

An investigation of magnetization transfer ratio and T1 hypointense lesion volume in secondary progressive multiple sclerosis.

Thomas Hayton 59067979

UCL

PhD Thesis

Declaration statement

I, Thomas Hayton, confirm that the work presented in this thesis is my own. Where information has been derived from other sources, I confirm that this has been indicated in the thesis and acknowledgements.

Abstract

Background

Multiple sclerosis (MS) is a chronic inflammatory demyelinating condition of the central nervous system (CNS). The majority of people with MS have a relapsing onset with recurrent episodes of acute or sub-acute onset neurological impairment interspersed with periods of functional recovery and relative clinical stability. Many people with relapse-onset MS will eventually develop progressive, irreversible disability, termed secondary progressive MS.

Pathological studies suggest that there is substantial neuroaxonal loss in MS. Data from animal models indicate that this may in part be due to the toxic accumulation of sodium in chronically demyelinated axons and that blockade of voltage gated sodium channels with drugs such as lamotrigine is potentially neuroprotective.

Quantitative magnetic resonance imaging (MRI) is a powerful tool which allows investigators to monitor CNS pathology *in vivo* and consequently has been integrated into clinical treatment trials. Several techniques have been developed which are thought to correlate with neuroaxonal loss. These include: volume of T1 hypointense lesions (T1LV); whole brain, regional and spinal cord atrophy; and magnetization transfer ratio (MTR), a technique that gives a measure of the macromolecular content of tissue, such that higher MTR may indicate higher myelin and intact axon content.

The purpose of this study was to evaluate the potential for T1LV and MTR to quantify clinically important brain pathology in secondary progressive MS and evaluate the

effect on these measures of treatment with lamotrigine.

Methods

118 people with secondary progressive MS were recruited into a double-blinded, placebo controlled, randomized trial of neuroprotection with lamotrigine. Clinical assessment included the multiple sclerosis functional composite (MSFC), expanded disability status scale (EDSS) multiple sclerosis impact scale (MSIS-29) and were collected at five timepoints: baseline, 6, 12, 18 and 24 months. The incidence of relapses was also checked at three monthly intervals during the course of the trial. MRI assessment included: central cerebral volume collected every 6 months; MTR of normal appearing white matter, normal appearing grey matter (NAWM; NAGM) and T2 hyperintense lesions; whole and regional brain volume; spinal cord cross sectional area; T1LV and T2 lesion volume (T2LV) collected at three timepoints – baseline, 12 and 24 months – and whole brain atrophy, detected using a coregistration-subtraction protocol (Structural Image Evaluation, using Normalisation, of Atrophy; SIENA) over the 24 months of the study.

The correlation of T1LV and brain MTR with brain volume and clinical measures was carried out at baseline on the whole cohort while longitudinal correlations were assessed in the placebo arm only. Comparison of change in MTR and T1LV measures in the verum arm was made with those in the placebo arm by intention to treat and two per protocol analyses: ‘serum compliant’ (subjects in whom lamotrigine was detected in the serum at 24 months) and ‘tablet compliant’ (subjects who were estimated to have taken at least 80% of the prescribed tablets).

Results

There were moderate cross-sectional correlations of MTR measures and T1LV with MSFC and component measures and normalized brain volume (NBV), with lower MTR values and higher T1LV associated with more severe brain atrophy and neurological impairment. In multiple regression models T1LV emerged as the only independently significant cross-sectional correlate of both NBV and clinical measures. Only NAGM MTR mean correlated with all three components of the MSFC. There was no association of any MRI measure with higher or lower EDSS.

The responsiveness of all the MRI measures was limited. In the placebo arm T1LV changed significantly over the 24 months of the trial ($p < 0.0001$) and, although the responsiveness was comparable to T2LV, it was lower than SIENA. Only three out of nine MTR measures – NAGM mean ($p < 0.0001$), lesion peak location (PL) ($p = 0.018$) and mean ($p < 0.0001$) – changed significantly over the 24 months.

Comparing the MTR and T1 hypointense lesion volume measures in the verum and placebo arms of the study did not show that lamotrigine was neuroprotective. Of nine MTR measures evaluated, only two – NAGM and lesion peak height (PH) - differed significantly between the two groups. Lesion PH increased over 24 months, by a greater magnitude in the placebo arm ($p = 0.004$), while NAGM PH fell more in the verum arm ($p = 0.036$).

The longitudinal correlations were limited. There were no significant correlations of

change in MRI measures with change in clinical measures. Using a mixed effect linear regression model it was possible to show that the correlations were consistent across all three timepoints, but there was no correlation of MRI measures with an interaction variable of [clinical measure*timepoint].

A fall in lesion PL MTR and an increase in lesion mean MTR was associated with an increased risk of experiencing a sustained increase in EDSS ($p=0.049$; $p=0.002$) but none of the other MRI measures were associated with a change in EDSS.

T1 to T2 lesion volume ratio was the only independently significant cross-sectional MRI correlate of the psychological component of the MSIS-29 ($R^2=0.13$, $p<0.0001$). In the longitudinal analysis change in timed walk correlated modestly with the physical component of the MSIS-29 ($R^2=0.09$, $p=0.047$) while change in timed walk, NAWM MTR mean and NAGM PH correlated modestly with the psychological component ($R^2=0.32$, $p=0.007$, $p=0.003$, $p=0.048$). The proportion of variability of the MSIS explained by these models was small.

Conclusions

The data suggest that MTR and T1 lesion volume are measures of clinically important brain pathology in secondary progressive MS. T1LV may be a more specific measure of clinically important brain pathology than T2LV. The results of this study highlight the value of NAGM MTR for identifying clinically important pathology, perhaps indicating that NAGM pathology is important in determining clinical status in secondary progressive MS. The data presented here do not show any neuroprotective

effect of lamotrigine treatment in secondary progressive MS.

Contents

List of publications and presentations	10
List of figures and tables	12
List of abbreviations	14
Acknowledgements	16
1. Introduction	19
1.1. Multiple sclerosis (MS)	19
1.2. Pathology of MS	10
1.3. Pathophysiology of MS	23
1.4. Quantifying neurological deficit in MS	28
1.5. Magnetic resonance imaging (MRI) in MS	33
1.6. Treatment of MS	49
1.7. Conclusion	55
2. Methods	57
2.1. Introduction: A randomized, double-blind, placebo-controlled trial of neuroprotection with lamotrigine in secondary progressive MS	57
2.2. Subjects	58
2.3. Clinical data in the lamotrigine trial	66
2.4. MRI data in the lamotrigine trial	71
2.5. Statistical analysis and dissemination of results	88
3. Results	90
4. Grey matter magnetization transfer ratio independently correlates with neurological deficit in secondary progressive multiple sclerosis	92
4.1. Introduction	92
4.2. Methods	89
4.3. Results	93
4.4. Discussion	103
5. Longitudinal changes in magnetisation transfer ratio in secondary progressive multiple sclerosis	108
5.1. Introduction	108
5.2. Methods	109
5.3. Results	111

5.4.Discussion	122
6. Clinical and radiological correlates of T1 and T2 lesion volume measures in secondary progressive multiple sclerosis	128
6.1.Introduction	128
6.2.Methods	129
6.3.Results	132
6.4.Discussion	138
7. Clinical and imaging correlates of quality of life in secondary progressive multiple sclerosis: a multimodal study of the multiple sclerosis impact scale	144
7.1.Introduction	144
7.2.Methods	144
7.3.Results	148
7.4.Discussion	153
8. Conclusion	158
References	174
Appendix 1: The Multiple Sclerosis Impact Scale (MSIS-29)	232

List of publications and presentations

The following publications and presentations were produced using work done as part of this project:

PUBLICATIONS

1. **Hayton T**, Furby J, Smith KJ, Altmann DR, Brenner R, Chataway J, Hunter K, Tozer DJ, Miller DH, Kapoor R. Longitudinal changes in magnetization transfer ratio in secondary progressive multiple sclerosis: data from a randomized placebo controlled trial of lamotrigine. *J Neurol.* (2012) 259, 505-514.
2. **Hayton T**, Furby J, Smith KJ, Altmann DR, Brenner R, Chataway J, Hunter K, Tozer DJ, Miller DH, Kapoor R. Clinical and imaging correlates of the multiple sclerosis impact scale in secondary progressive multiple sclerosis. *J Neurol* (2012) 259, 237-245
3. Furby J, **Hayton T**, Altmann D, Brenner R, Chataway J, Smith KJ, Miller DH, Kapoor R. A longitudinal study of MRI-detected atrophy in secondary progressive multiple sclerosis. *J Neurol* (2010) 257, 1508-1516
4. Kapoor R, Furby J, **Hayton T**, Smith KJ, Altmann DR, Brenner R, Chataway J, Hughes RA, Miller DH. Lamotrigine for neuroprotection in secondary progressive multiple sclerosis: a randomised, double-blind, placebo-controlled, parallel-group trial. *Lancet Neurol* (2010) 9, 681-688
5. **Hayton T**, Furby J, Smith KJ, Altmann DR, Brenner R, Chataway J, Hughes RAC, Hunter K, Tozer DJ, Miller DH, Kapoor R. Grey matter magnetization transfer ratio independently correlates with neurological deficit in secondary progressive multiple sclerosis. *J Neurol* (2009) 256, 427-435
6. Furby J, **Hayton T**, Altmann D, Brenner R, Chataway J, Smith KJ, Miller DH, Kapoor R. Different white matter lesion characteristics correlate with distinct grey matter abnormalities on magnetic resonance imaging in secondary progressive multiple sclerosis. *Mult Scler* (2009) 15, 687-694
7. Furby J, **Hayton T**, Smith KJ, Anderson V, Altmann D, Brenner R, Chataway J, Hughes R, Smith K, Miller D, Kapoor R. Magnetic resonance imaging measures of brain and spinal cord atrophy correlate with clinical impairment in secondary progressive multiple sclerosis. *Mult Scler* (2008) 14,1068-1075
8. **Hayton T**, Furby J and Kapoor R. The demyelinated axon. Review. *ACNR* (2007) 5, 10-13

PRESENTATIONS

1. **Hayton T**, Furby J, Smith KJ, Altmann DR, Brenner R, Chataway J, Hughes,

- R, Hunter K, Miller DH, Kapoor R. T1 hypointense lesion volume predicts localized and global brain pathology and correlates with upper limb function in secondary progressive multiple sclerosis. Abstract and Poster ECTRIMS 2009
2. **Hayton T**, Furby J, Smith KJ, Altmann DR, Brenner R, Chataway J, Hughes R, Hunter K, Miller DH, Kapoor R. Longitudinal changes in magnetization transfer ratio in secondary progressive multiple sclerosis. Abstract and Poster ECTRIMS 2009
 3. Furby J, **Hayton T**, Altmann DR, Brenner R, Chataway J, Smith KJ, Miller DH, Kapoor R. A longitudinal study of volumetric MRI in secondary progressive multiple sclerosis. Abstract and Poster WCTRIMS 2009
 4. Kapoor R, Furby J, **Hayton T**, Smith KJ, Altmann DR, Brenner R, Chataway J, Hughes R, Hunter K, Miller D, Kapoor R. Outcomes of a phase II randomized controlled trial of neuroprotection with lamotrigine in secondary progressive multiple sclerosis. Abstract ECTRIMS 2009
 5. Furby J, **Hayton T**, Smith KJ, Altmann D, Brenner R, Chataway J, Hughes RA, Miller D, Kapoor R. White matter lesion characteristics predict types of gray matter abnormality on magnetic resonance imaging in secondary progressive multiple sclerosis. Abstract ECTRIMS 2008
 6. **Hayton TD**, Furby J, Smith KJ, Altmann D, Brenner R, Chataway J, Fox N, Hughes R, Hunter K, Tozer DJ, Miller DH, Kapoor R. Predictors of disability in secondary progressive MS: a multimodal MRI study - Abstract and Poster AAN 2008
 7. **Hayton T**, Furby J, Smith KJ, Altmann DR, Brenner R, Chataway J, Hunter K, Tozer DJ, Miller DH, Kapoor R. Correlation between brain magnetization transfer ratio and clinical disability measures in secondary progressive multiple sclerosis. Abstract and Poster ECTRIMS 2007
 8. Furby J, Hayton T, Smith KJ, Altmann DR, Brenner R, Chataway J, Fox NC, Hughes RAC, Miller DH, Kapoor R. The correlation between MR measures of atrophy and disability in secondary progressive multiple sclerosis. Abstract and poster ECTRIMS 2007
 9. **Hayton T**, Furby J, Smith KJ Altmann DR, Brenner R, Chataway J, Hunter K, Tozer DJ, Miller DH, Kapoor R. The correlation between MR measures of atrophy and disability in secondary progressive multiple sclerosis – Poster MS Frontiers 2007
 10. Furby J, Hayton T, Smith KJ, Altmann D, Brenner R, Chataway J, Fox NC, Hughes RAC, Miller DH, Kapoor R. A randomized controlled trial of neuroprotection with lamotrigine in secondary progressive multiple sclerosis Abstract and Poster ECTRIMS 2006

List of figures and tables

Figure 1. A summary of the recruitment and screening process for the lamotrigine trial	63
Figure 2. The effect of practice on PASAT 3 scores	69
Table 1. MRI characteristics and acquisition parameters used in the lamotrigine trial	72
Figure 3. The outcome of segmentation with SPM99	82
Figure 4. Examples of regions used to generate MTR histograms	83
Figure 5. A typical MTR histogram of the whole brain	85
Table 2. Coefficients of variance for semi-automated MRI analysis	87
Table 3. Summary of clinical and MRI data collected from subjects in the SPMS study group at each timepoint	88
Table 4. Clinical characteristics for subjects in baseline analysis	95
Figure 6. MTR Histogram profiles for NAWM, grey matter and lesions at baseline	97
Table 5. Cross sectional correlation between magnetization transfer (MTR) measures for normal appearing white matter (NAWM) grey matter and T2 high-signal lesions, normalized brain volume (NBV) and T2 high-signal lesion volume	98
Table 6. Linear regression analysis with MSFC-based clinical measures as the outcome variable and a single MR measure as the predictor, adjusted for age, gender, disease duration and duration of SPMS at baseline	99
Figure 7. Scatter plot of grey matter MTR histogram mean vs. MSFC composite showing regression line at baseline	100
Table 7. Multiple linear regression models for predicting the MSFC and its components with MTR peak height, peak location and histogram mean for grey matter, NAWM and lesions; NBV, T2LV disease duration, duration of SPMS, age and gender as independent variables	102
Figure 8. Trial participant flow chart	112
Table 8. Clinical and MRI measures for both verum and placebo arm at baseline	113
Table 9. Change in MTR measures over 24 months	114
Table 10. Scan-reposition-rescan reproducibility of the MTR measures	115

Figure 9. MTR peak height.	116
Table 11. Longitudinal correlation of MTR measures with MSFC and component measures in the placebo arm	119
Figure 10. Mean lesion MTR measures	121
Table 12. Lesion volume measures at baseline	133
Table 13. Mean change in lesion volume measures	134
Table 14. Cross-sectional correlation of lesion volume measures with other MRI measures, MSFC and component measures	136
Table 15. Longitudinal correlation of lesion measures with other MRI measures, MSFC and components	137
Table 16. Baseline demographic characteristics for both the placebo and verum arms combined	148
Table 17. Clinical and MRI characteristics at baseline and change over 24 months	149
Table 18. The results of stage 1 cross-sectional analysis for MSIS-29 correlation	150
Table 19. The results of stage 2 cross-sectional analysis for MSIS-29 correlation	151
Table 20. The results of stage 2 longitudinal analysis for MSIS-29 correlation	152
Table 21. Multimodal ‘best predictor’ analysis for MSFC	164

List of abbreviations

2D – two dimensional
3D – three dimensional
9HPT – 9 Hole Peg Test
BET – brain extraction tool
CD – cluster of differentiation
CCV – central cerebral volume
Cho – choline containing compounds
CIS – clinically isolated syndrome
CNS – central nervous system
Cr – creatine/phosphocreatine
CSF – cerebro-spinal fluid
DTI – diffusion tensor imaging
DWI – diffusion weighted imaging
EAE – experimental allergic encephalomyelitis
EDSS - Expanded Disability Status Scale
FLAIR – fluid attenuated inversion recovery
FSPGR-IR – fast-spoiled gradient recall inversion recovery
GMF – grey matter fraction
IMP – investigational medicinal product
LFT – liver function test
MHC – major histocompatibility complex
MIDAS – Medical Image Display and Analysis System
MNI152 – Montreal Neurological imaging group 152 template
MR – magnetic resonance
MRI – magnetic resonance imaging
MS – multiple sclerosis
MSFC - Multiple Sclerosis Functional Composite
MSIS-29 – Multiple Sclerosis Impact Scale
MSIS-phys – MSIS-29 physical component
MSIS-psych – MSIS-29 psychological component
MT – magnetization transfer
MTR – magnetization transfer ratio
NAA – N-acetyl aspartate
NABT – normal appearing brain tissue
NAGM – normal appearing grey matter
NAWM – normal appearing white matter
NBV - normalized brain volume
NCX – Na⁺/Ca²⁺ exchanger
NHNN – National Hospital for Neurology and Neurosurgery
PASAT-3 - Paced Auditory Serial Addition Test – 3 minute version
PD – proton density
PH – peak height
PL – peak location
PML – progressive multi-focal leukoencephalopathy
pu – percent units
QA – quality assurance
RFH – Royal Free Hospital
SCCA – spinal cord cross-sectional area

SIENA – Structural Image Evaluation using Normalization of Atrophy [Longitudinal method]

SIENAX – Structural Image Evaluation using Normalization of Atrophy [Cross-sectional method]

SPM – statistical parametric mapping

T1LV – T1 hypointense lesion volume

T2LV – T2 hyperintense lesion volume

TE – echo time

TI - inversion time

TR – repetition time

TW – 25 foot timed walk

WMF - white matter fraction

Acknowledgements

The data that are presented in this thesis were taken from ‘A randomized controlled trial of neuroprotection with Lamotrigine in secondary progressive MS’. The protocol for the trial was devised by a steering committee comprising Dr D Altmann, Dr R Brenner, Dr J Chataway, Prof N Fox, Prof RAC Hughes, Prof K Smith and Prof DH Miller. It was chaired by Dr R Kapoor who was the principal investigator. The trial was funded by the MS Society of Great Britain and Northern Ireland.

