Magnetization Transfer Imaging to Investigate Tissue Structure and Optimise Detection of Blood Brain Barrier Leakage in Multiple Sclerosis

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Abstract

Magnetic resonance imaging (MRI) has become a powerful research tool for in vivo evaluation and monitoring of multiple sclerosis (MS). Conventional MRI techniques detect changes in the density or relaxation characteristics of “free” water protons. They are sensitive but lack pathological specificity. Magnetization transfer (MT) imaging provides a method for evaluating those protons “bound” to macromolecular structures.

Part one of this thesis outlines the clinical and pathological features of MS and discusses the importance of demyelination and blood-brain barrier breakdown. An introduction to MRI and MT imaging techniques is provided. Issues related to quality assurance and standardization for MT imaging are explored. In part two, MT ratio (MTR) is explored as a putative quantitative marker of demyelination and associated tissue destruction. In part three, MT contrast (MTC) is explored as a novel mechanism for improving the detection of focal contrast-enhancing lesions.

A normative database for MTR in healthy white matter is presented in chapter four. Highest values are found where myelin density is greatest. Minor age-related MTR reduction is observed. In chapter five, MTR is evaluated in central pontine myelinolysis, a rare neurological condition characterised pathologically by severe demyelination. The results support myelin as the predominant contributor to MTR values in white matter. In chapter six, MTR is employed as a putative marker of demyelination to explore the relationships between demyelination and blood-brain barrier damage in acute MS lesions. New techniques for registering two-dimensional images were implemented to allow reliable measurement of MTR prior to visible lesion formation. No evidence was found to suggest significant demyelination prior to opening of the blood-brain barrier. Chapter seven details the novel application of MTR measurement in the cervical spinal cord and preliminary data are presented showing reduction in MS. Further studies in this clinically eloquent region will be of interest.

The potential for MTC to improve gadolinium enhancing lesion detection in MS is explored in the chapters eight and nine. First, a cross-sectional study explores how the detection of enhancing lesions may be improved by MTC; in conjunction with a triple-dose gadolinium and subsequent delay prior to imaging, sensitivity was more than doubled. Triple-dose improved sensitivity more than MTC. Finally, a serial study is presented that confirms a significant increase in longitudinal sensitivity for such techniques. The potential benefits for monitoring phase II exploratory treatment trials in MS are evaluated.
Acknowledgements

Thanks to my supervisor David Miller for his guidance and unfailing support throughout my time in research. I have benefited from both his global vision and attention to detail. I would especially like to thank Ian McDonald for his clarity, wisdom and kindness, all of which have provided a continued source of inspiration. Thanks also to Alan Thompson for his input.

Many thanks to Paul Tofts and Gareth Barker for their theoretical and practical input. I am indebted to Tina Good for her radiological expertise, enthusiasm, and friendship. Thanks also to Ivan Moseley and Mary Gawne-Caine. I have enjoyed the international collaboration afforded by MAGNIMS and would particularly like to thank Achim Gass and Wolfgang Schreiber for their helpful suggestions and Massimo Fillipi and Maria Pia Sormani for their collaboration. I am grateful to Mark Symms for his genuine enthusiasm and novel ideas. This thesis would not have been possible without the dedication and hard work of all of the radiographers in the unit. Special thanks to Dave MacManus and also to Alison Fletcher, Beth Gunn, and Andrew Howe.

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A special thanks to the Multiple Sclerosis Society of Great Britain and Northern Ireland for their generous support throughout my research studentship. Finally, thank you everyone who volunteered to be studied - my heartfelt gratitude to you all for your unerring dedication.
Statement of Authorship

All projects outlined in this thesis represent my original and personal work. This thesis would not however have been possible without specific individual contributions from collaborators within the NMR Unit and other research centres. With the following exceptions and those listed in the text of this thesis, I was responsible for the initiation and design of all project protocols, recruitment of subjects for study, acquisition of all clinical and radiological data (with help from the individual radiographers who performed all scanning), analysis of all image data, statistical testing, and interpretation of the subsequent results: In Chapter 3, Drs. John Mottershead, Geoff Keir, and Alison Green helped design and manufacture the MTR phantoms. In Chapter 5, Dr Charlie Davie acquired the proton MRS control data from four healthy volunteers. In Chapter 6, Dr Ming Lai recruited and acquired data from three of the subjects. In Chapter 9, Dr Maria Pia Sormani performed the sample size calculations in conjunction with Dr Massimo Filippi.
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<table>
<thead>
<tr>
<th>Abbreviation</th>
<th>Description</th>
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<tbody>
<tr>
<td>BBB</td>
<td>blood-brain barrier</td>
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<tr>
<td>CADASIL</td>
<td>cerebral autosomal dominant arteriopathy with subcortical infarcts</td>
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<td>CNS</td>
<td>central nervous system</td>
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<td>CPM</td>
<td>central pontine myelinolysis</td>
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<tr>
<td>CPMG</td>
<td>Carr-Purcell-Meiboom-Gill</td>
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<td>CSF</td>
<td>cerebrospinal fluid</td>
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<td>CT</td>
<td>computerised tomography</td>
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<td>DNA</td>
<td>deoxyribonucleic acid</td>
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<td>DTPA</td>
<td>diethylene triaminepentaacetic acid</td>
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<td>EAE</td>
<td>experimental allergic encephalomyelitis</td>
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<td>EDSS</td>
<td>expanded disability status scale</td>
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<tr>
<td>ETL</td>
<td>echo train length</td>
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<td>f&lt;sub&gt;off&lt;/sub&gt;</td>
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<td>FLAIR</td>
<td>fluid attenuated inversion recovery</td>
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<td>Gd-DTPA</td>
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<td>MAG</td>
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<td>proteolipid protein</td>
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<td>PML</td>
<td>progressive multifocal leukoencephalopathy</td>
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<td>radiofrequency pulse</td>
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<td>SRCC</td>
<td>Spearman's Rank Correlation Coefficient</td>
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<td>T-cell receptor</td>
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List of Publications

NC Silver, GJ Barker, DG MacManus, JW Thorpe, RS Howard, DH Miller.
Decreased magnetisation transfer ratio due to demyelination: A case of central pontine myelinolysis.

NC Silver, CD Good, GJ Barker, DG MacManus, AJ Thompson, IF Moseley, WI McDonald, DH Miller.
Sensitivity of contrast enhanced MRI in multiple sclerosis: effects of gadolinium dose, magnetisation transfer contrast and delayed imaging.
*Brain* 1997;120:1149-1161.

Magnetisation transfer ratio measurement in the cervical spinal cord: a preliminary study in multiple sclerosis.

NC Silver, GJ Barker, DG MacManus, PS Tofts, DH Miller.
Magnetisation transfer ratio of normal brain white matter: a normative database spanning four decades of life.
*J Neurol Neurosurg Psych* 1997;62:223-228.

NC Silver, RA Barker, DG MacManus, GJ Barker, M Thom, DCT Thomas, DH Miller.
Proton magnetic resonance spectroscopy in a pathologically confirmed acute demyelinating lesion.
*Journal of Neurology* 1997;244:204-207.

NC Silver, M Lai, MR Symms, GJ Barker, WI McDonald, DH Miller.
Serial magnetization transfer imaging to characterize the early evolution of new MS lesions.
M Filippi, NC Silver, TA Yousry, DH Miller.
Newer magnetic resonance techniques and disease activity in multiple sclerosis: new concepts and new concerns.
*Multiple Sclerosis* 1998;4:469-470

NC Silver, GJ Barker, DH Miller.
Standardization of magnetization transfer imaging for multicenter studies.

NC Silver, M Lai, MR Symms, GJ Barker, WI McDonald, DH Miller.
Serial gadolinium-enhanced and magnetization transfer imaging to investigate the relation between the duration of blood-brain barrier disruption and extent of demyelination in new multiple sclerosis lesions.
*J Neurol* 1999;246:728-730.

CA Davie, NC Silver, GJ Barker, PS Tofts, AJ Thompson, WI McDonald, DH Miller.
Does the extent of axon loss and demyelination from chronic lesions in multiple sclerosis correlate with the clinical subgroup?
*J Neurol Neurosurg Psychiatry* 1999;67;710-715.

Silver NC, Good CD, Sormani MP, MacManus DG, Thompson AJ, Filippi M, Miller DH.
An optimised protocol for detection of enhancing brain and spinal cord lesions in MS.
*J Neurol* (in press).
PART ONE

BACKGROUND
1.1 Personal and social cost implications of multiple sclerosis

It is estimated that there are 87,000 people in the United Kingdom with multiple sclerosis (MS) [Holmes et al., 1995]. Although it may appear at any age, MS most commonly presents in early adult life. As such, MS represents a significant cause of personal morbidity with implications for both immediate family and society. For an affected individual, MS may have widespread impact on physical, cognitive, emotional and social functions, thereby limiting normal activity.

Life expectancy is only slightly reduced in MS, by approximately six to seven years [Sadovnick et al., 1992]. This study showed disability to be the greatest risk factor for death, with pneumonia as a common immediate cause. Suicide represented a significant cause of death, especially in the less disabled patients - overall rates approached seven and a half times that of the general healthy age- and sex-matched population.

Community based studies suggest that approximately 50% of patients will require assistance for mobility within 15 years of onset [Weinshenker et al., 1989]. There is great variability of disease expression. Indeed, people have been recognised where the disease only manifested itself at post-mortem [Mackay et al., 1967; Ghatak and Hirano, 1974]. As MS may affect all parts of the central nervous system (CNS), the range of possible physical symptoms is extensive. Often affected are vision, sensation, power, coordination and balance. Common distressing symptoms include fatigue, spasm, pain, sleep disorder and sexual dysfunction.

In addition to physical disability, more than 40% of community based patients show evidence of cognitive impairment on formal neuropsychological testing [Rao, 1997]. Cognitive impairment may result in unemployment, social dysfunction and loss of independence [Rao et al., 1991].

It has long been realised since Charcot's early descriptions of the disease that psychiatric abnormalities as well as cognitive impairment occur as part of MS. Studies from the early 20th Century commented that the majority of patients with MS were affected by depression, euphoria
(a prevailing mood of serenity and cheerfulness), eutonia (a sense of physical wellbeing) and disorders of emotional expression [Cottrell and Wilson 1926]. Although more recent studies with improved methodology have shown a lower incidence of psychiatric morbidity, depression is still very common, with a prevalence of 40-45% [Ron and Logsdail 1989]. When compared with other (non-cerebral) neurological and rheumatological causes of disability, the prevalence of depression appears much higher; while cerebral involvement may contribute, it may also reflect the reaction to a disease which has a major adverse impact on activities of daily living and quality of life [Ron and Logsdail 1989].

A recent community-based study in the United Kingdom (UK) looked at data gathered from a questionnaire circulated to 999 people with MS, of which 672 subjects (67%) responded [Holmes et al., 1995]. This broadly stratified subjects according to mobility into 3 groups: (A) ambulatory, (B) requiring unilateral support for ambulation, and (C) wheelchair-dependent. The annual per capita cost to the NHS was £350 (group A), £650 (group B), and £4000 (group C). The total UK annual cost from MS was £1.2 billion, with little more than 12% of this (£153 million) spent in the National Health Service (NHS). The NHS expenditure on MS amounted to less than 2% of total NHS expenditure. The major financial burden of £536 million per year was borne by individuals and families living with MS, with the greatest single loss being foregone earnings of more than £395 million per year. Individuals were also shown to bear a heavy cost of £140 million for expenses which include child care, house alterations, and purchase of special equipment and supplies. State costs (in financial benefits and lost tax revenue) were £435 million per year and industrial costs amounted to over £76 million per year (including absence from work by family carers).

1.2 Background

The cause or causes of the clinical condition termed MS remain uncertain. Basic observations that allow insight into the mechanisms of pathological evolution and symptom production / resolution are therefore very important in helping our understanding of the condition. Such observations can help distinguish the features of the disease that are responsible for its diverse clinical manifestations. In turn, increased knowledge may improve our ability to prevent or slow the disease process using therapeutic strategies that selectively target the appropriate abnormalities.
This thesis describes work using magnetic resonance imaging (MRI), a technique that may help to characterise and quantify changes in the CNS responsible for the clinical manifestations of the disease. Because it is safe and essentially non-invasive, MRI is ideally situated to allow repeated snapshots into the natural history of MS.

While clinical outcome measures remain the gold standard for determining therapeutic response in clinical trials, they are hampered by difficulties associated with the diversity of the disease process and the protean problems associated with disability scales, namely poor responsiveness, poor reproducibility [Frances et al., 1991], and a tendency to be affected by day to day fluctuations in function [Whittaker et al., 1995]. As such, definitive clinical therapeutic trials in MS require large patient numbers and a long study duration, thereby requiring major expenditure and resources. It is therefore not surprising that considerable interest exists in the use of quantitative MRI outcome measures for therapeutic trials [Miller et al., 1996]. However, it is also recognised that MRI techniques in current routine clinical use have poor ability to predict clinical outcome. There is therefore demand for sensitive and reproducible quantitative MRI measures that are able to assess global pathological changes in the CNS with greater pathological specificity.

MRI has greatly contributed to our understanding of MS and there is rapid development in this field. Early studies concentrated on the visible focal abnormalities (i.e. lesions) [Young et al., 1981] and correlated these with underlying pathological abnormalities [Stewart et al., 1984; Ormerod et al., 1987; Newcombe et al., 1991]. The use of conventional cranial MRI to detect lesions is useful for clinical diagnosis [Poser et al., 1983] and for prediction of those patients with clinically isolated syndromes who will go on to develop definite MS [Lee et al., 1991; Morrissey et al., 1993; O’Riordan et al., 1998]. The relation of lesion evolution, as described by conventional non-enhanced MRI, to mechanisms of disability and recovery appears more complex and is at best limited [Koopmans et al., 1989; Miller et al., 1996]. Reasons for this include (1) difficulties associated with both clinical and MRI measurements, (2) the inability of conventional qualitative MRI to differentiate the pathological substrates of lesions (e.g. oedema, demyelination, axonal loss, gliosis), (3) lesions will exert different clinical effects according to anatomical localization, (4) invisible pathology in the normal-appearing white matter also contributes to disability, and (5) conventional MRI detects changes in structure rather than functional change (e.g. due to the metabolic environment, brain plasticity or sodium channel insertion). Fortunately, MRI techniques such as gadolinium contrast-enhanced imaging,
magnetization transfer, proton spectroscopy, diffusion, and functional imaging show promise to help discriminate the relevant pathological substrates of clinical impairment and recovery.

Unlike all other MRI techniques, magnetization transfer (MT) imaging utilises the interaction between “free” water protons and those “bound” to macromolecular structures. This provides a novel form of tissue contrast that may be utilised either in a quantitative manner to assess the structural elements of tissue (e.g. measurement of the MT effect with magnetization transfer ratio (MTR)), or qualitatively to improve discrimination between different tissue components (i.e. magnetization transfer contrast (MTC)).

1.3 Aims

In this thesis, I explore the potential application of MT imaging to investigation of the underlying pathogenetic basis of MS and determine how MT imaging may be utilised to help monitor the natural history of the disease or its response to therapy.

For successful implementation of MT imaging techniques into clinical and research practice, issues related to quality assurance and standardization first require consideration. Such issues are discussed in chapter three and reference is made to original data.

In chapter four, a normative MTR database is established in healthy subjects to determine the anatomical variation of MTR within the normal brain and the variations that may arise as a result of age, gender or cerebral hemisphere dominance. To help evaluate the pathophysiological substrates of MTR measurement, in chapter five I investigate how MTR may be affected in central pontine myelinolysis, a condition characterised by severe demyelination as the predominant pathological abnormality. In chapter six, the natural history of MTR within MR visible lesions in MS is determined; regions of normal-appearing white matter are assessed at weekly intervals during their evolution into new and visible MS lesions. In chapter seven, MT imaging is successfully implemented to study the cervical spinal cord. A preliminary investigation is performed to compare MTR values in MS with those of healthy control subjects. The role for further studies in this clinically eloquent CNS site is determined.

In chapter eight, I investigate MTC as a method to optimise enhancing lesion conspicuity with gadolinium contrast-enhanced MRI. Comparison is made with two other potential optimization techniques, the use of a larger (triple) dose of gadolinium and delayed imaging. Chapter nine details the application of these optimization techniques to more accurately
determine disease activity in longitudinal imaging studies. In particular, their potential ability to simplify exploratory treatment trials in MS is explored.

1.4 Multiple Sclerosis

1.4.1 Clinical background and definitions

Multiple sclerosis is an acquired primary demyelinating disease of the CNS in which myelin is a main target of autoimmune inflammatory processes. The condition is typically characterised by multifocal episodic disturbances of CNS function, resulting in lesions that are of varied age and differing anatomical location. In this thesis, the terms “lesion” and “plaque” are used interchangeably.

Traditionally, MS has been referred to as a primary demyelinating condition. This is because (i) the disruption and loss of normal myelin is an acquired process and not due to abnormal myelinogenesis (i.e. dysmyelination), and (ii) demyelination has been considered as an event that is not dependent on axonal damage. However, it is increasingly recognised that axonal damage may represent an important feature of acute lesions [Trapp et al., 1998] early in the disease and play an important role in the development of persistent and progressive disability.

Multiple sclerosis is just one of a number of primary demyelinating conditions of the CNS [Table 1.1].

1.4.2 Diagnosis

The diagnosis of MS was previously made purely on clinical grounds. The ability to accurately diagnose MS has become increasingly important as therapeutic options have become more readily available. In addition, more accurate diagnosis has been made possible with the development of more sensitive paraclinical investigations to help support the diagnosis and exclude alternative neurological conditions.

Diagnostic criteria. The first attempt at precision of diagnosis came in the mid 1960's when the Schumacher committee established criteria with which to diagnose clinically definite MS [Schumacher et al., 1965] [Table 1.2].
Acquired, inflammatory or infectious demyelinating conditions

Multiple sclerosis (acute, chronic, and variants):
- benign
- relapsing-remitting (RR)
- secondary progressive (SP)
- primary progressive (PP)
- transitional
- Marburg variant of multiple sclerosis

Subacute sclerosing panencephalitis
Acute disseminated encephalomyelitis
Acute haemorrhagic leukoencephalopathy
Human immunodeficiency virus (HIV) encephalopathy
Human immunodeficiency virus (HIV) vacuolar myelopathy
HTLV-1 associated myelopathy (tropical spastic paraparesis)
Progressive multifocal leukoencephalopathy (PML)

Monosymptomatic demyelinating syndromes
- Optic neuritis
- Acute transverse myelitis
- Sacral myeloradiculitis

Demyelinating disease with restricted distribution
- Neuromyelitis optica (Devic's syndrome)
- Ballo's concentric sclerosis (tumour-like)

Acquired toxic-metabolic disorders of myelin
- Central pontine myelinolysis
- Machiafava Bignami syndrome
- Vitamin B12 deficiency (subacute combined degeneration)
- Hexachlorophene intoxication
- Periventricular leukoencephalopathy (associated with anti-mitotic and radiotherapy)
- Heroin pyrolysate leukoencephalopathy

Traumatic disorders of myelin
- Trauma
- Compression adjacent to tumour

Hereditary metabolic disorders of myelin
- Metachromatic leukodystrophy
- Adrenoleukodystrophy, adrenomyeloneuropathy
- Globoid cell leukodystrophy (Krabbe's disease, galactosylceramide lipidosis)
- Spongiform leukodystrophy (Canavan's disease)
- Pelizaeus-Merzbacher disease
- Dysmyelinogenetic lipodystrophy (Alexander disease)
- Phenylketonuria

Table 1.1 Primary disorders of myelin affecting the central nervous system.
Neurological examination reveals objective abnormalities of CNS function

Neurological history indicates involvement of at least two CNS regions

CNS disease predominantly reflects white matter involvement

Involvement of CNS follows one or two patterns

- two or more distinct episodes with significant symptoms, each of at least 24 hours duration and 1 month apart
- slow or stepwise progression of signs and symptoms in a disseminated pattern for at least 6 months

Patient 10-50 years old at onset

Symptoms and signs can not be better explained by an alternative disease process (diagnosis must be made by a competent clinician)

Table 1.2 Schumacher Criteria for diagnosis of multiple sclerosis [from Schumacher et al., 1965].

These criteria recognised both relapsing-remitting and progressive forms of the disease. In 1983, Poser and colleagues modified these criteria to help identify those patients who could be appropriately involved in research studies and clinical trials [Poser et al., 1983]. They expanded the age of onset to 59 years and recognised the incorporation of data derived from laboratory investigations (evoked potentials and neuroimaging to show evidence for dissemination in space and cerebrospinal fluid (CSF) analysis to confirm inflammatory reaction localised to the CNS). The new criteria now included categories for Clinically Definite and Laboratory Supported MS [Table 1.3].

All patients who have contributed data to the studies in this thesis have clinically definite or, in the case of patients with primary progressive disease, laboratory supported definite MS.
### Table 1.3

<table>
<thead>
<tr>
<th>Category</th>
<th>Attacks</th>
<th>Clinical evidence</th>
<th>Paraclinical evidence</th>
<th>CSF oligoclonal bands</th>
</tr>
</thead>
<tbody>
<tr>
<td>Clinically definite MS</td>
<td>2</td>
<td>2</td>
<td>1 and 1</td>
<td>1</td>
</tr>
<tr>
<td>Clinically probable MS</td>
<td>2</td>
<td>1</td>
<td>2</td>
<td>2</td>
</tr>
<tr>
<td></td>
<td>1</td>
<td></td>
<td>1 and 1</td>
<td>1</td>
</tr>
<tr>
<td>Laboratory supported</td>
<td>2</td>
<td>1 or 1</td>
<td>present</td>
<td>present</td>
</tr>
<tr>
<td>definite MS</td>
<td>1</td>
<td>2</td>
<td>present</td>
<td>present</td>
</tr>
<tr>
<td>Laboratory supported</td>
<td>2</td>
<td></td>
<td>present</td>
<td>present</td>
</tr>
<tr>
<td>probable MS</td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
</tbody>
</table>

*Table 1.3*  
Poser Committee Criteria for diagnosis of multiple sclerosis [from Poser et al., 1983].

**Laboratory investigations.** Although confirmation of the diagnosis does not necessarily require evidence from investigations, it is usual for neurologists to undertake one or more tests. Evoked potentials (visual, somatosensory and brainstem) were the first non-invasive investigations available and their use became well-established during the 1970's. Visual evoked potentials remain the most useful, particularly to document evidence of previous optic neuritis in patients who have no visual symptoms at time of investigation.

During the past 15 years, MRI has become established in confirming the diagnosis of MS [Paty et al., 1988; Fazekas et al., 1988; Offenbacher et al., 1993] and it is generally accepted that MRI is the most sensitive paraclinical test [Paty et al., 1991]. Several criteria have been
developed for the interpretation of brain MRI. The first, devised by Paty and colleagues recommended the presence of four lesions, with three of these in a periventricular location; while sensitivity was relatively good (85%), specificity was shown to be less than 60% [Paty et al., 1988]. The specificity of diagnosis using MRI can be increased by requiring the presence of an ovoid lesion or (sub)callosal lesions. Fazekas and colleagues proposed that a brain MRI scan is strongly suggestive of MS when two of three specified criteria (a periventricular lesion, a lesion larger than 6mm., or an infratentorial lesion) are present [Fazekas et al., 1988]. A retrospective study showed these criteria to have high specificity (almost 100%) and sensitivity of 88% [Offenbacher et al., 1993]. Cerebral MRI may also offer a useful role for excluding alternative differential diagnoses (e.g. vascular disease, lymphoma, metastases, vasculitis, granulomatous disorders, adrenoleukodystrophy, acute disseminated encephalomyelitis). Spinal MRI may offer useful additional information as lesions due to MS are commonly seen in the cervical spine and alternative diagnoses may be excluded. Repeated brain MRI with or without gadolinium contrast-enhanced T1-weighted images help show evidence of dissemination in space and time, although this approach is not generally employed unless diagnosis is difficult (e.g. differentiating MS from acute disseminated encephalomyelitis).

Examination of CSF provides the most persuasive evidence for an inflammatory immune disease affecting the CNS. Changes that may be observed in MS include a mild increase in the level of cellular reaction or protein and an increase in the level of immunoglobulin, the latter having various methods for detection. The European consensus identified the presence of oligoclonal bands isolated to cerebrospinal fluid as the most useful indicator of localised inflammatory disease [Andersson et al., 1994]. Oligoclonal bands are present in over 90% of patients with clinically definite MS, but are not specific to this condition and may be found in a large number of inflammatory and immune-mediated diseases of the CNS, some of which must be considered in the differential diagnosis [Table 1.4]. Newer techniques for immunoelectrophoresis may allow higher sensitivity and these include isoelectric focussing, the technique of choice.
### Inflammatory disorders
- multiple sclerosis
- systemic lupus erythematosus
- primary Sjögren's syndrome
- Behçet’s disease (rare)
- polyarteritis nodosa

### Infectious diseases
- viral encephalitis (e.g. herpes simplex, human immunodeficiency virus)
- transverse myelitis
- Lyme disease (neuroborreliosis)
- neurosyphilis
- chronic fungal meningitis
- subacute sclerosing panencephalitis
- progressive rubella panencephalitis
- progressive multifocal leukoencephalopathy
- toxoplasmosis
- cysticercosis
- trypanosomiasis
- chronic fungal meningitis
- yaws

### Granulomatous disease
- sarcoidosis (rare)

### Others (rare)
- Guillain-Barré syndrome
- cerebrovascular disease
- adrenoleukodystrophy (including adrenomyeloneuropathy)
- CNS lymphoma
- cerebral neoplasia

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**Table 1.4** Neurological disorders that may cause CSF oligoclonal bands.

A combination of MRI and CSF oligoclonal bands can be used to virtually rule out MS when both tests are negative, as their high sensitivities allow a high negative predictive value. Particular care is required when imaging shows multifocal white matter lesions, but oligoclonal bands are absent from the cerebrospinal fluid. Fieschi and colleagues extensively reinvestigated 18 such patients from a cohort of 405 consecutive patients and found new diagnoses for six; vasculitis in two, and one patient each with mitochondrial encephalomyelopathy with lactic acidosis and stroke-like episodes (MELAS), atrial septal aneurysm with embolic cerebrovascular episodes, olivopontine cerebellar degeneration and Lyme disease [Fieschi *et al.*, 1995].
Therefore, the presence of positive oligoclonal bands help support the diagnosis, although their presence is not specific to MS. When absent, this test alerts the clinician to the possibility of alternative diagnoses, although MS is not excluded [Zeman et al., 1996].

It should be remembered that, even with the diagnostic techniques available, the role of an experienced neurologist in diagnosis remains pivotal.

**Differential diagnosis.** A considerable number of conditions may result in multifocal deficits of the CNS and require consideration in the differential diagnosis of MS. They include inflammatory disorders, infectious diseases, post-infectious demyelinating conditions, inherited disorders (of myelin and mitochondria), structural disorders, vitamin deficiencies, and toxic disorders. They may be divided into those with a monophasic, multiphasic or progressive course [Table 1.5].

Certain clinical features may be particularly helpful when reaching a diagnosis of MS. The majority of patients present with one or more of the signs of optic neuritis, limb weakness, paraesthesiae, diplopia, ataxia, vertigo and disturbance of micturition. Certain symptoms are rare in alternative diagnoses and these include (i) heat intolerance with or without precipitation of transient focal deficit (e.g. Uhthoff’s phenomenon), (ii) Lhermitte’s sign (transient electric shock-like feeling, tingling, or sensation of vibration that travels down the back on neck flexion) in the absence of cervical disc herniation or cervical trauma, (iii) girdle-like dysesthesias, and (iv) certain paroxysmal symptoms (e.g. trigeminal neuralgia, tonic spasms, diplopia, neuralgic pain, transient limb weakness, hemiataxia). Multiple sclerosis may occur at any age and may occur in the presence of a strong family history. However, it is very uncommon for MS to present in childhood, over the age of 60, or where there is a clear pattern of inheritance. Patients with MS rarely have rheumatological or constitutional symptoms; their occurrence, especially with abnormal blood markers for disease such as positive autoantibodies should cause suspicion for an alternative diagnosis. Certain clinical features may occur as a result of MS but are rare at presentation; these include aphasia, significant cognitive dysfunction, psychosis, and extreme anxiety. Likewise, certain symptoms are recognised but rarely occur in the course of MS; these include hemianopic visual disturbance, movement disorders such as parkinsonism or dystonia, and the persistence of a single lesion without evidence of demyelination elsewhere in the CNS.
Monophasic course

- acute disseminated encephalomyelitis (ADEM)
- isolated optic neuritis

Multiphasic course

- **Inflammatory disorders**
  - e.g. systemic lupus erythematosus, primary Sjögren’s syndrome, Behçet’s disease, polyarteritis nodosa, Whipple’s disease, isolated angiitis of the CNS
- **Granulomatous disorders**
  - e.g. Sarcoidosis, Wegener’s granulomatosis
- **Vascular disease**
  - e.g. arteriovenous malformations, cerebral autosomal dominant arteriopathy with subcortical infarcts (CADASIL)
- **Prothrombotic disorders**
  - e.g. protein S deficiency, protein C deficiency, antithrombin III deficiency, antiphospholipid syndrome, thrombotic thrombocytopenic purpura
- **Infectious diseases**
  - e.g. Lyme disease, Brucellosis
- **Structural disorders**
  - e.g. Arnold-Chiari malformation
- **Mitochondrial encephalomyopathies**
  - e.g. mitochondrial encephalomyelopathy with lactic acidosis and stroke-like episodes (MELAS), myoclonic epilepsy with ragged-red fibres (MERRF)

Progressive course

- hereditary spastic paraparesis
- Leber’s hereditary optic neuropathy (subacute, irreversible visual loss)
- B12 deficiency
- tobacco-alcohol amblyopia
- tropical spastic paraparesis (especially in patients of Japanese or Caribbean origin)
- adrenoleukodystrophy (including adrenomyeloneuropathy)
- progressive multifocal leukoencephalopathy (PML)
- compressive lesion (e.g. disc herniation, rheumatoid spondylopathy, meningioma, neurofibroma, primary cerebral lymphoma, metastatic or primary malignant tumour)

Table 1.5 Differential diagnosis of multiple sclerosis.
1.4.3 Clinical manifestations

Presentation and disease course. Multiple sclerosis is classically characterised by a relapsing-remitting course due to lesions in many regions of the CNS, with onset most common in young adulthood and the development of progressive disability occurring in the majority of patients at some stage.

Relapses are described as acute or subacute episodes of neurological dysfunction that usually reach a peak in a few days to weeks, followed by relatively little change, and finally remission to a variable degree. Isolated or mixed motor or sensory symptoms are the most frequent presenting features, with less involvement of visual, cerebellar, and brain stem structures. 85% of patients commence with such a relapsing-remitting disease course [Runmarker and Andersen, 1993].

After this initial relapsing-remitting course, the majority of patients will enter a progressive phase of the disease (50% within 10 years) [Runmarker and Andersen, 1993]. Such patients are described as having a secondary progressive disease course and may, in addition to their disease progression, continue or cease to have superimposed relapses.

There is also a small group of patients in whom the onset of clinical disease is followed by a progressive decline without remission or superimposed relapse. Such patients are often described as having primary progressive MS and they account for approximately 10% of cases [Runmarker and Andersen, 1993; Thompson et al, 1997]. In these patients, the condition often predominantly involves one part of the CNS, and patients typically present at an older age than those with relapsing-remitting onset; the commonest mode of presentation is with a spastic paraparesis, as opposed to the more common sensory and visual disturbances seen at presentation in relapsing-remitting disease [Thompson et al, 1997]. In this thesis, patients have also been studied who have been classified as having a transitional disease course; in these cases, patients have had a primary progressive course with the addition of a single relapse either prior to or superimposed on their progressive course [Thompson et al, 1997].

The last subgroup of patients with MS studied in this thesis are more difficult to categorise and have been termed as having benign MS. For the purposes of all work presented in this thesis, this refers to patients with a disease duration greater than or equal to 10 years who have not developed significant disability, with a score of 3 or less on Kurtzke’s Expanded Disability Status Scale [EDSS, Kurtzke, 1983]. Despite this relatively benign early course, patients may enter a subsequent aggressive or progressive phase of disease and this may lead to...
certain difficulties in evaluating research results in this group.

**Clinical prognostic factors.** There remains uncertainty about those factors that pose risk either for development of MS, or for causing relapse or disease progression in established disease.

The population that is at highest risk for development of MS are women of childbearing age. Pregnancy has been shown to significantly reduce the frequency of relapse (approximate 30% risk reduction), although this is counteracted by a similar increase in relapse rate in the early puerperium [Confavreux et al., 1998]. However, pregnancy is not thought to have an overall bearing on long term prognosis.

For a long time, infection has been implicated in the pathogenesis of MS; indeed, this represented the original rationale behind the use of interferons in MS, although their mechanism of action is now thought to be independent of infection prevention. Several prospective studies have suggested a correlation between relapse and common infections, especially minor infections of the upper respiratory tract or chronic sinusitis [Sibley et al., 1985; Gay et al., 1986]. Mechanisms whereby MS activity may be precipitated by infections include the host production of γ-interferon [Panitch et al., 1987].

Other proposed precipitants of relapse in MS have included immunization, trauma, exposure to general anaesthetic agents, and stress. In many cases, data is not definitive and cannot rule out such associations, although the accumulated evidence to date does not convincingly support such hypotheses.

Studies have addressed the relation between demographic and clinical features of the disease. It is generally agreed that certain features are associated with more favourable outcome and these include: younger age at onset, female gender, onset with purely sensory symptoms or optic neuritis, complete remission from first episode, low attack frequency and long interval between first and second episodes. Conversely, a poorer outcome may be associated with disease presentation at an older age (>40 years), male gender, prominent cerebellar component, insidious pyramidal involvement, high attack frequency, and early fixed disability [Weinshenker and Ebers 1987; Weinshenker et al., 1991a; Runmarker et al., 1994; Weinshenker 1995].

**Laboratory prognostic factors.** It is notoriously difficult to predict outcome in MS and various studies have attempted to determine whether laboratory measures may be of assistance.
For clinically isolated syndromes suggestive of MS (isolated optic neuritis, acute partial myelitis, or acute brain stem syndromes suggestive of demyelination), MRI has some predictive role. Approximately 50-70% of such patients will have asymptomatic white matter lesions on MRI [Miller, 1997]. The risk of conversion to clinically definite MS is low where no asymptomatic lesions are seen, whereas multiple asymptomatic lesions increase both the risk of conversion to MS and also for greater disability at 10 years [O’Riordan et al., 1998].

In established MS, the predictive value of MRI for future clinical disability has so far been limited by the disappointingly poor correlations between lesions (as demonstrated on conventional T2-weighted sequences) and clinical features [Miller, 1997].

Cerebrospinal fluid may also yield prognostic information. A long term study by Sandberg-Wollheim and colleagues in acute optic neuritis demonstrated a low probability of remaining free of neurological symptoms 18 years later if cerebrospinal fluid abnormalities were noted at presentation [Sandberg-Wollheim et al., 1990].

However, despite data from large natural history studies and the appearance of new or improved techniques to provide in vivo markers of disease, it remains impossible to provide an accurate prognosis for individual patients with MS.

Clinical features and disability. One of the outstanding characteristics of MS is its variability, both between individuals and in the same individuals over time. Early in the disease, relapses commonly are followed by complete remission, although this is not invariable. Later in the course of the disease, incomplete recovery or disease progression are more usual, with gradual accumulation of neurological impairment. The most common deficits associated with MS are listed in Table 1.6.

<table>
<thead>
<tr>
<th>Presentation*</th>
<th>During disease course*</th>
</tr>
</thead>
<tbody>
<tr>
<td>visual /oculomotor</td>
<td>49</td>
</tr>
<tr>
<td>paresis</td>
<td>43</td>
</tr>
<tr>
<td>paraesthesiae</td>
<td>41</td>
</tr>
<tr>
<td>incoordination</td>
<td>23</td>
</tr>
<tr>
<td>bladder/bowel</td>
<td>10</td>
</tr>
<tr>
<td>cerebral</td>
<td>4</td>
</tr>
</tbody>
</table>

* expressed as a percentage (the total is greater than 100%, allowing for multiple symptoms at presentation)

Table 1.6 Common symptoms in multiple sclerosis (adapted from Poser et al., 1979).
There are certain clinically eloquent sites of the CNS that are preferentially affected by MS, thereby resulting in certain common symptoms [Table 1.7]. Other clinical features may be more difficult to clearly associate with individual affected sites of the CNS, especially where lesions are located within the cerebral hemispheres (e.g. cognitive impairment, depression, fatigue, sleep disturbance, movement disorders). Discrete hemisphere cortical lesions may rarely give rise to clearly associated symptoms (e.g. epilepsy, dysphasia).

A detailed account of disability and impairment in MS, in particular focusing on the problems associated with their measurement, is given in chapter two.

<table>
<thead>
<tr>
<th>Region</th>
<th>Clinical features</th>
</tr>
</thead>
<tbody>
<tr>
<td>Optic nerve</td>
<td>optic neuritis (blurred vision, scotoma, pain)</td>
</tr>
<tr>
<td>Spinal cord</td>
<td>paraesthesiae, numbness, weakness, spasticity, disturbance of bladder / bowel function, impotence, Lhermitte’s phenomenon</td>
</tr>
<tr>
<td>Brainstem</td>
<td>vertigo, diplopia, oscillopsia, dysphagia, incoordination, trigeminal neuralgia</td>
</tr>
<tr>
<td>Cerebellum</td>
<td>ataxia</td>
</tr>
</tbody>
</table>

*Table 1.7* Commonly affected clinically eloquent sites of the central nervous system in multiple sclerosis and their associated clinical features.

1.4.4 Aetiology

The cause of MS remains undetermined. Epidemiological studies have however provided some insight.

The prevalence of MS varies across the world. It appears to be influenced by a complex interaction of ethnic background and geographical position. In general, prevalence increases with increasing distance from the equator (<1 per 100,000 in equatorial regions, 6-14 per 100,000 in southern United States and southern Europe, 30-80 per 100,000 in parts of northern Europe and northern United States, and >100 per 100,000 in the United Kingdom and Canada), thus supporting an environmental influence. A number of important exceptions exist (e.g. the low
incidence of MS in Japan). Important differences may also be seen amongst populations containing established homogenous groups of different racial origin (e.g. lower incidence in indigenous South Africans, Maoris, Lapps, Inuit, Hungarian gypsies, and American Indians), implicating additional genetic factors. The role of environmental aetiological factors is supported by migration studies. These have shown that migration between two countries of varied incidence has no effect on disease susceptibility if migration occurred after 15 years of age (i.e. if migration occurs after the age of 15, the susceptibility for the country of origin is maintained [Dean and Kurtzke, 1971].

The role of genetic factors in the aetiology of MS is supported by the increased incidence in blood relatives and much higher concordance between monozygotic than dizygotic twins (i.e. 25-30 versus 4%) [Sadovnick et al., 1993; Mumford et al., 1994]. However, the study of candidate genes in MS has largely been unrewarding, although significant genetic influence appears to come from the Major Histocompatibility Complex (MHC), particularly the HLA-DR2 allele [McFarland et al., 1997]. Recently, several groups of investigators have initiated large detailed analyses of the entire genome in families with MS to help identify regions of the genome contributing to susceptibility. Results from 3 such studies, looking at 324 sib pairs, showed no single gene/region with strong linkage to MS susceptibility; 2 of the studies identified a gene in the MHC region of chromosome 6 as having the strongest association [Sawcer et al., 1996; Ebers et al., 1996; The MS Genetics Group, 1996]. It is therefore likely that the genetic predisposition to MS results from several genes of moderate effect with a polygenic mode of inheritance.

The existing data suggests the combination of (i) a polygenic inherited predisposition, and (ii) exposure to an environmental factor in childhood that, after years of latency, either evokes the disease or contributes to its causation. It is quite likely that the environmental factor in question represents an infective agent [Kurtzke, 1997]. This hypothesis is supported by three areas of investigation [Gilden, 1999]. First, epidemiological analysis of all cases on MS on the Faroe Islands between 1920 and 1977 indicated a point source epidemic of the disease, probably introduced by stationed British troops, suggesting a transmissible and probable infective origin. Second, where subjects share identical DNA (i.e. monozygotic twins), only 30% of those affected will have a similarly affected twin. Third, although normal cerebrospinal fluid contains up to 13% IgG, this is significantly raised in subjects with MS, usually with the presence of oligoclonal bands. Such IgG elevations are often found in infectious disorders affecting the CNS [Table 1.4]. When analysed for specificity, oligoclonal bands have been shown to be directed against the
causative virus in such infections. Although several viruses (e.g. Measles virus, Herpes Simplex virus, Varicella, Epstein-Barr virus, Parainfluenza viruses, Paramyxovirus, and various retroviruses) and the bacterium Chlamydia Pneumoniae [Sriram et al., 1999] have been implicated in the aetiology of MS, a universal role for a single agent has yet to be demonstrated.

1.4.5 Neuropathology

White matter components. Neurons are the fundamental constituents of the CNS. They form an intricate network of pathways that function by conduction of electrical impulses to synapses, where they communicate with other neurons or effector cells. The gray matter is principally composed of neuronal cell bodies and the white matter mainly of nerve fibres (axons) covered by myelin.

Myelin is the major structural component of healthy white matter, accounting for approximately 25% of the dry weight of the brain and 50% of the dry weight of cerebral white matter [Valk and van der Knaap, 1989]. The multilayered membraneous structure surrounds axons and is composed of condensed plasma membrane with alternating protein-lipid-protein-lipid-protein-lipid-protein lamellae as the repeating subunit of each membrane bilayer. In comparison with other membranes, myelin has a high ratio of lipid to protein (approximately 70-80% vs. 20-30% by dry weight). Small amounts of carbohydrate are also present. The predominant lipid components are phospholipids, glycolipids, and cholesterol, with gangliosides and several other minor lipids at very low concentrations. Of the total protein present in myelin, proteolipid protein and basic protein account for 70-80%. With a high lipid concentration and predominant composition of saturated fatty acids, the lipid bilayer structure may warp tightly around the axon to form a compact myelin sheath that is relatively dehydrated (water content is approximately 40 %). Individual structures within myelin include the major membraneous organelles (e.g. mitochondria, rough endoplasmic reticulum, golgi apparatus, lysosomes, peroxisomes, and smooth endoplasmic reticulum), non-membraneous organelles (free ribosomes, microtubules, centrioles and filaments), cell nucleus (containing ribonucleic acid (RNA) and deoxyribonucleic acid (DNA)), and cytoplasm matrix (containing enzymes required for cell metabolism). Myelin insulates axons, allowing rapid (saltatory) electrical conduction. In addition, it is thought to play a structural and nutritional functional role in supporting the axon. Oligodendrocytes are responsible for production and maintenance of myelin, their cell processes wrapping around axons to form myelin sheaths, where each unit (internode) is separated from each other by a node
of Ranvier. Each oligodendrocyte is thought to maintain approximately 30-50 internodes [Raine, 1997]. Damage to myelin and oligodendrocytes is a key feature of the MS lesion. Where demyelination occurs, remyelination may follow, especially in small lesions, albeit in thin sheaths. With the normal ageing process, human brain weight decreases and water content increases [Valk and van der Knaap, 1989]. The myelin content of white matter is reduced in old age, probably as a result of continuous neuronal loss with subsequent axonal and myelin degeneration. The composition of myelin may also alter, especially the fatty acid composition of myelin phosphoglycerides and cerebrosides [Valk and van der Knaap, 1989].

Astrocytes are found amongst the myelinated nerve fibres and act as the predominant supporting and structural elements of healthy white matter. They are involved in CNS repair, responding readily to injury by proliferation, with synthesis of glial fibrils. This is responsible for the typical scarring or “sclerosis” associated with MS lesions, otherwise known as astrocytosis or gliosis. Other white matter components include microglia, ependymal cells lining the ventricles, and blood vessels.

The CNS possesses a unique blood-brain barrier (BBB) that regulates the passage of cells and molecules from blood vessels. Cerebral microvessels differ both functionally and structurally from blood vessels in other organs. A complex relationship exists between endothelial cells, basement membrane, and associated cells such as perivascular macrophages and astrocytes. Tight junctions with extremely high electrical resistance join the endothelial cells. The endothelial cells are almost completely surrounded by the foot processes of astrocytes and some microglia. A further line of defence comes from the presence of highly phagocytic perivascular macrophages.

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The interaction between these structural components and the immune system influences the evolution of MS lesions, with progressive tissue loss (myelin with or without associated axons), scarring, and subsequent tissue atrophy [Dawson, 1916].

The multiple sclerosis plaque. The principal histopathological features of the MS lesion have been recognised since the early descriptions of Carswell in the early 19th Century: they are those of predominant demyelination with associated oedema, inflammation, blood vessel wall change and glial proliferation. Although lesions are predominantly found in white matter, it has long been realised that they are also commonly found in gray matter [Dawson 1916]. Typical sites for plaques include the spinal cord, medulla, pons, cerebellar white matter, optic nerves, corpus callosum, and cerebral white matter. They are commonly located adjacent to the fluid-
filled ventricular system. Inflammatory lesions may also occur in MS in regions devoid of myelin, such as the retina [Lightman et al., 1987].

In pathological specimens, MS plaques are often easily visible. They appear as well demarcated areas of demyelination that are not confined to specific regions or anatomical tracts; instead they are typically located in relation to central small blood vessels, as initially pointed out in the early pathological observations by Rindfleisch. At any given time, plaques will be variable in size, extent and stage of evolution. Old plaques will appear gray, translucent, gelatinous and firm (sclerosed), whereas newer ones appear soft and pink. Plaques grow either by coalescence of adjacent lesions, or with finger-like extensions (Dawson’s fingers) from the plaque edge [Dawson, 1916]. Plaques probably evolve through multiple demyelinating and remyelinating episodes [Prineas et al., 1993], the end result being a chronic demyelinated plaque with loss of oligodendrocytes. Post mortem examination frequently shows both generalised atrophy and focal plaques at varying stages of evolution. In addition, microscopy of the macroscopically normal-appearing white matter shows the presence of more diffuse lesions (shadow plaques) that have abnormally thin myelin but no evidence of ongoing inflammation or demyelination; these are generally regarded as “single-hit” lesions, afflicted just once by the disease process and then left to repair with remyelination and gliosis [Prineas et al., 1993; Raine, 1997]. Perivascular inflammation and astrocytic hyperplasia has also been reported in the macroscopically normal white matter [Allen et al., 1981].

Evolution of the multiple sclerosis plaque - the acute lesion. Myelin and oligodendrocytes appear to be the principal targets of attack in MS. Early events include splitting of myelin lamellae and formation of intracytoplasmic vesicles. Destruction of myelin is effected with phagocytosis by macrophages and, to a lesser degree, hypertrophic astrocytes. This results in abundant myelin and lipid degradation products. In comparison to this constant myelin destruction, the degree of oligodendrocyte damage is more variable [Ozawa et al., 1994].

