MAGNETIC RESONANCE IMAGING EVALUATION AND THERAPEUTIC TRIALS IN PRIMARY PROGRESSIVE MULTIPLE SCLEROSIS

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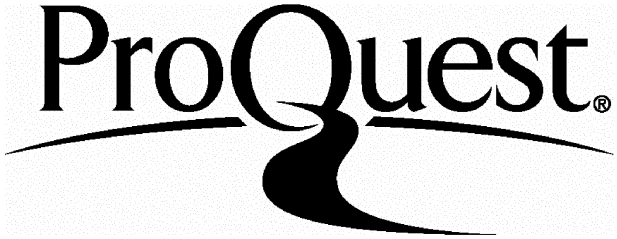
A thesis submitted to the University of London for the degree of

Doctor of Medicine

2002



ABSTRACT

Primary progressive multiple sclerosis is the central theme of this thesis which includes the first randomised controlled trial to be specifically designed for this group. The difficulties in diagnosis, classification and choice of outcome measures for therapeutic trials in this group are considered, and the role of magnetic resonance imaging (MRI) in monitoring disease progression is explored. Two recently developed and more pathologically specific MRI techniques, magnetisation transfer ratio (MTR) and ^{1}H magnetic resonance spectroscopy (MRS), are evaluated in cross-sectional studies. To investigate the marked discrepancy between focal cerebral lesions and disability in primary progressive multiple sclerosis, these techniques are applied to normal appearing white matter (NAWM) and provide evidence for the hypothesis that intrinsic changes, including axonal damage, are occurring in NAWM. Consideration is also given to methodological issues in serial MRI measurement and studies of reproducibility of spinal cord area measurement and of physiological variation in MRS are presented. Finally, the first randomised controlled study of a beta interferon in primary progressive multiple sclerosis is presented. This is an exploratory study and its objectives are to explore the safety of and look for hint of efficacy of interferon beta-1a in primary progressive multiple sclerosis and to identify potentially useful outcome measures in this group. This study demonstrates that it is entirely feasible to carry out therapeutic trials in primary progressive multiple sclerosis and appropriate outcome measures are available. Overall this thesis provides evidence to facilitate continuing increase in interest and research activity in primary progressive multiple sclerosis.

DECLARATION

The work presented in Chapters 3, 4 and 5 of this thesis is original. I was the lead investigator for the studies in Chapters 3 and 4 and was responsible for the study design, subject recruitment, MRI data collection, post-imaging analysis and statistical analysis. The EDSS assessments in the MTR and MRS studies in Chapter 3 were performed by VL Stevenson. The controls in the MTR study were provided by NC Silver. The quality of spectra in the MRS study was assessed by CA Davie. In the MRS study of aging in Chapter 4 subjects were also recruited by PA Brex.

I was the treating physician for the interferon beta-1a trial presented in Chapter 5 and was responsible for the recruitment, treatment and monitoring of all subjects. The EDSS assessments were performed by VL Stevenson, CM Griffin, SJ Hickman and N Tubridy. I collected the MRI data, which was blinded by DG MacManus, and carried out the post-imaging analysis with the following exceptions. Hard copy marking of cerebral T_2 lesions was performed jointly by DH Miller and myself, spinal T_2 lesions by DH Miller and, T_1 lesions by PA Brex. Measurement of whole brain atrophy was performed by DC Chard. Statistical analysis of the interferon beta-1a trial was carried out by M King.

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LIST OF ABBREVIATIONS

ADC	Apparent diffusion coefficient
BBSI	Brain boundary shift integral
Cho	Choline / choline containing compounds
CNS	Central nervous system
Cr	Creatine / phosphocreatine
CSE	Conventional spin echo
CSF	Cerebrospinal fluid
CV	Coefficient of variation
DTPA	Diethylene triamine pentaacetic acid
EAE	Experimental allergic encephalomyelitis
EDSS	Expanded Disability Status Scale
EPI	Echoplanar imaging
FLAIR	Fluid attenuated inversion recovery
FOV	Field of view
FS	Functional system
FSE	Fast spin echo
ICAM	Intercellular adhesion molecule
IL	Interleukin
MHC	Major histocompatability complex
mI	Myo-inositol
MRI	Magnetic resonance imaging
MRS	Proton magnetic resonance spectroscopy

MTR	Magnetisation transfer ratio
MSFC	Multiple sclerosis functional composite
NA	N-acetyl derived groups
NAA	N-acetyl aspartate
NAWM	Normal appearing white matter
Nex	Number of excitations
PRESS	Point-resolved spectroscopy
PROBE/SV	Proton brain exam / single voxel
QA	Quality assurance
SD	Standard deviation
SIENA	Structural image evaluation using normalisation of atrophy
SRCC	Spearman's rank correlation coefficient
T	Tesla
TE	Echo time
TI	Inversion time
TNF	Tumour necrosis factor
TR	Repetition time

PUBLICATIONS ARISING FROM THIS THESIS

Leary SM, Davie CA, Parker GJM, Stevenson VL, Wang L, Barker GJ, Miller DH, Thompson AJ (1999) ^{1}H magnetic resonance spectroscopy of normal appearing white matter in primary progressive multiple sclerosis. *J Neurol* **11**:1023-1026.

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ACKNOWLEDGEMENTS

The work in this thesis was carried out in the NMR Research Unit, Institute of Neurology which is supported by the Multiple Sclerosis Society of Great Britain and Northern Ireland. The interferon beta-1a trial was financially supported by Biogen.

I received support from numerous colleagues in the Institute of Neurology. Physics support and development was provided by GJ Barker, GJM Parker and L Wang. Radiograhy was performed by DG MacManus, AS Lowe and colleagues and MRI scans were reviewed by IF Moseley. The brain atrophy measurement software was provided by the Dementia Research Group and I am grateful for the help of NC Fox, WR Crum and R Gordon. Pharmacy support was provided by SP Dyson and the Pharmacy Department of the National Hospital for Neurology and Neurosurgery. Moral support was provided by too many people to mention but I would particularly like to thank PA Brex, DJ Werring, AS Lowe, VL Stevenson and GT Ingle. I would like to thank my supervisors DH Miller and especially AJ Thompson who expertly guided me through what at times appeared an unsurmountable task. Finally, I thank the patients and their families who participated in the study for their outstanding and inspirational commitment.

CHAPTER 1: INTRODUCTION

1.1 Background

Although multiple sclerosis has been clinically characterised for over 150 years, its aetiology and pathophysiology are still poorly understood. Therapeutics in multiple sclerosis has therefore remained in its infancy and until recently only symptomatic treatments were available. However, over the last 15 years several therapeutic agents have been shown to be partially effective in modifying the disease course of multiple sclerosis. The most thoroughly evaluated of these have been the beta interferons which have been reported to be of benefit in relapsing remitting and secondary progressive multiple sclerosis and more recently in clinically isolated syndromes suggestive of multiple sclerosis. However, until now beta interferons have not been evaluated in primary progressive multiple sclerosis. Patients with primary progressive multiple sclerosis have been excluded from the majority of therapeutic trials to date because they have atypical clinical and magnetic resonance imaging (MRI) characteristics which pose unique problems in carrying out trials (Leary *et al.* 1999a). However, with the advent of disease modifying drugs it is now an obligatory and worthwhile challenge to address these problems. This thesis presents the first randomised, controlled trial of a beta interferon in primary progressive multiple sclerosis and explores the problems posed by carrying out trials in this group. Monitoring disease progression is a particularly important issue and so MRI techniques novel to primary progressive multiple sclerosis are evaluated and particular consideration is given to methodological issues in collecting serial MRI data relevant to this study.

As background to the work presented in this thesis a brief overview of multiple

sclerosis and the basic principles of MRI is now given and the clinical application of MRI in multiple sclerosis is then reviewed.

1.2 Multiple Sclerosis

1.2(a) Clinical classification

Multiple sclerosis is an inflammatory, demyelinating disorder of the central nervous system (CNS). It typically presents with a relapsing and remitting course. Relapses are characterised as episodes of acute or subacute neurological dysfunction (lasting a minimum of 24 hours) usually evolving over days or weeks, plateauing for a short period and then remitting to a variable degree, from no resolution to complete recovery. Relapses may therefore result in accumulating neurological deficit or disability. Over half of patients with relapsing remitting multiple sclerosis enter a progressive phase characterised by a progressive accumulation of disability with or without superimposed relapses defined as secondary progressive multiple sclerosis (Lublin & Reingold 1996).

Approximately 10% of patients have primary progressive multiple sclerosis, characterised by continuous accumulation of neurological deficits from symptom onset, without relapse or remission (Thompson *et al.* 1997). There is also a small group of patients with predominantly progressive disease and minimal relapse activity who have been defined in two ways: progressive relapsing multiple sclerosis, characterised by progressive disease from onset with superimposed relapses (Lublin & Reingold 1996), and transitional progressive multiple sclerosis, characterised by a single relapse before or after the onset of disease progression (Stevenson *et al.* 1999). Patients with a single relapse before the onset of progression have also been classified as single attack progressive multiple sclerosis

(Cottrell *et al.* 1999a).

1.2(b) Diagnosis

The diagnosis of multiple sclerosis is a clinical diagnosis requiring evidence of CNS lesions disseminated in time and space and the exclusion of other diseases. Diagnostic criteria have been developed to improve the certainty of diagnosis. Schumacher *et al.* in 1965 deemed six criteria to be essential in characterising clinically definite multiple sclerosis - examination evidence of CNS dysfunction, involvement of two or more separate parts of the CNS, predominantly white matter involvement, either two or more episodes of worsening lasting at least 24 hours separated by one month or more or progression over at least six months, age of onset within 10 to 50 years and no better explanation by a physician competent in neurology. In 1983 Poser *et al.* modified these criteria to incorporate paraclinical and laboratory evidence and to extend the age of onset to 59 years. These criteria classify definite multiple sclerosis in two categories: clinically definite multiple sclerosis requires two disseminated attacks with clinical evidence of two lesions or clinical evidence of one lesion and paraclinical evidence of another lesion. Laboratory supported definite sclerosis requires two attacks with clinical or paraclinical evidence of one lesion and oligoclonal bands in the cerebrospinal fluid (CSF), or one attack with clinical evidence of two lesions or clinical evidence of one lesion and paraclinical evidence of another plus oligoclonal bands in the CSF. Patients with primary progressive multiple sclerosis do not conform readily to Poser criteria and classification of primary progressive multiple sclerosis will be discussed further in chapter 2.

1.2(c) Epidemiology

Multiple sclerosis affects approximately 87 000 people in the United Kingdom with an annual incidence of 1 in 2 500. It typically presents between 20 and 40 years of age and is the most common cause of neurological disability in young adults in this country. There is a female preponderance with twice as many affected as males. The prevalence of multiple sclerosis varies with geographical location; broadly speaking it is high in temperate and Western European populations and is low in tropical and subtropical areas (Ebers 1994). However, there are clear exceptions to this and racial genetic factors also appear to play a role in susceptibility to multiple sclerosis. Migration studies have also suggested that environmental factors in childhood may have an aetiological role (Dean & Kurtzke 1971).

1.2(d) Genetics

The contribution of genes and environment to the aetiology of multiple sclerosis remains a source of debate. Twin studies have demonstrated much higher concordance rates in monozygotic than dizygotic twins but nowhere near the 100% rate which would be expected if it was solely a genetic disease; in a Canadian study of twins with a mean age exceeding 50 years the monozygotic concordance rate was 30.8% compared to the dizygotic concordance rate of 4.7% (Sadovnick *et al.* 1993). Family studies have also demonstrated that relatives of multiple sclerosis patients are at a greater risk for developing the disease than the general population (Sadovnick 1993).

Another approach to address the role of genetic factors has been to look for candidate genes. An association has been demonstrated with the class II major histocompatibility complex (MHC) haplotype DR15 (Compston *et al.* 1995) but otherwise candidate gene studies have provided little information. Systematic genome screening has also been carried

out and, although it has not elucidated the genetic basis of multiple sclerosis, it has identified regions of interest where future work may be focused (Chataway *et al.* 1998). It appears likely that, rather than there being a single or a few major genes controlling susceptibility to multiple sclerosis, there are several genes with small susceptibility effects.

1.2(e) Pathology

The characteristic pathological feature of multiple sclerosis is the focal plaque or lesion. The lesions of multiple sclerosis were first depicted by Carswell in 1838 (Compston 1988). The principal elements of the lesion were described by Charcot in 1868 - demyelination, relative preservation of axons, gliosis and a variable amount of inflammation (McDonald 1994). Over 130 years later this histological overview remains just as accurate but the precise roles and inter-relationships of these elements in the pathogenesis of multiple sclerosis are yet to be clearly elucidated.

Demyelination is the hallmark of the multiple sclerosis lesion. Demyelination concerns the loss of myelin from areas of white matter with relative sparing of axons (Raine 1997). In multiple sclerosis the demyelinated lesions occur throughout the CNS but with a predilection for the optic nerves, periventricular white matter and corpus callosum, cerebellum and cervical cord (Lassmann 1998). Macroscopic examination of the post-mortem brain reveals disseminated plaques of varying morphology which, broadly classifying, may be pink and soft, representing acute lesions, or grey and firm, representing chronic lesions. Atrophy, particularly of the spinal cord and optic nerves, may also be apparent. The histopathological features of the active and chronic lesions are now discussed in more detail.

The acute or active lesion is characterised by marked inflammation. The inflammatory infiltrates predominantly consist of lymphocytes and macrophages and are associated with

small veins. The boundary of the lesion is indistinct and the centre of the lesion is oedematous with expansion of the extracellular space. The axons are demyelinated and are also reduced in number. Hypertrophic astrocytes are seen commonly, often associated with proliferated oligodendrocytes, but marked fibrous gliosis is not seen (Raine 1997).

The demyelinated chronic lesion is characterised by astrocytic gliosis which is abundant throughout the lesion. The number of axons is reduced, being more depleted in the centre than the periphery. Oligodendrocytes are depleted in the centre of the lesion although proliferated oligodendrocytes may be seen around the lesion margin (Raine 1997). Low-grade inflammation and macrophage activity may also be seen particularly towards the edge of the lesion.

Classifications of lesion activity also refer to chronic active lesions. The chronic active lesion histologically lies between the acute and chronic lesion.

Despite the earlier mention of relative sparing of axons in lesions it should be emphasised that axonal damage or loss clearly occurs in lesions (Barnes *et al.* 1991; Ferguson *et al.* 1997; Trapp *et al.* 1998). Axonal damage has been shown to occur in the presence of active inflammation (Ferguson *et al.* 1997; Bitsch *et al.* 2000) with a recent study, which quantified axonal transection, identifying the greatest number of transected axons in active lesions and a smaller number in chronic active lesions (Trapp *et al.* 1998). However, another recent study, which demonstrated axonal loss in chronic demyelinated lesions (Lovas *et al.* 2000), reminds us that continuous axonal degeneration may also be occurring in isolation.

Remyelination is also found in lesions, commencing from about one month after its development (Prineas *et al.* 1993). Shadow plaques, lesions with thinly myelinated fibres, are thought to be areas of complete remyelination of previously demyelinated lesions (Lassmann 1998).

Alongside the pathological abnormalities of focal lesions, abnormalities are seen in the so-called normal appearing white matter in multiple sclerosis. Normal white matter consists of myelinated axons, oligodendrocytes, astrocytes and microglia as well as blood vessels. A pathological study of macroscopically normal white matter in multiple sclerosis revealed that 72% of samples were histologically abnormal (Allen & McKeown 1979). The most common abnormality found was gliosis, often attributed to proliferation of astrocytes, as well as demyelination and perivascular inflammation. Axonal damage as demonstrated by transected axons has also been found in normal appearing white matter though to a much smaller extent than in lesions (Trapp *et al.* 1998). Interestingly, another study showed a significant reduction in axonal density in normal appearing white matter which was not significantly different to the reduction seen in lesions (Lovas *et al.* 2000).

1.2(f) Immunology

A number of points of evidence indicate that multiple sclerosis is an immunologically mediated disease (Martin & McFarland 1997; Wekerle 1998). Firstly, the inflammatory lesion is composed of several immunological components. Secondly, multiple sclerosis is genetically associated with MHC class II which plays an important role in immune responses. Thirdly, multiple sclerosis bears similarities to the immunologically mediated experimental allergic encephalomyelitis (EAE). Lastly, therapeutic effect in multiple sclerosis has been seen with immunosuppressive and immunomodulatory therapies.

The immunological components of the multiple sclerosis lesion have been well described and include adhesion molecules, T lymphocytes, macrophages, B lymphocytes and cytokines (Esiri & Gay 1997). Adhesion molecules play a role in the migration of leucocytes from the blood into the CNS; there is increased expression of intercellular adhesion molecule

(ICAM-1) in lesions and also expression of vascular cell adhesion molecule (VCAM-1) in chronic lesions. E-selectin, which provokes slowing of leukocytes in the blood and rolling against the endothelium, is also expressed in some lesions. Numerous T lymphocytes are found in lesions, particularly in active and chronic active lesions. There are two main classes of T lymphocytes - helper/inducer T cells which express the surface marker CD4 and cytotoxic/suppressor cells which express CD8. CD4 cells can further be subdivided into the T helper phenotypes TH-1, which preferentially secrete interferon gamma and interleukin (IL) -2, and TH-2, which secrete IL-4 and IL-5 (Wekerle 1998). Most studies report that overall CD8 cells are more plentiful than CD4 cells but in acute lesions CD4 cells generally outnumber CD8 cells. A separate subset of T cells, the γ/δ T cells, are also found but their significance is not clear. MHC class I antigens, which are recognised by cytotoxic T cells, are normally only found on endothelial cells but are diffusely expressed in multiple sclerosis lesions. Macrophages are also found in abundance in acute lesions. Activated microglial cells are present at the borders of acute and chronic active lesions. Both macrophages and microglia express MHC class II which is required for antigen presentation to helper T cells. B lymphocytes and plasma cells are also found in lesions but make up only a very small proportion of infiltrates. B cells are generally not found in the parenchyma of lesions but are confined to the perivascular spaces of acute lesions.

Numerous cytokines, which are mostly produced by macrophages and lymphocytes, have been detected in lesions and can be broadly classified as pro- and anti-inflammatory (Esiri & Gay 1997). Pro-inflammatory cytokines include tumour necrosis factor alpha (TNF-α), IL-1 and IL-2 and interferon gamma (IFN-γ). Anti-inflammatory cytokines include interferons alpha and beta (IFN-α, IFN-β), transforming growth factor beta (TGF-β) and IL-4. IL-6, which has mixed activity, is also found.

The study of the immunology of multiple sclerosis may be facilitated by animal models. EAE is an acute or chronic relapsing inflammatory white matter disease of the CNS which can be induced in a number of susceptible inbred animal strains by injection of whole white matter or single myelin proteins (Martin & McFarland 1997). Similar to multiple sclerosis, disease susceptibility is associated with MHC class II. Transfer experiments have established that EAE is a T cell-mediated disease with the encephalitogenic T cells being CD4 cells, usually expressing the TH-1 phenotype. From studies of EAE the following model of pathogenesis of demyelination has been proposed (Martin & McFarland 1997). An exogenous inducing event activates autoantigen-specific T cells in the periphery. Adhesion molecules on T cells and cerebrovascular T cells are up-regulated. Activated T cells transmigrate through the blood brain barrier into the CNS where they activate resident microglial cells which secrete cytokines and attract further inflammatory cells. Cytokines such as TNF-α/β as well as toxic oxygen and nitrogen metabolites, released by activated monocytes, probably have direct toxic effects on myelin sheaths and oligidendrocytes. Antibodies against myelin may also contribute to demyelination. Down-regulation of inflammatory activity in the lesion probably involves both programmed cell death and anti-inflammatory cytokines.

In multiple sclerosis there is also evidence to support T cell mediated disease activity. T cells with specific reactivity to the myelin basic protein, have been found more frequently in patients than controls in most studies and often express the TH1 CD4 subtype. T cell reactivity to other myelin proteins including proteolipid protein has also been found. However, CD4 T cells are unlikely to directly mediate oligodendrocyte damage as oligodendrocytes do not express MHC class II. TH1 cytokines also appear to be of importance; increased levels of TNF-α and IL-2 in multiple sclerosis have been reported and

a therapeutic trial of interferon gamma increased relapse rate (Panitch *et al.* 1987).

T cells have been hypothesised to play a central role in the pathogenesis of multiple sclerosis (Martino & Hartung 1999). It is believed that CD4 T cells provide the antigen-specificity of the pathogenic process and regulate the activity of other T cell subsets, B cells and macrophages that exert myelinotoxic activity. The initial phase of T cell stimulation is represented by the trimolecular complex consisting of the antigen, T cell receptor and MHC (Brosnan *et al.* 1997).

There is also evidence for antibody mediated immune responses in multiple sclerosis. Oligoclonal antibodies are found in the cerebrospinal fluid of over 90% of patients and are used as a diagnostic tool. B cells with reactivity to myelin protein are also found. In EAE administration of antibodies to myelin oligodendroglia glycoprotein has been reported to lead to widespread demyelination (Martin & McFarland 1997).

In conclusion, the immunopathogenetic mechanisms of multiple sclerosis are still not clear. However, the current understanding is still of clinical significance. Firstly, immunological parameters have the potential to be surrogate markers of disease activity. For example, soluble ICAM-1 has been reported to correlate with gadolinium enhancement on MRI (Giovannoni *et al.* 1997). Secondly, and more importantly, new targets for therapeutic agents have been identified. To date the majority of disease-modifying agents, including the beta interferons, have had relatively generalised immunomodulatory activity and at best have only been partially effective. New agents are now being developed with more focused immunomodulatory activity. In particular, there has been much interest in targeting the components of the trimolecular complex (Hohlfeld 1999).

1.2(g) Prognosis

The course of multiple sclerosis is extremely variable. One of the largest natural history studies showed that the median time to reach a level of disability requiring assistance for walking was 15 years (Weinshenker *et al.* 1989). Survival is not dramatically affected with the overall life expectancy for multiple sclerosis being reported as only about 6 to 7 years less than a control population (Sadovnick *et al.* 1992). Individual prognosis is very difficult to predict but suggested predictive factors for a poor prognosis include male sex, older age of onset, motor onset, progressive course, short time to reach Expanded Disability Status Scale (EDSS) score 3 and high relapse rate within the first 2 years (Paty *et al.* 1999).

1.2(h) Symptomatology

Multiple sclerosis may cause numerous and diverse symptoms reflecting involvement of any part of the CNS. Common areas to be involved that cause clinical symptoms are the spinal cord, optic nerves and brainstem. In relapsing remitting multiple sclerosis the commonest presenting symptoms are sensory and visual. Through the course of the disease a multitude of symptoms may occur and functional deficits and disability may develop. These symptoms include spasticity, weakness, ataxia, fatigue, sphincter and sexual dysfunction, pain, paroxysmal symptoms, visual dysfunction, vertigo, dysphagia, dysarthria, respiratory dysfunction, temperature sensitivity and cognitive and psychiatric dysfunction.

1.2(i) Management

Multiple sclerosis requires a comprehensive management approach addressing medical, functional, psychological and social aspects of the disease. Current management strategies must incorporate four main areas: i) education and support, ii) disease-modifying

therapies, iii) management of acute relapses and iv) symptomatic treatments and rehabilitation. Only disease-modifying therapies are discussed further here but it must be emphasised that current therapies have no impact on existing neurological deficits and so supportive and symptomatic treatment remain at the core of management.

In attempting to modify the disease process in multiple sclerosis a knowledge of the underlying disease mechanisms is required. Neurological deficit appears to occur in two ways - from incomplete remission from relapses and from disease progression. The probable underlying correlate of fixed neurological deficit is axonal loss (Trapp *et al.* 1998) but whereas in the former axonal loss may be related to acute inflammatory demyelination (Ferguson *et al.* 1997), in the latter it may be a more diffuse process less dependent on inflammation. Therefore, therapeutic agents directed at both inflammation and axonal protection may be helpful in multiple sclerosis; to date the majority of agents have focused on the inflammatory process. Therapies aimed at promoting remyelination and repair are also being developed (Compston 1997; Duncan 1999; Schwab 1999).

During the last decade several potential disease-modifying therapies have been developed and more therapies are currently being researched. Some of these therapies are partially effective, some have been ineffective or poorly tolerated and others remain unproven. Only the beta interferons and glatiramer acetate are currently licensed in the United Kingdom and prescribing must comply with strict criteria (Polman *et al.* 1999).

Interferon Beta

Natural interferon beta is a glycosylated protein which, among other properties, has immunomodulatory effects. The mechanism of action of interferon beta in multiple sclerosis is not clear but relevant biological effects include antagonism of the effects of interferon

gamma, inhibition of T cell proliferation and improvement of suppressor cell function (Weinstock-Guttman *et al.*1995). There are currently three recombinant preparations of interferon available - interferon beta-1b (Betaferon™/ Betaseron™), which is non-glycosylated and differs slightly in amino acid sequence from natural interferon beta, and two preparations of interferon beta-1a (Avonex™, Rebif™), which are glycosylated and identical in sequence to interferon beta. All are licensed for ambulatory patients with relapsing remitting multiple sclerosis and interferon beta-1b has recently been licensed for secondary progressive multiple sclerosis though it is not widely available in the United Kingdom.

Interferon Beta-1b (Betaferon™/ Betaseron™). A double-blind, placebo-controlled trial of subcutaneous interferon beta-1b in 372 patients with relapsing remitting multiple sclerosis was the first major interferon beta trial to be completed. There was a 34% reduction in annual relapse rate over two years, a reduction in relapse severity and an increased proportion of patients remained relapse free on treatment (IFNB Multiple Sclerosis Study Group 1993). No significant effect was seen on progression of disability. On MRI an 80% reduction in disease activity, as measured by number of active scans and appearance of new lesions, was seen. A reduction in lesion load was also seen (Paty *et al.* 1993). Recently a double-blind, placebo-controlled trial of interferon-beta-1b in Europe involving 718 patients with secondary progressive multiple sclerosis found a delay in the progression of disability of 9-12 months in a study period of two to three years in patients both with and without superimposed relapses (European Study Group 1998). On MRI a reduction in lesion load and new lesion activity was seen (Miller *et al.* 1999). However, a North American trial in secondary progressive multiple sclerosis found no effect on disease progression though positive effects were seen on relapse rate and MRI activity (Paty *et al.* 2000).

Interferon Beta-1a (Avonex™). A double-blind, placebo-controlled trial of

intramuscular interferon beta-1a in 301 patients with relapsing remitting multiple sclerosis also demonstrated a reduction of relapse rate by one-third in the treated group (Jacobs *et al.* 1996) although the reduction was only 18% in the smaller subset of patients who completed two years of follow-up. The primary endpoint of the study was time to sustained disability progression and this was significantly greater in patients treated with interferon beta-1a. The Kaplan-Meier estimate of the proportion of patients progressing by the end of two years was 34.9% in the placebo group and 21.9% in the interferon beta-1a group. A reduction in enhancing lesions was seen on MRI though there was no significant effect on lesion load. Publication of the results of a randomised controlled trial of interferon beta-1a in 436 patients with secondary progressive multiple sclerosis is awaited (Cohen *et al.* 2001).

Interferon Beta-1a (Rebif™). A double-blind, placebo-controlled trial of subcutaneous interferon beta-1a in 560 patients with relapsing remitting multiple sclerosis again demonstrated a reduction in relapse rate by one-third and also a delay in progression of disability (PRISMS 1998). On MRI a reduction in active lesions and lesion load was also seen. A randomised controlled trial in 618 patients with secondary progressive multiple sclerosis found no effect on progression of disability (SPECTRIMS 2001) though positive effects were seen on relapses and MRI measures (Li *et al.* 2001).

The most common side effects of interferon beta are flu-like symptoms and injection site reactions (with subcutaneous injections); liver enzymes and white blood cell abnormalities are also seen. The presence of neutralising antibodies has been demonstrated in a proportion of patients on all types of interferon beta though their clinical significance is still not clear.

