

Image Analysis

An experienced neuroradiologist, who was blinded to the clinical diagnosis, reviewed all the images. The number of high signal lesions on the PD/T2 weighted scans (called T2 lesions) was noted in each of the following regions: infratentorial, periventricular, juxtacortical, and discrete (in cerebral hemispheres but neither juxtacortical nor periventricular). All T2 lesions were marked on the PD-weighted scan and confirmed on the T2-weighted scan. Gadolinium enhancing lesions identified on the T1-weighted scans were confirmed on both the PD- and T2-weighted scans. The MRI brain and cord images were reported as normal if they had no lesions compatible with demyelination, or if only the symptomatic lesion, as determined by a separate unblinded observer, was visible. They were reported as abnormal if one or more asymptomatic lesions compatible with demyelination were present.

In order to evaluate the new diagnostic criteria, the baseline, three-month, one year and three year proton density/T2-weighted and gadolinium enhanced T1-weighted scans were retrospectively analyzed for evidence of DIS and DIT.

Dissemination in space based on the criteria of Barkhof and Tintore [Barkhof, *et al.* 1997][Tintore *et al.* 2000] consisted of three out of four of the following:

1. At least one gadolinium enhancing or nine T2 lesions.
2. At least one infratentorial lesion
3. At least one juxtacortical lesion

4. At least three periventricular lesions

One cord lesion was allowed to replace one brain lesion. Figure 4.1 is a clinical example of the use of MRI as evidence for DIS.

Dissemination in time consisted of either one or more gadolinium enhancing lesions at least three months after the attack or one or more T2 lesions subsequent to a scan at least three months after the attack. Figure 4.2 is a clinical example of MRI as evidence for DIT.

Statistical Analysis

The validity of the new “test” – i.e. the McDonald diagnostic criteria for MS - was assessed by comparing it with the “gold standard” for the disease which was defined as CDMS using the Poser criteria. Based on the clinical outcome at one year and three years the number of True Positives (TP: McDonald MS positive, CDMS positive), True Negatives (TN: McDonald MS negative, CDMS negative), False Positives (FP: McDonald MS positive, CDMS negative) and False Negatives (FN: McDonald MS negative, CDMS positive) were calculated and used to determine: Sensitivity, Specificity, Positive predictive value, Negative predictive value, and Accuracy.

4.1.4. Results

Baseline clinical data (Table 4.1)

The study is ongoing. Currently, 119 patients have been studied at baseline, 95 at three months, 79 at one year and 50 at three years. One patient died of an asthmatic attack prior to three years of follow up. Eighty-seven patients presented with acute

unilateral optic neuritis, two patients had bilateral consecutive optic neuritis, 19 had a brain stem syndrome, 10 patients had a spinal cord syndrome and one patient had a hemianopia attributable to a lesion of the optic tract. The median age at onset of symptoms was 31 years (range 16-50). The median EDSS was 1 at baseline (range 0-8; one patient with a spinal cord syndrome had an EDSS of 8).

Baseline MRI data (Table 4.2)

The median time delay between onset of symptoms and baseline scan was five weeks (range 1-12). At baseline 85/119 patients (71%) had an abnormal brain MRI (i.e. one or more T2 lesions), and 36/119 (30%) had an abnormal cord scan. Thirty-three/36 patients (92%) with cord lesions also had brain lesions; only three had cord lesions with a normal brain scan. Using the McDonald criteria, DIS was present in 43/119 (36%) when brain MRI findings alone were considered; this rose to 47/119 (40%) when spinal cord findings were included.

Development of MS at follow-up

The median intervals between the baseline and follow up evaluations were: (i) three months – 12 weeks (range 8-22); (ii) one year – 12 months (range 11-19); three years – 37 months (range 29-67). Table 4.2 shows, for each follow up time point, the number of patients developing MS using the McDonald criteria and the numbers who developed CDMS using the Poser criteria. Patients with MS diagnosed using the new criteria are further subdivided to show the numbers with either an MRI based diagnosis, or CDMS, or both. Table 4.2 also shows the number of patients with an abnormal scan, MRI evidence of DIS and DIT, and a diagnosis of MS using the new

MRI criteria alone, at each time point - these data are presented for brain MRI findings alone and for combined brain and cord findings.

Predictive value of the new diagnostic criteria for CDMS

Of those developing CDMS at each time point, the majority also had a diagnosis of MS based on the McDonald MRI criteria of DIS and DIT, i.e. 6/7 (86%) at three months, 13/16 (81%) at year one, and 17/19 (90%) at year three. At all time points, the addition of spinal cord MRI findings had a minimal impact on the proportion of patients with a diagnosis of MS using the new criteria.

The sensitivity, specificity, positive and negative predictive value and accuracy of the new diagnostic criteria for the development of CDMS after one and three years are presented in Tables 4.3 and 4.4. The predictive value of the baseline MRI findings is also provided.

CDMS at one year

A diagnosis of MS based on MRI evidence for DIS and DIT at three months provided a substantially higher specificity (87%) for Poser CDMS at one year than did baseline evidence for DIS whether using brain MRI alone (68%) or combined brain and spinal cord findings (67%). The positive predictive value and overall accuracy was also higher for MRI diagnosed MS at three months (56% and 83% respectively) than for either baseline brain MRI DIS (36% and 68% respectively) or baseline brain and cord DIS (40% and 71% respectively). A high sensitivity for CDMS at one year was

achieved only for baseline DIS when using combined brain and cord MRI findings (88%).

CDMS at three years (Table 4.4)

A diagnosis of MS using the McDonald MRI criteria at one year provided a sensitivity of 83%, specificity of 83%, positive predictive value of 75% negative predictive value of 89% and overall accuracy of 83% for CDMS at three years. The specificity and positive predictive values of McDonald MRI criteria MS at three months were also high (93% and 83% respectively) but sensitivity (59%) was lower than specificity. Baseline DIS on brain MRI had a lower specificity and positive predictive value for CDMS at three years (77% and 63% respectively); the sensitivity of DIS was also low for brain MRI findings (63%), but was increased substantially by incorporation of spinal cord lesions (79%). Although an abnormal baseline scan had 100% sensitivity for CDMS, the specificity (36%) and positive predictive value (49%) of this finding were much lower. Five patients were treated with beta interferon following diagnosis of MS. In all five, both CDMS and MRI DIS and DIT had developed before treatment was started. Eleven patients received short courses of steroids for relapses: five prior to their baseline scan, three between their baseline and three month scans, two between their three month and one year scans and one between their one and three year scans.

4.1.5. Discussion

An accurate and early diagnosis of MS is important in order to allow for the provision of information and discussion of prognosis and potential therapeutic interventions.

The earliest clinical event in most MS patients is a CIS, and the presence of multifocal MRI abnormalities in such individuals confers a relatively high likelihood for the development of further clinical relapses leading to a diagnosis of CDMS. Because MRI reveals many clinically silent lesions, it is logical to integrate MRI evidence of DIS and DIT in order to achieve an earlier diagnosis of MS. The International Panel of McDonald and colleagues has recently developed such diagnostic criteria. In recent years, we have been recruiting CIS patients into a prospective follow up study involving serial clinical and MRI evaluations with the aim of identifying prognostic imaging markers early in the disease course. Patients were included based on clinical features alone; MRI findings were not a part of the inclusion criteria and patients with a normal scan were included. This dataset thus provides an opportunity to investigate the frequency with which such patients develop MS using the new criteria and the predictive value of those criteria for CDMS.

Using the new criteria, almost half the patients had a diagnosis of MS after one year compared with only one fifth using traditional clinical criteria. More frequent scanning between month three and month 12 may have further increased the diagnostic yield - additional new enhancing or T2 lesions may have occurred that were no longer visible on the month 12 scan.

The main restriction on an MRI-based diagnosis at the three month follow up was the criterion of DIT which at this time requires the presence of a gadolinium enhancing lesion since a new T2 lesion cannot be verified as having appeared more than three months from clinical onset. MRI diagnosed MS was nevertheless almost three times more common than CDMS at this stage (20% versus 7%).

Spinal cord imaging had little effect on the proportion of patients who developed MS using the new criteria. This is partly because follow up cord imaging was obtained less often (specifically not at three months) but mainly because new lesions occur more often in the brain than the cord.[Brex *et al.* 2001c] Of the ten (8%) patients presenting with spinal cord syndromes, seven had spinal cord lesions and six had brain lesions suggesting that imaging of the spinal cord may be slightly more useful in this group. Further studies are needed of a larger cohort with isolated cord syndromes. Using the new criteria for the diagnosis of MS, cord imaging does not appear useful in patients with CIS presenting with optic neuritis or brain stem syndromes.

The McDonald criteria also include CSF findings in the diagnostic framework. The presence of CSF oligoclonal bands in addition to two MRI lesions can be used as an alternative criterion for DIS. Because lumbar puncture was only performed in a small number of patients with brain stem or spinal cord syndromes, the effect of CSF findings could not be evaluated in our study. In one recent report, the combination of MRI abnormalities using the criteria proposed by Paty, Fazekas or Barkhof and oligoclonal bands resulted in slight increase in accuracy for a diagnosis of CDMS compared to MRI data alone.[Paty *et al.* 1988][Fazekas *et al.* 1988][Tintore *et al.* 2001] Future CIS cohorts who have systematic CSF and MRI studies will be required to address the impact of CSF findings on the new diagnostic criteria.

While an earlier diagnosis using the McDonald criteria is welcome to enable accurate counseling and management advice for patients, confirmation that they reliably predict the development of MS using traditional clinical criteria is necessary to confirm their clinical relevance. This requires a reasonable length of follow up, and

for this reason we place more emphasis on the three year than one-year data for CDMS. In the present study, to date, fewer than half the patients have been followed for three years. Nevertheless the three-year cohort is similar to the original group in terms of age at onset, gender, type of CIS, frequency of MRI abnormalities and DIS at baseline (data not shown). The overall frequency of CDMS after three years (38%) is also similar to a previous study of 74 CIS patients (44%) who were followed for a similar period and in whom the predictive value of baseline MRI features were investigated [Barkhof *et al.* 1997].

In making a diagnosis of a lifelong condition such as MS, a high specificity for a test is more important than a high sensitivity, and the new criteria for MS at three months and one year were highly specific for CDMS at three years. A high positive predictive value for CDMS at three years was also seen for subjects who were diagnosed with MS using the new criteria after three months and one year (ranges 75-85%, Table 4.4). These observations confirm the new criteria as being clinically relevant, an impression consolidated by the overall robust overall accuracy of the criteria for CDMS (ranges 80-87%, Table 4.4).

While the sensitivity of the new criteria at one year is high for the development of CDMS after three years, the sensitivity of the criteria at three months is lower. This reflects the lower frequency (23%) of MRI DIT at this early stage of follow up whereas almost 40% had CDMS after three years. The potential exists for more frequent interval scanning during the first year of follow up to increase sensitivity while maintaining a high specificity and positive predictive value. Further prospective studies are needed to address this.

The new McDonald criteria do not allow the diagnosis of MS on a single MRI scan obtained within three months of a CIS as the criterion of DIT cannot be met (it is possible for a single scan obtained more than three months after the episode to satisfy the new criteria). A question arises whether, in future diagnostic criteria, baseline MRI findings alone, within the first three months, might be reliably used to diagnose MS. The simple presence of T2 lesions is inadequate since many such patients do not develop CDMS within three years, thus resulting in a low specificity, positive predictive value and accuracy in spite of 100% sensitivity. Baseline MRI evidence for DIS improved specificity and positive predictive value, but not to the level achieved by the new diagnostic criteria after three months and one year of follow up. Our results therefore suggest that the additional requirement of DIT is warranted to give a more confident and accurate diagnosis. The relatively short delay may also optimize the timing of discussions of diagnosis and prognosis that have a major impact on the patient.

It is our understanding that the new criteria were developed in order to allow an earlier, yet reliable diagnosis,[McDonald *et al.*2001] and were not intended to guide the timing of introduction of disease modifying treatments, though it is possible that some neurologists will apply them in this manner. There is currently no evidence that the long term prognosis is more favourably modified by therapeutic intervention at first presentation with a CIS - whether or not the new diagnostic criteria are fulfilled - than at the later stage of established relapsing remitting disease. Furthermore, a significant number of patients with CIS and baseline MRI abnormalities have a favorable long-term course without disease modifying therapy [Brex *et al.* 2002].

The development of disabilities in MS goes well beyond the three-year time frame reported in this study, and prolonged follow up studies are necessary to clarify the prognosis for the subgroup of CIS patients who fulfill the new diagnostic criteria at an early stage. A recent 14-year follow up of the first CIS cohort that was recruited in the mid 1980s has shown a significant relationship between T2 volume increase during the first 5 years and disability at year 14.[Brex *et al.* 2002] The pathological MRI changes during these early years are of prognostic significance and more detailed analysis using a range of modern, in vivo MR techniques is required. The McDonald criteria have also been applied by the Barcelona Group. Despite not having an MRI scan at three months, the results are comparable: One year after symptom onset more than three times as many CIS patients were diagnosed with MS using the new criteria (sensitivity 74%, specificity 86% and accuracy 80%).[Tintore *et al.* 2003].