Acute plaques contain large numbers of inflammatory cells arranged around veins, including macrophages, T-cells, antibody-producing plasma cells, and large mononuclear cells; inflamed areas are dominated by T-cells and macrophages. The type of inflammation varies according to disease activity and disease duration [Ozawa et al., 1994]. Perivascular and interstitial oedema is often prominent and is associated with an expanded extracellular space; the margin of the acute lesion is typically indistinct and associated with ongoing macrophage-
mediated demyelination. Surrounding the small central venules, there is intense inflammatory reaction with perivascular lymphocyte cuffing and diffuse infiltration by lipid-laden “foamy” macrophages. Astrocytes may extend beyond the plaque margin by several centimetres. The degree of inflammatory response may vary according to the clinical phenotype of the disease; one study has shown significantly less inflammation in primary than secondary progressive MS [Revesz et al., 1994].

Although axonal disruption occurs commonly in MS lesions [Dawson 1916], and transection may commonly occur in acute lesions [Trapp et al., 1998], there is relative axonal preservation with respect to the degree of disruption to normal myelin. Parenchyma that previously contained well myelinated axons is reduced to a loosely packed oedematous zone containing “naked” axonal sheaths stripped of myelin, damaged axons (with the appearance of retraction balls and beading), myelin debris and fat-laden macrophages.

The first event in the evolution of a new lesion is not known, although the cellular immune response is believed to be a consistent early feature. Whether this is in response to antigen production within the CNS or whether it is triggered by primary events outside the brain has not been resolved. Most investigators assume it to represent a response to one or more antigens associated with myelin.

**Evolution of the multiple sclerosis plaque - the chronic lesion.** Chronic MS lesions show variable degrees of demyelination, axonal loss, gliosis and oligodendrocyte loss. Myelin staining reveals these as plaques of white matter that are devoid of myelin and demarcated from adjacent myelinated tissue by a sharp edge. This gives the chronic lesion a “punched out” appearance. The parenchyma shows the normal myelinated fibres to be replaced with a network of astroglial scar tissue; axonal loss is prominent, particularly at the centre of the plaque [Lassmann et al., 1994]. Astrocytes are well separated, often multi-nucleated, and sharply demarcate the plaque from surrounding white matter. In most chronic plaques, fibrous astroglisis is intense, with glial processes forming parallel rows. Oligodendrocytes are typically absent in the centre of the chronic MS lesion, where there is associated absence of remyelination. Venules have thickened walls due to extensive deposition of collagen with some duplication of basement membrane material; the appearances are consistent with widespread and persistent blood-brain barrier damage in the chronic MS plaque [Broman 1964; Barnes et al., 1991; Kwon and Prineas, 1994]. Low-grade inflammatory activity is a common finding, even in otherwise silent-appearing lesions.
[Raine, 1997]. The histological findings suggest increased vascular permeability as a major perpetuating feature of the established MS lesion.

The edge of a chronic lesion may display evidence of ongoing cellular activity, with reactive astrocytes and sometimes evidence of proliferating oligodendrocytes, especially early in the disease. Remyelination may be a feature, especially in those patients with short disease duration [Prineas et al., 1993]. In more chronically active lesions, there may be a superimposed prominent inflammatory component upon a previously demyelinated plaque, with astroglial hypertrophy, oligodendrocyte hyperplasia, and ongoing demyelination. The features of chronic versus acute MS lesions are compared in Table 1.8.

<table>
<thead>
<tr>
<th>Acute (active)</th>
<th>Chronic (inactive)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Demyelination</td>
<td>Demyelination</td>
</tr>
<tr>
<td>(breakdown products present)</td>
<td>(breakdown products absent)</td>
</tr>
<tr>
<td>Variable oligodendrocyte loss</td>
<td>Marked oligodendrocyte loss</td>
</tr>
<tr>
<td>Hypercellular plaque edge</td>
<td>Hypocellular plaque</td>
</tr>
<tr>
<td>(infiltration of tissue with inflammatory cells)</td>
<td></td>
</tr>
<tr>
<td>Perivenous inflammatory infiltrate</td>
<td>Variable inflammatory infiltrate</td>
</tr>
<tr>
<td>(predominantly macrophages and lymphocytes)</td>
<td></td>
</tr>
<tr>
<td>Extensive blood-brain barrier dysfunction</td>
<td>Minor to moderate blood-brain dysfunction</td>
</tr>
<tr>
<td>Reactivated older plaques may have central gliosis</td>
<td>Plaques gliosed</td>
</tr>
</tbody>
</table>

*Table 1.8* The pathological features of acute and chronic lesions in multiple sclerosis.

*Early and late phases of multiple sclerosis.* Certain important pathological differences
exist between the plaques that occur early and late in the disease course. These are summarised in Table 1.9.

<table>
<thead>
<tr>
<th>Early</th>
<th>Late</th>
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<tbody>
<tr>
<td>Demyelination</td>
<td>Demyelination</td>
</tr>
<tr>
<td>Little or no oligodendrocyte loss</td>
<td>Extensive oligodendrocyte loss</td>
</tr>
<tr>
<td>Extensive remyelination (shadow plaques)</td>
<td>Sparse remyelination</td>
</tr>
<tr>
<td>Myelin destruction accompanies or precedes oligodendrocyte destruction</td>
<td>Oligodendrocyte destruction accompanies or precedes myelin destruction</td>
</tr>
<tr>
<td>Axons relatively preserved</td>
<td>More extensive axonal loss</td>
</tr>
<tr>
<td>Perivascular inflammation</td>
<td>Inflammation less pronounced</td>
</tr>
<tr>
<td>Few plasma cells</td>
<td>More plasma cells</td>
</tr>
<tr>
<td>Gliosis follows demyelination</td>
<td>Gliosis occurs early in lesion development</td>
</tr>
</tbody>
</table>

Table 1.9  The features of pathological lesions early and late in the course of multiple sclerosis.

Immunopathology. The aetiology of MS remains incompletely understood, although it is widely agreed that autoimmune mechanisms are fundamental to the pathogenesis. This is supported by the following observations: (i) in MS, white matter infiltrates chiefly consist of lymphocytes and monocytes, (ii) there is an association with genes relevant to immune responses, and (iii) there is a partial response to both immunosuppressive and immunomodulatory therapy.

Multiple sclerosis may be regarded as an organ-specific autoimmune disease mediated by autoreactive CD4+ T-cells [Giovannoni and Miller, 1999] [Figure 1.1].
Pathological autoreactive T-cells are established by an environmental mechanism, probably infective, in those people with a genetic predisposition early in childhood, probably between 5 and 15 years. They are activated by an environmental trigger (e.g. viral infection, superantigen) after a variable latency period, usually 10 to 20 years. Activated T-cells circulate systemically to selectively cross the blood-brain barrier. Once in the CNS they are exposed to a putative autoantigen and initiate a cell-mediated (TH 1) inflammatory reaction. A complex inflammatory cascade ensues with activation of T-cells, B-cells, macrophages and vascular endothelium. The induction of cytokines and inflammatory mediators results in damage to myelin and axons, with further release of sequestered autoantigens; such release may be considered to initiate further episodes of autoimmune inflammation, via intra- and/or intermolecular spread. An attractive alternative hypothesis implicates neurotropic viruses (e.g. Human...
Herpesvirus 6) as local triggers for inflammation, sparking a secondary autoimmune response.

The exact mechanisms of autoimmunity in MS are unknown. Activation of T-cells is dependent on an antigen-specific signal and this is transduced via a T-cell receptor (TCR)/CD3-CD4 complex interacting with MHC class II molecules. This probably takes place in the perivascular space, where macrophages are the most likely candidates for antigen-presentation. Other possible antigen-presenting cells include astrocytes and pericytes, both of which also express MHC class II molecules. To ensure T-cell activation, a certain threshold of TCRs is required in addition to alternative costimulatory signals via interaction of accessory molecule ligand pairs (e.g. B7/CD28). Current evidence points towards MS being mediated by Th1-CD4+ αβ T-cells, although other cells have also been implicated (e.g. CD8+ T-cells, natural killer cells, B-cells). Putative autoantigens include myelin proteins; myelin basic protein (MBP), proteolipid protein (PLP), myelin-associated glycoprotein (MAG), and myelin oligodendrocyte protein (MOG). Other possible autoantigens include αβ-crystallin and transaldolase.

Activated T-cells produce pro-inflammatory cytokines that activate macrophages and microglia. These cells play a central role in amplification of the inflammatory cascade, by producing chemokines and inflammatory monokines to activate astrocytes and endothelial cells, upregulating adhesion molecule expression, and disrupting the BBB. They are also responsible for antigen presentation, myelin phagocytosis and possibly assist remyelination via production of local growth factors.

Pro-inflammatory cytokines that appear pivotal in the inflammatory processes associated with MS include interferon-γ (IFN-γ), interleukin (IL)2 and tumour necrosis factor (TNF) α and β. Prior to relapse, stimulated peripheral blood cells of patients with MS produce increased quantities of IFN-γ and TNF α. Pro-inflammatory cytokines (as well as their messenger ribonucleic acid, mRNA) have been demonstrated in MS plaques. Increased levels of IL2, IL1, TNF α/β have also been demonstrated in the cerebrospinal fluid of patients with MS. These pro-inflammatory cytokines are potent activators of macrophages, microglia, and astrocytes, inducing and greatly augmenting their own cytokine production, stimulating oxygen and nitrogen free radical release. They also increase MHC, Fc receptor and adhesion molecule expression.

Chemokines such as macrophage inflammatory protein (MIP)-1α are chemoattractant cytokines that are responsible for the recruitment of specific subsets of inflammatory cells. Levels of MIP-1α appear elevated in the cerebrospinal fluid of patients with MS. Once cells have been attracted to and adhered to the vascular endothelium, they penetrate the basal lamina and
extracellular matrix. Lysis of the extracellular matrix is achieved by a large group of enzymes known as matrix metalloproteinases (MMP). Increased levels of MMP9 have been found in the cerebrospinal fluid of patients with MS and have been correlated with disturbance of BBB integrity, as evidenced by gadolinium contrast-enhanced MRI.

Non-specific mediators of inflammation (e.g. oxygen and nitrogen free radicals, proteases, eicosanoids) are all capable of damaging myelin and oligodendrocytes. The death of oligodendrocytes in MS appears to occur via apoptosis; similar mechanisms may be responsible for axonal and neuronal loss and contribute to irreversible clinical deficit.

Upregulated expression of adhesion molecules in MS allows recruitment of circulating leukocytes into the CNS, thereby assisting antigen presentation.

Other cytokines appear to have anti-inflammatory properties, counteracting the effects of pro-inflammatory cytokines, thereby inducing and maintaining remission. These include IL4, IL10, IL13, and transforming growth factor (TGF) β. Increased levels of IL10 and increased TGFβ mRNA expression in peripheral mononuclear blood cells have been associated with the recovery phase of relapses, periods of remission, and possibly a more benign disease course.

The humoral, B-cell, response is also very important in the process of demyelination. B-cells produce complement-fixing anti-myelin antibodies, especially to MOG, that are required to induce demyelination. Other important properties include Fc-receptor stimulation, chemotaxis and myelin opsonisation.

Mechanisms of relapse. The characteristic MS relapse is due to a failure or slowing of normal axonal conduction, i.e. conduction block. This is, in part, a direct result of disruption to the normal myelin within lesions [McDonald, 1996]. It is also likely that inflammation itself contributes to conduction block [McDonald, 1996]. Electrophysiological and magnetic resonance spectroscopy studies have shown that demyelination occurs early during the inflammatory phase of a lesion [Youl et al., 1991; Davie et al., 1994]. Proposed mechanisms for both demyelination per se and induction of conduction block include the production of reactive oxygen and nitrogen species as part of the inflammatory process (e.g. nitric oxide, superoxide, and peroxynitrite) [Redford et al., 1997; Smith et al., 1999]. Nitric oxide is produced by macrophages and there is in vivo evidence of this occurring within MS lesions [Giovannoni et al., 1997]. In an acute MS lesion, it is the demyelinated and early remyelinated nerve fibres that are likely to be most susceptible to these reactive species [Redford et al., 1997].
**Mechanisms of repair.** Remission is clearly associated with resolution of inflammation and oedema, with associated repair of the blood-brain barrier [Willoughby et al., 1989; Youl et al., 1991]. Other factors are also important, as demonstrated by the usual persistence of demyelination in the recovered optic nerve after an inflammatory episode [Youl et al., 1991].

Remyelination is also known to occur within MS lesions and is capable of restoring conduction [Prineas and Connell, 1979; McDonald, 1999]. Remyelination may be important for maintenance of axon integrity [McDonald, 1999].

Conduction may be restored early, within days of acute toxic damage to small nerves [Bostock and Sears, 1978; Smith et al., 1982]. There is evidence that slow conduction is also restored in persistently demyelinated fibres and it is likely that the restoration of axonal function is, at least in part, also due to insertion of new sodium channels in the exposed internodal membrane [Smith et al., 1981; Moll et al., 1991; Black et al., 1991].

A further potential mechanism for recovery is that of brain plasticity. In patients with optic neuritis, functional MRI has shown that new regions of the brain may assume activity following damage to critical neuronal pathways [Werring et al., 2000a; Lee et al., 2000].

**Mechanisms of permanent disability.** Complete remission from attacks is a usual early feature in the course of MS. Permanent disability is more typical in the later stages. One mechanism likely to be responsible for the development of permanent disability is a gradual failure of the repair processes. It is also likely that lesions sustain increasing damage with repeated episodes of reactivation and pathological studies confirm axonal loss, complete demyelination and gliosis. However, their individual roles in the development of fixed clinical deficit has been less clear.

Magnetic resonance imaging has provided valuable in vivo information relating to the pathogenesis of MS. However, while the appearance of new lesions correlates well with relapse activity, the relationship between MRI measures and development of disability or irrecoverable deficit is poor [Thompson et al., 1999].

One of the potential reasons for the poor correlations between MRI lesion volume and disability is that many studies have ignored the contribution of lesions in the spinal cord, a site where lesions may be (i) more easily correlated with the clinical features, and (ii) responsible for considerable disability. Lesions are commonly seen in the cervical spinal cord in MS, although their presence has not been shown to correlate with disability [Kidd et al., 1993]. Further
A longitudinal evaluation has shown very few new spinal lesions despite developing disability [Kidd et al., 1996]. A strong correlation has however been seen between cervical spinal cord cross-sectional area (at C2 level) and disability, and changes have been seen in less than a year that are particularly prominent in those with primary progressive MS [Losseff et al., 1996a; Stevenson et al., 1998]. Development of cerebral atrophy has also been shown to correlate with worsening disability [Losseff et al., 1996b]. Atrophy may occur as a result of destruction of tissue components (e.g. axonal loss, demyelination).

Other MRI techniques have been developed that show promise to provide greater pathological specificity. These include MTR, T2 relaxation curve analysis, T1 hypointense lesion load, proton magnetic resonance spectroscopy (MRS), and diffusion imaging. The use of MT imaging to evaluate pathological change is discussed in other sections of this thesis.

The analysis of T2 relaxation curves has revealed more biexponential lesions in patients with secondary progressive MS than in those with relapsing-remitting disease. This finding implies an expanded extracellular space associated with tissue loss [Filippi et al., 1994].

The volume of T1 hypointense lesions has been shown to have good correlation with disability. Increased T1 lesion load correlates with disability, especially in secondary progressive MS [Truyen et al., 1996]. Pathological study shows that lesions with T1 hypointensity are more destroyed, with greater axonal loss and expansion of the extracellular space [van Walderveen et al., 1998].

Diffusion imaging exploits a novel form of contrast that is dependent upon the restriction by fibre tracts of water molecules to their normal random diffusion. Their orientation may be used to locate lesions more precisely and changes in diffusion may help clarify the severity of structural damage within the brain. Changes to the normal diffusion pattern have been demonstrated in normal appearing white matter and lesions, appearing most abnormal in T1 hypointense and enhancing lesions [Werring et al., 1999]. Candidate substrates for this change in normal diffusion include axonal loss, demyelination, and gliosis.

Whilst limited by poor resolution, proton MRS may possibly yield most information of all MRI techniques, allowing concentrations of proton metabolites to be accurately measured. In cerebral tissue, these metabolites have been shown to correspond to various pathological changes: N-acetylaspartate (NAA) provides a marker for neuronal density/function, choline represents myelin turnover, inositol is a possible marker for gliosis, and lactate and free lipid probably represent macrophage metabolism and myelin breakdown respectively. Of these
metabolites, measurement of NAA appears particularly exciting as this is the only available in vivo neuronal marker, and it is the loss of neuronal function that ultimately results in neurological deficit. Marked reductions in NAA are seen within acute lesions and these are usually followed by a degree of recovery [Davie et al., 1994]. In the cerebellum, persistent NAA reduction has been shown to correspond to the severity of cerebellar impairment [Davie et al., 1995]. Other studies have shown NAA reductions in normal-appearing white matter of patients with clinically definite MS, more marked in patients with secondary progressive disease, but not evident at presentation with a clinically isolated episode suggestive of demyelination (i.e. optic neuritis) [Davie et al., 1997; Fu et al., 1998; Leary et al., 1999; Brex et al., 1999].

MRI studies have therefore suggested that little if any diffuse axonal damage has occurred at disease onset, but that damage gradually accumulates within lesions and normal appearing white matter over time, being most marked in patients experiencing disease progression. The consistent findings of structural abnormalities and reduced NAA in normal-appearing white matter could be explained by the presence of microscopic lesions (as observed in pathological studies), or may imply Wallerian degeneration (secondary to the axonal loss that occurs within lesions). MRI findings to date are consistent with the hypothesis that axonal damage and loss plays a central role in the development of permanent disability in MS.

1.4.6 Treatment - disease modification

Early therapies for MS did not distinguish between the aim of removing a “causative agent” and modification of its pathological consequences in order to slow the disease process. Attempts at treating the disease included the use of leeches, arsenicals, silver, iodides, fever therapy, and electroconvulsive therapy [McDonald, 1983]. Anticoagulation was tried but based upon erroneous pathological descriptions of venous thrombosis within plaques [McDonald, 1983].

The introduction of theories relating to MS having an autoimmune pathogenesis, and the similarities observed between this condition and an animal model of demyelination, experimental allergic encephalomyelitis (EAE) [Lassman, 1983], led to the introduction of therapies aimed at modifying the immune response that had already proved successful in EAE. Three main approaches have been used: immunosuppression, induction of tolerance, and immune modulation. The earliest approach was non-specific immunosuppression with agents such as azathioprine and cyclophosphamide, and later with cyclosporin and total body lymphoid
irradiation. At best, these agents resulted in a marginal effect. The first clear evidence of success came from a trial of adrenocorticotropic hormone (ACTH) in 1970 [Rose et al., 1970], but this was limited to the demonstration that relapse duration could be shortened. The publication of the trial of interferon beta 1-b in 1993 demonstrated for the first time an effect of treatment on the longer term course of disease [The IFNB Multiple Sclerosis Study Group, 1993; Paty, 1993].

**Targeted immunotherapies. Interferon beta.** IFN-β, an immunomodulatory agent, is in many countries the treatment of choice in relapsing-remitting MS [The IFNB Multiple Sclerosis Study Group, 1993; Jacobs LD, et al., 1996; PRISMS Study Group, 1998]. In the United Kingdom, three preparations are approved in relapsing-remitting patients, and one is licensed for secondary progressive MS [European Study Group on Interferon beta-1b in secondary progressive MS, 1998]. All preparations of Interferon beta appear to have similar effects on relapse rate, decreasing this by approximately 30%. The rate of severe relapse is reduced by about 50%. Magnetic resonance imaging studies have shown that these drugs are able to reduce the appearance of new lesions by approximately 80%. Effects on disease progression are less evident - two of three trials in secondary progressive MS have been negative. The long-term benefits of such therapy remain uncertain, as does the effect and significance of the development of neutralising antibodies that occur in 20-30% of patients within two years of commencing treatment.

**Glatiramer acetate.** Glatiramer acetate (formerly known as copolymer-1) is a mixture of random synthetic polypeptides. It was synthesised as an immunological mimic of MBP and was found to inhibit EAE, presumably by inducing immune tolerance with blocking of T-cell activation. Similar effects on relapse rate to interferon beta have been demonstrated in relapsing-remitting patients [Johnson et al., 1995]. Favourable effects on delaying disability have also been suggested [Johnson et al., 1998].

**Azathioprine.** Azathioprine, a purine analogue, depresses both cellular and humoral immunity. Recent meta-analyses of randomised, double-blind, placebo-controlled trials have supported (i) a relapse reduction with azathioprine that is comparable with interferon beta and glatiramer acetate [Yudkin et al., 1991], and (ii) a moderate effect on the progression of disability [Palace and Rothwell, 1997]. These data support the need for a prospective large trial of azathioprine in MS.

**Intravenous immunoglobulin.** Intravenous immunoglobulin has been successfully used
in a number of neuroimmunological disorders, including chronic inflammatory demyelinating polyneuropathy and myasthenia gravis. Its role in MS is not clear, although a preliminary trial of intravenous immunoglobulin has demonstrated favourable effects on relapse rate in relapsing-remitting MS [Fazekas et al., 1997]. A larger double-blind, placebo-controlled multicentre phase III study is underway.

**Cyclosporin A.** Cyclosporin is a potent immunosuppressant that inhibits activation of T-cells at several stages. Although this drug has been shown to have modest effects on disability in MS, the benefits appear to be outweighed by significant renal toxicity and hypertension [The Multiple Sclerosis Study Group, 1990].

**Non-targetted immunotherapies. Methotrexate.** Methotrexate inhibits dihydrofolate reductase. At low dose, it is relatively non-toxic. Its benefits in inflammatory rheumatological disorders (e.g. rheumatoid arthritis, psoriasis) have been thought to result from inhibition of cellular and humoral immunity. In addition it has anti-inflammatory properties. Low dose oral methotrexate (7.5 mg/week) has been shown to reduce progression of upper limb impairment in a small study of 60 patients with MS [Goodkin et al., 1995]. No effects were seen on more conventional clinical measures of ambulation and disability, and perhaps more importantly in a study of this size, the drug did not affect MRI.

**Cyclophosphamide.** Cyclophosphamide is an alkylating agent with potent cytotoxic and immunosuppressive effects. High dose cyclophosphamide with or without booster injections has not met with much success in studies of patients with progressive MS and its use in clinical practice is limited by an unfavourable side-effect profile [Rudick et al., 1997a].

**Potential future therapies.** There are many potential new therapies and it is not clear how best to screen and evaluate them. Effectiveness in EAE has been considered an essential prerequisite, although many such therapies have not been helpful in MS. Serial gadolinium contrast-enhanced MRI has been recommended as a screening tool [Miller et al., 1996].

A number of putative therapies are currently in different stages of testing and include drugs with a variety of different potential mechanisms for altering the pathogenesis of the disease. Examples of such agents include Campath-1H (a potent general immunosuppressant), Antegren (anti-VLA4 antibody, to decrease migration of lymphocytes into the CNS and interfere with T-cell activation), T-cell vaccination, oral myelin and myelin peptides conjugated to major
MHC molecules (to inhibit myelin reactive T-cells), and Thalidomide (to act as a TNFα antagonist).

A number of therapeutic strategies are also being considered that might help augment the repair processes of MS following damage. These are in a much earlier stage of development and include remyelination strategies, glial cell transplantation, and treatment with recombinant growth factors.

Finally, established drugs used in the treatment of clinically definite MS have been tested in patients with clinically isolated syndromes to determine their potential ability to delay the onset of MS [Jacobs et al., 2000].

1.4.7 Treatment - symptomatic strategies and rehabilitation.

With current therapies that only exert a modest effect on the disease process in MS, it is important to maximise the symptomatic and functional benefits with alternative strategies. Some of these are listed in Table 1.10. A multidisciplinary approach is helpful and, in certain circumstances, a period of intensive neurorehabilitation may improve disability, handicap, emotional well-being and health-related quality of life, independent of neurological impairment, upon which there is no effect [Freeman et al., 1999].
Therapy

<table>
<thead>
<tr>
<th>Condition</th>
<th>Therapeutic Strategies</th>
</tr>
</thead>
<tbody>
<tr>
<td>Acute relapse</td>
<td>high-dose intravenous or oral steroids</td>
</tr>
<tr>
<td>Spasticity</td>
<td>therapy input</td>
</tr>
<tr>
<td></td>
<td>removal of trigger source</td>
</tr>
<tr>
<td></td>
<td>- urinary tract infection, pressure sores, constipation</td>
</tr>
<tr>
<td></td>
<td>drugs - baclofen (oral/intrathecal), tizanidine, dantrolene,</td>
</tr>
<tr>
<td></td>
<td>diazepam</td>
</tr>
<tr>
<td></td>
<td>peripheral nerve blocks (botulinum toxin, phenol)</td>
</tr>
<tr>
<td>Fatigue</td>
<td>drugs - amantadine, aminopyridine, pemoline, calcium</td>
</tr>
<tr>
<td></td>
<td>antagonists</td>
</tr>
<tr>
<td></td>
<td>practical management advice</td>
</tr>
<tr>
<td>Thermal sensitivity</td>
<td>drugs - aminopyridine</td>
</tr>
<tr>
<td></td>
<td>cooling suits</td>
</tr>
<tr>
<td>Bladder disturbance</td>
<td>drugs - oxybutinin, desmopresin, intravesical capsaicin</td>
</tr>
<tr>
<td></td>
<td>local measures (suprapubic vibrator)</td>
</tr>
<tr>
<td></td>
<td>intermittent clean self catheterisation</td>
</tr>
<tr>
<td></td>
<td>effective management of constipation</td>
</tr>
<tr>
<td>Sexual dysfunction</td>
<td>sex therapy and counselling</td>
</tr>
<tr>
<td></td>
<td>attention to other factors (e.g. incontinence, spasticity)</td>
</tr>
<tr>
<td></td>
<td>drugs - sildenafil, papaverine, yohimbine (in men)</td>
</tr>
<tr>
<td></td>
<td>- lubricants (in women)</td>
</tr>
<tr>
<td>Mood disorders and emotional lability</td>
<td>antidepressants</td>
</tr>
<tr>
<td></td>
<td>counselling</td>
</tr>
<tr>
<td>Paroxysmal sensory or motor symptoms</td>
<td>drugs - carbamazepine, phenytoin, baclofen, bromocriptine</td>
</tr>
<tr>
<td>Chronic dysaesthetic pain</td>
<td>drugs - carbamazepine, clonazepam, tricyclic antidepressants,</td>
</tr>
<tr>
<td></td>
<td>misoprostol</td>
</tr>
<tr>
<td></td>
<td>topical capsaicin</td>
</tr>
<tr>
<td>Ataxia / tremor</td>
<td>therapy input</td>
</tr>
<tr>
<td></td>
<td>- posture, computer-controlled mechanical damping</td>
</tr>
<tr>
<td></td>
<td>drugs - isoniazid, clonazepam, primidone, propranolol,</td>
</tr>
<tr>
<td></td>
<td>gabapentin, carbamazepine</td>
</tr>
<tr>
<td></td>
<td>surgery (stereotactic thalamotomy / thalamic stimulation)</td>
</tr>
<tr>
<td>Oscillopsia</td>
<td>drugs - baclofen, valproate, carbamazepine, clonazepam,</td>
</tr>
<tr>
<td></td>
<td>gabapentin</td>
</tr>
<tr>
<td></td>
<td>botulinum toxin</td>
</tr>
<tr>
<td>Bulbar symptoms</td>
<td>surgery (percutaneous gastrostomy, tracheostomy)</td>
</tr>
<tr>
<td></td>
<td>speech therapy</td>
</tr>
</tbody>
</table>

Table 1.10 Therapeutic strategies to alleviate symptoms and optimise function.
1.5 MRI to monitor putative therapies in multiple sclerosis

Sensitive and reproducible clinical measures of neurological impairment and disability have been difficult to establish. In part, this is due to the wide degree of variation in disease expression between patients and the tendency for MS to follow an unpredictable and fluctuating disease course. It has become increasingly clear that a valid and sensitive surrogate measure of the disease process would be helpful to monitor the disease and evaluate the response to potential new treatments.

The essential requirements for a surrogate outcome measure are that (i) it is sensitive and changes more often than the current clinical outcome measures (e.g. change in EDSS), (ii) it is biologically related to the primary clinical outcome, and (iii) an effect of treatment on this measure must predict an effect on the primary outcome. MRI represents the most plausible surrogate outcome measure at this time.

Guidelines have been formulated by a task force of the United States National MS Society for the use of MRI in monitoring therapeutic trials in MS [Miller et al., 1996]. For the purpose of phase II exploratory treatment trials, they recommend that MRI measures alone may provide a suitable primary outcome measure. The chief purpose of phase II studies is to demonstrate an impression of efficacy or lack of efficacy to determine whether phase III studies are warranted. Phase III definitive treatment trials determine the clinical effect of a drug and it is important that the primary outcome (at least for the time being) be a relevant clinical measure (e.g. progression in disability, relapse rate).

Recommended protocols for phase II exploratory treatment trials involve the use of both T2-weighted and gadolinium contrast-enhanced T1-weighted MRI at monthly intervals for periods of 6-12 months, using either a parallel groups or cross-over study design. Parallel groups studies require slightly larger sample sizes because of greater inter- than intra-patient variability. However, they are likely to provide more robust assessment of therapeutic efficacy. It is recommended that MRI also be used in phase III studies, in this case to provide a secondary outcome measure. This is because MRI is able to give direct objective evidence of treatment effect on the pathological process of the disease. T2-weighted imaging has been recommended at 6-12 month intervals, with determination of change in lesion load as the most appropriate outcome. Frequent gadolinium contrast-enhanced MRI in a subgroup over a period of at least 2 years is also helpful, providing additional information relating to the effect of treatment on new or reactivated inflammatory lesions.
Newer MRI techniques (e.g. atrophy, T1 hypointense lesions, MTR) may have a future role as outcome measures as they are able to obtain information with greater pathological specificity. In addition, such techniques need to provide reproducible measures, and preferably should be easy to implement or standardise across different evaluating centres. Methods to simplify data acquisition and analysis are of particular importance. Such issues are central to the work detailed in this thesis.
CHAPTER 2
Common Methodology

2.1 Introduction
This chapter details methodology common to experiments described later in this thesis. It discusses the nuclear magnetic resonance (NMR) properties of hydrogen nuclei and how these may be exploited by magnetic resonance imaging (MRI) to provide both qualitative and quantitative information relating to the various properties of tissue. In particular, the concepts of proton T1 and T2 relaxation theory are explored. A more detailed discussion of the mechanisms of magnetization transfer and contrast-enhanced imaging follows. Finally, a discussion of the principles and difficulties associated with measurement, both clinical and technological, is presented.

2.2 Basic Principles of Nuclear Magnetic Resonance
2.2.1 Introduction
The first successful demonstrations of nuclear magnetic resonance (NMR) in bulk matter were carried out in 1946 by two independent groups, led by Bloch and Purcell; both noted that a pure substance could be magnetically energised and the atoms stimulated by a radiofrequency wave allowing a characteristic signal to be recorded [Roemer, 1995]. Initial applications of NMR were confined to the laboratory, where resonant properties were found for many different materials; narrow resonances were noted for liquids and gases whereas in solids these were observed to be broader. Early on, NMR was detected using continuous wave radiofrequency radiation. In the 1950's the discovery of spin echoes by Hahn stimulated the widespread used of pulsed NMR for purposes of physical chemistry and analytical spectroscopy [Roemer, 1995]. It was not until the 1970's that work began on the first clinical body NMR system, when Damadian and co-workers were inspired by the observation that rat tumour cells showed different relaxation properties from those seen in health [Roemer, 1995]. The first in vitro NMR images were obtained in 1973 by Lauterbur who devised a system of superimposed orthogonal gradients to allow frequency dispersion; in 1976, the first human in vivo image was reported by Mansfield and Maudsley [Roemer, 1995]. In the early 1980's, satisfactory images in humans were achieved
with the introduction of large-bore magnets with homogenous magnetic fields and efficient methods of spatial localisation.

The NMR experiment relies upon detection of atomic nuclei that have certain properties, namely that they are mobile and contain an odd number of protons. These are selectively excited by a short radiofrequency pulse that disturbs their energy state and orientation in an external magnetic field. Their subsequent relaxation results in the induction of an electrical current that allows information to be collected that can be used in the construction of either an image or NMR spectrum depending on the experiment performed.

2.2.2 Quantum mechanical description of magnetic resonance

Many atomic nuclei exhibit the property of spin. For any particular nucleus, the spin is characterised by the spin quantum number (I); this may be either an integer or half-integer. The nuclei most commonly studied in the NMR experiment are those with an odd mass number (i.e. an odd number of protons), where \( I = \frac{1}{2} \). This is the case for hydrogen (\(^1\)H), the most abundant naturally occurring nucleus within the human body (others also studied include \(^{31}\)P, \(^{13}\)C, \(^{23}\)Na, and \(^{19}\)F). Nuclei with an even number of protons and neutrons do not exhibit spin, whereas those where the spin \( I \) is one or greater, exhibit more complicated patterns of NMR behaviour. For the purposes of this overview, information relating predominantly to hydrogen (\(^1\)H) nuclei is presented.

As a consequence of spin, such nuclei possess a magnetic moment along the axis of spin. The inherent spin angular momentum (\( P \)) is given by the following formula:

\[
P = hI, \quad \ldots 2.1
\]

where \( h = \text{Planck's constant divided by } 2\pi. \)

The possession of both spin and charge results in a nucleus having a magnetic moment (\( \mu \)) associated with it. The magnetic moment (\( \mu \)) is related to the spin angular momentum as follows:

\[
\mu = \gamma P, \quad \ldots 2.2
\]

where \( \gamma \) is the gyromagnetic ratio and is constant for a particular nucleus (e.g. \( 26.7 \times 10^7 \text{ rad s}^{-1} \text{T}^{-1} \) for hydrogen). The hydrogen proton has the largest magnetic moment of any stable nucleus;
this combined with its natural abundance in human tissue make it particularly suitable for NMR study. When the hydrogen nucleus is placed in an external magnetic field ($B_0$), the spin and consequent magnetic moment will allow it to act like a bar magnet and align itself along the direction of the field. In the quantum mechanical model, a nuclear magnetic moment may take up a number ($2I + 1$) of orientations with respect to the applied external magnetic field. For the hydrogen nucleus, this equates to two Zeeman energy levels ($E_m$). These levels are influenced by the strength of the magnetic field as shown:

$$E_m = -m\gamma B_0,$$

...2.3

where $m$ is a quantum number that assumes the values $-I, (-I + 1) \ldots \ldots (I - 1), +I$. For hydrogen, where $I = \frac{1}{2}$, $m = -\frac{1}{2}$ or $\frac{1}{2}$. These 2 energy levels are separated by an amount of energy, $\Delta E$ [Figure 2.1].

![Energy level diagram for the hydrogen nucleus where $I = \frac{1}{2}$.](image)

When $m = +\frac{1}{2}$, the hydrogen magnetic moment lies parallel to the external magnetic field and the system is in a low energy state. When $m = -\frac{1}{2}$, the system is in a high energy state and the magnetic moment lies antiparallel. Normally when there are a large number of nuclei in a sample, spins will distribute themselves randomly between the high and low energy states. For a collection of hydrogen nuclei, there will be a bias towards a low energy state, with more protons aligned parallel to the external magnetic field. This bias results from thermal motion that can induce transitions from the low to the high energy state. The ratio of the number of spins in the lower ($N_L$) to upper ($N_U$) energy state is dependent on the absolute temperature ($T$) of the sample in Kelvin and the energy difference between the two states ($\Delta E$); this can be calculated from Boltzmann’s equation:
\[ \frac{N_L}{N_U} = \exp \left( \frac{\Delta E}{kT} \right), \]  \[ \text{...2.4} \]

where \( k \) is the Boltzmann constant. If the total number of spins in a sample is known, the excess aligned parallel to the external magnetic field can be calculated to give the net magnetization of the sample (e.g. at 1 Tesla, the effective fraction of spins aligned parallel is only 1 in 280,000). This net magnetization (\( M_o \)) is proportional to the applied field, with higher field strengths giving greater sensitivity.

Transitions between the two energy levels can be induced by a magnetic field of energy equivalent to \( \Delta E \) that oscillates at the Larmor precession frequency, \( \omega_L \):

\[ \Delta E = h \omega_L \]  \[ \text{...2.5} \]

The resonant frequency at which transitions can occur can be calculated by the Larmor equation; this can be derived from the expression for \( \Delta E \) given in figure 2.1 and equation 2.5:

\[ \omega_L = \gamma B_0 \]  \[ \text{...2.6} \]

After the oscillating magnetic field is switched off, the temporary excess of spins at a higher level will return to their lower energy states, emitting energy of \( \Delta E \) at the precession frequency, \( \omega_L \). This emission of energy will return the spin system to its former equilibrium state and this forms the basis of the signal detected in the NMR experiment.

Whilst the quantum model should strictly be used to investigate all NMR phenomena, the classical model can be used to describe a macroscopic spin system. For spins where \( I = \frac{1}{2} \), the quantum and classical models agree; the latter however allows clearer visualisation of the perturbation of spin magnetic moments during the NMR experiment and is now discussed.

2.2.3 Classical description of magnetic resonance

Classically, when a spin is placed within an external magnetic field (\( B_0 \)), the magnetic moment associated with that spin will experience a torque (\( L \)), where:

\[ L = \mu \times B_0 \]  \[ \text{...2.7} \]
The effect of the torque is to align the magnetic moment with the external field and cause the spin to move at right angles to its own direction; this results in a motion known as precession [Figure 2.2].

![Figure 2.2 Precession of a spin in a magnetic field.](image)

The equation of motion for the spin is found by equating the torque \( L \) with the rate of change of angular momentum \( P \):

\[
L = \frac{dP}{dt}
\]  

...2.8

By combining equations 2.7 and 2.8, and given equation 2.2, it can be shown that the rate of precession of the magnetic moment around the external magnetic field \( B_e \) is given by:

\[
\frac{d\mu}{dt} = \mu \times (\gamma B_e)
\]  

...2.9

In this equation of motion for a spin in a magnetic field \( B_e \), it can be seen that the rate of precession is given by the Larmor frequency \( \omega_e \).

A sample containing many nuclei can now be considered. The net magnetic moment (or
net magnetization, \( M \) is the vector sum of all individual magnetic moments (\( \mu \)):

\[
M = \mu \quad \text{(2.10)}
\]

At equilibrium, the net magnetic moment will be aligned with the external magnetic field (\( B_o \)). If this is assigned to be the \( z \) direction, then there will be no component of \( M \) in the xy plane (i.e. no phase coherence). This is represented in Figure 2.3.

![Diagram](image)

**Figure 2.3** Diagrammatic representation of the net magnetization \( (M) \) of individual magnetic moments at equilibrium.

The net magnetic moment \( (M) \) can precess around \( B_o \) in the same way as a single spin, thereby producing an observable rotating magnetic field at the Larmor frequency. In the normal equilibrium state \( (M_o) \), where \( M \) is aligned with \( B_o \), the net magnetic moment will not precess. In the NMR experiment, an additional magnetic field is applied to rotate the net magnetic moment (or net magnetization) into the xy plane. This additional field \( (B_r) \) is orientated perpendicular to \( B_o \) (i.e. in the xy plane) and oscillates at the Larmor frequency \( (\omega_r) \). This field is often referred to as the radiofrequency (RF) pulse. Because the rotating xy component of \( M \) now gives rise to an oscillating magnetic field, it will now have the ability to induce a current in
a suitably placed coil via electromagnetic induction. This is the basis of NMR signal measurement.

It is easier to visualise any perturbation of a spin system if it is depicted in the rotating frame of reference, defined by axes \( x, y, z \). If the frame is chosen to oscillate at the same angular frequency as the RF field \( B_1 \), then \( B_1 \) will appear stationary in the frame. Since the phase between \( B_1 \) and the frame is arbitrary, the frame may be chosen so that \( B_1 \) is directed along the \( x \) axis [Figure 2.4].

![Figure 2.4 The rotating frame.](image)

In practice, the \( B_1 \) field is applied using a RF coil which surrounds the sample under observation. The angle \( (\alpha) \) through which the net magnetization is rotated by this field is determined by the duration \((t_p)\) and magnitude of the field:

\[
\alpha = \gamma t_p B_1 \quad \text{...2.11}
\]

Two common angles of rotation used in NMR are 90° and 180°. These result in the net magnetization being tipped into the \( xy \) plane (along the positive \( y \) axis, with no \( z \) component) and along the negative \( z \) axis (antiparallel with \( B_0 \)) respectively.
2.2.4 Factors affecting the NMR signal: Spin-lattice and spin-spin relaxation

Having applied an RF pulse to the sample and perturbed the net magnetic moment, the magnetization vector \( \mathbf{M} \), will revert to its equilibrium state \( \mathbf{M}'' \), aligned along \( \mathbf{B}_0 \). There are two distinct relaxation processes occurring in the spin system and each are characterised by their own time constant.

**Spin-lattice relaxation, with time constant \( T_1 \).** This describes the restoration of the \( z \) component of the magnetization \( \mathbf{M}_z \) and is caused by an exchange of energy between the spin states of the proton and the surrounding molecular environment, often referred to as the lattice [figure 2.5].

![Figure 2.5](image)

*Figure 2.5* Diagram showing return of longitudinal magnetization in the rotating frame of reference (due to spin-lattice relaxation).

The characteristic time for relaxation of the longitudinal magnetization is given by the time constant, \( T_1 \) [Figure 2.6]. Longitudinal (T1) relaxation will occur after a 90° pulse tips the net magnetization into the transverse plane; with the application of a second 90° pulse, tissues with different T1 relaxation properties will show differences in resultant longitudinal magnetization that has been restored between the pulses. The time between pulses (the repetition time, TR) can be exploited to allow different tissues to exhibit different signal intensity, resulting in a mechanism for generating image contrast that is dependent on T1 relaxation properties (i.e. a T1-
weighted sequence). Where the TR is short, tissues with different T1 relaxation properties will give different signal. If the TR is lengthened, both tissues will have relaxed more fully and to a similar extent between RF pulses; the contrast mechanism will therefore disappear and both tissues will be difficult to distinguish on the basis of T1 relaxation.

Figure 2.6  Spin-lattice relaxation: exponential decay of longitudinal magnetization back to its equilibrium value, with time constant T1. Two tissue types with different relaxation properties are shown, showing the potential for generating “T1-weighted” image contrast.

*Spin-spin relaxation, with time constant T2*. Here the spin system loses no energy as a whole, but spins exchange energy amongst themselves. In the rotating frame, the net magnetization is first tipped into the transverse plane by a 90° pulse. Groups of magnetization vectors, known as isochromats, may be considered. Each of these experience different local fields after the pulse is switched off. The change in local field may result either from fluctuating changes within the tissue (i.e. a result of thermal motion of neighbouring electrons and nuclei) or from local static field inhomogeneities (e.g. susceptibility variations within the sample or in the laboratory magnet). The isochromats will rotate at different frequencies and the overall effect is a loss of phase coherence in the precessional motion of the spins; such dephasing results in a
loss of transverse magnetization [Figure 2.7].

![Diagram showing decay of transverse magnetization due to loss of phase coherence between the spins in the rotating frame of reference (due to spin-spin relaxation).](image)

**Figure 2.7** Diagram showing decay of transverse magnetization due to loss of phase coherence between the spins in the rotating frame of reference (due to *spin-spin* relaxation).

The characteristic time for relaxation of the transverse magnetization is given by the time constant, T2 [Figure 2.8]. The observed T2 relaxation ($T2^*$) is a combination of tissue "real" T2 effects and T2 inhomogeneity effects ($T2_i$):

$$
\frac{1}{T2^*} = \frac{1}{T2} + \frac{1}{T2_i}
$$  \[2.12\]

It is difficult to pinpoint the exact time at which relaxation is complete. In practice, T1
is defined as the time taken for 63% of the longitudinal magnetization to recover and T2 as the
time when the initial transverse magnetization has decreased by 37%. In biological tissues, T1
values are typically 300-2000 milliseconds (ms) and T2 values 30-150ms.

![Figure 2.8](image)

**Figure 2.8** Spin-spin relaxation: exponential decay of transverse magnetization back
to zero, with time constant T2.

2.2.5 *Free induction decay and signal detection*

So far, the phenomenon of NMR and the processes of relaxation have been described. An
account of how a simple NMR experiment may be performed to allow detection of signal now
follows. In this experiment, a sample is placed in a uniform static magnetic field, $B_o$ and
subjected to a $90^\circ$ RF pulse. This tips the net magnetic moment into the transverse $(xy)$ plane.
The pulse is switched off, allowing both spin-lattice and spin-spin relaxation to take place.
Changes in magnetization perpendicular to the static main field will induce an electric current
in a receiver coil placed nearby in the transverse plane, according to Faraday's law of
electromagnetic induction. During this post excitation period, the spins remain in a static field
and precess at the Larmor frequency. The current induced in the coil will be of the same
frequency. Due to spin-spin relaxation, the transverse magnetization and induced current both
decay with an exponential time course. An oscillating sine wave which decays to zero with a
characteristic time constant (T2) is obtained. This is known as the free induction decay [Figure

66
2.9]. At time zero, before the signal decays, the amplitude is proportional to the transverse magnetization and is also a measure of the number of excited nuclei present within the volume of interest. This is referred to as the proton density and, together with T1 and T2, forms one of the three fundamental NMR parameters.

![Free induction decay](image)

*Figure 2.9   Free induction decay*

2.2.6 Spin echoes

The spin echo sequence originally devised by Hahn [Hahn, 1950] is one upon which many modern NMR sequences are based. After applying a 90° pulse to tip the net magnetization into the transverse plane, various processes result [Figure 2.10]. Initially, the individual magnetic moments are aligned along the y axis. As a result of spin-spin relaxation, they start to dephase. A second pulse with a tip angle of 180° is now applied which has the effect of rotating the magnetization so that it points along the y-axis. The individual magnetic moments travel towards each other and rephase, re-establishing transverse magnetization. This refocused signal is known as an echo, hence the name "spin-echo". Once again, spin-spin relaxation will result in dephasing of these individual magnetic moments.

If the sequence is modified by repeating it a number of times, the time between the start of the sequence and the beginning of the next is called the repetition time (TR). The time between the 90° pulse and the initial dephasing of spins is known as the dephasing time. The time between
subsequent echoes is twice the duration of the dephasing time (i.e. time taken to rephase and dephase) and this is known as the echo time (TE). Most imaging sequences in clinical practice involve such repetition, and these parameters may therefore also be used to describe the timing of such sequences.

Figure 2.10  The spin echo.

The spin echo sequence may be modified by the application of further 180° pulses; this generates more echoes and allows calculation of T2. The sequence may be represented as:

\[ 90° - \text{TE/2} - 180° - \text{TE/2} - \text{echo} - \text{TE/2} - 180° - \text{TE/2} - \text{echo} - \text{TE/2} - 180° - \ldots \text{etc.} \]

Alternate echoes are opposite in phase (i.e. directed along -y or y), at a time TE apart. If there are
imperfections in the 180° pulse, these will be magnified and result in inaccurate calculation of T2. To avoid such problems, the sequence may be modified as in the Carr-Purcell-Meiboom-Gill (CPMG) sequence. This modification involves changing the direction of the 180° pulse, so that it is applied along the y direction, allowing the net magnetization to converge along y at every echo. This sequence is more tolerant of imperfections in the 180° pulse produced by B1 inhomogeneity. The intensity of the refocused signal will decay over time as a result of a change in the precession frequency of individual magnetic moments during the dephasing and refocusing of spins.

For imaging purposes, the manipulation of the time between each echo (TE) will allow contrast between tissues with different T2 relaxation curves [Figure 2.11]. It is apparent that the highest signal occurs with the shortest echo times. However, the two different tissues will show similar signal intensity. At longer echo times the differences become greater, resulting in variations in image contrast dependent on the T2 relaxation properties.