Glatiramer Acetate (Copaxone™)

Glatiramer acetate is composed of a mixture of polypeptides. Its mechanism of action is not clear but may involve induction of suppressor T cells, MHC blocking and T cell receptor antagonism (Aharoni *et al.* 1999). A double-blind, placebo-controlled trial of glatiramer acetate in 251 patients with relapsing remitting multiple sclerosis demonstrated a 29% reduction in relapse rate in the treated group (Johnson *et al.* 1995). A large randomised controlled MRI study also reported a reduction in enhancing lesions and accumulation of lesion load as well as a reduction in relapse rate (Comi *et al.* 2001a).

Intravenous immunoglobulin

IVIG has been studied in multiple sclerosis as it has been successful in other immunologically mediated disorders. A double-blind, placebo-controlled trial of IVIG in 148 patients found a greater than 50% reduction in relapse rate and an improvement in clinical disability (Fazekas *et al.* 1997) Smaller studies with MRI have shown no effect on lesion load (Achiron *et al.* 1998; Sorensen *et al.* 1998) but a significant reduction in enhancing lesions has been reported (Sorensen *et al.* 1998). IVIG has been reported to promote remyelination but it does not reverse neurological deficit (Noseworthy *et al.* 2000a, 2001).

Mitoxantrone

Mitoxantrone is an antineoplastic agent with immunomodulatory effects. A placebo-controlled trial of intravenous mitoxantrone in 51 patients with relapsing remitting multiple sclerosis found a reduction in relapse rate but no significant effects on MRI activity (Millefiorini *et al.* 1997) A randomised trial of mitoxantrone and methylprednisolone versus methylprednisolone alone in relapsing remitting and secondary progressive patients with very

active disease showed a significant reduction in relapse frequency, improvement in change in disability and a dramatic reduction in enhancing lesions on MRI (Edan *et al.* 1997). A phase III trial of mitoxantrone in progressive multiple sclerosis has recently been completed and preliminary reports indicate a beneficial effect on disease progression and relapse frequency (Hartung *et al.* 1999).

Azathioprine

Several randomised controlled trials of azathioprine have been carried out in multiple sclerosis. A meta-analysis of these trials confirmed a slight clinical benefit but debated whether this may be outweighed by side-effects (Yudkin *et al.* 1991) However, azathioprine may be as effective as newer treatments in increasing the proportion of patients who remain relapse free at two years (Palace & Rothwell 1997).

Methotrexate

A double-blind, placebo-controlled trial of low dose oral methotrexate in 60 patients with chronic progressive multiple sclerosis reported significantly less progression in the methotrexate compared to the placebo group, but the composite measure used has not been validated (Goodkin *et al.* 1995).

2-chlorodeoxyadenosine (Cladribine)

Cladribine is a purine nucleoside analogue with lymphocytotoxic activity. A double-blind, placebo-controlled, crossover study of intravenous cladribine in 51 patients with chronic progressive multiple sclerosis reported that clinical and MRI parameters remained stable in patients on cladribine but deteriorated in patients on placebo (Sipe *et al.* 1994).

However, a larger trial of subcutaneous cladribine in 159 patients with chronic progressive multiple sclerosis found no clinical efficacy although significant effects were demonstrated on MRI parameters (Rice *et al.* 2000). A double-blind, placebo-controlled trial of subcutaneous cladribine in 52 subjects with relapsing remitting multiple sclerosis had a favourable effect on both clinical and MRI parameters (Romine *et al.* 1999).

Anti-α4 integrin (Antegren™)

α4β1 integrin is an adhesion molecule which acts as a mediator of immune cell migration into the CNS. An exploratory study of anti-α4 integrin in relapsing remitting and secondary progressive (with superimposed relapses) multiple sclerosis reported a short-term reduction in the number of active lesions on MRI (Tubridy *et al.* 1999) though an increase in relapse rate was seen immediately post-trial. Preliminary results from a second larger study report a reductions in relapses and a profound reduction in gadolinium enhancing lesions (Miller *et al.* 2001a).

Campath-1H

An exploratory study of the humanised anti-leukocyte (CD52) monoclonal antibody Campath-1H in 27 patients with secondary progressive multiple sclerosis demonstrated a dramatic reduction in relapse rate and enhancing lesions but about half the patients experienced progressive disability and increasing brain atrophy (Coles *et al.* 1999). The first dose was associated with a transient reversible exacerbation. Autoimmune hyperthyroidism developed in one-third of patients.

Negative studies

A double-blind, placebo-controlled study of sulfasalazine, an oral agent with anti-inflammatory and immunomodulatory properties, in 199 active relapsing remitting and secondary progressive multiple sclerosis patients was negative after three years of follow-up despite positive effects being seen at the 18 month interim analysis (Noseworthy *et al.* 1998). Preliminary reports of the double-blind, placebo-controlled study of oral myelin, proposed to induce immune tolerance, in 515 patients with relapsing remitting multiple sclerosis indicate that there was no clinical or MRI benefit (Francis *et al.* 1997; Panitch *et al.* 1997).

A double-blind, placebo-controlled trial of anti-CD4 antibody in 72 patients with active relapsing remitting and secondary progressive multiple sclerosis did not reduce active lesions on MRI, despite reducing the number of circulating CD4-positive T cells, and would appear unlikely to have a beneficial clinical effect (van Oosten *et al.* 1997). A double-blind, placebo-controlled trial of lenercept, a tumour necrosis factor-α neutralising agent, in 168 patients mostly with relapsing remitting multiple sclerosis had no effect on MRI activity and there was a significant increase in number of exacerbations in the treated group (Lenercept Multiple Sclerosis Study Group 1999).

Linomide, an oral quinolone with immunomodulatory activity, reduced disease activity on MRI in phase II trials (Andersen *et al.* 1996; Karussis *et al.* 1996) but phase III trials were terminated due to an increased incidence of ischaemic heart disease (Noseworthy *et al.* 2000b).

Other

Several other potential disease-modifying therapies are currently under investigation. These include other focused immunomodulatory therapies such as T cell vaccination, altered

peptide ligands, matrix metalloproteinase inhibitors and autologous haemopoietic stem cell transplantation, and remyelination therapies such as transplantation of oligodendrocyte progenitor cells.

1.3 Basic Principles of MRI

1.3(a) Introduction

Magnetic resonance imaging is based on the physics of what happens to an atomic nucleus when placed in a magnetic field and radiofrequency electromagnetic radiation applied. The atomic nucleus must contain an odd number of protons; hydrogen nuclei (^1H), which are in abundance in body water, are conventionally used.

Protons possess both positive charge and motion, known as spin, and therefore generate a magnetic field. The protons in the body are normally randomly aligned and so there is no overall magnetic field. However, when placed in an external magnetic field the protons align themselves parallel or anti-parallel to the external field. As it requires less energy to be aligned parallel slightly more protons are aligned parallel than anti-parallel. The protons also exhibit motion around the direction of the magnetic field, identical to that of a top spinning under the influence of gravity, called precession. The frequency of precession depends on the strength of the external magnetic field as calculated by the Larmor equation:

$$\omega_0 = \gamma B_0$$

where ω_0 is the precession frequency (radions s^{-1}), γ is the gyro-magnetic ratio and B_0 is the

external magnetic field strength (Tesla).

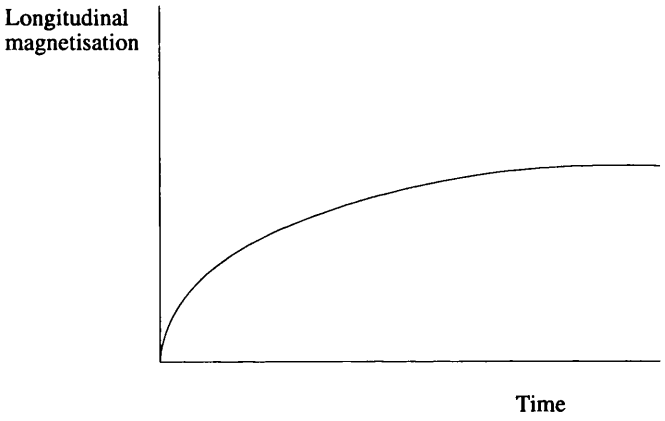
As there is a slight preponderance of parallel protons in the field a net magnetic vector is produced which is called longitudinal magnetisation. This cannot be measured directly and therefore has to be translated into a form which will generate an output. This is achieved by applying a radiofrequency pulse. When a radiofrequency pulse of the same frequency as the protons (given by the Larmor equation) is applied, an exchange of energy occurs (also known as resonance) and some protons move to the higher energy, anti-parallel, alignment. This results in a decrease in longitudinal magnetisation and also causes the protons to precess in phase transversely creating a net transverse magnetisation. This transverse magnetisation is rotating at the precession frequency and induces an electrical current in the receiver coil which can be amplified to give a measurable signal; this is the basis of an MR image.

If the radiofrequency pulse is then switched off, the protons 'relax' to their original state. The longitudinal magnetisation increases as protons return to the lower energy parallel state (longitudinal relaxation) and the transverse magnetisation decreases as the protons dephase due to local magnetic field inhomogeneities (transverse relaxation). Longitudinal relaxation occurs gradually following the exponential T_1 curve and transverse relaxation follows the exponential T_2 curve (Figure 1.1). It is impossible to identify the precise time at which relaxation is complete and so the longitudinal relaxation time, T_1, is defined as the time for 63% of the original longitudinal magnetisation to be reached and transverse relaxation time, T_2, is defined as the time for transverse magnetisation to decrease to 37% of its original value. For biological tissues T_1 is about 300-2000 ms whereas T_2 is shorter at about 30-150 ms. Relaxation times are different in different tissues, for example, water has a long T_1 and T_2 whereas in fat they are short.

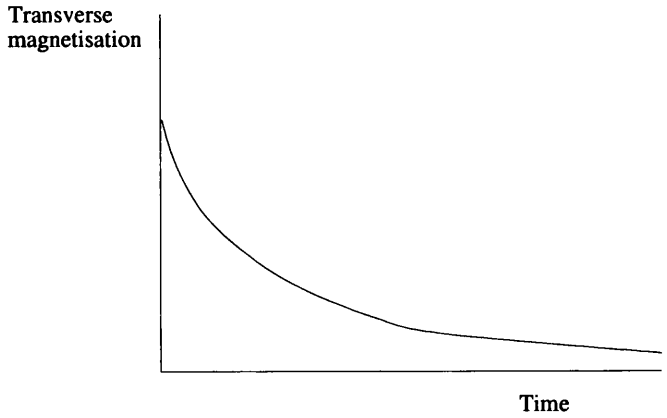
Figure 1.1

T_1 and T_2 relaxation curves

(a) T_1 relaxation curve



(b) T_2 relaxation curve



1.3(b) Pulse Sequences

The properties and sequence of radiofrequency pulses can be varied to have different effects on protons. For example, a pulse can be designed to flip half the protons from a parallel to anti-parallel state producing only transverse magnetisation and effectively tilting the longitudinal magnetisation through 90° (known as a 90° pulse). However, following a single radiofrequency pulse the protons quickly relax providing little information on tissue contrast. The combination of more than one radiofrequency pulse, as part of a 'pulse sequence', can provide more tissue contrast. According to the types of pulse and the time between pulses, i.e the time to repeat (TR), the magnetisation and signal intensity of various tissues can be modified. A time to repeat of $\simeq T_1$ (typically 500 ms) is considered to be a short TR and of $\simeq 2T_1$ (typically 1500 ms) a long TR.

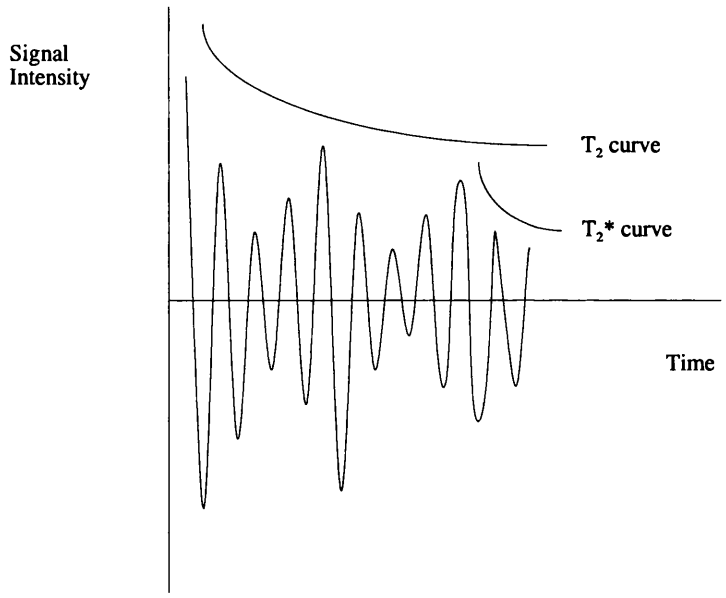
Spin echo sequence

In this sequence a 90° pulse is first applied. Initially the protons will precess in phase but, as some precess slightly faster than others, they lose synchronisation with each other and the signal decreases. A 180° pulse is then applied which reverses their position in the transverse plane; as the faster precessing protons are now 'catching-up' with the slower protons they start to rephase resulting in increased transverse magnetisation and so a stronger signal. This signal is called a spin echo. The time from the 90° pulse to the maximum of this signal is known as the echo time (TE) and so the time between the 90° and the 180° pulses is TE/2. Having rephased, the fast protons then overtake the slow protons and the signal decreases once more. If another 180° pulse is then applied the signal again increases and so the sequence can continue with a repeating 180° pulse. However, the 180° pulse only reverses the effects of the inhomogeneities in the external magnetic field and the signal is still affected

by inhomogeneities in the local magnetic field caused by interactions with the molecule. The signal intensity therefore decreases from echo to echo and this is described by the T_2 curve; if no 180° pulse is used the signal is affected by external and local field inhomogeneities resulting in a more rapid transverse relaxation which is described by the T_2* curve (Figure 1.2). The entire sequence can be repeated with the TR being the time from one 90° pulse to the next 90° pulse.

By looking at the combined T_1 and T_2 curve of a tissue it is possible to establish the signal intensity of that tissue and by varying the TE and the TR different characteristics of the tissue can be explored. A TE of ≈ T_2 (typically 30 ms) is considered to be short and a TE of ≈ $2T_2$ (typically 80 ms) long. The shorter the TE the stronger the signal from a tissue but the smaller the contrast between tissues. The longer the TE the more marked the signal contrast between tissues but the smaller the signal to noise. Using a short TR, where T_1 differences are apparent, and a short TE, which shows little T_2 contrast, T_1 weighted images are obtained. With a long TR, where difference in T_1 are not seen, and a long TE, where there is pronounced T_2 contrast, the images obtained are T_2 weighted. With a long TR and a short TE there is little T_1 or T_2 contrast and so the signal is just largely reflecting the density of the protons in the tissue and the resulting images are known a proton density images.

Figure 1.2 T_2* curve



Inversion recovery sequence

In this sequence a 180° pulse is first applied inverting the longitudinal magnetisation. A 90° pulse is then applied resulting in transverse magnetisation and producing a signal. The signal depends on the time between the 180° and 90° pulses, which is known as the inversion time (TI). The inverted longitudinal magnetisation recovers at a rate according to the T_1 of the tissue which in turn determines the amount of transverse magnetisation and the signal intensity. The resultant images are therefore generally very T_1 weighted.

The fluid attenuated inversion recovery (FLAIR) sequence combines an inversion recovery sequence with a long inversion time and a long TE. The long TI suppresses the CSF, due to its long T_1, but leaves tissue with shorter T_1 relatively unaffected. The long TE results in heavily T_2 weighted images together with suppression of the CSF signal. These images are useful for detecting pathological features which would otherwise be swamped by the bright CSF signal.

Fast imaging sequences

The aim of fast imaging sequences is to decrease the acquistion time of images. The clinical benefits of this are to reduce the time subjects spend in the scanner and hence increase compliance and reduce motion artefact. Acquisition time is conventionally determined by the following equation:

$$\text{Acquisition time} = TR \times N \times Nex$$

where N is the number of rows in the image (often known as the matrix size) and Nex is the number of excitations. Reducing any of these parameters will shorten acquisition time but

each has disadvantages. Shortening the TR reduces the signal and, considering a spin echo sequence, is limited by the time required to apply the 180° pulse. Reducing the number of rows decreases image resolution. Reducing the number of excitations, i.e. the number of signals to be averaged, reduces the signal to noise ratio.

One approach to faster imaging is to use a gradient echo instead of a spin echo, which allows a shorter TR. In a gradient echo sequence a magnetic field gradient is applied instead of a 180° pulse. This introduces greater magnetic field inhomogeneities and so the protons dephase more quickly. The gradient is switched off and then switched back on in the opposite direction so that some protons rephase. The maximum signal before the signal starts to decrease again is called the gradient echo. In the gradient echo sequence the flip angle of the initial pulse may also be reduced to less than 90° (typically 10-35°) which does not completely destroy the longitudinal magnetisation, leaving some signal to be measured by later pulses, even if the signal does not have time to recover via T_1 relaxation. so that it is readily available to be converted to signal. The atrophy techniques employed in this thesis utilise a fast gradient echo sequence and, because of the short TR, include radiofrequency spoiling to compensate for the signal from one TR period interfering with signal from the next.

Another approach is to collect multiple echoes, an echo train, during each repetition time rather than just the conventional one echo per TR. These extra echoes can be used to encode parts of the image that would otherwise have to be collected in separate TR periods. The longer the echo train length the shorter the acquisition time. Examples of multiple echo techniques include fast spin echo (FSE) and echoplanar imaging (EPI). In FSE sequences data is acquired as spin echoes but multiple echoes are collected during each repetition time and so more lines of the image are encoded per TR. Combining an FSE sequence with an

inverting pulse creates a fast-FLAIR sequence. In EPI gradient echoes are used and depending on the length of the echo train the sequence may be repeated several times (multi-shot EPI) or all the data may be collected in one acquisition (single-shot EPI). EPI is the fastest of the multiple echo techniques and is widely used in diffusion weighted imaging and functional MRI.

1.3(c) Spatial Information & Image Reconstruction

In order to create an image, the location of each signal generated has to be established. This can be considered in two steps. Firstly a 'slice' has to be selected. A magnetic field gradient, with different strengths at different locations or 'slices', is added to the external magnetic field. Within each slice there will therefore be different precession frequencies. If a radiofrequency pulse of the same precession frequency is then applied only the protons in a specific slice resonate. Therefore a slice is located by its designated radiofrequency pulse. The thickness of the slice can be determined either by using a range of frequencies so that the wider the range the thicker the slice, or by using the same frequency range and altering the slope of the gradient field so that the steeper the field the thinner the slice.

The second step is to determine where within a slice a signal is located. This is achieved by applying a second magnetic field gradient which creates a gradient of precession frequencies (the frequency encoding gradient) so that different 'columns' of protons have different frequencies. A third magnetic field gradient is also briefly applied along the column of protons; when this gradient, the phase encoding gradient, is switched off the protons within a column have phases which depend on their position. Therefore, a specific location is encoded by signals with different frequencies and signals with the same frequency but different phases.

The signals from each location are received into a grid of unprocessed signal data, known as k-space. This data is processed by a Fourier transformation to give a measure of the signal intensity for each location form which an image can be constructed.

The above description applies to two dimensional sequences. It is also possible to acquire three dimensional sequences by the addition of another phase encoding gradient in a third plane and such sequences can be used to provide volumetric information.

1.3(d) Contrast Media

Certain substances have paramagnetic properties, i.e, they have small local magnetic fields. If these substances are introduced into tissue they cause shortening of the T_1 and T_2 relaxation times of the protons in the surrounding tissue. Gadolinium (chelated to DTPA) is widely used in MRI. It can enhance tissue contrast in two ways - by increasing the signal on T_1 weighted images and by reducing the signal on T_2 weighted images. As the former is easier to discern, gadolinium is largely used in conjunction with T_1 weighted imaging.

1.4 Clinical Applications of MRI in Multiple Sclerosis

1.4(a) Introduction

It is now 20 years since MRI was first used to demonstrate the lesions of multiple sclerosis (Young *et al.* 1981). Since then MRI has provided considerable insight into the natural history of the disease and more pathologically specific techniques have been developed. Over the last ten years MRI has been increasingly used in the evaluation and monitoring of new treatments. The necessity for MRI techniques to be sensitive to change and reproducible over time has therefore also become an increasingly important issue. In this

section various MRI techniques are presented with particular emphasis on those techniques which are employed in this thesis. The basic principles of the techniques, their clinical significance and their role as therapeutic markers are discussed.

1.4(b) T_2 weighted imaging

The basic principles of conventional T_2 weighted imaging have already been discussed. In multiple sclerosis the typical finding on T_2 weighted imaging is of multiple areas of increased signal, known as lesions, in the white matter. Such lesions have been shown to correlate with areas of demyelination on pathological examination (Stewart *et al.* 1984). However, their appearance is not pathologically specific and may not only reflect demyelination but also inflammation, oedema, axonal loss and gliosis. Lesions are not static phenomena and may appear and disappear over time. Dynamic changes in the size of lesions are also seen (Isaac *et al.* 1988) with new lesions typically increasing in size over two months and then decreasing over two to three months to leave a smaller residual abnormality.

One of the most important applications of T_2 weighted imaging is in the diagnosis of multiple sclerosis. Although this is ultimately a clinical diagnosis MRI is now a standard investigation which can provide paraclinical evidence of disseminated white matter disease. MRI is probably the most sensitive test for demonstrating spatial dissemination (Paty *et al.* 1988). However, although MRI is a very sensitive test, white matter lesions are not specific to multiple sclerosis and so MRI criteria to aid diagnosis have been suggested. Fazekas *et al.* (1988) aimed to distinguish the lesions of multiple sclerosis from non-specific abnormalities and suggested that the presence of two of the following three features - size $\geq$6mm, abutting the ventricular body and infratentorial location - is specific for multiple sclerosis. Paty *et al.* (1988) also suggested a classification of MRI-supported definite multiple sclerosis

incorporating criteria for an MRI strongly suggestive of multiple sclerosis - requiring four predominantly white matter lesions (> 3mm diameter) or three with one being periventricular to be present. More recently, Barkhof *et al.* (1997) have developed an MRI model in clinically isolated syndromes which more accurately predicts the conversion to clinically definite multiple sclerosis. This model is based on the presence of four abnormal criteria - gadolinium-enhancement, juxtacortical, infratentorial and periventricular lesions.

The predictive role of T_2 weighted imaging in prognosis has also been an important issue. In established multiple sclerosis it does not appear to be of significant predictive value but in clinically isolated syndromes suggestive of multiple sclerosis T_2 weighted brain abnormalities are predictive of the risk of developing multiple sclerosis. A 10 year follow-up study found that 83% of patients with T_2 abnormalities at presentation progressed to clinically definite multiple sclerosis compared to 11% of patients with a normal MRI (O'Riordan *et al.* 1998a); MRI was also predictive for the severity of disability. Quantitative measurement of T_2 weighted lesion load in clinically isolated syndromes is also predictive of development of multiple sclerosis and level of disability (Sailer *et al.* 1999).

The role of T_2 weighted imaging in the monitoring of disease activity is more limited. There are marked discrepancies between T_2 weighted MRI findings and clinical status and MRI is not a routine part of the clinical management of established multiple sclerosis. New lesions on T_2 weighted images are seen about five times as often as clinical relapses (Miller & Frank 1998) and so may be a sensitive marker of disease activity. However, T_2 weighted new lesions and lesion loads show at best only modest correlations with disability (Filippi *et al.* 1995a; van Walderveen *et al.* 1995; IFNB Multiple Sclerosis Study Group 1995; Gawne-Cain *et al.* 1998). Currently T_2 weighted imaging is still widely used in the evaluation of new treatments. Guidelines on MRI outcomes in therapeutic trials recommend

the use of new T_2 weighted cerebral lesions in preliminary trials and T_2 weighted lesion loads in definitive trials (Miller *et al.* 1996).

The standard sequence used in T_2 weighted imaging is the conventional spin echo (CSE). FSE sequences produce T_2 weighted and proton density images with similar contrast to CSE but with much shorter acquisition times. FSE has been found to be at least as sensitive as CSE in the detection of lesions (Thorpe *et al.* 1994; Tubridy *et al.* 1998) but lesion volumes have been reported as lower for FSE than for CSE (Rovaris *et al.* 1997; Gawne-Cain *et al.* 1998). However, the gain in terms of acquisition time makes FSE a reasonable alternative to CSE.

Fast-FLAIR sequences produce heavily T_2 weighted images with suppression of the CSF signal. Acquistion time is again shorter than CSE. Fast-FLAIR may be more sensitive in detecting cerebral lesions than CSE, particularly subcortical lesions, (Filippi *et al.* 1996a; Tubridy *et al.* 1998) but it is less sensitive in detecting lesions in the posterior cranial fossa (Tubridy *et al.* 1998). Lesion volumes for fast-FLAIR have been reported as similar or larger than for CSE (Filippi *et al.*1996a; Gawne-Cain *et al.* 1998) but again are lower in the posterior fossa (Gawne-Cain *et al.*1998). Fast-FLAIR lesion volume has also been shown to have a modest correlation with disability (Gawne-Cain *et al.* 1998).

1.4(c) T_1 weighted imaging

The basic principles of T_1 weighted imaging have already been discussed. The T_1 weighted images referred to in this context are conventionally spin echo images. On T_1 weighted images multiple sclerosis lesions may appear as areas of signal hypointensity, commonly referred to as 'black holes'. Only about 20% of lesions appear hypointense on T_1 weighted images (Barkhof *et al.* 1998). Black holes appear to be more pathologically specific

than T_2 weighted lesions. In acute lesions oedema or demyelination may contribute to the hypointensity (Miller *et al.* 1998); the hypointensity of acute lesions may then persist or may become isointense over the following months (van Waesberghe *et al.* 1998). In chronic lesions hypointensity is more likely to indicate permanent tissue destruction including axonal loss (van Walderveen *et al.* 1998). In a recent pathological study, including both active and chronic lesions, T_1 hypointensity was found to strongly correlate with axonal density (van Waesberghe *et al.* 1999). T_1 hypointense lesion load has been reported to correlate with disability in cross-sectional and longitudinal studies (van Walderveen *et al.* 1995; Truyen *et al.* 1996) with the T_1 lesion load in secondary progressive multiple sclerosis showing a stronger correlation with disability than the T_2 lesion load though this has not been universally proven (O'Riordan *et al.* 1998b).

1.4(d) Gadolinium enhancement

The contrast enhancing agent gadolinium-DTPA, employed in conjunction with T_1 weighted imaging, has provided considerable insight into the natural history of multiple sclerosis. Gadolinium enhancement indicates an increase in the permeability of the blood-brain barrier. Initial studies revealed that lesions are heterogeneous with some lesions enhancing and others not and different patterns of enhancement being seen (Grossman *et al.* 1986; Kermode *et al.* 1990a). Serial studies went on to demonstrate that enhancement may precede the development of T_2 weighted new lesions and may precede the clinical expression of a lesion (Kermode *et al.* 1990b). Animal and pathological studies have reported that inflammation is the correlate of enhancement (Hawkins *et al.* 1991; Katz *et al.* 1993) and so it appears that inflammation is occurring early in the evolution of a new lesion. Enhancement usually resolves within one month but may persist for three to four months (Miller *et al.*

1998). With frequent scanning enhancement appears to be a consistent feature of all new lesions (Lai *et al.* 1996) but enhancement may also occur without a visible T_2 lesion and some older lesions may enhance (Miller *et al.* 1993). However, a recent cross-sectional and longitudinal study reported an asymmetric distribution between T_2 lesions and enhancing lesions with more T_2 lesions being found in the periventricular white matter raising the possibility that some lesions arise by a different mechanism not associated with early blood-brain barrier breakdown and inflammation (Lee *et al.* 1999). The size of contrast enhancing lesions may be relevant to the pathological progression of lesions as small contrast-enhancing lesions are less likely to progress to T_1 hypointense lesions (Brex *et al.* 2000a).