All patients in the trial were recruited by Dr J Furby and myself, but we are indebted to the consultants at the National Hospital for Neurology and Neurosurgery (NHNN), particularly Prof G Giovannoni and Prof AJ Thompson, MS research nurse specialists Sara Soudain and Maria Espasandin, MS nurse specialist for St Albans Hospital Loraine Lenahan and neurologists from all over the UK, particularly Dr J Chataway and Dr R Brenner, for their kind assistance with this.

The trial was sponsored by UCL Research and Development (R&D) department; randomization was done by T Brady at ‘The Sealed Envelope’ and the investigational medicinal product was supplied by GlaxoSmithKline (GSK). We are particularly grateful to Dr R Lai at GSK and Y Enever in the UCL (R&D) department for their assistance with safety monitoring.

Clinical care during the trial was provided by Dr J Furby and me, with close assistance from Dr R Kapoor and Prof DH Miller. All clinical data were collected by Dr J Furby and me, with help from Dr S Gnanapavan, who also processed all the blood tests and biomarkers for the study, Dr A Henderson and Dr M Kolappan, who

performed ocular coherence tomography. Training in the use of the clinical data tools was provided by Dr D Soon, Dr R Farrell and Dr Z Khaleeli, and advice on correcting for the learning effect in paced auditory serial addition test (PASAT-3) was provided by Dr J Cohen. Advice on dosage and escalation of the investigational medicinal product was provided by Prof J Duncan.

Magnetic resonance imaging (MRI) data was collected by the radiographers in the NMR unit scanner at the Institute of Neurology – R Gordon, C Benton, K Wimpey, A Berry, K Chappell and M Yiannakos. AB, KC and MY were also kind enough to have their brains scanned for quality assurance (QA). MRI data was anonymized and archived by K Hunter, who also created and maintained the database for clinical data and automated some of the analysis protocols.

Manual stages of all T1 hypointense lesion volume and magnetization transfer ratio (MTR) analysis were done by me, along with half of the analysis of T2 hyperintense lesion volume, active T2 lesion number and central cerebral volume. The manual components of the remaining analysis were done by Dr J Furby. Automated parts of the analysis were based on programmes written by Dr D Tozer, who also provided physics advice, and adapted by K Hunter.

We are indebted to Dr D Soon and Prof DH Miller for their training and advice on lesion volume analysis, to Dr K Mizkiel for her advice and assistance with active lesion identification and to Dr V Anderson for her training and advice on brain volume measures. Additional advice on MTR analysis was provided by Dr Z Khaleeli and Dr M Cercignani.

All statistical analyses presented in this thesis were performed by me with extensive input from Dr D Altmann. All text and figures were written or constructed by me with editorial input from my co-supervisors Prof D H Miller and Dr R Kapoor. Finally I would like to thank the 120 people with MS and their partners, friends and families for all their efforts taking part in the study and my wife Anita who has put up with more six hundred late dinners and Lamotrigine related conversations during the course of the trial.

Copyright note: chapters 4, 5 and 7 have been adapted from journal articles:

1. **Hayton T**, Furby J, Smith KJ, Altmann DR, Brenner R, Chataway J, Hughes RAC, Hunter K, Tozer DJ, Miller DH, Kapoor R. Grey matter magnetization transfer ratio independently correlates with neurological deficit in secondary progressive multiple sclerosis. *J Neurol* (2009) 256, 427-435 - <http://www.springerlink.com/content/u15u7n446x052185/?MUD=MP>
2. **Hayton T**, Furby J, Smith KJ, Altmann DR, Brenner R, Chataway J, Hunter K, Tozer DJ, Miller DH, Kapoor R. Longitudinal changes in magnetization transfer ratio in secondary progressive multiple sclerosis: data from a randomized placebo controlled trial of lamotrigine. *J Neurol* (2012) 259, 505-514. - <http://www.springerlink.com/content/n6j2g72g76q877u6/>
3. **Hayton T**, Furby J, Smith KJ, Altmann DR, Brenner R, Chataway J, Hunter K, Tozer DJ, Miller DH, Kapoor R. Clinical and imaging correlates of the multiple sclerosis impact scale in secondary progressive multiple sclerosis. *J Neurol* (2012) 259, 237-245 - <http://www.springerlink.com/content/t8k6410733055885/>

The final publications are available at www.springerlink.com

1. Introduction

1.1. Multiple sclerosis (MS)

Multiple sclerosis (MS) is a chronic demyelinating disease of the central nervous system (CNS) and one of the commonest causes of neurological disability among young people in the western world (Sadovnick and Ebers 1993). There are two major clinical phenotypes: relapse-onset, which accounts for approximately 85% of cases and primary progressive MS which accounts for the remaining 15% (Weinshenker et al 1989).

People diagnosed with relapse-onset MS experience recurrent episodes of acute, focal neurological dysfunction (relapse) with a variable level of recovery separated by periods of relative functional stability (remission) whereas the clinical course of primary progressive MS is the insidious development of progressively severe irreversible disability from the start (Thompson et al 2000).

In the majority of people with relapse-onset MS the relapsing-remitting stage is followed by the gradual accumulation of irreversible neurological disability (Weinshenker et al 1989) which is termed secondary progressive MS (Lublin and Reingold 1996). The majority of long term disability arises in this secondary progressive phase (Confavreaux et al 2000, Leray et al 2010, Scalfari et al 2010).

The age at the onset of progression in relapse onset MS is approximately the same as the age of onset for primary progressive MS and the rate at which progression occurs

is very similar in both conditions (Confavreaux et al 2000, Tremlett et al 2005, Tremlett et al 2008, Leray et al 2010). Furthermore the rate of progression in secondary progressive MS is the same irrespective of the incidence of superimposed relapses, perhaps suggesting that the pathophysiological process giving rise to progression may be different from that giving rise to relapses (Confavreaux et al 2000).

1.2. Pathology of MS

MS is a pathologically heterogeneous condition, with focal lesions in both grey (Kidd et al 1999, Peterson et al 2001, Bo et al 2003a, Bo et al 2003b Kutzelnigg et al 2005) and white matter (Lucchinetti et al 2000), diffuse white matter inflammation (Kutzelnigg et al 2005), diffuse axonal loss (Evangelou et al 2000, Evangelou et al 2005), global brain atrophy and widespread grey matter demyelination (Kutzelnigg et al 2005) and B-cell infiltration of the leptomeninges (Serafini et al 2004). The features of relapsing-remitting, primary progressive and secondary progressive MS differ, both in terms of the characteristics of focal white matter lesions and in the burden of non-lesion pathology (Kutzelnigg et al 2005).

White matter lesions

White matter lesions may demonstrate evidence of: inflammatory activity - activated microglia and macrophages, T-cells and plasma cells (although the latter are less common) - and demyelination with or without partial remyelination (Luchinetti et al 2000). Lesions can be described in terms of severity of inflammatory activity as acutely active, chronically active and chronic inactive lesions. This has been done

using immunohistochemistry major histocompatibility complex (MHC) class II markers (van der Valk and der Groot 2000) and qualitatively, defining active lesions as those with large numbers of macrophages containing early oligodendrocyte breakdown products (Bruck et al 1995); chronic active lesions as those demonstrating mild to moderate microglial activation and scattered macrophages especially at the outer edge of lesions; and chronic inactive lesions as those which have a well defined edge, devoid of macrophage infiltration (Kutzelnigg et al 2005).

Focal white matter lesions also show evidence of axonal damage, dysfunction (Diem et al 2007), with build up of amyloid precursor protein (Ferguson et al 1997), transection and retraction (Trapp et al 1998, Bitsch et al 2000), with axonal transection being most prominent in acute active lesions and around the edge of chronic active lesions, where the level of inflammatory activity is highest (Ferguson et al 1997, Trapp et al 1998).

Grey matter pathology

Lesions occur in both cortical grey matter (Kutzelnigg et al 2005, Bo et al 2003a, Bo et al 2003b, Peterson et al 2001, Kidd et al 1999) and deep grey structures (Brownell and Hughes 1962, Cifelli et al 2002). The number of lesions detected in cortical pathological samples can be substantial with one study identifying 478 lesions in 12 post mortem cases (Kidd et al 1999). Cortical lesions have also been sub-classified according to site and structural characteristics. One proposed system identifies 4 different lesion types: type 1, involving both grey matter and subcortical white matter; type 2, small intracortical lesions; type 3, superficial, sub-pial lesions; and type 4,

extending from the pial surface up to, but not beyond, the grey matter-white matter border (Bo et al 2003a, Bo et al 2003b). Type 3 and 4 lesions may be extensive, covering adjacent gyri and effectively causing general cortical demyelination (Bo et al 2003a, Bo et al 2003b).

In contrast to focal white matter lesions, grey matter lesions show very little infiltration with inflammatory cells (Kidd et al 1999, Bo et al 2003a, Bo et al 2003b). In fact the majority of inflammatory activity is within the white matter portion of type 1 lesions (Guerts et al 2005). On the other hand, grey matter lesions are associated with dendritic and neuroaxonal transection (Peterson et al 2001). In secondary progressive forms of the condition B-cell infiltration of the leptomeninges has also been found and may be involved in the development of sub-pial grey matter lesions (Serafini et al 2004, Magliozzi et al 2007, Serafini et al 2007, Magliozzi et al 2010, Howell et al 2011, Lovato et al 2011, Lucchinetti et al 2011).

Differences between relapsing-remitting and progressive MS

A recent study compared pathological findings from 52 people with MS (Kutzelnigg et al 2005). Although the total volume of white matter lesions was significantly higher in samples from people with progressive MS than in relapsing-remitting samples, the vast majority of the lesions in progressive MS were inactive, whereas the samples from relapsing-remitting patients had a significantly higher proportion of active lesions. Grey matter lesions, activated white matter microglia and transected axons in non-lesion white matter were significantly more prominent in samples taken from people with progressive MS.

In the samples from people with progressive MS there were significant correlations between the extent of diffuse white matter pathology and grey matter demyelination. However there were no significant correlations between focal white matter pathology and diffuse white matter pathology or grey matter demyelination (Kutzelnigg et al 2005). A further pathological study demonstrated relatively limited correlation of the burden of focal MS plaques with both brain weight and axonal content in the spinal cord (De Luca et al 2006).

A third study comparing cord tissue samples from people with primary and secondary progressive MS showed that while the level of demyelination was higher in secondary progressive MS, the degree of axonal loss was comparable in both groups, perhaps suggesting that axonal loss may not be entirely dependent on demyelination (Tallantyre et al 2009).

1.3. Pathophysiology of MS

The mechanisms by which the pathological changes seen in MS give rise to symptoms are incompletely understood. Data from animal models of MS, both *in vitro* and *in vivo* (using experimental allergic encephalomyelitis; EAE), along with pathological and MRI data in humans, have allowed investigators to form hypotheses of the pathophysiology responsible for relapses, paroxysmal symptoms and neurological progression some of which are detailed below.

Relapses

Acute focal MS lesions show demyelination, inflammation and neuroaxonal damage (Bruck et al 1995, Trapp et al 1998, Bitsch et al 2000, Luchinetti et al 2000, Barnett and Prineas 2004) and all three of these processes may contribute to relapse-related neurological impairment.

In healthy white matter the intact myelin sheath provides an insulating layer that allows rapid propagation of the action potential. Voltage gated sodium channels are almost exclusively located at the nodes of Ranvier with very low levels on the internodal membrane (Ritchie and Rogert 1977). Acutely demyelinated internodes are therefore essentially devoid of sodium channels (Shrager 1989). Nodal sodium currents may be insufficient to depolarise the denuded axolemma, leading to a conduction block in affected axons and dysfunction in affected pathways (McDonald and Sears 1969, Waxman 1989, Youl et al 1991).

Products of inflammation may also contribute to conduction block (Moreau et al 1996). One of the more prominent inflammatory molecules implicated in conduction block is nitric oxide (NO) which is present in MS lesions (Bagasra et al 1995, Johnson et al 1995a), is raised in the cerebrospinal fluid (CSF) in MS (Giovannoni et al 1997) and has been shown to induce conduction block in vitro (Redford et al 1997). The mechanism by which NO may contribute to conduction block is not fully understood but possibilities include interaction with sodium channels (Ahern et al 2000, Renganathan et al 2002), mitochondria (Bolanos et al 1997) or with the sodium-potassium ATP-ase (Guzman et al 1995) with consequent axonal depolarization (Garthwaite et al 2002).

Recovery of function - remission

Following the resolution of acute inflammation (Miller et al 1988, Larsson et al 1988) there is restored conduction but persistent electrophysiological evidence of conduction delay (Halliday et al 1972, Small et al 1978, Robinson and Rudge 1977, Youl et al 1991).

The resolution of oedema (Larsson et al 1988, Miller et al 1988) with reduction in potentially harmful inflammatory products may contribute to restoration of conduction. Immunological studies in both EAE (Craner et al 2003, Craner et al 2004a) and MS (Moll et al 1991, Craner et al 2004b) indicate that there may also be a proliferation and redistribution of voltage gated sodium channels on sections of chronically demyelinated axolemma which may help improve action potential propagation. Finally in new lesions there is evidence of partial remyelination (Prineas et al 1993, Patrikios et al 2006, Patani et al 2007, Goldshmidt et al 2009) which should contribute to restoring normal axonal function.

Intermittent symptoms

Paroxysmal functional deficit can occur in previously affected pathways, possibly through temporary conduction block. For example small increases in body temperature can shorten sodium channel opening time, decreasing the likelihood that the membrane will depolarise sufficiently to reach the firing threshold, potentially resulting in heat sensitive symptoms such as Uhthoff's phenomenon (Uhthoff 1890). Sustained electrical activity may impair depolarization through induction of the

electrogenic Na/K ATPase pump which hyperpolarizes the membrane (Bostock and Grafe 1985) which may result in exercise induced neurological deficit.

The restructured axolemma may also be prone to ectopic activity (Smith and McDonald 1982), leading to paroxysmal positive symptoms including: neurogenic pain, positive sensory phenomena and tonic spasms; mechanosensitive discharges such as Lhermitte's phenomenon (Lhermitte 1920) or visual phosphenes.

Progression

The lesion burden seen on T2 weighted images increases with time (Rudick et al 2006a, Fisniku et al 2008a) and there is a correlation of lesion burden with disability (Li et al 2006). Progressive disability may therefore arise partly from the progressive acquisition of a greater burden of focal demyelinated lesions. However there is a growing body of evidence indicating that progressive neuroaxonal loss may also be partly responsible for progressive disability.

The correlation of focal lesion burden with disability is modest (Miller et al 2002) and is weaker in people who have higher levels of disability (Li et al 2006). Furthermore medications that have been shown to reduce the number of new focal lesions and relapses (Paty and Li 1993, Kappos et al 1998, Coles et al 1999, Jacobs et al 2000, Comi et al 2001a, Kappos et al 2001, Li et al 2001, Cohen et al 2002, Panitch et al 2004, Kappos et al 2006) have limited effect on brain atrophy (Coles et al 1999, Molyneux et al 2000a) or progressive disability (Coles et al 1999, Kappos et al 2001).

Natural history studies also indicate that after the first few years, the impact of relapses on progressive disability is limited (Weinshenker et al 1989, Scalfari et al 2010), raising the possibility that a second pathological process may in part be responsible for progressive MS.

Pathological studies show axonal transection in focal lesions (Trapp et al 1998), diffuse axonal loss in normal appearing white matter (Evangelou et al 2000, Evangelou et al 2005) and gross generalized brain atrophy (Kutzelnigg et al 2005). Although brain atrophy occurs in even the earliest stages of MS (Dalton et al 2004) the extent of atrophy is greater in progressive forms of the condition (Kutzelnigg et al 2005) and it correlates with the level of neurological deficit and disability (Miller et al 2002). Although there is axonal damage in acute lesions (Ferguson et al 1997, Trapp et al 1998, Bitsch et al 2000) it is possible that there is also gradual progressive loss of chronically demyelinated axons.