![Potential for image contrast](image)

*Figure 2.11* Generation of T2 contrast with T2-weighted sequences. Two tissue types with different T2 curves are shown, showing the potential for generating "T2-weighted" image contrast.

Once a spin system has been perturbed, T1 and T2 relaxation occur simultaneously although they remain independent of each other. With spin echo sequences, it is therefore
possible to determine how signal differences due to the characteristics of tissue (e.g. T1, T2 and proton density) may be affected by the TE and TR sequence parameters. It has already been mentioned how these might help provide contrast in T1-weighted and T2-weighted sequences; a sequence with a long TR and long TE will be predominantly “T2-weighted” and one with a shorter TR and short TE will be predominantly “T1-weighted”. However, if a long TR is chosen, T1 effects are minimised and if a short TE is used, then the signal obtained will be relatively independent of tissue T2 characteristics. The amplitude will now depend predominantly on the spin density of the tissue, i.e. “proton-density weighted”.

2.2.7 Relaxation mechanisms

For the purposes of clinical imaging, NMR is utilised to provide images where tissues with individual biophysical properties may be distinguished from each other by variations in image signal intensity. The generation of image contrast relies to a certain extent upon the type of imaging sequence and the equipment used. Most importantly however it depends on the inherent properties of the tissue under investigation and their influence on proton relaxation. The mechanisms for proton relaxation are now discussed.

Dipole-dipole interactions. The main processes responsible for the T1 and T2 relaxation characteristics of water protons within tissue are known as dipole-dipole interactions. These cause the spin magnetic moments associated with hydrogen nuclei to experience both static and fluctuating magnetic fields arising from the presence of neighbouring spin magnetic moments (on either the same or different nuclei). The ability of such nuclear magnetic dipoles to induce significant relaxation upon each other is largely a result of their close proximity. This can cause magnetic field perturbations large enough to result in significant dephasing of phase-coherent neighbouring protons. A simplified account of the source of such fluctuating magnetic fields follows.

An individual hydrogen proton magnetic moment will result in a local magnetic field [Figure 2.12]. The net field, when placed in an external magnetic field \( B_0 \), will be greater in positions 2 and smaller in positions 1. Water molecules contain 2 hydrogen protons in close proximity; the effects of a neighbouring magnetic moment can now be considered. The water molecule can assume different configurations within the external magnetic field \( B_0 \). Two such configurations are shown [Figure 2.13].
Figure 2.12  Diagram showing a hydrogen proton magnetic moment and both its own associated magnetic field lines and those of the external magnetic field, $B_0$. 
Figure 2.13 Diagram illustrating how molecular rotation within the external magnetic field (\(B_0\)) can result in the hydrogen proton, \(H_a\), experiencing different local magnetic fields as a result of its neighbouring hydrogen proton, \(H_b\).

For the purposes of simplifying this description, the local magnetic field lines associated with the magnetic moment of \(H_b\) are not shown. The magnetic moment associated with the hydrogen proton, \(H_b\), will experience a smaller local magnetic field in the position shown in figure A than that in figure B. As the water molecule rotates, the magnetic field experienced by the hydrogen proton, \(H_a\), will therefore fluctuate as a result of the magnetic field associated with the hydrogen proton, \(H_b\). Fluctuations occurring at either the Larmor frequency or twice this value will encourage the processes of T1 and T2 relaxation. A measure known as the correlation time \(\tau_c\) is used to describe proton motion and interaction with other protons. This is a measure of the time a proton moves in a given direction before colliding and changing direction; as such it may characterise a material.
There is also a contribution to T2 relaxation from static local magnetic fields. If the position of the water molecule is now assumed to be fixed in the above model, the hydrogen proton, $H_b$, will experience a local static magnetic field due to the presence of its neighbouring proton, $H_a$. Such field differences will result in different precessional frequencies for each of the protons. As mentioned in 2.2.4, T2 relaxation results from a loss of phase coherence, whereby a collection of spins start to precess at varying frequencies with respect to each other. In this way, static field variations within a sample may also influence T2 relaxation.

In addition to dipole-dipole interactions, other interaction processes may contribute to T1 and T2 relaxation. These are briefly described:

**Scalar coupling.** This describes the interaction between 2 nuclear spins on the same molecule by means of distortions in their electron clouds. This interaction has negligible effect when compared with the dipole-dipole interactions already described.

**Chemical exchange.** This occurs when atoms such as hydrogen are physically transferred between molecules. This can also occur between protons and macromolecules. This interaction is thought to exhibit only a minor contribution to relaxation processes.

**Chemical shift anisotropy.** The gyromagnetic ratio, $\gamma$, is not only different for different nuclei; for any one particular type of nucleus, $\gamma$ is modified to a small extent by the environment of that nucleus. The electron cloud surrounding a nucleus may slightly shield against the applied field, reducing its effect and thereby reducing the Larmor resonant frequency. Protons with different degrees of shielding will exhibit different resonant frequencies and these are known as chemical shifts. As the chemical shielding experienced by a nucleus relies upon its orientation with respect to the external magnetic field, the interaction mechanism is referred to as chemical shift anisotropy. Chemical shifts (usually expressed in parts per million or ppm) can be utilised to differentiate different molecules and form the basis of NMR spectroscopy. However, in the context of conventional magnetic resonance imaging, the chemical shifts are small and are not a dominant relaxation mechanism.

**Quadrupole coupling.** This is a mechanism by which nuclei with spin number equivalent to 1 or more interact with electric fields of other nuclei. This therefore does not affect hydrogen protons, where the spin number is $\frac{1}{2}$.
2.2.8 Tissue NMR relaxation properties

Most tissues in the body consist of $75 \pm 15\%$ water; the proton NMR signal from tissues is primarily due to protons located on small, mobile molecules (e.g. water and certain lipids). In general, water contributes most to the NMR signal from the majority of body tissues. This water is not entirely a free liquid but, to some extent, is affected by the surrounding tissues which constrain the motion of some of the water molecules. This results in temporary binding of a layer or few layers of water molecules over the surface of a macromolecular structure. In principle, NMR signals from such protons within larger macromolecular structures (e.g. protein, polysaccharide or other types of lipid) should also be readily observable. However, the NMR properties of these protons resemble those of solids and their very short T2 relaxation times (typically less than 1 millisecond) make them practically "invisible" as their signal decays before the imaging observations. Most of the directly observable signal seen with NMR comes therefore from the protons of water and certain lipids.

The exact mechanisms responsible for NMR relaxation in tissues are not fully understood. It is clear that intrinsic properties of tissues have important effects on relaxation, and a brief discussion relating to these is presented.

Water structuring. As mentioned above, proton dipole-dipole interactions determine to a large extent the relaxation properties for a given tissue. Such interactions may be expressed in terms of their correlation time (a measure of proton mobility). In tissues, an interaction exists between water molecules and chemical groups at water:macromolecule interfaces; this interaction results from the presence of hydrogen bonds between water molecules and such chemical groups (or other water molecules). Structural groups of macromolecules may have hydrophilic or hydrophobic properties resulting in different structuring of water at such interfaces. For example, proteins may have both hydrophilic (e.g. ionic groups such as COO\(^-\)) and hydrophobic regions (e.g. CH\(_3\)). In regions where there is hydrogen bonding, the water molecules at the surface of the macromolecule (i.e. hydration or surface water) will have reduced mobility. The correlation times of water molecules in the hydration layer are longer than those of free water and vary according to the water structuring by the macromolecule (typically between $10^{-4}$ and $10^{-9}$ seconds). Movement of these water molecules will be slow and the precession frequency will be low. The ability for macromolecules to partially structure water in this manner decreases with increasing distance from their surface. Water protons closely associated with hydrophilic groups in this hydration layer may therefore be referred to as "tightly bound", as opposed to water molecules
further away that are “free” of such bonds. Water molecules a large way from molecular surfaces behave as bulk or “free” water and have shorter correlation times (of the order of $10^{12}$ seconds). Movement of these “free” water molecules will be fast and the precession frequency will be high. Water molecules of intermediate mobility will be found between these “bound” and “free” water protons.

Dipole-dipole interactions may occur between macromolecular protons and hydration water and these are discussed below. In general, these interactions are only present close to the interface between macromolecules and adjacent water.

Considering bulk water, molecules rotate in a rapid isotropic fashion (with a corresponding short correlation time). In general, the static field contribution to T2 interactions averages to zero and does not affect T2 relaxation mechanisms. This rapid motion of water molecules relative to the Larmor frequency results in inefficient NMR relaxation, resulting in long T1 and T2 relaxation times (approximately 3 seconds each).

Considering protons at macromolecular surfaces, these have restricted motion; this will enable a significant static field contribution, allowing efficient T2 relaxation (associated with dipole-dipole interactions). For these slow moving molecules, an absence of fluctuating magnetic fields at such a frequency (to encourage transitions between spin energy levels) will result in slow T1 relaxation processes. Overall, this will account for relatively shorter T2 than T1 relaxation times in tissue.

Water content. Early theories regarding the relaxation times of various biological tissues considered that these differed due to differences in water content. It is likely that the bound water fraction (i.e. the proportion of protons in the hydration layer of macromolecules) has an important effect on relaxation. However, the exact relationship between water and NMR relaxation time remains unclear. In general, tissue T1 relaxation times increase with increasing water content but are also affected by the specific macromolecules present within the sample.

Paramagnetic ions. The presence of transition metal ions (e.g. iron, copper, and manganese) or organic free radicals (e.g. paramagnetic molecular oxygen) may significantly affect NMR relaxation. These paramagnetic ions have unpaired electrons in one or more orbitals. When a pair of electrons exist in the same orbital, their opposed spins result in no net magnetic moment. An unpaired electron will have a relatively large magnetic moment (approximately 1000 times greater than a proton). This may produce a relatively large fluctuating magnetic field that is able to significantly affect the relaxation of neighbouring spin systems via dipole-dipole
interactions. In general, the ability for paramagnetic ions to shorten relaxation times is greater for T1 than T2. To reduce T1 values significantly, concentrations approaching the order of millimolar are required; the normal concentration of these substances in the body (e.g. Mn\(^{2+}\) as an enzyme cofactor in the pancreas and intestinal mucosa) is very low as such ions may be toxic in larger concentrations. In health, the effect of naturally occurring paramagnetic ions is therefore minimal. The effect of paramagnetic ions may be enhanced if attached to larger molecules. These properties form the basis for the use of gadolinium chelates as contrast agents in MRI; if these are present in tissue, the T1 shortening will result in an increased signal intensity on T1-weighted imaging. The particular properties of the chelate will have important effects on image contrast, the ability for the paramagnetic ion to reach certain body compartments and the overall safety of the contrast agent.

2.2.9 Obtaining spatial information with magnetic resonance imaging

To utilise NMR information in vivo and determine changes due to pathology, spatial localisation is required. This may be achieved by utilising the fact that the resonant frequency of protons within the tissue depends on the strength of the magnetic field. By varying the field throughout the tissue sample in a predictable way, the resonant frequencies of protons will also differ in a predictable manner. To achieve this, a second magnetic field is superimposed on the static external magnetic field; this additional field varies in strength across the sample and is therefore known as a gradient field and is produced by gradient coils. This gradient field allows the magnetic field strength to increase across a sample so that protons in different positions (e.g. within an individual slice) experience different magnetic fields and have different resultant precession frequencies. Because of this, the radiofrequency pulses must also have different frequencies. Sequences of differing radiofrequency and gradient pulses may be combined to produce a variety of MR sequences capable of producing spatial information with different image contrast properties.

The use of gradient fields results in a free induction signal with a spread of frequencies in which the amplitude of each frequency component is proportional to the amount of that nuclear species experiencing the corresponding magnetic field. In other words, information is represented in the form of a spectrum; in practice this is most easily obtained as the Fourier transform of the free induction signal [Figure 2.14].
Figure 2.14 A diagram illustrating how a superimposed field gradient may allow spatial information to be obtained in the form of a spectrum.

The mechanisms whereby spatial information may be sought from an individual slice have been briefly discussed. It is possible to select image slices of different thickness. In theory this can be achieved by varying the range of frequencies applied (i.e. the bandwidth of the radiofrequency pulse). In practice this is more easily achieved by keeping the bandwidth the same but varying the slope of the gradient field; the steeper the gradient field over a specific distance, the greater the variation in precession frequency and the narrower the resultant slice. This slice-selecting gradient is only switched on during the radiofrequency pulse. Having selected a slice and determined its thickness, it is next necessary to know where in the particular slice information has actually come from. This can be achieved in a similar manner to the slice-selecting gradient. After the radiofrequency pulse has been applied, another gradient field (the frequency encoding gradient) may be applied in an orthogonal direction to the first gradient pulse, so that precession varies across the slice. To obtain positional information across the slice in the perpendicular direction, a phase encoding gradient may next be used that causes protons in this direction across the slice to precess with different phases (i.e. before the gradient pulse, all protons are precessing in phase and when this is switched off they will experience the same field again and be out of phase but still have the same frequency). Finally, to obtain an idea as to the position and relaxation properties of spins at different individual points in a tissue sample,
it is necessary to use *Fourier transformation*; with this mathematical method, computers are able to analyse the mixture of signals from a particular slice by determining the intensity of those components of the free induction decay with different phase and frequency. This relies on initial acquisition of data in \( k \)-space (a spatial frequency domain where the raw MR signals are collected in the computer system prior to such image reconstruction). In an individual slice, it is now possible to allocate a signal intensity to each point of particular phase and frequency and therefore construct a 2-dimensional gray scale image representing a known slice thickness. For such images, the signal intensity will vary according to the transmit and receiver gains of the MR imaging system (i.e. the power of radiofrequency deposition and amplification of the received signal). The signal will be proportional to the density of protons unless modified to be \( T_1 \)- or \( T_2 \)-dependent (i.e. \( T_1 \)- or \( T_2 \)-weighted images). For individual subjects and the same subjects imaged on different occasions, the signal intensities will differ according to the experimental conditions and the chosen MR system settings. Two-dimensional images represent data in 3 dimensions and are typically composed of a grid of individual three-dimensional *voxels* of data (represented in the two-dimensional image by square *pixels*), of predetermined thickness (equal to that of the image slice) and uniform signal intensity (a typical *image matrix* would be 256 x 256 pixels). This image grid covers a *field of view* containing the sample under investigation (typically of the order of 24 x 24cm for brain imaging). In clinical practice, a typical slice thickness would be 3-5mm, often with a gap between slices to prevent interference (i.e. "cross-talk"). So, for a typical MR image one might expect an in plane resolution of the image of the order of 1mm x 1mm, with each pixel of data representing approximately 3-5mm\(^3\) of tissue.

For the purposes of clinical imaging, the time of data acquisition and the quality of the image obtained (as influenced by the signal-to-noise ratio) are particularly important. The time it takes to acquire data for a conventional two-dimensional Fourier transform MR image is dependent on the repetition time interval (TR), number of phase encodings and number of excitations (Nex):

\[
\text{Scan time} = TR \times \text{number of phase encodings} \times \text{Nex} \quad \ldots 2.13
\]
In general, longer times for data acquisition result in images with either improved signal-to-noise (e.g. as a result of more excitations) or greater image resolution (e.g. as a result of more phase encode steps). If the time of acquisition is too long however, degradation of image quality might result from involuntary patient motion during the scan. Individual pixels in the image matrix represent the average signal intensity from a designated tissue volume; depending on the size of this voxel, they may contain data relating to more than one tissue type (i.e. partial volume averaging effect). This is particularly important in multiple sclerosis, where lesions are often small and therefore difficult to sample accurately. To reduce partial volume and better delineate lesions and individual tissue types, the use of narrow slices is particularly helpful. Imaging time becomes a major trade-off when decreasing slice thickness. Signal-to-noise (SNR) decreases with respect to the amount of reduction and, to maintain this, a corresponding increase in averaging is required (i.e. halving slice thickness would require quadrupling of scan time). Contrast-to-noise is also important for the detection of small lesions. Where a lesion only fills part of the pixel, a good contrast-to-noise is required to ensure that the average signal from the pixel is greater than surrounding tissue in order to generate enough contrast for the lesion to be seen. Thus the smaller the lesion, the greater contrast-to-noise required. Furthermore, variability of volumetric measurements will occur with varying pixel size and contrast-to-noise. The SNR for a tissue is calculated by the mean signal intensity for a region divided by the standard deviation in the background (that represents only noise). As mentioned, this decreases for decreasing slice thickness; for similar reasons, it is also reduced with greater image resolution. In general, the use of higher field strengths allows greater SNR for the same image resolution. In clinical and research practice there are therefore certain inevitable trade-offs between data acquisition time, signal-to-noise, contrast-to-noise and image resolution and a compromise is required to achieve the most appropriate use of scanner and subject time.
2.2.10 MRI instrumentation:

Despite the many variations in imaging techniques, most MR systems share fundamental basic hardware:

i. The magnet,

ii. 3 sets of field gradient windings and their drivers,

iii. The RF transmit and receive coil(s) and associated electronics,

iv. Data acquisition and processing system, and

v. An image display.

*The magnet.* Two types of magnet are most commonly used, namely air-cooled resistive and superconducting. Other types include iron-assisted and permanent magnets. For all the studies in this thesis, a superconducting system with a field strength of 1.5 Tesla was used. This type of system relies on the properties of certain metals and alloys that exhibit no resistance at certain temperatures. This type of magnet allows a high field strength, good access for whole body or spinal imaging and typically has lower running costs than a resistive system.

*Gradient windings.* In most systems, 3 orthogonal field gradients are provided and may be of many different designs. For the purposes of this description, it is not necessary to discuss the various features of such gradient windings.

*The radiofrequency system.* The function of this part of the system is to apply the radiofrequency pulse and receive the weak free induction signal emanating from the patient. Both processes may be carried out by the same coil, as is typically the case with the body and head coils. All imaging systems contain a body coil for RF deposition which completely surrounds the part of the body to be examined. Receiver coils are usually modelled to the part of the body under examination (e.g. a helmet type of headcoil or a surface coil placed over the part of the body under investigation such as is used for imaging the spinal cord). Surface coils rely on the need for less RF deposition and therefore may safely produce images within the specific absorption rate (SAR) with good signal-to-noise. Caution should be applied if surface coils are used to transmit as well as receive signal, as this may result in difficulties associated with field inhomogeneity and uneven RF pulse deposition. For the purposes of quantitative imaging techniques, this limitation may be important. For high resolution spinal imaging, the use of phased array coils (where each coil is independent of the other coils) may be particularly helpful and, as such, was used for all spinal imaging studies described in this thesis. The lack of
interaction between coils results in maintenance of signal-to-noise with wide coverage, allowing whole spine examinations. For all brain imaging, a transmit and receive quadrature design of headcoil was used. This receives signal at both orthogonal axes to the main field ($x$ and $y$) and allows good reception of signal.

*Data processing and image display.* After amplification of the received signal, this is converted from analogue to digital form, so that it is suitable for further computer processing. This typically involves the use of a Fourier transform technique, as described. The resultant image data is transferred to a computer, where it may be displayed as a gray scale image using image display software.

2.2.11 Alternative pulse sequences for imaging

Various pulse sequences have been described that offer in some circumstances advantages over the conventional spin echo sequence, such as a faster speed of image acquisition or improved image contrast. The basic sequence of events in a conventional spin echo sequence is a $90^\circ$ RF pulse excitation, spatial encoding, a $180^\circ$ refocusing pulse, and signal readout. The speed of conventional spin echo image acquisition is limited by the TR used (sufficient time is required for longitudinal relaxation of the spins); as such this tends to make imaging relatively slow. In addition, the minimum TE achievable is dependent on the length of the $180^\circ$ pulse and associated gradient pulses.

*Gradient echo (GRE)* pulse sequences may provide images with contrast similar to those obtained using spin echo sequences but are generally faster. In addition, they may allow the use of shorter echo times. This is possible because gradient echo sequences use a smaller RF pulse (with a flip angle less than $90^\circ$, typically $10\text{-}35^\circ$) and, instead of using a $180^\circ$ pulse to refocus the spins, use gradients applied sequentially in opposite directions. The ability to rapidly switch between the polarity of the gradient pulses allows echo times as short as 4ms. Without a $180^\circ$ pulse, the signal decays faster and there is a greater dependence on $T2^*$ effects; the image contrast generated may therefore differ slightly from conventional spin echo sequences. Advantages of a gradient echo sequence (e.g. reduced imaging time, lower RF deposition as measured by the specific absorption rate (SAR), or increased coverage) may be offset by the additional burden on the system hardware caused by the intensive demand on the gradients and lower signal to noise.

*Fast spin echo (FSE)* techniques may also allow faster image acquisition than
conventional spin echo sequences. These allow multiple lines of data to be acquired for each repetition time. As with the conventional spin echo sequence, a $90^\circ$ RF pulse initiates the sequence. This is followed by multiple $180^\circ$ pulses to generate echoes (many more than used with conventional spin echo sequences). The number of echoes used is referred to by the echo train length (ETL). Multiple echoes are acquired with successive $180^\circ$ pulses, each of which are preceded by a separate phase encoding gradient. Each echo represents a different line in the same $k$-space file; image reconstruction will convert each echo into a separate image. By acquiring several lines of $k$-space (and therefore several images) for each repetition time, the scan time may be considerably shortened. These time-saving benefits may also be used to increase the TR without resulting in prohibitively long acquisition times. Because separate phase encoding gradients are applied prior to each echo, the echo time for all echoes in a single acquisition will differ. To describe the echo times of such a sequence, the term effective echo time ($TE_{eff}$) is used; this refers to the echo placed in the centre of $k$-space which contributes most to the resulting image's contrast. The resulting image will have contrast equivalent to a conventional spin echo using a TE equal to the effective TE, but more heavily T1-weighted and T2-weighted information will be mixed in due to the inclusion of shorter and longer echoes within the image. The beneficial time-saving effect of a longer ETL may however be offset by (1) reduced coverage (due to a smaller number of slices achievable for the same TR), (2) poorer signal-to-noise (due to the inclusion of later echoes with T2 decay effects into the map of $k$-space), and (3) image artefacts that result in blurring of the image (as a result of T2 decay effects which lead to successive echoes of weaker signal being mapped into $k$-space; steps occur between each echo that create an error when processed with the Fourier transform).

*Inversion recovery (IR)* sequences use a $180^\circ$ pre-pulse before the spin echo sequence, flipping the spins into a negative longitudinal direction, thereby providing a greater dynamic range in the relaxation process. This allows a greater degree of T1-weighting to be achieved. The time between the pre-pulse and the following sequence is called the inversion time (TI); manipulation of the TI may allow different effects to be achieved. Inversion recovery techniques may allow selective suppression of fat signals by use of a short TI, as in the short tau inversion recovery (STIR) sequence, or of water signal with a long TI, as with the fluid attenuated inversion recovery (FLAIR) sequence.

*Three-dimensional imaging* techniques acquire data from a contiguous volume of tissue rather than by using individual slices. The excitation pulse is not slice selective and at the end of
the acquisition the volume is divided into discrete locations by the slice select gradient. High resolution images may be achieved as slice thickness is determined by encoding and, unlike 2-dimensional sequences, is not dependent on the maximum gradient strength. With 3-dimensional techniques, it is possible to achieve a resolution of the order of 1 millimetre (slice excitation in 2-dimensional imaging reaches a practical limit at two to three times that thickness). If the sequence is set up to provide image pixels that are isotropic, the image may be reformatted following acquisition to allow any plane to be assessed. A particular advantage of 3-dimensional imaging techniques is that they provide data that is particularly amenable to methods that allow accurate anatomical co-registration. Registration techniques may be useful to accurately evaluate serial changes even when patient repositioning has not been perfect.

2.3 Magnetization transfer: principles and techniques

2.3.1 Introduction

Contrast in conventional MR sequences is generated by variations in the density and relaxation characteristics of individual protons within a particular tissue. In a homogenous sample, the relaxation of the observed magnetization is described by two monoexponentially decaying functions corresponding to the longitudinal and transverse magnetization (T1 and T2). Measurement of such relaxation times makes the implicit assumption that relaxation behaviour can be described in terms of a monoexponential decay. However, biological tissues are complex structures that contain a variety of macromolecular structures as well as free water protons. The structuring of water within such tissues will affect the proton mobility and T1 relaxation mechanisms, as discussed above (Section 2.2.8). Restricted motion of protons bound to the surfaces of such macromolecular structures may result in rapid T2 relaxation, too fast to allow their detection by conventional MR imaging methods. Whilst the restricted protons in this “bound” pool are not directly represented with conventional imaging techniques, they may interact with more mobile protons in the “free” water proton pool and thereby make a significant contribution to the observed dynamic behaviour of their relaxation. As a result, the observed relaxation behaviour is not expected to be monoexponential, or even a sum of exponentials, as might result from a mixture of independent non-exchanging samples. The complex interactions between mobile protons in the “free” water pool and restricted protons in the “bound” water pool form the basis for magnetization transfer (MT) imaging [Wolff and Balaban 1989].
In this section, the basic principles behind the MT effect will be discussed. Methods to study and exploit such effects will be described and the potential applications for clinical imaging outlined.

2.3.2 Basic principles of magnetization transfer

In biological tissues, cross relaxation may arise due to magnetic coupling between protons in the hydration water sheath and the more restricted protons of macromolecules, allowing magnetization to be transferred between the two proton pools [Figure 2.15].

![Diagram showing water structuring with magnetic interactions between water and macromolecular protons.](modified from Balaban and Ceckler (1992)). R in the diagram denotes side groups within the macromolecules.

This transfer of magnetization provides another pathway for protons to lose energy to the lattice, thereby contributing to T1 relaxation mechanisms. In tissues, the magnetic coupling between macromolecular and free water protons is thought to occur mainly through space dipole-dipole interactions, although chemical exchange is also likely to contribute. This results in magnetization exchange, or transfer, between the “free” and “bound” proton pools. Due to this
coupling, the relaxation in one pool will influence the relaxation in the other; this is termed cross-relaxation. Similarly, selective saturation of one pool will affect the saturation of the other. Molecular diffusion occurs at a rapid rate compared with the duration of the NMR experiment and ensures that the result of this magnetization transfer is experienced by the entire water proton pool. If measurements of this relaxation pathway could be isolated and quantitated in biological tissues, it is possible that the specificity of the MR exam could be significantly improved.

2.3.3 Saturation transfer

The ability of MT to change the saturation of one spin pool, by selective saturation of a second pool was first described for chemically exchanging species by Forsen and Hoffman in 1963. These investigators described the saturation transfer technique as a method for measuring the rate of chemical exchange under steady state conditions. The experimental design involves two spin populations, A and B, both with distinct chemical shifts. If the two pools are in slow chemical exchange with one another, it is possible to selectively irradiate one of the pools (e.g. pool A) by applying continuous RF irradiation at the appropriate resonant frequency. As these saturated spins equilibrate with those of the non-irradiated pool (pool B) via chemical exchange, the net magnetization observed from the non-irradiated pool B will decrease. This results in a decrease in signal intensity from the B resonance [Figure 2.16].

![Saturation transfer experiment showing the effect of saturating one of 2 pools (spin pool A) within a chemically exchanging system (modified from Balaban and Ceckler (1992)).](image)

Figure 2.16 Saturation transfer experiment showing the effect of saturating one of 2 pools (spin pool A) within a chemically exchanging system (modified from Balaban and Ceckler (1992)).
The decrease in observed signal intensity of the non-irradiated pool may be used to help
determine the exchange occurring between the two pools. The authors showed that, if the
longitudinal magnetization of the non-irradiated spin population is known, the pseudo first order
rate constant \( k_f \) governing the exchange between the 2 pools could be determined from equation
2.14, where \( T_1^b \) is the longitudinal relaxation of the non-irradiated pool in the absence of
exchange, and \( M_s \) and \( M_o \) are the longitudinal equilibrium magnetizations of the non-irradiated
pool in the presence and absence of exchange:

\[
\frac{M_s}{M_o} = \frac{1}{1 + k_f T_1^b}
\]  

...2.14

In other words, the magnitude of the MT effect is dependent on the rate constant of the exchange
\( k_f \) as well as the \( T_1 \) of the non-irradiated spin population in the absence of exchange \( (T_1^b) \). The
constant \( k_f \) is the intrinsic chemical exchange rate constant and is independent of the NMR
system magnetic field strength. The dependence on \( T_1^b \) is due to the fact that the saturation
moving in from the irradiated spin population persists for times defined by its \( T_1 \). Therefore, with
this approach, \( k_f \) and \( T_1^b \) will ultimately control the tissue signal intensity in an MR image. Since
the chemical exchange processes underlying the MT effect depend ultimately on \( k_f \) rather than
\( T_1^b \), it would be useful to know the value of \( T_1^b \) so that the rate constant could be determined.
However, it is experimentally difficult to measure \( T_1^b \) since the contribution of exchange to
longitudinal relaxation times is difficult to assess. Another approach to determine \( k_f \) involves
measuring the \( T_1 \) of the non-irradiated pool while its exchange partner is saturated. This \( T_1 \) value
has been termed \( T_1^{sat} \). \( T_1^{sat} \) is easy to measure experimentally and is a function of \( k_f \) and \( T_1^b \):

\[
\frac{1}{T_1^{sat}} = \frac{1}{T_1^b} + k_f
\]  

...2.15

Combining equations 2.14 and 2.15 results in an expression for the chemical exchange rate in
terms of the measurable parameters of the system:

\[
k_f = \frac{(1/T_1^{sat}) (1-M_s / M_o)}{(1-M_s / M_o)}
\]  

...2.16

The rate constant \( k_f \) is a pseudo first order rate constant that will include all kinetic constants
involved in the MT interactions occurring at the hydration water layer of macromolecules. As
such, it includes the rate constants for chemical exchange, dipolar coupling and diffusion. Although this work details saturation transfer techniques applied to chemical exchange, similar results would be obtained from dipolar exchange which is considered to be the dominant exchange process in tissues. As already described, such coupling occurs when two protons are sufficiently close that the magnetic field associated with one proton may influence that of its neighbour and induce transitions in spin states. Effects on the equilibrium proton magnetization of one pool may be transferred to the other in a manner analogous to chemical exchange. The effects of diffusion are rapid and, as such, make no significant contribution to the rate of the exchange process.

The effect of saturating one of the exchange partners not only decreases the equilibrium magnetization in its exchange partner, but also decreases the observed $T_1$ ($T_{1\text{sat}}$) of the non-irradiated spin pool. $T_{1\text{sat}}$ is therefore always shorter than $T_1$ observed in the absence of irradiation. In contrast, $T_2$ is not usually changed under the saturating conditions generally employed to manipulate MT.

In summary, the selective irradiation of one spin pool in a pair of exchanging species (where exchange may be chemical or dipolar in origin) results in a decrease in both the net equilibrium magnetization and the observed $T_1$ of the non-irradiated spin pool. These effects can be used for quantitative measurement of the rate of MT or may be utilised to provide qualitative variations in image contrast dependent on the exchange properties of tissues under investigation.

### 2.3.4 Magnetization transfer in biological tissue

The saturation transfer experiment of Forsen and Hoffman [1963] outlined above exploited the different resonant frequencies of the two spin pools to achieve selective saturation of only one of the pools. In biological tissues, no such separation exists between the resonant frequencies of the “bound” and “free” water proton pools, both of which share nearly identical centre frequencies. However, due to their different $T_2$ relaxation times, the resonances of the two proton populations differ with respect to linewidth; the restricted protons of the “bound” pool have very efficient spin-spin relaxation with a short $T_2$ relaxation time and this results in a broad resonance lineshape compared with the much narrower linewidth of the “free” water proton resonance [Figure 2.17]. To achieve selective saturation in biological tissues, this difference may be exploited by using RF irradiation that is applied sufficiently off-resonance to avoid direct saturation of the free water resonance. This is especially effective in solids as all the spins are
coupled by spin diffusion. Thus, RF irradiation applied at any frequency point along the broad resonance line will be experienced by the whole macromolecular spin population. All techniques for measuring and imaging MT are based upon a selective saturation of the “bound” macromolecular spins, while leaving unbound “free” spins unperturbed. This in effect turns the solid-like “bound” proton pool into a “sink” for accepting magnetization transferred from “free” protons in the liquid-like pool. Transfer of magnetization between the 2 pools is detected via a signal decrease in the observed magnetization (from the “free” spins) and a measure of this MT may be achieved by comparing the observed “free” water MR signal in conditions with and without saturation [Figure 2.17].

![Diagram showing the individual lineshapes associated with the macromolecular and free water proton pools and how these differences may be exploited to result in selective saturation with a consequent reduction in the free water signal using off-resonance RF irradiation.](image)

**Figure 2.17** Diagram showing the individual lineshapes associated with the macromolecular and free water proton pools and how these differences may be exploited to result in selective saturation with a consequent reduction in the free water signal using off-resonance RF irradiation.

Selective saturation of spins may be achieved using the off-resonance saturation techniques described (where the off-resonance saturation is applied either continuously throughout the experiment or as a pulse) or via on-resonance techniques that exploit a differential
effect of slow and fast relaxing spins.

*Continuous wave saturation transfer* techniques use RF irradiation typically 5-10 kHz from the “free” water proton resonance, taking advantage of the broader linewidth of the macromolecular “bound” proton resonance. To minimise any effects of direct saturation of the “free” water protons, these off-resonance MT pulses are of narrow bandwidth. This is generally accomplished using a relatively long RF pulse (hence the term continuous wave). Therefore, while the main RF channel is used to pulse and acquire signal, an auxiliary RF channel is required to provide off-resonance saturation. The first demonstration of saturation transfer as a novel contrast mechanism within biological tissues was by Wolff and Balaban [1989] using continuous wave saturation irradiation applied prior to each sampling of data in a conventional spin echo sequence. Because this method requires long duration RF pulses, this approach may be time consuming and may be technically difficult to implement on clinical MR systems. High specific absorption rates also become a concern, especially when imaging at higher field strengths.

*Pulsed saturation transfer* techniques were developed as an alternative to continuous wave techniques [Hu et al., 1992; Yeung and Aisen, 1992] in order to avoid long image acquisition times and high energy deposition within tissue (i.e. SAR). With these, saturation of the “bound” proton resonance may be achieved using a brief off-resonance RF pulse applied at strategic points in the sequence (e.g. with each TR). This approach has certain advantages over continuous wave techniques. (1) It is more amenable to commonly used MR hardware; most typical MR systems are not designed to provide a sustained RF output of more than several tens of milliseconds. (2) It is more time efficient; saturation pulses may be interleaved with pulses for spatial encoding and data acquisition making them more amenable to inclusion in fast imaging sequences. (3) Pulsed methods are less likely to exceed SAR limits; as such these sequences may allow greater subject coverage. Off-resonance pulsed methods use a frequency offset ($f_{\text{off}}$) that is far enough away from the “free” water resonance to avoid or minimise any direct saturation effects on this resonance. Direct saturation effects are greatest at low offset frequencies particularly when irradiating with large off-resonance pulse strengths. The power and offset frequency of the off-resonance pulse for any particular sequence are therefore chosen to provide a compromise between any unwanted effects of direct saturation and the ability to provide an adequate MT effect via saturation of the macromolecular pool.

Pulsed saturation transfer may also be applied using *on-resonance* techniques. These
involve the application of RF irradiation near the “free” water resonance; however, this is
designed to provide a net rotation of zero degrees to the protons in this pool. The strong spin-spin
(T2) relaxation of the macromolecules is exploited here to achieve selective saturation. A pulse
is applied with a small flip angle and followed closely by one of the same magnitude but opposite
phase. Only those spins on-resonance will be returned by this pulse, resulting in selective
saturation of off-resonance components. The major advantage of on-resonance techniques is that
the application of pulses near resonance means that low power pulses may be used which have
their SAR well within designated limits. These sequences are also easy to implement on clinical
systems. Disadvantages of this technique are that the effects seen will be more dependent on T2*
(with greater sensitivity to B1 and B0 inhomogeneities) and the techniques will be more sensitive
to motion.

For all experiments in this text, pulsed off-resonance saturation techniques have been
applied and the off-resonance pulse will be referred to here as either an MT pulse or MT
presaturation.

2.3.5 Preliminary in vivo demonstration of MT effects in biological tissue

The first in vivo demonstration of MT effects within biological tissues was in 1989 by
Wolff and Balaban, who demonstrated results in rabbit kidney, cardiac and skeletal muscle. They
demonstrated a reduction in the steady-state water proton signal and a corresponding reduction
in its T1 relaxation rate in the presence of continuous wave irradiation. The lipid and
trimethylamine resonances in the spectra appeared unaffected. The authors observed
magnetization exchange rates between “free” and “bound” water pools that appeared tissue
specific. These were of the order of 0 - 3 sec⁻¹, indicating that it takes seconds for the energy
levels of the two spin populations to equilibrate (if short off-resonance pulses are used, it may
therefore take several pulses before equilibrium is achieved between the two pools). They also
applied this technique to imaging, showing that the contrast between renal cortex and medulla
may be significantly improved by the addition of continuous wave irradiation to a proton density-
weighted pulse sequence. In contrast, the urine and fat signals in the image appeared unchanged
by the irradiation. These experiments therefore suggested that the MT effect is indeed an
interaction between water and specific macromolecules, and as such may be tissue specific. The
successful application of the Forsen and Hoffman saturation transfer experiment to biological
samples by Wolff and Balaban in 1989 provoked interest in the technique by demonstrating that
saturation transfer might be used to provide a novel method of generating tissue contrast in MR images.

2.3.6 In vivo applications of magnetization transfer imaging

Magnetization transfer imaging encompasses a family of techniques that generate contrast in MR images of water-containing macromolecular structures. Applications of MT may be divided into two categories: (1) those which exploit the properties of MT contrast (MTC) for qualitative imaging, and (2) those which derive quantitative information from the MT study.

(1) Magnetization transfer contrast:

Magnetization transfer contrast is the contrast generated in tissue signal intensity during irradiation of the macromolecular proton pool. Under ideal conditions, the tissue signal intensity is inversely proportional to $k_r T_{1,sat}$ as given by equation 2.16. Since measurement of $T_{1,sat}$ is time-consuming and therefore not generally determined in clinical studies, most of the effects used involve the relative changes in signal intensity in the “free” water pool brought about by saturating protons in the “bound” pool. As such, MTC may be applied to clinical imaging by providing an additional tissue-specific contrast mechanism when used in conjunction with either proton density or T2-weighted imaging. An example of how additional contrast may be generated by the addition of MTC to a proton density-weighted image is given above [Wolff and Balaban 1989]. When applied to brain imaging, the greatest signal suppression occurs in structures which exhibit a strong MT effect (i.e. greater suppression in cerebral white matter than gray matter) with little or no suppression from structures lacking such an effect (e.g. cerebrospinal fluid, extracerebral fat). The amount of MT displayed by a given sample will depend on the quantity and nature of macromolecules participating in an exchange with surface water protons. Changes in tissues due to disease processes may alter the number or nature of such interactions and result in different tissue MT properties, thereby providing the potential for greater pathological specificity. Similarly, MTC may complement T2-weighted imaging as most structures that are bright in long TE sequences (with the notable exception of fat) do not have large MT effects and remain bright when MT presaturation is applied [Wolff and Balaban 1989]. Despite the similarity between contrast generated by T2 relaxation and MT mechanisms, the latter appears more closely related to T1 than T2 relaxation [Ceckler and Balaban 1991, Grad et al., 1991]. A clear example of the lack of association between T2 and MTC was shown by studies demonstrating that the
relaxation behaviour of $[^3H]$-labelled water in lipid suspensions does not appear significantly affected by MT compared with that of $[^1H]$-labelled water, despite both sharing identical T2 values [Ceckler and Balaban 1991]. The fact that MTC and T2 are independent suggests that they may be complementary to each other. As such, MT presaturation may allow generation of images with contrast similar to long TE T2-weighted images but with use of a shorter echo time, thereby allowing faster acquisition, greater signal-to-noise and decreased motion constraints.

Magnetization transfer contrast may also be useful for suppressing background signal from those tissues that are not the focus of interest in a particular study. This potential is exploited in magnetic resonance angiography, where reduction of tissue intensity occurs while leaving blood relatively unaffected yielding images with “bright” blood. The magnitude of the MT effect in blood is small due to a low concentration of macromolecules. In addition, blood flow will cause blood that has been affected by the MT presaturation to be replaced, making it even more difficult to achieve saturation of any macromolecular pool, thereby adding to the overall contrast between blood vessels and surrounding tissue.

Background signal suppression with MTC is also a potentially useful tool for increasing the contrast levels between focal areas of gadolinium enhancement and surrounding tissue in T1-weighted imaging [Tanttu et al., 1992]. The increased T1 shortening caused by focal leakage of gadolinium into a particular region of tissue is due to a direct water-gadolinium interaction and does not depend upon macromolecular interactions. As such, MR signal in such a region is not appreciably suppressed by MT presaturation. Increased contrast with greater conspicuity of the enhancing region results from preferential suppression of signal from non-enhancing surrounding tissue. A similar phenomenon occurs in normal brain where the signal from normally enhancing structures (e.g. choroid plexus, deep cerebral veins, leptomeningeal vessels, dural sinuses and pineal and pituitary glands) are rendered more conspicuous by the application of MT presaturation. By virtue of their higher capillary density, deep and cortical gray matter structures may also appear slightly more hyperintense than white matter.

Additional suppression of normal intracranial signal has been reported when MT presaturation pulses have been applied close to the “free” water resonance [Moran and Hamilton, 1995; Ulmer et al., 1996]. Mechanisms proposed for this additional suppression include spin-tip and rotating frame relaxation (spin-lock) effects. Spin-tip effects tend to result in universal signal suppression and typically occur at offset frequencies of only a few hundred Hz from the water resonance. On the other hand, spin-lock effects can result in selective signal suppression and
result in additional alterations in image contrast to those resulting from the MT effect. With off-
resonance pulse amplitudes typically used in clinical imaging sequences, spin-lock may occur
where the MT presaturation pulse offset frequency less than 2kHz and, as with MT, suppression
is greater for lower offset frequencies or greater pulse amplitudes [Mathews et al., 1997]. These
saturation effects are accentuated in tissues or substances where T1 is much longer than T2 (e.g.
cerebral white matter). The addition of near-resonance spin-locking to MT contrast potentiates
the differential suppression of non-enhancing tissue with respect to gadolinium (where T1 and
T2 are nearer unity making it relatively insensitive to spin-locking) [Mathews et al., 1997]. For
all MT presaturated T1-weighted experiments outlined in this thesis, a spin echo sequence has
been used that has employed a prepulse that is 1kHz off-resonance; as such, this would be
expected to show MT and spin-lock saturation effects in tissue.

The exact mechanisms responsible for the generation of MTC remain unclear. However,
a few simple concepts may easily explain the signal changes brought about by the addition of MT
presaturation to T1-weighted cranial imaging. Signal suppression should be greatest in tissues
where there is a major water-macromolecular interaction. Conversely, fluids such as
cerebrospinal fluid or blood contain low concentrations of macromolecules and should appear
relatively unaffected. In addition, they have low T1/T2 ratios resulting in minimal spin-lock
saturation. The signal from fat in adipose tissue appears also relatively unaffected by MT
presaturation as it is principally stored as medium- and long-chain triglycerides; these do not
behave as macromolecules as their T2 is not particularly short. In addition, these molecules are
located in hydrophobic droplets, thereby having little interaction with water, and their low T1/T2
ratio results in minimal spin-lock saturation. In practice therefore, the addition of MT
presaturation to non-contrast T1-weighted images of the normal brain results in a reduced signal
from all tissues except cerebrospinal fluid and fat, with a preferential loss of signal from white
matter (that may result in relative brightness of deep and cortical gray matter structures). In the
presence of pathological tissue disruption, MT effects may be reduced. Spin-lock effects may
also be reduced if T2 is lengthened with respect to T1. The addition of MT presaturation may
therefore result in relative lesion hyperintensity and simulate enhancement on post-contrast
images. In practice, a corresponding pre-contrast image is necessary to be certain that bright
lesions on post-contrast images are indeed due to gadolinium leakage.

MT presaturation pulses may improve visualisation of enhancing lesions on both spin
echo and gradient echo images although it is not clear which of these approaches is most sensitive
[Mathews et al., 1997]. They have been used to improve visualisation of lesions in stroke [Mathews et al., 1994; Finelli et al., 1994; Mehta et al., 1995a], primary brain tumour [Tanttu et al., 1992; Kurki et al., 1992; Finelli et al., 1994], brain metastases [Kurki et al., 1992; Finelli et al., 1994; Mehta et al., 1995a; Ulmer et al., 1996], infection [Finelli et al., 1994; Burke et al., 1996] and inflammation / demyelination [Tanttu et al., 1992; Finelli et al., 1994; Mehta et al., 1995a].

(2) Quantitative magnetization transfer imaging:

A quantitative measure of the MT effect may be easily obtained by applying proton density-weighted imaging with and without MT presaturation and calculating the MT ratio (MTR), as defined by Dousset et al [1992]:

\[
MTR = \left( \frac{[S_I] - [S_o]}{[S_o]} \right) \times 100 \%
\]

where \( S_I \) and \( S_o \) represent signal intensities with and without the saturation pulse respectively. As such, MTR provides a quantitative measure of the MT effect in individual tissues. As the MT effect increases, so does MTR. In tissue not exhibiting such water-macromolecular interactions, MTR will be zero. To avoid confusion between absolute and percentage changes in this value in this text, MTR is expressed in this thesis as percent units (pu) rather than %.

Measurement of MTR provides a quantitative index of the MT effect in tissue that depends upon the quantity and nature of water-macromolecular interactions within tissue. Because this mechanism differs from conventional contrast mechanisms (e.g. T1, T2 and proton density), MTR measurements may be advantageous to increase the specificity of the MR exam. Another advantage of such measurements is that, for any particular MR system and imaging sequence, MTR values should remain constant within a particular tissue, providing stability to allow comparison between individual subjects or studies. However, MTR is not an absolute measure and the value varies according to the particular MR system / sequence used. The value obtained is dependent on various factors including parameters related to (1) the MT presaturation pulse (e.g. offset frequency, bandwidth, pulse shape, duration, and time interval between presaturation pulse applications), (2) the MR hardware (e.g. \( B_1, B_o \)), and (3) the main imaging pulse sequence (e.g. type of sequence (spin echo vs. gradient echo), degree of T1 weighting, number of slices). The individual effects of these parameters on the measurement of MTR are not
fully understood. This, combined with differences between MR manufacturers, makes incorporation of MTR into multicentre studies and direct comparisons of individual results from different groups difficult. One other potential problem lies in the need to obtain images with and without presaturation; subject motion between these acquisitions may lead to inaccurate calculation of MTR within pixels. However, sequences have been designed that interleave MT presaturated and unsaturated acquisitions thereby reducing any effect of motion in the final calculated MTR image [Barker et al., 1996].

To date, various investigators have applied quantitative MTR measurements to the evaluation of intracerebral disease. Pathological reductions in MTR have been demonstrated in temporal lobe epilepsy [Tofts et al., 1995], primary brain tumours [Lundbom, 1992; Kurki et al., 1995], brain metastases [Boorstein et al., 1994], adrenoleukodystrophy [Melhem et al., 1996], HIV encephalitis [Doussset et al., 1997], progressive multifocal leukoencephalopathy [Doussset et al., 1997], optic neuritis [Thorpe et al., 1995], cerebral infarction [Prager et al., 1994], head injury [Bagley et al., 1997] and multiple sclerosis [Doussset et al., 1992; Gass et al., 1994; Loevner et al., 1995; Mehta et al., 1996]. Pathological increase in MTR has also been seen initially as part of a biphasic response to axonal damage in a feline model of Wallerian degeneration [Lexa et al., 1994]. This temporary increase in MTR has been postulated to be due to an increase in the number of macromolecular sites available for exchange after breakdown of myelin.

