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CLINICAL AND MRI FEATURES OF
PRIMARY PROGRESSIVE
MULTIPLE SCLEROSIS

A thesis submitted by
Gordon Thorpe Ingle BSc MBChB MRCP(Ed.)
for the degree of Doctor of Medicine

Institute of Neurology
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July 2005
Abstract

In approximately 10-15% of cases Multiple Sclerosis follows a progressive rather than a relapsing course and this is known as Primary Progressive Multiple Sclerosis (PPMS). In this thesis previous clinical, pathological and Magnetic Resonance Imaging (MRI) studies of PPMS are reviewed and new studies using two cohorts of patients with PPMS are presented. In the first of these studies an existing cohort of patients with PPMS are re-examined at first two, and then five years, clinically and with MRI, to provide the longest period of MRI follow up in the condition to date. Changes in clinical and MRI measures over this time, and their correlation, are described. Over this extended period, some limited correlation can be found between clinical and MRI measures in PPMS. It is also seen that there is great variability in the rate of MRI and clinical progression between individuals with PPMS, although for a given individual progression is relatively constant. The possible implications of this observation for the nature of the underlying disease process are discussed. The second part of this thesis describes the clinical and MRI features of a second cohort of patients with clinically early PPMS, examined within five years of the first onset of symptoms, the first study to examine this stage of the condition. It is seen that much of the MRI variation seen in established PPMS is already present at this time and that the degree of MRI abnormality, even at this early stage, can be substantial. The specific question as to whether a distinct, early, inflammatory phase occurs in the condition (on the model of the more fully studied relapsing MS subtype) is addressed by the use of triple dose Gadolinium in a subgroup of this cohort examined over six months and evidence for the possible existence of such a phase in some patients with PPMS is found.
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Acknowledgements

I would like to give particular thanks to Valerie Stevenson who originally recruited the first of the two PPMS cohorts described in this thesis and who, together with Siobhan Leary, provided such a good introduction to the NMR unit. I must also thank David MacManus, Ros and Chris for their patience, professionalism and good humour as radiographers in acquiring MRI data from patients with high levels of disability in what were sometimes less than ideal circumstances. Thanks too to Claudia Kingshott-Wheeler and Gareth Barker who provided high quality physics support at all times and to Professor David Miller for many helpful suggestions. On a more personal note I would like to thank my office colleagues, Olga Ciccarelli and Ahmed Toosy, for their practical help and cheerful company and Jaume Sastre Garriga for his understanding, generosity and truly invaluable assistance with every aspect of the later stages of the early PPMS project. Finally, I must give my special thanks to my principal supervisor, Professor Alan Thompson, for his sound counsel and untiring support throughout, to my parents and to The Wellcome Trust and The MS Society of Great Britain who funded this work.
Declaration

I declare that I am the sole author of this thesis and it contains my own work except where indicated.

Gordon Thorpe Ingle
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Publications

Papers

Two-year follow-up study of primary and transitional progressive multiple sclerosis.

Magnetic resonance imaging in primary progressive multiple sclerosis.

Primary progressive multiple sclerosis: a 5-year clinical and MR study.
Ingle GT, Stevenson VL, Miller DH, Thompson AJ. *Brain*. 2003; 126:2528-36


**Book Chapter**

Conventional MRI in PPMS Ingle GT, Miller DH, Thompson AJ in “Primary Progressive Multiple Sclerosis” editors Filippi M and Comi G Springer Verlag 2002

ISBN 88-470-0167-6, pp 63-76
## Abbreviations

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<td>BBB</td>
<td>Blood Brain Barrier</td>
</tr>
<tr>
<td>Cr</td>
<td>Creatinine</td>
</tr>
<tr>
<td>EDSS</td>
<td>Expanded Disability Status Scale</td>
</tr>
<tr>
<td>CSF</td>
<td>Cerebrospinal Fluid</td>
</tr>
<tr>
<td>CV</td>
<td>Coefficient of Variation</td>
</tr>
<tr>
<td>DTI</td>
<td>Diffusion Tensor Imaging</td>
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<tr>
<td>fMRI</td>
<td>Functional MRI</td>
</tr>
<tr>
<td>FS</td>
<td>Functional Score</td>
</tr>
<tr>
<td>Gd</td>
<td>Gadolinium</td>
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<tr>
<td>MAGNIMS</td>
<td>Magnetic Resonance Network in Multiple Sclerosis</td>
</tr>
<tr>
<td>MIDAS</td>
<td>Medical Image Display and Analysis Software</td>
</tr>
<tr>
<td>MRI</td>
<td>Magnetic Resonance Imaging</td>
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<tr>
<td>MRS</td>
<td>Magnetic Resonance Spectroscopy</td>
</tr>
<tr>
<td>MS</td>
<td>Multiple Sclerosis</td>
</tr>
<tr>
<td>MTI</td>
<td>Magnetisation Transfer Imaging</td>
</tr>
<tr>
<td>MTR</td>
<td>Magnetisation Transfer Ratio</td>
</tr>
<tr>
<td>MSFC</td>
<td>Multiple Sclerosis Functional Composite</td>
</tr>
<tr>
<td>NAA</td>
<td>N-acetyl aspartate</td>
</tr>
<tr>
<td>9HPT</td>
<td>Nine Hole Peg Test</td>
</tr>
<tr>
<td>NAWM</td>
<td>Normal Appearing White Matter</td>
</tr>
<tr>
<td>NAGM</td>
<td>Normal Appearing Grey Matter</td>
</tr>
<tr>
<td>PASAT</td>
<td>Paced Auditory Serial Addition Test</td>
</tr>
<tr>
<td>PPMS</td>
<td>Primary Progressive Multiple Sclerosis</td>
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</table>
RF Radio frequency

RRMS Relapsing Remitting Multiple Sclerosis

RT Repetition Time

SD Standard Deviation

SE Spin Echo

SPMS Secondary Progressive Multiple Sclerosis

TE Echo Time

TPMS Transitional Progressive Multiple Sclerosis

TMW Timed Ten Metre Walk
Chapter 1

Magnetic Resonance Imaging

in Primary Progressive Multiple Sclerosis
1.0 Introduction

The earliest clinical accounts of multiple sclerosis (MS) recognised that it could follow a progressive as well as a relapsing course [1] but it was not until much later that a clear distinction was made between those patients whose condition is progressive from onset (primary progressive multiple sclerosis, PPMS) and those who develop progressive deterioration after an initial relapsing and remitting phase (secondary progressive multiple sclerosis, SPMS) [2]. Even then, for some time PPMS and SPMS tended to be regarded as a single entity; “chronic progressive MS”. However, the demonstration of clear differences in the magnetic resonance imaging (MRI) appearances of PPMS and SPMS suggested that there might be important differences in underlying disease mechanisms [3]. Subsequently, the trend has increasingly been to distinguish and consider separately the progressive MS subtypes both clinically and in research. This process has been assisted by the development of clear clinical definitions of PPMS and specific diagnostic criteria [4;5].

Patients affected by PPMS frequently experience an early onset of disability and, unlike patients affected by relapsing remitting Multiple Sclerosis (RRMS), have yet to be offered an effective disease modifying treatment. These are good reasons why it is important to try and understand the disease mechanisms that underlie the condition, in particular the mechanism of progressive disability. Not only may this help in the development of new therapies for PPMS, but it may also provide insights into the mechanism of progressive disability in MS more generally. There is also a need to give more accurate prognostic information to affected individuals early in their disease course as there is a wide range of clinical outcomes. This introductory chapter
considers in detail the contribution already made by MRI to the understanding of
disease mechanisms in PPMS and begins with a brief general description of the
clinical and paraclinical phenotype of PPMS.

1.1 Primary Progressive MS

The percentage of patients with MS whose condition is progressive from onset is
between 10 and 15% [6]. Distinctive features of the PPMS clinical phenotype, in
comparison with relapse onset MS, include an older age at onset, more prominent
motor symptoms at presentation and a similar incidence in males and females [7;8].
Diagnostic criteria for PPMS include a minimum period of clinical progression of at
least 12 months (with thorough investigation to exclude other progressive
neurological conditions), the presence of abnormal intrathecal synthesis of IgG
(oligoclonal bands in cerebrospinal fluid which are not present in the serum) and a
minimum number of nine brain lesions on T2-weighted MRI images of the brain or
two cord lesions. If there is one cord lesion, four to eight brain lesions are required
[5;7].

Fundamentally, PPMS, in common with all other forms of MS, is a chronic
inflammatory demyelinating disease of the central nervous system (CNS) of unknown
cause. A multifactorial aetiology is thought likely with contributions from inherited
and acquired dysfunction of the immune system and environmental factors. A genetic
contribution to aetiology was first suggested when it was observed that there is
increased familial risk of developing MS when another family member was affected
[9]. Genetic factors associated with susceptibility to MS, outcome and clinical course
have been studied over many years. One of the earliest, and most robust, of these associations is between HLA-DR2 status and risk of developing of MS [10]. Initially, it appeared that this association was not found in patients with PPMS but this is not now thought to be the case [11-13]. There is however, some evidence to suggest that HLA-DR4 may be under-represented in patients with PPMS [14]. There have also been reports that certain polymorphisms, for example of the interleukin 4 receptor [15]and of the Fc portion of IgG [16] differ in frequency between PPMS and other subtypes.

In PPMS, as in all MS, there is evidence for an abnormal immune response that leads to loss of myelin, axons and oligodendrocytes. Subtle differences between PPMS and other MS subtypes in have been reported in pathology, cell biology and immune response but interpretation of findings in this area is difficult as only a small number of studies have clearly differentiated PPMS from SPMS and initial findings have not always been replicated.

Pathological studies suggest that there is a less marked inflammatory response in PPMS. Brain lesions in PPMS have less lymphocytic perivascular cuffing and less parenchymal cellular density within them in comparison to brain lesions in SPMS [17]. If inflammatory T cells are less common in PPMS, the cells that are present may be unusually persistent as they appear to over express an anti-apoptotic protein, bcl-2 [18]. Conversely, bcl-2 may be under-expressed in oligodendrocytes in PPMS leading to early depletion [19]. Differences in oligodendrocyte behaviour have also been described with depletion and renewal of oligodendrocytes being seen in RRMS, but permanent loss in PPMS [20]. Specifically, Luchinetti et al reported a pattern of
Oligodendrocyte recruitment and extensive remyelination (which occurs in what they call group I lesions) that was typically associated with RRMS and SPMS and a pattern of relative loss of oligodendrocytes and lack of remyelination (group II lesions) typically associated with acute and PPMS. This pathological classification of patterns of demyelination was later revised into four types, I, II, III and IV. In this classification it is the type IV pattern where there is primary oligodendrocyte loss that appears to be associated with PPMS [21;22]. The occurrence of axonal damage in PPMS and other MS subtypes has been reported by Trapp [23]. On the limited histopathological evidence to date, the degree of axonal damage does not appear to be greater in PPMS than in other subtypes and may even be less than that seen in SPMS [24].

Immunologically it has been reported that patients with PPMS have a decrease in CD4+ cells producing IL-2, IL-13 and TNF-alpha and an increase in CD8+ cells producing IL-4 and IL-10 [25]. However, studies looking at the cytokine production of peripheral blood mononuclear cells in response to mitogenic stimulation did not find clear differences between PPMS and other subtypes [26]. Differences in levels of soluble adhesion molecules (for example, raised intracellular adhesion molecule-1, ICAM-1) have been reported for PPMS although this has not been shown in all studies [27]. Higher levels of antiganglioside antibodies have also been reported in PPMS; these antibodies may be involved in mediating axonal damage, (although as previously histopathological evidence of greater axonal damage in PPMS is, as yet, lacking) [22;28]. Other subtle differences in the immunology of PPMS include the finding of an association between insulin-like binding protein-3 and disability that is not seen for other subtypes [29], lower levels of interleukin-6 in cerebrospinal fluid in
comparison with RRMS [30], and decreased matrix metalloprotein-9 levels [31]. An association between anti myelin oligodendrocyte glycoprotein and disability in PPMS has also been reported [32]. Such an association is also seen in SPMS. Immunologic factors in PPMS have recently been reviewed [33].

1.2 MRI studies of PPMS

Against this background, magnetic resonance imaging (MRI) provides a safe and non-invasive way to study nervous tissue without the use of ionising radiation. As mentioned already, early MRI studies provided the first evidence that important differences in underlying disease mechanism might be present in PPMS when compared to relapsing MS subtypes. Subsequent studies have confirmed early observations and made use of new MRI techniques that have improved tissue characterisation. As the resolution of structural imaging has improved, it has become possible to use MRI to obtain information about nervous tissue, including its chemical composition and structure, previously available only by direct examination of pathological material.

The remainder of this chapter reviews the results of MRI studies in patients with PPMS. The results of so-called conventional MRI studies will be considered first. Conventional MRI techniques were the first to be developed and are characterised by short acquisition times and relatively simple analysis techniques. They also relate closely to the diagnostic sequences routinely carried in clinical practice. Newer, more pathological specific, non-conventional MRI techniques will then be discussed. Finally, the emerging model of disease suggested by these studies will be discussed.
and some of the unresolved issues to be addressed in subsequent chapters will be highlighted.

1.3 Conventional MRI

1.3.1 Brain: T2 and T1 load

MS is associated with areas of signal abnormality ("lesions") on both T2 and T1 weighted conventional MRI. On T2 weighted images abnormalities are hyperintense, on T1 weighted images abnormalities are hypointense. Only a proportion of T2 hyperintense areas are T1 hypointense, but all T1 hypointense areas are T2 hyperintense. The pathological basis and origin of these abnormalities is discussed in Chapter 2. It was not long after the first observation of MRI signal abnormalities in MS that quantification of signal abnormalities or lesion load began to be used as an objective measure of the disease process. Differences in lesion load and location were among the first MRI differences to be reported for PPMS. The first study to specifically examine PPMS compared MRI appearances with those of SPMS and benign MS [34]. Patients with PPMS had the fewest lesions on T2 weighted MRI of the brain and those lesions that were present were small, with 85% being less than 5mm in size. By comparison, in SPMS, lesions tended to be large and confluent. Distribution of lesions did not differ between the groups although there was a tendency for patients with PPMS to have fewer lesions in the periventricular area (see Table 1.1). Cortical atrophy (qualitatively assessed) was seen in one of 13 patients with PPMS in comparison with one of 12 patients with benign MS and five of 16 with SPMS.
This observation that there is a reduced frequency of T2 lesion load in PPMS has been confirmed by many subsequent studies using quantitative measures of T2 load (calculated using computer-assisted visual or semi-automated algorithms). These studies have also shown lower levels of T1 hypointensity lesion load (see Table 1.2) [34-40]. It should be noted that two early studies which used a scoring system based on lesion size are not included [41;42]. In these studies, T2 load was greater in SPMS than PPMS with a ratio of, PPMS: SPMS, 1:1.6 and 1:2.2.

Table 1.1: Lesion distribution (mean numbers) in primary progressive and secondary progressive multiple sclerosis (PPMS, n=14 and SPMS, n=20) compared (32)

<table>
<thead>
<tr>
<th></th>
<th>PPMS</th>
<th>SPMS</th>
<th>Level of significance</th>
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<tbody>
<tr>
<td>Periventricular</td>
<td>19.2</td>
<td>34.9</td>
<td>p=0.003</td>
</tr>
<tr>
<td>Discrete cerebral</td>
<td>15.6</td>
<td>22.0</td>
<td>p=0.01</td>
</tr>
<tr>
<td>Internal capsule</td>
<td>1.5</td>
<td>1.9</td>
<td>p=0.29</td>
</tr>
<tr>
<td>Brainstem</td>
<td>3.6</td>
<td>3.5</td>
<td>p=0.74</td>
</tr>
<tr>
<td>Cerebellum</td>
<td>1.0</td>
<td>1.9</td>
<td>p=0.21</td>
</tr>
<tr>
<td>Total</td>
<td>39.4</td>
<td>62.3</td>
<td>p=0.028</td>
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Table 1.2: T2 load and T1 hypointensity load in relapsing remitting multiple sclerosis (RRMS) secondary progressive multiple sclerosis (SPMS) and primary progressive multiple sclerosis (PPMS) in cm³

<table>
<thead>
<tr>
<th>Study</th>
<th>Year</th>
<th>RRMS</th>
<th>SPMS</th>
<th>PPMS</th>
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<tr>
<td><strong>T2 load</strong></td>
<td></td>
<td></td>
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<tr>
<td>Nijeholt [40]</td>
<td>1998</td>
<td>4.1</td>
<td>11.0</td>
<td>3.2</td>
</tr>
<tr>
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<td>1999</td>
<td>-</td>
<td>27.7</td>
<td>12.0</td>
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<tr>
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<td>Foong [37]</td>
<td>2000</td>
<td>-</td>
<td>39.8</td>
<td>10.7</td>
</tr>
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<td>2001</td>
<td>4.7</td>
<td>11.7</td>
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<td>Wolinsky [39]</td>
<td>2001</td>
<td>15.4</td>
<td>16.5</td>
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<td><strong>T1 hypointensity load</strong></td>
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</tbody>
</table>
Changes in lesions over time in PPMS have also been studied. In terms of lesion number it was found that 43.6% of patients with PPMS demonstrated one or more new brain lesions over a one-year period [43], a mean of 0.88(SD 1.56) new lesions a year. In terms of total lesion load, over the same period, T2 lesion load increased by a median of a 0.33ml (-6.0 to +20.3ml) a year (7.3%), and T1 lesion load increased by a median of 0.11ml (-10.0 to +11.3ml) a year (12.6%). Using image registration 91% of the total new T2 lesion load in the PPMS cohort appeared to come from the enlargement of existing lesions and only 9% from new, discrete lesions [44]. Not all serial studies however have shown an increase in lesion load. An automated segmentation analysis of data from the PROMISe randomised control trial of glatiramer acetate in PPMS showed an intriguing fall in T2 load over one year with a parallel rise in CSF segmented volumes [45]. This difference from the findings of studies using semi-automated outlining techniques is probably methodological (it may relate to the difference from using the image characteristics of the entire lesion rather than just its outer edge) but shows that there are complexities in the MRI maturation of lesions in PPMS that conventional MRI quantitative measures do not fully reflect.

Two further observations relating to T2 and T1 abnormalities have been made in PPMS. Firstly, a lower ratio of T1 hypointensity to T2 load has been reported; the significance of this observation is unclear. Secondly, it is known that in addition to clearly defined areas of T2 hyperintensity ("lesions") less clearly defined areas of T2 hyperintensity can also be seen in MS and it has been reported that the incidence of such diffuse hyperintense brain abnormalities is higher in PPMS than in other subtypes [40]. However, by their nature such abnormalities are difficult to quantify.
and again the significance of this observation in pathological terms is not known [38;40].

The relation between lesion load and clinical measures is poor in MS in general, and this is particularly the case in PPMS. Studies that have examined the relation between lesion load and disability in PPMS are shown in Table 1.3. In one study neither T2 lesion or T1 hypointensity volume correlated with any clinical parameter in PPMS, although in RRMS and SPMS T1 hypointensity volume was weakly associated with the Expanded Disability Status Scale (EDSS) score [46] and disease duration [38]. Ukkonen et al examined lesion load measures in relation to one specific aspect of disability in PPMS, namely voiding dysfunction, and found an association [47]. In a large multi-centre study, lesion load measures in the PPMS group did not correlate with either EDSS or disease duration [35;48]. However, correlations were seen between T2 and T1 lesion load and 9-hole peg test score and a measure of cognitive impairment (the Paced Auditory Serial Addition Test or PASAT [49]), these two tests together forming two of the three components of the Multiple Sclerosis Functional Composite (MSFC) [50]. When this study was extended to two years, no additional correlations were found between absolute or percentage change in clinical outcomes and MRI [51]. Another set of multi-centre data, that from the PROMISe study of glatiramer acetate, found no simple direct correlation between MRI and clinical measures but did find differences when the groups was divided into high and low EDSS strata [45].
Table 1.3: Relation between cross sectional MRI and disability in PPMS

<table>
<thead>
<tr>
<th>Study</th>
<th>Year</th>
<th>MRI-Clinical association</th>
</tr>
</thead>
<tbody>
<tr>
<td>Fillipi[42]</td>
<td>1995</td>
<td>Lower cerebral lesion loads in patients presenting with spinal cord syndrome</td>
</tr>
<tr>
<td>Lycklama a Nijeholt[40]</td>
<td>1998</td>
<td>No relation between EDSS and lesion load</td>
</tr>
<tr>
<td>Stevenson[35]</td>
<td>1999</td>
<td>Lower cerebral lesion loads in patients presenting with spinal cord syndrome, relation between T2 and T1 load and nine hole peg test performance, r=0.33</td>
</tr>
<tr>
<td>Van Walderveen[38]</td>
<td>2001</td>
<td>No relation between EDSS and lesion load</td>
</tr>
<tr>
<td>Wolinsky[39]</td>
<td>2001</td>
<td>Cerebral lesion load not associated with EDSS, relation with nine-hole peg test (r=0.31) and PASAT (r=0.33)</td>
</tr>
</tbody>
</table>
Differences in lesion load with clinical presentation have also been reported. Patients with a progressive spinal cord syndrome, the most common clinical presentation in PPMS, have been shown to have significantly lower cerebral lesion loads than those with evidence of cerebral or brainstem involvement [35;42].

1.3.2 Brain: Gadolinium studies

Lesion load studies suggest a lesser role for inflammation in the pathogenesis of PPMS. The role of inflammation is however more specifically assessed using the contrast agent gadolinium-DTPA (Gd) which detects areas of disruption to the blood brain barrier (disruption of the blood brain barrier is known to be associated with inflammation, as discussed in Chapter 2). PPMS has a lower frequency of lesion enhancement following administration of Gd than other MS subtypes [41]. An early study followed 12 patients with SPMS and 12 patients with PPMS monthly for six months. In addition to finding a lower rate of new lesion formation in PPMS, it found that of 105 new lesions scanned with Gd in the SPMS group, 91 (87%) showed enhancement with Gd compared to only one of 20 new lesions seen in the PPMS group. A reduced frequency of Gd enhancement has subsequently been reported by several other authors [39;52].

The use of a higher dose of Gd (triple dose) increases the number of enhancing lesions seen in RRMS and two authors have explored the effects of triple dose Gd in PPMS [53;54]. In the study of Filippi et al, ten patients with PPMS were examined over two sessions with early and delayed imaging after the administration of Gd. Four enhancing lesions were detected in two patients when the standard dose of Gd was
used. The numbers of enhancing lesions increased to 13 and the numbers of patients with such lesions to five when triple dose Gd-DTPA was used and to 14 lesions and six patients when, in addition to triple dose, there was a one hour delay before scanning. In the study of Silver et al, fifty patients were studied including 16 with PPMS. Imaging was performed on two occasions with single and triple dose Gd. Patients were imaged within early (0-20mins), short delay (20-40mins) and long delay (40-60mins) time windows. In this study, triple dose and delay increased the yield of enhancing lesions in patients with RRMS and SPMS but not in patients with PPMS. In a separate study, quantitative signal changes were measured in seven PPMS patients, in lesions conventionally regarded as non-enhancing, a significant signal increase was found [55]. This suggests the presence of a low-grade degree of blood brain barrier (BBB) leakage.