The possible role of nitric oxide, sodium and calcium metabolism in progressive disease

A number of hypotheses have been developed to explain why chronically demyelinated axons could gradually degenerate. One of these involves the interaction between inflammatory molecules, including NO, and adaptive processes, particularly proliferation of sodium channels on the denuded axolemma.

In experimental models of inflammation, axons can degenerate when exposed to nitric oxide, particularly if they are electrically active (Smith et al 2001). In models of ischaemia, axons loaded with sodium are at risk of degeneration because of the secondary influx of calcium ions through the reverse function of the membrane Na⁺/Ca²⁺ exchanger (NCX) (Stys et al 1992, Bechtold and Smith 2005). This may be exacerbated by the presence of nitric oxide and notably axonal degeneration due to nitric oxide can be blocked by inhibition of sodium channels or of the NCX (Kapoor et al 2003).

It is known that there is a proliferation of sodium channels on chronically demyelinated axons (Craner et al 2004a) with colocalisation of NCX (Craner et al 2004b) and it is possible that this can lead to increased intracellular sodium concentration, reversal of the NCX and an influx of cytotoxic calcium. While many other potentially cytotoxic processes have been identified that could also give rise to progressive disability in MS, the wide availability of relatively safe sodium channel blocking drugs means that inhibition of toxic sodium loading is an attractive target for potentially neuroprotective medication.

1.4. Quantifying Neurological Deficit in MS

Several clinical scales have been developed to quantify MS related neurological impairment based on disability, function and quality of life. A few of these are in widespread use and it is necessary to be familiar with them in order to make sense of much of the data published from MS studies. This section gives an introduction to the three clinical scales that have been used in this study.

Expanded Disability Status Scale (EDSS)

The most commonly used tool for quantifying clinical disability in MS is the expanded disability status scale (EDSS) (Kurtzke 1983). This is an ordinal scale in which an assessing clinician examines 8 functional systems – visual, brainstem, pyramidal, cerebellar, sensory, bowel/bladder, cerebral and ambulatory function - and assigns a score of 0-5 for each one (0-6 in the case of the visual system) according to defined examination findings. An overall score from 0-10 is assigned based on the combined scores from each functional system and an estimation of mobility and independent function: zero represents no detectable neurological deficit and 10 death due to MS.

Despite being widely used, the EDSS is heavily weighted towards mobility and relatively insensitive to upper limb, cognitive and visual dysfunction (Cutter et al 1999); has relatively poor intra- and inter-rater reproducibility (Noseworthy et al 1990); and is vulnerable to subjective investigator bias (Noseworthy et al 1994). Because of this the National MS Society established a task force to develop a more objective and reproducible assessment scale. The result was the multiple sclerosis functional composite (MSFC).

Multiple Sclerosis Functional Composite (MSFC)

In 1999 the National MS Society Task Force on Clinical Outcomes Assessment analyzed 14 large treatment trials in MS, evaluating 10 separate measures of neurological impairment. Of these 3 were selected on the basis that they best fit the

criteria of:

- reflecting the major dimensions of multiple sclerosis
- avoiding redundancy
- being simple to administer
- being sensitive to change over time

The three chosen outcome measures were: a measure of mobility, the 25 foot timed walk (TW); a measure of upper limb function, the 9-hole peg test (9HPT); and a measure of cognitive function, the paced auditory serial addition test – three minute version (PASAT-3). For each of these measures a score is calculated, reflecting how far an individual's performance deviates from the mean (z-score) for either the study group in question or for the MS population used by the task force (Fischer et al 1999). A combined z-score can be calculated from all 3 components, the MSFC.

The MSFC and component measures have since been validated by comparing scores against both the EDSS (Cutter et al 1999, Cohen et al 2001, Kalkers et al 2000, Fischer et al MS 1999) and a self reported quality of life questionnaire (Miller et al 2000). Significant correlations have been found between the MSFC and EDSS (Cutter et al 1999, Fischer et al 1999, Kalkers et al 2000, Cohen et al 2001, Rudick et al 2001, Miller et al 2000) with moderate to strong associations of performance in the timed walk and the 9-hole peg test with EDSS and more modest correlation of performance in the PASAT-3 with the EDSS (Cutter et al 1999, Cohen et al 2001, Kalkers et al 2000). In a longitudinal study, MSFC at baseline correlated with both EDSS and MSFC at 2 years and change in MSFC correlated with change in EDSS over the same time (Rudick et al 2001).

Although performance in components of the MSFC (PASAT-3 and 9HPT) are subject to a substantial learning effect (Cohen et al 2001, Solari et al 2005), inter-rater and intra-rater reproducibility was found to be good when compared to the EDSS (Cohen et al 2000).

The range of values for the timed walk, the 9-hole peg test and the MSFC was greater in people with progressive MS than in those with relapsing-remitting MS (Kalkers et al 2000) and greater in people with higher EDSS (Miller et al 2000, Cutter et al 1999), while the range of the PASAT-3 was similar in all clinical phenotypes and EDSS groups, perhaps indicating that the MSFC may be the more sensitive measure.

The MSFC does have some limitations. Notably the PASAT-3 is subject to a marked learning effect, which can make the interpretation of longitudinal data difficult (Polman and Rudick 2010); it is also relatively unpopular with subjects (Aupperle et al 2002) and the Symbol-Digit Modalities test has been considered as a possible replacement for this component of the MSFC (Drake et al 2010). The MSFC also does not include a test of visual function although future revisions may include the Low Contrast Letter Acuity Test (Balcer and Frohman 2010).

Overall in its present form the MSFC is highly reproducible, correlates with established objective and subjective measures of disability, is predictive of and sensitive to change in neurological deficit and may be more sensitive than the EDSS in people with substantial motor disability and progressive forms of the condition.

However whereas the EDSS can be easily and intuitively interpreted by any clinician familiar with the standard neurological examination, the connection between MSFC and clinical status is not immediately apparent and the interpretation is not so straightforward. To allow data from clinical trials to be widely interpreted it is convention to include and compare both measures in clinical studies.

Multiple Sclerosis Impact Scale (MSIS-29)

Investigators' assessment and patients' perception of the impact of neurological impairment can differ (Rothwell et al 1997). When EDSS has been tested against quality of life measures the results have been mixed, some showing no correlation of EDSS with quality of life (Simeoni et al 2008) some showing correlations with elements specifically dealing with physical function (Amato et al 2001, Gold et al 2001, Lobentanz et al 2004) and some showing correlation with several aspects of quality of life (Janhardan and Bakshi 2000, Nordvedt et al 2000, Benito-Leon et al 2002, Isaksson et al 2005). Several MS specific questionnaires have been proposed all with the aim of evaluating the impact of MS on patient experience and quality of life (Mitchell et al 2005).

The Multiple Sclerosis Impact Scale MSIS-29 consists of 29 items; 20 related to the physical impact of MS (MSIS-phys) and 9 related to the psychological impact (MSIS-psych). It was generated through a combination of structured interviews and surveys of 1530 randomly chosen people with MS, identifying 29 key questions from an initial battery of 129. The questionnaire was compared with widely used generic health impact scales and validated with a second sample of 1250 people with MS with

responsiveness being gauged by studying 50 patients being treated for acute relapses (Hobart et al 2001).

Subsequent studies have shown the MSIS-29 to correlate with: clinician assessed disability in patients being treated for acute relapses or for inpatient rehabilitation (Riazi et al 2002); and with objective measures of mobility, cognitive and upper limb impairment, the MSFC (Hoogervorst et al 2004, Costelloe et al 2008).

1.5. Magnetic resonance imaging (MRI) in MS

MRI allows investigators to detect and quantify pathology *in vivo* and so can provide extensive information regarding the natural history of MS and how different pathological processes may contribute to the development of neurological deficit. In clinical practice MRI has become an integral part of the diagnosis of MS (Polman et al 2005) and in the UK has been included as part of the decision making process for treatment with one of the disease modifying agents (Multiple sclerosis - natalizumab for the treatment of adults with highly active relapsing-remitting multiple sclerosis: Final appraisal determination – NICE 2007).

Quantitative MRI has become one of the most widely used tools in both natural history studies and treatment trials. International consensus committees on the investigation and treatment of multiple sclerosis have recommended that MRI outcomes be used to evaluate the efficacy of new treatments (Miller et al 1991, Whitaker et al 1995, Miller et al 1996, Filippi et al 1998a, Filippi et al 2002). The

following section is a brief review of some of the quantitative MRI modalities used in MS.

T2 weighted imaging

The most commonly used diagnostic MRI modalities are T2-weighted fast spin echo and fast fluid attenuated inversion-recovery (FLAIR) imaging sequences. Combined pathological and radiological studies have shown high signal lesions on T2 weighted imaging to be sensitive for the detection of focal white matter lesions (Stewart et al 1984, Ormerod et al 1987). The presence of T2 hyperintense lesions is central to the current, widely accepted diagnostic criteria for both primary progressive (Thompson et al 2000, McDonald et al 2001, Polman et al 2005, Polman et al 2011) and relapse-onset MS (McDonald et al 2001, Polman et al 2005, Polman et al 2011).

T2 hyperintense lesion burden, number or total volume, is also used as a quantitative measure of MS pathology. One long term follow up study of clinically isolated syndrome (CIS) suggestive of demyelination indicates that during the first 15 years of follow up there was a significant correlation between the increase in lesion volume and disability (Fisniku et al 2008a). However the correlation between lesion volume measures and measures of clinical impairment is poor and the discrepancy between lesion volume and disability, the so-called 'radiological-clinical paradox' gets more substantial as the condition progresses (Li et al 2006). Some cross-sectional studies comparing people with progressive MS with age and gender matched patients with a more benign disease course have shown that both groups can have similar lesion

volumes but radically different levels of disability (Thompson et al 1990, Filippi et al 1994). Furthermore in a trial of interferon β -1b in 718 subjects with secondary progressive MS, the correlation between change in T2 weighted lesion volume and change in disability over 3 years was not significant (Kappos et al 1998, Molyneux et al 2001).

T2 lesions can be histologically heterogeneous comprising varying levels of inflammation, axonal loss (Newcombe et al 1991) and remyelination (Barkhof et al 2003). Thus if a person has a large volume of less severely damaged or remyelinating lesions they may be less neurologically compromised than an individual with a smaller number of more severe lesions.

In addition detection of focal lesions on T2 weighted imaging gives limited information about surrounding white matter and, using a standard sequence at 1.5 Tesla (the magnetic field strength of many standard MRI scanners), is relatively insensitive for grey matter lesions (Guerts et al 2005). Since extensive pathology is seen in both of these regions, particularly in secondary progressive MS (Kutzelnigg et al 2005), much of the brain pathology that may be contributing to neurological deficit, may be overlooked by basic T2 lesion burden measures.

Although recent meta-analysis has identified that compound measures combining the number of new/active lesions and relapse rate can explain a large proportion of the variability in EDSS progression (Sormani et al 2009, Sormani et al 2011), much effort

has been invested in devising more sensitive and specific MRI markers of brain and cord pathology.

Gadolinium DTPA enhancing lesions

Gadolinium DTPA (Gd) is a paramagnetic contrast agent that crosses an abnormal blood-brain barrier (Grossman et al 1986, Miller et al 1988, Hawkins et al 1990, Katz et al 1993, Soon et al 2007) to give a high signal on T1 weighted imaging.

The incidence of Gd enhancing lesions correlates with relapse rate in secondary progressive MS (Tubridy et al 1998) and the incidence of enhancing lesions over 6 months is weakly predictive of disability at 24 months, but longitudinally there is no significant correlation between the incidence of enhancing lesions and change in disability (Kappos et al 1999).

Gd enhancing lesion number is felt to be a good MRI marker of acute focal neuroinflammation, so is widely used in studies of immunomodulatory treatments (for recent examples see Polman et al 2006, CAMMS223 Trial Investigators 2008, Giovannoni et al 2010, Kappos et al 2010). However it is not thought to be a sensitive measure of neurodegeneration and so measures such as brain atrophy (see section 1.5.4) have been recommended as more suitable MRI outcomes in studies of potential neuroprotective agents (Filippi et al 2005).

Magnetic Resonance (MR) Spectroscopy

Magnetic resonance (MR) spectroscopy enables quantification of several specific organic compounds within a chosen region of the brain or spinal cord. Examples include compounds such as N-acetyl aspartate (NAA), an amino acid derivative that is relatively specific for neurons and axons (Tallan et al 1956, Petroff et al 1995, Urenjak et al 1993); creatine/phosphocreatine (Cr.), which is present in many cell types and gives a strong, easily detected signal (Urenjak et al 1993); and choline containing compounds (Cho) which are also detected in a range of neural cells (Urenjak et al 1993), but are found at high concentration in newly enhancing MS lesions and so are thought to represent acute demyelination or inflammation (Davie et al 1994, Brenner et al 1993). MR spectroscopy has the potential to give additional information about the level of demyelination and axonal loss in brain tissue, particularly that which is normal appearing on standard T2 weighted imaging, and may be more suitable than T2 lesion burden for monitoring neurodegeneration.

Low global NAA/Cr ratio may reflect neuronal and axonal loss with relative preservation or increase of interstitial cells such as macrophages or reactive microglia. NAA/Cr ratio is low in people with MS compared with controls and is most marked among people with secondary progressive MS (De Stefano et al 1998, De Stefano et al 2001). In a study comparing chronic white matter lesions in people with relapsing remitting MS with a group of people with secondary progressive MS and significantly greater disability, NAA/Cr and NAA/Cho ratios were significantly lower in subjects with secondary progressive MS (Falini et al 1998) possibly indicating more severe axonal loss. However in other studies the cross-sectional and longitudinal correlations

with disability were stronger in the relapsing-remitting subgroup (De Stefano et al 1998, De Stefano et al 2001).

Overall normal appearing white matter (NAWM) NAA concentration has been investigated numerous times in relapsing-remitting (Chard et al 2002a, Helms et al 2000, Schubert et al 2002, van Walderveen 1999a, Vrenken et al 2005, Sarchielli et al 1999) secondary progressive (Helms et al 2000, Schubert et al 2002, van Walderveen et al 1999a, Vrenken et al 2005, Sarchielli et al 1999) and primary progressive MS (Vrenken et al 2005, Davie et al 1997, Suhy et al 2000). Some studies show lower NAA levels in MS than in normal controls (Leary et al 1999a, Sarchielli et al 1999, van Walderveen et al 1999a, Cucurella et al 2000), while others show no significant difference (Chard et al 2002a, Davie et al 1997, Helms et al 2000, Schubert et al 2002). Sarchielli and colleagues reported that in their cohort of 40 people with MS, lower NAWM NAA correlated with higher EDSS (Sarchielli et al 1999).

Grey matter NAA concentration has also been shown to be lower in secondary progressive MS than in relapsing-remitting subjects (Adalsteinsson et al 2003). However there was no significant correlation between grey matter NAA concentration and disability.

MR spectroscopy has been shown to be reproducible across several centres (Geurts et al 2004, Benedetti et al 2007) so has generated interest as a potential marker for brain pathology in future treatment trials. However problems with low signal to noise ratio

(De Stefano et al 2007), questions over the specificity of NAA (Bhakoo and Pearce 2000), the relatively limited correlation with disability in people with progressive MS (De Stefano et al 1998), and the observation that NAA/Cr ratio can increase following treatment (Narayanan et al 2001) indicating that observed decreases may be reversible, mean that it may not be the best technique for use in trials of possible neuroprotective agents in progressive MS.

Brain atrophy

Pathological studies have indicated that there may be significant brain and spinal cord tissue loss in MS (Evangelou et al 2000, Kutzelnigg et al 2005) which is more marked in progressive forms of the condition (Kutzelnigg et al 2005). To evaluate whether this tissue loss has any impact on disability, investigators have assessed brain and spinal cord atrophy on T1 weighted MRI. Techniques developed include regional two and three dimensional volume measures, global volume measures and more sophisticated techniques involving co-registration of serial images (Miller et al 2002). Whatever measure is used, rates of cerebral atrophy are higher in people with multiple sclerosis than in normal controls (Kalkers et al 2001a, Fox et al 2000, Anderson et al 2006).

MRI detectable brain atrophy has been observed in the very earliest stages of MS. For example in people with CIS there was significantly more brain atrophy in those subjects who subsequently went on to develop MS over one or three years compared to those who did not (Brex et al 2000, Dalton et al 2004). Although some studies have

suggested that the rate of atrophy is greater in people with relapsing-remitting MS than in those with secondary progressive MS (Redmond et al 2000, Pagani et al 2005, Filippi et al 2000a), the other studies indicate that rates of change are similar in both groups (Fox et al 2000, Kalkers et al 2002, Turner et al 2003). In the case of ventricular enlargement (a two dimensional measure of brain atrophy) the rate of change has been found to be greater in people with secondary progressive MS (Dalton et al 2006).

Many studies have shown correlation between brain atrophy measures and measures of neurological deficit in MS. People with MS who show greater disease progression also show greater central cerebral atrophy (Losseff et al 1996a, Coles et al 1999, Molyneux et al 2000a, Ingle et al 2002) regional atrophy (Gasperini et al 2002) and whole brain atrophy (Rudick et al 1999). In a mixed group of 28 people with MS including six people with secondary progressive MS there was significant correlation between change in brain volume and change in EDSS (Fox et al 2000). A similar result was found in a large study assessing central cerebral volume in a group of 239 people with relapsing-remitting MS (Rovaris et al 2001) while in a group of 160 patients with relapsing-remitting MS, change in whole brain volume over two (Fisher et al 2000), six (Rudick et al 2001) and eight years (Fisher et al 2002) correlated with both EDSS and, at the six year timepoint, MSFC.