The sensitivity of gadolinium enhancement for acute lesions has made it a key tool in the evaluation of new treatments. Gadolinium markedly increases the number of new active lesions detected compared to T_2 weighted scans alone (Miller *et al.*1993). Sensitivity may be further improved by frequent scanning, introducing a delay between contrast injection and imaging, the use of magnetisation transfer (discussed later in this chapter) T_1 weighted images and particularly the use of triple dose gadolinium. In one study the use of magnetisation transfer imaging with a long delay following triple dose gadolinium resulted in the detection of 126% more enhancing lesions than in standard single-dose imaging (Silver *et al.* 1997a). Gadolinium enhancement in the short-term has also been shown to be predictive of future clinical disease activity (Losseff *et al.* 1996a). Gadolinium enhancement is recommended and widely used as a primary outcome in short-term preliminary trials in multiple sclerosis and may also be used a secondary outcome in definitive trials (Miller *et al.* 1996).

1.4(e) T_1 and T_2 relaxation times

T_1 and T_2 relaxation times have been defined earlier. T_1 and T_2 relaxation times can be measured in vivo and provide information about the tissue water environment (Larsson *et al.* 1998). An increase in T_1 and T_2 times reflects a decrease in tissue organisation. Increases in both T_1 and T_2 relaxation times have been found in both lesions and normal appearing white matter in multiple sclerosis (Ormerod *et al.* 1986a, b). The pathological significance of these findings is not clear as relaxation times have not been proven to be pathologically specific; oedema, expanded extracellular space, demyelination, gliosis, axonal loss and remyelination may all contribute.

1.4(f) Spinal cord imaging

The spinal cord is clinically a common site of involvement in multiple sclerosis. Imaging of the spinal cord is therefore an important focus but its structure and location have made it difficult to image. However, spinal cord imaging has considerably improved with the use of phased array coils and the FSE sequence (Miller *et al.* 1998). Imaging of the spinal cord has been reported to be abnormal in three quarters of patients of patients with multiple sclerosis (Kidd *et al.*1993). Although not routinely required in diagnosis (except when excluding other causes of cord pathology) spinal cord imaging is of considerable value in patients with suspected multiple sclerosis with normal brain imaging and may increase sensitivity of CNS imaging to almost 100% (Thorpe *et al.* 1996a). It is also more specific than brain imaging as areas of high signal are not seen as a result of normal aging in the spinal cord (Thorpe *et al.* 1993). The value of spinal cord imaging in monitoring disease activity is more disappointing as cord lesion load does not correlate with disability cross-sectionally or longitudinally (Kidd *et al.*1993, 1996). The addition of gadolinium reveals

relatively few enhancing lesions in the spinal cord compared to the brain in relapsing remitting multiple sclerosis (Thorpe *et al.* 1996b) and almost none in progressive multiple sclerosis and is therefore unlikely to play a significant role in monitoring disease activity. Attempts have also been made to improve the sensitivity of the sequences used. Fast FLAIR, despite improving lesion detection in the cerebral hemispheres, has been shown to be much inferior in detecting lesions than FSE in the spinal cord (Stevenson *et al.* 1997). However, three dimensional FSE may be a more promising technique as it has been shown to detect almost twice as many lesions as two dimensional FSE (Stevenson *et al.* 1998a).

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1.4(g) Spinal cord atrophy

Spinal cord atrophy is a potentially more pathologically specific measure than conventional spinal cord imaging, possibly indicating axonal and myelin loss. Initial studies, using manual outlining of axial spinal cord slices acquired with a two-dimensional gradient echo sequence, demonstrated a correlation of spinal cord area with disability (Kidd *et al.* 1993; Filippi *et al.* 1996b). However, serial studies made apparent that the scan-rescan reproducibility of such measurement techniques was poor; their clinical application was therefore limited as in vivo changes due to disease progression were likely to be less than the errors of measurement variation (Losseff *et al.* 1996b). A new MRI method was subsequently developed to enable accurate and reproducible quantification of spinal cord atrophy (Losseff *et al.* 1996b). This utilised a volume acquired inversion prepared fast spoiled gradient echo T_1 weighted sequence which provided excellent CSF suppression and improved cord to CSF contrast. Axial slices from the caudal landmark of C2/3 were reformatted post-acquisition for more precise repositioning. This level was chosen as there is little anatomical variation, minimising the effects of repositioning errors, and there is a capacious CSF space optimising

cord conspicuity. The scan-rescan reproducibility of this method was excellent and a cross-sectional clinical study demonstrated a strong correlation between spinal cord cross-sectional area and disability. A follow-up serial study also demonstrated spinal cord atrophy occurring over only one year (Stevenson *et al.* 1998b); no correlation was seen with change in disability but clinical progression was detected in less than a third of the patients. Longer follow-up and/or more responsive clinical scales are required to assess the longitudinal relationship of spinal cord atrophy with disability. Nevertheless, spinal cord atrophy remains a promising MRI marker in the monitoring of disease progression.

An alternative method of measuring spinal cord volume has recently been described. This technique applies the Cavalieri method of modern design stereology in combination with point counting to three dimensional acquired images and has confirmed a significant reduction in upper cervical cord volume (C1-3) in multiple sclerosis and a correlation with disability (Liu *et al.* 1999). However, the reproducibility of this technique (Edwards *et al.* 1999) may limit its use in serial studies. More recently a three dimensional global measure of cervical spinal cord volume has been developed and preliminary results report a strong correlation with disability (Hickman *et al.* 2001). Further evaluation of this technique is required in serial studies.

1.4(h) Cerebral atrophy

Cerebral atrophy is similarly a potentially pathologically specific measure which may reflect loss of axons and myelin. The first MRI study to quantify cerebral atrophy in multiple sclerosis used a measure of partial brain volume calculated from four T_1 weighted axial slices at the level of the lateral ventricles (Losseff *et al.* 1996c); progressive cerebral atrophy was demonstrated over an 18 month period. Using the same method in combination with ^1H

magnetic resonance spectroscopy, cerebral atrophy has been shown to correlate with N-acetyl aspartate, a neuronal marker, suggesting that axonal loss is an important cause of atrophy (Coles *et al.* 1999).

Measures of whole brain volume have also confirmed cerebral atrophy in multiple sclerosis. Cerebral atrophy, as well as individual hemispheric, brainstem and cerebellar atrophy, has been demonstrated in a cross-sectional study of multiple sclerosis patients compared to controls using a manual segmentation method on volume acquired images (Filippi *et al.* 1998a). Another cross-sectional study, applying the stereological and point-counting technique, found significant infratentorial, cerebral white matter and corpus callosal atrophy in multiple sclerosis (Liu *et al.* 1999); modest correlations between atrophy and disability using this technique have also been reported (Liu *et al.* 1999; Edwards *et al.* 1999).

Measurement of ventricular volume has also been used as a marker of cerebral atrophy. Increased ventricular volumes have been reported in patients with multiple sclerosis compared to controls (Matthews *et al.* 1996; Philips *et al.* 1998). Differences in ventricular volumes between disease subgroups, with larger ventricular volumes in secondary progressive compared to relapsing remitting and primary progressive multiple sclerosis, have also been reported (Lycklama a Nijeholt *et al.* 1998).

A recent large serial study of cerebral atrophy in relapsing remitting multiple sclerosis over two years demonstrated increases in third ventricle and lateral ventricle width and decreases in brain width and corpus callosum area on T_1 weighted images (Simon *et al.* 1999). The same investigators also confirmed progressive cerebral atrophy in relapsing remitting multiple sclerosis by applying a more reproducible three dimensional segmentation technique to T_2 weighted images and calculating the brain parenchymal fraction, the ratio of brain parenchymal volume to the total volume within the brain surface contour (Rudick *et*

al. 1999).

For serial studies of brain atrophy reproducibility of the measurement technique is a key issue. With the techniques described above there are several potential sources of reproducibility error including serial positioning and segmentation errors. A registered, three dimensional volumetric technique, has been developed for research in Alzheimer's disease which minimises some of these errors (Fox *et al.* 1996). This technique allows whole brain MRI volumes to be registered with subvoxel accuracy (Freeborough *et al.* 1996) and so reduces positioning error. The cerebral volume change can then be measured directly from the registered scans by integrating the shifts in brain-CSF boundaries due to change in cerebral volume. The resulting integral, called the brain boundary shift integral (BBSI), is therefore a direct measure of the change in cerebral volume and is not affected by small segmentation errors. Applying this technique to a cohort of patients with multiple sclerosis demonstrated that significant cerebral atrophy is occurring at over twice the rate of that of age- and gender-matched controls (Fox *et al.* 2000). Ventricular volumes were also segmented on the registered images using a semi-automated technique and the rate of ventricular enlargement was five times greater then the controls. This technique appears to be a very sensitive and reproducible method and may potentially be useful as a marker of disease progression in therapeutic trials. SIENA (Structural Image Evaluation, using Normalisation, of Atrophy) is another recently developed registration method which can detect cerebral atrophy with sub-voxel accuracy (Smith *et al.* 2001) and has shown to be a sensitive measure of atrophy in primary progressive multiple sclerosis (Stevenson *et al.* 2000a).

1.4(i) Magnetisation transfer ratio

Conventional MRI largely reflects the relaxation properties of free water protons. However, it does not detect the water protons bound to macromolecular structures as they have very short T_2 relaxation times. In magnetisation transfer imaging the bound water protons can be saturated with an off-resonance radiofrequency pre-pulse; these exchange magnetisation with the protons in the free water pool resulting in an indirect reduction in tissue signal intensity. This effect can be quantified by calculating the magnetisation transfer ratio (MTR) of saturated and non-saturated spin echo images (Wolff & Balaban 1989). The MTR reflects the integrity of the macromolecular structure and a reduction in MTR would be expected if there is tissue destruction. The pathological substrate of a reduction in MTR is discussed further in Chapter 3.

In patients with multiple sclerosis MTR is significantly reduced in lesions (Dousset *et al.* 1992; Gass *et al.* 1994) and, to a lesser degree, in NAWM (Dousset *et al.* 1992; Loevner *et al.* 1995; Filippi *et al.* 1995b). In one cross-sectional study a significant correlation between lesion MTR and disability was reported (Gass *et al.* 1994). Theses initial studies of MTR used a region of interest approach which only assesses small volumes of brain tissue but more recent studies have employed a histogram approach which can provide global or compartmental measures of brain MTR. With this approach a reduction in the peak histogram height, thought to reflect the residual amount of normal brain tissue, was found in multiple sclerosis patients compared to controls (van Buchem *et al.* 1996). Further reductions in peak height have been demonstrated serially (van Buchem *et al.* 1996; Patel *et al.* 1999) and this may well be a sensitive marker of disease progression in multiple sclerosis. MT histogram parameters have been reported to have some modest clinical correlations (van Buchem *et al.* 1998) and have been suggested to be more sensitive to disease progression

than T_2 weighted lesion loads (Phillips *et al.* 1998; Filippi *et al.* 2000a). Differences in MTR histogram parameters have also been found between the major subgroups of multiple sclerosis in both the brain (Filippi *et al.* 1999; Tortorella *et al.* 2000; Dehmeshki *et al.*2001) and the cervical cord (Filippi *et al.* 2000b).

1.4(j) ^{1}H Magnetic resonance spectroscopy

Unlike conventional MRI, which acquires structural images derived from the magnetic resonance of protons in free water, ^{1}H magnetic resonance spectroscopy (MRS) can detect and quantify the resonances of protons in certain other brain metabolites. The free water is first suppressed with an on-resonance saturation pre-pulse. The pulse sequence then used differs between methods. The method used in this thesis is Point-RESolved Spectroscopy (PRESS) which employs a double spin echo, i.e., a 90° pulse followed by two180° pulses; each pulse is slice selective along a different axis. Only the second echo, containing signal from the intersection of all three planes, is collected. No spatial localising gradient is applied during readout and so instead of a structural image a spectrum of metabolite resonances in the selected volume is acquired.

The major metabolic peak in MRS is produced by N-acetyl aspartate (NAA) which is almost specific to neurons (Birken & Oldendorf 1989; Urenjak *et al.* 1993). NAA is also expressed in oligodendrocyte progenitor cells but these are only present in small numbers in adult human brains (Scolding *et al.* 1995). NAA is therefore a surrogate marker of neuronal function and permanently decreased concentrations would be expected in areas of irrecoverable axonal loss.

In patients with multiple sclerosis decreased levels of NAA have been demonstrated in both lesions (Arnold *et al.* 1990; Davie *et al.* 1994; Husted *et al.* 1994) and in areas of

NAWM(Davie *et al.* 1994; Husted *et al.* 1994). In serial studies decreases in NAA consistent with progressive axonal loss have been demonstrated (Arnold *et al.* 1992, 1994) but reversible changes in NAA, indicating recovery from axonal dysfunction, may also occur (Arnold *et al.* 1994; Davie *et al.* 1994). Inverse correlations between NAA and disability have been demonstrated in cross-sectional studies (Davie *et al.* 1997; Fu *et al.* 1998). Change in NAA has also been reported to correlate with change in disability in patients with relapsing remitting multiple sclerosis in a longitudinal study (de Stefano *et al.* 1998). Quantification of NAA remains a promising marker of disease progression and this may be facilitated by the recent development of techniques to measure total brain NAA (Gonen *et al.* 2000).

1.4(k) Diffusion weighted imaging

Diffusion weighted imaging concerns the random motion of water molecules in tissues. As the motion of water molecules is restricted by cellular structures diffusion weighted imaging can be used to measure structural properties of the tissue (Horsfield *et al.* 1998). The magnitude of diffusion is quantified by the apparent diffusion coefficient (ADC); an increase in ADC would be expected in the presence of inflammation or tissue destruction. Diffusion anisotropy provides a measure of the directionality of diffusion; highly directional tissues such as white matter tracts have high diffusion anisotropy and a decrease in diffusion anisotropy would be expected if there is neuronal loss or damage. In multiple sclerosis an increase in ADC has been demonstrated in both lesions and NAWM compared to control white matter which is higher in acute than chronic lesions and higher in lesions than NAWM (Larsson *et al.* 1992; Christiansen *et al.* 1993a). A reduction in diffusion anisotropy has also been reported in both lesions and NAWM (Werring *et al.* 1999).

1.4(l) Functional MRI

Functional magnetic resonance imaging is a non-invasive method that can investigate functional localisation with a high degree of anatomical spatial resolution (Yousry *et al.* 1998). It is based on the principle that there is increased blood flow in areas of brain activation resulting in decreased blood concentrations of deoxyhaemoglobin which is paramagnetic and causes MRI activation. Its application to multiple sclerosis is not yet well validated but motor and visual paradigms have been studied (Lee *et al.* 2000a; Werring *et al.* 2000a). It potentially may be of particular value in the investigation of mechanisms of recovery in multiple sclerosis.

1.4(m) Conclusion

MRI has provided considerable pathogenetic insights into the mechanisms of disease in multiple sclerosis. In the context of this thesis what is perhaps a more important issue is the role of MRI in the monitoring of disease progression. With the lack of reliable and responsive clinical scales MRI has become a key part of therapeutic monitoring in multiple sclerosis. The role of MRI in therapeutic monitoring in primary progressive multiple sclerosis has not yet been validated and this will be discussed further in the next chapter.

CHAPTER 2: PRIMARY PROGRESSIVE MULTIPLE SCLEROSIS - A UNIQUE CHALLENGE IN TRIAL METHODOLOGY

2.1 Introduction

Patients with primary progressive multiple sclerosis are a unique group with atypical clinical and magnetic resonance imaging (MRI) characteristics which may suggest a different mechanism of disease progression to relapsing remitting multiple sclerosis. As a result they have been excluded from the majority of therapeutic trials. In this thesis the first randomised controlled trial of beta interferon in primary progressive multiple sclerosis is presented. The execution of this trial has also provided further insight into the difficulties in designing and recruiting to therapeutic trials in this group. In this chapter the characteristics of primary progressive multiple sclerosis and their implications for treatment and clinical trials, particularly in regard to patient selection and outcome measures, are discussed. Therapeutic trials in primary progressive multiple sclerosis to date are also reviewed.

2.2 Characteristics of Primary Progressive Multiple Sclerosis

2.2(a) Classification

Patients with primary progressive multiple sclerosis comprise about 10% of all patients with multiple sclerosis (Thompson *et al.* 1997). Their clinical course is characterised by a progressive accumulation of neurological deficit from onset without relapse or remission although occasional plateaus and temporary minor improvements are allowed (Lublin & Reingold 1996). There is also a small group of patients with predominantly progressive

disease defined earlier as progressive relapsing multiple sclerosis and transitional progressive multiple sclerosis. Recent natural history studies have shown that the clinical characteristics and disability progression of patients with progressive relapsing multiple sclerosis are similar to primary progressive multiple sclerosis with only mild occasional or single relapses and suggested that the classifications could be merged (Kremenchutzky *et al.* 1999; Andersson *et al.* 1999). Transitional progressive multiple sclerosis has been suggested to lie between primary and secondary progressive multiple sclerosis (Gayou *et al.* 1997; Stevenson *et al.* 1999); separate classification may therefore not be helpful and reclassification of patients using Lublin & Reingold criteria, according to the timing of their relapse, may be more appropriate.

2.2(b) Clinical features

Aside from the clinical course several other differences are seen in primary progressive multiple sclerosis compared to the relapsing remitting / secondary progressive group. Their mean age of presentation is later (Confavreux *et al.* 1980; McDonnell & Hawkins 1996; Cottrell *et al.* 1999a), approximately 40 years compared to 30 years in relapsing remitting multiple sclerosis (Thompson *et al.* 1997). Relatively more men are affected resulting in a loss of the usual female preponderance (Thompson *et al.*1997). Mode of presentation is most commonly motor (McDonnell & Hawkins 1998; Stevenson *et al.* 1999; Cottrell *et al.* 1999a) with a progressive paraparesis whereas visual and sensory presentations are most common in relapsing remitting disease.

2.2(c) Cognitive profile

There have been relatively few studies of cognitive function in primary progressive

multiple sclerosis. The largest study to date found significant cognitive dysfunction compared to matched controls with impairment of verbal memory, attention, verbal fluency and spatial reasoning (Camp *et al.* 1999); cognitive impairment correlated modestly with MRI parameters. A previous smaller study had reported that cognitive dysfunction was rare in primary progressive multiple sclerosis and was significantly less extensive than in secondary progressive multiple sclerosis (Comi *et al.* 1995). However, a more recent study comparing primary and secondary progressive multiple sclerosis reported only subtle differences between the two groups and found significant cognitive impairment in both groups (Foong *et al.* 2000).

2.2(d) Prognosis

Prognosis has been considered poorer in primary progressive multiple sclerosis as time from disease onset to reach advanced disability is shorter than in relapsing remitting multiple sclerosis (Runmarker & Andersen 1993; Weinshenker *et al.* 1989; Confavreux *et al.* 2000). However, compared with the progressive phase in secondary progressive multiple sclerosis, both the rate of progression (Runmarker & Andersen 1993) and the age of onset of progression are similar (Minderhoud *et al.* 1988). A recent study assessed several clinical parameters as predictors of long-term prognosis (Cottrell *et al.* 1999a); shorter time to disability status score 3 and involvement of three or more neurological systems at onset were identified as adverse prognostic factors.

2.2(e) MRI characteristics

Conventional cerebral MRI is atypical in the primary progressive group with a relative paucity of lesions, less gadolinium enhancement (suggesting less inflammation), fewer new

lesions developing over time and, as a consequence, a more striking discrepancy between MRI activity and clinical deterioration than is seen in other groups (Thompson *et al.* 1990, 1991; Stevenson *et al.* 1999). This discrepancy raises two possibilities: 1) that spinal cord involvement is more clinically relevant in this group, and 2) that changes in the normal appearing white matter may be particularly important. The findings from spinal cord imaging are discussed later in this chapter. Abnormalities in the normal appearing white matter of primary progressive multiple sclerosis have been demonstrated using more pathologically specific MRI measures and these will be discussed in chapter 3.

2.2(f) Pathology

The limited enhancement on MRI suggests that primary progressive multiple sclerosis may be a less inflammatory disease. A pathological study analysed 578 lesions from patients with primary progressive and secondary progressive multiple sclerosis (Revesz *et al.* 1994). Inflammation, as judged by the frequency of perivascular cuffing and cellularity of the parenchyma, was seen in primary progressive disease but there was significantly more inflammation in secondary progressive disease which is consistent with the MRI findings. It has also been reported that very little remyelination occurs in primary progressive multiple sclerosis (Lassmann *et al.* 1997) which is a possible explanation for the non-remitting course of the disease. A recent study, identifying distinct patterns of oligodendrocyte pathology in lesions, reported that lesions with a relative loss of oligodendrocytes and lack of remyelination were found more often in primary progressive disease compared to relapsing remitting and secondary progressive disease in which lesions with oligodendrocyte recruitment and extensive remyelination predominated (Lucchinetti *et al.* 1999). Other differences between multiple sclerosis subgroups, including more prolonged T-cell

infiltration in primary progressive disease, have been suggested but the data available is limited (Bruck *et al.* In press).

The relative lack of focal cerebral inflammation, as demonstrated pathologically and on MRI, in primary progressive multiple sclerosis may suggest that the greater burden of the disease lies in the spinal cord. A recent pathological and MRI study of the spinal cord was carried out in patients with primary progressive and secondary progressive multiple sclerosis (Lycklama a Nijeholt *et al.* 2001). In primary progressive disease there was extensive spinal cord involvement with predominantly a mild increase in signal intensity on proton density images; histopathologically this correlated with partial demyelination. Secondary progressive disease was associated with focal high signal abnormalities which represented typical demyelinated plaques. This suggests that there may be a more diffuse disease process affecting the spinal cord in primary progressive multiple sclerosis.

2.2(g) Immunology

Establishing the immunological profile of primary progressive multiple sclerosis may potentially play an important role in understanding the mechanism of disease progression in this group. Immunological differences between primary progressive multiple sclerosis and other subgroups of the disease have been reported but there is a wide overlap and no distinct immunogenetic mechanisms have as yet been identified.

Differences in antibody profiles between primary progressive and other subgroups of multiple sclerosis have been reported. Unfortunately data on intrathecal IgG production is difficult to interpret as most studies have not clearly distinguished between primary progressive and secondary progressive multiple sclerosis. Increased frequencies of anti-ganglioside antibodies, suggested to be a marker of axonal damage, have been demonstrated

in primary progressive multiple sclerosis (Acarin *et al.* 1996; Sadatipour *et al.* 1998).

Studies of soluble adhesion molecules have produced conflicting results. sICAM-1 and sVCAM-1 levels in serum have been reported to be both normal and raised in primary progressive patients compared to controls and to be both significantly and not significantly different to the other subgroups of multiple sclerosis (Giovannoni *et al.* 1997; Duran *et al.* 1999; McDonnell *et al.* 1999a). More notably serum levels of sE-selectin appear to be increased in primary progressive disease compared to other subgroups (Giovannoni *et al.* 1996; McDonnell *et al.* 1999a). In a CSF study sVCAM-1 and sICAM-1 were increased in all subgroups but sE-selectin was significantly increased in primary progressive compared to relapsing remitting disease (McDonnell *et al.* 1998).

The cytokine profile of primary progressive multiple sclerosis has also been studied but no distinct pattern has emerged (Neuhaus & Hartung 2001). Increased levels of IL-1β, IL-6 and TNF-α have been reported but were not significantly different from other subgroups of multiple sclerosis (Rovaris *et al.* 1996). A recent study found patients with primary progressive disease to have less pro- and more anti-inflammatory cytokine producing T cells than relapse onset disease (Killestein *et al.* 2001) but this has not been confirmed in another study (Duran *et al.* 2001). Heterogeneity of T cell function within primary progressive multiple sclerosis has been reported with increased interferon gamma production in patients with high lesion loads though this was in a small number of patients (Prat *et al.* 2000).

2.2(h) Genetics

The genetic profile of primary progressive also remains unclear. Relatively few genetic studies have been carried out specifically in primary progressive multiple sclerosis and patient numbers have often been small. Despite suggestion to the contrary from earlier

studies primary progressive multiple sclerosis appears to have the same association with the DR2 haplotype DR15 as relapsing remitting multiple sclerosis (Olerup *et al.* 1989; McDonnell *et al.* 1999b). However, different HLA associations have been reported between primary progressive and relapsing remitting multiple sclerosis suggesting immunogenetic heterogeneity. The DRw17,DQw2 haplotype has been found five times more commonly in relapsing remitting than primary progressive disease (Olerup *et al.* 1989; Hillert *et al.* 1992); in the same studies an association of the DQB1 restriction fragment seen in DRw4,DQw8, DR7,DQw9, DRw8,DQw4 with primary progressive disease was initially reported but later not verified. More recently an association between DR4 and primary progressive disease has been again reported (Weinshenker *et al.* 1998) but this has not been confirmed (Hillert 2000).

2.2(i) Disease mechanisms

If the mechanisms underlying impairment and disability are considered, more fundamental differences may become apparent. Whereas neurological deficit in relapsing remitting multiple sclerosis appears to result from incomplete remission from relapses, in primary progressive multiple sclerosis deficit arises from disease progression. These differences may relate to mechanisms of axonal loss, the probable correlate of fixed neurological deficit (Trapp *et al.* 1998). In relapsing remitting disease axonal loss may be related to acute inflammatory demyelination (Ferguson *et al.* 1997), whereas in primary progressive disease, it may result from a more diffuse process with low-grade inflammation. This is supported by the MRI finding that diffuse abnormalities of brain and spinal cord are more common in primary progressive than relapsing remitting or secondary progressive multiple sclerosis (Lycklama a Nijeholt *et al.* 1998); this finding has also been confirmed pathologically in the spinal cord (Lycklama a Nijeholt *et al.* 2001). Although inflammation

is less in primary progressive disease, it clearly occurs (Revesz *et al.* 1994) and it may be that there is a different relationship between inflammation and axonal loss, perhaps with axons being more susceptible to damage. Unfortunately, pathological studies have not looked at axonal loss specifically in primary progressive multiple sclerosis but there is MR evidence for axonal loss or dysfunction in both lesions and normal appearing white matter in this group as judged by a decreased NAA on spectroscopy (Davie *et al.* 1997).

2.2(j) A distinct entity?

It has been a source of debate as to whether primary progressive multiple sclerosis is a separate disease entity distinct from relapsing remitting and secondary progressive multiple sclerosis. The atypical characteristics of primary progressive multiple sclerosis have ordained that at the present time it should be considered as a distinct subgroup of multiple sclerosis particularly in the context of clinical trials. However, there is undoubtedly overlap between subgroups - patients with primary progressive disease may still have a relapse even decades after disease onset (Kremenchutzky *et al.* 1999) - and it seems most useful to consider primary progressive multiple sclerosis as one end of the spectrum of the disease of multiple sclerosis.

2.2(k) Therapeutic implications

Therapeutics in primary progressive multiple sclerosis has been a neglected area. The classification of primary progressive multiple sclerosis has only come into regular use in the last decade and was reinforced by the Lublin & Reingold consensus definitions (1996). Previously, poorly specific terms such as 'chronic progressive' multiple sclerosis have been used to describe patients in any progressive phase of the disease. Although there have been

trials in chronic progressive multiple sclerosis few trials have specifically addressed primary progressive multiple sclerosis. Despite the unique characteristics already discussed the question may still be raised as to whether or not specific trials for primary progressive multiple sclerosis are required. The answer to this is probably 'yes', if two issues are considered. First, the proposed differences in the mechanisms underlying impairment and disability may have implications for the choice of therapeutic agent; if axons in primary progressive multiple sclerosis are more susceptible to damage then therapeutic agents directed at axonal protection as well as at inflammation may be particularly useful in this group. Second, the atypical clinical and MRI characteristics pose particular problems in selecting patients for and designing therapeutic trials. The implications of these characteristics for patient selection, choice of outcome measures for therapeutic monitoring and duration and size of study are now discussed.

2.3 Unique Problems in Trial Recruitment and Design

2.3(a) Patient selection

When selecting patients for therapeutic trials in primary progressive multiple sclerosis their diagnosis has to be clearly established, at three levels. 1) The diagnosis of multiple sclerosis has to be made excluding other diseases which may present a similar clinical picture. 2) The certainty of the diagnosis has to be defined, conventionally according to Poser Criteria (Poser *et al.* 1983). 3) The correct classification of primary progressive multiple sclerosis has to be made.