Table 4.1 Baseline clinical features of patients presenting with CIS (n=119)

Age of onset (years)	32 (mean) 31 (median) range (16-50)
Sex M/F	49/70
Clinical Syndrome	
Optic Neuritis	87
Bilateral Optic Neuritis	2
Brainstem Syndrome	19
Spinal Cord Syndrome	10
Demyelinating Optic Tract Lesion	1
Baseline EDSS	1 (mean) 1 (median) Range (0-8)
Onset-baseline scan (weeks)	6 (mean) 5(median) Range (1-12)
Abnormal Baseline brain MRI	85/119

Table 4.2: Frequency of abnormal MRI, DIS, DIT and MS diagnosed using the McDonald criteria and Poser criteria at three months, one and three years

	MRI data	3 months n=95	1 year n=79	3 years n=50
Abn MRI	Brain	67 (71%)	58 (73%)	40 (80%)
	Brain+Cord	70 (74%)	62(79%)	42 (84%)
MRI DIS	Brain	40 (42%)	40 (51%)	26 (52%)
	Brain+Cord	42 (44%)	42 (53%)	29 (58%)
MRI DIT	Brain	22 (23%)	42 (53%)	34 (68%)
	Brain+Cord	-----	46 (58%)	36 (72%)
McDMRI+	Brain	18 (19%)	34 (43%)	24 (48%)
	Brain+Cord	19 (20%)	35 (44%)	27 (54%)
McDMS	Brain	19 (20%)	38 (48%)	28 (56%)
	Brain+Cord	20 (21%)	38 (48%)	29 (58%)
CDMS		7 (7%)	16 (20%)	19 (38%)
McDMRI + CDMS-		13	22	10
McDMRI+ CDMS+		6	13	17
McDMRI-CDMS+		1	3	2

Abn MRI = 1 or more lesions

MRI DIS = MRI dissemination in space

MRI DIT = MRI dissemination in time

McDMRI+ = MRI evidence of DIS and DIT as defined by McDonald criteria

McDMS = MS diagnosed using McDonald criteria

CDMS = clinically definite MS by Poser criteria

Table 4.3 Sensitivity, specificity, positive and negative predictive value and accuracy of the new diagnostic criteria at 3 months and baseline MRI findings for the development of CDMS at 1 yr (n=79)

	McDMS at 3 months (all cases)	McDMRI+ at 3 months	DIS baseline brain only	DIS baseline brain & SC
Sensitivity	73%	67%	69%	88%
Specificity	87%	87%	68%	67%
PPV	58%	56%	36%	40%
NPV	93%	91%	90%	96%
Accuracy	84%	83%	68%	71%

McDMS (all cases) = MS diagnosed by MRI and/or clinical evidence for dissemination in space and time using the new McDonald criteria

McDMRI+ = MS diagnosed by MRI evidence of dissemination in space and time using the new McDonald criteria

DIS baseline brain only = Dissemination in space on brain MRI at baseline

DIS baseline brain & SC = Dissemination in space on brain and spinal cord MRI at baseline

PPV = positive predictive value

NPV = negative predictive value

Table 4.4: Sensitivity, specificity, positive, negative predictive value and accuracy of the new diagnostic criteria at 3 months & one year & baseline MRI findings for the development of CDMS at 3 years (50 patients)

	McDMS at 3 months		McDMS at 1 year			Abnormal baseline MRI	
	All cases	McDMRI+	All cases	McDMRI+	≥1 lesion	DIS brain	DIS B&SC
Sensitivity	65%	59%	94%	83%	100%	63%	79%
Specificity	93%	93%	83%	83%	36%	77%	77%
PPV	85%	83%	77%	75%	49%	63%	68%
NPV	82%	79%	96%	89%	100%	77%	86%
Accuracy	83%	80%	87%	83%	60%	72%	78%

McDMS = MS diagnosed by McDonald criteria

All cases = MS diagnosed using MRI and/or clinical evidence for DIS and DIT

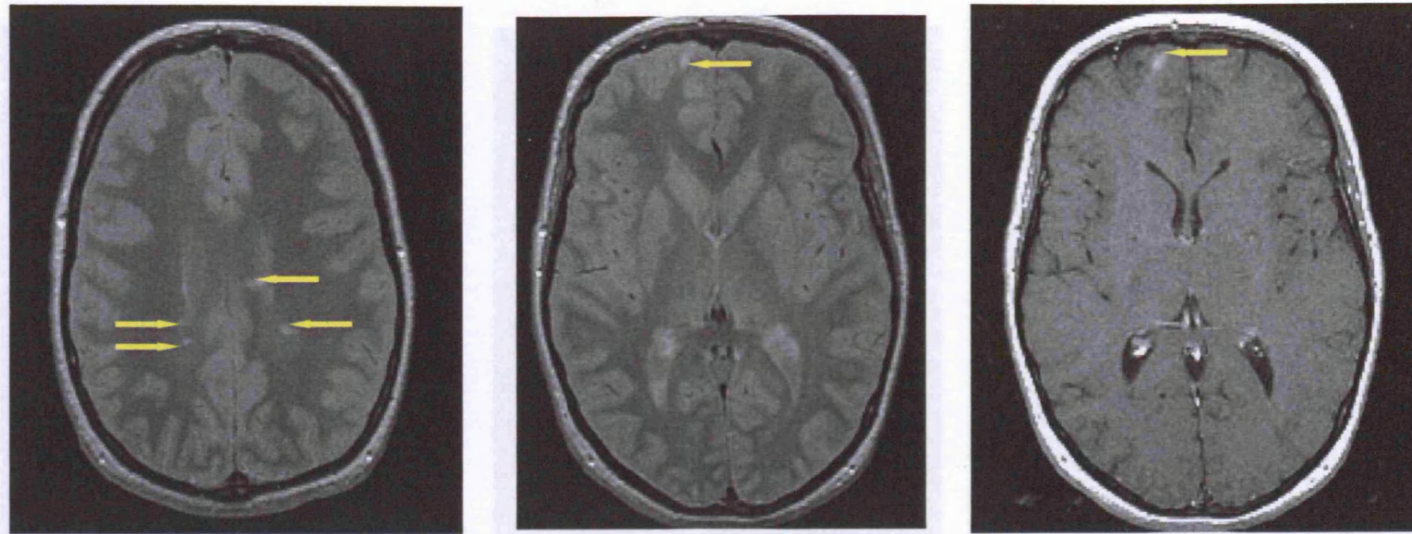
McDMRI+ = MS diagnosed using MRI evidence of DIS and DIT

≥1 lesion = 1 or more lesions consistent with MS on brain MRI at baseline

DIS brain = Dissemination in space on brain MRI at baseline, DIS B&SC = Dissemination in space on brain and spinal cord MRI at baseline

PPV = positive predictive value, NPV = negative predictive value

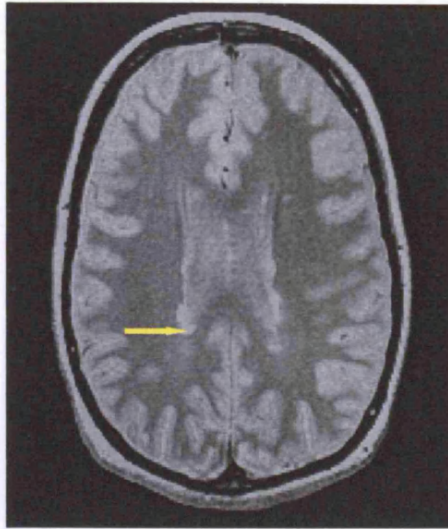
Figure 4.1 CIS patient: At baseline DIS (Barkhof) criteria are positive



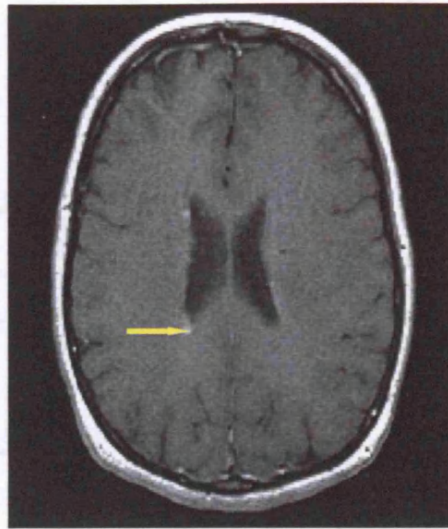
37 year old lady with left optic neuritis

4 periventricular lesions and 1 enhancing subcortical lesion.

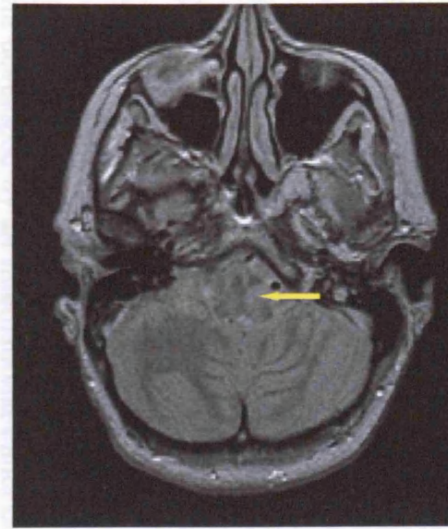
Figure 4.2 CIS patient: At 3 months, McDonald Poser criteria for MS are positive



Ventricular T2 lesions



New Gadolinium
enhancing ventricular
lesion



New symptomatic brainstem
lesion

4.2. Evaluation of new T2 lesions to enable an earlier diagnosis of MS in patients with CIS suggestive of MS

4.2.1. Aims

In CIS patients, new T2 lesions are detected more often than new gadolinium enhancing lesions after three months. Including new T2 lesions for MRI Dissemination in Time (DIT), may improve sensitivity but specificity must also remain high for the criteria to give an accurate diagnosis. Chapter 4.2 addresses the accuracy of new T2 lesions at a three month follow up visit in making a diagnosis of MS.[Dalton *et al.* 2003]

4.2.2. Introduction

New diagnostic criteria developed by an International Panel on MS [McDonald *et al.* 2001] allow MRI evidence of DIT [Brex *et al.* 2001c] and Dissemination in Space (DIS) [Barkhof *et al.* 1997][Tintore *et al.* 2000] enabling a diagnosis of MS to be made in patients with CIS. This contrasts with previous criteria that have required clinical evidence for DIS and DIT in order to diagnose CDMS.[Poser *et al.* 1983][Schumacher *et al.* 1965] In Chapter 4.1, we reported application of the McDonald criteria to a cohort of CIS patients followed clinically and with MRI within three months of symptom onset, and again three months, one year and three years later.[Dalton *et al.* 2002b] If a first MRI is performed less than three months after the onset of the clinical event, a second scan performed three months or more after the clinical event must show a new gadolinium enhancing lesion in order to fulfill the MRI criteria for DIT. After three months of follow up, specificity of the new criteria for the development of CDMS at three years was high (93%). Sensitivity was low (59%), reflecting the low proportion of patients developing new gadolinium enhancing lesions.[Dalton *et al.* 2002b] Diagnosis of MS in patients with a new lesion

that appears on a T2 weighted scan at the three month follow up scan is not permitted by the current criteria.

4.2.3. Methods:

Patients

Since 1995, patients between 16 and 50 years of age with CIS suggestive of MS have been recruited consecutively and imaged within three months of symptom onset. The majority of patients were recruited from a Neuro-ophthalmology clinic in Moorfields Eye Hospital. A CIS is defined as an acute isolated presumed demyelinating event of any part of the central nervous system, with no previous history of demyelinating events. Patients were assessed clinically and with MRI at baseline (within three months of clinical onset), and again three months, one year and three years later. Those with further relapses with new signs clinically disseminated in space and time were diagnosed as having CDMS [Poser *et al.* 1983].

Methods MRI

Patients underwent T2-weighted and gadolinium enhanced T1-weighted brain MRI at each visit. A fuller description of the imaging protocol is provided in Chapter 4.1. An experienced neuroradiologist, blinded to the clinical diagnosis, recorded the number and location of high signal T2/proton density and gadolinium enhancing lesions. The baseline and three-month scans were analyzed for MRI DIS and DIT using the McDonald criteria.[McDonald *et al.*2001] In addition to the existing criteria for MRI DIT (gadolinium enhancing lesions at three months), the alternative of new T2 weighted lesions at three months was investigated while retaining the original criteria for MRI DIS. Performance was assessed by comparing with the “gold standard”

CDMS after three years. The performance of a new T2 lesion per se was also evaluated.

4.2.4. Results

Baseline clinical and MRI data

This study is based on the 56 patients who have been followed clinically for three years, after having MRI at baseline and three months later. Thirty-seven patients had optic neuritis, fourteen a brain stem syndrome, four a spinal cord syndrome and one had a demyelinating lesion of the optic tract. The median age of onset of symptoms was 32 years (range 17-50). There were 30 females and 26 males. The median number of weeks from onset of symptoms to the baseline scan was 5 (range 1-12). Thirty eight/56 (68%) had an abnormal baseline T2-weighted brain MRI, and 18 (32%) had MRI DIS [Barkhof *et al.* 1997][Tintore *et al.* 2000].

Performance of the McDonald criteria & new T2 lesions at three months (Tables 4.5 and 4.6)

The median number of weeks, from the baseline scan to the three month follow up scan, was 12 (range 10-20). Although 20 patients developed new T2 lesions at three months (Table 4.5), only 14 had new gadolinium enhancing lesions. Specificity of the McDonald criteria at three months for CDMS at three years was 95% but sensitivity was only 58% (Table 4.6). Eight patients, who did not fulfill the McDonald criteria at three months, developed CDMS at three years. Addition of a new T2 lesion to the criterion of MRI DIT at three months also had high specificity (92%) and better sensitivity than the McDonald criteria (74% versus 58%). Only five patients who failed to fulfill these MRI criteria at three months developed CDMS after three years

(Table 4.6). A new T2 lesion, irrespective of the number of lesions on the baseline scan, was sensitive (84%) and specific (89%) for CDMS (Table 4.6; all patients with new T2 lesions at three months had one or more baseline MRI brain lesions). Four patients received IFNBeta during the three years of follow up but only after the three month MRI follow up and after they had developed CDMS. Only four patients received a course of steroids for their CIS, two before and two after the baseline scan.

4.2.5. Discussion

The present report extends our previous application of the McDonald criteria to patients with CIS in chapter 4.1.[Dalton *et al.* 2002b] In Chapter 4.1, the new criteria were shown to have a high specificity and positive predictive value for CDMS when applied after three months of follow up. Low sensitivity was due to the low frequency of new gadolinium enhancing lesions required to fulfill the criterion of MRI DIT. A new T2 lesion for MRI DIT increases the number diagnosed with MS after three months (Table 4.5) and the sensitivity for CDMS increases (from 58% to 74%) with a maintained high specificity (95% versus 92%). Thus, modifying the existing McDonald criteria to include a new T2 lesion at three months is both sensitive and specific in the early diagnosis of patients with CIS.

