EARLY RELAPSING REMITTING MULTIPLE SCLEROSIS: A MAGNETIC RESONANCE IMAGING STUDY INVESTIGATING NORMAL APPEARING BRAIN TISSUE AND LESION CHANGES AND THEIR RELATIONSHIP TO CLINICAL DISABILITY.

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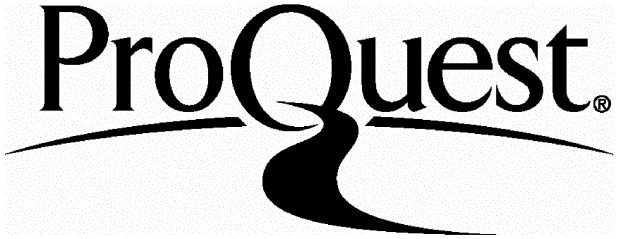

Abstract

The aim of this thesis is to study patients with early relapsing remitting multiple sclerosis (MS) using radiological and clinical methods. I have studied a cohort of patients with early disease (within three years of neurological symptom onset). I have used various MR imaging sequences in order to assess both lesions and normal appearing brain tissue (NABT). I have used quantitative techniques such as magnetisation transfer imaging (MTI) and T1 relaxation times that provide a sensitive measure of abnormalities in lesions and NABT. I have also used Gadolinium (Gd-DTPA) enhancement of T1 weighted images in order to assess lesion inflammation at this early stage. Diffusion tensor imaging (DTI) measurements also give an indication of changes in diffusivity and fibre tract integrity both within lesions and NABT. I have used these techniques both in isolation and in combination to assess early changes in this cohort of patients. In addition to using different MR sequences, I have compared region of interest (ROI) and histogram techniques in detecting change. I have assessed the relationship between MR parameters of disease activity and clinical scales (both well established and more novel scores). I have examined the relationship between MTR and T1 relaxation times in lesions and normal appearing white matter (NAWM) and found that this relationship differs between the different tissue types. I have detected NAWM and NAGM changes in this group of patients with early disease using DTI, MTR and T1 relaxation times. The most sensitive MR technique appears to be that of T1 relaxation times using a histogram approach and DTI using a ROI approach appears to be the least sensitive method for detecting changes in NABT in this cohort of patients.

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PUBLICATIONS AND PRESENTATIONS ARISING FROM THIS THESIS

CM Griffin, GJM Parker, AJ Thompson, DH Miller. MTR and T1 provide complementary information in MS NAWM, but not in lesions.

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Abbreviations

BBB	Blood brain barrier
CIS	Clinically Isolated Syndrome
CNS	Central Nervous System
CoV	Coefficient of Variation
CSF	Cerebrospinal fluid
CT	Computerised Tomography
Gd-DTPA	Gadolinium-Diethylene-triaminepenta-acetic acid
EDSS	Expanded Disability Status Scale
FOV	Field of view
FSE	Fast spin echo
FSPGR	Fast spoiled gradient echo
MRI	Magnetic Resonance Imaging
MS	Multiple Sclerosis
MTI/R	Magnetisation transfer imaging/ratio
NAA/ t NAA	N-acetyl aspartate / total N-acetyl derived groups
NAWM	Normal appearing white matter
NAGM	Normal appearing grey matter
NABT	Normal appearing brain tissue
PD	Proton density
r	Spearman Rank Correlation Coefficient
RF	Radio-frequency
ROI	Region of interest
SD	Standard Deviation
SNR	Signal to noise ratio

SSEP	Somato sensory evoked potentials
T	Tesla
TE	Echo time
TI	Inversion time
TR	Repetition time
VEP	Visual evoked potential

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Thesis summary

The work contained in this thesis forms part of a larger longitudinal natural history study of early relapsing remitting MS. This study is presently ongoing at the Institute of Neurology, London.

Multiple sclerosis (MS) is an acquired primary demyelinating disease in which central nervous system (CNS) lesions are thought to develop as a result of immune-mediated inflammation. It has a wide spectrum of clinical presentation and evolution. Magnetic resonance imaging (MRI) is a very important diagnostic tool, and has become one of the most widely utilised surrogate markers of disease activity in MS.

In this thesis, I have studied a group of patients with early relapsing remitting MS. I have used various MR imaging sequences in order to assess both lesions and normal appearing brain tissue (NABT). I have used quantitative techniques such as magnetisation transfer imaging (MTI) and T1 relaxation time measurements that provide a sensitive measure of abnormalities in lesions and NABT. I have also used Gadolinium-diethylene-triaminepenta-acetic acid (Gd-DTPA) enhancement of T1 weighted images in order to assess lesion inflammation at this early stage. Diffusion tensor imaging (DTI) measurements also give an indication of changes in diffusivity and fibre tract integrity both within lesions and NABT.

I have used these techniques both in isolation and in combination to assess early changes in this cohort of patients. In addition to using different MR sequences, I have also compared region of interest (ROI) and histogram techniques in detecting change. I have also assessed

the relationship between MR parameters of disease activity and clinical scales (both well established and more novel scores).

This thesis is divided into four parts:

Part 1 gives an introduction to MS, and describes the origins of the aims of these studies. The principles of MRI are described, with specific attention to their application to the study of MS. I have also described in detail the various MR sequences used in this thesis.

Part 2 describes a series of studies examining magnetisation transfer ratio (MTR) and T1 relaxation time abnormalities both in NABT and in lesions. One of the aims of these studies was to examine the relationship between MTR and T1 relaxation times in lesions and normal appearing white matter (NAWM) and to assess whether this relationship differs between the different tissue types. In a multiparametric MRI longitudinal study such as this, it is also important to assess the complementary nature of the MRI parameters being used, in order to enable optimal tissue characterisation.

Another of the aims was to examine the MTR and T1 relaxation time changes in normal appearing grey matter (NAGM) using a ROI approach. I was also able to further examine the relationship between lesion load measurements, clinical scores and the abnormalities occurring in NABT. By using the technique of T1 histogram analysis, I have been able to further assess these abnormalities in NABT and assess whether this may be a more accurate and sensitive technique for future use when compared to the more widely used ROI approach.

By further examination of the relationships between clinical markers of disease activity and MRI parameters, it has also been possible to assess the ability of the latter to reflect clinical status in the long term. Further long term follow up will allow an assessment of the prognostic role of the MR measures obtained in early MS.

Part 3 describes the role of diffusion tensor imaging (DTI) in the assessment of patients with early relapsing remitting MS, and examines its sensitivity to subtle changes within both the NAWM and the NAGM. I have also examined the relationship between clinical disability scales and DTI parameters.

Part 4 consists of a summary of the principle findings and the conclusions of this thesis. It also describes future directions for research in this ongoing study. I have detected NAWM and NAGM changes in this group of patients with early disease using DTI, MTR and T1 relaxation time measurements. The most sensitive MR technique appears to be that of T1 relaxation time measurement using a histogram approach. Overall, diffusion tensor imaging using a ROI approach appears to be the least sensitive method for detecting changes in NABT in this cohort of patients. One reason for this may be technical limitations encountered using the ROI approach to measure diffusion parameters in patients with early disease. The techniques of DTI and MTR histogram analysis may in the future prove to be a more sensitive method of analysis in this particular group.

By the long term follow up of these patients using radiological, clinical and immunological methods, it will also be possible to further define these early changes and observe their development over time. It will also be possible to further assess their relationship with clinical markers of disease activity. It may be possible to create an "MRI Composite score",

similar in nature to the MS clinical composite score, by combining scores for each individual MRI parameter. This would provide a total score that may be more completely indicative of the overall pathology. This would therefore provide both an accurate assessment of disease activity at one point in time, and enable a more accurate long term prognosis of future disability.

PART 1. INTRODUCTION, BACKGROUND AND INITIAL CLINICAL AND LESION LOAD DATA.

Chapter 1 Multiple Sclerosis

1.1 MS: an introduction

Multiple sclerosis (MS) is an acquired demyelinating disease that involves the central nervous system (CNS). It is a disease in which many aetiological factors have been postulated, one of which is that of an immune mediated process. CNS lesions are thought to develop as a result of immune-mediated inflammation. Loss of myelin (demyelination) leads to a delay in nerve conduction or conduction block, and manifests itself clinically in MS with abnormalities of CNS function that are disseminated in time (more than 1 month between episodes) and place (more than 1 site affected in the CNS) [Poser *et al.* 1983, McDonald *et al.* 2001]. MS is the most common primary demyelinating disorder in the CNS and is a common cause of neurological disability in young adults in the UK.

Myelin surrounds nerve fibres and serves to isolate the axon functionally. It also increases the velocity at which nerve impulses are conducted along its length. MS primarily involves destruction of this myelin sheath, often with relative preservation of axons. Charcot is thought to have given the first complete clinico-pathological account of typical MS [Charcot 1877]. Plaques seen in the disease have been noted to be highly variable in appearance, texture, size and shape, depending upon age and activity [Raine 1997].

It has been suggested that neurological deficit in relapsing remitting disease may result from incomplete recovery from relapses due to persistent demyelination in lesions, whereas in steadily progressive disease, deficits may arise from progressive axonal loss. This is, however, somewhat conjectural, especially the mechanism of incomplete recovery from

relapse. Trapp [Trapp *et al.* 1998] hypothesised that there is a threshold of axonal loss above which progressive disability occurs.

Pathological and MRI studies have demonstrated abnormalities in normal appearing white matter (NAWM) [Allen *et al.* 1981], [Christiansen *et al.* 1993], [Husted 1994], [Filippi *et al.* 1995a], [Fu *et al.* 1998] and normal appearing grey matter (NAGM) [Cercignani et al 2001], [Kapeller *et al.* 2001], [Ge *et al* 2001], [Ciccarelli *et al.* 2001] in patients with established MS.

By studying the normal appearing brain tissue (NABT) (NAWM and NAGM), and macroscopic lesions in patients with early relapsing remitting disease, it may be possible to gain further insights into the temporal relationship, location and nature of pathological processes which include inflammation and tissue damage with demyelination and axonal loss.

1.2 Pathology

In the acute stage of the disease, it is thought that T cell mediated inflammatory activity occurs within the immune system. This is initiated by auto reactive T lymphocytes. These lymphocytes become activated outside the CNS, and when activated, they increase their expression of adhesion molecules. Adhesion molecules are expressed on endothelial cells and there is a local breakdown of the blood brain barrier (BBB). Inflammation begins when there are a sufficient number of activated T lymphocytes within the CNS. Pro inflammatory cytokines such as interferon gamma and tumour necrosis factor alpha are released, and this in turn attracts more T lymphocytes. This causes the activation of macrophages, which

phagocytose myelin. The inflammatory reaction causes damage to myelin and oligodendrocytes.

There are a number of distinct components to the pathological evolution of lesions in multiple sclerosis: a breakdown of the blood-brain-barrier (BBB): an inflammatory process which is usually perivascular, and which may be associated with oedema: and demyelination which probably follows inflammation. Axonal loss is likely to result in irreversible disability and may be associated with an expanded extra-cellular space. Gliosis occurs in chronic lesions and its functional effect is presently unknown. In summary, it is known from pathological studies that inflammation, demyelination, gliosis, astrocyte hyperplasia, oedema, axonal damage and loss all contribute to the pathology of the disease.

Within many MS plaques, axonal loss is a feature. It is often said that there is relative axonal preservation, but the extent of axonal loss in chronic plaques may sometimes be profound [Lassmann *et al.* 1994]. Recently it has been shown that there may be considerable axonal damage and loss even in acute plaques [Trapp *et al* 1998]. The causes of axonal damage and loss are relatively unclear. It may partly result from a direct immune attack, but may also be due to the loss of protection that myelin would normally provide from inflammatory molecules such as cytokines, proteolytic enzymes, oxidative products and free radicals.

With the recent evolution of more specific imaging techniques, axonal damage has become a more important focus of research. Current evidence suggests that acute inflammation and demyelination within lesions may, at least in part, be responsible for axonal loss [Ferguson *et al.* 1997]. In one study, axonal damage was assessed by looking for amyloid precursor protein (APP). It was found that within acute plaques, axons stained strongly for APP, and

the number of axons seemed relatively well preserved. However, in chronic lesions, axonal loss was observed, but APP staining was not observed. This may indicate that axonal loss is initiated in the acute lesion, and may continue to develop subsequently. Trapp et al detected axonal transection in acute lesions, and also detected axonal transection to a lesser extent in chronic lesions. It would thus appear that axons are sensitive to acute inflammation but may not be the direct target of attack.

Kornek et al [Kornek *et al.* 2000] also noted that axonal loss appeared to be ongoing in acute and chronically demyelinated lesions, but in shadow plaques (with evidence of remyelination) no such continuing changes were observed. The theory that axons are not directly attacked by the immune response has recently been challenged [Bitsch *et al.* 2000]. It is, however, clear that axonal damage is a significant component in the pathology of both acute and chronic disease.

The pathological processes within acute and chronic MS plaques differ somewhat. Within an acute MS plaque, the edge of the plaque tends not to be well demarcated, due to ongoing demyelinating activity. The centre of the lesion is often oedematous, reflecting impairment of the BBB [Gay and Esiri 1991]. Lesions are associated with an acute inflammatory response involving extensive macrophage infiltration with perivenous deposition of fibrin and complement, [Raine1997]. Perivenular lymphocytic cuffs are often found in active plaques and myelin breakdown products are apparent in macrophages. Within chronic plaques however, there is generally decreased myelin and the plaques are well demarcated from adjacent myelinated white matter by a sharp edge. At the edge of the plaque there is often an abundance of T lymphocytes. In the chronic stages of the disease, plaques are often numerous and may involve large areas of the hemispheric white matter. Certain regions are

more likely to be affected, most notably the periventricular white matter, brainstem, optic nerves and spinal cord. While most chronic plaques are completely demyelinated, some demonstrate incomplete myelin loss and ill-defined margins, and are referred to as "shadow plaques". This may be partly due to remyelination.

The NAWM has been noted to be abnormal histopathologically in cases of established MS [Allen and McKeown 1979]. These changes include diffuse astrocyte hyperplasia, perivascular inflammation, gliosis, small focal areas of demyelination, lipofuscin deposition and collagenised veins. These changes have also been demonstrated in cases with predominately spinal involvement [Allen *et al* 1981]. This therefore suggests that a diffuse involvement of mild abnormality can occur without focal plaque formation.

Recent studies however, have found no abnormalities in the NAWM of patients with clinically isolated syndromes, using magnetisation transfer imaging (MTI) [Brex *et al.* 2001] and MR spectroscopy [Brex *et al.* 1999]. Plaques were present in a percentage of these patients. These findings support the theory that focal damage may occur prior to more diffuse and global damage of the normal appearing tissues. It has also been shown that focal NAWM changes can occur up to several months before the development of lesions in patients with established MS [Werring *et al.* 2000], [Filippi *et al* 1998]. Other investigators have reported diffuse NABT abnormalities in patients with clinically isolated syndromes (CIS) [Iannucci *et al.* 2000]. Recent work from our group [Dr A Traboulsee: personal communication] has shown that by using the technique of MTR histogram analysis, it is possible to detect early yet subtle NABT abnormalities in patients with CIS. The exact time course of early changes is therefore open to further debate.

1.3 Clinical manifestations and diagnostic criteria

A relapsing remitting course is the most common onset of MS. This is defined as disease characterised by relapses with a rapid onset of symptoms in hours to days and remissions (which typically follow days to weeks later). Ninety percent of all MS patients have a relapsing onset. Remission periods between disease relapses are characterised by a lack of disease progression but there may not be a full recovery between attacks. The period of remission may be weeks, months or years. Secondary progressive disease is defined as an initial relapsing remitting course in which the subsequent development of a steadily progressive course occurs, with or without occasional relapses or minor remissions. Sixty percent of patients with relapsing remitting disease will progress to the secondary progressive phase of the disease. In ten to fifteen percent of patients, the disease is defined as being primary progressive. This is disease that is slowly progressive from the onset with occasional plateaus and only temporary minor improvements, if any. Benign MS is defined as disease in which the patient has an EDSS score of less than or equal to 3 at least 15 years after the onset of the disease [Lublin and Reingold 1996]: as defined, it occurs in about 30% of patients.

The presenting neurological symptoms can be varied. Symptoms arise from CNS demyelination and consequent axonal conduction block. The most common deficits involve sensory, motor or visual impairment. For the 10% who have a primary progressive onset, a spastic and/or ataxic paraparesis is most common. As well as major clinical features such as weakness, sensory disturbances, cerebellar dysfunction, visual impairment and genitourinary problems, patients with MS may have a variety of paroxysmal symptoms. These include trigeminal neuralgia, tonic spasms, episodic dysarthria and ataxia, episodic pruritis and neuralgic pain.

Disability in MS is usually measured using the Kurtzke expanded disability status scale (EDSS) [Kurtzke 1983]. Neurological impairment results from the cumulative sequelae of recurrent attacks over years, or from slow progression. The relapse rate is usually 0.5-1 per patient per year, with higher rates in younger patients and in relapsing remitting disease [Weinshenker and Ebers 1987]. Between 30-50% of patients will switch from the relapsing remitting to the secondary progressive phase within 10 years of symptom onset [Wolfson and Confavreux 1987]. Even in the progressive phase, the change in disability over time is variable [Weinshenker *et al.* 1989]. The mean rate of change, in one large series, was 0.53 EDSS points per patient per year in the first 5 years after the onset of the progressive phase [Weinshenker *et al* 1989].

In one large natural history study, at 15 years from disease onset, 80% of patients had an EDSS>3 (i.e. at least moderate disability with minimal restriction on walking), 50% had an EDSS of 6 or more (able to walk about 100 meters with unilateral aid), 10% had an EDSS of 8 or more (wheelchair bound), and 2% were dead as a result of MS [Weinshenker *et al* 1989].

There are various features that are generally thought to be associated with a good prognosis. These include an early age of disease onset (i.e. less than 40 years), relapsing remitting disease at onset, female sex, optic neuritis or sensory symptoms at onset, and relatively few attacks in the first 2 years of the disease. A less favourable outlook is thought to be associated with onset over 40 years, male sex, onset with motor or cerebellar signs, progressive course from the onset, and a high number of attacks early in the course of the disease. The time to reach an EDSS of 3 may also identify those at higher risk of early progression of the disease. All of these clinical features are only weakly predictive. A

somewhat better predictor of outcome is disability status after 5 years [Kurtzke *et al.* 1977, Miller *et al.* 1992a].

Patients with MS have slightly shorter survival rates compared to that of the general population [Phadke 1990]. Most of the excess mortality is seen in patients with an EDSS >7.5 (able to walk a few steps with bilateral assistance). In another study the cause of death in MS was determined for 145 out of 3126 patients attending MS clinics. 47% of deaths were MS related, 29% were suicides (7.5 times that of the age matched general population) and other causes of death occurred at the same frequency as in the general population [Sadovnick *et al.* 1992].

In order to make a clinical diagnosis of MS, current criteria require there to be evidence of dissemination of disease in both time and space [Schumacher 1966] (see Table 1.1). The criteria set by the Poser committee [Poser *et al* 1983] (see Table 1.2), allow para clinical evidence of disease to be used to establish the diagnosis. These include demonstration of intrathecal synthesis of oligoclonal bands, abnormalities of evoked potentials (Eps) and MRI studies. The diagnosis of clinically definite MS made using the Poser criteria was found to be correct in 94% of 518 patients in one post mortem study [Engell 1988].

New criteria allow the use of MRI to diagnose dissemination in both time and space, and thus establish a diagnosis of MS in patients presenting with a single clinical episode [McDonald *et al* 2001]. These revised criteria were produced by the International Panel on the Diagnosis of Multiple Sclerosis. The main focus is still the objective demonstration of dissemination of lesions in both time and space. The revised criteria facilitate the diagnosis of MS in patients with a variety of presentations, including "monosymptomatic" disease suggestive of MS,

disease with typical relapsing remitting course, and disease with insidious progression, without clear attacks and remissions. Previously used terms such as "clinically definite" and "probable MS" are no longer recommended. The outcome of a diagnostic evaluation is either MS, "possible MS" (for those at risk of MS, but for whom diagnostic evaluation is equivocal), or "not MS".

Table 1.1 Schumacher criteria for the diagnosis of definite MS

- Neurological examination reveals objective abnormalities of CNS function.
- History indicates involvement of two or more parts of the CNS.
- CNS disease predominantly reflects white matter involvement.
- Involvement of the CNS follows one or two patterns:
    a. Two or more episodes each lasting at least 24 hours and being more than one month apart.
    b. Slow progression of signs and symptoms over at least 6 months.
- Signs and symptoms cannot be better explained by another disease process.
- Patient 10 – 50 years old at onset.

Table 1.2 Poser criteria for the diagnosis of Multiple Sclerosis

Category	Attacks	Clinical evidence		Para clinical evidence	CSF
A. Clinically definite (CD)					
CD MS A1	2	2			
CD MS A2	2	1	and	1	
B. Laboratory supported definite MS (LSD)					
LSD MS B1	2	1	or	1	OCB
LSD MS B2	1	2			OCB
LSD MS B3	1	1	and	1	OCB
C. Clinically probable (CP)					
CP MS C1	2	1			
CP MS C2	1	2			
CP MS C3	1	1	and	1	
D. Laboratory supported probable (LSP)					
LP MS D1	2				OCB

Evoked potentials (EPs) can provide evidence of a clinically silent lesion. EPs are the electric potentials evoked by brief sensory stimuli and are abnormal along central nervous system pathways when an area of demyelination in the pathway causes delayed axonal conduction, or conduction block. EPs can be visual, auditory or somatosensory. Visual evoked potentials (VEPs) are more sensitive to demyelination than examination of visual function. They are almost always abnormal in patients with a clear history of optic neuritis. Evoked potentials have a role to play as adjunctive tools in the diagnosis of MS (in confirming the presence of CNS disease and detecting clinically silent lesions), but have little scope for use in treatment trials.

The CSF examination has an important role in the diagnosis of MS, although it is not always required. On examining the CSF in a patient with MS, there is not infrequently evidence of a low-grade inflammatory reaction within the CNS. About a third of patients will have a CSF white cell count >5 cells/microlitre and glucose levels are invariably normal. The total protein concentration (normal <54mg/dl) is raised in about one third of cases. A raised CSF immunoglobulin G (IgG) concentration with oligoclonal bands is present in over 90% of cases of clinically definite MS. This is considered abnormal only if there is intra-thecal synthesis (i.e. bands are not present in a simultaneously collected serum sample). CSF markers are more relevant to the diagnosis of MS rather than to the prognosis of the disease. Most studies have shown MRI to be as good as, or more accurately predictive of, subsequent development of MS than CSF in patients with clinically isolated syndromes [Filippini *et al.* 1994], [Soderstrom *et al.* 1998].

1.4 Epidemiology

MS is a common cause of neurological disability in young adults in the UK, where its incidence is approximately 7/100 000 with a prevalence of 130/100 000 [Compston 1998a]. There is a difference in sex distribution, with women being more likely to be affected than men, in a ratio of approximately 2:1. There is, however, a more equal sex distribution in later life [Compston 1998b]. The mean age of onset in both sexes is 31-33 years, with the mean age slightly lower in females. MS rarely presents before the age of 16 years or after the age of 59 years (about 10% have onset of disease after age 50) [Noseworthy *et al.* 1983]. Those patients with onset before 16 years show a female preponderance and a tendency to present with visual and sensory symptoms before developing a relapsing remitting pattern of disease [Sindern *et al.* 1992].

Geographical areas can be divided into areas of high, medium or low incidence, as the incidence and prevalence of MS varies according to geographical location. All high and medium risk areas are among predominantly white populations. High frequency areas with prevalence rates of 30 and above per 100 000 population include most of Europe, Canada, northern USA, Israel, New Zealand and south-eastern Australia. Medium (5-29/100,000 population) frequency rates comprise most of Australia, South Africa, southern USA, south west Norway, northern Scandinavia and Russia. Low (<5/100,000) frequency rates occur in Asia, Africa, the Caribbean, and northern South America [Kurtzke and Page 1997]. Migrants from areas of different risk generally retain much of the risk of their birthplace. A prevalence study for migrants from a high (UK) to a low (South Africa) risk area indicated that those who migrated after the age of 15 retained the risk of their birthplace, and those migrating

before the age of 15 acquired the lower risk of the area into which they moved [Kurtzke *et al.* 1970, Dean and Kurtzke 1971]. However, epidemiological studies from Australia do not suggest an age cut-off [Hammond *et al.* 1988]. There have been possible epidemics that have been described in Iceland and the Faros. None of these have been confirmed, but the Faros is the most likely to be convincing. It has been suggested, as a result of these epidemiological studies, that MS is not only an acquired disease but also a transmissible one. Kurtzke [Kurtzke and Page1997] suggests that the transmissible agent is a widespread, specific (but unknown) persistent infection of adolescents and young adults, which only rarely leads to clinical neurological MS after years of incubation. Prolonged exposure to this undefined agent may be required to develop the disease.

There is also evidence of there being a genetic pre-disposition to developing MS. Certain individuals may develop an abnormal reaction to an environmental event, which, in turn, leads to an autoimmune process causing demyelination. Gaudet et al [Gaudet *et al.* 1995] found that the risk of developing MS appeared to be more closely related to genetic background than to environmental factors. It is, however, likely that both contribute. The genetic component is reflected in familial recurrence risk data and the higher rates of concordance in monozygotic than dizygotic twins [Ebers 1994]. Twin studies have shown concordance rates of 20 - 30% in monozygotes and 3.3 - 4.7% in dizygotes [Mumford *et al.* 1994]. Family studies have shown that up to 19% of patients have an affected family member [Sadovnick *et al.* 1993]. For first degree relatives of a patient with MS, the lifetime risk of developing the disease is about 1-5% (compared to the risk in the general population of 0.1% for those without a family history). The risk seems higher for siblings, and especially sisters of MS patients. The increased risk in second and third degree relatives (not living with the index case) favours a genetic factor in explaining the increased familial

incidence of MS [Sadovnick *et al* 1993], as does the much lower risk for MS in adoptive siblings of index cases [Ebers *et al.* 1995]. The incidence of unaffected close relatives having MRI abnormalities may be as high as 10% [Thorpe *et al.* 1994]. This may raise important questions as to whether they should be considered unaffected. The presence of an abnormal MRI, however, would obviously not establish a diagnosis of MS.

Putting all the evidence together, it has been postulated that an environmental factor (e.g. a viral infection) in MS induces an abnormal immune response in a certain genetically predisposed individual. A viral infection during childhood or early adulthood might cause an immune response, which potentially might induce MS after a long incubation period. The evidence is circumstantial, but destruction of myelin is a feature of a number of viral infections of the CNS (although none produces a relapsing-remitting course). Viruses may cause demyelination either by directly infecting oligodendroglia (as with progressive multifocal leucoencephalopathy) [Goswami *et al.* 1987], or due to a reaction of lymphocytes or macrophages with heterologous viral antigens [Wisniewski and Bloom 1975] where myelin is damaged by lymphokines or proteases released by the activated macrophages. It may well be that the immunopathogenic steps leading to MS follow a similar pattern, but that they are initiated by a variety of agents. This concept is compatible with the clinical and epidemiological data.

1.5 Clinical outcome measures

1.5.1 Expanded disability status scale (EDSS)

The Kurtzke EDSS score [Kurtzke1983] is an established clinical score widely used in the clinical assessment of MS patients (see Table 1.3). It is used as a primary outcome measure to assess neurological deficit and disease activity in MS clinical trials. It does, however, have

problems with standardisation, resulting in suboptimal inter-rater reliability: bias towards locomotor function, and limited sensitivity to change over time [Whitaker *et al.* 1995]. Correlation with other markers of disease activity such as lesion loads measured on T1 and T2 weighted images is also limited in established MS [Filippi *et al* 1995a].

Correlations between EDSS and MRI findings have previously been illustrated using various MR techniques. Good correlations between disability and average lesion magnetisation transfer (MT) ratio [Gass *et al.* 1994] and hypo intense lesion load on T1- weighted images [Truyen *et al.* 1996] have been found. Other studies have shown an inverse relationship between NAA peaks and disability. Kidd [Kidd *et al.* 1993] reported that more disabled patients with MS had a greater likelihood of spinal cord atrophy: two studies [Filippi *et al.* 1996a], [Losseff *et al.* 1996c] found that the degree of disability was inversely correlated with the cross sectional area of the cervical spinal cord. These non conventional measures are more specific for tissue disruption due to demyelination or axonal loss, whereas T2 lesions are pathologically non specific. This might account for the better clinical correlations of the former approaches.

Table 1.3 Kurtzke Expanded Disability Status Scale (EDSS)

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

*FS= functional score

1.5.2 MS FUNCTIONAL COMPOSITE MEASURE (MSFC)

In 1995, an International Taskforce set guidelines for the development of the MSFC. The six guiding principles were: 1. To use measures which reflected the major clinical dimensions of MS. 2. To avoid redundancy. 3. To use simple rather than complex measures. 4. To improve on the valuable characteristics of the EDSS. 5. To emphasize measures sensitive to change. 6. To develop an outcome measure that will be useful in clinical trials that may or may not also be useful in clinical practice.

The three components of the Composite score are: 1) The nine hole peg test [Goodkin *et al.* 1988]: which is a measurement of arm/hand function. 2) The timed 25 foot walk [Bohannon 1997] which measures ambulation/leg function, and 3) The PASAT (Paced Auditory Serial Addition Task) which is one of the many measures of cognitive function. The Composite score is calculated using a standard formula [Fischer *et al.* 1999]. A recent study [Kalkers *et al.* 2000] found that the leg and arm function tests showed a good correlation with the EDSS, whereas the cognitive test showed no correlation. The intra and inter observer variability of the MSFC would appear to be less than that for the EDSS [Cohen *et al.* 2000]. Recently, correlations have been observed between the MSFC and T1 and T2 lesion loads [Kalkers *et al.* 2001a]. The correlations were most pronounced in the relapse onset groups (relapsing remitting and secondary progressive MS).

With an increasing use of partially effective disease modifying therapies, future treatment trials using a placebo control group may become more difficult to perform, and trials may in future consist of two treatment arms. Hence the numbers of patients needed to produce adequate statistical power will need to increase. It will therefore become desirable to produce more responsive clinical outcome measures. In retrospective and cross-sectional data, the

MSFC has shown good concurrent and construct validity [Kalkers *et al* 2000], [Rudick *et al.* 1997],[Cutter *et al.* 1999]. Changes in the MSFC over the first year of observation predicted subsequent change in the EDSS, suggesting that the MSFC may well be as sensitive, if not more so, to change than the EDSS [Cutter *et al* 1999].

1.6 Treatment options

The treatment of MS is generally considered in three categories: 1) Disease modifying therapies 2) Treatment of acute exacerbations 3) Management of the symptoms of established disease.

1.6.1 Disease modifying therapies

Several disease modifying therapies have been developed over the last decade. These are predominantly targeted towards the immune system, and target inflammatory processes known to be involved in the pathology of the disease. The Interferons (beta interferon 1a and 1b) and glatiramer acetate are licensed for ambulant patients with relapsing remitting disease in the UK, and mitoxantrone is licensed for use in the USA.

Interferon beta occurs naturally and is known to have immunomodulatory effects. Its precise mechanisms of action are unclear, but it is known to have several biological effects. These include inhibitory effects on leucocyte proliferation and antigen presentation, inhibition of T cell migration across the blood brain barrier and modulation of cytokine production to produce an anti inflammatory environment. The interferon products used are made by recombinant DNA technology and they are highly purified before use. There are presently three recombinant interferon beta preparations in use – Avonex (Interferon beta 1a), Betaferon/Betaseron (Interferon beta 1b) and Rebif (Interferon beta 1a). Interferons were

originally administered intrathecally and showed fewer exacerbations in a study of 20 MS patients [Jacobs *et al.* 1987].

Avonex - A double blind placebo controlled phase III clinical trial of intramuscular Interferon- beta-1a in relapsing remitting disease reported a significant treatment benefit favouring active therapy when time to sustained progression was measured [Jacobs *et al.* 1996]. At two years after commencing treatment, benefits were also seen in the proportions of patients with sustained progression, the relative risk of having three or more exacerbations, the number and volume of new T1 gadolinium enhancing lesions, and the percentage change in T2 weighted lesion volume at 1 year. The relapse rate was decreased by approximately one third. Drug related toxicity was mild and fewer treated patients had developed neutralising antibodies at 2 years compared to Interferon beta 1b. However, the reduction in MRI activity was smaller with Interferon beta 1a (Avonex) than with Interferon beta 1b (Betaferon).

Betaferon/Betaseron - A multicentre North American phase III placebo controlled trial of subcutaneous Interferon beta 1b in relapsing remitting disease, showed a 34% reduction in annual on-study exacerbation rate compared to placebo in the first two years of the study [Paty and Li 1993, The IFNB Multiple Sclerosis Study Group 1995] with a higher dose preparation (8 million IU). It also increased the time to first relapse and the proportion of patients who were relapse free. There was no significant difference in neurological disability between treatment groups. A European trial examining the use of Betaferon in secondary progressive disease showed a delay in disability progression of 9 – 12 months over 2 – 3 years. There were also favourable effects on relapse rate and MRI parameters.

Rebif - The PRISMS study [PRISMS Study Group 1998], [Li and Paty 1999] was a double-blind randomized, placebo controlled study of Interferon 1a in RR MS patients. Over the two years of the study, the placebo group showed an increase in disease burden of 10.9%, whereas the treated group showed a decrease of 1.2% (22 micrograms IFN), and 3.8% (44 micrograms IFN). There was no significant difference between the 2 doses during the 2 years. The number of T2 active lesions and percentage of T2 active scans were significantly reduced in both the treatment groups. In the subgroup undergoing monthly scanning, the treatment effect became apparent two months after treatment commenced. The time interval between relapses was also increased, and the relapse severity decreased. The proportion of patients becoming more disabled was significantly reduced by both doses of Rebif. A recent four year extension study suggests some marginal benefits of the 44mcg versus the 22mcg dose [PRISMS-4, 2001]

Glatiramer Acetate (Copaxone) is a compound comprising of a mixture of synthetic polypeptides. These are designed to mimic myelin basic protein (MBP). It has a mode of action that is thought to involve the inhibition of lymphocyte migration and suppression of T lymphocyte activation. A 2 year study demonstrated a reduction of 29% in relapse rate in the treatment group of patients with relapsing remitting disease [Johnson *et al.* 1995]. A clinical trial of Copaxone in primary progressive MS is currently being conducted.

Anti-alpha4 integrin (Antegren). Antegren is a humanized monoclonal antibody directed against alpha4 integrin, a cell adhesion molecule involved in immune cell migration. A trial of patients with active relapsing remitting and secondary progressive MS reported a significant reduction in the number of active lesions on MRI over the short term [Tubridy *et al.* 1999a]. Further studies of this agent are presently being conducted.

Campath-1H is a humanised anti-leucocyte (CD52) monoclonal antibody that is administered as an intravenous infusion. A reduced relapse rate has been demonstrated, with a reduction in the number of inflammatory lesions seen on MRI. However, many treated patients experienced continued disability, and a significant number developed hyperthyroidism [Coles *et al.* 1999]. Many patients also experienced a worsening of neurological symptoms immediately after injection.