Recently interest has been focused on grey matter atrophy (Bermel et al 2003, De Stefano et al 2003, Sailer et al 2003, Amato et al 2004, Houtchens et al 2007). Selective grey matter atrophy has also been observed in CIS (Dalton et al 2004) and

early relapsing-remitting MS (Chard et al 2002b, Chard et al 2004, Tiberio et al 2005). Selective grey matter atrophy has also been identified in people with early primary progressive MS (Sastre-Garriga et al 2004) and secondary progressive MS (Sanfilippo et al 2005, Tedeschi et al 2005, Furby et al 2008). In addition grey matter atrophy correlates with both higher EDSS (Sanfilippo et al 2005, Tedeschi et al 2005) and neurological deficit measured with the MSFC (Furby et al 2008).

Brain atrophy measures are responsive, reproducible, well correlated with disability, and in the case of newer techniques, semi- or fully automated (Anderson et al 2006). As a consequence brain atrophy techniques are now commonly included as an endpoint when testing the neuroprotective potential of any medication. In this study a number of regional and whole brain atrophy measures were used, both to evaluate the effect of presumed neurodegeneration on clinical function and to assess the neuroprotective potential of lamotrigine.

Spinal cord atrophy

Much of the clinical impairment in people with MS is thought to be due to pathology of the spinal cord, which is known to be a common site for neuroinflammation and axonal loss (Oppenheimer 1978, Lovas et al 2000). Pathological studies of human tissue and studies in animal models of MS have confirmed that axonal loss is responsible for much of the observed spinal cord atrophy (Lovas et al 2000, McGavern et al 2000).

There is considerable evidence that clinical disability, estimated using the EDSS, correlates with spinal cord cross-sectional area (SCCA) (Losseff et al 1996b) and that changes in SCCA over time correlate with changes in EDSS (Stevenson et al 1998, Lin et al 2003a, Lin et al 2003b).

Measures of spinal cord atrophy have been identified as potentially sensitive marker of axonal loss in chronic MS. An international consensus group report has recommended that measures of spinal cord atrophy be included in trials evaluating proposed treatments in progressive MS (Filippi et al 2005).

T1 hypointense lesions

Part or all of some lesions that are high signal on T2 weighted MRI are hypo-intense on T1 weighted MRI. In a proportion of T2 lesions the hypointensity on T1 weighted imaging represents acute inflammation and is associated with Gd enhancement (van Waesberghe et al 1998a, Rovira et al 1999). A proportion of these lesions will become isointense as the enhancement wears off, a process that may represent a relative resolution of the inflammation and some remyelination (Bagnato et al 2003). Persistent T1 hypointensity, however, appear to characterize those focal lesions where the level of demyelination and axonal damage/loss is most severe (van Walderveen et al 1998, van Waesberghe et al 1999, Fisher et al 2007).

Although it is difficult to identify T1 hypointense lesions on post-mortem imaging, one study of five samples from people with progressive MS demonstrated that the lesions which were most hypointense on T1 weighted imaging tended to be hypo-

cellular, with a rim of reactive astrocytes (van Walderveen et al 1998). In combined pathological and radiological studies in progressive MS, lesions that were hypointense on T1 weighted imaging were more likely to be demyelinated (Fisher et al 2007), have lower axonal density (van Walderveen 1998, van Waesberghe et al 1999, Fisher et al 2007) and have greater axonal cross-sectional area, possibly due to pathological swelling (Fisher et al 2007), than those which were isointense with normal appearing white matter.

Finally an MR spectroscopic study of 12 people with secondary progressive MS and four with relapsing remitting MS found that NAA concentration was significantly lower in severely hypointense T1 lesions, whereas the concentration of Cho and Cr was relatively preserved (van Walderveen et al 1999a). The authors felt that this indicated a reduction in axonal density with proliferation of inflammatory and glial cells.

In cross-sectional studies the mean T1 lesion volume in subjects with secondary progressive MS was higher than in people with primary progressive (Stevenson et al 1999) or relapsing-remitting MS (Rovaris et al 1999, Tortorella et al 2000). Significant correlations of T1 lesion volume with EDSS (van Walderveen et al 2001) and upper limb function have been observed (Stevenson et al 1999). However in the latter study the correlation of T1 lesion volume with clinical function was no stronger than that of T2 lesion volume.

In a longitudinal study, comparing 46 people with secondary progressive MS with 29 people with relapsing-remitting MS who had an equivalent level of disability, T1 lesion load and ratio of T1/T2 lesion volume correlated with EDSS at baseline and 18 months. The increase in T1 lesion volume was significantly higher in the progressive group and this change correlated with change in EDSS. Finally both change in T1 and T2 lesion load were compared in multiple linear regression analysis and change T1 lesion load was found to be the only MR variable that correlated independently with change in EDSS (Truyen et al 1996). Similar results were obtained in a mixed group of 19 people with relapse-onset MS (van Walderween et al 1995). In this case there was a significant correlation between increase in EDSS and increase in T1 lesion volume, but not T2 lesion volume. Furthermore T1 lesion volume at baseline correlated with change in EDSS over 24 months.

T1 hypointense lesion burden may give a more specific measure of demyelination and neuroaxonal damage than T2 lesion volume so is potentially a better measure for use in studies of neurodegeneration and neuroprotection. However there are a number of factors which make brain volume measures more suitable as primary end-points: T1 lesion volume remains a relatively localized measure, giving little direct information about neuronal loss at distant sites; lesions which are acutely hypointense sometime represent areas of acute neuroinflammation or remyelination (Waesberghe et al 1998a, Rovira et al 1999, Bagnato et al 2003) and as such may not be as specific for neurodegeneration as brain atrophy; finally although automated techniques for contouring T1 hypointense lesions exist (Fisher et al 2007) these are not widely available; in this study a semi-automated technique was used, which was associated

with some intra-rater variability. T1 hypointense lesion volume measures were obtained to evaluate the correlation with other MRI measures, measures of clinical impairment and to quantify, what effect, if any, lamotrigine had on this measure.

Magnetization transfer ratio (MTR)

Magnetization transfer (MT) imaging is another quantitative MRI technique that, like MR spectroscopy, is thought to give a measure of the level of neuroaxonal damage and demyelination in a given tissue. MT imaging involves the use of an off-water-resonance radiofrequency pulse to saturate the nuclei of hydrogen atoms bound to large complex molecules - the "bound pool" - which are invisible using conventional MRI techniques. Magnetization is transferred from the saturated hydrogen atoms in the bound pool to hydrogen atoms in adjacent free water - the "unbound pool". This reduces the intensity of the signal seen in the image as the atoms in the free pool which previously contributed to the returned signal are now saturated. The greater the concentration of complex molecules in a tissue, the greater the reduction of the returned signal (Wolff and Balaban 1989). The ratio of pre- to post- saturation signal intensity from a given tissue, the magnetization ratio (MTR) therefore gives a measure of the macro-molecular content of the tissue.

An MRI study in early life demonstrated that MTR increases with maturation through childhood and that the sites of MTR increases corresponded to the sequence of myelination obtained from previous postmortem studies (Rademacher et al 1999). Furthermore pathological studies in MS have also shown that low MTR correlates

with low myelin content in both T2 high-signal lesions (Schemierer et al 2004, Barkhof et al 2003, Fisher et al 2007) and white matter that appears normal on T2 weighted MRI (van Waesberghe 1999, Vrenken et al 2006). MTR is also low in CNS demyelinating diseases other than MS, e.g. central pontine myelinolysis or adrenoleukodystrophy (Davie et al 1999, Fatemi et al 2005, Melhem et al 1996).

However low MTR may also denote neuroaxonal loss. In pathological studies low MTR correlates with low axonal content in both T2 lesions and normal appearing brain tissue (van Waesberghe et al 1999). In vivo T1 hypointense lesions, those more likely to show higher levels of axonal damage, have been shown to have low MTR compared to surrounding tissue (Heihle et al 1995, Filippi et al 1999, Fisher et al 2007), while low MTR has also been shown to correlate with other putative measures of axonal loss such as low NAA concentration (Davie et al 1999) and global and regional brain atrophy (Phillips et al 1998, Traboulsee et al 2003, Khaleeli et al 2007).

Overall MTR in whole brain (Kalkers et al 2001a, Rovaris et al 2001), normal appearing brain tissue (Traboulsee et al 2003) NAWM (Cercignani et al 2001, Ge et al 2002a, Dehmeshki et al 2003, Davies et al 2004, Beniek et al 2006, Vrenken et al 2007) and grey matter (Agosta et al 2006, Cercignani et al 2001, Davies et al 2004, Davies et al 2005a, Dehmeshki et al 2001, Ge et al 2002a, Griffin et al 2002) is lower in people with MS than age and gender matched controls.

Although not all studies have found significant correlations between MTR measures and disability (Rovaris et al 2001, Tortorella et al 2000, Davies 2005a, Davies et al 2005b Oreja-Guevara et al 2006), highly significant cross-sectional correlations have been identified of whole brain (Dehmeshki et al 2001, Kalkers et al 2001a, Inglese et al 2003), lesion (Gass et al 1994, Traboulsee et al 2003), normal appearing brain tissue (Traboulsee et al 2003, Rammio-Torrenta et al 2006), NAWM (Leary et al 1999b, Ramio-torrenta et al 2006) and normal appearing grey matter (NAGM) (Dehmeshki et al 2003, Davies et al 2004, Ramio-torrenta et al 2006) MTR measures with EDSS. Whole brain matter MTR measures have been found to correlate with cognitive function (Kalkers et al 2001a), grey matter MTR measures have been correlated with cognitive function (Ramio-torrenta et al 2006, Khaleeli et al 2007) upper limb function and mobility (Vrenken et al 2007, Davies et al 2004), while NAWM MTR has been shown to correlate with upper limb function and mobility (Ramio-torrenta et al 2006). MTR changes in normal appearing brain tissue and lesion MTR measures at baseline can predict later disability (Santos et al 2002, Rovaris et al 2003a) and correlate with changes in disability over time (Filippi et al 2004, Santos et al 2002).

In short, MTR appears to give a measure of both myelin and axonal content, is lower in people with MS than in age and sex matched controls with no known neurological deficit, changes with time, and correlates with disability and neurological deficits both cross-sectionally and longitudinally. MTR may be sensitive to change in subjects where a change in T2 lesion volume is not (Filippi et al 2000b). It is also a highly reproducible measure (Rovaris et al 1997, Sormani et al 2000, Inglese et al 2001) so is

appealing as a potential marker of disease progression. An international consensus conference has recommended that MTR measures be included as an adjunctive tool to monitor disease in clinical trials (Filippi et al 2005). In this study a large number of MTR measures have been acquired to evaluate the correlation with other MRI measures of pathology and to help elucidate the potential neuroprotective effects of lamotrigine in MS.

Diffusion tensor imaging (DTI)

Diffusion is the term used for the random motion of free water molecules. In intact neural tissue, particularly white matter, this motion is thought to be somewhat restricted and tends to be anisotropic with major axonal tracts. Pathological processes such as inflammation, demyelination and axonal transection will lift some of this restriction and allow localized relative isotropic diffusion of free water molecules. This can be detected with and quantified using MRI sequences termed diffusion weighted imaging (DWI) (Le Bihan et al 2001) one of which is diffusion tensor imaging (DTI) (Pierpaoli et al 1996).

The DTI characteristics of each tissue type – T2 hyperintense lesions, T1 hypointense lesions, grey matter and NAWM – differ from each other and the DTI characteristics of MS tissue differs from equivalent tissue types, where applicable, in non-MS controls (Rovaris et al 2005).

Studies have identified some correlation between diffusion imaging measures and clinical status and so it has the potential for use as an MRI endpoint in clinical trials (Rovaris et al 2005). However at the present time the exact nature of the pathological substrate underlying DTI abnormalities is not fully understood. DTI has not been used as an endpoint in this study, in which the principal focus is neurodegeneration, but may well appear as a secondary endpoint in future treatment trials.

1.6. Treatment of MS

At the moment the treatment of progressive MS is largely symptomatic. Although corticosteroids can accelerate recovery from acute relapses (Filippini et al 2008) and immunomodulatory drugs are used to effectively reduce relapse frequency, no medication has yet been developed which has been shown to provide long-term protection from progressive disability without the risk of serious side-effects (Edan et al 1997, Hartung et al 2002, Marriott et al 2010).

One of the aims of this study was to evaluate lamotrigine as a potential neuroprotective drug, with the hope that it could be shown to ameliorate some of the pathological processes that are thought to give rise to progressive disability. The following section is a brief review of the immunomodulatory drugs that are currently available followed by a summary of rationale behind testing the neuroprotective potential of lamotrigine.

Immunomodulatory drugs

Interferon β 1a and 1b have been tested extensively in both relapsing-remitting and progressive MS. Both compounds reduce the frequency of acute relapses in relapsing-remitting and secondary progressive MS by about 30% (Paty and Li 1993, Kappos et al 1998, Jacobs et al 2000, Comi et al 2001a, Li et al 2001, Cohen et al 2002, Panitch et al 2004, Kappos et al 2006) and reduce the number of new, radiologically detectable inflammatory lesions during the trial period (Panitch et al 2004, Li et al 2001, Cohen et al 2000, Leary et al 2003) and can reduce the proportion of subjects with sustained progression to a higher EDSS (Kappos et al 1998, Kappos et al 2004). Unfortunately neither have been shown to have any effect on the rate of progression of irreversible disability in entirely progressive forms of the condition (Panitch et al 2004, Li et al 2001, Cohen et al 2000, Leary et al 2003) and the apparent effect on disability in the relapsing cohorts may in fact be due to an effect on disability due to relapse (Ebers et al 2008).

One of the proposed MRI markers of neurodegeneration is brain atrophy (see section 1.7.4). People with relapsing-remitting MS in the verum arm of a trial of interferon β 1a had significantly less brain atrophy over the course of the study than those in the placebo group (Zivadinov et al 2007). However in a similar cohort these differences were not sustained in the long-term (Kappos et al 2006) and have not been seen in studies of primary (Montalban et al 2009) or secondary MS (Molyneux et al 2000a).

A second compound, glatiramer acetate, has also been shown to reduce relapse rate

and MRI activity in people with relapsing-remitting MS (Johnson et al 1995b, Johnson et al 1998, Comi et al 2001b) but, like interferon, has yet to be shown to ameliorate progressive disability (Wolinsky et al 2007)

Mitoxantrone, an antineoplastic agent, has been shown to reduce relapse rate in relapsing remitting and secondary progressive MS by approximately 66-76% (Hartung et al 2002, Edan et al 1997) with an associated mean improvement in EDSS compared with baseline (Hartung et al 2002). However potentially serious side-effects such as cardiotoxicity with a lifetime cumulative dose of $>100\text{mg/m}^2$ (Ghalie et al 2002) and the risk of promyelocytic leukaemia (Voltz et al 2004) have meant that in the UK the use of mitoxantrone is limited to people with rapidly progressive MS with both clinical and MRI evidence of active inflammation.

Several new compounds have been developed in recent years which may be more effective than interferon, with a less prohibitive side-effect profile than mitoxantrone. Natalizumab is a monoclonal antibody to the α_4 subunit of $\alpha_4\beta_1$ and $\alpha_4\beta_7$ integrins, molecules that facilitate binding of leukocytes to inflamed brain endothelium (Yednock et al 1992). It is thought to limit the migration of T cells across the blood brain barrier and hence has been shown to reduce frequency of relapses by up to 68% over two years (Polman et al 2006). A trial of monotherapy with natalizumab in 942 people with MS reported a generally favourable safety profile (Polman et al 2006). However in a parallel trial of combined therapy in 1171 people with MS, where natalizumab was administered with interferon β 1a, 2 subjects developed progressive multifocal leukoencephalopathy (PML), a potentially fatal and rapidly progressive

demyelinating condition (Rudick et al 2006b, Yousry et al 2006). At the time of writing (October 2011) 170 further cases of PML have been reported from the 88,100 people treated with Natalizumab worldwide (data supplied by Biogen Idec Inc.). At the present time in the UK the use of natalizumab is restricted to people with rapidly evolving severe relapsing–remitting multiple sclerosis or those who were previously participating in clinical trials of natalizumab.

Promising results have been obtained with several other immunomodulatory compounds. Alemtuzumab, a monoclonal antibody directed towards the lymphocyte surface protein CD52, which causes prolonged lymphocyte depletion (Coles et al 2006), has been shown to reduce the frequency of relapses in early relapse-onset MS by up to 74% with an associated improvement in EDSS over the course of the trial (CAMMS223 Trial Investigators 2008). However an earlier study in secondary progressive MS showed that the effect was principally on acute inflammatory activity, with less effect on progressive disability, or on MRI measures of neurodegeneration (Coles et al 1999).