The higher sensitivity of T2 versus gadolinium enhanced lesions on a 3 month scan is because a new T2 lesion usually persists whereas a new area of enhancement lasts, on average, one month [Miller *et al.* 1993][Thompson *et al.* 1992][Smith *et al.* 1993]. New T2 lesions reflect activity over the whole interval, whereas gadolinium enhancing lesions reflect only the previous month. The McDonald criteria require enhancement at this stage as the writing panel considered MRI DIT needed new

activity to occur at least three months after the clinical episode. A new T2 lesion could arise at any time during follow up, including within three months of the episode. However, the sensitivity and specificity of new T2 lesions appears sufficient to warrant their inclusion in the criteria.

On their own, new T2 lesions at three months were both sensitive (84%) and specific (89%) for predicting CDMS at three years. All 20 patients with new T2 lesions had an abnormal baseline scan, but three did not have MRI DIT. Further evaluation to determine the optimal MRI criteria for DIS may be warranted.

As MRI criteria can now be used to make a diagnosis of MS in patients presenting with CIS a number of caveats are suggested to ensure reliable application. First, the diagnosis should be made by a clinician experienced in CNS demyelinating disease. Secondly, MRI evaluation should be performed by a neuroradiologist or other clinician, with extensive experience of MS and other white matter disorders. Thirdly, T2 lesions should be unequivocal - normally lesions are at least 3mm in diameter, observed on two different types of T2 sequence (e.g. on proton density and heavily T2 weighted fast spin echo or on T2-weighted spin echo and FLAIR), and determined from high quality images. Fourthly, as our study was restricted to those aged between the age of 16 and 50 years, the accuracy of the MRI criteria outside these ages is uncertain. In children, acute disseminated encephalomyelitis (ADEM) is relatively common [Dale *et al.* 2000] and the specificity of the criteria in MS versus ADEM is unknown. In older adults, white matter lesions due to small vessel disease have the potential to reduce MRI specificity for MS. Fifthly, spinal cord MRI should also be performed when the CIS involves the cord, to exclude other disorders and to identify

demyelinating cord lesions [Miller et al. 1987]. Finally, the detection of cerebrospinal fluid oligoclonal bands is sometimes useful. Both oligoclonal bands and MRI (Paty's and Fazekas' criteria) have high sensitivity for CDMS [Paty et al 1988][Fazekas et al 1988][Tintore et al 2001].

In conclusion, we propose that in adults aged 16 to 50 years presenting with a CIS suggestive of MS, the McDonald MRI criteria for DIT are expanded to include new T2 lesions seen on a three month follow up scan when the first scan is obtained within three months of symptom onset.

Table 4.5 Patients with a diagnosis of MS at three months using MRI or clinical criteria

	McDMS	McDMS and new T2 lesion	Abnormal T2 scan, new T2 lesion and CDMS
MRI diagnosis only	8	12	15
CDMS only	1	0	0
MRI diagnosis and CDMS	4	5	5

Table 4.6 Performance of the McDonald criteria and new T2 lesions at 3 months in relation to the development of CDMS at 3 years

At 3 Months	McDMS	McDMS & new T2 lesion	Abnormal scan, new lesion and CDMS
CIS/Criteria+	2	3	4
CDMS/Criteria+	11	14	16
CIS/Criteria-	35	34	33
CDMS/Criteria-	8	5	3
Sensitivity(95% CI)	58% (34%-80%)	74% (49%-91%)	84 % (60%-97%)
Specificity (95% CI)	95% (82%-99%)	92% (78%-98%)	89% (75%-97%)
PPV (95% CI)	85 % (55%-98%)	82% (57%-96%)	80% (56%-94%)
NPV (95% CI)	81% (67%-92%)	87 % (73%-96%)	92 % (78%-98%)
Accuracy (95% CI)	82% (70%-91%)	86% (74%-94%)	87% (76%-95%)

CIS = clinically isolated syndrome at 3 years,

CDMS = clinically definite MS at 3 years

McDMS = Diagnosis of MS based on the McDonald criteria at 3 months

Criteria+/- = fulfill/ do not fulfill the relevant diagnostic criteria at 3 months

CI = Confidence Interval

Sensitivity, specificity, positive predictive value (PPV), negative predictive value (NPV) and accuracy refer to the performance of the diagnostic criteria at 3 months in relation to the development of CDMS.

Chapter 5

5. Atrophy: Ventricular volumes (MIDAS) and brain segmentation (SPM99)

5.1 Progressive ventricular enlargement in patients with CIS is associated with the early development of MS

5.1.1. Aims

The aims of the study were to confirm a preliminary study in a subgroup of this cohort of patients which showed measurable Ventricular Enlargement (VE) over one year in some patients with CIS. We also wanted to clarify whether VE is associated with the early development of MS, using conventional clinical criteria and the new McDonald criteria and finally we wanted to determine the relationship between VE and MR measurements of lesion load at this early stage of disease.

5.1.2. Introduction

Previous radiological and pathological studies have established that whole brain [Gross *et al.* 1993][Davie *et al.* 1995][Liu *et al.* 1999][Ge *et al.* 2000] and spinal cord atrophy [Filippi *et al.* 1997a][Losseff *et al.* 1996c] occur in MS in excess of that expected with age. In one study, progressive cerebral atrophy over one year has been detected using registered, volumetric MRI to measure both brain and ventricular volumes [Fox *et al.* 2000], with a rate of cerebral atrophy in MS shown to be twice that seen in normal controls and the rate of ventricular enlargement five times that of controls. There was also a good correspondence between global brain atrophy and ventricular enlargement [Fox *et al.* 2000].

While the mechanisms of atrophy in MS are not well understood, it probably indicates loss of functionally important structures: myelin and axons, which are the majority bulk of brain white matter, although variable effects on tissue volumes may also arise

from glial cell proliferation or loss, gliosis, inflammation and oedema.[Miller *et al.* 2002] A correlation between reduced cerebral white matter N-acetyl aspartate (a neuronal marker) and cerebral atrophy suggests that axonal loss may make a contribution.[Coles *et al.* 1999] However the relationship between atrophy and decreased NAA is non linear,[Collins *et al.* 2000] and factors other than axonal loss are also likely to effect these MR measures. In general, disability in MS has been more strongly correlated with measures of atrophy than lesion load.[Kalkers *et al.* 2001a][Losseff *et al.* 1996b] However, only modest correlations between atrophy and lesion load have been observed.[Losseff *et al.* 1996b][Paolillo *et al.* 2000] This could indicate that atrophy develops by mechanisms, some of which are independent of lesions, or that there is a temporal dissociation between lesion accumulation which occurs early in the disease process and atrophy, which occurs late.

To investigate the onset of atrophy and its relationship to lesions and clinical events, we studied patients presenting with CIS suggestive of MS. The study of this cohort allows an investigation into the earliest clinical stages of MS, since long term follow-up indicates that the majority of CIS patients with MRI brain lesions will go on to develop CDMS.[O'Riordan *et al.* 1998b][Brex *et al.* 2002][Ghezzi *et al.* 1999] In the present report, atrophy was evaluated using a measure of VE.[Dalton *et al.* 2002a] This study follows a previous preliminary pilot study of a small, selected group of 17 from within the present larger cohort of 55 patients.[Brex *et al.* 2000]

5.1.3. Methods

Patients

We recruited adult patients, aged between 17 and 50 years, who presented to our clinics at Moorfields Eye Hospital or the National Hospital for Neurology and Neurosurgery with CIS suggestive of MS. Appropriate investigations including full blood count, ESR, autoantibody screen, syphilis serology, vitamin B12, and MRI brain were used to exclude other diagnoses. Fifty five patients were recruited consecutively from January 1996 until June 1999 (16 patients, recruited prior to a hardware upgrade to the scanner in January 1996 were excluded from this study because serial evaluations pre and post upgrade revealed instability of volume measures. On going quality assurance protocols have confirmed the stability of measurements since January 1996). A CIS was defined as a single event of acute onset in the central nervous system suggestive of demyelination, e.g. unilateral optic neuritis, brainstem and partial spinal cord syndromes. Individuals with a history of previous neurological symptoms suggestive of demyelination were excluded. Other diagnoses were excluded by appropriate investigations.

Overall, 38 patients had optic neuritis, 11 a brainstem syndrome, 5 a spinal cord syndrome (spinal MRI excluded an alternative structural cause in these cases) and 1 patient had a hemianopia attributable to an MRI lesion in the optic tract. The age range at baseline was 17 to 50 years with a median age of 31 years. The upper age limit of 50 years was applied in order to reduce the likelihood of including patients with white matter lesions due to small vessel disease and because onset of MS above this age is less common. Local ethics committees approved the study and informed consent was obtained from each patient before entry. Patients were imaged on three

occasions: (i) at baseline, which in all instances was within 3 months of the onset of symptoms, (ii) approximately 3 months later, and (iii) approximately one year later. Clinical examination and EDSS [Kurtzke 1983] were performed at each visit. The diagnosis of clinical MS (clinically definite or probable) was based on the Poser criteria,[Poser *et al.* 1983] using clinical criteria alone (MRI or CSF findings were not incorporated). CDMS was diagnosed when a second relapse occurred more than one month after the CIS, which involved a separate part of the CNS and was accompanied by new findings on examination. Clinically probable MS was diagnosed when there was a history indicating a new relapse involving a separate location but there were no new signs on examination. The new McDonald MRI criteria, which additionally allow a diagnosis of MS in CIS patients based on MRI evidence of Dissemination of lesions in Time (DIT) and space (DIS), were also applied.[McDonald *et al* 2001]

Image acquisition

MRI was performed using a 1.5 Tesla GE scanner. At baseline, 3 months and one year each, a proton density/T2 weighted fast spin-echo (FSE) sequence (repetition time [TR] 3200 msec, effective echo time [TE] 15/95 msec) and a T1 weighted spin echo sequence (TR 600 ms TE 14 ms) were acquired in each patient, with 3mm contiguous, axial slices. The matrix used was 256x256 with a field of view of 24 cm. Each patient was given an intravenous bolus of 0.1 mmol/kg gadolinium DTPA 5-7 minutes prior to the commencement of image acquisition.

Ventricle Volume (VV) measurement

The ventricles were measured on baseline and one year follow up T1-weighted scans using the MIDAS interactive image analysis package by a single observer blinded both to patient details and scan acquisition order (the 3 month scan was not analyzed

using MIDAS as it was considered unlikely that change would be detectable over this short period). The initial step was segmentation of the whole brain using a semi automated interactive morphologic technique with the image intensity threshold for the boundary between the CSF and brain set at 60% mean signal intensity. The inferior cut-off was taken at the lowest point of the cerebellum. VV consisted of the lateral ventricles and temporal horns but excluded the third and fourth ventricles. This was measured using a semi-automated seed placing technique, involving voxels with an image intensity of less than 60% of the mean (Figure 5.1). MIDAS also provides a measure of change in whole brain volume, but on the 2-dimensional images available for this study, this measure required considerable time and manual editing. The analysis was therefore confined to VV only.[Freeborough *et al* 1997]

Reproducibility of the ventricular measurement technique

Ten VVs were measured and re-measured after 7 days by a single observer blinded to both patient details and scan acquisition order. The mean coefficient of variation was 0.89% prior to starting image analysis.

Lesion identification and lesion load measurement

The MRI scans were reviewed by an experienced neuroradiologist (KAM), who was blinded to the patients' clinical status at follow up. T2 weighted lesions were identified on the PD weighted images with confirmatory support from the long TE image, on both the baseline and one year follow up scans. T1 hypointense and gadolinium enhancing lesions were identified on the T1 weighted scans. T1 hypointense lesions were identified at baseline and one year follow up. Enhancing lesions were identified on all 3 scans (baseline, 3 months and 1 year). The volumes of

T2 and T1 hypointense lesions at baseline and one year were subsequently calculated from electronic data on a Sun Workstation using a semi automated local thresholding technique to contour the lesions.[Plummer 1992]

Statistical analysis

Comparison was made between the baseline and one year VVs using the Wilcoxon signed rank test (Tables 5.2 and 5.4). The Mann Whitney U test was used to compare baseline lesion load measures in patients who developed MS and those who had no further symptoms (Table 5.3). Correlations between lesions measures and VV change were performed using Spearman's non parametric correlations. The Bonferroni correction was applied by dividing the number of analyses into 0.05. The p value for significance was then calculated at 0.003 (Table 5.5).

5.1.4. Results

Baseline MRI findings (Table 5.2)

The median time delay between the onset of symptoms and baseline scan was 6 weeks, with a range of 1 to 12 weeks. At baseline, 40/55 (73%) patients had one or more lesions detected on T2-weighted MRI, of whom 21 also exhibited one or more hypointense lesions on T1-weighted images and 15 displayed one or more gadolinium enhancing lesions. VVs varied markedly between subjects (range 1.2-39.2cm³; see Table 5.2, Figures 5.2 and 5.3). There was no significant difference in baseline VVs in patients depending on gender (p=0.8), with or without T2 lesions (p=0.3), T1 lesions (p=0.9) and gadolinium enhancing lesions (p=0.2) (Mann Whitney U test for all

comparisons). There was no correlation between baseline VV and subsequent atrophy ($r=0.17$ and $p=0.23$).

Follow up findings (Tables 5.1-5.5)

Clinical outcome

The median time between the baseline and one-year follow up was 12 months (range 11-16 months). Thirty-seven patients remained asymptomatic, while 18 patients developed CDMS ($n=14$) or probable MS ($n=4$) during the 1-year follow-up period. Sixteen of the 18 patients who developed clinical MS had abnormalities on the T2 scan at baseline.

The relationship between lesions and clinical outcome

The baseline number of T2, T1 hypointense and gadolinium enhancing lesions was significantly higher in those who went on to develop clinical MS compared with those who did not (Table 5.3). The baseline volumes of T2 and T1 hypointense lesions were also significantly higher in those who developed MS (Table 5.3).

The relationship between VE and clinical outcome

There was a significant increase in VV over one year in the 18 patients who developed clinical MS during the 1-year follow-up period ($p=0.006$, Table 5.2). A significant increase in VV was also seen in the 16 patients with T2 lesions at baseline who developed clinical MS at 1 year (median increase 0.9 cm^3 , $p=0.002$). There was no significant correlation between VE and baseline EDSS, month 12 EDSS or change in EDSS over the year, using the Bonferroni correction (Table 5.5).