Two other quantitative MT imaging techniques that may be used to provide absolute measures representative of the MT effect are (1) $T_1$ sat and (2) the Z-spectrum. $T_1$ sat measurements involve measuring $T_1$ relaxation (through inversion recovery or other techniques) in the presence of RF irradiation that is assumed to completely saturate the macromolecular pool. This is time-consuming and often difficult to implement in a clinical setting. The Z-spectrum is the term given to the variation in MT effect with saturation offset frequency at a constant average saturation power. Investigation of the shape of curve forms the basis of ongoing research into the mechanisms of MT and it is thought that such information may potentially allow greater tissue specificity. Successful analysis of the Z-spectrum should enable calculation of new types of image based upon MT. A major disadvantage is that current acquisition techniques are time-consuming and application to faster imaging sequences may be limited by increasing SAR constraints.
2.4 MRI to assess blood-brain barrier integrity

2.4.1 The blood-brain barrier

The central nervous system requires a very stable environment to maintain normal function and certain substances that will normally pass easily elsewhere from capillary blood into non-nervous tissues are denied access here. This stability is maintained by the presence of a blood-brain barrier (BBB) that separates capillaries from brain parenchyma. It consists of a layer of endothelial cells in the capillary wall that are joined by tight junctions, a continuous basement membrane surrounding the capillary outside the endothelial cells and the perivascular end-feet of astrocytic processes that adhere to the outer surface of capillary walls [Figure 2.18].

![Figure 2.18](image)

Cross-section of blood capillary of central nervous system showing blood-brain barrier to be comprised of endothelial cells with tight junctions between, basement membrane and astrocyte foot processes.

This acts to provide a selective barrier to the passage of certain large molecules (e.g. plasma proteins). Free diffusion across these structures and the presence of active transport mechanisms within the endothelial cells allow the free passage of substances such as water, gases, lipid-soluble substances, amino acids and glucose as well as other molecules necessary for the healthy functioning of neuronal and neuroglial cells. A similar barrier exists in the spinal cord that shares the same properties as the blood-brain barrier. In a few small regions such as the area postrema in the medulla, the pineal gland and the neurohypophysis, the barrier is lacking and
large molecules may pass unrestricted. Early pathological studies in the nineteenth century first demonstrated the presence of a blood-brain barrier in animals using intravascular injection of dyes such as trypan blue that attached to proteins in the blood and entered all body tissues with the exception of the brain and spinal cord. In those areas lacking such a barrier, dye was shown to pass freely.

Pathological changes in the blood-brain barrier may occur following a variety of cerebral insults (e.g. trauma, infection, inflammation and neoplasia). In many of these conditions this deficiency is temporary, with repair within a few weeks. However, during this time, the permeability of the barrier may be altered, allowing the passage of larger molecules. This change in permeability may be exploited to allow in vivo detection by using tracers that would, on account of their large size, normally be excluded from entering the parenchymal tissue of the central nervous system. When injected intravenously they leak into and accumulate in these areas, exhibiting certain properties that allow their visibility with different imaging techniques. Examples of such tracers include radioisotopes whose radioactive emissions may be detected with scintigraphy and iodine-containing compounds that are radio-opaque to X-rays and may be visualised using computerised tomography (CT). Similarly, compounds with magnetic properties that alter tissue magnetization and relaxation may be used as contrast agents for magnetic resonance imaging, allowing assessment of blood-brain barrier integrity.

2.4.2 Contrast agents for MRI

Contrast agents are pharmaceutical compounds that provide additional information in images by manipulation of tissue signal intensity. Indications for their use include (1) visualisation of lesions that would otherwise be undetectable, (2) better delineation of the extent of disease, (3) improved diagnostic ability, and (4) assessment of disease activity. The ideal contrast agent is one that is well tolerated and safe, has a desirable excretion pathway, and where possible can be targeted to a particular area or organ of interest. Image contrast may be enhanced by increasing or decreasing the signal intensity of one tissue relative to another. Whereas X-ray contrast media have a direct effect of absorbing or scattering X-ray photons, the agents used as exogenous contrast media for MRI function indirectly through their alteration of the local magnetic environment of the tissue. These agents act primarily by altering relaxation rates of tissues in their immediate vicinity. Different substances may have different magnetic properties which may or may not be useful in the context of MRI.
If a substance is placed in a magnetic field, magnetization is induced within the substance resulting in a change in the nuclear magnetization that is additive to that of the applied field. This magnetic susceptibility is a fundamental property of matter, the magnitude of which may be either negative or positive. The induced magnetization that results will be antiparallel (diamagnetic) or parallel (paramagnetic, superparamagnetic and ferromagnetic) to the external magnetic field respectively.

Diamagnetism arises from the orbiting electrons surrounding each atomic nucleus. When an external field is applied, the orbits are shifted in such a way that the atoms set up their own magnetic field in opposition to the applied field. Diamagnetism is present in all materials, is weak, and exists only in the presence of an applied field. Diamagnetic compounds such as gold and silver show mild negative effects on the local magnetic field within the nucleus.

Paramagnetic, superparamagnetic and ferromagnetic compounds are all characterised by the predominant effects of unpaired electron spins and have positive magnetic susceptibilities and positive induced magnetization, augmenting the net magnetization in the direction of the applied field. Superparamagnetic agents have large magnetic moments and create large disruptive changes in the local magnetic fields. Ferromagnetic substances acquire large magnetic moments when placed within a magnetic field and retain this magnetization when the field is removed. However, most contrast agents used in MRI act as paramagnetic agents.

Paramagnetism results from the electron spin of unpaired electrons (i.e. those that have not been paired off in a chemical bond with a spin of the opposite character). Unpaired electrons have magnetic dipoles that respond to the external magnetic field in a similar manner to the weaker nuclear magnetic dipoles. As with nuclear spins, only a fraction of electron spins will contribute to the induced net magnetization. However, on the atomic scale, paramagnetic agents generate extremely strong local magnetic fields (the electron spin dipole is 658 times greater than the proton because electrons are smaller but have the same charge, so any approaching water molecules will experience an intense interaction that promotes relaxation). Like diamagnetism, paramagnetism only exists in the presence of an applied field. Paramagnetic materials have a dramatic effect on proton relaxation times when these are in solution. Bloch initially recommended the use of paramagnetic agents to reduce T1 in 1946 [Gore et al., 1993]. Bloembergen, Purcell, and Pound subsequently derived a formula that showed the T1 relaxation rate ($1/T1$) to increase in direct proportion to the ion concentration; subsequent studies confirmed this linear relationship in both solution and in whole tissue for a variety of agents [Gore et al.,
Most elements (including the transition and rare earth metals) and certain compounds are paramagnetic. Strong paramagnetism is exhibited by compounds containing iron, platinum, palladium, and rare-earth elements such as gadolinium.

In NMR, the contrast agent itself is not visualised, but its effects on proton behaviour are observed. T1 relaxation rate has been found to be proportional to the size of the magnetic moment (which itself is largely determined by the number of unpaired electron spins). Paramagnetic ions in simple solutions most effectively relax the protons in water molecules in close proximity, in the first molecular monolayer around the ion. As the correlation time for the ion increases, there is a dramatic increase in the relaxation efficiency. Most of the lanthanides and atoms such as cobalt and nickel have short electron spin relaxation times, generating locally fluctuating fields that are too fast to be effective. In comparison, manganese and gadolinium have relatively long correlation times and are therefore more effective.

Significant enhancements in relaxation may be achieved by binding these to proteins and other high molecular weight structures (e.g. by a factor of 10 for gadolinium when chelated with diethylene triaminepentaacetic acid (DTPA)). Binding multiple ions to a larger carrier molecule may also be an appropriate strategy for keeping the contrast agent within certain compartments (e.g. intravascular, or outside the blood-brain barrier). Such binding is useful to avoid toxicity of unbound ions, as these will otherwise accumulate within tissues that have high affinity for metals (e.g. lungs, liver, spleen, bone). Such chelating agents are therefore used to deliver paramagnetic ions to tissues, reduce metal toxicity and improve suitability for in vivo use. There are also undesirable effects of using chelates on the relaxation properties of metal ions, as the cage-like chelating structure restricts access of water molecules.

Metal ions are the commonest paramagnetic ions in use as contrast agents for MRI: iron, chromium, manganese and gadolinium cannot be used in free form because of toxicity. Various chelates have been used (EDTA, DTPA, DOTA, EHPG). Choice of contrast agent depends on effectiveness as a relaxation agent (this depends on the number of unpaired electrons, molecular size and mobility, and the number of binding sites available for water). Of all the contrast agents used for MRI, gadolinium is the most commonly used. It is a trivalent rare earth lanthanide element of transition group IIIb of the periodic table (atomic number 64, 7 unpaired electrons). Discovered in 1880 (JCG Marignac and PE Lecoq de Boisbaudran) and originally named

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1 The word chelate is derived from the Greek word "chele" for claw
Gadolinia, after a Finnish Chemist Johan Gadolin, it was found in many different minerals, the primary source being monazite. Other uses for gadolinium include its use in nuclear reactor control rods, certain electronic components, and as an alloying agent. Below 17°C it is ferromagnetic, and at very low temperatures, it is superconducting. Although gadolinium does not possess the largest magnetic moment, it does exert the strongest effect on spin-lattice relaxation of all known elements. Although it is not as effective as manganese for shortening transverse relaxation, gadolinium is favoured as its complexes are more stable and it is more effective at shortening $T_1$. Gadolinium has similar effect on both $T_1$ and $T_2$ shortening. In biological fluids, however, $T_1$ is much longer than $T_2$ and a much higher concentration than that used in clinical practice is required before significant $T_2$ shortening or image attenuation is observed on $T_1$-weighted images. Chelation with the linear chelate diethylene triaminepentaacetic acid (DTPA) forms a particularly stable complex, binding 8 of the 9 binding sites of the gadolinium ion while leaving the ninth free for water molecules approaching the paramagnetic centre. This is gadopentate (Gd-DTPA), a stable water soluble molecule. The gadopentate molecule has 2 negative charges and must be balanced in solution by 2 positively charged ions (e.g. meglumine to make gadopentate dimeglumine). For all work detailed in this thesis, Gd-DTPA is used to refer to gadopentate dimeglumine. A useful property for neuroradiology is the predominant inability for Gd-DTPA to pass into tissue possessing an intact blood-brain barrier. Visible enhancement in such regions is therefore likely to indicate blood-brain barrier breakdown or abnormal vascularity.

Other contrast agents will only briefly be mentioned, as all contrast-enhanced studies in this thesis have been performed using Gd-DTPA (gadopentate dimeglumine, Magnevist®). Other chelates of gadolinium in common use include Gd-DTPA-BMA (gadodiamide) which is a non-ionic derivative of Gd-DTPA, and Gd-HP-DO3A (gadoteridol), another non-ionic contrast agent with a macrocyclic ligand (affording it greater stability and a reduced tendency for release of the toxic gadolinium ion). Non-ionic compounds have a practical advantage of lower viscosity, allowing faster intravenous administration and better tolerance, especially at higher contrast doses. All of these gadolinium based contrast agents however have similar enhancement effects at equal doses [Yuh et al., 1991].

Bulk susceptibility contrast agents (e.g. superparamagnetic iron oxide particles coated with inert materials) are also used in MRI studies. They may be used orally or intravenously and distribute within the blood and reticuloendothelial system (depending on their size) with a
predominant effect on T2 and T2* imaging. A variety of different agents are also available that may be administered orally to delineate the bowel on abdominal imaging. These agents are not discussed further, as they have not been used in the following studies.

2.4.3 Pharmacokinetics of gadopentate dimeglumine

After intravenous injection, plasma concentration Gd-DTPA reaches an initial peak before the contrast agent undergoes rapid distribution within intravascular and extravascular compartments (following a two compartment model). This occurs for all tissues with exception of those protected by the blood-brain barrier. Small concentrations of intravascular Gd-DTPA are present in the brain, with highest concentration immediately following intravenous injection. Such changes are not readily apparent on qualitative T1-weighted images, although reduced gray:white matter contrast is often seen as a result of a relatively higher Gd-DTPA concentration within the more vascular gray matter. Following the initial peak, plasma concentration rapidly diminishes with biexponential decay. An 80% reduction in plasma concentration occurs within 10 minutes of contrast injection and the terminal half-life of Gd-DTPA within the body is approximately 90 minutes (with 91±13% excreted within 24 hours)[Niendorf et al., 1993]. No protein binding has been observed. Gadolinium-DTPA does not undergo metabolism, but is excreted unchanged with no dose dependence. The predominant excretion pathway is renal and dependent on the glomerular filtration rate, with no tubular sequestration or absorption. Extrarenal excretion (into the faeces) accounts for less than 1% of the administered dose [Niendorf et al., 1993]. These findings suggest that Gd-DTPA is safe in man. Poor diffusion through the placental barrier results from the extreme hydrophilicity, with only traces reaching the foetus. Only traces are secreted into human breast milk.

With disease, the normal tissue distribution of Gd-DTPA may be altered. Occlusion of blood vessels may lead to failure of the contrast agent to reach certain tissues whereas increased blood flow may result in more rapid focal contrast delivery, with or without related flow effects. In the central nervous system, an increase in blood-brain barrier permeability may arise from certain pathologies (e.g. infection, inflammation and neoplasia), allowing enhancement of brain or spinal cord parenchyma.

2.4.4 Safety and tolerance of gadopentate dimeglumine

Early animal studies showed low toxicity and good tolerance. The median lethal dose
(LD50) for Gd-DTPA in animals is approximately 100 times that of the dose commonly administered in human clinical studies. More recent animal studies in rats have shown Gd-DTPA to have neurotoxic potential when injected intraventricularly at very high doses (i.e. simulating an absent or damaged blood-brain barrier) [Ray et al., 1996]. This effect was shown to be independent of its osmotic properties. However, studies in pigs using varying doses of intrathecal Gd-DTPA have shown that neurotoxicity only occurs at doses considerably greater than those used for human studies [Skalpe and Tang, 1997]. Neural tolerance is also far better than that of both ionic and non-ionic X-ray contrast media. In humans, one consistent finding is a 15-30% transient increase in iron concentration that has generally persisted for less than 24 hours, returning to normal in all reported cases. The proposed mechanism has been an increased rate of sequestration of ageing red blood cells within the spleen and it is not thought to be of clinical consequence. Deposition of free gadolinium or Gd-DTPA has never been demonstrated in humans (Schering, personal communication).

In 1983, Gd-DTPA became the first parenteral paramagnetic agent to be studied in human volunteers. Initial studies showed high efficacy in detecting lesions within the central nervous system as well as systemic malignant disease. Phase I studies confirmed the pharmacodynamics and properties of Gd-DTPA (as outlined above). Phase I-IIIa studies involved 2,154 volunteers and patients, the vast majority receiving 0.1mmol/kg. From a meta-analysis of the data, it has been concluded that the overall incidence of adverse events in these studies is of the magnitude of 1% [Niendorf et al., 1993]. The first approval for general use was in 1988 (USA, Germany and Japan). Licences up to a dose of 0.2mmol/kg have since been granted in many other countries, including the United Kingdom. In IIIb-IV (pre- and post-marketing) studies, 13,439 patients were assessed using doses of 0.1 - 0.2mmol/kg Gd-DTPA according to a standardised assessment protocol [Niendorf et al., 1993]. Excluding sensation of warmth at the injection site, the overall incidence of adverse events was 1.15% (upper confidence limit estimated at 1.3%). Commonest adverse events included nausea and vomiting (incidence = 0.4%), local warmth/pain (0.4%), headache (0.3%), paraesthesiae (0.1%), dizziness (0.1%), and flushing (0.06%).

2 Adverse events were shown to occur in 0.63% of subjects in European and Japanese studies (these excluded the sensation of warmth either systemically or locally at the injection site as an adverse event). Without exclusion of systemic warmth, 7.6% of subjects complained of adverse events within the American studies. However, double-blind comparison of placebo (saline) showed an equal, albeit much higher incidence of side-effects (21.4% for placebo and 21.7% for Gd-DTPA).
Convulsions were seen in four patients (0.03%) following injection, all of whom had a previous history of epilepsy or of cerebral tumor. Other serious adverse events included urticaria (0.03%), skin and mucosal allergic reaction (0.1%), and cardiovascular reactions / arrhythmia (0.06%). Patients known to have a history of allergy had 3.7 times more adverse events than those without such history. This incidence is still low (2.6% with Gd-DTPA) relative to that of adverse events related to the use of non-ionic X-ray contrast media [Niendorf et al., 1993]. Post-marketing surveillance (with voluntary notification of adverse events by practitioners) has been reported for more than 5 million intravenous applications of Gd-DTPA [Niendorf et al., 1993]. Among these, there were 16 reported deaths, only one of which was likely to be directly related to Gd-DTPA, where an asthmatic maintained on β-agonist therapy died from anaphylactic shock. In a single case, a patient underwent MRI for back pain, which in retrospect was due to a myocardial infarction that subsequently proved fatal. In all other cases, patients were shown to have advanced terminal or malignant disease and the treating physicians thought that Gd-DTPA was unlikely to be associated with their demise (in 5 such reported subjects, death followed Gd-DTPA administration by more than 3 weeks).

2.4.5 Sensitivity of contrast-enhanced MRI

For detection of a focal enhancing lesion, the signal intensity must differ substantially from that of the background, non-enhancing tissue. Typically, for gadolinium contrast-enhanced MRI, this difference is likely to be of the order of approximately 30% [Bradley et al., 1997].

Various factors may be controlled for in the MR study to alter the sensitivity of focal enhancing lesion detection. The resolution and parameters of the imaging sequence are important, with shorter echo times allowing greater sensitivity to T1 shortening. Methods that increase the lesion concentration of contrast agent include the use of a higher bolus dose of contrast agent and, in certain pathologies, the introduction of a delay between contrast administration and data acquisition. Another method to improve enhancing lesion conspicuity is to suppress the signal arising from non-enhancing background tissue using MT presaturation, as discussed above [Tanttu et al., 1992]. Finally, image post-processing is important; qualitative analysis depends on optimal windowing and ideal viewing conditions, although registration of serial images with image histogram-matching may be useful to identify subtle regions of change [Holmes et al., 1999].

Naturally, detection of focal enhancement also depends upon intrinsic factors related to
the degree of contrast leakage into the lesion (e.g. blood-brain barrier permeability and lesion size) [Tofts and Kermode, 1989]. The size of the lesion relative to the image matrix is also important. However, if the concentration of contrast agent is high enough within tissue, this should allow enhancing lesions even smaller than an individual pixel to still be visualised [Bradley et al., 1997]. Using phantom simulations for enhanced MRI, large lesions have been shown to be more likely to be observed as enhancing than small lesions for a particular concentration of gadolinium contrast [Bradley et al., 1997]. The apparent size of an enhancing lesion will depend on the dose of contrast and concentration within the lesion. Lesions appear smaller at lower contrast doses or lesion concentrations, as partial volume averaging between enhancing and non-enhancing tissue results in poorer delineation of the lesion border [Bradley et al., 1997]. Conversely, the apparent size of enhancing lesions will increase with increasing contrast agent dose. This is because small lesions comprise a greater proportion of partial volume pixels. Small lesions therefore require greater doses of contrast agent to allow their detection on MRI.

2.5 MRI post processing to quantify pathological change

2.5.1 Qualitative MRI.

Images may be viewed using either in electronic format using a computer display or as hard copy films viewed on a light box. To produce an image that best represents the electronic data, the contrast level is manipulated by intensity windowing. Image windowing is subjective and, to my knowledge, no process has been reported that addresses ways of introducing more objective or automated methods that might allow greater reproducibility. Windowing therefore remains a potential source of variation in the NMR study that needs to be assessed for the purposes of standardisation. Before producing the final image, various changes may be made. To avoid abrupt change of signal intensity at the pixel margins (i.e. “pixelation”), the image can be smoothed by methods such as interpolation; while this does not increase the actual spatial resolution, the appearance may be improved so that regions of abnormality can be more easily demarcated.

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Assumptions regarding total enhancing lesion area and individual enhancing lesion size are complicated by both artefactual and “real” biological factors and therefore need caution if they are to be interpreted reliably.
2.5.2 **Quantitative MRI**

Quantitative measurements in MRI may be useful to characterise the pathological and anatomical changes that take place in disease, and in response to therapy. Quantitation is an essential component of many MR techniques, including magnetic resonance spectroscopy, MT imaging, diffusion imaging, and functional MRI.

Magnetic resonance imaging offers an advantage over many other imaging techniques in disease such as multiple sclerosis, in that it may be safely repeated over time, allowing both insight into the natural history of disease and a potential method for determining therapeutic response. Studies often require large numbers of patients to undergo serial evaluation over time in a number of evaluating centres. There is therefore an increasing need for accurate and reproducible measurement that was not previously an essential requirement for qualitative MRI studies.

If a particular MRI method is to provide a useful measurement instrument, it needs to be both practical in clinical terms and scientifically sound. Issues to be considered with regards to clinical practicality include (a) whether the technique is appropriate for the group of subjects being studied (e.g. difficulties may arise imaging the optic nerves in patients with severe nystagmus or the brain in those with severe titubation), (b) duration of subject study (long imaging sessions may be difficult in those with discomfort, spasm or urinary urgency and may result in motion that affects the study quality), (c) ease of administration (study protocols that are too complicated will lead to difficulties acquiring full studies without protocol violation, and (d) cost effectiveness. With regard to the scientific “quality” of the measure, it is important that it provides data that is reliable, valid and responsive:

Reliability considers whether the measurement instrument is accurate, stable over time, and precise. Accuracy refers to how close the mean of a number of a group measurements is to the “truth”; this therefore requires an alternative “gold standard” to allow comparison. Many NMR techniques have no such comparison. An example where accuracy may be important is in determining the size of a particular object from the image (e.g. to assess lesion or cerebral volume). The precision (or reproducibility) of a technique refers to the variation in repeated measurements (e.g. “scatter”). It is therefore possible for a technique to be accurate but not

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4All quantitative analysis in this thesis has been performed using a Sun workstation (Sun Microsystems, Mountain View, CA) with Dispeimage image display software [Plummer, 1992].
precise; such a technique will be relatively insensitive and show poor responsiveness. Precision may be easily assessed by carrying out repeated measurements (e.g. scan-rescan, or analysis-reanalysis). Reproducibility may refer to the whole experiment, although it is often helpful to consider the individual components of the experiment that may lead to variation (e.g. changes in the subject of the study, experimental setup, scanner performance, and data analysis). To ensure reliability over time for both cross-sectional and longitudinal MR studies, quality assurance is necessary to ensure that the measurement tool remains accurate and precise. Quality assurance is only just starting to be addressed in MRI, although this will be expected to play a greater part in future studies [Tofts, 1998]. Such issues have been addressed in this thesis with respect to MT imaging and discussed in more detail in Chapter 3.

Validity considers whether the instrument measures the concept it is intended to measure. This is difficult to ensure for many MR techniques, as this will often require pathological correlation and such data is relatively scarce and not usually available over time. Magnetic resonance imaging has been unique in providing clear natural history data over time in multiple sclerosis and validity of the techniques will require many pieces of evidence (often anecdotal) to be considered. The methodology required to help determine the validity of MTR as a useful marker of myelin is that of construct validity [Cronbach and Meehl, 1955; Hobart et al., 1996]. Construct validity considers whether an instrument does the following (a) measures what it is supposed to measure (convergent validity), (b) doesn’t measure what it is supposed not to measure (divergent validity), (c) distinguishes between groups in predictable ways (group differences), and (d) produces results consistent with theoretical prediction (hypothesis testing). Studies presented in this thesis contribute to the existing literature on the validity of MTR as a measure of pathological change.

Responsiveness is also a necessary attribute for serial measurements and considers whether the instrument is sensitive enough to detect clinically important change. This is important in conditions such as multiple sclerosis, where the clinical course may be highly variable and relatively unpredictable and where it often requires a particularly long time period before clinical change can be reliably confirmed. Responsiveness is therefore essential if a particular MR method is to be usefully employed to serve as a useful substitute for clinical measurement of disease (i.e. “surrogate” marker).

Approaches to image quantification. The most basic method of quantification in imaging involves a simple number count for a particular feature or abnormality (e.g. lesion number). If
desired, a visual estimate of the size of each abnormality can be performed from which an ordinal score can be derived [Thompson et al., 1990]. A more common application of quantitative image analysis involves image segmentation, allowing an image to be divided into a set of meaningful regions (e.g. marking out those pixels representative of lesions, cerebrospinal fluid, white matter etc.). Quantitative estimates of various parameters such as volume or average signal intensity may then be made for the individual regions. The simplest method of segmentation involves an observer manually tracing around a structure to create a region of interest (ROI), from which the area or volume may be computed [Isaac et al., 1988]. Disadvantages of manual outlining are that it is both time consuming and subjective. Automated approaches to image segmentation have been devised with the aims of improving the objectivity and reproducibility of measurement and allowing faster analysis. A semi-automated threshold technique, applied after correction for image non-uniformity, has been shown to improve reproducibility [Wicks et al., 1992; Filippi et al., 1995]. This technique is very threshold dependent and requires considerable editing to rule out false positives (areas of normal brain that have been segmented) and false negatives (low signal lesions that have not been included). Other approaches include the semi-automated lesion threshold or edge detection techniques that are applied to individual regions or lesions (e.g. seed-growing or contour). Although relatively time-consuming, these are more objective than manual outlining, as the program detects the edge of the lesion. For all studies presented in this thesis, regions have been defined using either the manual tracing technique or the contour (with manual edit) approach, which is now discussed [Plummer, 1992; Grimaud et al., 1996]. Here the operator selects a pixel within the image which is considered to lie at the border of the ROI and a computer algorithm then searches within two neighbouring pixels for a pixel of image intensity most removed from the selected pixel. A line is then formed between these two pixels which represents the steepest edge gradient present in the area selected. The mean of this gradient is then used as a seed point and a line is generated by searching for pixels with identical or most similar intensities. The steps in the analysis that are user dependent include initial lesion recognition and choice of edge pixel. Training has been consistently shown to improve both the inter-observer and intra-observer reproducibility [Filippi et al., 1998a]. Compared with manual outlining techniques, the intra-rater and inter-rater reproducibilities of the Contour technique appear considerably higher [Grimaud et al., 1996]. Other techniques have been developed that use multi-parametric data to achieve full automation; none of these techniques has yet been fully validated to provide reliable full automation, although they do offer promise for the future.
Many factors may affect lesion volume measurements, although image resolution is likely to be the most important. These are most reliable for images with high resolution, as this reduces partial volume averaging. Data related to the mean signal intensity within lesions will also be affected by partial volume averaging effects with surrounding tissue (e.g. for quantitative estimation of mean lesion MTR). The degree of bias will depend on lesion size, being most significant for small lesions as these contain a greater proportion of partial volume pixels. Histograms may provide a useful alternative method for image analysis, as they may provide useful additional information about the distribution of data within a particular region. In particular, these techniques may provide additional information about microscopic changes within the “normal-appearing” tissue.

It is often necessary to compare several different sets of MRI data. To optimise such comparisons, precise anatomical co-registration is required. This is best achieved using high resolution 3-dimensional imaging. However, if standardised methods are employed for subject positioning and image slice prescription [Gallagher et al., 1997], co-registration techniques may be successfully applied to 2-dimensional data [Woods et al., 1992; Symms et al., 1997; Holmes et al., 1999]. This is particularly useful for evaluating serial data and, in Chapter six this has been used to allow accurate delineation of regions of tissue that subsequently evolve into visible lesions.

2.6 Clinical measurement

2.6.1 Approaches to disease quantification.

Multiple sclerosis is one of a spectrum of inflammatory neurological disorders whose aetiology remains uncertain. Within the umbrella diagnosis of multiple sclerosis are a variety of subgroups defined currently on a clinical basis by differences in their disease course. To better understand the aetiology and pathogenetic mechanisms of multiple sclerosis, and to arbitrate the outcome of clinical trials, it is necessary to have a reliable method with which to quantitate pathological or clinical change. Realistic goals for therapy in multiple sclerosis include a prevention or reduction in the number, duration or severity of clinical relapses and/or the prevention, postponement, or reduction in the development of permanent impairment or disability.

A comprehensive quantitation instrument has not yet been universally accepted and it is unlikely that a single clinical instrument can be used effectively in a disease with such a myriad
of presentations and clinical features. As discussed above in the context of MRI measurement, an optimal measurement should be one that responsive, reliable (i.e. objective and reproducible), and valid (i.e. measures clinically important changes that are caused by the disease). It should measure components of the disease that reflect independent deficits (e.g. cognition, sphincter disturbance, coordination etc.) and it should be applicable to the full range of deficits encountered to avoid "floor" and "ceiling" effects. For practical utility, it should be easy to administer and cost-effective.

Some of the limitations in quantitation are disease-inherent. These include the variability in symptoms and signs, the variable and unpredictable natural history of the disease, the chronicity of the disease, and the apparent poor correlation between pathology and clinical signs.

2.6.2 Impairment and disability

In 1980, the World Health Organisation (WHO) proposed 3 categories of dysfunction in their International Classification of Impairments, Disabilities, and Handicaps. This describes the consequences of diseases and disorders at (1) the level of the body (impairment⁵ - the clinical symptoms and signs produced by a disease), (2) the person (disability⁶ - the personal limitations imposed upon the activities of daily living by the impairment), and (3) the person as a social being (handicap⁷ - the social and environmental effects of the disability). Whereas patients are often most concerned by "quality of life" issues (i.e. disability or handicap), the physician involved in assessing the progression of disease is likely to find impairment a more useful concept for measurement as this is less readily influenced by environmental factors and is therefore a more accurate reflection of the pathological processes involved in the disease.

Although clinical measurement tools generally have a number of limitations associated with their use in multiple sclerosis, they are accepted as the most important and relevant outcome measure for clinical treatment trials. MRI has been considered to be an alternative "surrogate"

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⁵ Defined by the WHO as “the loss or abnormality of psychological, physiological or anatomical structure or function.”

⁶ Defined by the WHO as “any restriction or lack of ability, resulting from an impairment, to perform an activity in the manner, or within the range, considered to be typical for human beings.”

⁷ Defined by the WHO as “a disadvantage for an individual resulting from an impairment or disability that limits or prevents the fulfilment of an individual’s typical role.”
marker of impairment; to justify its use as such, validation is required against clinical measures. This introduces a paradox, in that MRI promises to provide a more objective and sensitive measure of disease activity/progression than clinical assessment, yet it is required to show a close association with clinical measures before its use is justified.

A number of clinical measurement scales have been described that attempt to measure impairment and disability. Experiments in this thesis have used the Expanded Disability Status Scale (EDSS) in the evaluation of all patients [Kurtzke, 1983]. This is distinguished from other scales by virtue of its widespread use, extensive natural history studies and a common awareness of its disadvantages as well as advantages.

2.6.3 The Expanded Disability Status Scale

Early ordinal scales to monitor multiple sclerosis were heavily orientated to motor function and hindered by a lack of sensitivity. In 1955, a 10 step Disability Status Scale (DSS) was devised to monitor the clinical progress of patients in a clinical trial of isoniazid in multiple sclerosis [Kurtzke, 1955]. This scale was later expanded to include a set of 8 complementary objectively verifiable system measures (called the functional systems), each with a possible score of 0 (normal) to 5 or 6 (maximal impairment). These system scores are based upon the objective examination of pyramidal, cerebellar, brainstem, sensory, visual, and cerebral (“mood” and “cognitive”) systems, including assessment of “other” functional impairments (e.g. spasticity). Only one system score relies upon symptom description, measuring sphincter function. In 1983, this was further expanded to the Expanded Disability Status Scale (EDSS) following criticisms that the original DSS scale lacked sensitivity [Kurtzke, 1983]. The EDSS is divided into 20 half point steps from 0 (normal) to 10 (death from multiple sclerosis). Between 0 and 4, the scale is scored purely on functional system scores and is therefore an impairment scale. Higher values are more reliant on the patterns of disability and appear heavily influenced by ambulation and tend to be relatively insensitive to clinical changes that do not impair gait. Above EDSS 6.0, the scale takes account of the distance that can be walked and the aids required to accomplish this task. At EDSS 8.0 - 9.0, lower extremity function is generally lost and the score is mainly determined by upper limb function, with brainstem disturbance being the main determinant at EDSS 9.0. The full scale is listed in Appendix A.

If the EDSS is to perform as a useful measurement tool, it should ideally be appropriate for the study of patients with multiple sclerosis, easy to administer, practical, reproducible,
accurate, valid and responsive. The EDSS was designed specifically for the monitoring of multiple sclerosis. Although the scale is relatively easy to administer and practical, it is not altogether objective and does suffer to some extent from difficulties with intra- and inter-rater reproducibility.

Reproducibility of the EDSS has been extensively studied in several hundred patients, with most investigators showing similar levels of inter- and intra-rater agreement [Noseworthy, 1994]. Perfect inter-rater agreement occurs in 50-66% of the major divisions of the functional systems, and for the EDSS perfect agreement is greater towards the higher end of the scale. Due to the variable nature of multiple sclerosis, it is harder to assess reproducibility over time. One study has attempted this, showing an intra-rater reproducibility of approximately 75% in the 10 step DSS for 1,300 patients thought to be “clinically stable” over a period of 90 days between visits [Myers et al., 1993]. Reliability of EDSS assessment may be enhanced by having a single evaluating physician within a study, using standardised documentation and protocols, defining in advance the terms used for assessment, using measured distances to assess mobility, and employing standardised times of assessment.

Accuracy and validity are attributes that are difficult to assess for clinical scales, as no definitive standard exists with which to draw comparison. The EDSS may however be compared either with other clinical measures or with alternative assessment tools such as MRI. Magnetic resonance imaging techniques may demonstrate various aspects of the disease process (e.g. new regions of blood-brain barrier breakdown, change in lesion volume, atrophy, etc.), each reflecting different components of biological abnormality, all of which have an uncertain relationship with the consequent clinical deficit.

The responsiveness of the EDSS has been assessed in a single large study, where clinicians judged patients on two occasions 3 months apart. Of the patients classified as having current disease activity, many showed no change in their DSS score [Ellison et al., 1993]. Other studies have shown MRI to be up to ten times more sensitive than clinical methods in detecting evidence of disease activity [Willoughby et al., 1989; Thompson et al., 1991]. As such, it appears that the EDSS is somewhat limited in sensitivity.

A further limitation of the EDSS is its non-linearity [Weinshenker et al., 1991b]. The EDSS represents an ordinal scale composed of different steps that may not be equated with each other, as each represents quite different changes in impairment or disability. In natural history studies, peaks are noted at EDSS 1 and EDSS 6 (the latter possibly reflecting the automatic
inclusion of any person requiring unilateral assistance for ambulation into this category; various reasons may however exist for use of such aid that may be difficult to allow for despite a disciplined assessment). The average time spent at each point of the DSS and EDSS for those that progress to the next level has also been shown to be quite variable [Weinshenker et al., 1991b] [Table 2.1].

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\begin{array}{cccccccc}
DSS & 1 & 2 & 3 & 4 & 5 & 6 & 7 & 8 & 9 \\
\hline
\text{Time (years)} & 4.1 & 2.8 & 2.0 & 1.2 & 1.3 & 3.1 & 3.8 & 2.4 & 2.5 \\
\end{array}
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*Table 2.1* Mean time spent in years at a given point of the DSS for patients who later progress to the next level.

The EDSS has other inherent disadvantages. Because it is highly dependent on ambulation in the higher ranges, it tends to under-represent both upper limb and cognitive function. In addition, it is possible that some measures are redundant by virtue of their close correlations with each other. An example of this is the high correlation seen between mobility and bladder function. Finally, patients may exhibit day to day or diurnal fluctuation that is to some extent dependent on the environment (e.g. temperature). As such, it is useful in clinical trials to evaluate sustained changes in the EDSS, rather than relying on a single score at each time point.

Despite the disadvantages, the EDSS has been extensively used and investigated. Indeed, it is because of this wide experience (both positive and negative) that the EDSS remains the most widely used clinical scale for assessing multiple sclerosis in natural history and clinical trials. For this reason, clinical status has been classified using the EDSS for all studies described in this thesis.
CHAPTER 3

Quality Assurance and standardisation for magnetization transfer imaging

3.1 Introduction

The generation of contrast in conventional MRI relies upon the density and relaxation characteristics of free water protons. In homogenous tissue samples, the relaxation of the observed magnetization may be described by two monoexponential decaying functions corresponding to the longitudinal and transverse magnetization (T1 and T2). As described in Chapter 2, biological tissues are complex heterogeneous structures and proton mobility may be either restricted (e.g. bound to macromolecular structures) or free (e.g. water). This interaction between free and bound protons results in relaxation that is neither monoexponential (as might be expected from a homogenous sample) nor a sum of exponentials (as might be expected from a mixture of independent non-exchanging samples). The complex interactions that result in modification of the MR visible water free proton signal by protons in the bound pool form the basis of MT imaging [Wolff and Balaban, 1989].

A number of different imaging methods have been devised to utilise the MT effect, all of which rely upon the application of MT saturation pulses to conventional MRI sequences. To differing degrees, they are all based upon the saturation transfer techniques of Forsen and Hoffman that have been described in chapter 2. They may utilise either continuous wave or pulsed saturation transfer. Continuous wave saturation transfer techniques are rarely used for in vivo imaging as they are time-consuming, result in high energy deposition and are difficult to implement on clinical imaging systems, requiring a separate auxiliary amplifier. Pulsed saturation techniques achieve selective saturation of the “bound” proton resonance using either brief off-resonance RF pulses (applied at a frequency that is offset from the “free” water resonance) or on-resonance pulses (applied near the “free” water resonance). Off-resonance pulsed saturation transfer techniques are amenable to commonly used MRI hardware, time-efficient and result in low energy deposition. Direct saturation may be problematic if low offset frequencies or high
power pulses are employed. The design of pulse sequence is important to allow the most appropriate compromise between an adequate MT effect and the degree of unwanted direct saturation. On-resonance pulsed saturation techniques are easy to implement on clinical systems, result in low energy deposition and may suffer less from direct saturation effects. Disadvantages include a greater dependence on T2* effects (with greater sensitivity to $B_1$ and $B_0$ inhomogeneities) and a greater sensitivity to motion.

Magnetization transfer imaging applications based on off-resonance pulsed saturation transfer techniques are employed throughout this thesis. They are the predominant MT method in current use in clinical practice and in vivo research [Hu et al., 1992; Yeung et al., 1992]. Issues related to standardisation and quality assurance of MT imaging are discussed in this chapter; in general these relate to off-resonance pulsed saturation transfer techniques.

3.2 Applications of magnetization transfer

Various applications for MT imaging have been reported and issues related to standardisation of such methods are briefly discussed:

3.2.1 Magnetization transfer contrast

The simplest application of MT involves acquisition of a single qualitative image that utilises MT saturation to provide an additional contrast mechanism (MTC). Work presented in this thesis explores the potential application of MTC to improve the conspicuity and detection of enhancing MS lesions with gadolinium contrast-enhanced T1-weighted imaging [Tanttu et al., 1992; Finelli et al., 1994; Van Waesberge et al., 1997]. If such techniques are to be successfully incorporated into multicenter studies, standardisation of the MT saturation pulse parameters requires consideration and such issues are described in the following discussion. The standard imaging parameters (e.g. TE, TR, etc.) also require standardisation as the behaviour of the MT presaturation pulse varies according to the degree of T1-weighting.

3.2.2 Magnetization transfer ratio

A quantitative measure of the MT effect in tissues, the MT ratio (MTR), may be simply obtained by applying a pair of identical proton density-weighted images, one with and one without MT saturation [Dousset et al., 1992]. As described in Chapter 2, MTR is calculated for each pixel from the 2 images using the formula:
MTR = ([SI_o - SI_d] / [SI_o]) x 100 %

where SI_d and SI_o represent signal intensities with and without the saturation pulse respectively.

A number of factors relating to scanner performance cancel out in this calculation. It should therefore be possible to obtain MTR measurements that are (a) highly reproducible, and (b) sensitive to small physiological or pathological differences between subjects or within individuals over time, that would otherwise be undetectable with conventional qualitative MRI techniques [Bakker et al., 1997; Dousset et al., 1992; Filippi et al., 1995b; Silver et al., 1997a; Silver et al., 1998].

Whilst small reductions in MTR may be non-specific and occur in association with various pathological entities (e.g. axonal loss, demyelination, oedema associated with inflammation), there is good evidence that large MTR reductions indicate the presence of significant demyelination [Dousset et al., 1992; Dousset et al., 1994; Dousset et al., 1995; Dousset et al., 1997; Kimura et al., 1996; Lai et al., 1997; Lexa et al., 1994; Silver et al., 1995; Thorpe et al., 1995]. In MS, correlations have been reported between MTR and both physical and psychological deficits in MS, suggesting the potential value of MTR to monitor the disease [Gass et al., 1994; van Buchem et al., 1998; Rovaris et al., 1998; Silver et al., 1998; van Waesberghe et al., 1998, Filippi et al., 1999a]. However, while MTR may allow a quantitative measure for any individual imaging sequence and MRI system, it does not represent an absolute measure and its value is dependent on a variety of physical and biological factors that have not yet been fully elucidated.

Practical limitations of sequences to measure MTR include (a) the need for a pair of images to be acquired, making data acquisition more time-consuming than conventional or MT contrast imaging, although useful additional data may be acquired during this time (e.g. proton density or T2-weighted images), and (b) the addition of MT saturation may lead to difficulties remaining within SAR limits, especially when used in conjunction with certain fast imaging sequences such as fast spin echo; a compromise is therefore needed between slice coverage, MT saturation pulse power and image acquisition time. These technical limitations need to be addressed if MTR is to be successfully incorporated in multicenter studies. The availability of a standardised sequence that is easy to implement, providing adequate slice coverage within a clinically acceptable time that is able to perform identically on the various MR systems from different manufacturers remains an important challenge.
3.2.3 Absolute quantitative measures of the magnetization transfer effect

Absolute quantitative measures of the MT effect should theoretically be reproducible for different MRI scanners and imaging sequences. However, they require complicated measurements that rely on the acquisition of at least three different images. As a result, time required for data acquisition is increased. Such measures include (a) T1_sat (the observed T1 of the non-irradiated “free” water proton pool in the presence of “bound” proton pool saturation), (b) the rate constant (k) for magnetization transfer between the 2 pools, and (c) acquisition of the Z-spectrum. Because of increased demands on the MR system and the increase in time needed for data acquisition (with potential inaccurate parameter calculation secondary to motion), the in vivo application of these MT parameters is less certain than that of MTR measurement. New faster imaging sequences such as echo-planar imaging may make their application more practical in the future [Ranjeva et al., 1997; Zhang et al., 1998]. While their potential use in MS remains undefined, the future development and evaluation of these techniques will be of considerable interest.

3.3 Standardisation of MTR data acquisition

Of the different quantitative MT imaging techniques, most clinical experience has been gained with MTR as this can be implemented on standard clinical imaging systems to provide reproducible data within acceptable acquisition times. The discussion and experiments described in this chapter focus on MTR measurement. Many of the issues discussed will also be applicable to both MT contrast imaging and absolute measurements of MT effect.

Ideally, multicenter studies would incorporate a standard MR system and imaging protocol specifically designed to obtain MTR data that is precise (i.e. reproducible with lack of random error) and accurate (i.e. close to the truth with lack of systematic error). In practice, this is unrealistic and standardisation procedures are required either during the stages of data acquisition or postprocessing to help ensure that comparable measures of MTR are achieved over time and at different centers. To understand how this may be achieved, it is helpful to understand how different factors influence the degree of MT effect and resultant MTR values obtained using a particular MR system.

3.3.1 Factors that affect MT saturation

If the RF receiver gain remains identical for each pair of image acquisitions with and
without MT saturation, it can be assumed that the majority of variation in resulting MTR values is due to the degree of MT saturation experienced within a particular tissue region. Certain features of the MR system that influence the degree of MT saturation are likely to be fixed and not easily subject to manipulation (e.g. strength of the $B_0$ field, coil hardware, prescan function). Other features may be subject to alteration or variation, providing the potential for compensation or variable reproducibility respectively.

The degree of MT saturation depends predominantly on (i) the individual properties of the tissue under investigation, (ii) the amplitude (i.e. effective flip angle) of the saturation pulse and the interpulse interval (duty cycle), and (iii) the presence or absence of unwanted direct saturation effects on the "free" water resonance [Wolff and Balaban, 1989; Balaban and Ceckler, 1992; Brooks et al., 1994; Yeung and Aisen, 1992; Wang et al., 1997; Berry et al., 1997].

1. **Tissue properties**
   The ability for different tissues to exhibit different degrees of MT provides the rationale for using these techniques. However, the degree of uncertainty in the relationship between tissue properties and MTR complicates the process of standardisation and more information is needed before it is possible to predict the exact behaviour of individual tissues in different MR systems.

2. **Amplitude of the MT saturation pulse and interpulse interval**
   The dependence of MT saturation on individual pulse parameters has not been fully elucidated. A number of studies have addressed this issue for off-resonance pulsed saturation transfer imaging techniques [Berry et al., 1997; Finelli et al., 1997; Hamatake et al., 1997; Quesson et al., 1998]. Important determinants include the effective flip angle (which may be calculated from knowledge of the offset frequency, pulse shape (e.g. gaussian or sinc), duration, bandwidth and maximum amplitude) and the interpulse interval (defined as the repetition time divided by the number of slices). Berry and colleagues, using 1.5 Tesla MR systems from different manufacturers, reported a close correlation between MTR in healthy white matter and a ratio of the calculated effective flip angle to interpulse interval; the authors concluded that this may provide a correction factor for standardisation of multicenter data [Berry et al., 1997]. Significant variation may still remain despite this standardisation procedure. As a result, Barker and colleagues attempted to define a standard pulse sequence (Euro-MT) for implementation on scanners from GE (General Electric Medical Systems, Milwaukee, Wisconsin) and Siemens
This gradient echo sequence implemented a TR that minimised T1-weighting. The results showed small variation across sites which the authors attributed to possible differences in RF amplifier calibration, pulse shape (e.g. due to apodisation), subject positioning within the headcoil, MTR calculation, or region of interest (ROI) positioning on the calculated MTR image.

Measurement of MTR is highly dependent on the precision of the saturation pulse flip angle and thus on B1 calibration. On clinical MR systems, this calibration is usually part of an automatic prescan procedure and not under direct operator control. The algorithm used to set the power of RF pulses is scanner dependant and is likely to be set up to produce acceptable image quality, as opposed to precise flip angles as required for reproducible and standardised MTR measurements [King et al., 1995; Brookes et al., 1996]. As such, manufacturers may need to address this issue at the stages of scanner design or upgrade.