1.3.3 Brain: Atrophy

Measures of tissue volume and area can be made from conventional MRI and these can be used to detect atrophy, either by comparing values in a single subject over time, or by making comparisons with a control population at a single time point. Atrophy measures reflect changes in central nervous system white and grey matter and, in theory, are relatively specific markers of tissue loss. In practice, tissue loss is not the only process that will affect volumetric measures. Inflammation can cause increases in tissue volume, through increased water and cellular content. Conversely, dehydration can decrease tissue volume. Volume measures therefore represent the net effect of all these processes.
Ventricular and CSF volumes can be used as measures of cerebral atrophy as they reflect loss of central white matter and multiple techniques have been developed for their measurement [56]. Several studies have examined volume measures of this kind cross-sectionally in PPMS. A much smaller number have monitored longitudinal changes. Cross sectional studies will be considered first.

Wolinsky et al studied CSF volume normalised to total segmented intra-cranial contents [39]. In this study the least degree of atrophic change was seen in the PPMS group. Average CSF volume in PPMS was 15.8 ml compared to 17.1 ml in RRMS and 17.8 ml in SPMS. Patients affected by PPMS with higher EDSS were found to have larger CSF volumes. Ventricular volume was measured on T1 weighted images by Lycklama a Nijeholt et al [40]. They found that mean ventricular volume was greatest in SPMS at 31.9 ml in comparison to 21.3 ml in PPMS and 22.3 ml in RRMS. In PPMS an association was seen between ventricular volume and pyramidal functional systems score. Regional atrophy has also been studied; De Stefano et al found lower normalised cortical volumes in patients with PPMS in comparison with controls, even when disease duration was less than 5 years. In this study, a moderately strong association with disability was also found which was stronger for PPMS than RRMS [57]. Associations between brain atrophy and disability measures were also reported in the study of Ukkonen [58].

Stevenson et al studied brain atrophy measures both cross-sectionally and longitudinally [35;43]. Partial brain volume above the level of the third ventricle was used as a measure of partial cerebral atrophy [59]. No differences were seen between groups at baseline. Over one year a median change of -2.3% in partial brain volume
was seen. There was no correlation with clinical measures and patients presenting with cord syndromes did not differ from those presenting in other ways in terms of brain atrophy.

Recently new, tissue specific measures of volume (e.g. white matter, grey matter, cortical volume) have been developed with one study to date comparing patients with PPMS with other subtypes [57]. Cortical volumes were found to be less in PPMS than in both normal controls and patients with RRMS. A strong association between cortical volume and disability (EDSS) was also reported for PPMS (r=0.64).

1.4 Conventional MRI: Spinal Cord

PPMS frequently presents as a spinal cord syndrome and in addition to the brain abnormalities that have already been described MRI abnormalities are seen within the spinal cord. As in the brain these lesions are of two types: discrete lesions and diffuse change. These will be considered separately.

1.4.1 Spinal cord: Lesions

For technical reasons, lesion load is more difficult to quantify in the spinal cord compared with the brain but several studies have compared focal signal abnormalities between MS subtypes, including PPMS. The first observation to be made about cord lesions in PPMS is that they make up a slightly greater percentage of total load than is seen in SPMS (11.8% to 8.2%) [40]. The importance of cord lesions in MS when the number of brain lesions is low has been shown more generally by Thorpe et al [60].
11 patients with suspected MS were studied and it was found that where the brain MRI was normal or near normal there was usually at least one lesion visible in the spinal cord. The second observation to make is that, in spite of this, there does not appear to be any increased frequency of lesions within the spinal cord in PPMS in comparison with other subtypes. This was first studied by Kidd et al [52] who found roughly equal numbers of new cord lesions (3 each) occurring over 1 year in 10 patients affected by PPMS and 9 affected by SPMS. Lycklama a Nijeholt et al studied spinal cord appearances in 31 patients with PPMS, 28 with RRMS and 32 with SPMS [40] and found that the number of focal spinal T2 lesions was similar between clinical subtypes. Conventional MR appearances of the cervical cord were studied by Filippi et al as part of an MTR study considered in more detail in the section on newer MRI techniques below [61]. Nine patients with PPMS were studied together with 41 with RRMS and 31 with SPMS. 81.8% of patients with PPMS had abnormal cervical cord scans (i.e. lesions were present) compared to 78.8% of patients with RRMS and 94% of patients with SPMS. Patients with PPMS had a mean of 1.8 cervical cord lesions compared to a mean of 1.7 lesions in RRMS and 2.5 lesions in SPMS. The extent of cord damage was assessed by using the mean number of cervical cord slices showing lesions. This was 3.2 for PPMS, 4.4 for SPMS and 2.7 for RRMS. Therefore, patients with SPMS had a higher number of lesions and greater number of slices involved than both other groups (p=0.04 in each case). The number of focal spinal lesions in PPMS was also found to be lower than in SPMS in the study of Stevenson et al (means of 1.9 and 3.2 respectively, p=0.04) [35]. At baseline, there was no correlation between spinal cord lesion load and disability. Over one year, 25.5% of patients had one or more new cord lesions. Change in EDSS correlated weakly with percentage increase in number and load of spinal cord lesions (r=0.19,
p=0.005). No correlation was seen with the number of brain lesions. At baseline, when patients with PPMS who had presented with spinal cord symptoms were compared with those who had presented in other ways, they were not found to differ in terms of cord lesion load. Over one year there was a suggestion that the number of new cord lesions was higher in the cord presentation group compared to the non-cord presentation group, but this did not reach significance [43].

The study of Lycklama a Nijeholt, discussed above, also reported diffuse signal change on proton density weighted images within the spinal cord. These were seen mainly in SPMS and PPMS: ten of 32 (30%) and nineteen of 31 patients (60%) respectively [40]. By comparison, diffuse abnormalities were present in only six out of 28 patients (21%) with RRMS. The presence of diffuse spinal cord abnormalities without focal lesions is also more common in PPMS (P<0.05). It was seen in ten of 31 (30%) patients with PPMS but in only four of 32 (12%) patients with SPMS and was entirely absent in 28 patients with RRMS.

Information about the relationship between MR and histological abnormalities in the spinal cord in PPMS is provided by a study that compared MR appearances of the cord at post mortem at two field strengths (4.7 Tesla and 1 Tesla) with histological appearances [62]. Seven patients with PPMS were included in this study and MR appearances suggested extensive involvement of the spinal cord. In comparison with SPMS cords, only a mild increase in signal intensity was seen and there was little involvement of grey matter. Interestingly, a greater degree of abnormality was present on the MR images than was detected histopathologically. Areas of high signal intensity on MR corresponded with areas of complete demyelination, identified
histologically, while areas of mildly increased signal corresponded with areas of partial demyelination.

In summary, PPMS may show abnormalities in the cord when none are detectable in the brain but there does not seem to be a particularly high or extensive focal lesion load compared to other MS subtypes. A greater degree of diffuse abnormality (corresponding histologically to partial demyelination) may be a characteristic feature. As with cerebral lesion load, the relation between disability and cord lesion load is unclear. Other evidence relating to cord changes in PPMS comes from studies of atrophy and is considered next.

1.4.2 Spinal cord: Atrophy

Relatively few studies have examined cord atrophy in PPMS. Most of these have used cross sectional cord area measurements [35;40;52]. The important observation is that, in terms of atrophy, PPMS occupies a position between SPMS (most atrophy) and RRMS (least atrophy). In the study of Losseff et al, using a technique that studied spinal cord area at the anatomically consistent C2 level in 15 patients with PPMS, [63] median cord area was 73.1 mm$^2$ in PPMS compared to 61.2 mm$^2$ in SPMS, 85.6 mm$^2$ in RRMS and 84.7 mm$^2$ in controls. In the study of Stevenson et al, using the same technique, mean spinal cord area was 64.1 mm$^2$ in SPMS and 72.7 mm$^2$ in PPMS (although average EDSS was also greater in the SPMS group) [35]. A larger median spinal cord area in PPMS compared to SPMS was also found by Lycklama a Nijeholt et al [40]. In this study an association was also seen between cross sectional cord area and the number of spinal cord segments showing diffuse involvement in PPMS. In
these studies, and that of Ukkonen et al [58], no cross-sectional association between spinal cord atrophy and EDSS was found (although in the study of Ukkonen an association was found between the number of spinal T2 lesions and EDSS sensory subscore).

Change in cord area in PPMS was studied by Kidd et al and Stevenson et al. Kidd et al studied the cross sectional area of the spinal cord at four levels (C5, T2, T7 and T11) in patients with PPMS and SPMS over a period of one year [52]. A reduction in cord area was seen in both groups that was most pronounced at the C5 level and was greatest in SPMS, with a median change of $-5.39 \text{mm}^2$ compared $-2.62 \text{mm}^2$ in PPMS. In this study there was no correlation between change in cord area at any of the four levels and change in EDSS, although there was a trend towards greater reduction in cord area at C5 in those who changed more than one EDSS point compared to those who did not. Stevenson et al, examining changes at the C2 level over one year found a median change of $-2.9\%$ in cord area, corresponding to an absolute median reduction of $2.85 \text{mm}^2$ (-13.0 to +4.2mm) [43]. There was no correlation with clinical measures and patients presenting with cord syndromes did not differ from those presenting in other ways in terms of brain or cord atrophy.

1.4.3 Spinal cord: Gadolinium

No studies to date have reported on the comparative frequency of enhancing cord lesions in PPMS in comparison to other MS subtypes.
1.5 Conventional MR techniques: conclusion

The major limitation of conventional MRI techniques, especially non-enhancing lesion load measures, is a lack of pathological specificity. So while lesion load studies suggest that there are differences in disease mechanism between PPMS and other subtypes they do not make explicit what this difference might be. This is because the lesions seen on conventional T2 and T1 weighted MRI scans are pathologically non-specific and can reflect chronic as well as acute processes. Gliosis would be an example of the former and acute inflammation an example of the latter; these distinct processes, potentially very different in their clinical implications, are indistinguishable on conventional MRI (82). The need for greater pathological specificity has led to the development of newer, “non-conventional” techniques and these will now be considered.

1.6 Newer MRI techniques

Within the last ten years new MR methods have been developed to provide better ways of characterising nervous tissue. These include Magnetisation Transfer Imaging (MTI), Diffusion Weighted Imaging (DWI), Magnetic Resonance Spectroscopy (MRS) and functional MRI (fMRI). One of the most important general contributions made by these techniques to date is to highlight a range of abnormalities within tissues that appear normal on conventional MRI. The most studied of these tissues is normal appearing white matter or NAWM. An early hint that subtle abnormalities might be present in NAWM came from measurement of T1 relaxation times (see
Chapter 2), when as early as 1991 it was found that in frontal normal appearing white matter (NAWM) was slightly higher in PPMS than in control subjects [41].

In the section on conventional MRI, findings in brain and spinal cord were considered separately. Newer techniques however have only been significantly applied to the spinal cord in PPMS in the case of MTR. Brain and spinal cord findings are therefore considered together in the MTR section. For other techniques it is brain that is being discussed in each case.

1.6.1 Magnetisation Transfer in PPMS

The contribution of free water protons dominates the conventional MR image but there is another population of protons, bound to macromolecules, which can also be visualised. This is done by measuring the amount of signal suppression following off-resonance irradiation. The resulting measure is known as the magnetisation transfer ratio (MTR) and reflects characteristics of the macromolecular environment. Initially, it was hoped that reduction in MTR would provide a specific MR marker of demyelination but subsequent histopathological correlation studies suggest this is not the case. Reductions in MTR ratio in MS probably represent a combination of demyelination, matrix destruction and axonal loss.

MTR studies in PPMS have looked at lesions, NAWM and whole brain. The first MT study in PPMS was by Gass et al [64] who studied 10 patients with PPMS using a region of interest based analysis. PPMS lesions were compared with lesions from patients affected by small vessel disease and found to have a lower MT. No difference was found between subtypes in terms of their lesion MT characteristics.
Leary et al studied MTR in NAWM in 52 patients with PPMS and 26 healthy controls [65]. Absolute values of MTR were obtained from a number of brain regions including the genu of the corpus callosum and the 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. Median MTR was significantly lower in the corpus callosum, frontal white matter and centrum semiovale, providing further support for the existence of widespread abnormalities in normal appearing tissues in PPMS.

A novel technique for the interpretation of MTR data involves the use of whole brain MTR histograms. Filippi et al compared MT histogram peak height and position in MS subtypes and found that patients with PPMS had lower peak height compared to control subjects [36]. MTR histograms were used to study normal appearing cerebral tissue by Tortorella et al and lower histogram peak height and lower average histogram MTR were found [66]. The relation between MTR histogram measures and disability was studied by Kalkers et al [67]. Associations between clinical and MTR parameters were not seen in PPMS although they were seen in RRMS. In the study of Dehmeshki et al, 46 patients with PPMS were studied together with a number of normal controls and patients with MS of other types [68]. Average MTR was found to differ in PPMS in comparison with controls and there was an association with disability when a principal components analysis was carried out. The association with disability was stronger for other MS subtypes (RRMS, SPMS) than for PPMS. An extension of this work investigated segmented histograms and revealed abnormality in both grey matter and NAWM [69].
MTR has also been used to study the cervical spinal cord where patients with PPMS were seen to have lower average cord MTR and peak height in comparison to other subtypes [70].

1.6.2 Diffusion Weighted Imaging in PPMS

Diffusion weighted imaging (DWI) is based on the application of MR gradient pulses which result in the dephasing of signal intensity due to the Brownian motion of water protons. Randomly moving spins (in contrast to stationary spins) do not completely refocus and therefore attenuate the signal. Since water protons diffuse faster along myelinated fibres than across them, the apparent diffusion coefficient is directionally restricted, or anisotropic. By measuring the amount of anisotropy, DWI provides a measure of tissue integrity. Droogan et al, in 1999, studied diffusion measures in nine patients with PPMS together with a number of other MS subtypes [71]. In this study, no differences were found between the diffusion measures of the different subtypes and no association was found with disability. A later study confirmed this finding although some diffusion measures were found to differ between patients with PPMS and controls in the regions of the corpus callosum and internal capsule where this was not seen for patients with RRMS or SPMS [72]. In the study of Ciccarelli et al, an association was found between diffusion measures and disease duration in patients with PPMS [73]. In a study of DWI histograms in PPMS whole brain analysis did not show differences between subtypes though there were differences in diffusion measures in lesions between PPMS and SPMS [72;74]. Differences in diffusion measures have also been reported in the corpus callosum between patients with relapsing MS and PPMS [75]. Diffusion measures have also recently been shown to
change over time [76]. In this study a region of interest analysis was used and changes in diffusion measures over 12 months in patients with PPMS were found that were not seen in control subjects. Additionally, an association was found between diffusion measures in normal appearing tissue in at least one area and disability. Using a histogram based approach, changes in diffusion measures over time have also been shown in lesion containing tissue and in normal appearing grey matter in PPMS [77]. The use of MTR and DWI in PPMS is considered in depth in the review of Rovaris et al [78].

1.6.3 Magnetic Resonance Spectroscopy in PPMS

In MRS the protons of the free water pool that are used to generate the conventional MR image are suppressed and this allows separation of resonances from various brain metabolites including N-acetyl aspartate (NAA), creatinine, myoinositol, lactate, choline-containing compounds and mobile lipids. NAA, as a substance, is almost exclusively present in neurons and axons and can therefore be used as a marker for neuro-axonal loss. Davie et al in 1997 measured NAA in a number of MS subtypes, including PPMS [79]. Reduced NAA was seen in T2 lesions in all subtypes, including PPMS. Reduced NAA was also seen in NAWM in PPMS and there was a relation between reduced NAA and EDSS in the population as a whole.

Leary et al studied NAWM in twenty-four patients with PPMS with 16 age-matched controls and found lower levels of NAA in NAWM [80]. Similar results were found when 17 patients with PPMS were studied by Cucurella et al [81]. Differences between patients with PPMS and SPMS were not found. Lesions and NAWM in
patients with PPMS and RRMS were compared by Suhy et al [82]. The NAA/creatine ratio of NAWM in patients with PPMS was decreased compared with both RRMS and controls (NAA levels were similar in the two subtypes but creatine levels were higher in PPMS NAWM). The NAA/creatine ratio in lesions was similar for the two subtypes. 53 patients with PPMS, drawn from the multi-centre study of glatiramer acetate, were studied using a multivoxel slab of dimensions 10cm by 10cm by 1.5 cm [83]. Reduced levels of NAA were once again found in comparison with control subjects but segmentation analysis failed to show any difference in resonances between lesion containing and normal appearing tissue, both of which were abnormal. The use of MRS in PPMS has recently been reviewed [84].

1.6.4 Functional MRI in PPMS

Functional MRI is a relatively new technique that uses signal changes with associated blood oxygenation to detect localised brain activity when stimuli are presented or tasks performed. Two studies looking specifically at patients with PPMS have been published. In the first study, twenty six patients with PPMS carried out a task consisting of flexion and extension of the last four fingers of the right hand [85]. These patients had no clinical involvement of the right upper limb. In comparison with control subjects, patients with PPMS had greater activation bilaterally in the superior temporal gyrus, ipsilaterally in the middle frontal gyrus and contralaterally in the claustrum. Associations were also seen between relative activation of cortical areas and both diffusion and MT measures in normal appearing brain. There was also an association between activation and MT measures in the cervical cord. In the second study, a strong correlation was found between T2 lesion load and extent of activation
in 30 patients with PPMS [86]. In comparison with control subjects there was also increased activation in “nonmotor” areas when a simple motor task was carried out.

1.7 Summary: Questions

What can be concluded about the disease process in PPMS from existing MRI studies and what are the most important areas where further clarification is needed? The first, and most robust, finding in PPMS is that in general patients with PPMS have fewer cerebral T2 lesions and areas of T1 hypointensity and have less active inflammation than patients with RRMS and SPMS, despite comparable levels of disability. This was reported in the earliest MRI studies and has been supported in every study since. Lower lesion loads have been interpreted to mean a lesser, or at least different, role for inflammation in PPMS in comparison to other MS subtypes, and this interpretation now receives some support from histopathological studies [17]. If inflammation is less marked, it has been suggested that there may be a correspondingly greater role for neurodegeneration in PPMS than in other subtypes but, at least in MRI terms this has yet to be demonstrated. A comparative summary of the conventional MRI findings in PPMS, adapted and expanded from Lycklama a Nijeholt et al [40], is shown in Table 1.4.

Newer MRI techniques offer the possibility of greater pathological specificity and their use seems very likely to increase understanding of underlying disease processes. Their major contribution so far has been to highlight the degree to which subtle and diffuse abnormalities are present outside of the conventional lesion or plaque. However, even in relation to conventional MRI in PPMS there remain many
unresolved issues. The first of these is the relation of MR abnormalities to clinical status. Conventional MR abnormalities are undoubtedly present in PPMS and measurable changes in MR measures can be detected over quite short periods of time in natural history studies. Critically, however no clear relationship with clinical change has been shown over study periods. Until now it has not been clear whether this is because this relationship does not exist (perhaps because of the poor pathological specificity of conventional MRI measures) or alternatively because follow up has been too short for such a relationship to become apparent. To address this issue an extended clinical and MRI follow up over 5 years of a cohort of patients with PPMS was undertaken and will be described (see Chapter 3 and 4).

Another unresolved issue relates to the nature of MR findings in the earliest clinical stages of the condition, particularly with respect to the presence of inflammation. Studies of enhancement in PPMS have examined patients with established disease. In RRMS, which has been more extensively studied, there is some evidence for differences in disease pattern between early and late disease. For example, in a cohort of patients with RRMS recruited within three years of first symptoms 81% showed some enhancement [87] It has also be shown for RRMS patients in a 14 year follow up study that change in lesion load over the first five years correlated closely to disease related brain atrophy at 14 years [88]. Therefore, if enhancement appears to be not prominent in patients with PPMS might this be due to an early enhancing phase having been missed? The role of inflammation within the cord in PPMS has also received little attention although, has been seen the question of whether there is preferential involvement of the spinal cord is in PPMS is unresolved, with an apparent paradox between marked clinical involvement of the spinal cord and only moderate
MRI involvement in several studies. These issues will be addressed by a serial triple dose Gd study of brain and cord in a cohort of patients with early PPMS and is described in Chapter 5.

Finally, measures of tissue loss have already been shown to be clinically relevant in PPMS (for example, cervical cord area). There is however an increasing range of such measures and it remains to be determined which are the most reliable and which the most clinically useful and informative. This subject will be considered in detail in Chapter 6.
Table 1.4: Summary table of conventional MR features of PPMS (adapted from Lycklama a Nijeholt et al [40])

<table>
<thead>
<tr>
<th>Measure</th>
<th>RRMS</th>
<th>SPMS</th>
<th>PPMS</th>
</tr>
</thead>
<tbody>
<tr>
<td><strong>Brain</strong></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Focal T2 lesions</td>
<td>Many</td>
<td>Many</td>
<td>Moderate or few</td>
</tr>
<tr>
<td>Enhancing lesions</td>
<td>Often</td>
<td>Often (if also relapsing)</td>
<td>Seldom</td>
</tr>
<tr>
<td>Focal T1 lesions</td>
<td>Few or moderate</td>
<td>Many</td>
<td>Few</td>
</tr>
<tr>
<td>Diffuse abnormalities</td>
<td>Seldom</td>
<td>Variable</td>
<td>Frequent</td>
</tr>
<tr>
<td>Ventricular enlargement</td>
<td>Mild</td>
<td>Moderate or marked</td>
<td>Mild</td>
</tr>
<tr>
<td><strong>Spinal cord</strong></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Focal T2 lesions</td>
<td>Frequent</td>
<td>Frequent</td>
<td>Frequent</td>
</tr>
<tr>
<td>Focal T1 lesions</td>
<td>Never</td>
<td>Never</td>
<td>Never</td>
</tr>
<tr>
<td>Diffuse abnormalities</td>
<td>Seldom</td>
<td>Variable</td>
<td>Frequent</td>
</tr>
<tr>
<td>Spinal cord atrophy</td>
<td>Mild</td>
<td>Marked</td>
<td>Moderate</td>
</tr>
</tbody>
</table>
Chapter 2

Using Magnetic Resonance Imaging to study PPMS:

methodological aspects
2.0 Introduction

Chapter 1 described the contribution that MRI studies have made to the understanding of PPMS. This contribution has been threefold. Firstly, to emphasize the distinctiveness of PPMS from other forms of MS, secondly to suggest that PPMS might have a less inflammatory underlying pathology than relapsing MS and lastly to highlight, once again, the diffuse nature of these disease processes as in other forms of MS. Nevertheless there are a number of aspects of the PPMS disease process that remain to be addressed and these include the following.

Do MRI measures in PPMS relate to clinical progression?

Existing studies have shown little relationship between clinical and MRI measures in PPMS, either cross-sectionally or longitudinally over periods of one or two years. However, as clinical change can be relatively slow, it is not known whether the period of follow up of previous studies was too short to make any relation apparent. Additionally, clinical measures have limited sensitivity and MRI measures limited pathological specificity. With longer periods of observation a greater clinical change is expected to occur and this may make correlation with MRI measures more obvious.
Is the pathological process in PPMS linear?

The cellular details of the pathological process in PPMS are not yet known although this must involve some combination of inflammatory and neurodegenerative processes. Whatever this disease process is, it clearly operates over some time but the relation between time course and pathology has received relatively little attention. Even without knowledge of the precise details of cellular pathology documenting the evolution of MRI changes over time may provide insights into the disease process in general terms. For example, does it appear that one pathological process operates continuously throughout the course of the illness, or alternatively do different processes operate at different times? This is an issue with potentially important implications for therapeutic intervention.