A monoclonal antibody that causes depletion of CD20+ B cells, Rituximab, has been tested in a small cohort of people with relapsing-remitting MS over a short time-scale and been shown to reduce relapse rate by approximately half (Hauser et al 2008). However larger, longer studies will be needed to evaluate the efficacy and safety profile of Rituximab, particularly since there are case reports of PML in patients being treated with rituximab albeit for conditions other than MS

(<http://www.fda.gov/Drugs/DrugSafety/PostmarketDrugSafetyInformationforPatientsandProviders/ucm126519.htm>).

All of the above medications are parenterally administered, which limits their acceptability to some patients. Positive trials have recently been published for several oral compounds: cladribine and fingolimod both reduce relapse rate by approximately half compared to placebo (Giovannoni et al 2010, Kappos et al 2010), although concerns over safety mean that cladribine is unlikely to be granted marketing authorization by the Committee for Medicinal Products for Human Use (<http://guidance.nice.org.uk/TA/Wave20/69>). Teriflunomide has been found to reduce relapse rate by approximately 30% (O'Connor et al 2011); phase III trials of laquinimod and dimethyl fumarate have recently been completed with initial reports suggesting annualized relapse reduction rates of approximately 30 and 50% respectively (Comi et al 2011, http://www.biogenidec.com/PRESS_RELEASE_DETAILS.aspx?ID=5981&ReqId=1621631). People in the verum arm of the fingolimod and teriflunomide trials had significantly less brain volume loss and a smaller increase in T1 hypointense lesion volume than the placebo group perhaps suggesting that these substances may have some neuroprotective effect.

Neuroprotection

Based on the fact that the majority of immunomodulatory compounds that have been tested in MS have significant effect on both the clinical and radiological stigmata of

acute neuroinflammation without seeming to influence clinical and radiological evidence of progressive neurodegeneration (see section 1.3a). Investigators have therefore started to consider whether other compounds, with a primary action that is not immunomodulatory, may be neuroprotective and hence slow down the development of progressive disability.

A large number of different compounds are currently under investigation as potential neuroprotective agents. Among these are drugs which block voltage gated sodium channels since it is possible that intracellular sodium accumulation can damage chronically demyelinated axons (see section 1.3.5).

In EAE, the rodent model of MS, several sodium channel blockers including lamotrigine, flecainide and phenytoin have been shown to prevent axonal loss and reduce disability (Lo et al 2003, Bechtold et al 2004, Bechtold et al 2006). Of these lamotrigine was felt to be the most suitable candidate for the first clinical trial in human subjects; compared to phenytoin, lamotrigine had a greater effect on preserving axonal density and clinical status in EAE (Kapoor, personal correspondence), while flecainide was felt to have an unfavorable side-effect profile which would limit its widespread use (CAST investigators 1989).

A phase II clinical trial of neuroprotection with lamotrigine in people with secondary progressive MS at the Institute of Neurology in London forms the basis of this thesis. The trial was completed in early 2009.

1.7. Conclusion

MS is a chronic neurological condition that in many cases is associated with significant disability. It is associated with inflammation, demyelination and neurodegeneration, the last of which may be largely responsible for progressive neurological disability. Although a range of treatments have been developed that can reduce the incidence of acute inflammatory lesions and relapses, there is little evidence that they confer any significant protection against the development of progressive disability.

Some evidence exists suggesting that sodium loading of chronically demyelinated axons may give rise to some neurodegeneration and that sodium channel blockers such as Lamotrigine could be neuroprotective.

A number of clinical assessment tools have been developed which allow investigators to quantify levels of neurological function, disability and MS related reduction in quality of life. However even the most reliable clinical measures in MS are subject to a high degree of longitudinal variability, both between and within individuals.

Quantitative MRI is a powerful tool which gives investigators *in vivo* measures of brain pathology. MRI is reproducible, carries little risk, is well tolerated and can be used to obtain serial data points making it ideal for longitudinal monitoring. Even when trials are blinded, clinical measures are vulnerable to investigator and subject

bias (Noseworthy et al 1994). By contrast MRI analysis can be blinded and, provided adequate quality assurance procedures are followed, can be standardized longitudinally and between centres.

In this study clinical and MRI data was collected from a large group of people with secondary progressive MS who were taking part in a double-blinded, randomized, placebo-controlled trial of lamotrigine. The MRI techniques chosen were those that were felt to best capture neurodegeneration. Cross-sectional and longitudinal correlations between the MRI and clinical data were assessed to gain further insight into which pathological changes give rise to clinical impairment and which MRI modalities are most useful for monitoring progressive MS. The main focus of this thesis is the clinical correlation of MTR and T1 hypointense lesion measures. However atrophy and T2 hyperintense lesion volume data is presented where relevant. The effect of lamotrigine on MRI and clinical measures was also assessed to evaluate the neuroprotective potential of this compound.

2. Methods

2.1. Introduction: A randomized, double-blind, placebo-controlled trial of neuroprotection with lamotrigine in secondary progressive MS.

Data presented in this thesis are taken from a randomized, double-blinded, placebo-controlled, parallel arm trial of neuroprotection with lamotrigine in secondary progressive MS. Central cerebral volume (CCV) (see section 1.5) has been shown to be a reproducible and sensitive measure (Losseff et al 1996a) which changes in secondary progressive MS over 24 months (Molyneux et al 2000a) so was chosen as the primary end-point. The study was powered to detect a difference in change in CCV between verum and placebo arms.

Several other MRI endpoints, which were felt may be more sensitive (Anderson et al 2006), or to give different information, including MTR and T1 hypointense lesion volume (see section 1.5), were also measured.

Informed consent was taken from all subjects prior to the acquisition of any clinical or MRI data. Ethical approval for the lamotrigine trial was given by the Joint UCL/UCLH Committee on the Ethics of Human Research (Committee A). The trial was registered with the US National Institute of Health (<http://clinicaltrials.gov/ct2/show/NCT00257855?term=lamotrigine+multiple+sclerosis&rank=1>) and the UK Medicines and Healthcare Regulatory Authority and was conducted according to International Conference on Harmonisation of Technical Requirements for Registration of Pharmaceuticals for Human Use Good Clinical Practice (ICH-GCP) guidelines. Recruitment and data collection was monitored by

UCL Research and Development Department and was reviewed by an independent data and ethics monitoring committee.

The trial was funded by the MS Society of Great Britain and Northern Ireland and Sponsored by University College London. Neither party had any involvement in data collection, analysis, interpretation or the dissemination of results.

2.2. Subjects

The subjects in this study comprised 118 people with secondary progressive MS who were recruited into a randomised, placebo controlled trial of neuroprotection with lamotrigine.

A sample size of 120 subjects, 60 in the verum arm and 60 in the placebo arm was calculated. This was based on a power of 80%, to detect a 60% reduction in the change of CCV, with a significance level of 5% and combined loss to follow up and non-compliance of 20%. The sample size calculation was based on change in CCV from 17 people who took part in a natural history study (data from Professor Alan Thompson) and 46 people from the placebo arm of a trial of beta-interferon in secondary progressive MS (Molyneux et al 2000a, Molyneux et al 2000b). The mean rate of brain atrophy from these two groups was estimated at approximately 1% per annum. A treatment effect of 60% was extrapolated from EAE data, in which lamotrigine treatment gave rise to a 50% reduction in axonal loss. The 20% drop-out rate was estimated from clinical experience of sodium channel blocker treatment in MS (RK and DHM). ANCOVA and multi-level, random-intercept linear regression models were simulated and gave sample sizes of n=57 and n=48 respectively for each

arm, thus a sample size of $n=60$ in each arm was felt to be sufficient for the more powerful model, allowing for equal drop-out in each arm.

The sample size calculations were done by DA.

Recruitment into the lamotrigine trial was conducted by two investigators (myself and JF). A list of 354 potential subjects was generated from consultant referrals and by surveying all the hospital letters of three of the multiple sclerosis specialists at the National Hospital for Neurology and Neurosurgery (NHNN), London (DHM, RK and GG); two specialists at the Royal Free Hospital Hampstead (RFH) (RB and AG) and one at St Albans Hospital Hertfordshire (RB)

Subject information leaflets were posted to all potential subjects. All subjects were then contacted by telephone and screened to ensure that they met the inclusion/exclusion criteria for the trial. The inclusion criteria were:

- Age 18-60 years.
- Subjects should have a clinical history including one or more episodes of acute neurological deficit with full or partial recovery and a subsequent history of progressive neurological deterioration, the latter being the main cause of disability over the preceding 24 months. Evidence of clinical progression was taken from medical records or from prospective subjects reporting a reduction in mobility equating to a one point increase in EDSS over two years.
- EDSS 4 to 6.5 – this was checked by determining that a subject had some limitation of mobility (a limited exercise tolerance – EDSS 4.0) but were

able to walk at least 10m with bilateral assistance (EDSS 6.5) (See appendix 3).

The exclusion criteria comprised:

- Eligibility for disease-modifying treatment under 2001 recommendations of the Association of British Neurologists.
- Pregnancy.
- Progression of ≥ 2 EDSS points over the preceding 12 months.
- The use of sodium or calcium channel blockers during the two weeks preceding the baseline scan; corticosteroids in the preceding two months; beta interferon, glatiramer acetate or other immunomodulatory drugs in the preceding six months or mitoxantrone in the preceding year.
- History of serious systemic illness other than multiple sclerosis or any evidence of abnormal renal or hepatic function.
- Previously documented adverse reaction to lamotrigine.
- Disabling temperature dependent exacerbations of neurological impairment.
- Contraindications to MRI scanning e.g. pacemakers, cerebrovascular aneurysm clips or a previous penetrating injury with metal objects.

A total of 130 eligible subjects were invited for interview of whom 10 declined or were rendered unsuitable because of abnormal blood test results.

120 subjects gave informed consent were randomly assigned to either active treatment or placebo using minimization. Minimization is a form of stratified randomization

process, whereby the probability of an individual being assigned to a given group is weighted according to the characteristics of the subjects who have already been allocated to each group. Although less suitable for large studies, in smaller trials, such as the lamotrigine trial, it improves the likelihood that the two groups will be balanced. The minimization criteria used in the lamotrigine trial were:

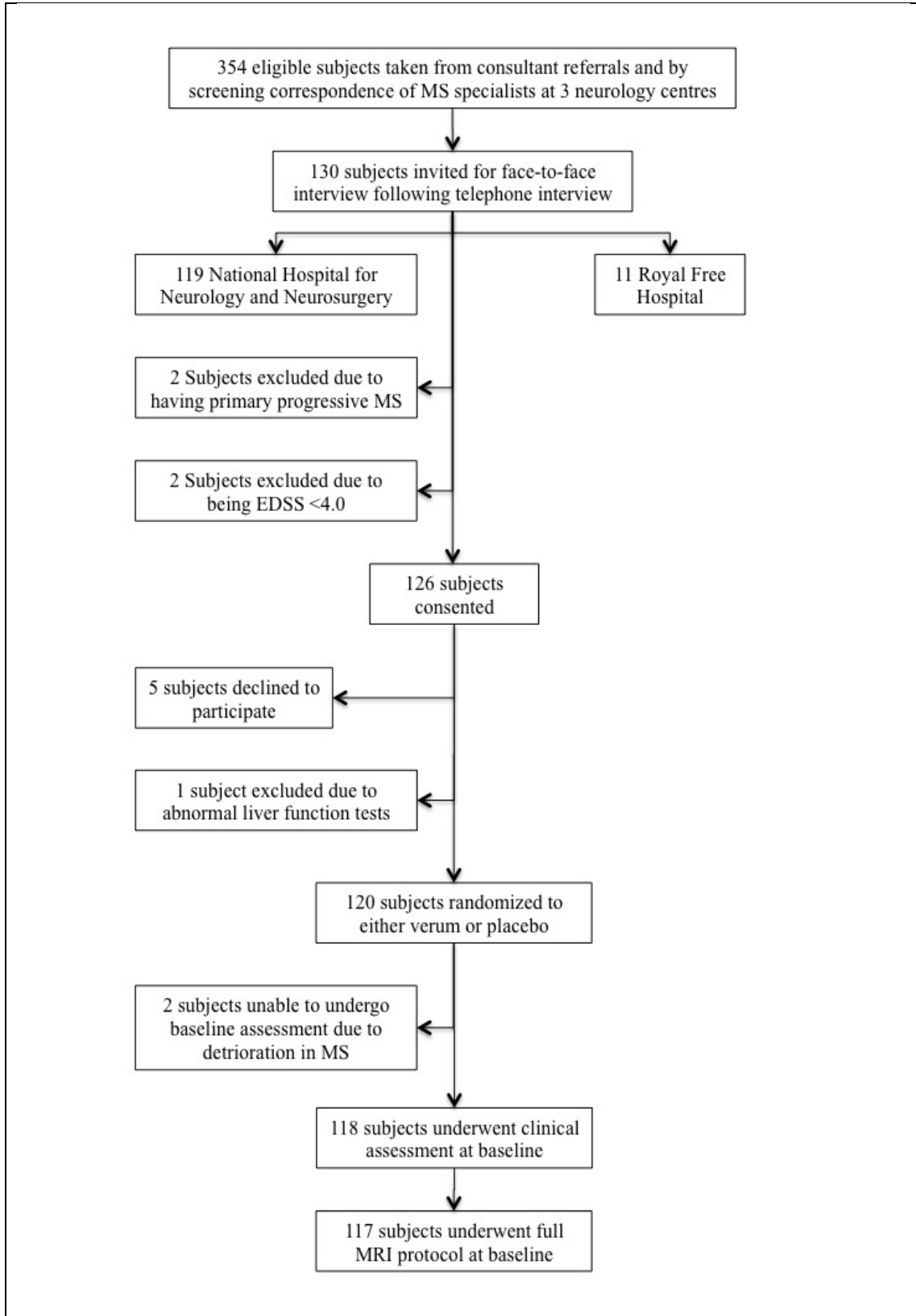
- Sex
- Age \geq 50
- EDSS \geq 5.5
- Site recruited from (NHNN or RFH)
- Clinical fellow seen at screening

By including the recruiting clinical fellow in the minimization criteria any differences in patient outcome related to the intervention of the treating physician (see section 2.3) or to inter-rater differences in clinical assessment or MRI analysis should be evenly spread between the two groups.

Minimization was done with concealed allocation using a fully automated, web-based protocol www.SealedEnvelope.com. (Sealed Envelope Ltd. London. UK). Patients were given a randomization number which was matched to a confidential treatment number by the study pharmacist assigning patients to either lamotrigine (in a sustained release form, Lamictal XR, GlaxoSmithKline, UK) or to a visually identical placebo tablet. Only the pharmacist was aware of the treatment allocation throughout the study; both subjects and investigators were blinded to treatment allocation for the complete duration of the trial. All MR images were analyzed and all statistical analysis protocols were established prior to unblinding.

Following randomization, two subjects experienced acute neurological deterioration which meant that they were unable to attend for baseline assessment. One subject was unable to tolerate MRI scanning. 117 subjects underwent the full MRI and clinical assessment at baseline. A summary of the recruitment and screening process is shown in Table 1.

Figure 1. A summary of the recruitment and screening process for the lamotrigine trial. One subject discovered that she was too claustrophobic to tolerate the full MRI protocol.



After randomization subjects attended for baseline assessment, which included clinical assessment (see section 2.3) and acquisition of MRI images.

The investigational medicinal product (IMP) was dispensed by the treating physician. The dose of the IMP was escalated using the following regime, which was recommended by the Department of Epilepsy at NHNN (Prof John Duncan):

25mg daily for two weeks, 50mg daily for two weeks, 100mg daily for one week, 200mg daily for one week, 300mg daily for one week, 400mg daily for one week.

Subjects were encouraged to find the highest dose at which they did not suffer unacceptable side-effects and this was established as the maintenance dose for the rest of the trial. Subjects met with their treating physician at three-monthly intervals until the end of the trial to report on side-effects, return un-used IMP and collect a new supply for the following three months. Once established on the maintenance dose of the IMP, subjects continued with that dose until a date approximately 24 months after the baseline visit, at which point the IMP was de-escalated over three months.

When assessing the effects of lamotrigine treatment on MRI and clinical measures both intention to treat (including all subjects randomized) and per-protocol (including only those subjects who were judged to be compliant) analyses were employed.

Compliance with treatment was evaluated in two ways:

1. By calculating the number of tablets that a subject should have consumed since the preceding visit, calculating the number of tablets that should be returned, counting back the number of tablets that were actually returned and comparing the two values. Subjects were considered to be 'tablet compliant' if they appeared to have consumed $\geq 80\%$ of the tablets and were still taking the tablets at 24 months. An 80% threshold for treatment adherence is widely used in clinical trials (Robiner 2005) and is in line with the lower levels of adherence seen with established disease modifying treatments in MS (Bruce et al 2010).
2. By measuring serum lamotrigine levels at 6, 12, 18 and 24 months. Subjects were considered to be 'serum compliant' if they had detectable serum lamotrigine levels at 24 months.

A study, published after all the subjects in the lamotrigine had been started on the trial medication, showed that in experimental allergic encephalomyelitis sudden withdrawal of two sodium channel blockers, carbamazepine and phenytoin, lead to rebound CNS inflammation and clinical deterioration (Black et al 2007).

