The Natural History of Primary Progressive Multiple Sclerosis: Serial Clinical and MRI Evaluation and Application of New Spinal Imaging Techniques

Valerie L Stevenson, MBBS, MRCP
NMR Research Unit
Institute of Neurology
Queen Square
London WC1N 3BG

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Abstract

This thesis describes the clinical and Magnetic Resonance Imaging (MRI) characteristics of patients with primary progressive multiple sclerosis (PP MS). These patients make up less than 10% of the MS population and because of their relative rarity, few studies have looked at this group and only three small therapeutic trials have been undertaken to date. By definition their course is purely progressive and therefore they are an ideal group to study disease progression independent from the effect of relapses. In a cohort of 60 patients, cross sectional analysis revealed that patients with PP MS have lower T2 and T1 hypo-intensity lesion loads than other patient groups. Serial analysis revealed that despite these low lesion loads, measurable changes were seen after relatively short time periods. Both brain and cord atrophy was also shown to develop. These results have implications for trial design particularly in the choice of appropriate outcome measures. As many of these patients have a predominant spinal cord syndrome which is responsible for much of their locomotor disability, the development of several techniques aimed at optimising spinal cord imaging are described. These include two new MR sequences, fast FLAIR and 3- dimensional fast spin echo (3D FSE). Although the 3D FSE sequence did increase lesion detection, the relationship between spinal cord lesion load and disability remained poor. The fast FLAIR sequence failed to detect lesions in the cord despite increased lesion detection in the brain, thus indicating differences in lesion composition dependent on lesion site. This was confirmed by the subsequent quantification of T1 and T2 relaxation times in both lesions and normal appearing white matter in MS. This work is important in improving our understanding of the pathological processes responsible for disease progression in MS and aids in the choice of appropriate outcome measures for monitoring change, particularly important in therapeutic trials.
Declaration

The work described in parts B and C of this thesis is all original.

Part B documents a cross sectional and serial study of patients with primary and transitional progressive MS. These patients were recruited as part of the MAGNIMS (Magnetic resonance network in multiple sclerosis) study group, an EC initiative which brings together centres throughout Europe with an interest in both MS and MRI. I was involved in designing the protocol and organising the data transfer from the six participating centres. One hundred of the 211 patients recruited were seen by myself, this involved annual clinical assessments and MRI. All of the data, from the six centres, were analysed by myself.

I was the lead investigator for the studies outlined in chapters four, seven and eight. This involved study design, clinical assessments, the overseeing of MRI and data analysis.
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Abbreviations

2D  two dimensional
3D  three dimensional
$B_0$  magnetic field strength
$\gamma$  gyro-magnetic ratio
$\omega_0$  frequency of precession
ADC  apparent diffusion coefficient
CDMS  clinically definite multiple sclerosis
cm  centimetre
CNS  central nervous system
COV  coefficient of variation
CPMS  clinically probable multiple sclerosis
CSE  conventional spin echo
CSF  cerebrospinal fluid
CT  computerised tomography
DSS  disability status scale
DTPA  diethylene triaminepentaacetic acid
EC  european community
EDSS  expanded disability status scale
FID  free induction decay
FLAIR  fluid attenuated inversion recovery
FSE  fast spin echo
HLA  human leucocyte antigen
<table>
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<td>ICAM-1</td>
<td>intercellular adhesion molecule-1</td>
</tr>
<tr>
<td>IFNB-1a</td>
<td>interferon beta-1a</td>
</tr>
<tr>
<td>IFNB-1b</td>
<td>interferon beta-1b</td>
</tr>
<tr>
<td>IR FSPGR</td>
<td>inversion recovery fast spoiled gradient echo</td>
</tr>
<tr>
<td>IVIG</td>
<td>intravenous immunoglobulin</td>
</tr>
<tr>
<td>k-space</td>
<td>the area where data is stored</td>
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<td>LSDMS</td>
<td>laboratory supported definite multiple sclerosis</td>
</tr>
<tr>
<td>LSPMS</td>
<td>laboratory supported probable multiple sclerosis</td>
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<tr>
<td>MAGNIMS</td>
<td>magnetic resonance network in multiple sclerosis</td>
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<tr>
<td>MBP</td>
<td>myelin basic protein</td>
</tr>
<tr>
<td>MHz/T</td>
<td>mega hertz per tesla</td>
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<tr>
<td>mm</td>
<td>millimetre</td>
</tr>
<tr>
<td>ms</td>
<td>millisecond</td>
</tr>
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<td>MRI</td>
<td>magnetic resonance imaging</td>
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<td>MRS</td>
<td>magnetic resonance spectroscopy</td>
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<tr>
<td>MS</td>
<td>multiple sclerosis</td>
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<tr>
<td>MT</td>
<td>magnetisation transfer</td>
</tr>
<tr>
<td>MTR</td>
<td>magnetisation transfer ratio</td>
</tr>
<tr>
<td>NAA</td>
<td>N-acetyl aspartate</td>
</tr>
<tr>
<td>NAWM</td>
<td>normal appearing white matter</td>
</tr>
<tr>
<td>NS</td>
<td>not significant</td>
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<tr>
<td>PD</td>
<td>proton density</td>
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<tr>
<td>PLP</td>
<td>proteo-lipid protein</td>
</tr>
<tr>
<td>Abbreviation</td>
<td>Definition</td>
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<tr>
<td>PP MS</td>
<td>primary progressive multiple sclerosis</td>
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<tr>
<td>RARE</td>
<td>rapid acquisition with relaxation enhancement</td>
</tr>
<tr>
<td>RF</td>
<td>radio-frequency</td>
</tr>
<tr>
<td>ROI</td>
<td>region of interest</td>
</tr>
<tr>
<td>RR MS</td>
<td>relapsing-remitting multiple sclerosis</td>
</tr>
<tr>
<td>SD</td>
<td>standard deviation</td>
</tr>
<tr>
<td>SNR</td>
<td>signal to noise ratio</td>
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<tr>
<td>SP MS</td>
<td>secondary progressive multiple sclerosis</td>
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<tr>
<td>T1</td>
<td>longitudinal relaxation time</td>
</tr>
<tr>
<td>T2</td>
<td>transversal relaxation time</td>
</tr>
<tr>
<td>T2*</td>
<td>loss of phase due to inhomogeneities within the magnetic field</td>
</tr>
<tr>
<td>TE</td>
<td>echo time</td>
</tr>
<tr>
<td>TEeff</td>
<td>effective echo time</td>
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<tr>
<td>TI</td>
<td>inversion time</td>
</tr>
<tr>
<td>TR</td>
<td>repetition time</td>
</tr>
<tr>
<td>TLV</td>
<td>total lesion volume</td>
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<tr>
<td>TP MS</td>
<td>transitional progressive multiple sclerosis</td>
</tr>
<tr>
<td>VCAM-1</td>
<td>vascular adhesion molecule-1</td>
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<tr>
<td>yrs</td>
<td>years</td>
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Papers arising from this work


Stevenson VL, Parker GJM, Barker GJ, Birnie K, Tofts PS, Miller DH, Thompson AJ. Quantification of T1 and T2 relaxation times in the normal appearing white matter and lesions of multiple sclerosis. Submitted to J Neurol Sci.


Part A  Background
Chapter 1: Multiple Sclerosis

Multiple Sclerosis (MS) is an extremely variable disease both within the patient population and within each patient over time. It is characterised by multiple episodes of demyelination throughout the neural axis over many years. These demyelinating lesions or 'plaques' can affect any area of the central nervous system (CNS) and therefore produce a multiplicity of symptoms. These range from the obvious problems of weakness and spasticity, ataxia and sphincter disturbance, to less frequent but equally important problems such as visual, cognitive, swallowing and respiratory difficulties. Some of the commonest and to the patient most disabling symptoms, are fatigue, thermal sensitivity and pain, areas often neglected by the physician in the face of more obvious physical needs. MS is a disease that strikes young people in the prime of their life and is then present for perhaps thirty or forty years having little impact on longevity and consequently it is a major financial burden to society. In a large survey of members of the MS society in the UK, the estimated annual cost to society was in the order of £1.2 billion, with half of this being accounted for by lost earnings and state benefits (Holmes 1995).

Prognosis in an individual is unpredictable, most patients (~85%), follow an initial course of relapses (a neurological deficit lasting at least 24 hours) with complete or incomplete recovery, so called relapsing-remitting (RR) MS. Over half of these patients then go on to a progressive phase where disability accumulates steadily with or without superimposed relapses, secondary progressive (SP) MS (Runmarker and Andersen 1993). Approximately 10% of patients experience a progressive disease course from onset with
the absence of any relapses or remissions, these patients are classified as primary progressive (PP) MS. This leaves a small proportion who do not fit easily into any of these categories. Those who essentially exhibit a progressive course but who have a history of a single relapse (this may be many years before the onset of the disease progression or superimposed on the progression) have been denoted as transitional progressive (TP) MS. If there is a history of more than one relapse on a background of progression they are classified as progressive-relapsing (Lublin and Reingold 1996). The disease courses are depicted in figure 1.1.

![Disease course in multiple sclerosis](image)

**Figure 1.1:** Disease course in multiple sclerosis.

In addition to this classification, two terms have been applied to disease severity, these are Benign MS, in which the patient has minimal disability ten years after onset (Filippi 1996c, Losseff 1996b) and Malignant MS which is characterised by a rapid, progressive course leading to death or severe disability within five years.
1.1 Diagnosis

Multiple sclerosis is fundamentally a clinical diagnosis although investigations including magnetic resonance imaging (MRI), evoked potentials and cerebrospinal fluid (CSF) examination increase diagnostic accuracy and certainty. The role of such investigations in the confirmation of dissemination in space is particularly useful. The initial clinical criteria for diagnosis of MS were proposed by Schumacher and included three levels (Schumacher 1965):

i) Clinically definite MS. Two or more episodes of neurological dysfunction ≥ 1 month apart or slow or stepwise progression > 6 months, plus objective signs of neurological dysfunction on examination displaying dissemination in space.

ii) Probable MS. Relapsing-remitting symptoms with only one neurological sign or a documented single episode with signs of multifocal disease.

iii) Possible MS. Relapsing-remitting symptoms without documented or objective signs.

The Poser Committee revised these criteria to include paraclinical information obtained from the results of visual evoked potentials, CSF examination and MRI (Poser 1983). The Poser criteria consists of two major groups, definite and probable, each with two subgroups, clinically and laboratory (CSF) supported;

A. Clinically definite MS (CDMS). Two attacks and clinical evidence of two separate lesions, or, two attacks with clinical evidence of one lesion and paraclinical evidence of another, separate lesion.

B. Laboratory supported definite MS (LSDMS). Positive oligoclonal bands in the CSF plus either; i) two attacks and clinical or paraclinical evidence of one lesion, ii) one attack and clinical evidence of two separate lesions, or, iii) one attack with clinical evidence of
one lesion and paraclinical evidence of another, separate lesion.

C. Clinically probable MS (CPMS). Two attacks and clinical evidence of one lesion, one attack and clinical evidence of two separate lesions, or, one attack with clinical evidence of one lesion and paraclinical evidence of another, separate lesion.

D. Laboratory supported probable MS (LSPMS). Two attacks and positive oligoclonal bands in the CSF.

These criteria were established predominantly to restrict entrance to therapeutic and research trials, but patients also appreciate the end of uncertainty with a diagnosis of definite MS. With the recent advances in MRI, specificity may be increased by incorporating imaging characteristics into the diagnostic criteria.

1.2 Epidemiology

The prevalence of MS varies throughout the world (Kurtzke 1966). High risk areas with rates of 30-100 per 100,000 include northern and central Europe, Canada, United States and parts of Australia and New Zealand. All of the high risk areas are bounded by areas of medium risk (5-29 per 100,000) which include southern United States, northernmost Scandinavia and Russia. Low risk areas (1-4 per 100,000) include Asia, Africa, Alaska, Greenland and much of South America. All high and medium risk areas are among predominantly caucasian populations. In the USA, blacks, orientals and possibly native American Indians have lower rates of MS than caucasians but they still demonstrate the geographical gradients seen in the local caucasian populations.

These observations inevitably lead onto the question of the cause or origin of MS. Through migration studies it is clear that the risk to an individual of developing MS is not defined exclusively by their place of birth (although it is still dependent on race to
some extent). Those migrating after the age of 15 years retain the risk from their place of birth but those migrating before the age of 15 years acquire the risk of their new environment (Kurtzke 1971). The migrant data also support the theory that MS is usually acquired in adolescence with a lengthy incubation period before onset of symptoms. There is also some evidence to suggest epidemics of MS have occurred, the best example of which is in the Faroe Islands where no cases of MS were documented before the arrival of British troops in 1940. Subsequent to this there have been four peaks in incidence rate which can be considered to be secondary to susceptible populations (adolescent Faroese) being exposed to an unknown exogenous antigen (Kurtzke and Hyllested 1988, Kurtzke 1993a and 1993b). The evidence of epidemics, prevalence and migration studies, suggests that the antigen responsible is a transmissible agent, perhaps a retrovirus, but only a small proportion of people exposed to it will later go on to develop MS which in itself is not transmissible.

1.3 Genetics

Although epidemiological studies support the theory of an environmental trigger, not all exposed individuals will develop MS, susceptibility is influenced by their genetic makeup. This genetic susceptibility is supported by evidence from family and twin studies. In a large family study in Canada, 19% of the MS patients had an affected relative (Sadovnick 1988 and 1993) and the lifetime risk for a first-degree relative of an affected person was 5% compared to an individual without a family history of only 0.2%. The risk was greatest for siblings, especially sisters, of an affected person and for daughters. Many of the twin studies in MS have been criticized because of ascertainment bias resulting in an excess of monozygotic and of concordant pairs. There are however
five recent studies which approximate to a population study (Compston 1995) and show consistency with an increased rate in monozygotic twins of approximately 25% compared to 3% in dizygotic twins. The results of family and twin studies do not fit with any accepted model of inheritance but point to a polygenetic influence with multiple genes possibly exerting a threshold effect on susceptibility.

The only consistent finding in studies searching for susceptibility genes has been in the HLA region on chromosome 6, in particular the area encoding class II molecules. As the techniques of molecular genetics have progressed the genomic associations with MS have been refined and strengthened with consequent changes in the nomenclature. The susceptibility alleles have now been reclassified as the DR15 and DQ6 subtypes of DR2 and Dqw6 respectively (Compston 1995). Other areas that look promising include the gene encoding the immunoglobulin heavy chain on chromosome 14 (Pandy 1981) and following recent reports of large family genomic screens, areas of interest have been identified on chromosomes 2,3,5,7,11 and X (Ebers 1996, Sawcer 1996, The MS Genetics Group 1996).

It is clear that although the presence of the known susceptibility alleles increases the risk of MS, there must be many more genes that remain to be identified which make an even greater contribution to susceptibility. With the advent of collaborative random gene searches it is hoped our understanding of genetic factors in MS will evolve rapidly.

1.4 Immunology

The immunopathogenesis of MS is similar to other central nervous inflammatory
conditions with the exception that inflammation is more focal occurring within plaques and their immediate surroundings (Esiri and Gay 1997). Disease induction requires establishment and activation of auto-reactive T cells. In MS this may be by the exposure of a susceptible individual to an exogenous antigen, a long latency period then occurs before the T cells are activated against a CNS antigen. Myelin possesses a number of unique constituents which have been protected from the immune system by an intact blood-brain barrier, once this has been breached there is a failure to recognise these antigens as self and the inflammatory cascade is triggered involving many cell types and mediators.

Adhesion molecule expression is greatly increased in MS allowing recruitment of circulating leukocytes across the blood brain barrier and providing accessory molecules in antigen presentation. Increased levels of intercellular adhesion molecule (ICAM-1), vascular adhesion molecule (VCAM-1) and the selectins have been found in the CSF of patients with MS (Giovannoni 1996). As more cells are recruited into the area, increasing levels of cytokines are produced, these are both pro-inflammatory (interferon-γ, interleukin-2, tumour necrosis factor-α and tumour necrosis factor-β) and anti-inflammatory (interferons-α and -β, transforming growth factor-β and interleukin-4). Damage to oligodendrocytes occurs as a result of exposure to many of these cytokines and complement, as well as to non-specific mediators such as reactive oxygen and nitrogen free radicals produced by macrophages. Regeneration and recovery also occur (remission following an acute relapse) and may be influenced to some extent by products of inflammatory cells within the MS plaque. This may in itself be a factor in the lack of recovery in chronic lesions typical of PP MS, which have been found to be less inflammatory (Revesz 1994).
The auto-antigen responsible for this inflammatory response remains unknown although most work concentrates on the myelin specific proteins. However the complexity of the immune response in MS provides many avenues for therapeutic intervention capable of interrupting the inflammatory cascade.

1.5 Pathology

MS is a disease characterised by primary demyelination, that is the loss of myelin from areas of white matter with relative sparing of axons. It is important to use the term ‘relative’ as in most MS lesions there is some proclivity for an appreciable percentage of axons to be lost during the early stages of an MS plaque as well as a slower degree of axonal loss in the chronic fibrotic lesion. Extensive axonal transection has been demonstrated within both active and chronic active lesions, this was related to the degree of demyelination within the lesion (Trapp 1998). Examination of areas of normal appearing white matter (NAWM) also revealed more transected axons than seen in control brains (Trapp 1998). Work assessing immunoreactivity to amyloid precursor protein which is present in healthy axons has shown that axonal damage is linked to inflammation and occurs in the acute lesions of early MS (Ferguson 1997). This has implications for therapeutic interventions in that treating early MS may reduce subsequent axonal loss and consequent irreversible disability.

Plaques can occur anywhere within the white matter of the central nervous system, although there is a predilection for hemispheric periventricular zones. The optic nerves, brainstem and cervical cord are also common sites of involvement with no particular pattern in the level or tracts affected. The lesions may vary in size from less than one millimetre to several centimetres and often coalesce. Discrete white matter lesions at the
grey/white matter interface may extend into the adjacent grey matter as such areas also contain myelinated nerve fibres (Kidd 1999).

Oligodendrocytes are the cells responsible for myelination, each cell produces 30-50 cell processes which become flattened forming an internodal myelin sheath for as many axons (Raine 1990). If injured the oligodendrocyte does not respond by mitosis but frequently degenerates, a key feature in the MS plaque. Scattered throughout the bundles of myelinated nerve fibres are astrocytes, these are the major supporting cells of grey and white matter. If astrocytes are injured they react by rapid proliferation and by synthesising glial fibrils. This fibrillary astrogliosis leads to a state of sclerosis or scarring, also a characteristic of the MS plaque. The histopathology of a plaque is based largely on the extent of scarring and inflammatory activity. Plaques can be divided into several types according to these characteristics (Raine 1997).

**Acute MS lesion.** The margins of an acute lesion are indistinct, the centre is highly oedematous with an increase in extracellular space and frequent hypertrophic astrocytes. The entire area is infiltrated with perivascular and haematogenous cells, large numbers of macrophages laden with lipid or myelin debris occur throughout the lesion. Around venules there are deposits of fibrin and complement associated with haemorrhage and disruption of vessel basement membrane material. The demyelinated centre of the lesion is depleted of axons and occasionally signs of remyelination can be seen.

**Chronic MS lesion.** These are clearly seen by myelin staining to be areas devoid of myelin with clear edges separating them from adjacent myelinated parenchyma. Staining for glial fibres show the same area to be involved by intense fibrillary astrogliosis and scarring, at the edges of the lesion there are often a few reactive astrocytes. Axon stains show a moderate depletion at the edges with more severe depletion in the centre of the
lesions, long term demyelinated axons show a decrease in axonal diameter. Oligodendrocyte depletion is readily apparent through the centre of the lesion. Even in the oldest lesions some evidence of inflammatory activity (particularly at the lesion edges) is often noted, usually by the presence of small numbers of lymphocytes, macrophages, plasma cells and occasional mast cells.

**Chronic active MS lesion.** This lesion is half way between the characteristics of the chronic and acute lesions described above; a prominent inflammatory reaction on a background of a previously demyelinated, fibrous astrogliotic plaque. There is also evidence of astroglial hypertrophy, oligodendrocyte hypoplasia and ongoing demyelination. Perivascular cuffing is seen around venules and the lesion edge is relatively sharp with large numbers of lipid laden macrophages. The centre of the plaque can be identical to that of a chronic plaque or may only be distinguishable from that of acute lesions by prominent fibrillary gliosis. Evidence of remyelination is apparent around the edge of the lesion.

**Shadow MS lesion.** This lesion is seen most frequently as a diffusely staining pale myelinated area often in the spinal cord. The axons are thinly myelinated and hence it is thought to be an area of previous damage which has undergone repair and remyelination. This classification obviously represents a spectrum of histopathological appearances, as lesions develop they will often move between the groups, chronic silent lesions may reactivate even after many years. The nature of the trigger to this intense inflammatory reaction is as yet unknown, however the ability to induce in animals a similar myelin pathology by sensitising them against a number of myelin antigens supports the theory of an auto-immune process.
1.6 Pathophysiology of Symptoms

The acute lesion is characterised by inflammation and blood brain barrier breakdown which is seen by MRI as an area of gadolinium enhancement, this usually lasts between four and six weeks. These inflammatory changes are accompanied by demyelination, evidence for which comes from MR spectroscopy studies demonstrating lipid peaks (Davie 1994). There is also evidence of acute axonal loss at this time (Ozawa 1994, Ferguson 1997). During this period, function, depending on the site of the lesion, may be affected. This is readily demonstrable in studies of the optic nerve (Youl 1991). In a study of 18 patients with optic neuritis, gadolinium enhancement was associated with reduced visual acuity and a reduced amplitude of the P100 component of the visual evoked potential, indicating conduction block. When the patients were studied four weeks later, nine of 11 had ceased to show enhancement and both acuity and the amplitude of the P100 had improved. However the latency of the visual evoked potential was prolonged throughout. This indicates that although demyelination persisted some degree of functional recovery occurred, this is probably due to both the resolution of inflammatory changes which contribute to conduction block and to the proliferation of sodium channels restoring conduction. The clinical change seen during this process is that of an acute relapse with complete or incomplete recovery. Any resulting disability is fixed and tends to remain stable. However this process does not account for the major cause of disability in MS, that of insidious disease progression. It is thought that this may be a product of progressive axonal loss both in chronic MS lesions as they age (Barnes 1991) and diffusely within the NAWM. Evidence for this occurring comes from MR spectroscopy studies in PP and SP MS (Davie 1997, Leary 1998a, Matthews 1996, Fu 1998), the end result is apparent in the progressive atrophy of both the brain (Losseff 1996a) and of the
spinal cord (Losseff 1996b, Stevenson 1998a).

1.7 Management of Multiple Sclerosis

Management of the MS patient begins at presentation with the first symptom, investigation and subsequent diagnosis. This is a particularly crucial time and the key areas of management are education and support (Werring and Thompson 1998a). In established MS, patient management can be split into four main areas:

1) Anticipation and prevention of problems
2) Symptomatic treatment
3) Therapies aimed at reducing disease activity
4) Rehabilitation and service delivery

Each of these areas will be considered briefly, in turn.

1.7.1 Anticipation and Prevention of Problems

Maintaining good general health is of prime importance in MS, it has been suggested by Petajan and colleagues that regular aerobic exercise, when possible, increases physical and psychological well-being (Petajan 1996). The role of diet in MS remains an area of debate. Several studies have implicated the consumption of animal fat, others propose dairy products increase the risk of MS (Lauer 1997). A beneficial effect of increasing intake of $\omega$-3 and $\omega$-6 fatty acids has also been suggested to decrease the frequency and severity of relapses, although no effect on long term outcome has been found (Dworkin 1984, Bates 1989). However many of the epidemiological studies have been inconsistent and no definite relationship between MS and dietary intake has been elucidated. General dietary advice to patients is to decrease animal fat and increase both vegetable fat and
seafood intake (Lauer 1997).

As relapses may be precipitated by intercurrent infections, these should be treated promptly and more importantly prevented if possible, particularly in relation to the urinary and respiratory tracts. Large residual urinary volumes or poor intermittent self catheterisation techniques are often responsible for precipitating urinary tract infections. With regular assessments and training these can be avoided. In the respiratory tract early signs of aspiration should be noted and a speech therapist involved early for advice on practical management.

The question of whether patients with MS should receive vaccinations has been long debated, but recent work (Miller A 1997) suggests that the influenza vaccination does not increase the risk of disease exacerbation and thus should not be contra-indicated in MS. The risk of other vaccines have not been studied in MS however the risk to benefit ratio of these immunisations is probably low when used in ‘at risk’ patients and should always be offered (Panitch 1997).

Spasticity and weakness can also lead to problems with abnormal posture and gait. This can put excessive strain on the back and result in mechanical problems with considerable pain. Untreated spasticity can also lead to contractures with consequent increasing disability and permanent loss of function.

In the past patients were often advised not to become pregnant as this was associated with disease exacerbation, however the relationship between pregnancy and MS has become clearer more recently. Prospective studies have suggested that the relapse rate is decreased during pregnancy but raised during the three month puerperium, giving little difference in the overall relapse rate over the pregnancy year, or in the long term disability of the patient (Confavreux 1998). It is generally felt that patients wishing to
start a family should not be discouraged from doing so.

1.7.2 Symptomatic Treatment

Realistically, the recent advent of new immunosuppressant treatments will have little impact on existing problems and disability; consequently much of the management of a patient with MS relates to control of a vast array of symptoms (Thompson 1998a, Clanet 1997). Much can be done for the patient in all areas. A combination of education, physiotherapy and drug therapy is usually required but occasionally there is a place for more invasive treatments such as intrathecal baclofen administration for severe spasticity, thalamic surgery for cerebellar tremor or intravesical capsaicin for severe detrusor hyper-reflexia.

Acute relapses which are significantly affecting a patient's function can be treated with a short (three day) course of intravenous steroids, this has been shown to accelerate recovery although has no effect on long term outcome (Thompson 1989, Miller 1992).