Where does variation in PPMS arise?

Clinical outcome is known to vary in PPMS [6]. Some patients develop high levels of disability within a few years of symptom onset whilst others have minimal disability despite many years of disease. Cross-sectional studies have also shown variation in the MRI appearances of the condition; it is not known at what stage in the illness that these individual variations develop. A better understanding of this issue might also provide insights into the disease process in PPMS.
Is there an early inflammatory phase in PPMS?

As discussed in Chapter 1 inflammation is prominent in the early clinical stages of RRMS. Although it is known that inflammation in established PPMS clinical course is not prominent it is not known whether this is also true in the early stages of the condition.

2.1 Choice of techniques

The safe and non-invasive nature of MRI makes it a good tool to study the time course and variability of the disease process in PPMS. A number of MRI techniques are available and an explanation is therefore needed of the particular acquisitions and unique analysis techniques used in the studies described in this thesis. An important constraint on the choice of techniques was the decision to avail of the opportunity to use pre-existing MRI and clinical data from a PPMS cohort that was originally recruited in 1996 as the basis for a new follow up study. The MRI data acquired in this group was conventional in nature, meaning that the sequences that were acquired were similar to those acquired in routine clinical practice. Such sequences, as will be discussed below, have the disadvantage of being relatively non-specific in pathological terms. They have the advantage of robustness, being rapidly acquired, and suitable for analysis using well-validated and reproducible techniques. New MRI data acquisition in this cohort followed the plan of previous data acquisition in this group. A second cohort of patients, selected for early disease duration was recruited de novo. In this second group a wider range of MRI data was acquired. Conventional MRI sequences, similar to those acquired in the first cohort, were also acquired to
allow comparisons to be made between the cohorts. Additional non-conventional MRI data was acquired in this second, early, cohort. Of this data, results from studies using triple dose gadolinium will be included in this thesis.

The following sections will give a brief description of the physical basis of the MRI signal, followed by a more detailed description of the specific sequences acquired and the analysis techniques used.

### 2.2 The physical basis of MRI

MRI makes use of the fact that in when tissue is placed in a powerful magnetic field the free, spinning, protons of the tissue (mostly in water) align the axes of their spins with the field of the magnet. In a magnet tissue becomes magnetized in the direction of the field giving the protons a *nuclear magnetic* resonance signal of their own. While the axes are aligned in the direction of the external field this nuclear magnetic resonance signal cannot be detected. The signal can be detected though by turning the axes into the receiving plane of an aerial by applying a radio frequency (RF) pulse of the appropriate frequency to resonate with the proton spins. The signal returned from the resonating protons is in proportion to the number of protons resonating synchronously and how closely their axes point in the direction of the receiver coil.

A specific duration of RF pulse can be used to turn the axes of the protons through 180 degrees. When the transmission ceases the spinning protons relax back into their original position. The rebuilding of the initial magnetization by relaxation is exponential and takes a finite time. The measure of this time is known as T1 and is the...
time taken for 63% of the total magnetization to return to the original direction. To measure how much magnetization has returned at any moment additional RF pulses are used. An RF receiver coil is arranged to pick up signals from magnetization directed at right angles to the longitudinal magnetic axis. After the initial burst of RF that turns the spins through 180 degrees, subsequent bursts turn the returned magnetization through 90 degrees so that it can be measured. The magnitude of the signal picked up by the receiver coil will be proportional to the amount of magnetization that had returned by longitudinal relaxation before the second burst.

The speed of longitudinal relaxation of protons depends on the nature of the surrounding molecular lattice and is slower in fluids where inter-particular distances are greater than in solids where energy transfer is easier; protons in fluids have a longer T1 than protons in solids. When a measurement is made at 500ms relatively little magnetization will have returned in cerebrospinal fluid, but the protons in white matter will be approaching complete magnetization and will appear white.

The constituent parts of the image (the matrix of voxels) are made up of many signals; these are obtained by repeated application of excitation pulses. The repetition time (TR) is the time between excitation pulses and the inversion time the time after the excitation pulse when the measurement is taken. A typical T1 weighted sequence might have a repetition time of 1500-2000ms and an inversion time of 500ms.

There is another type of magnetic relaxation which takes place at the same time as longitudinal relaxation but is independent of it: transverse relaxation or T2 relaxation. Whereas T1 relaxation characterizes the rate of return (i.e. the increase of longitudinal
magnetization), T2 relaxation, characterizes the rate of loss of transverse magnetisation. The measurement of T2 relaxation makes use of the fact that the burst of radiofrequency energy that turns the direction of the magnetic moments of the protons also forces their positional rotations into exact phase with one another. Initially, all the protons will be spinning with the same speed so at this time the amplitude of the resulting signal is high. Depending on the molecular environment the speed of spin will be slowed in some protons more than others so that as the individual frequencies of rotation about the axis of the field (the precessional frequency) fall gradually out of phase the amplitude of the signal emitted at the original frequency decreases.

The rate of loss of transverse magnetization is measured in the transverse plane of the magnet by a sequence of radiofrequency pulses known as spin echo (SE). The first burst of radiofrequency in this sequence rotates the protons magnetization through 90 degrees. Then, after a chosen echo time the remaining signal is read (in fact by imposing a further 180 degrees turn to the direction of magnetization so that it is again at 90 degrees to the longitudinal field).

In an SE sequence if sufficient time (e.g. 2000ms) is allowed for the recovery of longitudinal relaxation and the echo time (TE) is short (e.g. 30ms) then during the recovery time a good deal of the longitudinal magnetization will have returned (virtually all of it except from that derived from the protons in the most fluid components like CSF). When the 90 degrees pulse is imposed, all the recovered longitudinal magnetisation will be turned to the transverse plane and all the protons forced to pause in their precession. When the signal is measured after only a short
echo interval allowing little T2 relaxation it will be large and significantly dependent on the concentration of protons in the tissue.

### 2.3 MRI signal abnormalities in MS: genesis and interpretation

When T2 weighted MRI, as described in the previous section, was first performed in patients with MS areas of focal signal abnormality in the central nervous system were seen [89]. These signal abnormalities, or MRI lesions, are the most characteristic MR abnormality in MS and appear to reflect subclinical as well as clinical pathology; when present in a characteristic distribution they can be important evidence in favour of a diagnosis of MS in the appropriate clinical context. Indeed, the presence of lesions is an important part of recently published diagnostic criteria for both PPMS and RRMS [5;90]. MRI lesions as such have been used as an objective measures of disease, and of treatment response, in a number of clinical trials. Figure 2.1 is an examples of typical MRI signal abnormalities in a patient with MS.

#### 2.3.1 The origin of MRI signal abnormalities in MS

The underlying pathology of the abnormalities that give rise to focal MRI lesions in MS has been studied by scanning formalin-fixed post mortem brains [91;92] and comparing the images with histological preparations cut in the imaging plane. In general, there is a good correspondence between MRI and histological lesions, in that it is unusual to find areas of MRI abnormality that are not also abnormal histologically. Clearly demarcated MRI lesions with a very high signal usually correspond to the archetypal, macroscopic, sclerotic MS lesions that are found at post
mortem. Smaller and brighter MRI lesions sometimes do not correspond to the macroscopic lesions. At a microscopic level such lesions have a range of appearances including partial demyelination or remyelination and early inflammation [93].

The areas of abnormal MRI signal occur because of changes in the number or physico-chemical environment of water protons. It is a limitation of conventional MRI that different pathological processes can produce similar MRI appearances of abnormality. It has been shown for example that whereas oedema is the main source of abnormal signal in acute MS lesions it is gliosis that makes the largest contribution in chronic lesions [91;93-97].

Some of the lesions that appear hyperintense on a T2 weighted image appear hypointense on a T1 weighted image: these are known as T1 hypointense lesions or "black holes". These lesions, which form a subset of all T2 lesions, appear on the basis of post-mortem studies to have a distinctive pathological basis. Whereas almost any alteration in brain tissue composition in MS leads to an increase T2 relaxation time, a more limited number of alterations in tissue produce a prolongation of the T1 relaxation time. Biopsy [98] and autopsy [34;93] studies have shown that T1 hypointensity corresponds with degree of expansion of matrix tissue (i.e. widening of the extracellular space) and with loss of nerve axons. The majority of hypointense lesions appear to represent areas of irreversible tissue destruction. There is also one other important sources of marked T1 hypointensity; active lesions, of the sort that also show enhancement with the contrast agent gadolinium which show gadolinium, can appear hypointense on precontrast T1 weighted images [99]. This phenomenon probably relates to the presence of oedema, inflammatory cells and incomplete
demyelination. It has been shown that approximately half of these acute T1 hypointense lesions are no longer visible at follow up. There has been speculation that this recovery relates to resolution of oedema and inflammation and, possibly, remyelination [23;99;100]. T1 hypointense lesions have been shown to have a better correlation with clinical disability than has been observed for T2 lesions [34;101;102]. Examples of the appearances of a single lesion in a patient with PPMS on T2 and T1 weighted scans are shown in Figure 2.2a and Figure 2.2b at the end of this chapter.

### 2.3.2 Lesion load

As MR lesions relate, at least in part, to tissue abnormality in MS, lesion quantification offers a measure of disease impact. There are several ways in which lesions can be quantified:

- By simple counting
- By manual contouring
- By semi-automatic contouring
- By automatic lesion segmentation techniques

Semi-automatic contouring is the technique used in this thesis and will be discussed in the following section.
2.3.3 Quantification of lesion load by semi-automatic contouring

The technique used to quantify lesion load makes use of semi-automatic contouring and is applied to axial images with slice thicknesses of 3 and 5 mm. Lesions were in the first instance marked on hard copies by an experienced marker (Professor D. H. Miller). Where previous imaging was available this was assessed together with the current scan to allow consistent decisions to be made on equivocal lesions on serial scans. Lesion identification and subsequent delineation was performed on proton density images.

For all the studies in this thesis contouring was performed on a Sun workstation and made use of a program package (Dispimage) that has an option for tracing the boundary of an image object (the object in this case being a lesion) using a local threshold algorithm [103;103]. The rater, by means of a mouse click, identifies a point on the lesion edge. The algorithm then finds the lesion edge by searching for the strongest local intensity gradient. The program then follows a contour of isointensity to delineate the lesion. The contoured lesion is then displayed for review by the rater. Occasionally, when lesion/background contrast is poor, other areas of increased signal near to the lesion will be included within the lesion boundary; manual editing is needed to delete these areas. Finally, lesion volume is calculated by multiplying the total lesion area of all lesions on all slices by the slice thickness.
2.3.4 Reproducibility

Measurement error is an important factor to bear in mind when quantifying lesion load in MS. In serial studies, lesion segmentation is only one of many potential sources of variability during image acquisition and analysis (other factors include variation in scanner performance and the effects of suboptimal repositioning).

Precision and reliability at the lesion segmentation stage is nevertheless important if small changes in lesion load over time are to be detected [104]. Precision, or reproducibility, is defined as the extent to which repeated measurements on the same object are in agreement. Reliability of a technique is a measure of measurement error as a proportion of variance between patients.

Several statistical techniques are available to define the precision of a measurement technique. The coefficient of variation (CV) is the most frequently used measure. This is calculated as the standard deviation of repeated measurements divided by their mean. A limitation of the use of CV as a measure of precision is that it dependent on the magnitude of the measured value. In the context of lesion load measurements the smaller the lesion volume is, the greater the CV. An alternative measure of precision is the repeatability coefficient. For this measure precision is expressed in terms of the unit of measurement. The difference between two measurements for the same subject is expected to be less than the reproducibility coefficient in 95% of observations.

The semi-automated lesion segmentation technique above has a significant element of human interaction and an assessment of measurement error is therefore important in their validation. Precision of measurement of lesion load has been assessed cross-
sectionally by several studies [101;103;105-107]. In the study of Filippi, where intra and inter observer agreement (expressed as one minus the coefficient of variation) of lesion volume assessment for a semi-automated technique were assessed for three raters on lesions from 20 subjects, intra-observer agreement was 96.3% (range 94.2% to 98.9%) and inter-observer agreement 93.7% (range 83.8% to 98.3%). Intra-observer variability was significantly lower than inter-observer variability. Precision and reliability of change in lesion load was assessed by Molyneux [104]. He assessed intra and inter observer agreement for a semi-automated technique for three raters on lesions from 16 subjects (affected by RRMS) scanned on two occasions at an interval of two years. For the change in lesion volume the intra-rater and inter-rater repeatability coefficients were 2.6cm$^2$ and 2.8cm$^2$ respectively. The values for intra-rater and inter-rater reliability for measuring change in lesion volume were 94.5% and 94.4% respectively (i.e. comparable to the values found cross-sectionally by Fillipi and others). It appears that using the semi-automated contouring technique variance due to random measurement error is small compared to that due to wide biological variability in changes in lesion load in MS.

### 2.4 Measures of atrophy

As discussed in the introductory chapter, destructive or degenerative change, through loss of axons, myelin and other cellular material, is thought to play an important role in pathological mechanisms relevant to the development of disability [56]. Accurate MRI volumetric measures of central nervous tissue structures such as the cerebral hemispheres, cerebral ventricles and spinal cord provide a means of quantifying regional and global atrophy. Characterisation of the dynamics of atrophic change is
therefore another aspect of characterizing the dynamics of the disease process in PPMS.

2.4.1 The pathological basis of atrophy

Atrophy refers to the loss of tissue. Nervous tissue consists of many elements (for example, nerve cell bodies, nerve axons, myelin, glial cells, blood vessels, blood and tissue fluid); loss of any of these elements may therefore result in a loss of total volume. Unfortunately, it is difficult to accurately assess the contribution made by loss in a specific tissue to overall atrophy but knowledge of the proportion of a particular tissue that is present in vivo provides some guide as its contribution. For example, loss of myelin is the classical pathological feature in MS but contributes only around 25% of the brain white matter volume in vivo. On this basis, loss of myelin alone, even if complete, would not be enough to account for the reduction in overall tissue volume that is found. It is likely therefore that destruction of other tissues is also important, for example of nerve axons. Axonal loss is certainly known to occur in plaques [108] and also has been observed in active inflammatory lesions [23].

Axonal loss has both local and distant atrophic effects. Locally, axonal loss in a lesion leads to a reduction in tissue volume at that site. Remotely, Wallerian degeneration along the fibre pathways that traverse a lesion can lead to atrophy in remote areas, including grey matter. There is also evidence to suggest that, where axonal loss occurs it is smaller axons that may have proportionately greater loss [23]. Another important pathological process with potential consequences for changes in tissue volume is
gliosis. The effect of gliosis on tissue volume are complex: on one hand cellular
destruction and condensing of tissue leads to loss of volume, while on the other
proliferation of astrocytes and activation of astroglia results in increased tissue bulk.

Finally, changes in tissue water content may also have effects on tissue volume. Acute
lesions are associated with breakdown of the blood brain barrier, inflammation and
vasogenic oedema. Sometimes this leads to visible swelling which may affect overall
brain volume.

Accurate measurements of the volume of nervous tissue structures using MRI can
provide a measure of tissue atrophy when compared either with reference or control
populations or with the individual’s own measurement when repeated over time.

Techniques used designed to accurately measure tissue volume using MRI and used in
this thesis will be discussed in the following section [56]. These techniques make use
of 2D data acquisitions. It should be noted that, when compared with 3D acquisitions,
these have the slight disadvantage of a greater frequency of partial volume effects.
However, the sequences chosen had high resolution and good CSF contrast, factors
important in ensuring accurate segmentation of tissue boundaries.

2.5 Atrophy analysis techniques

2.5.1 Partial brain volume

This technique provides a measure of cerebral white matter and quantifies a volume
of a region of the cerebral hemispheres, it was first described by Losseff [59]. The
technique makes use of a T1 weighted (TR=600ms, TE =20ms) sequence and was originally described using axial contiguous 5mm slices beginning at the velum interpositum cerebri and continuing rostrally. In this thesis this technique is used in this form and also in a modified form with 3mm slices. This technique calculates the volume of part of the brain only. The volume chosen contains a representative sample of the cerebral hemispheres at the level of the lateral ventricles. This is a region that is usually well characterized on conventional MRI images, with good CSF/tissue contrast and fewer partial volume effects than are seen in either basal or apical brain regions to make tissue segmentation easier. The technique consists of identifying upper and lower brain slices on the basis of an anatomical marker, extracting the brain between these slices from the skull and CSF spaces and quantifying their volume in the extracted image. With the slice containing the velum interpositum cerebri as the first slice, three further 5 mm slices (or five 3mm slices) are selected rostrally. Brain tissue from these slices is then extracted using a histogram based extraction algorithm.

The tissue extraction algorithm works as follows. Firstly, a histogram of image intensity of all the pixels in the image is made. As a first step in the segmentation process, the program then assumes that pixels in the image belong to one of two classes, either “background” or “brain”. A discriminant analysis then searches for the optimal intensity level that best separates these two classes by a process of variance optimization. Once this optimal threshold has been found it is then applied back to the image intensity histogram to create the final version of the “background” and “brain” classes. The resulting histogram corresponds to a binary image with two defined regions. At this stage there are imperfections in the thresholded binary image and these are corrected by a morphological opening operation; the brain element is first
eroded and then dilated and undesired, non-brain components (e.g. bone, muscle and scalp) are separated from the brain using a circular structuring element. The size of the structuring element is fixed throughout the whole process. The next stage is known as connected component analysis which consists of three main operations (1) connection of components with non-zero pixels (the CSF spaces and areas previously occupied by tissues associated with the skull now having zero image intensity) (2) labeling and counting the connected components (3) selecting the largest connected component as the brain. Lastly there is a masking operation whereby the resulting binary image of the brain is masked to the original grey image and the brain extracted from the head. Examples of extracted brain slices are shown in Figure 2.3.

Following extraction and editing the volume is calculated using a program that counts the number of non-zero pixels remaining and multiplies them by their size.

2.5.2 Ventricular volume

A different technique was used to measure ventricular volume. This made use of the MIDAS package (Medical Image Display and Analysis Software), an interactive segmentation technique designed to allow the accurate delineation of anatomical structures on MRI images [109]. The first step is to extract the brain from its surrounding tissues. In a complex process, morphological operators isolate the brain using the knowledge that it is the largest structure in the head and is only weakly connected to adjacent structures. Once the brain is extracted, the average intensity of its constituent pixels is calculated. 60% of this value is then used as an automatic threshold to identify the boundaries of the ventricular system. Ventricular size is
based on a 2D threshold-based growing technique. The ventricular volume of interest is calculated by recursive 2D growth of the seeds, placed in the ventricles by the operator. Growth is constrained such that it cannot occur by crossing a drawn edge or by adding a point outside the intensity threshold range. The edges of the grown region (i.e. the ventricular margin) are overlaid on the 2D cut using a different colour. Fine editing of the ventricular boundary, where necessary, is done by the user by drawing, erasing and changing seeds and by setting image intensity controls. Examples of ventricular margins outlined using this technique are shown in Figure 2.4.

2.5.3 Spinal cord atrophy

As discussed in the first chapter, spinal cord involvement is an important cause of disability in MS. Spinal cord lesion load (which, for technical reasons is more difficult to measure than brain lesion load) seems not to relate to disability either cross-sectionally or longitudinally [110]. Relationships have however been found between spinal cord atrophy and disability [63] (as previously discussed).

Early studies [110;111] used a technique that involved acquisition of axial sections of the spinal cord by a two dimensional gradient echo sequence with manual outlining to calculate the cord area. Losseff showed that this technique has poor scan-rescan reproducibility (a coefficient of variation of 6% in controls) [112] and subsequently a new technique was proposed [63]. The most important problem with the earlier technique was that it failed to take account of the marked local anatomical variability of the cord. For example at the level of C5, the cord is expanded (root outlets) and the CSF space is small leading to turbulent flow and CSF signal void. In the thoracic
region the cord abuts the bony canal (this makes segmentation difficult). The C2 cord level has three advantages (1) the CSF space is wide and it is easy to position the patient so that the cord lies in the middle of the CSF pool (maximizing cord/CSF contrast) (2) there is less cross-sectional variability in the area of the cord over this segment (3) the C2 level is an uncommon site for disc protrusion.

2.5.4 Losseff technique for measuring spinal cord atrophy

This technique is used to analyse cord data acquired using a Signa 1.5T system using phased array coils [110]. The specific sequence is a volume acquired inversion prepared fast spoiled gradient echo acquisition (this sequence has marked CSF suppression to enhance cord-CSF contrast and also results in homogeneity of the cord and CSF compartments) performed following an axial pilot at the level of the C7 vertebral body. Sixty-four partitions in the saggital plane (x direction) of 1mm thick equivalents are then acquired in a three-dimensional volume centered on the cervical spine, with the following parameters: $T1=450\text{ms}$, $TE=4.2\text{ms}$, $TR=17.8\text{ms}$ flip angle=$20^\circ$, 256 phase encodings in the z direction, one average, field of view 25cm, for both the z and y directions, matrix $256 \times 256$. An example from this acquisition is shown in Figure 2.5a

From the volume data set a series of five contiguous 3 mm pseudo axial slices are then reformatted using the centre of the C2/C3 intervertebral disc as a caudal landmark. The reformatted slices are perpendicular to the spinal cord.
Examples of the resulting images are shown in Figure 2.5b. It will be seen that there is a clear interface (a strong signal intensity gradient) between the brighter cord and the darker CSF. The actual border, at a high level of magnification, is in fact somewhat blurred. This is due to partial volume effects (as discussed above). The true boundary lies at a position halfway between the signal intensities of the cord and CSF and can therefore be simply calculated from the mean intensities of cord and CSF. To do this, images were transferred to a SUN workstation and uniformity corrected [64]. Images are then blinded and displayed using the image display program Dispimage (as described above). A region of interest is drawn around the cord in each slice followed by a region of interest around the cord and CSF space. From these two regions the mean CSF and cord intensities are calculated. The mean cord signal intensity is derived simply from the mean of the cord region of interest. The mean CSF signal is obtained by subtraction, i.e.

\[
\text{Mean CSF signal intensity} = \text{Mean (CSF + cord signal intensity)} - \text{Mean (cord signal intensity)}
\]

Once the mean signal intensity of the cord and CSF are known the signal intensity of the boundary (the mean of the two regions) can be calculated. A seed is planted manually near the cord/CSF boundary and the computer constructs an automated border at, or near to, the boundary signal intensity. This is then performed for each of five axial slices whose areas are then averaged.
2.6 Triple dose Gadolinium

As discussed in the introductory chapter, evidence from radiological and pathological studies suggests that there may be important differences in role for inflammation in PPMS in comparison with RRMS. In this study the presence of inflammation in the earliest clinical years of PPMS will be studied using contrast enhanced imaging, specifically triple dose gadolinium.