The use of **azathioprine** in MS has been studied in 21 trials over the last 3 decades. The results have generally been disappointing, but a meta-analysis of all the controlled trials showed that oral azathioprine (2 to 3 mg/kg per day) reduced the rate of relapse in MS, even in the first year of treatment. It had no effect on the progression of disability [Yudkin *et al.* 1991]. However, the effects on relapses appear generally modest, and there may be a slightly increased risk of neoplasia long term [Weinshenker and Sibley 1992], [Goodkin *et al.* 1991].

Cyclophosphamide and **plasma exchange**. These were studied in a placebo-controlled trial of patients with progressive MS [The Canadian Cooperative Multiple Sclerosis Study Group, 1991]. This study failed to detect any treatment benefits, and toxicity is frequently a problem with cyclophosphamide.

Mitoxantrone. This is a cytotoxic agent with potent immunosuppressant effects. It causes suppression of B cell immunity and reduction in T cell numbers. Mitoxantrone is usually reserved for suitable patients with clinically worsening relapsing remitting and relapsing secondary progressive MS who have failed to respond to first line disease modifying therapies. Millefiorini [Millefiorini et al 1997] showed a significant reduction in relapse rates

but not in MRI lesion load or progression of disability. Edan [Edan et al 1997] compared the effect of intravenous methylprednisolone to a combination of mitoxantrone and methylprednisolone over a six month period and showed a significant improvement in disability scores in patients treated with mitoxantrone, as well as showing a significant reduction in relapse rate and number of active lesions on MRI in this group.

Other therapies that have been studied and found to have, overall, little or only marginal benefit in MS, include cyclosporine [The Multiple Sclerosis Study Group, 1990], methotrexate [Goodkin *et al.* 1995] and OKT3 antibodies [Weinshenker *et al.* 1991].

1.6.2 Management of the acute exacerbation

Acute attacks are often treated with oral prednisolone or intravenous methylprednisolone (IVMP). High dose IVMP has been shown to shorten the duration of new symptoms when compared with placebo, but does not appear to alter the final outcome of the relapse. Gadolinium enhancement of lesions on MRI studies is ameliorated temporarily by IVMP [Miller *et al.* 1992, ;Barkhof *et al.* 1994]. In spite of sustained clinical improvement, however, many lesions may re-enhance within a few days of stopping IVMP [Miller *et al* 1992b]. There is no evidence to support the long term use of steroids to slow disease progression, although the results of the Optic Neuritis Treatment Trial showed a possible short term effect [Beck *et al.* 1993]. Patients receiving IVMP experienced a reduced incidence of clinically definite MS in the 2 years of the study after they had an attack of optic neuritis but this was not sustained at 3 years. In addition, the rate of recovery of vision was significantly faster in the IVMP group but there were no significant differences between groups in visual outcome at six months.

1.6.3 Management of the symptoms of established disease

Spasticity is one of the major symptoms of established disease. Baclofen remains the drug of choice. It is an analogue of GABA (gamma aminobutyric acid) and is thought to inhibit monosynaptic and polysynaptic transmission at the spinal level, and also depress the CNS. Tizanidine is an alternative treatment. Tizanidine is an alpha-2 adrenergic receptor agonist that acts at both supra-spinal and spinal levels. It inhibits spinal polysynaptic reflex activity. It has no direct effect on skeletal muscle. Benzodiazepines and dantrolene are other alternatives for the treatment of spasticity.

About 70% of patients experience cerebellar intention tremor or ataxia at some stage. It is a very difficult symptom to treat. Isoniazid, clonazepam, ondansetron, propranolol and carbamazepine are some of the treatment options. Surgical intervention using techniques such as thalamotomy or thalamic stimulation is also a possibility.

Neuropathic pain is another troublesome symptom, and carbamazepine, gabapentin and tricyclic antidepressants are used in its treatment. Bladder dysfunction is often responsive to oxybutinin or tolterodine, and/or intermittent self catheterisation.

Amantadine may ameliorate fatigue, and sildenafil is often effective in treating erectile dysfunction.

Once moderate or severe disabilities are established, a programme of multidisciplinary care including neurorehabilitation, physiotherapy and occupational therapy along with appropriate drug treatments can be beneficial.

CHAPTER 2 Magnetic Resonance Imaging: principles and applications in MS

2.1 MRI: Basic principles

The application of a radiofrequency (RF) excitation pulse to mobile hydrogen (H^1) nuclei or protons produces a nuclear magnetic resonance (NMR) signal. Protons align themselves in an external magnetic field in directions that are both parallel and antiparallel to that field. A small net parallel alignment occurs, and the net magnetic vector is therefore parallel to the external field. This net vector rotates around the main magnetic field. This motion is known as precession: the precession frequency is defined by the Larmor equation:

LARMOR EQUATION

$$W_o = \gamma B_o$$

Where Wo = Precession frequency (Hz), γ = Gyromagnetic ratio and Bo = Magnetic field strength (Tesla).

The precession of protons causes them to be misaligned with the external magnetic field resulting in both longitudinal (i.e. parallel to B_o) and transverse (i.e. perpendicular to B_o) magnetic properties. The combined longitudinal component of the protons produces a small magnetic field (longitudinal magnetisation), which cannot be directly measured. The transverse magnetic fields of the protons cancel each other out because the protons precess out of phase with each other.

In order to measure the magnetic properties of the protons, an RF pulse with the same frequency as the precessing protons is used to transfer energy to them (resonance). The power and duration of the RF pulse can be manipulated to produce specific effects on

protons. A 90° RF pulse will rotate the net magnetisation into the transverse plane, so that it produces a measurable magnetic effect (transverse magnetisation). The magnetisation vector precesses at the same frequency as the individual protons and induces a voltage in a receiver coil placed in the transverse plane, which constitutes the MR signal.

Two processes occur simultaneously after the application of the RF pulse. Longitudinal magnetisation increases as the net magnetisation returns to a parallel (to the external magnetic field) position. This is made possible by the protons transferring energy to their immediate environment (also called the "lattice") and is therefore termed spin-lattice relaxation. This is an exponential process defined by a time constant - the T1 relaxation time (T1). At the same time, the net transverse magnetic vector starts to decrease as the protons begin to precess out of phase with one another. This is due to the interaction of adjacent nuclei that produce small fluctuating alterations (inhomogeneities) in the magnetic environment local to each proton. This decay of transverse magnetisation is called spin-spin relaxation and occurs exponentially with another time constant- the T2 relaxation time (T2).

After RF excitation, the signal intensity that is observed from a sample is directly related to the degree of magnetisation (i.e. the magnitude of the net vector) achieved. The MR signal is measured when the individual nuclei are precessing in phase. This may be achieved by transmitting a second RF pulse (a 180° pulse), which re-focuses the protons to once again produce an in-plane net transverse magnetic vector (an "echo" of the situation immediately after the 90° pulse). This is known as the spin echo (SE).

Tissues have different T1 and T2 relaxation times, depending on the macromolecular structure of the tissue. These differences (averaged per voxel) in relaxation rates, and hence

MR signal intensity between voxels, are translated into an image. There are three main factors that influence the image contrast seen between structures. These are: proton density (PD), T1 and T2. Image contrast can be made predominantly T1-weighted by restricting the time available for T1 recovery after excitation (partial saturation) i.e. the repetition time (TR) is short. T1 depends on tissue composition, structure and the surroundings. When the surrounding lattice consists mostly of liquid/water, it is difficult for the protons to lose their energy (as the small water molecules move too rapidly to interact well). Thus, all of the protons will return to a parallel orientation (longitudinal magnetisation) more slowly. As a result, water/liquids have a long T1 in vivo. Fat has a short T1 as carbon bonds at the end of their fatty acids have a frequency nearer the Larmor frequency and so energy exchange is more efficient.

If contrast in MR imaging between two types of tissue is poor (because their PD, T1 and T2 values are similar), improvement of contrast can be obtained by selective manipulation of the relaxation characteristics of one of the tissues by means of a contrast agent. For clinical use, the paramagnetic agent gadolinium (a rare earth element, the ion of which has 7 unpaired electrons) is chelated (made non-toxic and stable) as gadolinium-diethylene-triaminepenta-acetic acid (Gd-DTPA). By introducing this contrast agent, T1 relaxation (or spin-lattice relaxation) will be more rapid in the region it enters. Gd-DTPA will also shorten the T2 relaxation time but to a smaller extent. Gd-DTPA strongly enhances T1 relaxation in vivo at a dose of 0.1-0.3 mmol/Kg. More recent studies have found that triple dose Gd-DTPA (0.3mmol/Kg) increases the sensitivity of MRI to contrast-enhancing multiple sclerosis lesions by over 70% [Filippi *et al.* 1996c], [Silver *et al.* 1997] when compared to a standard dose (0.1mmol/Kg).

For the acquisition of a T2- weighted image, a variable loss of phase coherence is allowed to occur before a refocusing pulse (180°) is applied to produce an echo (i.e. the echo time (TE)- the time between the excitation pulse and the echo- is long). T2* ("T2 star") relaxation occurs when protons lose phase coherence due to inhomogeneities of both the external magnetic field (i.e. the MR magnet) and the local magnetic field. The former, but not the latter is refocused by the spin echo. Different tissues restrict proton motion in different ways leading to different relaxation properties. To produce a T2-weighted image, a long TR is used (which minimises the effect of T1 weighting) and a signal (called the spin echo) is received at time TE (time to echo) after the 90° pulse. The shorter the TE, the stronger the signal received but, if it is too short, there will be little T2 contrast between the tissues. Under these circumstances, contrast becomes largely due to differences in proton density (PD) in different tissues. Thus a long TR and a short TE sequence can be considered PD-weighted. If the TE is longer, the difference in the T2 curves, and thus the difference in signal intensity (or contrast), is more pronounced. If a tissue has a shorter T2, it will lose transverse magnetisation faster. CSF has a longer T2 than brain tissue, so it appears bright on long TE, long TR images. With a shorter TE, the difference in signal intensity between CSF and brain tissue is less pronounced than after a longer TE.

2.2 MRI findings in MS and their role in diagnosis

MS lesions are visible as high signal areas on PD/T2 weighted images because of an increase in both PD and T2 relaxation times. A substantial increase in T1 relaxation time results in about 10 - 30% of MS lesions displaying low signal on T1-weighted images (T1 hypointense or "black hole" lesions). MS plaques are readily seen on PD and T2-weighted sequences because of a higher density and mobility of water protons than normal white matter.

However, any of the main pathological features of plaques (inflammation, oedema, demyelination, axonal loss), may directly, or indirectly, result in an increase in water content and mobility and an important limitation of PD/T2 weighted sequences is their low pathological specificity.

Multifocal white matter abnormalities occur in over 95% of patients with clinically definite MS [Ormerod *et al.* 1987]. MRI aids in the diagnosis of MS by providing paraclinical evidence for dissemination in space (and more recently, time [McDonald *et al* 2001]). The distribution of the lesions in central white matter is also distinctive in MS (though not specific). In one study, 98% of 314 patients had periventricular lesions especially in the regions of the body and trigone of the lateral ventricles. The size of the lesions varies, but they are usually less than 5mm in diameter and many are rounded/ovoid in shape [Miller *et al.* 1988]. Involvement of the corpus callosum is seen in 93% (in sagittal images) [Gean-Marton *et al.* 1991], this being an unusual site for ischaemic lesions. Lesions at the cortico-medullary junction are relatively common, and on MRI they may appear to selectively involve the subcortical u-fibres (which are characteristically spared in Binswanger's disease). Sixty seven per cent of patients in one series had lesions in the brain stem, especially in the region of the floor of the fourth ventricle. Fifty-four percent of patients in the same series had cerebellar lesions [Miller *et al* 1988].

In neuropathological studies, cortical lesions are common in MS, with up to 24% of lesions arising from the grey matter in one study [Kidd *et al.* 1999]. These lesions are rarely seen on conventional T2-weighted images, but detection is improved modestly with fluid-attenuated inversion-recovery (FLAIR) sequences [Tubridy *et al.* 1999b]. Focal lesions can also occur in the basal ganglia in MS. Mild T2 hypointensity of the basal ganglia in MS patients has also

been reported, especially in the thalamus, perhaps indicating increased iron content [Grimaud *et al.* 1995]. A recent study detected abnormalities in the thalamus using diffusion tensor imaging [Ciccarelli *et al* 2001].

MRI is very useful for diagnosing MS, but there are many other causes of brain white matter lesions, so MRI alone cannot make the diagnosis of MS. Even if the clinical evidence and paraclinical studies are strongly indicative of MS, there must be no better explanation for the clinical and paraclinical abnormalities than MS, for a secure diagnosis to be made [McDonald *et al* 2001]. Detection of spinal cord lesions in MS is of particular relevance, since they are more often associated with symptoms and with current techniques it is possible to detect multiple lesions in the majority of patients [Kidd *et al* 1993]. Cord lesions are also helpful in the diagnosis as they do not occur with aging per se in the way that brain lesions do. MRI of the cord reveals one or more intrinsic lesions in 75% of patients with clinically definite MS (CDMS), most of which are central or posterior in the cord, especially in the cervical region [Kidd *et al* 1993]. They occur in all clinical MS subgroups. New cord lesions are about one-fifth to one-tenth as common as new brain lesions in relapsing remitting MS [Thorpe *et al.* 1996a], [Silver *et al.* 2001].

The relapsing remitting group of MS patients have been studied on many occasions with serial MRI brain imaging [Barkhof *et al.* 1992a],[Thorpe *et al* 1996a], [Thompson *et al.* 1992]. In one study relapsing remitting MS patients displayed a mean of 18 new lesions per annum on monthly gadolinium-enhanced or T2-weighted images [Thompson *et al* 1992], but there was marked inter-individual variation. There is no regular predictable pattern, although, to some extent, there is an increase in the number of new lesions in association with clinical relapses [Frank *et al.* 1994].

In secondary progressive disease, a wide range of MRI findings occur. Early small studies indicated that the majority of secondary progressive MS patients continued to have as high a frequency of new lesions as RR patients, despite entering the progressive phase of the disease (a mean of 18.2 new T2 or enhancing MRI lesions per patient per annum, with enhancement being seen in 87% of new lesions) [Thompson *et al.* 1991]. It was suggested, in another small serial study, that patients who continued to have superimposed relapses displayed more new MRI activity [Kidd *et al.* 1996]. Tubridy et al [Tubridy *et al.* 1998] showed that MRI activity was more common in relapsing secondary progressive disease.

In a benign subgroup of MS patients (defined as having an EDSS less than or equal to 3 and a disease duration of greater than 10 years), fewer lesions are seen on MRI [Thompson *et al* 1992]. Only one-third of new lesions in this group show Gd-DTPA enhancement. With increasing duration of disease, it is suggested that patients will have fewer new lesions and those lesions that do occur may be less inflammatory (and thus less likely to enhance with Gd-DTPA) [Thompson *et al* 1992], [Kidd *et al.* 1994].

Primary progressive MS may differ from relapsing remitting and secondary progressive disease both immunologically and pathologically [Thompson *et al.* 1997]. These patients continue to deteriorate clinically, but few new lesions are seen on MRI, and those that do occur are often small (78% of lesions were less than 5mm diameter in one study) [Thompson *et al* 1991]. In the study by Kidd et al, [Kidd *et al* 1996] patients with PPMS displayed a mean of only 3.3 new lesions per patient per year on MRI, and in addition, little new enhancement was seen. The lesions seen in PPMS may be of a less inflammatory nature than those seen in the other sub-groups [Revesz *et al.* 1994]. There is a subgroup of PPMS that is

referred to as transitional progressive MS. These patients have features predominantly in keeping with primary progressive MS but also have a single episode of acute deterioration either at the onset or during the course of the disease. The MRI findings are very similar to those seen in primary progressive MS [Filippi *et al.* 1995b], [Stevenson *et al.* 1997].

It is apparent from these studies that the number of lesions and frequency of new lesion development varies between the different clinical subgroups. In patients with relapsing remitting and secondary progressive MS, between 5 and 10 new lesions are seen on proton density (PD) and T2-weighted images for each clinical relapse [Isaac *et al.* 1988], [Willoughby *et al.* 1989]. The lesions expand over a period of days to weeks and reach their maximum size within one month. They then shrink to a stable size at 2-3 months. A residual abnormality almost invariably persists thereafter [Thompson *et al* 1991]. Almost all new lesions show gadolinium enhancement that usually lasts for 2-6 weeks [Miller *et al* 1988], [Thompson *et al* 1992], [Lai *et al.* 1996]. Enhancement of new lesions may precede the appearance of an abnormality on a T2-weighted image [Kermode *et al.* 1990b]. Occasionally, lesions seen on previous T2 scans may also enhance but this is less frequent.

Cord lesions are especially helpful in the diagnosis of MS when brain MRI is normal and in older patients in whom changes on brain MRI are often non-specific [Thorpe *et al.* 1996b]. Silver [Silver *et al* 2001] found an increase of 154% in the total number of enhancing lesions and 107% increase in the number of new enhancing lesions detected using a modified approach, which consisted of combined brain and spinal cord delayed imaging and the use of triple dose Gd-DTPA. Delayed imaging was performed at 40 minutes post contrast administration for the brain images, and 30 minutes for the spinal cord. Triple dose Gd-DTPA (0.3mmol/Kg) and MT presaturated T1 weighted imaging of the brain was also used.

The most important factor in the increased sensitivity of the protocol was the use of triple dose Gd-DTPA.

The correlation between PD and T2-weighted brain MRI findings in established MS and clinical findings is generally weak. There are a number of theories as to why this may be the case (see Table 2.1).

Table 2.1 Factors influencing the correlation between brain MRI and clinical findings in MS

- EDSS disability scale is non-ordinal and heavily reliant on locomotor function
- MR sequences are not pathologically specific
- NAWM pathology contributes to disability but may not be detected on MRI
- NAGM pathology contributes to disability but may not be detected on MRI
- Spinal cord disease also contributes substantially to clinical disability

Relatively small numbers of patients have been enrolled in natural history studies at present, and this may mean a statistical association between brain lesion load and disability is not found as would be expected (with increased numbers of lesions, there is a greater chance that the relevant pathways would be involved). In short term studies (of 6-12 months duration), a lack of correlation is less surprising as disability alters little over such short time periods. In long term studies such as the beta-interferon trials, some correlation (albeit modest) between PD/T2 lesion loads and clinical disability has been found [Paty and Li1993]. To improve the

correlation, other MR strategies are being employed including newer techniques to improve pathological specificity and imaging of the spinal cord [Kidd *et al* 1996].

Newer techniques to improve pathological specificity include proton MR Spectroscopy which measures metabolites such as N-Acetyl Aspartate (NAA), choline-containing compounds, creatine/phosphocreatine and lactate, all of which contain mobile protons. Acute MS plaques may display decreased NAA, increased choline, creatine, myoinositol, lactate and lipid protons. Chronic plaques may also show decreased NAA peaks but generally display normal levels of choline, creatine, lactate and lipid protons. In the adult brain NAA is almost exclusively contained in neurones; thus a persistent reduction of NAA may be an indicator of axonal damage or loss.

Other techniques such as the measurement of cerebral and spinal cord atrophy are also becoming important in the long term follow up of patients with MS [Fox *et al.* 2000] [Losseff and Miller 1998], [Losseff *et al.* 1996b] [Rudick *et al.* 2000]. These techniques have been employed in relapsing remitting [Rudick *et al.* 1999, Simon *et al.* 1999], progressive [Stevenson *et al.* 2000a] and both [Fox *et al* 2000], [Liu *et al.* 1999], [Losseff *et al* 1996b] clinical subtypes of MS. The results suggest that atrophy occurs at a significantly faster rate in MS subjects compared with a normal healthy population, and that this can be correlated with clinical outcome. Using such techniques significant differences in brain volume have been demonstrated between control and MS populations, however it remains unclear how early in the disease atrophy appears and whether it is predominantly localised to white matter or grey matter. A recent study performed in the cohort of patients described in this thesis [Chard *et al* 2002] showed that atrophy was present at this early stage of the disease, and primarily affected white matter. It can therefore be hypothesised that the mechanisms

underlying atrophy in MS are not merely an acceleration of normal age related atrophy, which primarily involves grey matter.

MRI can be used to give an indication of prognosis at the clinical onset of disease. In over 90% of patients who subsequently develop MS, the first clinical manifestation is with an acute neurological disturbance that usually remits, in part or in whole, over a few weeks or months. This is described as a clinically isolated syndrome (CIS). Between 50 and 70% of patients presenting with a CIS show clinically silent cerebral white matter lesions on brain MRI at presentation [Miller *et al.* 1989]. The lesions are usually multiple and are in a pattern similar to that seen in established MS. In the Optic Neuritis Treatment Trial [Beck *et al* 1993], 31% of the placebo group who had an abnormal MRI brain went on to develop CD MS at 2 years, whereas 2% who had a normal MRI at presentation developed CD MS at 2 years.

In one report, those patients with a CIS who had four or more lesions in a brain MRI scan at presentation had an especially high risk for the early development of MS: 85% did so after 5 years [Bergstrom 2000]. This finding has importance in counselling patients and in selecting patients for trials of treatments aimed at preventing early conversion to clinically definite MS. A recent study [Brex *et al* 2001b] showed that serial imaging in patients with CIS improved the positive predictive value, sensitivity and specificity of MRI for the development of early MS, and also identified patients at lower risk of early MS than would have been expected from their abnormal baseline MRI. Selection of patients with CIS for therapeutic intervention or clinical trials may benefit from serial MRI to target those at greatest risk of early development of MS.

Brain MRI is now also widely used to monitor treatment efficacy in definitive phase II and III MS treatment trials [Jacobs *et al* 1996], [Simon *et al.* 1998]. A well established MRI outcome measure is T2 weighted brain lesion volume, which has been used to demonstrate a significant treatment effect in several multicentre trials in both relapsing remitting and secondary progressive disease. However, the lack of a strong relationship between T2 lesion volume and clinical state dictates that the primary outcome measures for such trials are based on clinical outcome measures [Miller *et al.* 1996]. A major reason for the modest nature of this clinical/MRI correlation is the lack of pathological specificity of increased signal on a T2 weighted sequence; such areas can reflect acute, potentially reversible oedema and inflammation, as well as the more destructive processes such as demyelination and axonal loss.

Individual MRI parameters are not generally pathologically specific, but various measurements may provide information regarding certain pathological processes. A marked decrease in MTR values in lesions probably involves demyelination, T1 hypointense lesions indicate gliosis, axonal loss and oedema, and T1 enhancing lesions are a reliable surrogate marker of inflammation and BBB leakage. Diffusion tensor imaging enables the measurement of the diffusion of water molecules in vivo and offers a valuable insight into fibre tract integrity.

There is much interest in the study of NABT: both NAWM and NAGM. Subtle but widespread changes in NABT may be responsible for clinical disability, and may help to explain some of the discrepancy between clinical outcome scores and conventional lesion measures such as total T2 lesion load. Recent studies have shown there to be subtle focal abnormalities in NAWM up to six months before the development of new lesions at the same

site [Werring *et al* 2000], [Filippi 1999a]. Changes in NAWM have been illustrated using MTR [Dehmeshki *et al.* 2001], [Silver *et al.* 1998], [Griffin *et al.* 2000], DTI [Werring *et al.* 1999], [Hickman *et al.* 2001] and T1 relaxation times [Stevenson *et al.* 2000b], [Griffin *et al* 2000]. NAGM changes have also been illustrated in patients with established disease, and these changes may be responsible, at least in part, for some of the cognitive decline seen in established MS [Filippi *et al.* 2000]. NAGM changes have been illustrated using DTI [Hickman *et al* 2001] and NMR Spectroscopy [Kapeller *et al* 2001].

Much of the white matter between the MS plaques appears macroscopically normal both at postmortem and on MRI. In a histopathological study of clinically severe cases of MS, [Allen and McKeown1979] about 70% of samples of macroscopically NAWM, taken at a distance from the plaques, showed histological abnormalities and histochemically showed increased acid-phosphatase containing cells with the morphology of astrocytes. In a further study of 20 cases of mild or spinal MS [Allen and McKeown1979] diffuse gliosis was seen in the NAWM that was nodular, pericapillary or finely diffuse. There was also evidence of vascular sclerosis and perivascular inflammation. Unsuspected demyelination was occasionally found. It was suggested that in MS there may be a BBB breakdown with resulting astrocytic activation, or the astrocytic activation may occur as a primary event. There are quantitative changes in T1 and T2 between the NAWM in MS and healthy individuals [Ormerod *et al.* 1986]. It has been suggested that the increase in T1 (by up to 3%), and T2 (by up to 12%) seen in the NAWM of MS subjects compared to healthy controls may be due to the subtle histopathological changes described by Allen or to the presence of a microscopic ("invisible") lesion load.

2.3 T2 weighted imaging and the measurement of total lesion load

The volume of lesions seen on conventional T2 weighted images (T2 lesion load) is commonly regarded as the MRI measure of disease burden, and is used as a surrogate outcome measure in phase III treatment trials [Miller 1996]. However, as previously discussed, the predictive value of changes in T2 lesion load is generally weak in established MS. Active lesions are usually defined as any new or enlarging lesions seen on serial (unenhanced) T2-weighted MRI and any gadolinium (Gd) enhancing lesion seen on post contrast T1-weighted images. Several studies report a correlation between clinical and MRI activity [Thompson *et al* 1991], [Filippi *et al* 1995d]. The frequency of active scans and active lesions increases shortly before and during clinical relapses [Willoughby *et al* 1989], [Smith *et al.* 1993]. During a spinal cord attack, most patients have active lesions in the spinal cord, 83% of patients at presentation with isolated spinal cord syndromes suggestive of MS have cord lesions consistent with the clinical manifestation. Conversely, 30-40% of the active lesions in the spinal cord are symptomatic [Wiebe *et al* 1992].

2.4 T1 weighted imaging, the assessment of hypo intensity and T1 relaxation time measurement

Although T1 relaxation is caused by an increase in free water (and only indirectly by matrix destruction), hypo intense lesions seen on T1 weighted images may represent more destructive lesions. In a study of 5 post mortem unfixed whole brains [van Walderveen *et al.* 1998] it was found that hypo intense lesions seen on T1 weighted SE MR images were associated histopathologically with more severe tissue destruction including axonal loss.

Hypo intense lesions were first described by Uhlenbrock and Sehlen [Uhlenbrock and Sehlen 1989] who noticed that hypo intense lesions were common in MS but less common in subcortical arteriosclerotic encephalopathy. They hypothesised that these lesions represented gliosis and axonal loss.

It has also been shown that the degree of hypo intensity in these lesions correlates significantly with MT ratios (see discussion of MTR in section 2.6), suggesting that increased lesion hypo intensity corresponds to more extensive breakdown in the macromolecular structure of myelin [Hiehle, Jr. *et al.* 1995], [van Waesberghe *et al.* 1997]. T1 hypo intensity is characteristic of 10 – 30% of MS lesions [Hiehle, Jr. *et al* 1995]. The term "black hole" is sometimes used to describe the hypo intense appearance of lesions on short TR/short TE spin echo images [van Walderveen *et al.* 1995]. Short TR/short TE images are commonly referred to as T1 weighted images, although the degree of T1 weighting is highly variable and very sequence dependent. Often on such images, white and grey matter can be almost iso intense, whereas they show greater contrast on more heavily T1 weighted images.

Compared with T2 lesion load, T1 lesion load (as measured on T1 weighted images) has shown a higher correlation with the Kurtzke EDSS score in patients with relapsing remitting or secondary progressive disease in a cross sectional analysis [van Walderveen *et al* 1995], [Truyen *et al.* 1990]. Recent longitudinal studies have shown annual increases of up to 20% in T1 hypo intense lesion volume in relapsing remitting and secondary progressive MS [Barkhof *et al.* 1998].

In secondary progressive disease, a relative increase in T1 lesion load (after three years of follow up) has been strongly correlated with disease progression ($r=0.81$, $p<0.001$). In these patients a larger percentage of T2 lesions was accompanied by hypo intense T1 lesions compared to relapsing remitting patients [Truyen *et al* 1996]. This could indicate that in secondary progressive disease the repair mechanisms fail, thus leading to an accumulation of more destructive lesions.

Hypo intensity is not an all or nothing phenomenon; in the fact that some lesions are nearly iso intense with CSF, and others are closer to grey matter signal. There is probably a range of severity of tissue destruction underlying these differences in appearance. The practical implication of these gradations of blackness is variation between observers in measuring black holes. The within observer variability for T1 hypo intense lesion (3-5%) is higher than that for T2 lesion load measured on proton density (PD) images (approx 2%) [van Walderveen *et al* 1995]. Another complication is that the hypo intense signal of CSF interferes with detecting and measuring adjacent hypo intense T1 lesions, and similarly "grey" holes close to the similar appearing grey matter may go undetected. Improvement of variability rates occurs when the lesions are first marked on hard copy images [Filippi *et al.* 1998a].

It has been noted that new enhancing lesions can be acutely hypo intense, especially when ring enhancing [Loevner *et al.* 1995b], [Nesbit *et al.* 1991]. Such acute hypo intense lesions are common, with 80% of new enhancing lesions being hypo intense. Over the course of subsequent months, these lesions either stay hypo intense or may revert to being iso intense [Lai *et al* 1996]. Such patterns might be consistent with the initial blackness representing

demyelination, this being followed either by remyelination (with a return to iso intensity) or persistent tissue destruction (with persistent hypo intensity). An alternative explanation for resolution in hypo intensity is the disappearance of oedema.

Lesion hypo intensity provides a MR parameter with higher histopathological specificity than T2 weighted images. The role of this simple parameter in monitoring treatment is less studied than T2 lesion load, although there is an indication that reduction in lesion load after interferon treatment, such as that found on T2, does not occur using T1 hypo intensity as a marker [Polman *et al.* 1995] [Hoogervorst *et al.* 2001]. Interferon beta has been found to have a stabilising effect on T1 weighted hypo intense lesion volume and to reduce its rate of increase [Gasperini *et al.* 1999], [Simon *et al.* 2000], [Hoogervorst *et al* 2001].

T1 relaxation time has previously been shown to illustrate changes in both lesions and NAWM [Stevenson *et al* 2000b]. T1 relaxation times are increased both in MS lesions [Larsson *et al.* 1988] and NAWM [Stevenson *et al* 2000b]. In using this sequence in patients with early disease, it is possible to further assess the changes in T1 relaxation time and the way in which these changes are related to other MRI abnormalities.

2.5 The measurement of MRI activity using Gadolinium enhancement

The intact BBB usually prevents the passage of Gd-DTPA into the CNS (so no enhancement is normally seen). MR imaging studies in MS have shown that disruption of the BBB occurs early in the development of new lesions [Kermode *et al.* 1990a, Barkhof *et al.* 1992b].

The presence of a new or enhancing lesion indicates that disease is active at the time the MRI is obtained, as enhancement in experimental acute encephalomyelitis and in MS is related to BBB damage associated with intense inflammation [Katz *et al.* 1993]. Gd-DTPA enhancement (see Chapter 2.1) may precede the onset of clinical symptoms [Kermode *et al* 1990a] and usually lasts for six weeks. The presence of new or enlarging T2 lesions also indicates that disease activity has occurred in the time that has elapsed between two consecutive scans.

The relationship between the frequency and extent of active lesions and long term evolution of the disease is unclear. Koudriavtseva [Koudriavtseva *et al.* 1997] reported that the presence of enhancing lesions is predictive of both clinical and MRI activity over the following six months. Stone [Stone *et al.* 1995a] found a modest correlation between the degree of clinical disability and the mean frequency of enhancing lesions over three months in a group of 68 patients with relapsing remitting disease. Losseff [Losseff *et al.* 1996a] found that the number of Gd enhancing lesions detected over a six month follow up correlated with clinical worsening five years later in patients with secondary progressive disease.

A comparison between the sensitivity of monthly un-enhanced and enhanced scans of the brain in detecting active MS lesions has shown that enhanced MRI is approximately twice as sensitive as un-enhanced MRI [Miller *et al.* 1993], whereas the combination of the two MRI techniques results in a sensitivity about 4 - 10 times higher than that of clinical measures [Miller *et al* 1988]. Finally, the number of active lesions detected by enhanced MRI is about 5-10 times higher in the brain than in the spinal cord [Thorpe *et al* 1996a] [Silver *et al* 2001].

More frequent scanning may increase the likelihood of detecting active lesions. However, this may be a minor issue as it has been reported that weekly scanning detects less than 10% more active lesions than monthly scanning [Lai *et al* 1996] at the expense of increased costs and patient discomfort [Silver *et al* 1997]. Increasing sensitivity of enhanced MRI can also be obtained by using thinner slices [Filippi *et al.* 1996d].

Imaging of the spinal cord is more difficult than the imaging of the brain. The cord has a small cross sectional area, and it lies at a depth of several centimeters from the surface. The spinal cord is also surrounded by CSF, which moves in a pulsatile manner and causes flow artifacts. Studies have shown that Gd-DTPA enhanced imaging of the spinal cord is helpful in the follow up of patients [Silver *et al* 2001], [Thorpe *et al* 1996a].

2.6 Magnetisation Transfer Imaging

Protons in tissues exist either as a "free pool" of mobile protons such as those in water and a "bound" pool of protons that are attached to proteins, other large macromolecules and lipid cell membranes. The former pool has a narrow spectral line (i.e. a relatively restricted resonance frequency range) and a long T2, so providing the majority of the signal detected with conventional imaging. The bound pool has a broader spectral line and a very short T2 (<1 msec) and is not "visible" on conventional MRI. MT utilizes the fact that magnetisation can be transferred between these two co-existing proton pools. After selectively saturating the bound water pool with an off resonance radio frequency pulse- "off" the resonance frequency of mobile water- the magnetisation in that pool is largely destroyed. Magnetisation

then "flows" to it from the free proton pool, resulting in a measureable loss of magnetisation, and therefore signal, from the latter.

MT images are derived from sequences that are acquired with and without the application of a prepulse to selectively saturate the broad spectrum resonance of immobile macromolecular protons. The magnetisation transfer ratio (MTR) measurements obtained are quantitative and highly reproducible.

The MTR is the percentage reduction in signal (from the free proton pool) as a result of the pre-saturation radio frequency pulse. The MTR of a given region is calculated using the formula:

MTR = (Mo – Ms) / Mo

where Mo and Ms are the mean pixel intensities of a region without and with pre-saturation, respectively.

The size of the MTR is an indicator of the amount and complexity of macromolecular structure present. The MTR value is dependent on the operating field strength and the pulse sequence, as well as the frequency, shape, amplitude and duration of the saturating pulse. Tissues or fluids that show little or no MT effect (such as CSF) have a low MTR. MTR images show white matter as areas of high signal (since the MTR is large due to dense macromolecular structures). The MTR for white matter is slightly greater than that for grey matter possibly due to the greater amount of myelin and thus a relatively greater bound proton pool.

Myelin is the most complex macromolecular structure in normal white matter and it has been proposed that the extent of demyelination in MS might be quantified by measuring MT ratios. Direct pathological confirmation of this is lacking, but experimental studies [Lexa *et al.* 1994]have demonstrated large reductions in MTR where there is loss of myelin or Wallerian degeneration (in the latter situation an early rise in MTR is followed by a fall). In central pontine myelinolysis, a pure demyelinating disorder, MTR is seen to decrease [Silver *et al.* 1996]. Lower MTR values have also been reported in MS lesions that are hypo intense on T1 weighted images [Hiehle, Jr. *et al* 1995], the latter appearance probably indicating a longer T1 relaxation time [Loevner *et al.* 1995a].