1.7.3 Therapies Aimed at Reduction of Disease Activity

In recent years considerable progress has been made in the understanding of the complex immunopathological mechanisms underlying MS, many of these discoveries have led to the investigation of new therapeutic strategies (see table 1.1). Beta interferons (1a and 1b) are now licensed worldwide and Glatiramer acetate (copaxone) is in use in the United States and Israel. Many others drugs are undergoing pivotal trials (Stevenson 1998b).

**Interferons.** There are three forms of beta interferon now available, interferon beta-1b (IFNB-1b, Betaseron in North America, Betaferon in Europe) given as a subcutaneous
injections every other day, at a dose of 300 micigrams or 9.6 million IU, interferon beta-1a (IFNB-1a, Avonex) as a once weekly intramuscular injection at a dose of 6 million IU (30 micrograms) and interferon beta-1a (IFNB-1a, Rebif) given as a subcutaneous injection three times a week at a dose of 6 million IU (22 micrograms). Common side effects include injection site reactions and flu-like symptoms including fever, chills and muscle aches. Considering the results of the pivotal trials in RR MS, all three interferons demonstrated a reduction of the relapse rate in the order of one third (Paty 1993, IFNB 1995, Jacobs 1995, Jacobs 1996, PRISMS 1998). In the Betaferon study there was no significant difference between the treated and placebo groups when considering progression of disability, both the Rebif and Avonex studies of IFNB-1a suggested lower rates of progression in the treated groups compared to the placebo groups. The Rebif study reported a reduction in total lesion load seen on T2 weighted MRI, the Betaferon a stabilisation of T2 lesion load and the Avonex study no effect. All three studies described a striking reduction of new gadolinium enhancing lesions as compared to the placebo controls. More recently a European multicentre study of IFNB-1b for SP MS has been completed, this demonstrated again a reduction in relapse rate by one third but more importantly a significant difference between the treated and placebo groups in time to confirmed disability progression and time to reach defined disability end-points (becoming wheel chair bound) (European study group on Interferon β-1b in secondary progressive MS 1998). These differences were in the order of 20-32% and were independent of baseline disability or superimposed relapses (Miller 1998a and 1998b). This study demonstrated for the first time that IFNB has an effect not only on the relapses of MS but also slows the insidious disease progression probably responsible for the majority of disability in MS.
**Table 1.1:** Therapeutic agents in multiple sclerosis.

<table>
<thead>
<tr>
<th>Licensed Treatment for Relapsing-Remitting MS</th>
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<tbody>
<tr>
<td>- Interferon beta-1b (betaferon)</td>
</tr>
<tr>
<td>- Interferon beta-1a (avonex, rebif)</td>
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<tr>
<td>- Glatiramer acetate (copaxone)</td>
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<table>
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<tr>
<th>Drugs Undergoing Pivotal Trials</th>
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<tr>
<td>- Interferon beta-1b (SP MS and clinically isolated syndromes)</td>
</tr>
<tr>
<td>- Interferon beta-1a (SP and PP MS, and clinically isolated syndromes)</td>
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<tr>
<td>- Intravenous immunoglobulin</td>
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<tr>
<th>Other Drugs Investigated</th>
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<tbody>
<tr>
<td>- Mitoxantrone (effective in RR MS and ‘active’ MS)</td>
</tr>
<tr>
<td>- Cladribine (of no proven benefit)</td>
</tr>
<tr>
<td>- Methotrexate (effective in small study of progressive patients)</td>
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<tr>
<td>- Oral tolerance: ingested myelin (negative study)</td>
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<tr>
<td>- Sulfasalazine (of no proven benefit)</td>
</tr>
<tr>
<td>- Linomide (adverse effect: ischaemic heart disease)</td>
</tr>
<tr>
<td>- Hyperbaric oxygen (of no proven benefit)</td>
</tr>
<tr>
<td>- Deoxyspergualine (of no proven benefit)</td>
</tr>
<tr>
<td>- Total lymphoid irradiation (of no proven benefit)</td>
</tr>
<tr>
<td>- Ginkolide B (of no proven benefit)</td>
</tr>
<tr>
<td>- Acyclovir (non significant reduction in relapse rate)</td>
</tr>
<tr>
<td>- Anti- CD4 (of no proven benefit)</td>
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<table>
<thead>
<tr>
<th>Future Therapies</th>
</tr>
</thead>
<tbody>
<tr>
<td>- Monoclonal antibodies (Campath -1H)</td>
</tr>
<tr>
<td>- Adhesion molecule therapies</td>
</tr>
</tbody>
</table>
Further studies with the interferons are ongoing, they include, IFNB-1a (Rebif) for SP MS and IFNB-1a (Avonex) for RR, SP and PP MS (Leary 1997). Both Rebif and Avonex are also being studied in patients with clinically isolated syndromes (monosymptomatic) to detect if the time to conversion to MS can be delayed.

**Glatiramer Acetate (Copaxone).** This is a mixture of several polypeptides (L-alanine, L-glutamic acid, L-lysine and L-tyrosine) in a specific ratio. Its therapeutic effect is thought to involve inhibition of the immune response to myelin basic protein. In 1991 a pivotal trial showed a reduction in relapse rate of 29% in the treated group (Johnson 1995, Wolinsky 1995). There was no difference in progression to disability and a very limited MRI study did not show any treatment effect on total T2 lesion load or in the number of new enhancing lesions (Cohen 1995). It is given as a daily subcutaneous injection. Side effects are rare but include injection site reactions and a rare transient systemic reaction consisting of flushing, chest tightness, shortness of breath and anxiety, lasting between 30 seconds and 30 minutes.

**Intravenous Immunoglobulin.** The mechanism of action of intravenous immunoglobulin (IVIG) in MS is unknown but is thought to be a combination of T cell receptor blockade, modulation of cytokine activity and induction of antigen specific suppressor cells. Animal studies have suggested that it might promote remyelination. A recent double-blind, placebo controlled study of monthly IVIG infusions in 150 patients for two years showed that IVIG treatment had a beneficial effect on the course of clinical disability (Fazekas 1997). It is well tolerated with very few side effects.

### 1.7.4 Rehabilitation and Service Delivery

A multi-disciplinary approach is required to deal with the wide range of disabling and
interacting symptoms seen in MS. Multi-disciplinary team assessments identify areas of potential functional improvement and patient-centred, goal-orientated rehabilitation programmes are initiated (Thompson 1996).

Short periods of intensive inpatient rehabilitation have been shown to reduce levels of disability and handicap (Freeman 1997), the benefit persisting for at least six months (Freeman 1999).

Ideally the patient with MS and their carers should be managed in a comprehensive, flexible, community based service with close links to a neuroscience centre. This is usually achieved by ensuring a link worker or community care co-ordinator is established to deal with both the purchaser and provider. Their role should ideally also include the continuation of education and training for both the patients and health professionals (Thompson 1997a).

1.8 Primary Progressive Multiple Sclerosis

Approximately 10% of patients with MS exhibit a progressive course from onset with no history of relapse or remission and are classified as PP MS. The classification of patients with progressive forms of MS is extremely difficult as noted in a recent survey of members of the International MS Clinical Research Community (Lublin and Reingold 1996). This classification included a group of patients classified as progressive-relapsing who are progressive from onset but who also have superimposed relapses. Patients with TP MS (Filippi 1995a, Thompson 1997b, Gayou 1997), who describe a progressive course with the exception of a single relapse or remission at any time before or after the onset of disease progression were not distinguished from these.
Difficulties in the diagnosis of PP MS both in terms of distinguishing it from other progressive neurological disorders and in excluding patients with previous relapse activity have recently been highlighted (Thompson 1997b, McDonnell and Hawkins 1997). The differential diagnosis must be considered, this is particularly important if the patient presents with a progressive paraparesis where cord compression and foramen magnum anomalies must be carefully excluded by detailed MR imaging. Other inflammatory conditions must also be screened for including human T-cell lymphocyte virus type 1, syphilis, borrelia, X-linked adrenomyeloneuropathy, systemic lupus erythematosis, Sjogrens disease and DNA analysis performed for Leber's optic neuropathy if the patient has presented with progressive visual failure.

Making the diagnosis of PP MS may be difficult. Progressive symptoms need to be present for a minimum of six months before the diagnosis can be considered though a longer time course of two years has been suggested to be more clinically appropriate (Thompson 1997b). Many patients present with a progressive but monosymptomatic course, often a spastic paraparesis, however the Poser criteria require evidence of disease dissemination both in time and space. Evidence from paraclinical tests (oligoclonal bands on CSF examination, delay in visual evoked potentials and abnormalities on MRI), are often helpful but fulfilling the criteria, particularly dissemination in time, can be difficult. This may involve repeating tests, for example, a visual evoked potential which is normal at presentation may later become delayed.

The frequency of PP MS is estimated at approximately 10%, giving a prevalence figure in the order of 35,000 in Europe or 8,000 in the UK (Thompson 1997b), however the precise proportion varies between studies from 7.7% (Weinshenker 1989) to 37% (Minderhoud 1988). This variation probably reflects the method of data collection. In
Weinshenker's study the proportion of patients with PP MS calculated from the retrospective data was 18%, whereas in the group of patients seen from presentation this fell to 7.7%. Initial relapses, particularly if they occurred many years before the onset of the progressive syndrome, are easily forgotten by the patient (Mathews 1998).

Few studies have looked at this patient group and because of their relative rarity patient numbers have been small. However differences between PP MS and RR and SP subtypes have been documented. The age of onset appears to be later, mean age at presentation 37.3 years (Confavreux 1980) and 43.6 years (Thompson 1986) in two such studies. The male to female ratio seems to be equal or even in favour of a slight male excess (Runmarker and Andersen 1993, Van Lambalgen 1986). This lack of female preponderance, which is usually seen in auto-immune conditions, may reflect the less inflammatory/immune nature of the disease course compared to other subgroups of MS. Symptoms at presentation also differ in that unlike RR MS patients who usually present with a visual or sensory disturbance, patients with PP MS most commonly present with a progressive paraparesis. However a small proportion will present with progressive cerebellar, brainstem, visual or hemiplegic syndromes. Very rarely progressive cognitive decline can occur. Cognitive dysfunction has not been thought to be a prominent feature in this patient group, particularly when compared to patients with SP MS (Comi 1995).

Differences in the genetic and immunological profiles of PP MS have been noted but the study numbers are small and results inconsistent (Thompson 1997b). As discussed previously the only consistent finding in studies searching for susceptibility genes has been in the HLA region on chromosome 6, more specifically with the susceptibility alleles DR15 and DQ6 subtypes of DR2 and Dqw6 respectively (Compston 1995). A similar incidence of DR2 has been shown in PP MS compared to RR and SP MS.
(McDonnell 1998a), however it has been suggested that PP MS may be associated with different DQB1 restriction fragments (Olerup 1989).

Little is known about the immunological differences between clinical subtypes of MS, the prevalence of positive oligoclonal bands in the CSF of patients with chronic progressive MS was shown to be equal to that in RR patients in two studies (Thompson 1985, Tourtelotte 1988) but less in a more recent Finnish study (Pirttila and Nurmikko 1995). Differences in the frequency of auto-antibodies have also been suggested. In one study two subgroups of MS were described, one associated with antibodies against myelin basic protein (MBP) and the other with anti-proteo-lipid protein (anti-PLP) antibodies, three of the five patients with anti-PLP antibodies had PP MS (Warren 1994). Raised anti-ganglioside antibodies particularly in MS patients with PP disease have been noted (Acarin 1996), pro-inflammatory cytokines and levels of interleukin-2 receptors have also shown to be higher in progressive patients than those with RR MS (Chalon 1993). There may also be differences in the concentration of adhesion molecules (Matsuda 1995) and, in particular, levels of soluble E-selectin, an endothelial adhesion molecule which is increased in patients with PP MS (Giovannoni 1996, McDonnell 1998b).

There is very little published concerning the pathological findings in PP MS, one study compared post mortem material of four patients with PP MS to five with SP MS, inflammatory changes were evident in all cases but were significantly more marked in the SP MS group (Revesz 1994). In another study of three patients with PP MS it was suggested that demyelination may occur as a secondary phenomenon to oligodendrogial damage (Lucchinetti 1996).

Prognosis in terms of progression to disability is poor in PP MS. The median time to
reach disability status scale (DSS) 6 (Kurtzke 1961) was only six years in a cohort of 36 patients (Runmarker and Andersen 1993), similar findings have been demonstrated in other studies (Weinshenker 1989). Prediction of prognosis is also very poor in this group, Losseff looked at 10 patients with PP MS and 12 with SP MS who had all undergone six monthly MRI scans five years previously. In the SP MS group three factors were found to be predictive of outcome; the number of enhancing lesions, relapse frequency and the development of disability over the six month period. In the PP MS group no MRI measures were predictive with only the development of disability showing any relationship (Losseff 1996c).

At present there are no licensed treatments to alter the clinical course of PP MS and management remains symptomatic and supportive. Due to the relative rarity and the less inflammatory nature of this disease which in itself causes difficulties in monitoring the effect of treatments, there has been very little interest in therapeutic trials for this group. To date there are only three small treatment trials underway in PP MS two with beta-interferon 1a (Leary 1997, Montalban 1998) and the other with riluzole (personal communication CH Polman).
Chapter 2: Magnetic Resonance Imaging

This chapter concentrates on the basic principles and technological aspects of MRI (Westbrook and Kaut 1993). Many of the pulse sequences and methods of quantification used in studies discussed later are described in detail.

2.1 Basic Principles of MRI

MRI enables high resolution pictures to be obtained with excellent discrimination between soft tissue structures. It does this by manipulating the different magnetic properties that tissues acquire when placed within an external magnetic field to produce an electrical signal, which can then be collected and transformed into an image. To understand how this image is formed it is important to consider the properties of the hydrogen nuclei present in the water and lipids of soft tissues.

In conventional MR imaging the hydrogen nucleus with its sole proton is the source of the signal from which an image may be formed. Hydrogen is present in abundance in the form of water and macromolecules within the human body. The hydrogen nuclei, or protons, possess a property named ‘spin’ which can be envisaged as each proton spinning around its axis, much the same as the Earth spins around its axis. As a consequence of this spin and their positive charge, they have their own magnetic moment which is along the direction of the axis. However the protons are all spinning in a random direction and therefore there is no net magnetic moment in the structure. If the structure is then placed in an external magnetic field, the proton spins align within this in either a parallel or anti-parallel fashion. There are always slightly more in a parallel direction than anti-parallel
as the former is the lowest energy state attainable. This results in a net magnetic moment in the direction of the magnetic field, (conventionally defined as the \( z \) axis), known as longitudinal magnetisation (see figure 2.1).

![Figure 2.1: Alignment of protons within a magnetic field.](image)

The protons, whilst aligned, continue spinning around their axis in a motion known as ‘precession’, this has been likened to a spinning top (see figure 2.2). The frequency of this precession (\( \omega_0 \)) is dependent on the strength of the magnetic field (\( B_0 \)) and the gyro-magnetic ratio (\( \gamma \)) which is different for different materials (eg. the value for protons is 42.5 MHz/T), \( \omega_0 \) can be calculated using the Larmor equation:

\[
\omega_0 = \gamma B_0
\]
As the protons do not precess in phase, under the conditions described so far, there is no net magnetic moment along the $x$ or $y$ axes (perpendicular to $z$).

To obtain a useful signal from the longitudinal magnetisation it is necessary to apply a radio-frequency (RF) pulse, this is transmitted from a coil which surrounds the part of the body being imaged. The RF pulse interacts with the precessing protons by transferring energy to them. To do this the RF pulse frequency needs to be the same as the proton precession frequency (calculated by the Larmor equation) allowing resonance to occur.

This exchange in energy has two main effects; firstly some of the protons move to a higher energy level, i.e. change from parallel to anti-parallel, thus resulting in a change in the net longitudinal magnetisation, and secondly the protons begin to precess in phase. This results in a magnetic moment moving around the $z$ axis in the $x$ and $y$ direction which is called transverse magnetisation (see figure 2.3). This rotating magnetic moment may be detected by its ability to induce a current in the RF coil, forming the MRI signal.
An RF pulse which results in no net longitudinal (equal protons in a parallel or anti-parallel direction) and maximum transverse magnetisation is called a 90° pulse.

![Diagram of RF pulse and magnetisation](image)

**Figure 2.3:** Transverse magnetisation following an RF pulse.

The RF pulse is short (~ 1ms) and as soon as it is switched off the protons try and return to their low energy state, they do this by transferring their excess energy to the surroundings (the lattice). As this occurs longitudinal magnetisation increases (more protons align in a parallel rather than anti-parallel direction), this process is called longitudinal or spin-lattice relaxation. At the same time due to inhomogeneities in the external magnetic field and the influence of neighbouring protons with their magnetic fields, the protons precess at different frequencies and consequently begin to lose phase; thus the transverse magnetisation decreases, this process is called transversal or spin-spin relaxation.

The time taken for the longitudinal magnetisation to recover back to its original value is
described by a time constant; the longitudinal relaxation time also called T1. Likewise there is a time constant to describe how long it takes the transverse magnetisation to disappear; the transversal relaxation time or T2. Both relaxation processes are exponential thus T1 is defined as when 63% of the original longitudinal magnetisation is reached and T2 as the time when transversal magnetisation decreases to 37% of the original value. The T1 of a substance is always longer than the T2. In biological tissues T1 ranges from about 300ms in fat to greater than 2000ms in CSF, the range for T2 is approximately 30 to 150ms (see figure 2.4).

![Figure 2.4: T1 and T2 relaxation time curves.](image)

The properties of the tissue effect the T1 and T2 values. Excess energy is more readily transferred from protons to bound water or fat molecules than free water, hence the T1 of low viscosity substances, for example CSF, is very long. In the same way if the molecules of the lattice are of different sizes and moving at different speeds (bound water
or fat), protons lose phase more quickly than if they are of a similar size and fast moving (free water). The T2 is therefore long for watery substances and shorter for fat and other complex structures. Protons also lose phase due to inhomogeneities within the magnetic field, this is termed the T2* effect. However T2* effects are always less than T2 and are reversible.

Both the longitudinal and transverse magnetisation make up the net magnetisation which can be depicted as a sum vector which in itself is precessing around its axis. However only the transverse magnetisation is available to produce the signal. This type of signal is called an FID signal (Free Induction Decay), although the amplitude decreases as relaxation occurs the frequency always remains constant (see figure 2.5).

![Decay governed by T2* effect](image)

**Figure 2.5:** Free induction decay signal.

To identify variations in tissues any difference in relaxation times may be exploited. If
the signal from a tissue is repeatedly sampled, the time delay between sampling (TR; time to repeat) will restrict the degree to which longitudinal relaxation occurs, depending on the T1 of the tissue. Likewise, the time delay between RF excitation and acquisition of signal (TE; time to echo) will alter the signal amplitude according to T2. A ‘T1 weighted’ image may be obtained by keeping TR short relative to typical T1 values. A ‘T2 weighted’ image may be obtained by keeping TE long relative to typical T2 values. If the TR is long but the TE is short the differences in T1 or T2 of the tissues cannot manifest themselves and the signal intensity depends on the number of protons present ie. the proton or spin density (PD weighted image).

The most basic method for obtaining an MR signal is to use a spin-echo sequence. This consists of two pulses; a 90° pulse followed by a 180° pulse. The 180° pulse, given at time TE/2, reverses the dephasing effects of T2*, leading to the formation of a signal echo at TE (see figure 2.6).

The T1 weighted images from a spin echo sequence have a high signal to noise ratio (SNR) and consequently demonstrate the anatomy of the brain and spinal cord extremely well. They may show some areas of pathology in multiple sclerosis as areas of hypointensity (so called black holes), although changes tend to be less dramatic than on T2 weighted images where diseased tissues which are often oedematous give high signal changes (see figure 2.7). The TR and TE of the T1 weighted sequence are both short and acquisition time for 44 contiguous 3mm slices through the brain is in the order of 6 minutes. The T2 weighted images from a conventional spin echo sequence (CSE) are also of high SNR but due to the long TR and TE the acquisition time can be over 15 minutes, making it impractical in many clinical situations.
In the spinal cord the images are often degraded by motion and flow effects. These are produced due to mismapping of signals from moving nuclei, the result are artefacts known as flow motion artefacts or flow-void phenomena.

To get round these problems faster sequences have been developed. Before these faster sequences are discussed it is important to consider how an image is created.

### 2.2 Creating an Image

Before an MR image can be constructed the spatial distribution of the signal needs to be known. This is done by firstly selecting a specific slice from which to collect information.
Figure 2.7: T1 (top) and T2 (bottom) weighted images in multiple sclerosis.
In addition to the main external field, additional gradient fields may be applied which modify the magnetic field strength (\(B_0\)) along their directions of action. The gradient field therefore influences the frequency of precession of all the protons along its length and only those with the same frequency as the RF pulse will be excited. By choosing an RF pulse with a certain central frequency and range of frequencies it is possible to determine both the site of the slice and its thickness. A slice selective gradient is usually applied with each RF pulse in a sequence.

To enable the precise location of a signal to be known we need to know the \(x\) and \(y\) coordinates within the chosen slice which involves the use of both frequency and phase encoding gradients. The frequency encoding gradient is a further gradient field which is applied after the RF pulse in the \(x\) axis, thus causing the protons along the \(x\) axis to have different precession frequencies. Consequently spatial distribution along \(x\) may now be inferred from the differences in the emitted signal frequencies (dependent on the precession frequencies). This now leaves protons in columns in the \(y\) direction with the same precession frequencies. \(Y\) positions are differentiated by using another magnetic gradient (the phase encoding gradient) along this direction. This causes the precessing protons to ‘speed up’ in accordance with the strength of the magnetic field to which they are being exposed. When this short gradient is switched off, all the protons once again experience the same magnetic field and thus have the same precession frequency, however they are now out of phase (due to some having speeded up more than others during the brief magnetic gradient).

Each signal is therefore of a different frequency or phase and these correspond to its location. These signals constitute what is known as ‘\(k\)-space’. With the application of a Fourier transformation, \(k\)-space is converted into the final MR image.
2.3 Contrast Media

The most commonly used contrast media is gadolinium, this is a rare earth metal which is toxic in its free state but when chelated to diethylene triaminepentaacetic acid (DTPA) is safe. Gadolinium-DTPA behaves as a paramagnetic substance when injected into the body and results in shortening of both T1 and T2 relaxation times in surrounding tissues (proton relaxation enhancement). This is best seen on T1 weighted images where areas of contrast enhancement are seen as areas of high signal (bright white) within the brain matter. In a normal brain no areas of high signal are seen, however if the blood brain barrier has been breached secondary to inflammation (or vascular damage due to strokes or tumours) enhancement will be visible (see figure 2.8).

2.4 Spinal Cord Imaging

The spine has several properties which render imaging more difficult. The spinal cord is mobile, very long and surrounded by CSF which is constantly flowing in an unpredictable fashion. To enable high resolution images of the whole cord to be obtained it is necessary to employ a series of six overlapping surface coils, the multi-array coil. These each receive the MR signal simultaneously and a composite image is constructed with a field of view of 48cm enabling the whole spinal cord to be imaged in a single acquisition (see figure 2.9). The pixel size should be as small as possible to reduce partial volume effects at the cord/CSF interface which results in blurring of the boundary. However reducing pixel size increases the acquisition time which in turn increases the flow effects seen in the CSF, this can also lead to blurring at the cord boundary and motion artefact within the cord. For these reasons the following fast sequences are used.
Figure 2.8: $T_2$ weighted (top) and post-gadolinium-DTPA $T_1$ weighted (bottom) images in multiple sclerosis.
Figure 2.9: Spinal cord image using the phased array coil (fast spin echo).
2.5 Pulse Sequences

2.5.1 Fast Spin Echo

This is a spin echo sequence but as its name suggests the acquisition time is significantly shorter than CSE. The increase in speed is due to the way k-space is filled by the two sequences, in CSE only one phase encoding gradient is applied per TR and therefore only one ‘line’ of k-space is filled per TR. However in fast spin echo (FSE) an echo train of several 180° rephasing pulses is applied after each 90° pulse and at each rephasing a different phase encoding step is used. The number of echoes produced (equivalent to the number of 180° pulses) corresponds to the number of lines of k-space filled during each TR. The echoes are generated at different TE’s and therefore have variable T2 weightings. An effective TE (TEeff) is selected and is achieved by ordering the phase encodings so that data from echoes close to the effective TE have more impact on the image contrast as they fill the central lines of k-space (see figure 2.10).

The FSE used in the spinal cord studies discussed in this thesis has an echo train length of 16, thus shortening the equivalent CSE acquisition time to 1/16th and reducing motion artifacts greatly. The markedly reduced acquisition time facilitates the application of high resolution matrices which are normally time consuming.

2.5.2 Three Dimensional Fast Spin Echo

Three dimensional fast spin echo (3D FSE) is a new technique in which the slice thickness can be decreased to 1mm and with this single volume acquisition the image can be reformatted into any plane. Its acquisition is similar to 2D FSE except that the multiple slices in 2D FSE are replaced by multiple slabs, each of which is partitioned into 1mm
slices using an additional phase encoding gradient. The main difficulty is in ensuring that the slabs are contiguous otherwise any reformats would have gaps in them. However forcing slabs together can result in crosstalk which results in loss of signal at the edges of slabs. One solution to this is to acquire the slabs in two interleaved acquisitions which eliminates crosstalk but any patient motion between the acquisition leads to slab misregistration. It is possible to acquire the odd slabs and then the even slabs on two passes within the same TR but again within the reformats artefacts can occur in a striped fashion due to patient motion (see figure 2.11).
Figure 2.11: Axial reformat of 3D FSE (top), demonstrating striped motion artefact (bottom).
2.5.3 Inversion Recovery Techniques

These are pulse sequences which begin with a 180° or inversion pulse. This inverts all the longitudinal magnetisation by 180° (all in an antiparallel direction) which then recovers according to the T1 of the tissue. If a 90° pulse is then applied at a time TI (time from inversion), the amount of transverse magnetisation obtained depends on the amount of longitudinal magnetisation recovery. After the 90° pulse, a 180° rephasing pulse may be applied at a time TE/2 and after a time TE the signal (echo) is received, as in a CSE acquisition. The signal obtained depends on the TI used. This property may be utilised to null the signal from specific tissues according to their T1 (see figure 2.12).