The majority of patients with primary progressive multiple sclerosis present with a

single progressive symptom, usually paraparesis implicating the spinal cord (McDonnell & Hawkins 1996; Stevenson *et al.* 1999). Therefore, other causes of progressive pathology need to be excluded, particularly compressive spinal cord lesions including Arnold Chiari malformations and, intrinsic cord lesions such as angiomas.

In relation to the certainty of diagnosis, inclusion criteria for therapeutic trials conventionally require a diagnosis of clinically definite or laboratory-supported definite multiple sclerosis as defined by the Poser criteria (IFNB Multiple Sclerosis Study Group 1993; Jacobs *et al.* 1996). However, patients with primary progressive multiple sclerosis do not readily conform to these criteria. A recent retrospective study of patients with a diagnosis of primary progressive multiple sclerosis did not allow classification of any patient as clinically definite multiple sclerosis and only one third were classified as laboratory-supported definite multiple sclerosis (McDonnell & Hawkins 1998). Patients with only a single clinical lesion cannot be classified as clinically definite multiple sclerosis and so more emphasis has to be put on the presence of oligoclonal bands and paraclinical evidence of dissemination in time and space. The difficulty of diagnosis is also highlighted when considering the five "red flags" described by Rudick *et al.* (1986) alluding to the possibility of an incorrect diagnosis of multiple sclerosis. Patients with primary progressive disease commonly raise two of these flags, absence of clinical remission and localised disease.

To address the problem of certainty of diagnosis specific diagnostic criteria are required for primary progressive multiple sclerosis and such criteria have recently been developed (Thompson *et al.* 2000). Three levels of diagnostic certainty are defined - definite, probable and possible - based on clinical, cerebrospinal fluid, MRI and neurophysiological findings. Evidence of intrathecal IgG synthesis is of central importance and must be present for a definite diagnosis together with one of the following MRI criteria, derived in part from

Barkhof *et al.* (1997): 1) nine brain lesions, 2) two spinal cord lesions, or 3) four to eight brain lesions and one spinal cord lesion. These criteria have as yet only undergone limited validation on retrospective data (Thompson *et al.* 2000; Brieva *et al.* 2000) and were not available at the time of recruitment to the study presented in this thesis and so are not considered further here. The sensitivity of MRI as a diagnostic tool in primary progressive multiple sclerosis is also supported by the finding that 92% of brain MRI scans in a large cohort of primary progressive patients fulfilled Fazekas / Paty criteria (Kremenchutzky *et al.* 2000).

In the study presented in this thesis patients were selected on the basis of a progressive history without relapse or remission, presence of at least two lesions on MRI and either evidence of intrathecal IgG production and/or abnormal visual evoked potentials. Particular emphasis was placed on the CSF findings; oligoclonal bands were positive in CSF and not serum in all of the 78% of patients from whom results were available. In an on-going phase III trial of glatiramer acetate in primary progressive multiple sclerosis all subjects were required to have had a CSF examination emphasising the importance of evidence of intrathecal IgG synthesis in certainty of diagnosis (Wolinsky *et al.* 2001).

Correct application of the classification of primary progressive multiple sclerosis may also present problems. Establishing a history of gradually progressive disease may be more difficult than it would appear; the details of the initial presentation may fade with the passage of time and it may be difficult to distinguish retrospectively between fluctuations in function and a true neurological relapse. It may not be possible to make the classification early in the disease as it has been suggested that a minimum disease duration of one year is required to establish with confidence that the course is progressive without superimposed relapses (Thompson *et al.* 2000).

These difficulties in diagnosis and classification were highlighted in the recruitment for the current study. Only 50 of 138 patients referred with a diagnosis of primary progressive multiple sclerosis were enrolled. All the referrals were from experienced neurologists. The reasons for not including 88 patients are listed in Table 2.1. Forty three patients could not be classified as definite primary progressive multiple sclerosis. Twenty-six of these patients had a history of single relapse prior to their progressive syndrome (up to 17 years previously), at the onset of their condition or later during its course and by definition were classified as transitional progressive. Seven patients had a history of more than one relapse and were classified as secondary progressive. Ten patients could not be securely diagnosed as primary progressive; seven of these had a history of a progressive illness consistent with multiple sclerosis but could not be confidently diagnosed due to negative or atypical MRI and/or CSF findings; three had a history of less than two years or did not have a clear history of progression.

The remaining 45 patients were not included on the basis of their own decision or due to other medical, psychiatric or social problems. The 11 patients not included on medical grounds highlighted another problem of therapeutic trials in this population. The higher mean age of presentation of primary progressive multiple sclerosis makes this group liable to a greater incidence of general medical problems. In this study the mean age at entry was 45.3 years with a range between 25 and 59 years. Two patients were not included due to ischaemic heart disease and two due to cord compression secondary to intervertebral disc prolapse. Cervical spondylosis is also common in this age group and spinal cord compression may be present at entry into a clinical trial or it may develop during the trial and confound assessment. Patients with general medical problems may also be more sensitive to any toxic effects of therapeutic agents. Several patients above the age of 60 were referred but had to

be excluded because the increased incidence of non-specific white matter lesions in this age-group would potentially confound the already limited MRI analysis (Fazekas *et al.* 1988).

Table 2.1 Reasons for exclusion of referrals to the randomised controlled trial of interferon beta-1a in primary progressive multiple sclerosis

Reason for exclusion	**Number of referrals**
Transitional progressive MS	26
Patient decision	18
Not definite primary progressive MS	10
Secondary progressive MS	7
Psychosocial	6
Abnormal liver function tests	4
Cardiovascular disease	3
Cord compression	2
Other medical	2
Miscellaneous	10
Total	88

Whether stage or severity of disease should influence patient selection is not clear. There is growing evidence that axonal loss is occurring early in the disease course of multiple sclerosis but if the diagnosis cannot be made before one year patients with very early primary progressive disease are by definition excluded from trials. In established disease, whereas in relapsing remitting multiple sclerosis patients with frequent or severe relapses or significant residual deficits may be targeted, the majority of patients with primary progressive disease have a gradual course. However, rate of disease progression may be a guide to 'active' patients as change in EDSS in the short term has been reported to predict faster disease progression in the longer term (Losseff *et al.* 1996a).

The difficulty in finding suitable candidates for a therapeutic trial in this group emphasises the rarity of primary progressive multiple sclerosis. Although this study is being carried out in a single centre in London, patients were recruited from all over England and Wales. Larger studies would require multiple centres and this has been illustrated by the phase III trial of glatiramer acetate which has had to recruit subjects from multiple centres on an international scale.

2.3(b) Outcome measures

The primary outcome of a definitive trial in the treatment of established multiple sclerosis has to be clinical (Whitaker *et al.* 1995). However, MRI is now widely accepted as a surrogate marker of disease activity, either as a primary outcome in preliminary short-term trials in relapsing remitting and secondary progressive multiple sclerosis or as a secondary outcome in definitive long-term trials in relapsing remitting, secondary progressive and primary progressive multiple sclerosis (Miller *et al.* 1996). The choice of clinical and MRI

outcome measures poses unique problems in the primary progressive group and also has implications for the duration and size of the study.

Clinical outcome measures

The conventional clinical outcome measures in treatment trials in established multiple sclerosis assess relapses and disease progression. Relapse frequency and severity are not applicable to the primary progressive population. Disease progression is a relevant outcome but there are limitations with the currently available clinical scales to monitor progression. The most widely used scale is the Kurtzke Expanded Disability Status Scale (EDSS) (Kurtzke 1983), which is based on the neurological examination, assessing eight functional systems (pyramidal, cerebellar, brainstem, sensory, sphincter, visual, cerebral, 'other') and disability (including ambulation) and is scored on an ordinal scale (Appendix). The EDSS can be applied to patients with primary progressive multiple sclerosis but there are problems with its validity and reliability (Willoughby & Paty 1988; Noseworthy *et al.* 1990) and its responsiveness is poor (Hobart *et al.* 2000). Responsiveness of a clinical scale is particularly important when disease progression is slow and when small changes may be clinically significant. The degree of change on the scale that reliably reflects clinical change has to be established. To allow for variability in the EDSS the progression of 1.0 point has been recommended as an outcome for clinical trials (Noseworthy *et al.* 1990) although a change of 1.5 points may be more accurate (Francis *et al.* 1991). For patients with an EDSS of 5.5 or above at entry, a progression of 0.5 points has been suggested to be sufficient as changes at these levels may be more easily discernible (Goodkin 1991) though this has not been validated.

Despite its limitations the EDSS has remained the first choice for clinical trials in

multiple sclerosis. However, a functional composite measure which incorporates quantitative tests of arm, leg and cognitive function - the multiple sclerosis functional composite (MSFC) - has recently been developed (Cutter *et al.* 1999). Early evaluation has confirmed its validity (Kalkers *et al.* 2000) but not whether it confers benefit over the EDSS (Ciccarelli *et al.* In press). The MSFC is now being included in longitudinal clinical trials which will provide more information on its sensitivity and reliability.

MRI outcome measures

MRI outcome measures can be divided into conventional measures which largely reflect non-specific inflammation and measures which are potentially more pathologically specific. Existing recommendations for MRI outcome measures in relapsing remitting and secondary progressive multiple sclerosis include the conventional measures of new T_2 weighted cerebral lesions and gadolinium enhancing lesions in preliminary trials and change in T_2 weighted cerebral lesion load in definitive trials (Miller *et al.* 1996). However, in primary progressive multiple sclerosis the rate of development of new lesions is low (Thompson *et al.* 1991; Stevenson *et al.* 1999) with only a small proportion enhancing, 5% compared to 87% in secondary progressive multiple sclerosis (Thompson *et al.* 1991). T_2 weighted cerebral lesion load may be a responsive measure, since significant change has been demonstrated over one and two years (Stevenson *et al.* 2000b; Ingle *et al.* In press), but no correlation has been shown to date between T_2 lesion load and EDSS in cross-sectional (Stevenson *et al.* 1999) or longitudinal studies (Stevenson *et al.* 2000b). Triple dose gadolinium may increase the yield of enhancing lesions (Filippi *et al.* 1995c), although this has not been confirmed (Silver *et al.* 1997a) and fast flair imaging may increase detection of subcortical lesions in this group (Gawne-Cain *et al.* 1997). However, even with such

optimisation, the role of conventional MRI measures as markers of disease activity remains limited in primary progressive multiple sclerosis.

The lack of clinical correlation of conventional MRI measures may be a reflection of the clinical presentation and/or the underlying pathological mechanisms in primary progressive multiple sclerosis. Considering the clinical presentation, the majority of primary progressive patients present with spinal cord syndromes, 83% in a recent study of 158 patients (Stevenson *et al.* 1999), and so cerebral imaging may be missing the clinically relevant lesion load. Disappointingly, however, there is no correlation between spinal cord lesion load and disability in cross-sectional studies (Kidd *et al.* 1993; Stevenson *et al.* 1999) and either no or weak correlations in longitudinal studies over one year (Kidd *et al.* 1996; Stevenson *et al.* 2000b).

The findings on conventional MRI are not surprising given the pathological finding of less inflammation in primary progressive disease (Revesz *et al.* 1994). It appears likely that pathologically specific MRI markers of tissue destruction, in particular of axonal loss, may be more clinically relevant in this group. Such markers include T_1 weighted hypointense lesions, spinal cord and cerebral atrophy, MTR, MRS and diffusion imaging; the putative pathological significance of these markers has been discussed elsewhere. T_1 hypointense lesion load may be a responsive measure in that significant change has been demonstrated in only one year but disappointingly it has not correlated with disability in primary progressive multiple sclerosis in cross-sectional or longitudinal studies (Stevenson *et al.* 1999; Stevenson *et al.* 2000b).

Measures of atrophy appear to be more promising markers of disease progression. Spinal cord cross-sectional area has been shown to correlate with disability in primary progressive multiple sclerosis in a cross-sectional study (Stevenson *et al.* 1999) and

significant spinal cord atrophy has been demonstrated in this group in only one year (Stevenson *et al.* 1998b; Stevenson *et al.* 2000b). However, spinal cord atrophy has not been shown to correlate with progression in disability even over two years (Ingle *et al.* In press) but a five year follow-up study is underway to evaluate this further. Using a measure of partial brain volume a correlation with disability cross-sectionally and significant cerebral atrophy over one year have been demonstrated in primary progressive multiple sclerosis (Stevenson *et al.* 1999, 2000b). A more sensitive and reproducible technique has also confirmed whole brain atrophy and ventricular enlargement occurring over one year in patients with primary progressive multiple sclerosis (Fox *et al.* 2000).

Owing to the relative paucity of lesions in primary progressive multiple sclerosis it appears likely that intrinsic changes in normal appearing white matter (NAWM) may make a major contribution to disability in this group. MTR and MRS are potentially powerful tools to study changes in NAWM as well as in lesions. A significant reduction in MTR in lesions but not NAWM was found in a study of a small number of primary progressive patients (Gass *et al.* 1994) although MTR has been reported to be lower in NAWM in patients with progressive multiple sclerosis (Filippi *et al.* 1995b). More recent studies using MTR histograms have identified differences in MTR parameters between primary progressive and other groups of multiple sclerosis in both the brain and cervical cord (Filippi *et al.* 1999; Tortorella *et al.* 2000; Filippi *et al.* 2000b). Further work including longitudinal studies is still required to evaluate the role of MTR in monitoring disease progression. Using MRS a preliminary study demonstrated a reduction in NAA in lesions and NAWM (Davie *et al.* 1997) and more recent studies will be discussed in chapter 3. Longitudinal studies of both MTR and MRS are required to evaluate their role in monitoring disease progression and this will be discussed further in chapter 6.

Finally, a preliminary study of diffusion imaging in primary progressive multiple sclerosis has shown increased apparent diffusion coefficient in lesions (Droogan *et al.*1999) but further work is required to evaluate this as a disease marker.

In summary, although these more pathologically specific measures are potentially useful tools to monitor disease progression, they are not yet well validated in serial studies and need further evaluation. Currently in primary progressive multiple sclerosis there is no proven MRI measure capable of reliably detecting short-term change which is predictive of longer term disability (Losseff *et al.* 1996a). There is therefore no valid MRI measure that can be used as a primary outcome in therapeutic trials.

Aside from clinical validation, consideration has to be given to the reliability of a technique. Therapeutic trials may run for several years, involving many centres and techniques must be able to reliably detect change above the limits of any possible measurement variation. Consequently, quality assurance is an essential part of any MRI measure used in clinical trials. The acquisition time of a technique is also important as there is a limit to how long patients, particularly with higher levels of disability, can tolerate lying in a scanner. Movement artefact during long scanning sessions will also adversely effect the quality and reproducibility of results. Other limiting factors are availability and cost of scanner time. A multiparametric MRI protocol may improve sensitivity and specificity but acquisition times may become unacceptably long. Further work is needed to evaluate multiparametric approaches which are practical, responsive and reliable over time.

2.3(c) Study duration and sample size

The choice of outcome measures is a major determinant of the duration and sample size of any study. In a trial of primary progressive multiple sclerosis the primary outcome

currently has to be disease progression and the study has to be sufficiently long to ensure that the disease will have progressed in a significant number of patients. Using the Kurtzke EDSS the rate of progression in multiple sclerosis is on average 0.5 points a year (Weinshenker *et al.* 1989) but varies at different levels of the scale, being slower at lower and higher scores. It has been reported that a statistically significant therapeutic effect in multiple sclerosis using the EDSS as the primary outcome measure can be demonstrated with approximately 150 patients per treatment arm and a study duration of two to three years (Rudick *et al.* 1996). Specific information on the rate of disease progression in primary progressive multiple sclerosis has recently been provided by a natural history study of 216 primary progressive patients (Cottrell *et al.* 1999a). From this data sample size calculations for clinical trials in primary progressive multiple sclerosis have been published (Cottrell *et al.* 1999b). However, this data was not available for the study presented in this thesis.

For the study presented in chapter 5 an estimate was made as to the percentage of subjects treated with placebo expected to progress within two years based on historical data from two small studies (Kidd *et al.* 1996; Losseff *et al.* 1996a). Combining the data from these studies 16 of 24 subjects (67%) were calculated to experience disease progression (as defined in chapter 5) within two years. As funding was only available for a pilot study, a sample size of 50 subjects was actually chosen as a practical number to recruit and manage at one site. Thirty-three subjects were therefore predicted to reach the primary clinical endpoint but with such a small number the study was not anticipated to detect significant treatment effect.

2.4 Therapeutic Trials

2.4(a) Introduction

Currently there is no definitively proven disease modifying treatment available for primary progressive multiple sclerosis. Several trials have been carried out in chronic progressive multiple sclerosis but without clear distinction between primary and secondary progressive disease. Some of these trials have included patients with primary progressive multiple sclerosis but there is insufficient evidence available to recommend their use in this group. More recently, a number of trials have been specifically designed for primary progressive multiple sclerosis. These trials are either not yet completed or peer-reviewed publication of results is awaited.

2.4(b) Trials including patients with primary progressive multiple sclerosis

Azathioprine

A randomised controlled trial of azathioprine included a subgroup of 51 patients with progressive disease from onset. Although analysis of the whole group showed a small beneficial effect, no significant effects were seen in the patients with progressive disease from onset (British and Dutch MS Azathioprine Trial Group 1988).

Methotrexate

Eighteen patients with primary progressive multiple sclerosis were included in the double-blind, placebo-controlled trial of low dose oral methotrexate in chronic progressive

multiple sclerosis (Goodkin *et al.* 1995). Although less progression was reported in the methotrexate group, the result was not significant when considering the primary progressive group alone.

2-chlorodeoxyadenosine (Cladribine)

In the double-blind, placebo-controlled trial of subcutaneous cladribine in progressive multiple sclerosis 48 patients had primary progressive disease (Rice *et al.* 2000). No clinical efficacy was apparent in the primary progressive group. A significant treatment effect on enhancing lesions was reported for the whole cohort but was not seen on subgroup analysis of the primary progressive group.

Intravenous Immunoglobulin

A double-blind, placebo-controlled study of intravenous immunoglobulin is currently underway in 110 patients with secondary progressive and 50 patients with primary progressive multiple sclerosis (Poehlau *et al.* 1997). The primary outcome measure is the EDSS but no MRI measures are included.

2.4(c) Trials specifically designed for primary progressive multiple sclerosis

Interferon Beta-1a

This study is presented in Chapter 5.

Interferon Beta-1b

Currently nearing completion is a double-blind, placebo-controlled trial of

subcutaneous interferon beta-1b (8 MIU alt dies) in 70 patients with primary progressive and transitional progressive multiple sclerosis (Montalban *et al.* 1998). Clinical outcome measures include the EDSS and MRI outcomes include lesion loads, cervical cord area, magnetization transfer ratio and spectroscopy.

Glatiramer Acetate

A double-blind, placebo-controlled trial of subcutaneous glatiramer acetate (20 mg daily) is currently underway (Wolinsky *et al.* 2001). It is the largest therapeutic trial to be carried out in primary progressive multiple sclerosis. Approximately 900 subjects have been recruited from North America and Europe with the use of strict clinical criteria. The primary outcome measure is the EDSS and secondary outcomes include the MSFC and MRI measures.

Mitoxantrone

A double-blind, placebo-controlled trial of mitoxantrone in 54 patients with primary progressive multiple sclerosis has recently got underway (Kita *et al.* 2000). Outcome measures include the EDSS, nine hole peg test and MRI measures.

Riluzole

A small pilot cross-over study of the neuroprotective agent riluzole has recently been completed (Kalkers *et al.* 1999). Sixteen patients had six monthly clinical and MRI evaluations for one year untreated and one year on treatment. Publication of results is awaited.

2.5 Conclusion

Until recently therapeutics in primary progressive multiple sclerosis has been a neglected area but with the advent of effective disease modifying drugs this group should no longer be excluded from therapeutic trials. Recruiting to and designing therapeutic trials in primary progressive multiple sclerosis presents unique difficulties but is an important and worthwhile challenge. Certainty of diagnosis has been a key problem but this can now be addressed by newly developed diagnostic criteria. Further work is required to validate reliable and responsive clinical and MRI markers of disease progression to facilitate therapeutic trials. Further investigation of the pathogenesis of disease progression is also required to guide the development of future therapeutic agents which should aim to target the underlying pathological process.

CHAPTER 3: NOVEL MARKERS OF DISEASE PROGRESSION IN PRIMARY PROGRESSIVE MULTIPLE SCLEROSIS

3.1 Introduction

One of the key problems in carrying out therapeutic trials in primary progressive multiple sclerosis is the lack of reliable and valid markers of disease progression. Conventional MRI measures are poorly responsive and have little clinical correlation in primary progressive multiple sclerosis. More pathologically specific measures are now available which potentially may be more clinically relevant. Atrophy measures look particularly promising and validation in primary progressive multiple sclerosis is already underway. Other potentially useful techniques include MTR and MRS, particularly when applied to NAWM, but their clinical correlation in primary progressive multiple sclerosis has not yet been fully investigated.

In this chapter the role of MRI examination of NAWM in evaluating disease progression in primary progressive multiple sclerosis is considered. The hypothesis that changes in NAWM contribute to disease progression in multiple sclerosis is supported by the presence of abnormalities in NAWM in pathological and MRI studies. On pathological examination, abnormalities in NAWM in multiple sclerosis have been found including gliosis, demyelination and inflammation (Allen & McKeown 1979) as well as axonal damage or loss (Trapp *et al.* 1998; Lovas *et al.* 2000). In MRI studies of patients with multiple sclerosis, longer relaxation times (Ormerod *et al.* 1986b; Miller *et al.* 1989) and increased apparent diffusion coefficient (Christiansen *et al.* 1993a; Droogan *et al.* 1999) have been reported, probably reflecting increased water content and expanded extracellular space. With

MRS, decreased levels of NAA, a potential marker of axonal loss or dysfunction, have been demonstrated in NAWM in multiple sclerosis (Husted *et al.* 1994; Davie *et al.* 1994; Fu *et al.* 1998). A reduction in MTR has also been demonstrated in NAWM (Dousset *et al.* 1992; Filippi *et al.* 1995b; Loevener *et al.* 1995).

Changes in NAWM also appear to be relevant to the pathogenetic evolution of lesions. Decreases in MTR in NAWM have been demonstrated to precede the appearance of enhancing lesions (Filippi *et al.* 1998b; Goodkin *et al.* 1998) though this has not been universally proven (Silver *et al.* 1998). An increase in apparent diffusion coefficient has also been reported to precede the development of enhancing lesions (Werring *et al.* 2000b) though preliminary results from another study found a pre-lesional decrease (Gass *et al.* 1999). Although there are discrepancies such studies suggest that structural abnormalities in pre-lesional NAWM are occurring before there is evidence of blood-brain barrier breakdown. Pathological processes other than inflammation may therefore be involved in the early evolution of lesions though it is also likely that subtle inflammation may be occurring without detectable gadolinium enhancement. Study of NAWM has also provided information on the remote effects of lesions. Increases in apparent diffusion coefficient (Werring *et al.* 2000b) and decreases in NAA (de Stefano *et al.* 1999) have been found in the NAWM contralateral to acute lesions. This indicates that neuronal damage or dysfunction in lesions causes similar effects in the contralateral NAWM perhaps by involvement of inter-hemispheric projections. Despite the recent interest in NAWM the majority of studies have not included patients with primary progressive multiple sclerosis and studies of NAWM in this group have been limited. In this chapter the largest cross-sectional studies carried out to date of MTR and MRS of NAWM in primary progressive multiple sclerosis are presented and discussed.

3.2 Magnetisation Transfer Ratio of Normal Appearing White Matter

3.2(a) Introduction

MTR provides an index of tissue structural integrity and a reduction in MTR is expected in the presence of tissue destruction. A preliminary study of MTR in primary progressive multiple sclerosis, using a region of interest approach, demonstrated a significant reduction in lesions but not in NAWM (Gass *et al.* 1994). However, the number of patients examined in this study was small (n=10) and so in this study MTR of NAWM was examined in a larger cohort of patients.

3.2(b) Methods

Fifty-two patients (32 male, 20 female) with primary progressive multiple sclerosis were recruited from the cohort being screened for the trial of interferon beta-1a before the onset of treatment. Twenty-six control subjects (15 male, 11 female) were also studied. As changes in MTR may occur with ageing (Silver *et al.* 1997b) the controls were age-matched as far as possible. All patients underwent a clinical examination and were scored on the EDSS by a single observer.

MRI was carried out using a 1.5 T Signa Echospeed Horizon system (General Electric, Milwaukee, Wisc., USA). A dual spin echo sequence (28 contiguous 5mm axial slices, TE 30/80ms, TR 1720ms, 0.75 NEx, 256x128 matrix, FOV 24x18cm) was performed with and without presaturation pulses (total acquisition time 20 minutes). The presaturation pulse was a Hamming-apodised three lobe sinc pulse with a duration of 16 ms and a peak

amplitude of 23.2 μT, giving a nominal bandwidth of 250 Hz, applied 1 kHz off water resonance. Scans with and without presaturation were interleaved for each TR period providing precise co-registration (Barker *et al.* 1996).

MTR was calculated for each pixel by the formula ($[M_0 - M_s] / [M_0]$) x 100 percent units where M_s and M_0 represent signal intensities with and without presaturation respectively. MTR analysis was carried out using image display software (DispImage, DL Plummer, University College, London, UK) by a single observer blinded to patient identity. A standard template for regions of interest in the pons (77.3 mm^2) and genu of the corpus callosum (22.9 mm^2) and for bilateral regions in the centrum semiovale (77.3 mm^2) , frontal white matter (49.2 mm^2), parieto-occipital white matter (49.2 mm^2) and posterior limb of the internal capsule (59.8 mm^2) was created. The template was applied in each subject to appropriate regions of NAWM on the non-presaturation proton density images, with cross-reference to the T_2 weighted images. Regions were also visualised in the slices above and below to ensure they remained in NAWM. Any regions containing focal lesion, grey matter, cerebrospinal fluid or contaminated by motion artefact were not included in the study. MTR measurements were then made on the corresponding regions on the inherently co-registered calculated MTR images. Absolute MTR values were obtained from the genu of the corpus callosum and pons, and mean values were calculated from bilateral regions in the centrum semiovale, frontal white matter, parieto-occipital white matter and posterior limb of the internal capsule.

Statistical analysis was carried out using the independent sample t-test as a parametric test and the Mann-Whitney test as a non-parametric test. Correlations were performed using the Spearman's Rank Correlation Coefficient.

3.2(c) Results

There was no significant difference between the mean ages of the patients (45.4 years, range 25-59 years) and controls (42.5 years, 25-56 years; p=0.19). The median disease duration was 6 years (range 2-21 years). The median EDSS score was 5.25 (range 2.0-7.0).

The median MTR was lower in all regions in patients compared to controls and this was statistically significant in the corpus callosum, centrum semiovale and frontal white matter (Table 3.1). MTR in the corpus callosum and parieto-occipital white matter correlated with disability as scored on the EDSS (Table 3.2). There was no correlation between MTR and disease duration.

3.2(d) Discussion

This study demonstrated small but widespread reductions in MTR in NAWM in primary progressive multiple sclerosis, consistent with previous studies in other groups of multiple sclerosis. These small reductions in MTR suggest that there may be a subtle but diffuse pathological process occurring in NAWM distinct from focal lesions. This may help to explain why patients with primary progressive multiple sclerosis often develop severe disability despite a paucity of lesions on conventional MRI and supports the hypothesis that intrinsic changes in NAWM contribute to disability in this group.

Table 3.1 MTR values (percent units) in controls and patients.

		Controls	Patients	
Pons	Number	26	41	
	Median	38.49	38.03	p=0.07
	Range	36.07-40.46	34.99-40.14	
Corpus callosum	Number	24	35	
	Median	40.63	39.73	p=0.01*
	Range	39.20-42.03	36.51-42.00	
Frontal	Number	26	33	
	Median	39.59	39.11	p=0.01*
	Range	37.75-40.86	36.25-40.49	
Parieto-occipital	Number	24	24	
	Median	37.58	37.53	p=0.43
	Range	36.38-39.11	36.34-38.70	
Internal Capsule	Number	26	33	
	Median	37.31	36.77	p=0.29
	Range	34.07-38.47	34.88-38.14	
Centrum Semiovale	Number	26	32	
	Median	37.82	37.21	p=0.045*
	Range	35.92-38.98	36.16-38.59	

*p-value<0.05

Table 3.2 Correlation between MTR and EDSS.