When T1 weighted MR imaging is carried out after the peripheral administration of the intravascular contrast agent Gadolinium diethylene triamine pentaacetic acid (Gd-DTPA, chelated gadolinium) focal extravasation of contrast is visible as an area of enhancement [113] under certain circumstances. These circumstances are that there is a local breakdown in the integrity of the blood brain barrier allowing passage of the large Gd-DTPA molecule. Gd-DTPA works as an MRI contrast medium as it is paramagnetic, and shortens the T1 relaxation time very strikingly (leading to an area of high signal on a T1-weighted image). Enhancing areas are thought to represent regions of focal inflammation where there has been breakdown of the blood brain barrier and that contrast enhancing lesions reflect the acute inflammatory step in lesion development [98;114]. In addition, both in experimental allergic encephalomyelitis (EAE) and MS, enhancement has been shown to correlate with the presence of active macrophages in relation to demyelination [114-116]. In relapsing MS new enhancing areas are often, but not always associated, with new symptoms and episodes of clinical relapse [117] but in PPMS the clinical significance of enhancement is less clear [41;52].
The dose of Gd-DTPA used was 0.6mmol/kg, administered through an antecubital vein. Post injection imaging was carried out immediately (i.e. within 5 minutes of the injection being completed). Enhancing lesions were identified on the hard copy image by an experienced observer (Professor D. H. Miller) who was blind to all clinical details. Enhancing lesion volumes were then calculated using Dispimage in the same way as for T2 hyperintensity and T1 hypointensity loads as described above. An example of an enhancing lesion in a patient with PPMS is shown in Figure 2.6.

The results of applying these techniques to two cohorts of patients with PPMS in order to address the questions outlined at the beginning of this chapter will now be presented.
Figure 2.1: An example of typical periventricular MRI signal abnormalities from a T2 weighted study in a patient with MS
Figures 2.2a and 2.2b: examples of T2 (above) and T1 (below) weighted images of the same periventricular, right parietal lesion in a patient with PPMS
Figure 2.3: Representative slices extracted from the brain at the level of the lateral ventricle to be used for the calculation of partial brain volume.
**Figure 2.4:** Using the MIDAS technique, and a threshold based on 60% of the signal intensity of the entire brain, ventricular volumes are highlighted using a semi-automatic technique.
Figures 2.5a and 2.5b. The upper image shows a sagittal section from the 60 slice volumetric acquisition from which the five lower axial sections are reconstructed prior to calculation of cord area using a semi automatic thresholding technique.
Figure 2.6: An example of an enhancing brain lesion. The upper and lower images are T1 weighted images in patient with PPMS before and after the administration of Gd-DTPA at a dose of 0.6mmol/kg.
Chapter 3

Serial studies of primary progressive multiple sclerosis.
3.0 Introduction

The MRI abnormalities present in PPMS show some quantitative differences from other MS subtypes, although there are no truly unique features, as discussed in Chapter 1 [3;41;42]. More generally, MRI is useful diagnostically in PPMS [5] and is now being widely used to study its underlying disease mechanisms. However, the relationship of MRI abnormalities to clinical activity in PPMS and their potential role as surrogate markers in clinical trials is less clear. This important issue will be the focus of this chapter, which aims to investigate the relationship between MRI abnormalities and clinical state in PPMS through clinical and MRI longitudinal natural history studies.

In PPMS an obvious relationship between the degree of MRI abnormality and clinical state is not found using conventional MRI measures in cross-sectional studies [35]. This appears initially counterintuitive; abnormalities in MRI reflect disruption to normal tissue, the greater the destruction, the greater the disability that might be expected to result. However, it has been shown that conventional MRI measures are pathologically non-specific [96;118] and areas of abnormality that appear radiologically similar may, in fact, be pathologically heterogeneous. So whereas an area of MRI abnormality in one subject may represent a region of damage to tissue (gliosis and axonal loss), an apparently similar area may in another subject represent a region of tissue repair (resolving inflammation and remyelination) [93]. Lesion location is also likely to be an important factor; lesions in areas not critical to function, are likely to have less impact than those in key functional areas, or in the nerve tracts.
between them. Diffuse abnormalities which may result in clinical deficit may also be difficult to detect and quantify with conventional MRI.

Beyond any cross-sectional relationship, a relation between MRI abnormalities and clinical state can also be sought by examining changes in these measures over time. Although limitations with respect to pathological non-specificity and insensitivity to lesion location apply equally to measures of MRI change it is conceivable that such measures might nevertheless reflect disease activity. This is an important issue for if MRI measures are to be used as surrogate markers of disease activity in PPMS it is necessary to first establish, by natural history studies, that they meaningfully reflect changes in clinical state [119]. Additionally, a better understanding of the natural history and evolution of MRI abnormalities in PPMS may, in itself, give insights into underlying pathology. For example, as discussed earlier, is the disease process a constant one, or alternatively is there evidence of fluctuation over time? Where there is change in more than one MRI measure is it of equal degree and does it occur in unison?

To begin to answer these questions, and to document for the first time the extended natural history of conventional MRI abnormalities in PPMS, two longitudinal studies are described in this chapter. The first examines a large internationally recruited cohort over two years, the second, a smaller cohort recruited from one centre and examined over five years.
3.1 Study one: A two year multicentre follow up study of PPMS

By 1996, a number of studies of MRI in PPMS using conventional measures had been published but there was a paucity of longitudinal data. Through an European Community funded initiative, MAGNIMS (Magnetic Resonance Network in Multiple Sclerosis), patients with PPMS were recruited from six European centres (Amsterdam, Barcelona, Bordeaux, Lisbon, London, Milan) to take part in a longitudinal clinical and MRI study. Cross-sectional analyses of the baseline and one year data were published in 1999 and 2000 respectively [35;43].

In addition to patients with PPMS, this cohort also included patients with so-called Transitional Progressive Multiple Sclerosis (TPMS). This term refers to a subgroup of patients that has an essentially progressive course but with the additional history of a single relapse before or during the progressive phase [4;120;121]. The term TPMS has not gained wide acceptance and its usefulness as a meaningful sub-grouping has been questioned [122]. The status of TPMS will be considered in more detail later in this chapter.

At baseline, patients with PPMS had a mean age of onset of 40.2 years, there were equal numbers of male and female subjects and their mean T1 and T2 brain lesion loads were low, in agreement with earlier studies [35] The only direct MRI/clinical correlations were between disability, measured by the EDSS [46] and brain and spinal cord atrophy (r=0.3, 0.2 respectively, p=0.006). When the cohort was subdivided by clinical presentation, those presenting with cord symptoms had significantly lower
cerebral T1 and T2 lesion loads in comparison to those with other presentations including progressive cerebellar, brainstem, and visual syndromes. Otherwise abnormalities in T2 load, T1 hypointensity load and partial brain volume were found to have no general relation to clinical status.

After one year change in several MRI measures was seen [43] Brain T1 and T2 lesion load increased by 12.6% and 7.3% respectively though few new lesions were seen in either brain or cord. Patients with PPMS developed brain and cord atrophy. No correlation between change in MRI measures and disability was found.

To examine whether a longer duration of follow up of this unique large international cohort of patients with PPMS would show MRI and clinical correlations not seen at one year patients returned for collection of further MRI and clinical data at two years. Identical methods of data collection and analysis were used as in the earlier cross-sectional and one year studies. These are described in detail in Chapter 2 but are summarised briefly in the “Imaging” and “Analysis” sections below.

3.2 Methods

3.2.1 Patients

Five of the six original centres were able to contribute clinical data at two years (Amsterdam, Barcelona, Bordeaux, London, Milan) and four provided MRI data (Amsterdam, Barcelona, London, Milan). At two years, clinical data were available
on 125 patients and MRI data on 113 patients.

3.2.2 Clinical measures

Clinical measures included EDSS, timed 10m walk and the nine-hole peg test. The nine-hole peg test was used as a measure of upper limb disability by averaging the times from both hands. When the patient was unable to perform the task, or took longer than five minutes to complete it, the time for that hand was recorded as 300 seconds. The timed ten metre walk was carried out by measuring the time taken and the number of completed steps used for the subject to cover a distance of 10 metres from a standing start. For ease of comparison with one year data, significant improvement or deterioration in either the nine-hole peg test or 10 metre timed walk was defined as a change equal or greater than 20% [35;43;123]. A one step change in EDSS was defined as a change of 1.0 if EDSS was 5 or less, or a change of 0.5 if EDSS was >5.0, as used in an earlier one year study of this cohort [43].

3.2.3 Imaging Protocol

At two years patients were examined using the same scanners that had been used for the baseline and one year studies. These had been serviced regularly over the two year period with no major upgrades. London scans were carried out using a Signa 1.5T system (General Electric, Milwaukee, Wisconsin, USA); the other three sites (Amsterdam, Barcelona and Milan) used Siemens, Magnetom 1.5T systems.
Each patient underwent T1 and T2 weighted spin echo imaging of the brain (3 mm contiguous axial slices. T2; TR 3000 ms, TE 15/90 ms. T1; TR 600 ms, TE 20 ms). All sequences were acquired as contiguous, 3mm thick axial slices (44 images in total). In the spinal cord, nine contiguous, 3mm, sagittal T2 hyperintense and T1 hypointense and proton density weighted slices were obtained (TR 2500 ms, TE 45/90 ms). A volume acquired inversion prepared gradient echo acquisition of the spinal cord (60 1 mm slices, TR 15.6 ms, TE 4.2 ms, T1 450 ms, FA 20°, matrix 256x256) was also performed (Signa scanners; fast spoiled gradient echo: FSPGR, Magnetom scanners; magnetisation prepared rapid acquisition gradient echo MPRAGE) and from the data set a series of five contiguous 3 mm axial slices (perpendicular to the spinal cord) were reformatted using the centre of the C2/C3 disc as the caudal land mark. The imaging parameters for each site are detailed in the baseline data paper [35].

3.2.4 Analysis

An experienced blinded rater (Professor DH Miller) marked T2 lesions on hard copy for all subjects at all three time points. Another experienced blinded rater (Dr SM Leary) used the marked films to calculate the number of new lesions for all subjects at year one and year two. Cerebral lesion and whole brain analysis was carried out at a SUN workstation using a local thresholding technique with manual editing. T2 and T1 hypointensity volume were calculated with reference to the marked hard copies. Dr VL Stevenson analysed cerebral data (including partial brain volume) for subjects from London at all three time points and data from Amsterdam, Barcelona and Milan at baseline and year one. I analysed cerebral data from Amsterdam, Barcelona and
Milan at year two. The measurements of VLS and GTI were found to correlate highly \( r=0.994 \). Spinal cord data from London was analysed at all three timepoints by Dr VL Stevenson and from Amsterdam, Barcelona and Milan by Dr M Rovaris

The measures of partial brain volume and cross sectional spinal cord area reflecting atrophy were calculated using the techniques described in Chapter 2. Brain volume was measured by extracting the brain images from the skull by means of a computer algorithm and by measuring the volume occupied by six 3mm slices the most caudal at the level of the velum interpositum cerebri.

Measurement reproducibility was assessed for brain lesion load, cerebral atrophy, and cross sectional cord area by repeating measurements on the baseline data set of 10 random subjects twice, at least one year apart. The coefficient of variation (CV) was calculated for each measure by dividing the SD by the mean. All statistical analyses employed non-parametric tests. The Mann-Whitney test was used to look for differences between the patient groups and the Wilcoxon signed ranks test to compare the three time points. Correlations were assessed using the Spearmann's rank correlation coefficient. To reflect the large number of statistical comparisons a p value of 0.01 was considered significant. Values between 0.01 and 0.05 were regarded as representing a trend. No mathematical correction of statistical significance was carried out to avoid inflating type II errors (the probability of accepting the null hypothesis when the alternative is true) and thus missing real differences.
3.3 Results

Of the 190 patients on whom MRI and clinical data were available at baseline, 125 returned at year two (66% of baseline). This consisted of 100 patients with PPMS and 25 with TPMS (64% and 73% of baseline populations respectively). The median time to follow up was 24 months (range 18-31 months). The mean disease duration at the time of entry into the study was 12.5 years (SD 7.1 years) and median EDSS for both groups was 6.0.

The reasons for non-attendance at year two were not individually recorded but it is likely that increasing disability was an important factor. The percentage of the baseline cohort returning by centre was 71% for Amsterdam, 67% for Barcelona, 53% for Bordeaux, 73% for London and 81% for Milan. To study possible reasons for non-attendance, and to quantify its effects on the character of the cohort as a whole, baseline patient data were retrospectively subdivided into two groups: those who had completed two years of follow (125 patients) and those who had dropped out of the study by year two (65 patients). When these groups were compared no significant difference was found in baseline EDSS but there was a trend to worse ten metre timed walk performance at baseline in those patients who did not return at two years (p ≤ 0.05) (Table 3.1). Differences were also seen with respect to age of onset (37 versus 42 years, p ≤ 0.01) and age at study entry (49 versus 54 years, p ≤ 0.01) with the defaulting group being older in each case. Median baseline T2 and T1 loads were higher in the defaulting group but these differences were not significant. There was a trend to lower median brain volumes in the defaulting group (p ≤ 0.05). Overall
therefore there is evidence that the patients who did not return at year two were older and had more limited mobility at baseline.

Intrarater reproducibility was assessed for the MRI measures: the mean coefficient of variation (CV) for brain lesion load analysis was 3.1% (SD 9.6), the more automated measures of brain atrophy and cord cross sectional area measurement produced CVs of 0.17% (SD 0.48) and 0.51% (SD 0.54) respectively.

**Table 3.1: Clinical and MR characteristics of patients lost to follow up at year two**

<table>
<thead>
<tr>
<th></th>
<th>Completed follow up N=125</th>
<th>Did not complete follow up N=65</th>
</tr>
</thead>
<tbody>
<tr>
<td><strong>Age</strong></td>
<td>48 (28-76)</td>
<td>56 (40-78)</td>
</tr>
<tr>
<td><strong>Age at onset</strong></td>
<td>35 (18-63)</td>
<td>43 (24-62)</td>
</tr>
<tr>
<td><strong>Duration</strong></td>
<td>11.0 (1-43)</td>
<td>12 (5-33)</td>
</tr>
<tr>
<td><strong>EDSS</strong></td>
<td>6.0 (2.9-8.5)</td>
<td>5.5 (2.0-8.0)</td>
</tr>
<tr>
<td><strong>9HPT</strong></td>
<td>29.5 (16.5-300)</td>
<td>28.7 (18-300)</td>
</tr>
<tr>
<td><strong>10m</strong></td>
<td>13.1 (5-300)</td>
<td>15.0 (7.5-70.0)</td>
</tr>
<tr>
<td><strong>Baseline T2 load (cm³)</strong></td>
<td>7.82 (0.1-72.9)</td>
<td>8.00 (0.2-102.8)</td>
</tr>
<tr>
<td><strong>Baseline T1 load (cm³)</strong></td>
<td>2.24 (0-33.2)</td>
<td>2.82 (0.1-54.2)</td>
</tr>
<tr>
<td><strong>Baseline brain volume (cm³)</strong></td>
<td>269.5 (196-335)</td>
<td>259.0 (198-316)</td>
</tr>
<tr>
<td><strong>Baseline cord area (mm²)</strong></td>
<td>71.9 (51.9-93.8)</td>
<td>76.0 (52.0-91.0)</td>
</tr>
</tbody>
</table>
3.3.1 Clinical measures: EDSS, timed ten metre walk and nine hole peg test

Although the median EDSS did not change over two years (Table 3.2), the mean EDSS (not shown) increased from 5.7 to 5.9 in the PPMS group and from 5.9 to 6.0 in the TPMS group. Considering those patients on whom one and two year data were available, at one year, 25 (20%) had deteriorated by one or more EDSS steps and at two years, 50 (40%) had deteriorated (for the UK patients at five years this figure was 65%). Seventeen (14%) and fifteen patients (12%) had improved by one and two years respectively. Over two years, significant changes were seen in EDSS and timed ten metre walk (p<0.01) for patients with PPMS and TPMS. No difference was found between the groups. Significant changes in the nine hole peg test score were seen for the TPMS group only.
Table 3.2: Clinical findings at baseline, one and two years (median values)

<table>
<thead>
<tr>
<th>measure</th>
<th>year</th>
<th>PP</th>
<th>TP</th>
</tr>
</thead>
<tbody>
<tr>
<td>EDSS</td>
<td>0</td>
<td>6.00 (2.0-8.5)</td>
<td>6.00 (2.5-8.5)</td>
</tr>
<tr>
<td></td>
<td>1</td>
<td>6.00 (2.0-9.0)</td>
<td>6.00 (2.5-8.5)</td>
</tr>
<tr>
<td></td>
<td>2</td>
<td>6.00 (2.0-9.0)</td>
<td>6.00 (3.0-9.0)</td>
</tr>
<tr>
<td>9HPT</td>
<td>0</td>
<td>27.9 (17-300)</td>
<td>30.1 (19-300)</td>
</tr>
<tr>
<td></td>
<td>1</td>
<td>28.0 (15-300)</td>
<td>31.7 (18-300)</td>
</tr>
<tr>
<td></td>
<td>2</td>
<td>29.5 (15-300)</td>
<td>35.7 (20-300)</td>
</tr>
<tr>
<td>TMW</td>
<td>0</td>
<td>12.0 (5-300)</td>
<td>17.1 (6-300)</td>
</tr>
<tr>
<td></td>
<td>1</td>
<td>13.6 (6-300)</td>
<td>19.0 (6-300)</td>
</tr>
<tr>
<td></td>
<td>2</td>
<td>16.1 (6-300)</td>
<td>21.0 (6-300)</td>
</tr>
</tbody>
</table>

Comparison with baseline measure (Wilcoxon signed ranks test) 1. p=0.002 2. p=0.001 3. p<0.001
3.3.2. MRI measures (Tables 3 and 4)

New lesions were seen in 26% of the PPMS group compared with 41% of the TPMS groups. No significant difference was found in the number of new lesions over two years in the two disease groups. In the PPMS and TPMS group T2 load increased at both one and two years (p<0.001). T1 load increased in PPMS but not TPMS patients at years one and two. There were no significant differences between the patient groups in either absolute change in lesion loads or in the percentage change on either of these measures.

The six slice measure of brain volume, reflecting atrophy, showed a significant decrease in PPMS and TPMS patient groups at years one and two. No differences between the patient groups were seen in either volumes at each timepoint or change between timepoints. A significant reduction in spinal cord area was seen in the PPMS and TPMS groups over two years.

No significant differences were found between PPMS and TPMS subgroups for change in any of the MR measures (see Table 3).
Table 3.3: MRI findings at baseline, one and two years (median values)

<table>
<thead>
<tr>
<th>Measure</th>
<th>Year</th>
<th>PP</th>
<th>TP</th>
</tr>
</thead>
<tbody>
<tr>
<td>Brain T2 Load (cm³)</td>
<td>0</td>
<td>6.62 (0.1-72.2)</td>
<td>10.7 (0.48-72.9)</td>
</tr>
<tr>
<td></td>
<td>1</td>
<td>7.41 (0.1-74.7) ¹</td>
<td>12.5 (0.39-85.4)³</td>
</tr>
<tr>
<td></td>
<td>2</td>
<td>9.04 (0.2-102.4)²</td>
<td>15.0 (0.28-93.7)⁴</td>
</tr>
<tr>
<td>New Brain Lesions (99:24)</td>
<td>At 1</td>
<td>0.0 (0-0) [1.04]</td>
<td>1.0 (0-2) [0.54]</td>
</tr>
<tr>
<td></td>
<td>At 2</td>
<td>1.0 (0-22) [1.48]</td>
<td>0.0 (0-5) [1.00]</td>
</tr>
<tr>
<td>Brain T1 Load (cm³)</td>
<td>0</td>
<td>1.68 (0.3-33.1)</td>
<td>3.25 (0.10-24.4)</td>
</tr>
<tr>
<td></td>
<td>1</td>
<td>2.41 (0.05-32.9)⁵</td>
<td>4.09 (0.07-32.3)</td>
</tr>
<tr>
<td></td>
<td>2</td>
<td>2.88 (0.05-49.9)⁶</td>
<td>3.54 (0.20-39.2)</td>
</tr>
<tr>
<td>6 Slice Brain Volume (cm³)</td>
<td>0</td>
<td>271.2 (210-320)</td>
<td>271.1 (238-297)</td>
</tr>
<tr>
<td></td>
<td>1</td>
<td>267.5 (208-315)⁷</td>
<td>267.6 (235-298)⁹</td>
</tr>
<tr>
<td></td>
<td>2</td>
<td>262.0 (202-316)⁸</td>
<td>266.0 (227-298)¹⁰</td>
</tr>
<tr>
<td>Cord Area (mm²)</td>
<td>0</td>
<td>71.9 (54-91)</td>
<td>68.6 (56-84)</td>
</tr>
<tr>
<td></td>
<td>1</td>
<td>69.3 (49-89)¹¹</td>
<td>63.6 (53-81)¹³</td>
</tr>
<tr>
<td></td>
<td>2</td>
<td>69.6 (47-91)¹²</td>
<td>63.0 (52 84)¹⁴</td>
</tr>
</tbody>
</table>

Comparison with baseline measure within groups (Wilcoxon signed ranks test)
1. p<0.001 2. p<0.001 3. p=0.001 4. p<0.001 5 p<0.001 6. p<0.001 7. p<0.001
8. p<0.001 9. p=0.005 10. p=0.001 11. p<0.001 12. p<0.001 13. p=0.001 14. p=0.001.

Note: For "new brain lesions" the mean value is given in square brackets.
### Table 3.4: Change in clinical and MR measures over first and second years (SD)

<table>
<thead>
<tr>
<th>Change in measure</th>
<th>PP</th>
<th></th>
<th>TP</th>
<th></th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>1 year</td>
<td>2 years</td>
<td>1 year</td>
<td>2 years</td>
</tr>
<tr>
<td>ΔEEDSS</td>
<td>20%</td>
<td>36%</td>
<td>20%</td>
<td>52%</td>
</tr>
<tr>
<td>Δ9HPT</td>
<td>15%</td>
<td>26%</td>
<td>23%</td>
<td>46%</td>
</tr>
<tr>
<td>ΔTMW</td>
<td>27%</td>
<td>41%</td>
<td>16%</td>
<td>32%</td>
</tr>
<tr>
<td>ΔT2 lesion load</td>
<td>0.41</td>
<td>0.94</td>
<td>0.74</td>
<td>1.30</td>
</tr>
<tr>
<td>(19.1%)</td>
<td>(36.1%)</td>
<td></td>
<td>(23.6%)</td>
<td>(46.5%)</td>
</tr>
<tr>
<td>ΔT1 hypointensity load</td>
<td>0.23</td>
<td>0.64</td>
<td>0.48</td>
<td>0.44</td>
</tr>
<tr>
<td>(39.8%)</td>
<td>(78.4%)</td>
<td></td>
<td>(20.2%)</td>
<td>(48.2%)</td>
</tr>
<tr>
<td>Δ6 slice brain volume</td>
<td>-2.12</td>
<td>-6.26</td>
<td>-2.31</td>
<td>-5.87</td>
</tr>
<tr>
<td>(-1.0%)</td>
<td>(-2.7%)</td>
<td></td>
<td>(-0.7%)</td>
<td>(-2.3%)</td>
</tr>
<tr>
<td>ΔCord area</td>
<td>-2.37</td>
<td>-3.03</td>
<td>-3.37</td>
<td>-5.27</td>
</tr>
<tr>
<td>(-3.7%)</td>
<td>(-3.8%)</td>
<td></td>
<td>(-5.7%)</td>
<td>(-6.7%)</td>
</tr>
</tbody>
</table>
3.3.3 Clinical/MRI correlations

No correlation was found between the absolute or percentage change in clinical outcomes and MRI measures at two years. The data were examined to see whether degree of change over the first year of the study correlated with degree of change over the second year. No correlation was found with the exception of change in T2 load; for which change at year one predicted change at year two (r=0.26, p=0.012). The relation between changes in MRI measures over two years was also compared (Table 3.5). A strong correlation was seen between change in T2 load and change in T1 load over two years (r=0.69, p<0.001). A weaker correlation was also seen between change in T2 and T1 load and change in partial brain volume.