In MS, NAWM has a reduced MTR, but the reduction is small and is probably pathologically non-specific. It is apparent that MT may increase the pathological specificity of MR [Lai *et al.* 1997], [van Waesberghe *et al.* 1999] but not to a perfect degree.

MTR therefore has the potential to provide an insight into changes both in lesions and normal appearing brain tissue in patients with MS. It provides more pathological specificity than more conventional MR techniques, and is useful in the long term radiological follow up of patients. By using both histogram and region of interest approaches, it may be possible to gain an insight into the pathological mechanisms underlying both the development of the disease and the progression to irreversible disability.

2.7 Diffusion Tensor Imaging

Molecules in a fluid system are subject to continuous random motion (diffusion). Water molecules within the brain may encounter structures that impede their motion in a particular direction. These structures include the myelin sheath, axonal membranes and subcellular organelles. In this case the diffusion is "hindered" and the value measured is known as the "apparent" diffusion coefficient (ADC). MR images can be sensitised to diffusion by means of large magnetic field gradient pulses, allowing a non-invasive measurement of the ADC. Disruption of the permeability or geometry of structural barriers by pathology alters the diffusion behaviour of water molecules.

Some biological tissues such as brain white matter contain oriented barriers to diffusion. This leads to a property known as anisotropy, and results in a variation in the measured diffusion with tissue direction. White matter fibre tracts consist of collections of similarly aligned myelinated axonal cylinders. Diffusion is much greater along these fibre tracts than across them, due to directional structures including the myelin sheath, axonal membranes, and the neurofilamentous cytoskeleton [Beaulieu 1994]. This directionality of water diffusion causes fibre tracts to exhibit anisotropic diffusion. Anisotropy is of interest as it is likely to be affected by pathological damage to white matter tracts.

Accurate quantification of diffusion anisotropy requires complete characterization of molecular motion in three directions. The most complete description is provided by a mathematical quantity (a nine element matrix) known as the diffusion tensor [Basser1994]. Measurement of the diffusion tensor is usually performed using echo-planar imaging (EPI) and the diffusion is measured using at least six directions of measurement. From the

diffusion tensor, measures of both the degree and direction of the diffusion throughout the brain may be quantified [Basser 1996], [Pierpaoli *et al* 1996]. The diffusion tensor has an advantage over simple ADC measurements due to the fact that it is rotationally invariant and independent of patient position or data sequence acquisition.

From the tensor a number of other quantities can be calculated including various anisotropy measures. In white matter, water molecules diffuse preferentially in the direction parallel to axons, being restricted in perpendicular directions [Pierpaoli *et al* 1996]. This property, termed diffusion anisotropy, may be quantified by two indices: the Fractional Anisotropy (FA) [Basser *et al* 1996] and Volume Ratio (VR) [Pierpaoli *et al* 1996]. FA increases with anisotropy and provides the most detailed spatial depiction of anisotropic areas. VR decreases with anisotropy and provides the strongest contrast between low- and high-anisotropy areas, but with decreased anatomical detail. In general, whereas white matter has an oriented microstructure due to similarly aligned fibre tracts with high anisotropy, gray matter is characterized by less ordered tissue and relatively low anisotropy.

Mean Diffusivity (MD) [Basser *et al* 1996] is another tensor index that measures the magnitude of water diffusion in the tissue without regard to its directonality. MD would therefore not be expected to discriminate between white and gray matter. Cellular structures in the CNS restrict water molecule motion. Pathological processes that modify size, shape and integrity of water filled spaces can result in increased diffusivity. Focal oedema is one of the most prominent changes occurring in acute MS lesions, and might increase diffusivity by reducing the amount of structural barriers to water molecule motion.

Previous studies have also shown an increase in ADC [Larsson *et al.* 1992b], [Christiansen *et al* 1993] suggesting a net loss of structural barriers to water motion in MS plaques. Inflammation, oedema, blood-brain barrier leakage and axonal loss may all contribute to the development of a high ADC in new enhancing lesions [Katz *et al* 1993], [Trapp *et al* 1998].

Initial diffusion studies reported higher water diffusion in MS plaques compared to NAWM, and also found evidence that early plaques had the highest diffusion values. The NAWM in MS patients had higher diffusion than normal control white matter. Early studies were limited by head motion, which is an inherent problem in diffusion imaging, and an inability to measure diffusion in more than three directions. Diffusion Tensor Imaging (DTI) overcame these problems by being theoretically independent of the position of the patient and the pulse sequence used [Basser 1996].

DTI characterizes the microstructural organization of brain tissue in a way that is not possible with other MRI techniques. However, experimental and postmortem studies correlating the DTI changes and histopathology in MS are needed to establish the pathologic basis of the diffusion MRI findings. DTI is likely to illuminate further the pathophysiological changes occurring within MS lesions, particularly when employed in longitudinal studies of lesion evolution together with other MRI techniques including NAA spectroscopy and magnetization transfer imaging. Future studies should examine the relationship of DTI changes to clinical deficit, and the natural history of diffusion changes within lesions.

Previous studies have shown fractional anisotropy (FA) to be significantly lower and mean diffusivity (MD) significantly higher in patient NAWM [Werring *et al* 1999], [Christiansen *et*

al 1993], [Larsson *et al* 1992b]. Structural abnormalities of NAWM may play a part in the development of irreversible disability in MS. Studies using spectroscopic measurements of NAA have suggested that axonal loss occurs in patient NAWM [Davie *et al.* 1997] and that this may contribute to progressive disability [Fu *et al* 1998]. Axonal loss can be expected to show a decrease in FA and an increase in MD.

One study has found that NAWM subsequently involved by enhancement show significantly increasing diffusivity starting up to six months before enhancement appearance [Werring *et al* 2000]. This suggests that dynamic events, which are closely and temporally related to lesion development, play a part in the changes seen in NAWM before lesion development.

By using DTI to study both lesions and normal appearing brain tissue in patients with early relapsing remitting MS, I hope to evaluate changes in anisotropy and diffusivity at an early stage, and to assess their development over time, as well as their relationship to clinical disability.

2.8 Assessment of normal appearing brain tissue (NABT)

2.8.1 Introduction

Pathological abnormalities found in the NAWM from patients with MS have the potential to modify the relative proportions of mobile and immobile protons of the diseased tissue. As a consequence, they can determine a decrease of the MTR from the white matter outside MS lesions visible on conventional MR images [Loevner *et al* 1995a], [Filippi *et al.* 1998c], [Filippi *et al* 1995a]. The MTR values of the NAWM away from, or around [Filippi *et al*

1995a] T2 visible lesions from patients with secondary progressive MS are lower than those of the corresponding NAWM regions of patients with less disabling relapsing remitting disease. However, these studies evaluated only a small part of the NAWM, whereas histogram analyses can provide an overall assessment of the cerebral tissue separate from T2 visible lesions.

Four longitudinal studies have looked at whether NAWM changes precede the formation of new enhancing lesions in MS [Pike *et al.* 2000], [Filippi *et al.* 1995a], [Goodkin *et al.* 1998], [Silver *et al* 1998]. Three of these studies appeared to show that this was the case: [Pike *et al* 2000], [Filippi *et al* 1995a], [Goodkin *et al* 1998], but the dynamics and nature of these changes are still unclear. Thus, by further studies such as this, in patients with early disease, it will be possible to further define the nature and time course of these early changes.

2.8.2 Region of interest (ROI) approach

Typically, mean MTR, T1 relaxation time and DTI values are collected from white matter lesions and small ROIs in NAWM [Gass *et al* 1994],[Lai *et al* 1997], [Dehmeshki *et al.* 2001b], [Ciccarelli *et al* 2001] and increasingly in NAGM [Griffin *et al* 2001]. Using a manual local thresholding technique [Plummer 1992] it is possible to identify lesions using a semi-automated method. By choosing a ROI in NABT, it is possible to study a particular anatomical area in greater detail. However, one of the potential drawbacks of this technique is that inter and intra rater variability depends on the exact placement of the ROI in exactly the same area on each image.

2.8.3 Histogram analysis

Histogram analysis allows evaluation of data from all the pixels of brain tissue, thus providing a complete assessment of macro- and microscopic disease burden in MS. MTR histogram derived measures from MS patients are different from those of healthy controls [Filippi *et al.* 1999]. In whole brain histograms, it is difficult to disentangle the relative contributions of changes occuring within or outside the MS lesions visible on conventional MR images.

A global approach based on histograms has been used to more accurately represent occult disease in NAWM [van Buchem *et al.* 1997], NAGM [Dehmeshki *et al* 2001], [Ge et al 2001] or NABT (NAWM and NAGM combined). Characteristics of whole brain histograms have also been correlated with cognitive and neuropsychological test results [van Buchem *et al* 1997] and have been used to evaluate changes in response to treatment [Richert *et al.* 1998].

In order to study NABT using a histogram approach, it is necessary to segment the images and remove both CSF and lesions. This can be done using a number of methods, including statistical parametric mapping (SPM) [Ashburner and Frintin 1994]. In my study using T1 relaxation time histograms (discussed in Chapter 6) I have used SPM 99 followed by a thresholding technique to extract brain tissue, thereby obtaining isolated whole brain tissue images.

SPM creates a probability map for brain tissue (after combining white and grey matter), CSF and "other" tissues. In order to extract brain tissue and reduce partial volume effects, only

white matter and grey matter voxels above 75% confidence (probability) were selected in my study. I also examined each image in order to ensure that there had been accurate removal of CSF. In order to obtain NABT histograms, lesion masks were then applied to the whole brain images, so that the lesions were excluded from the images.

Six histogram features are usually extracted from each normalised and smoothed histogram. For example, using T1 histograms: mean T1 measurement, peak height, peak location, MTR measurement value of the 25^{th}, 50^{th} and 75^{th} percentiles are all extracted. A T1 histogram of whole brain and NABT is calculated for each subject. Each histogram is normalised to the residual brain tissue volume by dividing the number of counts in each sampling bin by the total number of voxels. Thus the total area under the histogram is fixed at unity and the normalised histogram is therefore a frequency distribution.

There are, however, problems associated with this method. Using an automated segmentation technique, misclassification of lesions can occur, especially in patients with larger lesion loads. This can be overcome by careful manual inspection of the images and correction of any misclassification. Partial volume effects due to CSF and atrophy also need to be addressed.

In summary, there are various different techniques that can be used for the assessment of NABT. Each method has advantages and allows either the examination of anatomically distinct sites, or a more global assessment of tissue damage. Each method also brings with it its own challenges. By using both methods in a multiparametric MRI study such as this, it

should be possible to improve tissue characterisation due to the complementary nature of the two approaches.

Chapter 3 Study design and initial clinical and MRI lesion data

3A Study design

3A.1 Introduction

This thesis forms part of an ongoing prospective longitudinal natural history study. The study uses radiological, immunological and clinical methods to assess disease activity and progression in a cohort of patients with early relapsing remitting MS. The primary aim of the study is to use surrogate markers of disease activity to further examine the temporal relationship between the pathological processes that occur at an early stage in the disease. Other aims are to assess the roles of MRI, immunological and clinical markers in the prognosis and long term follow up of patients with relapsing remitting MS. One of the many problems with the long term follow up of these patients at present is the lack of definitive prognostic markers, and the lack of sensitive and specific markers of disease activity. A sensitive and specific marker of disease activity at any given point in time, and a prognostic indicator for long term disability would certainly add greatly to the role of MR imaging in the long term follow up of this disease.

As previously discussed in Chapter 1, it is known that axonal damage and loss [Trapp *et al* 1998], demyelination [Ferguson *et al* 1997] and gliosis [Larsson *et al.* 1989] all occur in the disease process. The exact stage in the disease evolution that these pathologies occur and their relationship to each other is less clear. Some previous studies looking at patients with clinically isolated syndromes (using a region of interest approach) have shown no significant changes in the NAWM of patients using MTR and MR spectroscopy, even though lesions may be present in patients at this stage [Brex *et al* 1999], [Brex *et al* 1999]. However,

another study has reported subtle MTR abnormalities in the normal appearing brain tissue (NABT) of CIS patients [Iannucci et al 2000]. Therefore the point at which changes begin to occur in the disease process is still open to debate.

Early changes in NABT are of interest, as they may have an important clinical impact. There is, however, a limited correlation between MRI lesion findings and clinical state. This may, in part, be due to the fact that there are changes in the NABT that are contributing to clinical disability, but that are too subtle to be detected using present MR techniques. By further examination of the early pathological events, it may also be possible to find pathological targets for new therapeutic strategies at a stage of the disease when the potential benefits are greatest.

3A.2 MRI

The imaging sequences used in this study are: DTI, MTR, chemical shift imaging (CSI), brain and spinal cord volume measurement, T1 weighted imaging of the brain and spinal cord pre and post triple dose Gd-DTPA (0.3mmol/Kg) using delayed imaging (20 minutes for brain and 35 minutes for spinal cord imaging) and FSE (proton density (PD) and T2 weighted imaging of the brain). PD and T1 weighted gradient echo images are also acquired, with the subsequent calculation of the T1 relaxation time maps [Parker et al 2001]. In this thesis, I will report the findings of DTI, MTR, T1 relaxation time measurement and brain and spinal cord T1 weighted imaging pre and post Gd-DTPA.

Patients were studied at baseline and six monthly thereafter, with additional T1 weighted imaging of the brain and spinal cord pre and post triple dose Gd-DTPA (0.3mmol/Kg) at

months 1, 2 and 3. The initial length of follow up of the cohort will be three years. All imaging was performed on a 1.5 Tesla Signa system (GE Medical Systems, Milwaukee, WI). Brain MRI was carried out using a standard quadrature head coil. Pilot scans were performed prior to each scan to ensure accurate repositioning of the patient according to previously defined rules [Gallagher *et al.* 1997] and all scans were of 5mm thickness and obtained in the axial plane. This was to aid the direct comparison of the different imaging techniques, and to allow potential co-registration of the different data sets in order to allow accurate cross sectional and longitudinal analyses. With a larger slice thickness, smaller lesions may go undetected [Filippi *et al.* 1998b], although acquisition of thinner slices is limited for 2D imaging by scan time [Molyneux *et al.* 1998b]. All acquisitions were thus kept at a comparable slice thickness. The following sequences were obtained:

3A.2.1 Fast spin echo (FSE)

Images consisting of T2 weighted and proton density (PD) weighted images (TR = 3030 ms, TE = 19/90 ms, 28 contiguous 5mm axial slices) were acquired. This sequence was acquired in order to identify lesions and measure total T2 lesion load. Studies using serial T2 weighted images have demonstrated that the disease is often active in the absence of clinical symptoms and relapses. Disease activity in patients with relapsing remitting MS is seen on average up to 10 times as frequently as a clinical relapse [Willoughby et al 1989], [Barkhof *et al* 1992a], [McFarland *et al.* 1992], [Stone *et al.* 1995b]. By using T2 and PD weighted images, it is possible to assess disease activity radiologically, and compare this with clinical evidence of activity. The acquisition time for this sequence was 15 minutes.

Figure 3.1 Fast spin echo sequence. Proton density image showing multiple white matter lesions in a patient with MS

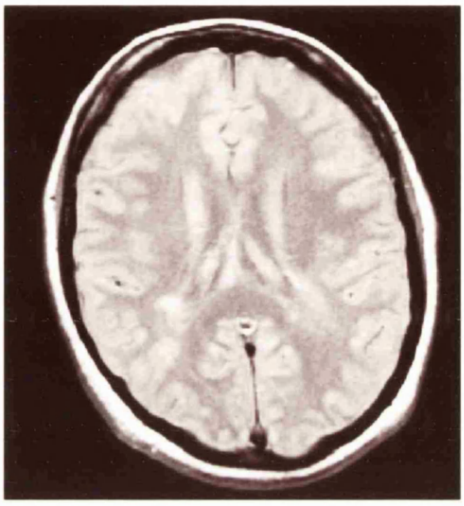
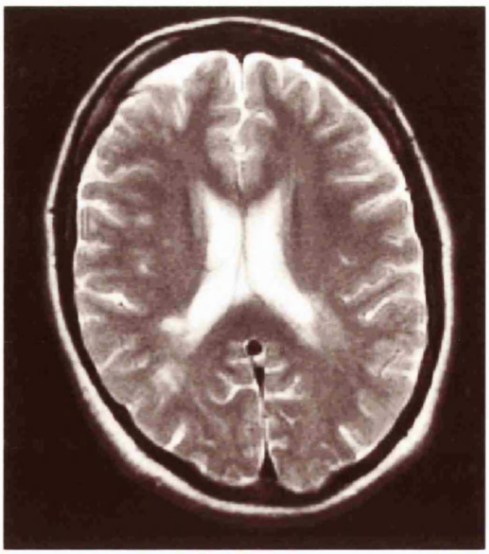


Figure 3.2 Fast spin echo sequence. Corresponding T2 image showing multiple white matter lesions in a patient with MS



3A.2.2 T1 imaging pre and post Gadolinium

2D T1 weighted images (TR=600ms, TE=17ms, 28 contiguous 5mm axial slices) of the brain and spinal cord were acquired pre and post administration of triple dose (0.3 mmol/Kg) Gd-DTPA. A delayed imaging technique was used for both the brain and the spinal cord (the brain was imaged 20 minutes after the administration of Gd-DTPA, and the spinal cord 35 minutes afterwards).

Previous studies have shown that the use of higher doses of Gd-DTPA may be beneficial for improved detection of enhancing lesions [Filippi *et al* 1996b]. It has also been shown that the combination of triple dose Gd-DTPA and delayed imaging increases the total number of enhancing lesions detected by up to 154% [Silver *et al* 1997]. The higher dose of Gd-DTPA does not seem to be associated with more side effects than the standard dose of 0.1mmol/Kg. The mechanisms whereby higher contrast-medium doses and delayed imaging result in improved detection are likely to rely on increased concentration of gadolinium chelates within lesions, resulting in an increased rate of T1 relaxation (i.e. a shorter T1 relaxation time, and a higher signal on T1 weighted images). These sequences allow the assessment of disease activity by assessing both number and volume of lesions.

Gd-DTPA images have become a useful marker of disease activity in the context of clinical trials [Miller *et al.* 1991], [Paty 1993]. A close relationship has been shown to exist between the number and volume of enhancing lesions. The frequency of Gd-DTPA enhancement correlates with clinical relapse activity [Smith *et al* 1993], [Molyneux *et al.* 1998a] and may

predict long term disease evolution [Giovannoni *et al.* 1997c], although the latter is still uncertain.

Alterations in the blood-brain-barrier (BBB) are thought to occur at an early stage in the disease process, and the presence of GD-DTPA enhancement correlates with BBB breakdown and inflammation [Kermode *et al* 1990a], [Hawkins *et al.* 1991]. Many enhancing lesions are evident on T1 weighted images before they are seen on un-enhanced T2 weighted images.

Spinal cord disease is clinically expressed more often than that affecting the brain [Kidd *et al* 1996], although the frequency of new spinal cord lesions is less than that for brain lesions [Thorpe *et al* 1996a]. Spinal cord imaging may therefore be valuable in the long-term follow up of MS patients and is also a very important initial diagnostic test.

Previous longitudinal studies have followed patients over time using spinal cord imaging. Capra [Capra *et al.* 1992] studied patients over 3 months. Thorpe [Thorpe *et al* 1996a] studied a group of 10 patients with relapsing remitting MS within 5 years of disease onset (mean disease duration 3 years). In this study, enhancing brain lesions were ten times as common as enhancing cord lesions, and there were many more active brain lesions than clinical relapses. 31% of the enhancing cord lesions produced symptoms. New spinal cord lesions were less likely to enhance (61%), particularly in the thoracic region (30%) than new brain lesions (94%) and unlike brain lesions never demonstrated enhancement on more than one study. This may be a sensitivity issue, with the greater technical difficulty involved in

obtaining high quality artefact free T1 weighted images of the spinal cord, and also the relatively small size of the spinal cord lesions.

In one recent study [Silver *et al* 2001], when using single dose Gd-DTPA (0.1mmol/Kg) the ratio of the number of enhancing cord versus brain lesions was relatively high (1:4) when compared to previous studies [Kidd *et al* 1996], [Thorpe *et al* 1996]. This may partly be a reflection of the differences that occur by chance between relatively small patient cohorts. It may also reflect an increased sensitivity of the technique using thinner slices (3mm) and a longer post-contrast delay (30minutes).

In summary, by using both brain and spinal cord and triple dose Gd-DTPA enhanced images, we are able to assess disease activity in a sensitive manner in this early cohort, and observe the changes in enhancement over time. This allowed a sensitive assessment of BBB leakage and inflammation in the CNS.

Figure 3.3 Pre Gd-DTPA T1 weighted image of the brain showing a T1 hypo intense lesion in a patient with MS

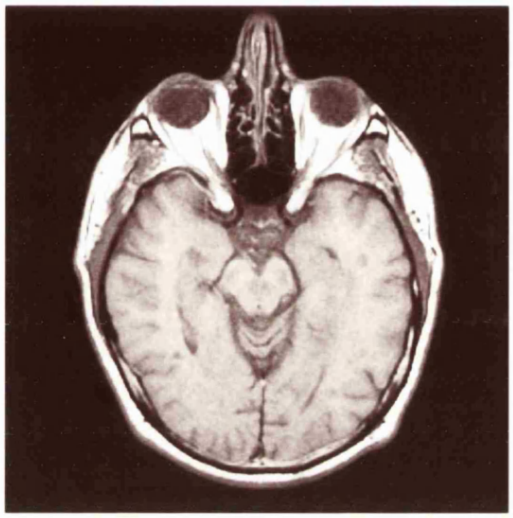


Figure 3.4 Corresponding post Gd-DTPA T1 weighted image of the brain showing Gd-DTPA enhancement of the hypo intense lesion

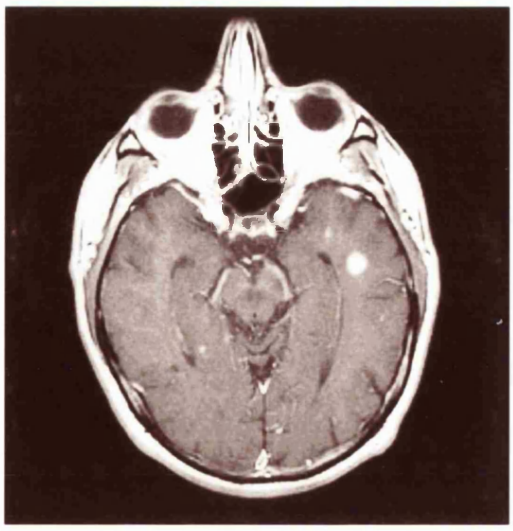


Figure 3.5 Pre Gd-DTPA T1 weighted image of the spinal cord in a patient with MS

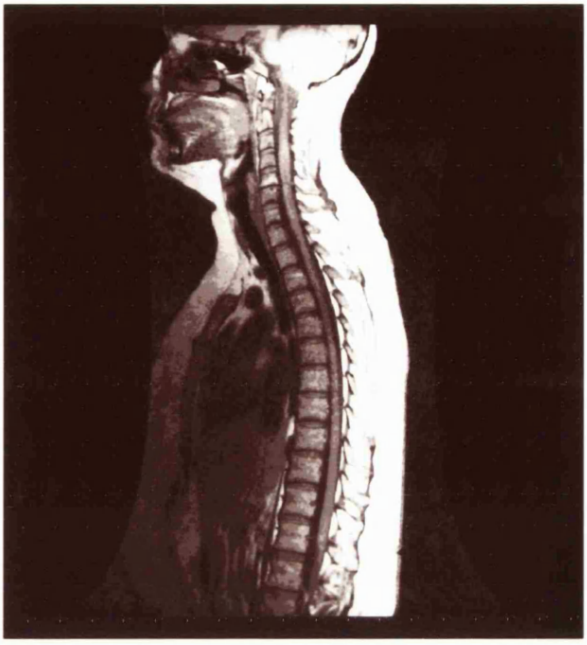
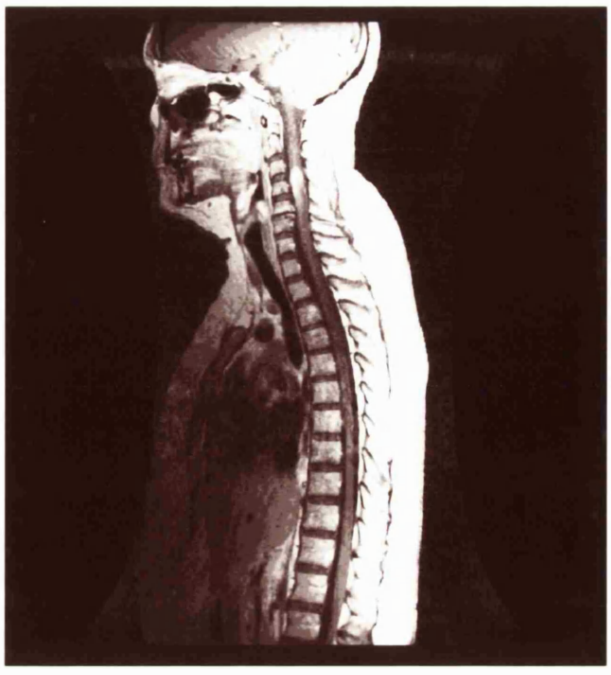


Figure 3.6 Corresponding post Gd-DTPA T1 weighted image of the spinal cord showing a Gd-DTPA enhancing lesion which is not visible on the pre-contrast images



3A.2.3 Magnetisation Transfer Imaging (MTI)

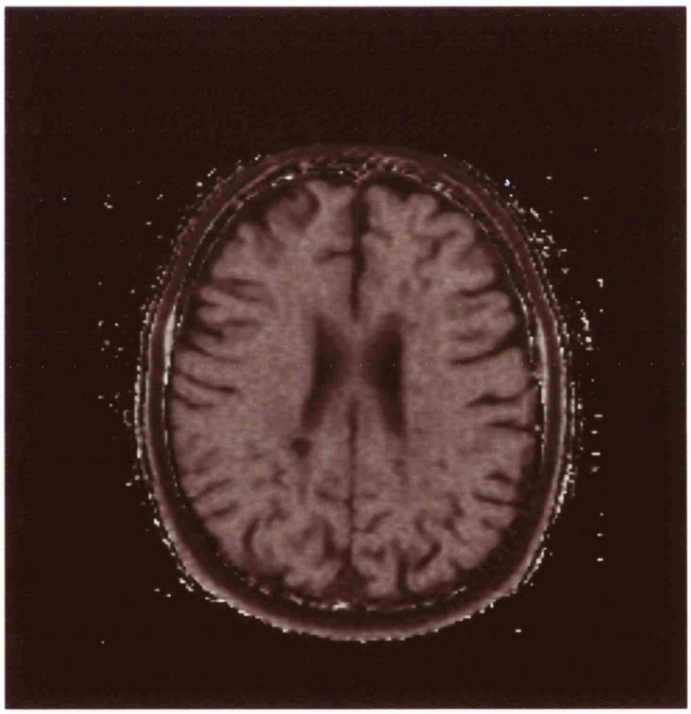
MTI was performed using a spin echo sequence (TR=1500ms, TE=19/90 ms, 28 contiguous 5mm axial slices). This was performed both with and without presaturation pulses - the former to saturate the broad resonance of immobile macromolecular protons. This was adapted from a previously described sequence [Barker *et al* 1996]. To ensure exact co-registration of the pixels on saturated and unsaturated images, scans with and without presaturation were interleaved for each TR period. In all subjects MTR was calculated from the short echo time (TE=19ms) data on a pixel by pixel basis from the formula:

{[Mo-Ms]/[Mo]} x 100 percent units (pu)

Mo and Ms represent the signal intensities without and with the saturation pulse respectively.

In this study, MTR provided a robust quantitative measure of tissue integrity in NAWM and NAWM. Marked reductions in lesion MTR are a potential marker of demyelination, but were not evaluated in the present study.

Figure 3.7 Magnetisation transfer sequence. Calculated magnetisation transfer image showing multiple white matter lesions in a patient with MS



3A.2.4 T1 relaxation time measurement

This protocol required the acquisition of two gradient echo data sets at different repetition times, to provide a PD weighted image and a heavily T1 weighted image. The acquisition parameters were (TR/TE/flip angle/number of averages): 1500ms/11ms/45°/2 and 50ms/11ms/45°/8 for the PD- weighted and the T1- weighted acquisitions respectively. Slice thickness was 5mm and the acquisition time 20 minutes.

T1 was determined from these images after accounting for the effects of head coil non-uniformity and excitation pulse profile [Parker *et al* 2001]. The accuracy and precision of the relaxation time measurement has been previously assessed with the aid of a quality assurance programme developed at our site [Stevenson *et al* 2000]. This involved repeated imaging of gel standards to assess the mean accuracy (the modulus of the percentage difference between the measured relaxation time and the nominal value, averaged over all measurements and samples) and the mean systematic error (the percentage difference between the measured relaxation time and the nominal value, averaged over all measurements and samples). The mean accuracy and the mean systematic error of the T1 measurement protocol were 5.42% and 1.23% respectively. The coefficient of variation in T1 within the scanning region was less than 5%.

T1 relaxation times are increased both in MS lesions [Larsson *et al* 1988] and NAWM [Stevenson *et al* 2000]. In using this sequence in patients with early disease, it is possible to further assess the changes in T1 relaxation time and the way in which these changes are related to other MRI abnormalities. By using techniques such as T1 histogram analysis, it is also possible to gain a more global assessment of changes in the normal appearing brain

tissue. Although the region of interest approach enables the study of anatomically specific sites, the use of histograms allows a much larger tissue sample to be studied.

Figure 3.10 Heavily T1 weighted image used in the calculation of T1 relaxation time maps showing multiple white matter lesions in a patient with MS

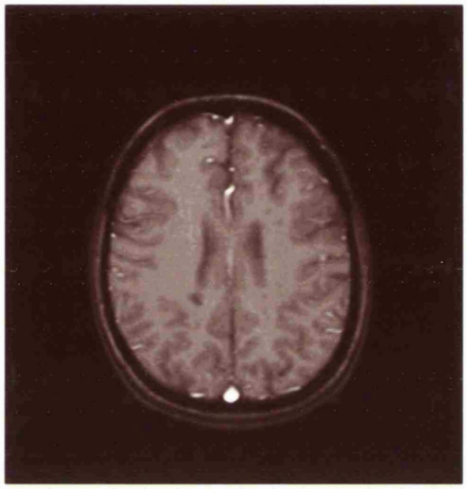


Figure 3.11 Corresponding proton density weighted image used to calculate T1 relaxation time maps showing multiple white matter lesions in a patient with MS

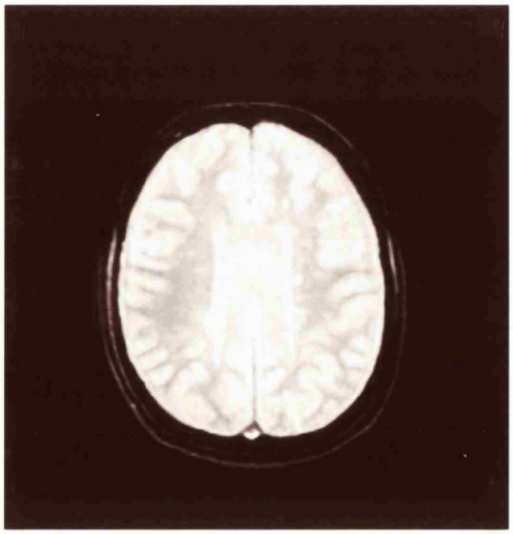
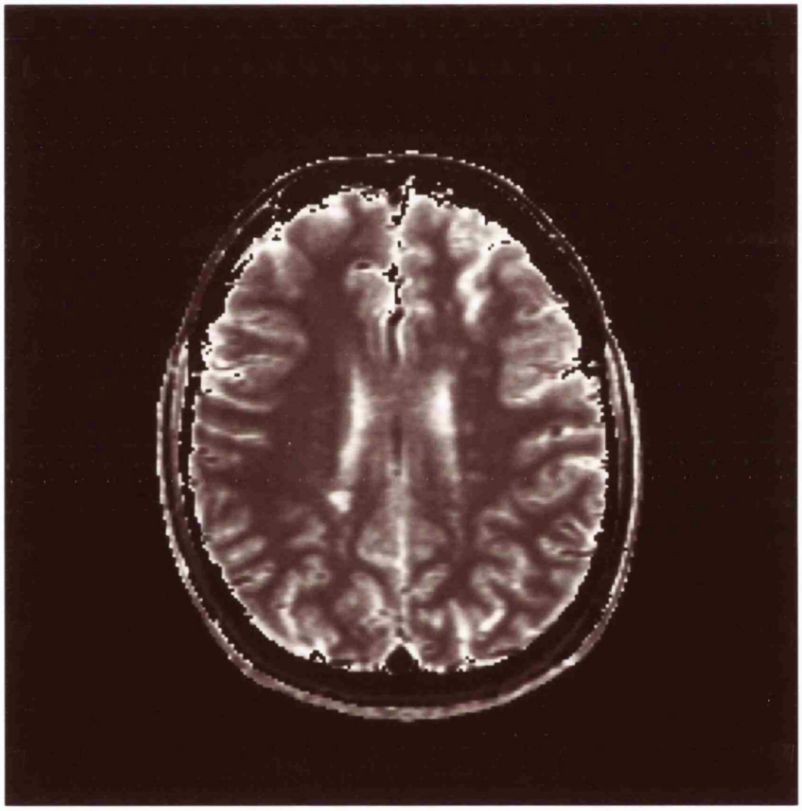


Figure 3.12 Corresponding T1 relaxation time map showing multiple white matter lesions in a patient with MS



3A.2.5 Diffusion Tensor Imaging (DTI)

The diffusion protocol consisted of 3 series of 7 interleaved slices, acquired with a single shot Diffusion Weighted Echo Planar Imaging (DW-EPI) sequence. Twenty one 5mm contiguous slices were compiled into a single file after reconstruction. The DW-EPI parameters were: TE 78ms, matrix size 96x96, FOV 240mm x 240mm and 4 b values, increasing linearly with G^2 (G=gradient amplitude) from 0 to 700s mm^{-2}, applied in each of seven non-collinear directions. The acquisition time for this sequence was 40 minutes.

In the presence of large diffusion gradients, on-line time domain averaging can cause disruption of the averaged signal due to large phase changes between successive shots. To abate this, a minimum of four signal averages was therefore collected and stored separately for subsequent off line averaging. The resulting magnitude images were averaged after reconstruction to improve the signal to noise ratio (SNR). Cardiac gating was also used to reduce motion artifact due to the pulsation of blood and cerebrospinal fluid.

DTI allows the assessment of fibre tract integrity, and allowed the investigation of early changes in MS, both in NAWM and in NAGM. Recent studies have shown abnormalities in the basal ganglia of patients with more advanced disease [Ciccarelli *et al* 2001] and diffusion abnormalities have been linked to the cognitive changes seen in established MS [Filippi 2000]. By using DTI in this cohort of patients, it enables the study of early changes in fibre tract integrity, and also the comparison of DTI abnormalities with clinical outcome measures.