Another factor that may or may not result in saturation pulse variation is the side of the water resonance to which the off-resonance pulse is applied. The “bound” proton spectral line has been shown to be asymmetric in feline white matter [Pekar et al., 1996]; if a similar asymmetry exists in humans then the sign of the offset frequency of the saturation pulse is likely to affect MTR. The European Commission funded MAGNIMS collaborative group (Magnetic Resonance Network in MS: ERBCHRXCT 940684 “Development of optimal magnetic resonance techniques to monitor treatments for preventing disability in multiple sclerosis”) have recommended a standard nomenclature for the sign of offset frequency ($F_{off}$), where the offset from the water resonance is either positive (positive $F_{off}$) and is applied to the water side or negative (negative $F_{off}$) and applied to the lipid side of the spectrum. Research from Dr Barker and colleagues at the NMR Research Unit, Queen Square, have addressed the issue of verifying the magnitude and sign of the offset frequency providing a test method that should be implementable on any commercial clinical scanner [Silver et al., 1999a]. All MT pulses used in this thesis were applied with a positive $F_{off}$.

3. Evaluation of effects of position in the headcoil on MTR measurement

Theoretically, the delivered power of the MT saturation pulse (the B1 field) that is experienced by the tissue should ultimately depend upon the uniformity of the transmitter coil. To address this issue, an experiment was devised to evaluate how MTR measurements within phantoms (in vitro) and brain (in vivo) depended upon their position within the headcoil. Data
were acquired using a GE Horizon Echospeed 1.5T system with standard quadrature headcoil.

Two phantoms containing different concentrations of bovalbumin were studied using a single slice gradient-echo sequence (TR 130ms, TE 4ms, 5mm slice thickness) with and without MT presaturation (equivalent to a 1200° flip angle applied 800 Hz off water resonance at the coil centre). All acquisitions were landmarked to the centre of the phantoms. These were maintained in a constant position within an annular loading phantom throughout the experiment. For each acquisition the headcoil was moved in the $B_0$ direction by 1 cm relative to the phantom [Figure 3.1].

![Diagram of phantom experiment](image)

**Figure 3.1** Phantom experiment to help determine the effect of $B_1$ inhomogeneity on MTR measurement in a standard imaging headcoil. The diagram portrays a number of separate data acquisitions where the phantom remains in the same place in the magnet and the headoil by 1 cm into the scanner at each acquisition.

For each coil position, MTR was calculated for an identical region of interest (ROI) within each phantom. Confidence intervals (95%) were evaluated for measurements at the centre of the coil where the greatest degree of homogeneity is expected (i.e. 10 to 20 cm from the caudal end). Using these confidence intervals, it was possible to demonstrate uniformity of phantom MTR.
values for a much wider region along the $B_o$ direction, between 7 and 28cm from the caudal end of the coil. Significant reduction in measured MTR was noted for objects placed within 7cm from the caudal end of the coil [Figure 3.2].

Phantom data is presented for 2 separate bovalbumin phantoms of differing concentration (PHANT 1 and PHANT 2). The MTR is shown with respect to the distance from the caudal end of the headcoil, while the headcoil is gradually moved with respect to the “fixed” magnet and phantom. 95% confidence intervals are shown for figures derived from the centre of the coil for comparison.

To confirm these results in the brain, five healthy volunteers (age 25-36 years) were studied using a dual spin-echo sequence (TR1730ms, TE31/80, 28 contiguous 5mm axial oblique slices) with and without MT presaturation (equivalent to a 1430° flip angle applied 1kHz off water resonance at the coil centre). Image data were first obtained with the head as far into the headcoil as possible and landmarked to a point 15cm from the end of the coil (approximate nasion). Image data were next obtained without moving the patient or repositioning the landmark; the only difference was movement of the headcoil 7cm into the MR scanner ("S" direction) [Figure 3.3].
Figure 3.3 In vivo experiment to help determine the effect of $B_1$ inhomogeneity on MTR measurement in a standard imaging headcoil. The diagram portrays 2 separate data acquisitions where the head remains in the same place in the magnet and the head coil by 7cm into the scanner at the second acquisition.

Three subjects were further imaged at the original position to determine 95% confidence limits for scan-rescan variation. For both data series, MTR was evaluated from 19 comparable anatomical white matter regions covering the whole brain. The position of each ROI in the $B_1$ direction was noted on the initial series. The percentage change in MTR brought about by moving the head coil 7 cm into the scanner was next calculated. Data is represented as the percentage difference in MTR between these two image acquisitions [Figure 3.4], showing MTR measurements to be reliable between 8 and 24 cm from the caudal end of the coil (i.e. within "normal" scan-rescan confidence limits). These in vivo findings for effects of coil inhomogeneity at the extremes of the head coil confirmed the in vitro phantom data.
In vivo data is presented to show the effect of a 7cm rostral headcoil shift on MTR measurements within similar brain regions. Findings are in keeping with the phantom data in Figure 3.3, showing increasing inhomogeneity for MTR measurements within regions of interest placed caudally in the headcoil.

Whole coverage of the brain was possible within homogenous regions of the coil when the head was placed as far as possible into the headcoil (all cerebral hemisphere and posterior fossa structures were between 11.6 and 24.4 cm from the caudal end of the coil). As this method of positioning is the one routinely used for brain MTR measurement in all experiments described in this thesis, effects of coil inhomogeneity may be ignored. Caution is however required when interpreting MTR within regions placed at the extremes of the transmitting coil (e.g. upper cervical spine), where potential effects of B1 field inhomogeneity can not be excluded. For comparative and longitudinal studies, accurate repositioning will be important as variation in the placement of a particular region of interest may lead to variation in MTR as a result of coil inhomogeneity. The influence of B1 inhomogeneity remains to be determined for other scanners using different coil designs.

To assess MTR within the cervical spine, the effects of such inhomogeneity may be avoided by using a system where the main body coil transmits the MT saturation pulse and a local spine phased array coil is used to receive signal. The implementation of this technique is described in Chapter 7.
4. Direct saturation effects

For off-resonance saturation transfer techniques, unwanted direct saturation effects may be reduced by either increasing the offset frequency or reducing the amplitude of the MT saturation pulse [Wolff and Balaban, 1989]. Whilst smaller degrees of MT saturation will result in lower MTR values, this will minimise direct saturation effects and such compromise may be worthwhile to reduce variability.

Other factors that may affect MT saturation

Other factors may also be responsible for variation in MT saturation and need to be considered. (1) MT saturation not only reduces the amplitude of the “free” water resonance, but may also result in shortening of T1 if the sequence has significant T1-weighting. It is therefore preferable to use MT imaging sequences that have minimal T1-weighting. (2) Different imaging sequences may behave in different ways. For instance, the use of gradient echo sequences may allow a short TR to be used without heavy T1-weighting. (3) The effect of subject motion may be reduced by interleaving acquisitions with and without MT presaturation [Barker et al., 1996].

3.4 MTR data acquisition and processing to achieve standardised measures

Conventional sequences are susceptible to patient motion. Calculation of MTR for individual pixels depends on precise registration of unsaturated and saturated images. A shift of 1mm or less may be enough to render invalid the calculated MTR value especially where image intensity varies over a small distance (e.g. with small lesions). Barker and colleagues have devised a spin echo sequence that interleaves these images during acquisition [Barker et al., 1996]. This approach may also theoretically be applied to other forms of imaging (e.g. gradient echo). Whilst large amounts of motion will affect the accuracy of MTR calculation, the interleaved acquisition can tolerate smaller shifts over a period of several minutes and only require that no significant motion occurs over the time to collect one phase encode step (which is of the order of a few seconds). One disadvantage of this technique is that data acquisition will be more time consuming due to the need to reach a steady state of magnetization. This is the predominant approach used for data acquisition in this thesis.

An alternative approach to overcome movement involves post-acquisition registration of data from independently acquired saturated and unsaturated images [Hajnal et al., 1997]. The application of normalised histogram analysis techniques may be useful to standardise MTR data.
acquired from different sites but is yet to be formally evaluated. Histogram analysis may be useful to provide information of the global changes in brain tissue structural integrity [van Buchem et al., 1997]. These techniques may however have certain limitations in that normalised histogram parameters provide no anatomical localisation, they provide information regarding the distribution of pixel MTR values rather than absolute number of pixels with given MTR values and may be significantly influenced by partial volume effects and the effects of atrophy [Silver et al., 1998b].

No standardised units of MTR currently exist and reporting of measurements may be confusing. This has been expressed in terms of a ratio (no units), a percentage (%), or percent units (pu). This last approach helps differentiate absolute from percentage changes in MTR and is one that would be recommended to avoid confusion and allow standardisation across the literature.

3.5 Quality assurance

Individual MR systems may perform differently over time leading to variation in MTR. In longitudinal studies (or cross-sectional studies carried out over a period of time), it is important to ensure that changes in MTR are due to the disease process rather than artificially introduced by variations in scanner performance. Quality assurance is therefore an important consideration.

3.5.1 Development of a phantom for MTR quality assurance

An homogenous gel phantom was developed that was easy to manufacture in large sterile batches (20% gelatin: 300g gelatin + 1.5g sodium azide + 1.5L water within a plastic universal container sealed with petroleum jelly and plastic sealant tape). The composition of the phantom was arrived at by a series of experiments to assess (1) ease of manufacture, (2) phantom stability, (3) MTR homogeneity within the phantom, and (4) amount of saturation that could be achieved with MT imaging. The ease of bulk manufacture was an important consideration in these tests. Of the various gel compounds tested, gelatin provided the best combination of ease of manufacture, homogeneity on cooling and MT effect (e.g. compared with bovalbumin, acrylamide, and other polymers). Sodium azide was incorporated into the phantom to prevent bacterial colonisation and potential effects on gel stability. The phantom containers were filled with solution that was bubble free and that had been heated for a prolonged period in a hot water
bath without allowing evaporation from the apparatus. The phantom containers used were plastic universal bottles. Cooling of the phantoms was carried out in a hot water bath at 65° centigrade that was subsequently switched off, so that cooling was slow and gradual. Once cooled to room temperature, the bottles were sealed to prevent any significant dehydration over time using petroleum jelly and the cap was externally sealed using plastic sealant tape. All gels were imaged to confirm MTR uniformity throughout and between each phantom. Gels were stored in a standard domestic fridge between MT measurements. No significant weight loss was observed in serial measurements over a period of more than 18 months, thereby confirming a tight seal and absence of dehydration. Phantom MTR uniformity was also maintained over this time (as assessed by the standard deviation of MTR values within the pixels of 3 predetermined regions of interest within each gel).

3.5.2 Developing and implementing a protocol for quality assurance in MT imaging

As the exact mechanisms of MT are incompletely evaluated, phantom measurements over time may have certain limitations in that they may be influenced by environmental conditions and phantom stability. Repeated study of healthy controls over time may be helpful to avoid such issues, as MTR measurements may be highly reproducible in normal white matter [See Chapter 4]. As part of the work involved in this thesis, a quality assurance protocol was devised and incorporated into the MT imaging program at the NMR Research Unit, Queen Square, to help ensure reliability of serial and cross-sectional MTR data acquisition and highlight potential problems in scanner or sequence reliability at the earliest opportunity. The aim was to develop a simple, reliable protocol that could in future be easily implemented in different MR evaluating centres. The protocol incorporates regular measurements from phantoms and control subjects. The results of an initial pilot study to test and validate this quality assurance protocol are presented.

All imaging was carried out using a 1.5 Tesla system (GE Echospeed Horizon) with standard quadrature headcoil. Two phantoms from an identical batch (and a third containing sterile water) were imaged at one to two weekly intervals for six months. Seven healthy controls (age 15-52, mean 33.7 years) were imaged at two to four monthly intervals for a period of 10 months that encompassed the time of phantom data acquisition. Control subjects were initially evaluated at baseline with scan-rescan data, where each subject was removed from the MRI system between scans and repositioned according to previously described techniques [Gallagher
et al., 1997]. This allowed an assessment of scanner performance and reproducibility independent of future potential scanner drift. A software scanner upgrade occurred halfway through the study whilst human and phantom data were being collected.

Phantoms and controls were each examined using 2 different sequences, one a gradient echo and the other an interleaved dual spin echo (as used for MTR measurement in in vivo studies within this thesis). The gradient echo sequence (Euro-MT) had the following parameters: TR=960ms, TE=12ms, matrix 128x256, FOV 24cm, 24 contiguous axial-oblique 5mm slices (healthy controls) and 12 axial 5mm slices with 5mm slice gap (phantoms), 2 NEX. Magnetization transfer presaturation involved the use of an off-resonance gaussian pulse of duration 7.68ms, bandwidth 250Hz, amplitude equivalent to a flip angle of 500°, that was applied 1kHz off-resonance. Acquisition time was 6 minutes for both controls and phantoms. The interleaved dual spin echo sequence (SE) had the following parameters: TR=1720ms, TE 30/80ms, matrix 128x256, FOV 24cm, 28 contiguous axial-oblique 5mm slices (controls) and 14 axial 5/5mm slices (phantoms), 3/4 NEX. Magnetization transfer presaturation involved the use of an off-resonance Hamming-apodised 3-lobe sinc pulse interleaved for each TR period, duration 16ms, bandwidth 250Hz, amplitude equivalent to a flip angle of 1430°, applied 1kHz off-resonance. Acquisition time was 19 minutes for controls and 10 minutes for phantoms. Accurate repositioning for controls and phantoms both within the scanner and the headcoil was ensured in addition to strict radiographical repositioning for each. For phantoms, a custom-designed holder was used within the headcoil. In addition, subject weight was arbitrarily entered as 10kg for all phantom studies and a tuning ring was used to load the headcoil.

Phantoms were visually assessed to ensure accurate repositioning within the headcoil and mean MTR was evaluated from three central standardised regions of interest (ROI). For healthy controls, mean MTR was measured from nine standardised 70mm³ brain regions (bilateral frontal, parietal, occipital and cerebellar regions and a single pontine region). Statistical analysis for control data involved analysis of median coefficient of variation and paired t-tests to distinguish whether changes over time differed in magnitude from those expected as a result of limitations in scanner and measurement precision, i.e. as determined by initial scan-rescan data.

Phantom data: For both sequences, mean MTR, standard deviation and MTR range (over six months) are shown for both phantom gels for the gradient and dual spin echo sequences [Table 3.1]. MTR fluctuations over time are shown for the Euro-MT gradient echo sequence in the 2 gels, where considerable instability was observed [Figure 3.5].
Figure 3.5 Quality control data for MTR measurements derived from 20% gelatin phantoms over six months. The inaccuracy of repeated measurements was realised to be secondary to “transmitter gain” fluctuations (i.e. flip angle calibration) and these were a result of poor auto prescan function with too small a figure used for the weight input.

<table>
<thead>
<tr>
<th></th>
<th>mean MTR (pu)</th>
<th>s.d. (pu)</th>
<th>range (pu)</th>
</tr>
</thead>
<tbody>
<tr>
<td>SE - gel A</td>
<td>24.7</td>
<td>0.5</td>
<td>23.0-25.6</td>
</tr>
<tr>
<td>SE - gel B</td>
<td>24.6</td>
<td>0.5</td>
<td>23.0-25.2</td>
</tr>
<tr>
<td>Euro-MT - gel A</td>
<td>31.2</td>
<td>4.2</td>
<td>19.9-37.2</td>
</tr>
<tr>
<td>Euro-MT - gel B</td>
<td>31.5</td>
<td>4.1</td>
<td>20.6-37.2</td>
</tr>
</tbody>
</table>

Table 3.1 Quality assurance data for phantoms.

In retrospect these changes were found to be due to “transmitter gain” (i.e. flip angle calibration) fluctuations as shown, and these were found to be secondary to poor auto prescan as a result of the arbitrary weight entered into the imaging prescription being too small. When taken into account, a correction was retrospectively applied and this resulted in results comparable with the dual spin echo data. On increasing the arbitrary gel phantom weight entered into the imaging prescription from 10 to 15kg, the problem disappeared.
**Control data:** No significant difference was observed between mean MTR (i.e. all regions of interest from all volunteers) at baseline and each of the 3 follow-up time points ($p>0.05$, paired t-test) [Table 3.2].

<table>
<thead>
<tr>
<th></th>
<th>spin echo</th>
<th>Euro-MT gradient echo</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>mean MTR</td>
<td>median COV</td>
</tr>
<tr>
<td>February*</td>
<td>38.2</td>
<td>0.8</td>
</tr>
<tr>
<td>May</td>
<td>38.0</td>
<td>0.8</td>
</tr>
<tr>
<td>July</td>
<td>38.2</td>
<td>0.9</td>
</tr>
<tr>
<td>November</td>
<td>38.1</td>
<td>1.1</td>
</tr>
</tbody>
</table>

*Table 3.2* Quality assurance data for healthy controls. The median coefficient of variation for each time point compared with baseline was compared with that for scan-rescan* to determine whether scanner performance varied over time.

This pilot study showed that a useful combined patient/phantom QA protocol could be implemented to measure scanner performance over time. The combination of different MT imaging sequences, phantoms and human controls provides information that is not only useful to detect any systematic errors that might arise from changes in scanner performance over time (or changes due to software/hardware upgrades) but that is also helpful to enable troubleshooting should any systematic variation occur, such as occurred in the pilot study with one imaging sequence for determining phantom MTR. For controls, this approach (i.e. comparisons for mean MTR and COV with baseline measures) allowed both loss of accuracy or loss of precision to be recognised. The study also confirmed that the phantoms developed for quality assurance purposes demonstrated reasonable stability. Finally, the implementation of a QA protocol may provide useful information relating to the effect of scanner upgrades on continuity of accurate MTR measurement. Here, no adverse effects were recognised following such a software upgrade.

### 3.6 Conclusion

Various applications of MT imaging may be useful to help define the pathological sequence of events and to monitor the clinical course of multiple sclerosis and its modification by therapy. Measurement of MTR provides a simple and robust (but not absolute) measure to quantitate the MT effect. To ensure comparable data from multicenter or longitudinal studies,
standardised and reproducible MTR data is required. Various factors affect the degree of MT saturation achieved and these need to be taken into account when designing such sequences to achieve a compromise that allows relatively standardised MTR measurements within a clinically acceptable imaging time. Future standardisation between independent MR system manufacturers and the facility for improved operator control would be welcome, but in the meantime MTR may be standardised to a reasonable degree by various acquisition or data post-processing procedures. To ensure stability of measurements over time (and troubleshooting in the event of change in scanner performance), the incorporation of a standardised quality assurance protocol across sites is essential. Future development of absolute measurements of the MT effect such as $T1_{sat}$ and acquisition of a Z-spectrum offer exciting future roles for standardised quantitative imaging in MS.
PART TWO

Magnetization Transfer to measure structural integrity
CHAPTER 4
A normative database for brain MTR measurements in health

4.1 Introduction
For interpretation of MTR in disease, it is important to understand the normal variation that may occur in healthy subjects as a result of normal ageing and gender. For studies investigating unilateral pathology that rely on obtaining control values from the unaffected contralateral side, it is necessary to know the potential extent of interhemispheric variation (i.e. lateralisation) within the normal brain. The study outlined in this chapter details the normal MTR variation that may be observed within normal brain white matter of young and middle-aged subjects.

4.2 Methods
Subjects Forty one healthy volunteers (between 16 and 55 years) were recruited: 16-25 years (5 male, 5 female), 26-35 years (5 male, 6 female), 36-45 years (5 male, 5 female) and 46-55 years (5 male, 5 female). The mean age of the male group (n=20) studied was 36.7 years and that of the female group (n=21) was 34.4 years. All subjects were entered into the study on the basis of a normal neurological history.

Handedness was assessed in all subjects using a questionnaire devised and validated by Annett [Annett, 1985]. This assesses hand preference for 12 manual tasks. For the purposes of this study, we assigned a value of +2 points for each task performed preferentially with the right hand and -2 points the left hand. Handedness was assigned according to an arbitrary total score (left-handed between -24 and -10, ambidextrous between -9 and +9, right-handed between +10 and +24).

The subjects of this and all other studies outlined in this thesis gave written, informed consent prior to participation. All studies described in this thesis were approved by the joint ethics committee of the Institute of Neurology and the National Hospital for Neurology and Neurosurgery.
MRI Protocol A dual spin echo sequence was performed (TR 1730, TE31/80, 28 contiguous 5mm axial slices, 3/4Nex, 256 x 128 matrix, 240 x 240mm field of view) both with and without presaturation pulses to saturate the broad resonance of immobile macromolecular protons, providing proton density, T2-weighted and calculated MTR images. This was adapted from a previously described sequence [Barker et al., 1996]. The applied presaturation pulse was a Hamming apodised 3-lobe sinc pulse, with a duration of 16 milliseconds and a peak amplitude of 23.2 μT (giving a nominal bandwidth of 250 Hz), applied 1kHz off water resonance. The energy deposited by this pulse provided measurable differences between saturated and unsaturated images and ensured a good signal-to-noise ratio in the calculated MT image. To ensure exact co-registration of the pixels on saturated and non-saturated images, scans with and without presaturation were interleaved for each TR period. The total time of acquisition was 17.7 minutes.

Magnetization transfer ratio analysis was carried out blinded to subject identity and demographic data. Regions of interest were outlined on the T2-weighted images. Care was taken to include only white matter and avoid partial volume averaging with surrounding gray matter or cerebrospinal fluid. No regions of interest included minor areas of T2 hyperintensity, a "normal" finding in healthy adults. For each anatomical area studied, a standardised region of interest was applied. Mean MTR measurements were then taken from the corresponding regions on calculated MTR images [Figure 4.1]. In both cerebral hemispheres regions were chosen in the centrum semiovale (216mm²), frontal white matter (70mm²) and parieto-occipital white matter (70mm²). In addition, midline regions were also evaluated in the genu of the corpus callosum (23mm²) and pons (70mm²).

Statistical analysis Statistical analysis was carried out using Levene’s test for equality of variances, Student’s t-test, paired t-test and Spearman rank correlation coefficient as appropriate.
Figure 4.1  Calculated MTR images showing regions of interest for mean MTR measurement.
4.3 Results

Significant regional variations in MTR values were noted throughout normal brain white matter [Table 4.1]. In the 41 subjects studied, highest values were observed in the corpus callosum (40.56 pu). This was significantly higher than any other region studied (p<0.001, paired t-test). For the 3 hemisphere regions studied, significantly higher MTR values were seen in frontal white matter (p<0.001, paired t-test).

<table>
<thead>
<tr>
<th>MTR (pu)*</th>
</tr>
</thead>
<tbody>
<tr>
<td>Corpus Callosum (genu)</td>
</tr>
<tr>
<td>Pons</td>
</tr>
<tr>
<td>Left Frontal</td>
</tr>
<tr>
<td>Right Frontal</td>
</tr>
<tr>
<td>Left Centrum Semiovale</td>
</tr>
<tr>
<td>Right Centrum Semiovale</td>
</tr>
<tr>
<td>Left Parieto-Occipital</td>
</tr>
<tr>
<td>Right Parieto-Occipital</td>
</tr>
</tbody>
</table>

* mean +/- standard error of the mean

Table 4.1 Regional variation of MTR measurement in normal brain white matter.

MTR values for each region were evaluated between the male and female subjects (Levene’s test for equality of variances and Student’s t-test) and no significant effect of gender was noted for any region [Table 4.2].

<table>
<thead>
<tr>
<th>MTR (pu)*</th>
</tr>
</thead>
<tbody>
<tr>
<td>Male (n=20)</td>
</tr>
<tr>
<td>Corpus Callosum (genu)</td>
</tr>
<tr>
<td>Pons</td>
</tr>
<tr>
<td>Left Frontal</td>
</tr>
<tr>
<td>Right Frontal</td>
</tr>
<tr>
<td>Left Centrum Semiovale</td>
</tr>
<tr>
<td>Right Centrum Semiovale</td>
</tr>
<tr>
<td>Left Parieto-Occipital</td>
</tr>
<tr>
<td>Right Parieto-Occipital</td>
</tr>
</tbody>
</table>

* mean +/- standard error of the mean

Table 4.2 Effect of gender on regional MTR measurements.
White matter regions (frontal, centrum semiovale and parieto-occipital) in the right and left sides of the brain were compared in all normal volunteers [Table 4.3]. In all these regions, MTR values were higher in the left hemisphere. In frontal white matter, the difference was not statistically significant (paired t-test). Differences between the hemispheres were statistically significant in the centrum semiovale (p<0.05, paired t-test) and parieto-occipital white matter (p<0.05, paired t-test).

<table>
<thead>
<tr>
<th></th>
<th>Left Hemisphere</th>
<th>Right Hemisphere</th>
<th>p value**</th>
</tr>
</thead>
<tbody>
<tr>
<td>Frontal</td>
<td>39.50 +/- 0.11</td>
<td>39.45 +/- 0.12</td>
<td>-</td>
</tr>
<tr>
<td>Centrum Semiovale</td>
<td>37.75 +/- 0.09</td>
<td>37.57 +/- 0.11</td>
<td>&lt;0.05</td>
</tr>
<tr>
<td>Parieto-Occipital</td>
<td>37.79 +/- 0.15</td>
<td>37.55 +/- 0.12</td>
<td>&lt;0.05</td>
</tr>
</tbody>
</table>

* mean +/- standard error of the mean, ** paired t-test

Table 4.3 Interhemispheric variation of white matter MTR values in all 41 healthy control subjects.

Assessment of handedness using the Annett handedness questionnaire [Annett, 1985] showed 35 normal volunteers to be right handed, one ambidextrous and five left handed. For the 35 right handed subjects, higher values were consistently seen in the left hemisphere regions, with significant differences noted in the centrum semiovale (p=0.05, paired t-test) [Table 4.4]. Although left hemisphere MTR values were also consistently higher than on the right side in the five left handed subjects [Table 4.5], none of these differences were statistically significant on paired t-test analysis.

<table>
<thead>
<tr>
<th></th>
<th>Left Hemisphere</th>
<th>Right Hemisphere</th>
<th>p value**</th>
</tr>
</thead>
<tbody>
<tr>
<td>Frontal</td>
<td>39.47 +/- 0.12</td>
<td>39.30 +/- 0.13</td>
<td>-</td>
</tr>
<tr>
<td>Centrum Semiovale</td>
<td>37.69 +/- 0.08</td>
<td>37.51 +/- 0.11</td>
<td>-</td>
</tr>
<tr>
<td>Parieto-Occipital</td>
<td>37.67 +/- 0.16</td>
<td>37.43 +/- 0.12</td>
<td>0.05</td>
</tr>
</tbody>
</table>

* mean +/- standard error of the mean, ** paired t-test

Table 4.4 Interhemispheric variation of white matter MTR values in the 35 right handed subjects studied.
To assess the effects of age on white matter MTR measurements, the normal volunteers were divided into two age groups (16-35 years, n=21 and 36-55 years, n=20). Five white matter regions (frontal, centrum semiovale, parieto-occipital, pons and the genu of the corpus callosum) were evaluated. For the hemispheric regions, both right and left hemisphere MTR values for each subject were considered in the analysis. The two age groups were compared for each region using a Student's t-test. Significantly lower MTR values were noted in all regions except for the pons in the older age group, as compared with the 16 to 35 year group [Table 4.6].

**Table 4.6**  Effect of age on white matter MTR measurements.

In addition a significant inverse correlation was noted between age and the MTR values observed in the corpus callosum and frontal, centrum semiovale and parieto-occipital white matter regions [Table 4.7].

**Table 4.7**  Correlation between white matter MTR and subject age.
4.4 Discussion

A number of studies support the role of MTR as a putative marker for myelin loss or disruption [Dousset et al., 1992; Gass et al., 1994; Ordidge et al., 1991; Thorpe et al., 1995; Dousset et al., 1995; Silver et al., 1996]. As such, this is likely to be a useful method for investigating dysmyelinating and demyelinating CNS conditions. In this chapter, I have explored the normal variations in MTR that may arise as a result of anatomical location, age, gender and handedness. As discussed in chapter three, the values of MTR are not absolute and are only applicable to the particular imaging system and sequence used for this study [Hajnal et al., 1992; Berry et al., 1996]. In addition to providing a normative database for MTR values to compare with pathological conditions, a number of observations are made that provide insight into both the normal brain architecture and factors that influence this in health.

Anatomical variation in MTR

In this study, the highest value for MTR was observed in the genu of the corpus callosum, as compared with white matter regions in the cerebral hemispheres and pons. In addition, frontal white matter showed significantly higher MTR values than those found in the centrum semiovale and parieto-occipital white matter. These regional variations are in agreement with previous studies evaluating MTR in the normal brain [Gass et al., 1994; Mehta et al., 1995b; Barker et al., 1996]. Possible explanations for such regional MTR variation include differences in the fibre packing density, degree of myelination, tissue hydration, and vascularity. The observation of high MTR values in the corpus callosum would appear to correlate with the histological appearance of a large number of myelinated fibres (about 300 million) in this structure [Barr and Kiernan, 1993]. As such, this study is in agreement with the hypothesis that MTR provides a putative marker for myelin integrity and demyelination.

Interhemispheric MTR variation

In addition to regional intrahemispheric MTR variations, significant interhemispheric differences have been observed. Higher MTR values were observed in the left hemisphere for all three regions studied (centrum semiovale, parieto-occipital and frontal white matter), although this difference was not significant in frontal regions. In a previous report, Mehta and coworkers did not show white matter asymmetry [Mehta et al., 1995b]. Several factors may account for this discrepancy. First, this study has relied on an interleaved spin echo sequence with predominant
proton density weighting as opposed to the separate acquisition of MT and non-MT prepared T1-weighted (TR 800ms) data, as performed by Mehta and colleagues [Mehta et al., 1995b]. The interleaved sequence has potential advantages of image co-registration between MT and non-MT images, theoretically allowing more accurate subtraction and consequent MTR calculation [Barker et al., 1996]. Secondly, it is less likely that T1 relaxation properties of tissue will affect MTR values to such a degree when data are obtained using a longer TR of 1730 ms. Thirdly, there are advantages to using a co-registered T2-weighted image for placing regions of interest, as discussed in Chapter 2. Fourthly, this study has relied on using controls with no neurological symptoms or previous history of neurological disease, as opposed to retrospective assessment of patients in whom imaging was normal, thereby supporting a non-neurological diagnosis [Mehta et al., 1995b]. All these factors may account for the wider ranges of MTR observed by Mehta and colleagues, which in turn may have obscured small interhemispheric differences.

Artefactual causes for such interhemispheric MTR differences may exist and are difficult to exclude (e.g. coil asymmetry with consequent inhomogeneities in the MT presaturation pulse). However, a natural asymmetry in the brain is well recognised. It is widely accepted that the two hemispheres of the brain are functionally specialised for different cognitive tasks. On a cellular and morphological level, various asymmetries exist in human cortical structures such as the planum temporale [Geschwind and Levitsky, 1968; Wada et al., 1975] and in parietal structures [Eidelberg and Galaburda, 1984]. Further evidence from in vivo imaging studies includes planum temporale and parietal operculum asymmetry noted with MRI [Habib et al., 1995] and hemisphere width, lateral ventricle, sylvian fissure and occipital petalia asymmetry all noted with computerised tomography [Le May and Kido, 1978; Shapiro et al., 1986]. Possible explanations for interhemispheric MTR asymmetry include variations in fibre packing density, the degree of myelination, or phospholipid metabolism between the two hemispheres. Interestingly, in rats, total phospholipid content and sphingomyelin concentrations have been shown to be higher in the left hemisphere with significant asymmetries in cortical phospholipid metabolism [Pediconi and Barrantes, 1990].

Many autopsy and in vivo studies have noted a relationship between handedness and hemisphere asymmetry, with reduced asymmetry in left handers [Beaton, 1985]. In the present study, MTR values in parieto-occipital white matter were significantly higher in the left hemisphere for right handed subjects, whereas no significant asymmetry was found in left handed subjects; no firm conclusions can be drawn however because of the very small number of left
handed subjects studied.

Effects of age on MTR

This study has demonstrated a relationship between MTR and age, where increasing age is associated with reduced MTR values in the corpus callosum and all three hemisphere white matter regions studied. This association has not previously been recognised. These preliminary findings require cautious interpretation due to the cross-sectional study design; unknown environmental factors could also account for such differences. Other studies provide support for subtle changes in brain architecture over time. Cross-sectional MRI studies have shown significant relationships between brain atrophy and age in similar age groups to those studied here [Condon et al., 1988; Blatter et al., 1995], and one longitudinal MRI study in 11 controls (mean age 55 years) has shown a small but significant reduction in brain volume over a single year [Fox et al., 1996]. It is likely that age-related variation in MTR is a real phenomenon and it is postulated that this reflects either structural change in brain white matter (e.g. neuronal loss and/or myelin loss, changes in glial tissue or altered water content) or change in phospholipid metabolism with increasing age.

Effect of gender on MTR

This study has shown no effect of gender on measurements of MTR for any region studied. This is in agreement with a previous study [Mehta et al., 1995b].

In conclusion, MTR measurement appears a robust in vivo technique with potential applications for evaluating structural changes in brain white matter occurring both in health and disease. Regional and age-related variations in MTR may be seen within normal brain white matter and it is important that such variation is considered when interpreting MTR changes in pathological states.
CHAPTER 5

MTR as a putative marker of demyelination: observations in central pontine myelinolysis

5.1 Introduction

In MS, MRI is widely used in diagnosis and evaluation of new therapies. Conventional MRI techniques do not however differentiate between demyelination and axonal loss, the major pathological processes that are thought most likely to result in functional impairment in MS. This lack of specificity contributes to the weak relationship between T2-weighted MRI abnormalities and disability. There is therefore a premium on developing techniques with greater pathological specificity. Potential techniques include MT imaging for assessment of myelin and proton MRS for evaluating axonal integrity.

As a quantitative measure of the MT effect, MTR may provide information about tissue structural integrity. In MS, reduction in lesion MTR has been shown to correlate more strongly with disability than T2-weighted lesion load [Gass et al., 1994], suggesting that MT imaging may be more specific to those pathological changes in MS that result in clinical deficit. In particular, it is likely that myelin provides the predominant contribution to MTR values in healthy brain white matter. This is supported by evidence from several sources. (1) In normal brain development, the value of MTR in white matter is lowest at birth; with maturity this more than doubles, following the normal known pattern for myelin development [Engelbrecht et al., 1997] with highest MTR values observed in the most heavily myelinated fibre tracts by adulthood [Silver et al., 1997a]. (2) Pronounced reductions in MTR have been found in an experimental model of demyelination induced by lysolecithin [Dousset et al., 1995] and in demyelinating lesions associated with chronic experimental allergic encephalomyelitis [Dousset et al., 1994]. (3) Profound reductions in MTR may be seen in patients with progressive multifocal leukoencephalopathy (a condition associated with predominant demyelination and relative absence of axonal degeneration or inflammation) [Dousset et al., 1997]. (4) In optic neuritis,
MTR reduction within the lesion correlates with the latency of the visual evoked potential, suggesting that a graded relationship exists between the extent of MTR reduction and demyelination [Thorpe et al., 1995]. Changes in MTR are not specific to demyelination; other pathological entities that may lead to reduction in MTR values in the brain include Wallerian degeneration, diffuse axonal injury following head trauma, and oedema. However, the changes seen appear much smaller than those seen as a result of complete demyelination [Lexa et al., 1994; Kimura et al., 1996; Dousset et al., 1992; Lai et al., 1997].

Proton MRS may be used to quantify the concentration of proton-containing chemicals within certain tissues. Of particular interest is the ability to detect in vivo axonal disruption. The normal proton spectrum is dominated by N-acetyl derived groups (N-acetyl aspartate and N-acetylaspartylglutamate). The predominant component of the NA peak is N-acetyl aspartate (NAA) [Birken and Oldendorf, 1989]. This amino acid of unknown function is found to be contained almost exclusively in neurons [Urenjak et al., 1993]. Although NAA may also be found in oligodendrocyte (O2A) progenitors, there are very few such cells present in healthy adult brains and this source is unlikely to contribute significantly to the overall concentration of cerebral NAA. Neuronal loss is predicted to result in permanent reduction in the tissue NAA concentration. Several studies have confirmed such changes in conditions associated with axonal loss [Gideon et al., 1992; Van der Knaap et al., 1992; Chong et al., 1993]. In MS, NAA reductions are found in normal-appearing white matter [Davie et al., 1994; Husted et al., 1994; Narayanan et al., 1997; Fu et al., 1998], acute lesions (where some of the changes may be reversible and suggest temporary axonal dysfunction rather than loss) [Mathews et al., 1991; Miller et al., 1991; Davie et al., 1994; De Stefano et al., 1995], and chronic lesions [Mathews et al., 1991; Arnold et al., 1990]. The degree of NAA reduction appears most marked in those with the greatest level of neurological disability [Fu et al., 1998; Davie et al., 1995; Davie et al., 1997; Davie et al., 1999]. In chronic MS lesions, there appears to be both marked demyelination and axonal loss with correlative MTR and NAA reduction [Davie et al., 1999]. Proton MRS may yield additional information about MS lesions. Acute demyelination is associated with the temporary presence of myelin breakdown products, thereby providing an indication of acute demyelination [Davie et al., 1994]. Other spectral regions are also of potential interest in the evaluation and potential differentiation of diseases of the CNS: lactate is a marker of ischaemia, inflammation or macrophage activity; elevation of the choline resonance is associated with membrane turnover, especially as a result of inflammation [Brenner et al., 1993] or
demyelination; inositol may indicate gliosis [Brex et al., 2000]. Creatine is often regarded as a relatively stable internal reference marker that is minimally affected by most neurological diseases.

To explore the hypothesis that demyelination per se has an important effect on MTR that is independent of axonal integrity, a comparative MT imaging and proton MRS study was performed in three patients with central pontine myelinolysis (CPM) - a relatively rare condition characterised pathologically by severe demyelination in the absence of inflammation, oedema or significant axonal damage [Adams et al., 1959; Endo et al., 1981].

5.2 Methods

Three patients admitted to the National Hospital for Neurology and Neurosurgery with a clinical diagnosis of CPM were studied using conventional proton density and T2-weighted MRI, MT imaging and proton MRS. Where possible, serial data were obtained to help evaluate the natural evolution of this condition. All subjects underwent a detailed history and a comprehensive neurological examination was performed prior to each MRI study by one observer (NCS).

Imaging was performed using a 1.5 Tesla superconducting system with standard quadrature headcoil (Signa, GE Medical Systems, Milwaukee, Wisconsin). Subjects were positioned and repositioned using previously described techniques [Gallagher et al., 1997].

Magnetization transfer imaging

A dual spin echo sequence was used (TR 1730, TE31/80, 28 contiguous 5mm axial slices, 3/4Nex, 256 x 128 matrix, 240 x 240mm field of view) both with and without presaturation pulses to provide proton density, T2-weighted and calculated MTR images (as described in Chapter 4).

All MTR analysis was performed by one observer (NCS) using region of interest (ROI) analysis on the proton density images. Mean MTR was evaluated for each ROI on the corresponding calculated MTR image.

Control data was obtained from the normative database of 41 healthy volunteers described in Chapter 4 (20 male, 21 female, age range 16-55 years, average age 35.6 years).

In control subjects and patients, calculated MTR images were assessed using ROIs
standardised for size and anatomical position. Sampled regions included pons, cerebral peduncles, substantia nigra, cerebellar hemisphere white matter, basal ganglia, internal capsules, cerebral white matter (frontal, parietal, occipital, centrum semiovale), and cortex. This was with the exception of mean MTR measurement within CPM lesions, where the ROI was obtained by a contour and manual edit approach.

**Proton magnetic resonance spectroscopy**

Proton density and T2-weighted axial brain images were acquired to allow voxel placement and localisation. Proton single voxel MRS (PROBE-PRESS, TR 3000ms, TE 30ms, 192 averages, 8 Nex) was performed in a designated volume in the basis pontis (3.2 - 6.2 ml). Spectra were analysed using an automated method (LC model) [Provencher, 1993]. This method uses a basis-set of known concentrations of the different metabolites as an internal reference. All metabolites are expressed as ratios to the absolute Creatine (Cr) concentration within the same voxel. Metabolite ratios were measured for NAA, choline (Cho), glutamate + glutamine (Gln), inositol, and lactate. Results were compared with spectra from 4 healthy controls obtained in an identical fashion (2 male, 2 female, average age = 37 years).

5.3 Case histories

**Case 1**

A 28 year caucasian man with a 12 year history of severe alcohol abuse presented with a progressive 4 day history of tremor, poor balance, falls, slurred speech and mild swallowing difficulty. The neurological disturbance commenced 10 days after initiating a regime for alcohol detoxification at home. Initially during detoxification he suffered symptoms of delirium tremens and generalised seizures. There was no history of previous serious illness.

On hospital admission (day four of his neurological illness), he appeared agitated, but alert and orientated. Systemic examination revealed signs of dehydration and mild hepatomegaly. He was unable to stand unsupported. Neurological examination revealed severe gait and limb ataxia, but no other abnormality. Investigations revealed hyponatraemia (127mmol/l), hypokalaemia (2.2mmol/l) and deranged liver function (bilirubin = 23 umol/l, gamma glutamyl transferase = 218 IU/l, aspartate transaminase = 342 IU/l).

The patient was treated with cautious nasogastric maintenance fluids; at no time did he
receive intravenous fluids. Two days later his illness was complicated by pneumonia and progressive neurological deterioration. He became less responsive and developed hypophonia with subsequent mutism, dysphagia, and severe generalised tremor at rest. Examination revealed bilateral ophthalmoplegia with bilateral horizontal gaze-evoked nystagmus, absent corneal reflexes, bilateral trigeminal and facial nerve pareses, loss of palatal sensation and absent gag reflex. A complete spastic quadriplegia ensued and he developed urinary retention. Spontaneous myoclonus was observed without any correlate on electroencephalogram. Re-measurement of electrolytes revealed correction of plasma sodium to normal levels (142mmol/l) and diminished urea (1.5mmol/l). Brain CT was unremarkable, although scans were degraded by motion artefact. Cerebrospinal fluid examination was unremarkable. On day 13 of his illness, he was electively sedated and ventilated for transfer to the National Hospital for Neurology and Neurosurgery. Sedation was discontinued after three days (day 16). He was conscious and obeyed commands, although neurological examination confirmed worsening of his condition with absence of horizontal eye movements. T2-weighted MRI demonstrated a single high signal symmetrical lesion confined to the basis pontis and surrounded by a rim of normal signal intensity; the lesion appeared hypointense on T1-weighted images and did not enhance following Gd-DTPA (0.1mmol/kg). There was partial sparing of the descending corticospinal tracts, which appeared less hypointense [Figure 5.1]. These appearances confirmed the clinical diagnosis of CPM.

At eleven weeks, he had made a partial recovery. He was self-ventilating and able to stand with bilateral support, despite persisting moderate quadripareisis. There was persistent ataxia affecting gait more than limbs. Pursuit eye movements were jerky although of full range. There was incomplete resolution of the trigeminal facial, glossopharyngeal and hypoglossal cranial nerve palsy. Further MRI studies at this stage demonstrated marked MTR reduction within the basis pontis. The greatest reduction in MTR was observed in the lesion centre, with values gradually increasing towards the lesion edge (this appeared sharply demarcated from the surrounding rim of apparently unaffected tissue) [Figure 5.2]. Relative preservation of MTR values was observed in the descending corticospinal tracts [Figure 5.2]. Normal MTR values were found elsewhere in the brain.

Proton MRS revealed lower NAA (NAA/Cr = 0.94) than that observed in any of the four healthy control subjects (range = 1.27-1.66) [Table 5.1, Figure 5.1]. Lactate was observed in the spectrum (Lac/Cr = 1.13). There was prominence of the free lipid peaks at 0.9 and 1.3p.p.m.
Figure 5.1  T2-weighted axial and T1-weighted coronal MRI, demonstrating a symmetrical lesion in the basis pontis (T2-weighted hyperintense, T1-weighted hypointense). The rim of the lesion appears of normal signal. On T2-weighted MRI, the lesion appears homogenous. On T1-weighted MRI, partial sparing of the corticospinal tracts is observed. Proton MRS from the lesion shows NAA reduction and elevated free lipid peaks.

Figure 5.2  Calculated MTR image showing appearances of the lesion within the basis pontis. A profile of MTR values across the lesion is shown, showing maximal MTR reduction in the lesion centre, relative sparing of MTR within the descending corticospinal tracts, radial increase in values from the centre of the lesion and normal values in the outer rim surrounding the lesion.
At twenty weeks there was minimal remaining neurological deficit. Mild heel-toe and limb ataxia were the only identifiable abnormalities. At his last assessment (64 weeks), the only neurological abnormality on full examination was minimal heel-toe ataxia.

Serial MT imaging was performed at 11, 13, 17, 22, 37 and 60 weeks of the neurological illness; proton MRS was performed at 11, 17, 37 and 60 weeks. Results showed gradual and marked recovery in MTR [Table 5.1]. Some pixels with MTR = 0 pu were seen on the study at 22 weeks, suggesting complete loss of macromolecular content. Follow-up study at 60 weeks revealed considerable MTR resolution; all pixels now had MTR values greater than 15.7 pu. There was associated mild pontine atrophy. Improvement in MTR was observed prior to resolution of abnormal signal on the conventional T1- and T2-weighted sequences. Proton MRS at 60 weeks revealed a persistent reduction of NAA/Cr (0.68) [Table 5.1].

<table>
<thead>
<tr>
<th></th>
<th>NAA / Cr *</th>
<th>Mean MTR (pu)**</th>
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<tbody>
<tr>
<td>Healthy controls</td>
<td>1.44 (1.27 - 1.66)</td>
<td>38.8 (38.0 - 39.6)</td>
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<tr>
<td>Case 1:</td>
<td>11 weeks</td>
<td>0.94</td>
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<tr>
<td></td>
<td>13 weeks</td>
<td>-</td>
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<td></td>
<td>17 weeks</td>
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<td></td>
<td>22 weeks</td>
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<td></td>
<td>37 weeks</td>
<td>0.79</td>
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<td></td>
<td>60 weeks</td>
<td>0.68</td>
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<tr>
<td>Case 2:</td>
<td>3 months</td>
<td>-</td>
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<tr>
<td></td>
<td>29 months</td>
<td>1.65</td>
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<tr>
<td>Case 3:</td>
<td>1 week</td>
<td>1.36</td>
</tr>
</tbody>
</table>

* control values for NAA/Cr are derived from 4 healthy volunteers (mean, range of values); ** for patients, average lesion MTR values are presented; the range of pixel MTR values within each lesion are shown in parentheses. Control values are derived from a standardised region of interest in 40 healthy volunteers (mean lesion MTR, 95% confidence interval for the mean)

Table 5.1 MTR and proton MRS data from three patients with central pontine myelinolysis.
Case 2

A 50 year Caucasian woman with a long history of severe alcohol abuse was admitted to hospital with delirium tremens and septicemia. Investigations revealed borderline hyponatraemia (134 mmol/L) and reduced urea (1.8 mmol/L) and creatinine (55 umol/L). Liver function was deranged (bilirubin = 26 umol/L, alanine transaminase = 65 U/L, gamma glutamyl transferase = 605 U/L). Full blood count was normal with the exception of a macrocytosis (107fl) and mild thrombocytopenia (118 x 10^9/L). She was treated with oral diazepam and thiamine, intravenous antibiotics and fluids. After two days, she discharged herself from hospital, following which she maintained abstinence from alcohol. From that time, she experienced progressive difficulties with walking and balance. She became increasingly forgetful and developed peripheral paraesthesiae, ankle oedema, poor appetite and weight loss. Her condition slowly deteriorated and she was admitted to hospital after three months. There was no other significant past medical history.