The relationship between VE and the McDonald criteria for MS

The baseline, three month and one year scans were retrospectively analyzed to see how many patients fulfilled the new McDonald criteria for the diagnosis of MS.[McDonald *et al.* 2001] Patients with CIS with MRI evidence of DIS and DIT were diagnosed as having McDonald MRI MS. Twenty-seven of the 55 patients were McDonald criteria positive at one year (Table 5.1).

The relationship between VE and lesion load measures

Subgroup comparisons (Table 5.4)

Significant VE was seen in the group of 40 patients with T2 lesions at baseline ($p=0.001$) but not in the 15 patients with normal scans at baseline ($p=0.46$, Table 4). The 15 patients with gadolinium enhancing lesions at baseline had significant VE at one year ($p=0.004$, Table 5.4).

The 14 patients who had gadolinium enhancing lesions at 3 months also showed significant VE at one year (baseline median 5.7cm^3 [2.6 – 25.9], one year median 6.7cm^3 [2.9 – 26.5], $p=0.006$). The 7 patients who had enhancing lesions at both baseline and 3 months exhibited a substantial increase in VV (baseline median 7.9cm^3 [3.2 – 25.9], one year median 12.9cm^3 [3.7 – 26.5], $p=0.02$).

VVs in the 21 patients with T1 hypointense lesions at baseline showed significant enlargement over the following year ($p=0.001$), but there was no significant change in the 19 patients who had T2 lesions but no T1 hypointense lesions (Table 5.4).

Correlations (Table 5.5)

Four correlations with VE were significant, namely the baseline T1 hypointense lesion number and volume and one year T1 hypointense and T2 lesions volumes.

5.1.5. Discussion

This study has confirmed previous reports in demonstrating that the presence and number of MRI lesions at presentation with a CIS influences the risk of future clinical relapses leading to an early diagnosis of CDMS or probable MS. Additionally, it revealed VE over one year in the subgroup of patients who developed clinical MS within that period. It also demonstrated VE in the larger cohort of patients with T2 MRI lesions at presentation, the presence of which is associated with a high probability for developing CDMS after prolonged follow-up.[O'Riordan *et al* 1998b][Brex *et al* 2002][Ghezzi *et al.* 1999] Finally, the study demonstrated significant, but only modest, correlations between T2 and T1 hypointense lesion load measures and subsequent VE.

A question arising is whether the VE might reflect a reduction in brain water content (pseudo atrophy) rather than true tissue loss. There was no significant difference in the gadolinium enhancing lesion number between the baseline and 1 year follow-up scans (data not shown), so that a spontaneous decrease in the number of inflammatory oedematous lesions did not account for the VE seen. Anti-inflammatory treatments such as corticosteroids [Hoogervorst *et al.* 2002] or IFNbeta can reduce the number of inflammatory oedematous lesions, but in the present study only three patients received steroids (one received IFNbeta) during the follow-up phase.

There have been few prior studies investigating atrophy in CIS patients. My group previously reported a preliminary study of a small, selected group from within the present larger cohort, consisting of 9 patients with T2 lesions at baseline who developed MS during 1 year of follow-up and 8 patients with a normal baseline scan who had no further clinical events. In that study Brex et al reported significant VE in the subgroup with T2 lesions.[Brex *et al.* 2000] Secondly, Brex et al recently investigated cervical cord in 43 CIS patients demonstrated mild but significant atrophy of the upper cervical cord in those patients who had T2 abnormalities on brain imaging [Brex *et al* 2001a].

The present investigation of a large cohort of consecutively recruited CIS cases confirms our preliminary observation in the smaller cohort that early development of VE is associated with further clinical relapses leading to a diagnosis of MS. The development of ventricular atrophy in association with early clinical relapses suggests a potential for this MR measure to provide prognostic data, which needs to be confirmed with long term follow-up. There is some evidence from the literature, albeit inconsistent, that relapse rate in the first 1-2 years after a CIS is related to long term disability.[Weinshenker *et al.* 1989] The relationship between early atrophy and long term disability should be investigated by prolonged follow up.

A possible mechanism for the observed VE would be loss of myelin and axons within lesions per se, and Wallerian degeneration secondary to axonal transection within the lesions. Consistent with this mechanism are the correlations, albeit weak, between the baseline and month 3 number of enhancing lesions and VE. Enhancement is associated with pathological evidence of active inflammation [Katz *et al.* 1993][Bruck

et al. 1997] and inflammation is associated with axonal damage and transection.[Ferguson *et al.* 1997][Trapp *et al.* 1998] The small group of patients with enhancing lesions at both baseline and month three exhibited more substantial VE. The more aggressive inflammatory disease may have contributed to this.

A modest correlation of VE was also found with baseline T2 and T1 hypointense loads but was somewhat stronger for T1 load. T1 hypointense lesions are associated pathologically with more severe axonal loss,[Van Waesberghe *et al.* 1999] and thus might be expected to result in a greater degree of secondary Wallerian degeneration and atrophy. It was notable that in the subgroup comparison of those with and without T1 hypointense lesions at baseline, significant VE was seen only in those with such lesions. T1 hypointense lesions in this study were detected on post contrast scans – they are thus likely to indicate irreversible changes associated with axonal loss rather than the reversible hypointensity sometimes seen in acute enhancing lesions. The lack of correlation between change in T1 hypointense load over the year and VE may reflect the fact that the magnitude of the former was very small.

Since none of the correlations between lesion measures and VE were strong, additional factors may contribute to atrophy. One possibility is a more diffuse process involving normal appearing tissues. To date, quantitative MR investigations of normal appearing brain tissues have provided conflicting evidence of abnormality in small CIS cohorts.[Brex *et al.* 1999a][Brex *et al.* 2001b][Kaiser *et al.* 2000][Iannucci *et al.* 2000] An MR spectroscopy report demonstrated no significant reduction of N-acetyl aspartate in the normal appearing white matter.[Brex *et al.* 1999a] Further quantitative

MR investigations of the normal appearing brain tissue are needed in larger CIS cohorts.

The technique used in the present study does not resolve the site of atrophy. While the MIDAS method used in this study provides a reliable measure of change in whole brain volume on 3D images, we found it less reliable with the present 2D dataset. It is not therefore certain whether atrophy occurs close to lesions, in normal appearing white matter, or in grey matter, although the fact that the ventricles are close to white matter suggests that there may be atrophy in this region. There are alternative strategies for measuring segmented grey and white matter volumes which have been applied in Chapter 5.2.[Chard *et al* 2002c] Approaches such as voxel based morphometry may depict the regions in which atrophy is occurring. Application of such methods in future studies in CIS patients will be of interest.

The study of atrophy may help to understand the clinical effects of disease modifying treatments for MS. In established disease, beta interferons and glatiramer acetate markedly reduce the rate of new MRI lesions (by 50-70%; IFNB study 1993[Paty and Li 1993][Jacobs *et al* 1996;1998a][Comi *et al* 2001b], but they have a smaller effect in preventing relapses, disability and atrophy [Rudick *et al.* 1999][Rudick *et al.* 1999b][Molyneux *et al.* 2000][Gasperini *et al.* 2002][Simon *et al.* 1999][Rudick *et al.* 2000][Rovaris *et al.* 2001][Fisher *et al.* 2002]. Future reports of the effect of treatment on atrophy in CIS patients are awaited, but the present work highlights the potential for a mechanism of clinical deterioration that is independent of lesions.

While the present knowledge suggests that atrophy is occurring at the earliest clinical stage of MS, a direct comparison with other studies at a later stage is not possible, since the methodology involved has differed. There are a number of sensitive and reproducible methods for detecting brain atrophy but they may produce quite a different absolute measure of change.[Miller *et al.* 2002] Further study of cohorts with later disease using the same MR acquisition and analysis method for measuring VE is now being undertaken. Further follow up of the CIS cohort is now needed to determine the longer term evolution of atrophy, and its relationship to lesion evolution, quantitative MR changes in the normal appearing tissues, and clinical outcome.

In conclusion, the present study reveals two MR features in patients with CIS who go on to develop early clinical MS - lesions and atrophy. While lesions contribute to atrophy, the correlation is modest, suggesting that the latter also develops by other mechanisms. Both measurement of lesions and atrophy are therefore complementary methods for monitoring the course of MS, even from its earliest stages.

Table 5.1 Patient details

Clinical status after one year	Early MS	Asymptomatic
Sex, M/F	6/12	18/19
Age in years median (range)	30.5(17-49)	31(18-50)
Weeks from onset to baseline scan median (range)	6.5(1-12)	5(2-11)
Months from baseline to year 1 scan median (range)	12(11-15)	12(12-16)
EDSS at baseline median (range)	1.5 (0- 8)	2 (0- 4)
EDSS at one year median (range)	2 (0- 8)	1 (0- 3)
Clinical status after one year	McD MS	McD MS negative
Sex, M/F	10/17	14/14
Age in years median (range)	31(17-47)	31(18-50)
Weeks from onset to baseline scan median (range)	5(1-12)	6(2-11)
Months from baseline to year 1 scan median (range)	12(11-16)	12(12-14)
EDSS at baseline median (range)	1 (0- 8)	2 (0- 4)
EDSS at one year median (range)	1 (0- 8)	1 (0- 3.5)

Table 5.2 Comparison of VV in patients who developed clinical MS and McDonald MS versus those who did not develop clinical and McDonald MS

Baseline VV cm ³ median (range)	Early MS n=18	Asymptomatic n=37	Total n=55
	*5.4(1.2- 39.2)	**6.0 (1.5- 23.1)	5.9 (1.2- 39.2)
1 year follow up VV cm ³ median (range)	*7.7(1.3- 40.6)	**6.2 (1.7- 24.1)	6.3 (1.3- 40.6)
***VV change cm ³ median (range)	+0.5 (-0.6- 7.2)	+0.06 (-0.9- 1.4)	+0.1 (-0.9- 7.2)

Comparing baseline versus one year VVs *p=0.006, **p=0.402

Comparing VV change in MS and those with no further symptoms ***p=0.007

	McDonald MS n=27	McDonald MS negative n=28
Baseline VV cm ³ median (range)	*6.0(1.2- 39.2)	**5.3 (1.7- 23.1)
1 year follow up VV cm ³ median (range)	*8.1(1.4- 40.6)	**5.2(1.7- 24.1)
***VV change cm ³ median (range)	+0.3 (-0.7- 7.2)	+0.05 (-0.9- 1.4)

Comparing baseline versus one year VVs *p=0.005, **p=0.632

Comparing VV change in patients with McDonald MRI MS (n=27) and those without (n=28) ***p=0.03.

Table 5.3: Comparison of baseline lesion measures in patients who developed MS and those who remained asymptomatic

	Early MS (18)	Asymptomatic (37)	P value
Number of T2 lesions at baseline	20 (0-70)	2 (0- 76)	p=0.001
Volume of T2 lesions at baseline cm ³ median (range)	1.7 (0-13.9)	0.3 (0- 5.2)	p=0.001
Number of Gd-DTPA lesions at baseline	1.5 (0- 21)	0 (0- 13)	p<0.001
Number of T1 hypointense lesions at baseline	1 (0-18)	0 (0-2)	P=0.001
Volume of T1 hypointense lesions at baseline cm ³ median (range)	0.09 (0-4.5)	0 (0-0.6)	p=0.001

Table 5.4: Ventricular volumes VV in patients with a normal and abnormal MRI, with and without Gadolinium (Gd) enhancing and T1 hypointense lesions at baseline.

		Normal & abnormal baseline MRI (n=55)		Abnormal baseline MRI (n=40)		Abnormal baseline MRI (n=40)	
		Normal MRI (n=15)	Abnormal MRI (n=40)	Gd lesions (n=15)	No Gd lesions (n=25)	T1 hypointense lesions (n=21)	No T1 hypointense lesions (n=19)
Baseline	VV cm ³	4.3 (2.6- 23.1)	6.4 (1.2- 39.2)	7.9 (3.1-25.9)	5.9 (1.2- 39.2)	6 (1.2-25.9)	6.9 (1.7- 39.2)
median (range)							
Year 1	VV cm ³	4.4 (2.4- 24.1)	6.8 (1.4- 40.6)	10.3 (3.2-26.5)	6.5 (1.4- 40.6)	6.7 (1.4-26.5)	7 (1.7- 40.6)
median (range)							
	VV change cm ³	-0.2 (-0.9- 1.0)	0.2 (-0.7 - 7.2)	+0.7 (-0.6- 7.2)	+0.1 (-0.7- 1.6)	+0.5 (-0.6- 7.2)	+0.03 (-0.7- 1.9)
median (range)							
Comparing baseline		P=0.46	P=0.001	P=0.004	P=0.069	P=0.001	P=0.376
and year 1 VV							

Table 5.5 Correlation of change in VV over one year with other MRI lesion measures and with EDSS

Correlation	R value	P value
Baseline T2 lesion number	0.339	0.011
Baseline T2 lesion volume	0.381	0.004
Change in T2 lesion volume over 1 year	0.202	0.138
Sum of baseline and new T2 lesion numbers at 1 year	0.386	0.006
Number of new T2 lesions at month 12 compared with baseline	0.254	0.061
1 year T2 lesion volume	0.447	0.001*
Baseline Gd lesion number	0.354	0.008
Month 3 Gd lesion number	0.367	0.007
Baseline and month 3 Gd lesion number	0.38	0.006
Month 12 Gd lesion number	0.206	0.132
Baseline, month 3 and month 12 Gd lesion number	0.385	0.005
Baseline T1 hypointense lesion number	0.495	<0.001*
Baseline T1 hypointense lesion volume	0.467	<0.001*
Change in T1 hypointense lesion volume over 1 year	0.05	0.735
1 year T1 hypointense lesion volume	0.421	0.001*
Baseline EDSS	0.132	0.335
EDSS at m12	0.317	0.019
EDSS change over 1 year	0.116	0.398