Figure 2.12: Inversion recovery sequence.
2.5.4 Flair Sequences

Fluid Attenuated Inversion Recovery sequences (FLAIR or fast FLAIR) are inversion recovery sequences in which the CSF signal is nulled by choosing a TI which corresponds to the time it takes the CSF to recover to the transverse plane with no longitudinal magnetisation. FLAIR is used to suppress the high signal in CSF of proton density or T2 weighted images to enable more clear demonstration of pathology adjacent to CSF (see figure 2.14).

Fast FLAIR is achieved in a similar way to FSE, instead of one 180° rephasing pulse being applied, an echo train of variable length is used (see figure 2.13).

Figure 2.13: Fast FLAIR sequence.
Figure 2.14: Fast FLAIR image of the brain in multiple sclerosis.
2.5.5 *Inversion Recovery Fast Spoiled Gradient Echo (IR FSPGR)*

In a gradient echo sequence the RF pulse, known as the ‘flip angle’, is not always 90° as it is in a spin echo sequence. The flip angle is often small (10°- 35°) and the TR subsequently short. Also instead of applying a 180° rephasing pulse to refocus the dephasing spins a magnetic field gradient is used. The degree of T1 weighting of the image can be varied by adjusting the flip angle or TR. The shorter TR’s used enable a 3D volume acquisition to be obtained in a reasonable time. The IR FSPGR used in the cord atrophy studies in chapters four, five and six has an acquisition time of 7 minutes and gives excellent SNR and T1 weighting, it is therefore ideal when considering anatomical detail such as the cord/CSF interface.
Chapter 3: Application of Magnetic Resonance Imaging to Multiple Sclerosis

Before the advent of MRI in the 1980's the only imaging technique capable of demonstrating the lesions of MS was computerised tomography (CT), this was not particularly sensitive and involved a considerable radiation dose to the patient. MRI enables high resolution images to be obtained without the use of ionising radiation. This has led not only to its routine use as a diagnostic tool in MS, but also with the development of new sequences, as a research tool to further our understanding of the underlying pathological processes. With the advent of therapeutic trials in MS, MRI has also become increasingly important as an outcome measure.

The first reported use of MRI in MS was in 1981 (Young 1981) and involved an inversion recovery sequence in which lesions appeared as low signal compared to normal brain. Further work demonstrated that T2 weighted sequences in which lesions are of higher signal than the rest of the brain were more useful (Ormerod 1987). These sequences often took a considerable length of time to acquire and therefore faster imaging techniques were employed as they were developed. Fast spin echo is now in widespread use both in the brain and spinal cord and has largely replaced CSE (Thorpe 1993, Kidd 1993).

3.1 MRI in the Diagnosis of Multiple Sclerosis

MS should always be diagnosed primarily on clinical grounds and requires clinical evidence of CNS lesions disseminated in both time and space (Poser 1983), however
often the patient presents with multiple symptomatic episodes but signs of only one lesion allowing a diagnosis of only clinically probable MS to be made. With the use of MRI, dissemination in space can be confirmed thus enabling a diagnosis of clinically definite MS rather than clinically probable. The ability to detect dissemination in space is also extremely valuable in the diagnosis of PP MS where the patient often presents with a progressive myelopathy but no other signs. MRI can be used to confirm dissemination in time by either the use of serial imaging or by using contrast media, enhancement occurs in most new lesions thus allowing new lesions to be distinguished from old.

The appearance and distribution of lesions can also be helpful in atypical cases. Several criteria to aid radiological diagnosis have been developed which utilise the number of lesions, their shape, location and enhancement properties (Fazekas 1988). These findings however, even with the use of the suggested diagnostic criteria are not specific for MS, many white matter lesions indistinguishable from those of MS can be found in normal volunteers and in patients with different diseases (Triulzi and Scotti 1998). The most commonly encountered differential diagnosis for MS is probably that of vasculitis associated with autoimmune disorders such as systemic lupus erythematosus, antiphospholipid syndrome or Behchet’s disease. Vascular causes, including migraine and hypertension may be considered but are usually distinguishable on clinical grounds. Similar radiological findings may be seen in neurosarcoidosis, progressive multifocal leukoencephalopathy, acute disseminated encephalomyelitis, the leukodystrophies and Lyme disease.

It must be remembered however, that a normal brain scan does not exclude a diagnosis of MS. Recent studies of fast FLAIR sequences have shown it to be superior in the
detection of cortical brain lesions (Gawn-Caine 1997a) than standard sequences, fast FLAIR may thus have a role in diagnosis if lesions are not apparent on routine FSE images. Alternatively spinal cord imaging may be useful, with the use of both brain and cord imaging the sensitivity of MRI to detect the lesions of MS is thought to approach 100% (Thorpe 1996a).

A further potential use of MRI is to provide prognostic information to individuals presenting with an isolated syndrome of the CNS suggestive of MS. A definite diagnosis of MS cannot be given after a single episode, as dissemination in both time and space is lacking. However we know from clinical studies that the risk of MS following for example, an isolated optic neuritis is between 30-70% (Miller 1997). The presence of MRI abnormality at presentation with a clinically isolated syndrome has been shown to increase the risk of progression to clinically definite MS. In a five year follow up study, 65% of patients with an abnormal brain scan had progressed to MS compared to only 3% with a normal scan. Furthermore the degree of MR abnormality at baseline was shown to increase the predictive value, 85% of patients with four or more lesions progressed to MS compared to 54% with one to three lesions (Morrissey 1993). Subsequently the same cohort was reviewed at ten years following their initial presentation, at which time 83% of patients with an abnormal baseline scan had progressed to clinically definite MS, a further 4% were classified as clinically probable MS, leaving only 13% with a diagnosis of a clinically isolated syndrome (O’Riordan 1998a). Only 11% of patients with a normal baseline scan had progressed to have MS. This prognostic information allows an optimistic approach to patients with normal scans at presentation and perhaps highlights which patients would be most appropriate for counselling and future therapeutic strategies to prevent conversion to MS.
3.2 MRI as an Outcome Measure in Multiple Sclerosis

Whether patients are in trials of new therapeutic agents or receiving established treatments, it is necessary to monitor their progress to assess efficacy. The primary outcome measure in all definitive phase III therapeutic trials is clinical change (Paty and McFarland 1998). The measures used include relapse rate and severity, and perhaps more importantly measures of disease progression, either sustained change in disability measures or the reaching of well defined disability end points. There are however problems with the clinical scales used to assess disability involving reproducibility, validity and sensitivity (Thompson 1998b) and consequently surrogate measures of disease progression allowing quantitative analysis are extremely valuable. However before a surrogate measure for disease activity can be used it must be shown to be accurate, reproducible, sensitive to change and clinically predictive. Accuracy is difficult to determine for many MRI measurements as it involves the use of phantoms of known volumes or chemical composition, most of which are quite unlike the human brain. The accuracy of volume measurements has been assessed and it is clear that there is considerable variation between real and measured lesion volumes (using contouring or manual outlining techniques), particularly in relation to small volumes when partial volume problems occur (Tofts 1997). However it is generally accepted that the manual tracing technique for volume assessments is the gold standard against which other more automated techniques should be compared. The reproducibility of an MRI measure is usually expressed as the intra- and inter-rater reliability. It must be remembered that this only describes the differences in repeat measures of a data set by an individual or pair of observers. It does not take into account factors such as patient repositioning (Gawne-Cain 1996), lesion identification or scanner performance over time which can only be assessed
by scan-rescan comparisons. Sensitivity to change is also important, MRI measures appear to be more sensitive than clinical measures although may be less specific.

MRI is extremely useful in the monitoring of disease activity as it shows higher sensitivity than clinical relapses, up to ten new lesions are seen for every clinical relapse (Miller 1998b). The rate of new lesion formation and the use of contrast enhanced images (using gadolinium-DPTA) have become routine in the assessment of activity in MS and in particular in the monitoring of phase II clinical trials where treatment effects on MRI can be seen in time periods of only six months. Although these measures are sensitive to acute inflammatory activity and blood brain barrier breakdown, they do not necessarily reflect the long term prognosis of MS which is often linked more to insidious disease progression than to the relapse rate. This is illustrated well by the small but important group of patients with PP MS. These patients are known to have extremely low rates of new lesion formation and gadolinium enhancement despite considerable rates of accumulating disability (Thompson 1997b). In a small follow up study of patients with SP and PP MS, a correlation was shown between gadolinium enhancement over a six month period and disability progression five years later in the SP patients but not in the PP group (Losseff 1996c). This poor predictive value of the rate of new lesion formation and gadolinium enhancement precludes the use of these measures in the monitoring of definitive treatment trials or in the use of established therapies, where clinical change and surrogate measures of disease progression are more important.

All of the recent large therapeutic trials with the beta-interferons attempted to address the problem of the monitoring of disease progression by using one such surrogate measure; that of serial MRI T2 lesion volume as a measure of the overall pathological extent of disease (IFNB MS study group 1995, Simon 1998, PRISMS 1998, Miller 1998b). This
and other MR measures are used in the studies in chapters four and five. Each is discussed in turn and the methodology outlined.

3.2.1 T2 Lesion Volume

The commonest measure for disease burden is the assessment of total brain lesion volume (TLV) on unenhanced T2 weighted (long TR, short TE) scans. There are several techniques available for TLV quantification, the simplest is manual outlining which, although considered to be the gold standard, is time consuming and reproducibility is not ideal. More automated methods include global thresholding or local thresholding (contour technique). The contour method has been shown to be the most reproducible (Filippi 1995b, Molyneux 1998a) and is the one used throughout these studies. Using the Dispunc display software for MR images (Plummer 1992) a point is chosen on the edge of the lesion, the computer algorithm then searches the 5x5 pixel area surrounding the selected point for the next pixel with the same (or similar) intensity, this continues until the contour returns to its start point. Sometimes manual editing is required to modify part of the boundary particularly in poorly defined lesions. The areas outlined are stored as regions of interest (ROI) and the TLV calculated by multiplying the total ROI area by the slice thickness. Serial assessments should always be carried out on the same scanner, since increasing the power of the magnet from 0.5 tesla to 1.5 tesla produces a 30% increase in measured lesion load (Filippi 1997), measurements should also ideally be by the same investigator. Likewise care must be taken to ensure that repositioning of the patient is accurate (Gawne-Cain 1996) and that slice thickness remains constant (the optimum thickness is probably 3mm), in order to improve the reproducibility and sensitivity of the technique (Filippi 1995b, Molyneux 1998b). With careful attention to
such factors the reproducibility of repeat lesion load measurements can be maintained at coefficients of variation of less than 5%.

TLV is usually measured annually and has been shown to exhibit changes, in placebo arms of previous trials, in the order of a median increase of 5-10% a year in RR MS (Paty 1994) and somewhat less in SP MS due to the larger baseline TLV in this patient group (Miller 1998a). The measurement of TLV appears to be more sensitive than clinical change; considerable lesion loads are seen after only short disease durations when only low levels of clinical impairment are measurable, however as with any surrogate measure of disease progression the measurement must reflect clinical change. The pathological correlations with individual lesions are good, demonstrating that demyelination corresponds to increased signal on T2 weighted scans (Stewart 1986). However the clinical correlations between disability scales and TLVs are disappointingly low (only $r=0.23$ in the large IFNB MS study group 1995), this has been accounted for in several ways. Firstly the TLV does not take into account the location of the lesion within the brain and ignores completely the presence of disease in the spinal cord, which in itself may account for the majority of disability measured by mobility weighted disability scales such as the expanded disability status scale (EDSS). Secondly, increase in T2 weighted signal is pathologically non specific. Areas of oedema, gliosis, demyelination or axonal loss can not be distinguished from each other. Lastly any pathological changes in the normal appearing white matter (NAWM) are ignored (Miller 1998b).

The lack of relationship between TLV and disability is particularly well illustrated by the PP MS patients who despite small TLVs have considerable disability. Whether changes in TLV can be quantified in PP MS is explored in chapters four and five but until recently these patients were excluded from therapeutic trials partly due to their rarity and also
because of the lack of a validated MR outcome measure.

3.2.2 T1 (Hypo-intensity or ‘Black Hole’) Lesion Volume

In an effort to find a surrogate measure which correlates more highly with disability than the TLV, investigators have studied the areas of hypo-intensity or ‘black holes’ observed on unenhanced T1 weighted images. It is thought that these areas, seen much less frequently than high signal areas on T2 weighted scans, are more pathologically specific. In a recent post mortem study, the degree of hypo-intensity correlated well with the degree of axonal loss within the lesion (van Walderveen 1998); it has also been shown to correlate with magnetisation transfer ratios implying demyelination is present (van Waesberghe 1997). In some studies the correlations between the EDSS and the T1 hypo-intensity lesion volume have been higher than with the TLV; in one serial study in particular the increase in T1 hypo-intensity lesion load was shown to correlate extremely highly (r= 0.81) with disease progression (Truyen 1996). In other studies T1 hypo-intensity lesion volume has not correlated with disability (or cognitive dysfunction) (O’Riordan 1998b, Rovaris 1998).

Like the measurement of TLV quantification of ‘black holes’ is time consuming and relies on accurate repositioning and reproducibility. The identification of hypo-intense lesions can be difficult as many lesions are degrees of ‘grey holes’, this difficulty in defining ‘black holes’ can lead to considerable inter-observer variation (Barkhof 1998). The results from trials in progress should clarify its value in the monitoring of disease progression in phase III studies.
3.2.3 Magnetisation Transfer Imaging

In the search for more pathologically specific measures there has been great interest in magnetisation transfer (MT) imaging. In conventional MRI the signal is dependent on the relaxation properties of free water protons; the much smaller number of bound protons have little effect on the signal. In MT imaging the bound water molecules (largely in myelin) are saturated with an off-resonance radiofrequency pulse. These then exchange magnetisation with free water protons resulting in an indirect reduction in tissue signal intensity. This effect can be quantified by calculating the MT ratio (MTR) of saturated and non saturated images. The MTR is thus an indirect measure of tissue integrity and will be decreased in an area of demyelination or tissue destruction (Wolff and Balaban 1989).

Reduced MTR values have been demonstrated in both the lesions (Dousset 1992, Gass 1994) and NAWM (Filippi 1995c) of MS and the mean lesion MTR has been shown to correlate moderately ($r= -0.44$) with the EDSS (Gass 1994). It is also possible to measure the MTR in the spinal cord, in a small study the mean cervical MTR of patients with MS was shown to be significantly lower than controls, however no relationship between MTR and disability was shown (Silver 1997a).

More recently new techniques based on the generation of histograms constructed from calculated MTR values have allowed the combination of information from both lesions and NAWM. These histograms demonstrate a single peak in the normal brain corresponding to white matter, in MS this peak has reduced amplitude and is shifted to lower MTR values. By quantifying the area under the curve it may be possible to monitor disease progression (van Buchem 1997). Further work is ongoing in this area to look at the relationship between histogram analysis and clinical change as well as improving and
standardising the measurement technique.

### 3.2.4 Brain and Spinal Cord Atrophy

As severe tissue destruction occurs the central nervous system may respond to this by shrinkage and reorganisation, changes that are only visible to us at the edges of a structure; widening of the sulci and ventricles in the brain and shrinkage of the circumference of the spinal cord. The measurement of the rate of shrinkage may therefore directly reflect the progression of the pathological process responsible for disease progression. The nature of the process underlying atrophy is uncertain but recent pathological and MR studies demonstrating axonal loss both in lesions and NAWM (Ferguson 1997, Fu 1998, Trapp 1998) make this the most likely candidate, although demyelination alone has been shown to result in a reduction of axonal diameter (Prineas and Connell 1978) and could contribute to atrophy.

**Spinal cord atrophy.** The first quantitative study of spinal cord atrophy employed a T2 weighted gradient echo sequence and evaluated 5mm axial slices taken at four vertebral levels; C5, T2, T7 and T11 (Kidd 1993). The cords were manually traced around and atrophy was considered to be present when the measured area was two standard deviations below that of the mean for healthy controls. The mean cord areas of the patients were significantly smaller than that of the controls at each of the four levels. Those patients with atrophy were found to have significantly higher levels of disability as measured by the EDSS than those without. A further study measuring cross sectional area at C5 showed a significant difference between patients with benign and SP MS (Filippi 1996c).

Subsequent studies concentrated on measuring serial cord cross sectional area at C5. In
a study comparing PP and SP MS, both groups showed a decrease in mean cord area over
one year, but there was no difference between the two groups and no significant
correlation with disease progression (Kidd 1996). The intra-rater reliability of the
measurement technique was 2%, but the scan-rescan variability was in the order of 6%
and changes detected were within the 95% confidence limits for measurement variation.
A similar study of RR MS patients over one year using the same technique also failed to
demonstrate a significant change in cord area but the mean intra-rater variability was high
at 4.8% (intra-rater limits of agreement -11.6 to 12.9%) (Thorpe 1996a).
All of these studies depended on two dimensional imaging with a T2 weighted gradient
echo sequence and a manual outlining technique for cross sectional area measurement.
The poor reproducibility of this technique made the detection of small change, an
essential prerequisite for serial studies, impossible.
More recently a new technique has been developed which addresses these difficulties
(Losseff 1996b). By using a volume acquired IR FSPGR acquisition the contrast between
the grey cord and nulled black CSF is markedly improved. The cord area is calculated
from axial reformats obtained from a T1 weighted IR FSPGR sequence at the level of
C2/3 (see figure 3.1). This level was chosen as there is little variability in cross sectional
area over this segment and the CSF pool is capacious thus optimising cord/CSF contrast.
The boundary between cord and CSF is defined on each of the five axial reformats by
using a threshold technique where the threshold is the mean signal intensity of cord and
CSF, thus reducing the partial volume effect (Losseff 1996b). The cord cross sectional
area is calculated for each of the reformats and a mean value obtained. Using this
methodology measurements can be obtained in a very short time (<10 minutes) with
excellent intra, inter-observer and scan-rescan reproducibility (<1%) (Losseff 1996b).
Figure 3.1: Axial reformat of an IR FSPGR sequence for cord atrophy assessment.
In a cross sectional study 30 controls and 60 patients (15 in each subgroup of RR, PP, SP and benign MS patients) were studied (Losseff 1996b). The cord cross sectional areas of the benign, PP and SP groups were significantly smaller than the controls, while the RR group had no significant atrophy. Cord cross sectional area correlated strongly with the EDSS (r= -0.7, p< 0.001) and with disease duration (r= -0.52, p< 0.001).

Despite the excellent reproducibility, including scan-rescan, when this technique was applied serially there were problems. During the study period there was a major hardware upgrade, which resulted in minor changes in the pulse sequence and spatial signal intensity uniformity. As a consequence all of the control subjects exhibited an artefactual increase in their measured cord areas (Losseff 1996d). Chapter four deals with the problems of serial cord atrophy measurements and assesses its use as a surrogate marker for disease progression and as an outcome measure for clinical trials in MS.

**Cerebral atrophy.** The measurement of cerebral atrophy is more complicated than cord atrophy as it involves both the extraction of the brain from the skull and the delineation of the lower border of the brain. An early study looked at a measure of partial brain volume which assessed serial change in four contiguous 5mm slices with the most caudal at the level of the velum interpositum cerebri (Losseff 1996a). These slices represent the ROI and were chosen as they cover most of the lateral ventricles, which along with the cortical surface are the areas most likely to exhibit atrophic changes. The velum interpositum cerebri is thought to be a stable landmark despite ongoing atrophy allowing repositioning for serial assessment. The brain is extracted from the skull using a computer algorithm (DS Yoo, UCL, London, UK) and the ROI volume calculated by multiplying the sum of the ROI areas by the slice thickness (see figure 3.2). Like the measurement of spinal cord atrophy this is extremely reproducible. In this study a
Figure 3.2: Brain atrophy assessment: before (top) and after (bottom) skull extraction.
decrease in cerebral volume beyond the 95% confidence limits was seen in 16 of the 29 patients in a time period of 18 months. The rate of atrophy was significantly higher in those with a definite change in disability compared to those without. Interestingly there was no relationship between the rate of atrophy and the change in TLV or volume of gadolinium enhancement, again highlighting the disparity between markers of inflammatory disease activity (new lesions, gadolinium enhancement) and disease progression (Losseff 1996c).

This measurement technique is used in the studies in chapters five and six, but utilises six 3mm slices, again with the most caudal at the level of the velum interpositum cerebri.

3.2.5 Magnetic Resonance Spectroscopy

Magnetic resonance spectroscopy (MRS) complements conventional MRI by allowing quantification of chemical pathology within lesions and NAWM thus increasing its pathological specificity. The dominant resonance peak in the normal brain is that of N-acetyl aspartate (NAA). This is almost exclusively contained within neurones in the adult brain, thus a reduction in NAA suggests loss or dysfunction of axons. Strong correlations have been shown with white matter NAA concentrations and disability (Arnold 1990, Davie 1995) and reduced levels of NAA have been shown to occur in the NAWM of patients (Fu 1998), even those with PP MS who are known to have small TLVs (Leary 1998a). Changes in the lipid portion of the spectra are also seen in acute or enhancing lesions probably reflecting inflammation and demyelination (Wolinsky 1990).

Short term serial studies of acute lesions have shown that changes in NAA concentration may recover suggesting that axonal damage or dysfunction is not always a permanent phenomenon (De Stefano 1995, Arnold 1992), however long term serial studies of
NAWM have shown a progressive decrease in NAA over time which probably reflects cumulative axonal loss (Arnold 1994). This finding suggests that the serial measurement of the NAA concentration in large areas of the brain would be a measure of progressive axonal loss or irreversible damage and could therefore be useful in the monitoring of disease progression. Further work is needed to look at the sensitivity and reproducibility of the techniques involved, as well as practical issues such as the acquisition time.

3.2.6 Diffusion Imaging

Diffusion-weighted imaging reflects the random motion of water molecules over short distances, resulting in the ability to quantify the amount of diffusion by the ‘apparent diffusion coefficient’ (ADC). Within biological tissue the movement of water molecules is hindered by both permeable and non-permeable barriers; the destruction, or alteration in permeability, of these barriers results in changes in the ADC. It is also possible to measure the degree of directionality of diffusion, known as diffusion anisotropy. In normal white matter fibre tracts, diffusion is greater along the nerve fibres than across them (Horsfield 1998). In the few reported studies of diffusion imaging in MS, the ADC of lesions has been shown to be higher than NAWM. Whilst studies suggest that acute lesions have higher ADC than chronic lesions due to the increase in extra-cellular water associated with oedema, it is possible that the influx of inflammatory cells and myelin breakdown products may reduce the ADC in acute lesions. A reduction in anisotropy indicates disruption of the structural integrity of nerve fibre tracts, a common finding in both lesions and NAWM (Werring 1998b). Measurements of the ADC of NAWM have also shown increases in the ADC of patients with MS compared to healthy controls (Larrson 1992a, Christiansen 1993).
At the present stage diffusion imaging remains technically challenging requiring specialised hardware and is sensitive to patient motion. However it adds further information on the pathological changes occurring in MS and may be useful in the future to aid in the monitoring of disease progression.

3.3 MRI Studies in Primary Progressive Multiple Sclerosis

Several studies have confirmed that patients with PP MS, regardless of disability, have smaller cerebral total lesion volumes than other subtypes of MS and the lesions present tend also to be smaller in size (Thompson 1990, Filippi 1995d). The MRI findings in TP MS have also been shown to differ from SP MS and appear similar to those in PP MS (Filippi 1995a, Gayou 1997).

Few serial studies have been carried out in PP MS. One such study followed 12 patients with PP MS and 12 with SP MS with frequent scanning for six months (Thompson 1991). The rate of development of new lesions was 3.3 lesions per patient per year in the PP group compared to 18.2 in the SP MS group. Enhancement with gadolinium-DTPA was seen in only 5% of new lesions in the PP group compared to 87% in the SP group. In a further study of ten patients with PP MS, monthly scanning for 12 months revealed 20 new brain lesions of which 14 enhanced (although 13 lesions, all of which enhanced, were in one patient) and three new cord lesions, none of which enhanced (Kidd 1996).

The low rate of new lesion formation and the rarity of gadolinium enhancement combined with the difficulties of defining disease progression has raised problems in the monitoring of treatment trials both clinically and by MRI in this patient group. As the presence of enhancement and hence an active scan is important for the monitoring of treatment trials, investigators have looked at improving this low enhancement rate by the use of triple
dose gadolinium. In a series of ten patients with PP MS four enhancing lesions (two active scans) were seen following standard dose gadolinium and 13 lesions (six active scans) following triple dose, an increase in both lesion detection and active scans of 300% (Filippi 1995d). However this increased pick up rate of subtle enhancement was not confirmed by a further study which showed no increase in lesion detection with the use of triple dose gadolinium (Silver 1997b).

The lack of relationship between low cerebral lesion loads, little inflammatory activity but considerable disability suggests that PP MS is a less inflammatory disease than other subtypes of MS. This was supported by a small pathological study comparing the lesions of PP MS with those of SP MS (Revesz 1994). However it does not explain the mechanism by which the disability in PP MS occurs. In the past this has been thought to be secondary to predominant spinal cord disease. However imaging with phased array coils demonstrated that spinal cord lesions were equally prevalent in disabled and non-disabled patients and there were no differences between clinical subtypes of MS (Kidd 1993 and 1996). Although the presence and degree of cord atrophy did correlate with disability, no differences between SP and PP MS have been shown (Losseff 1996b). More recently the presence of diffuse signal change on proton density weighted CSE images of the spinal cord in MS has been noted to be more common in the PP subtype, the degree of this signal change was shown to correlate with cord atrophy (Lycklama 1997a).

Further information regardin the mechanism of disability in this patient group comes from studies assessing the more pathologically specific MR measures of both lesions and NAWM. MR spectroscopy studies have demonstrated reductions of cerebral NAA in both lesions (Davie 1997) and NAWM in PP MS (Leary 1998a), suggesting that axonal loss is diffuse in this subgroup and is not restricted to the lesions identified by standard T2
weighted MRI.