On admission, systemic examination revealed spider naevi and ankle oedema. She was alert but not fully orientated. Neuropsychological evaluation revealed a severe global deterioration in intellect with specific difficulties for recent memory and formation of new memory; registration was intact. Gait was broad-based and ataxic. Romberg’s test was positive. Cranial nerve examination revealed bilateral non-sustained gaze-evoked horizontal nystagmus but no other abnormality. Tone was increased in the lower limbs, although power was normal throughout. There was limb ataxia, more pronounced in the lower limbs. Reflexes were symmetrically brisk and associated with bilateral extensor plantar responses. Vibration sense was absent in both legs and joint position sense was decreased in the toes. Light touch, pain and temperature sensation were reduced in the hands and feet.

Investigations revealed vitamin B12 deficiency (169 ng/L) and a positive gastric parietal cell antibody (titre 1/10). Biochemical screen showed an isolated reduced creatinine (54 umol/L) with no other abnormality. Cerebral MRI showed a sharply demarcated triangular “trident-shaped” symmetrical lesion in the basis pontis that appeared hyperintense on T2-weighted and hypointense on T1-weighted images. There was no enhancement following 0.1 mmol/kg Gd-DTPA. The descending corticospinal tracts appeared spared. There was mild generalised cerebral atrophy. Cerebrospinal fluid examination was unremarkable. Neurophysiology studies showed evidence of a predominant sensory axonal polyneuropathy.

On the basis of the clinical and MRI findings, a diagnosis of CPM was made (associated diagnoses included alcoholism, B12 deficiency, and possible recent Wernicke-Korsakoff syndrome).
syndrome). Further MRI studies at this stage demonstrated marked MTR reduction within the basis pontis [Figure 5.3, Table 5.1]. The greatest decrease in MTR was at the centre of the lesion, gradually increasing towards normal values in the surrounding rim of pontine tissue. Mean lesion MTR was 14.8 pu (pixel range = 5.9 to 29.5pu). Normal MTR values were found elsewhere in the brain. She was treated with vitamin replacement and made a reasonable recovery.

The patient was reviewed 29 months later. She reported general good health but recently, after a period of two years of abstinence, she had resumed her heavy alcohol intake. Residual problems included mild balance disturbance and persistent forgetfulness. Examination was normal with the exception of poor short term memory, mild gait and upper limb ataxia, extensor plantar responses, and persistent absence of vibration in the lower limbs. Mean lesion MTR had recovered to 31 pu (pixel range 18.6 to 42.9 pu) [Figure 5.3, Table 5.1]. Proton MRS performed at this stage revealed NAA/Cr at the upper end of normal control values (1.65) [Figure 5.4].

**Figure 5.3** T2-weighted axial MRI and calculated MTR images showing appearances of a symmetrical trident-shaped lesion confined to the basis pontis, surrounded by a rim of tissue with normal T2-weighted signal intensity and MTR values. Recovery is seen between the studies performed at 3 and 29 months, with shrinkage of the lesion, decreased T2 hyperintensity, and recovery of MTR values. Proton MRS at 29 months showed normal NAA.
Case 3

A 59 year caucasian lady with a long history of severe alcoholism for many years presented with a progressive neurological disorder during alcohol detoxification. In the year prior to detoxification, she had been diagnosed with mild alcohol-related liver disease. Recently she had been treated with selective serotonin reuptake inhibitors for depression and, for the previous year, she had been prescribed thioridazine for insomnia. Prior to introduction of neuroleptic medication she had noted difficulties with fine manual dexterity and symptoms suggestive of micrographia. These symptoms worsened on thioridazine and for six months she had experienced progressive gait disturbance, with a tendency to shuffle, lose her balance and fall. For a few months she had lost her appetite and experienced incontinence of both urine and faeces. For one week prior to detoxification she was unwell with repeated vomiting. On admission to the centre for alcohol detoxification she was inebriated but able to walk and climb stairs. She was noted to have features suggestive of parkinsonism, with poverty of movement, hypophonia and hypomimia. She was commenced on oral thiamine, phenytoin and a reducing regime of chlordiazepoxide. Despite this medication, she experienced worsening tremor and found it increasingly difficult to stand or walk. There were no seizures. Although clinical signs of dehydration were observed, she received no intravenous fluid replacement. On the third day of detoxification her condition deteriorated, with increased tremor, lower limb weakness, and inability to stand. At this stage she was transferred to hospital.

On admission, she reported symptoms of urinary urgency and hesitancy and was
incontinent of both urine and faeces. On examination, her appearance was dishevelled. Systemic examination revealed dehydration and mild pyrexia. She was unable to stand unsupported and exhibited marked retropulsion. She was alert but appeared slightly disorientated, bradyphrenic and vague. Frontal executive function was impaired and there was evidence of upper limb ideational and ideomotor apraxia. Glabellar tap was abnormal. Bilateral grasp reflexes and a left pout reflex were observed. A parkinsonian “pill-rolling” tremor was noted in the upper limbs and tardive dyskinesia was apparent in her chin and tongue. Hypomimia and hypophonia were apparent. There was full range of eye movements, although initiation of saccades was impaired with mild apraxia. Eye movements were otherwise normal without nystagmus. Blink was markedly reduced without apraxia of eyelid opening. Bilateral weakness of the lower face was observed. In the upper limbs, there was intermittent stimulus sensitive myoclonus. Tone was increased in both the arms (extrapyramidal) and legs (pyramidal). There was moderate bradykinesia of all limbs. Power was normal in the upper limbs but markedly reduced in a pyramidal distribution in the lower limbs. Sustained clonus was present at the knees and ankles. Reflexes were symmetrical and pathologically brisk throughout. Abdominal reflexes were absent. Plantar responses were extensor. There was reduced sensation to light touch and temperature in the hands and feet and subjective reduction of vibration sense at the halluces. There was bilateral reduction in stereognosis. Formal neuropsychological evaluation revealed evidence of widespread cognitive dysfunction, especially visual perception and executive dysfunction, and dyspraxia.

Investigations on admission to hospital revealed normal electrolytes, glucose, calcium, phosphate, B12, folate, and thyroid function. Full blood count was normal with the exception of a macrocytosis (116fL) and mild thrombocytopenia (138 x 10⁹/L). C-reactive protein was elevated (27.6mg/L). Liver function was deranged with elevation of bilirubin (28umol/L), gamma glutamyl transferase (601 IU/L), alkaline phosphatase (148IU/L), and alanine transferase (138IU/L). Chest X-ray was normal. Intravenous normal saline fluid replacement therapy was started within 7 hours of reaching hospital (maximal rate = 125 ml/hour).

Magnetic resonance imaging was performed one week after onset of the neurological disturbance. A sharply demarcated triangular “trident-shaped” symmetrical lesion was demonstrated in the basis pontis that appeared to spare the corticospinal tracts; this appeared hyperintense on T2-weighted and hypointense on T1-weighted images [Figure 5.5]. No enhancement was seen following 0.1mmol/kg Gd-DTPA.
Proton density, T2-weighted, and calculated MTR images depicting a symmetrical, trident-shaped lesion in the basis pontis surrounded by rim of tissue with normal signal characteristics, compatible with central pontine myelinolysis.

A few scattered white matter signal T2 hyperintense lesions were also seen, compatible with small vessel disease. No abnormalities were found in the basal ganglia, substantia nigra or cerebellum. Mean lesion MTR was 10.6pu. Maximal MTR reduction was observed in the centre of the lesion (where pixels were noted with values of 0 pu) with radial increase in values towards the rim, where MTR values were in keeping with normal healthy control values [Figure 5.6].

MTR profile of CPM lesion, demonstrating maximal reduction in lesion centre (where pixels with MTR values of 0 pu are observed) with radial increase to normal values at lesion edge. (a) right-left profile, (b) antero-posterior profile.
Normal MTR values were found elsewhere in the brain. Proton MRS showed NAA/Cr within the range of normal control values (1.36) [Table 5.1; Figure 5.7].

Provisional diagnoses were made of initial neuroleptic-exacerbated idiopathic Parkinsons disease and subsequent CPM (secondary to alcohol withdrawal and nutritional vitamin deficiency). Thioridazine and the antidepressant treatment were stopped. The initial pyrexia settled spontaneously. She was commenced on a dopamine agonist and treated with intravenous thiamine (Pabronex) and oral multivitamins with marked initial improvement. There was continued and almost complete recovery of the cognitive dysfunction, parkinsonism, and quadriparesis over a period of one month. Primitive reflexes disappeared and the dyspraxia resolved.

![Proton MRS demonstrating normal NAA in an acute CPM lesion, 1 week after onset of neurological disturbance. In the same lesion, mean MTR was 10.6 pu (normal range = 36.5 - 40.9 pu).](image)

**Figure 5.7** Proton MRS demonstrating normal NAA in an acute CPM lesion, 1 week after onset of neurological disturbance. In the same lesion, mean MTR was 10.6 pu (normal range = 36.5 - 40.9 pu).

### 5.4 Discussion

Central pontine myelinolysis characteristically occurs in the setting of severe illness, especially when associated with chronic alcohol abuse, malnutrition and/or electrolyte disturbance [Adams *et al.*, 1959; Messert *et al.*, 1979; Menger and Jorg, 1999]. The clinical and pathological features are discussed to allow interpretation of the MRI findings in this study.
Many patients with CPM become profoundly disabled with bulbar palsy, quadriplegia and a locked-in state during the acute phase of the illness. Additional features that were observed in our series included cognitive deficit, myoclonus, parkinsonism, and frontal lobe release signs. These are recognised in CPM, usually but not exclusively as a result of extrapontine involvement in regions such as the cortex, subcortical white matter, mamillary bodies, and basal ganglia [Okeda et al., 1986; Brunner et al., 1988; Salerno et al., 1993]. In the cases described here, there was no evidence from either T2-weighted MRI or calculated MTR images to support extrapontine involvement.

Typical pathological changes of CPM have also been found at post mortem where neurological deficit referable to the pontine lesion has been minimal or absent [Goebel and Herman-Ben Zur, 1972; Slager, 1986; Newell and Kleinschmidt-DeMasters, 1996]. With the advent of MRI, an increasing number of cases are seen in whom the characteristic radiological abnormality has been identified in the presence of minimal clinical disability [DeWitt et al., 1984; Pfister et al., 1985]. Since MRI and computerised tomography (CT) have provided a method for establishing this diagnosis in vivo, the condition is increasingly recognised and patients are observed with significant neurological deficit who make a significant or full recovery [Thompson et al., 1989; Menger and Jorg, 1999].

The pathology is remarkably homogenous: a single large symmetrical lesion is found in the basis pontis. This is typically ovoid, triangular or trident-shaped, with relative or complete sparing of the descending corticospinal tracts [Goebel and Herman-Ben Zur, 1976; Wright et al., 1979]. There is preferential involvement of fibres of transverse rather than longitudinal orientation. Such features have also been recognised in MRI studies [Thompson et al., 1988]. There is usually a rim of intact pontine tissue that is sharply demarcated from the lesion [Adams et al., 1959; Wright et al., 1979]. The characteristic microscopic abnormality is destruction of myelin and oligodendrocytes, with relative preservation of axonal and neuronal cell body integrity [Adams et al., 1959; Wright et al., 1979]. Typically, myelin is most destroyed at the centre of the lesion; the abnormality becomes progressively less pronounced from the median raphe to the lesion edge [Adams et al., 1959; Wright et al., 1979]. If axonal disruption is present, this is usually only minimal but most marked at the lesion centre [Wright et al., 1979]. Inflammatory changes and oedema are absent [Adams et al., 1959; Goebel and Herman-Ben Zur, 1976; Wright et al., 1979]. Blood vessels are typically unaffected [Adams et al., 1959], although acute lesions may show early capillary proliferation [Newell and Kleinschmidt-DeMasters,
Blood-brain barrier disruption may be present in the first few weeks, as evidenced by contrast-enhanced imaging studies [Menger and Jorg, 1999]. Large numbers of macrophages may be seen throughout the lesion and mild astrocytosis may sometimes be documented as an early phenomenon [Newell and Kleinschmidt-DeMasters, 1996].

**MTR as a putative marker of myelin integrity**

From the information available from pathological studies of CPM, it is likely that the large MTR reductions seen in these cases are the result of myelin damage or loss. In particular, the described anatomical distribution of myelinolysis corresponds well with the anatomical MTR variation observed in each of these cases. Further evidence of preserved NAA/Cr in two of the patients studied provides support for maintained axonal integrity. As such, this study provides strong supportive evidence for myelin as the major contributor to MTR values in health.

**MTR and proton MRS to assess the pathological mechanisms and evolution of CPM**

In two of the studied cases, serial MTR data were available. In case one, serial proton MRS data were also available. These data allow insight into the pathological mechanisms involved in the evolution of CPM. Mean lesion MTR was most reduced at the time of first study in all three cases. In case one, the slow and progressive recovery in MTR values mirrored the clinical recovery. Proton MRS showed a moderate reduction in NAA/Cr that was maintained throughout the period of study, even in the presence of minimal residual disability. This is likely to indicate persistent axonal loss; presumably the extent of axonal loss was insufficient to preclude a good clinical recovery. In case two, the clinical recovery was also paralleled by significant, albeit incomplete, recovery in MTR. In this case, proton MRS confirmed NAA at the upper level of the normal range; as such, this suggests maintained axonal integrity.

Improvement in MTR in case one preceded the visible recovery on conventional T1- and T2-weighted sequences. Here, marked tissue destruction was initially observed in pixels within the lesion centre, as demonstrated by the consistent extreme MTR reduction (by as much as 91 to 100%) when compared with healthy control subjects. When almost fully clinically recovered at 60 weeks, the maximal observed MTR reduction within any single pixel was 59%. This recovery in MTR was associated with visible pontine atrophy on the conventional images. It seems likely that the majority of MTR reversal represents remyelination. In the chronic lesion, intense fibrillary gliosis has been observed [Wright et al., 1979]. The proliferation of astrocytes
(with their abundant cytoplasm) in areas of gliosis would be expected to result in increased tissue water content and decreased density of bound protons, with consequent reduction in MTR. Persistent reduction in MTR is likely to result from incomplete remyelination, persistent gliosis and axonal loss (as suggested by the maintained reduction of NAA/Cr in this case); all such factors would be expected to contribute to the associated atrophy.

Lactate was observed in the initial study in one case of CPM, at 11 weeks. In the normal brain, about 15% of glucose metabolism leads to lactate formation; these low levels (<1mmol) are usually undetectable by proton MRS. Pathological conditions with increased glycolytic activity, and to a lesser extent normal cerebral activation, cause rapid saturation of the lactate transport mechanism, resulting in accumulation within the brain [Breiter et al., 1994; Prichard et al., 1991]. Serial proton MRS study of acute demyelinating lesions has reported elevated lactate during the first 6 to 10 weeks [Davie et al., 1994]. Three mechanisms might account for the elevation of lactate in this case. First, it is conceivable that, in large inflammatory lesions, lactate production is increased as a result of ischaemia, where locally raised pressure might impair local circulation. Because of the relative sparing of axons and neurons, most investigators have favoured a toxic-metabolic pathogenesis rather than a vascular process [Adams et al., 1959; Goebel and Herman-Ben Zur, 1972; Wright et al., 1979]. However, some investigators have suggested an ischemic component to CPM with pressure-related disruption of local blood supply to white matter tracts by adjacent vascular gray matter affected by vasogenic oedema. The particular susceptibility of the pons in CPM may result from the grid-like arrangement of longitudinal and transverse myelinated fibre tracts that circumscribe the pontine nuclei and are partly surrounded by a large volume of gray matter [Messert et al., 1979; Okeda et al., 1986]. Secondly, elevated lactate in acute CPM may result from the glycolytic activity of the activated macrophages abundant in CPM lesions [Newell and Kleinschmidt-DeMasters, 1996]. It is of interest that macrophages are probably implicated in prolonged lactate production in MS and in stroke after restoration of normal blood flow [Silver et al., 1997b; Petroff et al., 1992]. Thirdly, lactate may reflect neuronal mitochondrial dysfunction and in this respect the reduction in NAA in this case may be relevant [Brenner et al., 1994].

**Combined MTR and proton MRS in the diagnosis of CPM**

In the presented cases, the conventional MRI appearances are typical of CPM. In each, a symmetrical lesion is confined to the basis pontis, extending from the median raphe to a normal
pontine edge, with relative or complete sparing of the descending corticospinal tracts. Marked MTR reduction has been observed and, in each of the three cases, is maximal at the lesion centre. Magnetization transfer ratio values increase radially towards the edge, where there is a rim of unaffected tissue with normal MTR. In addition, where corticospinal tracts were affected (case one), the degree of MTR reduction was less marked than that of the remainder of the lesion. The distribution of MTR within the CPM lesions studied here is compatible with the characteristic appearances of myelin disruption in CPM [Adams et al., 1959; Wright et al., 1979]. As such, quantitative MRI studies with MTR assessment might be helpful in confirming a diagnosis of CPM.

These markers may provide useful information to help distinguish CPM from the main radiological differential diagnosis of ischemic stroke. The presence of normal NAA associated with significantly reduced MTR in two of the cases supports the presence of relatively isolated demyelination; in stroke, indiscriminate tissue damage would be expected to result in combined NAA and MTR reduction.

Proton MRS and MTR to assign prognosis in CPM and other demyelinating diseases

The presence of normal NAA suggests little if any significant axonal disruption in acute CPM. As such, this suggests NAA may serve as a useful prognostic marker if levels are not obviously reduced. However, the relatively wide range of NAA values in health makes it difficult to be certain of the absence of axonal damage in such cases. In addition, we have seen excellent clinical recovery even in the presence of NAA reduction, albeit to a moderate degree. As such, the presence of persistent low NAA does not necessarily imply a poor outcome, even in the presence of severe neurological deficit. Further studies are required to confirm the usefulness of NAA as a prognostic marker in acute CPM.

The findings of this study highlight some of the difficulties that may be associated with the use of myelin markers for prognosis in demyelinating diseases. The ability to remyelinate lesions in MS has been well described [Prineas and Connell, 1979]. Remyelination has not been reported in pathological studies of CPM, although recovery in auditory evoked potentials is suggestive [Paja et al., 1993]. The considerable degree of clinical recovery and parallel MTR increase that we have observed in CPM are extremely suggestive of remyelination. Very low values of MTR were observed in all of these cases during the period of early neurological impairment. Full or almost full clinical recovery was paralleled by substantial, albeit incomplete,
recovery in MTR. Study of CPM allows valuable insight as the condition is associated with a single lesion that (a) is of remarkably homogenous pathology, and (b) is sited in a clinically eloquent site. Persistent significant abnormalities of neuronal conduction related to this lesion should be relatively easy to detect clinically.

**MTR and NAA as independent markers of myelin and axonal integrity**

A wide range of values of MTR is seen in MS lesions, and a modest correlation exists between lesion MTR and disability [Gass *et al.*, 1994]. In patients with secondary progressive MS, a strong correlation is seen between MTR and NAA, supporting the hypothesis that demyelination and axonal loss occur together in the same chronic multiple sclerosis lesions [Davie *et al.*, 1999]. Hiehle and colleagues [1994] failed to find such a correlation in an earlier study, where the majority of patients studied had relapsing-remitting disease.

In this report, we have observed marked MTR reduction in two cases of CPM, where NAA appears normal. These findings therefore provide support for the roles for MTR and NAA as independent markers of myelin and axonal integrity. This is of interest to the future study of MS, where independent putative markers for demyelination and axonal loss may provide useful information about the natural history and response to treatment.

In conclusion, these data provide evidence to support the hypotheses that (a) myelin forms the predominant contribution to MTR values in healthy white matter, (b) demyelination per se can result in severe MTR reduction, (c) profound MTR reduction may be seen in conditions associated with little if any neuronal damage, (d) MTR and NAA provide relatively independent markers of myelin and axonal pathology, and (e) MTR and NAA may provide useful diagnostic information in CPM. Whereas normal NAA may help assign prognosis in CPM, the role of MTR appears less certain. Further work is required to clarify the abilities of MTR and NAA to provide prognostic information in MS and other CNS demyelinating diseases.
CHAPTER 6
A study to define the pathological evolution of new MS lesions

6.1 Introduction

While the pathological features of MS are well described (demyelination, relative axonal preservation, inflammation and gliosis) [Dawson 1916; Prineas and McDonald, 1997], the precise sequence of events relating to the formation and subsequent evolution of new lesions is less clear. Increased understanding of the relationship between such pathological processes and resulting clinical deficit will be of considerable importance in developing and assessing potential new therapies. Magnetic resonance imaging provides a safe and useful tool for following the natural history of MS. Conventional MRI sequences are extremely sensitive for detecting pathological changes in MS [Young et al., 1981]. They are however limited by their inability to differentiate the individual pathological features.

Measurement of MTR provides an indication of macromolecular structure present in a given volume. A loss of such structure with consequent MTR reduction is expected in disorders of the central nervous system where demyelination or Wallerian degeneration are prominent [Dousset et al., 1992; Dousset et al., 1994; Dousset et al., 1995; Dousset et al., 1997; Lexa et al., 1994; Thorpe et al., 1994; Silver et al., 1996; Kimura et al., 1996; Hanover et al., 1997].

Gadolinium enhancement detects blood-brain barrier breakdown and inflammation in new MS lesions [Hawkins et al., 1990; Kermode et al., 1990a; Katz et al., 1993]. Monthly studies have shown that blood-brain barrier breakdown precedes or coincides with new areas of signal change on proton density and T2-weighted images in relapsing-remitting and secondary progressive MS and the presence of such enhancement appears more frequent during periods of clinical activity [Thompson et al., 1991; Thompson et al., 1992; Gonzalez-Scarano et al., 1987; Smith et al., 1993; Frank et al., 1994].

This study addresses crucial questions regarding the pathogenesis of MS lesions:
(1) What is the primary event in the formation of a multiple sclerosis lesion, blood-brain barrier breakdown with inflammation or primary myelin disruption and demyelination stimulating a secondary blood-brain barrier leak with inflammation?
(2) Does inflammation (as evidenced by the duration of blood-brain barrier breakdown) influence the early structural changes associated with new MS lesions that might contribute to clinical deficit (e.g. demyelination)?

To help answer these questions, weekly studies have been performed for a period of 3 months using both gadolinium contrast-enhanced T1-weighted MRI and MT imaging.

6.2 Methods

Subjects Patients with clinically definite relapsing-remitting or secondary progressive multiple sclerosis [Poser et al., 1983] were recruited from the population of patients attending the outpatient department of the National Hospital for Neurology and Neurosurgery. Of the 11 patients who underwent an initial screening examination, only three (one with relapsing-remitting and two with secondary progressive multiple sclerosis) showed gadolinium enhancing lesions, fulfilling the criteria to proceed with the serial study. The entrance criteria were designed to maximise the yield of enhancing lesions during the study.

Clinical evaluation was undertaken by a single observer, with assessment at study entrance and exit using the EDSS and Kurtzke’s functional score [Kurtzke 1983].

MRI Protocol All subjects underwent serial weekly study with both MT and contrast-enhanced T1-weighted imaging over a period of 3 months. Standard repositioning techniques were used for all patient studies [Gallagher et al., 1997]. A set of three T1-weighted pilot scans (sagittal, axial and coronal) was initially performed to allow accurate repositioning for slice selection. For all imaging, sequences allowed the acquisition of 16 contiguous 5mm axial oblique slices (field of view 24cm, matrix 256 x 128).

At the initial MRI assessment, T1-weighted imaging was performed before and after a bolus intravenous injection of 0.1mmol/kg Gd-DTPA using a spin-echo sequence (TR=500ms, TE=14ms, 2 Nex). For all follow-up studies, post-contrast T1-weighted imaging was used without the additional acquisition of pre-contrast images. At each visit, proton density, T2-weighted and calculated MTR images of the brain were obtained prior to Gd-DTPA using an interleaved dual spin echo sequence (TR=1500ms, TE=32/80ms, 3/4 Nex). For presaturation, a Hamming-apodised 3 lobe sinc pulse (duration 64ms, peak amplitude 14.6 uT, nominal bandwidth 62.5Hz) was applied 2kHz off water resonance.
For each patient, the proton density images obtained at weekly intervals were co-registered using multi-slice registration software [Symms et al., 1997]. This applies a histogram-matched multi-scale approach to Woods' Automated Image Registration routines [Woods et al., 1992] to provide robust alignment of MRI data. Errors that can be caused by the relatively thick slices are reduced by the use of radiographical pre-positioning. The registration parameters obtained from proton density images were automatically applied to the calculated MTR images obtained from the same imaging dataset, allowing the inherent co-registration between proton density and calculated MTR images to be maintained during such post-processing.

Serial post-gadolinium T1-weighted images were displayed on a Sun workstation and assessed to determine the time of initial enhancement and the duration of visible lesion activity. Lesions were designated as showing "definite enhancement" only if there was a corresponding lesion noted at some stage on the proton density and T2-weighted series. For each active lesion, a corresponding ROI was applied to include the maximum area of signal change seen on the full series of co-registered proton density images. Serial mean MTR measures were obtained for each lesion by applying this ROI to the full series of registered calculated MTR images. This approach not only allowed accurate ROI placement and MTR measurement in visible lesions, but also allowed measurements to be obtained in regions of "normal appearing" tissue prior to their first appearance.

The primary event in the formation of a new lesion

To determine the relationship between blood-brain barrier breakdown (indicated by gadolinium enhancement) and MTR, only those lesions that started to enhance after the baseline study were included in the analysis. Lesions with an area smaller than 20mm² were excluded from this particular analysis, as were those adjacent to CSF spaces (to avoid potential measurement errors caused by slight misregistration or partial volume averaging effects).

Duration of gadolinium enhancement and maximal MTR reduction

To determine the relationship between the duration of gadolinium enhancement and the lesion maximal MTR reduction (as compared with the immediate pre-enhancement value), only lesions with a defined time course of enhancement (i.e. starting and ceasing to enhance within the study duration) were included in the analysis. For this analysis, lesions of all sizes were evaluated, although those adjacent to CSF spaces were excluded to avoid potential errors (as
Statistical analysis was carried out using a paired t-test to determine significant changes in lesion MTR related to gadolinium enhancement and Spearman’s Rank Correlation Coefficient (SRCC) to determine the relationships between duration of enhancement, maximal MTR reduction and maximal lesion area. For all such tests, a level of \( p<0.05 \) was taken to designate statistical significance.

6.3 Results

Patient 1 (aged 38 years) had relapsing-remitting MS. The EDSS score at study entrance was 6 and remained unchanged at exit despite a mild relapse in the fourth week. Patient 2 (aged 35 years) had secondary progressive MS. The initial EDSS score was 6, before worsening during the period of study to a score of 7. Patient 3 (aged 40 years) had secondary progressive multiple sclerosis. She remained clinically stable throughout the study and her EDSS was 7 at entrance and exit.

The primary event in the formation of a new lesion

Overall, in the three patients, 48 lesions enhanced during the period of study. Ten of these lesions were noted to be enhancing at study onset and these were therefore excluded from further analysis. All of the remaining lesions were associated with corresponding high signal on proton density and T2-weighted images at or after initial enhancement and none had pre-existing high signal. Twelve of the remaining 38 lesions had an area smaller than 20mm\(^2\) and three lesions were contaminated by partial volume with CSF; these were therefore also excluded. The mean area of the remaining 23 lesions that were included for this analysis was 40.6mm\(^2\).

For these 23 lesions, mean MTR decreased significantly during the week in which enhancement was first noted (29.6pu pre-enhancement vs. 28.2pu at initial enhancement, 23 lesions, \( p<0.001 \) paired t-test) [Figure 6.1].

However, no significant change in MTR was noted over the week preceding this (29.0pu two weeks before initial enhancement vs. 29.4pu one week before initial enhancement, 23 lesions, paired t-test). Following initial enhancement there was a steady and significant decline in mean lesion MTR for a further three weeks (28.1pu to 25.5pu, 19 lesions, \( p<0.001 \) paired t-
To determine the nature of MTR change within the acute lesion, a single large lesion (101 mm$^2$) that first enhanced at week 2 and continued to enhance throughout the study was evaluated [Figure 6.2]. The initial appearance on T1-weighted images was of a small homogenous enhancing region seen for two weeks; on later studies this enlarged with ring-enhancement of the advancing edge; this was first seen at three weeks following initial enhancement and was associated with concurrent cessation of enhancement in the older centre. Two ROI's were identified: a ring related to the corresponding area of ring-enhancement on T1-weighted images and a central ROI corresponding to the initial central area of enhancement. A small decrease in MTR was first seen in the centre of the lesion alone during the first week following initial enhancement (31.0 pu to 30.29 pu) [Figure 6.3].
Figure 6.2 Images showing the initial appearances of a large new enhancing lesion: (a) Post-gadolinium T1-weighted image showing a new lesion with focal enhancement, (b) Post-gadolinium T1-weighted image two weeks later showing the new appearance of ring-enhancement, (c) T2-weighted image corresponding to onset of ring-enhancing appearance, (d) Calculated MTR image corresponding to onset of ring-enhancing appearance.
Figure 6.3 Change in MTR in the central region and peripheral rim during the initiation and evolution of a large ring-enhancing lesion.

The most notable decreases in MTR were seen in the centre of the lesion and between one and three weeks following initial enhancement (to 21.6pu in the second and 12.9pu in the third week). Following this, a continued slower decline in MTR was observed in the lesion centre despite the cessation of enhancement in this region, reaching a minimum of 7.4pu (equivalent to 24% of the pre-enhancement value) five weeks after initial enhancement. The rim of the lesion showed no obvious decrease in MTR until two weeks after the initial appearance of central homogenous enhancement. This initial decrease in lesion rim MTR occurred later than in the centre of the lesion. A further decrease in MTR was seen in the rim, albeit at a slower rate than the lesion centre, reaching a minimum of 15.1 pu (equivalent to 48% of the pre-enhancement value) five weeks after initial enhancement. From the sixth week following initial enhancement until the final study, there was a gradual recovery in MTR both centrally and in the rim, reaching values of 19.9 pu and 26.0 pu respectively (equivalent to 63% and 82% recovery). In general, I observed that larger lesions and those showing evidence of ring-enhancement tended to be associated with greater decreases in MTR; in these cases in particular, this was often followed by a considerable gradual recovery [Figure 6.4].
Onset of enhancement

Figure 6.4  Mean lesion MTR (pu) for all lesions greater than 45 mm$^2$ (mean = 71.6 mm$^2$), showing a greater decrease in MTR and more significant resolution than seen with smaller lesions.

Duration of gadolinium enhancement and maximal MTR reduction

Of the 48 lesions seen to enhance during the period of study, 25 had a defined time course of enhancement and fulfilled the criteria for this particular analysis.

The mean duration of gadolinium enhancement was 3.7 weeks (standard deviation = 1.9 weeks; range = 1 - 8 weeks). The mean value for maximal lesion area was 26.2 mm$^2$ (standard deviation = 14.9 mm$^2$; range = 11.4 - 74.7 mm$^2$). The mean maximal MTR reduction was 3.3 pu (standard deviation = 2.2 pu; range = -0.2 - 9.2 pu). This corresponded to a mean percentage reduction of 11.1% compared with pre-enhancement MTR values (standard deviation = 7.3%; range = -0.7 - 30.9%).
A significant correlation was seen between the duration of gadolinium enhancement and maximal MTR reduction for all lesions (SRCC=0.52, \( p=0.008 \)) [Figure 6.5].

![Figure 6.5](image)

*Figure 6.5* Scatter plot showing the relation between the duration of gadolinium enhancement and maximal lesion MTR reduction (SRCC=0.52, \( p=0.008 \)).

A significant correlation was also seen between maximal lesion area and maximal MTR reduction (SRCC=0.41, \( p=0.04 \)) [Figure 6.6]. No significant correlation was observed between the duration of gadolinium enhancement and maximal lesion area (SRCC=0.28, \( p>0.05 \)).
Figure 6.6 Scatter plot showing the relation between maximal lesion area (as seen on the series of proton density-weighted images) and maximal MTR reduction (SRCC=0.41, p=0.04).

6.4 Discussion

Previous studies have either correlated MRI with pathological changes apparent at biopsy or post-mortem [Nesbitt et al., 1991; Katz et al., 1993], assessed differences in MTR between enhancing and non-enhancing lesions [Hiehle et al., 1995, Campi et al., 1996; Petrella et al., 1996], or evaluated change in MTR following enhancement [Lai et al., 1997; Alonso et al., 1997]. This is one of the first studies to assess MTR in an area of normal-appearing white matter and follow the changes as this evolves into a new MS lesion.

In the three patients studied, there was no instance of a detectable decrease in MTR prior to gadolinium enhancement. Following the first recognition of enhancement, there was a gradual
decrease in MTR for most lesions over a period of a few weeks. Not infrequently, this was followed by significant recovery, although prolonged follow up (beyond three months) was not performed. In addition, considerable recovery in MTR was shown to occur despite continuing evidence of blood-brain barrier breakdown, as evidenced by persisting enhancement. The mechanisms and significance of these findings are now discussed.

The primary event in the formation of a new MS lesion

It is clear from MRI studies that vascular events are prominent in the early evolution of MS lesions [McDonald et al., 1992]. Such studies have not resolved whether these events represent a primary and obligatory event for the initiation of an acute lesion, or whether they represent an epiphenomenon related to perivascular myelin breakdown initiated by some independent process [Calder et al., 1989]. In the peripheral nervous system, Wallerian degeneration may be associated with an increase in blood-nerve barrier permeability, suggesting that vascular events may occur secondary to either axonal or myelin breakdown [Seitz et al., 1989; Latker et al., 1991]. However, I am not aware of comparable data for the central nervous system and in cases of lysolecithin- or cuprizone-induced experimental demyelination, there is no related inflammation or blood-brain barrier deficit [Bakker et al., 1987; Dousset et al., 1995]. Retinal studies in acute optic neuritis associated with MS provide evidence that inflammatory lesions with blood-retinal barrier breakdown can occur in unmyelinated regions, suggesting that myelin breakdown need not necessarily be a prerequisite to inflammatory changes associated with the vascular endothelial barrier in the central nervous system [Lightman et al., 1987]. While the retina and brain share many similarities in their normal vascular structure and function and both undergo similar pathophysiological change in MS, these studies suggest but can not establish that new focal brain lesions are initiated by vascular events. In the present study, no observable change in MTR occurred prior to the first detectable gadolinium enhancement within MS lesions; the lack of change in MTR suggests that no significant demyelination develops in the weeks prior to blood-brain barrier breakdown.

A separate analysis of 15 regions of white matter that appeared "normal" on the baseline study and which subsequently evolved into lesions was carried out, comparing the mean MTR
from these areas with that of 15 equivalent contra lateral regions of "normal-appearing" white matter that remained unaffected throughout the study period. No significant differences were seen between regions that either did or did not evolve into lesions (30.3 pu vs. 29.9 pu; p>0.05, Wilcoxon matched pairs signed rank sum test). Thus, whilst it remains possible that a minor degree of demyelination is diffusely present in areas of "normal-appearing" tissue, it is unlikely that this specifically predisposes to subsequent focal breakdown of the blood-brain barrier.

The results do not preclude a mild degree of myelin disruption or damage (i.e. beyond the detectability of MTR measurement) or other subtle changes (e.g. microglial activation) as early events preceding blood-brain barrier breakdown. The report of apparent lipid peaks observed with proton magnetic resonance spectroscopy prior to the appearance of gadolinium-enhancing lesions could indicate early myelin breakdown [Narayana et al., 1998]. Also, recent serial studies using MTR or diffusion-weighted imaging have reported subtle alterations in the normal-appearing white matter preceding a lesion’s appearance [Filippi et al., 1998b; Pike et al., 1999; Werring et al., 2000b]. However, subtle changes in the blood-brain barrier may not be readily apparent even when the most sensitive of qualitative contrast-enhanced MRI techniques are used and quantitative studies have suggested the possible presence of small or subtle widespread foci of blood-brain barrier in the otherwise “normal-appearing” white matter [Silver et al., 1997c; Silver et al., 1999b]. The observations in the current study suggest that a major degree of demyelination occurs only after there is a frank disruption of the blood-brain barrier.

The evolution of an acute lesion

The major features of acute MS lesions include inflammatory infiltrate associated with blood-brain barrier breakdown and oedema, active demyelination and variable axonal loss; considerable remyelination may also be present [Lassman et al., 1994]. Chronic lesions are typically demyelinated (although remyelination is sometimes evident) with dense gliosis, selective oligondendrocyte damage and axonal loss to a variable but sometimes profound degree [Nesbitt et al., 1991; Lassman et al., 1994; Barnes et al., 1991].

Following initial gadolinium enhancement, a steady decrease in MTR was observed in the majority of lesions over approximately one month. Anecdotally, greater decreases in MTR
were observed in larger lesions and/or those with ring-enhancement. One such lesion which showed initial focal central enhancement at onset, followed by the appearance of ring-enhancement two weeks later that persisted for a further 10 weeks, provides insight into the pathological sequence of events. An initial decrease in MTR was seen only in the centre of the lesion, corresponding to the region where enhancement was first seen on gadolinium-enhanced images. During the first week following the initial appearance of enhancement only a mild reduction in MTR was seen (<4% of the initial value). This corresponds to the mild reductions reported in lesions where oedema occurs in the absence of demyelination or axonal loss [Dousset et al., 1992; Mehta et al., 1996]. Such changes support the hypothesis that the initial events in the formation of an acute lesion are inflammatory changes associated with opening of the blood-brain barrier. A more significant decrease in MTR was observed in the centre of the lesion over the following four weeks (76% reduction compared with pre-enhancement values). This reduction suggests a significant loss of structure within the lesion, more than could be accounted for by oedema alone. These findings are in agreement with pathological studies showing significant central demyelination within acute lesions. Whilst it is also possible that a loss of axonal integrity is contributing to this MTR reduction, studies of diffuse axonal injury caused by traumatic brain injury have shown only mild reductions in MTR [Kimura et al., 1996; Hanover et al., 1997]. It is therefore likely that, following an initial central inflammatory response to blood-brain barrier breakdown, demyelination occurs and these processes spread radially, as evidenced by the later but still substantial reductions in the rim of the lesion (52% reduction compared with pre-enhancement values). The rim may not reach such low MTR values as the centre for various possible reasons; (1) demyelination is less pronounced, perhaps because oligodendrocytes tend to be more preserved at the edge of the acute lesion, (2) oedema may be less marked away from the central area of the lesion, (3) remyelination is more prominent than at the centre of the lesion [Prineas and Connell, 1979], (4) macrophages containing myelin debris tend to accumulate at the lesion edge and these, being structural elements, might be expected to maintain MTR values to a certain degree, or (5) it is possible that the method of analysis is affected to some extent by partial volume effects with normal surrounding tissue.
*The resolving lesion*

It is not possible to comment on the long term evolution in the whole group of lesions, as only a small number had prolonged assessment up to 12 weeks following initial enhancement and there may well be remodelling or repair for a longer period. However, some lesions did show a prominent gradual recovery in MTR, although not usually to pre-enhancement values. The findings of the present study are in agreement with longer term follow-up MT imaging studies, where it has been suggested that various factors including remyelination, resolution of oedema and possibly gliosis may all to some degree be responsible for the significant but incomplete resolution of MTR values seen in large MS lesions [Lai et al., 1997; Alonso et al., 1997]. However, the relative contributions of these processes to the recovery in MTR remains uncertain.

*Duration of gadolinium enhancement and maximal MTR reduction*

A significant correlation has been observed between the duration of enhancement and maximal MTR reduction in new MS lesions. For these same lesions, a smaller correlation was seen between maximal MTR reduction and maximal lesion area. For a region of interest analysis approach, as used in this study, it might be expected that mean MTR measurements from smaller lesions would be affected to a greater degree by partial volume with adjacent unaffected tissue; this bias would be expected to result in small lesions showing less noticeable MTR reduction. Such a bias could possibly contribute to the significant positive association observed between maximal MTR reduction and maximal lesion area. However, the lack of a significant association between maximal lesion area and duration of gadolinium enhancement suggests that, at least in this sample of lesions, the effect of enhancement duration on MTR reduction is relatively independent of such bias.

A wide spectrum of pathological findings exists within MS lesions, most of which are likely to increase the average water content and consequently reduce MTR values. Whilst small reductions in MTR appear relatively non-specific, the large reductions shown to relate to more persistent blood-brain barrier disruption seen in this study are likely to indicate a significant degree of demyelination [Dousset et al., 1992; Dousset et al., 1994; Dousset et al., 1995; Dousset et al., 1997; Lexa et al., 1994; Thorpe et al., 1994; Silver et al., 1996].
It has previously been shown that immune-modifying agents such as the beta-interferons are able to reduce (1) the occurrence of focal gadolinium contrast-enhancing lesions and (2) the development of permanent disability in MS [European Study Group on interferon beta-1b in secondary progressive MS 1999; Rudick et al., 1997b; Simon et al., 1998]. Here, a positive association is shown between the duration of gadolinium enhancement and reduction in tissue structural integrity that is suggestive of demyelination. It is therefore possible that certain immune-modifying agents are able to modify the clinical course and reduce the progression to fixed disability by reducing the severity or duration of inflammation that might otherwise subsequently result in longstanding structural changes (e.g. persistent demyelination and axonal loss) within MS lesions. The potential mechanism can be explored by a serial study of the MTR outcome of new enhancing lesions which develop in patients on disease modifying treatments.
CHAPTER 7

Measurement of MTR in the spinal cord in MS

7.1 Introduction

Magnetic resonance imaging readily detects the lesions of MS and is increasingly used to help assess experimental therapies [Young et al., 1981; Miller et al., 1996]. However, a consistent correlation between the extent of brain MRI abnormalities and disability has not been found [Koopmans et al., 1989; Thompson et al., 1990; Thompson et al., 1992]. This may, in part, be a consequence of the poor pathological specificity of conventional proton density and T2-weighted images [Miller and McDonald, 1994]. The likely pathological substrates of functional deficit in MS are demyelination and axonal loss [Miller and McDonald, 1994]. As discussed in chapter five, myelin is the major contributor to MTR values in healthy white matter. It has been shown that MTR is reduced to a greater or lesser extent within MS lesions [Dousset et al., 1992; Gass et al., 1994; Tomiak et al., 1994], and that the degree of reduction shows a moderate correlation with clinical deficit [Gass et al., 1994].

A large part of the locomotor disability and sphincter disturbance often seen in MS is due to lesions in the spinal cord. At post mortem, multiple intrinsic cord lesions are found in most patients with MS; these are more common in the cervical region [Fog, 1950; Oppenheimer, 1978]. To date, an association between cord lesions, as detected by MRI, and disability has not emerged [Kidd et al., 1993]. There is however a relationship between cord atrophy and disability, suggesting that disability occurs when there is demyelination and axonal loss [Losseff et al., 1996a].

This work details the novel application of MT imaging to the cervical spinal cord to assess whether it is feasible to measure MTR in this clinically eloquent area and determine whether spinal cord MTR values are influenced by pathological changes commonly seen in MS.
7.2 Methods

Subjects Twelve patients with definite MS [Poser et al., 1983] were recruited from the population of patients attending the outpatient department of the National Hospital for Neurology and Neurosurgery (8 female, 4 male; average age 34.7 years, range 24-52 years). Of these, 7 patients had relapsing-remitting and 5 secondary progressive MS [Poser et al., 1983]. None of the patients studied had suffered a clinical relapse within the preceding month. Clinical evaluation was undertaken by a single observer (NCS) using the EDSS and Kurtzke’s functional score [Kurtzke, 1983]. In addition, 12 age- and sex-matched normal controls were studied (8 female, 4 male; average age 30.3 years, range 24-50 years). Of note, no control subjects had a history of previous neurological disease, spinal cord injury or degenerative disc disease.

MRI data acquisition All imaging was performed using a Signa 1.5 T superconducting system with local phased-array receiver coils (GE Medical Systems, Milwaukee). All subjects underwent identical cervical spine MT imaging protocols. Initially, a sagittal fast spin echo of the spine was performed (TR=2500ms, TE$_{ef}$=102ms, 3mm contiguous slices, acquisition time 2.5 minutes), from which the MT sequence was prescribed. These scans also provided T2-weighted cervical spine images to allow both exclusion of significant degenerative disease and analysis of intrinsic signal abnormality in the patient group. For MT imaging, scans were centred at the level of the second cervical vertebra, the middle slice passing through the centre of the spinal cord. For acquisition of MTR data, a sagittal fast spin echo sequence (TR=1600ms, TE$_{ef}$=17ms, 256 x 192 matrix, echo train length 6, 8 excitations, 5mm contiguous slices, acquisition time 17.7 minutes) was performed with and without the application MT presaturation pulses. For MT presaturation, a 3-lobe Hamming apodised sinc pulse of 20ms duration and with an amplitude equivalent to a flip angle of 1430 degrees was applied 1 Khz off water resonance. For accurate quantitation of MTR in the spinal cord, it is necessary to overcome or avoid problems resulting from motion or deformation of the spinal cord that may arise between data acquisition with and without MT presaturation. The effect of such motion may be overcome by using a spin echo based sequence with interleaved MT pulses [Barker et al., 1996]. However, spin echo sequences are relatively slow and a low spatial resolution is required if prohibitively long data acquisition times are to be avoided. An interleaved fast spin echo sequence produces slightly lower MT contrast than a
corresponding SE based sequence but data acquisition is much faster. The time saved can be used both to increase the resolution and to increase the number of excitations and thus improve the SNR of the calculated MT images. To assess MTR within the cervical spine, the effects of B1 field inhomogeneity require consideration, as discussed in chapter three. Such problems are avoided here, as the main body coil transmits the MT saturation pulse (rather than the local spine phased array coil that is used to receive signal).

**MRI data analysis** Inherently co-registered calculated MTR images were derived, displayed and analysed using a Sun workstation. All MTR analysis was carried out blinded to subject identity, demographic and clinical details. To assess intrarater reproducibility for measurement of MTR, all calculated MTR images were re-evaluated on a separate occasion (more than 3 days apart).

An elliptical ROI measuring between 114 and 121 mm² was carefully positioned along the central sagittal slice of cord. In all cases the lower limit of this ROI was placed opposite the intervertebral disc at C2/3 so that alignment of the long axis was parallel to the cord [Figure 7.1]. To minimise partial volume averaging effects, at least 2 pixels separated the elliptical ROI from the visible edge of the cord, as seen on the calculated MTR image. Mean cervical cord MTR values were obtained for each subject.

In the patient group, T2-weighted images were analysed by Dr Mary Gawne-Caine, who blinded to all clinical and demographic details. These were evaluated for evidence of degenerative spinal disease, and for evidence of intrinsic signal abnormality at the level of the second cervical vertebra (corresponding to the ROI from which MTR measurements were made).
Statistical analysis Data from patient and control groups were compared using Levene's test for equality of variances and the Mann-Whitney U test. Intrarater measurement:re-measurement reproducibility was assessed by analysis of covariance.

7.3 Results

Ten of the twelve patients showed evidence of high signal lesions on T2-weighted scans in the region from which MTR was measured [Table 7.1]. No significant degenerative disc disease was noted.

The patient group exhibited a wide range of disability, ranging from EDSS 2.0 to 8.0. Values for mean MTR according to EDSS and patient disease subgroup are shown [Table 7.1].
Table 7.1  Cervical cord mean MTR, EDSS, disease subgroup and T2 appearances in patients.