Table 3.5: Correlates between MRI parameters over two years for PPMS group

<table>
<thead>
<tr>
<th>MRI parameters</th>
<th>r</th>
<th>p</th>
</tr>
</thead>
<tbody>
<tr>
<td>Change in T2 load v Change in T1 load</td>
<td>r=0.69</td>
<td>p&lt;0.001</td>
</tr>
<tr>
<td>Change in T2 load v Change in brain volume</td>
<td>r=-0.25</td>
<td>p=0.024</td>
</tr>
<tr>
<td>Change in T2 load v Change in cord area</td>
<td>r=-0.01</td>
<td>p=0.97</td>
</tr>
<tr>
<td>Change in T1 load v Change in brain volume</td>
<td>r=-0.26</td>
<td>p=0.027</td>
</tr>
<tr>
<td>Change in T1 load v Change in cord area</td>
<td>r=-0.005</td>
<td>p=0.74</td>
</tr>
<tr>
<td>Change in brain volume v Change in cord area</td>
<td>r=0.002</td>
<td>p=0.90</td>
</tr>
</tbody>
</table>
3.3.4 Analysis by clinical presentation and clinical course

When the 100 patients with PPMS were subdivided on the basis of clinical presentation into those with a progressive cord syndrome (78 patients) and those with other presentations (ataxia, visual disturbance, hemiparesis or brainstem symptoms, 22 patients) significant differences were found in clinical and MRI measures (see Tables 3.6 and 3.7). Although the median EDSS (6.0) did not change in either group over two years, the mean EDSS (not shown) increased from 5.7 to 6.0 in the cord presentation group and from 5.6 to 5.7 in the non-cord presentation group. Thirty nine percent of patients with cord presentations underwent a significant step change in EDSS compared to 29% of patients with a non-cord presentation but this difference was not statistically significant. The cord presentation group did however have a lower cerebral T2 and T1 lesion load (p=0.0014 for T2 and 0.007 for T1) and median increase in T2 load was less than in the non-cord presentation group (p<0.001 at both years). Increases in T1 hypointensity load were seen for patients with both cord and non-cord presentations however the change from baseline was only significant in the cord group (p=0.001). There was no difference in the number of new cerebral lesions at two years between patients with cord and non-cord presentations. Few new lesions were seen in the spinal cord. Median partial brain volume decreased significantly in both the cord and the non-cord groups but there was no difference between the groups. There was a larger reduction in cord area in the group of patients with cord presentations, but this did not achieve significance.
Table 3.6: Clinical and MRI measures in PPMS by presentation (median values)

<table>
<thead>
<tr>
<th>Measure</th>
<th>Year</th>
<th>Cord Presentation</th>
<th>Other Presentation</th>
</tr>
</thead>
<tbody>
<tr>
<td><strong>EDSS</strong></td>
<td>0</td>
<td>6.00 (2.0-8.5)</td>
<td>6.00 (2.0-8.5)</td>
</tr>
<tr>
<td>(78:21)</td>
<td>1</td>
<td>6.00 (2.0-9.0)</td>
<td>6.00 (2.5-8.5)</td>
</tr>
<tr>
<td></td>
<td>2</td>
<td>6.00 (2.0-9.0)</td>
<td>6.00 (3.0-8.5)</td>
</tr>
<tr>
<td><strong>New Brain Lesions</strong></td>
<td>1</td>
<td>0.0 (0-9) (1.15)</td>
<td>0.0 (0-4) (0.60)</td>
</tr>
<tr>
<td>(78:20)</td>
<td>2</td>
<td>1.0 (0-22) (1.59)</td>
<td>0.5 (0-5) (1.05)</td>
</tr>
<tr>
<td><strong>Brain T2 Load (cm³)</strong></td>
<td>0</td>
<td>6.19 (0.1-62.6)a</td>
<td>12.7 (0.2-72.2)a</td>
</tr>
<tr>
<td>(76:18)</td>
<td>1</td>
<td>6.39 (0.2-56.5)b</td>
<td>13.9 (0.14-74.7)b</td>
</tr>
<tr>
<td></td>
<td>2</td>
<td>7.88 (0.2-55.8)c</td>
<td>15.5 (0.41-102.4)c</td>
</tr>
<tr>
<td><strong>Brain T1 Load (cm³)</strong></td>
<td>0</td>
<td>1.30 (0-33.1)d</td>
<td>6.42 (0.2-26.2)d</td>
</tr>
<tr>
<td>(66:16)</td>
<td>1</td>
<td>1.76 (0-32.9)e</td>
<td>7.99 (0.3-21.8)e</td>
</tr>
<tr>
<td></td>
<td>2</td>
<td>2.39 (0-34.0)f</td>
<td>9.07 (0.2-49.9)f</td>
</tr>
<tr>
<td><strong>6 Slice Brain Volume (cm³)</strong></td>
<td>0</td>
<td>272.2 (219-320)</td>
<td>262.1 (210-306)</td>
</tr>
<tr>
<td>(71:18)</td>
<td>1</td>
<td>268.8 (208-315)</td>
<td>258.2 (208-299)</td>
</tr>
<tr>
<td></td>
<td>2</td>
<td>266.2 (201-316)</td>
<td>252.5 (209-294)</td>
</tr>
<tr>
<td><strong>Cord Area (mm²)</strong></td>
<td>0</td>
<td>71.6 (54.4-90.8)</td>
<td>72.5 (65.4-88.4)</td>
</tr>
<tr>
<td>(49:13)</td>
<td>1</td>
<td>69.2 (48.5-89.0)</td>
<td>73.2 (60.6-86.8)</td>
</tr>
<tr>
<td></td>
<td>2</td>
<td>67.9 (47.2-91.1)</td>
<td>72.9 (60.3-91.1)</td>
</tr>
</tbody>
</table>

Comparison with baseline measure (Wilcoxon signed ranks test) 1. p<0.001 2. p<0.001 3. p<0.001 4. p=0.043 5. p=0.012 6. p<0.001 7. p=0.001 8. p<0.001 9. p<0.001 10. p=0.004 11. p<0.001 12 p<0.001 13 p<0.001 Comparison between groups (Mann-Whitney test) a=0.004, b=0.009, c=0.014, d=0.007, e=0.004, f=0.007

Note: For “new brain lesions” the mean value is given in square brackets
Table 3.7: Change in clinical and MR measures by presentation

<table>
<thead>
<tr>
<th>Measure</th>
<th>Cord Presentation</th>
<th>Other Presentation</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>1 year</td>
<td>2 years</td>
</tr>
<tr>
<td>Δ EDSS$^1$</td>
<td>22%</td>
<td>39%</td>
</tr>
<tr>
<td>Δ T2 load (cm$^3$)</td>
<td>0.40</td>
<td>0.94</td>
</tr>
<tr>
<td>Δ T1 load (cm$^3$)</td>
<td>0.21</td>
<td>0.57</td>
</tr>
<tr>
<td>Δ 6 slice brain volume (cm$^3$)</td>
<td>-1.77</td>
<td>-5.73</td>
</tr>
<tr>
<td>Δ Cord area (mm$^3$)</td>
<td>-2.50</td>
<td>-2.80</td>
</tr>
</tbody>
</table>

Note: 1 “Δ EDSS” refers to the percentage of patients showing a clinically significant change in the EDSS score as defined in the text.

When the PPMS group was subdivided on the basis of whether or not a step change in EDSS had occurred over a two year period a trend to a difference in change in brain atrophy was found (see Tables 3.8 and 3.9). Patients with deterioration in their EDSS score were found to have a trend to greater brain atrophy in comparison to those whose EDSS score was unchanged (p=0.05). Greater reduction in spinal cord area was also seen in this group, though this was not found to be significant (p=0.62).
Table 3.8: MRI measures in PPMS by clinical course (median values)

<table>
<thead>
<tr>
<th>Measure</th>
<th>Year</th>
<th>Change in EDSS</th>
<th>No change in EDSS</th>
</tr>
</thead>
<tbody>
<tr>
<td>New Brain Lesions (33:57)</td>
<td>At 1</td>
<td>0.0 (0-5) (1.36)</td>
<td>0.0 (0-9) (0.84)</td>
</tr>
<tr>
<td></td>
<td>At 2</td>
<td>1.0 (0-22) (2.64)</td>
<td>1.0 (0-7) (0.96)</td>
</tr>
<tr>
<td>Brain T2 Load (cm³) (33:52)</td>
<td>0</td>
<td>8.33 (0.4-64.0)</td>
<td>6.41 (0.1-72.2)</td>
</tr>
<tr>
<td></td>
<td>1</td>
<td>11.6 (0.4-74.7)¹</td>
<td>6.33 (0.1-74.0)²</td>
</tr>
<tr>
<td></td>
<td>2</td>
<td>13.6 (0.4-102.4)³</td>
<td>7.61 (0.2-71.3)⁴</td>
</tr>
<tr>
<td>Brain T1 Load (cm³) (31:44)</td>
<td>0</td>
<td>2.88 (0.0-33.1)</td>
<td>1.95 (0.1-33.1)</td>
</tr>
<tr>
<td></td>
<td>1</td>
<td>3.80 (0.1-32.9)⁵</td>
<td>2.33 (0.1-32.9)⁶</td>
</tr>
<tr>
<td></td>
<td>2</td>
<td>2.94 (0.1-34.0)⁷</td>
<td>2.94 (0.1-34.0)⁸</td>
</tr>
<tr>
<td>6 Slice Brain Volume (cm³) (28:52)</td>
<td>0</td>
<td>271.9 (226-305)⁹</td>
<td>270.4 (210-320)¹⁰</td>
</tr>
<tr>
<td></td>
<td>1</td>
<td>269.2 (216-302)¹¹</td>
<td>266.9 (207-315)¹²</td>
</tr>
<tr>
<td></td>
<td>2</td>
<td>267.5 (209-294)¹³</td>
<td>262.3 (202-316)¹⁴</td>
</tr>
<tr>
<td>Cord area (mm²) (21:40)</td>
<td>0</td>
<td>72.3 (65.4-88.4)</td>
<td>75.7 (54.4-90.8)</td>
</tr>
<tr>
<td></td>
<td>1</td>
<td>70.7 (60.1-86.8)¹⁵</td>
<td>72.2 (48.5-89.0)</td>
</tr>
<tr>
<td></td>
<td>2</td>
<td>71.6 (60.3-91.1)¹⁶</td>
<td>72.2 (47.2-91.1)</td>
</tr>
</tbody>
</table>

Comparison with baseline measure (Wilcoxon signed ranks test) ¹. p=0.04 ². p=0.04 ³. p=0.02 ⁴. p=0.004 ⁵. p<0.001 ⁶. p=0.012 ⁷. p<0.001 ⁸. p=0.002 ⁹. p<0.001 ¹⁰. p<0.001 ¹¹. p<0.001 ¹². p=0.001 ¹³. p<0.001 ¹⁴. p<0.001 ¹⁵. p<0.001 ¹⁶. p=0.011
Table 3.9: Change in MRI measures by clinical course

<table>
<thead>
<tr>
<th>Measure</th>
<th>Change in EDSS</th>
<th>No change in EDSS</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>1 year</td>
<td>2 years</td>
</tr>
<tr>
<td>Δ T2 load (cm³)</td>
<td>0.11</td>
<td>0.72</td>
</tr>
<tr>
<td>Δ T1 load (cm³)</td>
<td>0.35</td>
<td>0.86</td>
</tr>
<tr>
<td>Δ 6 slice brain volume (cm³)</td>
<td>-2.3</td>
<td>-8.9</td>
</tr>
<tr>
<td>ΔCord area (mm²)</td>
<td>-2.3</td>
<td>-3.7</td>
</tr>
</tbody>
</table>

Comparison between groups (Mann-Whitney test) a Change> No Change p=0.011

3.3.5 Discussion

The purpose of this two year study was to examine the relation between clinical and MRI measures in a large cohort of patients with PPMS. Before discussing the results it is important to note that one of the major limitations of this study is incomplete follow up. It might be expected that increasing disability would be an important factor in this respect, especially, as within this cohort, there were many patients with long disease durations and high baseline disability. In fact, baseline EDSS and disease duration were not higher in the defaulting group although this group did have a significantly higher mean age both at disease onset and study entry. Therefore the study population who returned at year two was, at baseline, younger but not less disabled than the study population who did not return. This does not exclude the possibility that patients who dropped out of the study had a more rapid rate of deterioration. However, patients who did not attend at year two, and on whom year one data was available, did not seem to have progressed more rapidly on sensitive
measures such as the timed ten metre walk over the first year. The principal reason for non-attendance at year two in this study is therefore unclear but from the available data the reason does not seem to be disability alone.

This two year dataset confirms the observation made over one year, that the rate of acquisition of new lesions in brain and spinal cord in these patient groups is low. In spite of this however, statistically significant change in several MRI measures is seen. In the case of T2 load, it is likely that the greatest contribution to change comes from enlargement of existing lesions. A previously published sub study of lesions in patients from the UK cohort of this study found that 91% of the total new T2 lesion volume was from enlargement of pre-existent lesions and only 9% from new, discrete, lesions [44]. Therefore, even with the generally lower level of both MRI abnormality and MRI activity in PPMS, it can be confidently stated that MRI is still sensitive to change over relatively short periods.

However, a more important question is whether this change in MRI measures relates to clinical change over the same period. In fact, the poor correlation seen cross-sectionally at baseline and longitudinally over the first year between MRI and clinical measures did not improve when data over two years were considered. There are two possible explanations for the failure to find a substantial relation between clinical and MRI change. It may be that conventional MRI measures, although in some way related to the disease process, do not reflect the processes that are most critical to the development of clinical disability. Alternatively, it may be that two years is not a sufficiently long period to make any relation apparent given both the slow evolution
of the condition and the relative insensitivity of clinical measures of disability. In relation to the latter point, the lack of change in EDSS, over two years, may be relevant.

Subgroup analysis in the PPMS group does however suggest that conventional MRI indices are providing at least some information that is clinically meaningful as suggested by earlier, smaller, studies in this patient group [42]. For example, this study suggests that there may be differences in MR findings between patients with cord and non-cord presentations, i.e. cerebral T2 and T1 lesion loads are significantly higher in patients with non-cord presentations and brain atrophy is greater. However there is considerable overlap in the range of values seen for each presentation.

Another issue raised by this study is the status of TPMS as a meaningful disease subtype [4]. Currently, two broad approaches have been adopted to cases where there is an essentially progressive disease course but in addition a clear cut relapse. One approach considers that those patients with no relapses at any time form the only distinct group whose identification for research purposes is practical. This is the approach taken in recently published diagnostic criteria [5]. The other approach proposes that patients with an essentially primary progressive course, but with superimposed relapses either early or late in the course of their progression, should be subsumed within the PPMS division as they do not appear to have clinical outcomes that differ from patients without relapses [122]. It is also proposed that patients in whom a single relapse occurs before the onset of progression might be subsumed within the SPMS division [124]. In the current study no clear differences were seen
between either clinical or MRI behaviour of the PPMS and TPMS subgroups, although numbers in the latter group were small. This study therefore provides some additional evidence that there is no striking difference in the MRI and clinical behaviour of TPMS in relation PPMS. Despite this, until underlying pathogenic mechanisms are more clearly understood, a strong case can still be made for regarding MS that is entirely progressive from onset as a separate entity.

3.4 Study two: A five year follow up study of PPMS

We have now seen that even when a very large number of patients with PPMS were examined in a multicentre study trial over two years, no relation was detected between MRI and clinical change. One possible reason for this could be that the period of follow up was too short for such a relation to become apparent. To explore this possibility we extended the follow up to five years. This was done for one centre only (UK) where a preliminary telephone survey had suggested that not only would a substantial proportion of patients be able to return for clinical and MRI assessment at five years but that almost all patients would be available for at least a telephone assessment of their clinical state.

This longer period of observation with multiple timepoints (four) also allows the question of disease dynamics to be addressed. This issue has received little attention in the past other than in clinical observational studies which have seemed to suggest that the rate of clinical progression is constant and relentless [124][19]. It is not known whether underlying pathological mechanisms also operate at a constant rate
throughout the course of the condition or, whether change is more marked in the early or late stages of the disease.

3.5 Methods

3.5.1 Patients and evaluation

Fifty-nine patients with PPMS who had participated in the earlier two year MRI study [35;43;51] were asked to return for a further clinical and MR examination at year five. By this time, four patients had died and one patient could not be contacted. Thirteen patients were unable to re-attend because of severe disability but 41 of 54 patients returned for clinical and MR reassessment at year five. Of these 41 patients, 15 took part in a phase two trial of interferon beta 1a during years two and three and nine were in the active, treatment, arm [125].

The same clinical measures were obtained at year five as had been collected at baseline, year one and year two: i.e. EDSS [46], timed ten metre walk, nine-hole peg test [123] and Paced Auditory Serial Addition Test, 3 second version (PASAT 3) [49]. For the analysis at five years however the latter three measures were combined to form the Multiple Sclerosis Functional Composite (MSFC) measure. As discussed in Chapter 2 this is a multidimensional measure that in recent years has become increasingly used as an objective measure of disability in MS. As its three component variables measure different entities (time for timed walk and nine hole peg test, number of correct answers for PASAT) a Z score is used to provide a common metric
[50]. Two observers were involved in the collection of clinical data: clinical examinations at baseline, year one and year two were performed by Dr VL Stevenson and at year five by myself. At the time of the year five assessments, I was blind to all MR measures. Clinical outcome data on the thirteen patients who were unable to re-attend were obtained by means of a structured telephone interview which allowed the EDSS to be calculated [126]. In order to assess the reliability of the telephone interview, eight patients had both phone EDSS and standard EDSS and the two measures were correlated highly, (r=0.92) as previously reported [126].

3.5.2 MRI: acquisition and analysis

As at baseline, year one and year two [35;43;51] lesions were marked on hard copy proton density weighted images for all subjects at year five with reference to T2 weighted images by a single, expert rater Professor D H Miller who was blind to all clinical measures. These films were then used to calculate the number of new lesions over five years. Individual rates of increase of T2 and T1 load were calculated by linear regression of the four time points for each patient. Cerebral lesion and whole brain analysis was carried out on a SUN workstation using a local threshold technique with manual editing. T2 and T1 hypointensity volume were calculated with reference to the marked hard copies. Baseline, year one, year two and year five data were analyzed by a single rater (GTI).

Derived MRI measures, partial brain volume and cross sectional spinal cord area were calculated as in the first study in this chapter together with an additional tissue volume
measure: ventricular volume, reflecting cerebral atrophy. Ventricular volumes were obtained by analyzing the T1-weighted images using the MIDAS package, as described in Chapter 2 [127].

The rate of change for each MR measure was found by averaging individual rates of change calculated by linear regression of the four time points for each patient. The effect of subdividing the group into clinically stable and clinically worsening groups was explored by logarithmic transformation of the data (to account for the wide variation in baseline values) and subsequent linear regression.

Measurement reproducibility was assessed for brain lesion load, cerebral atrophy, cross sectional cord area and ventricular volume by repeating measurements on a data set of 10 random subjects at an interval of two months. The coefficient of variation (CV) was calculated for each measure by dividing the SD by the mean.

3.5.3 Statistics

Differences between MR and clinical measures over time were assessed by means of the Wilcoxon signed rank test and correlations were assessed using Spearmann's rank correlation coefficient. The principal comparisons in the study were between change in each of five MR measures (T1 hypointensity, T2 load, partial brain volume, ventricular volume and cord area) and each of two disability measures (MSFC and EDSS). In view of the limited number of comparisons of the principal measures (ten), and because of inter-relation of the MR measures (T1 and T2 load, partial brain
volume and ventricular volume) it was felt that a correction for multiple comparisons was not appropriate. A larger number of comparisons was made with baseline MR, clinical and demographic measures and here it was also thought that it would be inappropriate to correct for multiple comparisons as the purpose of this analysis was to identify potentially confounding factors, should they be present. As multiple comparisons are being carried out the findings should be regarded as exploratory. Variability in the dynamics and consistency of MR behaviour over time between and within patients was assessed using a random intercepts regression model. The trajectories for the five MRI variables were checked for curvature over the time frame of the study with the MRI variable as response and linear terms in time as predictor.

3.6 Results

3.6.1 Clinical and demographic characteristics

The cohort of 41 patients who returned at year five was 54% male and had a mean age of 55.7 years (SD 10.9 years). The mean disease duration was 16.3 years (SD 6.8 years) and the mean time between initial and final assessment was 58.4 months (SD 5.2 months). The 13 patients who were unable to attend at year 5 had greater disability at baseline than those who did return. They had a baseline median EDSS of 7.0 compared to 6.0 (p=0.001), poorer MSFC (-3.5 as opposed to -1.8, p=0.006) and slower timed walk (Z score 7.7 as opposed to 3.7, p=0.006). Age, male-female ratio and disease duration were comparable in the two groups. The 15 patients who had participated in the two year trial of interferon beta 1a (nine in the active arm) were not
found to differ from other patients in the cohort on any demographic, clinical or MRI measure at baseline. This was also the case when the nine patients who had participated in the active arm of the trial were considered separately.

3.6.2 Clinical Change

The median EDSS at five years for the study group was 6.5 which represented a median increase of 0.5 points (range of EDSS change; -1 to 6.5) (Figure 3.1, Table 3.10). Change in EDSS over five years is shown in Figure 2f. For patients who could not attend, the median EDSS, by structured telephone interview was 7.5 (p<0.001). There was no difference in the median change in EDSS between the attending and non-attending groups. Patients with the greatest change in EDSS score had an earlier age of disease onset (r=-0.24, p=0.044) and higher baseline EDSS (r=0.48, p<0.001) but there was no relation to disease duration. The greatest changes in the Functional Scores (FS) of the EDSS were in the bowel-bladder, pyramidal and cerebellar components. The MSFC worsened over five years by 0.44 points (SD 1.1, p<0.05). Of its components, the mean changes in Z were as follows: timed walk 0.97 (SD 2.9, not significant), nine hole peg test 0.67 [SD 0.88, p<0.001] and PASAT -0.32 [SD1.1, p<0.05] (Table 3.10).
<table>
<thead>
<tr>
<th></th>
<th>EDSS</th>
<th></th>
<th></th>
<th></th>
<th></th>
<th></th>
<th></th>
<th></th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>Median</td>
<td>Range</td>
<td>Mean</td>
<td>SD</td>
<td>Mean Z score</td>
<td>SD</td>
<td>Mean Z score</td>
<td>SD</td>
</tr>
<tr>
<td>Baseline</td>
<td>6.0</td>
<td>(2.0-8.5)</td>
<td>-1.9</td>
<td>2.3</td>
<td>3.9</td>
<td>5.9</td>
<td>-1.0</td>
<td>1.6</td>
</tr>
<tr>
<td>One Year</td>
<td>6.0</td>
<td>(2.0-8.5)</td>
<td>-1.9</td>
<td>2.5</td>
<td>3.9</td>
<td>6.0</td>
<td>-1.1</td>
<td>1.8</td>
</tr>
<tr>
<td>Two Years</td>
<td>6.0</td>
<td>(2.0-9.0)</td>
<td>-2.1</td>
<td>2.4</td>
<td>4.6</td>
<td>6.1</td>
<td>-1.2</td>
<td>1.8</td>
</tr>
<tr>
<td>Five Years</td>
<td>6.5**</td>
<td>(3.0-9.0)</td>
<td>-2.3*</td>
<td>2.5</td>
<td>4.9</td>
<td>5.9</td>
<td>-1.6**</td>
<td>1.7</td>
</tr>
</tbody>
</table>

**Table 3.10:** Median EDSS and range, mean and standard deviation for MSFC and MSFC components (timed ten metre walk, nine hole peg test and PASAT 3 second). Values marked ** are significantly different from baseline at a level of p<0.001, values marked * are significantly different from baseline at a level of p<0.05 Wilcoxon signed rank test.
3.6.3 MRI measures

The mean CV for brain lesion load analysis was 3.1% (SD 9.6), the more automated measures of brain atrophy, cord cross sectional area and ventricular volume produced CVs of 0.17%, 0.51% and 0.26% respectively. The mean rate of change of T2 load was 1.0mls per year (SD 0.92) and of T1 hypointensity load was 0.4mls per year (SD 0.51) (Table 3.11). The individual variation in five year change in T2 hyper and T1 hypointensity load by subject with respect to disease onset is shown in Figure 3.2a and Figure 3.2b, respectively. Variability in the dynamics and consistency of MR behaviour over time between and within patients was investigated using a random intercepts regression model. In each case, only a small proportion of total variation was due to within patient variation for T2 load (2.5%), T1 hypointensity load (2.9%), ventricular volume (4.2%), partial brain volume (3.1%) and cervical cord volume (4.1%).