To evaluate whether a similar response may have been occurring the subjects on the active arm of the lamotrigine trial, further MRI and clinical data were acquired at 27 months, after subjects had withdrawn from the trial medication. In the absence of Gd enhancing lesion number, T2 lesion volume and the incidence of new T2 lesions were felt to be the best MRI markers of new inflammatory activity and so were acquired at 27 months.

There was also concern that lamotrigine may have direct, osmotic effect reducing overall brain and spinal cord volume in the subjects in the verum arm, which could potentially obscure a positive treatment effect. To evaluate this possibility CCV and whole brain atrophy estimated using Structural Image Evaluation using Normalisation of Atrophy – longitudinal method (SIENA) (see section 2.4) were also calculated at 27 months.

In total 69 subjects consented to have an MRI scan and full clinical assessments at 27 months. MTR and T1 lesion volume data were not acquired at 27 months. Data from the 27 month timepoint has been published elsewhere (Kapoor et al 2010).

2.3. Clinical data in the lamotrigine trial.

Clinical data comprised: baseline demographic details and clinical history, i.e. age, sex, date of first neurological symptom, and date of diagnosis; EDSS; MSFC and component measures; and MSIS-29. All adverse events experienced during the 24 month trial period, including clinical relapses, were also recorded. On four occasions clinical data were collected by colleagues (AH and SG), otherwise all clinical data were collected by the same two investigators (myself and JF)

To ensure, as far as possible, that the objective clinical endpoints were not influenced by the investigators knowledge of how a subject was reacting to the trial medication each subject was assigned a treating and assessing physician.

The treating physician was the investigator who saw the subject at screening, collected the demographic and clinical data at baseline, prescribed the trial medication

and saw the subject on a three monthly basis to discuss clinical problems, e.g. side-effects, relapses and other adverse events. The assessing physician's only role was to collect the EDSS, MSFC and MSIS-29 at baseline, six, 12, 18 and 24 months. In a subset of subjects (see above) these measures were also acquired at 27 months.

Each investigator was responsible for screening 60 subjects and so was automatically assigned as assessing physician for the other 60 subjects.

EDSS

The EDSS comprises a structured clinical history and examination performed by an investigator who had undergone prior training using a web based training protocol (www.neurostatus.net).

The range of EDSS values was narrow – 4.0 to 7.5 at baseline (see table 4). Therefore for cross sectional analysis subjects were classified as being in either a low disability group ($EDSS \leq 6.0$) or a high disability group ($EDSS \geq 6.5$), which ensured a relatively even split of subjects.

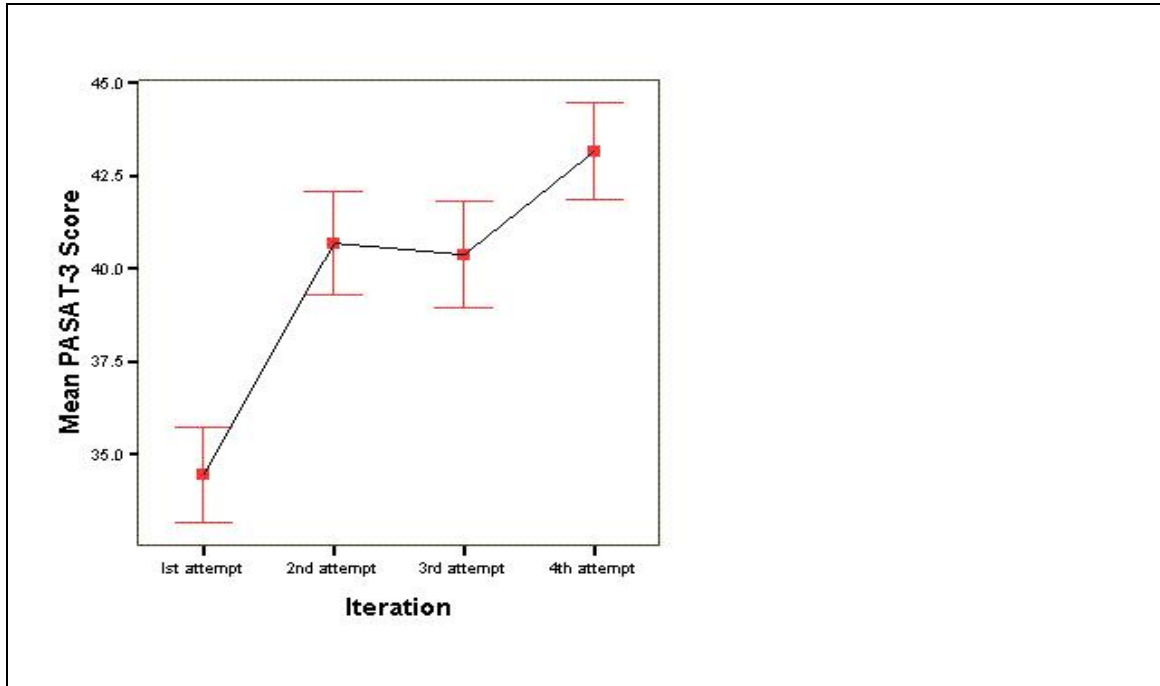
For longitudinal analysis patients were divided into groups depending on whether or not they had experienced sustained disability, defined as an increase of ≥ 0.5 points from baseline observed at two consecutive assessment visits in subjects with a baseline EDSS of ≥ 6.0 or a sustained increase of 1.0 in subjects with a baseline EDSS of ≤ 5.5 (Weinshenker et al 1991a, Weinshenker et al 1991b).

MSFC

The MSFC was conducted and recorded using an internationally agreed protocol (Fischer et al 2001; <http://nationalmssociety.org/for-professionals/researchers/clinical-study-measures/msfc/index.aspx>). Both investigators received training in administering the MSFC from an experienced practitioner (DS and RF) prior to collecting any data. The MSFC comprises three component tests: a test of ambulation - TW; a test of upper limb function – 9HPT and a test of cognitive function – the PASAT-3.

The PASAT-3 is known to be subject to a measurable learning effect which attenuates after three or four repetitions (Cohen et al 2001, Solari et al 2005). All subjects completed three iterations of the PASAT over a two week period prior to the collection of the baseline data. Results of these ‘Practice’ PASAT tests are shown in figure 2. The score in the first attempt was significantly lower than score used as the baseline PASAT value ($t= 4.832, p<0.0001$).

Figure 2. *The effect of practice on PASAT-3 scores. The dots and black line indicate the mean PASAT-3 score for the whole SPMS study group. The red lines show the standard error of the mean at each iteration.*



When a subject was unable to complete the 9HPT due to disability a score of 777 seconds was allocated (Cutter et al 1999). Where a subject was unable to complete the TW due to disability a score of 180 seconds was allocated. The latter was chosen because it is similar to the maximum time observed in the pooled dataset used by the National MS society to create the MSFC (Fischer et al 2001). None of the subjects were unable to complete the PASAT-3.

The three component measures were combined to generate the MSFC combined score using the following formula:

$$\text{MSFC} = (Z_{1/9\text{HPT}} + Z_{-TW} + Z_{\text{PASAT-3}})/3$$

Where Z is: (the mean score for a subject at a given timepoint - the mean score for the whole study group at baseline)/ the standard deviation of the scores for the whole study group at baseline. $1/9\text{HPT}$ here refers to: $(1/\text{mean score for the dominant hand} + 1/\text{mean score for the non-dominant hand})/ 2$.

In addition to the overall MSFC score, the component scores were considered individually. For cross-sectional statistical analysis the PASAT-3 was treated as a continuous variable. Reciprocals of the TW and 9HPT were used for all analysis, meaning that a higher score represented a better performance in the test and making the results easier to interpret.

Longitudinal change in MSFC or any of the component measures was calculated as the value at the second timepoint minus the value at the first, so a negative value represented a fall in that measure over time.

MSIS-29

The MSIS-29 is a self reported questionnaire about the impact of a subject's MS over the preceding two weeks. This was completed by the subject, with the assistance of one of two investigators (myself and JF) where the subject had marked upper limb impairment. It comprises 20 questions relating to physical impairment (MSIS-phys) and nine questions relating to psychological wellbeing (MSIS-psych) (See appendix 1). The physical and psychological scores for the MSIS-29 were recorded separately.

Relapses

Relapses were defined as a new neurological symptom, or deterioration of an existing neurological symptom, in the absence of any concurrent potentially confounding systemic medical conditions, such as infection, with an acute or sub-acute onset, duration of greater than 24 hours and partial or total recovery. Subjects were encouraged to contact the investigators as and when they experienced any neurological symptoms and the number of relapses was totalled at 24 months. It was decided that relapse rate should be converted to a binary variable with subjects divided into those who had experienced one or more relapses during the two year follow up period and those who had none.

2.4. MRI data in the lamotrigine trial

Five MRI sequences were used in the lamotrigine trial; a list of these with the acquisition parameters is shown in Table 1. All of these sequences have been previously used in human studies and been shown to have good reproducibility.

Table 1. MRI characteristics and acquisition parameters used in the lamotrigine trial. *PD = proton density, TR = repetition time, TE = echo time, μT = micro tesla, TI = inversion time, 2D = two dimensional, 3D = three dimensional, FOV = field of view.*

	<i>Sequence</i>	<i>MRI Characteristics</i>
1	<i>2D T1 weighted* - Brain (Losseff et al 1996a)</i>	<i>46 contiguous 3mm slices – TR 550ms, TE 15ms; FOV 240x180mm; Matrix 256x256; voxel size 1.98mm³</i>
2	<i>2D PD weighted/T2 dual fast spin echo* - Brain (Molyneux et al 2001)</i>	<i>2 interleaved sequences PD and T2 each 46 contiguous 3mm slices TR - 2500 ms, TE – PD 20ms T2 80m; , FOV 240x180mm; Matrix size 256x256; voxel size 1.98mm³</i>
3	<i>MTR* - Brain (Barker et al 1996)</i>	<i>4 interleaved sequences each of 28 x 5mm contiguous slices: PD, T2 weighted, with and without MT saturation – a total of 112 images. TR 1730ms, TE 30/80ms MT saturation pulse 14.6 μT, 64ms in duration, 1 kHz off resonance; FOV 240x180mm; Matrix 256x128; voxel size 6.59mm³</i>
4	<i>3D T1 weighted fast-spoiled gradient recall inversion recovery (FSPGR-IR)†- Brain (Fox et al 2000)</i>	<i>124 contiguous 1.5mm slices TR 10.9ms, TE 4.2ms, TI 450ms; FOV 250x190mm; , matrix 256x192 1.28 ; voxel size 1.28mm³</i>
5	<i>T1 FSPGR-IR – Cervical and upper thoracic spinal cord‡ (Losseff et al 1996b)</i>	<i>60 contiguous 1mm slices TR 13.2ms, TE 4.2ms; FOV 250x250mm; Matrix 256x256 ; voxel size 1.95mm³</i>

**acquired in the axial oblique plane to pass along the inferior borders of both the anterior genu and posterior splenium of the corpus callosum (Gallagher et al 1997).*

†acquired in the coronal plane, not oblique to any subject landmarks

‡acquired in the sagittal plane following an axial pilot at the level of the C7 vertebral body

All MRI data were acquired using the same Signa 1.5 Tesla scanner (General Electric, Milwaukee, WI, USA). Scan data was transferred from the scanner via a secure network and stored in a dedicated library. All subject identification data - name, date of birth, date of scan - was removed from the images prior to analysis.

All analysis was performed using Sun Blade 150 Workstations (Sun Microsystems, Mountainview, Ca, USA). All post acquisition MRI analysis for this study was performed by two investigators (myself and JF).

In accordance with departmental policy, control subjects with no known neurological condition and specially designed ‘phantoms’ with a set shape and proton density were scanned on a regular (monthly) basis for quality control and to ensure that there was no change in MRI derived measures.

Part way through the trial, between the 12 and 24 month timepoints, a major component of the scanner, the gradient coil, was replaced. To identify whether this was associated with any step-change in absolute values of any of the MRI measures three volunteers (AB, KC and MY) agreed to be scanned 10 times over a four month period before and after the replacement of the gradient coil. There was no significant change in the absolute values or the variability of any of the volume measures or normal appearing brain tissue MTR measures.

It was not possible to test in the controls whether lesion measures – volume, activity or MTR - changed significantly following the replacement of the gradient coil. An unselected sub-group of 27 lamotrigine trial subjects did agree to undergo a further scan at 18 months, with lesion volume and MTR analysis, to try and evaluate the effects of the equipment change on lesion measures. It was not possible to detect any significant change in these measures which could be attributed to the gradient coil replacement.

T2 hyperintense lesion volume (T2LV)

T2 hyperintense lesions were identified using the two dimensional (2D) T2/proton density (PD) weighted sequences (see table 1 row 2). Lesions were contoured on the PD images from using a semi-automated intensity-thresholding programme Dispimage (Plummer 1992, University College London, London, UK). An area of high signal was considered a lesion if it was within the brain parenchyma, was higher intensity than grey matter on both the T2 weighted and PD images, was visible on more than one slice and was felt to be in an anatomical region where MS lesions commonly occur (Polman et al 2005).

T2LV was calculated by multiplying the area within the resulting 'T2 lesion map' by the slice thickness. Investigators analyzed the T2LV of the subjects for whom they were the treating physician – approximately half each of the analysis was done by myself and JF

T1 hypointense lesion volume (T1LV)

T1 hypointense lesions were identified using the 2D T1 weighted sequence (see table 1 row 1) with the 'T2 lesion map' as a reference. T1 hypointense lesions were also contoured using Dispimage (Plummer 1992). An area of low signal on the 2D T1 images was designated T1 hypointense lesion if it corresponded to a contoured area on the 'T2 lesion map' and had lower signal intensity than the surrounding brain parenchyma. T1 lesion volume was calculated by multiplying the area within the 'T1 lesion map' by the slice thickness.

This analysis was done by me alone.

New and enlarging – ‘active’- T2 lesions

New and enlarging lesions were identified after 12 months and 24 months by comparing the corresponding slices from two serial 2D T2 weighted scans taken from the same subject (see table 1 row 2). They were marked on the PD images. The definition of what constituted new and enlarging lesions were taken from a previous consensus paper which allows good intra- and inter-rater reproducibility (Molyneux et al 1999). A sample of 30 subjects (15 from each investigator) was repeated by a consultant radiologist (KM) and concordance was found to be 100%. Investigators analyzed the new and enlarging lesion counts of the subjects for whom they were the treating physician – approximately half each of the analysis was done by myself and JF

Central cerebral volume (CCV)

Central cerebral volume was calculated from the 2D T1 weighted sequence (see table 1 row 1) the using a modified version of the ‘Losseff central cerebral volume technique’ (Losseff 1996a). Six contiguous slices were isolated, the most caudal at the level of the velum interpositum. A semi-automated intensity thresholding tool – Medical Image Display and Analysis System (MIDAS) (Freeborough et al 1997) – was used to extract brain tissue only. MIDAS allows investigators to select the largest contiguous region within a given intensity range. The upper and lower limits of this intensity range are chosen by a trained investigator. The resulting region of interest was then corrected manually. The volume was then calculated by multiplying the area of the extracted slices by slice thickness.

Investigators analyzed the CCV of the subjects for whom they were the treating physician – approximately half of the analysis was done by myself and JF

Normalized brain volume (NBV)

Normalized brain volume (NBV) is a technique which allows comparison of brain volumes between subjects with different head sizes. It was calculated using ‘Structural Image Evaluation, using Normalization of atrophy – Cross sectional method’ (SIENAX; FSL software Oxford UK) on the three dimensional (3D) T1 weighted fast-spoiled gradient recall inversion recovery (FSPGR-IR) sequence (see table 1 row 4). It is a fully automated technique that uses a two step process to identify brain and non-brain tissue, the Brain Extraction Tool (BET; Smith 2002, Smith et al 2002).

Step 1: the largest contiguous region is selected bounded by maximum and minimum brain tissue intensity thresholds. However rather than being chosen by a trained investigator, the thresholds are identified by a computer algorithm that generates a compound histogram of intensity of all the voxels in the image and sets the thresholds as approximately the 10th and 90th centiles. This region of interest is used to estimate the rough size of the brain and the centre of gravity.

Step 2: a spherical mesh of tessellating triangles is gradually expanded and deformed until the vertices of the triangles cross into CSF at which point local threshold and smoothing protocols are used to create a 3D brain map.

The BET derived region of interest and the subject's skull are registered to a standard space brain image. In the case of SIENAX the template used is the based on 152 normal controls scanned by the Montreal Neurological Imaging group (MNI152) (Evans et al 1993). The skull of a given subject is also segmented by BET, by projecting lines from the surface of the BET brain region of interest and using an intensity gradient to identify the (low intensity) skull and the (high intensity) scalp.

Where extra-axial structures, such as the optic nerve, are mis-identified as brain parenchyma by the BET, these can be manually removed. This analysis was conducted by a single investigator (JF).

Whole brain atrophy (SIENA)

Whole brain atrophy was calculated using Structural Image Evaluation, using Normalization of Atrophy – Longitudinal protocol (SIENA) (Smith et al 2002). This is a fully automated technique which employs stereotactic co-registration of serial images and identification of regions of change. It was conducted by a single investigator (JF) using the 3D T1 weighted FSPGR-IR images (see table 1 row 4).