MT imaging has also been applied to both lesions and NAWM in PP MS, the MTR correlated inversely with disability and was lower in progressive (PP and SP) MS than in RR MS. However there were no differences between the PP and SP subtypes (Gass 1994, Dousset 1992, Filippi 1995c). More recently in a study of 52 patients with PP MS, the MTR of NAWM was shown to be significantly lower than that of age matched controls (Leary 1998b).

Further studies of larger cohorts are essential to enable the selection of appropriate, validated MR parameters which are reproducible, sensitive and clinically predictive as outcome measures for the monitoring of treatment trials in PP MS. Measures that reflects axonal loss and/or intrinsic change in the NAWM would seem the most appropriate considering the studies to date.
Part B: Clinical Studies in Multiple Sclerosis
Chapter 4: Serial Assessment of Spinal Cord Atrophy

Although cross sectional studies have demonstrated a close relationship between spinal cord atrophy and disability the techniques used have not been sufficiently reproducible to allow serial assessment. Due to the extremely small changes expected in cord cross sectional area over time periods of only two or three years it is essential all parameters during data acquisition and analysis remain absolutely constant. During the following study an ongoing quality assurance programme was in place, which detects any drift in control measurements over time and in particular can document (and consequently correct) any effects secondary to hardware services or upgrades (Leary 1999).

4.1 Study Design

Patients were recruited from a cohort (Losseff 1996b) previously studied. Thirteen of the controls and 28 patients attended at baseline and one year follow up. Of the 28 patients 12 had PP, six SP, six RR and four benign MS (relapsing-remitting disease of at least ten years duration and a disability on the EDSS of three or less).

All patients underwent a neurological examination and evaluation of the EDSS before undergoing MRI of the cervical spine. A definite change in EDSS over the 12 month period was defined as an increase of 1.0 if the EDSS ≤ 5.0 or an increase of 0.5 if > 5.0.

MR imaging was carried out on a Signa 1.5T system (General Electric, Milwaukee, Wisc., USA). A volume IR FSPGR sequence was acquired (60 1mm slices, TI= 450ms, TE= 4.2ms, TR= 15.6ms, flip angle= 20°, matrix 256x256, acquisition time= 7 minutes) and from the data set a series of five contiguous 3mm axial slices (perpendicular to the
spinal cord) were reformatted using the centre of the C2/3 disc as the caudal landmark. All of the sagittal data sets were examined for the presence of spondylosis or disc protrusion. Each data set was scored according to a four point scale, 0 (normal), 1 (thecal indentation only), 2 (thecal indentation touching the cord) and 3 (cord compression). Measurement of the cord cross sectional area was achieved using the semi-automated methodology previously described in chapter three, following image uniformity correction (Tofts 1994) and blinding. Intra-rater and inter-rater reproducibility was assessed in ten random subjects and scan-rescan in five subjects. To ensure reproducibility over time a quality assurance protocol was designed which involved weekly scanning of controls (Leary 1999).

Statistical analysis involved the paired t test to assess significant changes within the groups over time and the independent t test to assess differences between the groups. Correlations between cord area, disease duration and the EDSS were calculated using Spearman's rank correlation coefficient.

4.2 Results

Intra-rater reproducibility was found to be 0.51%, inter-rater reproducibility was 1.75% and scan-rescan variation was 0.87%.

There was no significant difference between the age of patients and controls, though as expected patients with RR MS were the youngest of the patient subgroups. The benign and SP patients had significantly longer disease durations than the other patient groups and the PP and SP MS patients were significantly more disabled than the other groups. There was no significant difference in the scores for vertebral degenerative disease between the patients and controls (patient mean score 1.38, control mean score 1.23, p=
At baseline there was a significant difference in cord cross sectional area between controls and both PP and SP groups, but not between controls and the RR MS and benign groups. There was a significant difference in both the baseline cord area (p= 0.001) and the change in cord cross sectional area over the 12 months (p= 0.05) between the whole patient group and controls. In the whole patient group and in the PP and RR subgroups, the follow up measures were significantly smaller (p< 0.001). No significant difference in cord area at baseline and one year was seen in the controls, SP or benign patient subgroups (see tables 4.1, 4.2 and figure 4.1).

There was a strong correlation between baseline cord area and EDSS (r= -0.52, p= 0.005) and to a greater extent disease duration (r= -0.75, p< 0.001). The EDSS did not correlate significantly with disease duration (r= -0.34, p= 0.07).

Eight of the 28 patients had a definite increase in their EDSS over the 12 month period but they exhibited no significant differences in cord area at baseline (p= 0.69) or change in cord area over the year (p= 0.51) compared to the 20 patients without a definite increase in EDSS.
Table 4.1: Cord cross sectional area in controls and patients.

<table>
<thead>
<tr>
<th></th>
<th>Controls</th>
<th>Patients</th>
</tr>
</thead>
<tbody>
<tr>
<td>Number</td>
<td>13</td>
<td>28</td>
</tr>
<tr>
<td>Mean age (years)</td>
<td>46.3</td>
<td>45.1</td>
</tr>
<tr>
<td>Range (years)</td>
<td>30 - 59</td>
<td>27 - 65</td>
</tr>
<tr>
<td>Mean cord area; baseline (mm²)</td>
<td>80.95</td>
<td>71.25</td>
</tr>
<tr>
<td>Range (mm²)</td>
<td>73.32 - 86.80</td>
<td>42.02 - 90.84</td>
</tr>
<tr>
<td>Mean change in cord area (mm²)</td>
<td>-0.77 (-0.92%)</td>
<td>-2.26 (-3.71%)**</td>
</tr>
<tr>
<td>Range (mm²)</td>
<td>-4.60 - 2.60</td>
<td>-6.72 - 1.82</td>
</tr>
</tbody>
</table>

* significant difference between patients and controls, p = 0.01

** significant difference between baseline and 1 year data in patients (p< 0.001) and in change in cord area between patients and controls, p= 0.05 (% change p= 0.03)
Table 4.2: Cord atrophy study; patient subgroups.

<table>
<thead>
<tr>
<th></th>
<th>PP</th>
<th>SP</th>
<th>RR</th>
<th>Benign</th>
</tr>
</thead>
<tbody>
<tr>
<td>Number</td>
<td>12</td>
<td>6</td>
<td>6</td>
<td>4</td>
</tr>
<tr>
<td>Mean age (yrs)</td>
<td>49.3</td>
<td>43.7</td>
<td>36.7</td>
<td>47.8</td>
</tr>
<tr>
<td>Range (yrs)</td>
<td>40 - 65</td>
<td>37 - 55</td>
<td>27 - 52</td>
<td>44 - 51</td>
</tr>
<tr>
<td>Mean disease duration</td>
<td>10.9</td>
<td>19.3</td>
<td>5.6</td>
<td>17.3</td>
</tr>
<tr>
<td>Range (yrs)</td>
<td>4 - 22</td>
<td>17 - 24</td>
<td>2 - 9</td>
<td>13 - 22</td>
</tr>
<tr>
<td>Median EDSS</td>
<td>5.75</td>
<td>7.25</td>
<td>3.25</td>
<td>2.25</td>
</tr>
<tr>
<td>Range</td>
<td>3.0 - 8.5</td>
<td>6.0 - 8.0</td>
<td>1.5 - 6.5</td>
<td>2.0 - 3.0</td>
</tr>
<tr>
<td>Definite change in EDSS*</td>
<td>2</td>
<td>2</td>
<td>1</td>
<td>3</td>
</tr>
<tr>
<td>Mean cord size (mm²)</td>
<td>71.98</td>
<td>57.03</td>
<td>83.97</td>
<td>71.35</td>
</tr>
<tr>
<td>Range (mm²)</td>
<td>54.6 - 90.8</td>
<td>42.0 - 67.3</td>
<td>77.1 - 90.2</td>
<td>61.3 - 85.1</td>
</tr>
<tr>
<td>p= from controls</td>
<td>0.01</td>
<td>&lt;0.001</td>
<td>NS</td>
<td>NS</td>
</tr>
<tr>
<td>Mean change (mm²)</td>
<td>-3.52(-5.2%)</td>
<td>-0.26(-0.7%)</td>
<td>-2.98(-3.8%)</td>
<td>-0.41(-0.8%)</td>
</tr>
<tr>
<td>Range (mm²)</td>
<td>-6.7 - 1.7</td>
<td>-2.6 - 1.8</td>
<td>-4.6 - 1.4</td>
<td>-2.7 - 1.6</td>
</tr>
<tr>
<td>p= within subgroup</td>
<td>&lt;0.001</td>
<td>NS</td>
<td>0.001</td>
<td>NS</td>
</tr>
<tr>
<td>p= from control</td>
<td>0.005</td>
<td>NS</td>
<td>0.01</td>
<td>NS</td>
</tr>
</tbody>
</table>

*Definite change in EDSS; an increase of 1.0 if the EDSS ≤5.0 or an increase of 0.5 if > 5.0.

NS = Not significant.
**4.3 Discussion**

This study demonstrates that it is possible to reproducibly measure and detect change in cord cross sectional area over a time period of 12 months in MS patients. It also confirms the previous findings of a strong correlation between a clinical measure of disability (the EDSS) and spinal cord atrophy. The study has been possible due to improvements in the measurement technique. With the application of an IR FSPGR sequence and assessment at the C2/3 level, excellent cord/CSF contrast is obtained. This increase in contrast combined with a semi-automated contouring technique eliminates much of the measurement variability that occurred with the previous method (manual outlining of the...
cord at the C5 level using a gradient echo sequence).

These findings are independent of vertebral degenerative disease, which was mild and comparable in both the control and patient group. The commonest site of spondylosis was the C5/6 disc space, no subjects had abnormality at or above the C2/3 disc space where atrophy measures were obtained. The scoring system used was extremely sensitive and scores of 1 or 2 were not felt to be of clinical significance. Six subjects had evidence of mild cord compression at one or both of their examinations, two of these were controls and four were patients (15% of controls and 14% of patients).

There was no significant change in cord area over one year in the control group. Cord atrophy was present at baseline in the benign and PP groups, but was most marked in the SP MS group. Increasing atrophy over the 12 month period was only detectable in the RR and PP patient groups.

In order to understand the mechanisms and clinical importance of atrophy it is relevant to consider the pathophysiology of the development of disability, which results from two causes. Firstly from incomplete recovery following relapse and secondly, and most importantly, as the result of insidious disease progression. The RR MS patient group who are very early in their disease process have normal sized cords, but have a high rate of cord cross sectional area loss. These patients are experiencing relapses which are predominantly associated with conduction block secondary to demyelination although some axonal loss can occur acutely (Ozawa 1994, Ferguson 1997). Demyelination per se has been shown to result in a reduction of axonal diameter (Prineus and Connell 1978) and combined with an element of acute axonal loss could well result in atrophy of a structure such as the spinal cord following a relapse.

The benign patients begin with a similar relapsing course resulting in associated cord
loss, however minimal change in function occurs. This may be due to predominant
demyelination rather than axonal loss. Subsequently in their disease course, they
experience very few relapses and little further change in cord area.

The largest degree of cord atrophy over time was seen in the PP MS group, which is not
surprising if we consider their slowly progressing disability as a consequence not of
inflammatory demyelinating lesions but of progressive axonal loss. Certainly less
inflammation is seen in this group as shown by pathological (Revesz 1994) and MRI
studies (Thompson 1991).

The SP patient group who had the smallest cords may have a combination of both
demyelination and acute axonal loss secondary to relapses and subsequently progressive
axonal loss underlying the later progressive phase. The small sample in this study is not
entirely representative of SP MS as all four patients were very disabled and perhaps
consequently no further change in cord cross sectional area was detectable.

The suggestion that atrophy may have two mechanisms, one non disabling
(demyelination), the other disabling (axonal loss) is supported by the almost identical
atrophic cord areas seen in the disabled PP and non disabled benign MS cohorts. Another
factor that should be considered is the role of reactive gliosis; this in theory may partly
compensate for the loss of myelin by filling space but could also lead to contraction of
the underlying tissue. Evidence of gliosis comes from histological (Allen and McKeown
1979) and MR spectroscopy studies (Rooney 1997). A reduced NAA/ creatine ratio has
been demonstrated in areas of NAWM, which appeared to be produced by a rise in the
level of creatine and could be accounted for by an increase in gliosis. This study did not
find evidence of diffuse axonal loss (decreased NAA levels) although the patient group
was not defined and may have comprised only of early RR MS cases (Rooney 1997).
These arguments are hypothetical and could be investigated further by combining the assessment of atrophy with markers of demyelination (MT imaging) and axonal loss (MR spectroscopy). The reported reduction of NAA in both lesions and NAWM in PP MS (Davie 1997 and Leary 1998a) suggests that diffuse axonal loss is present in this subgroup. Further evidence for pathological change apart from new lesion formation in this patient group comes from the presence of diffuse signal change on proton density weighted conventional spin echo images of the spinal cord. The degree of this signal change has been shown to correlate with cord atrophy (Lycklama a Nijeholt 1997a). The combination of MR studies with post mortem data will further enable us to correlate changes in the size of the spinal cord with pathological changes (Lycklama a Nijeholt 1997b, Mottershead 1997).

The fact that significant changes in cord cross sectional area can be measured over only 12 months is important, both in increasing our understanding of the underlying mechanisms of disability and in the potential use of serial cord atrophy measures as a surrogate measure of disease progression in the monitoring of therapeutic trials. The findings in the PP MS group are of particular importance as this patient group do not show the rate of new lesion development and enhancement which is used to monitor activity in RR or SP MS. The use of cord and brain atrophy to monitor disease progression in PP MS is explored in detail in chapters five and six.

This study does have limitations, the sample size is small, in particular there are few patients in the SP and benign MS patient groups, and follow up is relatively short. Larger and longer serial studies are needed to confirm the detected differences between disease subgroups and ideally should commence in the early stages of MS allowing evaluation of cord atrophy as a potential prognostic marker.
Chapter 5: A Cross Sectional Study in Primary Progressive Multiple Sclerosis

Disability in MS accumulates both as a consequence of incomplete remission following relapses and more importantly from insidious disease progression. The lack of relapses in PP MS makes this patient group an ideal model for studying disease progression with the aim of improving our understanding of the underlying mechanisms of disability. As part of an EC initiative (MAGNIMS: Magnetic resonance network in multiple sclerosis), patients with PP MS were recruited from six centres within Europe, these patients were followed serially with annual MRI, clinical and cognitive assessments. By documenting the clinical course and MRI features of a large cohort of PP MS patients, it is hoped appropriate outcome measures (surrogate markers for disease progression) for therapeutic trials can also be identified. The small subgroup of patients with TP MS behave similarly to PP MS with the exception of a single relapse or remission during or prior to their progressive disease. Little is known about this patient group and therefore a smaller cohort of TP MS patients were also studied. To allow comparisons to be made with previous data a small group of SP MS patients were followed as a reference group. The data collected on 100 London patients, 60 with PP, 20 with TP and 20 SP MS patients is presented.

5.1 Study Design

Patients. All patients consented to the study and approval from the joint medical ethics committee of the National Hospital for Neurology and Neurosurgery and the Institute of
Neurology was granted. Eighty patients with PP or TP MS were recruited with no restriction on age or disability. A careful history was obtained to exclude patients with a non progressive disorder or, in relation to TP MS patients, those with more than one relapse in the course of their disease. A further 20 patients with SP MS were recruited as a reference group. Each patient underwent a clinical assessment, impairment and disability were assessed using the Kurtzke EDSS (Kurtzke 1983), ten metre timed walk and the 9-hole-peg-test (Goodkin 1988).

Imaging protocol. The examinations were performed on a 1.5T imager (Signa; GE Medical Systems, Milwaukee, Wis.). T2 weighted FSE and T1 weighted spin echo scans were acquired in the brain (3mm contiguous axial slices. T2; TR 3000ms, TE 15/90ms. T1; TR 600ms, TE 20ms).

In the spinal cord nine contiguous, 3mm, sagittal T2 and proton density weighted slices were obtained (TR 2500ms, TE 45/90ms). A volume IR FSPGR acquisition of the cervical cord was also performed (60 1mm slices, TR 15.6ms, TE 4.2ms, TI 450ms, flip angle 20°, matrix 256x256) and from the data set a series of five contiguous 3mm axial slices (perpendicular to the spinal cord) were reformatted using the centre of the C2/3 disc as the caudal landmark.

Analysis. The 9-hole-peg-test was used as an upper limb measure of disability by averaging the times from both hands. When the patient was unable to perform the task or took longer than five minutes to complete it the time for that hand was recorded as 300 seconds, likewise if the patient was unable to walk ten metres the time for the ten metre walk was recorded as 300 seconds.

Brain MRI lesions were identified and marked on the proton density weighted films with cross reference to the T2 weighted images by one observer who was blinded to patient
type and details. T1 hypo-intensities or ‘black holes’ (areas of decreased signal intensity
demarcated from surrounding tissue and corresponding to identified lesions on the T2
weighted films) were marked in a similar way. T2 and T1 hypo-intensity lesion volumes
of the brain were then assessed using the semi-automated contouring technique (Plummer

A measure of partial brain volume reflecting atrophy was acquired as outlined in chapter
three (Losseff 1996a). This technique measures the volume of brain covered by six
contiguous slices, the most caudal one at the level of the velum interpositum cerebri. This
area was chosen as it covers a large proportion of the lateral ventricles and cortical sulci
and the velum interpositum cerebri is thought to be a stable landmark despite ongoing
atrophy allowing repositioning for serial assessment.

A single observer marked spinal cord lesions identifying their size by multiplying the
length (number of vertebral bodies the lesion extended over) by the transverse size (the
number of slices the lesion appeared on). The total cord lesion load was determined by
the sum of the individual lesion sizes.

Cord cross sectional area at the C2/3 level was measured from the IR FSPGR reformats
using a semi-automated technique as described in chapter three (Losseff 1996b).

Reproducibility. Brain lesion load, cerebral atrophy measures and cross sectional cord
areas were repeated on ten random subjects two weeks apart. The coefficient of variation
(COV) was calculated for each measure by dividing the standard deviation by the mean,
to assess measurement reproducibility.

Statistical analysis. Non parametric statistical tests were used throughout. The Mann-
Whitney test was used to look for differences between the patient groups, correlations
were assessed using the Spearmans rank correlation coefficient. To reflect the large
number of statistical comparisons a p value of 0.01 was considered significant and a value between 0.01 and 0.05 a trend. No mathematical correction of statistical significance was carried out to avoid inflating type II errors (the probability of accepting the null hypothesis when the alternative is true) and thus missing real differences (Perneger 1998).

5.2 Results

A total of 100 patients (60 with PP, 20 with TP and 20 with SP MS) was recruited. The male:female ratio in the PP group was 31:29 (52% male), in the TP 10:10 (50% male) and in the SP group 5:15 (25% male). The PP patients were older at the onset of their disease than the other two groups (p < 0.05), while the SP group was more disabled and had the longest disease duration. The progression index (EDSS/disease duration) was greater in the PP group than either the TP (p = 0.04) or SP (p = 0.02) group (see table 5.1). Intra-rater reproducibility was assessed for the MRI measures, the COV for brain lesion load analysis was 2.48%, the more automated measures of brain atrophy and cord cross sectional area measurement produced COVs of 0.65% and 0.51% respectively. The PP group had significantly lower T2 and T1 hypo-intensity brain lesion loads than the SP group (p = 0.001), the TP group were intermediate. The T2/T1 mean lesion load ratios were similar in the three patient groups (PP 3.87, TP 3.94, SP 3.94). There was no significant difference in the measure of partial brain volume between the groups. In the spinal cord there were no differences in the number or volume of lesions. The measure of cord atrophy revealed that the SP MS group had a significantly smaller mean cord area than either the PP (p = 0.01) or TP (p = 0.05) MS groups (see table 5.2).
Table 5.1: Clinical findings in PP, TP and SP MS.

<table>
<thead>
<tr>
<th></th>
<th>(n)</th>
<th>PP (60)</th>
<th>TP (20)</th>
<th>SP (20)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Sex Ratio (M:F)</td>
<td></td>
<td>31:29</td>
<td>10:10</td>
<td>5:15</td>
</tr>
<tr>
<td>(52% male)</td>
<td></td>
<td>(50% male)</td>
<td>(25% male)</td>
<td></td>
</tr>
<tr>
<td>Mean Onset Age (yrs)</td>
<td></td>
<td>40.9</td>
<td>35.1</td>
<td>28.7</td>
</tr>
<tr>
<td>(SD 9.6)</td>
<td></td>
<td>(SD 10.6)</td>
<td>(SD 7.3)</td>
<td></td>
</tr>
<tr>
<td>Mean Disease Duration (yrs)</td>
<td></td>
<td>10.9</td>
<td>13.0</td>
<td>18.6</td>
</tr>
<tr>
<td>(SD 6.4)</td>
<td></td>
<td>(SD 7.5)</td>
<td>(SD 11.3)</td>
<td></td>
</tr>
<tr>
<td>Median EDSS (range)</td>
<td></td>
<td>6.0</td>
<td>5.75</td>
<td>7.0</td>
</tr>
<tr>
<td>(range 2-8.5)</td>
<td></td>
<td>(range 2.5-8.5)</td>
<td>(range 3-8.5)</td>
<td></td>
</tr>
<tr>
<td>Progression Index (Mean)</td>
<td></td>
<td>0.81</td>
<td>0.54</td>
<td>0.52</td>
</tr>
<tr>
<td>(SD 0.65)</td>
<td></td>
<td>(SD 0.31)</td>
<td>(SD 0.33)</td>
<td></td>
</tr>
</tbody>
</table>

SD= standard deviation

The entire group showed an extremely weak correlation between brain TLV and disease duration ($r= 0.22$, $p= 0.027$) but no correlation between either brain T2 or T1 hypo-intensity lesion load and the EDSS. The only clinical measure which did correlate with the T2 and T1 hypo-intensity brain lesion loads was the 9-hole-peg-test ($T2; r= 0.35, p< 0.001$. $T1; r= 0.34, p= 0.001$). Cord cross sectional area was the only MRI parameter which correlated with the EDSS ($r= -0.33, p< 0.001$). The clinical measures of upper limb function and ambulation both correlated strongly with the EDSS (9-hole-peg-test $r= 0.64$, ten metre timed walk $r= 0.88, p< 0.001$).
### Table 5.2: MRI findings in PP, TP and SP MS.

<table>
<thead>
<tr>
<th></th>
<th>(n)</th>
<th>PP (60)</th>
<th>TP (20)</th>
<th>SP (20)</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td></td>
<td>Mean (SD)</td>
<td>Median (Range)</td>
<td>Mean (SD)</td>
</tr>
<tr>
<td>Brain T2 Load</td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>(cm³)</td>
<td></td>
<td>11.85 (14.70)</td>
<td>15.70 (16.59)</td>
<td>27.74 (30.08)</td>
</tr>
<tr>
<td></td>
<td></td>
<td>6.29 (0- 72.2)</td>
<td>11.96 (0.5- 72.0)</td>
<td>20.68 (3.3- 138.0)</td>
</tr>
<tr>
<td>Brain T1 Load</td>
<td></td>
<td>3.06 (5.32)</td>
<td>3.98 (5.11)</td>
<td>7.04 (7.37)</td>
</tr>
<tr>
<td>(cm³)</td>
<td></td>
<td>1.07 (0- 33.1)</td>
<td>2.42 (0- 22.0)</td>
<td>4.24 (0.5- 22.1)</td>
</tr>
<tr>
<td>6 Slice Volume</td>
<td></td>
<td>269.28 (22.53)</td>
<td>271.33 (21.51)</td>
<td>261.37 (25.86)</td>
</tr>
<tr>
<td>(cm³)</td>
<td></td>
<td>269.41 (213- 320)</td>
<td>271.14 (238- 324)</td>
<td>257.3 (223- 335)</td>
</tr>
<tr>
<td>Number of Cord</td>
<td></td>
<td>2.9 (2.77)</td>
<td>3.9 (2.45)</td>
<td>3.2 (3.3)</td>
</tr>
<tr>
<td>Lesions</td>
<td></td>
<td>2.0 (0- 12)</td>
<td>3.0 (1- 9)</td>
<td>3.0 (3- 13)</td>
</tr>
<tr>
<td>Cord Lesion Load</td>
<td></td>
<td>3.8 (4.1)</td>
<td>4.8 (4.0)</td>
<td>3.5 (3.1)</td>
</tr>
<tr>
<td></td>
<td></td>
<td>3.0 (0- 18.5)</td>
<td>3.0 (0.5- 12.5)</td>
<td>2.5 (0- 8.0)</td>
</tr>
<tr>
<td>Cord Area (mm²)</td>
<td></td>
<td>70.6 (9.5)</td>
<td>69.6 (6.6)</td>
<td>64.1 (9.9)</td>
</tr>
<tr>
<td></td>
<td></td>
<td>71.0 (43.6- 90.8)</td>
<td>68.3 (58.2- 80.3)</td>
<td>63.6 (42.3- 80.3)</td>
</tr>
</tbody>
</table>

Considering the PP MS patients alone there was a moderate correlation between the EDSS and disease duration ($r= 0.46$, $p< 0.001$). As in the entire group analysis, the only clinical measure to correlate with either the T1 hypo-intensity or TLV was that of the 9-hole-peg-test (T2; $r= 0.26$, $p= 0.045$. T1; $r= 0.25$, $p= 0.05$).

All three of the clinical measures correlated with spinal cord cross sectional area (EDSS; $r= -0.43$ $p= 0.001$, 9-hole-peg-test; $r= -0.46$ $p< 0.001$, ten metre timed walk; $r= -0.41$ $p= -94-$
0.001). The measure of brain volume correlated with the ten metre timed walk only \((r=-0.36, p=0.005)\). There were no correlations between brain and cord MRI findings or indeed between the number or volume of spinal cord lesions and cord cross sectional area.