The ratio of T2 to T1 load changed during the study from 15.0 (SD 32.7) at baseline to 8.5 (SD 14.5) at year 5 (p=0.02) due to a higher percentage rise in T1 (see Table 3.11). By five years all measures were significantly different from baseline (Table 3.11). The mean number of new brain lesion seen over five years was 4.67 (SD 3.87) while the mean number of new cord lesions was only 0.35 (SD 0.63).
<table>
<thead>
<tr>
<th></th>
<th>T2 load</th>
<th></th>
<th>T2 lesion number</th>
<th></th>
<th>T1 hypointensity</th>
<th></th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>ml</td>
<td>SD</td>
<td>Change</td>
<td>number</td>
<td>ml</td>
<td>SD</td>
</tr>
<tr>
<td>Baseline</td>
<td>10.3</td>
<td>12.8</td>
<td></td>
<td>19.6</td>
<td>2.0</td>
<td>4.0</td>
</tr>
<tr>
<td>One Year</td>
<td>11.8**</td>
<td>13.5</td>
<td>↑ 14.6%</td>
<td>21.9**</td>
<td>2.4**</td>
<td>4.4</td>
</tr>
<tr>
<td>Two Years</td>
<td>12.7**</td>
<td>15.0</td>
<td>↑ 23.3%</td>
<td>23.2**</td>
<td>2.8* *</td>
<td>4.9</td>
</tr>
<tr>
<td>Five Years</td>
<td>15.0**</td>
<td>15.7</td>
<td>↑ 45.6%</td>
<td>26.2**</td>
<td>3.8 **</td>
<td>5.5</td>
</tr>
</tbody>
</table>

**Table 3.11 (part 1):** Mean T2 lesion load, T2 lesion number, and T1 hypointense lesion load over five years with standard deviation and percentage change from baseline. Values marked * * are significantly different from baseline at a level of p<0.001, Wilcoxon signed rank test.
<table>
<thead>
<tr>
<th></th>
<th>Ventricular volume</th>
<th></th>
<th>Brain volume</th>
<th></th>
<th>Cord area</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>ml</td>
<td>SD</td>
<td>Change</td>
<td>ml</td>
<td>SD</td>
</tr>
<tr>
<td>Baseline</td>
<td>16.5</td>
<td>13.0</td>
<td></td>
<td>271.8</td>
<td>20.8</td>
</tr>
<tr>
<td>One Year</td>
<td>17.7**</td>
<td>14.6</td>
<td>↑ 7.3%</td>
<td>268.2**</td>
<td>22.3</td>
</tr>
<tr>
<td>Two Years</td>
<td>19.9 **</td>
<td>15.4</td>
<td>↑ 20.6%</td>
<td>267.4**</td>
<td>23.4</td>
</tr>
<tr>
<td>Five Years</td>
<td>22.9 **</td>
<td>17.6</td>
<td>↑ 38.8%</td>
<td>262.2**</td>
<td>22.7</td>
</tr>
</tbody>
</table>

Table 3.11 (part 2): Ventricular volume, brain volume and cord area over five years with standard deviation and percentage change from baseline.

Values marked ** are significantly different from baseline at a level of p<0.001, Wilcoxon signed rank test.
3.6.4 Relation between clinical and MRI measures

Increasing disability was associated with changes in T2 load, ventricular volume and cord area (Table 3.12). Deterioration in MSFC was associated with increases in total T2 load ($r=0.31$, $p=0.038$) and ventricular volume ($r=0.31$, $p=0.044$). Change in cord area was the only measure to be associated with change in EDSS ($r=0.31$, $p=0.038$). Of MSFC components, worsening timed walk ($r=0.34$, $p=0.022$) was significantly associated with change in T2 load and change in PASAT score was associated with decrease in brain volume ($r=0.31$, $p=0.041$) and increase in ventricular volume ($r=0.36$, $p=0.020$). No clinical measure was found to change in association with increasing T1 load (see Table 3.12). No difference in rates was found when the cohort was subdivided into a clinically stable (no change in EDSS, $n=14$) and clinically deteriorating (worsening EDSS of 0.5 point or more, $n=27$) groups.

Higher rates of T2 load increase were associated with younger age at onset ($r=0.30$, $p=0.047$), shorter disease duration ($r=0.30$, $p=0.042$), higher baseline EDSS score ($r=-0.35$, $p=0.019$) and higher FS cerebellar scores ($r=0.37$, $p=0.013$). Patients with greater increases of T1 hypointensity load had higher baseline cerebellar and brainstem FS scores ($r=0.54$ and $p<0.001$, $r=0.41$ and $p=0.005$ respectively) and lower baseline PASAT score ($r=0.37$, $p=0.013$) but there was no association with the EDSS.

Greater rates of reduction in brain volume were associated with longer disease duration ($r=0.30$, $p=0.044$), higher baseline cerebellar FS scores ($r=0.30$, $p=0.043$), higher baseline EDSS ($r=0.43$, $p=0.002$), slower baseline timed walk score ($r=0.336$, $p=0.013$).
p=0.021) and higher baseline MSFC (r=-0.336, p=0.021). Higher rates of ventricular enlargement were associated with higher cerebellar and brainstem FS scores at baseline (r=0.46 & r=0.631, p=0.002 & p<0.001 respectively) and also with higher PASAT and peg test scores (r=0.51 & r=0.33, p<0.001 & p=0.032 respectively). Greater rate of reduction in cord area was also associated with younger age (r=0.42, p=0.004) and higher baseline FS sensory score (r=0.31, p=0.039).

No association was found between new lesion number over five years and any clinical measure.
<table>
<thead>
<tr>
<th></th>
<th>EDSS</th>
<th></th>
<th>MSFC</th>
<th></th>
<th>TTMW</th>
<th></th>
<th>9-HPT</th>
<th></th>
<th>PASAT 3</th>
<th></th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>R</td>
<td>p</td>
<td>R</td>
<td>p</td>
<td>R</td>
<td>p</td>
<td>r</td>
<td>P</td>
<td>r</td>
<td>P</td>
</tr>
<tr>
<td>T2 load</td>
<td>0.22</td>
<td>0.145</td>
<td>0.31*</td>
<td>0.038</td>
<td>-0.34*</td>
<td>0.022</td>
<td>0.029</td>
<td>0.853</td>
<td>0.060</td>
<td>0.654</td>
</tr>
<tr>
<td>T1 hypointensity load</td>
<td>0.22</td>
<td>0.153</td>
<td>0.05</td>
<td>0.740</td>
<td>-1.11</td>
<td>0.473</td>
<td>0.52</td>
<td>0.740</td>
<td>-0.003</td>
<td>.982</td>
</tr>
<tr>
<td>Ventricular volume</td>
<td>0.19</td>
<td>0.235</td>
<td>0.31*</td>
<td>0.044</td>
<td>0.150</td>
<td>0.344</td>
<td>0.145</td>
<td>0.360</td>
<td>0.36*</td>
<td>0.020</td>
</tr>
<tr>
<td>Brain volume</td>
<td>0.12</td>
<td>0.405</td>
<td>0.31*</td>
<td>0.041</td>
<td>0.245</td>
<td>0.105</td>
<td>0.222</td>
<td>0.143</td>
<td>0.31*</td>
<td>0.041</td>
</tr>
<tr>
<td>Cord area</td>
<td>-0.31*</td>
<td>0.038</td>
<td>-0.03</td>
<td>0.845</td>
<td>-0.150</td>
<td>0.344</td>
<td>0.080</td>
<td>0.608</td>
<td>-0.030</td>
<td>0.848*</td>
</tr>
</tbody>
</table>

**Table 3.12:** Associations between change in clinical and MR measures. Values marked * are significantly different from baseline at a level of p<0.05 using the Wilcoxon signed rank test.
3.6.5 Relation between MR measures

Cross sectional associations between T2 load, T1 hypointensity load, six slice brain volume and cord area have already been reported for the original cohort at baseline [5]. Larger ventricular volume was associated cross-sectionally with male sex (r=0.36, p=0.015), higher cerebellar FS score (r=0.31, p=0.040) and poorer performance on PASAT (r=0.34, p=0.023). The cross sectional MR associations of larger ventricular size were with high T2 load (r=0.48, p<0.001), high T1 hypointensity load (r=0.51, p<0.001) and smaller partial brain volume (r=0.46, p=0.001).

The strongest association between changes in MR measures was between T1 hypointensity and T2 lesion load (r=0.7, p<0.001) (Table 3.13). Changes in cord volumes appeared to be independent of other MR parameters. Ventricular volume change correlated with partial brain volume change but did not correlate with change in any other MR parameter. The association between baseline MR measures and subsequent MR change is shown in Table 3.14. Baseline T2 load is the measure that shows most correlation with subsequent change in other MR measures, with the exception of change in cord volume.
<table>
<thead>
<tr>
<th></th>
<th>$\Delta$T2 load</th>
<th>$\Delta$T1 hypointensity</th>
<th>$\Delta$partial brain volume</th>
<th>$\Delta$ventricular volume</th>
<th>$\Delta$cord area</th>
</tr>
</thead>
<tbody>
<tr>
<td>$\Delta$T1 hypointensity</td>
<td>$r=0.668$ p=0.000**</td>
<td>$r=0.359$ p=0.017*</td>
<td>$r=0.183$ p=0.251</td>
<td>$r=-0.125$ p=0.420</td>
<td></td>
</tr>
<tr>
<td>$\Delta$partial brain volume</td>
<td>$r=0.259$ p=0.696</td>
<td>$r=0.217$ p=0.173</td>
<td>$r=-0.091$ p=0.556</td>
<td></td>
<td></td>
</tr>
<tr>
<td>$\Delta$ventricular volume</td>
<td>$r=0.663$ p=0.000**</td>
<td>$r=0.018$ p=0.000**</td>
<td>$r=0.007$ p=0.967</td>
<td></td>
<td></td>
</tr>
</tbody>
</table>

** correlation is significant at the 0.01 level
* correlation is significant at the 0.05 level

**Table 3.13:** Associations between change in MR measures over five years.
<table>
<thead>
<tr>
<th></th>
<th>ΔT2 load</th>
<th>ΔT1 hypointensity</th>
<th>Δpartial brain volume</th>
<th>Δventricular volume</th>
<th>Δcord area</th>
</tr>
</thead>
<tbody>
<tr>
<td>Baseline T2 load</td>
<td>r=0.410 p=0.006**</td>
<td>r=0.529 p=0.000**</td>
<td>r=0.391 p=0.009**</td>
<td>r=0.508 p=0.001**</td>
<td>r=0.040 p=0.795</td>
</tr>
<tr>
<td>Baseline T1 hypointensity</td>
<td>r=0.194 p=0.207</td>
<td>r=0.451 p=0.002**</td>
<td>r=0.464 p=0.001**</td>
<td>r=0.687 p=0.000**</td>
<td>r=0.037 p=0.810</td>
</tr>
<tr>
<td>Baseline partial brain volume</td>
<td>r=0.197 p=0.200</td>
<td>r=0.285 p=0.060</td>
<td>r=0.204 p=0.162</td>
<td>r=0.345 p=0.020*</td>
<td>r=0.065 p=0.666</td>
</tr>
<tr>
<td>Baseline ventricular volume</td>
<td>r=0.150 p=0.054</td>
<td>r=0.207 p=0.183</td>
<td>r=0.521 p=0.001**</td>
<td>r=0.547 p=0.000**</td>
<td>r=0.060 p=0.697</td>
</tr>
<tr>
<td>Baseline cord area</td>
<td>r=0.292 p=0.054</td>
<td>r=0.206 p=0.181</td>
<td>r=0.023 p=0.879</td>
<td>r=0.689 p=0.662</td>
<td>r=0.173 p=0.236</td>
</tr>
</tbody>
</table>

** correlation is significant at the 0.01 level
* correlation is significant at the 0.05 level

Table 3.14: Associations between change in MR measures over five years and baseline
3.6.6 Discussion

In contrast to the two year data, the five year study demonstrates a relationship between clinical deterioration and change in MR measures in PPMS. Three important questions arise; how strong is this relationship, is it clinically meaningful, and what are the implications, if any, for disease monitoring in patients with PPMS? Before discussing these points in detail, it is important to bear in mind a number of limitations regarding the interpretation of this study. Patients were recruited from a hospital population and were unselected for disease duration. Despite best efforts, follow up was incomplete and non-attending patients differed from attending patients in having higher disability at both baseline and five years. This might limit the ability of the study to detect MRI changes associated with higher disability. Furthermore, the absence of a control group limits the ability of the study to determine whether subtle changes in MR measures are due to disease effects or aging.

In this study, there were three MR changes that correlated with clinical change; change in T2 load correlated with change in MSFC ($r=0.31$), while change in cord area correlated with change in EDSS ($r=0.31$) and change in brain atrophy correlated with change in PASAT score ($r=0.31$ and $r=0.36$). No relationship was found with change in T1 load, despite the largest relative changes over five years being seen in this measure. That a stronger correlation is not seen is perhaps not too surprising; most clinical scales in MS are relatively insensitive to clinical change [26] and, as discussed in the introduction to this chapter, conventional MR measures have limited...
histo-pathological specificity [128]. Furthermore, there may not be a direct temporal relationship between MR change and clinical change. Pathological changes occurring at one stage of the condition (with resulting changes in MR appearances) might conceivably have a clinical impact that only becomes apparent at a later stage. As such there may be periods when changes in MR would not associate strongly with clinical change (despite a true relationship). An example of this is provided by relapsing-remitting MS where T2 load in the earliest clinical stages of the disease correlated with long term clinical evolution [129].

How do the results of this study build on the results of earlier MRI studies in PPMS? Firstly, they confirm that changes in MRI measures can easily be detected in patients affected by PPMS over time. More importantly, they show that some relation between clinical and MRI change can be found but that it is necessary to wait for several years to elapse in order to allow for a sufficiently great degree of clinical progression to occur to make this relationship obvious. Specifically, whereas at one year only 25% of patients had shown a significant deterioration in EDSS [43], at five years this figure rose to 65%. These figures are consistent with the probabilities of progression for PPMS as calculated for patients in the London, Ontario natural history cohort [130].

This study also provides new information on the dynamics of MR change in patients with PPMS, and illustrates how these vary both between and within individuals over time. PPMS is considered to have a universally poor clinical outcome but the rate of clinical deterioration in this cohort over five years varied greatly. This variability is also seen in the MR measures, as seen in figure 3.2, with some patients showing large
amounts of change over the study period and others much less. In contrast, the rate of change in individual patients appears relatively constant over time. This relative consistency of both clinical and MR behaviour in PPMS within patients over time with variability in outcome between patients, suggests differences in underlying mechanisms of disease and repair between patients that would also seem to be relatively constant over time. Such constancy raises the possibility that useful prognostic information could be gained from the early assessment of the rate of MR change in PPMS and allow early identification of those patients at greatest risk of poor outcome. The question of whether the rate of change of MRI measures is constant at all stages of the condition will be considered again in later chapters where these same measures will be considered in a cohort of patients selected for early disease duration of five years or less.

Although both MR and clinical changes may be relatively consistent over time their relationship may not be straightforward and it is probably too simplistic to infer that patients with the greatest change on MR will always show the greatest clinical change. In this study, there is a wide variation in rate of increase of T1 hypointensity but no association with the rate of clinical progression. One could hypothesise that change in some MR measures, or more properly the underlying pathological processes which they imply, may be essentially continuous throughout the course of the disease but only cause disability at certain critical periods, or after certain threshold points have been reached. At a pathological level it has been suggested that such a threshold effect may operate with respect to axonal loss [131]. It will be seen from Figure3.2 that patients can have identical lesion loads at a single point in time and yet differ in terms
of their rate of change of MR abnormality. This study suggests that in certain cases it is the change over time of an MR variable, rather than its absolute value, that is the important measure. The predictive value of short term change measures in the longer term has, however, yet to be demonstrated.

Treatment trials in PPMS have to date used progression of disability as their primary outcome measure. However, as clinical evolution can be slow and its assessment difficult, there has been interest in identifying MRI outcome measures as so-called "surrogate markers". MRI measures have the advantage of being objective, sensitive and quantitative but to be useful as surrogates it needs to be shown that they accurately reflect differences in clinical behaviour between patients. It has been suggested that such measures need to be validated: the measure should predict future clinical disease, the effect of treatment on clinical disease must be explained by the effect on the surrogate and the surrogate should not be restricted to use with a single treatment but should ideally respond in a similar and predictable manner to all effective treatments [119]. This natural history study supports the view that conventional MR measures relate to clinical status and suggests that atrophy measures may provide additional information. While T2 load and brain atrophy measures are somewhat correlated, cord atrophy does not correlate with other MR changes. It therefore appears to be providing independent information suggesting that cord atrophy measures should be included in MR studies in this group. Alternative cord measures such as quantification of signal abnormality on T2 weighted MRI are poor markers of pathological change [132] Validation of the other criteria will need to await the development of effective treatments in full scale clinical trials. At present it
seems reasonable to expect that treatments which reduce the rate of increase of T2 load, ventricular volume and cord atrophy in the shorter term would, in the longer term, be shown to have clinical benefit.
Figure 3.1: Histograms showing EDSS of patients by number of patients at each EDSS point at a) baseline and b) five years

Figure 3.2a: T2 load (in ml) by patient over five years shown with reference to point in disease course as reflected in years since onset of first symptoms

Figure 3.2b: T1 load (in ml) by patient over five years shown with reference to point in disease course as reflected in years since onset of first symptoms

Figure 3.2c: Ventricular volume (in ml) by patient over five years shown with reference to point in disease course as reflected in years since onset of first symptoms

Figure 3.2d: Partial brain volume (in ml) by patient over five years shown with reference to point in disease course as reflected in years since onset of first symptoms

Figure 3.2e: Cervical cord area (in mm²) by patient over five years shown with reference to point in disease course as reflected in years since onset of first symptoms

Figure 3.2f: EDSS by patient over five years shown with reference to point in disease course as reflected in years since onset of first symptoms
Figure 3.1
Figure 3.2
Chapter 4

Conventional MRI abnormalities in early PPMS
4.0 Introduction: Clinical and MRI variability in PPMS

Chapter 3 examined longitudinal changes in conventional MRI measures in PPMS together with their relationship to clinical change in a cohort of patients unselected for disease duration and with individual clinical histories of between 3 and 34 years. There were two key observations. Firstly, that over extended periods a relationship can be shown between MRI and clinical change. Secondly, that change in MRI measures was relatively constant for a given individual over time but varied considerably between individuals. The second observation may offer an insight into the nature and dynamics of the disease process in PPMS and this will be explored further in this chapter using a second cohort of patients with PPMS recruited within five years of their first symptoms.

Chapter 1 introduced a number of studies that have examined conventional MRI abnormalities in PPMS [3;35;40-42]. A common feature of these studies, and one which has received relatively little attention, is the wide range of quantitative MRI abnormality that is observed among individuals [133]. For example, in the study of Fillipi, total T2 load in the PPMS group ranged from 3 ml to 121 ml, while in the study of Nijeholt total T2 load ranged from 0.4 ml to 32.1 ml, while total T1 hypointensity load ranged from 0 ml to 11.5 ml [3;4]. Therefore, although the degree of MRI abnormality in PPMS is on average less than in other MS subtypes, low levels of MRI abnormality are not universally found and the overall spectrum of abnormality is wide.
MRI abnormalities such as lesion load are known to show progressive change in PPMS over time [43]. A simplified, schematic representation of some of the ways in which cross sectional differences in a progressive MRI abnormality could occur is shown in Figure 4.1. The simplest models, shown in Figure 4.1b and 4.1c, assume a fixed rate of accrual of MRI abnormality. They imply that given sufficient time all patients will ultimately show the same degree of abnormality. However, these models do not fit the observed dynamic changes as described in Chapter 3. These are shown in a simplified version in Figure 4.2a. Therefore, the models shown in Figure 4.1c and 4.1d appear closer to the observed situation. The key feature of these models is that cross-sectional variation in MRI measures reflects a more fundamental variation in the rate at which MRI abnormality accrues and, by extension, the rate at which the disease process progresses between individuals.

The factors responsible for differences in the rate of MRI “progression” between individuals are unknown. It is also not known whether the features of MRI progression described in Chapter 3 are present in the earliest years of the condition or develop later (Figure 4.2b). This is potentially an important question in that if it is the case that disease course is set early on then it may be possible to anticipate clinical outcomes at or near to the time of presentation, if appropriate early disease markers can be identified. Alternatively, if disease course is not set in the early clinical years, but is somehow determined during this period, there may be an opportunity for outcomes to be modified by early therapeutic intervention. As a first step to addressing this question the study described in this chapter examines conventional MRI appearances in a cohort of patients in the earliest clinical stages of PPMS.
Figure 4.1: Cross sectional variation in the degree of a MRI abnormality, known to increase linearly over time, as observed in three individuals at a single timepoint is shown, (a). The observed variation seen in (a) could arise through the affected individuals being at different stages of a common trajectory (b). Alternatively, trajectories themselves could differ between individuals with differences in the stage reached on each individual trajectory introducing a further source of variation (c). If MRI abnormalities first appear at variable points in the overall disease process (i.e. individual trajectories do not necessarily originate at zero) this is a further potential source of variation (d) & (e).
Figure 4.2: (a) A simplified version of the changes in MRI measures, based on the changes actually observed in the cohort of patients described in Chapter 3, is shown for three individuals emphasising that patients with PPMS differ not only in their degree of cross sectional abnormality but also in the rate at which abnormality increases over time (b) The rate of MRI progression was seen to be relatively constant for a given individual in the longitudinal study described in Chapter 3. It is not known whether this is also true in the earliest clinical stages of the condition or whether significant inter-individual variation in conventional MRI measures is also present at this time.
4.1 Methods

Forty-two patients with PPMS with disease duration of five years or less were identified by referrals to Professor Thompson and by a direct appeal to local neurologists. They were asked to take part in a three-year MRI and clinical study involving three, monthly, gadolinium enhanced scans at baseline (see next chapter) and subsequently six monthly examinations. Confirmation that patients were within five years of first symptom onset was determined from clinical records and by interview. In all cases patients had a clinical diagnosis of PPMS. According to recent diagnostic criteria for PPMS [90] at baseline, 40 patients (95%) had definite PPMS and two had probable PPMS.