<table>
<thead>
<tr>
<th>Patient</th>
<th>MS subgroup*</th>
<th>EDSS</th>
<th>Mean MTR (%)</th>
<th>T2-weighted image</th>
</tr>
</thead>
<tbody>
<tr>
<td>1</td>
<td>RR</td>
<td>4.0</td>
<td>19.2</td>
<td>normal</td>
</tr>
<tr>
<td>2</td>
<td>RR</td>
<td>4.0</td>
<td>19.1</td>
<td>lesion</td>
</tr>
<tr>
<td>3</td>
<td>SP</td>
<td>8.0</td>
<td>19.0</td>
<td>lesion</td>
</tr>
<tr>
<td>4</td>
<td>RR</td>
<td>3.0</td>
<td>19.0</td>
<td>lesion</td>
</tr>
<tr>
<td>5</td>
<td>RR</td>
<td>3.5</td>
<td>18.8</td>
<td>normal</td>
</tr>
<tr>
<td>6</td>
<td>SP</td>
<td>6.0</td>
<td>18.4</td>
<td>lesion</td>
</tr>
<tr>
<td>7</td>
<td>RR</td>
<td>3.0</td>
<td>17.5</td>
<td>lesion</td>
</tr>
<tr>
<td>8</td>
<td>SP</td>
<td>8.0</td>
<td>17.4</td>
<td>lesion</td>
</tr>
<tr>
<td>9</td>
<td>RR</td>
<td>2.0</td>
<td>17.4</td>
<td>lesion</td>
</tr>
<tr>
<td>10</td>
<td>SP</td>
<td>5.5</td>
<td>17.2</td>
<td>lesion</td>
</tr>
<tr>
<td>11</td>
<td>RR</td>
<td>5.5</td>
<td>17.0</td>
<td>lesion</td>
</tr>
<tr>
<td>12</td>
<td>SP</td>
<td>8.0</td>
<td>16.5</td>
<td>lesion</td>
</tr>
</tbody>
</table>

* RR = relapsing-remitting, SP = secondary progressive

The patient and control groups were compared using Levene's test for equality of variances (F=19.1, p<0.0005). Because of the significant difference in the distribution of mean MTR values between the 2 groups, the data was further analysed using non-parametric tests. The median value for mean cord MTR in the patient group was 17.95 pu (interquartile range 17.25-19.00), compared with the control group where the median value for mean MTR was 19.30 pu (interquartile range 19.05-19.55) [Figure 7.2]. The differences between patient and control groups were significant (p<0.0005), Mann-Whitney U test), with significantly lower mean cervical spinal cord MTR values in MS. Two of the 12 patients had values for mean MTR within the interquartile range of normal controls, with one of these patients having coincident T2 high signal in this region. Intrarater variability for MTR measurement was 0.25% in the control group and 0.6% in the patient group. No significant correlation was noted between disability (EDSS) and mean cervical cord MTR.
7.4 Discussion

Multiple sclerosis commonly affects the spinal cord [Fog, 1950; Oppenheimer, 1978; Kidd et al., 1993]. The commonest site of spinal cord involvement is the cervical region, as demonstrated histologically at post mortem and in vivo using MRI [Oppenheimer, 1978; Kidd et al., 1993]. The presence of T2-weighted MRI spinal cord lesions has not been shown to correlate with disability [Kidd et al., 1993]. Pathological evidence of demyelination may be found in the cervical spinal cord at post mortem despite an apparent absence of neurological deficit during life [Ghatak et al., 1974]. In vivo studies using MRI and somatosensory evoked potentials have shown that, even in the presence of appropriately placed lesions, there may be no
demonstrable clinical or electrophysiological deficit [Turano et al., 1991]. There is however a significant association between cervical cord atrophy and disability [Kidd et al., 1993; Losseff et al., 1996], invoking permanent loss of neurons or myelin as a cause of persistent clinical deficit.

These observations raise important questions about the relationship between MRI findings, pathology and disability in MS. Disability in MS probably results partly from demyelination (with relative preservation of axons); this in turn causes conduction block [Bostock and Sears, 1978]. Demyelinated fibres may however restore conduction with insertion of sodium channels in the internodal membrane [Moll et al., 1991]. Chronic, irreversible disability in MS may therefore in a large part be due to loss of the nerve fibre itself- a finding that has been consistently noted in both active and chronic lesions at post mortem [Lassmann et al., 1994; Ferguson et al., 1997; Trapp et al., 1998].

The weak relationship between T2 lesion load and disability both in the brain and spinal cord may be due to the low pathological specificity of T2-weighted imaging. In particular, the extent of demyelination or axonal loss can not be quantified. On the other hand, these pathologies would be expected to result in cord atrophy, and the close relationship of the latter with disability makes good sense.

The loss of macromolecular structure associated with demyelination and/or axonal loss might also be expected to substantially reduce MTR. It is therefore perhaps surprising that despite showing cervical cord mean MTR to be significantly reduced in patients with MS, this reduction does not clearly relate to disability. Some significantly disabled patients had mean MTR values compatible with normality, but other patients with remarkably little clinical deficit showed marked reduction in this measurement. However, on closer consideration, this lack of association is perhaps, for several reasons, not too surprising. First, there is a moderate range of normal values for cervical cord mean MTR (ranging from 18.6 to 20.4 pu), thereby making cross-sectional analysis of such small numbers of subjects difficult to interpret. A further study involving a larger cohort will be of interest. Second, demyelination alone (with preserved axons) could conceivably cause a substantial MTR reduction and still be compatible with no associated functional disability. In other words, the relative effects of axonal loss versus demyelination alone on MTR reduction may not correspond to their individual contributions to clinical deficit.
Thirdly, other pathologies such as oedema and gliosis, which occur in MS lesions, may exert an effect on MTR independent of the extent of demyelination or axonal loss. Fourthly, the ROI will have included central gray matter as well as white matter tracts - a relationship with disability might be more apparent if white matter tracts alone could be studied. Finally, MTR reduction may be limited by coexistent atrophy of parallel fibre tracts, resulting in a relative maintenance in myelin density despite progressive damage. Further experimental studies are needed to illuminate the relationship between pathology and MTR in the spinal cord.

In conclusion, this study demonstrates that MTR measurement is feasible in the cervical cord and that a reduction clearly occurs in MS. The study failed to show a clear cross-sectional relationship between cervical cord MTR and disability and this deserves further investigation in a larger subject cohort. Indeed, since this work was done, a larger study of cervical cord MTR in MS patients, using a 2D-gradient echo sequence, has identified a relationship between MTR histogram parameter abnormalities and disability [Rovaris et al., 2000]. It will be important to evaluate other approaches for measuring MTR within the spinal cord (e.g. 3D-gradient echo, conventional spin echo) and to assess axial cord images, as these may allow more specific MTR measurement within specific white matter tracts. A positive longitudinal relationship of cord MTR and disability in such studies would suggest a potential role for monitoring treatment response.
PART THREE

Magnetization Transfer as a contrast mechanism
CHAPTER 8

A cross-sectional study to evaluate techniques to optimise detection of focal blood-brain barrier breakdown in multiple sclerosis

8.1 Introduction

The ability to alter the clinical course or eventual disability in MS is generally agreed to be the definitive outcome measure for assessment of potential new therapies [Rudick et al., 1996; Miller et al., 1996]. Nevertheless, because the clinical course is highly variable, markers of disease activity are required that are related to, but more sensitive than, clinical measures. Such techniques would allow more rapid screening of new therapies in exploratory (phase I / II) trials, in addition to acting as supplementary markers of disease activity in definitive (phase III) studies where a clinical endpoint is the primary outcome measure. In relapsing-remitting and secondary progressive MS, serial T2-weighted MRI reveals 5 to 10 times as many new lesions as there are clinical relapses [Willoughby et al., 1989; Thompson et al., 1991]. Gadolinium enhancement, by detecting blood-brain barrier breakdown and inflammation in new and reactivated chronic lesions [Hawkins et al., 1990; Kermode et al., 1990a, 1990b; Katz et al., 1993], further increases the reliability and sensitivity of detecting active lesions [Miller et al., 1993; Miller, 1994]. In relapsing-remitting and secondary progressive MS, the presence of such enhancement is more frequent during relapse and correlates well with clinical activity [Gonzalez-Scarano et al., 1987; Thompson et al., 1991, 1992; Smith et al., 1993; Frank et al., 1994]. In addition, for patients with relapsing-remitting and secondary progressive MS, short term serial measurement of the number of Gd-DTPA enhancing lesions may have a limited predictive value for long term clinical outcome [Losseff et al., 1996c, 1996d]. In benign MS, where there is relatively little clinical deterioration over time, longitudinal studies using conventional doses of Gd-DTPA have noted much lower rates of activity than seen in relapsing-remitting MS [Thompson et al., 1992; Kidd.
et al., 1994]. For patients with primary progressive MS, few new lesions are generally seen on monthly T2-weighted images and little or no enhancement is seen with conventional doses of Gd-DTPA, despite steady clinical deterioration [Thompson et al., 1991; Kidd et al., 1996].

Several methods have been proposed to increase the conspicuity of gadolinium-enhancing lesions in a variety of neurological diseases, including MS. These include the use of a higher (0.3mmol/kg) dose of Gd-DTPA, introduction of a delay between contrast medium injection and imaging, and the utilisation of MT contrast.

The mechanisms whereby higher contrast doses and delayed imaging result in improved detection are likely to rely on increased concentration of gadolinium chelates within the lesion, resulting in a faster rate of T1 relaxation (i.e. shorter T1 relaxation time) with brighter signal on T1-weighted images. With regard to the dose of gadolinium chelates used, several groups have shown that a dose higher than 0.1mmol/kg may be beneficial for improved detection of enhancing lesions in various neurological disorders, including MS [Yuh et al., 1991; Runge et al., 1991; Haustein et al., 1993; Mathews et al., 1994; Wolansky et al., 1994; Filippi et al., 1995c, 1996a, 1996b]. Although experience is relatively limited, this higher dose of Gd-DTPA has not to date appeared to cause more side effects than standard doses [Haustein et al., 1993]. The optimal time for imaging after contrast medium injection is not clear, although dynamic studies in MS with 0.1-0.2mmol/kg Gd-DTPA have shown marked lesion heterogeneity with maximal intensity occurring anywhere between 4 minutes and 2 hours [Kermode et al., 1990b]. Studies in primary progressive and benign MS have shown possible small advantages in using a 1 hour delay after contrast medium injection [Filippi et al., 1995, 1996b].

An alternative method for improving enhancing lesion conspicuity relies on decreasing the signal of surrounding brain parenchyma using MT contrast [Balaban and Ceckler., 1992]. Although MT presaturation will also reduce the signal intensity of free water protons adjacent to gadolinium, this reduction is small compared with that of normal tissue [Mehta et al., 1995a]. Several studies have reported improved conspicuity of gadolinium-enhancing lesions in MS using MT contrast [Tanttu et al., 1992; Finelli et al., 1994; Mehta et al., 1995a].

The aims of this study were to assess the relative merits of each individual optimisation technique and evaluate how they may be best applied in combination for the detection of focal
blood-brain barrier breakdown. The development and application of more sensitive techniques will be of potential value for (1) research into the pathogenesis of MS, (2) clinical practice (where diagnostic accuracy might be improved), and (3) therapeutic trials (possibly allowing shorter and/or smaller exploratory (phase I / II) studies).

8.2 Methods

Subjects Fifty patients aged 24 to 56 years with clinically definite MS [Poser et al., 1983] were recruited from the population of patients attending the outpatient department of the National Hospital for Neurology and Neurosurgery. Of these, 14 patients were classified as relapsing-remitting, 7 as benign, 10 as secondary progressive, 16 as primary progressive, and 3 as transitional MS [Poser et al., 1983; Kurtzke, 1983].

All patients underwent a detailed history and complete neurological examination by one observer (NCS) and disability was scored using the EDSS and Kurtzke’s functional score [Kurtzke, 1983]. Exclusion criteria for the study included pregnancy, breastfeeding, a history of allergy, any previous adverse reaction to contrast media, current asthma, or a history of asthma requiring previous hospital admission. No patients were admitted to the study within 1 month of a clinical relapse. In addition, no patients had received steroids within the previous month or other immunosuppressive therapy within the previous six months.

MRI Data Acquisition All imaging was carried out using a 1.5 Tesla superconducting system with standard quadrature headcoil to obtain 46 contiguous axial oblique 3mm slices (256 x 256 image matrix, 24 x 18cm field of view).

Patients were imaged with identical protocols on 2 separate days (24-72 hours apart, consecutive where possible). The only difference between these two sessions was the dose of Gd-DTPA used (day 1 = 0.3 mmol/kg; day 2 = 0.1 mmol/kg).

First, proton density and T2-weighted images of the brain were acquired using a dual fast
spin-echo sequence (TR = 3020ms, TE\_ef = 19 and 90ms, echo train length 8, acquisition time 8 minutes). The patient was then removed from the MRI scanner and an intravenous cannula was inserted. A long line was attached to the cannula and flushed with normal saline. Imaging resumed with a pair of T1-weighted spin-echo images (TR = 600ms, TE = 17ms, 1 excitation), acquired separately, with and without MT presaturation. Acquisition time was 8 minutes 11 seconds without and 10 minutes 18 seconds with MT presaturation. For presaturation, a Hamming apodised 3-lobe sinc pulse with peak amplitude equivalent to a 520° flip angle was applied for a duration of 12 ms at 1 kHz off resonance. [The MT presaturated sequence had previously been optimised in a separate non contrast-enhanced experiment to allow hypointense or isointense T1-weighted lesions to almost disappear when MT presaturation was applied, without resulting in pre-contrast hyperintense signal].

Without moving the patient, gadopentate dimeglumine (Gd-DTPA) contrast medium was administered as a bolus, followed by 5ml normal saline via the long line. Imaging resumed with T1-weighted sequences identical to those attained pre-injection, alternating MT and non-MT presaturated acquisitions over a one hour period. This resulted in pairs of MT and non-MT studies for the first 20 minutes (early), 20-40 minutes (early delay) and 40-60 minutes (late delay) for each dose of Gd-DTPA. Patients were alternated as to whether contrast-enhanced imaging commenced with or without MT and this sequence remained constant for both sessions.

All patients were initially blinded to Gd-DTPA dose administered for the first session (0.3mmol/kg) and were all asked an open-ended question regarding any adverse events after imaging and again at the second session 24-72 hours later. For the second session (0.1 mmol/kg Gd-DTPA), those patients who had suffered possible adverse events following triple-dose and were worried about further contrast administration were unblinded before having single-dose. All patients were asked to contact the department if they suffered any adverse events following the second session (0.1 mmol/kg).

For all imaging, patient repositioning was standardised [Gallagher et al., 1996]. To minimise artefactual contrast variations on the processed films, radiofrequency amplifier gains were manually held constant for both the MT and non-MT acquisitions at both sessions. In addition, window levels were manually set separately for MT and non-MT unenhanced images to allow optimal contrast for each type of scan. These window levels remained identical for the
comparable pre- and post-injection images at each session.

**MRI Data Analysis** All images were assessed by Dr Tina Good who was blinded to patient identity, all clinical information, Gd-DTPA dose and imaging details (delay and MT). Because MT images resulted in loss of contrast between grey and white matter, an element of unblinding was unavoidable. The following analyses were undertaken:

(a) Unenhanced non-MT T1-weighted images acquired at the second imaging session were assessed for all patients.

(b) Unenhanced MT presaturated T1-weighted images were assessed for evidence of high lesion signal that might make assessment of the contrast-enhanced study more difficult.

(c) Contrast-enhanced non-MT and MT presaturated T1-weighted images were analysed individually in a random order (patients, Gd-DTPA dose and acquisition modality). All contrast-enhanced studies were assessed with the comparable unenhanced MT/non-MT image for evidence of enhancing lesions. Before commencing this analysis, films from 15 patients studied were jointly assessed by Drs Tina Good and Ivan Moseley to help establish and standardise criteria for defining enhancing lesions and to help ensure reliable observer agreement in this study.

**Statistical analysis** For all statistical comparisons, MRI data were analysed using the Wilcoxon matched pairs signed rank sum test.

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\(^8\) Strict criteria were applied to the designation of an enhancing lesion: all "definite" enhancing lesions were included, whereas areas of bright signal indistinguishable from flow artefact or Gd-DTPA contrast within vessels without comparable high signal on T2- or proton density-weighted images were excluded.
8.3 Results

Clinical data

Of the 50 patients recruited [Table 8.1], 19 patients (3 with benign, 5 with relapsing-remitting, 5 with secondary progressive, and 6 with primary progressive MS) failed to complete the full protocol for various reasons including poor compliance, technical difficulties and medical adverse events.

<table>
<thead>
<tr>
<th></th>
<th>number*</th>
<th>mean age** (years)</th>
<th>mean disease duration (years)</th>
<th>median EDSS</th>
</tr>
</thead>
<tbody>
<tr>
<td>benign</td>
<td>7 (1:6)</td>
<td>43 (37-50)</td>
<td>18</td>
<td>2.5</td>
</tr>
<tr>
<td>relapsing-remitting</td>
<td>14 (3:11)</td>
<td>34 (24-51)</td>
<td>9</td>
<td>4.5</td>
</tr>
<tr>
<td>secondary progressive</td>
<td>10 (4:6)</td>
<td>39 (26-50)</td>
<td>12</td>
<td>7.5</td>
</tr>
<tr>
<td>primary progressive</td>
<td>16 (12:4)</td>
<td>44 (27-56)</td>
<td>9</td>
<td>7.5</td>
</tr>
<tr>
<td>transitional</td>
<td>3 (1:2)</td>
<td>42 (35-50)</td>
<td>17</td>
<td>8</td>
</tr>
</tbody>
</table>

* figures in parentheses represent number of males:females; ** figures in parentheses represent age range.

Table 8.1 Demographic data.

Overall, 7 of the 50 patients (14%) who received triple-dose Gd-DTPA suffered adverse events possibly related to the drug (allergic reaction, flushing with paraesthesiae, flushing and a sensation of facial congestion followed by headache, diarrhoea, diarrhoea and vomiting, vomiting following contrast medium injection, and dry mouth immediately following contrast medium injection). Only 1 of the 48 patients (2%) who received single-dose suffered an adverse event possibly related to Gd-DTPA, namely a dry mouth immediately following contrast medium injection.

MRI data

No patient showed evidence of residual Gd-DTPA enhancement on any of the post-contrast day 2 images that were obtained 24-72 hours following triple-dose Gd-DTPA.

Focal high signal on unenhanced MT presaturated T1-weighted images was observed in only 3 patients (1 each with transitional, relapsing remitting and secondary progressive MS). In each case, this affected only a single lesion. In 5 patients (1 with relapsing remitting, 3 with
secondary progressive and 1 with primary progressive MS) the normal-appearing white matter surrounding lesions (as assessed on the T2-weighted images) appeared as a faint bright rim. This phenomenon was also seen to a lesser extent on some of the comparable non-MT T1-weighted images. None of the high signal regions noted on unenhanced MT images were shown to change in signal intensity following contrast medium on either MT presaturated or conventional T1-weighted images, indicating that MT presaturation was not responsible for false positive reporting of focal enhancement in this particular study.

A comparison of all imaging modalities in the 31 patients (4 with benign, 9 with relapsing-remitting, 5 with secondary progressive, 10 with primary progressive and 3 with transitional MS) who completed the full study protocol is shown [Figure 8.1].

To assess the individual and combined effects of MT, delayed imaging and Gd-DTPA dose, data from all patients entered into the study is presented.

![Figure 8.1](image)

Figure 8.1 The individual and combined effects of Gd-DTPA dose, delay and MT contrast on detection of enhancing lesions in 31 patients who underwent the complete protocol. The dose of Gd-DTPA is in mmol/kg and the time of image acquisition following contrast injection is as follows: early: 0-20 minutes, early delay: 20-40 minutes, and late delay: 40-60 minutes following contrast administration.
**Gd-DTPA dose** Early contrast-enhanced T1-weighted images were assessed in all MS subgroups to evaluate the effect of Gd-DTPA contrast dose [Table 8.2]. Compared with conventional single-dose (0.1 mmol/kg) Gd-DTPA, triple-dose (0.3 mmol/kg) detected an additional 75% enhancing lesions (48 subjects; 132 with single-dose vs. 231 with triple-dose, \( p < 0.002 \)). Triple-dose imaging showed at least as many lesions as single-dose in all but 2 patients: one with relapsing-remitting MS showed 10 enhancing lesions with triple-dose and 11 with single-dose and a patient with primary progressive MS who underwent the full protocol showed a single enhancing lesion only on the conventional early single-dose study.

<table>
<thead>
<tr>
<th></th>
<th>number</th>
<th>0.1 mmol/kg*</th>
<th>0.3 mmol/kg*</th>
<th>( p )**</th>
</tr>
</thead>
<tbody>
<tr>
<td>benign</td>
<td>7</td>
<td>1 (1)</td>
<td>11 (4)</td>
<td>ns</td>
</tr>
<tr>
<td>relapsing-remitting</td>
<td>13</td>
<td>46 (7)</td>
<td>86 (7)</td>
<td>0.03</td>
</tr>
<tr>
<td>secondary progressive</td>
<td>9</td>
<td>83 (5)</td>
<td>133 (5)</td>
<td>ns</td>
</tr>
<tr>
<td>primary progressive</td>
<td>16</td>
<td>2 (2)</td>
<td>1 (1)</td>
<td>ns</td>
</tr>
<tr>
<td>transitional</td>
<td>3</td>
<td>0</td>
<td>0</td>
<td>ns</td>
</tr>
</tbody>
</table>

| total            | 48     | 132 (15)     | 231 (17)     | \( < 0.002 \) |

*numbers of patients with active scans are shown in parentheses; ** Wilcoxon matched pairs signed rank sum test (ns = not significant).

Table 8.2 Effect of Gd-DTPA dose (total number of enhancing lesions detected within 20 minutes of 0.1 mmol/kg and 0.3 mmol/kg bolus doses).

The number of patients with an active study, as defined by the presence of one or more enhancing lesions, increased from 15 to 17 (3 patients with benign MS were active only with triple-dose and 1 patient with primary progressive MS was active only with single-dose).

In individual MS subgroups, triple-dose imaging increased detection of enhancing lesions in patients with benign, relapsing-remitting and secondary progressive MS; this was significant only in patients with relapsing-remitting MS (86 vs. 46 enhancing lesions in 13 patients, \( p < 0.05 \)).

**Delayed imaging (20-40 minutes)** The effect of an early delay for both single and triple doses of Gd-DTPA was assessed in 44 patients who had all such studies (6 with benign, 12 with relapsing-remitting, 9 with secondary progressive, 14 with primary progressive and 3 with transitional MS).

Conventional early single-dose imaging showed 131 enhancing lesions (14 active studies). A delay in imaging by 20-40 minutes resulted in the detection of 165 enhancing lesions,
with 15 active studies (1 patient each with benign, relapsing-remitting and secondary progressive MS were active only with delayed imaging; 1 patient each with benign and primary progressive MS were active only with early imaging).

In the same group of patients, early triple-dose imaging showed 225 enhancing lesions (15 active studies). A delay in imaging by 20-40 minutes resulted in the detection of 243 enhancing lesions, with 18 active studies (1 patient each with relapsing-remitting, secondary progressive and transitional MS were active only with delayed imaging).

**Delayed imaging (40-60 minutes)** The effect of a late delay for both single and triple doses of Gd-DTPA was assessed in 35 patients who had all such studies (5 with benign, 9 with relapsing-remitting, 7 with secondary progressive, 11 with primary progressive and 3 with transitional MS).

Conventional early single-dose imaging showed 88 enhancing lesions (8 active studies). A delay in imaging by 40-60 minutes resulted in the detection of 113 enhancing lesions with no overall effect on the number of active studies (1 patient with benign MS was active only with delayed imaging and 1 with primary progressive MS showed a single enhancing lesion noted only with conventional early imaging).

In the same group of patients, early triple-dose imaging showed 152 enhancing lesions (9 active studies). A delay in imaging by 20-40 minutes resulted in the detection of 190 enhancing lesions and an additional 3 active studies.

The overall trend for both early (20-40 minute) and late delayed (40-60 minute) imaging to increase the number of enhancing lesions was not statistically significant for either single-dose or triple-dose studies.

**Early vs. late delay** There were no significant differences between early delayed and late delayed imaging for either single-dose (enhancing lesions: 88 (early) vs. 119 (early delay) vs. 113 (late delay), 35 patients) or triple-dose studies (enhancing lesions: 145 (early) vs. 170 (early delay) vs.186 (late delay), 38 patients).

**MT presaturation** The individual effect of MT imaging was assessed at the early time point (0-20 minutes post contrast) for both single- and triple-dose studies in 42 patients (6 with benign, 10 with relapsing-remitting, 7 with secondary progressive, 16 with primary progressive and 3 with transitional MS) [Figure 8.2].
Figure 8.2  Contrast-enhanced T1-weighted images from a patient with secondary progressive MS, showing differences in enhancing lesion conspicuity with Gd-DTPA dose and MT. All post-contrast images were obtained within 20 minutes of Gd-DTPA administration.

Pre-contrast images
(a) proton density, (b) T2-weighted,
(c) non-MT T1-weighted, (d) MT T1-weighted

Post-contrast images
(e) 0.1mmol/kg Gd-DTPA, (f) 0.1mmol/kg Gd-DTPA and MT,
(g) 0.3mmol/kg Gd-DTPA, (h) 0.3mmol/kg Gd-DTPA and MT.
With conventional single-dose imaging, 90 enhancing lesions was detected (10 active studies). By adding MT presaturation, 119 enhancing lesions were detected, with 12 active studies (1 patient each with benign, relapsing-remitting and primary progressive MS were active only with MT imaging; 1 patient with primary progressive MS showed a single enhancing lesion only with conventional imaging). In the same patients, triple-dose conventional imaging showed 156 enhancing lesions (12 active studies). With additional MT presaturation, 169 enhancing lesions were detected, with 15 active studies (1 patient each with relapsing-remitting, secondary progressive, primary progressive and transitional MS were active only with MT imaging; 1 patient with benign MS showed a single enhancing lesion only with non-MT imaging). The overall trend for MT imaging to increase the number of enhancing lesions detected was not statistically significant for either single-dose or triple-dose studies.

For individual patients in this group, the effect of MT imaging was variable. With single-dose studies, MT imaging increased detection of enhancing lesions in 7 patients and decreased detection in 3 others; for 3 patients with enhancement, no difference was observed. With triple-dose studies, MT imaging increased detection of enhancing lesions in 8 patients and decreased detection in 3 others; for 4 patients where enhancement was noted, no difference was observed.

MT versus triple-dose Gd-DTPA The effect of early single-dose MT presaturated imaging was compared with early triple-dose conventional (non-MT) T1-weighted imaging in 43 patients studied (6 with benign, 11 with relapsing-remitting, 7 with secondary progressive, 16 with primary progressive and 3 with transitional MS). The use of triple-dose resulted in the detection of a significantly greater number of enhancing lesions than MT presaturation (160 vs. 119, \( p < 0.05 \)). Whilst the overall number of active studies was also higher with the triple dose (13 vs. 12), 2 studies were active only with single-dose MT imaging (1 patient each with relapsing-remitting and primary progressive MS).

Effects of combined MT and delayed imaging The combination of MT with early delayed imaging was assessed for 40 patients with single-dose [Table 8.3] and 41 patients with triple-dose studies [Table 8.4].
Compared with conventional early T1-weighted imaging, significantly more enhancing lesions were detected with this combined approach (single-dose studies: \( p < 0.05 \), and triple-dose studies: \( p < 0.01 \)). For single-dose there was no difference in the overall number of active studies, although 1 patient with primary progressive MS showed activity only on the conventional early, non-MT images. For triple-dose, the combination of MT and early delay resulted in 3 additional
active studies (from 10 to 13).

Comparison between early and late delay for MT imaging at each Gd-DTPA dose showed no significant difference in the number of enhancing lesions (single-dose studies: 130 (early delay) vs. 126 (late delay), 34 patients; triple-dose studies: 186 (early delay) vs. 194 (late delay), 35 patients).

For individual benign, relapsing-remitting and secondary progressive subgroups, MT imaging with either early or late delay increased the overall number of enhancing lesions detected for both single- and triple-dose studies, although these increases were not statistically significant.

Delayed MT imaging (single-dose) versus triple-dose Gd-DTPA The effects of combined MT presaturation and early delayed imaging (the most sensitive of the single-dose studies) were compared with those of early non-MT imaging following triple-dose in 40 patients studied (5 with benign, 10 with relapsing-remitting, 7 with secondary progressive, 15 with primary progressive and 3 with transitional MS). The number of enhancing lesions detected with early delayed MT presaturated imaging following single-dose Gd-DTPA was 132 (with 10 active studies), compared with 154 enhancing lesions detected (and a single additional active study) with early non-MT triple-dose studies. These differences were not statistically significant.

Combined MT, triple-dose, and delayed imaging The combination of triple-dose, MT presaturation and late delay was compared with conventional, early single-dose non-MT imaging in 35 patients studied (5 with benign, 9 with relapsing-remitting, 5 with secondary progressive, 13 with primary progressive and 3 with transitional MS). The use of such a combination resulted in an overall 126% increase in the number of enhancing lesions detected, the greatest of any approach studied (88 with early single-dose non-MT imaging vs. 199 with late delayed triple-dose MT presaturated imaging, \( p < 0.05 \)). For these 35 patients, the number of active studies increased from 8 to 10 with this combined approach (2 patients with benign, 1 each with relapsing-remitting and transitional MS were active only with triple-dose, MT presaturation, and late delay; 1 patient each with primary progressive and secondary progressive MS were active only with early non-MT single-dose studies).

The combination of triple-dose, MT presaturation and early delay detected 118% more enhancing lesions than early non-MT single-dose studies \( (p < 0.002) \) [Table 8.5].
Table 8.5  Combined effect of MT presaturation, early delay and triple-dose Gd-DTPA.

<table>
<thead>
<tr>
<th></th>
<th>number</th>
<th>0.1mmol/kg*</th>
<th>0.3mmol/kg*</th>
<th>p**</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td></td>
<td>early, non-MT</td>
<td>early delay, MT</td>
<td></td>
</tr>
<tr>
<td>benign</td>
<td>5</td>
<td>0</td>
<td>7 (2)</td>
<td>ns</td>
</tr>
<tr>
<td>relapsing-remitting</td>
<td>10</td>
<td>24 (4)</td>
<td>55 (5)</td>
<td>&lt; 0.05</td>
</tr>
<tr>
<td>secondary progressive</td>
<td>7</td>
<td>64 (4)</td>
<td>130 (5)</td>
<td>&lt; 0.05</td>
</tr>
<tr>
<td>primary progressive</td>
<td>14</td>
<td>2 (2)</td>
<td>2 (1)</td>
<td>ns</td>
</tr>
<tr>
<td>transitional</td>
<td>3</td>
<td>0</td>
<td>2 (1)</td>
<td>ns</td>
</tr>
<tr>
<td>total</td>
<td>39</td>
<td>90 (10)</td>
<td>196 (14)</td>
<td>&lt; 0.002</td>
</tr>
</tbody>
</table>

* Total number of enhancing lesions (numbers of active scans are shown in parentheses); ** Wilcoxon matched pairs signed rank sum test (ns = not significant).

For these 39 patients, the number of active studies increased from 10 to 14 (2 patients with benign, and 1 each with relapsing-remitting, secondary progressive and transitional MS were active only with triple-dose, MT and early delay; 1 patient with primary progressive MS was active only with early single-dose, non-MT imaging).

In benign, relapsing-remitting, secondary progressive and transitional MS, delayed MT imaging following triple-dose Gd-DTPA detected more enhancing lesions than early non-MT imaging with single-dose. With late delay, this increase was not statistically significant for any MS subgroup. For early delay however, increases were significant for both relapsing-remitting and secondary progressive MS subgroups ($p < 0.05$).

8.4 Discussion

This study demonstrates that triple-dose (0.3mmol/kg) Gd-DTPA, MT presaturation and delayed imaging all affect the number of enhancing lesions detected in MS. The most sensitive combination, MT presaturated imaging between 40 and 60 minutes after administration of triple-dose Gd-DTPA, resulted in a 126% increase in the overall number of enhancing lesions detected over conventional non-MT early single-dose imaging (the conventional approach in current use); a shorter delay of 20 to 40 minutes resulted in only a marginally smaller increase (118%).

While the advantage of combining these techniques is clear, some MRI units normally participating in treatment trials may have difficulties using all three methods, because of
financial, time or software limitations. In addition, the post-marketing surveillance of triple-dose Gd-DTPA is extremely limited compared with that of single-dose administration. The study was designed to address the individual effects of each technique. In isolation, only triple-dose Gd-DTPA resulted in significantly increased enhancing lesion detection (75% more than with single-dose). Delayed or MT presaturated imaging alone resulted in more modest improvements in sensitivity for both single- and triple-dose studies. In combination, early delay and MT presaturation significantly increased the detection of enhancing lesions by 47% for single-dose and 27% for triple-dose. The number of enhancing lesions detected was not significantly further increased by a late delay, although there was a trend towards this with triple-dose.

Previous groups have suggested that, in MS, the use of MT with single-dose Gd-DTPA may result in an improvement in contrast comparable to that given by triple-dose Gd-DTPA, thereby avoiding the higher cost of the latter and the possibility of more side effects at this higher dose [Finelli et al., 1994]. However, in this study, non-MT imaging with a triple dose significantly increased the number of enhancing lesions detected by 34%, when compared with MT presaturated imaging following single-dose Gd-DTPA.

More adverse events were seen following the use of the higher triple (0.3mmol/kg) dose of Gd-DTPA: seven (14%) with triple-dose in 50 patients vs. one (2%) with single-dose in 48 patients. All these were relatively minor and self-limiting, and it is possible that some were incidental and unrelated to injection of contrast medium. Some of these reactions (i.e. urticaria, vomiting, flushing, paraesthesiae and headache) are well described with Gd-DTPA [Niendorf et al., 1993]. The frequency of 2% of possible/probable side effects associated with 0.1mmol/kg is comparable with that in other studies [Niendorf et al., 1993]. A review of studies investigating the safety of 0.1mmol/kg concluded that the overall frequency of adverse events is in the order of 1% and comparable to that of intravenous physiological saline [Niendorf et al., 1993]. In a randomised trial of 199 patients comparing 0.1mmol/kg and 0.3mmol/kg Gd-DTPA, a much higher frequency of adverse events with 0.1mmol/kg (6%) was observed, equal to that associated with 0.3mmol/kg Gd-DTPA [Haustein et al., 1993]. In that trial, there was no blinding and assessment of adverse events was carried out only immediately following imaging. In this work however, all subjects were initially blinded to dose for the first day (0.3mmol/kg) and all were assessed for possible adverse events not only after imaging but also at the second session 24-72 hours later. In addition, all patients were asked to contact the department if they suffered any adverse events following the second session (0.1mmol/kg). Without the late assessment at the
second imaging session three adverse events possibly related to triple-dose would have been missed. Previous authors have suggested that a history of allergy may predispose patients to a higher risk of adverse events following Gd-DTPA administration [Niendorf et al., 1993]. In addition, information from post-marketing surveillance revealed one death from anaphylactic shock in a patient with asthma [Niendorf et al., 1993]. Whilst previous reports suggest that adverse events are not dose-related [Haustein et al., 1993; Niendorf et al., 1993], caution should be maintained regarding those patients at potential risk (e.g. allergies, asthma) until more data is available. In this study, it is possible that the use of bolus Gd-DTPA rather than an infusion contributes to the increased frequency of adverse events noted with the higher contrast dose. In MS, where the time to reach maximal enhancement is relatively long compared with many other pathologies, the use of an infusion over a few minutes might not be too detrimental to sensitivity. Further studies into tolerance of triple-dose Gd-DTPA would be useful, and these might address such issues. If triple-dose Gd-DTPA is to be used for serial assessment in treatment trials, then any potential increase in power of the study should be weighed against a potential for increased side-effects that may reduce patient compliance and cause premature withdrawal.

In the present study, the MT sequence was designed to allow lesions to become less hypointense or disappear, compared with standard T1-weighted images. The MT presaturation pulse for this sequence resulted in a 16.8% mean reduction in signal intensity for normal white matter, as determined in a separate study of three healthy subjects. Other studies using more powerful MT presaturation pulses, where signal intensity of normal appearing white matter has been reduced by 35-37%, have reported frequent high signal from MS lesions prior to administration of gadolinium contrast medium [Mehta et al., 1995a, Finelli et al., 1994]. In order to define enhancing lesions in this situation, signal to noise evaluation before and after contrast medium is desirable, but may be time consuming. Where less powerful MT sequences are used, as in our study, this situation does not arise, and straightforward comparison of pre- and post-contrast images should suffice. This approach may also confer an advantage for acquisition, allowing a greater number of thinner slices without exceeding SAR limits. Whichever approach is used, acquisition of an unenhanced MT presaturated T1-weighted image is essential for analysis of MT-prepared contrast-enhanced images. In addition, imaging parameters before and after injection should ideally remain identical, with no changes in RF amplifier gain, and image window levels should remain constant for visual assessment of enhancing lesions. All these factors were carefully controlled in this study.
Within the context of treatment trials, it is important to consider the disease subgroups of MS. Whilst the overall detection of enhancing lesions was significantly improved using a combination of triple-dose and delayed MT imaging, up to 126% over standard single dose imaging, this effect differed between subgroups. Compared with conventional early non-MT imaging following single-dose Gd-DTPA, the combination of early delay, MT and triple-dose Gd-DTPA significantly increased detection of enhancing lesions in patients with relapsing-remitting and secondary progressive MS. In 22 patients with relapsing-remitting and secondary progressive MS it was noted that the triple dose alone resulted in a 70% increased yield in enhancing lesions over single-dose Gd-DTPA, which is comparable to the results of a previous study, where a 66% increase was observed [Filippi et al., 1996a]. In the present study, many more enhancing lesions were seen in patients with relapsing-remitting and secondary progressive MS than in those with benign disease, regardless of imaging modality or dose of Gd-DTPA. This is in keeping with previous studies using delayed imaging and triple dose Gd-DTPA [Filippi et al., 1996a, 1996b].

However, in the 16 patients with primary progressive MS, little enhancement was seen with any approach (total number of enhancing lesions < 2 for any particular combination). These findings are discordant with a previous study comparing the combined effects of delay and triple-dose Gd-DTPA with single-dose MRI in 10 patients with primary progressive disease, in which 4 enhancing lesions (2 active studies) were seen with single-dose Gd-DTPA, 13 enhancing lesions (5 active studies) with early triple-dose Gd-DTPA and 14 enhancing lesions (6 active studies) an hour after triple-dose Gd-DTPA [Filippi et al., 1995c]. The reasons for this discrepancy are not clear. It is unlikely that different imaging parameters contribute; the main difference was the use of 3 mm here (rather than 5 mm slices); however, this might be expected to increase rather than decrease detection, as has been shown with three-dimensional T1-weighted gradient echo sequences in MS [Filippi et al., 1996c]. With small studies there is a possibility of selection bias, although 16 patients have been studied with a negative result. There were differences in clinical parameters between the two studies, the subjects here having a longer disease duration (9 vs. 6.5 years) and being relatively more disabled (median EDSS 7.5 vs. 4.6). In addition, the diagnosis of primary progressive MS is historical, and therefore it is difficult to be absolutely certain about the absence of transient neurological episodes before onset of the progressive course.

The findings in the primary progressive group raise the possibility that the pathological
substrate of the slow progression in disability may be independent of blood-brain barrier disruption. Pathological studies have, however, shown inflammatory activity in patients with primary progressive MS, albeit less intense than in those with secondary progressive disease [Revesz et al., 1994]. In relapsing-remitting and secondary progressive MS, the presence of gadolinium enhancement within lesions has been correlated with evidence of inflammation and active demyelination, as demonstrated by pathological examination of tissue obtained from post-mortem or stereotaxic biopsy [Nesbit et al., 1991; Katz et al., 1993; Rodriguez et al., 1993]. It appears that, in primary progressive MS, the milder inflammatory changes are generally not associated with evident changes in blood-brain barrier permeability to gadolinium based contrast media. Studies of the blood-brain barrier using cerebrospinal fluid markers nevertheless suggest that some disruption is commonly present [McLean et al., 1993], but do not differentiate between focal or diffuse changes in blood-brain barrier disruption. Whereas focal changes might result in visible enhancing lesions (contrasting with surrounding non-inflammatory tissue), diffuse disruption might be expected to cause subtle widespread intensity changes not apparent on visual evaluation.

The study results have direct relevance to the use of gadolinium-enhanced MRI as an indicator of therapeutic efficacy in treatment trials. Whilst this has shown that the number of enhancing lesions detected may be significantly increased in relapsing-remitting and secondary progressive subgroups using the above methods, this has not been paralleled by such noticeable increases in the number of active studies. Indeed, some active studies with standard techniques were designated inactive with potentially more sensitive techniques. It is possible that minor amounts of patient movement during the study might have influenced the detection of small enhancing lesions. This may also reflect potential difficulties in interpreting areas of high signal and differentiating contrast enhancement from artefact. Such difficulties were highlighted by the results from the reported patient with primary progressive MS who showed activity with conventional early non-MT single-dose imaging alone. Retrospective analysis confirmed a small focal area of high signal in the left hemisphere on these images (with an underlying area of T2/proton density hyperintensity), whilst subsequent images from the completed series confirmed this as a likely false positive result, with more obvious flow artefact arising from the third ventricle in this same region. Whilst imaging with triple-dose Gd-DTPA would always be expected to increase visible enhancement compared with single-dose [Tofts, 1996], techniques such as MT and delayed imaging might be expected to have different effects upon different
lesions. This is especially likely with delayed imaging, where the time for optimal enhancement will depend on lesion size and the degree of blood-brain barrier deficit [Tofts, 1996]. In a study of patients with relapsing-remitting and secondary progressive MS, it has been shown that delayed imaging one hour following Gd-DTPA administration significantly increases the number of enhancing lesions detected that are over 10mm², whilst smaller lesions may be better detected if imaging immediately follows Gd-DTPA administration [Filippi et al., 1996a]. When considering “activity” (i.e. the presence of ≥1 enhancing lesion), it is likely that delayed imaging will have different effects for different lesions; this would account for the variable effect of delay on activity noted in this study, where certain patients were designated active only on early images. In this study, MT imaging appeared to result in an overall trend towards increased detection of enhancing lesions, although individual patients behaved differently. Whilst the individual effect of MT imaging might be expected to differ according to the amount of tissue disruption both within the enhancing lesion and surrounding tissue, the lesion conspicuity should theoretically always be comparable or improved. In this study, MT presaturated imaging appeared to show fewer enhancing lesions than non-MT imaging in only a minority of patients. Whilst differing degrees of flow artefact between the different imaging techniques might possibly account for this, it is more likely that small differences in imaging time following contrast medium injection are responsible. The study was designed to minimise such effects, in that corresponding MT and non-MT images were acquired within 10 minutes of each other for each time point and patients were alternated as to whether post-contrast imaging sequence commenced with MT or non-MT imaging. Such differences in time of acquisition might account for individual patient variability, whilst the overall effect of MT in the whole group should be minimally affected due to the number of patients studied.

The findings in this and other studies indicate that triple-dose Gd-DTPA, MT contrast and delayed imaging probably all increase the sensitivity of contrast-enhanced MRI studies in relapsing-remitting and secondary progressive MS, although the gain is greatest from the use of triple-dose. In contrast, in primary progressive MS, such methods are unlikely to be useful. Further studies are required to evaluate the reproducibility of such combinations; the choice of optimal technique will require consideration of multiple factors including cost, safety, sensitivity, reproducibility and clinical predictive value.
CHAPTER 9
A serial study to assess the longitudinal sensitivity of an optimised approach for contrast-enhanced imaging

9.1 Introduction

Frequent serial MRI studies have revealed a great deal of asymptomatic disease activity in patients with MS. In particular, monthly contrast enhanced imaging using a standard (0.1 mmol/kg) dose of gadolinium has revealed about 5 to 10 new areas of enhancement for every clinical relapse in patients with relapsing-remitting or secondary progressive MS [Harris et al., 1990; Bastianello et al., 1990; Thompson et al., 1991; Thompson et al., 1992; Barkhoff et al., 1992]. Such studies have also revealed that the great majority of new focal abnormalities appearing on conventional T2-weighted images display an initial phase of gadolinium enhancement [Miller et al., 1993], indicating a breach of the blood-brain barrier which generally lasts between two and six weeks, although it is sometimes seen for a shorter or longer time period [Lai et al., 1996; Silver et al., 1998c; Tortorella et al., 1999].

Because of the high sensitivity to asymptomatic disease, a monthly gadolinium contrast-enhanced brain MRI protocol using standard doses of contrast has become widely used as a means of obtaining a preliminary evaluation of efficacy in phase I/II trials [Miller et al., 1996], and indeed clear cut treatment effects have been demonstrated for a number of agents when using the number of enhancing lesions as the primary outcome [Moreau et al., 1994; Sipe et al., 1994; Stone et al., 1995; Karussis et al., 1996; Pozzilli et al., 1996]. However, the value of such a protocol in evaluating treatment effects has been questioned because of the limited correlations between enhancing lesion activity and clinical outcomes [Kappos et al., 1999] and because the number of patients that need to be studied is substantial, especially in parallel group placebo-controlled studies, as a result of the variability in MRI activity seen between patients [Tubridy et al., 1998].

With this background, a number of measures which increase the detection of enhancing
lesions have emerged that are of potential interest. These include: (i) spinal MRI which at standard doses of contrast, although less sensitive than brain imaging, reveals lesion activity which is more likely to be clinically eloquent [Thorpe et al., 1996a]; (ii) triple-dose contrast which substantially increases the detection of enhancing lesions in the brain [Filippi et al., 1996a; Silver et al., 1997c]; (iii) delayed imaging and MT presaturation, both of which moderately increase the detection of enhancing lesions in the brain [Mehta et al., 1995a; Filippi et al., 1997; Silver et al., 1997c; Bastianello et al., 1998]. A recent serial brain MRI study of 40 patients using triple-dose gadolinium over 3 months, with and without a delay, indicated that the increase in activity seen could reduce the sample sizes needed for a trial using enhancing lesions as the outcome [Filippi et al., 1998c].

In this study, 16 patients with relapsing-remitting or secondary progressive MS underwent monthly gadolinium contrast-enhanced imaging of the brain and spinal cord over a six month period using two approaches: (i) conventional T1-weighted brain imaging following a standard dose of Gd-DTPA; (ii) a brain and spine protocol modified to enhancing lesion detection, which included the use of triple-dose Gd-DTPA and, in the brain, delayed MT presaturated T1-weighted imaging. The aims of the study were to: (i) assess the overall gains in sensitivity that may result from the serial use of a modified brain protocol (as derived from the experiments outlined in Chapter 8), and to help determine the viability of additional spine imaging at the higher gadolinium contrast dose; (ii) determine potential reductions in the sample size requirement for therapeutic trials as a result of optimisation; (iii) further evaluate the safety and tolerability of a serial monthly triple-dose protocol over a six month period.

9.2 Methods

Subjects

Sixteen patients aged 23 to 47 years with clinically definite MS [Poser et al., 1983] were studied [Table 9.1]. Eight patients each with relapsing-remitting (RR) and secondary progressive (SP) MS were recruited from outpatient clinics at the National Hospital for Neurology and Neurosurgery [Poser et al., 1983; Kurtzke, 1983].