	SRCC	
Pons	-0.001	p=0.99
Corpus Callosum	-0.35	p=0.04*
Frontal	-0.13	p=0.47
Parieto-occipital	-0.47	p=0.02*
Internal Capsule	0.12	p=0.51
Centrum Semiovale	-0.24	p=0.18

SRCC = Spearman's Rank Correlation Coefficient

*p-value<0.05

MTR allows quantitative assessment of tissue destruction although the extent of its pathological specificity is not clear. It has been proposed that large reductions in MTR may reflect demyelination, distinguishing this from the smaller changes seen as a result of oedema (Dousset *et al.* 1992). Profound MTR reduction has been demonstrated in other CNS diseases in which the predominant pathology is demyelination (Silver *et al.* 1996; Dousset *et al.* 1997) and in an animal model of demyelination (Dousset *et al.* 1995). With spectroscopy, marker peaks thought to represent myelin breakdown products have been reported to correlate with MTR (Hiehle *et al.* 1994). However, axonal loss may also cause a reduction in MTR. In animal studies small reductions in MTR have been demonstrated in conjunction with the histopathological findings of axonal damage (Lexa *et al.* 1994; Kimura *et al.* 1996). More recently an MRI study of postmortem tissue from patients with multiple sclerosis confirmed that MTR strongly correlates with axonal density in both lesions and NAWM (van Waesberghe *et al.* 1999); a modest correlation was also seen between MTR and myelin density in active demyelinating lesions. Gliosis may conceivably have an effect on MTR but this is yet to be elucidated. The small reductions in MTR seen in this study may be consistent with any of these pathological changes.

The reductions in MTR seen in this study are much smaller than the reductions seen in lesions (Dousset *et al.* 1992; Gass *et al.* 1994) or in pre-lesional NAWM (Filippi *et al.* 1998b). It appears likely that the small but widespread reductions in this study represent a more diffuse abnormality in the NAWM; this may be due to a truly diffuse change, to small discrete lesions beyond the resolution of the images (Barbosa *et al.* 1994) or to changes in the periphery of visible focal lesions (Filippi *et al.* 1995b). This hypothesis is also supported by a recent study in a small group of primary progressive patients (n=10) using MTR histograms (Filippi *et al.* 1999) which, despite an almost normal average brain MTR and

normal peak position, found a reduction in peak height (indicating a reduction in the amount of tissue with normal MTR) and suggested that this may be due to diffuse changes in NAWM. An MTR histogram study of normal appearing brain tissue, predominantly consisting of NAWM, in primary progressive multiple sclerosis (n=13) also found a reduction in peak height as well as a reduction in average MTR with normal peak position again suggesting widespread but mild changes (Tortorella *et al.* 2000). Histogram analysis has subsequently been carried out on 46 of the primary progressive patients from the study presented here (Dehmeshki *et al.* 2001). A reduction in average brain MTR was again found and there was a slight left shift of peak position (indicating more tissue at a lower MTR). These changes are consistent with the finding from the study here of widespread reductions in MTR. The peak height was normal, though inspection of the group curve indicated a trend for this also to be lower. This may at least partly reflect the heterogeneity of this larger cohort in which there was a wide range of lesion loads.

It should be noted that regions of NAWM were not identified in all areas in all subjects, predominantly in the patient group. In seven patients no values for analysis were obtained. This reflects the stringency of the criteria applied in selecting NAWM. This was to avoid not only visible focal lesion but also the partial volume effects of grey matter and cerebrospinal fluid which may cause bias in measurements, particularly in patients with cerebral atrophy. The mean of bilateral values was taken from non-midline regions to minimise any effects of laterality (Silver *et al.* 1997b). The use of a region of interest approach rather than a histogram approach allowed detection of subtle regional reductions in MTR which may be lost in a global analysis.

The significance of MTR changes in NAWM in relation to disability is yet to be established. A correlation between lesion MTR and disability has previously been reported

(Gass *et al.* 1994). In this study there were limited correlations between MTR in NAWM and disability. The significance of the correlation in the parieto-occipital white matter is doubtful, there being no difference in MTR between the patient and control groups in this region, as is its biological significance as changes in this area would not be expected to make a major contribution to the EDSS score. It is perhaps surprising that no correlations were seen in the pons or internal capsule given the known locomotor bias of the EDSS. However, as only the brain was studied, the paucity of clinical correlation may reflect that changes in the spinal cord are more likely to significantly contribute to locomotor disability. Other factors to consider are that the study was restricted to a disability range of EDSS 2.0-7.0 and so patients with severe disability were not included and that cross-sectional studies are limited in evaluating relationships with disability. Change in EDSS may be a more robust measure of disability and so longitudinal studies are required to establish whether measurement of MTR in NAWM is useful in the monitoring of disease progression in this group. Interestingly, in the cohort of patients from this study on which histogram analysis was later performed, a significant correlation with disability ($r=0.40$, $p<0.025$) was seen on principal component analysis, a method which provides a global characterisation of variation in the MTR histogram (Dehmeshki *et al.* 2001).

In conclusion, this study supports the hypothesis that there is diffuse tissue damage in NAWM in primary progressive multiple sclerosis that may contribute to disability in this group.

3.3 ^{1}H Magnetic Resonance Spectroscopy of Normal Appearing White Matter

3.3(a) Introduction

Axonal loss is likely to be a major contributor to disease progression in primary progressive multiple sclerosis. MRS, through measurement of NAA, provides a unique tool to investigate this. A preliminary study found a reduction in N-acetyl derived groups in lesions and NAWM in patients with primary progressive multiple sclerosis (n=6) but not in patients with benign disease suggesting that axonal loss is relevant to the development of disability (Davie *et al.* 1997). This was the first study to provide evidence for axonal loss in NAWM in primary progressive multiple sclerosis. As this is an important issue in understanding the mechanisms of disease it was essential to carry out a study to verify these initial observations in a larger cohort of patients.

3.3(b) Methods

Twenty-four patients with primary progressive multiple sclerosis (16 male, 8 female) were recruited from the larger cohort being screened for the trial of interferon beta-1a before the onset of treatment. The mean age of the patients was 48 years (range 33-59). Sixteen healthy volunteer controls (9 male, 7 female) were also studied. As the concentration of metabolites in normal brains may change with aging (Soher *et al.* 1996; Pfefferbaum *et al.* 1999) controls were age-matched as far as possible (mean age 46 years, range 31-62). All subjects gave their informed consent prior to their inclusion in the study. All patients underwent a clinical examination and were scored on the EDSS by a single observer.

Magnetic resonance imaging and spectroscopy were carried out on a 1.5T Signa Horizon Echospeed system with a standard quadrature head coil (General Electric, Milwaukee, Wisc., USA). Nineteen 5mm axial slices with 1.5mm gap were acquired with a T_2 weighted dual echo FSE sequence (TE 14/84ms, TR 3000ms, matrix 256x192). A slice was then chosen at the level of the lateral ventricles and a volume of interest was selected (patients median 2.74ml, range 0.96-8.10ml; controls median 2.22ml, range 1.13-3.23ml; p=0.263) from an area of NAWM in a periventricular or posterior parietal region. The voxel was also visualised in the slices below and above to ensure it remained in NAWM. Voxels containing any visible lesion or grey matter were not included in the study. ^{1}H spectra were acquired using PROBE/SV (PROton Brain Exam / Single Voxel), a version of the PRESS sequence (Bottomley 1988; Ordridge *et al.* 1985), with automatic shimming and water suppression (TE 30ms, TR 3000ms; 192 averages collected using an eight step phase cycle in ten minutes).

Spectra were analysed using the LCModel method which analyses the in vivo spectrum as a linear combination of a basis set of complete model spectra of metabolite solutions (Provencher 1993). This method is almost fully automatic, only requiring the user to input acquisition parameters, thus minimising user variability. Quality of the spectra was reviewed by an observer blinded to clinical status and spectra with visually judged poor signal to noise or poor fitting by the LCModel were not included in the study. The metabolite to creatine (Cr) concentration ratios were calculated for all patients (n=24) and controls (n=16). Absolute quantification of metabolite concentrations was possible for spectra which were acquired following the institution of a protocol of quality assurance (patients n=18, controls n=16). Quality assurance (QA) was achieved by scanning a phantom, consisting of a 50 mM N-acetyl aspartate solution, weekly. These measurements also provided a

calibration factor required by the LCModel for absolute quantification (Provencher 1993). The concentration of N-acetyl derived groups, NA - the sum of NAA and N-acetyl aspartyl glutamate, a metabolically related compound found in much smaller concentrations (Birken & Oldendorf 1989) - was measured, as well as NAA individually, as the spectral peaks of these two compounds are similar and may be difficult to separate reliably on in vivo spectra. The concentration of creatine was also measured.

Statistical analysis was carried out by comparing the patient and control groups using the Mann-Whitney test. Correlations were performed using the Spearman's Rank Correlation Coefficient.

3.3(c) Results

The results are presented in Table 3.3. There was no significant difference between the ages of the patient and control groups (p=0.42). The median EDSS score of the patients was 4.5 (range 2.0-7.0). The median disease duration was 6 years (range 2-19 years).

In the whole patient group there was a significant reduction of NA/Cr and NAA/Cr compared to controls. There was also a significant reduction in absolute concentration of NA and NAA in patients compared to controls. There was no significant difference between the creatine concentration in patients and controls. There was no significant correlation between any metabolite ratio / concentration and EDSS or disease duration.

Table 3.3 Metabolite ratios and concentrations of controls and patients.

	Controls	Patients	
Number - Ratio	16	24	
Median NA/Cr	1.70	1.40	p=0.006
(Range)	(1.27-2.14)	(0.86-1.91)	
Median NAA/Cr	1.32	1.12	p=0.01
(Range)	(1.05-1.71)	(0.35-1.77)	
Number - Concentration	16	18	
Median [NA] (mM)	7.77	6.90	p=0.03
(Range)	(6.60-9.71)	(4.62-10.38)	
Median [NAA] (mM)	6.25	5.60	p=0.045
(Range)	(4.56-8.43)	(2.96-7.89)	
Median [Cr] (mM)	4.54	4.82	p=0.27
(Range)	(3.72-5.50)	(3.44-7.94)	

3.3(d) Discussion

This study has demonstrated a significant reduction in NA in NAWM of patients with primary progressive multiple sclerosis compared to controls; this finding has been confirmed in more recent studies (Cucurella *et al.* 2000; Suhy *et al.* 2000). This supports the hypothesis that there is axonal loss in NAWM in primary progressive multiple sclerosis and that, as it occurs despite the paucity of focal inflammation, there may be a more diffuse pathological process causing axonal damage.

Although the reduction of N-acetyl derived groups in this and previous studies (Husted *et al.* 1994; Davie *et al.* 1994; Fu *et al.* 1998) may well reflect axonal loss, the possibility that these reduced values are indicative of axonal dysfunction rather than loss cannot be discounted. In serial studies of lesions both progressive and reversible changes in NAA have been reported (Arnold *et al.* 1992; Arnold *et al.* 1994; Davie *et al.* 1994). Serial examination will show whether the changes in this study are progressive, static or reversible and this will be presented subsequently.

The pathological relationship between axonal damage in NAWM and focal inflammation is not clear. Focal inflammation in multiple sclerosis may cause axonal damage in distant NAWM as illustrated by the finding of a transient reduction in NAA in the NAWM contralateral to acute lesions (De Stefano *et al.* 1999). However, the paucity of lesions in primary progressive multiple sclerosis may suggest that axonal damage in NAWM occurs independently of focal inflammation. It may also be tempting to hypothesise that axonal damage in NAWM may be more predominant in primary progressive multiple sclerosis than in other groups which would explain the discrepancy between focal inflammation and disability. However, there is no evidence for this and a study comparing NAA in patients

with primary and secondary progressive multiple sclerosis, which demonstrated significant reductions in NAWM and to a greater degree in lesions in both groups, found no significant differences between the two groups (Cucurella *et al.* 2000). Similarly, no significant differences in NAA in either NAWM or lesions are seen between primary progressive and relapsing remitting multiple sclerosis (Suhy *et al.* 2000).

In this study there was no correlation between reduction in NA in NAWM and disability as measured by the EDSS. This is not surprising as single voxel MRS only studies a very small localized volume of the CNS and sampling in this study was from the periventricular and posterior parietal white matter which would not be expected to have a major impact on the locomotor biased EDSS. However, a study using single slice spectroscopic imaging demonstrated a significant correlation between NAA/Cr and disability in patients with relapsing remitting multiple sclerosis (Fu *et al.* 1998). This correlation remained significant when considering voxels within NAWM but not within lesions suggesting that axonal damage in NAWM may make a major contribution to disability.

In this study the concentration of NA was measured rather than NAA alone because the combined information could be determined with greater confidence. Metabolite concentration ratios were measured as well as absolute concentrations to include the larger cohort of patients for whom reliable QA of absolute measurements was not available. The absolute concentration of NA is more specific than the NA/Cr ratio as a decreased ratio may reflect decreased NA or increased creatine. However, there was no significant difference in absolute creatine concentrations between patients and controls, indicating that the reduction in NA/Cr was due to a reduction in NA.

It is of interest that this study demonstrated normal creatine concentrations in NAWM. This is in keeping with a pathological study which demonstrated reduced creatine

concentrations from multiple sclerosis lesions but normal concentrations from NAWM (Davies *et al.* 1995). Previous MRI studies have demonstrated an increase in creatine in NAWM in multiple sclerosis (Husted *et al.* 1994; Rooney *et al.* 1997) and it has been suggested that this may be a marker of gliosis. A recent study, which found creatine in NAWM to be increased in primary progressive multiple sclerosis and to be significantly greater than in relapsing remitting disease, suggested that diffuse gliosis may contribute to disability in primary progressive multiple sclerosis (Suhy *et al.* 2000). This study has found no evidence to support this hypothesis.

In conclusion, this study supports the hypothesis that axonal loss occurs in NAWM in primary progressive multiple sclerosis and this may well be a mechanism of disease progression in this group. MRS is a potentially pathologically specific technique which may be particularly useful in primary progressive disease where conventional MRI has provided little pathological insight. The lack of correlation of single voxel spectroscopy with disability suggests that this technique has a limited role in monitoring disease progression in clinical trials at present. However, single voxel spectroscopy of more clinically eloquent areas such as the spinal cord and internal capsule (Lee *et al.* 2000b), or chemical shift imaging techniques to study single / multiple brain slices or whole brain may be useful in the future. The latter approaches will be of particular value in elucidating the relationship between axonal loss in NAWM and focal lesions.

3.4 Conclusion

Patients with primary progressive multiple sclerosis have the most striking discrepancy between conventional MR activity and clinical deterioration compared with other

groups of multiple sclerosis. One possible explanation for this is that intrinsic changes in NAWM make a major contribution to disability in this group. The above studies support this hypothesis by demonstrating abnormalities in NAWM in primary progressive multiple sclerosis. There were either no or very modest clinical correlations as judged by the EDSS seen in either study and so further work is required to validate whether or not these methods have a role as surrogate markers of disease progression. Longitudinal data for these methods over two years has been provided from the therapeutic trial of interferon beta-1a in primary progressive multiple sclerosis and this will be presented subsequently.

CHAPTER 4: METHODOLOGICAL ISSUES RELATING TO SERIAL MRI MEASUREMENT

4.1 Introduction

In this thesis several MRI measures of disease progression in multiple sclerosis are presented and applied and their clinical relevance discussed. However, when using such measures consideration must also be given to the fundamental principles of measurement, accuracy and precision. Accuracy refers to the biological truth of the measurement. Precision or reliability refers to the extent the measurement is repeatable over time. In any scientific measurement, errors of measurement occur and are never completely eliminated (Nunnally 1978). Consideration of measurement error is of vital importance in natural history and therapeutic studies in multiple sclerosis, which may last for several years and/or involve several centres. In particular, in serial studies biological changes over time due to multiple sclerosis are likely to be small and so the measurement error of a technique must be known in order to interpret the significance of any measured change.

Several factors may contribute to the overall measurement error of a technique. Establishing the magnitude of the error is of prime importance although identifying and correcting for sources of error may then improve the measurement error of the technique. Possible sources of error in MRI studies in multiple sclerosis include physiological variation, subject positioning and movement, scanner software and hardware characteristics, post-imaging processing and analysis methods and intra- and inter-operator / observer variation. These sources of error are inevitably amplified the more scanners, methodological approaches, operators and observers that are involved and the longer the studies run. In

particular scanner upgrades, a potential source of considerable error, will inevitably occur during long-term serial MRI studies.

Accuracy of a MRI measurement can be difficult to establish as the true biological value of a parameter is usually not known but can often be estimated using phantom techniques (Tofts 1998). Reliability of measurements may also be evaluated by performing repeated measurements from phantoms or alternatively from human control subjects. It has been recommended that demonstrating the accuracy of a technique in phantoms and the precision of a technique in both phantoms and human subjects is a prerequisite for a technique to be included in long-term multicentre studies (Tofts 1998). Monitoring of reliability of the technique, or quality assurance, is then an ongoing requirement throughout any study and should be incorporated into its design. A standard of reliability is thereby provided against which change may be evaluated. The impact of the reliability of measurements on serial studies is illustrated by the experience of the Interferon Beta-1b Multiple Sclerosis Study Group. In the third year of the multicentre placebo-controlled trial of interferon beta-1b in relapsing remitting multiple sclerosis there was a step decrease in the lesion load in all treatment groups which was attributed to a step change in the observer's technique (Paty *et al.* 1993). Although this did not affect the inter-group differences and reanalysis, analysing all the scans for each patient at one time, removed the step (IFNB Multiple Sclerosis Study Group 1995) it did highlight the difficulty of performing reliable measurements over several years.

Throughout the trial presented in this thesis quality assurance of techniques was performed as a routine part of the MRI protocol. However, there were two techniques which required special consideration. Firstly, prior to the start of the trial it had become apparent in a serial study of spinal cord atrophy that a scanner upgrade had artefactually affected

measurements (Losseff *et al.* 1996d). Secondly, serial MRS had not previously been used as a therapeutic marker in multiple sclerosis and 'normal' physiological variation was not well established for this age group. Studies addressing both these methodological issues are now presented; in the former the overall reliability of the technique is evaluated whereas the latter focuses on just one potential source of measurement variation, increasing age.

4.2 Reliability of Magnetic Resonance Imaging Measurements of Spinal Cord Atrophy

4.2(a) Introduction

An accurate and reproducible MRI method to quantify spinal cord atrophy (Losseff *et al.* 1996b) has been described in Chapter 1. The reported scan-rescan reproducibility of this technique is excellent with a coefficient of variation of 0.79% and a strong correlation between spinal cord atrophy and disability has been demonstrated cross-sectionally. This method therefore appears to be a potentially powerful tool to monitor disease progression in multiple sclerosis. However, it became apparent during the first serial study of this method that a scanner hardware upgrade had affected measurement parameters resulting in an artefactual increase in the cord area of all the controls (Losseff *et al.* 1996d). From this experience it was clear that quality assurance of the reliability of measurement is an essential requirement for serial studies of spinal cord atrophy particularly as changes in cord area over time due to disease are likely to be small (Stevenson *et al.* 1998b). A protocol was therefore designed to evaluate the long term reproducibility of this method.

4.2(b) Methods

As there was no phantom available that accurately represented the human spinal cord, healthy volunteer controls were scanned. MRI of the spinal cord was carried out in five control subjects participating in a rota over one year. The characteristics of the controls are presented in Table 4.1.

Table 4.1. Characteristics of spinal cord controls

	Age (years)	Sex	Height (cm)	Weight (Kg)
Control A	30	F	155	55
Control B	25	M	185	75
Control C	29	F	181	70
Control D	47	M	185	80
Control E	28	F	175	55
Mean	31.8		176.2	67

Volunteers were scanned in rotation at least once every five weeks, providing a minimum of one scan per week. By interleaving several volunteers the effect of any volunteer being unavailable for a scan on the continuity of the protocol was minimised. Preliminary analysis after seven months indicated that measurements were stable week to week and so frequency of scans was reduced to a minimum of two scans per month during the following five months.

MRI was carried out on a 1.5T Signa Echospeed Horizon system (General Electric,

Milwaukee, Wisc., USA) with a standard phased array spinal coil. A volume acquired inversion prepared fast spoiled gradient echo was performed (60 1mm slices reformatted sagittally, TI 450ms, TE 4.2ms, TR 15.6ms, flip angle 20°, FOV 25x25cm, matrix 256x256, 1 Nex). Five contiguous 3mm axial slices from the caudal landmark of the C2/3 intervertebral disc were reformatted from the volume data set and a coil radiofrequency uniformity correction was applied (Tofts *et al.* 1994). Cord area was measured using a semi-automated method previously described (Losseff *et al.* 1996b).

All image processing and cord area measurements were performed by a single observer. Images were randomly blinded so the observer was unaware of their identity. Intra-observer variation for same scan analysis was assessed on 10 scans. Reproducibility was assessed by the coefficient of variation and the standard deviation of measurement variation.

4.2(c) Results

A total of 46 scans was performed over one year. The results are presented in Table 4.2. The mean cord area of all the scans was 82.87 mm^2 (range 73.42-100.72 mm^2). The mean coefficient of variation of all subjects over one year was 1.35% (range 0.71-1.87%) and the mean standard deviation of measurement variation was 1.14 mm^2 (range 0.53-1.60 mm^2). The intra-observer coefficient of variation for same scan analysis was 0.63% (range 0-2.16%), standard deviation 0.51 mm^2 (range 0-1.65 mm^2).

To evaluate whether there was any drift in measurements over time, cord area was plotted against time (Figure 4.1). The mean regression coefficient for all subjects was 0.002 mm^2/week indicating that there was no significant drift in measurements. A scanner upgrade was carried out in week 20 but there was no apparent subsequent change in measurements.

Table 4.2. Serial cord area reproducibility measures

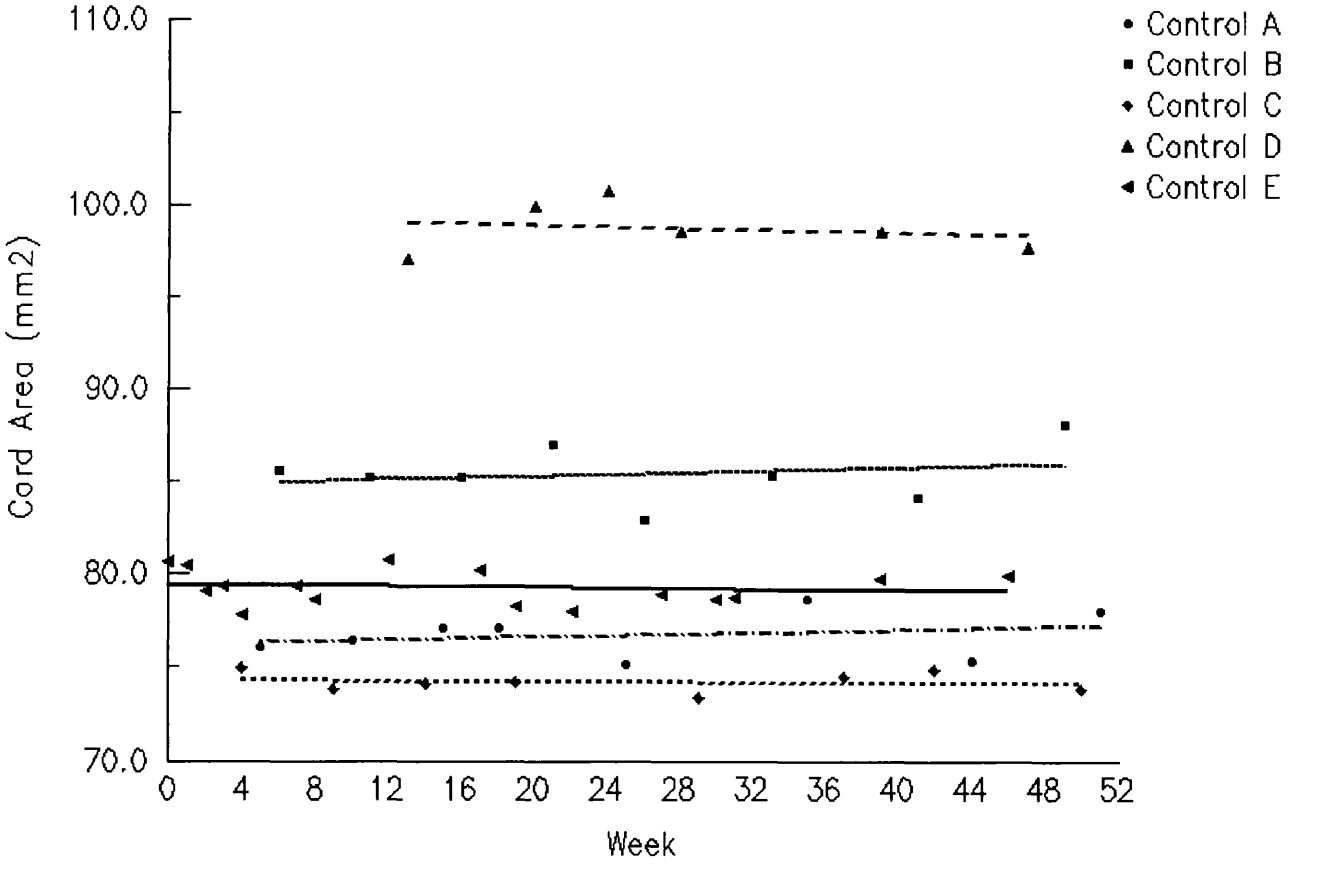
Control	No. of Scans	Mean Area (mm^2)	Range (mm^2)	SD (mm^2)	CV (%)	Slope (mm^2/week)
A	8	76.72	75.16-78.68	1.23	1.6	0.012
B	8	85.41	82.92-88.10	1.6	1.87	0.023
C	8	74.2	73.42-74.96	0.53	0.71	-0.004
D	6	98.71	97.04-100.72	1.39	1.4	-0.02
E	16	79.3	77.86-80.78	0.94	1.18	-0.008

SD = standard deviation of measurement variation

CV = coefficient of variation

Slope = regression coefficient

Figure 4.1 Plot of cross-sectional spinal cord area (mm^2) versus time. Linear regression to the data is shown for each subject.



4.2(d) Discussion

Evaluation of test-retest reliability, the stability of a measuring instrument over time, is a fundamental requirement in any scientific study (Nunnally 1970). In serial studies of multiple sclerosis, changes in MRI parameters may be small and the effect of any measurement variation may be great. Quality assurance of the reproducibility of serial measurements is therefore vital for the correct interpretation of any change. In particular, quantification of cord atrophy is a promising surrogate marker of disease progression, but quality assurance must be an integral part of this method in order to be confident of results.

Practical experience has shown that upgrading of scanners can affect measurements. As upgrades will unavoidably occur during long term serial studies, quality assurance is an ongoing requirement. The scanner used in this study undergoes a service once a month and, as this may introduce measurement error, quality assurance should be performed at least as frequently. This protocol will also detect drift in measurements which may occur due to scanner dependent variables. Early identification of measurement changes by quality assurance will enable an appropriate and rapid rectification of a problem and prevent the results of a serial study from being jeopardised.

In quality assurance of MRI, phantom measurements can usually be employed as surrogate markers of human measurements. However, in measurement of spinal cord area, the 'partial volume' effect at the cord/CSF boundary has to be considered. The method of Losseff *et al.* (1996b) utilised an algorithm to minimize this effect on boundary delineation and phantom measurements do not give a representative indication of the algorithm's performance. Therefore, this study used human controls.