Clinical examination consisted of neurological examination with calculation of EDSS. Nine hole peg test, timed 25 foot walk and PASAT were also measured and used to calculate the Multiple Sclerosis Functional Composite (MSFC) (Chapter 2) [50].

The data presented in this chapter make use of the conventional MRI imaging of brain and spinal cord carried out at baseline and at again at six months. All sequences were acquired as contiguous, 5 mm thick axial slices (T2; TR 3000 ms, TE 15/90 ms. T1; TR 600 ms, TE 20 ms). Cerebral lesion loads were quantified together with partial brain volume, ventricular volume and spinal cord area using the methods described in Chapter 2. Lesions were identified by a single, experienced rater (Professor DH Miller) and image analysis was carried out at a SUN workstation using a local thresholding
technique with manual editing. T2 and T1 hypointensity volumes were calculated with reference to marked hard copies.

4.2 Statistics

Non-parametric tests of statistical significance were used throughout. Cross-sectional comparisons between groups were carried out using the Mann-Whitney U-test and changes in MRI and clinical measures over time were assessed using the Wilcoxon signed rank test. Correlations were assessed using Spearman's rank correlation coefficient.

4.3 Results

4.3.1 Demographic and disease characteristics

Demographic and clinical characteristics of this cohort of patients with early PPMS are shown in Table 4.1. For comparison, demographic and clinical characteristics for the cohort of patients with PPMS unselected for disease duration described in Chapter 3 are also included. It will be seen that the main differences between the cohorts are in their average disease duration, 3.2 years versus 16.3 years, and in the average age of subjects, 44.9 versus 55.7 years. Although, the cohort of patients with early PPMS has lower levels of disability than the unselected PPMS cohort (e.g. median EDSS 4.5 versus median EDSS 6.0) a wide range of disability is nevertheless present with EDSS ranging from 1.5 to 7.0. Based on a comparison of the normalized Z scores for
each MSFC component the greatest clinical difference between these two cohorts was in their level of motor disability.

<table>
<thead>
<tr>
<th></th>
<th>average</th>
<th>SD</th>
</tr>
</thead>
<tbody>
<tr>
<td><strong>Age</strong></td>
<td>44.9 years</td>
<td>11.0 years</td>
</tr>
<tr>
<td><strong>Sex</strong></td>
<td>30 male 20 female</td>
<td></td>
</tr>
<tr>
<td><strong>Disease duration</strong></td>
<td>3.2 years</td>
<td>0.9 years</td>
</tr>
<tr>
<td><strong>EDSS</strong></td>
<td>4.5</td>
<td>1.5 -7.0</td>
</tr>
<tr>
<td><strong>MSFC</strong></td>
<td>-0.8</td>
<td>1.4</td>
</tr>
<tr>
<td><strong>Z score NHPT</strong></td>
<td>-0.9</td>
<td>1.2</td>
</tr>
<tr>
<td><strong>Z score TMW</strong></td>
<td>1.1</td>
<td>3.5</td>
</tr>
<tr>
<td><strong>Z score PASAT</strong></td>
<td>-0.3</td>
<td>1.3</td>
</tr>
</tbody>
</table>

**Table 4.1:** Demographic and clinical characteristics of 50 patients with early PPMS at baseline.
4.3.2 Conventional MRI characteristics

Summary measures of conventional MRI characteristics for the early PPMS cohort are shown in Table 4.2. The range of conventional MRI abnormality present was wide and this is shown by means of histograms in Figure 4.3.

Any direct comparison with these same measures as found in the cohort of patients unselected for disease duration described in Chapter 3 has to be done with caution as MRI acquisition parameters (with respect to both relaxation and echo times and slice thickness) differed significantly between the two studies (with the exception of the sequence from which cord area is calculated). With this caveat, an average T2 load of 15.1ml in the early cohort compares with an average T2 load of 10.1ml in the unselected cohort at baseline and 15.0ml in this cohort at 5 years. Similarly with respect to T1 hypointensity load an average T1 hypointensity load of 2.0 ml at baseline and 3.8 ml at five years in the cohort unselected for disease duration compares with an average T1 hypointensity load of 4.8ml in the early cohort.

From Figure 4.3 it can be seen that the distribution of both T2 and T1 lesion loads is heavily skewed with the majority of patients having low lesion loads and a few patients having very high values. This may mean that the mean value for these measures is a misleading description of the cohort as a whole. If median values are considered instead, a median T2 load of 5.2ml in the unselected cohort (rising to 8.4 ml at 5 years) compares with a median T2 load of 6.8ml in the early cohort. With respect to T1 hypointensity load, a median value of 0.6ml rising to 1.9ml at five years
compares with a median T1 load of 1.2ml in the early cohort. The median ratio of T2 to T1 hypointensity load was 4.1:1. This compares with a median ratio of 6.4:1 in the cohort unselected for disease duration.

<table>
<thead>
<tr>
<th></th>
<th>average</th>
<th>SD</th>
</tr>
</thead>
<tbody>
<tr>
<td>T2 load</td>
<td>15.1ml</td>
<td>22.0ml</td>
</tr>
<tr>
<td>T1 load</td>
<td>4.8ml</td>
<td>9.4ml</td>
</tr>
<tr>
<td>Partial Brain Volume</td>
<td>260.4ml</td>
<td>23.5ml</td>
</tr>
<tr>
<td>Ventricular Volume</td>
<td>23.0ml</td>
<td>17.4ml</td>
</tr>
<tr>
<td>Cord area</td>
<td>73.5mm²</td>
<td>8.6 mm²</td>
</tr>
</tbody>
</table>

**Table 4.2:** Conventional MRI characteristics of patients with early PPMS at baseline.
Figure 4.3: Histograms showing the range of values obtained for the MRI indices T2 load (A), T1 hypointensity load (B), partial brain volume (C), ventricular volume (D) and cord area (E) in a cohort of patients with early PPMS.
In terms of atrophy measures, the mean ventricular and partial brain volumes of 23.0 ml and 260.4 ml respectively in the early cohort compare with values of 16.5ml and 271.8ml respectively in the unselected cohort at baseline and 22.9ml and 262.2 ml respectively at five years. Mean partial brain volume in an age and sex matched population of control subjects was 272.1ml (ns, early cohort). For cord area, where identical MRI acquisitions were used in both cohorts, a mean cord area of 73.5 mm² in the early cohort compared to a mean cord area of 71.4 mm² at baseline in the cohort unselected for disease duration (ns) and 64.0 mm² at five years (P<0.001). Mean cord area in both cohorts was less than in an age and sex matched population of control subjects (79.6 mm²; SD 7.5 mm², p=0.007 (early cohort) p=0.001 (unselected cohort)).

4.3.3 Conventional MRI abnormalities and clinical measure in early PPMS

The relation between conventional MRI abnormalities and clinical and demographic measures in this early cohort is shown in Table 4.3. This analysis is regarded as exploratory and no correction was therefore made for multiple comparisons. The strongest associations (r=0.6) were seen for lesion load measures. Higher T2 and T1 hypointensity loads and larger ventricular volumes were associated with higher EDSS scores and higher T2 and T1 hypointensity loads and smaller partial brain volumes were associated with more negative (i.e. more abnormal) MSFC scores. A trend to higher T2 lesion load in younger patients was also seen.
Table 4.3: Correlation between clinical and demographic measures and conventional MRI measures for patients with early PPMS.

<table>
<thead>
<tr>
<th>Measure</th>
<th>T2 load</th>
<th>T1 load</th>
<th>Partial brain volume</th>
<th>Ventricular volume</th>
<th>Cord volume</th>
</tr>
</thead>
<tbody>
<tr>
<td>Age</td>
<td>r=-0.293 p=0.041*</td>
<td>r=-0.128 p=0.352</td>
<td>r=-0.225 p=0.120</td>
<td>r=-0.108 p=0.960</td>
<td>r=0.001 p=0.960</td>
</tr>
<tr>
<td>Disease duration</td>
<td>r=-0.098 p=0.503</td>
<td>r=-0.233 p=0.108</td>
<td>r=-0.093 p=0.552</td>
<td>r=0.106 p=0.497</td>
<td>r=0.093 p=0.552</td>
</tr>
<tr>
<td>EDSS</td>
<td>r=0.352 p=0.013*</td>
<td>r=0.403 p=0.004**</td>
<td>r=-0.241 p=0.096</td>
<td>r=0.408 p=0.007**</td>
<td>r=-0.267 p=0.084</td>
</tr>
<tr>
<td>MSFC</td>
<td>r=-0.613 p&lt;0.001**</td>
<td>r=-0.512 p=0.002**</td>
<td>r=0.309 p=0.031*</td>
<td>r=-0.213 p=0.171</td>
<td>r=0.179 p=0.250</td>
</tr>
<tr>
<td>Z score NHPT</td>
<td>r=-0.559 p&lt;0.001**</td>
<td>r=-0.496 p&lt;0.001**</td>
<td>r=0.003 p=0.983</td>
<td>r=-0.471 p=0.001**</td>
<td>r=0.109 p=0.487</td>
</tr>
<tr>
<td>Z score TMW</td>
<td>r=0.404 p=0.004**</td>
<td>r=0.410 p=0.003**</td>
<td>r=-0.318 p=0.026*</td>
<td>r=0.046 p=0.772</td>
<td>r=-0.180 p=0.248</td>
</tr>
<tr>
<td>Z score PASAT</td>
<td>r=-0.416 p=0.001**</td>
<td>r=-0.414 p=0.003**</td>
<td>r=0.179 p=0.220</td>
<td>r=-0.200 p=0.197</td>
<td>r=0.015 p=0.925</td>
</tr>
</tbody>
</table>

**p<0.01 *p<0.05
In comparison, in the cohort of patients unselected for disease duration, no cross-sectional association was seen for either EDSS or MSFC with any MRI conventional measure, although associations were seen between T2 load and Z scores for nine hole peg test and PASAT ($r=-0.359$, $p=0.016$; $r=-0.317$, $p=0.034$, respectively), between T1 hypointensity load and PASAT Z score ($r=0.346$, $p=0.017$), and between cord area and nine hole peg test Z score ($r=0.324$, $p=0.026$). In contrast to the early cohort where no associations were seen with disease duration, in the cohort of patients unselected for disease duration there was a trend towards smaller cord area and longer disease duration ($r=-0.375$, $p=0.010$).

### 4.3.4 Relations between conventional MRI abnormalities

Cross-sectional associations between the MRI measures are shown for the early cohort in Table 4.4. The same correlations for the cohort of patients unselected for disease duration described in Chapter 3 are also shown in this table for comparison. The associations between T2 load and T1 hypointensity load and between higher lesion loads and greater ventricular volume are present in both cohorts. In both cases the association between lesion load measures is strong ($r=0.9$, 0.8) and the association with ventricular volume is slightly stronger with T1 hypointensity than T2 load. An association between lesion load and partial brain volume, seen in the cohort of patients unselected for disease duration, is not found in the early cohort. In neither cohort is any association found between spinal cord area and any cerebral measure.
Table 4.4: Associations between conventional MRI measures in patients with early PPMS

<table>
<thead>
<tr>
<th></th>
<th>T1 hypointensity</th>
<th>partial brain volume</th>
<th>ventricular volume</th>
<th>cord area</th>
</tr>
</thead>
<tbody>
<tr>
<td><strong>T2 load</strong></td>
<td><strong>r=0.938, p&lt;0.001</strong></td>
<td><strong>r=-0.149, p=0.314</strong></td>
<td><strong>r=0.399, p=0.009</strong></td>
<td><strong>r=0.002, p=0.991</strong></td>
</tr>
<tr>
<td>T1 hypointensity</td>
<td><strong>r=-0.207, p=0.154</strong></td>
<td><strong>r=0.468, p=0.002</strong></td>
<td><strong>r=-0.067, p=0.675</strong></td>
<td></td>
</tr>
<tr>
<td>partial brain volume</td>
<td><strong>r=0.111, p=0.485</strong></td>
<td></td>
<td><strong>r=0.244, p=0.120</strong></td>
<td></td>
</tr>
<tr>
<td>ventricular volume</td>
<td></td>
<td></td>
<td><strong>r=-0.151, p=0.340</strong></td>
<td></td>
</tr>
</tbody>
</table>

** correlation is significant at the 0.01 level
* correlation is significant at the 0.05 level
4.3.5 Change in MRI measures over six months in early PPMS

The change in conventional abnormalities in 40 patients over six months is shown in Table 4.5. Over this short period the only measures that showed statistically significant changes were T2 lesion load and cord volume. Caution is needed when comparing these changes to those observed in the cohort of patients unselected for disease duration because of the much shorter period of observation (6 months versus 60 months) and smaller number of observations (two versus four). With this caveat, the median change in T2 load of 2.8 ml (range -5.0-15.8 ml) over 6 months was comparable to that seen over 6 months (average value calculated from four observations over five years) in the cohort of patients unselected for disease duration (2.9 ml (0-29.5ml)). However, the median change in cord volume over six months in the early cohort at 1.9 mm$^2$ (range -0.3- 11.5mm$^2$) was approximately double the 0.8 mm$^2$ average 6 monthly change seen over five years in the cohort unselected for disease duration. No significant changes in disability measures were found over the six-month period and no association between clinical and MRI change.
<table>
<thead>
<tr>
<th>Measure</th>
<th>Month</th>
<th>Mean</th>
<th>SD</th>
</tr>
</thead>
<tbody>
<tr>
<td>T2 load (ml)</td>
<td>0</td>
<td>14.0</td>
<td>21.6</td>
</tr>
<tr>
<td></td>
<td>6</td>
<td>15.3 *</td>
<td>22.4</td>
</tr>
<tr>
<td>T1 load (ml)</td>
<td>0</td>
<td>4.9</td>
<td>9.9</td>
</tr>
<tr>
<td></td>
<td>6</td>
<td>4.5</td>
<td>9.0</td>
</tr>
<tr>
<td>Partial brain volume (ml)</td>
<td>0</td>
<td>257.7</td>
<td>23.7</td>
</tr>
<tr>
<td></td>
<td>6</td>
<td>257.4</td>
<td>25.0</td>
</tr>
<tr>
<td>Ventricular volume (ml)</td>
<td>0</td>
<td>19.4</td>
<td>12.5</td>
</tr>
<tr>
<td></td>
<td>6</td>
<td>20.1</td>
<td>13.1</td>
</tr>
<tr>
<td>Cord Area (ml)</td>
<td>0</td>
<td>73.5</td>
<td>9.1</td>
</tr>
<tr>
<td></td>
<td>6</td>
<td>70.9 **</td>
<td>8.2</td>
</tr>
</tbody>
</table>

*p<0.05, **p<0.01

**Table 4.5** Changes in conventional MRI measures over six months.
4.4 Discussion

This chapter describes the nature of conventional MRI abnormalities in a population of patients with PPMS with similar disease durations, early in the clinical course of their illness. Only one other study to date has specifically examined patients with clinically early PPMS [57]. In this study grey matter atrophy was found to be present in patients with PPMS with a short history, even when the clinical history was five years or less. This extends the characterization of early PPMS by demonstrating that, even at this early stage, patients can vary substantially in their clinical state and their degree of conventional MRI abnormality. In other words, much of the variation that is seen between individuals with established disease is already present at the point where symptoms first appear and patients make their first clinical presentation. Assuming that conventional MRI measures do have some relation to the underlying disease process [128], this variation suggests that important aspects of the disease course in an affected individual are also determined at an early stage.

Not only is variation in conventional MRI abnormality present at this early stage, but the degree of MRI abnormality present can be considerable. This implies that, at least in some cases, the disease process is active for some time before clinical symptoms first appear. This has been shown in at least one clinical case report where a patient with PPMS had abnormalities on an incidental MRI ten years before the onset of clinical symptoms [134]. This may turn out to be an important limitation on the ability of disease modifying treatments (which can only be started after the condition
manifests clinically) to alter disease outcomes, as significant damage may already have occurred at the point at which patients present.

In view of the generally held characterization of PPMS and SPMS as conditions with low and high levels of conventional MRI abnormality respectively [135] the question arises as to whether those patients with higher lesion loads included in this early PPMS study cohort were correctly classified. However, clinical histories in these patients were consistent with a diagnosis of PPMS and, as stated in the introduction, high lesion loads have been described for some patients with PPMS in all cross-sectional studies. If such patients are distinct from those with the “classical” conventional MRI appearances of PPMS, then this is not apparent on clinical grounds alone. One study to date has addressed the issue of whether patients with PPMS and high lesion loads might differ fundamentally from those without [133]. This study divided a cohort of patients with PPMS (unselected for disease duration) into “low” and “high” T2 load subgroups. These subgroups did differ in their clinical characteristics or in their degree of abnormality on a spectroscopic measure in normal appearing brain tissue.

In terms of the nature of conventional MRI abnormalities present in early clinical disease no unexpected features were seen. The appearance and distribution of lesions, the ratio between T2 and T1 lesion load, the relation between lesion load and atrophy measures and the absence of a relation between cord and brain measures all appeared similar to that seen in the PPMS cohort unselected for disease duration described in Chapter 3. This may mean that there is no pathological process that is unique to the
early clinical stages of the condition. However, as stated previously, conventional MRI measures are pathologically non-specific, and therefore caution is needed in interpreting them in these terms. However, one difference that does appear to be present between this early cohort and the established cohort described in Chapter 3 is that a stronger association is found between MRI and clinical measures. It may therefore be that the impact of disease on clinical state is more direct at this early stage before compensatory mechanisms have become established.

In conclusion, conventional MRI measures in early PPMS have shown much of the variation, both cross-sectionally and longitudinally that is seen in MRI abnormality in established PPMS cohorts. The presence of substantial MRI abnormality in some cases near to the time of clinical presentation argues strongly for an onset of a disease process that may predate the onset of clinical symptoms by many years. This process, on the basis of the conventional MRI evidence, seems to be more quantitative than qualitative and overall there are no particularly “early” features have been identified.

Given the pathological non-specificity of conventional MRI techniques, it is difficult to specify the precise nature of the pathological changes occurring at this time, such as the relative contribution of inflammation and neurodegeneration, including demyelination and axonal loss. To do this requires the use of more specific MRI techniques and in the next chapter we address the first, and possibly most important, of these: the nature and role of inflammation at this time as studied by the use of triple dose Gadolinium DTPA.
Chapter 5

The role of inflammation:

enhancement in early PPMS
5.1 Introduction

In Chapter 3 it was seen that long-term changes in MRI measures related, albeit weakly, to clinical change in PPMS. The serial studies described showed that there is considerable variation not only cross-sectionally in MRI measures but also in the rate at which MRI measures change over time between patients. In Chapter 4 there was evidence of cross-sectional variation in MRI measures even in the earliest clinical stages of the condition. It may be that processes at work in these early stages are important in setting the pattern of the later disease course. In this chapter the nature of inflammation in the early stages of PPMS, as assessed by MRI, is considered.

One of the characteristic MRI features of PPMS is the relative rarity of enhancement with Gd-DTPA on T1 weighted MRI (Chapter 1) [41;52;136]. Gd-DTPA is an MRI contrast agent that indicates areas of blood brain barrier breakdown and inflammation (Chapter 2) [98;113;114]. With triple dose Gd-DTPA, it may be possible to detect more enhancing lesions in PPMS, but the overall frequency of enhancement is still less than in other subtypes of MS [54;137]. In RRMS, enhancing lesions have a significant association with clinical relapse [117]. In PPMS, the clinical significance of enhancement is less clear. MRI evidence suggesting a lesser role for inflammation in PPMS is supported by pathological studies [17]. This may help explain why treatment trials in PPMS with agents known to suppress inflammation, such as interferon beta and glatiramer acetate, have given negative results [125;138].
Based on existing studies, inflammation would appear to play a relatively minor role in PPMS. In RRMS, there is evidence that the predominant pathological process that operates early in the condition differs from those that occur later; in early phases, inflammation is predominant; in later phases, degeneration is more important [139]. If a similar pattern were to exist in PPMS this would potentially have important implications for treatment. The study described in this chapter aims to assess the presence, characteristics and consequences of inflammation in the early stages of PPMS in a cohort of patients seen within five years of symptom onset. Specifically, the following questions will be addressed:

- Is there more enhancement in early PPMS than has been reported for established disease?
- How do enhancing lesions evolve?
- Do PPMS patients with enhancement differ from those without?
- Do the clinical and MR associations of enhancement provide any insights into the pathological role of inflammation in PPMS?
5.2 Methods

Patients with PPMS with disease duration of five years or less were studied. They were asked to take part in a three year MRI and clinical study involving three, monthly scans at baseline and subsequently six monthly examinations. As described earlier, confirmation that patients were within five years of first symptom onset was determined from clinical records and by interview. In all cases, patients had a clinical diagnosis of PPMS. According to recent diagnostic criteria for PPMS [5] at baseline, 40 patients (95%) had definite PPMS and two had probable PPMS.

Clinical examination consisted of neurological examination with calculation of EDSS. Nine hole peg test, timed 25 foot walk and PASAT were also measured and used to calculate the Multiple Sclerosis Functional Composite (MSFC) (Chapter 2) [50].

Imaging of brain and spinal cord was carried out on three occasions at 0, 1 and 2 months and again at a follow up visit at 6 months. Cerebral lesion loads were quantified together with partial brain and ventricular volume using the methods described in Chapter 2. The same scanner was used throughout the study. Each patient underwent T1 imaging of the brain and cord before and immediately after administration of triple dose Gd-DTPA at a dose of 0.3 mmol per kg. T2 weighted spin echo imaging of the brain (T2: TR 3000 ms, TE 15/90 ms; T1: TR 600 ms, TE 20 ms) was also carried out after Gd-DTPA administration. All sequences were acquired as contiguous, 5 mm thick axial slices. All enhancing lesions were identified by a single experienced rater (Professor DH Miller). On follow-up scans lesions were
classified as new (first appearance) or persistent (seen on previous scan). Image analysis was carried out at a SUN workstation using a local thresholding technique with manual editing. T2 and T1 hypointensity volumes were calculated with reference to marked hard copies.

5.3 Statistics

Non-parametric tests of statistical significance were used throughout. Cross-sectional comparisons between groups were carried out using the Mann-Whitney U-test and changes in MRI and clinical measures over time were assessed using the Wilcoxon signed rank test. Correlations were assessed using Spearman's rank correlation coefficient.

5.4 Results

Forty two patients were enrolled into the study and, of these, 23 agreed to attend for three scans at 0, 1 and 2 months. Follow up data on 36 patients was available at six months (mean 6.1 months, SD 1.2 months). For six patients no follow up data was available. This was because of death from non-MS related cause (one case), dislike of MRI (one case), intercurrent non-MS related surgical illness (one case), personal reasons (two cases) and non-residency in UK (one case).
5.4.1 Baseline: Clinical

The 42 patients studied at baseline consisted of 24 males and 18 females with an age range of 22 to 65 years. Mean age was 45.1 years (SD 10.8) and mean disease duration 3.3 years (SD 0.9 years). Thirty four of the 42 cases presented with a cord syndrome (81%). The median EDSS was 4.5 (range 3.0 to 7.0) and the mean MSFC was -0.8 (SD 1.5). EDSS or MSFC (EDSS and MSFC were highly correlated, r=0.7, p<0.001). Younger patients had shorter disease duration (r=0.5, p=0.001). Patients with cord and non-cord presentations did not differ significantly in age, disease duration or EDSS but there was a trend to greater disability in patients with cord presentations as measured by the MSFC (p=0.05).