3A.2.6 Lesion identification methods

Patients had conventional spin echo Proton Density (PD) and T2 weighted images (TR 2000ms, TE 30ms/120ms; matrix size 256x256; FOV 240mm; 28 contiguous axial slices of 5mm thickness) acquired prior to DTI. Lesions were initially identified on the hard copy images of the PD and T2 sequences. Lesions are conventionally marked on the PD image of the FSE with reference to the T2 image of the same sequence. Lesions were then contoured using a semi automated local thresholding technique [Plummer 1992]. This uses a contour approach [Grimaud *et al.* 1996]. This technique requires input for lesion identification, and then uses an algorithm to delineate lesion boundaries according to the local intensity environment. Manual editing is sometimes required to modify part of the boundary of poorly defined lesions, or to occasionally outline fully lesions where the algorithm fails. Consensus guidelines for the application of the contour method have been published [Filippi *et al* 1998a] and were used as the guideline for contouring lesions in this study.

3A.2.7 Assessment of NABT

I have used both a region of interest (ROI) and a histogram approach in the study of NABT in these patients. A ROI approach allows the study of anatomically eloquent sites, but histogram analysis allows a much larger area of tissue to be sampled, as a more global measure of tissue involvement (see chapter 2.6.2).

3A.3 Clinical scores

The clinical assessment of patients with MS is an essential part of the assessment of the progression of disease. However, all the clinical scales presently in use have limitations. The

EDSS is not an ordinal scale, and has poor inter and intra rater variability. The MSFC is a newer clinical scale which has been developed in order to try to address some of these issues. However there are a limited number of studies available that specifically look at the MSFC and its various components, either over time or in relation to MR measures [Kalkers *et al* 2001a].

By using both a well established (EDSS) and a newer (MSFC) clinical outcome measure, it is possible to evaluate and compare the two measures, both in a cross sectional and a longitudinal setting. Clinical outcome measures are still the primary outcome measure in definitive phase III treatment trials, and it is important to evaluate newer outcome assessments in patients with early disease. Present and future treatments are likely to be directed towards suppressing the disease early on, and may be therefore directed at patients with minimal disability.

This cohort of patients has also been assessed using subjective measurements in the form of questionnaires. The questionnaires I have used are the Queen Square Hospital Disability Status Scale, the Modified Fatigue Impact Scale and the 36 item Short Form Health Survey. By performing subjective assessments of disease activity as well as objective assessments, it is possible to further monitor disease activity over time.

The Queen Square Disability Status Scale is a newly developed clinical scale that uses both acute and chronic assessments of disease. It assesses the impact of disease on the activities of daily living, as well as assessing the long-term issues. It is hoped that it may prove to be a sensitive and specific assessment of disability over time.

The SF36 is a more widely used subjective clinical scale, and it assesses short term disease impact [Freeman *et al.* 2000]. The Modified Fatigue Impact Scale [Fisk *et al.* 1994] assesses fatigue and the impact on daily life. It may be able to compare the newer techniques to the more established scores, in order to rationalise the subjective assessment of patients, both in research and in clinical practice. The subjective data collected in the form of questionnaires will be assessed at the end of the study, when all the data has been collected and it will therefore be possible to assess the ability of the various questionnaires to detect change over time.

The data available from these questionnaires will not be discussed further in this thesis. This is because the complete data sets are not available at present, and in order to fully analyse the questionnaire data it will be necessary to compare data from several different longitudinal time points.

A careful general medical and neurological history was also obtained from all patients at each visit, with special reference to relapses. A relapse was defined as the occurrence of a symptom of neurological dysfunction that lasted for more than 24 hours [Poser *et al* 1983] and a remission was defined as an improvement in signs or symptoms, or both, that lasted for at least one month. A full neurological assessment was also performed at each visit. If a relapse had occurred between visits, this was documented from a clear history given by the patient, as there may have been no new clinical signs on examination.

Healthy normal controls were also studied. A full general medical and neurological history was taken from all controls, and the FSE images were reviewed by a consultant neuroradiologist (Dr K Miszkiel) in order to exclude any abnormalities. Any normal controls with neurological abnormalities either clinically or radiologically were excluded from the study. Normal controls were recruited in order to match the age and sex distribution of the patient cohort. The normal controls are presently being studied using an identical MRI protocol to that used in the patients except for the use of Gd-DTPA. The controls are also being studied at identical time points. Healthy normal control data acquisition is vital in a longitudinal study such as this. It is necessary in order to examine the stability of the MR measurements over time, and also to compare patient data at each time point to that of healthy age and sex matched control subjects.

3A.4. Immunological assessment

The immunological markers of disease activity assessed in this study consist of both serum and urine markers of inflammation. The serum markers are soluble adhesion molecules (SVCAM and SICAM) [Khoury *et al.* 1999], tumour necrosis factor alpha [Martino *et al.* 1997] and nitric oxide metabolites (nitrates and nitrites) [Giovannoni *et al.* 1997a]. Serum is obtained at each MRI visit, at months 0, 1, 2, 3, 6 and 6 monthly thereafter. Urinary assessment consists of measuring neopterin and free light chain levels. Urine samples are collected once a week by the patients throughout the study. They are stored in a home freezer and analysed at 6 monthly intervals. These inflammatory markers are raised in demyelination, and by measuring them over time, it will be possible to further investigate their role as sensitive markers of acute inflammatory activity in MS.

Increased levels of serum nitric oxide metabolites have been observed in patients with MS [Giovannoni 1998]. Inflammatory markers such as neopterin also increase during both relapses and acute infections [Giovannoni *et al.* 1997b]. The difference is that the increase due to acute infections tends to be intermittent and short lived, but the increase due to acute neurological relapses tends to be of a longer duration. Soluble adhesion molecules may also be increased in acute relapse [Giovannoni *et al* 1997c]

By measuring these inflammatory markers over time, it will be possible to assess their future role as prognostic indicators of future disability, and their role in the long term monitoring of patients with MS [Giovannoni *et al.* 1998]. MRI and immunological correlations will assist in understanding the immunopathological events occurring in early relapsing remitting MS. The inflammatory markers being measured in this study will be analysed at the end of the study, when all the longitudinal data is available. As such, I will not report the results here.

3A.5. Recruitment

All patients have been recruited from the National Hospital for Neurology and Neurosurgery, Queen Square, London and have given their written informed consent to participate in the study. The study has been approved by the joint ethics committee of the National Hospital for Neurology and Neurosurgery and the Institute of Neurology, London. All patients were within 3 years of initial neurological symptom onset at the point of recruitment, and were aged between 18 – 60 years when recruited. No patients had received disease-modifying treatments such as beta interferon prior to enrolment in the study. Over the course of the three years of the study, some of the patients in the cohort will commence treatment with beta-Interferon. These subjects will then form a separate sub-group, and will enable the

subsequent evaluation of MRI, clinical and immunological evaluation while on Interferon treatment.

Patients with relapsing remitting MS have often had disease for more than 3 years, and as such many patients who had been referred for entry into the study were found not to be suitable as they had experienced neurological symptoms over a longer time period. The recruitment of patients at this early stage for longitudinal, prospective natural history studies brings its own challenges. Patients who are minimally disabled often have difficulty with compliance in longitudinal studies, especially those that are time consuming. This is primarily because they have work and family commitments.

The total scanning time for each 6 monthly visit in this study was 4 hours. The clinical assessment took a further hour. Therefore, each patient made two visits within one week at each six monthly time point, so that the length of each visit was made more tolerable. Each patient also had breaks within the scanning protocol, depending on the individual patient needs. Great care was also taken in order to cause minimal disruption to the daily routine of both the patients and the control volunteers. This involved careful planning to fit appointments around work schedules and other family commitments such as collecting children from school. All patients and control subjects have tolerated the scans extremely well, and of the original 31 patients studied at baseline, 27 are in long term follow up. The patients who did not continue with the study dropped out because one patient became too disabled to tolerate the scans, one went on a long traveling holiday, and two found the journey to the hospital too difficult as they lived a long distance from the Institute.

Table 3.1 Study entry and exclusion criteria

Entry criteria

- Age 18 – 60 years old.
- Within 3 years of first neurological symptom.
- EDSS score less than or equal to 3.
- No treatment with disease modifying drugs such as beta interferon.
- Clinically definite relapsing remitting MS [Poser 1993].

Exclusion criteria

- Female patients pregnant or breast feeding.
- Presence of other neurological conditions.
- Past medical history of severe asthma or Gd-DTPA allergy.
- Treatment with beta-interferon.

3B Initial clinical and MRI lesion data

3B.1 Clinical data

Thirty one patients (22 female, 9 male) have been studied at baseline at the time of writing. The mean age at the point of recruitment is 35.2 years (range 24.0 – 54.0 years), mean disease duration 1.7 years (range 1.0 – 3.0 years). The mean total number of relapses per patient prior to the study was 2.74 (range 2 – 5), giving a mean annual relapse rate prior to recruitment of 1.87 relapses per year (range 1-3 per year).

During the first 6 months of the study the mean number of relapses was 1.23 (range 0 – 3) giving an annualised relapse rate of 2.46 relapses per year. Fourteen age and sex matched healthy controls (9 female and 5 male, mean age 34.7 years, range 25 –55 years) have also been studied at the time of writing. At present, the number of patients having completed the time points is as follows: 19 patients have completed the 6 month time point, fifteen month 12, thirteen month 18, eleven month 24 and five month 30.

3B.2 Lesion load measurements

It has previously been noted that the measurement of lesion *number* may be as accurate a method of assessing disease activity and response to treatment as enhancing lesion *volume.* In many treatment trials, enhancing lesion number is used as one of the primary end points [Miller *et al.* 1999, Miller *et al* 1996]. In our study the correlation between lesion volume and number was very strong ($r = 0.98$, $p < 0.0001$).

The median T2 lesion load at baseline was 4.32cc (range 0.31 – 51.29cc) and at month 6 this was 6.39cc (range 0.63 – 53.79cc). The median T1 hypo intense lesion load at baseline was 0.40cc (range 0 – 27.46cc) and at six months 0.31cc (range 0 – 29.90cc). The median Gd-DTPA enhancing lesion load was 0.17cc (range 0 – 2.50cc) at baseline and 0.15cc (range 0 – 2.95cc) at month six (see Table 3.2). However, it should be noted that of the original 31 patients studied at baseline, only 19 have so far reached the 6 month time point, so these values at months 0 and 6 are not directly comparable.

This is an active cohort of patients: 81% (25 patients out of a total of 31) had an active scan at baseline (defined as either brain or spinal cord enhancement). Of the 19 patients who have been assessed at month six, the degree of activity remained high with 63 % of patients showing activity (12 patients out of 19). The number of Gd-DTPA enhancing lesions has remained fairly constant in both the brain and the spinal cord over the first 6 months of the study (see Table 3.3). The number of new Gd-DTPA enhancing lesions has also stayed fairly static, with most of the lesions being new rather than persistent enhancing lesions (see Table 2). However, it should be noted that persistence of enhancement was seen equally in the brain and the spinal cord using triple dose Gd-DTPA.

The mean ratio of brain to cord lesions at baseline was 5.93 (range 1-10). The ratio of total Gd-DTPA enhancing lesions (brain and cord combined) over the first six months of the study to the total number of relapses within the first six months was 8.40. We would expect enhancement to occur more frequently than clinical relapse [Thorpe *et al* 1996a].

There has been minimal change in any of the clinical scores in the group of patients studied so far, over the first 6 months. Both the EDSS and MSFC and all the individual components of the MSFC have not significantly changed over the six month time period (see Table 3.2). This is not surprising, over such a short follow up, only 19 patients have so far been studied at six months.

3B.3 MRI-Clinical correlations

The MSFC (previously discussed in Chapter 1.5.2) at baseline correlated with both the T1 lesion load ($r = -0.44$, $p = 0.02$) and the T2 lesion load ($r = -0.48$, $p = 0.01$). The 25 foot timed walk correlated with all three lesion load measurements (T1LL: $r = 0.43$, $p = 0.02$, T2LL: $r = 0.40$, $p = 0.02$ and Gd-DTPA enhancing LL: $r = 0.42$, $p = 0.03$) see Table 3.4.

The total number of enhancing lesions over the entire first 6 months of the study correlated with annual relapse rate prior to recruitment ($r = 0.60$, $p = 0.01$) and the number of relapses within the first 6 months of the study ($r = 0.60$, $p = 0.002$). The Kappos study [Kappos *et al.* 1999] found Gd-DTPA to be a predictor of the occurrence of relapses, but not of the development of cumulative impairment or of disability. The relapse rate in the Kappos study was predicted with moderate ability by the mean number of Gd-DTPA enhancing lesions in monthly scans during the first six months of the study ($p=0.023$).

The strong correlation between enhancing lesion number and relapse rate in the present group could reflect the very homogeneous short disease duration cohort, and the use of triple dose Gd-DTPA. Enhancing lesion number is therefore a relatively useful surrogate marker of relapse activity in early disease.

In summary, this group of patients has a high degree of activity as measured using Gd-DTPA enhanced T1 weighted brain and spinal cord images. Although the overall total T2 lesion load has increased over the first six months of the study there has not been an increase in either T1 hypo intense lesion load, or Gd-DTPA enhancement. As expected, the number and volume of enhancing lesions were fairly consistent from month to month.

Assessment of clinical parameters using EDSS and MSFC has failed to show a change. However, it is difficult to interpret these findings, as only 19 patients have so far been studied at the six month time point and previous work has indicated poor sensitivity to change over such a time frame. When more data becomes available, both clinical and MRI, it will be possible to more realistically assess the change over time in the entire group.

Table 3.2 Lesion load and clinical data at baseline and month six

	Baseline mean (SD) (n = 31)	Baseline median (range) (n = 31)	Month 6 mean (SD) (n =19)	Month 6 median (range) (n = 19)
Total T2 LL (cc)	8.72 (10.86)	4.32 (0.31 -51.29)	9.37 (11.96)	6.39 (0.63 – 53.79)
T1 LL (cc)	1.84 (4.95)	0.40 (0 – 27.46)	2.04 (6.61)	0.31 (0 – 29.90)
Enhancing LL (cc)	0.45 (0.65)	0.17 (0 – 2.50)	0.30 (0.66)	0.15 (0 – 2.95)
EDSS	1.2 (0.8)	1.0 (0 – 3.0)	1.6 (1.0)	1.50 (0 – 3.5)
EDSS cerebellar score	0.19 (0.48)	0 (0 – 2)	0.1 (0.5)	0 (0 – 2.0)
9 hole peg test (sec)	23.11 (6.51)	20.49 (16.75 – 50.0)	22.86 (7.92)	21.88 (16.42 – 52.63)
Timed walk (sec)	4.68 (0.72)	4.60 (3.80 – 7.00)	4.75 (1.11)	4.70 (3.50 – 7.39)
PASAT	44.48 (15.31)	50.00 (0 – 60)	45.7 (13.1)	48.0 (21.0 – 60.0)
MSFC	2.2 x 10^{-4} (0.61)	0.16 (-1.57 – 1.23)	3.0 X 10^{-4} (0.75)	0.12 (-1.43 – 1.05)
EDSS	1.21 (0.86)	1.00 (0-3.00)	1.60 (2.16)	1.00 (0 – 3.5)

Table 3.3 Enhancing lesion load data

	Month 0	Month 1	Month 2	Month 3	Month 6
Total number of enhancing brain lesions mean (SD)	3.26 (2.71)	2.33 (3.21)	2.70 (3.79)	3.0 (5.05)	1.60 (2.16)
Total number of enhancing brain lesions median (range)	1.0 (0 – 10)	1.0 (0 – 13)	2.0 (0 – 15)	1.0 (0 – 20)	1.0 (0-8)
Number of new enhancing brain lesions mean (SD)	N/A	1.52 (2.24)	1.59 (2.36)	1.86 (4.19)	1.45 (1.84)
Number of new enhancing brain lesions median (range)	N/A	1.0 (0-8)	1.0 (0-10)	0 (0-19)	1.0 (0-7)
Number of persistent enhancing brain lesions mean (SD)	N/A	0.82 (1.21)	1.14 (1.77)	1.14 (2.24)	0.15 (0.37)
Number of persistent enhancing brain lesions median (range)	N/A	0 (0-5)	0 (0-7)	0 (0-10)	0 (0-1)
Total number of enhancing cord lesions mean (SD)	0.55 (1.58)	0.48(0.98)	0.48 (0.70)	0.24 (0.70)	0.30(0.73)
Total number of enhancing cord lesions median (range)	0.0 (0 – 8)	0.0 (0 – 4)	0.0 (0 – 2)	0.0 (0 – 3)	0.0 (0 – 3)
Number of new enhancing cord lesions mean (SD)	N/A	0.29 (0.61)	0.33 (0.62)	0.09 (0.30)	0.25 (0.55)
Number of new enhancing cord lesions median (range)	N/A	0 (0-2)	0 (0-2)	0 (0-1)	0 (0-2)
Number of persistent enhancing cord lesions mean (SD)	N/A	0.18 (0.48)	0.14 (0.36)	0.14 (0.48)	0.01 (0.23)
Number of persistent enhancing cord lesions median (range)	N/A	0 (0-2)	0 (0-1)	0 (0-2)	0 (0-1)

Table 3.4 The relationship between clinical and lesion load data.

	T1 LL Baseline	T2 LL Baseline	Enhancing LL Baseline
MSFC BASELINE	r = -0.44 p = 0.02	r= -0.48 p= 0.01	NS
9 HOLE PEG TEST BASELINE	NS	NS	NS
TIMED WALK BASELINE	r = 0.43 p = 0.02	r= 0.40 p= 0.02	r= 0.42 p= 0.03
PASAT BASELINE	NS	NS	NS
EDSS BASELINE	NS	NS	NS
DISEASE DURATION	NS	NS	r= 0.40 p= 0.02
RELAPSES PRIOR TO STUDY	NS	NS	r= 0.50 p= 0.04

PART 2. MTR AND T1 RELAXATION TIME MEASUREMENTS IN EARLY DISEASE.

Chapter 4 MTR and T1 provide complementary information in MS NAWM, but not in lesions

4.1 Introduction and study aims

The aim of this study was to document the relationship between MTR and T1 relaxation time in MS lesions and NAWM. This was in order to determine whether the combination provides a more comprehensive tissue characterisation than either measure in isolation. In prospective longitudinal studies such as this, multiple MR parameters are frequently used to assess disease activity and progression. As such, it is important to document any potential redundancy of information, and to assess the complementary nature of the MR parameters. Theoretically it should be possible to develop a composite MR "score" for patients with MS, using various complementary MR techniques to fully assess the pathological and physiological "activity" of the disease at any point in time.

The patient demographics of the patients used in this study were: 5 male, 5 female, mean age 37.6 years, (range 30-48 years). The patients were in the early relapsing remitting cohort of patients (as described in Chapter 3). Ten age matched healthy controls were also studied. All subjects underwent imaging using a protocol that included the measurement of both MTR and T1 relaxation times (as described in Chapter 3). The MTR and T1 values were compared statistically using a commonly adopted correlation approach and a mixed-model regression approach. The age matched healthy control demographics were: 7 male, 3 female, average age 36.0 years, (range 29-44 years).

4.2 MRI acquisition protocol

Imaging was carried out according to the protocols described in Chapter 3. The following sequences were used:

1. Fast spin echo (FSE)

2. MTR

3. T1 relaxation time measurement

4. T1 weighted images pre and post triple dose Gd-DTPA administration

4.3 Analysis of MTR, T1 relaxation time and evaluation of T1 hypo intensity

The MTR images and the calculated T1 relaxation time maps were co-registered using a method described by Symms [Symms *et al* 1997]. Calculated MT images were displayed on a Sun workstation (Sun Microsystems, Mountain View, CA) using Dispimage image display software [Plummer 1992]. This software was used to obtain MTR within 2D regions of interest (ROIs) with reference to MS lesions marked on the FSE hard copy images. Lesion ROIs were defined according to previously defined parameters [Filippi *et al* 1998a]. ROIs were also defined in NAWM in areas of the cerebellum, pons, occipital, frontal, parietal regions, internal capsule (anterior and posterior limbs), and the genu and splenium of the corpus callosum. Two symmetrical regions (left and right) were taken from all the areas listed, except the pons, genu and splenium of the corpus callosum, where one central region was sampled. The individual ROI areas (in mm^2) were; pons 60, genu and splenium 20, internal capsule 24, and all other areas 36. The regions were made as large as was possible in order to sample as much of the relevant white matter areas as possible. The regions of interest were placed in the middle of areas of NAWM in order to minimise partial volume

effects of grey matter or CSF. ROIs were placed in the same anatomical positions for the control white matter. Identical ROIs were then placed on the calculated MT images and the calculated T1 relaxation time maps. Lesions were defined as enhancing (acute) or non enhancing (chronic) according to their appearances on 2D T1 weighted Gd-DTPA enhanced images. Non enhancing lesions were further classified according to their degree of hypo intensity relative to surrounding NAWM on the unenhanced 2D T1 weighted image as follows:

- Iso intense: visible on T2 weighted and PD weighted images and iso intense with surrounding normal appearing white matter on T1 weighted images.

- Hypo intense: seen on T2 weighted and PD weighted images and hypo intense with regard to normal appearing white matter on T1 weighted images.

4.4 Statistical methods

Two methods were employed for the statistical analysis of the MTR and T1 relaxation rate ($R_1 = 1/T1$) data. Firstly, Spearmans rank correlation coefficient was calculated between MTR and R_1 for the different tissue types. This approach was adopted to allow comparison with the methods of previous studies with broadly similar aims to our own [van Waesberghe *et al* 1997].

A more advanced statistical analysis was then performed by Dr Martin King. As the analysis described above does not take account of the possible contribution of within versus between subject variation, and of tissue type and anatomical position to the MTR-R_1 relationship, this second, more rigorous analysis was included. This aimed to provide a model that best

describes all observed variation in MTR in terms of the variation in R_1 according to the anatomical location (SITE) of each sample ROI, and the type of tissue (TISSUE) encompassed (lesion, NAWM, and control white matter). To this end, a mixed-model regression analysis was performed [Hand *et al* 1996], [Sullivan *et al* 1999]. A random coefficients model was adopted. Fixed effects terms were selected using the likelihood ratio test to compare various nested models.

A model of the form MTR = SITE + TISSUE + R_1 + SITE x TISSUE + R_1 x TISSUE + R_1 x SITE was found to be adequate. Akaikes Information Criterion [Sullivan *et al* 1999] was used to determine which random effects should additionally be included in the model, with the result that a random subjects (i.e. individual patients or controls) term was included. This term accounts for expected random inter-subject variation in MTR, which is independent of SITE, TISSUE, or R_1. Probability values were obtained subsequent to a conversion of the restricted maximum likelihood (REML) Wald statistics to F ratios, using denominator degrees of freedom (ddf) equal to the difference between the number of observations and the rank of the fixed effects design matrix. (No inferences were altered by adopting alternative specifications for the ddf). The calculations were performed using SAS Version 6.11 PROC MIXED [SA Institute Inc 1996].

4.5 Results

There was a strong correlation between MTR and R_1 (1/T1) in MS lesions observed using the basic correlation analysis ($r = 0.74$). The correlation was similar in all lesion subtypes: ie hypo intense non enhancing ($r = 0.62$) and iso intense non enhancing ($r = 0.67$), all non enhancing ($r = 0.68$) and enhancing lesions ($r = 0.81$). The correlation seen in patient

NAWM appeared weaker (r = 0.24). In control white matter there was no observed correlation (r = 0.06) (see Tables 4.1 and 4.2, Figures 4.1 and 4.2).

The mixed-model regression analysis showed that MTR has a significant dependence on T1 relaxation rate (and hence T1) and that this dependence is tissue type specific (R_1 x TYPE = 18.6, ndf = 2, ddf = 452, $p < 0.0001$). The result obtained from the significance test on the R_1 x SITE interaction term is equivocal (F = 1.76, ndf = 6, ddf = 452, prob. value = 0.106) and the question concerning the presence of anatomical site differences in the MTR - T1 relaxation rate relationship therefore remains unanswered. The SITE x TISSUE interaction term is significant (F = 2.31, ndf = 11, ddf = 452, prob. value = 0.009), indicating that, for a given T1 relaxation time, the MTR depends on the combination of anatomical site and lesion type.

4.6 Discussion

The striking finding of this study is that the relationship between T1 and MTR is very different in lesions, patient NAWM and control white matter. This is apparent from Figures 4.1 and 4.2 and supported by the statistical analysis previously described.

Although the simpler, more commonly adopted, statistical procedure of calculating Spearman's rank correlation coefficient shows an apparent difference in the MTR-T1 relationship between tissue types, no significance can be assigned to this difference using this approach. This is due to the potential effects of not only tissue type, but also of site, multiple comparisons, and within and between subject variation on the observed distributions.

However the mixed-model analysis explicitly accounts for these variables where necessary, and confirms that it is highly probable that the MTR-T1 relationship is tissue type dependent, while the influence of anatomical position is likely to be small, although some influence cannot be ruled out altogether.

The strong relationship between the two parameters in lesions would be compatible with a common pathological feature affecting both. Demyelination is the hallmark of MS lesions and should cause a major reduction in the pool of bound protons thus reducing MTR. The same process is likely to result in increased free water content relative to tissue structure which will increase T1.

The lack of relationship between MTR and T1 in control white matter is in sharp contrast with the lesion findings. On inspecting the control white matter data it is evident that the range of MTR measurements is small whereas the range of T1 relaxation times is quite large. MTR in healthy white matter is dominated by the effect of protons bound to myelin, the latter being the major structural element. There are however, regional differences in the compaction and orientation of myelinated fibres, which leads to slight differences in MTR. For example, there are slightly higher MTR values in corpus callosum than in other white matter regions [Silver *et al* 1998]. While such anatomical variations have a minor effect on the overall bound proton pool, they may have a greater impact on the mobile proton pool in adjacent extracellular spaces - this in turn could account for the larger T1 variations seen in different white matter regions.

A weak MTR/T1 relationship in patient NAWM falls between the pattern observed in control white matter and lesions. Pathological studies of NAWM in MS reveal a variety of subtle changes, such as astrocyte hyperplasia, inflammatory infiltrates and myelin breakdown products [Allen and McKeown1979]. Water content is also increased [Tourtellotte and Parker 1968]. These subtle changes might extend the range of T1 relaxation times and lead to slight reductions in MTR - indeed comparing control and patient NAWM there were group differences in these two parameters (see Table 4.1). A modest relationship between MTR and T1 thus emerges (see Table 4.2 and Figure 4.2).

Although the pathological and anatomical basis of the changes in MTR and T1 measurements is to an extent conjectural, it is evident that their combination provides a more complete characterisation of different tissues than either measure alone. In MS, the complementary information obtained from MTR and T1 is most apparent in NAWM. The results emphasise the potential of multiparameter MR data in general to improve tissue characterisation. Such approaches may be valuable in improving diagnosis, understanding disease pathogenesis and monitoring treatment. For example, the application of multiparameter MR measurements in MS, using appropriate techniques for specific pathological features (e.g. gadolinium enhancement for inflammation marked MTR reduction for demyelination and NAA measurement for axonal damage) may elucidate the mechanisms of myelin and axonal loss, which ultimately lead to irreversible disability in many individuals.

Table 4.1 MTR and T1 values for different tissue types

	Control white matter	Patient NAWM	Iso intense lesions	Hypo intense lesions	Enhancing lesions
MTR (pu)					
Mean	38.1	37.6	31.2	29.7	31.5
Median	38.4	37.8	31.8	30.2	32.7
Range	35.3 – 43.8	33.5 – 40.9	22.2 - 36.7	19.2 – 36.2	20.1 - 37.1
T1 (ms)					
Mean	677	749	891	967	912
Median	650	732	873	948	887
Range	522 – 971	532 – 1008	607 – 1514	655 – 1693	675 – 1292

Table 4.2 MTR and T1 correlations for control white matter, NAWM and all lesions

	Number of ROIs	r (Spearman's rank correlation coefficient)
Control white matter	120	0.06
Patient NAWM	73	0.24
All lesions	312	0.74

Figure 4.1 The relationship between MTR and T1 relaxation time in MS lesions

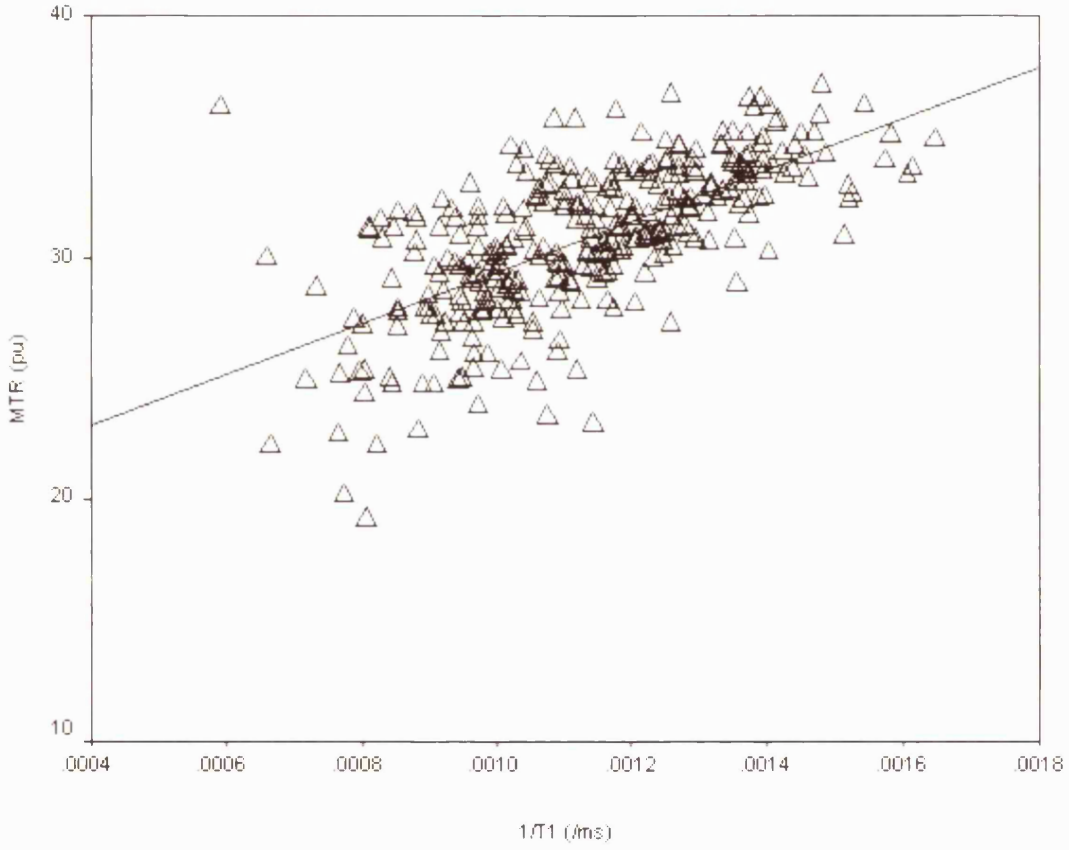
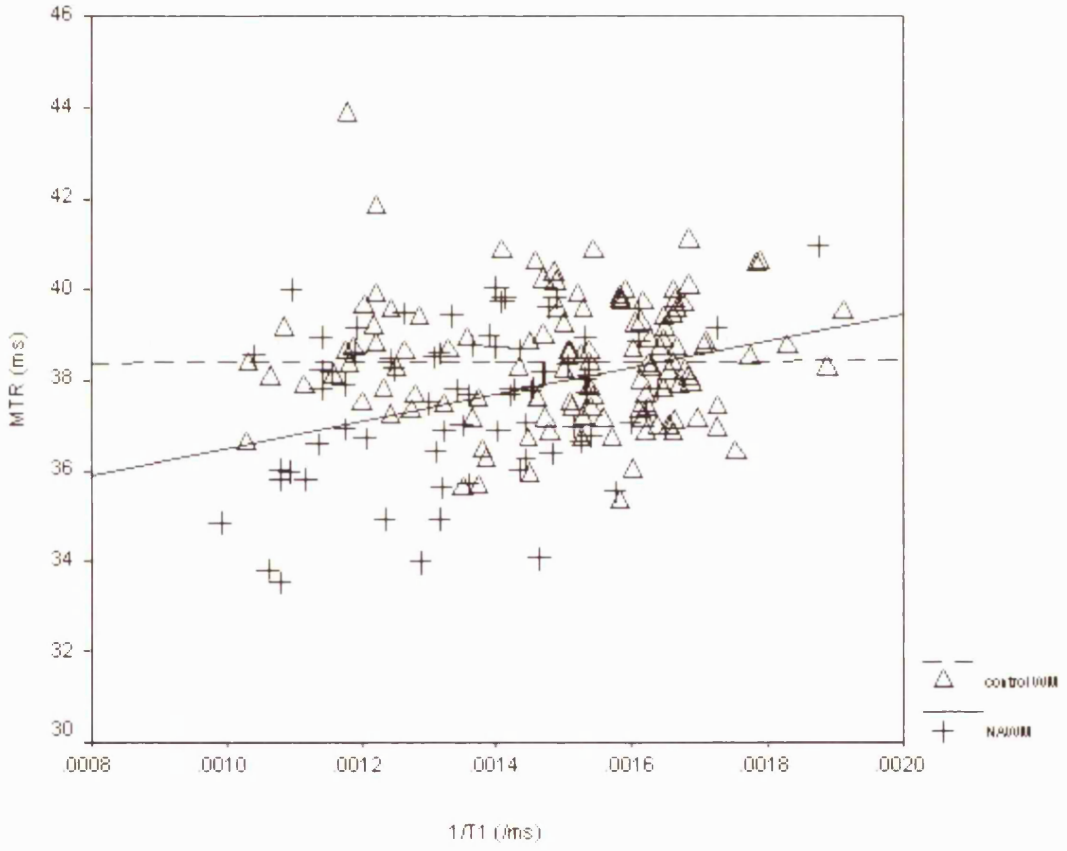


Figure 4.2 The MTR/T1 relationship seen in control white matter and patient NAWM



Chapter 5 An investigation of T1 and MTR in normal appearing brain tissue

5.1 Introduction and study aims

This study has used the MR techniques of MTR and T1 relaxation time measurements in early MS to study normal appearing tissues and lesions in this cohort of patients with early relapsing remitting MS. The purpose of this study was to determine whether abnormalities are already detectable in normal appearing tissues (including NAGM) in early MS, and if so how they correlate with lesion characteristics. As previously discussed in Chapter 2, MTR and T1 relaxation times are abnormal in both lesions and NAWM in established MS. Chapter 4 has shown that the relationship between MTR and T1 varies between the different tissue types.

A low MTR indicates a reduced capacity of the macromolecules in brain tissue to exchange magnetisation with the surrounding water molecules, suggesting that there is structural tissue damage [Grossman 1994], [Lexa *et al* 1994]. A recent preliminary post mortem report found a correlation between low MTR and the percentage of residual axons in MS lesions [van Waesberghe *et al* 1998]. Animal studies have shown that a substantially lowered MTR correlated with histopathologic findings of myelin loss and axon destruction [Lexa *et al* 1994], whereas oedematous lesions resulted in only slightly decreased MTR values.