In this study there was a high standard of reliability of spinal cord cross-sectional area

measurements over one year. Although only five volunteers were studied, they covered the wide range of spinal cord areas previously described in a 'normal' population (Losseff *et al.* 1996b), and are therefore robust data to confirm the reliability of the method for clinical studies. The quality assurance protocol provided ongoing evaluation during the studies presented in this thesis giving confidence to the results. During the three years of the interferon beta-1a trial presented in this thesis a total of seven subjects participated in the rota for a mean of 23 months, range 10-34 months. The mean coefficient of variation for all subjects during the trial was 1.72%, range 0.62-2.49%, but it should be noted that this routine analysis was not blinded and was potentially subject to drift in analysis technique.

In conclusion, quality assurance is an essential and practical component of all serial MRI studies without which the clinical implications of change cannot be reliably evaluated. A protocol of quality assurance should be incorporated in studies of spinal cord atrophy in all centres.

4.3 ^{1}H Magnetic Resonance Spectroscopy of Aging in Parietal White Matter

4.3(a) Introduction

The clinical application of MRS has already been discussed in the earlier chapters. The study presented in chapter 5 includes MRS as a novel serial marker of disease progression in a therapeutic trial. However, in order to evaluate the clinical significance of changes in brain metabolites it is first essential to establish any normal physiological changes in metabolites including those relating to increasing age.

Several MRS studies of aging in the adult human brain have been carried out which have examined a wide range of regions, both grey and white matter, using different methods

and giving a variety of results (Christiansen *et al.* 1993b; Moats *et al.* 1994; Charles *et al.* 1994; Soher *et al.* 1996; Chang *et al.* 1996; Lim & Spielman 1997; Pfefferbaum *et al.* 1999; Saunders *et al.* 1999). As multiple sclerosis is predominantly a white matter disease this study has examined only white matter. Most studies have reported stable NAA in white matter (Charles *et al.* 1994; Soher *et al.* 1996; Chang *et al.* 1996; Pfefferbaum *et al.* 1999; Saunders *et al.* 1999) but recent studies have shown an increased creatine/phosphocreatine (Cr) in white matter in older age groups (over 60 years of age) (Pfefferbaum *et al.* 1999; Saunders *et al.* 1999). However, these studies do not provide an ideal reference for trials in multiple sclerosis as they have examined wide age ranges including elderly subjects. Multiple sclerosis affects a younger population, usually first presenting between 20 and 40 years of age, and clinical studies therefore typically include young to middle aged subjects. This study has therefore examined a large cohort of normal controls with an age range representative of the multiple sclerosis population included in clinical trials to assess the effect of aging on metabolite concentrations.

4.3(b) Methods

Forty-four healthy volunteer controls (22 female, 22 male) were recruited from the staff of the Institute of Neurology and the National Hospital and partners of patients participating in research trials. Subjects were recruited within a potential age range of 18 to 65 years. Recruitment was stratified in four age bands - 29 years or under, 30 to 39, 40 to 49 and 50 or over - with a minimum of ten subjects in each band to ensure an even distribution of ages. Subjects were excluded if there was a history of neurological disease. All subjects gave their informed consent to participate in the study.

MRI and MRS were carried out according to the methods protocol described in chapter 3. A spectroscopic volume of interest (median 2.20ml, range 1.13-3.35ml) was selected in parietal white matter at the level of the lateral ventricles. Parietal white matter, a common area affected in multiple sclerosis, was chosen as the optimal site to select a white matter voxel without contamination from grey matter or cerebrospinal fluid. The voxel was selected from either hemisphere as no effects of laterality on metabolite concentrations have been demonstrated (Tedeschi *et al.* 1995; Pouwels & Frahm 1998). MRI slices above and below the voxel were also visualised in order to ensure that no voxel contained grey matter or cerebrospinal fluid. ^{1}H spectra were acquired as described earlier.

Sixteen subjects underwent repeat examination with the same imaging parameters after an interval of one year (median 12 months, range 8-15 months) to look for any physiological or artefactual short term changes. The same volume of interest as baseline was used (median 2.26ml, range 1.45-3.35ml) and the voxel was visually positioned as closely as possible to the baseline voxel.

Spectra were analysed using the LCModel method as described in chapter 3. Absolute concentrations of NA, NAA, Cr, choline containing compounds (Cho) and *myo*-inositol (mI) were measured. Quality assurance was performed as described earlier.

Statistical correlations were performed using the Spearman's Rank Correlation Coefficient. Statistical analysis of the serial data was carried out using the Wilcoxon Signed Ranks Test and coefficients of variation were also calculated.

4.3(c) Results

The mean age of the whole group was 39.5 years (range 22-62 years). No significant differences in metabolite concentrations were seen between females and males. There were significant correlations between age and the concentrations of Cr ($r=0.43$, $p=0.004$) and Cho ($r=0.38$, $p=0.01$) (Figure 4.2) but not NA, NAA or mI (Table 4.3).

The mean age of the serial group was 42.5 years (range 24-62 years). There were no significant changes in any of the metabolite concentrations over one year (Table 4.4).

A typical voxel and spectrum are illustrated in Figure 4.3.

4.3(d) Discussion

This study has demonstrated stability of NAA and mI in parietal white matter over four age decades but increasing Cr and Cho with age in a large cohort of adults representative of the age range of subjects included in studies of multiple sclerosis. This confirms that aging does affect certain brain metabolites, albeit modestly; serial examination did not demonstrate significant changes in metabolite concentrations over one year.

A number of previous studies have quantified brain metabolites with respect to age in normal white matter. Although there have been reports of NAA decreasing with age in grey matter (Charles *et al.* 1994; Lim & Spielman 1997), NAA appears to remain stable in white matter (Charles *et al.* 1994; Soher *et al.* 1996; Chang *et al.* 1996; Pfefferbaum *et al.* 1999; Saunders *et al.* 1999). Cr has previously been reported to remain stable in white matter (Charles *et al.* 1994; Chang *et al.* 1996) but more recent studies have demonstrated increases with age (Pfefferbaum *et al.* 1999; Saunders *et al.* 1999). Cho has usually been found to be

Figure 4.2

(a) Plot of Cr concentration versus age.

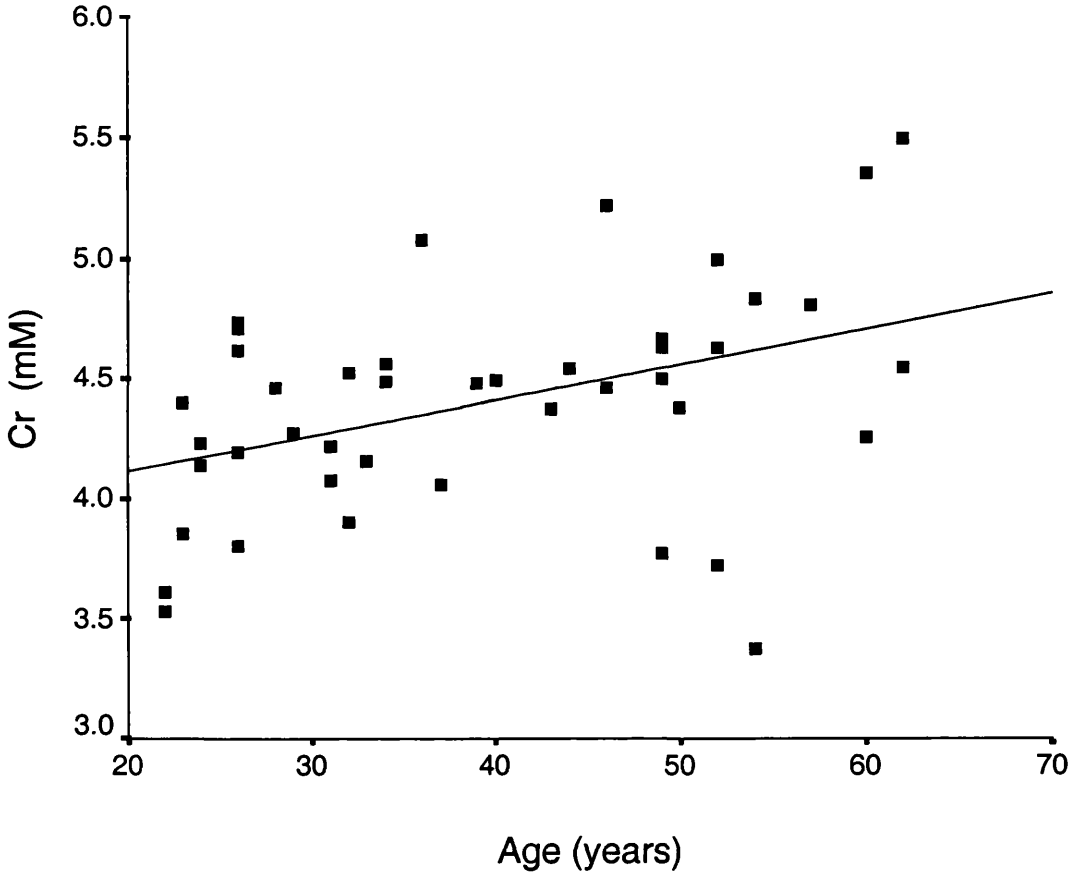


Figure 4.2

(b) Plot of Cho concentration versus age.

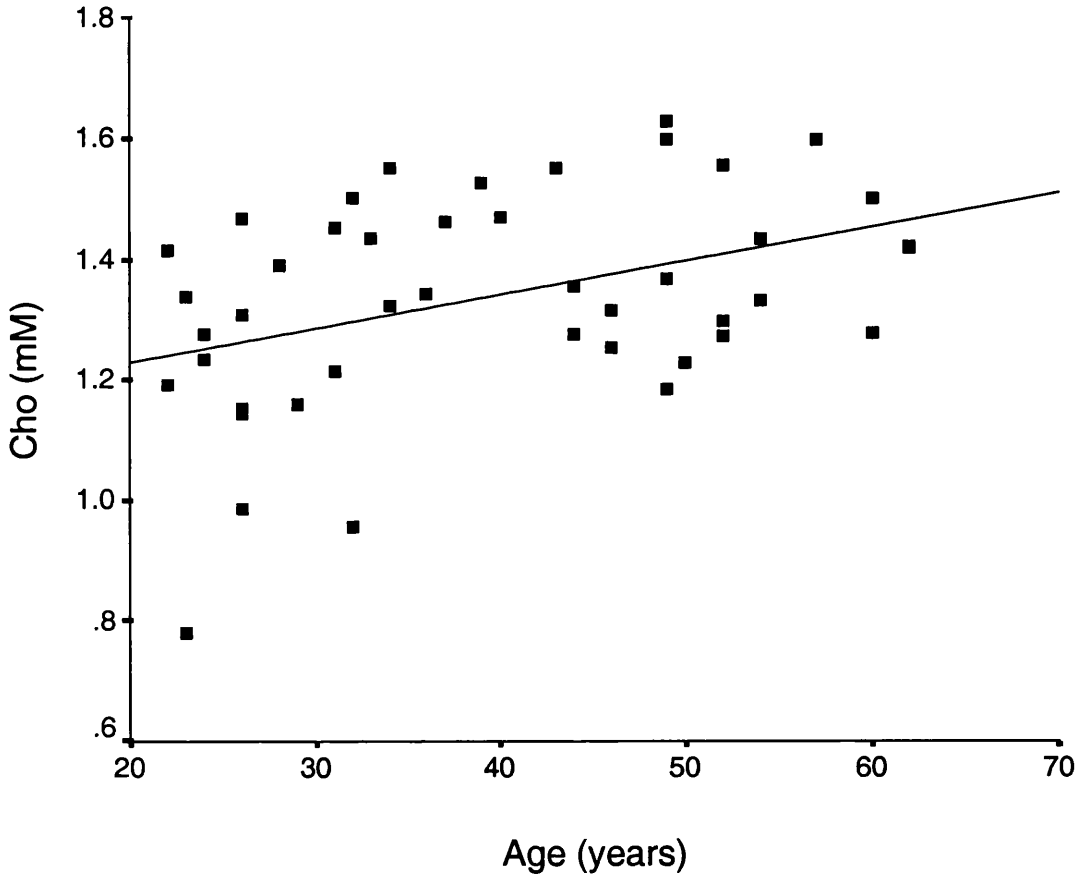


Table 4.3 Metabolite concentrations and correlations with age

	[Median] (mM) (Range)	SRCC	
NA	7.84 (6.46-9.71)	0.13	ns
NAA	6.15 (4.56-8.43)	-0.05	ns
Creatine	4.47 (3.37-5.50)	0.43	p=0.004
Choline	1.34 (0.78-1.63)	0.38	p=0.01
Myo-inositol	3.81 (2.22-6.81)	0.06	ns

SRCC - Spearman's Rank Correlation Coefficient

ns - not significant ($p>0.05$)

Table 4.4 Serial metabolite concentrations

	Year 0 [Median] (mM) (Range)	Year 1 [Median] (mM) (Range)		Mean CV (%) (SD)
NA	7.84 (6.46-9.71)	7.71 (6.17-8.81)	ns	5.60 (3.80)
NAA	6.15 (4.83-8.43)	6.07 (4.67-7.57)	ns	8.35 (5.85)
Creatine	4.51 (3.72-5.50)	4.38 (3.69-5.54)	ns	5.78 (4.78)
Choline	1.28 (1.14-1.50)	1.33 (1.06-1.56)	ns	7.45 (4.21)
Myo-inositol	3.82 (2.38-6.81)	4.05 (2.67-7.67)	ns	18.28 (10.91)

CV - coefficient of variation

SD - standard deviation

ns - not significant ($p>0.05$)

Figure 4.3

MRI and spectrum from a 26 year old female subject.

(a) T_2-weighted scan showing the voxel from which the spectrum was obtained.

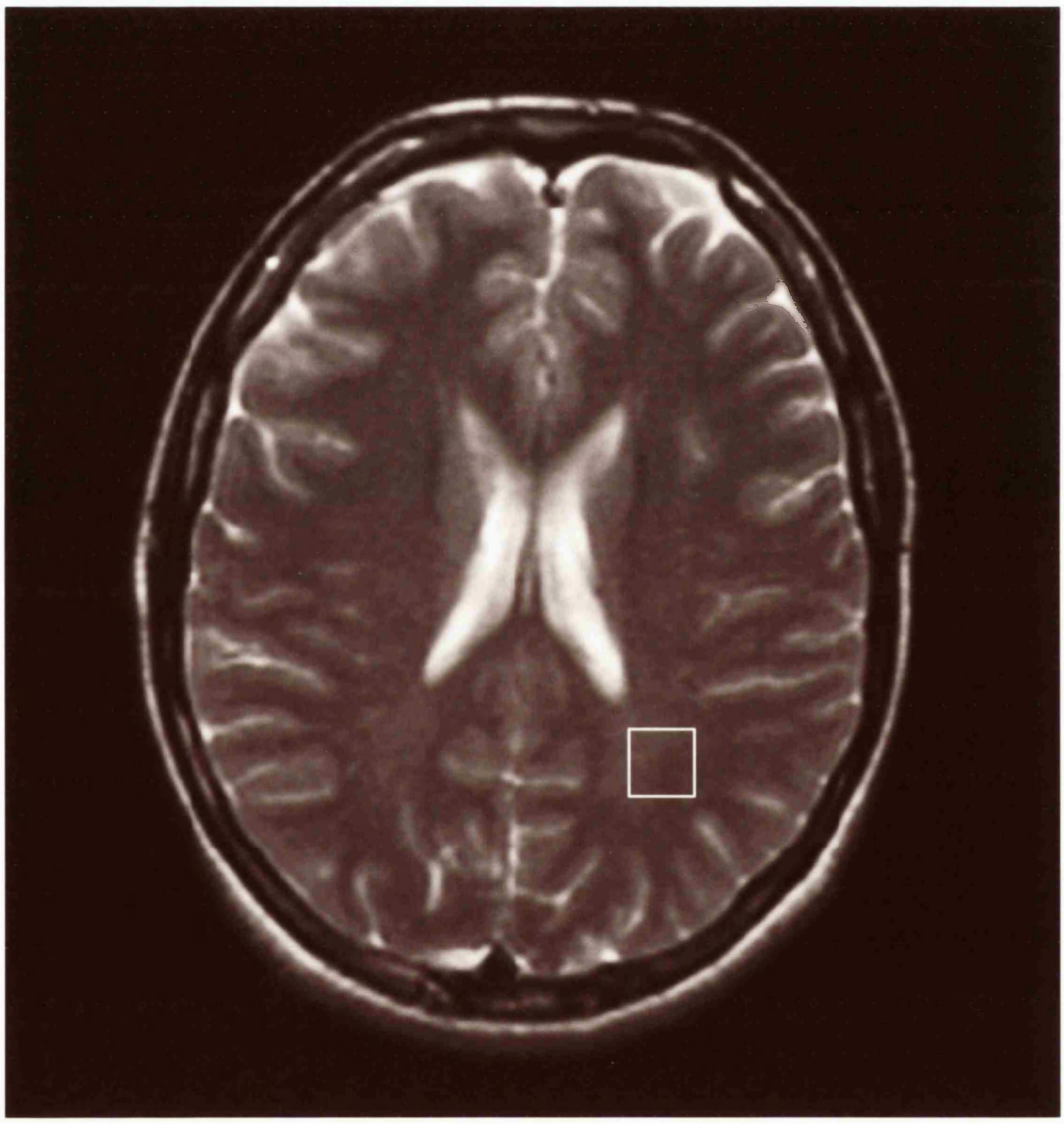
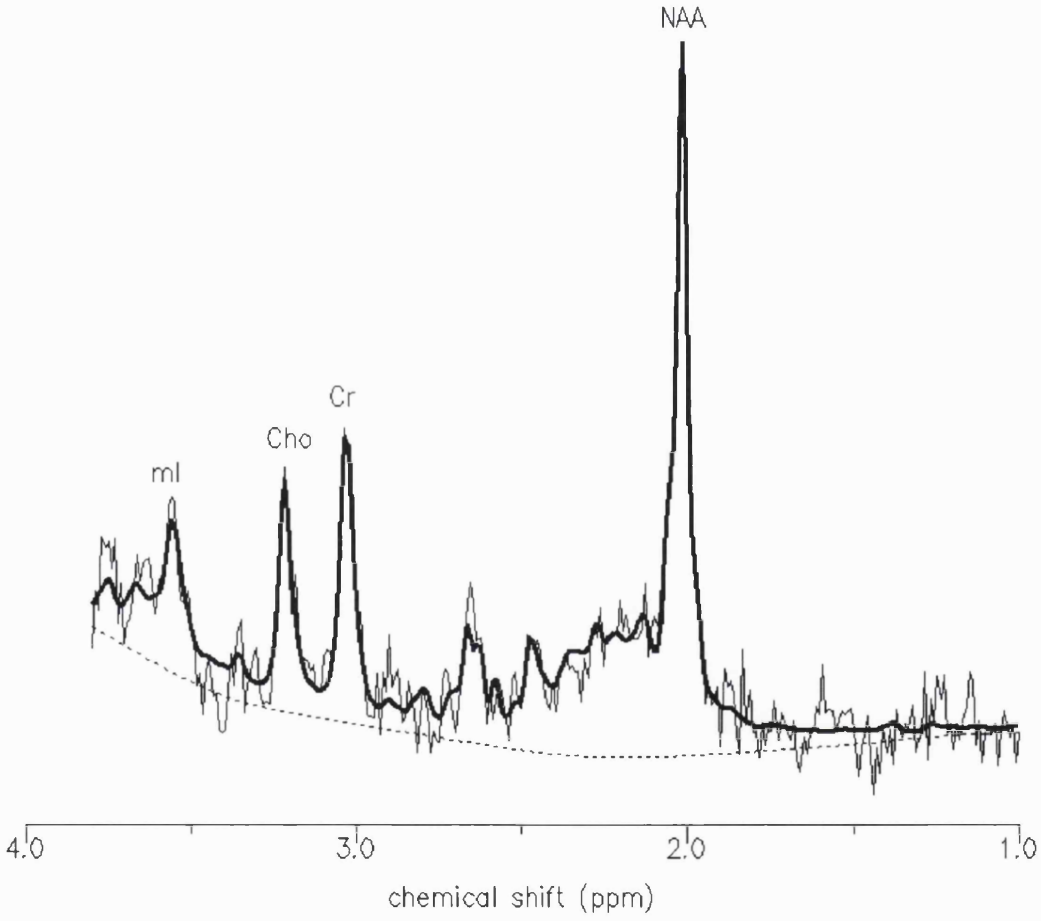


Figure 4.3

(b) The acquired and processed spectrum as generated by the LCModel (thin line - original phased spectrum, thick line - LCModel fitted data, dashed line - baseline as determined by LCModel).



stable in white matter (Charles *et al.* 1994; Chang *et al.* 1996; Pfefferbaum *et al.* 1999; Saunders *et al.* 1999) but has also been reported to increase with age (Moats *et al.* 1994). Regional differences in aging have also been demonstrated with one study finding age-related increases of Cho and Cr in frontal but not posterior white matter (Soher *et al.* 1996) but the reasons for this are not clear. mI has been reported to remain stable with age in white matter (Charles *et al.* 1994; Saunders *et al.* 1999).

The results of this study for NAA, Cr and mI are consistent with those of recent studies. The finding of a correlation of Cho in white matter with age, albeit very modest, conflicts with most but not all studies. Increases of choline with age have been more commonly reported in grey matter (Chang *et al.* 1994; Pfefferbaum *et al.* 1999) but due to the stringency in voxel selection and the use of small volumes of interest in this study there does not appear to be contamination from grey matter. Whether methodological differences, for example, the use of the LCModel which minimises user variability and improves reproducibility, explains the discrepancy is impossible to assess without direct comparisons of the various techniques. However, one possible explanation is that this study examined a larger number of subjects over a narrower age range than other studies which may have allowed the detection of more subtle changes.

The stability of NAA suggests that significant age-related axonal loss in white matter is not occurring in this age group. Whereas NAA is only found in neurons in the adult human brain, Cr and Cho are found in all neural cell types with their concentrations being highest in glial cells (Urenjak *et al.* 1993). Increased Cho has been reported in association with increased cell membrane turnover and breakdown but the biological significance of increasing Cr and Cho with age is not clear. However, these changes have significant implications for the methodology of MRS studies in multiple sclerosis. Metabolite

concentration ratios, assuming Cr or Cho to be an 'internal standard', have frequently been used to measure metabolites such as NAA in clinical studies. A reduction in NAA/Cr or NAA/Cho may reflect increases in Cr or Cho rather than a reduction in NAA. Absolute quantification of metabolites should therefore be performed where possible. Furthermore, for group comparisons, age-matching is essential.

In conclusion this study has demonstrated age-related changes in brain metabolite concentrations even in a relatively young adult population and these changes must be taken into account in MRS studies of multiple sclerosis or other diseases affecting young to middle aged adults.

CHAPTER 5: AN EXPLORATORY TRIAL OF INTERFERON BETA-1A IN PRIMARY PROGRESSIVE MULTIPLE SCLEROSIS

5.1 Introduction

Patients with primary progressive multiple sclerosis have been excluded from the majority of therapeutic trials because of the unique problems of carrying out trials in this group which have been described earlier. In recent years beta interferon has been extensively investigated in the other subgroups of multiple sclerosis. It has a partial effect on relapse frequency and severity in relapsing remitting (IFNB Multiple Sclerosis Study Group 1993; Jacobs *et al.* 1996; PRISMS 1998) and secondary progressive multiple sclerosis (European Study Group 1998; SPECTRIMS 2001). It reduces the rate of development of clinically definite multiple sclerosis in patients with clinically isolated syndromes suggestive of multiple sclerosis (Jacobs *et al.* 2000; Comi *et al.* 2001b). The effect of beta interferon on disease progression is less clear. Of two published studies in secondary progressive multiple sclerosis one reports a delay in the accumulation of disability (European Study Group 1998) while the other finds no significant clinical effect (SPECTRIMS 2001). Preliminary reports of two further studies also present conflicting results (Paty *et al.* 2000; Cohen *et al.* 2001). All studies have demonstrated a positive effect of beta interferon on MRI markers of inflammation. Although inflammation is less prominent in primary progressive disease compared to other subgroups it has been clearly demonstrated and so a trial of beta interferon in this group was justified.

The first randomised controlled trial of beta interferon in primary progressive multiple sclerosis is presented here. This is an investigator lead, exploratory trial and has been carried

out at a single centre with a small sample size. Based on other studies, and assuming that beta interferon is not more effective in this group than in other subgroups, it was not expected to demonstrate a treatment effect. The three main objectives of the trial were i) to investigate safety and tolerability, ii) to identify potentially useful outcome measures, and iii) to look for a hint of efficacy. The trial studied two doses of intramuscular interferon beta-1a, the standard 30 μg dose and a 60 μg dose. The high dose has been included as it is conceivable that stronger immunosuppression may be required in primary progressive multiple sclerosis given the low grade nature of inflammation seen in this group.

5.2 Methods

Subjects

Fifty subjects with primary progressive multiple sclerosis were enrolled at a single clinical centre (The National Hospital for Neurology and Neurosurgery, London, UK). All subjects gave informed written consent and the study was approved by the local ethics committee. At the time of study recruitment no specific diagnostic criteria for primary progressive multiple sclerosis existed though subsequently criteria have been published (Thompson *et al.* 2000). The diagnosis was therefore ascertained on the basis of a progressive history without relapse or remission, at least two typical lesions on MRI and the presence of oligoclonal bands in the cerebrospinal fluid not present in parallel serum and/or abnormal visual evoked potentials. Inclusion criteria were: i) primary progressive multiple sclerosis of at least two years duration, ii) age 18 to 60 years, iii) Expanded Disability Status Scale (EDSS) (Kurtzke 1983) score of 2.0 to 7.0 inclusive, and iv) at least two lesions on T_2 weighted MRI of the brain and/or spinal cord. Exclusion criteria were: i) interferon,

immunosuppressant or chronic steroid therapy within three months prior to study entry, ii) pregnancy, breast-feeding or unwillingness to practise contraception in female subjects unless post-menopausal or surgically sterile, iii) seizure within the previous three months, and iv) a history of severe depression.

Study Design and Treatment

This was a double-blind, placebo-controlled trial of two doses of interferon beta-1a (Avonex, Biogen, Cambridge, Mass., USA). Subjects were randomised according to the block method to receive intramuscular interferon beta-1a 30 μg (IFN30), interferon beta-1a 60 μg (IFN60) or placebo once a week with 15 subjects in each of the active treatment arms and 20 subjects in the placebo arm. The study period was 24 months. All subjects and study personnel, including the treating physician, were blinded to treatment status. EDSS assessments were performed by an independent evaluating physician blinded to all clinical information. A questionnaire to test the blinding was given to all subjects and the treating physician at the end of the study.

Intramuscular injections were administered by the treating physician and local medical/nursing practitioners or, after training, the subject or a family member. Non-steroidal anti-inflammatory drugs and/or paracetamol were recommended for prophylaxis of interferon associated flu-like reactions. In the event of intolerance to the study drug there was an option to halve the dose.

Efficacy Evaluation

Clinical efficacy evaluation visits took place at baseline and every three months until month 24. The primary clinical endpoint was time to sustained progression in disability;

progression was defined as ≥1.0 point increase in EDSS score for subjects with a baseline EDSS score ≤5.0 or a ≥0.5 point increase for subjects with a baseline ≥5.5. Progression was considered sustained if documented at two consecutive visits three months apart; the time of the first visit was recorded as the time to progression. Secondary clinical outcome measures included the timed ten metre walk (TTMW) and the nine hole peg test (NHPT) which were also performed at each visit. The time to walk ten metres, with assistance as required, was recorded on one attempt which was performed on the same course at each visit. The time to perform the NHPT was recorded on two consecutive trials for both the right and left arms. If a subject was unable to perform or complete the TTMW or NHPT within five minutes a time of 300 seconds was recorded. A quality of life questionnaire (SF-36 Health Survey) was completed at baseline and months 12 and 24 as a tertiary clinical outcome and will be reported subsequently.

Efficacy was also evaluated by MRI. Secondary outcome measures included: i) T_2 weighted brain lesion load, ii) new T_2 brain lesions, iii) new T_2 spinal cord lesions, iv) T_1 weighted brain lesion load, v) new T_1 brain lesions, vi) spinal cord cross-sectional area, vii) whole brain volume and, viii) ventricular volume. Tertiary MRI outcome measures, which will be reported subsequently, included magnetisation transfer ratio (MTR) of lesions and normal appearing white matter (NAWM) and ^1H magnetic resonance spectroscopy (MRS) measurement of N-acetyl derived groups in NAWM. T_2 and T_1 weighted imaging were performed at baseline and months 6, 12, 18 and 24. Spinal cord area, brain volumes, MTR and MRS were performed at baseline and months 12 and 24.