The brain and skull are segmented using the BET. These regions of interest are then co-registered, not with a standard template, but rather with a skull and generated region of interest from the same subject at a different timepoint. Regions where the two brains do not correspond exactly are identified as positive or negative change in brain volume. Results are expressed as a percentage of the volume of the earlier image.

Grey matter fraction (GMF) and white matter fraction (WMF)

The proportions of intracranial volume occupied by white matter and grey matter were calculated using Statistical Parametric Mapping (SPM; University College London, London, UK) – segmentation. This employed the 3D T1 weighted FSPGR-IR images, which had been reformatted into 28 5mm axial slices as part of the MTR analysis protocol (see section 2.4). SPM is a fully automated system in which images are co-registered into MNI152 standard space brain template. The intensity and position of each voxel is compared to adjacent voxels and the MNI152 template and assigned a probability of being white matter, grey matter, CSF or extra-axial tissue based on site and intensity (Ashburner and Friston 1997, Ashburner and Friston 2000).

Binary masks for white matter, grey matter, CSF and other tissue can then be generated using a probability cut-off. In this study a value of 75% was assigned. The volume of each segment can then be calculated by multiplying the area of the binary mask by the slice thickness.

Grey matter and white matter parenchymal fraction (GMF;WMF) were calculated by dividing the grey matter and white matter volumes by the total intracranial volume (the sum of grey matter, white matter and CSF volumes).

Several versions of the SPM programme are available (see www.fil.ion.ucl.ac.uk/spm). At the start of this study SPM99 (Ashburner and Friston 1997) was used and some baseline data was published using this programme. Later SPM05 was made available, which was felt to be superior (Ashburner and Friston

2005) so all data used in longitudinal analysis was analyzed using this. This involved re-analyzing the baseline images with SPM05

Even moderate lesion volumes are known to effect segmentation and give rise to significantly different grey matter and white matter volumes (Chard et al 2002c). In order to try to minimize the effect of this, T2 high-signal lesions were ‘masked-out’ prior to segmentation. The lesion mask was then added back into the white matter mask post segmentation. This is the same technique that was used to generate the NAGM and NAWM masks as part of the MTR analysis and is described in more detail in the relevant section (see below).

Spinal cord cross-sectional area (SCCA)

This analysis was using the 3D T1 weighted FSPGR-IR images of the spinal cord (see table 1 row 5). The protocol used was a modified version of the ‘Losseff cervical spinal cord technique’ (Losseff et al 1996b).

The scan was acquired in the sagittal plane. From the sagittal images, five contiguous pseudo-axial 3mm thick slices were created using the scanner console with the centre of the C2/3 intervertebral disc as the caudal landmark. Around the circumference of the cord there is a spectrum of intensity values, ranging from high (cord parenchyma) to low (CSF), and it is difficult to determine where the true boundary between cord and CSF lies.

Using Dispimage two regions of interest, one within the cord and one within the CSF were manually selected for each slice. The mean signal intensity for cord and CSF

were duly estimated from the two regions and the cord CSF threshold set as midway between these two values. The largest contiguous region with signal intensity greater than the threshold value was then selected automatically and non-cord material removed manually using a mouse driven cursor. The spinal cord cross sectional area was the mean of the five slices.

All the SCCA analysis was done by JF.

Brain MTR

The MTR image (see table 2 row 3) was analyzed using histogram analysis. This involved the generation of a binary mask, corresponding to a region of interest or tissue type – lesions, NAGM, NAWM, normal appearing brain tissue (NABT) or whole brain parenchyma – which was then applied to the with- and without-saturation pulse images.

1. Lesion masks were created by contouring T2 high-signal lesions on the PD images of the MTR sequence with the T2 images of the MTR sequence as a reference; this was done using Dispimage and the same rules as for T2LV analysis. The regions of interest were converted into an intrinsically co-registered binary mask and MTR histograms were derived from any voxels which were covered by the mask.

2. Normal appearing grey and white matter masks were created using SPM. The intention at the outset was to segment the T2-weighted images from the MTR sequence (see table 1 row 3). However in eight of 20 scans in which segmentation of the T2-images was attempted, the resulting tissue masks were clearly incorrect (an

example of this is shown in figure 3). The 3D T1-weighted FSPGR-IR images (see table 1 row 4) had a higher grey matter-white matter contrast and so a technique was devised whereby the NAGM and NAWM masks could be generated using this sequence and then applied to the MTR sequence.

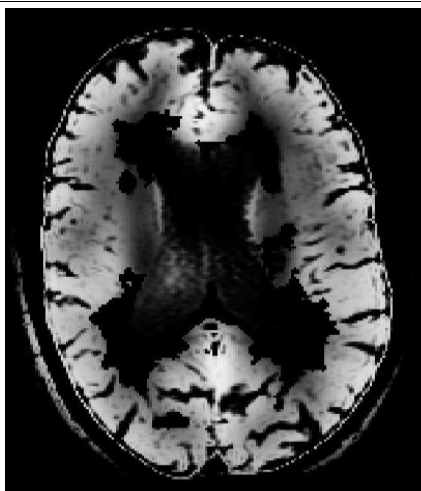

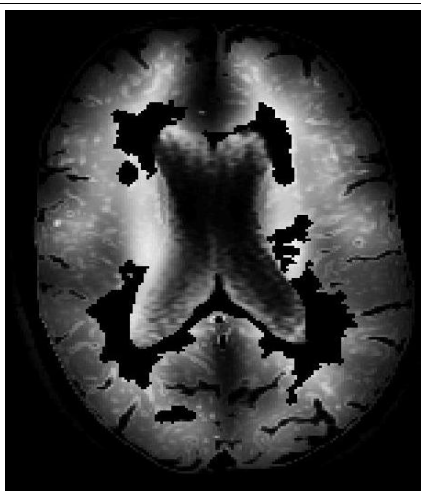
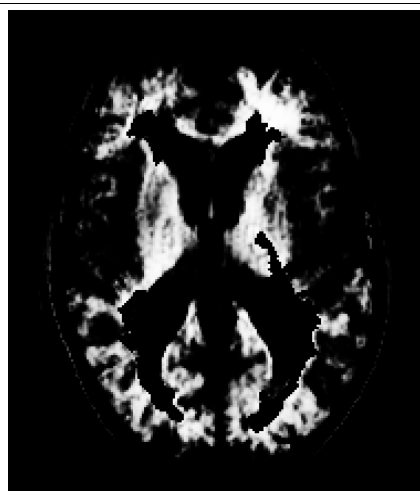
The 3D T1 weighted FSPGR-IR sequence was first re-formatted into the same virtual space as the MTR images using a specially written programme (DT and KH). This process converted the 124 x1.5mm coronally oriented slices into 50x5mm axially oriented slices. These images were qualitatively reviewed by a single rater (myself) and the 28 slices which most closely corresponded to the 28 slices of the MTR sequences were isolated and co-registered with the MTR sequence. It was these images that were segmented with SPM. A minimum tissue probability threshold of 75% was used.

Because even moderate lesion volumes are known to affect segmentation and give rise to significantly different grey matter and white matter volumes (Chard et al 2002c) the T2 lesion mask was applied to the re-formatted, co-registered T1 images prior to segmentation. A signal intensity of 0 was allocated to the lesion mask which meant that it could not be identified as grey matter or white matter.

In order to minimize the contribution of partial volume voxels, a two voxel erosion was applied to the white matter mask and a one voxel erosion was applied to the grey matter masks. On a subset of 10 scans I removed two voxels from the grey matter mask and calculated the volume change; the mean value was 66.37% (standard deviation 3.13%) so it was felt that a one voxel erosion would be more suitable.

Examples of the SPM output generated using this protocol are shown in figure 3.

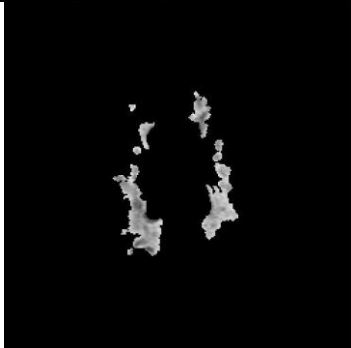


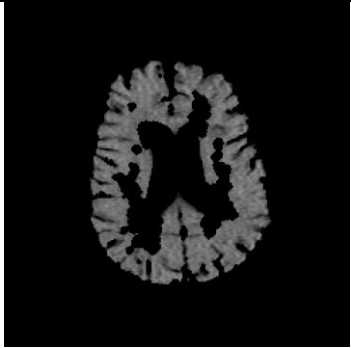
Figure 3. *The outcome of segmentation with SPM99. Using the T2-weighted images from the MTR sequence (left hand column) and the re-formatted, co-registered 3D T1 weighted FSPGR-IR sequence (right hand column)*

	<i>Segmentation of T2 weighted images from the MTR sequence</i>	<i>Segmentation of the re-formatted, co-registered 3T1-weighted FSPGR-IR sequence</i>
<i>Grey Matter</i>		
<i>White matter</i>		

3. NABT masks were generated by combining the NAGM and NAWM output from SPM and then applying a one voxel erosion to the whole mask.

Example of the binary masks used to generate MTR histograms are shown in figure 4.

Figure 4. Examples of regions used to generated MTR histograms

<i>Lesions</i>	<i>NAGM</i>	<i>NAWM</i>
		
<i>NABT</i>		
		

NAGM – normal appearing grey matter, NAWM – normal appearing white matter, NABT- normal appearing brain tissue.

MTR, expressed as per cent units (pu) was then calculated for each voxel in the region covered by the mask, using the formula:

$$MTR = ([MO-MS]/[MO]) \times 100 \text{ pu}$$

where MO is the intensity of the image with no saturation and MS is the intensity of the with saturation image. Histograms were then generated, with MTR values along

the x-axis at a resolution, or bin width, of 0.1 pu and the proportion of the total number of voxels at a given MTR value on the y-axis. To circumvent the problem of variable brain size, and to make values between individuals comparable, the histogram was normalized by dividing all the values by the total number of voxels. Thus the units of the y-axis are normalized brain volume units (or percentage of brain volume per pu).

Although it is possible to generate values which describe the overall shape of the histogram (Dehmeshki et al 2001), several localized descriptors are typically used to summarize the histogram. These are: histogram mean; peak location (PL) (the modal MTR value); peak height (PH) (the number of normalized brain volume units at the modal MTR value); and 25th, 50th and 75th centiles.

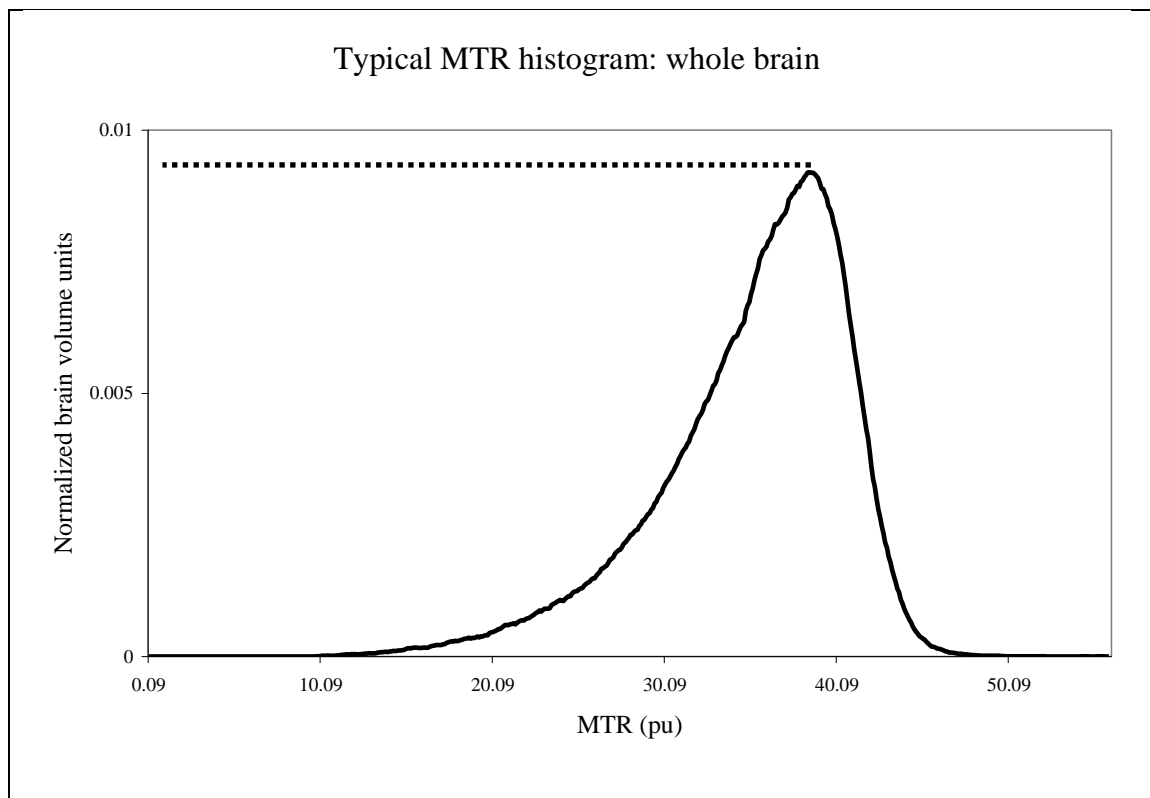
The initial MTR histogram appears spiky. These spikes arise in part from noise in the images, but also come from the use of a division in the formula for MTR and the integer signal intensities in the images. This results in an uneven distribution of MTR values (Tozer and Tofts 2003). It is important to smooth out the histogram as much as possible because spikes could give rise to erroneous localized descriptors, with peak height and peak location being particularly vulnerable to this.

To smooth out spikes caused by the division of integers, random noise with a value within the range $-0.5/+0.5$ was added to each voxel (Tozer and Tofts 2003). Spikes caused by non-uniformity in the magnetic field were removed by applying a moving average smoothing window of 0.7 pu to the histogram. For more details see Tofts et al 2003.

To minimize the contribution from partial volume voxels containing CSF or other non-brain tissue, any voxels with an MTR value of $<10\text{pu}$ were excluded from the analysis.

A typical final MTR histogram is shown in figure 5.

Figure 5. A typical MTR histogram of the whole brain. Taken from one of the subjects in the lamotrigine trial. The dotted line indicates the value of the peak height.



MTR – magnetization transfer ratio

Validation of semi-automatic analysis techniques.

For all techniques that include manual editing, investigators (myself and JF) received training from experienced practitioners: T2LV (DS, DHM); T1LV (DS, DHM); CCV (VA); SCCA (WR).

The MTR analysis included lesion contouring, for which training was provided by DS and DHM, and selecting the slices of the re-formatted, co-registered images which corresponded to the MTR sequence, which was essentially a new technique.

To quantify intra-rater and inter-rater variability a set of five test scans was analyzed on two separate occasions and the data were used to calculate coefficients of variance.

Intra-rater variability values were quite high for techniques that required the highest level of manual input –T2LV, T1LV, CCV – and for NAWM PH. However for the majority of the MTR measures the coefficient of variance was reassuringly small (see table 2).

Table 2. Coefficients of variance for semi-automated MRI analysis. Data shown is the coefficient of variance as a percentage of the mean value for each measure.

<i>MRI measure</i>		<i>Intra-rater coefficient of variance.</i>	<i>Inter-rater coefficient of variance.</i>
T2LV		6.63%	8.61%
T1LV		10.09%	n/a
CCV		0.82%	1.40%
		SPM99	SPM05
NAGM	PH	7.58%	7.52%
	PL	1.88%	2.78%
	Mean	0.47%	0.28%
NAWM	PH	11.08%	11.28%
	PL	1.26%	1.42%
	Mean	3.89%	3.87%
Lesions	PH	4.14%	4.15%
	PL	1.43%	1.43%
	Mean	1.37%	1.37%

MRI – magnetic resonance imaging, T2LV – T2-weighted high signal lesion volume, T1LV – T1 hypointense lesion volume, CCV – central cerebral volume, NAGM – normal appearing grey matter, MAWN – normal appearing white matter, PH - peak height, PL – peak location, SPM – statistical parametric mapping.

A summary of the data collected during the trial is shown in table 3.

Table 3. Summary of clinical and MRI collected from subjects in the SPMS study group at each timepoint. Month 0 is baseline. ✓ data collected on all subjects, ✓^s data collected on a sub-set of patients.