Pathological processes such as axonal damage and loss, gliosis, inflammation and oedema can all potentially cause an alteration in T1 relaxation time measurements through an increase in the pool of mobile water protons [Barnes *et al.* 1991]. These processes occur in NAWM [Evangelou *et al.* 2000a], [Allen and McKeown1979] and in lesions [Barnes *et al* 1991], [Bronwell *et al* 1962], [Bruck *et al* 1997]. Cortical lesions are commonly seen in MS [Kidd

et al. 1999] and recent quantitative MR spectroscopy studies have documented the presence of NAGM changes in MS [Kapeller *et al* 2001]. Quantitative T1 relaxation time measurement may be more sensitive to the various subtle forms of both grey and white matter pathology.

From these observations, two questions arise which are relevant to the pathogenesis of MS:

(1) In addition to lesions, is there evidence for pathological changes in the NAWM and NAGM in the early stages of MS?

(2) Do abnormalities in lesions, NAWM and NAGM develop by related or independent processes?

To further explore these questions I have studied a group of patients from the early relapsing remitting MS cohort of patients [Griffin *et al* 2000]. In these patients, total T2, enhancing and T1-hypointense lesion loads were measured, and the MTR and T1 relaxation time of NAWM and NAGM investigated.

5.2 Methods

Twenty two patients (7 male, 15 female, mean age 36.6 years, range 30-50) with a median disease duration 2 years (range 7 months – 3 years) from the clinically definite early RR MS [Lublin and Reingold1996, Poser *et al* 1983] cohort were studied. All had a Kurtzke expanded disability status scale (EDSS) [Kurtzke1983] of less than or equal to 3 and all had neurological symptoms for less than 3 years. All patients underwent a full neurological examination and evaluation of the EDSS by me before undergoing MRI of the brain. None of the patients were receiving disease modifying drugs and none were in acute relapse at the time of examination. No patients had been treated with steroids in the preceding month.

Eleven age matched healthy controls (4 male, 7 female, mean age 37.0 years, range 29 - 44) were also studied.

5.3 MRI acquisition protocol

The following sequences were obtained (according to the protocols discussed in Chapter 3).

1. Fast spin echo (FSE)
2. MTR
3. T1 relaxation time measurement
4. T1 weighted images pre and post Gd-DTPA

5.4 MR Analysis

All images were transferred to and displayed on a Sun workstation (Sun Microsystems, Mountain View, CA) using Dispimage image display software [Plummer 1992]. This software was used to define regions of interest (ROIs) from lesions, NAWM and NAGM. Lesions were identified on the PD-weighted FSE hard copy images and outlined using previously defined rules [Filippi *et al* 1998a]. Additional ROIs were placed in the middle of NAWM and NAGM regions with care to minimise partial volume effects. When placing an ROI, care was taken to place it as far away from contaminating grey matter, white matter and CSF as possible, depending on whether it was a NAWM or a NAGM ROI respectively. The slices above and below each ROI were also examined to ensure there was no contamination. MTR and T1 relaxation times were measured in 9 regions of NAWM and 7 of NAGM. As the patients studied have relatively low lesion loads, it was possible to place each ROI in an area of tissue without visible lesion contamination (examples shown in figure 5.1a).

ROIs were defined in 9 NAWM regions: the cerebellum, pons, frontal, temporal and parieto-occipital lobes, internal capsule (anterior and posterior limbs), the genu and splenium of the corpus callosum and the centrum semiovale. Symmetrical regions (left and right) were taken from all areas, except the pons, genu and splenium of the corpus callosum, where one midline region was sampled. To achieve a greater number of samples, 2 ROIs were taken bilaterally from parieto-occipital, frontal and centrum semiovale regions. The ROI areas (in mm^2) for NAWM regions were: pons 60, genu and splenium 20, internal capsule 24, and all other areas 36.

ROIs were placed in 7 NAGM regions: the cerebellar cortex, caudate, putamen, thalamus, frontal, temporal and parieto-occipital cortex. One area was sampled bilaterally from all regions except the temporal area, from which 2 ROIs were taken bilaterally, and the frontal and parieto-occipital areas, where 3 ROIs were sampled bilaterally. The ROI areas (in mm^2) were: 36 for the cerebellum, caudate, putamen and thalamus and 20 for frontal, temporal and parieto-occipital grey matter. The smaller cortical grey matter ROIs were necessary in order to minimise partial volume effects from neighboring CSF and NAWM.

Co-registration of the PD-weighted, MTR and calculated T1 relaxation time maps was ensured using a previously described method [Symms *et al* 1997], [Wood *et al* 1997]. This allowed ROIs defined on the PD-weighted images to be placed on the MT images and the MTR calculated. The same ROIs were placed on the T1 relaxation time maps and the T1 relaxation times obtained (figure 5.1b).

Total T2 lesion load was measured from lesions identified on the PD-weighted images. These lesions were outlined on a Sun workstation using a semi-automated local threshold method [Plummer 1992]. Volumes were calculated by multiplying the lesion area by slice thickness. Non enhancing lesions were further classified according to their appearances on the T1-weighted image as iso intense or hypo intense with regard to surrounding NAWM. Gd-DTPA enhancing lesions were identified on the T1 weighted Gd-DTPA enhanced images. The volume of Gd-DTPA enhancing lesions and hypo intense lesions was subsequently determined using the same method as that for T2 lesions.

5.5 Statistical analysis

Comparisons between patient and control groups were performed using the Mann-Whitney test. Where there was more than one ROI in an anatomical region, the mean value from the multiple regions was calculated and used for the comparisons. The Bonferroni method was used to correct for multiple comparisons between the two groups. Using a per family error rate of 0.05, with 16 ROI comparisons, a p value of 0.003 was derived for determining statistical significance for both T1 and MTR measures. The Spearman rank correlation coefficient (r) was calculated to investigate the correlation of global NAWM and NAGM MTR and T1 with enhancing, T1 hypo intense and T2 lesion volumes.

5.6 Results

The median EDSS of patients was 1.0 (range 0 - 2.5), with a median disease duration of 2.0 years (mean 2.0 years, range 7 months - 3 years). The mean number of relapses experienced since onset was 2.3 (range 2 - 4). Eighteen patients (81%) had enhancing brain lesions; the median number of enhancing lesions was 1.0 (mean 2.3, range 0 – 9). The median total T2

lesion load (LL) was 4.21cc (range 0.31 – 29.02cc), T1 hypo intense LL 0.33cc (range 0 - 6.36cc) and Gd-DTPA enhancing LL 0.16cc (range 0 – 2.50).

In patients, T1 was significantly higher in both NAWM and NAGM: MTR was significantly reduced in NAWM only: tables 5.1 and 5.2 show regional comparisons between patients and controls. In NAWM, increased T1 was found in 6/9 regions (including all supratentorial regions apart from the splenium of the corpus callosum). MTR was reduced in 3/9 NAWM regions: frontal, temporal and parieto-occipital. In NAGM, significantly increased T1 was found only in frontal cortex with a trend to increase in several other deep grey matter regions. No NAGM region exhibited abnormal MTR.

There was no correlation between number or volume of enhancing lesions and patient global NAWM or NAGM MTR or T1. Nor was there a significant correlation between T1 hypo intense or T2 lesion volumes and NAWM or NAGM MTR and T1, although there was a non significant trend for a correlation between NAWM T1 and T1 hypo intense lesion load ($r = 0.41$, $p = 0.09$).

5.6 Discussion

In this study, we have found: 1. The NAWM and NAGM are abnormal in early RRMS, and 2. That NAWM/NAGM abnormalities are not correlated with lesion load measures. The significance of these findings will now be discussed, but to provide a context, some observations on the lesion load results will first be considered.

Lesion load findings

This study recruited patients with strictly defined clinically definite early RR MS (disease duration less than 3 years). Few previous studies have investigated a cohort with such early disease. No patient had a normal brain scan, confirming the low frequency of the latter findings in patients with clinically definite RR MS [Thorpe *et al* 1996b]. The median T2 lesion load (4.2cc) is intermediate between that found in patients with longer duration RR MS and in those presenting with a clinically isolated syndrome (CIS) and T2 abnormalities, the presence of which is known to confer a high risk for subsequent development of CDMS [Optic Neuritis Study Group 1997]. Thus, in the PRISMS trial of beta interferon-1a in RR MS, median T2 lesion load at entry was approximately 10cc and median disease duration 8 years [Li and Paty1999]. In the CHAMPS study of beta interferon-1a in patients with a CIS and at least two T2 lesions, median T2 lesion volume at entry was 2cc [Jacobs *et al.* 2000]. Most patients (81%) exhibited one or more Gd enhancing lesions. This high frequency reflects a clinically active cohort [Grossman *et al.* 1986, Smith *et al* 1993]; the use of triple dose Gd may also have increased the number of enhancing lesions [Silver *et al* 1997, Filippi *et al* 1996c]. The T1- hypo intense lesion volume was less than 10% of total T2 lesion volume, which is considerably less than the T1/T2 volume ratio seen in secondary progressive MS which is about 30 %[Katz *et al* 1993, Truyen *et al* 1996]. The combination of frequent enhancement and low T1/T2 ratio suggests that there is considerable inflammation and oedema but relatively little axonal damage in early RR MS lesions. However, there were marked inter-individual variations in both Gd-enhancing and T1-hypointense lesion volumes implying that the amount of inflammation and axonal damage

varies considerably between patients. Long term follow up studies are needed to determine their prognostic significance.

NAWM and NAGM are abnormal in early RRMS

Both T1 and MTR were abnormal in the NAWM, confirming that NAWM abnormalities occur early in the course of MS. Since both lesions and NAWM abnormalities are present, the study does not clarify which appears first. Serial studies in established MS have shown quantitative MR abnormalities appearing in pre-lesional NAWM for several months prior to lesion appearance [Werring *et al* 2000;Filippi *et al* 1998c;Goodkin *et al* 1998].

However, such studies concentrated only on small focal regions that subsequently became lesions, whereas the present study suggests a more diffuse process involving the NAWM. To investigate NAWM at an earlier stage, our group has recently studied 27 CIS patients with cerebral T2 lesions [Brex *et al* 2001]. Using an identical ROI method, there was no difference in individual regions or globally when comparing patient versus control MTR. This suggests that lesions evolve before diffuse NAWM abnormality, with the latter developing soon after. However, further investigation of CIS NAWM is needed, with potentially more sensitive methods such as T1 measurement or MTR histogram analysis, before drawing firm conclusions. We have previously reported that there is only a weak correlation between T1 and MTR in NAWM [Griffin *et al* 2000], and in Chapter 4, suggesting that they provide complementary information. The present study also showed more extensive abnormalities of T1 than of MTR, suggesting that the former is more sensitive to the subtle pathological changes occurring in NAWM, at least using the sequences

applied in this study. T1 relaxation time thus appears a promising tool for monitoring MS NAWM.

Three recent studies have used MTR to examine patients with clinically isolated syndromes (CIS) [Iannucci *et al* 2000] [Kaiser *et al* 2000], [Brex *et al* 2001], two using histograms [Iannucci *et al* 2000] [Kaiser *et al* 2000] and one using a ROI approach [Brex *et al* 2001]. Iannucci et al showed a lower normal appearing brain tissue (NABT) average MTR and a lower peak position in patients with CIS compared to controls. Patients who went on to develop clinically definite MS (CDMS) showed a significantly lower average NABT MTR and a lower peak position than those who did not develop CDMS. However, Kaiser et al showed no difference in MTR histograms between CIS patients and controls. Brex et al studied 27 patients with CIS and found no NAWM abnormalities detectable with MTR using a ROI approach. Hence, the time scale of the earlier changes in the normal appearing tissues would appear to remain uncertain.

Using MR spectroscopy, Kapeller et al [Kapeller *et al* 2001] have shown decreased N-acetyl aspartate (NAA) in both NAWM and cortical grey matter, and increased myo-inositol in NAWM in a sub-group of the present cohort of patients. Whereas MTR and T1 are investigating changes in water proton content and structure, MRS investigates other metabolites. The reduced NAA suggests that some neuronal dysfunction or loss is occurring, which in turn could contribute to a subtle change in T1.

The nature of the NAWM pathology seen in our cohort is however uncertain. The changes in patient MTR (decrease) and T1 relaxation time (increase) were of a small degree, when compared with control values. Such changes are pathologically non-specific: they suggest a

decrease in the pool of bound water protons (reducing MTR) and an increase in the pool of mobile water protons (increasing T1). In short, these changes may be accounted for by most of the pathological findings reported in MS NAWM such as oedema [Dousset 1992], inflammation and demyelination [Allen and McKeown1979] astrocyte hyperplasia and gliosis [Barnes *et al* 1991] and axonal loss [Lexa 1994].

Cortical plaques are less evident than white matter plaques on routine macroscopic inspection of post mortem brain slices, and are even less often visualised using conventional MR sequences. However, careful microscopic examination has shown that the cortex is frequently involved by demyelinating lesions [Kidd *et al* 1999]. Conventional MR sequences show a degree of mixed weighting i.e. T1, T2 and PD all affect the scan contrast to a greater or lesser degree. Their lack of sensitivity in depicting grey matter lesions probably reflects the fact that normal grey matter has combined PD, T1 and T2 characteristics similar to many MS lesions. Quantitative techniques such as MTR and T1 measurement are specific to individual MR properties, and may be more sensitive to subtle changes due to grey matter pathology. Two groups have recently reported abnormalities occurring in MS grey matter using and MTR histogram analysis [Cercignani *et al.* 2001], [Ge *et al* 2000]. These studies investigated patients with a longer duration disease. In the present cohort, we have demonstrated unequivocal evidence of abnormality in frontal NAGM using a quantitative measure of T1. While this suggests that grey matter pathology is present, the changes were much less extensive than those seen in NAWM. A caveat, however, is that ROI analysis, by sampling selected regions only, may be less sensitive to abnormality than histogram analysis of the entire segmented tissue type. We are currently investigating the cohort using segmented histogram analysis.

Lack of correlation between NAWM/NAGM measures and lesion load

This study showed no significant correlation between NAWM T1 and MTR and any of the three lesion load measures. This suggests that the lesion and NAWM abnormalities have developed by at least partly independent mechanisms. This suggests that measurement of NAWM and lesions will provide complementary information in studying the disease course and treatment effects. There was nevertheless a trend for T1-hypointense lesion load to correlate weakly with NAWM T1 ($r = 0.55$, $p = 0.09$). This subgroup of lesions exhibit more axonal loss, and Wallerian degeneration in connected fibre tracts in the NAWM may explain the weak correlation. Since the overall load of T1-hypointense lesions at this stage of MS is small, the extent of Wallerian degeneration is likely to be modest. A corollary is that as the disease progresses, with more axonal loss in lesions and Wallerian degeneration in NAWM, a stronger correlation between lesion and NAWM parameters may emerge. Long term follow up of the present group will address this hypothesis.

In summary, both MTR and T1 relaxation times may be useful in the long term follow up of patients with early relapsing remitting MS since they detect abnormalities in normal appearing tissues, especially NAWM. We have previously shown that the two techniques add complementary information (Chapter 4: [Griffin *et al* 2000]) and they both appear to be sensitive to early pathological change in the disease, especially T1.

The cohort we have recruited will be expanded to confirm the present observations. They are also being followed up in order to (1) clarify the relationship between lesion and NAWM

abnormalities over time; (2) determine when more extensive NAGM abnormalities appear, and (3) relate the serial MR changes to the patients clinical course and functional status.

Table 5.1 T1 relaxation time of NAWM and NAGM in patients and controls: regional data

AREA	NAWM/NAGM	MEDIAN PATIENT T1 (SD)	MEDIAN CONTROL T1 (SD)	p VALUE
CEREBELLUM	NAGM	1074.70(176.73)	1042.50(103.34)	0.48
CAUDATE	NAGM	1107.95(82.88)	1034.60(80.24)	0.02
PUTAMEN	NAGM	930.40(59.15)	890.45(68.47)	0.009
THALAMUS	NAGM	958.80(66.22)	925.80(54.25)	0.08
FRONTAL	NAGM	1110.55(98.25)	1053.80(85.67)	0.001
TEMPORAL	NAGM	1093.15(91.02)	1064.00(104.80)	0.11
PARIETO-OCCIPITAL	NAGM	1021.55(88.23)	1004.50(101.03)	0.11
PONS	NAWM	908.90(62.91)	907.10(41.60)	0.81
CEREBELLUM	NAWM	806.55(78.63)	775.80(109.83)	0.14
TEMPORAL	NAWM	705.45(59.79)	648.80(40.78)	<0.001
FRONTAL	NAWM	658.25(63.76)	609.00(47.55)	<0.001
GENU	NAWM	691.95(59.94)	623.00(72.64)	0.002
SPLENIUM	NAWM	692.65(75.40)	629.00(28.29)	0.007
INTERNAL CAPSULE	NAWM	708.90(49.72)	686.10(38.18)	0.001
PARIETO-OCCIPITAL	NAWM	674.00(57.40)	614.05(34.97)	<0.001
CENTRUM SEMIOVALE	NAWM	654.60(47.75)	610.90(33.06)	<0.001
GLOBAL	NAGM	1048.85 (113.15)	1014.65 (106.07)	0.001
GLOBAL	NAWM	687.00 (85.38)	642.40 (90.02)	<0.001

Table 5.2 MTR of NAWM and NAGM in patients and controls: regional data

AREA	NAWM/NAGM	MEDIAN PATIENT MTR (SD)	MEDIAN CONTROL MTR (SD)	p VALUE
CEREBELLUM	NAGM	32.45(1.98)	32.55(1.33)	0.91
CAUDATE	NAGM	33.08(1.03)	33.62(0.92)	0.06
PUTAMEN	NAGM	33.77(0.96)	34.13(0.70)	0.02
THALAMUS	NAGM	36.25(0.98)	36.32(0.78)	0.24
FRONTAL	NAGM	33.62(1.54)	34.33(1.65)	0.12
TEMPORAL	NAGM	34.10(1.87)	34.21(1.79)	0.82
PARIETO-OCCIPITAL	NAGM	33.45(1.50)	34.01(1.76)	0.02
PONS	NAWM	37.46(1.00)	38.55(0.76)	0.04
CEREBELLUM	NAWM	37.22(1.70)	37.54(2.18)	0.54
FRONTAL	NAWM	38.78(1.23)	39.29(0.95)	<0.001
TEMPORAL	NAWM	37.52(1.11)	38.25(0.86)	0.001
GENU	NAWM	39.96(1.71)	40.12(0.74)	0.18
SPLENIUM	NAWM	37.97(2.20)	39.25(0.96)	0.11
INTERNAL CAPSULE	NAWM	36.61(1.36)	37.29(1.43)	0.009
PARIETO-OCCIPITAL	NAWM	36.90(1.50)	37.58(1.34)	0.001
CENTRUM SEMIOVALE	NAWM	37.17(1.29)	38.02(1.03)	0.006
GLOBAL	NAGM	33.83 (1.71)	33.98 (1.72)	0.336
GLOBAL	NAWM	37.52 (1.60)	38.25 (1.50)	<0.001

Figure 5.1a PD image with NAWM and NAGM ROIs.

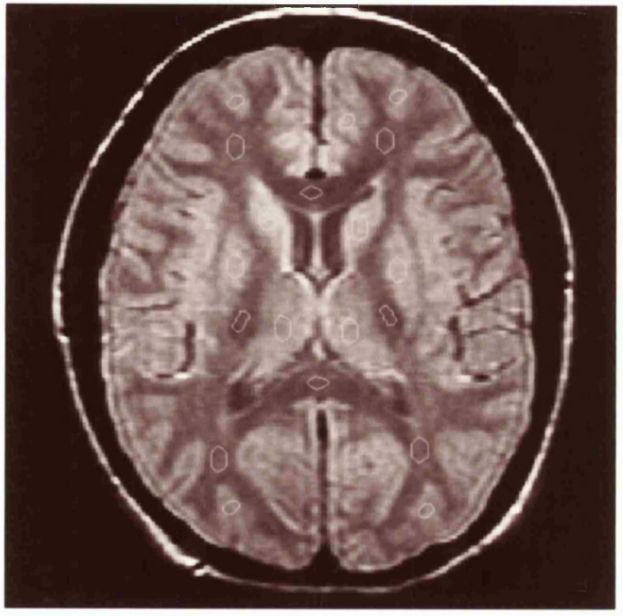
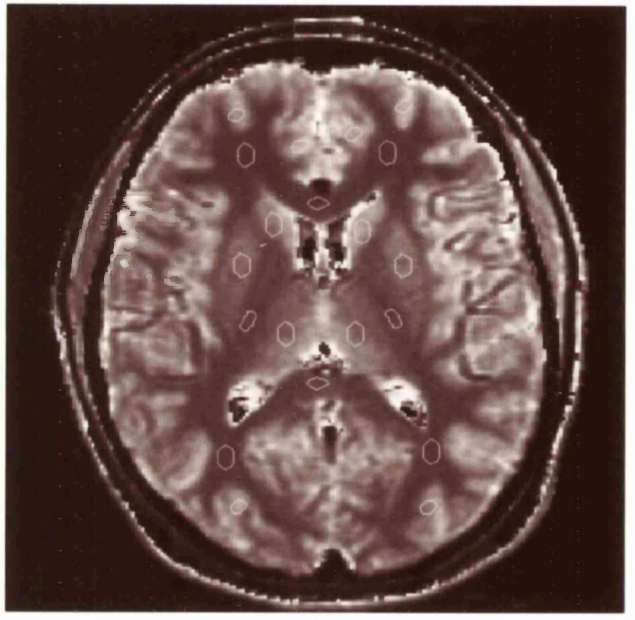


Figure 5.1b T1 relaxation time map with NAWM and NAGM ROIs.



Chapter 6 T1 histograms of normal appearing brain tissue are abnormal in early relapsing remitting MS

6.1 Study aims and methods

The aim of this study was to use both whole brain and NABT T1 relaxation time histograms to investigate abnormalities in this cohort of patients. In patients with established MS, both lesions and NABT exhibit an increase in T1 relaxation time. By using T1 histogram analysis in early disease, it is hoped that such changes can be detected. Histogram measurements are becoming more widely applied in the radiological assessment of patients with MS. Most of these studies have involved analysis using magnetisation transfer imaging (MTI) histograms [Kalkers *et al.* 2001b], [Ge *et al.* 2001], [Tortorella *et al.* 2000], but histogram abnormalities have also been detected using diffusion tensor imaging (DTI) [Cercignani *et al.* 2000], [Nusbaum *et al.* 2000]. Histograms appear sensitive in examining changes in both NAWM and NAGM, as they sample a much larger volume of tissue compared to the more conventional region of interest (ROI) approach. Histograms do not allow the specific sampling of individual anatomical areas of interest, but they do give a more global assessment of change.

T1 histograms have not been widely studied to date in MS, although some preliminary work exists in abstract form [Parry *et al* 2000], [van Walderveen *et al* 2000]. Region of interest (ROI) studies have reported increased T1 relaxation times in MS lesions [Larsson *et al.* 1992a], [Miller 1989] and NAWM [Stevenson *et al* 2000b], [Parry *et al* 2000], [van Walderveen *et al* 2000] when compared to the white matter of healthy controls. T1 relaxation time measurements appear sensitive for identifying tissue damage in MS, and T1

may be increased by axonal loss, oedema and gliosis [van Walderveen *et al* 1998], [Barnes *et al* 1991], [Barnes *et al* 1998].

In this study I have investigated 27 patients with early relapsing remitting disease, with a median disease duration of 1.7 years. By examining NABT as well as whole brain histograms in this group of patients, it is possible to assess whether there are observable changes affecting the normal appearing brain, and the extent to which lesions and NABT abnormalities contribute to global changes.

6.2 Subjects

Twenty seven patients (8 male, 19 female, mean age 35.7 years, range 24 - 54 years) again from the cohort of patients with early relapsing remitting MS [Poser *et al* 1983] were studied. All had a Kurtzke expanded disability status scale (EDSS) [Kurtzke1983] of less than or equal to 3 and all were within 3 years of first neurological symptom onset. Median disease duration was 1.7 years (range 1.0 to 3.0 years). All patients underwent a full neurological examination and evaluation of the EDSS and the MS Functional Composite Measure before undergoing MRI of the brain. The Functional Composite Score consists of three components: the nine hole peg test (a measure of upper limb function), the timed 25 foot walk (a measure of lower limb function) and the Paced Auditory Serial Addition Test (PASAT: a measure of cognitive function). The final Composite Measure was calculated using a standard protocol [Rudick *et al* 1997]. None of the patients were receiving disease modifying drugs and none were in acute relapse at the time of examination. No patients had been treated with steroids in

the preceding month. Fourteen age and sex matched healthy controls (5 male, 9 female, mean age 34.7 years, range 25 - 55) were also studied.

6.3 MRI acquisition protocol

The following data sets were obtained according to the protocols described in Chapter 3:

1 Fast spin echo (FSE)

2 T1 relaxation time

3 T1 weighted images pre and post Gd-DTPA

6.4 MRI data post-processing

6.4.1 Extraction of whole brain and NABT

Statistical Parametric Mapping (SPM) 99 segmentation followed by a likelihood thresholding step was used to extract brain tissue [Ashburner and Friston 1997], [Ashburner and Friston 2000], thereby obtaining whole brain tissue binary images. This was done using the heavily T1 weighted images obtained from the T1 relaxation time protocol. SPM creates a probability map for brain (white matter and grey matter), CSF and other tissues as its standard segmentation output. In order to extract brain tissue and reduce partial volume effects from CSF, only voxels above 75% confidence (probability) were selected as representative of each tissue. Voxels representing white or grey matter were then combined to produce a single binary image. I examined each resulting image in order to ensure that there had been accurate removal of CSF. In order to obtain NABT histograms, lesion masks were applied to the whole brain images, so that the lesions were excluded from the segmentation.

6.4.2 Lesion identification on T1 maps

Lesions were identified and outlined using a semi-automated local thresholding technique [Plummer 1992] on a PD weighted SE sequence (TR = 1500ms, TE = 19ms), which had been obtained as part of a parallel MTR acquisition [Griffin *et al* 2000] . This sequence was chosen for lesion outlining since the PD-weighting provided the high sensitivity required for lesion identification and the images could be co-registered with the PD-weighted image acquired for the T1 map calculation, which was of identical slice thickness. Co-registration was performed using a previously documented method [Symms *et al* 1997], [Woods *et al* 1997]. The regions of interest defined on the PD-weighted SE images were then placed on the T1 relaxation time maps. For examples of the images, see Figure 6.1.

6.4.3 Histogram analysis

As in previous studies [Dehmeshki *et al* 2001], [Filippi *et al* 1999] six histogram features were extracted from each normalised and smoothed histogram: mean T1 relaxation time measurement, peak height, peak location, T1 relaxation time measurement value of the 25^{th}, 50^{th} and 75^{th} percentiles. A T1 relaxation time histogram (i.e. T1 relaxation time frequency spectrum) of whole brain and NABT was calculated for each subject. Each T1 histogram was normalised to the residual brain tissue volume by dividing the number of counts in each sampling bin by the total number of voxels. Thus the total area under the histogram is fixed at unity and the normalised histogram is therefore a scaled frequency distribution. From each normalised histogram, the six histogram features as described above, were extracted.

6.4.4 Lesion load measurement

Total T2 lesion load was measured from lesions identified on the PD-weighted images of the FSE sequence. These lesions were outlined using a semi-automated local threshold technique [Plummer 1992]. Volumes were calculated by multiplying the lesion area by slice thickness. Non enhancing lesions were further classified according to their appearances on the T1-weighted image as iso intense or hypo intense with regard to surrounding NAWM. Gd-DTPA enhancing lesions were identified on the T1 weighted Gd-DTPA enhanced images. The volume of Gd-DTPA enhancing lesions and T1 hypo intense lesions was subsequently determined using the same method as that for T2 lesions.

6.5 Statistical analysis

All statistical analysis was performed using SPSS 9.0 (SPSS Inc, Chicago, Illinois, USA). Comparisons between patient and control groups were performed using the Mann-Whitney test. The Spearman rank correlation coefficient (r) was calculated to investigate correlations between histogram features and other parameters of disease activity, both clinical and radiological. In an exploratory analysis, $p < 0.05$ was regarded as significant.

6.6 Results

6.6.1 Clinical features

The median EDSS was 1.0 (range 0-3.0). The median PASAT score was 50 (range 0 – 60: SD = 15.25, although all patients with the exception of one scored between 30 and 60 correct answers, with one outlier who was unable to perform the test). The median 25 foot timed

walk was 4.70 seconds (range 3.80 – 7.00 seconds, SD = 0.74) and the median 9 hole peg test score was 21.03 seconds (range 15.9 – 70.10 seconds, SD = 6.96).

6.6.2 MRI

The T1 histograms showed the characteristic form expected from whole brain data, in both normal controls and patients [Parker 2001]. The highest peak of the histogram occurs at low T1, and represents white matter. A broad plateau extends to higher T1, and reflects the higher and more diverse T1 values seen in grey matter (see Figure 6.2).

6.6.3 Whole brain histograms

All 6 T1 histogram parameters were significantly different between patients and controls. Mean T1 value ($p=0.003$), peak location ($p=0.009$) and 25^{th} ($p=0.002$), 50^{th} ($p=0.008$) and 75^{th} ($p=0.01$) centile locations were all significantly higher in patients compared to controls, and the peak height was significantly lower ($p<0.001$) in patients (see Table 6.1). The mean T1 relaxation time histograms for the patient and control groups are illustrated in Figure 6.2.

6.6.4 Whole brain histogram correlations

The nine hole peg test score correlated moderately with all the histogram features, apart from peak height: mean T1 relaxation time ($r = 0.46$, $p = 0.01$), peak location ($r = 0.41$, $p = 0.04$), 25^{th} percentile ($r = 0.39$, $p = 0.05$), 50^{th} percentile ($r = 0.44$, $p = 0.02$) and 75^{th} percentile ($r = 0.56$, $p = 0.002$). There was no correlation of the T1 histogram measures with the 2 other components of the MSFC or with the EDSS. Both T2 total lesion load ($r = -0.57$, $p = 0.002$), and T1 hypo intense lesion load ($r = -0.48$, $p = 0.01$) correlated with histogram peak height, but not with the other histogram parameters.

6.6.5 NABT histograms

All 6 T1 histogram parameters were significantly different between patients and controls. Mean T1 value (p=0.003), peak location (p=0.01) and 25th (p=0.002), 50th (p=0.007) and 75th (p=0.009) percentiles were all significantly higher in patients, and the peak height was significantly lower (p<0.001) in patients (see Table 6.2).

6.6.6 NABT histogram correlations

The nine hole peg test score correlated moderately with all the histogram features, excluding the peak height. Mean T1 relaxation time (r = 0.47, p =0.01), peak location (r = 0.41, p = 0.04), 25th percentile (r = 0.39, p = 0.05), 50th percentile (r = 0.44, p = 0.02) and 75th percentile (r = 0.56, p = 0.02). There was no correlation of the NABT T1 histogram measures with the 2 other components of the MSFC or with the EDSS. The T1 hypo intense lesion load correlated with the histogram peak height (r = -0.48, p = 0.01).

6.6.7 Lesion Load measurements

The median T2 total lesion load was 4.20ml; T1 hypo-intense lesion load 0.33ml and the Gd-DTPA enhancing lesion load 0.16ml. The MS Functional Composite Score correlated moderately with T2 total lesion load (r = -0.48, p = 0.01) and T1 hypo-intense lesion load (r = -0.44, p = 0.02). Of its components, only the 25 foot timed walk correlated with all lesion load measurements: T2 lesion load (r = 0.46, p = 0.02), T1 hypo intense lesion load (r = 0.43, p = 0.02) and Gd-DTPA enhancing lesion load (r = 0.42, p = 0.03). There was no correlation between EDSS and any of the lesion load measures.

6.7. Discussion

I have found significant differences in all six histogram features between patient and control groups in both whole brain and NABT histograms. Mean T1 relaxation time, peak location and all percentile location values were increased and the peak height lower in patients when compared to controls. As the whole brain and NABT histograms are very similar, lesions would appear to make little contribution to the global histogram abnormalities. This is not surprising in view of the very small lesion load seen in these patients with early disease (median T2 lesion load was only 4.2ml which represents approximately 0.25% of total brain volume). Peak height was the only measure that appreciably changed when lesions were included (the value dropped from 18 to 17 x 10^{-4}). One possible explanation for this could be the fact that lesions often predominantly involve the white matter and the peak of the histogram occurs due to white matter: therefore the lesions will have a predominant effect at that position, even though they only constitute a small fraction of brain volume.

Pathological processes such as axonal damage and loss, gliosis, inflammation and oedema can all potentially cause an alteration in T1 relaxation time measurements through an increase in the pool of mobile water protons [Barnes *et al* 1991]. These processes occur in NAWM [Evangelou *et al.* 2000b], [Allen and McKeown1979] and in lesions. Cortical lesions are commonly seen in MS [Kidd *et al* 1999], and recent quantitative MR spectroscopy studies have documented the presence of NAGM changes in MS [Kapeller *et al* 2001]. Quantitative T1 relaxation time measurement should thus be sensitive to the various subtle forms of both grey and white matter pathology, resulting in the abnormal NABT histograms.

I extracted brain tissue using SPM 99, and then applied a threshold technique using a 75% probability threshold for voxels to be classified as brain tissue (white matter or grey matter combined). This will have diminished the effects from CSF containing pixels at the edge of the brain. This makes it more likely that intrinsic tissue changes rather than partial volume effects from atrophy are causing the NABT and whole brain histogram abnormalities.

A correlation between lesions and NABT T1 was only seen for the T1 hypo intense lesion load versus peak height. T1 hypo intense lesions are correlated pathologically with more severe axonal loss and this association may reflect lesions with axonal transection leading to secondary Wallerian degeneration in NAWM. However, as it is only a modest correlation, there are probably mechanisms for the NABT changes that are independent of lesions.

There were correlations between the nine hole peg test and all NABT histogram features, except for peak height. The nine hole peg test was the only clinical measurement that correlated with NABT histogram features. Other studies have found it to be a sensitive marker of disease activity [Kalkers *et al* 2001a]. In our cohort, all patients had an EDSS of less than or equal to 3.0, and as such did not show a great deal of clinical heterogeneity on this scale. However, the nine hole peg test was the clinical measure which showed a greatest range of clinical diversity (median 21.03 seconds, range 15.9 – 70.10 seconds, SD = 6.96). By following this cohort of patients longitudinally, it will be possible to further assess whether the nine hole peg test remains a sensitive marker of disease activity over time.

I have studied a group of patients with relapsing remitting disease, that have a shorter disease duration (median 1.7 years) than previous T1 relaxation time histogram studies which have been reported in abstract form only [Parry *et al* 2000], [van Walderveen *et al* 2000]. The application of histogram analysis techniques to T1 relaxation time measurements appears promising for a number of reasons. Firstly, it reflects global change within tissues, allowing assessment of subtle changes in NABT that would otherwise be "invisible". Secondly, the technique is largely automated, and the resulting measurements are objective, thus reducing the potential for inter-rater variability. Previous ROI approaches require manual intervention to place the ROI. Thirdly, histograms evaluate all of the tissue available where an ROI method samples smaller sub regions.