The majority of patients with PP MS (48 patients, 80%) presented with a progressive cord syndrome, the TP and SP MS patients were more varied (see table 5.3).

**Table 5.3:** Presenting clinical syndromes.

<table>
<thead>
<tr>
<th></th>
<th>PP</th>
<th>TP</th>
<th>SP</th>
</tr>
</thead>
<tbody>
<tr>
<td><strong>Spinal Cord</strong></td>
<td>48 (80%)</td>
<td>6 (30%)</td>
<td>7 (35%)</td>
</tr>
<tr>
<td><strong>Cerebellar</strong></td>
<td>8 (13%)</td>
<td>3 (15%)</td>
<td>1 (5%)</td>
</tr>
<tr>
<td><strong>Hemiparesis</strong></td>
<td>3 (5%)</td>
<td>3 (15%)</td>
<td>0</td>
</tr>
<tr>
<td><strong>Visual</strong></td>
<td>1 (2%)</td>
<td>4 (20%)</td>
<td>6 (30%)</td>
</tr>
<tr>
<td><strong>Brainstem</strong></td>
<td>0</td>
<td>4 (20%)</td>
<td>6 (30%)</td>
</tr>
<tr>
<td><strong>Total</strong></td>
<td>60 (100%)</td>
<td>20 (100%)</td>
<td>20 (100%)</td>
</tr>
</tbody>
</table>

When the 48 patients presenting with a cord syndrome were compared to the 12 with any other presentation no difference in disease duration or EDSS was seen (see table 5.4).
Table 5.4: Presentation at onset in primary progressive multiple sclerosis.

<table>
<thead>
<tr>
<th></th>
<th>Cord Presentation</th>
<th>Other Presentation</th>
<th>Significant differences</th>
</tr>
</thead>
<tbody>
<tr>
<td><strong>(n)</strong></td>
<td>(48)</td>
<td>(12)</td>
<td></td>
</tr>
<tr>
<td>Mean Disease Duration (yrs)</td>
<td>11.0 (SD 6.7)</td>
<td>10.5 (SD 5.1)</td>
<td>NS</td>
</tr>
<tr>
<td>Median EDSS (Range)</td>
<td>6.0 (2.0-8.5)</td>
<td>6.5 (4.0-8.5)</td>
<td>NS</td>
</tr>
<tr>
<td>Brain T2 Load (cm³)</td>
<td>9.50 (SD 12.34)</td>
<td>21.26 (SD 19.68)</td>
<td>(p=0.029)</td>
</tr>
<tr>
<td></td>
<td>4.50 (0-62.6)</td>
<td>23.08 (0.4-72.2)</td>
<td></td>
</tr>
<tr>
<td>Brain T1 Load (cm³)</td>
<td>2.37 (SD 5.14)</td>
<td>5.81 (SD 5.34)</td>
<td>(p=0.009)</td>
</tr>
<tr>
<td></td>
<td>0.70 (0-33.1)</td>
<td>5.02 (0-17.8)</td>
<td></td>
</tr>
</tbody>
</table>

NS= not significant

However the brain T2 and T1 hypo-intensity lesion loads of the cord onset group were considerably smaller than those with other presentations (p=0.002). There was no difference in the cord parameters between the two groups although within the cord presentation group spinal cord cross sectional area correlated significantly with the EDSS (r= -0.49, p<0.001), this relationship was not present in the other smaller group (r= 0.006, p=0.99).
5.3 Discussion

This cross sectional study confirms that patients with PP MS differ clinically from the other subtypes of progressive MS in several ways. The late age of onset (40.9 years) is in agreement with previous studies (Confraveux 1980, Thompson 1986, Leibowitz 1964, Weinshenker 1989) and is significantly later than all other subgroups. The incidence appears to be equal in males and females; this lack of female preponderance, which is usually seen in auto-immune conditions, may reflect the less inflammatory/immune nature of the disease course compared to other subgroups of MS.

The MRI findings in this study are also consistent with previous findings (Thompson 1990). Patients with PP MS had low brain T2 and T1 hypo-intensity lesion loads and no correlation was found with the EDSS. As with other studies the number and volume of spinal cord lesions differed little between the subgroups and there was no correlation with disability (Kidd 1993 and 1996, Lycklama 1998). This may in part be explained by the pathological heterogeneity of T2 lesions and the effects of atrophy. As atrophy progresses lesions may be lost due to partial volume effects on sagittal images (Kidd 1993) or the whole cord may become diffusely abnormal with loss of contrast between lesions and abnormal white matter (Lycklama 1998).

Most patients (80%) with PP MS presented with a progressive spastic paraparesis. These patients did not differ from other presentations in either disease duration or disability as measured by the EDSS. However their brain T2 and T1 hypo-intensity lesion loads were significantly lower than the ‘other presentations’ group (though no difference in the number or volume of cord lesions was seen). Despite there being no difference between the cord presentation group and the others in cord cross sectional area there was a significant correlation within the cord group with the EDSS which was not present in the
other group. This may reflect the smaller sample size in the other presentation group or the influence of ataxia or brainstem scores in the EDSS in this group.

The prognosis in PP MS is thought to be poor in relation to disability (Runmarker and Andersen 1993, Weinshenker 1989), this is supported by the findings in this study. The progression index (EDSS/disease duration) is significantly higher in the PP MS patients than in the other two groups. This finding is similar to that of previous workers (Gayou 1997), and is seen despite the low number of lesions in the brain and spinal cords of patients with PP MS, suggesting that the mechanism of disability in this group cannot be attributed to lesion formation. The only MR measure which correlated significantly with the EDSS was the spinal cord cross sectional area. Atrophy occurs secondary to a combination of demyelination (Prineas and Connell 1978) and axonal loss (Trapp 1998), suggesting that in patients with PP MS the development of disability may be a consequence of diffuse axonal loss. This hypothesis is supported by MR spectroscopy work showing reduced levels of the axonal marker NAA in NAWM of patients with PP MS (Davie 1997, Arnold 1990, Leary 1998a). A recent study in RR MS demonstrated a correlation between change in the EDSS and the NAA: Creatine ratio of NAWM (no such correlation was seen with the T2 lesion load) (Fu 1998). This suggests that diffuse axonal loss may be an important factor in the accumulation of disability in MS even in early RR MS. Areas of NAWM have also been studied using MT imaging to assess the degree of demyelination present, the MTR has been found to be significantly lower in PP MS patients than controls (Leary 1998b) and in chronic progressive MS patients than those with RR MS although patients with PP MS were not distinguished from SP MS (Dousset 1992, Filippi 1995c).

One of the other frequently asked questions with regard to PP MS, is whether it is a
distinct disease entity or merely a part of the spectrum of MS (Thompson 1997b). Although there is no doubt that the PP MS group show marked differences in their clinical, pathological and MRI features, there is still considerable overlap in both the brain T2 and T1 hypo-intensity lesion loads and the spinal cord findings between MS subtypes preventing distinction on radiological grounds. In fact when this large cohort is separated into modes of presentation one subgroup of PP MS shows larger brain lesion loads (approaching those of the SP MS group) supporting the view that PP MS is indeed at one end of a spectrum, a point which is supported by the findings in TP MS, a patient group which has many similarities to PP MS patients but which has MRI findings mid way between PP and SP MS.

With regard to the monitoring of disease activity necessary for therapeutic trials the most promising markers appear to be those of brain and spinal cord atrophy as these have been shown to correlate with clinical measures of impairment and disability in this cross sectional study, however it is essential to study their responsiveness over time. It is also important to assess serial change in the TLV in this patient group as this is already a validated marker which is universally used in both RR and SP MS therapeutic trials. Chapter six presents the results of a two year follow up of this cohort.
Chapter 6: A Longitudinal, Two Year, Study of Primary Progressive Multiple Sclerosis

Cross sectional analysis of a large cohort of PP and TP MS patients and a smaller group with SP MS was presented in chapter five. The findings were similar to previous smaller studies and confirmed that patients with PP MS have a relatively older age of onset and an equal sex ratio. The MRI findings were also consistent with previous data demonstrating that patients with PP MS have low mean T2 and T1 hypo-intensity brain lesion loads. The number and volume of cord lesions differed little between the subgroups and there was no correlation with disability. The MRI findings in TP MS appeared to be similar to those of PP MS. The correlations between clinical and MR parameters were moderate for measures of atrophy but poor for the T2 and T1 hypo-intensity brain lesion loads. Within the MRI data there was no correlation between brain and cord parameters or between the number or volume of spinal cord lesions and cord cross sectional area.

Many of the problems in the planning of appropriate therapeutic trials in PP MS, including difficulties in the measurement of clinical progression and the lack of a validated surrogate marker for disease progression, are outlined in chapter five. In this chapter serial data of this large cohort of PP MS patients is presented. This study provides an opportunity to assess a) whether there is change clinically or on MRI, and b) if there is change in these, whether there is a correlation between them.
6.1 Study Design

The 100 patients previously studied (chapter five) were approached for repeat evaluation at one and two years following their initial assessment. At each visit the following clinical measures were recorded; Kurtzkes EDSS, the ten metre timed walk and the 9-hole-peg-test. Patients underwent the same imaging protocol as outlined in chapter five at each visit.

Analysis. All of the analysis techniques are documented in chapter three. A significant deterioration in either the 9-hole-peg-test or ten metre timed walk was defined as prolongation by 20% or more (Goodkin 1988).

Brain lesions on year one and two scans were identified and marked with reference to the baseline images before lesion load measurement. The number of new brain and spinal cord lesions was recorded.

Measurement reproducibility was assessed for brain lesion load, cerebral atrophy and cross sectional cord area by repeating measurements on the same data set of ten random subjects at least one year apart. The COV was calculated for each measure by dividing the standard deviation by the mean.

A significant change in the degree of brain or cord atrophy was defined as a reduction by more than twice the COV.

Statistical analysis. As in chapter five non parametric statistical tests were used throughout. The Mann-Whitney test was used to look for differences between patient groups and the Wilcoxon signed ranks test to compare patient group results at different time points. Correlations were assessed using the Spearmans rank correlation coefficient. As before, to allow for the large number of statistical comparisons, a p value of 0.01 was considered significant and a value between 0.01 and 0.05 a trend.
6.2 Results

Of the 100 patients originally studied 84 returned for re-assessment at one year (49 (82%) patients with PP, 18 (90%) with TP and 17 (85%) with SP MS) and 75 patients returned at two years (42 (70%) with PP, 16 (80%) with TP and 17 (85%) with SP MS). Of the 25 patients who did not complete the study, one patient had died from an unrelated cause, two others became unwell, all from other conditions. A further patient was imprisoned and two moved away from the area. Five patients found the travelling too arduous due to increasing disability, the remaining 14 patients declined to give a reason for leaving the study.

The mean time to follow up was 11.4 months (8-17) and 22.4 months (18-28) for each assessment. On review at one year, two patients had experienced a relapse during the first year of the study, one initially classified as TP became SP MS, the other, classified initially as PP, became TP MS for the follow up analysis.

Intra-rater reproducibility was assessed for the MRI measures, the mean COV for brain lesion load analysis was 3.18% (SD 2.62), the more automated measures of brain atrophy and cord cross sectional area measurement produced COV’s of 0.13% (SD 0.38) and 0.51% (SD 0.54) respectively.

Clinical Measures: EDSS. Of the 75 patients who completed the study, 18 (24%) at year one and 29 (39%) at year two, had a one (or more) step deterioration in the EDSS (an increase of 1.0 if the EDSS < 5.0 or an increase of 0.5 if > 5.0). Considering the PP population, the majority of patients showed no change (27 patients (64%) at year one and 24 (57%) at year two). Nine patients (21%) at year one and 11 patients (26%) at year two deteriorated by at least one step on the EDSS scale. Proportionately more patients in the
TP and SP groups exhibited a deterioration in their EDSS score (TP; three (19%) at year one and ten (63%) at year two. SP; six (35%) at year one and eight (47%) at year two). Seven patients (9%) had improved by one step from baseline at both time points (six PP and one TP MS patients at year one and seven PP MS patients at year two).

**9-Hole-Peg-Test.** Only ten (13%) of the 75 patients deteriorated on the 9-hole-peg-test at one year and 20 (27%) at two years. Of these, four at one year and nine at two years were patients with PP MS. The majority of patients demonstrating deterioration in the 9-hole-peg-test were patients with TP MS (five patients (31%) at year one and seven patients (44%) at year two). Three patients at both time points (all PP MS at year one and two PP and one SP MS at year two) improved on the 9-hole-peg-test.

**Ten Metre Timed Walk.** This showed a similar effect with a 20% prolongation in 11 patients (15%) at year one and 19 (25%) at year two. Most of these patients had PP or TP MS (seven (17%) PP MS patients deteriorated at one year and 11 (26%) at two years. Within the TP MS group, three (19%) deteriorated at year one and seven (44%) at year two). Six patients (five PP and one TP MS) showed significant improvement in their ten metre walk at one year and five (four PP and one TP MS) at two years.

The baseline and two year follow up measures of the EDSS and 9-hole-peg-test scores were significantly different in the TP and SP groups (p< 0.01) but not in the PP MS patient group (EDSS p= 0.27, peg test p=0.65). The follow up measures of the ten metre walk were not significantly different in any of the patient groups however there was a marked floor effect. Twenty four patients (32%) at baseline and 26 (35%) at one year had a maximum score of 300 seconds and therefore were unable to demonstrate change. When these patients were excluded from the analysis the entire group showed a significant change over two years (p= 0.004), the now smaller PP and TP patient groups
demonstrated a trend (PP; p = 0.037, TP; p = 0.021) (see table 6.1).

**Table 6.1: Clinical measures at baseline, year 1 and year 2 (median values).**

<table>
<thead>
<tr>
<th></th>
<th>PP (42)</th>
<th>TP (16)</th>
<th>SP (17)</th>
</tr>
</thead>
<tbody>
<tr>
<td><strong>EDSS</strong></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>0</td>
<td>6.00 (2.0-8.5)</td>
<td>6.00 (2.5-8.5)</td>
<td>7.00 (3.0-8.5)</td>
</tr>
<tr>
<td>1</td>
<td>6.00 (2.0-9.0)</td>
<td>6.00 (2.5-8.5)</td>
<td>7.50 (3.5-8.5)*</td>
</tr>
<tr>
<td>2</td>
<td>6.25 (2.0-9.0)</td>
<td>6.00 (3.0-9.0)*</td>
<td>7.00 (3.5-8.5)*</td>
</tr>
<tr>
<td><strong>Definite Change</strong></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>at 1</td>
<td>9 patients (21%)</td>
<td>3 patients (19%)</td>
<td>6 patients (35%)</td>
</tr>
<tr>
<td>at 2</td>
<td>11 patients (26%)</td>
<td>10 patients (63%)</td>
<td>8 patients (47%)</td>
</tr>
<tr>
<td><strong>9-Hole-Peg-Test</strong></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>0</td>
<td>32.7 (17-300)</td>
<td>36.0 (19-300)</td>
<td>36.1 (18-188)</td>
</tr>
<tr>
<td>1</td>
<td>28.9 (15-300)</td>
<td>40.7 (18-300)</td>
<td>40.5 (19-185)</td>
</tr>
<tr>
<td>2</td>
<td>34.4 (15-300)</td>
<td>43.6 (21-300)*</td>
<td>40.3 (18-184)*</td>
</tr>
<tr>
<td><strong>Definite Change</strong></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>at 1</td>
<td>4 patients (10%)</td>
<td>5 patients (31%)</td>
<td>1 patients (6%)</td>
</tr>
<tr>
<td>at 2</td>
<td>9 patients (21%)</td>
<td>7 patients (44%)</td>
<td>4 patients (24%)</td>
</tr>
<tr>
<td><strong>Ten Metre Walk</strong></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>0</td>
<td>13.0 (7-300)</td>
<td>11.7 (6-300)</td>
<td>189 (8-300)</td>
</tr>
<tr>
<td>1</td>
<td>15.5 (6-300)</td>
<td>13.4 (6-300)</td>
<td>300 (6-300)</td>
</tr>
<tr>
<td>2</td>
<td>18.0 (6-300)</td>
<td>19.0 (6-300)</td>
<td>237 (6-300)</td>
</tr>
<tr>
<td><strong>Definite Change</strong></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>at 1</td>
<td>7 patients (17%)</td>
<td>3 patients (19%)</td>
<td>1 patients (6%)</td>
</tr>
<tr>
<td>at 2</td>
<td>11 patients (26%)</td>
<td>7 patients (44%)</td>
<td>1 patients (6%)</td>
</tr>
</tbody>
</table>

Definite change in EDSS is defined as at least an increase of 1.0 if the EDSS ≤5.0 or an increase of 0.5 if > 5.0. Change in 10m walk or 9-hole-peg-test is defined as a 20% change from baseline.

Mann-Whitney test used to detect differences between the patient groups;

- PP < TP in change in 9-hole-peg-test (p = 0.003) and EDSS (p = 0.006) at two years.

* Wilcoxon signed ranks test used to assess difference from baseline (p < 0.01).
Magnetic Resonance Imaging: New Lesions. One or more new brain lesions (range 0-6) were seen in 52% of the PP MS patients and 17% demonstrated a new cord lesion (range 0-1); combining these, 64% of the PP patients had a new lesion in either brain or cord over the two year study period. Within the TP group 50% of patients demonstrated a new brain lesion but no new cord lesions were seen. The SP group demonstrated a slightly higher frequency of new lesions with 53% of patients with at least one new brain lesion and 35% with new cord lesions, this gives a combined activity of 71% (of patients with a new lesion in either brain or cord) over the two year study period (see tables 6.2 and 6.3).

Brain Lesion Loads. The MRI measure of T2 lesion load showed an increase over the two years in all patient groups (PP; 17.2% median change, p= 0.003. TP; 13.3% median change, p= 0.004. SP; 15.4% median change, p= 0.002), this change was not significant at the one year assessment, even if the full cohort of 84 patients was considered. The T1 hypo-intensity lesion load increased significantly in all groups at year two (PP; 33.7% median change, p< 0.001. TP; 25.2% median change, p= 0.007. SP; 33.1% median change, p= 0.004) and in the PP and SP groups at year one (PP; 31.5%, p< 0.001. SP; 29.2%, p= 0.008). The SP patient group had a larger absolute change (p= 0.012) in T2 lesion load than the PP group over the two years (PP 0.43cm³, TP 2.22cm³, SP 2.46cm³). The changes in T1 hypo-intensity loads were of smaller magnitudes (PP 0.29cm³, TP 0.86cm³, SP 1.05cm³) but followed a similar pattern. Except for the absolute change in T2 lesion load, there were no other significant differences in either the rates of change or absolute change in MRI measures between the patient groups.

Brain Atrophy. The six slice measure of brain volume reflecting atrophy showed a significant change between baseline and two years in the PP and SP MS patient groups,
Table 6.2: MRI brain findings at baseline, year 1 and year 2 (median values).

<table>
<thead>
<tr>
<th></th>
<th>year</th>
<th>PP (42)</th>
<th>TP (16)</th>
<th>SP (17)</th>
</tr>
</thead>
<tbody>
<tr>
<td><strong>New Brain Lesions</strong></td>
<td>at 1</td>
<td>0.0 (0-6) [mean 0.79]</td>
<td>0.0 (0-1) [mean 0.44]</td>
<td>0.0 (0-8) [mean 1.18]</td>
</tr>
<tr>
<td></td>
<td>at 2</td>
<td>1.0 (0-6) [mean 1.21]</td>
<td>0.5 (0-4) [mean 0.94]</td>
<td>1.0 (0-9) [mean 2.12]</td>
</tr>
<tr>
<td><strong>Brain T2 Load</strong></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>(cm&lt;sup&gt;3&lt;/sup&gt;)</td>
<td>0</td>
<td>6.29 (0.2-72.2) [4.4%]</td>
<td>14.04 (0.5-72.9) [10.9%]</td>
<td>18.92 (3.6-48.8) [7.6%]</td>
</tr>
<tr>
<td></td>
<td>1</td>
<td>6.31 (0.3-73.9) [11.9%]</td>
<td>15.56 (0.4-85.4) [13.3%]</td>
<td>18.99 (6.6-54.3) [15.4%]</td>
</tr>
<tr>
<td></td>
<td>2</td>
<td>7.88 (0.2-71.3)* [17.2%]</td>
<td>16.37 (0.3-93.7)* [10.9%]</td>
<td>22.87 (5.8-55.3)* [15.4%]</td>
</tr>
<tr>
<td><strong>Change</strong></td>
<td>at 1</td>
<td>0.09 (-6.0-15.4) [4.4%]</td>
<td>1.03 (-0.6-12.6) [10.9%]</td>
<td>1.03 (-9.5-5.6) [7.6%]</td>
</tr>
<tr>
<td></td>
<td>at 2</td>
<td>0.43 (-6.8-16.4) [17.2%]</td>
<td>2.22 (-0.3-20.9) [13.3%]</td>
<td>2.46 (-3.6-7.6) [15.4%]</td>
</tr>
<tr>
<td><strong>Brain T1 Load</strong></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>(cm&lt;sup&gt;3&lt;/sup&gt;)</td>
<td>0</td>
<td>1.07 (0-33.1) [31.5%]</td>
<td>3.28 (0.0-22.0) [20.2%]</td>
<td>3.30 (0.4-20.5) [29.2%]</td>
</tr>
<tr>
<td></td>
<td>1</td>
<td>1.53 (0-32.9)* [33.7%]</td>
<td>4.09 (0.04-32.3) [25.2%]</td>
<td>4.97 (0.6-24.3)* [33.1%]</td>
</tr>
<tr>
<td></td>
<td>2</td>
<td>1.34 (0-34.0)* [33.7%]</td>
<td>4.05 (0.1-39.3)* [25.2%]</td>
<td>3.64 (0.7-26.7)* [33.1%]</td>
</tr>
<tr>
<td><strong>Change</strong></td>
<td>at 1</td>
<td>0.27 (-0.5-11.3) [31.5%]</td>
<td>0.71 (-0.7-10.3) [20.2%]</td>
<td>0.87 (-2.0-3.7) [29.2%]</td>
</tr>
<tr>
<td></td>
<td>at 2</td>
<td>0.29 (-0.6-10.8) [33.7%]</td>
<td>0.86 (-1.4-17.2) [25.2%]</td>
<td>1.05 (-1.4-6.1) [33.1%]</td>
</tr>
<tr>
<td><strong>6 Slice Brain Volume</strong> (cm&lt;sup&gt;3&lt;/sup&gt;)</td>
<td>at 1</td>
<td>273.2 (219-320) [-1.07%]</td>
<td>272.3 (238-297) [-0.66%]</td>
<td>255.9 (223-335) [-0.58%]</td>
</tr>
<tr>
<td></td>
<td>at 2</td>
<td>270.0 (208-315)* [-1.07%]</td>
<td>270.8 (235-298) [-0.66%]</td>
<td>253.0 (219-334) [-0.58%]</td>
</tr>
<tr>
<td></td>
<td></td>
<td>268.5 (202-316)* [-1.07%]</td>
<td>266.4 (229-298) [-0.66%]</td>
<td>251.0 (215-326)* [-0.58%]</td>
</tr>
</tbody>
</table>

-PP< SP (p= 0.012) in absolute change in T2 lesion load at year two.

* Wilcoxon signed ranks test used to assess difference from baseline (p< 0.01).
Table 6.3: MRI cord findings at baseline, year 1 and year 2 (median values).

<table>
<thead>
<tr>
<th>(n)</th>
<th>year</th>
<th>PP (42)</th>
<th>TP (16)</th>
<th>SP (17)</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Number of Cord Lesions</td>
<td>0</td>
<td>2.0 (0-12)</td>
<td>3.0 (1-9)</td>
<td>3.0 (0-13)</td>
</tr>
<tr>
<td></td>
<td>1</td>
<td>2.5 (0-12)</td>
<td>3.0 (1-9)</td>
<td>4.0 (0-13)</td>
</tr>
<tr>
<td></td>
<td>2</td>
<td>3.0 (0-12)</td>
<td>3.0 (1-9)</td>
<td>4.0 (0-13)</td>
</tr>
<tr>
<td>New Cord Lesions</td>
<td>at 1</td>
<td>0.0 (0-1) [mean 0.11]</td>
<td>0.0</td>
<td>0.0 (0-1) [mean 0.33]</td>
</tr>
<tr>
<td></td>
<td>at 2</td>
<td>0.0 (0-1) [mean 0.20]</td>
<td>0.0</td>
<td>0.0 (0-2) [mean 0.33]</td>
</tr>
<tr>
<td>Cord Lesion Load</td>
<td>0</td>
<td>3.25 (0-18.5)</td>
<td>3.00 (5-11)</td>
<td>2.50 (0-10.5)</td>
</tr>
<tr>
<td></td>
<td>1</td>
<td>3.25 (0-18.5)</td>
<td>3.00 (5-11)</td>
<td>2.50 (0-10.5)</td>
</tr>
<tr>
<td></td>
<td>2</td>
<td>3.50 (0-18.5)</td>
<td>3.00 (5-11)</td>
<td>2.50 (0-10.5)</td>
</tr>
<tr>
<td>Increase in Cord Load</td>
<td>at 1</td>
<td>0.0 (0-2) [mean 0.19]</td>
<td>0.0</td>
<td>0.0 (0-2) [mean 0.37]</td>
</tr>
<tr>
<td></td>
<td>at 2</td>
<td>0.0 (0-2) [mean 0.27]</td>
<td>0.0</td>
<td>0.0 (0-2) [mean 0.37]</td>
</tr>
<tr>
<td>Cord Area (mm²)</td>
<td>0</td>
<td>70.8 (54-91)</td>
<td>68.6 (58-78)</td>
<td>63.6 (52-80)</td>
</tr>
<tr>
<td></td>
<td>1</td>
<td>66.7 (49-87)*</td>
<td>63.6 (54-75)*</td>
<td>58.4 (50-76)*</td>
</tr>
<tr>
<td></td>
<td>2</td>
<td>65.8 (47-85)*</td>
<td>63.1 (52-72)*</td>
<td>56.0 (50-77)*</td>
</tr>
<tr>
<td>Change</td>
<td>at 1</td>
<td>-4.3 (-11.8-0.3) [-5.66%]</td>
<td>-4.0 (-7.1-0.6) [-5.56%]</td>
<td>-2.7 (-9.7-3.3) [-4.68%]</td>
</tr>
<tr>
<td></td>
<td>at 2</td>
<td>-4.5 (-14.4-0.7) [-6.11%]</td>
<td>-5.3 (-8.5-3.0) [-7.41%]</td>
<td>-4.2 (-11.5-1.8) [-6.34%]</td>
</tr>
</tbody>
</table>

The Mann-Whitney test did not detect any differences (p<0.01) between the patient groups in absolute or percentage change of MR parameters.