*significant correlations using Bonferroni correction $p < 0.003$

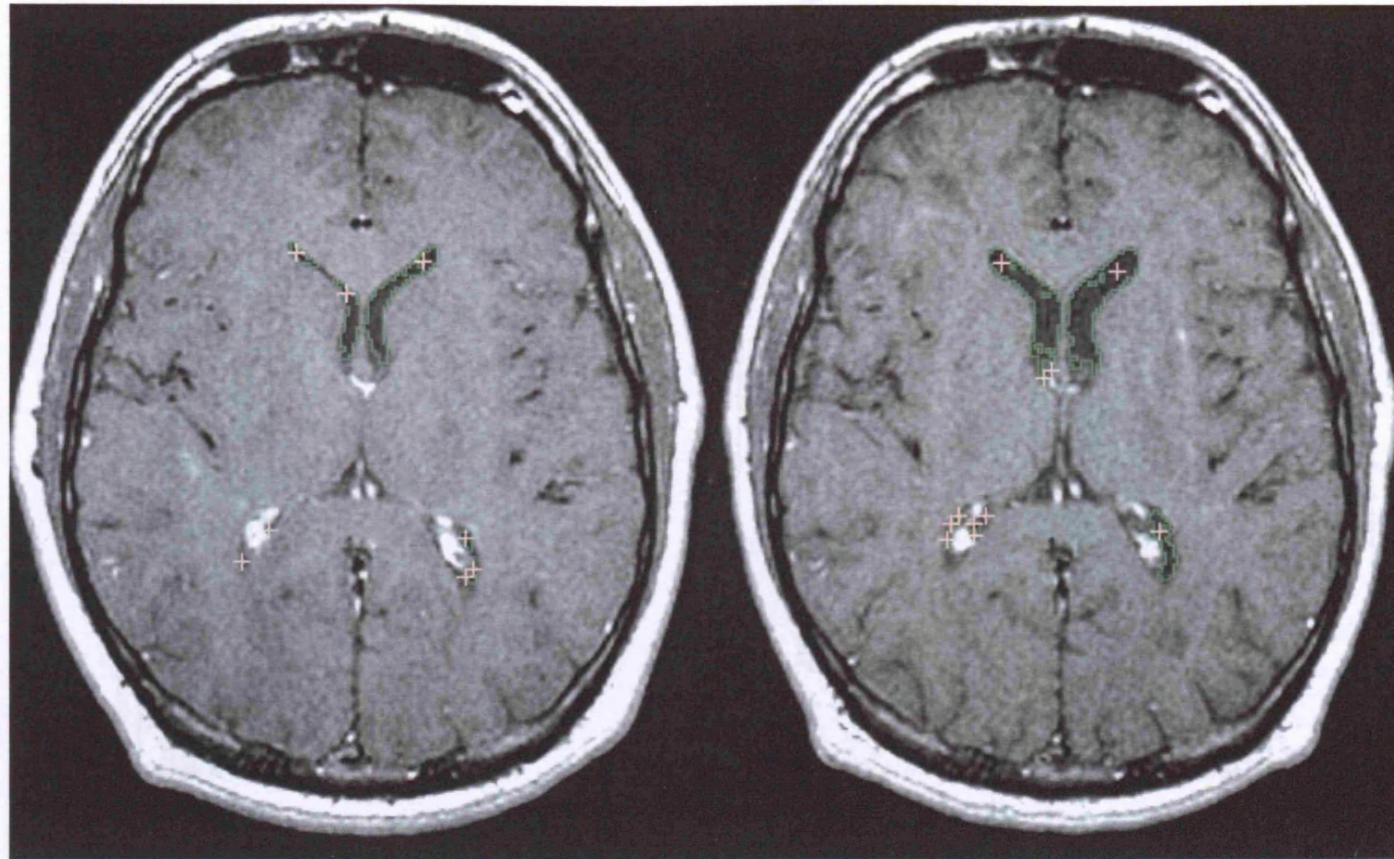


Figure 5.1 Baseline and 1 year ventricular volumes in MS

Baseline Ventricular Volume = 5.6 cm³

One Year Ventricular Volume = 12.8 cm³

Ventricular segmentation: seeds are inserted into the lateral ventricles and temporal horns. High signal choroid is excluded. This 29 year old male presented with left optic neuritis in 1998. Two months later he developed numbness on the right side of his face. He had 3 relapses between his baseline and 1 year scans.

Figure 5.2: Scatter plot of VVs at baseline and 1 year in the 37 patients with CIS

at one year

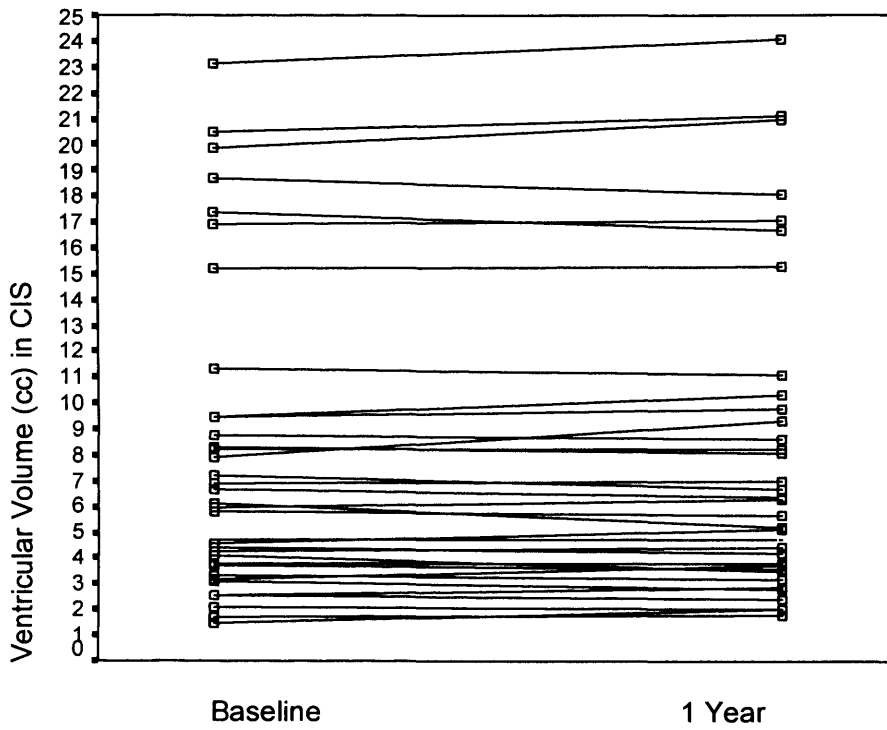


Figure 5.3: VVs at baseline and one year in the 18 MS patients

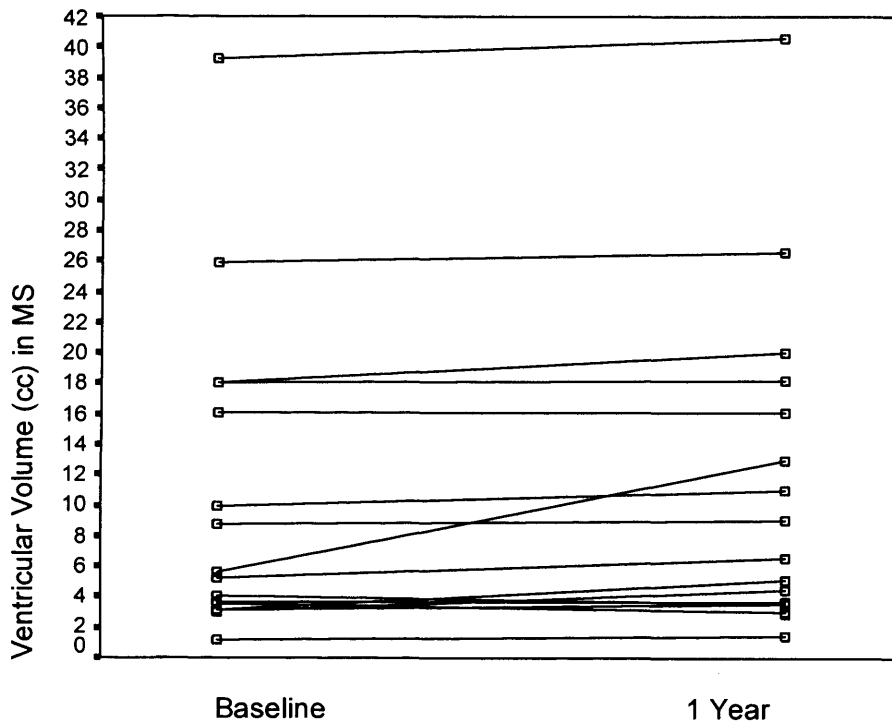
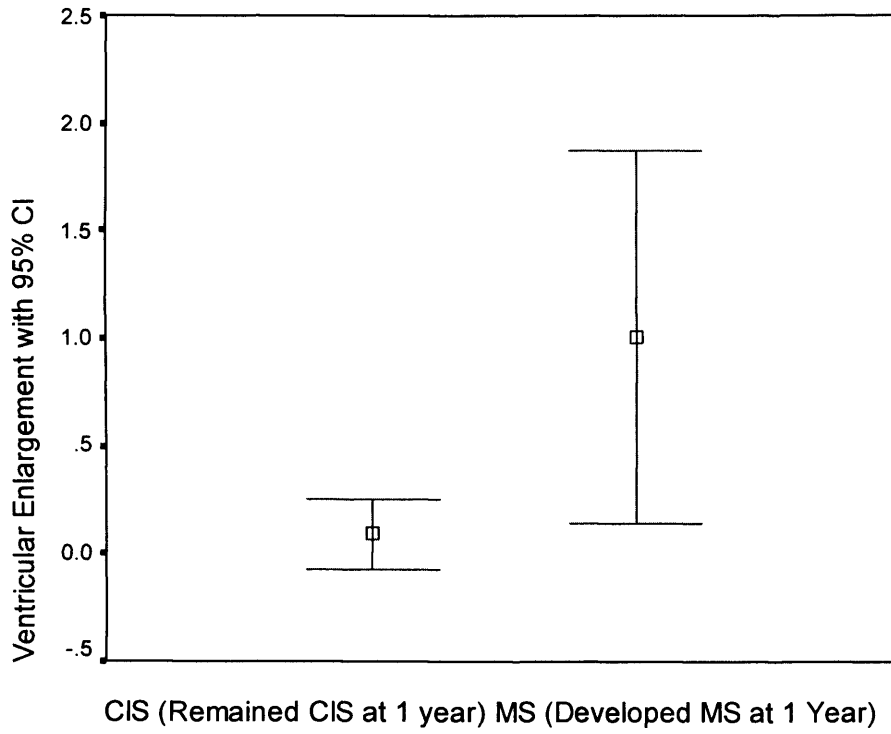


Figure 5.4: Error bar plot of VE in patients with CIS and MS.



5.2. Early development of MS is associated with progressive grey matter atrophy in patients presenting with CIS

5.2.1. Aims

The aim of this study was to determine which tissues are affected by axonal loss or atrophy by segmentation into Grey Matter (GM), White Matter (WM) and CSF.

5.2.2. Introduction

MS initially presents as a CIS such as optic neuritis, brain stem or spinal cord syndromes in 85% to 90% of patients. Sixty-80% of patients presenting with CIS suggestive of MS who have MR visible brain lesions go on to develop CDMS.[Brex *et al* 2002][Beck *et al.* 2003] However, while lesions are associated with the development of MS, there is only a limited relationship between lesion accrual and subsequent disability. This may reflect pathological heterogeneity within lesions, with variable effects on nerve conduction and clinical function. Axonal and neuronal damage have become the focus of significant attention [Trapp *et al.* 1998][Bjartmar *et al.* 2001][Bjartmar and Trapp 2001][Cifelli *et al.* 2002] and may be more salient when considering long-term outcomes, with atrophy measures potentially acting as a more specific marker of such pathology than lesion volume measures [Miller *et al.* 2002]. This is supported by the observation that in established MS, atrophy of the brain and spinal cord is more noticeable with increasing disability [Losseff *et al.* 1996b][Losseff *et al.* 1996c].

Relatively little investigation of atrophy has been undertaken in patients in the earliest stages of MS. Studies at the early stage of disease have potential to identify prognostic markers and techniques for therapeutic monitoring and may provide insights in to

disease pathogenesis. In previous studies in patients with CIS, progressive Ventricular Enlargement (VE) was detected over one year in those with an abnormal MRI [Brex *et al.* 2000]. In this study we report global (brain parenchymal fraction (BPF)) and regional (grey matter (GM) fraction (GMF), white matter (WM) fraction (WMF)) measures of brain volume changes, as well as a measures of ventricular volume (VV) change in 58 subjects with CIS, scanned within three months of the onset of the CIS and again three years later, when 31 (53%) of patients had developed MS as defined by the McDonald criteria.[McDonald *et al.* 2001] The clinical and MRI *lesion* findings of most subjects in the present cohort have been previously reported [Dalton *et al.* 2002b][Dalton *et al.* 2003a]. The purposes of the present study were to determine: (i) the location of early brain atrophy; (ii) the relationship of early brain atrophy - both global and tissue specific - with the development of MS, and (iii) the relationship of early brain atrophy with focal lesion load measures.[Dalton *et al.* 2004]

5.2.3. Methods

Patients

Subjects aged between 17 and 50 years presenting at Moorfields Eye Hospital or the National Hospital for Neurology and Neurosurgery with CIS suggestive of MS were invited to participate in this study. Fifty-eight patients were recruited from 1995 to 1999, and followed up for approximately 3 years. A CIS was defined as the first acute neurological event suggestive of CNS demyelination, e.g. unilateral optic neuritis, brainstem and partial spinal cord syndromes, where no alternative diagnosis was identified upon appropriate investigation. Overall, 40 patients presented with optic neuritis, 13 a brainstem syndrome, four a spinal cord syndrome (spinal MRI excluded

an alternative structural cause in these cases) and one patient had a hemianopia attributable to an optic tract lesion. Patients were clinically assessed using the Kurtzke expanded disability status scale (EDSS) [Kurtzke 1983] and MRI scans obtained upon entry to the study (within 3 months of symptom onset) and approximately three months, one and three years later. A diagnosis of MS was determined according to the McDonald criteria and included both MRI and clinical evidence for Dissemination in Space (DIS) and Time (DIT).[McDonald *et al.* 2001] The study was undertaken with approval by the local ethics committee and written informed consent was obtained from all the study subjects.