T1 relaxation time histograms may provide a sensitive method for assessing disease evolution in NABT. Serial studies are now underway to assess sensitivity to change over time. Given the evidence for extensive NABT abnormality in early relapsing remitting MS, which is at least partially independent of lesions, serial T1 histogram analysis of NABT should provide valuable new insights into the pathological natural history of MS and the effect of treatment interventions. Further studies investigating separately segmented histograms of NAWM and NAGM will also be of interest.

Table 6.1 Whole brain histogram features: patients compared to controls

	Mean T1 Relaxation time (ms)	Peak height (x 10^{-4})	Peak location (ms)	25th percentile (ms)	50th percentile (ms)	75th percentile (ms)
Patient mean (SD)	1026 (73)	17.1 (2.8)	681 (107)	749 (58)	967 (65)	1208 (83)
Patient median (range)	1008 (937 – 1251)	17.1 (10.9 – 22.6)	661(604 – 1087)	736 (683 – 923)	948 (899 – 1164)	1182 (1116-1461)
Control mean (SD)	969 (40)	20.8 (1.8)	619 (32)	698 (38)	919(43)	1152 (48)
Control median (range)	953 (922 – 1055)	20.5 (18.5 – 23.0)	610 (582 – 681)	683 (649 – 770)	906 (864 – 998)	1129 (1103 – 1245)
Patient vs Control	$p = 0.003$	$p < 0.001$	$p = 0.009$	$p = 0.002$	$P = 0.008$	$p = 0.01$

Table 6.2 NABT histogram features: patients compared to controls

	Mean T1 Relaxation time (ms)	Peak height (x 10^{-4})	Peak location (ms)	25th percentile (ms)	50th percentile (ms)	75th percentile (ms)
Patient mean (SD)	1027 (74)	18.0 (2.6)	680 (107)	751(59)	968 (66)	1209 (83)
Patient median (range)	1010 (938 – 1245)	18.0 (12.5 - 22.5)	659 (604 – 1087)	736 (685 – 923)	949 (900 – 1162)	1183 (1117 – 1451)
Control mean (SD)	969 (41)	20.8 (1.8)	619 (32)	699 (38)	920 (43)	1153 (48)
Control median (range)	954 (923– 1055)	20.5 (18.5 - 23.8)	610 (582 – 681)	683 (649 – 770)	906 (864 – 998)	1129 (1103 – 1245)
Patient vs Control	$p = 0.003$	$p < 0.001$	$p = 0.01$	$p = 0.002$	$p = 0.007$	$p = 0.009$

Figure 6.1

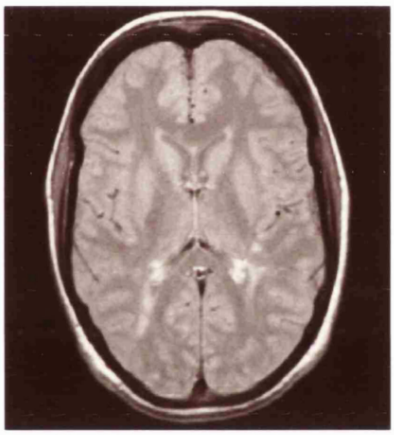


Figure 1a Proton density image of a patient showing white matter lesions

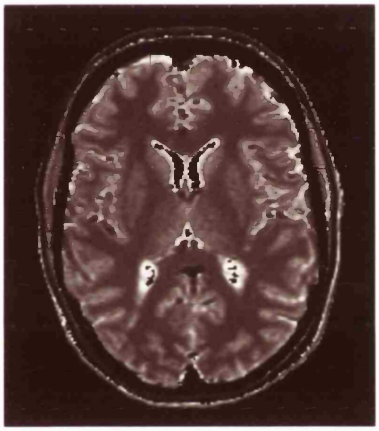
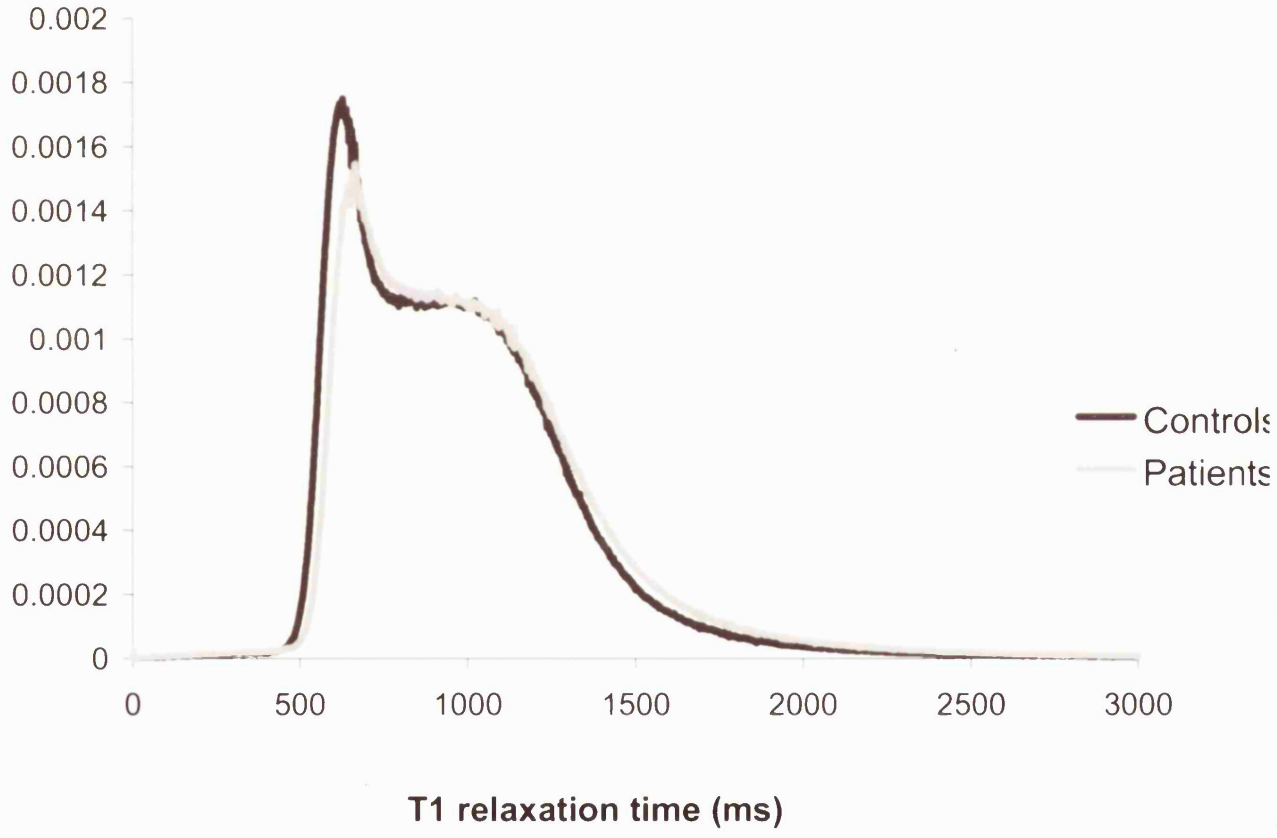


Figure 1b Corresponding T1 relaxation time map. Some of the pixels within the CSF appear black. This is because very high T1 values are not calculated (and are physiologically meaningless). These pixels are not included in any subsequent analysis.

Figure 6.2 Mean whole brain patient and control T1 relaxation times



PART 3. DIFFUSION TENSOR IMAGING IN EARLY DISEASE.

Chapter 7 Diffusion tensor imaging in early relapsing remitting Multiple Sclerosis

7.1. Study aims

Diffusion tensor magnetic resonance imaging (DTI) indices are abnormal in patients with established MS. The objective of this study was to examine the diffusion characteristics of MS lesions, NAWM and NAGM in MS patients in this cohort of patients. A further objective was to investigate the relationship between three DTI parameters (fractional anisotropy [FA], mean diffusivity [MD] and volume ratio [VR]) and clinical outcome measures (Kurtzke EDSS and MS Functional Composite Measure) in early disease.

Diffusion weighted imaging can detect abnormalities in lesions and NAWM in MS [Larsson *et al* 1992b], [Christiansen *et al* 1993], [Horsfield *et al.* 1996]. Pathological studies have also shown diffuse NAWM changes [Allen and McKeown1979], [Allen *et al* 1981]. Although the disease predominantly affects white matter, up to 24% of lesions can be found in the grey matter [Kidd *et al* 1999].

Abnormalities in MTR and mean diffusivity have been detected in NAGM using histogram analysis [Cercignani *et al* 2001] in patients with MS. Positron Emission Tomography (PET) studies [Paulesu *et al.* 1996], [Bakshi *et al.* 1998] and conventional MR techniques [Grimaud *et al* 1995], [Russo *et al.* 1997] have demonstrated both functional and structural abnormalities in NAGM. However, the functional significance of grey matter abnormalities has not been fully established.

The present study has investigated DTI in a group of patients with early relapsing remitting MS (mean disease duration 1.7 years). The purpose was to further define changes in macroscopically normal appearing brain tissue at this early stage in the disease process

7.2 Methods.

7.2.1 Patients

Twenty eight patients (mean age 35.5 years, 9 males and 20 females) from the early relapsing remitting MS cohort (median disease duration 1.7 years, range 1.0 to 3.0 years) were studied. The median Kurtzke EDSS score was 1.0 (range 0-3.0). Twenty seven healthy age and sex matched controls were also studied (mean age 33.9 years, 9 males and 18 females). The Kurtzke EDSS [Kurtzke1983] and MS Functional Composite Measure were assessed in each patient by myself. The timed walk was performed twice, and the mean of these times (in seconds) was used for analysis. The 9 hole peg test was performed twice for each hand, the mean of the values for each hand being used for the analysis. The 3 second version of the PASAT was performed once (scored by total number of correct answers).

The following image sequences were obtained as per the protocols described in Chapter 3:

1. Diffusion tensor imaging
2. Fast spin echo

7.3 Image processing

The data was processed to determine the diffusion tensor on a pixel by pixel basis for each of the 21 slices. FA, MD and volume ratio (VR) were calculated from the principle diffusivities of the diffusion tensor. All images were displayed on a Sun workstation (Sun Microsystems, Mountain View, CA), using the DispImage software package.

ROIs were placed in 12 NAWM regions and 9 NAGM regions (for examples see figures 7.1 and 7.2). These were placed in the following NAWM areas: middle cerebellar peduncle, superior cerebellar peduncle, cerebral peduncle, temporal, parietal, occipital and frontal lobes, anterior limb, genu and posterior limb of the internal capsule, and the genu and splenium of the corpus callosum. The following NAGM areas were studied: cerebellar grey matter, hippocampus, temporal, frontal, occipital and parietal grey, and the caudate, putamen and thalamus.

ROIs were a uniform size (9 pixels, equivalent to 31.72 mm^2) in all areas except the posterior limb of the internal capsule, where a larger region was sampled in order to attempt to study this anatomically eloquent site in greater detail. Bilateral ROIs were analysed for all regions except the genu and splenium of the corpus callosum where a single central ROI was placed. For the purposes of the analysis, a mean value was calculated for each anatomical area. ROIs were outlined on the b0 (b=0) images of the DW-EPI data set, with reference to the corresponding slices of the T2 and PD weighted images.

Lesion ROIs were defined according to previously defined criteria [Filippi *et al* 1998a]. The ROIs were then automatically transferred to the FA, VR and MD maps. Careful attention was paid to the placement of the ROIs, with the FA, VR and MD maps being examined after the placement of the ROIs to ensure accuracy and to minimise CSF contamination. Total T2 lesion load (on the PD-weighted images), T1 hypo intense lesion load and Gadolinium enhancing lesion load (on the post-contrast T1-weighted images) were also calculated.

The coefficient of variation was measured in 5 control subjects (3 females and 2 males, mean age 36.0 years) over a period of 6 months. Six NAWM and 6 NAGM areas were analysed. The mean coefficient of variation was 2.8%. The coefficient of variation was calculated separately for each region and in each region the coefficient of variation was calculated separately for MD, FA and VR. The final value was the mean of all the individual values. Note that due to geometrical distortions and low resolution unique to the EPI based DTI scans, the NABT and lesion ROI definitions used previously in chapters 3 – 6 could not be used here.

7.4 Statistical analysis

Differences between the patient and control groups for NAWM and NAGM regions were assessed using the Mann-Whitney test. The Bonferroni correction for multiple comparisons was applied. The corrected level of significance was set at p=0.002 in view of the fact that there were 21 separate comparisons made, assuming an initial level of significance of p=0.05. Differences between lesions and NAWM in patients were assessed using the Wilcoxon signed rank test. The relationship between

diffusion parameters and clinical outcome measures was assessed using Spearman's correlation coefficient.

7.5 Results

7.5.1 Lesions

The mean T2 lesion load was 8.09 ml (range 0.31-51.29 ml), the mean T1 hypointense lesion load was 1.77 ml (range 0-5.11ml). The mean Gadolinium enhancing lesion load was 0.46 ml (range 0-2.50 ml).

All three DTI indices showed a significant difference between lesions seen on T2 weighted images and NAWM.

Fractional anisotropy: lesion mean 0.31 (SD = 0.03), NAWM mean 0.59 (SD = 0.02), $p<0.001$.

Mean diffusivity: lesion mean 1.19 (SD = 0.18), NAWM mean 0.87 (SD = 0.05), $p<0.001$.

Volume ratio: lesion mean 0.88 (SD = 0.04), NAWM mean 0.59 (SD = 0.04), $p<0.001$.

7.5.2 NAWM and NAGM DTI measures

No statistically significant differences were found between patients and controls in any of the individual NAWM or NAGM regions (see Tables 7.1 and 7.2). However, several NAWM areas approached significance for FA and VR:

Fractional anisotropy: cerebral peduncle (p=0.01), temporal (p=0.01), and occipital (p=0.02): all were lower in patients.

Volume ratio: cerebral peduncle (p=0.005), temporal (p=0.02), occipital (p=0.01): all were higher in patients.

No correlation was found between clinical outcome measures (EDSS and MS Functional Composite Measure) and the three DTI indices in lesions, NAWM or NAGM (see Table 3). No correlation was found between lesion FA, VR and MD and the lesion load measurements.

7.6 Discussion

Measurements of molecular self diffusion in vivo yield an "apparent" diffusion coefficient (ADC). The ADC in vivo reflects the structural properties of the cellular compartments from which tissue is composed. Diseases that modify the size, spacing or integrity of CNS structures may therefore be evaluated by diffusion measurements. In brain regions in which the diffusion of water varies significantly with direction, the tissue exhibits the property of anisotropy. By contrast, regions where diffusion is similar in all directions have low anisotropy.

FA increases with anisotropy and provides the most detailed depiction of anisotropic areas. VR decreases with anisotropy and provides the best differentiation between high and low anisotropy areas. VR produces less well defined anatomical information than FA. Generally, white matter structures have a higher anisotropy due to the fact that the structures are more highly ordered, and grey matter has a lower anisotropy due to the decreased order of the fibres. The two anisotropy measures FA and VR both reflect this degree of tissue ordering, but differ in their sensitivity to changes at different anisotropy values, making them potentially sensitive to different pathological changes. Previous diffusion weighted imaging studies showed abnormalities in diffusion parameters in both lesions [Larsson *et al* 1992b] and

NAWM [Christiansen *et al* 1993], [Horsfield *et al* 1996]. However the acquisition of the diffusion tensor using diffusion tensor imaging (DTI) is more accurate as it is rotationally invariant, being independent of patient position and data acquisition method [Basser 1994].

One recent study using histogram analysis has shown that DTI histograms have different properties in patients with relapsing remitting MS (median disease duration 3.5 years) compared to controls. The patients showed a significantly elevated mean whole brain diffusion trace value and significant shift of the peak location to higher diffusivity values [Cercignani *et al* 2000]. However, a second study using whole brain diffusion histograms showed a significant difference between patients with secondary progressive MS and controls, but no significant difference was seen between patients with relapsing remitting disease (mean disease duration 2.8 years for patients with relapsing remitting disease) and controls [Nusbaum *et al* 2000]. This may, at least in part, have been due to the fact that the relapsing remitting MS patients in the first study had longer disease duration, and that they were a larger group (35 patients in the first study, 9 in the second).

A recent study looking at diffusion changes in both NAWM and grey matter [Ciccarelli *et al* 2000] found a significant decrease in anisotropy and a trend towards increased diffusivity in both the infratentorial and supratentorial NAWM, and a significant increase in anisotropy in the basal ganglia. The patients in this study had more established disease (median disease duration 13 years) and had a greater degree of disability than the present study (median EDSS 4.0).

Why were no significant DTI abnormalities detected in the NAWM and NAGM in our patient group? Several factors may be relevant. First, the group of patients we studied had early relapsing remitting disease and were mildly disabled. They had short disease duration (mean 1.7 years). Changes in NAWM and NAGM at this stage of MS may be subtle, and DTI may not be sensitive to them. Secondly the ROI methodology may lack sensitivity in NAWM, due to variable directions and crossing of multiple white matter fibre tracts and the subsequent heterogeneity of diffusion tensor maps. In this setting, slight shifts in ROI placement will give variable results, particularly for the anisotropy measures. This limitation is greater when the image pixel is large, as was the case for the present DTI sequence (2.5 x 2.5 mm in plane).

This particular ROI method applied to diffusion tensor imaging may not be as sensitive as when applied to other MR sequences for picking up early changes in normal appearing tissues. Indeed, in the same cohort, we have observed significant increases in NAWM T1 and decreases in NAWM MTR using a similar ROI method [Griffin *et al* 2000] as described in previous chapters. A recent study [Filippi *et al.* 2001] using a similar ROI approach, found diffusion abnormalities in NAWM of patients with more established MS (median disease duration 10 years). The same study reported a significant correlation between lesion MD and EDSS in patients with secondary progressive MS, but not in those with relapsing remitting disease, which may indicate that DTI is a more sensitive marker of disease activity in more established disease.

Although none of the NAWM regions reached statistical significance after Bonferroni correction, the trend towards significance was generally greater for FA and VR than for MD. This may be compatible with subtle interruption of fibre tract integrity and

axonal damage (which will decrease FA) but without significant oedema and inflammation (which will cause a non-directional increase in MD). This possibility is supported by MR spectroscopy studies in the same cohort, which have reported a mild but significant reduction in NAA, a neuronal marker, in the NAWM [Kapeller *et al* 2001]. Wallerian degeneration in NAWM may result from fibre transection in lesions [Trapp *et al* 1998]. The low FA seen in many lesions would be consistent with axonal damage, although loss of myelin per se may also contribute.

The lack of correlation between DTI indices and clinical outcome scores may again illustrate the relative lack of sensitivity and specificity of the technique in early disease. However, all patients were minimally disabled, thus the range of clinical abnormalities was very limited, which may have precluded a meaningful correlation.

The fact that MTR, T1 relaxation time (reported in this thesis) and NAA changes (reported in the literature [Kapeller *et al* 2000]) have been previously described in this cohort, suggests that this particular ROI method of diffusion imaging is not as sensitive to early and subtle change as other MR parameters. The greater sensitivity of the former technique may reflect higher tissue resolution for ROI analysis (for MTR and T1) or more global coverage (MRS).

Other methods of studying diffusion tensor abnormalities in early disease, such as whole brain and segmented DTI histograms, or the use of higher field MRI to improve the resolution and signal to noise ratio of DTI, may prove to be more sensitive and may better detect early and subtle changes in normal appearing tissues. Although the present study did not reveal significant changes in the normal appearing tissues, follow up will be of interest to determine when such abnormalities appear (as

are known to be present in longer duration MS cohorts [Filippi *et al* 1995a], [Cercignani *et al* 2001]) and to relate changes in DTI measures to the evolving clinical state.

Table 7.1 DTI parameter values in patient NAWM and control white matter

	FA patients median (range)	FA controls median (range)	FA patients vs controls (p value)	MD ($x10^{-3}mm^2/sec$) patients median (range)	MD ($x10^{-3}mm^2/sec$) controls median (range)	MD patients vs controls (p value)	VR patients median (range)	VR controls median (range)	VR patients vs controls (p value)
Middle cerebellar peduncle	0.56 (0.40-0.74)	0.56 (0.33-0.72)	0.99	0.83 (0.62-1.00)	0.79 (0.59-1.14)	0.07	0.65 (0.41-0.82)	0.63 (0.35-0.86)	0.81
Superior cerebellar peduncle	0.51 (0.34-0.73)	0.53 (0.41-0.65)	0.23	1.0 (0.73-1.40)	0.91 (0.60-1.30)	0.06	0.72 (0.47-0.93)	0.65 (0.44-0.83)	0.09
Cerebral peduncle	0.67 (0.56-0.79)	0.72 (0.61-0.80)	0.01	0.92 (0.62-1.43)	0.89 (0.53-1.20)	0.41	0.49 (0.28-0.67)	0.39 (0.34-0.60)	0.01
Temporal	0.51 (0.43-0.65)	0.55 (0.45-0.69)	0.01	0.88 (0.76-1.00)	0.84 (0.73-1.07)	0.17	0.72 (0.58-0.80)	0.69 (0.47-0.77)	0.02
Occipital	0.49 (0.40-0.63)	0.54 (0.46-0.65)	0.02	0.86 (0.77-1.00)	0.85 (0.68-1.08)	0.84	0.72 (0.60-0.83)	0.66 (0.57-0.78)	0.01
Ant. Internal capsule	0.55 (0.42-0.76)	0.57 (0.46-0.72)	0.57	0.83 (0.67-0.95)	0.83 (0.67-1.01)	0.93	0.67 (0.40-0.82)	0.63 (0.38-0.78)	0.92
Genu internal capsule	0.65 (0.52-0.87)	0.66 (0.49-0.80)	0.43	0.81 (0.61-0.97)	0.81 (0.64-0.97)	0.92	0.52 (0.28-0.71)	0.50 (0.31-0.73)	0.46
Post. Internal capsule	0.66 (0.53-0.73)	0.66 (0.38-0.74)	0.79	0.78 (0.62-0.92)	0.77 (0.60-0.97)	0.85	0.49 (0.36-0.70)	0.48 (0.31-0.70)	0.51
Genu corpus callosum	0.74 (0.52-0.87)	0.76 (0.55-0.95)	0.52	0.85 (0.65-1.12)	0.82 (0.68-1.07)	0.35	0.37 (0.23-0.71)	0.35 (0.25-0.59)	0.55
Splenium corpus callosum	0.71 (0.51-0.84)	0.74 (0.55-0.95)	0.14	0.96 (0.79-1.4)	0.91 (0.65-1.19)	0.06	0.44 (0.22-0.71)	0.41 (0.30-0.61)	0.23
Frontal	0.48 (0.38-0.62)	0.48 (0.39-0.62)	0.80	0.88 (0.72-1.11)	0.87 (0.75-0.97)	0.74	0.75 (0.61-0.97)	0.72 (0.56-0.84)	0.11
Parietal	0.53 (0.42-0.62)	0.53 (0.43-0.62)	0.63	0.80 (0.67-0.93)	0.80 (0.67-1.13)	0.81	0.69 (0.57-0.98)	0.68 (0.56-0.82)	0.82
Global NAWM	0.60 (0.53-0.65)	0.61 (0.55-0.67)	0.09	0.87 (0.78-1.01)	0.85 (0.74-0.96)	0.13	0.59 (0.51-0.68)	0.58 (0.50-0.66)	0.09

Table 7.2 DTI parameter values in patient NAGM and control grey matter

	FA patients median (range)	FA controls median (range)	FA patients vs controls (p value)	MD (x 10^{-3} mm^2/sec) patients median (range)	MD (x 10^{-3} mm^2/sec) controls median (range)	MD patients vs controls (p value)	VR patients median (range)	VR controls median (range)	VR patients vs controls (p value)
Cerebellar peduncle	0.21 (0.15-0.28)	0.19 (0.12-0.27)	0.13	0.87 (0.69-1.10)	0.87 (0.68-1.40)	0.56	0.95 (0.86-0.99)	0.95 (0.85-0.98)	0.79
Hippocampus	0.26 (0.15-0.41)	0.26 (0.16-0.41)	0.93	1.01 (0.75-1.30)	1.02 (0.79-1.44)	0.76	0.91 (0.82-0.97)	0.91 (0.79-0.97)	0.99
Temporal grey	0.18 (0.13-0.35)	0.17 (0.13-0.27)	0.35	0.90 (0.68-1.20)	0.96 (0.73-1.40)	0.47	0.96 (0.85-0.98)	0.96 (0.90-0.97)	0.89
Frontal grey	0.19 (0.14-0.25)	0.17 (0.13-0.23)	0.08	1.00 (0.87-1.20)	1.00 (0.87-1.20)	0.84	0.96 (0.93-0.97)	0.96 (0.89-0.97)	0.89
Caudate	0.32 (0.16-0.55)	0.31 (0.21-0.43)	0.93	0.82 (0.69-0.99)	0.84 (0.69-0.97)	0.86	0.88 (0.65-0.97)	0.88 (0.78-0.96)	0.76
Putamen	0.30 (0.20-0.42)	0.30 (0.20-0.46)	0.76	0.80 (0.63-0.94)	0.80 (0.62-0.87)	0.70	0.90 (0.80-0.97)	0.90 (0.75-0.96)	0.79
Thalamus	0.35 (0.25-0.48)	0.34 (0.23-0.46)	0.77	0.84 (0.63-0.94)	0.85 (0.66-1.19)	0.83	0.86 (0.73-0.93)	0.86 (0.74-0.94)	0.68
Occipital grey	0.18 (0.13-0.25)	0.17 (0.13-0.26)	0.53	0.97 (0.77-1.30)	0.97 (0.82-1.20)	0.78	0.96 (0.92-0.98)	0.97 (0.92-0.98)	0.63
Parietal grey	0.17 (0.12-0.26)	0.17 (0.11-0.25)	0.69	1.00 (0.81-1.33)	1.00 (0.71-1.20)	0.86	0.96 (0.80-0.98)	0.97 (0.93-0.98)	0.60
Global NAGM	0.23 (0.19-0.29)	0.23 (0.18-0.28)	0.53	0.92 (0.74-1.09)	0.92 (0.80-1.09)	0.45	0.93 (0.89-0.96)	0.93 (0.90-0.96)	0.83

Table 7.3 Spearman's correlations between DTI parameters and clinical outcome measures

	EDSS	COMPOSITE SCORE
Lesion FA	R = -0.36 p = 0.07	r = 0.13 p = 0.57
Lesion MD	R = 0.24 p = 0.24	r = -0.33 p = 0.14
Lesion VR	R = 0.42 p = 0.06	r = -0.34 p = 0.13
NAWM FA	r = -0.17 p = 0.39	r = 0.08 p = 0.72
NAWM MD	R = 0.01 p = 0.95	r = -0.04 p = 0.86
NAWM VR	R = 0.17 p = 0.38	r = -0.01 p = 0.97
NAGM FA	R = 0.04 p = 0.86	r = -0.05 p = 0.83
NAGM MD	R = -0.24 p = 0.21	r = 0.34 p = 0.10
NAGM VR	R = -0.08 p = 0.67	r = 0.04 p = 0.87

Figure 7.1 Fractional anisotropy map of a control subject showing NAWM ROIs.

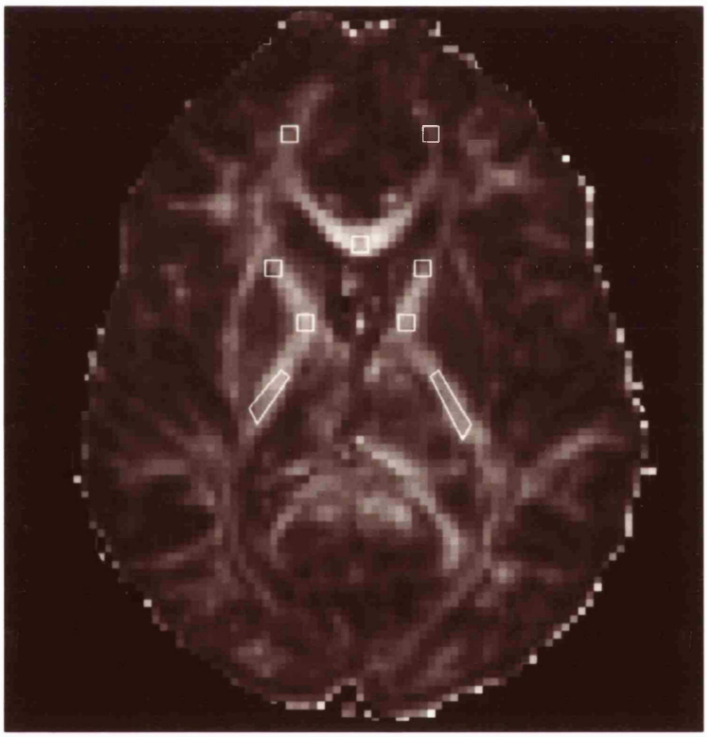
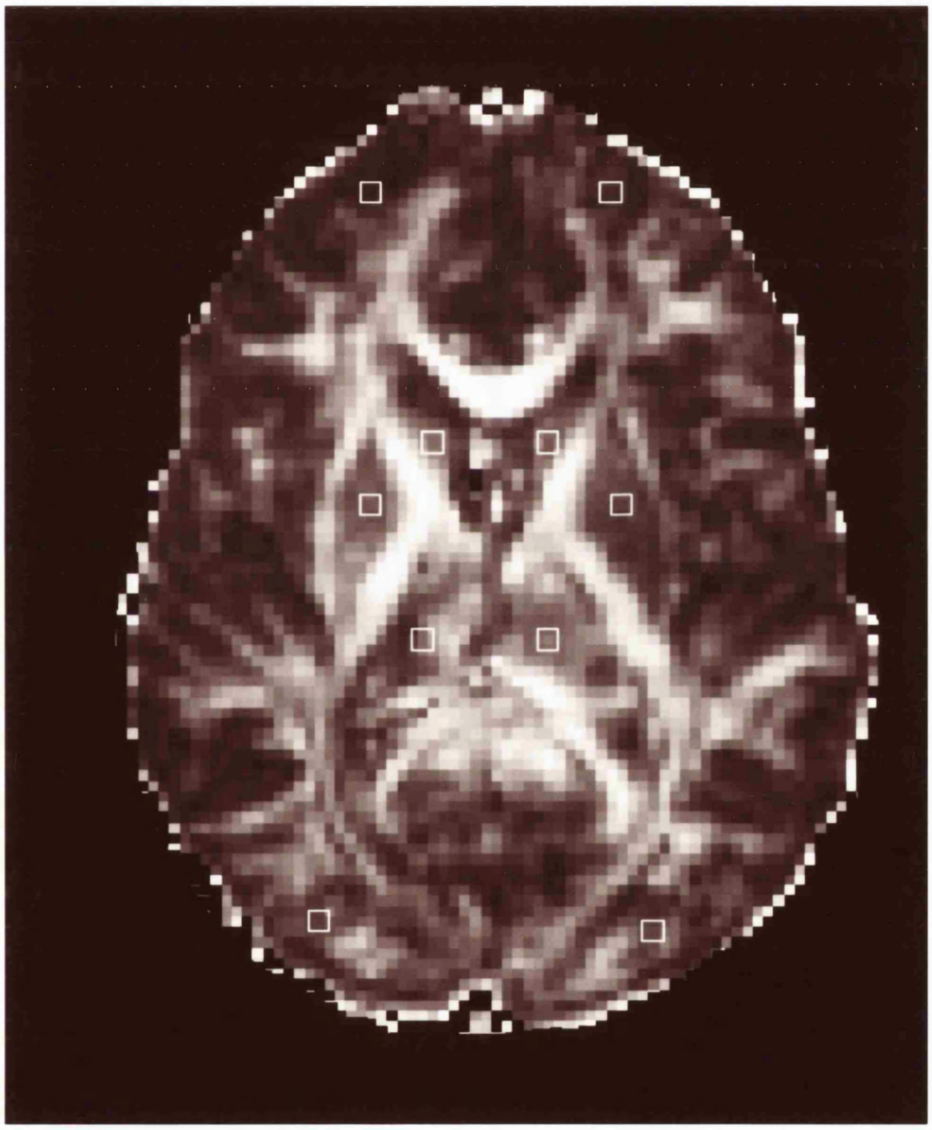


Figure 7.2. Fractional anisotropy map showing NAGM ROIs.



PART 4. SUMMARY AND CONCLUSIONS.

Chapter 8 Summary and conclusions

8.1 Study aims

The aims of this thesis were to examine changes in both NABT and lesions in patients with early relapsing remitting MS using both MR imaging techniques and clinical outcome scores. MR imaging techniques have become important outcome measures used in MS treatment trials, and as such we have used various specific techniques to observe the changes that occur at this early stage in the disease evolution. These studies have recruited patients with strictly defined [Poser *et al* 1983] clinically definite early relapsing remitting MS (disease duration less than 3 years). There are presently relatively few studies that have investigated a cohort with such early disease.

8.2 Lesion changes

By using techniques such as MTR, DTI, T1 relaxation time measurements, FSE and pre and post Gd-DTPA T1 weighted imaging of the brain and spinal cord, I have been able to identify changes occurring at this early stage, and also compare the changes in NABT to those within MS lesions. Most patients (81%) in this cohort exhibited one or more Gd-DTPA enhancing lesion. This high frequency reflects a clinically active cohort [Grossman *et al* 1986, Smith *et al* 1993] although the use of triple dose Gd-DTPA may also have modestly increased the number of patients with enhancing lesions [Silver *et al* 1997, Filippi *et al* 1996c]. The T1- hypo intense lesion volume was less than 10% of total T2 lesion volume, which is considerably less than the T1/T2 volume ratio seen in secondary progressive MS, this being about 30 % [Katz *et al* 1993, Truyen *et al* 1996].

The combination of frequent enhancement and low T1/T2 ratio suggests that there is considerable inflammation and oedema but relatively little axonal damage in early relapsing remitting MS lesions. However, there were marked inter-individual variations in both Gd-DTPA enhancing and T1-hypointense lesion volumes implying that the amount of inflammation and axonal damage varies considerably between patients. Long term follow up studies are needed to determine the prognostic significance of these features.

I have also shown significant structural changes within MS lesions using MTR (decrease), T1 relaxation time measurement (increase), fractional anisotropy (decrease), volume ratio (increase) and mean diffusivity (increase). Such changes are consistent with the well known pathological features: demyelination, axonal damage and inflammation in this early cohort.

8.3 NABT changes

I have shown the relationship between MTR and T1 relaxation time to be different in lesions and patient NAWM, thus illustrating that the pathological changes occurring are different within the two tissue types. A weak MTR/T1 relationship in patient NAWM falls between the patterns observed in control white matter (no correlation) and lesions (moderate correlation). This tells us that the pathological changes that occur in patient tissue may have a different relationship to each other when compared to their relationship in normal controls.