* Wilcoxon signed ranks test used to assess difference from baseline (p<0.01).
this difference was also seen at one year in the PP MS group (median change 1.07%, p< 0.001). The change in brain volume in the TP group was not sufficient to reach the significance level of p< 0.01 (at two years; 1.19%, p= 0.023) (see table 6.2).

**Spinal Cord Lesion Load.** Neither the number of lesions or the lesion load increased significantly over the study period in any patient group (see table 6.3).

**Spinal Cord Atrophy.** All groups showed a significant reduction in spinal cord area over a time period of only one year and no differences in the degree or rate of change were seen between the three groups.

Ninety percent of all patients demonstrated a significant change in the degree of cord atrophy (reduction in cord cross sectional area by more than twice the COV) and 64% a significant change in degree of brain atrophy at one year. At two years 95% had significant change in cord cross sectional area and 79% a significant loss of brain volume, there were no differences in clinical measures or disease type between those with atrophy and those without.

**Clinical Presentation.** When the PP MS patients were divided into those who presented with a progressive cord syndrome (33 patients) and those with any 'other presentation' (9 patients), the changes in the clinical measures were not significantly different between the two groups (table 6.4). Both showed changes in T1 hypo-intensity load over the two years (cord p< 0.001, other p= 0.008), with no difference in the absolute or percentage change. However only the cord presentation group showed a significant change in T2 lesion load (p= 0.003). There was no difference in the number of new brain or cord lesions identified. No significant increase in spinal cord lesion number or load were seen in either group over the two years and no differences in absolute or percentage change between the two groups were noted (see table 6.5).
Table 6.4: PP MS according to presenting symptom; clinical and brain MRI findings.

<table>
<thead>
<tr>
<th>(n)</th>
<th>year</th>
<th>Cord Presentation (33)</th>
<th>Other Presentation (9)</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td></td>
<td>EDSS 0</td>
<td>EDSS 0</td>
</tr>
<tr>
<td></td>
<td></td>
<td>6.00 (2.0- 8.5)</td>
<td>6.00 (4.0- 8.5)</td>
</tr>
<tr>
<td></td>
<td></td>
<td>6.00 (2.0- 9.0)</td>
<td>6.00 (4.0- 8.5)</td>
</tr>
<tr>
<td></td>
<td></td>
<td>6.00 (2.0- 9.0)</td>
<td>6.50 (4.0- 8.5)</td>
</tr>
<tr>
<td></td>
<td></td>
<td>7 patients (21%)</td>
<td>2 patients (22%)</td>
</tr>
<tr>
<td></td>
<td></td>
<td>8 patients (24%)</td>
<td>3 patients (33%)</td>
</tr>
<tr>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td></td>
<td></td>
<td>Brain T2 Load (cm³)</td>
<td>Brain T2 Load (cm³)</td>
</tr>
<tr>
<td></td>
<td></td>
<td>0.73 (0-33.1)</td>
<td>0.73 (0-33.1)</td>
</tr>
<tr>
<td></td>
<td></td>
<td>1.13 (0-32.9)*</td>
<td>1.13 (0-32.9)*</td>
</tr>
<tr>
<td></td>
<td></td>
<td>0.95 (0-34.0)*</td>
<td>0.95 (0-34.0)*</td>
</tr>
<tr>
<td></td>
<td></td>
<td>0.09 (-6.0-15.4) [2.9%]</td>
<td>0.08 (-3.6-8.3) [5.9%]</td>
</tr>
<tr>
<td></td>
<td></td>
<td>0.55 (-6.8-16.4) [18.1%]</td>
<td>0.23 (-4.9-9.0) [7.1%]</td>
</tr>
<tr>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td></td>
<td></td>
<td>Brain T1 Load (cm³)</td>
<td>Brain T1 Load (cm³)</td>
</tr>
<tr>
<td></td>
<td></td>
<td>0.17 (-0.5-11.3) [29.1%]</td>
<td>0.17 (-0.5-11.3) [29.1%]</td>
</tr>
<tr>
<td></td>
<td></td>
<td>0.27 (-0.6-10.8) [31.0%]</td>
<td>0.27 (-0.6-10.8) [31.0%]</td>
</tr>
<tr>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td></td>
<td></td>
<td>6 Slice Brain Volume (cm³)</td>
<td>6 Slice Brain Volume (cm³)</td>
</tr>
<tr>
<td></td>
<td></td>
<td>275.0 (219-320)</td>
<td>262.5 (247-306)</td>
</tr>
<tr>
<td></td>
<td></td>
<td>272.2 (208-315)*</td>
<td>257.1 (241-299)*</td>
</tr>
<tr>
<td></td>
<td></td>
<td>269.8 (202-316)*</td>
<td>250.4 (243-294)*</td>
</tr>
<tr>
<td></td>
<td></td>
<td>-1.63 (-12.5- 7.5) [-0.61%]</td>
<td>-6.32 (-9.0- 0.5) [-2.42%]</td>
</tr>
<tr>
<td></td>
<td></td>
<td>-4.61 (-21.0- 8.1) [-1.60%]</td>
<td>-6.69 (-12.1- 1.6) [-2.61%]</td>
</tr>
</tbody>
</table>

The Mann-Whitney test did not detect any differences (p< 0.01) between the patient groups in absolute or percentage change of MR parameters.

* Wilcoxon signed ranks test used to assess difference from baseline (p< 0.01).
Table 6.5: PP MS according to presenting symptom; spinal cord MRI findings.

<table>
<thead>
<tr>
<th></th>
<th>Cord Presentation (33)</th>
<th>Other Presentation (9)</th>
</tr>
</thead>
<tbody>
<tr>
<td><strong>Number of Cord Lesions</strong></td>
<td></td>
<td></td>
</tr>
<tr>
<td>0</td>
<td>2.0 (0-12)</td>
<td>3.5 (0-7)</td>
</tr>
<tr>
<td>1</td>
<td>2.0 (0-12)</td>
<td>4.0 (0-7)</td>
</tr>
<tr>
<td>2</td>
<td>2.0 (0-12)</td>
<td>4.0 (0-7)</td>
</tr>
<tr>
<td><strong>New Cord Lesions</strong></td>
<td></td>
<td></td>
</tr>
<tr>
<td>at 1</td>
<td>0.0 (0-1) [mean 0.07]</td>
<td>0.0 (0-1) [mean 0.25]</td>
</tr>
<tr>
<td>at 2</td>
<td>0.0 (0-1) [mean 0.19]</td>
<td>0.0 (0-1) [mean 0.25]</td>
</tr>
<tr>
<td><strong>Cord Lesion Load</strong></td>
<td></td>
<td></td>
</tr>
<tr>
<td>0</td>
<td>2.75 (0-16)</td>
<td>4.75 (0-18.5)</td>
</tr>
<tr>
<td>1</td>
<td>3.00 (0-16)</td>
<td>5.50 (0-18.5)</td>
</tr>
<tr>
<td>2</td>
<td>3.00 (0-16)</td>
<td>5.50 (0-18.5)</td>
</tr>
<tr>
<td><strong>Increase in Load</strong></td>
<td></td>
<td></td>
</tr>
<tr>
<td>at 1</td>
<td>0.0 (0-2) [mean 0.13]</td>
<td>0.0 (0-2) [mean 0.38]</td>
</tr>
<tr>
<td>at 2</td>
<td>0.0 (0-2) [mean 0.24]</td>
<td>0.0 (0-2) [mean 0.38]</td>
</tr>
<tr>
<td><strong>Cord Area (mm²)</strong></td>
<td></td>
<td></td>
</tr>
<tr>
<td>0</td>
<td>70.8 (54.4-90.8)</td>
<td>70.7 (65.4-85.6)</td>
</tr>
<tr>
<td>1</td>
<td>66.7 (48.5-86.6)*</td>
<td>67.3 (60.6-73.9)</td>
</tr>
<tr>
<td>2</td>
<td>65.4 (47.2-85.4)*</td>
<td>66.8 (60.3-74.0)</td>
</tr>
<tr>
<td><strong>Change</strong></td>
<td></td>
<td></td>
</tr>
<tr>
<td>at 1</td>
<td>-4.3 (-9.7-0.3) [-5.66%]</td>
<td>-4.3 (-11.8--0.2) [-6.20%]</td>
</tr>
<tr>
<td>at 2</td>
<td>-4.5 (-12.7-0.7) [-5.95%]</td>
<td>-4.6 (-11.6--1.2) [-6.79%]</td>
</tr>
</tbody>
</table>

The Mann-Whitney test did not detect any differences (p< 0.01) between the patient groups in absolute or percentage change of MR parameters.

* Wilcoxon signed ranks test used to assess difference from baseline (p< 0.01).

Both groups demonstrated measurable brain atrophy over the two years, significant differences were also seen at one year (‘cord onset’ p= 0.006, ‘other onset’ p= 0.008).

The cord onset group demonstrated significant spinal cord atrophy at one year (p<0.001), the ‘other onset’ group showed less change even at two years (p= 0.012), although no differences in the degree or rates of change were observed between the two groups.

-110-
Treatment Status. Of the 42 patients with PP MS, five patients at year one and 18 at year two were participating in a double-blind placebo controlled trial of interferon beta-1a (Leary 1997). Their treatment status is unknown but as the study has three arms (one placebo, two treatments) approximately two thirds (twelve patients) are likely to be receiving treatment. These eighteen patients were compared to those without treatment, there was no difference in the change in EDSS or 9-hole-peg-test between the two groups. There was a significant difference in the rate of deterioration in the ten metre timed walk (p< 0.001) in favour of the non treated group. However 11 (46%) of the non treated patients had a maximum ten metre time (300 seconds) at baseline and therefore were unable to progress on this test, none of the treatment trial patients had a maximum score at any time during the study. Both groups demonstrated a significant increase in T1 brain hypo-intensity lesion load (p≤ 0.002) and decrease in cord cross sectional area (p< 0.001) over the two years, the untreated group also showed a significant change in brain T2 lesion load (p= 0.001, treatment trial patients p= 0.42). However there were no significant differences in the absolute or percentage changes between the two groups and there was no significant difference in the number of new brain or cord lesions identified.

Correlations Between MRI and Clinical Measures. Correlations between the absolute or percentage change in clinical (EDSS, 9-hole-peg-test and ten metre timed walk) and MRI measures in PP MS were limited. The degree of change in EDSS correlated with the total number (brain and cord) of new lesions (r= 0.47, p= 0.005) and with the volume of new spinal cord lesions (r= 0.35, p= 0.039) over two years. There was no relationship with change in the T2 lesion load or the measures of atrophy but the number of EDSS step changes did correlate with the percentage change (but not the absolute change) in T1 hypo-intensity lesion load (r= 0.33, p= 0.04). The change in ten metre walk correlated
weakly with the change in the T1 hypo-intensity lesion load ($r = 0.34$, $p = 0.035$) at two years. No correlations were seen with the 9-hole-peg-test.

The only baseline MR measure which was predictive of clinical change in PP MS was the T2 lesion load, this was limited to a correlation with the subsequent two year change in the ten metre walk ($r = 0.43$, $p = 0.007$).

There were several interesting relationships between the MR parameters; the baseline T2 and T1 hypo-intensity load correlated with subsequent brain atrophy (T2; $r = 0.44$, $p = 0.003$. T1; $r = 0.42$, $p = 0.005$) but not with change in T2 or T1 lesion loads. The baseline cord area correlated with change in the T1 hypo-intensity load ($r = 0.36$, $p = 0.037$) and the rate of cord atrophy correlated with both the change in T2 and T1 hypo-intensity lesion loads (T2; $r = 0.39$, $p = 0.017$. T1; $r = 0.38$, $p = 0.028$) and with the total (brain and cord) number of new lesions ($r = 0.41$, $p = 0.025$).

### 6.3 Discussion

This study aims to characterise the serial changes in clinical and MR parameters in both PP and TP MS with a view to gaining insights into the pathological processes which result in disease progression and disability and guiding appropriate trial design, particularly in relation to the selection of outcome measures.

Previous studies have demonstrated that the lesion load in patients with PP MS is low, few new lesions develop over time and enhancement with gadolinium is rare compared to patients with secondary progressive or relapsing-remitting disease. This has raised problems in monitoring such patients with MRI in therapeutic trials. Most trials rely on measures of disease activity in the short term (new lesions, gadolinium enhancement) and
include levels of disease burden or lesion load for long term monitoring, along with clinical measures (Miller 1998b, Paty 1994). In PP MS it has been thought that these measures would be unlikely to change significantly over the usual time period associated with clinical trials and consequently these patients have been excluded from therapeutic trials. The results of this study suggest that this is not necessarily the case as measurable changes in several MR parameters have been demonstrated in this large cohort over the relatively short time period of two years.

Clinical change remains the primary outcome measure in all definitive therapeutic trials (Paty 1998). To date the EDSS has been relied upon for this although it is known to demonstrate poor sensitivity to change and like any clinical measure can be dependent on other factors (temperature, time of day etc)(Thompson 1998). Unfortunately 25% of patients in this longitudinal study were lost to follow up at two years. The dropout rate was highest in the PP MS group (30%). The majority of patients did not give a reason for leaving the study but increasing disability was certainly a factor in some cases. Twenty one percent of the PP MS patients completing the study showed a one step deterioration in EDSS over one year and 26% at two years. This was less than the TP and SP MS groups in which 63% and 47% progressed on the EDSS at two years, respectively. The lack of change in the PP MS group may be a consequence of an increased proportion of PP patients having a baseline EDSS of 6.0 (PP 29%, TP 13%, SP 12%), the level which has been shown to have the longest staying time and consequently the least likelyest to demonstrate change (Weinshenker 1989). Despite this being a cohort of purely progressive patients the scores of seven patients improved. This illustrates the variability in the EDSS measure where scores of 5.5 or 6.0, and 7.0 and 7.5 are particularly vulnerable to effects of temperature or fatigue. This study also documented two other
clinical measures, the ten metre timed walk and the 9-hole-peg-test both of which detected slightly lower rates of deterioration. Whilst the TP and SP patient groups showed significant change in the 9-hole-peg-test over the two years, the change in the PP group did not reach significance. This test is particularly sensitive to loss of coordination, the lack of change in the 9-hole-peg-test in the PP MS group may be a consequence of the lower frequency of cerebellar dysfunction in this group (38% scored zero on the cerebellar function sub-score of the EDSS compared to 12% of the TP and 25% of the SP MS patients). The ten metre walk only demonstrated deterioration in 25% of patients at two years, this is probably due to the marked floor effect, with 35% of patients unable to demonstrate change.

These findings emphasise the importance of selecting clinical measures which are appropriate to the sample under study (Van der Putten 1999). The ten metre walk was useless in over one third of patients due to the floor effect and therefore should probably not be relied upon in this patient group. The 9-hole-peg-test was more appropriate with only 4% of patients at baseline and 8% at year one scoring maximum and therefore unable to demonstrate change.

In this study the rate of new lesion formation was very low with less than one new brain lesion per year in both the PP and TP groups, the SP group was slightly higher, although not significantly. In the spinal cord few new lesions were seen in the PP group and none were identified in the TP patients. The changes in T2 and T1 hypo-intensity lesion loads over the study period were small but significant change was seen at two years in all patient groups. This study demonstrates the importance of considering absolute rather than percentage change in lesion load when making comparisons between patient groups. As the PP MS patients have small baseline lesion loads, greater percentage changes are
seen with only small increases in absolute lesion volume. A few small new lesions in the PP MS patient group will be reflected in a large percentage gain whereas the same amount of pathological change in SP MS patients may be inconsequential. This point is illustrated by the similar percentage changes in T2 lesion load in the PP and SP patient groups at two years, however the PP group had a significantly smaller absolute change. The PP MS group also had significantly smaller change in absolute T1 hypo-intensity lesion load. Like the T2 lesion load the TP group were intermediate to the PP and SP median lesion loads but were closer to the SP group, however none of these differences were significant. Spinal cord imaging did not reveal any differences in the number of new lesions or change in lesion load between the patient groups. The fact that new lesions were so rare implies that spinal cord imaging in clinical trials is probably not worthwhile.

The measure of cerebral atrophy demonstrated significant change in the PP group at one year and in the SP group at two years, although no definite change in the TP group was seen. However in agreement with a previous study (chapter four, Stevenson 1998a) evaluation of spinal cord cross atrophy appeared more sensitive to change and was reduced in all groups after only one year. In view of the similar reproducibility of the brain and cord techniques this is most likely a true reflection of differing rates of atrophy.

In the brain, tissue loss can be seen as atrophy or as ‘black holes’ (T1 hypo-intensities). Due to the ordered structure of the spinal cord black holes are extremely rare but atrophy is commonly seen.

The correlations between change in the clinical and MRI measures in PP MS are poor. Change in the EDSS did correlate with the number of new lesions which is perhaps surprising as 15 (36%) of the 42 patients did not develop a single new lesion in either brain or cord during the two year study period, however only three of those patients
demonstrated a definite change in their EDSS. Despite only 17% of PP MS patients developing new cord lesions the EDSS did correlate with change in cord lesion load, this is perhaps accounted for by the mobility bias of the EDSS. Both changes in the EDSS and ten metre walk correlated with change in the T1 hypo-intensity lesion load but not with the T2 lesion load. Despite encouraging correlations with measures of cord atrophy in cross sectional data, the degree of ongoing atrophy did not correlate with changes in any of the clinical measures. This may be due to the high sensitivity of the cord area measurement which demonstrated significant atrophy in 88% of PP patients whilst the EDSS only detected clinical deterioration in 26%. Larger studies are needed to investigate this further.

The relationships between the MR measures, in particular T2 and T1 hypointensity lesion loads and atrophy are interesting in view of the suggested mechanisms. T2 lesion loads are not pathologically specific, reflecting inflammatory and gliotic changes as well as demyelination and axonal loss. T1 hypo-intensity has been shown to reflect axonal loss which is also likely to be the main contributor to atrophy.

When evaluating the PP patients according to presentation the sample sizes become small however the cord onset group have considerably lower T2 and T1 hypo-intensity brain lesion loads, both groups showed a definite increase in T1 hypo-intensity lesion load over the two years. Only the cord group demonstrated a definite increase in T2 lesion load, but this may be a consequence of the different sample sizes, as the rate and degree of change of T2 lesion load was similar in both groups. This increase in brain T2 and T1 weighted disease burden with little change in the EDSS illustrates the well established limited MR/clinical relationship. Both groups exhibited measurable degrees of brain and cord atrophy, although the p value for cord atrophy in the ‘other presentation’ group was only
0.012 (probably a sample size effect), no differences between the two groups were seen in the rate of change.

The only differences detected between the group (18 patients, 43%) of PP MS patients participating in a treatment trial for interferon beta-1a (placebo controlled, treatment status unknown) and the non treated patients was firstly in the rate of deterioration of the ten metre timed walk and secondly in the T2 lesion load measurements. The ten metre walk results actually favoured the non treated patients but can be explained by the higher level of disability in this group, which prevented 46% of the patients being able to progress due to the floor effect of the test. Although the non treated group demonstrated a significant difference in their baseline and follow up T2 lesion loads whereas the trial group did not, this is probably an effect of the differing sample sizes and not due to any real treatment effect. The absolute changes and rates of change did not differ between the two groups.

The results from this large cohort of PP MS patients suggest for the first time that there are measurable changes in several MR parameters over a time period of only two years. However most of these changes have not been shown to correspond with definite clinical change. This may be due to the relatively short study time and to the baseline disability level, this was higher than most therapeutic trials and consequently more patients were on the portion of the EDSS scale which is slower to change (Weinshenker 1989). It is well known that MRI parameters are much more sensitive than clinical measures in detecting change (Filippi 1998). Comparing this data to several recent therapeutic trials in RR MS the median percentage change in T2 lesion load (17.2% over two years in PP MS) was not dissimilar to the RR results, which are in the order of 5-12% (Paty 1998, Simon 1998, Filippi 1998, Paty 1993, IFNB MS Study Group 1995). The recent
European multicentre study of Interferon beta-1b in SP MS reported a median increase in T2 lesion load of only 1.64% in the placebo group over the first year, this is much lower and probably reflects the larger baseline lesion loads of these patients (Miller 1998). The absolute changes in lesion volumes are rarely quoted but Simon et al. make the point that particularly in those patient groups with low initial disease burden, this may be more relevant than percentage change (Simon 1998). In that particular study (IFNB-1a in RR MS, Simon 1998) the median absolute change in the placebo arm was 0.46cm$^3$ (median 12.6% change) at one year compared to 0.09cm$^3$ in the PP patients in this study and 0.30cm$^3$ in the secondary progressive study (Miller 1998).

This data demonstrates that although clinical change is difficult to monitor over a time period of two years in PP MS patients, changes are measurable in several MR parameters. Annual lesion load measurements do reflect change and may be a more useful secondary outcome measure than previously anticipated for clinical trials in PP MS. The rate of development of new lesions in both the brain and spinal cord is extremely low and probably will be of minimal use even in large cohorts. The measurements of both brain and spinal cord atrophy correlate well with the EDSS in cross sectional studies (Losseff 1996b, Stevenson 1999, Filippi 1994) and demonstrate change over one year (Stevenson 1998a). Longer studies are needed to assess their clinical correlations but considering the extremely high reproducibility and level of automation compared to the measurements of brain lesion loads they have considerable potential in future therapeutic trials.
PART C: Advances in Magnetic Resonance Imaging
Involvement of the spinal cord in MS has long been known to be extremely common and of particular importance in the development of disability (Oppenheimer 1978). With MRI, lesions are demonstrable within the spinal cord in 75% of patients with MS, more commonly in the cervical than the thoracic cord (Kidd 1993). Serial imaging has shown that the frequency of new lesions in the spinal cord is approximately 10% that of the brain, but spinal cord lesions are more symptomatic and more likely to result in a clinical relapse (31% compared to approximately 10% in the brain) (Thorpe 1996a). MRI of the spinal cord has also been shown to be useful in both the diagnosis of MS and in the exclusion of dual pathology such as cord compression. Accurate definition of cord lesions may be of particular value in patients very early in the disease process (when brain MRI abnormalities are often less extensive), in patients presenting over the age of 50 years when brain white matter lesions become less specific (Fazekas 1988, Kidd 1993) and in the estimated 1-5% of patients in whom MS is strongly suspected on clinical grounds but brain imaging is normal (Runge 1984, Thorpe 1996b).

Spinal cord imaging in patients with PP MS is of particular importance for several reasons. Many of these patients present with a progressive spastic paraparesis with very few other signs but significant disability. They often have few brain abnormalities. In an early study looking at MS patients with normal brain imaging, 11 of the 20 patients followed a primary progressive disease course (Thorpe 1996b). Also as the age of onset in PP MS is considerably older, cerebral white matter changes on MRI become more difficult to interpret due to the known occurrence of areas of high signal associated with
normal ageing. There is no evidence however to suggest age related changes occur within the spinal cord thus cord imaging is an important tool in confirming the diagnosis in this age group (Thorpe 1993). For these reasons the spinal cord is an ideal site to study the mechanisms of disability.

Although spinal cord imaging does detect lesions in the majority of patients with MS, neither the number or extent of lesions has corelated with disability in cross sectional or longitudinal studies (Kidd 1993 and 1996). This may be a consequence of poor pathological specificity of lesions or of small lesions being missed, changes within the NAWM are also going undetected. It is therefore desirable to optimise spinal imaging both to improve lesion detection and pathological specificity within the lesions and NAWM of MS. Advances in spinal cord imaging should improve diagnostic certainty and increase our knowledge of the pathogenesis of MS. New techniques may also aid in the monitoring of change over time, particularly important in therapeutic trials.

This chapter describes two new methods aimed at increasing lesion detection, fast FLAIR and three-dimensional FSE imaging, and their validation against standard sequences in patients with MS. Subsequently in chapter eight work which quantifies the T1 and T2 relaxation times of MS lesions and normal appearing brain tissue is presented. This demonstrates the heterogeneity of lesions and the presence of changes within the NAWM, both of which contribute to the poor relationship between T2 lesion volume and measures of disability.
7.1 The Use of Fast FLAIR in Multiple Sclerosis

FLAIR sequences were developed to improve lesion conspicuity. Lesions in the brain are best seen on heavily T2 weighted CSE or FSE images, however the high signal from CSF obtained with a heavily T2 weighted sequence creates partial volume effects and flow artefacts which can cause problems in lesion detection, particularly in the periventricular and subcortical regions of the brain and in the spinal cord. The use of shorter echo times reduces CSF signal but there is a loss of T2 weighting which is an important element of the contrast between lesions and normal brain or cord. FLAIR sequences combine the suppression of CSF signal (due to a long inversion time) with heavy T2 weighting (long echo time).

Recent reports have suggested that the use of FLAIR sequences improves the detection of MS lesions in the brain (De Coene 1992, Hajnal 1992), brainstem (De Coene 1993) and spinal cord (White 1992, Thomas 1993). The main problem of FLAIR is the long acquisition time, typically 12 minutes or more, hence fast FLAIR sequences have been developed. Preliminary studies of fast FLAIR (defined as inversion recovery prepared fast spin echo) in MS have had conflicting results; some reporting a greater sensitivity to brain lesions compared with FSE or CSE (Rydberg 1994, Hashemi 1995, Filippi 1996a) and others reporting no difference in sensitivity (Barratti 1995, Thorpe 1994). Experience with fast FLAIR in the spinal cord in MS has not been reported previously. The purpose of the present study was to compare the sensitivity of fast FLAIR with FSE in both the brain and cord of patients with MS.