Throughout the study, clinical evaluation was performed by one observer (NCS), allowing initial classification and scores of disability at study entry, exit and during periods of new clinical
activity using the EDSS and functional scores [Kurtzke, 1983]. Relapses as described by the Poser criteria were recorded [Poser et al., 1983].

Exclusion criteria for entry into the study included pregnancy, breastfeeding, history of significant allergy, previous adverse reaction to contrast media, current asthma, or any history of asthma requiring hospital admission. No patients entered the study within a month of significantly increased clinical activity or steroid therapy. No immunomodulatory therapy was received prior to or during the study period with the exception of steroids which were allowed during periods of significant clinical exacerbation. To minimise potential suppression of gadolinium enhancement, an obligatory 2 week steroid-free interval was ensured prior to all imaging studies.

**MRI Data Acquisition**

*Summary of protocol and its rational* The modified protocol in the brain involved the use of triple dose Gd-DTPA, 40 minute delay and MT presaturation of the T1-weighted image, as described in Chapter 8. In the spinal cord, the only difference between the conventional and modified protocol was the dose of Gd-DTPA.

*Detailed protocol* Imaging was carried out using a 1.5 Tesla superconducting system (Signa, GE Medical Systems, Milwaukee, Wisconsin). Subjects were positioned and repositioned using previously described techniques [Gallagher et al., 1997]. Brain images were acquired using a standard quadrature headcoil (GE Medical Systems, Milwaukee, Wisconsin) and sequences were prescribed with 46 contiguous axial oblique 3mm slices (256 x 256 image matrix; 24 x 18cm field of view). Spinal cord images were acquired using a phased array spinal coil (GE Medical Systems, Milwaukee, Wisconsin), and sequences were prescribed as 9 contiguous sagittal 3mm slices (512 x 512 image matrix; 48cm field of view).

Patients were studied over a six month period, each month undergoing brain and spine

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9Little previous work has been carried out to study strategies for improving detection of cord lesions. From experience gained in brain enhanced imaging studies, the main gain is likely to result from triple dose Gd-DTPA rather than effects of MT presaturation or delay [Silver et al., 1997c]. The potential addition of MT presaturation to whole cord T1-weighted imaging would require considerable further development, especially where phased array coils are required for pulse delivery [Silver et al., 1999a]. In this study, the choice of a 30 minute delay in all cord studies was arbitrary.
imaging on 2 separate occasions 24 to 72 hours apart. This allowed comparison of modified (day 1) versus conventional (day 2) imaging protocols for sensitivity to the detection of brain and spinal cord gadolinium enhancement. Proton density and T2-weighted images of the brain and spinal cord were obtained each month to facilitate accurate interpretation of post-contrast changes on T1-weighted images. To avoid false positive reporting of gadolinium enhancement, pre-contrast T1-weighted images of the spine were acquired at the study baseline and pre-contrast T1-weighted brain images with and without MT presaturation were acquired each month. To allow accurate comparison of contrast-enhanced and non contrast-enhanced images, all T1-weighted images were acquired using sequences standardised for all parameters.

On day 1 of each monthly assessment, a bolus intravenous dose of 0.3 mmol/kg ("triple-dose") Gd-DTPA was initially administered. Proton density and T2-weighted images of the spinal cord were next acquired using a dual fast spin echo sequence (TR = 2500ms, TE = 42 and 90ms; echo train length 16; acquisition time 5.5 minutes). Contrast-enhanced T1-weighted images of the spinal cord were acquired 30 minutes following Gd-DTPA using a spin echo sequence (TR = 500ms, TE = 14ms; 2 excitations; acquisition time 4.5 minutes). Contrast-enhanced T1-weighted images of the brain were acquired 40 minutes following Gd-DTPA using a spin echo sequence with MT presaturation (TR = 600ms, TE = 17ms; one excitation; acquisition time 8 minutes). Presaturation was applied 1kHz off-resonance using a Hamming-apodised 3-lobe sinc pulse of 12 ms duration and peak amplitude equivalent to a 520 degree flip angle. Finally, proton density and T2-weighted images of the brain were acquired using a dual fast spin echo sequence (TR = 3020ms, TE = 19 and 90ms, echo train length 8; acquisition time 8 minutes).

On day 2, unsaturated and MT presaturated T1-weighted brain images were acquired prior to a bolus intravenous dose of 0.1 mmol/kg ("single-dose") Gd-DTPA. Conventional contrast-enhanced T1-weighted images of the brain and spine were acquired at 7 minutes and 30 minutes following Gd-DTPA respectively.

All subjects were blinded to the dose of Gd-DTPA administered at each session. To assess the safety and tolerability of the higher (0.3mmol/kg) versus conventional (0.1mmol/kg) dose of Gd-DTPA, subjects were questioned about the occurrence of unexpected symptoms during or following each imaging study. Blood samples were analysed for serum electrolytes, calcium and phosphate, iron studies (ferritin, serum iron and total iron binding capacity), liver function, and full blood count at months 0, 3, and 6.
Data analysis

All images were assessed by Dr Tina Good using hardcopy images. Analysis was blinded to subject identity, demographic and clinical data. To reduce potential bias, the conventional image series for each subject was analysed at least a month apart from the modified image series.

To delineate the presence of enhancing lesions on each brain and spine series, the rater had access to all pre- and post-contrast T1-weighted images and all fast spin echo data. The appearance of enhancing lesions was documented for each series. Separate note was made of those enhancing lesions whose onset could be defined (i.e. new enhancing lesions). Individual monthly MRI studies for each subject were classified as either “active studies” (i.e. the presence of either new or persisting enhancement) or as “new active studies” (i.e. the presence of new enhancement).

Unenhanced T1-weighted pre-contrast images from day 2 (i.e. 24 to 72 hours following 0.3mmol/kg Gd-DTPA) were assessed to ensure the absence of potential residual enhancement.

Statistical analysis

MRI data were analysed using the Wilcoxon matched pairs rank sum test for paired and Mann-Whitney test for non-paired comparisons.

Sample size calculations for clinical trials using new enhancing lesions as the outcome measure were carried out by Drs Maria Pia Sormani and Massimo Filippi using the data from the 12 patients with complete studies (5 with relapsing-remitting and 7 with secondary progressive MS). A negative binomial model was applied to the data to simulate treatment effects [Sormani et al., 1999]. Two trial designs (parallel groups and cross over) were incorporated into the model and the Wilcoxon Rank Sum test was used to assess significance. The parallel design involves a comparison between an active treatment group and placebo, with the placebo group assumed to behave like the 12 patients from the present study; the period of study is 6 months with monthly imaging and it is assumed that the treatment is immediately effective. The crossover design compares a 3 month period of run in (untreated) with a 3 month period of active treatment.
9.3 Results

Clinical data

The clinical and demographic data are shown [Table 9.1]. Of the 16 patients studied, 12 (5 with relapsing-remitting and 7 with secondary progressive MS) completed the full protocol [Table 9.2]. Reasons for incomplete studies were (1) an allergic reaction to Gd-DTPA, (2) a severe relapse leading to hospitalisation and subsequent death from MS, (3) poor compliance in an individual with severe fatigue, and (4) incidental detection of a developmental venous anomaly, large enough to cause difficulty with interpretation of enhancing lesions.

<table>
<thead>
<tr>
<th>Subject</th>
<th>MS course*</th>
<th>Age</th>
<th>Disease duration</th>
<th>Duration of progression</th>
<th>Number of new or superimposed relapses**</th>
<th>EDSS (Month 0)</th>
<th>EDSS (Month 6)</th>
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<td>2</td>
<td>-</td>
<td>1 (month 2)</td>
<td>8.0</td>
<td>.***</td>
</tr>
<tr>
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<td>32</td>
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<td>-</td>
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</tr>
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<td>RR</td>
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<td>-</td>
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</tr>
<tr>
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<td>-</td>
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<td>3 (months 1,3,4)</td>
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* where RR = relapsing-remitting, and SP = secondary progressive MS; ** relapses are classified in the secondary progressive (SP) patient group as a temporary disturbance of new neurological deficit with acute or subacute onset and at least partial recovery, and do not include new symptoms with insidious onset and/or lack of recovery (i.e. disease progression); *** EDSS not determined as study incomplete, and patient not studied at month 6.

Table 9.1 Demographic data

Safety and tolerability of frequent monthly study with triple-dose Gd-DTPA

Adverse events following triple-dose Gd-DTPA were rare and minor. One subject experienced nausea, dizziness and pain at the injection site within minutes of injection on one
occasion; another subject experienced a single episode of headache and a third felt a sensation of flushing in the left face and lower limb within 2 minutes of Gd-DTPA injection on a single occasion.

Following single-dose Gd-DTPA, a single patient experienced nausea and a widespread urticarial rash over the upper limbs, neck and trunk followed the next day by vomiting and diarrhoea, leading to her withdrawal from the study. This patient had previously received single-dose and triple-dose Gd-DTPA on numerous occasions without adverse effect.

There was no significant abnormality of any of the blood or serum parameters measured during the study.

**MRI data**

MRI activity data for each patient is summarised [Table 9.2]. Data from all available scans is included. Overall, there were 97 completed monthly studies, of which 81 allowed assessment of new enhancing lesions. No residual enhancement was detected on day 2 pre-contrast T1-weighted images (i.e. 24 to 72 hours following triple-dose Gd-DTPA).

### i Enhancing lesions

Modified brain MRI revealed 754 enhancing lesions whereas conventional imaging revealed 347 (119% increase, \(p<0.0005\), Wilcoxon Rank Sum Test). Modified cord MRI revealed 123 enhancing lesions compared to 75 on conventional imaging (64% increase, \(p<0.0005\)). Compared with conventional brain MRI alone, the combined modified brain and spine imaging protocol increased sensitivity to enhancing lesion detection by 154% (877 vs. 347 enhancing lesions, \(p<0.0005\)) [Figure 9.1].

### ii New enhancing lesions

Modified brain MRI revealed 276 new enhancing lesions whereas conventional imaging detected 168 (64% increase, \(p<0.0005\)). Modified cord MRI revealed 71 new enhancing lesions, compared with 40 on conventional imaging (75% increase, \(p=0.002\)). Compared with conventional brain MRI alone, the modified brain/spine imaging protocol increased detection of new enhancing lesions by 107% (\(p<0.0005\)). No new or enlarging T2 lesions were detected in the absence of prior or corresponding enhancement on modified gadolinium enhanced studies.
Figure 9.1  Conventional versus optimised approaches to gadolinium enhanced imaging

(A) conventional T1-weighted MRI following 0.1mmol/kg Gd-DTPA
(B) optimised T1-weighted MRI following 0.3mmol/kg Gd-DTPA (and additional delayed MT presaturated imaging for brain studies)
<table>
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<td>No. of new Gd enhancing lesions</td>
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<td>SP</td>
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|            |            |                          | 347  | 75     | 168  | 40    | 754  | 123   | 276  | 71     |

*where RR = relapsing-remitting, and SP = secondary progressive MS.

**Table 9.2** MRI lesion activity summary for individual patients.

**iii Active studies**

For the conventional protocol, spinal MRI yielded only a 5% increase in the number of active studies (63 for brain/cord vs 60 for brain alone). Compared with conventional combined brain/spine imaging, the modified protocol detected 6% more active studies (67 vs. 62). For brain alone, the increase was 7% (64 vs. 60), and for spine 9% (36 vs. 33). Compared with conventional brain MRI alone, combined modified brain and spine imaging increased the number of active studies by 12% (67 vs. 60).

**iv New active studies**

For the conventional protocol, spinal MRI yielded a 7% increase in the number of new active studies (45 for the brain and cord and 42 for the brain alone). Compared with conventional
combined brain/spine imaging, the modified combined protocol detected an additional 11% new active studies (50 vs. 45). For brain alone, the increase was 14% (48 vs. 42), and for spine 12% (28 vs. 25). Compared with conventional brain imaging alone, combined modified brain/spine imaging increased the number of new active studies by 19% (50 vs. 42).

The effects of a modified protocol upon required sample sizes for clinical trials

Power calculations are presented for each type of scan (i.e. brain, cord, combined brain and cord) using the conventional and modified protocol [Tables 9.3 and 9.4]. In each row, there is a different presumed treatment effect (i.e. a 50, 60 and 70% reduction in the number of new enhancing lesions for parallel groups design and a 30, 40, 50% reduction for cross over design). The comparison involved conventional versus modified imaging protocols, as outlined above.

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</tr>
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<td>49 74 84 51</td>
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</table>

Table 9.3 Statistical power calculations for the parallel groups trial design. [Based on a 6 month study using monthly MRI comparing a placebo versus active treatment group. The treatment effect (percentage reduction in new enhancing lesions) is in the left hand column. The sample size is the top row of each table (in bold). A power of more than 80% is generally considered adequate for determining the sample size required to show a particular treatment effect].
### Table 9.4

Statistical power calculations for the cross over trial design [Based on a 6 month study using monthly MRI comparing 3 months run in (untreated) followed by 3 months of active treatment. The treatment effect (percentage reduction in new enhancing lesions) is in the left hand column. The sample size is the top row of each table (in bold). A power of more than 80% is generally considered adequate for determining the sample size required to show a particular treatment effect].

<table>
<thead>
<tr>
<th></th>
<th>BRAIN IMAGING</th>
<th></th>
<th></th>
<th>SPINAL CORD IMAGING</th>
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<th>COMBINED BRAIN AND SPINAL CORD IMAGING</th>
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The main observations are: (i) spinal cord MRI alone is much less powerful than brain MRI whether using a conventional or modified protocol; (ii) the addition of spinal to brain MRI does not improve power using either a conventional or modified protocol; (iii) compared to conventional brain MRI, a modified brain protocol does not significantly improve power using the parallel group design but does using the baseline crossover design.
9.4 Discussion

The study has revealed that a modified protocol - which used combined brain and cord imaging, triple dose Gd-DTPA, delayed imaging and, in the case of the brain, MT presaturation - markedly increased the amount of detectable enhancement compared with conventional single-dose enhanced T1-weighted imaging of the brain alone. Compared to the standard approach, the modified approach yielded 154% increase in the total number of enhancing lesions and 107% increase in the number of new enhancing lesions. This indicates that there is a substantial degree of focal lesion activity associated with BBB impairment that remains undetected with the current standard approaches to enhanced imaging which are widely used in clinical trial and natural history studies.

Using single dose contrast, the ratio of the number of enhancing cord versus brain lesions was relatively high when compared with previous studies [Kidd et al., 1996; Thorpe et al., 1996b]. This may partly be a reflection of the differences that occur by chance between relatively small patient cohorts. It may however reflect an increased sensitivity of the techniques which used thinner slices (3mm versus 4mm) and a longer post contrast delay (30 minutes versus 7 minutes) compared with the earlier studies. The gains in the cord using triple dose, while clearly apparent, were slightly less than those seen in the brain - the lack of a pre-saturation MT pulse and a shorter delay in the cord (30 versus 45 minutes) might account for this. The different timing was necessitated by the fact that only one region can be scanned at a time. The cord scans were thus not as fully modified as those from the brain; however, the extra time and effort involved in such optimisation would not seem worthwhile given the likelihood of minimal gains.

It was also notable that in almost all instances where there were enhancing cord lesions, enhancement was also seen in the brain - cord imaging therefore has little effect on the overall number of scans or patients that show activity using either the conventional or modified protocol. The overall increase, for combined brain and cord images, in the number of active scans using the modified protocol was also much less impressive than that for active lesions, with only 12% increase in the number of scans displaying enhancement and a 19% increase in the number of scans showing new enhancing activity. The majority of additional lesion activity detected thus occurs in scans and in patients already shown to have some degree of activity using the standard protocol. Looked at in another way, 30/37 (81%) scans that contained no enhancing lesions using the standard protocol continued to be inactive using the modified protocol. This indicates that the modified protocol is still associated with a high variability in the amount of inter-scan and inter-

212
patient lesion activity. Indeed, the inter-patient variability increases and this explains why there was no reduction in sample sizes required for parallel group design trials even though the overall sensitivity of the modified protocol was higher. A reduction in sample sizes was apparent when using the baseline versus treatment crossover design - whereas inter-patient variation is a limiting factor in sample sizes for parallel group designs, the intra-patient variability over time is the only source of variation influencing the sample sizes of crossover studies. In addition, completely inactive patients inevitably lead to a reduction of the power of crossover studies (since inactive patients can only remain stable or worsen); by reducing the number of inactive patients, the modified protocol will further improve the power.

A way of reducing intra-patient variability and amplifying sensitivity is to recruit only patients who have enhancing lesions on baseline MRI. Such patients are considerably more active on MRI over the next 3-6 months than those without enhancing baseline lesions. In this situation, the gain in sensitivity is more likely to improve the power of the modified over the conventional protocol in a parallel groups, placebo-controlled design. Such a gain may be useful, given the decreasing number of patients who can be recruited in to placebo-controlled trials, even for short periods, in an environment where increasing numbers of patients are being treated with disease-modifying therapies. The increase in sensitivity may also be useful for future studies which use an active control arm as a comparator, which results in a lower level of activity with which to compare the new therapy. The more activity that can be found in the active control arm, the greater the opportunity of being able to detect a stronger treatment effect in the new agent being tested.

In considering the application of a modified protocol in therapeutic trials, it is relevant to consider its individual components more closely. In particular, the addition of spinal cord imaging does not seem cost effective as it is time consuming to perform and offers negligible gains in terms of sample size requirements for either parallel groups or crossover designs. Furthermore, the use of MT presaturation for brain imaging could introduce difficulties in sequence standardisation between sites, and extra care is needed to compare pre- and post-contrast images since the MT pulse alone can sometimes result in a hyperintense lesion. The major gains in sensitivity and sample size requirements seen in our study reflect the effect of the higher dose of contrast applied to the brain. Previous cross-sectional work in the brain reveals that the increase in sensitivity of triple dose alone considerably exceeds that obtained by MT presaturation or delay [Silver et al 1997c]. A previous study using data from monthly MRI over
3 months in 40 patients compared triple dose versus single dose brain MRI directly and showed overall gains in sensitivity not dissimilar to those seen in the present study [Filippi et al 1998b]; sample size calculation in that study actually showed a reduction when using triple versus single dose, probably because the cohort exhibited less inter-patient variation in activity in comparison with the present cohort.

In the present study, a parametric simulation procedure based on the negative binomial model was used to derive sample size calculations. This model has been recently validated for enhancing lesion counts in a large cohort of patients with MS [Sormani et al., 1999] and it has been shown that, when using such an approach, sample size calculations are not dependent on the size of the data set used to estimate the parameters of the distribution. The present calculations, based on 12 cases with complete data, should therefore be reliable.

Taking all the data together, it seems that triple dose brain MRI alone is the most suitable method for short term MRI outcome studies using monthly imaging and a crossover design; the case for triple dose over single dose in parallel group studies is less convincing, but is probably warranted when selection is confined to those with enhancing lesions on a baseline scan. Another potential advantage of using triple dose contrast in treatment trials is the possibility that it will depict differential treatment effects on lesions with different enhancing characteristics. For example, the additional lesions seen only using triple dose are smaller and probably less destructive than those already seen using single dose [Filippi et al., 1998d], and as shown in a study of beta interferon, may be differentially suppressed by treatment [Filippi et al., 1999b]. However, such differential treatment effect on lesions can only be assessed when both single and triple dose images are obtained, which in the execution of a treatment trial is unlikely to be feasible.

Serial studies using conventional protocols have revealed a moderate correlation between the amount of enhancing lesions activity and clinical relapse [Kappos et al 1999], but little if any correlation with change in disability on the EDSS. A question arising is whether the additional activity seen using a modified protocol might improve clinical correlations. The follow up period of the present study was insufficient to evaluate these relationships. Although larger, longer duration studies are needed to definitively characterise the relationship between modified protocols and clinical outcomes, the finding that most of the additional activity seen using the modified protocol was at times when activity was already apparent using the conventional protocol, suggests that the correlations with clinical outcomes will not be much different.
Although their application in clinical trials may be limited, implementation of a wider range of techniques than triple dose brain MRI alone (e.g. MT presaturation, delay, cord imaging) does undoubtedly increase the overall sensitivity to detection focal areas of BBB breakdown, many of which are likely to be associated with inflammatory activity; such techniques may therefore have a role in selected studies evaluating natural history and pathogenesis. Because of the logistic requirements, such studies are likely to be useful in only a few specific investigative settings.
PART FOUR

Conclusion
10.1 MTR as a robust measure of structural change in multiple sclerosis

Myelin fulfills important roles for maintenance of normal axonal integrity and neuronal conduction. Demyelination is the hallmark pathological feature of MS, a common neurological cause of disability in young adults. It is increasingly apparent that myelin disruption is only one of the pathological substrates of permanent disability in MS; others include axonal loss and inflammation. Pathological processes implicated in subsequent tissue repair and restoration of neuronal function include remyelination, sodium channel insertion, resolution of inflammation, and brain plasticity. Future therapeutic strategies may be targeted to these processes in the hope of slowing the natural tendency towards disease progression. It is therefore of increasing importance to have techniques with which to probe and monitor the pathological processes that result in disability or restoration of function in MS and their modulation by such experimental therapies. Magnetization transfer imaging is of interest as this technique may provide a quantitative index of tissue structure. The most commonly used measure of the MT effect is MTR. Myelin is the major white matter component that contains structural components with bound protons. As such, measurement of MTR appears promising as a method for assessing the state of myelin in CNS demyelinating disorders such as MS.

In this thesis, I have explored the potential of MTR as a putative marker of myelin integrity. Previous studies have provided preliminary evidence for such a role; reductions in MTR have been observed in MS, EAE, and PML (a condition characterised by predominant demyelination). In the healthy developing child, white matter MTR has been shown to increase in accordance with the normal time course and anatomical distribution of myelination. In a study of optic neuritis, reduction of MTR has been shown to correlate with physiological evidence of demyelination. However, it is also clear that MTR does not have absolute specificity as a marker for myelin integrity; much smaller reductions than seen with predominant demyelination may also occur as a result of other pathological states such as axonal injury or oedema.

In chapter four of this thesis, the normal anatomical variation of MTR within the brain is
described. In agreement with previous studies, the highest values for MTR were observed in the corpus callosum; no other structure in the healthy brain has such tightly packed myelin. This provides supporting evidence for myelin as a contributor to MTR values. However, it does not exclude the contributions to MTR from other structures such as axons and glial tissue. Likewise, the observed association between increasing age and mild decreases in MTR could be explained by demyelination, axonal loss, gliosis or any combination of such features. These data therefore provide only limited evidence to support a contribution of myelin to MTR values in health. They do however provide a useful database for normal MTR within healthy tissue structures. For studies incorporating MTR, it is important to be aware of the small reductions that may result with increasing age. Such changes are however very small and probably of only little significance for the purpose of many studies.

Data presented in chapter five provides greater insight into the relative contributions of myelin and axons to MTR values. Here, patients with central pontine myelinolysis (a non-inflammatory condition characterised by predominant demyelination and relative lack of axonal disruption) were studied with both MTR and proton MRS. Proton MRS confirmed an absence of significant disruption to axonal function or integrity in 2/3 cases. Early in the disease, MTR values were markedly reduced. These recovered in parallel with clinical improvement. This recovery in MTR could not be suitably explained by atrophy (and associated increase in tissue density) and/or gliosis, as pontine atrophy was minimal when observed. It may indicate remyelination. Severe MTR reduction, as much as 100% within pixels within the centre of the lesion, was noted in all cases. Mean MTR for the whole lesion was reduced in one case by as much as 73%. This is in keeping with pathological descriptions that frequently report absence of myelin or severe demyelination that is most profound at the lesion centre. Here, the normal NAA resonance on proton MRS confirmed the absence of significant axonal disruption. These data provide strong support for myelin as the predominant contributor to MTR values in health. Unlike previous studies that have addressed this question, the concurrent use of proton MRS excluded a significant axonal influence on the reduced MTR values in two of the three cases studied.

The natural evolution of MTR within acute MS lesions is described in chapter six. Here, MTR was measured prior to and during the evolution of new MS lesions. A weekly study interval was employed to reduce the limits of temporal resolution. Contrast-enhanced T1-weighted MRI allowed the onset of blood-brain barrier disruption to be defined. MTR was
normal in the region prior to the appearance of a visible lesion, decreased over the first few weeks and subsequently underwent partial recovery. The duration of enhancement correlated with the degree of MTR reduction. The limited time of study did not allow demonstration of the actual degree of recovery, although a number of other studies have suggested persistent mild to moderate MTR reduction in MS lesions. A number of conclusions may be drawn. First, the initial change in MTR is subsequent to enhancement which is the initial visible change in all lesions studied. This suggests that BBB breakdown is not preceded by substantial demyelination. As such, demyelination may not be a necessary initiating factor in MS lesions and it may be that demyelination occurs as a result of initial BBB disruption and inflammation. Although it is not possible in this study to exclude a small degree of myelin disruption as the primary event in the formation of a lesion, observations of blood-retinal barrier breakdown in optic neuritis support the hypothesis that inflammatory BBB barrier breakdown may occur in the absence of myelin or myelin breakdown products. Second, the degree of MTR reduction observed is greater than that previously observed in any isolated pathological entity other than demyelination. A number of additional factors are likely to contribute, especially during the phase of BBB disruption where one would expect oedema, inflammatory infiltrate and a minor degree of axonal disruption. Third, the degree of MTR reduction is in some way associated with the duration of BBB breakdown, suggesting a graded relationship between the degree of inflammation and subsequent demyelination. This would support the hypothesis that demyelination is mediated by inflammatory responses. Fourth, assuming the main reduction in MTR to be a result of demyelination, the predominant recovery probably reflects subsequent remyelination. Again, other factors such as resolution of oedema may contribute. Gliosis is commonly present in older lesions and would be expected to contribute to persistent MTR reduction. Finally, it may seem difficult to reconcile these and other investigators’ findings with reports of subtle quantitative abnormalities in white matter (lipid peaks, reduced MTR or abnormal diffusion) that predate visible lesion development in MS by some months. Possible explanations for the different results are differences in: (i) the frequency of scanning; (ii) the sensitivity of sequences used to detect blood-brain barrier leakage or subtle quantitative change in other MR measures; pathogenic mechanism (some patient groups having blood-brain barrier leakage as the first event and others having a subtle pathological change preceding it).

In this section of the thesis, the potential application of MTR as a putative marker of demyelination has been evaluated and applied to MS. Evidence has been provided to support the
hypothesis that myelin provides the predominant contribution to MTR values in healthy white matter. However, it is also clear from other works that MTR is not specific to demyelination but may be influenced to far smaller degrees by a variety of other pathological processes found both in MS and other CNS disorders. However, anything other than small reductions in MTR is suggestive of disruption to the normal state of myelin. In combination, MT imaging and proton MRS may be particularly useful for evaluating the natural history of demyelinating conditions. Future combined use of diffusion imaging and functional MRI may allow further insight into the precise mechanisms of neurological impairment and recovery.

These studies have also highlighted potential difficulties associated with the use of myelin markers for prognosis in MS and other demyelinating conditions. The importance of demyelination and the natural ability to remyelinate lesions in MS has been well described in the contexts of neurological impairment and subsequent clinical recovery. As such, measurements related to the state of myelin might be assumed to be of importance in prognosis and response to therapy in MS. The serial study of new MS lesions confirms that it is possible to observe, at least to a certain extent, the time course and degree of demyelination and remyelination that may occur within such acute lesions. The recorded MTR will also be influenced to some extent by those changes other than demyelination that are known to occur in new MS lesions. Study of CPM has allowed valuable insight into the relationships between MTR, demyelination, and clinical course because this condition is associated with a single lesion that is (a) of remarkably homogenous pathology, and (b) sited in a clinically eloquent site. Extremely low values of MTR were observed in these cases during the period of early neurological impairment. Clinical recovery was paralleled by recovery in MTR. The observed large MTR reductions and marked but incomplete recovery may be ascribed with a degree of confidence to demyelination and remyelination. Although I am not aware of pathological reports confirming the presence of remyelination in CPM, the considerable degree of clinical recovery that may occur in CPM and published evidence of acute reduction and subsequent recovery in auditory evoked potentials provide support for remyelination. It is of note that full or almost full clinical recovery occurred despite considerable persistent MTR reduction. These data could indicate that persistent partial demyelination and incomplete remyelination can be associated with normal neurological function. A parallel example of such discrepancy is optic neuritis, where persistent lengthening of the latency may be associated with the absence of neurological deficit, or a state where the deficit is too subtle to interfere with normal function. As such, the ability of a myelin marker to
monitor and assign prognosis to more complex conditions such as MS is in doubt. In MS particularly, lesions are more pathologically heterogenous and may occur in any site. Pathological changes vary according to the duration and clinical course of the disease. Lesions are not static but evolve and vary over time. Different lesions in the same patient are typically at varying stages of evolution. Finally, much of the pathology is subtle and diffuse and beyond the resolution of conventional MRI. As such, certain limitations are to be expected for using MTR measurements as a surrogate marker of disability or prognosis in MS.

10.2 Future development of MTR techniques

One major advantage for MRI is the ability to safely repeat examinations, allowing repeat study over short time periods and also combination of different techniques. With increasing time and parallel improvement in other MRI techniques, the relationship of MTR to lesion pathology should become more clear. New techniques for MTR data acquisition such as echoplanar imaging and three-dimensional image acquisition are likely to provide shorter imaging times and improved resolution respectively. In chapter six, registration of two-dimensional serial MTR data allowed useful insight into serial change. The study showed limitations in this registration technique for very small lesions or regions of interest. Three-dimensional techniques have smaller pixels and may be re-formatted in any plane; they are therefore amenable to more accurate registration. In addition to image acquisition, developments in data processing are evolving. These include histogram techniques to look at global changes in structure of MR visible and invisible lesions and improved mechanisms to classify such change, e.g. principal component analysis (PCA). Such techniques lack anatomical specificity and therefore the ability to look at specific regions may require improved segmentation techniques to initially delineate a particular region of interest. With three-dimensional MTR data acquisition, and also with use of methods such as statistical parametrical mapping (SPM), there will be the ability to compare groups of subjects for anatomical distribution of change. With improved clinical-radiological correlation, it may be possible to monitor clinically eloquent regional changes associated with MS with greater precision. Standardisation may be applied at both the data acquisition and processing stages to allow multi-site data to be collected and compared. This will be important if MTR is to be used as a surrogate marker of disability to monitor clinical trials. Finally, the development of alternative quantitative measures of the MT effect such as the Z-spectrum will be of interest;
these techniques first need to be more amenable to clinical imaging systems if they are to be of benefit to the study of diseases such as MS.

10.3 The role of MT as a technique for improving sensitivity of contrast-enhanced MRI

Blood-brain barrier breakdown is an important component of the inflammatory response associated with development of new MS lesions and reactivation of chronic lesions. The majority of therapies in use or in development for the treatment of MS are directed at the immune response. By detecting BBB breakdown, contrast-enhanced MRI may provide indirect information about inflammatory activity within lesions.

In chapter eight, three different techniques to improve sensitivity of contrast-enhanced MRI were evaluated. The study design was cross-sectional. This study confirmed that at least 50% of active inflammatory lesions associated with BBB breakdown are likely to be missed using conventional techniques of the type normally used in clinical and research studies. For any pathological study that requires accurate delineation of active versus inactive lesion, the most sensitive test is optimal if conclusions are to be drawn about the inactive lesions, as their inactivity may be artefactual. When comparing individual techniques to optimise sensitivity, the use of a higher “triple” dose of Gd-DTPA appeared more useful than either MT contrast or delayed imaging. Whilst there are disadvantages of cost, and possibly of an increased tendency towards unwanted side-effects, this is potentially the easiest optimisation technique to apply in clinical trials. Delayed imaging has disadvantages of increasing the time of study unless the protocol is carefully planned to use the delay for the purpose of acquisition of other necessary images (e.g. T2 and proton density). Another problem is that all lesions have their own unpredictable optimal delay to allow optimal detection. Any pre-defined delay has to take into account that this will be optimal for most but by no means all of the lesions. Such limitations are insurmountable unless a number of images are acquired with different degrees of delay to ensure as many enhancing lesions may be detected. Magnetization transfer adds a small benefit for increasing the detection of enhancing lesions, comparable with the benefits of delayed imaging. It is important to use relatively small presaturation pulses that do not result in significant false positive enhancing lesions (i.e. bright prior to gadolinium). Difficulties exist for standardisation across sites and it could be argued that the use of MT generates an additional need for quality assurance to ensure no change occurs in scanner performance over time. Bearing in mind these
limitations, the combined use of triple-dose gadolinium, magnetization transfer and delayed imaging does provide an increase in cross-sectional sensitivity that is significantly more than that of triple-dose alone. This was seen in all but the patients with primary progressive MS, where it is likely that discrete inflammatory lesions are a relatively rare occurrence; these findings support the hypothesis that progressive disability may occur independent of focal BBB breakdown and inflammation. Overall, these results suggest that the combined approach may be useful to increase the sensitivity to detection of enhancing lesions and should be considered in situations where there are disadvantages to missing some inflammatory lesions (e.g. in the research setting, where optimal accuracy and sensitivity are considered to be important).

In chapter nine, the optimisation strategies for enhanced brain imaging (triple-dose gadolinium, magnetization transfer, and delayed imaging) were further explored in a longitudinal study that also evaluated the potential advantages of adjunctive spinal cord imaging with triple-dose gadolinium. Power calculations were applied to the serial data to help evaluate the potential for such techniques to reduce sample size or study duration in MS treatment trials. The modified brain and spine protocol detected more than two and a half times the number of enhancing lesions and more than double the number of new enhancing lesions than conventional single-dose gadolinium T1-weighted brain imaging, the technique in current use for most treatment trials. It was notable that in almost all instances where there were enhancing cord lesions, there were also enhancing brain lesions. As such, the addition of cord imaging is not particularly useful in detecting otherwise undetectable lesion activity. Only 19% of negative conventional brain studies were associated with evidence of focal enhancement when imaged with the more sensitive modified brain and spine protocol. Disadvantages of these optimisation techniques include increased time of study (with increased cost and the need for increased patient compliance), the theoretical increased risk of adverse effects with a higher triple-dose of gadolinium (further studies are required to confirm the increased risk of minor reactions shown here) and the increased cost of gadolinium. With MT presaturation, there is an increased risk of false positive reporting of enhancing lesions with lesions appearing bright prior to administration of gadolinium - this potential problem may be avoided if pre-contrast films are also obtained. If MT presaturation is employed, this requires careful standardisation for the purposes of multi-site trials. Benefits other than increased sensitivity to detection of enhancing lesions include the potential increase in specificity - a decrease in vascular markings is observed at the delayed time point making the differentiation between vessels and lesions clearer.
When power calculations were applied to the data, it was clear that there was no reduction in sample sizes required for parallel group design trials even though the overall sensitivity of the modified protocol was higher. A marginal but statistically significant reduction in sample sizes was however apparent when using the baseline versus treatment crossover design. Such small benefits need to be weighed against potential disadvantages. Overall, it appears that triple-dose brain imaging is the most useful optimisation technique for such purposes, whereas delayed imaging and magnetization transfer may have more disadvantages than advantages for the purpose of clinical trials.

Although their application in clinical trials may be limited, implementation of a wider range of techniques than triple dose brain MRI alone (e.g. MT presaturation, delay, cord imaging) does undoubtedly increase the overall sensitivity to detection of focal areas of BBB breakdown, many of which are likely to be associated with inflammatory activity; such techniques may therefore have a role in selected studies evaluating natural history and pathogenesis. Because of the logistic requirements, such studies are likely to be useful in only a few specific investigative settings.

10.4 Future development of contrast-enhanced MRI

In designing an optimal contrast-enhanced study, various factors need to be addressed. What is optimal for one study may not be optimal for another and the design will depend upon the individual clinical or research requirements. As shown in chapter nine, the most sensitive sequence does not necessarily impart significant benefit in power of the study. If only small gains are seen in sensitivity, these need to be carefully evaluated against any increase in cost or study time.

Despite such considerations, certain parts of the MRI process may be amenable to further optimisation. In the process of data acquisition, the use of newer fast imaging techniques may be useful, especially for patients undergoing detailed imaging protocols. This may reduce sensitivity to motion artefact. Simple imaging protocols are necessary to ensure reproducibility over time and also continued patient compliance in what may appear increasingly demanding studies. Standardisation is essential for multi-centre studies and such issues have been addressed for MT presaturation in chapter three. Standardisation may also be important in the other parts of the data acquisition, production of hardcopy images (e.g. windowing) and in the analysis of data if different raters are employed to analyse the data. It may be possible to further improve sensitivity
by improving spatial resolution of the T1-weighted sequence; in particular, three-dimensional imaging with the ability to study thinner image slices looks promising. Reformatting of three-dimensional data in different planes also may allow greater confidence in reporting of enhancing lesions and this should reduce false positive reporting of enhancing lesions. Changing to a three-dimensional sequence will only be of use if optimal T1-weighting is retained. Sensitivity may be further increased by improving the temporal resolution, e.g. weekly versus monthly study. This is important when attempting to correlate imaging parameters against other measures that may fluctuate rapidly (e.g. measures of inflammatory markers, clinical measures). The considerable increase in demand on patients may however be impractical, especially for large studies.

Improvements in contrast-enhanced studies may also be achieved at the post-processing stage. One potential method that will be of considerable interest is the use of registered serial data to determine change over time. It will be important for standardised imaging protocols to have precise delays between contrast administration and data acquisition, if blood vessels are to cancel out enough to allow recognition of subtle changes in enhancing lesions over time. Such strategies offer the potential for future automation, with reduced trial costs and possible improvements in reproducibility.
APPENDIX A

A I. The Expanded Disability Status Scale (EDSS) [Kurtzke, 1983]

0.0 Normal neurological exam (all grade 0 in functional score, FS).
1.0 No disability, minimal signs in one FS (i.e. grade 1).
1.5 No disability, minimal signs in more than one FS (more than one grade 1).
2.0 Minimal disability in one FS (one FS grade 2, others 0 or 1).
2.5 Minimal disability in two FS (two FS grade 2, others 0 or 1).
3.0 Moderate disability in one FS (one FS grade 3, others 0 or 1) or mild disability in three or four FS (three or four FS grade 2, others 0 or 1), though fully ambulatory.
3.5 Fully ambulatory but with moderate disability in one FS (one grade 3) and one or two FS grade 2; or two FS grade 3, or five FS grade 2 (others 0 or 1).
4.0 Fully ambulatory without aid, self-sufficient up and about some 12 hours a day despite relatively severe disability consisting of one FS grade 4 (others 0 or 1) or combinations of lesser grades exceeding limits of previous steps; able to walk or rest some 500m.
4.5 Fully ambulatory without aid, up and about much of the day, able to work a full day, may otherwise have some limitation of full activity or require minimal assistance, characterised by relatively severe disability usually consisting of one FS grade 4 (others 0 or 1) or combinations of lesser grades exceeding limits of previous steps; able to walk without aid or rest some 300m.
5.0 Ambulatory without aid or rest for some 200m; disability severe enough to impair full daily activities (e.g. to work a full day without special provisions); (usual FS equivalents are one grade 5 alone, others 0 or 1, or combinations of lesser grades usually exceeding specifications for step 4.0).
5.5 Ambulatory without aid or rest for about 100m, disability severe enough to preclude full daily activities (Usual FS equivalents are one grade 5 alone, others 0 or 1, or combinations of lesser grades usually exceeding specifications for step 4.0).
6.0 Intermittent or unilateral constant assistance (cane, crutch, brace) required to walk about
100m with or without resting (Usual FS equivalents are combinations with more than two FS grade 3).

6.5 Constant bilateral assistance (canes, crutches, braces) required to walk about 20m without resting (usual FS equivalents are combinations with more than 2 FS grade 3).

7.0 Unable to walk beyond approximately five meters even with aid, essentially restricted to wheelchair; wheels self in standard wheelchair and transfers alone, up and about in wheelchair some 12 hours a day. (Usual FS equivalents are combinations with more than one FS grade 4+, very rarely pyramidal grade 5 alone).

7.5 Unable to take more than a few steps; restricted to wheelchair; may need aid in transfer, wheels self but cannot carry on in standard wheelchair a full day; may require motorised wheelchair. (Usual FS equivalents are combinations with more than one grade 4+).

8.0 Essentially restricted to bed or chair or perambulated in wheelchair, but may be out of bed itself much of the day; retains many self-care functions; generally has effective use of arms (Usual FS equivalents are combinations, generally grade 4+ in several systems).

8.5 Essentially restricted to bed much of the day; has some effective use of arm(s); retains some self-care functions. (Usual FS equivalents are combinations, generally grade 4+ in several systems).

9.0 Helpless bed patient; can communicate and eat (Usual FS equivalents are combinations, mostly grade 4+).

9.5 Totally helpless bed patient, unable to communicate effectively or eat/swallow. (Usual FS equivalents are combinations, almost all grade 4+).

10.0 Death due to MS.

Pyramidal Functions
0. Normal.
1. Abnormal signs without disability.
2. Minimal disability.
3. Mild or moderate paraparesis or hemiparesis; severe monoparesis.
4. Marked paraparesis or hemiparesis; moderate quadriparesis; or monoplegia.
5. Paraplegia, hemiplegia, or marked quadriparesis.
6. Quadriplegia.
v. Unknown.

Cerebellar Functions
0. Normal.
1. Abnormal signs without disability.
2. Mild ataxia.
  ii Moderate truncal or limb ataxia.
  i i Severe ataxia, all limbs.
  ii Unable to perform coordinated movements due to ataxia.
  i i Unknown.
  ii Is used throughout after each number when weakness (grade 3 or more on pyramidal)
interferes with testing.

Brain Stem Functions
0. Normal.
1. Signs only.
  ii Moderate nystagmus or other mild disability.
  ii Severe nystagmus, marked extraocular weakness or moderate disability of other cranial
nerves.
  ii Marked dysarthria or other marked disability.
  ii Inability to swallow or speak.
Sensory Functions

0. Normal
1. Vibration or figure-writing decrease only, in one or two limbs.
2. Mild decrease in touch or pain or position sense, and/or moderate decrease in vibration, in one or two limbs; or vibratory decrease alone in three or four limbs.
3. Moderate decrease in touch or pain or position sense, and/or essentially lost vibration in one or two limbs; or mild decrease in touch or pain and/or moderate decrease in all proprioceptive tests in three or four limbs.
4. Marked decrease in touch or pain or position sense, alone or combined, in one or two limbs; or moderate decrease in touch or pain and/or severe proprioceptive decrease in more than two limbs.
5. Loss (essentially) of sensation in one or two limbs; or moderate decrease in touch or pain and/or loss of proprioception for most of the body below the head.
6. Sensation essentially lost below the head.
V. Unknown.

Bowel and Bladder Functions

0. Normal
1. Mild urinary hesitancy, urgency, retention of bowel or bladder.
2. Moderate hesitancy, urgency, retention of bowel or bladder, or rare urinary incontinence.
3. Frequent urinary incontinence.
4. In need of almost constant catheterisation.
5. Loss of bladder function.
V. Unknown.

Visual (or Optic) Functions

0. Normal
1. Scotoma with visual acuity (corrected) better than 20/30.
2. Worse eye with scotoma with maximal visual acuity (corrected) of 20/30 to 20/59.
3. Worse eye with large scotoma, or moderate decrease in fields, but with maximal visual acuity (corrected) of 20/60 to 20/99.

4. Worse eye with marked decrease of fields and maximal visual acuity (corrected) of 20/100 to 20/200; grade 3 plus maximal acuity of better eye of 20/60 or less.

5. Worse eye with maximal visual acuity (corrected) less than 20/200; grade 4 plus maximal acuity of better eye of 20/60 or less.

6. Grade 5 plus maximal acuity of better eye of 20/60 or less.

V. Unknown.

X. Is added to grades 0 to 6 for presence of temporal pallor.

Cerebral (or Mental) Functions

0. Normal.

1. Mood alteration only (Does not affect DSS score).

2. Mild decrease in mentation.

3. Moderate decrease in mentation.

4. Marked decrease in mentation (chronic brain syndrome - severe or incompetent).

V. Unknown.

Other Functions

0. None.

1. Any neurologic findings attributed to MS (specify).

V. Unknown.
## APPENDIX B

### B1 Conference proceeding abstracts associated with this thesis

**NC Silver, GJ Barker, DG MacManus, DH Miller.**
Magnetisation Transfer ratio in the cervical cord in multiple sclerosis.
*Multiple Sclerosis Society Grantholders meeting: Multiple Sclerosis 1996;2 (1): 4.27.*

**NC Silver, GJ Barker, DG MacManus, DH Miller.**
Magnetisation transfer ratio of brain white matter: a normative database spanning 4 decades of life.

**NC Silver, CD Good, GJ Barker, DG MacManus, AJ Thompson, DH Miller.**
Evaluation of Gd-DTPA dose, magnetisation transfer imaging and delayed scanning on detection of multiple sclerosis enhancing lesions.

**NC Silver, GJ Barker, DG MacManus, DH Miller.**
Magnetisation transfer ratio of the cervical cord in multiple sclerosis.

**NC Silver, GJ Barker, DG MacManus, JW Thorpe, RS Howard, DH Miller.**
Decreased magnetisation transfer ratio due to demyelination: a case of central pontine myelinolysis.

**NC Silver, CD Good, GJ Barker, DG MacManus, AJ Thompson, IF Moseley, DH Miller.**
Enhancing lesion detection in multiple sclerosis: effects of gadolinium dose, magnetisation transfer and delayed scanning.

**NC Silver, GJ Barker, DG MacManus, DH Miller.**
Magnetisation transfer ratio of the cervical cord in multiple sclerosis.

**NC Silver, CD Good, GJ Barker, DG MacManus, AJ Thompson, IF Moseley, DH Miller.**
Sensitivity of contrast enhanced MRI in multiple sclerosis: effects of gadolinium dose, magnetisation transfer contrast and delayed imaging.
*British Chapter of the Society of Magnetic Resonance in Medicine 1996; 38.*


NC Silver, E Hughes, K Birnie, PD Molyneux, DG MacManus, GJ Barker, PS Tofts, DH Miller. Magnetization transfer imaging provides a highly reproducible quantitative measure of brain tissue structural integrity. *International Society for Magnetic Resonance in Medicine* 1998;2162.


Bibliography


Balaban RS and Ceckler TL. Magnetization transfer contrast in magnetic resonance imaging. Magnetic Resonance Quarterly 1992;8:116-137.

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Ellison GW, Myers WL, Leake BD. Responsiveness of the disability status scale (DSS) [abstract]. Neurology 1993;43 (suppl 2):A204.


McDonald WI. From pathogenesis to symptomatology (pp. 1-4). Key advances in the effective management of multiple sclerosis, Royal Society of Medicine Press 1999.


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Myers LS, Ellison GW, Leake BD. Reliability of the disability status scale (DSS) [abstract]. Neurology 1993;43 (suppl 2):A204.


Paty DW, McFarlin DE, McDonald WI. Magnetic resonance imaging and laboratory aids in the diagnosis of MS. Ann Neurol 1991;29:3-5.
Paty DW, Li DKB, the UBC MS/MRI Study Group, and the IFNB Multiple Sclerosis Study Group. Interferon beta-1b is effective in relapsing-remitting multiple sclerosis. II. MRI analysis results of a multicenter, double-blind, placebo-controlled trial. Neurology 1993;43:662-667.


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Tofts PS. Standardisation and optimisation of magnetic resonance techniques for multicentre studies. J Neurol Neurosurg Psychiatry 1998;64:S37-S43.