Image acquisition

Brain MRI was performed using a 1.5 Tesla GE scanner (General Electric Medical Systems, Milwaukee, WI, USA). At baseline, three months, one and three years, a proton density/T2 weighted fast spin-echo (FSE) sequence (repetition time [TR] 3200 msec, effective echo time [TE] 15/95 msec) and a T1 weighted spin echo sequence (TR 600 ms, TE 14 ms) were acquired in each patient after IV administration of 0.1 mmol/kg gadolinium (Gd) DTPA 5 to 7 minutes prior to the start of image acquisition. Patients were repositioned using a reproducible technique for serial imaging studies.[Gallagher *et al.* 1997] Forty-six, 3mm contiguous axial slices were obtained with an in-plane resolution of 0.9 by 0.9 mm.

Lesion identification and measurements

The MRI scans were reviewed by an experienced neuroradiologist (KAM), who was blinded to the patients' clinical status at follow-up. T2, Gd-enhancing and T1 hypointense lesions were identified at baseline, three months, one and three years of

follow-up. Using a semi-automatic local thresholding technique, lesions were segmented on the PD-weighted FSE images and T1-weighted post gadolinium enhanced images, which provided T2 hyper-intense and T1 hypo-intense lesion volumes respectively.[Plummer 1992]

Brain tissue segmentation

The baseline and 3-year follow-up T2-weighted images were segmented using a fully automated technique into images representing the probability of any given voxel containing GM, WM, CSF and other tissues using SPM99.[Chard, *et al.* 2002c] Lesions were identified and contoured using the same semi-automated lesion segmentation technique as noted above and used to allow for SPM99 misclassification of WM lesions as GM, CSF or other tissues. The lesion mask over-rode all SPM99 tissue classifications, otherwise a voxel was classified as GM, WM, CSF or other tissue, dependent on which mask had the greatest probability at that location. Results were assessed as fractions of total intracranial (TI) volume, determined by adding GM, WM, lesion and CSF volumes. BPF was calculated as GM, WM plus lesion volume divided by TI volume. WMF was calculated as WM plus lesion volumes divided by TI volume. GMF was calculated as the GM volume divided by the TI volume.

The segmented brains were then visually checked for quality assurance to ensure GM, WM and CSF had been correctly segmented. In 13 scans, two measurements of the segmented were made at least one week apart in order to assess reproducibility of the method. The mean coefficients of variation for GM, WM and CSF were respectively 0.0002%, 0.0003%, 0.0006%.

To investigate whether the higher lesion loads in the MS group when compared with the CIS group (see Table 4) might have influenced the segmented atrophy measures, simulated lesions were added by Gerard Davies to the three year scans of the CIS group such that the group had a median and range of lesion load increase similar to the MS group. The size and signal intensity of the simulated lesions were characteristic of MS lesions seen on T2-weighted scans. The signal intensity of lesions was set at a level half way between GM and CSF intensity. The lesions were placed at multiple locations in the cerebral white matter of the T2-weighted scans used for subsequent atrophy analysis. Measurement of the GMF and WMF of the CIS group with simulated lesions were obtained and compared with the measures made without such lesions.

Ventricle volume measurement

The ventricles were measured on baseline and 3-year follow-up T1-weighted scans using the MIDAS interactive image analysis package by a single observer blinded to patient details in a previously described method [Freeborough *et al.* 1997][Fox *et al.* 2000][Dalton *et al.* 2002a]. VV was estimated for the lateral ventricles and temporal horns but excluding the third and fourth ventricles; this approach has been previously applied in multiple sclerosis and demonstrated to have a good reproducibility and sensitivity to change [Fox *et al.* 2000].

Statistical Methods

Patients were divided into one of two principal groups according to their 3-year follow-up status:

- i. those with a diagnosis of MS by the McDonald criteria using both clinical and MRI evidence for DIS and DIT (31 subjects)
- ii. those who still had a diagnosis of a CIS (27 subjects); for some analyses this group was further divided in to those who had MR visible brain lesions (13 subjects) and those without MR visible brain lesions (14 subjects) at any time during follow-up.

Mean fractional volume changes over time within groups were estimated by linear regression adjusting for baseline value. Comparisons of differences in mean changes in fractional volumes over time between groups were assessed by adding a group indicator to the regression with baseline covariate. Baseline adjustment did not alter the point estimates, but improved precision. Where there was evidence of non-Normality the validity of inference was checked using a non-parametric bias-corrected bootstrap with 1000 replicates,[Carpenter and Bithell 2000] and where the bootstrap estimates differed these are reported. Non-parametric tests of change within group over time used the Wilcoxon signed rank test, and of differences in lesion volumes and changes between groups used the Mann-Whitney U test. Between group comparison in lesion numbers used negative binomial regression. Correlations between lesion load and atrophy measures were assessed using Spearman rank correlation.

5.2.4. Results

Baseline clinical and MRI findings

The median time delay between the onset of symptoms and the baseline scan was five weeks, (range of 1 to 12 weeks). Thirty-nine patients (67%) had an abnormal baseline T2-weighted MRI scan with one or more focal high signal lesions. Of those, 16 had Gd-enhancing and 23 had T1 hypo-intense lesions. The median EDSS at baseline in the group of patients with an abnormal baseline T2-weighted MRI scan was 2 (0 – 8) and 1 (0 – 3) in those with a normal scan.

Diagnosis at three years

The median time between the baseline and the three-year follow-up scan was 37 months (range of 31 to 72 months). At three years 31/58 (53%) had developed MS (13 males and 18 females), and 27 had not. Of the latter group, 13 had a diagnosis of CIS with brain lesions (six males and seven females) and 14 had a diagnosis of CIS without brain lesions (eight males and six females). The median EDSS at 3 years was 1 (0 – 8) in the MS group, 0 (0 – 1) in the CIS group with brain lesions and 0 (0 – 2) in the CIS group without brain lesions. The duration of follow up was not significantly different between those developing MS and those with a remaining CIS (median 1125 vs 1111 days, $p = 0.47$).

The relationship between atrophy and clinical outcome (Tables 5.6-5.8)

Table 5.6 shows global and regional brain and VV measures at baseline and 3 years for the MS and CIS subgroups. Table 5.7 shows absolute volume changes – over 3 years - in volume measures and Table 5.8 shows median and mean percentage volume changes for the MS and CIS subgroups. No statistically significant differences were

found in mean 3yr – baseline changes in BPF, GMF, WMF and VV between the CIS subgroups with and without lesions, and these two groups were combined for statistical comparisons.

A significant decrease in BPF over 3 years was seen in both the MS and combined CIS groups but the decrease was significantly larger in the MS group (Tables 5.7 & 5.8). There was a significant increase in VV between baseline and year 3 in those who developed MS but not in the combined CIS group; the difference between the groups was significant. There was a significant decrease in GMF between baseline and year 3 in both the MS and combined CIS groups but the decrease was significantly larger in the MS group (Tables 5.7 & 5.8). There was a weakly significant increase in mean WMF at 3 years compared to baseline in the patients who developed MS (Table 5.7; $p=0.023$); the median percentage change in these patients was +1.2% (Table 5.8; $p = 0.046$). Though there was no significant change in WMF in the combined CIS group.

Seven patients were treated with short courses of steroids (three prior to the baseline scan; four between the baseline and year 3 scan); two of these were also treated with IFNbeta between baseline and year 3, and one patient received IFNbeta but not steroids. Five of these eight were in the group who developed MS, three were in the CIS non-lesion group. Exclusion of these eight patients did not affect MS versus CIS comparisons. Nor did exclusion of the patient with an EDSS of 8 affect these comparisons.

Lesion measures in MS group versus CIS group with abnormal MRI (Table 5.9)

The MS group had higher T2 and T1 hypointense lesion volumes and higher numbers of Gd enhancing lesions at baseline and year 3. The MS group also displayed a greater increase in T2 volume between baseline and year 3 but no difference was observed between the two groups in change in T1 hypointense volume. Excluding patients treated with steroids and IFNbeta did not alter comparisons except for numbers of Gd enhancing lesions, which failed to reach significance.

Effect of simulated lesions on the segmented atrophy measures in the CIS group

When the three year CIS group segmented atrophy measures, with and without simulated lesions, were compared, the measures with simulated lesions displayed slightly higher GMFs and lower WMFs, but the differences were not significant (mean increase in GMF = +0.0032, 95% confidence intervals -0.00037, +0.0067, $p=0.077$, 2-tailed paired t-test; mean decrease in WMF = -0.00096, 95% confidence intervals -0.0022, +0.0003, $p = 0.135$, 2-tailed paired t-test).

Associations between lesion and atrophy measures (Table 5.10)

Change in BPF (3 year – baseline) correlated negatively with the baseline number of Gd-enhancing lesions, and changes (3 year – baseline) in T1 hypo-intense and T2 lesion volume. Change in VV (3 year – baseline) correlated positively with baseline T1 hypo-intense and T2 lesion volumes and Gd-enhancing lesion number, and with change (3 year – baseline) in T1-hypointense and T2 lesion volumes. Change in GMF (3 year – baseline) was modestly correlated negatively with changes (3 year – baseline) in T1 hypo-intense and T2 volume. There were no significant correlations between lesion volumes and WMF changes.

Associations between VV change and other tissue volume measurements

Three year – baseline changes in VV correlated with changes in GMF ($r_s = -0.393$, $p = 0.002$) and BPF ($r_s = -0.453$, $p < 0.001$), but not with changes in WMF ($r_s = +0.027$, $p = 0.839$).

5.2.5. Discussion

Previous cross sectional studies have reported both total and neocortical GM atrophy – in excess of that seen in healthy controls – in early relapsing-remitting MS with disease durations of less than 3,[Chard *et al.* 2002b] 5,[De Stefano *et al.* 2003] and later RR and SPMS of disease duration 6.1 [Sailer *et al.* 2003] years respectively. The present study extends these observations in demonstrating that there is increasing GM atrophy in patients developing MS over 3 years following presentation with a CIS. The MS cohort also exhibited a significant increase in VV during the follow-up period. Although decreasing GMF was also seen in the 27 patients in the remaining CIS group, the decrease was significantly less than that observed in the MS group.

While it is likely that some individuals in the CIS group will develop MS with longer follow-up, the present study shows that progressive GM atrophy is more prominent in those CIS patients who develop MS within 3 years using the McDonald criteria. Although the small sample size limits the sensitivity of the analysis, no significant atrophy was detected in the 14 CIS patients with normal imaging, this being a group that has a relatively low risk for developing MS [Brex *et al.* 2002]. There was significantly greater VE seen in the MS versus the remaining CIS group, and the amount of increase in the MS group is also more than that reported from healthy young adult populations in the literature.[Fox *et al.* 2000].

This study investigates tissue volume changes in MS at an early stage. This has been enabled by the recently developed McDonald criteria which allow a diagnosis based on MRI evidence for DIS and DIT in patients with single clinical episodes [McDonald *et al.* 2001]. Studying early MS has potential to identify markers which predict the future clinical course, and to find useful techniques for treatment trial monitoring that can be applied prior to the emergence of persistent neurological deficits.

In contrast to the development of GM atrophy, there was no decrease in WM volume in the MS group over the 3 years. Indeed there was a suggestion of an increase in the amount of WM. The MS group had a large increase in WM lesion load during the study. Lesions in early MS often display inflammation, and increased glial cellularity is also reported in MS normal-appearing WM (NAWM).[Allen *et al.* 2001] Such processes might contribute to an increase in tissue volume. Alternatively, loss of axons and myelin within inflammatory lesions,[Trapp *et al.* 1998] and axonal loss in NAWM would be expected to cause tissue loss. The observed result would be compatible with WM volume gain from inflammation compensating for loss due to axonal degeneration. Two previous MR spectroscopy studies of NAWM in CIS have shown no significant reduction of N-acetyl-aspartate, an axonal marker, which suggests that substantial WM axonal loss is not prominent this early stage.[Brex *et al.* 1999a][Tourbah *et al.* 1999] Other techniques that assess intrinsic tissue characteristics may be of greater use than atrophy measures when evaluating longitudinal WM processes in early MS.

Because in the present study no healthy control group was available for comparison using an identical image acquisition sequence, we were unable to directly investigate

whether the CIS cohort already had GM or WM atrophy at presentation. However, in a previous study of 40 CIS patients that used a different (5 mm thick) acquisition sequence for tissue volume analysis, we observed a significant decrease in WMF but not GMF compared with healthy controls, within 6 months of onset of the CIS.[Traboulsee *et al.* 2002] Taking that observation together with the present study raises the possibility that there are differences in the temporal evolution of GM and WM atrophy in CIS and early relapse onset MS, with GM atrophy emerging later and then evolving more rapidly. Further studies of other patient and control cohorts are warranted to define the temporal evolution of GM and WM atrophy in early MS.

GM atrophy might reflect neuronal degeneration secondary to anterograde or retrograde degeneration from WM lesions in which there has been axonal transection. Alternatively it could reflect the development of focal lesions within GM in which there is axonal loss. Current MRI methods are largely unable to detect focal GM lesions, although they are well recognised pathologically.[Kidd *et al.* 1999][Peterson *et al.* 2001] GM lesions exhibit less inflammation than those seen in WM.[Kidd *et al.* 1999] Volume changes in GM may thus provide a more direct measure of the neurodegenerative component of MS pathology, being relatively unaffected by fluctuations due to inflammation.

Perhaps surprisingly, the increase in VV was not accompanied by a decrease in WM volume. Possibly ventricular enlargement is more related to the focal effects of tissue loss in periventricular lesions rather than to global WM volumes.[Kalkers *et al.* 2002] The robust correlation of VE with lesion volume measures indicates that there is a notable relationship between focal lesions and this particular measure of atrophy. VE

was also correlated with GMF changes, and a generalised remodeling of the brain secondary to GM atrophy may contribute to VE. In addition, the GMF includes deep periventricular structures such as thalamus and caudate nucleus, and atrophy in these regions may have contributed more directly to the VE.