7.1.1 Study Design

The fast FLAIR technique used in this study is a development of that described by
Rydberg which consisted of 5mm slices and a slice selective inversion pulse (Rydberg 1994). A standard inversion recovery sequence with interleaving of two sections (sequential interleaving) and a 16 echo Rapid Acquisition with Relaxation Enhancement (RARE) readout was utilised to decrease acquisition time. In order to reduce flow artefact caused by the pulsatile nature of non nulled CSF, a chopping phase encoding technique was applied and the inversion pulse slice width was increased.

Ten patients with clinically definite MS (two RR, six SP and two PP; EDSS 2-8) and previously documented cord lesions were imaged with conventional T2 weighted FSE in the axial plane resulting in 46 contiguous 3mm slices (TR= 2500ms, TEeff=48 and 96ms, echo spacing 12ms, in-plane resolution 0.9x1.25mm) in the brain and nine contiguous 3mm slices (TR=2500ms, TEeff=52 and 104ms, echo spacing 13ms, in-plane resolution 0.9x0.9mm) in the cervical cord. The fast FLAIR sequence acquired an equivalent number of 3mm contiguous slices (TR=11,000ms, TI=2150ms, TEeff=144ms, echo spacing 13ms, in-plane resolution 0.9x0.9mm), the acquisition time was 6 minutes 5 seconds in the brain and 7 minutes 44 seconds in the cervical cord.

Analysis. The FSE and fast FLAIR images were mixed and blinded for patient name. Lesions were identified on the proton density images with cross reference to the T2 weighted images by two observers independently, who then reached a consensus on number and site of lesions. Five sites were considered: spinal cord, posterior fossa, and in the cerebral hemisphere, discrete, periventricular and subcortical. Discrete lesions were defined as those which were not contiguous with either the ventricles or cortex. The scans were then compared for each patient and scored depending on whether lesions were seen on both sequences, FSE only, fast FLAIR only, FSE in retrospect or fast FLAIR in retrospect for each of the four regions on every slice. Lesion identification was aided by
guidelines developed following examination of 40 fast FLAIR scans in normal controls (Gawne-Cain 1997b). These primarily excluded symmetrical areas of increased signal in the posterior internal capsule and periventricular areas, regions which are known to exhibit high signal on FLAIR.

**Statistical analysis.** Lesion detection rates in both sequences in different regions were analysed using the non parametric Wilcoxon signed-ranks test.

### 7.1.2 Results

Analysis of the brain images showed additional cerebral hemisphere lesions in the periventricular, discrete and subcortical areas in all patients with the fast FLAIR sequence compared with FSE (p< 0.03). On comparing the total number of lesions seen on each sequence for each area (ie. FSE only + FSE in retrospect vs. fast FLAIR only + fast FLAIR in retrospect) fast FLAIR still demonstrated significantly more lesions in the periventricular and subcortical areas (p< 0.02). However in the posterior fossa more lesions were seen on the FSE images than on the fast FLAIR (p= 0.02, total including lesions seen in retrospect p= 0.01) (see tables 7.1 and 7.2). Lesions in the cerebral hemispheres, which appeared discrete on the FSE images were often shown on the fast FLAIR to be more extensive and confluent (see figure 7.1).

In the spinal cord, a total of 33 lesions were identified on the FSE images but only one of these was seen on fast FLAIR alone and a further one on reviewing the FSE and fast FLAIR images together (p< 0.001) (see table 7.2).
Table 7.1: Lesion detection in the brain and cord by FSE and fast FLAIR.

<table>
<thead>
<tr>
<th></th>
<th>FSE and fast FLAIR</th>
<th>FSE only</th>
<th>fast FLAIR only</th>
<th>FSE in retrospect</th>
<th>fast FLAIR in retrospect</th>
<th>Total</th>
</tr>
</thead>
<tbody>
<tr>
<td>Discrete</td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Periventricular</td>
<td>266 (59.1%)</td>
<td>64 (14.2%)</td>
<td>120 (26.7%)</td>
<td>46</td>
<td>17</td>
<td>450 (100%)</td>
</tr>
<tr>
<td>Subcortical</td>
<td>142 (56.8%)</td>
<td>18 (7.2%)</td>
<td>90 (36.0%)</td>
<td>14</td>
<td>8</td>
<td>250 (100%)</td>
</tr>
<tr>
<td></td>
<td>62 (35.4%)</td>
<td>20 (11.4%)</td>
<td>93 (53.1%)</td>
<td>26</td>
<td>0</td>
<td>175 (100%)</td>
</tr>
<tr>
<td>Posterior Fossa</td>
<td>4 (7.4%)</td>
<td>39 (72.2%)</td>
<td>11 (20.4%)</td>
<td>3</td>
<td>6</td>
<td>54 (100%)</td>
</tr>
<tr>
<td>Spinal Cord</td>
<td>1</td>
<td>32</td>
<td>0</td>
<td>0</td>
<td>1</td>
<td>33</td>
</tr>
</tbody>
</table>

FSE only vs fast FLAIR only;
Discrete: p= 0.01, Periventricular: p< 0.001, Subcortical: p= 0.003, Posterior Fossa: p= 0.02, Spinal Cord: p< 0.001.

(FSE+FSE retro) vs (fast FLAIR+fast FLAIR retro);
Discrete: p= 0.1, Periventricular: p= 0.001, Subcortical: p= 0.01, Posterior Fossa: p= 0.01, Spinal Cord: p< 0.001.
**Table 7.2:** Lesion number by patient in the posterior fossa and spinal cord detected by FSE and fast FLAIR.

<table>
<thead>
<tr>
<th>Patient</th>
<th>Posterior Fossa</th>
<th>Spinal Cord</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>FSE and fast FLAIR</td>
<td>FSE only</td>
</tr>
<tr>
<td>A</td>
<td>0</td>
<td>0</td>
</tr>
<tr>
<td>B</td>
<td>0</td>
<td>2</td>
</tr>
<tr>
<td>C</td>
<td>1</td>
<td>22 (1)</td>
</tr>
<tr>
<td>D</td>
<td>1</td>
<td>13</td>
</tr>
<tr>
<td>E</td>
<td>0</td>
<td>1</td>
</tr>
<tr>
<td>F</td>
<td>0</td>
<td>0</td>
</tr>
<tr>
<td>G</td>
<td>0</td>
<td>1</td>
</tr>
<tr>
<td>H</td>
<td>2</td>
<td>0 (2)</td>
</tr>
<tr>
<td>I</td>
<td>0</td>
<td>0</td>
</tr>
<tr>
<td>J</td>
<td>0</td>
<td>0</td>
</tr>
<tr>
<td>Total</td>
<td>4</td>
<td>39</td>
</tr>
</tbody>
</table>

( ) refer to lesions seen in retrospect when the two sequences are compared directly.
Figure 7.1: FSE (left) and fast FLAIR (right) images of the cerebral hemispheres (top), posterior fossa (middle) and spinal cord (bottom).
7.1.3 Discussion

By decreasing the signal from CSF, FLAIR sequences allow the use of longer TEs and hence more T2 weighting without compromising lesion visibility in the periventricular or subcortical areas. Furthermore image degradation is decreased by reducing flow artefacts. Using sequential interleaving and a 16 echo RARE readout the acquisition time can be decreased to a suitable level whilst maintaining high resolution 3mm thick slices. Optimisation of contrast is achieved by using longer TRs and TIs. The fast FLAIR sequences have been shown to be superior to standard FSE sequences in detecting lesions in areas often difficult on FSE scans, particularly those at the grey/white matter interface and around the ventricles where partial volume effects and higher CSF signal become significant. As in other studies of both FLAIR and fast FLAIR (Gawne-Cain 1997b), there was reduced grey/white matter contrast and diffuse high signal around the ventricles particularly the frontal and occipital horns, which did cause some difficulty with identification of lesions within these areas, nevertheless CSF suppression was excellent and flow effects were minimal.

The aim of this study was to assess the relative efficacy of fast FLAIR imaging in the spinal cord and brain. Due to the problem of significant CSF flow artefacts, particularly in the cord, the sequence used by Reiderer was modified by increasing the inversion pulse width to 2.2 times the slice thickness and changing the inversion time to 2150ms to allow for the double inversion that then occurs. As in most previous studies fast FLAIR proved to show significantly more lesions than FSE in the brain. However this study demonstrates fast FLAIR to be inferior to standard FSE imaging in the detection of MS lesions in both the posterior fossa and spinal cord.

This finding is particularly relevant to the recent suggestion that fast FLAIR sequences
could supplant the conventional T2 weighted spin echo sequence as the basic screening sequence for imaging of the brain and cord (De Coene 1993) and indicates that further consideration is required before CSE is replaced.

Apart from poor lesion identification in the posterior fossa and spinal cord a further issue which may be relevant to the fast FLAIR sequence is that of measuring TLV, a key component in many MS treatment trials. Quantitative lesion load measurement has not been undertaken in this study but a potential difficulty with fast FLAIR could be the diffuse high signal found around the frontal and occipital horns, which depending on lesion identification guidelines may either result in over-reporting of lesions or even under-reporting as small lesions may become lost amongst increased background signal. Also as the RARE technique uses variable echo times, slight blurring of lesion edges in the phase encoded direction of an image may occur which in theory means small lesions could be lost, although this may be balanced by increased intrinsic contrast of lesions in the heavily T2 weighted images (Constable and Gore 1992).

Although in terms of improving spinal cord imaging, this is a negative study, the result is important in identifying the fact that lesions can not be presumed to be similar throughout the central nervous system. Consequently new MR sequences should not be introduced into clinical practise until they have been validated throughout both the brain and spinal cord. The fact that lesions are detected much less frequently in the posterior fossa and cord indicates that the lesions themselves are of different composition in different sites. This observation is explored further in chapter eight, where T1 and T2 relaxation times are compared in controls and MS patients. By quantifying the actual characteristics of lesions through relaxation time measurements pathological specificity will be improved enabling MRI sequences to be optimised for lesion detection.
7.2 Three Dimensional Fast Spin Echo Imaging of the Spinal Cord

Two dimensional (2D) FSE sequences are the standard technique to detect the lesions of MS in the spinal cord for both diagnostic and monitoring purposes. They may however be missing small lesions which could in part explain the lack of correlation with disability as measured by Kurtzkes EDSS (Thorpe 1993).

The present study reports the use of a new 3D FSE sequence comparing it to standard 2D FSE in ten patients with clinically definite MS and in ten normal controls.

7.2.1 Study Design

Ten patients with clinically definite MS (four RR, three SP and three PP MS, age 43-59 years) and a range of disabilities (EDSS range 3.5-8.5) attended for a clinical examination including assessment of the EDSS before undergoing MR imaging. The examinations were performed on a 1.5T imager (Signa; GE Medical Systems, Milwaukee, Wis.) and the protocol included both conventional 2D T2 weighted FSE and the new 3D FSE in the sagittal plane.

The 3D FSE sequence (provided by GE Medical Systems) has an effective slice thickness of only 1.5mm; 60 contiguous sagittal slices of the cervical cord are acquired in 15:06 minutes (TR=2000ms, T_Eff=50.6ms, echo train length 24, field of view 24cms, matrix 256 x 256, 10 ‘slices’ per slab, 2 ‘slices’ per overlap, 10 ‘slices’ per location, 2 averages). The 2D FSE has a slice thickness of 3mm and the acquisition time for two sets of nine contiguous sagittal slices (short and long echo) is 11:24 minutes (TR=2500ms, T_Eff=52 and 104ms, echo spacing 13ms, in-plane resolution 0.9x0.9mm). Ten healthy volunteers (age 24-49 years) with no history of neurological events also underwent imaging by 3D
FSE.

As the 3D FSE sequence is a volume acquisition it is possible to reformat the data sets in any plane, this allows the localisation of lesions within fibre tracts of the spinal cord. All of the patient data sets were reformatted in the axial plane (perpendicular to the axis of the cord) using a slice thickness of 1.5mm and covering the cord between the upper border of C1 and the lower border of C7 (see chapter three).

**Analysis.** The twenty sagittal 3D FSE films were firstly scored as either normal or abnormal by a blinded experienced observer. All the films (2D and 3D) were then mixed and blinded for patient name. Lesions were marked on both the 2D and 3D images, the level of the lesion, transverse size and length of the lesion were recorded. Transverse size was estimated according to the number of sagittal slices on which the lesion appeared and the length by the number of corresponding vertebral bodies. Lesions were identified on the 2D images by cross reference between the proton density and T2 weighted images. Lesions were also identified by a blinded observer on the axial reformats and characterised as either anterior, posterior, lateral-left, lateral-right, central or full thickness. The number of slices on which each lesion was visible was recorded and individual lesion loads summed for each region; anterior, posterior, lateral (left + right), central or full thickness (see figure 7.2).

**Statistical analysis.** The number, transverse size and length of lesions seen on sagittal 3D and 2D FSE were compared using the independent t-test. Correlations were looked for between number of lesions and lesion load (length of lesion x transverse size) on both sequences and the EDSS, using Spearmans rank correlation coefficient.
7.2.2 Results

All the 3D FSE images from the normal controls were reported as normal. However it was noted that all showed a distinct line of high signal within the anterior part of the high cervical spinal cord which was considered a linear artefact. This was not typical of pathological change and did not lead to confusion. It was also noted that the whole cord appeared to have a mild diffuse inhomogeneity but this again did not lead to any false positive interpretations (see figure 7.3).

On analysis of the 2D FSE images 29 focal lesions in total were seen (range 1-5 per patient, mean 2.9) compared to 53 on the 3D FSE images (range 2-8, mean 5.3); this difference was significant (p= 0.05). Lesion length was found to be significantly smaller on the 3D FSE images than on the 2D FSE (3D: range 0.5-8, mean 1.36. 2D: range 0.5-7, mean 2.0, p= 0.03). As expected (given that 3D slice thickness is half that of 2D) the lesions detected were seen on significantly more sagittal 3D FSE slices (3D: range 1-7, mean number of slices 3.17. 2D: range 1-4, mean 2.07, p= 0.001) (figure 7.4).
Figure 7.3: 3D FSE in a normal control.
There was no significant correlation between lesion load on either sequence with EDSS (2D; r= 0.185, p= 0.61. 3D; r= 0.142, p= 0.70). However when looking at lesion number there was a significant negative correlation with EDSS in the 2D group ie. the more lesions, the less disabled (r= -0.802, p= 0.005), which was not seen in the 3D group (r= 0.047, p= 0.897).

**Axial reformats.** Two of the ten data sets were degraded by motion artefact and were excluded from the analysis. In total 38 lesions were identified on the remaining eight axial data sets, compared to 41 lesions identified on the corresponding sagittal images. There were no significant correlations between the EDSS and the regional area lesion loads. Considering the EDSS functional scores for pyramidal, sensory and sphincter dysfunction there were no correlations with the pyramidal or sphincter scores but there was a significant correlation between the sensory functional score and the full thickness area lesion load (r= 0.82, p= 0.02).

**7.2.3 Discussion**

Three dimensional imaging of the spinal cord has the advantage of higher signal to noise ratios per unit time and higher spatial resolution. These features enable the use of thinner contiguous slices (1.5mm) resulting in improved resolution. It is also possible to reformat the data set in any plane, however as discussed earlier in chapter three the main problem with reconstructing a volume acquisition is its sensitivity to motion artefact. The data is acquired in two interleaved acquisitions in the sagittal plane as slabs, but any patient motion during the acquisition leads to slab mis-registration.
**Figure 7.4:** 2D FSE (top) and 3D FSE (bottom) images in a patient with MS.
The findings in normals suggest that it is a reliable technique with no false positives; however the observer must be aware of the minor artefacts described to avoid confusion. More lesions were detected using the 3D than the 2D FSE sequence. This may be due, in part, to the separation of confluent abnormal signal seen on 2D into several lesions (the fact that the mean lesion length was less with 3D FSE would support this) and in part to the detection of other small lesions not visible with 2D FSE. Not surprisingly, lesions on average were seen on more slices of the 3D FSE than the 2D FSE images, reflecting the smaller slice thickness, but the latter probably also accounts for some small lesions being missed on the 3mm thick 2D slices. This is illustrated by three lesions which were visible on only one 3D FSE slice, one of these was missed completely by the 2D FSE, the other two were indistinguishable from neighbouring lesions. The small discrepancy in the number of lesions identified on the sagittal and axial image sets is expected and accounted for by the often snake like appearance of lesions running through the spinal cord often on many slices of the image set.

The relationship between lesion load and disability in MS is complex, this data set is too small to explore this further. The three most disabled patients in this study (EDSS 8.5, 7.5 and 7.0) all had only one lesion visible on 2D FSE imaging, this was a long lesion in a thin cord, which when imaged by sagittal 3D FSE became clearly a series of lesions (two, five and seven lesions respectively). These patients help to explain the strongly negative correlation seen with EDSS in the 2D group, but there is still a lack of correlation between lesion number on the high resolution 3D FSE sequence and disability. Even when lesions were documented in the axial plane according to their location, the relationships with disability measures were disappointing. Several factors probably contribute to this, firstly the degree of atrophy which is not taken into account
by looking at lesion load, but has been shown to correlate closely with disability (Losseff 1996b). Atrophy may evolve independently of new lesion formation and may be associated with loss of focal lesions. Secondly the pathological nature of the lesions, high signal on T2 weighted images does not differentiate between areas of oedema, gliosis or axonal loss. In the same way diffuse pathological change in the normal appearing cord is not reflected, but may also be a significant factor (Lycklama 1997a). The use of quantifiable techniques (eg. T1 and T2 relaxation time measurements or magnetisation transfer ratio) may help to characterise lesions further and to improve correlations with disability.

This study demonstrates that this new three dimensional FSE sequence is more sensitive in detecting lesions than the standard 2D sequence and should be considered in both the monitoring and diagnosis of MS. It perhaps is particularly applicable in patients very early in the disease process or in older patients and in PP MS, when brain MRI abnormalities are often less extensive. It could also be potentially useful in patients with other pathologies, for example motor neurone disease, hereditary spastic paraparesis or vitamin B12 deficiency where degeneration is in specific tracts of the spinal cord. However neither increasing lesion detection or attempting to localise lesions within the cord has helped define the relationship with disability. This work highlights the need to study the whole cord with more pathologically specific techniques to increase our understanding of the development of disability which clearly is not simply due to the degree of T2 hyper-intensity present.
Chapter 8: Quantification of T1 and T2 Relaxation Times in Multiple Sclerosis

T2 weighted MRI is used routinely for both the diagnosis and monitoring of MS, where lesions are clearly visible as areas of high signal. However this increase in T2 weighted signal is not pathologically specific. Areas of oedema, gliosis, demyelination or axonal loss can not be distinguished from each other and the relationship between lesion load and disability measures is poor (Miller 1998b). In an effort to increase pathological specificity, investigators have studied the areas of hypo-intensity or ‘black holes’ observed on unenhanced T1 weighted images. The correlations between the EDSS and the T1 hypo-intensity lesion load appear higher than with the TLV in some studies (Truyen 1996), although this has not been a consistent finding (O’Riordan 1998b). The identification of hypo-intense lesions is subjective and can be difficult, however this increase in pathological specificity seen with an MR measure dependent on the T1 of tissues can be explored further by the quantification of the T1 and T2 relaxation times in NAWM and lesions. Previous workers in the late 1980's and early 90's have shown it is possible to accurately and precisely measure the T1 and T2 relaxation times of cerebral tissue with coefficients of variation ranging from less than 5% (Breger 1989, Miller 1989, Steen 1994) to 9% (Larrson 1992b). Studies of the NAWM of patients with MS compared to controls have consistently demonstrated significantly prolonged T1 and T2 relaxation times in MS patients (Armspach 1991, Barbosa 1994, Lacomis 1996, Larrson 1988, Miller 1989, Ormerod 1987, Rumbach 1991). When these were compared to other disease groups, they were significantly longer in MS than in patients with systemic lupus
erythematosis or cerebral sarcoidosis (Miller 1989), although the ranges of T1 and T2 in MS lesions were found to be large and overlapped with the lesions of other disease states (Larrson 1988, Larrson 1992b, Miller 1989). Some of the variation in relaxation times has been shown to be due to the age of the MS plaques (Ormerod 1987). In the acute MS lesions there is firstly a prolongation of T1 and T2, thought to be due to acute oedema, but this is soon followed by shortening of both T1 and T2 relaxation times (Larrson 1988). However within chronic stable plaques the scatter of relaxation times is large and overlaps with those of acute lesions (Larrson 1988 and 1989) indicating considerable differences in the tissue composition of lesions despite their age or activity. Lesions also exhibit considerable pathological heterogeneity, those with severe tissue destruction and axonal loss will have expanded extracellular spaces and hence exhibit longer T2 relaxation times than those which are predominantly cellular with gliosis. In an earlier study patients in different clinical subtypes of MS and at different levels of disability were compared (Kidd 1997). Patients with PP MS showed the greatest proportion of lesions with prolonged T2 relaxation times however there was no difference between the lesions of the disabled SP patient group and the non disabled benign group.

Few studies have considered the effect of location on lesion composition and consequent T1 and T2 relaxation times. An early study demonstrated that periventricular lesions had significantly higher relaxation times than discrete lesions within the cerebral white matter although there was considerable overlap (Ormerod 1987). The finding that the pulse sequence fast FLAIR detects more lesions in total than either CSE or FSE, though fewer lesions in either the posterior fossa or spinal cord (Gawne-Cain 1997a, Stevenson 1997) prompted investigators to measure T2 relaxation times. T2 was significantly lower in lesions of the posterior fossa (Gawne-Cain 1997a, Stevenson 1997) and spinal cord -139-
(Stevenson 1997) when compared to cerebral white matter lesions. It was hypothesised that this reduction may account for the poor detection of infra-tentorial lesions by the heavily T2 weighted fast FLAIR sequences.

This study aims to quantify the T1 and T2 relaxation times of normal white matter in control subjects and the NAWM, grey matter and lesions in MS patients. This will enable a comparison to be made between white matter and lesions in different regions of the brain, which will hopefully aid in the optimisation of pulse sequences for lesion detection in the posterior fossa and spinal cord. The possible role of relaxation time quantification as a surrogate measure of disease progression is also addressed.

8.1 Study Design

Ten patients with clinically definite MS and ten healthy controls with no history of neurological or psychiatric problems were recruited for the study. Of the ten patients five had SP, three RR and two benign MS (relapsing-remitting disease of at least ten years duration and an EDSS of 3 or less). All patients underwent a neurological examination and evaluation of the EDSS before undergoing MRI of the brain, none were in acute relapse at the time of assessment.

Imaging. All MR imaging was carried out on a Signa Horizon Echospeed 1.5 Tesla system with standard quadrature head coil (General Electric, Milwaukee, Wisc.). Each subject first underwent sagittal and axial scout imaging followed by a 2D FSE sequence (28 contiguous 5 mm thick axial slices, 8 echo train length, 2 echoes, TEeff 35 ms and 90 ms, TR 2100 ms). From these images, 14 alternate (odd or even) slice positions were selected according to which set had the most posterior fossa lesions, as these
lesions were of the greatest interest.

The 14 chosen slice positions were then used for the quantitative T1 and T2 relaxation time measurements and the fast FLAIR acquisition. The number of slices imaged was restricted to 14 to allow the entire study to be performed in a period of less than one hour. Consequently fast FLAIR, T1 measurement, T2 measurement and FSE acquisitions were obtained such that all had the same axial slice positions, slice thickness (5 mm thick, with a separation of 5 mm), field of view (280 mm x 210 mm) and covered the full extent of the brain (the FSE additional slice positions were subsequently ignored).

The fast FLAIR acquisition parameters were: echo train length 8, TEeff 135 ms, TR 11000 ms, TI 2600ms. The T1 measurement protocol consisted of the acquisition of two gradient echo data sets at different repetition times, to provide a proton density-weighted image and a heavily T1-weighted image. The acquisition parameters were (TR/TE/flip angle/number of averages): 1500 ms/10 ms/90°/2 and 360 ms/10 ms/90°/8 for the proton density-weighted and the T1-weighted acquisitions, respectively. Both acquisitions used a 256 x 192 imaging matrix, giving 1.1 mm x 1.1 mm in-plane resolution with interpolation. T1 was determined from these images using an in house technique (Parker 1999). The T2 measurement protocol consisted of a double echo spin echo acquisition (TR/TE1/TE2/number of averages): 2000 ms/30 ms/120 ms/1 and again a 256 x 192 imaging matrix was used giving an in-plane resolution of 1.1 mm x 1.1 mm. The theoretical relationship between signal intensity and echo time was found not to hold (an effect likely to be due to inexact spin refocusing due to imperfect slice profiles in the 180° pulses in the double spin echo). An empirical calibration was
therefore applied (between the ratio of the second echo signal intensity to the first echo signal intensity and T2) with the use of high accuracy T2 standards (Eurospin Test Object 5, Diagnostic Sonar Ltd., Livingston, Scotland).

From the relationships derived for the T1 and T2 measurement protocols, quantitative T1 and T2 maps were constructed using software written in-house for this purpose. These maps provide a direct relationship between pixel intensity and relaxation times in milliseconds.

**Accuracy and Quality Assurance.** The reliability of the T1 and T2 relaxation time measurements was assessed with the aid of the in-house quality assurance program. This involves repeated imaging of gels with known relaxation times to assess the mean accuracy (the modulus of the percentage difference between the measured relaxation time and the nominal relaxation time value, averaged over all measurements and samples) and the mean systematic error (the percentage difference between the measured relaxation time and the nominal relaxation time value, averaged over all measurements and samples).

Errors in the relaxation time measurements due to coil radiofrequency transmission non-uniformities were determined by measuring the gels at varying positions throughout the coil.