Pathological studies of NAWM in MS reveal a variety of subtle changes, such as astrocyte hyperplasia, inflammatory infiltrates, myelin breakdown products [Allen

and McKeown1979] and axonal loss [Evangelou *et al* 2000b]. Water content is also increased [Tourtellotte and Parker1968]. These subtle changes might extend the range of T1 relaxation times further but also lead to slight reductions in MTR - indeed comparing control and patient NAWM there were group differences in these two parameters. As there was only a weak relationship between MTR and T1; it is evident that the combination of MTR and T1 relaxation time measurement provides a more complete characterisation of different tissues than either measure alone. In MS, the complementary information obtained from MTR and T1 is most apparent in NAWM. I have shown more extensive abnormalities of T1 than of MTR, suggesting that the former is more sensitive to the subtle pathological changes occurring in NAWM, at least using the sequences applied in this study. T1 relaxation time thus appears a promising tool for monitoring MS NAWM. Subtle changes may also be detected by other techniques such as magnetic resonance spectroscopy, which allows an accurate and sensitive measurement of various metabolite concentrations within both normal appearing tissues and lesions.

Increasingly, grey matter pathology has been studied and thought to contribute to both neurological and psychological disability in MS. Quantitative techniques such as MTR and T1 measurement are specific to individual MR properties, and may be sensitive to subtle changes due to grey matter pathology. Two groups have recently reported abnormalities occurring in MS grey matter using MTR histogram analysis [Cercignani *et al* 2001], [Ge *et al* 2000]. Both these studies investigated patients with a longer duration of disease. In the present cohort, I have demonstrated unequivocal evidence of abnormality in frontal NAGM using a quantitative measure of T1. While this suggests that grey matter pathology is present, the changes were much less extensive than those seen in NAWM.

Restricting sampling to selected regions may be less sensitive to abnormality than histogram analysis of the entire segmented tissue type and I have found significant differences in all six T1 relaxation time measurement histogram features between patient and control groups in both whole brain and NABT histograms. Mean T1 relaxation time, peak location and all percentile location values were increased and the peak height was lower in patients when compared to controls. As the whole brain and NABT histograms are very similar, lesions would appear to make little contribution to the global histogram abnormalities. This is not surprising in view of the very small lesion load seen in these patients with early disease (median T2 lesion load was only 4.2ml which represents approximately 0.25% of total brain volume). Peak height was the only measure that appreciably changed when lesions were included (the value dropped from 18 to 17 x 10^{-4}). A possible explanation for this is the fact that lesions often predominantly involve the white matter and the peak of the histogram occurs due to white matter: therefore the lesions will have a predominant effect at that position, even though they only constitute a small fraction of brain volume. T1 relaxation time histograms may prove to be a very sensitive marker of disease activity over time, but serial studies will be required to investigate this issue.

In the studies detailed within this thesis, DTI appeared to be the least sensitive MRI parameter at detecting change in NABT. This could be due to a number of reasons. Changes in NAWM and NAGM at this stage of MS may be subtle, and DTI may not be sensitive to them. The ROI methodology may lack sensitivity in NAWM, due to variable directions and crossing of multiple white matter fibre tracts and the subsequent heterogeneity of diffusion tensor maps. In this setting, slight shifts in ROI placement will give variable results, particularly for the anisotropy measures. This

limitation is greater when the image pixel is large, as was the case for the present DTI sequence (2.5 x 2.5 mm in plane).

A recent study [Filippi *et al* 2001] using a similar ROI approach, found diffusion abnormalities in NAWM of patients with more established MS (median disease duration 10 years). The same study reported a significant correlation between lesion MD and EDSS in patients with secondary progressive MS, but not in those with relapsing remitting disease, which may indicate that DTI is a more sensitive marker of disease activity in more established severe disease.

Although the DTI analysis of NAWM regions did not reach statistical significance after Bonferroni correction, the trend towards significance was generally greater for FA and VR than for MD. This may be compatible with subtle interruption of fibre tract integrity and axonal damage (which will decrease FA) but without significant oedema and inflammation (which will cause a non-directional increase in MD). This possibility is supported by MR spectroscopy studies in the same cohort, which have reported a mild but significant reduction in NAA, a neuronal marker, in the NAWM [Kapeller *et al* 2001]. Wallerian degeneration in NAWM may result from fibre transection in lesions [Trapp *et al* 1998]. The low FA seen in many lesions would be consistent with axonal damage, although loss of myelin per se may also contribute.

Given that unequivocal abnormalities of MTR, T1 relaxation time (reported in this thesis) and NAA (reported in the literature [Kapeller *et al* 2001]) have been described in the NAWM of the same cohort, it would appear that the ROI method of diffusion analysis is not as sensitive to early and subtle change as the other MR parameters. The greater sensitivity of the former techniques may reflect higher tissue resolution

for ROI analysis (for MTR and T1) or more global coverage (MRS). It will be of future interest to assess DTI using both ROI and histogram approaches over a longer time course.

8.4 Clinical correlations and scales

The lack of correlation between DTI indices and clinical outcome scores may illustrate a relative lack of sensitivity and specificity of the technique in early disease. However, all patients were minimally disabled, thus the range of clinical abnormalities was very limited, which may also have precluded a meaningful correlation. On the other hand, a correlation observed between NABT T1 measures and the 9HPT suggests that not only is the former a more sensitive measure of abnormality than diffusion but it may also be detecting functionally relevant abnormalities.

In the future, it will be important to assess both the EDSS and MSFC clinical scores in the long term, in order to evaluate their sensitivity to clinical change. The relationship between the older and newer clinical scores will also be of interest, as the EDSS score is presently widely used in clinical trials at present, yet it does have many limitations. By the longitudinal assessment of this cohort of patients, it will be possible to gain further valuable insights into the natural history of this disease and its progression to irreversible disability. It should also be possible to comment further on the prognostic value of each clinical and radiological parameter over time and their relationship to relapse and disability. By examining the clinical, immunological and radiological data of the sub-group of patients who will be treated with Interferon-beta over the course of the study, it will be possible to further assess the effects of this therapeutic intervention.

8.5 Future prospects

The results of the studies within this thesis emphasise the potential of multi parameter MR data in general to improve tissue characterisation. Such approaches may be valuable in improving diagnosis, understanding disease pathogenesis and monitoring treatment. For example, the application of multi parameter MR measurements in MS, using appropriate techniques for specific pathological features (e.g. gadolinium enhancement for lesion inflammation, MTR and T1 to monitor NABT and to evaluate tissue damage in lesions and NAA measurement for axonal damage) may elucidate the mechanisms of myelin and axonal loss, which ultimately lead to irreversible disability in many individuals.

By continuing this study over a longer time period, it will be possible to assess these MR imaging methods and their sensitivity to change over time. Other MR imaging data being collected from this cohort, such as brain and spinal cord atrophy and MR spectroscopic imaging, will also provide further information, both cross-sectional and over a longitudinal time course. The combination of measures will further elucidate the relationship between the neurodegenerative and inflammatory aspects of MS pathology. As part of this longitudinal study, we are also collecting immunological and questionnaire data (as discussed in chapter 3). The assessment of the prognostic role of all these measures will also be of great interest. This study is continuing at present, and these important questions concerning pathogenesis and prognosis will be addressed by longitudinal analysis of the MR, immunological and clinical data.

REFERENCES

Efficacy and toxicity of cyclosporine in chronic progressive multiple sclerosis: a randomized, double-blinded, placebo-controlled clinical trial. The Multiple Sclerosis Study Group. *Ann.Neurol.* 1990;**27**:591-605.

The Canadian cooperative trial of cyclophosphamide and plasma exchange in progressive multiple sclerosis. The Canadian Cooperative Multiple Sclerosis Study Group. *Lancet* 1991;**337**:441-6.

Interferon beta-1b in the treatment of multiple sclerosis: final outcome of the randomized controlled trial. The IFNB Multiple Sclerosis Study Group and The University of British Columbia MS/MRI Analysis Group. *Neurology* 1995;**45**:1277-85.

Placebo-controlled multicentre randomised trial of interferon beta-1b in treatment of secondary progressive multiple sclerosis. European Study Group on interferon beta-1b in secondary progressive MS. *Lancet* 1998;**352**:1491-7.

Randomised double-blind placebo-controlled study of interferon beta-1a in relapsing/remitting multiple sclerosis. PRISMS (Prevention of Relapses and Disability by Interferon beta-1a Subcutaneously in Multiple Sclerosis) Study Group. *Lancet* 1998;**352**:1498-504.

PRISMS-4: Long-term efficacy of interferon-beta-1a in relapsing MS. *Neurology* 2001;**56**:1628-36.

Allen IV, McKeown SR. A histological, histochemical and biochemical study of the macroscopically normal white matter in multiple sclerosis. *J.Neurol.Sci.* 1979;**41**:81-91.

Allen IV, Glover G, Anderson R. Abnormalities in the macroscopically normal white matter in cases of mild or spinal multiple sclerosis (MS). *Acta Neuropathol.Suppl (Berl)* 1981;**7**:176-8.

Ashburner J and Friston K. Multimodal Image Coregistration and Partitioning - A Unified Framework. *Neuroimage* 1997;**6**:209-217.

Ashburner J and Friston K. Voxel-Based Morphometry - The Methods. *Neuroimage* 2000;**11**:805-821.

Bakshi R, Miletich RS, Kinkel PR, Emmet ML, Kinkel WR. High-resolution fluorodeoxyglucose positron emission tomography shows both global and regional cerebral hypometabolism in multiple sclerosis. *J.Neuroimaging* 1998;**8**:228-34.

Barker GJ, Tofts PS, Gass A. An interleaved sequence for accurate and reproducible clinical measurement of magnetisation transfer ratio. *Magn Reson Imaging* 1996;**14**: 403-405.

Barkhof F, Scheltens P, Frequin ST, Nauta JJ, Tas MW, Valk J, Hommes OR. Relapsing-remitting multiple sclerosis: sequential enhanced MR imaging vs clinical findings in determining disease activity. *AJR Am.J.Roentgenol.* 1992a ;**159**:1041-7.

Barkhof F, Valk J, Hommes OR, Scheltens P, Nauta JJ. Gadopentetate dimeglumine enhancement of multiple sclerosis lesions on long TR spin-echo images at 0.6 T. *AJNR Am.J.Neuroradiol.* 1992b ;**13**:1257-9.

Barkhof F, Tas MW, Frequin ST, Scheltens P, Hommes OR, Nauta JJ, Valk J. Limited duration of the effect of methylprednisolone on changes on MRI in multiple sclerosis. *Neuroradiology* 1994;**36**:382-7.

Barkhof F, McGowan JC, van Waesberghe JH, Grossman RI. Hypointense multiple sclerosis lesions on T1-weighted spin echo magnetic resonance images: their contribution in understanding multiple sclerosis evolution. *J.Neurol.Neurosurg.Psychiatry* 1998;**64 Suppl 1**:S77-S79.

Barnes D, Munro PM, Youl BD, Prineas JW, McDonald WI. The longstanding MS lesion. A quantitative MRI and electron microscopic study. *Brain* 1991;**114 (Pt 3)**:1271-80.

Basser PJ, Pierpaoli C. Microstructural and physiological features of tissues elucidated by quantitative-diffusion-tensor MRI . *J Magn Reson B* 1996; **111**: 209-219.

Beaulieu C, Allen PS. Determinants of anisotropic water diffusion in nerves. *Magn Reson Med* 1994; **31**:394-400.

Beck RW, Cleary PA, Trobe JD, Kaufman DI, Kupersmith MJ, Paty DW, Brown CH. The effect of corticosteroids for acute optic neuritis on the subsequent development of multiple sclerosis. The Optic Neuritis Study Group. *N.Engl.J.Med.* 1993;**329**:1764-9.

Bergstrom T. Several options for antiviral treatment trials in multiple sclerosis-- but which targets should be selected? *Expert.Opin.Pharmacother.* 2000;**1**:1087-90.

Bitsch A, Schuchardt J, Bunkowski S, Kuhlmann T, Bruck W. Acute axonal injury in multiple sclerosis. Correlation with demyelination and inflammation. *Brain* 2000;**123 (Pt 6)**:1174-83.

Brex PA, Gomez-Anson B, Parker GJ, Molyneux PD, Miszkiel KA, Barker GJ, MacManus DG, Davie CA, Plant GT, Miller DH. Proton MR spectroscopy in clinically isolated syndromes suggestive of multiple sclerosis. *J.Neurol.Sci.* 1999;**166**:16-22.

Brex PA, Leary SM, Plant GT, Thompson AJ, Miller DH. Magnetization transfer imaging in patients with clinically isolated syndromes suggestive of multiple sclerosis. *AJNR Am J Neuroradiol* 2001a ;**22(5)**: 947 – 51.

Brex PA, Miszkiel KA, O'Riordan JI, Plant GT, Moseley IF, Thompson AJ, Miller DH. Assessing the risk of early multiple sclerosis in patients with clinically isolated syndromes: the role of a follow up MRI. *J.Neurol.Neurosurg.Psychiatry* 2001b ;**70**:390-3.

Capra R, Marciano N, Vignolo LA, Chiesa A, Gasparotti R. Gadolinium-pentetic acid magnetic resonance imaging in patients with relapsing remitting multiple sclerosis. *Arch.Neurol.* 1992;**49**:687-9.

Cercignani M, Iannucci G, Rocca MA, Comi G, Horsfield MA, Filippi M. Pathologic damage in MS assessed by diffusion-weighted and magnetization transfer MRI. *Neurology* 2000;**54**:1139-44.

Cercignani M, Bozzali M, Iannucci G, Comi G, Filippi M. Magnetisation transfer ratio and mean diffusivity of normal appearing white and grey matter from patients with multiple sclerosis. *J.Neurol.Neurosurg.Psychiatry* 2001;**70**:311-7

Charcot JM. Lectures on the diseases of the nervous system. London. The New Sydenham Society, 1877.

Chard DT, Griffin CM, Parker GJM, Kapoor R, Thompson AJ, Miller DH. Brain atrophy in clinically early relapsing remitting multiple sclerosis. *Brain* 2002;**125**: 327 – 337.

Christiansen P, Gideon P, Thomsen C, Stubgaard M, Henriksen O, Larsson HB. Increased water self-diffusion in chronic plaques and in apparently normal white matter in patients with multiple sclerosis. *Acta Neurol.Scand.* 1993;**87**:195-9.

Ciccarelli O, Werring DJ, Wheeler-Kingshott CA, Barker GJ, Parker GJ, Thompson AJ, Miller DH. Investigation of MS normal-appearing brain using diffusion tensor MRI with clinical correlations. *Neurology* 2001;**56**:926-33.

Cohen JA, Fischer JS, Bolibrush DM, Jak AJ, Kniker JE, Mertz LA, Skaramagas TT, Cutter GR. Intrarater and interrater reliability of the MS functional composite outcome measure. *Neurology* 2000;**54**:802-6.

Coles AJ, Wing M, Smith S, Coraddu F, Greer S, Taylor C, Weetman A, Hale G, Chatterjee VK, Waldmann H, Compston A. Pulsed monoclonal antibody treatment and autoimmune thyroid disease in multiple sclerosis. *Lancet* 1999;**354**:1691-5.

Compston DAS Distribution of multiple sclerosis. In Compston DAS *et al* (eds). *McAlpine's Multiple Sclerosis.* Third edition. Churchill Livingstone, 1998a: 63 – 100.

Compston DAS Genetic susceptibility to multiple sclerosis. In Compston DAS *et al* (eds). *McAlpine's Multiple Sclerosis.* Third edition. Churchill Livingstone, 1998b: 101 – 142.

Cutter GR, Baier ML, Rudick RA, Cookfair DL, Fischer JS, Petkau J, Syndulko K, Weinshenker BG, Antel JP, Confavreux C, Ellison GW, Lublin F, Miller AE, Rao SM, Reingold S, Thompson A, Willoughby E. Development of a multiple sclerosis functional composite as a clinical trial outcome measure. *Brain* 1999;**122 (Pt 5)**:871-82.

Davie CA, Hawkins CP, Barker GJ, Brennan A, Tofts PS, Miller DH, McDonald WI. Serial proton magnetic resonance spectroscopy in acute multiple sclerosis lesions. *Brain* 1994;**117 (Pt 1)**:49-58.

Davie CA, Barker GJ, Thompson AJ, Tofts PS, McDonald WI, Miller DH. 1H magnetic resonance spectroscopy of chronic cerebral white matter lesions and normal appearing white matter in multiple sclerosis. *J.Neurol.Neurosurg.Psychiatry* 1997;**63**:736-42.

Dean G, Kurtzke JF. On the risk of multiple sclerosis according to age at immigration to South Africa. *Br.Med.J.* 1971;**3**:725-9.

Dehmeshki J, Silver NC, Leary SM, Tofts PS, Thompson AJ, Miller DH. Magnetisation transfer ratio histogram analysis of primary progressive and other multiple sclerosis subgroups. *J.Neurol.Sci.* 2001;**185**:11-7.

Dousset V, Grossman RI, Ramer KN, Schnall MD, Young LH, Gonzalez-Scarano F, Lavi E, Cohen JA. Experimental allergic encephalomyelitis and multiple sclerosis: lesion characterization with magnetization transfer imaging. *Radiology* 1992;**182**:483-91.

Ebers GC. Genetics and multiple sclerosis: an overview. *Ann.Neurol.* 1994;**36 Suppl**:S12-S14.

Ebers GC, Sadovnick AD, Risch NJ. A genetic basis for familial aggregation in multiple sclerosis. Canadian Collaborative Study Group. *Nature* 1995;**377**:150-1.

Engell T. A clinico-pathoanatomical study of multiple sclerosis diagnosis. *Acta Neurol.Scand.* 1988;**78**:39-44.

Evangelou N, Esiri MM, Smith S, Palace J, Matthews PM. Quantitative pathological evidence for axonal loss in normal appearing white matter in multiple sclerosis. *Ann.Neurol.* 2000a ;**47**:391-5.

Evangelou N, Konz D, Esiri MM, Smith S, Palace J, Matthews PM. Regional axonal loss in the corpus callosum correlates with cerebral white matter lesion volume and distribution in multiple sclerosis. *Brain* 2000b ;**123 (Pt 9)**:1845-9.

Ferguson B, Matyszak MK, Esiri MM, Perry VH. Axonal damage in acute multiple sclerosis lesions. *Brain* 1997;**120 (Pt 3)**:393-9.

Filippi M, Campi A, Dousset V, Baratti C, Martinelli V, Canal N, Scotti G, Comi G. A magnetization transfer imaging study of normal-appearing white matter in multiple sclerosis. *Neurology* 1995a ;**45**:478-82.

Filippi M, Campi A, Martinelli V, Pereira C, Scotti G, Comi G. Transitional progressive multiple sclerosis: MRI and MTI findings. *Acta Neurol.Scand.* 1995b ;**92**:178-82.

Filippi M, Paty DW, Kappos L, Barkhof F, Compston DA, Thompson AJ, Zhao GJ, Wiles CM, McDonald WI, Miller DH. Correlations between changes in disability and T2-weighted brain MRI activity in multiple sclerosis: a follow-up study. *Neurology* 1995c ;**45**:255-60.

Filippi M, Horsfield MA, Tofts PS, Barkhof F, Thompson AJ, Miller DH. Quantitative assessment of MRI lesion load in monitoring the evolution of multiple sclerosis. *Brain* 1995d ;**118 (Pt 6)**:1601-12.

Filippi M, Campi A, Colombo B, Pereira C, Martinelli V, Baratti C, Comi G. A spinal cord MRI study of benign and secondary progressive multiple sclerosis. *J.Neurol.* 1996a ;**243**:502-5.

Filippi M, Yousry T, Horsfield MA, Alkadhi H, Rovaris M, Campi A, Voltz R, Comi G. A high-resolution three-dimensional T1-weighted gradient echo sequence improves the detection of disease activity in multiple sclerosis. *Ann.Neurol.* 1996b ;**40**:901-7.

Filippi M, Capra R, Campi A, Colombo B, Prandini F, Marciano N, Gasparotti R, Comi G. Triple dose of gadolinium-DTPA and delayed MRI in patients with benign multiple sclerosis. *J.Neurol.Neurosurg.Psychiatry* 1996c ;**60**:526-30.

Filippi M, Yousry T, Campi A, Kandziora C, Colombo B, Voltz R, Martinelli V, Spuler S, Bressi S, Scotti G, Comi G. Comparison of triple dose versus standard dose

gadolinium-DTPA for detection of MRI enhancing lesions in patients with MS. *Neurology* 1996d ;**46**:379-84.

Filippi M, Gawne-Cain ML, Gasperini C, vanWaesberghe JH, Grimaud J, Barkhof F, Sormani MP, Miller DH. Effect of training and different measurement strategies on the reproducibility of brain MRI lesion load measurements in multiple sclerosis. *Neurology* 1998a ;**50**:238-44.

Filippi M, Horsfield MA, Ader HJ, Barkhof F, Bruzzi P, Evans A, Frank JA, Grossman RI, McFarland HF, Molyneux P, Paty DW, Simon J, Tofts PS, Wolinsky JS, Miller DH. Guidelines for using quantitative measures of brain magnetic resonance imaging abnormalities in monitoring the treatment of multiple sclerosis. *Ann.Neurol.* 1998b ;**43**:499-506.

Filippi M, Rocca MA, Comi G. Magnetization transfer ratios of multiple sclerosis lesions with variable durations of enhancement. *J.Neurol.Sci.* 1998c ;**159**:162-5.

Filippi M, Rocca MA, Martino G, Horsfield MA, Comi G. Magnetization transfer changes in the normal appearing white matter precede the appearance of enhancing lesions in patients with multiple sclerosis. *Ann.Neurol.* 1998d;**43**:809-14.

Filippi M. Magnetization transfer imaging to monitor the evolution of individual multiple sclerosis lesions. *Neurology* 1999a ;**53**:S18-S22.

Filippi M, Iannucci G, Tortorella C, Minicucci L, Horsfield MA, Colombo B, Sormani MP, Comi G. Comparison of MS clinical phenotypes using conventional and magnetization transfer MRI. *Neurology* 1999b ;**52**:588-94.

Filippi M, Tortorella C, Rovaris M, Bozzali M, Possa F, Sormani MP, Iannucci G, Comi G. Changes in the normal appearing brain tissue and cognitive impairment in multiple sclerosis. *J.Neurol.Neurosurg.Psychiatry* 2000;**68**:157-61.

Filippi M, Cercignani M, Inglese M, Horsfield MA, Comi G. Diffusion tensor magnetic resonance imaging in multiple sclerosis. *Neurology* 2001;**56**:304-11.

Filippini G, Comi GC, Cosi V, Bevilacqua L, Ferrarini M, Martinelli V, Bergamaschi R, Filippi M, Citterio A, D'Incerti L, . Sensitivities and predictive values of paraclinical tests for diagnosing multiple sclerosis. *J.Neurol.* 1994;**241**:132-7.

Fischer JS, Rudick RA, Cutter GR, Reingold SC. The Multiple Sclerosis Functional Composite Measure (MSFC): an integrated approach to MS clinical outcome assessment. National MS Society Clinical Outcomes Assessment Task Force. *Mult.Scler.* 1999;**5**:244-50.

Fisk JD, Pontefract A, Ritvo PG, Archibald CJ, Murray TJ. The impact of fatigue on patients with multiple sclerosis. *Can.J.Neurol.Sci.* 1994;**21**:9-14.

Fox NC, Jenkins R, Leary SM, Stevenson VL, Losseff NA, Crum WR, Harvey RJ, Rossor MN, Miller DH, Thompson AJ. Progressive cerebral atrophy in MS: a serial study using registered, volumetric MRI. *Neurology* 2000;**54**:807-12.

Frank JA, Stone LA, Smith ME, Albert PS, Maloni H, McFarland HF. Serial contrast-enhanced magnetic resonance imaging in patients with early relapsing-remitting multiple sclerosis: implications for treatment trials. *Ann.Neurol.* 1994;**36 Suppl**:S86-S90.

Freeman JA, Hobart JC, Langdon DW, Thompson AJ. Clinical appropriateness: a key factor in outcome measure selection: the 36 item short form health survey in multiple sclerosis. *J.Neurol.Neurosurg.Psychiatry* 2000;**68**:150-6.

Fu L, Matthews PM, De Stefano N, Worsley KJ, Narayanan S, Francis GS, Antel JP, Wolfson C, Arnold DL. Imaging axonal damage of normal-appearing white matter in multiple sclerosis. *Brain* 1998;**121 (Pt 1)**:103-13.

Gallagher HL, MacManus DG, Webb SL, Miller DH. A reproducible repositioning method for serial magnetic resonance imaging studies of the brain in treatment trials for multiple sclerosis. *J.Magn Reson.Imaging* 1997;**7**:439-41.

Gasperini C, Pozzilli C, Bastianello S, Giugni E, Horsfield MA, Koudriavtseva T, Galgani S, Paolillo A, Haggiag S, Millefiorini E, Fieschi C. Interferon-beta-1a in relapsing-remitting multiple sclerosis: effect on hypointense lesion volume on T1 weighted images. *J.Neurol.Neurosurg.Psychiatry* 1999;**67**:579-84.

Gass A, Barker GJ, Kidd D, Thorpe JW, MacManus D, Brennan A, Tofts PS, Thompson AJ, McDonald WI, Miller DH. Correlation of magnetization transfer ratio with clinical disability in multiple sclerosis. *Ann.Neurol.* 1994;**36**:62-7.

Gaudet JP, Hashimoto L, Sadovnick AD, Ebers GC. Is sporadic MS caused by an infection of adolescence and early adulthood? A case-control study of birth order position. *Acta Neurol.Scand.* 1995;**91**:19-21.

Gay D, Esiri M. Blood-brain barrier damage in acute multiple sclerosis plaques. An immunocytological study. *Brain* 1991;**114 (Pt 1B)**:557-72.

Ge Y, Grossman RI, Udupa JK, Babb JS, Kolson DL, McGowan JC. Magnetization transfer ratio histogram analysis of gray matter in relapsing-remitting multiple sclerosis. *AJNR Am.J.Neuroradiol.* 2001;**22**:470-5.

Gean-Marton AD, Vezina LG, Marton KI, Stimac GK, Peyster RG, Taveras JM, Davis KR. Abnormal corpus callosum: a sensitive and specific indicator of multiple sclerosis. *Radiology* 1991;**180**:215-21.

Giovannoni G, Heales SJ, Silver NC, O'Riordan J, Miller RF, Land JM, Clark JB, Thompson EJ. Raised serum nitrate and nitrite levels in patients with multiple sclerosis. *J.Neurol.Sci.* 1997a ;**145**:77-81.

Giovannoni G, Lai M, Kidd D, Thorpe JW, Miller DH, Thompson AJ, Keir G, Feldmann M, Thompson EJ. Daily urinary neopterin excretion as an immunological marker of disease activity in multiple sclerosis. *Brain* 1997b ;**120 (Pt 1)**:1-13.

Giovannoni G, Lai M, Thorpe J, Kidd D, Chamoun V, Thompson AJ, Miller DH, Feldmann M, Thompson EJ. Longitudinal study of soluble adhesion molecules in multiple sclerosis: correlation with gadolinium enhanced magnetic resonance imaging. *Neurology* 1997c;**48**:1557-65.

Giovannoni G. Cerebrospinal fluid and serum nitric oxide metabolites in patients with multiple sclerosis. *Mult.Scler.* 1998a ;**4**:27-30.

Giovannoni G, Kieseier B, Hartung HP. Correlating immunological and magnetic resonance imaging markers of disease activity in multiple sclerosis. *J.Neurol.Neurosurg.Psychiatry* 1998b ;**64 Suppl 1**:S31-S36.

Goodkin DE, Hertsgaard D, Seminary J. Upper extremity function in multiple sclerosis: improving assessment sensitivity with box-and-block and nine-hole peg tests. *Arch.Phys.Med.Rehabil.* 1988;**69**:850-4.

Goodkin DE, Bailly RC, Teetzen ML, Hertsgaard D, Beatty WW. The efficacy of azathioprine in relapsing-remitting multiple sclerosis. *Neurology* 1991;**41**:20-5.

Goodkin DE, Rudick RA, VanderBrug MS, Daughtry MM, Schwetz KM, Fischer J, Van Dyke C. Low-dose (7.5 mg) oral methotrexate reduces the rate of progression in chronic progressive multiple sclerosis. *Ann.Neurol.* 1995;**37**:30-40.

Goodkin DE, Rooney WD, Sloan R, Bacchetti P, Gee L, Vermathen M, Waubant E, Abundo M, Majumdar S, Nelson S, Weiner MW. A serial study of new MS lesions and the white matter from which they arise. *Neurology* 1998;**51**:1689-97.

Goswami KK, Randall RE, Lange LS, Russell WC. Antibodies against the paramyxovirus SV5 in the cerebrospinal fluids of some multiple sclerosis patients. *Nature* 1987;**327**:244-7.

Griffin CM, Parker GJ, Barker GJ, Thompson AJ, Miller DH. MTR and T1 provide complementary information in MS NAWM, but not in lesions. *Mult.Scler.* 2000;**6**:327-31.

Grimaud J, Millar J, Thorpe JW, Moseley IF, McDonald WI, Miller DH. Signal intensity on MRI of basal ganglia in multiple sclerosis. *J.Neurol.Neurosurg.Psychiatry* 1995;**59**:306-8.

Grimaud J, Lai M, Thorpe J, Adeleine P, Wang L, Barker GJ, Plummer DL, Tofts PS, McDonald WI, Miller DH. Quantification of MRI lesion load in multiple sclerosis: a comparison of three computer-assisted techniques. *Magn Reson.Imaging* 1996;**14**:495-505.

Grossman RI, Gonzalez-Scarano F, Atlas SW, Galetta S, Silberberg DH. Multiple sclerosis: gadolinium enhancement in MR imaging. *Radiology* 1986;**161**:721-5.

Grossman RI. Magnetization transfer in multiple sclerosis. *Ann.Neurol.* 1994;**36 Suppl**:S97-S99.

Hammond SR, English D, de Wytt C, Maxwell IC, Millingen KS, Stewart-Wynne EG, McLeod JG, McCall MG. The clinical profile of MS in Australia: a comparison between medium- and high-frequency prevalence zones. *Neurology* 1988;**38**:980-6.

Hand D. Practical longitudinal data analysis. Chapman and Hall, London 1996.

Hawkins CP, Mackenzie F, Tofts P, du Boulay EP, McDonald WI. Patterns of blood-brain barrier breakdown in inflammatory demyelination. *Brain* 1991;**114 (Pt 2)**:801-10.

Hickman SJ, Brex PA, Brierley CM, Silver NC, Barker GJ, Scolding NJ, Compston DA, Moseley IF, Plant GT, Miller DH. Detection of optic nerve atrophy following a single episode of unilateral optic neuritis by MRI using a fat-saturated short-echo fast FLAIR sequence. *Neuroradiology* 2001;**43**:123-8.

Hiehle JF, Jr., Grossman RI, Ramer KN, Gonzalez-Scarano F, Cohen JA. Magnetization transfer effects in MR-detected multiple sclerosis lesions: comparison

with gadolinium-enhanced spin-echo images and nonenhanced T1-weighted images. *AJNR Am.J.Neuroradiol.* 1995;**16**:69-77.

Hoogervorst EL, van Winsen LM, Eikelenboom MJ, Kalkers NF, Uitdehaag BM, Polman CH. Comparisons of patient self-report, neurologic examination, and functional impairment in MS. *Neurology* 2001;**56**:934-7.

Horsfield MA, Lai M, Webb SL, Barker GJ, Tofts PS, Turner R, Rudge P, Miller DH. Apparent diffusion coefficients in benign and secondary progressive multiple sclerosis by nuclear magnetic resonance. *Magn Reson.Med.* 1996;**36**:393-400.

Husted C. Contributions of neuroimaging to diagnosis and monitoring of multiple sclerosis. *Curr.Opin.Neurol.* 1994;**7**:234-41.

Iannucci G, Tortorella C, Rovaris M, Sormani MP, Comi G, Filippi M. Prognostic value of MR and magnetization transfer imaging findings in patients with clinically isolated syndromes suggestive of multiple sclerosis at presentation. *AJNR Am.J.Neuroradiol.* 2000;**21**:1034-8.

Isaac C, Li DK, Genton M, Jardine C, Grochowski E, Palmer M, Kastrukoff LF, Oger J, Paty DW. Multiple sclerosis: a serial study using MRI in relapsing patients. *Neurology* 1988;**38** :1511-5.

Jacobs L, Salazar AM, Herndon R, Reese PA, Freeman A, Jozefowicz R, Cuetter A, Husain F, Smith WA, Ekes R, . Intrathecally administered natural human fibroblast interferon reduces exacerbations of multiple sclerosis. Results of a multicenter, double- blind study. *Arch.Neurol.* 1987;**44**:589-95.

Jacobs LD, Cookfair DL, Rudick RA, Herndon RM, Richert JR, Salazar AM, Fischer JS, Goodkin DE, Granger CV, Simon JH, Alam JJ, Bartoszak DM, Bourdette DN, Braiman J, Brownscheidle CM, Coats ME, Cohan SL, Dougherty DS, Kinkel RP, Mass MK, Munschauer FE, III, Priore RL, Pullicino PM, Scherokman BJ, Whitham RH, . Intramuscular interferon beta-1a for disease progression in relapsing multiple sclerosis. The Multiple Sclerosis Collaborative Research Group (MSCRG). *Ann.Neurol.* 1996;**39**:285-94.

Jacobs LD, Beck RW, Simon JH, Kinkel RP, Brownscheidle CM, Murray TJ, Simonian NA, Slasor PJ, Sandrock AW. Intramuscular interferon beta-1a therapy initiated during a first demyelinating event in multiple sclerosis. CHAMPS Study Group. *N.Engl.J.Med.* 2000;**343** :898-904.

Johnson KP, Brooks BR, Cohen JA, Ford CC, Goldstein J, Lisak RP, Myers LW, Panitch HS, Rose JW, Schiffer RB. Copolymer 1 reduces relapse rate and improves disability in relapsing- remitting multiple sclerosis: results of a phase III multicenter, double-blind placebo-controlled trial. The Copolymer 1 Multiple Sclerosis Study Group. *Neurology* 1995;**45**:1268-76.

Kalkers NF, de G, V, Lazeron RH, Killestein J, Ader HJ, Barkhof F, Lankhorst GJ, Polman CH. MS functional composite: relation to disease phenotype and disability strata. *Neurology* 2000 ;**54**:1233-9.

Kalkers NF, Bergers L, de G, V, Lazeron RH, van Walderveen MA, Uitdehaag BM, Polman CH, Barkhof F. Concurrent validity of the MS Functional Composite using MRI as a biological disease marker. *Neurology* 2001a ;**56**:215-9.

Kalkers NF, Hintzen RQ, van Waesberghe JH, Lazeron RH, van Schijndel RA, Ader HJ, Polman CH, Barkhof F. Magnetization transfer histogram parameters reflect all dimensions of MS pathology, including atrophy. *J.Neurol.Sci.* 2001b ;**184**:155-62.

Kapeller P, McLean MA, Griffin CM, Chard D, Parker GJ, Barker GJ, Thompson AJ, Miller DH. Preliminary evidence for neuronal damage in cortical grey matter and normal appearing white matter in short duration relapsing-remitting multiple sclerosis: a quantitative MR spectroscopic imaging study. *J.Neurol.* 2001;**248**:131-8.