VV, GMF and BPF are potentially useful outcome measures for monitoring disease modifying therapies in early MS, as in this study they were sensitive to progressive tissue loss. The GMF change correlated only modestly with lesion volume changes (Strongest correlation with T2 volume change: $r=0.428$; Table 5) suggesting that it provides additional information in understanding disease evolution and monitoring treatment effects. The Limited correlation between GMF and lesion load, accounting for <20% of variability in either measure ($r^2=0.17$), also suggested segmentation bias associated with focal signal abnormalities does not alone account for the observed tissue specific volume changes.[Chard *et al.* 2002b][Quarantelli *et al.* 2003] Furthermore, we found only minimal changes to the 3 year GMF and WMF measures in the CIS group when simulated lesion loads approximating the increases seen in the MS cohort were introduced. The small changes observed were to slightly increase GMF and decrease WMF thus *increasing* the differences between the MS and CIS groups.

Further studies are warranted to confirm the present findings using different scan acquisitions and segmentation methodologies. Future studies should investigate the potential for early VV and GM volume measures to predict disability or cognitive impairment, and thereby help to identify patients in whom early disease modifying treatments are most needed. It will also be relevant to investigate the effect of current or future disease modifying treatments on these atrophy measures.

Tables 5.6 Brain and VV measures at baseline and 3 years. Mean, median (range).

	Total (n=58)	cohort McDonald (n=31)	MS CIS With lesions (n=13)	Without lesions (n=14)	CIS Combined (n=27)
BPF Year 0	0.864, 0.860 (0.80, 0.91)	0.863, 0.856 (0.80, 0.91)	0.868, 0.860 (0.85, 0.90)	0.862, 0.861 (0.83, 0.91)	0.865, 0.860 (0.83, 0.91)
BPF Year 3	0.855, 0.853 (0.80 – 0.91)	0.851, 0.848 (0.80 – 0.91)	0.862, 0.856 (0.85 - 0.89)	0.858, 0.855 (0.83 - .91)	0.860, 0.855 (0.83, 0.91)
GMF Year 0	0.494, 0.490 (0.440 – 0.570)	0.490, 0.489 (0.44 – 0.55)	0.504, 0.500 (0.47 – 0.54)	0.494, 0.486 (0.47 - 0.57)	0.499, 0.491 (0.47, 0.57)
GMF Year 3	0.483, 0.479 (0.430 – 0.550)	0.473, 0.473 (0.43 – 0.54)	0.497, 0.497 (0.46 – 0.55)	0.490, 0.482 (0.46 – 0.55)	0.493, 0.493 (0.46, 0.55)
WMF Year 0	0.370, 0.369 (0.33 – 0.41)	0.373, 0.369 (0.35 – 0.41)	0.363, 0.363 (0.33 – 0.40)	0.368, 0.372 (0.33 – 0.40)	0.366, 0.365 (0.33, 0.40)
WMF Year 3	0.372, 0.371 (0.33 – 0.40)	0.377, 0.373 (0.33–0.40)	0.365, 0.363 (0.33 – 0.40)	0.368, 0.368 (0.34 – 0.39)	0.367, 0.365 (0.33, 0.40)
VV (ml) Year 0	8.605, 6.286 (0.610 – 26.86)	9.718, 8.569 (1.22 - 26.86)	6.844, 4.572 (1.68 – 19.88)	7.776, 6.563 (0.61 – 23.12)	7.327, 5.848 (0.61, 23.12)
VV (ml) Year 3	9.972, 7.499 (0.740 - 28.91)	12.128, 11.198 (1.42-28.91)	7.335, 5.247 (1.79 – 22.31)	7.647, 6.307 (0.74 – 23.49)	7.497, 5.701 (0.74, 23.49)

Table 5.7: Year three minus baseline changes in BPF, GMF, WMF and VV in the MS and CIS Groups

Atrophy	MS	CIS (combined)	Baseline adjusted between
	Mean change (CI) and p value	Mean change (CI) and p value	group difference, Mean MS – CIS difference (CI) and p value
BPF	-0.012 (-0.016, -0.008) p = 0.001	-0.005 (-0.010, -0.0003) p = 0.038	-0.008 (-0.014, -0.001) p = 0.022
GMF	-0.017 (-0.022, -0.011) p = 0.001	-0.005 (-0.011, 0.004) p = 0.03*	-0.014 (-0.021, -0.006) p = 0.001
WMF	0.005 (0.0007, 0.009) p = 0.023	0.0005 (-0.004, 0.005) p = 0.820	0.006 (0.0003, 0.0123) p = 0.040
VV (mls)	2.410 (1.301, 3.518) p = 0.001	0.170 (-1.018, 1.358) p = 0.775	2.305 (0.651, 3.958) p = 0.007

No statistical differences were noted in mean differences in BPF, GMF, WMF and VV changes from baseline to year 3 between the CIS groups with and without lesions so these groups were combined for statistical purposes.* Bootstrap derived p value, CI = 95% Confidence Interval

Table 5.8: Median and mean % change in 3-year BPF, GMF, WMF and VV (relative to baseline values)

MRI parameter	MS (n=31)	CIS		
	Median, Mean	With MRI lesions (n=13) Median, Mean	Without MRI lesions (n=14) Median, Mean	CIS Combined (n=27) Median, Mean
BPF	-0.8% -1.4%	-0.4 % -0.7%	-0.6 % -0.5%	-0.5 % -0.6%
GMF	-2.1% -3.3%	-1.4 % -1.4%	-1.2 % -0.7%	-1.4 % -1.1%
WMF	+1.2% +1.3%	+1.1 % +0.4%	+0.2 % +0.1%	+0.8 % +0.2%

Table 5.9 Comparison of lesion load measures and changes in lesion load measures (median plus ranges) in MS subjects versus CIS group with MRI lesions.

MRI parameter	MS (n=31)	CIS with MRI lesions (n=13)	p-value (Mann-Whitney U)
Baseline T2 lesion volume (ml)	1.1 (0.0 to 13.9)	0.2 (0.0 to 0.5)	<0.001
3 year T2 lesion volume (ml)	2.5 (0.3 to 58.5)	0.2 (0.03 to 1.4)	<0.001
T2 lesion volume change 0-3 (ml)	1.2 (-1.5 to 44.6)	0.1 (-0.3 to 1.0)	<0.001
Baseline T1 lesion volume (ml)	0.1 (0.0 to 4.5)	0.0 (0.0 to 0.3)	0.008
3 year T1 volume (ml)	0.1 (0.0 to 18.3)	0.0 (0.0 to 0.4)	0.006
T1 lesion volume change 0-3 (ml)	0.1 (-2.0 to 13.8)	0.0 (-0.3 to 0.2)	0.196
Baseline Gd lesion number	0 (0 to 21)	0 (0 to 3)	0.013 ^a
3 Year Gd lesion number	1 (0 to 6)	0 (0 to 1)	0.016 ^a

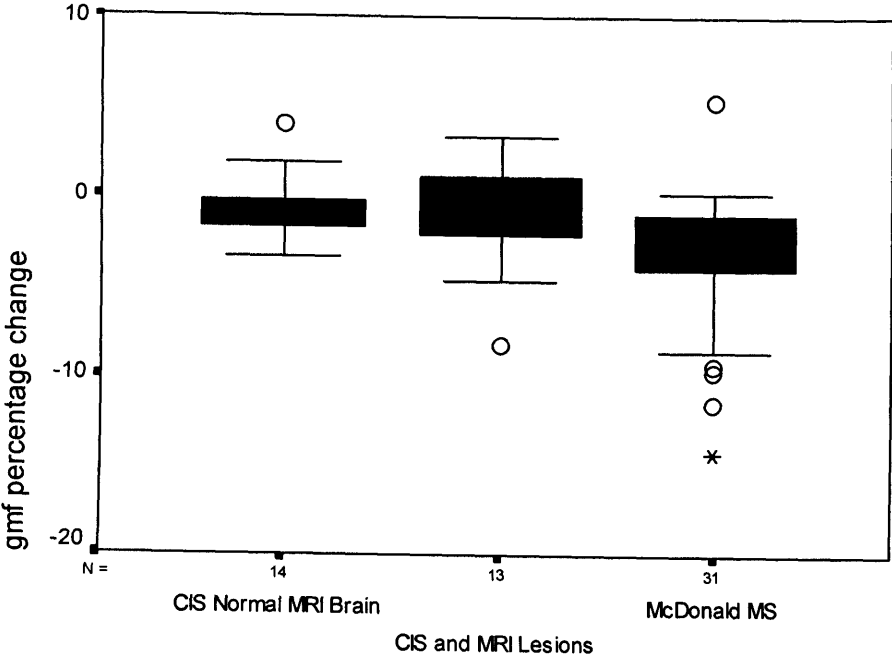
^aNegative binomial regression

Table 5.10: Spearman correlations between lesion load and atrophy measures.

Atrophy measures	Lesion load measures	r _s	P
VV change year 3 – 0	Volume of T2 lesions at baseline	0.528	0.0002
	Number of Gd-enhancing lesions at baseline	0.514	0.0004
	Volume of T1 hypo-intense lesions at baseline	0.529	0.0002
	T2 lesion volume change year 3-0	0.440	0.0028
	T1 lesion volume change year 3-0	0.369	0.0137
BPF change year 3 – 0	Volume of T2 lesions at baseline	-0.258	0.091
	Number of Gd-enhancing lesions at baseline	-0.440	0.0028
	Volume of T1 hypo-intense lesions at baseline	-0.167	0.2791
	T2 lesion volume change year 3-0	-0.410	0.0057
	T1 lesion volume change year 3-0	-0.306	0.0432
GMF change year 3 – 0	Volume of T2 lesions at baseline	-0.0003	0.9986
	Number of Gd-enhancing lesions at baseline	-0.1193	0.4405
	Volume of T1 hypo-intense lesions at baseline	-0.0049	0.9751
	T2 lesion volume change year 3-0	-0.4280	0.0037
	T1 lesion volume change year 3-0	-0.3071	0.0426

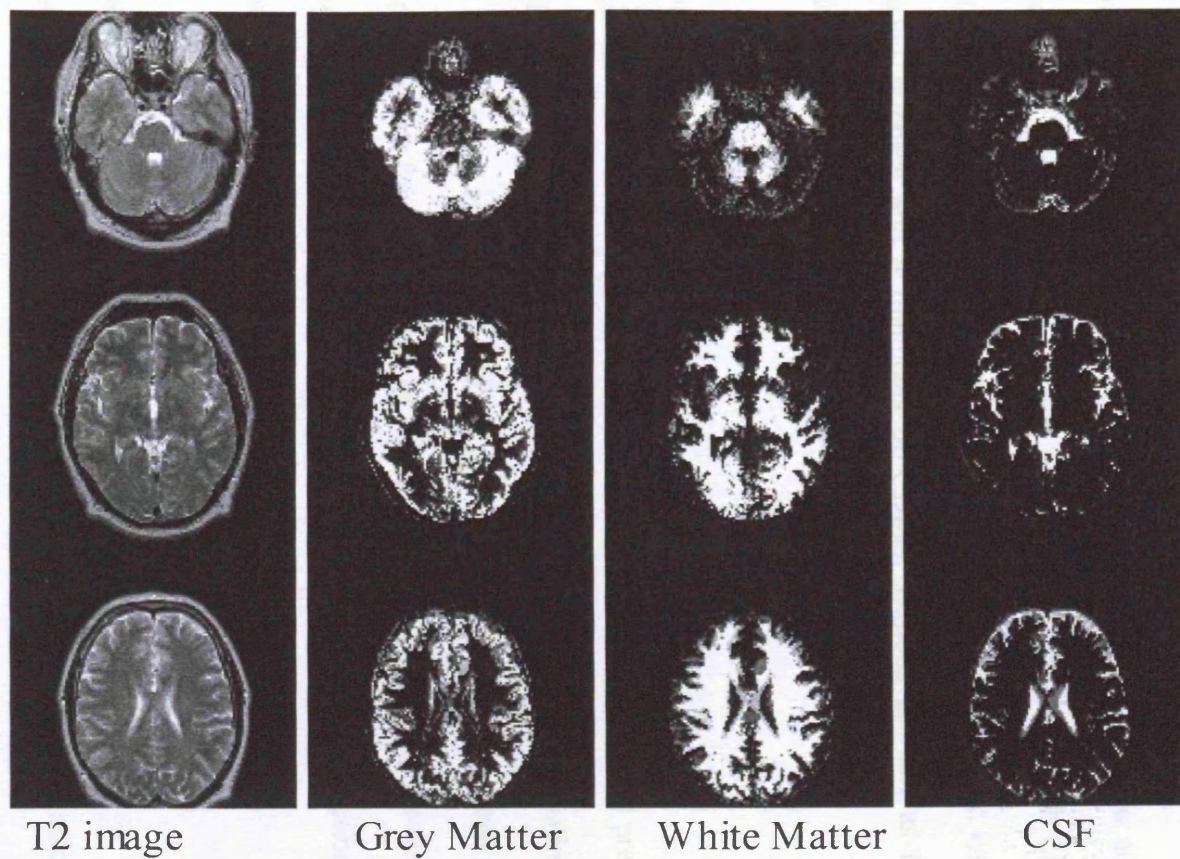
(No significant correlations between the change in WMF and lesion load changes)

Figure 5.5: Box plot showing the medians, interquartile ranges (box), highest and lowest values, excluding outliers (whiskers), outlier and extreme values (circles) for GMF percentage change in CIS patients with and without MRI lesions, and MS patients.



Diagnosis at three years

Figure 5.6 SPM99 T2 image segmentation: Grey Matter, White Matter and CSF



Chapter 6

6.1 Discussion and conclusions

6.1.1 Background to MRI and the diagnosis of MS

This thesis has focused on MRI in the earliest phase of MS. Although MRI abnormalities were present in over 62% of patients with CIS followed for 3 years, the specificity of an abnormal MRI for a diagnosis of CDMS at 3 years is low at 56%. The McDonald MRI criteria enable an earlier MS diagnosis in patients with CIS and an abnormal MRI scan. Conventional MRI techniques using lesion number, type and location predict relapses and a diagnosis of CDMS. Lesions are not as helpful predicting disability where atrophy may be a more useful surrogate marker.