**Analysis.** Areas of normal appearing brain tissue were identified on the T1 maps with cross reference from the FSE images. Thirteen ROIs were identified in each subject comprising the spinal cord (level of Cl/2), medulla, cerebellum, pons, midbrain, frontal white matter, occipital white matter, genu and splenium of the corpus callosum, caudate nucleus, putamen, thalamus and cortex. Care was taken to place the ROIs in the centre
of these areas away from lesions and borders of adjacent structures to minimise partial volume effects. The ROIs were then transferred onto the T2 maps and the mean T1 and T2 values within the ROIs recorded.

Lesions were identified in the patients on the FSE and fast FLAIR images as visible on both sequences, on FSE only or on fast FLAIR only. ROIs were placed in the centre of lesions again to minimise partial volume effects, all lesions visible on only one scanning sequence were assessed. Of the lesions visible on both sequences a maximum of five were assessed for each of five regions (brainstem, cerebellar, periventricular, discrete white matter and subcortical) in each patient. When more than five lesions were present in a region, those seen most clearly on both sequences were chosen. Discrete cerebral hemisphere lesions were defined as those which were not contiguous with either the ventricles or cortex.

To assess the reproducibility of measurement of the T1 and T2 relaxation times the COV was calculated (standard deviation/ mean) for each of the 13 ROIs across the controls. The mean of these COVs was then calculated for both T1 and T2 measurements.

Statistical analysis included the independent t test to assess differences in the relaxation times of NAWM and lesions between patients and controls and within the patient group. Spearman's rank correlation coefficient was applied to both mean NAWM and lesion relaxation times with the EDSS. To reflect the large number of statistical comparisons the p value had to reach 0.01 to be considered significant.

8.2 Results

The control group (mean age 27.3 years, SD 9.7) was significantly younger than the
patient group (mean age 41.0 years, SD 6.2). The group of MS patients was chosen to reflect a wide range of disability (median EDSS 5.0, range 2.5- 8.0) with the SP patients being the most disabled.

The mean accuracy and the mean systematic error of the T1 measurement protocol were 5.42% and -1.23%, similarly for the T2 measurement protocol 4.62% and -2.93% respectively. There were no apparent systematic drifts in the measured T1 and T2 over time. Variation in T2 within the scanning region due to coil radiofrequency transmission non-uniformities was estimated to be less than 2%. The errors in T1 measurement were less than 5% (Parker 1999). Assessment of measurement reproducibility in the ten control subjects across all ROIs resulted in mean COVs of 8.5% (range 5.5- 13.2) for T1 and 5.4% (range 2.9- 9.7) for T2 relaxation times.

On comparing the relaxation times of normal tissue in the control subjects, the basal ganglia and cortex exhibited significantly longer T1 relaxation times than other areas. In the white matter, T1 and T2 were significantly longer ($p \leq 0.01$) in the infra-tentorial regions compared to the corpus callosum, frontal and occipital white matter regions. There was no correlation between age and T1 or T2 relaxation times within areas of white matter with the notable exception of the splenium which demonstrated a strong negative correlation with the T1 relaxation time ($r = -0.95$, $p = 0.001$).

Comparison of normal appearing brain between patients and controls revealed several significant differences in both T1 and T2 (see table 8.1). Patients had significantly longer T1 relaxation times in the spinal cord, frontal white matter, occipital white matter and in the thalamus. T2 relaxation times of the patients were prolonged in the frontal white matter and in the genu and splenium of the corpus callosum.
Table 8.1: Comparison of NAWM and grey matter in patients and controls. Mean (SD).

<table>
<thead>
<tr>
<th></th>
<th>Controls &lt;br&gt; T1</th>
<th>Patients &lt;br&gt; T1</th>
<th>Controls &lt;br&gt; T2</th>
<th>Patients &lt;br&gt; T2</th>
</tr>
</thead>
<tbody>
<tr>
<td>Cord</td>
<td>895 (94)</td>
<td>1073 (132)*</td>
<td>111 (7)</td>
<td>114 (5)</td>
</tr>
<tr>
<td>Medulla</td>
<td>978 (63)</td>
<td>1043 (99)</td>
<td>101 (6)</td>
<td>104 (4)</td>
</tr>
<tr>
<td>Pons</td>
<td>938 (69)</td>
<td>1021 (70)</td>
<td>96 (5)</td>
<td>99 (4)</td>
</tr>
<tr>
<td>Midbrain</td>
<td>908 (51)</td>
<td>946 (60)</td>
<td>103 (9)</td>
<td>96 (8)</td>
</tr>
<tr>
<td>Cerebellum</td>
<td>824 (58)</td>
<td>931 (198)</td>
<td>96 (4)</td>
<td>97 (8)</td>
</tr>
<tr>
<td>Frontal White</td>
<td>668 (37)</td>
<td>712 (34)*</td>
<td>87 (3)</td>
<td>92 (4)*</td>
</tr>
<tr>
<td>Occipital White</td>
<td>676 (39)</td>
<td>727 (34)*</td>
<td>99 (3)</td>
<td>92 (32)</td>
</tr>
<tr>
<td>Genu</td>
<td>669 (42)</td>
<td>738 (103)</td>
<td>85 (4)</td>
<td>94 (6)*</td>
</tr>
<tr>
<td>Splenium</td>
<td>705 (70)</td>
<td>841 (149)</td>
<td>96 (6)</td>
<td>104 (6)*</td>
</tr>
<tr>
<td>Caudate</td>
<td>1360 (132)</td>
<td>1332 (127)</td>
<td>92 (4)</td>
<td>93 (5)</td>
</tr>
<tr>
<td>Thalamus</td>
<td>1073 (142)</td>
<td>1285 (136)*</td>
<td>88 (3)</td>
<td>92 (7)</td>
</tr>
<tr>
<td>Putamen</td>
<td>1101 (120)</td>
<td>1171 (107)</td>
<td>83 (4)</td>
<td>83 (5)</td>
</tr>
<tr>
<td>Cortex</td>
<td>1362 (167)</td>
<td>1247 (288)</td>
<td>109 (11)</td>
<td>105 (9)</td>
</tr>
</tbody>
</table>

*Significant (p ≤ 0.01) difference between patients and controls, independent t-test.

-145-
A total of 151 lesions was analysed, of these 47 (31.1%) were periventricular, 42 (27.8%) discrete, 40 (26.5%) subcortical, 18 (11.9%) cerebellar and four (2.6%) brainstem. The four brainstem lesions (in three patients) had significantly longer T1 relaxation times than lesions in any other area. The cerebellar lesions demonstrated significantly shorter T2 but similar T1 relaxation times than periventricular, discrete or subcortical lesions (see table 8.2).

Table 8.2: Comparison of lesions defined by site within the brain. Mean (SD).

<table>
<thead>
<tr>
<th></th>
<th>T1</th>
<th>T2</th>
</tr>
</thead>
<tbody>
<tr>
<td>Brainstem</td>
<td>1786 (659)</td>
<td>123 (15)</td>
</tr>
<tr>
<td>n= 4</td>
<td></td>
<td></td>
</tr>
<tr>
<td>Cerebellar</td>
<td>1228 (239)</td>
<td>109 (16)</td>
</tr>
<tr>
<td>n= 18</td>
<td></td>
<td></td>
</tr>
<tr>
<td>Periventricular</td>
<td>1271 (323)</td>
<td>159 (38)</td>
</tr>
<tr>
<td>n= 47</td>
<td></td>
<td></td>
</tr>
<tr>
<td>Discrete</td>
<td>1110 (267)</td>
<td>144 (41)</td>
</tr>
<tr>
<td>n= 42</td>
<td></td>
<td></td>
</tr>
<tr>
<td>Subcortical</td>
<td>1124 (337)</td>
<td>135 (28)</td>
</tr>
<tr>
<td>n= 40</td>
<td></td>
<td></td>
</tr>
</tbody>
</table>

Significant differences, p< 0.001, independent t-test.

T1; Brainstem > all other areas.

T2; Cerebellar < periventricular, discrete and subcortical (< periventricular).
There was no relationship between NAWM relaxation times and disability as measured by the EDSS, however there was a significant correlation between the mean lesion T1 relaxation time and the EDSS \((r= 0.63, p= 0.05)\). No relationship was seen with T2.

Of the 151 lesions 139 were visible on both the FSE and fast FLAIR images, eight on the FSE only (all cerebellar) and four on the fast FLAIR only (all subcortical). The T2 relaxation times were significantly longer in the lesions seen on both sequences than those on only one. Although the mean lesion T1 relaxation times were not significantly different, again the lesions seen on both sequences tended to have longer T1 relaxation times (see table 8.3).

**Table 8.3:** Comparison of lesions defined by MR sequence visibility.

<table>
<thead>
<tr>
<th></th>
<th>Mean T1 (SD)</th>
<th>Mean T2 (SD)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Seen on Both</td>
<td>1209 (340)</td>
<td>144 (37)</td>
</tr>
<tr>
<td>(n= 139)</td>
<td></td>
<td></td>
</tr>
<tr>
<td>Seen on FSE Only</td>
<td>1107 (134)</td>
<td>102 (5)</td>
</tr>
<tr>
<td>(n= 8)</td>
<td></td>
<td></td>
</tr>
<tr>
<td>Seen on Fast FLAIR Only</td>
<td>903 (186)</td>
<td>123 (17)</td>
</tr>
<tr>
<td>(n= 4)</td>
<td></td>
<td></td>
</tr>
</tbody>
</table>

Significant differences, \(p< 0.001\), independent t-test.

T1; No significant differences.

T2; FSE < both and fast FLAIR only.
8.3 Discussion

Previous studies have demonstrated that there is considerable variation in the T1 and T2 relaxation times of lesions which cannot be accounted for by the age or activity of the lesion (Larrson 1988 and 1989, Ormerod 1987). This study demonstrates that the site of the lesion is another factor which appears to affect the T1 and T2 of lesions and consequently, their appearance on both T1 and T2 weighted imaging. This effect may be responsible for the poor sensitivity of FLAIR sequences in detecting lesions within the posterior fossa and spinal cord.

The methods used to measure both T1 (Parker 1999) and T2 relaxation times have been shown to be both precise and accurate. The mean accuracy values were comparable with previous studies (Breger 1989, Larrson 1992b, Miller 1989, Steen 1994). The assessment of measurement reproducibility despite being across different controls scanned at different times, resulted in COVs consistent with the mean accuracy values and variation within the scanning region due to coil radiofrequency transmission non-uniformities was minimal.

Differences were seen in the brain relaxation times of healthy controls according to location, in keeping with the findings of MTR studies in the normal brain. The MTR has been shown to be highest in the corpus callosum which would equate to a shortened T1 relaxation time measurement (Silver 1997c). However these differences cannot account for the findings within lesions in different regions as although the posterior fossa lesions demonstrated prolonged T1, the T2 relaxation times were shorter. T1 was also much more prolonged than can be accounted for by the variations in the underlying normal tissue. Therefore other factors need to be considered including increasing age of the
patient and the chronicity of the lesion. Age related changes in T1 are known to occur. In a large study of 115 healthy subjects (age range 4.5 - 71.9 years) the T1 relaxation time was shown to decrease with age until a critical age (35-60 years for different structures) was reached, at which point it began to slowly increase (Cho 1997). In this study an age related effect was seen only in the splenium. This is not surprising as although the patient group is significantly older than the control group most subjects will in fact lie along the flat portion of the U shaped age/T1 graph. Furthermore, if age was the dominant factor the younger control subjects would be expected to have the higher T1 relaxation times. The variability of mean T1 and T2 relaxation times of MS lesions has been attributed to differences in the age of the lesion (Ormerod 1987), however even when comparing acute and chronic lesions the overlap is considerable (Larrson 1989), suggesting that the pathological heterogeneity of lesions must be of importance (McDonald 1992). It is this heterogeneity which is thought to be, at least in part, responsible for the poor relationship between the EDSS and TLV on standard T2 weighted sequences, although the EDSS is well known to have limitations in responsiveness, reliability and validity (Thompson 1998b). In this study there is an impressive significant correlation between the mean lesion T1 relaxation time and the EDSS (r= 0.63, p= 0.05). This is consistent with the correlations reported between the EDSS and T1 hypointensity or ‘black hole’ lesion load (Truyen 1996), a measure which appears to be more pathologically specific than TLV in that it is associated with severe tissue destruction, in particular axonal loss (van Walderveen 1998). The degree of hypointensity on T1 weighted images has also been shown to correlate with magnetisation transfer ratio implying the presence of demyelination (van Waesberghe 1997).

The measurement of TLV not only lacks pathological specificity but by definition
excludes the well documented pathological changes in NAWM (Allen and McKeown 1979, Trapp 1998). As in previous investigations this study demonstrates prolonged T1 and T2 relaxation times in the NAWM of MS patients compared to controls. The construction of pixel by pixel maps of the NAWM of MS patients (using control NAWM values of T1 and T2 as reference) in an earlier study demonstrated multiple small abnormal areas often consisting of only one or two pixels (Barbosa 1994). Further evidence for the existence of microscopic plaques comes from a study which demonstrated a significant correlation between the T1 of NAWM and the measured lesion load suggesting that the ‘invisible’ (microscopic) lesion load is proportionate to the ‘visible’ measured lesion load (Thompson 1991).

When lesions were considered by location, those in the cerebellum were found to have significantly shorter T2 relaxation times than lesions elsewhere in the brain, while the relaxation times of periventricular lesions were the longest. The differences were less marked in T1 excepting the four brainstem lesions (in three patients) which showed markedly prolonged T1 relaxation times; however this may be an effect of the small sample size. Although the variation demonstrable in T1 and T2 relaxation times according to location in normal brains is of insufficient magnitude to explain the differences in lesions, it does suggest that differences in the underlying brain tissue structure may effect the pathological response to a lesion within that area of the brain. This is in keeping with the observation of the relative rarity of hypo-intense lesions (black holes) in certain areas, particularly the optic nerves, brainstem and spinal cord (Gass 1996). In theory, densely packed fibres or a rigid structure may result in less extracellular water accumulating and consequently shorter T1 and T2 relaxation times in lesions within these areas. However in this study the T1 relaxation times of lesions in
the cerebellum were prolonged and similar to those in periventricular or discrete lesions. This disparity of prolonged T1 but near normal T2 may partly be attributable to gliosis. Experimental studies following cortical freezing injuries in the cat demonstrated acute changes of vasogenic oedema characterised by prolonged T1 and T2, which then normalised before the development of astrocytic gliosis three months later. Areas of gliosis demonstrated normal T2 but prolonged T1 relaxation times (Barnes and McDonald 1988, Barnes 1988). The similar prolongation of T1 in cerebellar and periventricular lesions may be due to gliotic changes. However less change in T2 is seen in the lesions of the cerebellum and brainstem compared to other regions, this may be a consequence of the more ordered structure within these regions which restricts the degree of accumulation of extracellular water, hence differences in T2 are seen despite similar T1 relaxation times.

Most (92%) of the lesions analysed were visible on both the FSE and fast FLAIR sequences. As described in chapter seven the FSE sequence proved superior in detection of posterior fossa lesions whilst the fast FLAIR detected more subcortical lesions. It has been recently suggested that the poor performance of fast FLAIR in the posterior fossa may be due to incomplete suppression of CSF at the inferior extremity of the head coils and by using an adiabatic pulse (one which is insensitive to field inhomogeneities) this effect can be reduced resulting in increased lesion conspicuity (Hajnal 1998). Poor CSF suppression may be a contributing factor, particularly in the cerebellum where a combination of atrophy and high signal CSF could decrease lesion conspicuity, but this does not explain the failure of fast FLAIR imaging to detect lesions in the spinal cord where CSF suppression is excellent (Filippi 1996b, Stevenson 1997).

This study demonstrates that the relaxation times and thus pathological characteristics of
lesions in MS are dependent not only on their age and activity but also on their location within the central nervous system. It may be possible, by utilising this information, to optimise fast FLAIR sequences (by altering the TE/TR combination) to improve detection of pathological changes within the posterior fossa and spinal cord. The relationship between mean lesion T1 and the EDSS is also interesting and suggests a possible role for relaxation time quantification as a surrogate measure of disease progression. This may be a more sensitive and reproducible technique than measuring areas of hypo-intensity on T1 weighted images and should be investigated further.
Chapter 9: Conclusions

Primary progressive MS is relatively rare in the MS population, for this reason few studies have been carried out in this patient subgroup. Due to the perceived problems in monitoring PP MS, namely the lack of clinical relapses and the supposed minimal change in T2 lesion volume, as well as the uncertainty of the underlying pathological process, these patients have been excluded from most therapeutic trials to date. There was thus a need to carry out a large systematic serial study of these patients to document the natural history of PP MS.

In agreement with previous small studies, patients with PP MS were shown to differ clinically from other subtypes of progressive MS in several ways. The age of onset was later and the incidence appeared to be equal in males and females. MRI quantification of T2 and T1 hypo-intensity lesion loads revealed smaller lesion loads in patients with PP MS than in SP MS. The TP MS patients were intermediate but appeared to be closer to the PP group. In the spinal cord no differences were shown between the patient groups. As in other studies no relationship between the measures of brain lesion load (T2 and T1 hypo-intensity) or spinal cord lesion load and disability as measured by the EDSS was shown. However the measure of spinal cord atrophy did correlate with the EDSS as well as the other two clinical measures; the 9-hole-peg-test and ten metre walk. The lack of relationship between the measures of brain lesion load and disability was reflected in the progression indices (EDSS/disease duration) of the three patient groups. Despite the PP MS group having the smallest lesion loads their progression index was higher than either the TP or SP MS groups. This suggests that the mechanism of disability in PP MS can not
be attributed to lesion formation, but must be associated with atrophy of both the brain and cord.

Serial assessment of this cohort demonstrated less clinical change in the PP MS group than expected (only 21% progressed by one EDSS step at one year). The reasons for this are multiple but probably include both the higher dropout rate in PP MS and the fact that more patients in this group were at a stage of the EDSS scale which is slower to change. Particularly problematic is the segment of the EDSS scale which spans 6.0 to 6.5. Disease progression which results in a patient changing from being able to walk 100m with unilateral assistance to only 20m with bilateral assistance takes considerably longer than other incremental changes. One of the other clinical scales used, the ten metre walk, was also unhelpful in detecting change due to the considerable floor effect. The difficulties in measuring clinical change over short time periods were also demonstrated in the recently published London, Ontario natural history cohort of PP MS (Cottrell 1999a) which by their definition included patients with both pure PP MS and the progressive-relapsing MS subgroup (Lublin and Reingold 1996). Progression probabilities (the probability of progression to the next DSS level in one year) were calculated for patients at each DSS level. Even when patients were at the DSS level of 4 or 5 the progression probability was only 40% and 33% respectively. At DSS 6 and 8 the probabilities of progression in one year fell to 4% and 2%. Consequently when sample size calculations were undertaken, large samples, even if highly selected (progression by one DSS level in the preceding year), were needed to show a treatment effect on clinical progression (Cottrell 1999a).

With regard to serial MRI changes, as in previous studies the rate of new lesion formation (brain and cord) was extremely low, however despite this there was a measurable change
in both the T2 and T1 hypo-intensity lesion volumes over the time period of two years, albeit much smaller than published values for other MS subgroups. Significant changes were also measurable in the degree of both brain and cord atrophy after a time period of only one year.

The correlations between change in MRI measures and the clinical measures of disability were however poor. This was in part due to the lack of change in the clinical disability scales due to their poor responsiveness. Longer serial studies are essential to explore these relationships further and ideally would follow patients from their first presentation, before the development of disability. Despite these poor MRI-clinical relationships the results of this study are extremely encouraging, measurable changes in several MR parameters have been demonstrated in this cohort over the relatively short time periods of one and two years.

9.1 Clinical Trials in Multiple Sclerosis

If large therapeutic trials are to be set up for PP MS there must be agreement on definitions. This study looks at two progressive groups in detail; purely PP MS and TP MS. The London Ontario study (Cottrell 1999b) includes under the title of PP MS, patients with pure PP MS and those with so called progressive-relapsing MS (Lublin and Reingold 1996), which by our definition would fall into the SP MS category. Furthermore the London Ontario study excluded patients with what they called single-attack-progressive MS (SAP MS), defined as a single relapse preceding the onset of progression (TP MS by our definition), and reclassified such patients as SP MS. However they also state that ‘a substantial minority (28%) of the PP MS cohort had a distinct relapse even decades after onset of progressive deterioration’ (Cottrell 1999b). This situation is
extremely confusing and leads to the question of whether these phenotypic subdivisions are useful or indeed at all relevant if patients can move between MS subgroups. The fact that there is considerable overlap in both the brain and spinal cord MRI findings between MS subtypes preventing distinction on radiological grounds also supports the theory that PP MS is not a separate disease entity but can be thought of as at one end of a spectrum. This point is supported by the findings in the TP MS patients, a group which has many similarities to PP MS, but MRI findings midway between PP and SP MS. Interestingly when the PP patients were subdivided into two groups according to their clinical presentation the ‘cord’ onset group had significantly smaller brain lesion loads than patients with other presentations, which approached the level of disease burden (lesion load) seen in SP MS patients. The main reasons for subdividing MS into clinical phenotypes are to aid in the choice of therapeutic agents or for entry into clinical trials. At the present time we are unable to distinguish subgroups by genetic, immunological or imaging techniques. Unless this becomes a reality in the future it may be more relevant to simplify the classification scheme to three groups; 1) Relapsing-remitting MS, 2) Secondary progressive MS: defined as an initial relapsing phase (one or more relapses), followed by a progressive phase with or without subsequent relapses, and 3) Progressive MS: characterised by a progressive picture from onset with or without superimposed relapses. This would avoid patients moving between groups and prevent unclassifiable patients being denied treatments which are licensed only for particular clinical subgroups. Once the patient group is defined, appropriate techniques or measures which are capable of detecting disease progression must be employed. The choice of clinical measures is extremely important, these need to be appropriate for the study group and floor effects avoided. As entrance criteria for trials are widened to include higher levels of disability,
the choice of clinical measures becomes more difficult. This is particularly important when considering composite measures, for example the composite clinical measure developed by the National MS Clinical Outcomes Assessment Task Force (Rudick 1997, Cutter 1999) combines measures of upper limb function, ambulation and cognitive function. If one component of the composite measure has floor or ceiling effects its overall sensitivity will be markedly reduced.

The use of MRI as a surrogate measure of disease progression is invaluable as the range of techniques available are objective and more sensitive to change than clinical measures, thus shortening trial duration. Annual lesion load measurements are already an accepted validated secondary outcome measure in clinical trials of RR and SP MS. This two year study has demonstrated change in the T2 lesion load in PP MS and consequently it is hoped this will prove to be a more useful secondary outcome measure than previously anticipated. The change in cord lesion load and the rate of development of new lesions in both the brain and spinal cord is low and probably will be of minimal use even in large cohorts. However the quantification of brain and spinal cord atrophy, in contrast to lesion load measurements, correlated well with the EDSS in cross sectional studies and demonstrated change in only one year. The techniques available to measure atrophy are automated, fast and have greater reproducibility than lesion volume assessment. Spinal cord atrophy assessment is particularly sensitive to change as unlike the brain, where loss of tissue may be seen as ‘black holes’ (T1 hypo-intensities) or atrophy, any loss of tissue in the spinal cord results in atrophy, due to the intrinsic parallel infrastructure of nerve fibres. The fact that changes in the degree of both brain and spinal cord atrophy are measurable in only twelve months suggest that atrophy is directly reflecting the pathological process responsible for disease progression and consequent disability in PP
MS. This is reflected in the stronger correlations between atrophy and disability in PP MS compared to other MRI measures.

Assessing prognosis in PP MS is difficult, it has recently been suggested that two clinical factors may be of prognostic value; multiple system (three or more) involvement (eg. motor, sensory, cerebellar, visual etc), and the rate of early disability (reaching DSS 3 within two years) (Cottrell 1999b). The development of spinal cord or brain atrophy may also be useful as a pre-clinical marker of disease progression. All of these factors could be used as selection criteria for therapeutic trials or for aggressive therapies if available.

9.2 Pathological Changes of Disability

The study of patients with PP MS highlights the discrepancy between MRI changes on conventional imaging and the clinical picture. Despite low lesion loads and few new lesions over time these patients accrue significant disability. As many of these patients have considerable spinal cord involvement clinically the studies in chapters four, seven and eight concentrated on the spinal cord with a view to gaining insights into the pathological processes which result in disease progression and disability. Using standard imaging techniques the relationship between cord lesion load and disability is poor. Two new techniques aimed at improving lesion detection were described in chapter seven. The use of 3D FSE demonstrated increased lesion detection, this may be of particular use in the diagnosis of MS in older patients, where vascular changes make interpretation of brain imaging difficult, or in those with little brain abnormality. However although resolution is improved the information gained lacks pathological specificity. This is demonstrated by the lack of relationship between lesion volumes, even when defined by the site within the spinal cord, and disability. In contrast the fast FLAIR sequence when
applied to the spinal cord failed to demonstrate lesions despite improved detection in the brain. This was an interesting finding as it demonstrated lesion heterogeneity within the central nervous system according to site. By measuring the T1 and T2 relaxation times (chapter eight) this heterogeneity was confirmed and was shown to be dependent on the site of the lesion within the central nervous system, this has implications both for future optimisation of MRI sequences for different areas of the CNS and for the possible role of relaxation time measurements as a surrogate measure of disease progression in MS. The measurement of brain lesion loads not only lacks pathological specificity but by definition excludes any changes in the NAWM. By measuring the mean T1 and T2 relaxation times of large areas of the brain and spinal cord, the effect and degree of pathological changes in both the NAWM and lesions will be reflected. Such techniques would be faster and more automated than lesion load measurements and may be useful in the future monitoring of disease progression in MS.

At the present time the serial measurement of atrophy appears to be the most appropriate outcome measure for PP MS in terms of reproducibility, sensitivity and validity. If used alongside the more validated measure of T2 lesion volume, the quantification of atrophy will be extremely useful as both a primary outcome measure in phase II trials and as secondary outcome measures in phase III trials in PP MS. Further studies are however needed to confirm the longitudinal relationship between ongoing atrophy and changes in clinical measures as well as exploring new measures such as relaxation time quantification techniques.
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