Kappos L, Moeri D, Radue EW, Schoetzau A, Schweikert K, Barkhof F, Miller D, Guttmann CR, Weiner HL, Gasperini C, Filippi M. Predictive value of gadolinium-enhanced magnetic resonance imaging for relapse rate and changes in disability or impairment in multiple sclerosis: a meta-analysis. Gadolinium MRI Meta-analysis Group. *Lancet* 1999;**353**:964-9.

Katz D, Taubenberger JK, Cannella B, McFarlin DE, Raine CS, McFarland HF. Correlation between magnetic resonance imaging findings and lesion development in chronic, active multiple sclerosis. *Ann.Neurol.* 1993;**34**:661-9.

Kermode AG, Thompson AJ, Tofts P, MacManus DG, Kendall BE, Kingsley DP, Moseley IF, Rudge P, McDonald WI. Breakdown of the blood-brain barrier precedes symptoms and other MRI signs of new lesions in multiple sclerosis. Pathogenetic and clinical implications. *Brain* 1990a; **113 (Pt 5)**:1477-89.

Kermode AG, Tofts PS, Thompson AJ, MacManus DG, Rudge P, Kendall BE, Kingsley DP, Moseley IF, du Boulay EP, McDonald WI. Heterogeneity of blood-brain barrier changes in multiple sclerosis: an MRI study with gadolinium-DTPA enhancement. *Neurology* 1990b ;**40**:229-35.

Khoury SJ, Orav EJ, Guttmann CR, Kikinis R, Jolesz FA, Weiner HL. Changes in serum levels of ICAM and TNF-R correlate with disease activity in multiple sclerosis. *Neurology* 1999;**53**:758-64.

Kidd D, Thorpe JW, Thompson AJ, Kendall BE, Moseley IF, MacManus DG, McDonald WI, Miller DH. Spinal cord MRI using multi-array coils and fast spin echo. II. Findings in multiple sclerosis. *Neurology* 1993;**43**:2632-7.

Kidd D, Thompson AJ, Kendall BE, Miller DH, McDonald WI. Benign form of multiple sclerosis: MRI evidence for less frequent and less inflammatory disease activity. *J.Neurol.Neurosurg.Psychiatry* 1994;**57**:1070-2.

Kidd D, Thorpe JW, Kendall BE, Barker GJ, Miller DH, McDonald WI, Thompson AJ. MRI dynamics of brain and spinal cord in progressive multiple sclerosis. *J.Neurol.Neurosurg.Psychiatry* 1996;**60**:15-9.

Kidd D, Thompson PD, Day BL, Rothwell JC, Kendall BE, Thompson AJ, Marsden CD, McDonald WI. Central motor conduction time in progressive multiple sclerosis. Correlations with MRI and disease activity. *Brain* 1998;**121 (Pt 6)**:1109-16.

Kidd D, Barkhof F, McConnell R, Algra PR, Allen IV, Revesz T. Cortical lesions in multiple sclerosis. *Brain* 1999;**122 (Pt 1)**:17-26.

Kornek B, Storch MK, Weissert R, Wallstroem E, Stefferl A, Olsson T, Linington C, Schmidbauer M, Lassmann H. Multiple sclerosis and chronic autoimmune encephalomyelitis: a comparative quantitative study of axonal injury in active, inactive, and remyelinated lesions. *Am.J.Pathol.* 2000;**157**:267-76.

Koudriavtseva T, Thompson AJ, Fiorelli M, Gasperini C, Bastianello S, Bozzao A, Paolillo A, Pisani A, Galgani S, Pozzilli C. Gadolinium enhanced MRI predicts clinical and MRI disease activity in relapsing-remitting multiple sclerosis. *J.Neurol.Neurosurg.Psychiatry* 1997; **62**:285-7.

Kurtzke JF, Beebe GW, Nagler B, Nefzger MD, Auth TL, Kurland LT. Studies on the natural history of multiple sclerosis. V. Long-term survival in young men. *Arch.Neurol.* 1970;**22**:215-25.

Kurtzke JF, Beebe GW, Nagler B, Kurland LT, Auth TL. Studies on the natural history of multiple sclerosis-8. Early prognostic features of the later course of the illness. *J.Chronic.Dis.* 1977a ;**30**:819-30.

Kurtzke JF, Page WF. Epidemiology of multiple sclerosis in US veterans: VII. Risk factors for MS. *Neurology* 1997b ;**48**:204-13.

Kurtzke JF. Rating neurologic impairment in multiple sclerosis: an expanded disability status scale (EDSS). *Neurology* 1983;**33**:1444-52.

Lai M, Hodgson T, Gawne-Cain M, Webb S, MacManus D, McDonald WI, Thompson AJ, Miller DH. A preliminary study into the sensitivity of disease activity detection by serial weekly magnetic resonance imaging in multiple sclerosis. *J.Neurol.Neurosurg.Psychiatry* 1996;**60**:339-41.

Lai HM, Davie CA, Gass A, Barker GJ, Webb S, Tofts PS, Thompson AJ, McDonald WI, Miller DH. Serial magnetisation transfer ratios in gadolinium-enhancing lesions in multiple sclerosis. *J.Neurol.* 1997;**244**:308-11.

Larsson HB, Frederiksen J, Kjaer L, Henriksen O, Olesen J. In vivo determination of T1 and T2 in the brain of patients with severe but stable multiple sclerosis. *Magn Reson.Med.* 1988;**7**:43-55.

Larsson HB, Frederiksen J, Petersen J, Nordenbo A, Zeeberg I, Henriksen O, Olesen J. Assessment of demyelination, edema, and gliosis by in vivo determination of T1 and T2 in the brain of patients with acute attack of multiple sclerosis. *Magn Reson.Med.* 1989;**11**:337-48.

Larsson HB, Christiansen P, Zeeberg I, Henriksen O. In vivo evaluation of the reproducibility of T1 and T2 measured in the brain of patients with multiple sclerosis. *Magn Reson.Imaging* 1992a ; **10**:579-84.

Larsson HB, Thomsen C, Frederiksen J, Stubgaard M, Henriksen O. In vivo magnetic resonance diffusion measurement in the brain of patients with multiple sclerosis. *Magn Reson.Imaging* 1992b ;**10**:7-12.

Lassmann H, Suchanek G, Ozawa K. Histopathology and the blood-cerebrospinal fluid barrier in multiple sclerosis. *Ann.Neurol.* 1994;**36 Suppl**:S42-S46.

Lexa FJ, Grossman RI, Rosenquist AC. Dyke Award paper. MR of wallerian degeneration in the feline visual system: characterization by magnetization transfer rate with histopathologic correlation. *AJNR Am.J.Neuroradiol.* 1994;**15**:201-12.

Li DK, Paty DW. Magnetic resonance imaging results of the PRISMS trial: a randomized, double-blind, placebo-controlled study of interferon-beta1a in relapsing-remitting multiple sclerosis. Prevention of Relapses and Disability by Interferon-beta1a Subcutaneously in Multiple Sclerosis. *Ann.Neurol.* 1999;**46**:197-206.

Liu C, Edwards S, Gong Q, Roberts N, Blumhardt LD. Three dimensional MRI estimates of brain and spinal cord atrophy in multiple sclerosis. *J.Neurol.Neurosurg.Psychiatry* 1999;**66**:323-30.

Loevner LA, Grossman RI, Cohen JA, Lexa FJ, Kessler D, Kolson DL. Microscopic disease in normal-appearing white matter on conventional MR images in patients with multiple sclerosis: assessment with magnetization-transfer measurements. *Radiology* 1995b ;**196**:511-5.

Loevner LA, Grossman RI, McGowan JC, Ramer KN, Cohen JA. Characterization of multiple sclerosis plaques with T1-weighted MR and quantitative magnetization transfer. *AJNR Am.J.Neuroradiol.* 1995a ;**16**:1473-9.

Losseff NA, Kingsley DP, McDonald WI, Miller DH, Thompson AJ. Clinical and magnetic resonance imaging predictors of disability in primary and secondary progressive multiple sclerosis. *Mult.Scler.* 1996a ;**1**:218-22.

Losseff NA, Wang L, Lai HM, Yoo DS, Gawne-Cain ML, McDonald WI, Miller DH, Thompson AJ. Progressive cerebral atrophy in multiple sclerosis. A serial MRI study. *Brain* 1996b ;**119 (Pt 6)**:2009-19.

Losseff NA, Webb SL, O'Riordan JI, Page R, Wang L, Barker GJ, Tofts PS, McDonald WI, Miller DH, Thompson AJ. Spinal cord atrophy and disability in multiple sclerosis. A new reproducible and sensitive MRI method with potential to monitor disease progression. *Brain* 1996c ;**119 (Pt 3)**:701-8.

Losseff NA, Miller DH. Measures of brain and spinal cord atrophy in multiple sclerosis. *J.Neurol.Neurosurg.Psychiatry* 1998;**64 Suppl 1**:S102-S105.

Lublin FD, Reingold SC. Defining the clinical course of multiple sclerosis: results of an international survey. National Multiple Sclerosis Society (USA) Advisory Committee on Clinical Trials of New Agents in Multiple Sclerosis. *Neurology* 1996;**46**:907-11.

Martino G, Consiglio A, Franciotta DM, Corti A, Filippi M, Vandenbroeck K, Sciacca FL, Comi G, Grimaldi LM. Tumor necrosis factor alpha and its receptors in relapsing-remitting multiple sclerosis. *J.Neurol.Sci.* 1997;**152**:51-61.

McDonald WI, Compston A, Edan G, Goodkin D, Hartung HP, Lublin FD, McFarland HF, Paty DW, Polman CH, Reingold SC, Sandberg-Wollheim M, Sibley W, Thompson A, Van Den NS, Weinshenker BY, Wolinsky JS. Recommended diagnostic criteria for multiple sclerosis: guidelines from the International Panel on the diagnosis of multiple sclerosis. *Ann.Neurol.* 2001;**50**:121-7.

McFarland HF, Frank JA, Albert PS, Smith ME, Martin R, Harris JO, Patronas N, Maloni H, McFarlin DE. Using gadolinium-enhanced magnetic resonance imaging lesions to monitor disease activity in multiple sclerosis. *Ann.Neurol.* 1992;**32**:758-66.

Miller DH, Rudge P, Johnson G, Kendall BE, MacManus DG, Moseley IF, Barnes D, McDonald WI. Serial gadolinium enhanced magnetic resonance imaging in multiple sclerosis. *Brain* 1988;**111 (Pt 4)**:927-39.

Miller DH, Ormerod IE, Rudge P, Kendall BE, Moseley IF, McDonald WI. The early risk of multiple sclerosis following isolated acute syndromes of the brainstem and spinal cord. *Ann.Neurol.* 1989;**26**:635-9.

Miller DH, Barkhof F, Berry I, Kappos L, Scotti G, Thompson AJ. Magnetic resonance imaging in monitoring the treatment of multiple sclerosis: concerted action guidelines. *J.Neurol.Neurosurg.Psychiatry* 1991;**54**:683-8.

Miller DH, Hornabrook RW, Purdie G. The natural history of multiple sclerosis: a regional study with some longitudinal data. *J.Neurol.Neurosurg.Psychiatry* 1992a ;**55**:341-6.

Miller DH, Thompson AJ, Morrissey SP, MacManus DG, Moore SG, Kendall BE, Moseley IF, McDonald WI. High dose steroids in acute relapses of multiple sclerosis: MRI evidence for a possible mechanism of therapeutic effect. *J.Neurol.Neurosurg.Psychiatry* 1992b ;**55**:450-3.

Miller DH, Barkhof F, Nauta JJ. Gadolinium enhancement increases the sensitivity of MRI in detecting disease activity in multiple sclerosis. *Brain* 1993;**116 (Pt 5)**:1077-94.

Miller DH. Guidelines for MRI monitoring of the treatment of multiple sclerosis: recommendations of the US Multiple Sclerosis Society's task force. *Mult.Scler.* 1996a ;**1**:335-8.

Miller DH, Albert PS, Barkhof F, Francis G, Frank JA, Hodgkinson S, Lublin FD, Paty DW, Reingold SC, Simon J. Guidelines for the use of magnetic resonance techniques in monitoring the treatment of multiple sclerosis. US National MS Society Task Force. *Ann.Neurol.* 1996b ;**39**:6-16.

Miller DH, Molyneux PD, Barker GJ, MacManus DG, Moseley IF, Wagner K. Effect of interferon-beta1b on magnetic resonance imaging outcomes in secondary progressive multiple sclerosis: results of a European multicenter, randomized, double-

blind, placebo-controlled trial. European Study Group on Interferon-beta1b in secondary progressive multiple sclerosis. *Ann.Neurol.* 1999;**46**:850-9.

Molyneux PD, Filippi M, Barkhof F, Gasperini C, Yousry TA, Truyen L, Lai HM, Rocca MA, Moseley IF, Miller DH. Correlations between monthly enhanced MRI lesion rate and changes in T2 lesion volume in multiple sclerosis. *Ann.Neurol.* 1998a ;**43**:332-9.

Molyneux PD, Tubridy N, Parker GJ, Barker GJ, MacManus DG, Tofts PS, Moseley IF, Miller DH. The effect of section thickness on MR lesion detection and quantification in multiple sclerosis. *AJNR Am.J.Neuroradiol.* 1998b ;**19**:1715-20.

Mumford CJ, Wood NW, Kellar-Wood H, Thorpe JW, Miller DH, Compston DA. The British Isles survey of multiple sclerosis in twins. *Neurology* 1994;**44**:11-5.

Nesbit GM, Forbes GS, Scheithauer BW, Okazaki H, Rodriguez M. Multiple sclerosis: histopathologic and MR and/or CT correlation in 37 cases at biopsy and three cases at autopsy. *Radiology* 1991;**180**:467-74.

Noseworthy J, Paty D, Wonnacott T, Feasby T, Ebers G. Multiple sclerosis after age 50. *Neurology* 1983;**33**:1537-44.

Nusbaum AO, Tang CY, Wei T, Buchsbaum MS, Atlas SW. Whole-brain diffusion MR histograms differ between MS subtypes. *Neurology* 2000;**54**:1421-7.

Ormerod IE, Johnson G, MacManus D, du Boulay EP, McDonald WI. Relaxation times of apparently normal cerebral white matter in multiple sclerosis. *Acta Radiol.Suppl* 1986;**369**:382-4.

Ormerod IE, Miller DH, McDonald WI, du Boulay EP, Rudge P, Kendall BE, Moseley IF, Johnson G, Tofts PS, Halliday AM, . The role of NMR imaging in the assessment of multiple sclerosis and isolated neurological lesions. A quantitative study. *Brain* 1987;**110 (Pt 6)**:1579-616.

Parker GJM, Barker GJ, Tofts PS. Accurate multislice T1 measurement in the presence of non ideal RF pulse profiles and RF field inhomogeneity. *Magn Reson Med* 2001;**45**:838-845.

Parry A, Clare S, Matthews PM. Total white matter T1 values correlate with disability in patients with multiple sclerosis. *Revue Neurologique* 2000;156:102.

Paty DW. Magnetic resonance in multiple sclerosis. *Curr.Opin.Neurol.Neurosurg.* 1993a ;**6**:202-8.

Paty DW, Li DK. Interferon beta-1b is effective in relapsing-remitting multiple sclerosis. II. MRI analysis results of a multicenter, randomized, double-blind, placebo-controlled trial. UBC MS/MRI Study Group and the IFNB Multiple Sclerosis Study Group. *Neurology* 1993b ;**43**:662-7.

Paulesu E, Perani D, Fazio F, Comi G, Pozzilli C, Martinelli V, Filippi M, Bettinardi V, Sirabian G, Passafiume D, Anzini A, Lenzi GL, Canal N, Fieschi C. Functional basis of memory impairment in multiple sclerosis: a[18F]FDG PET study. *Neuroimage.* 1996;**4**:87-96.

Phadke JG. Clinical aspects of multiple sclerosis in north-east Scotland with particular reference to its course and prognosis. *Brain* 1990;**113 (Pt 6)**:1597-628.

Pierpaoli C, Basser PJ. Toward a quantitative assessment of diffusion anisotropy. Magn Reson Med 1996;36:893-906.

Pike GB, De Stefano N, Narayanan S, Worsley KJ, Pelletier D, Francis GS, Antel JP, Arnold DL. Multiple sclerosis: magnetization transfer MR imaging of white matter before lesion appearance on T2-weighted images. *Radiology* 2000;**215**:824-30.

Plummer DL. Dispimage: a display and analysis tool for medical images. *Riv Neuroradiol* 1992;**5**:489-495.

Polman CH, Dahlke F, Thompson AJ, Ghazi M, Kappos L, Miltenburger C, Pozilli C. Interferon beta-1b in secondary progressive multiple sclerosis - outline of the clinical trial. *Mult.Scler.* 1995;**1 Suppl 1**:S51-S54.

Poser CM, Paty DW, Scheinberg L, McDonald WI, Davis FA, Ebers GC, Johnson KP, Sibley WA, Silberberg DH, Tourtellotte WW. New diagnostic criteria for multiple sclerosis: guidelines for research protocols. *Ann.Neurol.* 1983;**13**:227-31.

Raine CS. The Norton Lecture: a review of the oligodendrocyte in the multiple sclerosis lesion. *J.Neuroimmunol.* 1997;**77**:135-52.

Revesz T, Kidd D, Thompson AJ, Barnard RO, McDonald WI. A comparison of the pathology of primary and secondary progressive multiple sclerosis. *Brain* 1994;**117 (Pt 4)**:759-65.

Richert ND, Ostuni JL, Bash CN, Duyn JH, McFarland HF, Frank JA. Serial whole-brain magnetization transfer imaging in patients with relapsing-remitting multiple

sclerosis at baseline and during treatment with interferon beta-1b. *AJNR Am.J.Neuroradiol.* 1998;**19**:1705-13.

Rudick R, Antel J, Confavreux C, Cutter G, Ellison G, Fischer J, Lublin F, Miller A, Petkau J, Rao S, Reingold S, Syndulko K, Thompson A, Wallenberg J, Weinshenker B, Willoughby E. Recommendations from the National Multiple Sclerosis Society Clinical Outcomes Assessment Task Force. *Ann.Neurol.* 1997;**42**:379-82.

Rudick RA, Fisher E, Lee JC, Simon J, Jacobs L. Use of the brain parenchymal fraction to measure whole brain atrophy in relapsing-remitting MS. Multiple Sclerosis Collaborative Research Group. *Neurology* 1999;**53**:1698-704.

Rudick RA, Fisher E, Lee JC, Duda JT, Simon J. Brain atrophy in relapsing multiple sclerosis: relationship to relapses, EDSS, and treatment with interferon beta-1a. *Mult.Scler.* 2000;**6**:365-72.

Russo C, Smoker WR, Kubal W. Cortical and subcortical T2 shortening in multiple sclerosis. *AJNR Am.J.Neuroradiol.* 1997;**18**:124-6.

Sadovnick AD, Ebers GC, Wilson RW, Paty DW. Life expectancy in patients attending multiple sclerosis clinics. *Neurology* 1992;**42**:991-4.

Sadovnick AD, Armstrong H, Rice GP, Bulman D, Hashimoto L, Paty DW, Hashimoto SA, Warren S, Hader W, Murray TJ, . A population-based study of multiple sclerosis in twins: update. *Ann.Neurol.* 1993;**33**:281-5.

SAS Institute Inc., SAS/STAT software. Changes and enhancements through release 6.11, SAS Institute, Cary, NC, 1996.

Schumacher GA. Multiple sclerosis. *Arch.Neurol.* 1966;**14**:571-3.

Silver NC, Barker GJ, MacManus DG, Miller DH, Thorpe JW, Howard RS. Decreased magnetisation transfer ratio due to demyelination: a case of central pontine myelinolysis. *J.Neurol.Neurosurg.Psychiatry* 1996;**61**:208-9.

Silver NC, Good CD, Barker GJ, MacManus DG, Thompson AJ, Moseley IF, McDonald WI, Miller DH. Sensitivity of contrast enhanced MRI in multiple sclerosis. Effects of gadolinium dose, magnetization transfer contrast and delayed imaging. *Brain* 1997;**120 (Pt 7)**:1149-61.

Silver NC, Lai M, Symms MR, Barker GJ, McDonald WI, Miller DH. Serial magnetization transfer imaging to characterize the early evolution of new MS lesions. *Neurology* 1998;**51**:758-64.

Silver NC, Good CD, Sormani MP, MacManus DG, Thompson AJ, Filippi M, Miller DH. A modified protocol to improve the detection of enhancing brain and spinal cord lesions in multiple sclerosis. *J.Neurol.* 2001;**248**:215-24.

Simon JH, Jacobs LD, Campion M, Wende K, Simonian N, Cookfair DL, Rudick RA, Herndon RM, Richert JR, Salazar AM, Alam JJ, Fischer JS, Goodkin DE, Granger CV, Lajaunie M, Martens-Davidson AL, Meyer M, Sheeder J, Choi K, Scherzinger AL, Bartoszak DM, Bourdette DN, Braiman J, Brownscheidle CM, Whitham RH, . Magnetic resonance studies of intramuscular interferon beta-1a for relapsing multiple sclerosis. The Multiple Sclerosis Collaborative Research Group. *Ann.Neurol.* 1998;**43**:79-87.

Simon JH, Jacobs LD, Campion MK, Rudick RA, Cookfair DL, Herndon RM, Richert JR, Salazar AM, Fischer JS, Goodkin DE, Simonian N, Lajaunie M, Miller DE, Wende K, Martens-Davidson A, Kinkel RP, Munschauer FE, III, Brownscheidle

CM. A longitudinal study of brain atrophy in relapsing multiple sclerosis. The Multiple Sclerosis Collaborative Research Group (MSCRG). *Neurology* 1999;**53**:139-48.

Simon JH, Lull J, Jacobs LD, Rudick RA, Cookfair DL, Herndon RM, Richert JR, Salazar AM, Sheeder J, Miller D, McCabe K, Serra A, Campion MK, Fischer JS, Goodkin DE, Simonian N, Lajaunie M, Wende K, Martens-Davidson A, Kinkel RP, Munschauer FE, III. A longitudinal study of T1 hypointense lesions in relapsing MS: MSCRG trial of interferon beta-1a. Multiple Sclerosis Collaborative Research Group. *Neurology* 2000;**55**:185-92.

Sindern E, Haas J, Stark E, Wurster U. Early onset MS under the age of 16: clinical and paraclinical features. *Acta Neurol.Scand.* 1992;**86**:280-4.

Smith ME, Stone LA, Albert PS, Frank JA, Martin R, Armstrong M, Maloni H, McFarlin DE, McFarland HF. Clinical worsening in multiple sclerosis is associated with increased frequency and area of gadopentetate dimeglumine-enhancing magnetic resonance imaging lesions. *Ann.Neurol.* 1993;**33**:480-9.

Soderstrom M, Ya-Ping J, Hillert J, Link H. Optic neuritis: prognosis for multiple sclerosis from MRI, CSF, and HLA findings. *Neurology* 1998;**50**:708-14.

Stevenson VL, Gawne-Cain ML, Barker GJ, Thompson AJ, Miller DH. Imaging of the spinal cord and brain in multiple sclerosis: a comparative study between fast FLAIR and fast spin echo. *J.Neurol.* 1997;**244**:119-24.

Stevenson VL, Parker GJ, Barker GJ, Birnie K, Tofts PS, Miller DH, Thompson AJ. Variations in T1 and T2 relaxation times of normal appearing white matter and lesions in multiple sclerosis. *J.Neurol.Sci.* 2000;**178**:81-7.

Stone LA, Smith ME, Albert PS, Bash CN, Maloni H, Frank JA, McFarland HF. Blood-brain barrier disruption on contrast-enhanced MRI in patients with mild relapsing-remitting multiple sclerosis: relationship to course, gender, and age. *Neurology* 1995a ;**45**:1122-6.

Stone LA, Albert PS, Smith ME, DeCarli C, Armstrong MR, McFarlin DE, Frank JA, McFarland HF. Changes in the amount of diseased white matter over time in patients with relapsing-remitting multiple sclerosis. *Neurology* 1995b ;**45**:1808-14.

Sullivan L. Tutorial in biostatistics. An introduction to hierarchical linear modelling. *Statist. Med.* 1999; **18**: 855 – 888.

Symms MR, Barker GJ, Wang L, Tofts PS. Improving spatial registration of multislice two dimensional MR images with a radiographical repositioning technique. *Proc Int Soc Magn Reson Med* 1997;689.

Thompson AJ, Kermode AG, Wicks D, MacManus DG, Kendall BE, Kingsley DP, McDonald WI. Major differences in the dynamics of primary and secondary progressive multiple sclerosis. *Ann.Neurol.* 1991;**29**:53-62.

Thompson AJ, Miller D, Youl B, MacManus D, Moore S, Kingsley D, Kendall B, Feinstein A, McDonald WI. Serial gadolinium-enhanced MRI in relapsing/remitting multiple sclerosis of varying disease duration. *Neurology* 1992;**42**:60-3.

Thompson AJ, Polman CH, Miller DH, McDonald WI, Brochet B, Filippi MM, X, de Sa J. Primary progressive multiple sclerosis. *Brain* 1997;**120 (Pt 6)**:1085-96.

Thorpe JW, Mumford CJ, Compston DA, Kendall BE, MacManus DG, McDonald WI, Miller DH. British Isles survey of multiple sclerosis in twins: MRI. *J.Neurol.Neurosurg.Psychiatry* 1994;**57**:491-6.

Thorpe JW, Kidd D, Moseley IF, Kenndall BE, Thompson AJ, MacManus DG, McDonald WI, Miller DH. Serial gadolinium-enhanced MRI of the brain and spinal cord in early relapsing-remitting multiple sclerosis. *Neurology* 1996a ;**46**:373-8.

Thorpe JW, Kidd D, Moseley IF, Thompson AJ, MacManus DG, Compston DA, McDonald WI, Miller DH. Spinal MRI in patients with suspected multiple sclerosis and negative brain MRI. *Brain* 1996b ;**119 (Pt 3)**:709-14.

Tortorella C, Viti B, Bozzali M, Sormani MP, Rizzo G, Gilardi MF, Comi G, Filippi M. A magnetization transfer histogram study of normal-appearing brain tissue in MS. *Neurology* 2000;**54**:186-93.

Tourtellotte WW, Parker JA. Some spaces and barriers in postmortem multiple sclerosis. *Prog.Brain Res.* 1968;**29**:493-525.

Trapp BD, Peterson J, Ransohoff RM, Rudick R, Mork S, Bo L. Axonal transection in the lesions of multiple sclerosis. *N.Engl.J.Med.* 1998;**338**:278-85.

Truyen L, Gheuens J, Van de Vyver FL, Parizel PM, Peersman GV, Martin JJ. Improved correlation of magnetic resonance imaging (MRI) with clinical status in multiple sclerosis (MS) by use of an extensive standardized imaging-protocol. *J.Neurol.Sci.* 1990;**96**:173-82.

Truyen L, Gheuens J, Parizel PM, Van de Vyver FL, Martin JJ. Long term follow-up of multiple sclerosis by standardized, non-contrast- enhanced magnetic resonance imaging. *J.Neurol.Sci.* 1991; **106**:35-40.

Truyen L, van Waesberghe JH, van Walderveen MA, van Oosten BW, Polman CH, Hommes OR, Ader HJ, Barkhof F. Accumulation of hypointense lesions ("black holes") on T1 spin-echo MRI correlates with disease progression in multiple sclerosis. *Neurology* 1996;**47**:1469-76.

Tubridy N, Behan PO, Capildeo R, Chaudhuri A, Forbes R, Hawkins CP, Hughes RA, Palace J, Sharrack B, Swingler R, Young C, Moseley IF, MacManus DG, Donoghue S, Miller DH. The effect of anti-alpha4 integrin antibody on brain lesion activity in MS. The UK Antegren Study Group. *Neurology* 1999a ;**53**:466-72.

Tubridy N, Molyneux PD, Moseley IF, Miller DH. The sensitivity of thin-slice fast spin echo, fast FLAIR and gadolinium- enhanced T1-weighted MRI sequences in detecting new lesion activity in multiple sclerosis. *J.Neurol.* 1999b ;**246**:1181-5.

Tubridy N, Coles AJ, Molyneux P, Compston DA, Barkhof F, Thompson AJ, McDonald WI, Miller DH. Secondary progressive multiple sclerosis: the relationship between short-term MRI activity and clinical features. *Brain* 1998;**121 (Pt 2)**:225-31.

Uhlenbrock D, Sehlen S. The value of T1-weighted images in the differentiation between MS, white matter lesions, and subcortical arteriosclerotic encephalopathy (SAE). *Neuroradiology* 1989;**31**:203-12.

van Buchem MA, Udupa JK, McGowan JC, Miki Y, Heyning FH, Boncoeur-Martel MP, Kolson DL, Polansky M, Grossman RI. Global volumetric estimation of disease

burden in multiple sclerosis based on magnetization transfer imaging. *AJNR Am.J.Neuroradiol.* 1997;**18**:1287-90.

van Waesberghe JH, Castelijns JA, Scheltens P, Truyen L, Lycklana ANG, Hoogenraad FG, Polman CH, Valk J, Barkhof F. Comparison of four potential MR parameters for severe tissue destruction in multiple sclerosis lesions. *Magn Reson.Imaging* 1997;**15**:155-62.

van Waesberghe JH, Kamphorst W, De Groot CJ, van Walderveen MA, Castelijns JA, Ravid R, Nijeholt GJ, van d, V, Polman CH, Thompson AJ, Barkhof F. Axonal loss in multiple sclerosis lesions: magnetic resonance imaging insights into substrates of disability. *Ann.Neurol.* 1999;**46**:747-54.

van Walderveen MA, Barkhof F, Hommes OR, Polman CH, Tobi H, Frequin ST, Valk J. Correlating MRI and clinical disease activity in multiple sclerosis: relevance of hypointense lesions on short-TR/short-TE (T1-weighted) spin-echo images. *Neurology* 1995;**45**:1684-90.

van Walderveen MA, Kamphorst W, Scheltens P, van Waesberghe JH, Ravid R, Valk J, Polman CH, Barkhof F. Histopathologic correlate of hypointense lesions on T1-weighted spin- echo MRI in multiple sclerosis. *Neurology* 1998;**50**:1282-8.

van Walderveen MAA, Van Schijndel RA, Pouwels PJW, Polman CH, Barkhof F. Multi slice T1 relaxation time measurements in the brain using IR-EPI: T1 histogram analysis in multiple sclerosis patients. *Revue Neurologique* 2000;156:99.

Weinshenker BG, Ebers GC. The natural history of multiple sclerosis. *Can.J.Neurol.Sci.* 1987;**14**:255-61.

Weinshenker BG, Bass B, Rice GP, Noseworthy J, Carriere W, Baskerville J, Ebers GC. The natural history of multiple sclerosis: a geographically based study. 2. Predictive value of the early clinical course. *Brain* 1989;**112 (Pt 6)**:1419-28.

Weinshenker BG, Bass B, Karlik S, Ebers GC, Rice GP. An open trial of OKT3 in patients with multiple sclerosis. *Neurology* 1991;**41**:1047-52.

Weinshenker BG, Sibley WA. Natural history and treatment of multiple sclerosis. *Curr.Opin.Neurol.Neurosurg.* 1992;**5**:203-11.

Weinshenker BG. Natural history of multiple sclerosis. *Ann.Neurol.* 1994;**36 Suppl**:S6-11.

Werring DJ, Clark CA, Barker GJ, Thompson AJ, Miller DH. Diffusion tensor imaging of lesions and normal-appearing white matter in multiple sclerosis. *Neurology* 1999;**52**:1626-32.

Werring DJ, Brassat D, Droogan AG, Clark CA, Symms MR, Barker GJ, MacManus DG, Thompson AJ, Miller DH. The pathogenesis of lesions and normal-appearing white matter changes in multiple sclerosis: a serial diffusion MRI study. *Brain* 2000;**123 (Pt 8)**:1667-76.

Whitaker JN, McFarland HF, Rudge P, Reingold SC. Outcomes assessment in multiple sclerosis clinical trials: a critical analysis. *Mult.Scler.* 1995;**1**:37-47.

Wiebe S, Lee DH, Karlik SJ, Hopkins M, Vandervoort MK, Wong CJ, Hewitt L, Rice GP, Ebers GC, Noseworthy JH. Serial cranial and spinal cord magnetic resonance imaging in multiple sclerosis. *Ann.Neurol.* 1992;**32**:643-50.

Willoughby EW, Grochowski E, Li DK, Oger J, Kastrukoff LF, Paty DW. Serial magnetic resonance scanning in multiple sclerosis: a second prospective study in relapsing patients. *Ann.Neurol.* 1989;**25**:43-9.

Wisniewski HM, Bloom BR. Primary demyelination as a nonspecific consequence of a cell-mediated immune reaction. *J.Exp.Med.* 1975;**141**:346-59.

Wolfson C, Confavreux C. Improvements to a simple Markov model of the natural history of multiple sclerosis. I. Short-term prognosis. *Neuroepidemiology* 1987;**6**:101-15.

Woods RP, Cherry SR, Mazziotta JC. Rapid automated algorithm for aligning and reslicing PET images. *J Comput Assist Tomogr* 1992;16:620-633.

Yudkin PL, Ellison GW, Ghezzi A, Goodkin DE, Hughes RA, McPherson K, Mertin J, Milanese C. Overview of azathioprine treatment in multiple sclerosis. *Lancet* 1991;**338**:1051-5.

Addendum to thesis:

1) **Page 20, paragraph 2, sentence 1** should read:

Myelin surrounds nerve fibres and plays an integral part in the function of the axon.

2) **Page 25 paragraph 1** should read:

A relapsing remitting course is the most common onset of MS. This is defined as disease characterised by relapses with a rapid onset of symptoms in hours to days and remissions (which typically follow days to weeks later). Ninety percent of all MS patients have a relapsing onset. Remission periods between disease relapses are characterised by a lack of disease progression but there may not be a full recovery between attacks. The period of remission may be weeks, months or years. Secondary progressive disease is defined as an initial relapsing remitting course in which the subsequent development of a progressive course occurs, with or without occasional relapses or minor remissions. Many patients with relapsing remitting disease will progress to the secondary progressive phase of the disease. In ten to fifteen percent of patients, the disease is defined as being primary progressive. This is disease that is slowly progressive from the onset with occasional plateaus and only temporary minor improvements, if any. Benign MS is defined as disease in which the patient has an EDSS score of less than or equal to 3 at least 15 years after the onset of the disease [Lublin and Reingold 1996]: as defined, it occurs in about 30% of patients. However, patients with so-called benign disease may obviously go on to develop more active disease and greater disability at a future time point.

3) **Page 30 paragraph 2 sentence 6** should read:

This is considered abnormal only if there is intra-thecal synthesis. This can either be illustrated by bands which are not present in a simultaneously collected serum sample, or when there are unmatched quantities of bands in the CSF and serum.

4) **Page 72 section 3A.1 Additional sentence at the beginning of the first paragraph** should read:

All the data reported in this thesis is work which I have personally carried out as part of a larger study which is presently being conducted in the NMR Unit at The Institute of Neurology. I have reported the data I personally analysed but other studies are also currently underway, studying the same cohort of patients.