6.1.2 Aims of this thesis

The Aims of this thesis were two fold. Firstly, we wanted to evaluate MRI predictors for the diagnosis of CDMS at 3 years. To this end, we evaluated baseline MRI imaging of the brain and spinal cord and the combination with imaging three months later as predictive tests for the diagnosis of CDMS. Secondly, we wanted to determine whether atrophy occurred from onset of CIS and how atrophy related to lesions. This was done using two techniques: MIDAS and SPM99.

6.1.3 Baseline MRI in patients with CIS

Conventional T2 lesions at baseline MRI scan remain the most sensitive indicator for CDMS at 3 years (95%). High specificity using a baseline MRI brain scan could only be achieved by the requirement of 9 or more Gadolinium enhancing lesions (100%). This is rarely present in patients presenting with CIS, hence the poor sensitivity (14%).

6.1.4 Spinal cord MRI in patients with clinically isolated optic neuritis

This thesis has clarified the limited usefulness of spinal cord imaging in patients with Clinically Isolated Optic Neuritis (although imaging of the Spinal Cord is essential in Spinal Cord Syndromes). Using the existing McDonald criteria, which allow one cord lesion to substitute for one brain lesion, cord lesions are of limited value only in patients with between one and eight brain lesions. Any expansion in the use of spinal cord lesions in updated MS diagnostic criteria should first be validated to ensure accuracy for predicting a second relapse.

6.1.5 Accurate criteria for MS diagnosis in patients with CIS

We have clarified the accuracy of the new McDonald diagnostic criteria by applying them in a prospective study of 119 patients with CIS suggestive of MS, 50 of whom were followed for 3 years. The specificity of the criteria at 3 months of follow-up was high (93%), sensitivity was lower (65%). Specificity is more important than sensitivity in diagnosing a lifelong condition such as MS. Low sensitivity means patients may be given a falsely negative diagnosis, or that the diagnosis is delayed.

New T2 lesions are detected more often than new Gadolinium enhancing lesions on the three month follow up scan. Using the existing criteria for DIS, a new T2 lesion applied for DIT was more sensitive (74%) without loss of specificity (92%) for the diagnosis of CDMS at 3 years.

A new T2 lesion per say at three months was both sensitive (84%) and specific (89%) for predicting CDMS at three years. A number of caveats must be reiterated, before

considering modifications of the existing criteria most importantly that the MRI is performed in an experienced centre and read by a clinician or neuroradiologist with extensive experience with MRI and MS as well as other white matter disorders to ensure all lesions are unequivocal.

6.1.6 Atrophy in CIS patients associated with MS diagnosis

This thesis has confirmed VE in patients with CIS is associated with the development of CDMS as early as one year from the onset of initial symptoms. The correlations between VE and lesion load parameters were modest (Baseline T1 Hypointense Lesion Volume $r = 0.495$ and $p < 0.001$), suggesting that additional factors may contribute to atrophy. Further investigations using quantitative MT and NMR spectroscopy are needed in large CIS cohorts to evaluate the causal relationship between factors in the NAWM and the evolution of atrophy.

In a cohort of 58 CIS patients followed for 3 years, this thesis has confirmed the early development of MS is associated with progressive GM but not WM atrophy. Again the correlations between lesion load measures and atrophy were only modest (Baseline T1 Hypointense lesion volume $r = 0.5$ and $p < 0.001$).

6.1.7 Concluding remarks

Baseline MRI brain and cord lesions are highly sensitive for the development of CDMS, but lack specificity. Early follow up MRI brain was used to evaluate MRI evidence of DIT and DIS using the McDonald MRI criteria and provides specificity for CDMS at three years. Application of the criteria in their present form has revealed they are specific but less sensitive for CDMS at three years. Longer follow up is required to

evaluate disability. Non conventional MRI techniques including atrophy may be more helpful predicting longer term disability.

6.1.8 Future Work

Longer term follow up is required to evaluate disability, in the first instance at 5 years. MRI markers for disability may be used to evaluate therapeutic and rehabilitation strategies for treatment of MS. Finally we are auditing patients' perceptions of research. The future aim is the dissemination of the information which patients would like to receive as part of this research project.

Appendix 1: Optic Neuritis Patient Information Sheet

We think the symptoms you are having are due to optic neuritis. This is the medical term used for inflammation of your optic nerve. The optic nerve is the nerve of vision and carries images of what we see coded as electrical impulses from the eye to the brain. If the nerve fibers do not do their job, vision becomes blurred.

Most patients with optic neuritis complain of painful subacute visual loss aggravated by eye movements, which progresses over a period of a few days to two weeks. The pain is variable in severity but usually does not interfere with sleep. The maximal visual loss varies from blurred to complete loss of vision in the affected eye, but recovery always occurs. 80% of patients show signs of improvement by three weeks and 90% by five weeks from the onset of symptoms. In 95% the recovery is to near normal vision and only 5% have significant loss of vision. Slower visual recovery can occur for up to 2 or 3 years. Some patients develop symptoms such as deterioration of vision with exercise or in bright light or difficulty judging direction of moving objects. Routine blood tests will be performed in clinic in order to exclude Optic Neuritis associated with infections or inflammatory conditions. Occasionally intravenous methylprednisolone (steroid) is used for optic neuritis; however steroids can cause side effects. It has been shown that steroids speed up the recovery but do not improve the eventual outcome. In some patients with optic neuritis treatment with steroids is essential.

There is an association between Optic Neuritis and Multiple Sclerosis (MS). MS can be diagnosed clinically after the occurrence of a second episode of inflammation at a

different site in the central nervous system, for example numbness in both legs lasting three weeks. More recently MRI scans of the brain are being used to make an earlier diagnosis of MS. About 50-70% of patients have areas of inflammation on their MRI brain, when they present with optic neuritis. In long term studies of patients with Clinically Isolated Syndromes including optic neuritis, over 80% of patients with an abnormal scan developed MS and less than 20% of patients with a normal scan developed MS. MS may not become clinically apparent for many years and does not inevitably lead to disability. Earlier diagnosis in the future may lead to earlier treatment.

In clinical practice, if there is any doubt about the diagnosis a scan may be carried out to look for another cause of visual loss. Otherwise the only reason for carrying out a scan is to more accurately predict the likelihood of the development of MS and this issue will be discussed with you.

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Appendix 2: Addendum

The publishing of the McDonald criteria of the International Panel almost four years ago has been a starting point for efforts to evaluate their robustness and reliability. There are a number of issues raised in this thesis, which need to be addressed in a final discussion.

1. The studies in this thesis focus on application of the McDonald criteria in with monosymptomatic CIS (presenting in the majority with optic neuritis). Reassuringly the results subsequently from another cohort of CIS patients - in whom optic neuritis, brainstem and spinal cord syndromes were equally represented - are very similar, suggesting the robustness of the McDonald criteria for DIS and DIT in patients with CIS.[Tintore *et al.* 2003] Future MRI studies should concentrate on larger cohorts of patients with polysymptomatic CIS with clinical DIS, which may be indicative of a higher risk of MS. They might also concentrate on modifications that improve sensitivity in making an early diagnosis, this still being a limitation of the McDonald criteria.
2. EDSS is the only measure of clinical disability used in this thesis. Evaluation of parameters used to measure clinical severity of optic neuritis, including visual acuity, visual fields, colour vision and fundoscopic findings, were not evaluated in this thesis as tests predicting clinical outcome at three years.
3. The presence of CSF oligoclonal bands can significantly change the requirements for the diagnosis of MS. The MRI components are less strict, with only two or more lesions consistent with MS rather than the full imaging DIS criteria being required. The majority of patients in the studies in this thesis did not have lumbar punctures performed. There are a number of

patients who may only fulfill the new McDonald criteria by the inclusion of the oligoclonal bands. This addition could improve sensitivity to the values given in this thesis. However, in another cohort, CSF findings within the DIS criteria were evaluated and were found to lower specificity and accuracy of the full imaging DIS criteria, suggesting limited usefulness when considered in isolation. [Tintore *et al.* 2003]

4. Few patients in the cohort reported in this thesis received steroids or disease modifying agents. In the Optic Neuritis Treatment Trial, high-dose intravenous steroids were shown to have a short-term beneficial effect, delaying the time to the development of a second demyelinating event. However, there was no proven long-term benefit from a single course of steroid treatment. [Optic Neuritis Study Group 1997] There is no large trial of yearly or recurrent pulses of steroids in patients with CIS given with the aim of preventing a second relapse. There is evidence that IFN β -1a delays the development of MS in patients with CIS. [Jacobs *et al.* 2000, Comi *et al.* 2001] IFN β -1a in RRMS reduces relapses by approximately 30% [Jacobs *et al.* 1996, PRISMS study 1998] It is not surprising therefore that IFN β -1a delays a second relapse in those with a CIS. The effect of steroids and IFN β -1a were not evaluated in this thesis.
5. Data used to create the McDonald criteria for dissemination in time may have been partly based on an earlier short term follow up study of patients from the cohort reported in this thesis [Brex *et al.* 2001] which was published around the time that the Panel convened, although it is not clear that this is actually the case from either reading the International Panel's paper or from discussion with members of the Panel. The DIS criteria were certainly not derived from

our cohort and were indeed based on earlier reports of other cohorts studied by Barkhof et al (1997) and Tintore et al (2000). [Barkhof *et al.* 1997, Tintore *et al.* 2000] I have now followed the majority of these patients for three years and report the findings in this thesis. Validation studies imply use of a different population. Chapters 4.1 and 4.2 are studies on the application of the new criteria, rather than validation studies, *per se*. Similar results achieved in a patient group from Spain with a different genetic background are, however, very reassuring. [Tintore *et al.* 2003]

Proposed Revisions of the McDonald Criteria

While the McDonald criteria have by and large been seen to be a useful improvement in clinical practice, they have a number of limitations that have thoroughly reviewed recently by Polman et al (2005), and in the near future an International Panel will be reconvening to consider whether they should be revised. [Polman *et al.* 2005] This thesis presents data arguing for expansion of the existing criteria to include a new T2 lesion 3 months after a CIS as evidence of DIT. However, unlike the more unequivocal nature of the gadolinium enhancing lesion, the detection of a new T2 lesion is sometimes harder to be sure about. In this respect, I must reiterate the advice given in Chapter 4.2, namely that MRI evaluation should be performed by a neuroradiologist or a clinician with experience of MS. Also, T2 lesions should be unequivocal, at least 3mm in diameter, and observed on two different types of high quality T2 sequence. Furthermore, it is recommended that the application of MRI criteria is restricted to those aged between 16 and 50 years in order to maintain a high diagnostic accuracy. Finally, spinal cord MRI should also be performed when the CIS

involves the cord, to exclude other disorders and to identify demyelinating cord lesions.[Miller *et al.* 1987]

Proposed evaluation of grey matter atrophy in patients with CIS

The significant decrease in GMF between baseline and year 3 in MS contrasts with the weakly significant increase in mean WMF at 3 years compared to baseline. Addition of simulated WM lesions to healthy control images resulted in slightly higher GMFs and lower WMFs, suggesting that lesions *per se* are not causing this GM and WM volume abnormalities seen in early MS. It is possible that there are a number of distinct pathological processes at work, including an increased glial cellularity in the NAWM [Allen *et al.* 2001] due to inflammation causing volume gain, and also grey matter neuroaxonal loss causing volume loss. Also, demyelinating grey matter lesions, which are not visible on MRI, are less inflammatory and may be important in the development of grey matter tissue loss. A multifactorial regression approach may help to determine the importance of each of the new non-conventional MRI techniques as predictors of disability in future longer term follow up studies (few patients were disabled after 3 years). Information gained from measuring grey matter atrophy and inflammatory lesion load accumulation following CIS might – in future - be used to select groups of CIS patients with the greatest inflammation and atrophy who can be targeted for intervention in clinical trials of novel MS therapies that aim to arrest the MRI-detected pathological processes at an early stage, and thereby prevent disability.

Proposed Clinical Practice in the Management of Patients with Optic Neuritis

The MAGNIMS network evaluated studies on the role of MRI within diagnostic criteria. [Miller *et al.* 2004] The following is a summary of the main

recommendations of the MAGNIMS group, with additional comment included from a current UK perspective:

1. At the time of diagnosis of optic neuritis, or any other CIS, patients should be told of the risk of MS. Many patients attending clinics in the UK have read of the association between optic neuritis and MS on the Internet and will want to discuss the risk. In this respect, it is important to reiterate in clinic that the course of MS may be mild and non-disabling. In my experience, only a very small percentage of patients will not want further discussion.
2. An MRI of the brain should be offered, including T2 weighted and gadolinium-enhanced sequences. It should be explained that an abnormal MRI brain scan is associated with a 60-80% likelihood of developing MS. [Brex *et al.* 2002, Beck *et al.* 2003]. If the MRI brain imaging is normal, the patient can be reassured the likelihood for developing MS is approximately 20%. [Brex *et al.* 2002][Beck *et al.* 2003]
3. If the MRI brain scan is performed more than 3 months after the onset of the attack and the McDonald MRI criteria are fulfilled, then a diagnosis of MS can be made.
4. If the MRI brain scan performed less or more than 3 months after the attack is abnormal (but at this stage it is not possible to fulfill the DIT criteria for MS), then a repeat MRI scan can be performed 3-6 months after the original scan to see whether MS can be diagnosed. If the criteria are not fulfilled, further scanning is not recommended.
5. At present, two significant relapses in the previous two years are required in the UK before patients are commenced on disease-modifying drugs. Earlier diagnosis with MRI does not therefore influence the treatment decision.

However, if the UK guidelines (developed by the Association of British Neurologists) are revised in the future to include CIS patients who fulfill the McDonald criteria for MS, the use of MRI to investigate CIS patients would take on a more pressing role.

References to Appendix 2: Addendum

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