Safety evaluation

Safety evaluation took place throughout the study and for three months post-study.

Adverse events, physical examination findings and haematological and biochemical laboratory tests were monitored. An electrocardiogram was also performed at baseline and the end of study. An interim safety review by an independent assessor was performed at the mid-point of the trial.

Neutralising antibodies

Serum was collected for interferon beta-1a neutralising antibody assay at baseline and every three months during the trial. Results were not revealed to study personnel before the trial was completed. Subjects were reported to be neutralising antibody positive if an antibody titre was positive (>1/10) on any assay during the study.

MRI and Image Analysis

MRI was carried out on a 1.5T Signa Horizon Echospeed system (General Electric, Milwaukee, Wisc., USA). Brain imaging was performed with a standard quadrature head coil and spinal cord imaging with a standard phased array spinal coil. Imaging and post-imaging analysis was carried out according to the following protocols (all observers/raters were blinded to subject identity):

T_2 weighted brain imaging

Forty-six contiguous 3mm slices were acquired using a dual echo fast spin echo sequence (TE 15/90ms, TR 3200ms, echo train length 8, FOV 24x24cm, matrix 256x256, 1 signal average). All images were copied to film (hard copy) and electronic disc. Identification and marking of every brain lesion was then carried out on the hard copy proton density weighted images with cross-reference to the T_2 weighted images to aid lesion identification. Hard copy marking was performed by two observers with all scans for a given

subject being marked by the same observer. Scans were marked serially, in chronological order, with reference to the preceding scans. New lesions were also marked and the number of new lesions documented. The electronic copy was displayed using DispImage software (DL Plummer, University College London, UK). All lesions marked on the corresponding hard copy were contoured by a single rater using a semi-automated local thresholding technique plus manual editing, with reference to published guidelines (Filippi *et al.* 1998c). Individual lesion volumes were calculated as the lesion area multiplied by the slice thickness and the total lesion volume was calculated as the sum of all the individual lesion volumes.

T_1 weighted brain imaging

Forty-six contiguous 3mm slices were acquired using a spin echo sequence (TE 17ms, TR 600ms, FOV 24x24cm, matrix 256x256, 1 signal average). Post-imaging analysis was carried out according to the same protocol as the T_2 weighted brain imaging analysis with the exception that all the hard copy marking was performed by a single observer.

T_2 weighted spinal cord imaging

Nine contiguous 3mm sagittal slices of the whole spinal cord were acquired using a dual echo FSE sequence (TE 45/90ms, TR 2500ms, echo train length 16, FOV 48x48ms, matrix 512x512, 2 signal averages) and copied to film. With reference to both scans, every spinal cord lesion was marked on the baseline scan by a single observer and the number of lesions documented. All new focal lesions on the corresponding serial scans were marked by the same observer and the number of new lesions documented.

Spinal cord cross-sectional area

The imaging and post-imaging analysis protocol is detailed in section 4.2(b).

Brain/Ventricular volume

A whole brain, volume acquired, inversion prepared, fast spoiled gradient echo was

performed (128 1.5mm slices acquired coronally, TE 4.2ms, TI 450ms, TR 15.6ms, flip angle 20°, FOV 24x18cm, matrix 256x192, 1 signal average). Image analysis was carried out using a semi-automated method previously described (Fox & Freeborough 1997). The inferior cutoff for the whole brain volume was at the level of the lowest point of the cerebellum. Serial scans were accurately registered to the baseline scan. The change in brain volume was measured from the registered scans by integrating the shifts in brain-CSF boundaries. The resulting brain boundary shift integral (BBSI) is therefore a direct measure of change in cerebral volume (a positive value indicates atrophy). The absolute ventricular volume (comprising the lateral ventricles including the temporal horns) was also measured on the baseline and registered serial images.

Due to the sensitivity of atrophy measures to movement artefact all spinal cord and brain volume images were systematically checked by a blinded observer and rejected if there was significant movement artefact.

MRI Reproducibility

Intra-observer variation for same scan analysis was assessed for T_2 lesion volume (n=10), T_1 lesion volume (n=10), spinal cord area (n=10), BBSI (n=6) and ventricular volume (n=10) using the coefficient of variation.

Statistical analysis

The statistical analysis was carried out on an intention to treat basis. As this was an exploratory trial primary and secondary statistical methods were included (Table 5.1). All methods were performed using SAS version 6.12 (SAS Institute Inc., Cary, North Carolina, USA). The primary statistical method for the primary clinical endpoint was the Mantel-Cox

Table 5.1 Statistical methods

Efficacy outcome	Primary statistical method	Secondary statistical method
Time to sustained progression	Mantel-Cox log-rank test	Piecewise exponential modelling
Timed 10 metre walk	Nonparametric analysis of covariance	Mixed model regression analysis
Nine hole peg test	Nonparametric analysis of covariance	Mixed model regression analysis
T_2 cerebral lesion load	Nonparametric analysis of covariance	Mixed model regression analysis
T_1 cerebral lesion load	Nonparametric analysis of covariance	Mixed model regression analysis
New T_2 cerebral lesions	CDA - Mean score statistic	-
New T_1 cerebral lesions	CDA - Mean score statistic	-
New T_2 spinal cord lesions	CDA - Mean score statistic	-
Spinal cord area	Nonparametric analysis of covariance	Mixed model regression analysis
BBSI (cerebral volume)	MANOVA - Wilk's lambda statistic	-
Ventricular volume	Nonparametric analysis of covariance	Mixed model regression analysis

CDA - categorical data analysis

MANOVA - multivariate analysis of variance

log-rank test and the secondary method was piecewise exponential modelling.

For the secondary clinical and the majority of the secondary MRI outcomes the primary statistical method was nonparametric (rank) analysis of covariance and the secondary method was mixed model regression analysis; these analyses adjusted for any differences at baseline. The raw data for these analyses were the absolute measurements recorded but the mixed model regression analysis was performed using a log (y+constant) transformation. For the NHPT the times for both trials for each arm were included in the analyses. The nonparametric analysis of covariance results are given for month 24; if a treatment difference was identified the directionality of the effect was determined by using the group mean residuals and by inspection of the data (including residual plots). The mixed model regression analysis results for efficacy are restricted to the rates of progression during the interval from the first to the last treatment observation. Due to the use of log transformation the mixed model regression analysis results are given in terms of p-values only, since the log space regression coefficient estimates and confidence intervals have little clinical meaning. In addition, raw data space estimates of the rates of change at month 24 and their confidence of intervals are given; the rates were obtained for subjects having a baseline value equal to the least-squares mean value.

For new lesions the cumulative number was calculated and categorical data analysis using the mean score statistic was performed. The BBSI measure of brain atrophy, which provided a bivariate change score, was analysed with multivariate analysis of variance using the Wilks' Lambda statistic.

In addition to comparisons of the individual treatment groups, the active treatment groups were combined and compared to placebo using all statistical methods for the primary clinical endpoint and using the primary statistical method for the secondary outcome

measures. P-values <0.05 were considered significant.

Comparisons of baseline data were carried out using the parametric two-sample t-test or the non-parametric Mann-Whitney test. Analysis of adverse event data was performed using Fisher's exact test.

5.3 Results

Study completion / Compliance

Fifty subjects were enrolled. Forty-nine subjects completed 24 months of follow-up. Forty-three subjects completed 24 months on study drug; seven patients had their dose reduced. The majority of treatment withdrawals and dose reductions (Table 5.2) occurred in the IFN60 group with only six of the 15 patients in this group completing the study on full dose; 12 of the 15 patients in the IFN30 group completed the study on full dose. Patients in the active treatment groups withdrew due to flu-like reactions and in the placebo group due to perceived lack of benefit. The most common reason for dose reduction was a rise in liver enzymes.

Baseline characteristics

There were no significant differences in age, disease duration or EDSS score between treatment arms (Table 5.3) and no significant differences in baseline MRI characteristics (Table 5.4).

Table 5.2 Reasons for treatment withdrawal and dose reduction

	Entire Cohort	Placebo	IFN30	IFN60
Treatment Withdrawal				
Flu-like reactions	5	-	1	4
Lack of benefit	2	2	-	-
Total	7	2	1	4
Dose Reduction				
Liver enzyme rise	4	-	1	3
Flu-like reactions	2	-	-	2
Fatigue	1	-	1	-
Total	7	-	2	5

Table 5.3 Baseline clinical characteristics

	Entire Cohort	**Placebo**	**IFN30**	**IFN60**
M/F	32/18	15/5	10/5	7/8
Mean age (years)	45	43	46.5	47
range	25-59	30-59	29-58	25-59
Mean disease duration	8	8	8	8
(years)	2-21	2-19	2-17	3-21
range				
Median EDSS	5.25	4.5	5.5	5.5
range	2.0-7.0	2.0-7.0	3.5-7.0	2.0-6.5
Median TTMW (secs)	11	9.5	11	12
range	4-137	6-137	6-75	4-132
Median NHPT - right (secs)	28.85	30.15	28.75	28.85
range	17.05-300.00	17.05-300.00	18.90-75.50	19.85-50.85
Median NHPT - left (secs)	28.65	29.70	26.80	28.35
range	16.85-300.00	16.85-132.00	18.85-124.05	18.45-300.00

Table 5.4 Baseline MRI characteristics

		Entire Cohort	Placebo	IFN30	IFN60
Number of subjects		50	20	15	15
T_2 lesion load (cm^3)	median	12.49	9.51	11.48	15.83
	range	0.29-77.57	0.29-77.57	0.98-64.12	0.69-67.58
T_1 lesion load (cm^3)	median	1.67	1.17	1.29	3.26
	range	0-33.79	0-27.21	0-33.79	0-24.54
No. T_2 cord lesions	median	4	2.5	4	5
	range	0-8	0-8	1-8	1-8
Cord area (mm^2)	median	69.15	69.15	70.36	65.32
	range	47.48-83.58	57.96-81.70	47.78-83.58	47.48-82.66
		(n=48)	(n=20)	(n=13)	(n=15)
Brain volume (cm^3)	median	1154.35	1179.3	1145.6	1112.9
	range	755.0-1436.5	1025.1-1436.5	1029.2-1255.4	755.0-1326.8
Ventricular volume	median	19.47	23.26	23.52	17.67
(cm^3)	range	4.73-82.45	7.34-63.64	6.27-82.45	4.73-44.74

MRI Reproducibility

The mean coefficients of variation for same scan analysis were as follows: T_2 lesion volume 2.63%, T_1 lesion volume 4.08%, spinal cord area 0.48%, BBSI 2.13% and ventricular volume 0.15%.

Primary clinical endpoint

The primary clinical endpoint was reached in 48% of subjects. No significant difference in time to sustained disease progression was seen on either the primary or secondary statistical analyses. The time to progression data for the individual treatment arms and for the combined interferon arm are shown in the form of survival curves (Figure 5.1).

Secondary clinical outcomes

The medians and ranges of the raw data are given in Table 5.5 and the estimates of rates of change at month 24 are shown in Table 5.6. No treatment effect was seen on the TTMW. On nonparametric analysis of covariance of the NHPT a treatment group difference (on three group comparison) was suggested for the right side (p=0.09); considering the individual groups, differences were suggested between the IFN30 group and both the control and IFN60 groups (p=0.08 and p=0.09 respectively). The mixed model regression analysis suggested a treatment group difference in rate of progression that depended on side (p=0.07). There were no significant differences between the active treatment groups compared to controls but the IFN30 group had a lower rate of deterioration in right side performance than the IFN60 group (p=0.02).

Figure 5.1

Survival curves for time to sustained disease progression.

(a) Placebo and combined interferon groups.

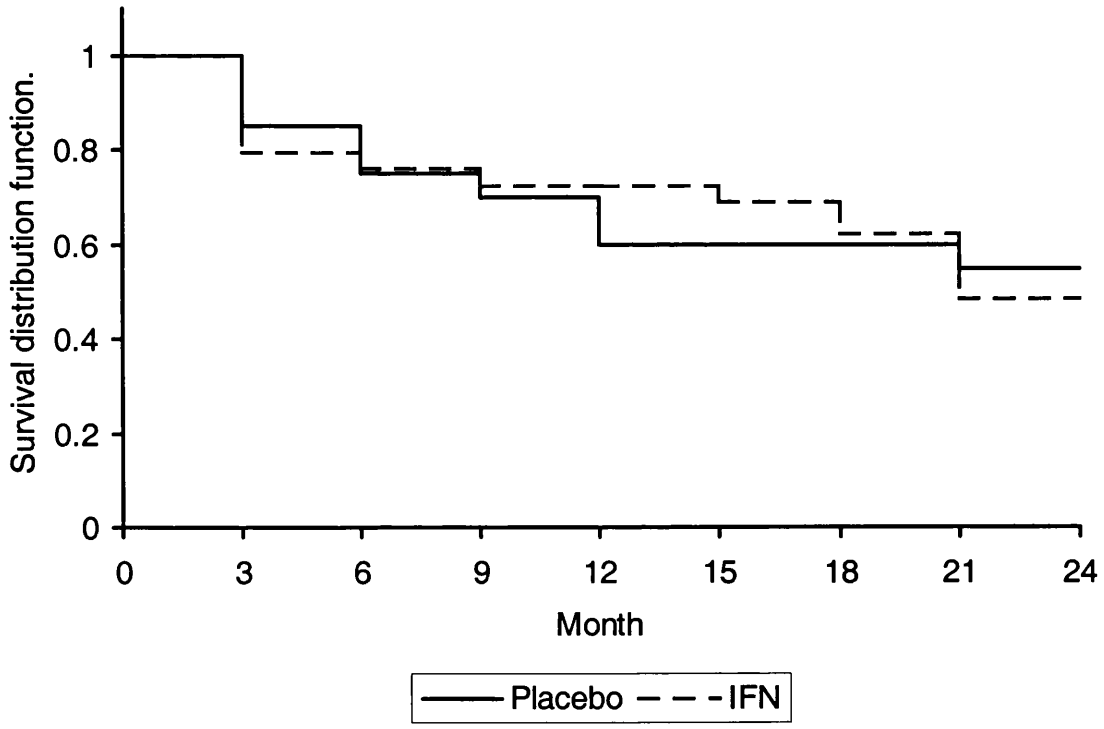


Figure 5.1

(b) Placebo, IFN30 and IFN60 groups.

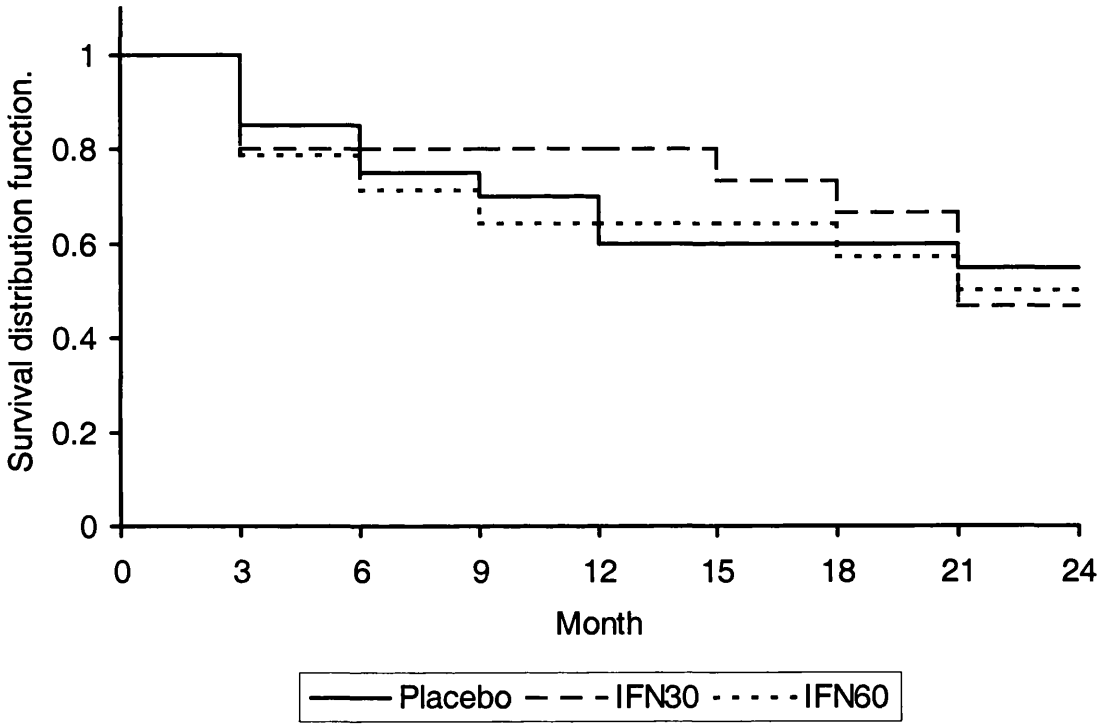


Table 5.5 Secondary clinical outcome measures (time to perform in seconds) throughout study

		Placebo	IFN30	IFN60
TTMW				
Baseline	n	20	15	15
	median (range)	9.5 (6-137)	11 (6-75)	12 (4-132)
Month 12	n	20	15	14
	median (range)	11 (6-300)	12 (7-300)	13 (4-164)
Month 24	n	19	15	14
	median (range)	14 (6-300)	19 (8-300)	13 (5-300)
NHPT - right				
Baseline	n	20	15	15
	median (range)	30.15 (17.05-300)	28.75 (18.90-75.50)	28.85 (19.85-50.85)
Month 12	n	20	15	14
	median (range)	30.30 (17.45-300)	23.55 (18.80-135.00)	28.60 (17.40-71.70)
Month 24	n	19	15	14
	median (range)	31.05 (15.20-300)	23.80 (17.65-93.90)	29.00 (18.15-113.95)
NHPT - left				
Baseline	n	20	15	15
	median (range)	29.70 (16.85-132.00)	26.80 (18.85-124.05)	28.35 (18.45-300)
Month 12	n	20	15	14
	median (range)	29.93 (17.50-82.55)	27.10 (18.80-135.00)	27.93 (18.50-300)
Month 24	n	19	15	14
	median (range)	31.20 (15.15-95.95)	27.20 (17.15-300)	30.93 (18.95-300)

Table 5.6 Estimates of rates of change and confidence intervals at time=24 months (for a baseline value equal to the least-squares mean value)

	Treatment Group	Rate of Change	Confidence Interval
T_2 lesion load	Placebo	85.5	33.7 to 147.8
(mm^3/month)	IFN30	1.7	-39.1 to 52.7
	IFN60	71.2	14.2 to 143.1
T_1 lesion load	Placebo	18.6	5.2 to 35.2
(mm^3/month)	IFN30	13.6	-0.2 to 31.5
	IFN60	12.3	-1.7 to 30.5
Spinal cord area	Placebo	-0.10	-0.18 to -0.03
(mm^2/month)	IFN30	-0.12	-0.21 to -0.03
	IFN60	-0.09	-0.19 to 0.01
Ventricular volume	Placebo	136.3	73.1 to 199.4
(mm^3/month)	IFN30	76.4	11.5 to 141.4
	IFN60	149.5	68.2 to 230.8
TTMW	Placebo	0.52	0.10 to 0.94
(seconds/month)	IFN30	0.46	0.03 to 0.90
	IFN60	0.72	0.16 to 1.29
NHPT - right	Placebo	0.07	-0.04 to 0.18
(seconds/month)	IFN30	-0.02	-0.11 to 0.08
	IFN60	0.22	0.03 to 0.40
NHPT - left	Placebo	0.03	-0.07 to 0.14
(seconds/month)	IFN30	0.07	-0.07 to 0.21
	IFN60	0.12	-0.05 to 0.28

MRI outcomes

The medians and ranges of the raw MRI data are given in Tables 5.7-5.9. Where applicable the estimates of rates of change at month 24 are shown in Table 5.6.

T_2 Lesion Load (Table 5.7)

No significant differences were seen on comparison of individual groups (IFN30 v placebo, p=0.19; IFN60 v placebo, p=0.19) on nonparametric analysis of covariance but comparing the placebo and the combined interferon group there was a non-significant difference (p=0.075) favouring the interferon group. On mixed model regression analysis there was a trend to a treatment group difference (p=0.06) with a significantly lower rate of increase in lesion load in the IFN30 group compared to the placebo group (p=0.025) but no significant difference between the IFN60 and placebo groups (p=0.74).

T_1 Lesion Load (Table 5.7)

No significant treatment differences in T_1 lesion load were seen on nonparametric analysis of covariance (IFN30 v placebo, p=0.12; IFN60 v placebo p=0.96) or on mixed model regression analysis.

New Lesions (Table 5.8)

Few new lesions were seen and there was no significant treatment effect on new T_2 and T_1 brain lesions or new T_2 spinal cord lesions.

Table 5.7 T_2 and T_1 brain lesion loads throughout study

		Placebo	IFN30	IFN60
T_2 lesion load (cm^3)				
Baseline	n	20	15	15
	median (range)	9.51 (0.29-77.57)	11.48 (0.98-64.12)	15.83 (0.69-67.58)
Month 6	n	19	15	14
	median (range)	13.65 (0.30-81.58)	10.53 (1.07-66.95)	14.62 (0.50-70.54)
Month 12	n	20	15	14
	median (range)	9.61 (0.41-79.50)	10.99 (0.83-74.44)	13.1 (0.47-69.23)
Month 18	n	19	15	14
	median (range)	12.50 (0.51-77.26)	11.45 (0.79-76.68)	15.81 (0.56-65.09)
Month 24	n	19	15	14
	median (range)	12.70 (0.45-79.16)	10.96 (0.81-81.60)	16.29 (0.50-65.13)
T_1 lesion load (cm^3)				
Baseline	n	20	15	15
	median (range)	1.17 (0-27.21)	1.29 (0-33.79)	3.26 (0-24.54)
Month 6	n	19	15	14
	median (range)	1.04 (0-24.83)	1.19 (0-37.31)	3.26 (0-21.07)
Month 12	n	20	15	14
	median (range)	1.30 (0-25.82)	1.43 (0-42.45)	3.27 (0-23.60)
Month 18	n	19	15	14
	median (range)	0.99 (0-23.03)	1.98 (0-41.63)	3.08 (0-24.55)
Month 24	n	19	15	14
	median (range)	1.62 (0.12-27.13)	1.67 (0-48.47)	3.64 (0-26.84)

Table 5.8. Number of cumulative new cerebral and spinal cord lesions

		Placebo	IFN30	IFN60
Cerebral T_2				
Month 12	n	19	15	14
	median (range)	1 (0-37)	1 (0-6)	0 (0-4)
Month 24	n	18	15	14
	median (range)	1 (0-44)	1 (0-10)	1 (0-10)
Cerebral T_1				
Month 12	n	19	15	14
	median (range)	0 (0-7)	0 (0-2)	0 (0-3)
Month 24	n	18	15	14
	median (range)	0 (0-10)	0 (0-6)	0 (0-4)
Spinal T_2				
Month 12	n	19	15	14
	median (range)	0 (0-2)	0 (0-1)	0 (0-1)
Month 24	n	18	15	12
	median (range)	0 (0-2)	0 (0-1)	0 (0-2)

Spinal Cord Area & Brain Volume (Table 5.9)

No significant treatment differences were seen in spinal cord area or BBSI. On nonparametric analysis of covariance of ventricular volume there was a significant treatment difference between the IFN60 and placebo groups (p=0.025) with the IFN60 group having a worse outcome. However, on mixed model regression analysis no significant differences were seen in rates of progression (IFN30 v placebo, p=0.20; IFN60 v placebo, p=0.97).

Table 5.9 Spinal cord area, BBSI and ventricular volume throughout study

		Placebo	IFN30	IFN60
Cord area (mm^2)				
Baseline	n	20	13	15
	median (range)	69.15 (57.96-81.70)	70.36 (47.78-83.58)	65.32 (47.48-82.66)
Month 12	n	19	14	13
	median (range)	69.52 (56.36-81.68)	69.90 (59.00-83.18)	64.28 (46.90-84.12)
Month 24	n	19	14	12
	median (range)	67.86 (50.36-83.76)	66.73 (57.16-85.08)	66.76 (55.70-82.16)
BBSI (cm^3)				
Month 0 to 12	n	19	14	12
	median (range)	12.1 (-21.2-40.1)	8.75 (-13.2-30.3)	9.05 (1.2-75.5)
Month 0 to 24	n	17	14	14
	median (range)	15.5 (-0.5-36.4)	12.75 (5.6-35.7)	14.3 (6.7-92.5)
Ventricular Vol (cm^3)				
Baseline	n	20	15	15
	median (range)	23.26 (7.34-63.64)	23.52 (6.27-82.45)	17.67 (4.73-44.74)
Month 12	n	19	15	12
	median (range)	23.81 (7.14-67.36)	22.85 (6.10-88.78)	19.92 (4.70-65.23)
Month 24	n	18	15	14
	median (range)	26.08 (7.36-67.21)	21.98 (6.35-97.50)	21.44 (5.21-74.00)

Adverse events

The most common adverse event was flu-like reactions, reported in 100% of the IFN60 group, 87% of the IFN30 group and 55% of the placebo group (Table 5.10). Mood disturbance occurred more commonly on interferon than placebo. Rises in alanine transaminase occurred more commonly in the IFN60 group. Four subjects, in the placebo group, had acute neurological relapses. There were no significant differences in serious adverse events requiring hospital admission in the treatment arms compared to placebo (Table 5.11).

Neutralising antibodies

One subject, in the IFN30 group, became positive for neutralising antibody during the study.

Blinding

58% (28/48) of patients who completed the blinding questionnaire correctly identified whether they received placebo or interferon beta-1a. The treating physician correctly identified placebo versus interferon treatment in 86% (43/50) of patients.

Table 5.10 Common adverse events (number of subjects affected on one or more occasions)

*p<0.05, **p<0.01 (pairwise comparisons between treatment arm and placebo)

Adverse Event	Placebo (n=20)	IFN30 (n=15)	IFN60 (n=15)
fatigue / malaise	5	7	3
flu-like reaction	11	13	15**
haematological			
anaemia < 10 g/dl	-	1	-
lymphopaenia < 1.0×10^9	-	-	3
neutropaenia < 1.5×10^9	-	-	2
injection site reaction	2	1	2
liver enzyme rise >2xULN			
alanine transaminase	-	-	5**
gamma glutaryl transferase	2	3	3
mood / behavioural disturbance	2	7*	6
(mild/moderate)	(2/-)	(5/2)	(6/-)
relapse	4	-	-

Table 5.11 Events requiring hospital admission (number of subjects admitted).

All p-values >0.05 (pairwise comparisons between treatment arm and placebo).

	Placebo	IFN30	IFN60
Acute cholecystitis	1	-	-
Acute deterioration due to pain	-	-	2
Chest infection	2	1	1
Elective neurorehabilitation	1	3	-
Flu-like reactions (elective management)	-	-	2
Fracture	-	-	2
Gallstone pancreatitis	-	-	1
Gastroenteritis	1	-	-
Medical investigations	-	2	-
Urinary tract infection	-	1	1
Number of subjects admitted to hospital (for any event)	3	6	5

5.4 Discussion

This is the first randomised controlled trial of a beta interferon devoted solely to primary progressive multiple sclerosis. Although only an exploratory study it has demonstrated that therapeutic studies in primary progressive multiple sclerosis are possible. Subject compliance was excellent with 98% of subjects completing the study. Disease progression was clearly evident with 48% of subjects reaching the primary clinical endpoint. The study has provided information on the safety and tolerability of interferon beta-1a and the usefulness of outcome measures in this group.