5.4.2 Baseline MRI

Of the 42 patients studied at month zero, 18 (43%) had at least one enhancing lesion (Figure 5.1a). In patients with enhancement, the average number of enhancing lesions seen was 2.2 (SD 2.0) and the average enhancing lesion volume was 0.3 ml (SD 0.4 ml). Three patients had enhancing cord lesions (7%) at baseline (Figure 5.1b), all of whom also had enhancing cerebral lesions. The number of enhancing lesions correlated inversely with age (r=-0.5, p=0.003) and directly with T2 load (r=0.5, p=0.02). When subdivided on the basis of the presence or absence of enhancement (Table 5.1), patients with enhancement had greater disability (EDSS, p=0.011; MSFC, p=0.004), higher T2 and T1 hypointensity loads (p=0.005, p=0.001 respectively) and a trend to greater atrophy as assessed by partial brain volume (p=0.049). Gd-DTPA
enhancement was seen in 35% of patients with cord symptoms at initial presentation and in 75% of those without. This difference was not significant.
Figure 5.1: Examples in early PPMS of a) an enhancing brain lesion b) an enhancing cord lesion
<table>
<thead>
<tr>
<th></th>
<th>Enhancing (18)</th>
<th>Non-enhancing (24)</th>
<th>p values</th>
</tr>
</thead>
<tbody>
<tr>
<td>Age</td>
<td>42 years (SD 11.9)</td>
<td>47.3 years (SD 9.9)</td>
<td>p=0.14</td>
</tr>
<tr>
<td>Sex</td>
<td>10 males: 8 females</td>
<td>14 males: 10 females</td>
<td></td>
</tr>
<tr>
<td>Disease duration</td>
<td>3.2 years (SD 0.8)</td>
<td>3.5 years (SD 0.9)</td>
<td>p=0.14</td>
</tr>
<tr>
<td>Presentation</td>
<td>12 cord: 6 non-cord</td>
<td>22 cord: 2 non-cord</td>
<td></td>
</tr>
<tr>
<td>EDSS</td>
<td>6.0 (range 3.5)</td>
<td>4.3 (range 3.5)</td>
<td>p=0.011*</td>
</tr>
<tr>
<td>MSFC</td>
<td>-1.5 (SD 1.8)</td>
<td>-0.4 (SD 1.1)</td>
<td>p=0.004**</td>
</tr>
<tr>
<td>T2 load</td>
<td>22.5ml (SD 25.0ml)</td>
<td>9.0ml (SD 20.1ml)</td>
<td>p=0.005**</td>
</tr>
<tr>
<td>T1 load</td>
<td>7.3ml (SD 10.0ml)</td>
<td>3.2ml (10.0ml)</td>
<td>p=0.001**</td>
</tr>
<tr>
<td>Ventricular volume</td>
<td>23.4.ml (SD 19.0ml)</td>
<td>22.0ml (SD 16.8ml)</td>
<td>P=0.81</td>
</tr>
<tr>
<td>Partial Brain Volume</td>
<td>248.3ml (SD 23.8ml)</td>
<td>264.1ml (SD 19.5ml)</td>
<td>p=0.049*</td>
</tr>
<tr>
<td>Whole Brain Volume</td>
<td>1110.1ml (SD136.8ml)</td>
<td>1196.4ml(SD137.8m)</td>
<td>p=0.072</td>
</tr>
</tbody>
</table>

** significant at the level of p=0.005 or below
* significant at the level of p= 0.05 or below

**Table 5.1** Comparison of clinical and MRI features of patients with and without enhancing lesions
Twenty-three patients (15 males, 8 females) attended for monthly serial examination at baseline. Within this group, 12 patients had enhancement on the baseline scan and seven (64%) enhanced at every time point (Figure 5.2a). Of the remaining five patients, four showed enhancement on the month zero scan only and one showed enhancement at months one and two. Of the 11 patients not showing enhancement at baseline, none showed subsequent enhancement. When enhancement was present, the mean number of enhancing lesions was 2.4 per patient (SD 2.5) and the mean enhancing lesion volume per patient was 0.4 ml (SD 0.5 ml), values similar to the estimates obtained from the larger month zero cohort.

The duration of enhancement of individual lesions over the first three months is shown graphically in Figure 5.3. Of the 23 enhancing lesions that were seen at month zero, four were still enhancing at month one and only one was still enhancing at month two. Thirteen new lesions were seen in month one and, of these, two were still enhancing at month two. Therefore, 11 out of the 13 new enhancing lesions (85%) enhanced for between one and two months. There were 22 new enhancing lesions at month two. Overall, only six lesions were seen on two successive monthly scans and only one on all three. Thus the majority of lesions enhanced on only one scan and only one lesion definitely showed enhancement lasting more than two months.

Serial examination at months one and two confirmed the low frequency of spinal cord enhancement seen at month zero. From a total of 69 monthly cord observations, only four instances of cord enhancement were observed (6%) (Figure 5.2b). Of two patients with enhancing spinal cord lesions at month zero, one showed further spinal
cord enhancement at month one and month two while the other enhanced at month zero only. In each case cerebral enhancing lesions were also present.

5.4.3 Six month follow-up

The 36 patients (22 males and 14 females) on whom six month data were available did not differ from the baseline cohort. At six months, the scans of 11 patients showed enhancement (31%) and all of these had enhanced at baseline. (There were four patients who enhanced at baseline but had no enhancement at six months). No patient without initial enhancement showed enhancement at six months. Thus enhancement status did not change in 32 out of 36 cases (89%) and the presence of enhancement at baseline was strongly associated with enhancement at six months (r=0.8, p<0.001). Mean enhancing lesion number (3.0) and volume (0.3 ml) remained constant between baseline and six months. The one patient with an enhancing cord lesion at six months (3%) also had an enhancing brain lesion at six months and an enhancing cord and brain lesion at baseline. None of the enhancing lesions seen on the baseline scans was still enhancing at six months; 64% became T2 lesions and 39% became T1 lesions. 55% of the lesions that became T2 lesions (and 35% of all enhancing lesions) also became T1 lesions.

Over six months, the only MR measure, which showed a significant increase, was mean T2 load (14.3 ml to 15.6 ml, p=0.013). The presence of enhancement at baseline was not associated with greater or lesser change in any MR measure over 6 months.
Figure 5.2: A graphical representation of the pattern of a) cerebral and b) spinal cord enhancement by patient at the baseline, month one and month two timepoints. Patients with enhancement at that timepoint are shown in dark grey.
Figure 5.3: A graphical representation of enhancing lesions at baseline, month one and month two. Enhancing lesions present at baseline are shown in dark grey, new enhancing lesions appearing at month one and two are shown in light grey and white respectively.
5.5 Discussion

The study described in this chapter aimed to address four questions relating to inflammation in early PPMS. The first and most fundamental question is whether there is an early enhancing phase in PPMS as is seen in RRMS. This study finds no evidence of enhancement as a universal feature in early PPMS. Even when triple dose gadolinium and serial examination are used to detect inflammation, it is still absent in a majority of cases. However, 43% of patients had some enhancement and while this is much less than the 75% of patients who enhanced in a comparative, clinically early, RRMS cohort examined under identical circumstances [140] it is more than is seen in established disease. For example, using entry data from a recent clinical trial in PPMS (where the median time since first symptoms was 10.9 years ± 7.5 years) 14.1% of patients had enhancing lesions [136]. However, a complicating factor is that this clinical trial, together with the majority of studies of enhancement in PPMS, used single and not triple dose Gd-DTPA. It is still not clear whether the use of triple dose Gd-DTPA leads to detection of a greater number of enhancing lesions in PPMS, as the two small studies that have examined this question draw different conclusions. In these studies 10 and 16 patients with PPMS with disease durations of six and nine years respectively were examined [54;137]. Whereas one study found that use of triple dose increased the number of enhancing lesions from four to 13 and the number of patients showing enhancement from two to five [137][7], the other found no increase [54]. It was speculated that differences in disease duration, disability, patient selection, or small sample size might explain these differences [54].
In the spinal cord, little enhancement is seen (7% of patients, compared to 33% of patients in a comparative, clinically early, RRMS cohort) [140]. Therefore although, cord syndromes are a common presentation of PPMS, they do not appear to arise from extensive cord inflammation in the early clinical stages of the condition.

Given that cerebral inflammation is present in a considerable proportion of patients with early PPMS, the next question is, does this inflammation have any distinguishing features? The answer is apparently not. Enhancing lesion number, volume, location and duration are all similar to those reported in RRMS and SPMS [141;142]. The only previous comparable study in PPMS, using triple dose gadolinium, studied ten patients with a mean disease duration 6.5 years and showed very similar results [137]. The evolution of enhancing lesions into T2 areas of hyper-intensity and/or T1 areas of hypo-intensity is also similar to that seen in other clinical subgroups [99]. This suggests that the underlying disease mechanism at least with respect to focal blood-brain-barrier breakdown may be common to all types of MS.

One of the striking findings in this study is the relative consistency of enhancement among patients. This can be seen in two ways. First, cord enhancement was not seen in the absence of enhancing lesions in the brain. Secondly, in a subset of 12 patients who enhanced at month zero, eight had further enhancement over the next two months (67%), which for the majority was as a result of new enhancing lesions. Finally, all 11 patients who enhanced at six months had baseline enhancement. This observation may have treatment implications for PPMS that will be discussed later.
The relative consistency in enhancement leads on to the third question, namely whether the subset of patients with enhancement has any other distinguishing features. In our study, patients with enhancing and non-enhancing scans did not differ in presenting history but did on other clinical and MR measures. Enhancing patients had greater disability, higher lesion loads and evidence of greater brain atrophy, this suggests that the presence of enhancement is an adverse feature in PPMS and future follow up of this cohort will determine whether this is truly the case. The presence or absence of enhancement may also reflect important differences in immunopathogenesis within PPMS. In this study all patients had oligoclonal bands in their cerebrospinal fluid, but in a recent clinical trial 21% of patients did not have oligoclonal bands at entry. There were strong trends to increased enhancement in patients with oligoclonal bands together also with significantly higher lesion loads (p=0.1, p<0.001 respectively) [136].

Finally, do the clinical and MRI findings of this study provide any insights into the pathological role of inflammation in PPMS? Inflammation, where present, seems to be associated with adverse features though it does not necessarily follow that this relationship is causal. To date, inflammation-suppressing therapies such as interferon beta and glatiramer acetate appear to have little impact on disease progression when used in patients with PPMS. Might a greater clinical effect be seen in the subset of patients with evidence of inflammation? This question may be answered by the recently terminated large international trial of glatiramer acetate in PPMS where single dose gadolinium was given to all subjects [136]. Equally, the question remains as to the nature of the non-inflammatory mechanism of disability that is operating in
the majority of patients who do not show enhancement. At this point it is relevant to consider a potential limitation of this study; namely that, although patients may be in the early clinical stage of their condition, they may not necessarily be in the early stages of their disease process. The considerable degree of MR abnormality seen on some of the initial MR scans, even when the clinical history is short, suggests that this may be the case. This point has been well illustrated in a recent case report of a patient with primary progressive MS who had abnormalities on brain MRI 10 years prior to the onset of clinical symptoms [134]. Therefore, the presence of an inflammatory process in a preclinical phase (which persists into the clinical phase in a proportion of patients) cannot be excluded. Ongoing follow up of this cohort will provide further data which will help determine if inflammation is a transient phase in early PPMS (i.e. will cessation of enhancement be seen in some patients over time as they become clinically established) and will explore the pathological associations and consequences of enhancement in greater detail using a wider range of newer and pathologically more specific MRI techniques.
Chapter 6

Overview of results and conclusions
6.0 Introduction

This thesis describes MRI studies that provide insights into the underlying pathology and disease dynamics of PPMS. This final chapter considers the findings from the studies described in Chapters 3 to 5 and sets these in context of the wider PPMS literature, as described in Chapter 1.

There have been many MRI studies that have examined cross-sectional abnormalities in patients with PPMS, comparisons being made with control subjects and with individuals affected by other MS subtypes. Over time these studies have employed increasingly sophisticated techniques of MRI analysis and acquisition. Key observations from such studies would include the relatively lower level of inflammation in PPMS [41], the demonstration of abnormalities within tissues that appear normal on conventional MRI [143], the demonstration of what appears to be widespread neuro-degeneration in the condition (although this has also been shown for RRMS) [57] and, more recently, the observation that there are changes in the patterns of cortical activation which may form part of a compensatory mechanism [86].

These cross-sectional observations tell us little about the dynamics of the disease process, how it varies between individuals and over time, and how this process might relate to progression in clinical disability. This thesis contains data on the pattern of MRI changes over periods longer than one year in PPMS, the relation of MRI measures to clinical disability and the nature of MRI abnormalities in the earliest
clinical years of PPMS. A better understanding of the MRI findings in PPMS in these areas will hopefully provide insights into the disease mechanisms that underlie the condition and improve the clinical utility of MRI by aiding the identification of effective disease markers for the purposes of prognosis and disease monitoring.

This overview chapter will consider the following subjects:

- The nature of longitudinal changes in conventional MRI measures
- The relation between MRI and clinical disability in patients with PPMS
- The conventional MRI features of early PPMS
- The role of inflammation in PPMS
- Individual variation in PPMS

6.1 Longitudinal change in conventional MRI measures: implications for the nature of the underlying disease process

The study described in Chapter 3 extended MRI observations in a cohort of patients with PPMS to first two, and then five years, and is the first account of how MRI measures change in PPMS over such an extended period. It was seen that for all the MRI measures observed, whether lesion load measures (T2 and T1 hypointensity), or tissue volume measures (partial brain, ventricular and cervical cord volume) a progressive change towards greater abnormality occurred over time. This MRI progression is not surprising given that PPMS is a clinically progressive condition and
existing studies, over shorter periods, have already shown change in measures of this kind occurring over a period of one year [43].

What was less predictable about the longitudinal MRI behaviour observed in this cohort was that a wide variation exists in the rate of change of MRI abnormality between individuals. In addition, there was an absence of a clear relation between the length of the clinical history and the amount of MRI change seen. For some individuals, therefore, there is evidence of considerable disease activity many years into the clinical course. Furthermore, whatever changes were seen (whether large or small) seemed to be seen across measures for a given individual as was demonstrated by the strong associations found between changes in MRI abnormalities (including between change in lesion load and change in tissue volume measures).

Before considering further the possible implications for PPMS pathology of these observations a caution is needed. MRI measures are pathologically non-specific and it may be the case that the processes leading to increase in T2 load at one stage in an individual's disease course may be different from the factors causing the same MRI change at a later stage. Assuming however that similar factors are in operation at each stage, there are two implications for the nature of the underlying disease process: firstly, that it has characteristics that are specific to the affected individual (i.e. its rate or degree of aggressiveness), secondly that the process occurs at a relatively steady rate over relatively long periods.
It should be noted that there was an important exception to the observation that most MRI measures appeared to change in parallel, and that was the change in cervical cord area, which although obeying the same general rule of being relatively constant over time for a given individual but varying considerably between individuals, did not mirror change in cerebral measures. This was shown by the failure to find an association between cervical cord change and any cerebral MRI measure.

6.2 Relation between MRI and clinical disability in patients with PPMS

The most important practical implication of the studies described in this thesis is to show that at least some conventional MRI measures do relate, albeit weakly, to clinical change in the condition. The importance of this observation is that it provides support for the use of such measures in clinical trials examining the potential effectiveness of disease modifying treatments in the condition. Specifically, it provides some grounds for believing that any therapeutic agent that can be shown to convincingly reduce the accrual of T2 or T1 hypointensity load, or prevent tissue loss as measured by partial brain volume, ventricular volume or cord volume might ultimately also have an effect on the rate of clinical progression. Several trials have already taken place using these measures, by extension from trials in RRMS and SPMS, and therefore the demonstration of an association between conventional MRI and clinical measures has an immediate application in the possible interpretation of these studies [125;138].
This work can however only be regarded as one step towards the development of a truly valid disease surrogate for PPMS. As discussed in Chapter 3 the ideal surrogate is required to meet a number of conditions, of which only the first, the observation of the association between the surrogate and the clinical outcome in natural history studies is made here [119]. Therefore it remains to be shown that an effect on a T2 load surrogate marker in PPMS will actually translate into clinical benefit.

There is now an increasing list of more novel MRI measures that have been shown to relate cross-sectionally with disability measures in PPMS. These include newer atrophy measures, DTI, MT and fMRI [55;57;61;86]. In theory these measures have increased pathological specificity but it remains to be seen whether changes in these measures will associate with clinical change to a greater extent than the simpler measures described here. It is notable that the clinical associations seen for the conventional measures described in Chapter 3 differed between measures. Change in some measures (for example, ventricular volume and partial brain volume) correlated mainly with change in cognitive function, as measured grossly by performance on the PASAT test, but changes in other measures (for example cord area) correlated with change in global disability (EDSS) only. This may imply that pathological processes that affect certain areas may leave other area relatively unaffected. In the context of newer MRI measures, this may mean that, despite their greater pathological and anatomical specificity, there is no universal single MRI surrogate that will adequately reflect all clinically relevant aspects of the disease process. Instead, a number of different measures may be needed.
The last observation to make about the relation between clinical and MRI abnormality in PPMS is the unexpected *cross-sectional* association seen between conventional MRI measures and disability in the cohort of patients with early PPMS. One potential explanation for the difference in cross-sectional clinical associations seen between the two cohorts is that it reflects a more direct effect on the disease process on disability at this time. In turn it could be postulated that this is because intrinsic compensatory mechanism have not yet had time to come fully into effect. However, in the context of the literature of PPMS more generally cross sectional associations between conventional MRI measures and measures of disability are occasionally shown (for example, between T2 load and ambulation index, r=0.51, p<0.001 in 28 patients with PPMS in the recent study of Ukkonen [58]) and therefore it may be that the MRI associations described in Chapter 4 reflects some other characteristic of the cohort other than its short clinical histories (for example the wide spectrum of disease severity represented or the relatively young age of subjects).

6.3 **Early PPMS: conventional MRI features**

The conventional MRI features of clinically early PPMS are described for the first time in Chapter 4. Only one previous MRI study has examined patients with PPMS selected for short disease duration (the study of cortical atrophy of De Stefano [57]). It was seen that even when the clinical history was short, the degree of MRI abnormality present could be still be marked. As discussed in Chapter 4, this implies that the disease process may be active for some time before clinical symptoms become apparent. This observation is supported by a case report where incidental MRI was
performed many years before the development of symptoms of PPMS in a single individual [134]. The implication is potentially an important one both for PPMS research more generally and for the specific prospect of developing effective treatments for the condition. For PPMS research more generally, it may mean that the term “clinically early PPMS” might potentially have little meaning in terms of underlying pathology, with patients who are similar in terms of length of their clinical course potentially differing widely in terms of the length of their actual disease process. Further, the observed variation in MRI measures also suggests that the development of clinical symptoms does not occur on the basis of gross disease burden alone and is instead dependent on other, as yet unknown, factors. Therapeutically, the relevance of this observation is to raise the possibility that “effective” treatments (even when available) begun at or close to the time of clinical presentation might ultimately still fail to prevent clinical progression through the existence of already established subclinical disease processes.

Another observation made in the cohort of patients with short clinical history that has more general implications is that there is no evidence, at least in terms of MRI conventional measures, of any unique process occurring at this time. Although this may mean that there are no new sites for therapeutic intervention at this time, it also suggests that treatments, once available, might be equally effective at all stages in the clinical history.
6.4 Early PPMS: inflammation

The role of inflammation in the pathogenesis of PPMS has been a subject of interest for some time. Early observations that the degree of enhancement after injection with gadolinium DTPA was less for PPMS than for other MS subtypes offered both an explanation for the defining clinical features of the condition (namely, the absence of relapses) and an insight into a possible key difference in the underlying pathophysiology (namely where, in contrast to RRMS, neurodegenerative as opposed to neuroinflammatory processes dominate from the beginning) [41]. The observation of a reduced enhancement frequency in PPMS compared to other MS subtypes has been confirmed in every subsequent comparative study and the original clinico-pathological interpretation placed enhancement findings is now well established.

However, in Chapter 5 it was seen that in the cohort of patients with early PPMS almost half had enhancement following administration of triple dose Gd. How can this be explained? Firstly, it is important to note that in each of the studies that have examined enhancement in patients with PPMS, although the relative frequency of enhancement is always less than in other subtypes, a proportion of patients with PPMS have still shown enhancement. Secondly, it should be noted that the number of existing studies using triple dose Gd to study this question in PPMS is very small (two only, [54;137]) and that in one of these a comparable enhancement frequency was seen.
The observations regarding the frequency of Gd enhancement in the early PPMS cohort suggest that the role of inflammation in PPMS may need, in part, to be reconsidered. One possibility is that the enhancement data reflect the operation of an inflammatory process that is prominent in the early clinical stages of the condition. This would be a finding of some practical interest in that inflammatory activity is the one aspect of MS pathology that we can currently influence by therapeutic intervention (for example, with the use of interferon beta or glatiramer acetate). The importance of inflammatory processes may furthermore, on the basis of at least one of the other studies of triple dose Gd in an established PPMS population, not be limited to patients in the early part of their clinical course and further study of established patients (together with ongoing follow up of the cohort described here) may be indicated.

It should be noted too that enhancement was also associated with adverse clinical and MRI features, suggesting that inflammation in these patients is a genuine factor of both clinical and pathophysiological importance.

6.5 Individual variation in PPMS

One common theme that emerges from all the studies described in this thesis is that there appears to be an important role for individual variation in PPMS. This is manifested in the nature of the dynamic MRI changes described in Chapter 3, in the variation in conventional measures in patients with similar (early) disease duration in Chapter 4 and with respect to enhancement status as described in Chapter 5. A better
understanding of the factors responsible for the variation between individuals with PPMS might therefore be useful in helping to understand disease mechanisms. Practically, this might mean trying to identify factors associated with rapid or slow progression of MRI abnormality, or factors common to patients whose clinical condition remains stable despite progressive MRI change or who experience rapid clinical progression irrespective of MRI findings, or who show enhancement.

6.6 Conclusion

The most important observation from these studies is probably that correlation between MRI and clinical measures in PPMS can be found when the period of observation is long. Much existing research, including therapeutic trials, depends on the existence of such a relationship and the demonstration of longitudinal correlation is, perhaps, overdue.

For future research the most important contribution of the studies described in this thesis is to suggest that simplistic assumptions about the unitary nature of the disease process in PPMS may be incorrect. There appears not only to be considerable variation in the rate (and possibly even nature) of the disease process between individuals but also some suggestion, from the studies of inflammation in early PPMS, of a qualitative difference in the disease process in the earliest clinical stages. The twin findings of substantial MRI abnormality at clinical presentation in some cases and the demonstration of steady progression in abnormality throughout the disease
course in others provide further evidence for a significant preclinical evolution as suggested by case reports [134].
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   Ref Type: Abstract