In regard to safety, there were no life-threatening adverse events in any treatment group and there were no significant differences in serious adverse events between treatment groups. The commonest adverse event associated with interferon treatment was flu-like reactions. Mood disturbance was also more common with interferon treatment though in the majority of subjects was mild (not requiring treatment) and no cases were severe. Rises in alanine transaminase were seen but were reversible and there were no clinical sequelae. The interferon beta-1a 30 μg dose was well tolerated with only one treatment withdrawal and two dose reductions. In contrast, the interferon beta-1a 60 μg dose was poorly tolerated, due to flu-like reactions and raised liver enzymes, and only six of the 15 patients completed the study on full dose; this poor treatment compliance means that the data from this group needs to be interpreted with great care. The recent dose comparison study of intramuscular interferon beta-1a in relapsing-remitting multiple sclerosis also reported an increased incidence of flu-like reactions and raised liver enzymes in the 60 μg compared to the 30 μg group but overall both doses were well tolerated (Clanet *et al.* 2001). The discrepancy in tolerance of the 60 μg dose between the studies may partly reflect the younger age and lower

disability of patients in the relapsing-remitting study.

Four subjects (8%) experienced acute neurological relapses during the two years of the study. In all cases the relapses were mild and resolved without steroid treatment. Although all subjects had a clear history of primary progressive disease without relapses prior to the study this is not surprising. A recent natural history study reported that 28% of patients with primary progressive multiple sclerosis had a relapse at some point even decades after disease onset (Kremenchutzky *et al.* 1999).

Disease progression, as judged by the primary clinical endpoint, was confirmed in 48% of subjects and progression was also evident on the secondary outcome measures. Wide variability in rates of progression, often dependent on baseline value, was seen between individual patients and so the rates of progression in Table 5.6 are given for an average baseline value. In the placebo group there was a significant increase in the time to walk ten metres and a trend to increase in the time to perform the NHPT. On MRI there were significant increases in T_2 and T_1 brain lesion loads. Spinal cord atrophy was detectable though changes were small and there was also significant brain atrophy. These findings are consistent with those of a large European natural history study in primary progressive multiple sclerosis which demonstrated significant increases in T_2 and T_1 lesion loads and significant spinal cord and brain atrophy after two years of follow-up (Ingle *et al.* In press). Both the study presented here and the European study found few new brain and spinal cord lesions (Stevenson *et al.* 2000b) which may suggest that the role of new lesions as a marker of disease progression is limited in primary progressive multiple sclerosis. However, as demonstrated in the five year follow-up study of the European data, new brain and cord lesions may predict disease progression over a longer period (Stevenson *et al.* 2001).

In view of the small sample size of this study any discussion of efficacy must be

circumspect. No treatment effect was seen on the primary clinical endpoint. There was a suggestion of a treatment difference on right side performance on the NHPT. Patients with primary progressive multiple sclerosis frequently have a paraparesis or quadraparesis with the legs being much more affected than the arms. A measure of upper limb function may therefore be more sensitive to change than measures of ambulation in patients who have already accrued significant lower limb dysfunction. The NHPT has previously been shown to be more sensitive to change than the EDSS in multiple sclerosis (Goodkin *et al.* 1988). In the recent study of intramuscular interferon beta-1a in secondary progressive multiple sclerosis (Cohen *et al.* 2001) no effect was seen on the EDSS and the treatment effect on the Multiple Sclerosis Functional Composite (Cutter *et al.* 1999) was almost entirely due to change in the NHPT.

A differential treatment effect was seen on two of the eight MRI outcome measures, T_2 lesion load and ventricular volume. Subjects on interferon beta-1a 30 μg showed only a minimal increase in T_2 lesion load with a significantly lower rate of increase than control subjects. This observation raises two issues. Firstly, T_2 lesion load, although thought to be a poor surrogate marker of disability in primary progressive multiple sclerosis (Filippi *et al.* 1995a), may be a more responsive measure than previously appreciated as suggested by Stevenson *et al.* (2000b). Secondly, it suggests the possibility of an anti-inflammatory effect in primary progressive multiple sclerosis despite it being considered a disease characterised by low grade inflammation (Revesz *et al.* 1994; Silver *et al.* 2001). This observation suggests that a therapeutic role for anti-inflammatory agents in primary progressive multiple sclerosis cannot be outruled.

The group receiving interferon beta-1a 60 μg appeared to do worse in terms of ventricular enlargement. One possible explanation is that this group had a higher lesion load

at baseline, though not reaching significance, and atrophy may be greater in patients with a higher burden of disease (Paolillo *et al.* 2000). Another possible explanation is beta interferon may contribute to brain atrophy due to its anti-inflammatory effect as suggested recently in a study in secondary progressive multiple sclerosis (Molyneux *et al.* 2000). However, as previously discussed, it is difficult to draw conclusions regarding the IFN60 group as treatment was so poorly tolerated. What is perhaps more important is that it was possible to detect a differential effect on ventricular volume and this may therefore be a useful outcome measure in primary progressive multiple sclerosis though further evaluation of its relationship with disease progression is required. Change in ventricular volume has recently been shown to be a sensitive measure of atrophy in patients with multiple sclerosis (Fox *et al.* 2000) and in clinically isolated syndromes predicts conversion to definite multiple sclerosis (Dalton *et al.* 2001a).

Subsequent to the start of this study beta interferon has been reported to worsen spasticity in primary progressive multiple sclerosis (Bramanti *et al.* 1998). Following completion of the study a post-hoc analysis of spasticity was carried out. The clinical notes were reviewed and the occurrence of spasticity was noted and divided into that occurring post-dose in association with flu-like reactions and that reported as a sustained increase in background level of spasticity. Any increase in anti-spasticity treatment was also noted. No significant increase was seen in post-dose spasticity, background spasticity or anti-spasticity treatment on interferon. These findings therefore do not support the hypothesis that beta interferon enhances spasticity in primary progressive multiple sclerosis.

This study has demonstrated that it is entirely feasible to carry out therapeutic trials in primary progressive multiple sclerosis. A pilot trial of riluzole has now also been completed in this group (Kalkers *et al.* 1999) and exploratory studies of interferon beta-1b

(Montalban *et al.* 1998) and mitoxantrone (Kita *et al.* 2000) and a large multi-centre phase III trial of glatiramer acetate (Wolinsky *et al.* 2001) are underway. The lack of reliable MRI markers of disease progression remains a challenge for therapeutic trials in primary progressive multiple sclerosis but this study has shown that T_2 lesion load is a useful measure and ventricular volume also has potential in this group. Finally, although this study has provided some information on which a phase III study might be based, the advisability of doing such a trial remains uncertain.

CHAPTER 6: CONCLUSIONS AND FUTURE DIRECTIONS

This thesis has focused on primary progressive multiple sclerosis, exploring newly developed imaging techniques and the information they may provide on the disease process, and has reported the first randomised controlled trial specifically designed for this group. This has highlighted several areas of interest related to therapeutic trials in primary progressive multiple sclerosis and these are now discussed, with an emphasis on possible future directions, under three main headings. Firstly, in regard to patient selection, the unique difficulties in diagnosis, classification, recruitment and sample size are addressed. Secondly, the role of MRI, both as a tool to investigate pathogenesis and as an outcome measure, is explored. Finally, the results of the randomised controlled trial of interferon beta-1a in primary progressive multiple sclerosis are discussed.

Patient Selection

The difficulties in diagnosis and classification of primary progressive multiple sclerosis were highlighted in the recruitment of this study. Of the 88 patients referred with a diagnosis of primary progressive multiple sclerosis but not included in the study, 50% either did not have primary progressive multiple sclerosis or the diagnosis was not secure. This at least partly reflects the lack of specific diagnostic criteria for primary progressive multiple sclerosis at the time of recruitment. To address this problem diagnostic criteria have recently been proposed (Thompson *et al.* 2000). As patients with primary progressive multiple sclerosis do not have 'attacks' these criteria essentially differ from the Poser criteria in that they do not require evidence of attacks disseminated in time, though evidence of

clinical progression for at least one year is mandatory. Three levels of certainty of primary progressive multiple sclerosis are defined: 1) definite - adequate evidence clinically and from investigations to be confident that the diagnosis is sufficiently definite, 2) probable - strong clinical suspicion of the diagnosis but insufficient evidence to be definite, and 3) possible - only limited evidence suggests the diagnosis. Investigations considered to provide supportive evidence are CSF examination, MRI and visual evoked potentials with central importance placed on evidence of intrathecal synthesis of IgG in the CSF. Further details of the diagnostic criteria are given in Table 6.1. As yet these criteria have only undergone limited validation on retrospective data (Thompson *et al.* 2000; Brieva *et al.* 2000) and prospective validation is awaited. For future clinical trials the inclusion of only patients with definite primary progressive multiple sclerosis may provide more robust cohorts.

Difficulties in certainty of diagnosis are not only limited to primary progressive multiple sclerosis but also to other clinical presentations of multiple sclerosis. Diagnostic criteria for multiple sclerosis have recently been revised (McDonald *et al.* 2001). These criteria still place an emphasis on dissemination of lesions in time and space and incorporate both clinical and paraclinical evidence. Magnetic resonance imaging, which is recognised as the most sensitive and specific paraclinical test, has been incorporated formally into the diagnostic scheme with defined MRI criteria for both dissemination in space and time. Diagnostic criteria have been recommended for different clinical presentations. If the criteria are fulfilled, the diagnosis is 'multiple sclerosis'; if the criteria are not completely fulfilled, the diagnosis is 'possible multiple sclerosis'; if the criteria are fully explored and not fulfilled, the diagnosis is 'not multiple sclerosis'. The terms clinically definite / probable and laboratory-supported definite / probable are no longer recommended. The criteria for patients with insidious neurological progression suggestive of multiple sclerosis essentially equate

Table 6.1. Diagnostic criteria for primary progressive multiple sclerosis

Definite primary progressive multiple sclerosis
Clinical progression for at least one year, and
Positive CSF evidence, and
Positive MRI evidence, or equivocal MRI evidence and a delayed VEP
Probable primary progressive multiple sclerosis
Either:
Clinical progression for at least one year, and
Positive CSF evidence, and
Equivocal MRI evidence, or delayed VEP
Or:
Clinical progression for at least one year, and
Positive MRI evidence, or equivocal MRI evidence and a delayed VEP
(CSF evidence either unavailable or negative)
Possible primary progressive multiple sclerosis
Clinical progression for at least one year, and
Equivocal MRI evidence or delayed VEP

to the criteria of Thompson *et al.* (2000) for definite primary progressive multiple sclerosis. Preliminary validation of the McDonald criteria in a prospective study of patients with clinically isolated syndromes suggestive of multiple sclerosis show that they have a high specificity and high positive predictive value (Dalton et al 2001b); further validation is required for other clinical presentations.

Another problem in recruiting patients to therapeutic trials in multiple sclerosis is the relative rarity of the disease. For the small exploratory trial presented in this thesis patients were recruited from all over England and Wales. For the current phase III trial of glatiramer acetate in primary progressive multiple sclerosis patients were recruited from multiple centres in North America but due to a shortfall in numbers recruitment had to be extended to Europe. Large phase III trials may therefore virtually 'exhaust the supply' of patients for treatment trials making it difficult to recruit for trials of other therapeutic agents simultaneously. It is therefore important in the planning of phase III trials that there is an international investigator led consensus as to which therapeutic agents are best explored so that patient resources are not diverted from key trials.

At the time of recruitment for the study presented here, limited natural history data was available on which to base sample size calculations. A geographically based multiple sclerosis study in Ontario, Canada has subsequently extended the natural history data on primary progressive multiple sclerosis (Cottrell *et al.* 1999a). This study provides clinical data on 216 patients with primary progressive multiple sclerosis with a mean longitudinal follow-up of 23 years and on a second series of 165 patients seen over five years. From this database the number of patients who would be trial eligible were determined for a series of prognostically defined hypothetical entry criteria (Cottrell *et al.* 1999b). Sample size tables were then developed giving the number of patients and length of follow-up required to detect

a significant result for a percentage decrease in the median time to progression on the disability status score. For example, in a two year trial, 543 patients per group would be necessary to have an 80% chance of detecting an increase in median staying time of 25%. These sample size calculations will be useful in the planning of future therapeutic trials in primary progressive multiple sclerosis. The magnitude of sample sizes indicated again reinforces the need for large multicentre trials.

MRI

MRI has been used extensively in this thesis, both as a tool to further the understanding of disease pathology in primary progressive multiple sclerosis and as a surrogate marker of disease progression in therapeutic monitoring. In particular, this thesis has incorporated recently developed imaging measures that may be more pathologically specific than conventional measures. When evaluating an MRI measure three issues need to be considered, i) the insight it provides into pathogenesis of the disease, ii) its validity as a marker of disease progression which may be judged by its correlation with disability, and iii) its responsiveness to change. The latter two issues are particularly relevant when assessing the usefulness of an MRI measure as an outcome in clinical trials. However, consideration must also be given to the reliability of a measure and this is first briefly discussed.

Methodological issues relating to the serial MRI measurement including reliability have been discussed in Chapter 4. The importance of evaluating the reliability of a measurement technique was exemplified in the serial study of spinal cord atrophy and as a result a protocol of quality assurance is now an integral part of all on-going studies at this centre. Quality assurance is an essential requirement for all serial studies and should be

incorporated into the design of all clinical trials. In the evaluation of new MRI techniques consideration must also be given to possible sources of measurement variation including physiological variation. The study of the effect of aging on MRS metabolite concentrations emphasises the need for age-matching and also indicates that metabolite concentration ratios are inferior to absolute concentrations in the accurate interpretation of results. Future MRS studies in multiple sclerosis should therefore ideally quantify absolute concentrations of NAA.

Considering the role of MRI in understanding the pathogenesis of primary progressive multiple sclerosis, one of the outstanding questions that remains unanswered is why there is such a marked discrepancy between clinical status and focal lesions on MRI. In this thesis the disease process in NAWM has been investigated using the MRI techniques of MTR and MRS which have not previously been thoroughly evaluated in primary progressive multiple sclerosis.

As presented in Chapter 3 small but widespread reductions in MTR of NAWM were seen using a region of interest approach. This suggests that there is diffuse disease involvement of the NAWM in primary progressive multiple sclerosis but it is difficult to draw further conclusions regarding pathogenesis; although recent studies have provided important information on the pathological substrate of a reduction in MTR (van Waesberghe *et al.* 1999) it may still reflect a range of disease processes. Recent studies of MTR have employed a histogram approach which can provide more global measures of MTR. It would be hoped that global measures of MTR, containing information on large areas of tissue, would be more sensitive to pathological changes. Therefore, such measures, particularly if used in combination with pathological studies, may be more helpful in providing information on pathogenesis.

In this region of interest study correlations of MTR of NAWM with disability were limited. Despite larger reductions of MTR in lesions than in NAWM, average lesion MTR in primary progressive multiple sclerosis also did not correlate with disability (Leary *et al.* 1999b). In histogram studies abnormalities in MTR histogram parameters in primary progressive multiple sclerosis have been demonstrated in both brain (Filippi *et al.* 1999; Dehmeshki *et al.* 2001) and cervical spinal cord (Filippi *et al.* 2000b) cross-sectionally. In the largest of these studies, which studied 46 patients (from the cohort presented in this thesis), there was no correlation between individual brain MTR parameters and disability (Dehmeshki *et al.* 2001). However, a significant correlation with disability was seen with principal component analysis. Principal component analysis provides a more global characterisation of the histogram variation and so may be a more sensitive measure than conventional MTR histogram parameters. This is therefore a promising method which requires further evaluation.

MTR histogram analysis can also be used in conjunction with segmentation techniques to investigate the contribution of different tissue compartments to pathology. Histogram analysis has been carried out on normal appearing brain tissue (NABT), by segmenting out lesions, and has shown a significant reduction in average MTR and a reduction in peak height in patients with primary progressive multiple sclerosis consistent with widespread but mild changes (Tortorella *et al.* 2000). The authors suggested that these changes were due to white matter rather than grey matter abnormalities because NAWM represents the largest part of the normal appearing brain tissue. However, a recent study in 30 patients with primary progressive multiple sclerosis (also from the cohort studied in this thesis) not only showed a reduction in mean normal appearing grey matter (NAGM) MTR but also a significant correlation of mean NAGM MTR with EDSS (Miller *et al.* 2001b). The

clinical significance of this is not clear but further investigation is warranted.

Further information on the role of MTR as a marker of disease progression will be provided from the placebo serial data from the therapeutic trial of interferon beta-1a presented in this thesis. Disappointingly, using a region of interest approach, preliminary analysis indicates that there was no significant change in average lesion MTR or MTR of NAWM over the two years of the trial. (SM Leary, unpublished data). This is perhaps not surprising as it is likely that changes over time are small and so may not be detected by the limited sampling of a region of interest approach. As yet there is limited longitudinal MTR histogram data available. Filippi *et al.* (2000a) studied nine patients with primary progressive multiple sclerosis over one year and found that whereas in other subgroups of multiple sclerosis, particularly secondary progressive multiple sclerosis, significant changes in MTR histogram parameters were seen, no significant changes were detected in primary progressive multiple sclerosis. However, preliminary results from 16 patients in the placebo arm of the interferon beta-1a trial show a significant decrease in mean normal NABT MTR over two years (Traboulsee *et al.* 2001). Further serial studies are required to evaluate whether MTR histogram analysis is sensitive enough to be useful as an outcome measure in clinical trials.

This thesis also includes data on MRS in NAWM in primary progressive multiple sclerosis. A significant reduction in NA was seen in primary progressive patients, and this finding has since been confirmed in other studies (Cucurella *et al.* 2000; Suhy *et al.* 2000). This is consistent with the hypothesis that axonal loss or damage is the pathological substrate of disability in primary progressive multiple sclerosis. No correlation with disability was seen in this study which is not surprising considering only a single voxel was studied. However, in a more recent study using single slice spectroscopic imaging a significant relationship was found between NAA/Cr and EDSS ($r=0.67$, $p=0.025$) in primary progressive but not

relapsing remitting patients (Suhy *et al.* 2000). The role of single voxel MRS seems limited as a marker of disease progression and preliminary analysis from serial follow-up of this study indicates no significant change in NA over one year (SM Leary, unpublished results). Single and multiple slice spectroscopic imaging, which provide more global quantification of brain NAA, remain promising disease markers. Measurement of whole brain NAA has recently been described (Gonen *et al.* 2000) and this may potentially be the most promising spectroscopic marker of disease progression. However, further validation of these techniques is required particularly in regard to reproducibility. Considering single voxel spectroscopy, even in an experienced centre the scan-recan reproducibility is in the order of 6% (DG MacManus, personal communication). As changes in global NAA due to disease progression are likely to be small the reproducibility of the measurement technique must be known in order to correctly interpret any change.

The study of interferon beta-1a has been one of the first clinical trials to use recently developed measures of spinal cord and brain atrophy and has provided further information on their usefulness as outcome measure. Although the spinal cord atrophy technique has previously been shown to be a sensitive and reproducible measure (Losseff *et al.* 1996b), the changes in cord area seen over time in this study are small. Similarly, in the European natural history study a reduction in cord area of only 3% was seen at two years (Ingle *et al.* In press). This technique may therefore only be expected to detect a large treatment effect in a study of this duration. However, at five years in the European study a reduction of cord area of 9.5% was seen with a weak correlation between change in cord area and disability (Ingle *et al.* 2001a) and so cord atrophy may be a useful marker of disease progression in the longer term. In a recent study in multiple sclerosis of the whole brain atrophy technique used in this trial, the rate of brain atrophy was only 0.8% per year, though this was twice that of controls

(Fox *et al.* 2000). This technique may again only be expected to detect a large treatment effect. However, in the same study the rate of ventricular enlargement was five times greater than that of controls. This is entirely consistent with the results of the interferon beta-1a trial which showed only a significant differential treatment effect on ventricular enlargement and not on spinal cord or whole brain atrophy. Ventricular enlargement therefore appears to be a responsive measure though its validity as a marker of disease progression may be less clear; brain atrophy appears to not only reflect disease progression but also the presence of inflammatory lesions and the effects of anti-inflammatory drugs (Molyneux *et al.* 2000). The value of brain atrophy as a short-term marker of disease progression in therapeutic trials may therefore be confounded by anti-inflammatory effects though its role in the longer term is yet to be evaluated.

Returning to the question of why there is such a marked discrepancy between clinical and MRI findings in primary progressive multiple sclerosis it must be concluded that the studies presented here have not provided an answer; although abnormalities have been identified in NAWM they are not disproportionately greater than those seen in the other subgroups of multiple sclerosis. Other approaches to this question must therefore be considered. The hypothesis that patients with primary progressive multiple sclerosis may have a greater disease burden in the spinal cord has previously been discussed and there is no clear evidence to support this hypothesis. Another approach is suggested by the different clinical courses of primary progressive and relapsing remitting / secondary progressive multiple sclerosis. Patients with primary progressive multiple sclerosis have a relentlessly progressive course despite relatively little evidence of inflammation compared to patients who present with relapses. Acute inflammatory demyelination appears to be the pathological substrate of relapses and so it is consistent that less inflammation is seen in patients who do

not have relapses. As discussed earlier the proposed pathological correlate of disease progression is axonal loss and investigation of the natural history of axonal loss is the logical next step in furthering our understanding of pathogenesis. This is already underway and studies in clinically isolated syndromes, i.e. at the earliest presentation of multiple sclerosis, have demonstrated that atrophy, a surrogate marker of axonal loss, is occurring (Brex *et al.* 2000b; Brex *et al.* 2001). MRS has also provided evidence of widespread axonal damage in early relapsing remitting multiple sclerosis (Kapeller *et al.* 2001). Similar study in early primary progressive multiple sclerosis is required to evaluate the extent and progression of axonal loss in this subgroup and this is now underway (Ingle *et al.* 2001b).

Interferon Beta-1a

The first randomised controlled trial of a beta interferon in primary progressive multiple sclerosis has been presented in this thesis. At the outset of this trial it was considered a challenge due to the unique problems outlined earlier. However, the need for such a trial was clearly evident as therapeutics in primary progressive multiple sclerosis was such a neglected area. Despite the difficulties in identifying appropriate patients already described, recruiting individual patients was simple as it was the first opportunity for all the patients involved to participate in a treatment trial. The commitment that the patients showed to the trial was outstanding, particularly as most patients had significant disability which made the frequent visits and long scanning protocols even more inconvenient, and this was exemplified by the excellent compliance with follow-up.

The main objectives of the trial were to explore the safety of and look for hint of efficacy of interferon beta-1a in primary progressive multiple sclerosis and to identify

potentially useful outcome measures in this group. In regard to safety, as would be expected from previous trials in multiple sclerosis, no serious adverse events were significantly associated with interferon beta-1a therapy. However, the interferon beta-1a 60 μg dose was poorly tolerated and so this dose not appear to be a suitable dose for the majority of patients with primary progressive multiple sclerosis. In respect to efficacy the trial was negative for the primary clinical endpoint though there was a suggestion of a differential effect on one of the secondary clinical outcomes, the NHPT. A positive effect was also suggested on T_2 lesion load but not on any of the other MRI outcomes and a negative effect was seen on ventricular volume. In view of the small sample size it is not meaningful to comment further on the significance of the treatment effects seen. However, the fact that certain outcome measures detected differential effects implies that they are potentially responsive measures. Outcome measures have already been extensively discussed but this study has provided particularly useful information on T_2 lesion load.

T_2 lesion load has previously been considered to be a poorly responsive measure in primary progressive multiple sclerosis. However, the European natural history study has shown significant increases in T_2 lesion load after one and two years (Stevenson *et al.* 2000b; Ingle *et al.* In press). Further follow-up is now required to evaluate whether changes in T_2 lesion load are predictive of long term disability and this information will be provided by the ongoing European study. Despite the recent interest in more pathologically specific measures there is currently more evidence to support T_2 lesion load as a useful outcome in primary progressive multiple sclerosis than there is for MTR or MRS. In a recent longitudinal study in primary progressive multiple sclerosis comparing the sensitivities of MTR measures and conventional lesion loads, an increase was seen in T_2 lesion load but no significant changes were seen in any MTR parameters in primary progressive multiple sclerosis (Filippi *et al.*

2000a).

An interesting issue reiterated by this trial is the question of whether primary progressive multiple sclerosis is a distinct disease entity or just part of the spectrum of disease of multiple sclerosis. Four patients had acute neurological relapses during the course of the trial and relapses have also been reported to occur in primary progressive multiple sclerosis in natural history studies (Kremenchutzky *et al.* 1999). However, all the relapses were mild and the predominant character of the patients' disease course remained progressive. Although it therefore seems that there is at least overlap between disease subgroups the question of why some patients have a predominantly progressive disease remains unanswered and requires further investigation.

Probably the main achievement of this trial has been to prove that it is entirely feasible to carry out therapeutic trials in primary progressive multiple sclerosis. Despite the small sample size there was clear evidence of disease progression with 48% of patients reaching the primary clinical endpoint and differential treatment effects were seen on three outcome measures. Whether or not interferon beta-1a is an effective treatment in primary progressive multiple sclerosis cannot be answered by this trial. With the currently available outcome measures it is unlikely that preliminary phase II trials would further predict the answer to this question and this would require a phase III trial with approximately 500 patients per group. Whether a phase III trial should be carried out is a difficult question. The results of phase III trials in secondary progressive multiple sclerosis suggest that the effect of beta interferons on disease progression is probably marginal. With the difficulty in recruiting primary progressive patients for trials a consensus decision should be made as to which of the current therapeutic agents under exploration including interferon beta-1a should be investigated further. What is probably more important is that when more effective

treatments are developed for multiple sclerosis there should be no hesitation in proceeding with trials in primary progressive multiple sclerosis and so these patients should no longer be 'at the back of the queue'. Recruitment for the first trial of beta interferon in primary progressive multiple sclerosis commenced eight years after the start of recruitment for the first trial in relapsing-remitting multiple sclerosis. Since then two therapeutic trials in primary progressive multiple sclerosis have been completed and three are presently underway. It is hoped that therapeutics in primary progressive multiple sclerosis will continue to expand and, by investigating further the underlying pathogenetic mechanisms, more effective therapeutic agents will be developed.

Concluding Remarks

At the outset of the work presented in this thesis relatively little was known about MRI evaluation and therapeutic trials in primary progressive multiple sclerosis. Subsequently there has been an upsurge in interest in this patient group. This is exemplified by the international workshop on primary progressive multiple sclerosis held in Barcelona in May 2000 at which data on all aspects of the disease were presented and discussed (Montalban & Thompson In press). It is hoped that the data provided by this thesis facilitates a continuing increase in interest and research activity in primary progressive multiple sclerosis.

Appendix

Kurtzke Expanded Disability Status Scale

0.0 Normal neurological examination

1.0 No disability, minimal signs in one functional system (FS)

1.5 No disability, minimal signs in more than one FS

2.0 Minimal disability in one FS

2.5 Minimal disability in two FS

3.0 Moderate disability in one FS or mild disability in three or four FS, though fully ambulatory

3.5 Fully ambulatory but with moderate disability in one FS and mild disability in one or two FS; or moderate disability in two FS; or mild disability in five FS

4.0 Fully ambulatory without aid, self-sufficient up and about some 12 hours a day despite relatively severe disability in one FS or combinations exceeding limits of previous steps; able to walk without aid or rest some 500 m

4.5 Fully ambulatory without aid, up and about much of the day, able to work a full day, may otherwise have some limitation of full activity or require minimal assistance; able to walk without aid or rest some 300 m

5.0 Ambulatory without aid or rest for about 200 m; disability severe enough to impair full daily activities (e.g. to work a full day without special provisions)

5.5 Ambulatory without aid or rest for about 100 m, disability severe enough to preclude full daily activities

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

6.5 Constant bilateral assistance (canes, crutches, braces) required to walk about 20 m with or without resting

7.0 Unable to walk beyond approximately 5 m even with aid, essentially restricted to wheelchair; wheels self in standard wheelchair and transfers alone, up and about in wheelchair some 12 hours a day

7.5 Unable to take more than a few steps; restricted to wheelchair; may need aid in transfer, wheels self but cannot carry on in standard wheelchair a full day; may require motorised wheelchair

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

8.5 Essentially restricted to bed much of day; has some effective use of arm(s); retains some self-care functions

9.0 Helpless bed patient; can communicate and eat

9.5 Totally helpless bed patient; unable to communicate effectively or eat/swallow

10.0 Death due to MS

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