Month Data	0	1	2	3	6	9	12	15	18	21	24	27
Clinical Data												
<i>Adverse events new relapses</i>	✓	✓	✓	✓	✓	✓	✓	✓	✓	✓	✓	✓
<i>Compliance data</i>	✓	✓	✓	✓	✓	✓	✓	✓	✓	✓	✓	✓
<i>EDSS, MSFC and MSIS-29</i>	✓				✓		✓		✓		✓	✓ ^s
<i>Serum lamotrigine levels</i>	✓				✓		✓		✓		✓	
MRI Data												
<i>CCV</i>	✓				✓		✓		✓		✓	✓ ^s
<i>T2LV; T1LV; T1 to T2 lesion volume ratio</i>	✓						✓		✓ ^s		✓	✓ ^s
<i>Active T1 and T2 lesions</i>							✓				✓	✓ ^s
<i>NBV, GMF, WMF</i>	✓						✓		✓ ^s		✓	✓ ^s
<i>Brain MTR</i>	✓						✓		✓ ^s		✓	
<i>SCCA</i>	✓						✓		✓ ^s		✓	

EDSS – expanded disability status scale, MSFC multiple sclerosis functional composite, MSIS-29 – multiple sclerosis impact scale, CCV – central cerebral volume, T2LV – T2-weighted high signal lesion volume, T1LV – T1 hypointense lesion volume, NBV – normalized brain volume, GMF – grey matter fraction, WMF – white matter fraction, MTR- magnetization transfer ratio, SCCA – spinal cord cross sectional area

2.5 Statistical analysis and dissemination of results

Statistical analysis of the effects of lamotrigine on CCV, whole brain, white and grey matter volume, T1 and T2 lesion volume and all clinical measures was designed and performed by DA. Details of these analyses were published, following peer review, in 2010 (Kapoor et al 2010). Additional statistical analysis regarding the effect of lamotrigine treatment on MTR measures, T1 and T2 lesion volume were designed and conducted by me and are detailed in chapters 3.2 and 3.3 of this thesis.

Cross-sectional and longitudinal correlation of whole brain and regional volume and atrophy measures with clinical measures in the placebo group were evaluated by JF and have been published following peer review (Furby et al 2008, Furby et al 2010).

The correlation of baseline lesion measures with grey matter MTR and volume measures was also performed by JF and has been published following peer review (Furby et al 2009). The cross-sectional and longitudinal correlation of MTR measures with clinical measures and the cross-sectional and longitudinal correlation of all MRI measures with MSIS-29 scores were performed by me, are detailed in chapters 3.1, 3.2 and 3.4 and have been published following peer review (Hayton et al 2009, Hayton et al 2011a, Hayton et al 2011b).

3. Results

The main aims of this study were:

- to evaluate the correlation of brain MTR measures and T1 hypointense lesion measures with measures of clinical status in secondary progressive MS and so derive some insight into the types of pathological processes that give rise to disability in this condition.
- To evaluate the neuroprotective potential of lamotrigine in secondary progressive MS by observing the effects of treatment with this drug compared to a placebo on brain MTR and T1 lesion volume measures.

With regard to the first aim, both cross-sectional and longitudinal correlations were considered. A strength of cross-sectional studies is that they provide an indication of all the MS pathology that has accumulated over each subject's clinical course. Longitudinal studies are potentially more specific, showing that change in one measure can be directly correlated with change in another, and may also be more sensitive, showing pathological change before a subject manifests relevant clinical changes.

For quantifying the effect of lamotrigine treatment only longitudinal analysis is suitable, comparing the verum arm with the placebo arm.

To get some indication of how MTR and T1 lesion volumes are related to neurodegeneration and neuroinflammation respectively, brain volume or atrophy

measures and T2 hyperintense lesion measures were frequently included in the analysis. Furthermore by using ‘best-predictor’ stepwise multiple regression analysis to compare the clinical correlates of all the MRI measures it was possible to determine to what extent MTR and T1 lesion volumes measured clinically relevant pathological processes that were not captured by brain atrophy or T2 hyperintense lesion volume.

In addition to testing the correlation of MRI measures with ‘objective’ clinical measures such as EDSS and MSFC, the relationship with the MSIS-29 was also assessed. This gives an indication of how the identified pathological processes impacted on quality of life.

4. Grey matter magnetization transfer ratio independently correlates with neurological deficit in secondary progressive multiple sclerosis

4.1. Introduction

Grey matter involvement in MS is well recognised (Brownell and Hughes 1962, Lumsden 1970). It affects deep brain nuclei (Cifelli et al 2002) and cortical grey matter, (Bo et al 2003a, Geurts et al 2005, Kidd et al 1999, Kutzelnigg et al 2005, Peterson et al 2001). A recent quantitative pathological study has suggested that cortical lesion load is much greater in progressive MS than in relapsing remitting MS (Kutzelnigg et al 2005).

Limited correlations have been observed between grey matter MTR and measures of neurological deficit in early relapsing-remitting MS (Davies et al 2004), primary progressive MS (Dehmeshki et al 2003, Ramio-Torrenta et al 2006) and a mixed group including 19 people with SPMS (Traboulsee et al 2003). However there are as yet no large-scale, single centre and MTR sequence studies examining the correlation between grey matter MTR measures and neurological deficit in SPMS.

In this study the correlation was assessed of brain grey matter MTR measures with the EDSS and MSFC. To establish whether or not grey matter pathology had a greater impact on clinical status than white matter or lesion pathology, the correlation of NAWM and lesion MTR measures with EDSS and MSFC was also calculated.

Established measures of neuro-axonal loss (NBV) and focal neuroinflammation (T2LV) were also included in this study to identify how closely related these measures are and perhaps give some indication of the pathological process that gives rise to low MTR.

4.2. Methods

Subjects

This study comprises 113 people with secondary progressive MS, taking part in the lamotrigine trial (see section 2.1.1), who had MTR imaging at baseline.

Clinical data

Clinical data included here are EDSS and MSFC. For details of how these measures were collected see section 2.2.

MRI acquisition and analysis

Details of the MRI acquisition parameters used in this study are given in table 1. Data presented here are:

- T2LV
- NBV
- MTR histograms for T2 hyperintense lesions, NAWM and NAGM. The data presented here were generated from tissue segments derived using SPM99 (Ashburner and Friston 1997)

Statistics

Statistical analyses were performed using the statistical package for social sciences (version 11.5; SPSS Inc. Chicago, IL, USA) and Stata 9.2 (Stata Corporation, College Station, TX, USA).

Within-patient differences between NAWM, grey matter and lesion MTR measures were assessed using multiple paired Student's t-tests. To evaluate the association between different MRI values and the MSFC and its components, we used multiple linear regression of the clinical variable as the outcome for each MTR measure with age, gender, disease duration and duration of secondary progressive MS as covariates.

A two-stage set of multiple linear regression models was used also to determine best predictors of MSFC and its components, using the following potential predictors: mean, peak location and peak height from each of NAWM, grey matter and lesion MTR; NBV; T2 lesion volume; age; gender; disease duration and duration of secondary progressive MS. In the first stage best predictors were obtained separately within each of the NAWM, grey matter and lesion MTR measures, retaining significant variables ($p < 0.05$); in a manual forward stepwise procedure, the best predictors from each of these tissue classes were then entered together, and after removing non-significant variables, the remaining covariates were added singly. Discarded variables were then re-entered singly in the final models, and retained only if significant.

We used binary logistic regression to assess whether MRI variables could predict whether a subject would be in the lower (EDSS \leq 6.0 n=45) or higher (EDSS \geq 6.5, n=68) disability group. Age and gender were covariates in the model.

Spearman-rank coefficients were used to assess the correlation between MSFC components and EDSS and between MRI measures and EDSS.

4.3. Results

Clinical features

The clinical features of the subject group are detailed in table 4. There were significant correlations between the EDSS and the MSFC composite, 1/TW and 1/9HPT (r_s -0.39 p <0.0001, r_s -0.81 p <0.0001, r_s -0.36 p <0.0001). There was no significant correlation between the EDSS and the PASAT-3.

Table 4. Clinical characteristics for subjects in baseline analysis. Values expressed as mean (standard deviation; range) unless otherwise stated

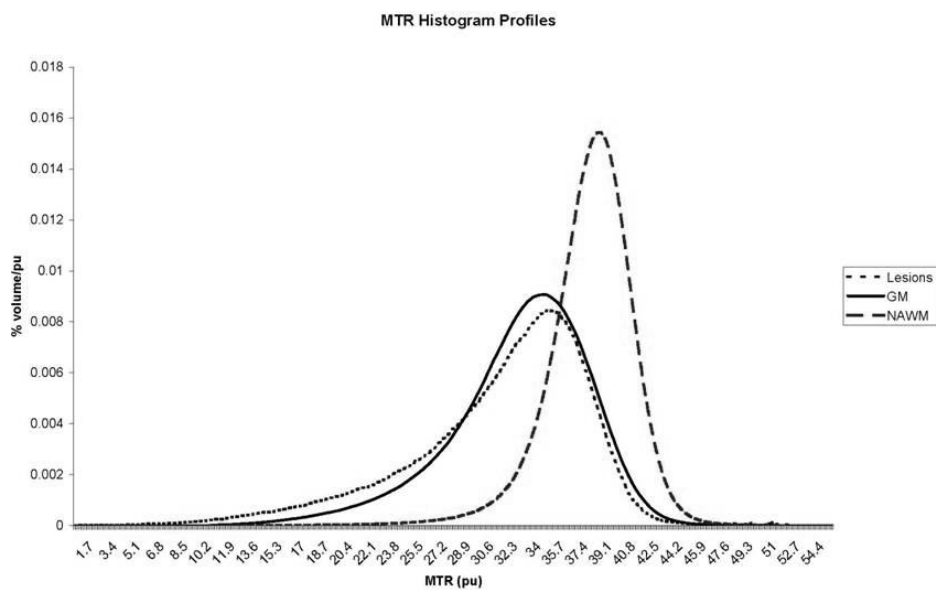
Characteristics	Secondary Progressive MS subjects, n=113
Sex, F/M (%)	80/33 (70.8/29.2)
Age, yrs (range)	50.2 (29 to 60)
Disease duration, yrs (range)	19.9 (3 to 41 yrs)
Duration of secondary progressive MS, yrs (range)	7.8 (1 to 26 yrs)
EDSS, median (IQR; range)	6.0 (6.0 to 6.5; 4.0 to 7.5)
PASAT-3	43.44 (13.65; 2 to 60)
1/9HPT	0.034 (0.010; 0.001 to 0.052)
1/TW	0.092 (0.061; 0.006 to 0.25)

EDSS – expanded disability status scale, PASAT-3 paced auditory serial addition test, 9HPT - 9 hole peg test, TW – timed 25 foot walk.

MRI findings

Mean MTR for NAWM was 37.36 pu (standard deviation 1.06, range 33.15 to 38.97), for grey matter was 32.43 (standard deviation 1.03, range 29.37 to 34.04) and for lesions was 30.70 (standard deviation 1.98, range 24.90 to 35.09). Histogram profiles for grey matter, NAWM and lesions are shown in figure 6. NAWM peak height, peak location and histogram mean were significantly higher than the corresponding grey matter or lesion measures ($p < 0.0001$). Grey matter MTR histogram mean was significantly higher and peak height significantly lower than the corresponding lesion measures ($p = 0.010$, $p < 0.0001$) but there was no significant difference between grey matter and lesion peak location.

Figure 6. Histogram profiles for NAWM, grey matter and lesions at baseline. These comprise the average MTR histograms for lesions (dotted line), NAWM (dashed line) and grey matter (solid line) for all subjects. The peak location is the modal MTR value in pu. The peak height is the number of normalized brain volume units at the modal MTR value.



The mean NBV was 1478.915 ml (SD99.87 ml, range 1215.79 to 1712.97 ml) and mean T2 lesion volume was 25.44 ml (SD18.0 ml, range 0.35 to 87.97 ml). There were significant correlations between all MTR measures and both T2 lesion volume and NBV (Table 5).

Table 5. Cross-sectional correlation between magnetization transfer (MTR) measures for normal appearing white matter (NAWM) grey matter and T2 high-signal lesions, normalized brain volume (NBV) and T2 high-signal lesion volume.

Significant correlations are shown in bold

	<i>NBV, Pearson correlation coefficient (p value)</i>	<i>T2LV, Pearson correlation coefficient (p value)</i>
<i>T2 lesion volume</i>	-0.478 (<0.0001)	-
<i>NAWM peak height</i>	0.319 (0.008)	-0.36 (<0.0001)
<i>NAWM peak location</i>	0.381 (<0.0001)	-0.445 (<0.0001)
<i>NAWM histogram mean</i>	0.417(<0.0001)	-0.420(<0.0001)
<i>NAGM peak height</i>	0.379 (<0.0001)	-0.481(<0.0001)
<i>NAGM peak location</i>	0.355 (<0.0001)	-0.506 (<0.0001)
<i>NAGM histogram mean</i>	0.625 (<0.0001)	-0.713 (<0.0001)
<i>Lesion peak height</i>	0.511(<0.0001)	-0.635 (<0.0001)
<i>Lesion peak location</i>	0.350 (<0.0001)	-0.523 (<0.0001)
<i>Lesion histogram mean</i>	0.567 (<0.0001)	-0.692 (<0.0001)

NAWM - normal appearing white matter, NAGM – normal appearing grey matter

Correlation between EDSS and MRI variables

Significant inverse correlations existed between EDSS and grey matter peak location (r_s -0.19, $p < 0.046$). No other MRI measures significantly correlated with EDSS.

Logistic regression analysis for EDSS using MRI variables

None of the MRI measures significantly predicted whether a subject was in the higher EDSS group.

Linear regression analyses for MRI measures with MSFC measures (Table 6)

Table 6. Linear regression analysis with MSFC-based clinical measures as the outcome variable and a single MR measure as the predictor, adjusted for age, gender, disease duration and duration of secondary progressive MS at baseline.

Statistically significant results in bold * $p \leq 0.01$, ** $p \leq 0.001$, *** $p < 0.0001$

		<i>MSFC</i> standardised β	<i>PASAT-3</i> standardised β	<i>1/9 HPT</i> standardised β	<i>1/TW</i> standardised β
NAWM MTR	PH	0.280*	0.338***	0.201	0.076
	PL	0.337***	0.368***	0.285*	0.194
	Mean	0.327***	0.399***	0.283*	0.163
NAGM MTR	PH	0.265*	0.435***	0.182	-0.032
	PL	0.354**	0.280*	0.250 *	0.225*
	Mean	0.460***	0.543***	0.391***	0.144
Lesion MTR	PH	0.304**	0.334***	0.279*	0.057
	PL	0.368***	0.374***	0.371***	0.116
	Mean	0.394***	0.429***	0.376***	0.135
T2LV		-0.360***	-0.425***	-0.295**	-0.062
NBV		0.447***	0.373***	0.505***	0.177

MSFC – multiple sclerosis functional composite, PASAT-3 – paced auditory serial addition test, 9HPT – 9 hole peg test, TW – 25 foot timed walk, NAWM – normal appearing white matter, MTR – magnetization transfer ratio, NAGM – normal appearing grey matter, T2LV – T2 weighted hyperintense lesion volume, NBV – normalized brain volume

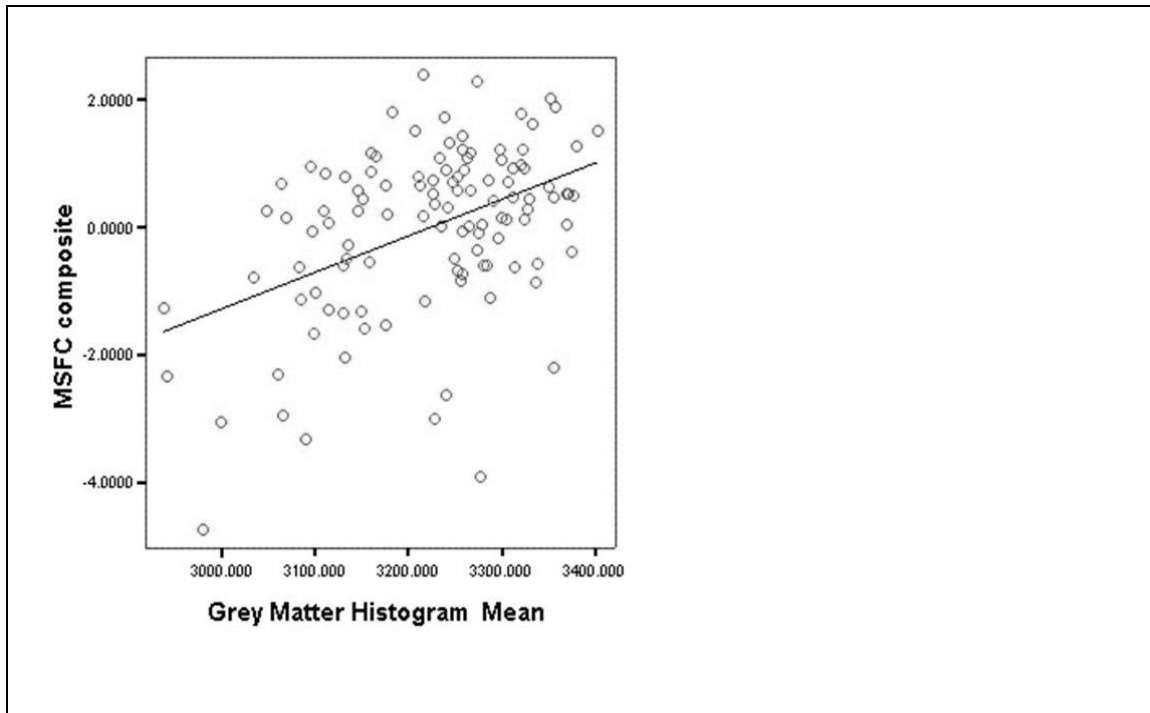
NAWM MTR

NAWM MTR peak height, peak location and histogram mean correlated with MSFC score, PASAT-3 and 1/9HPT. There was a significant, though weaker, correlation between NAWM MTR peak location and 1/TW.

NAGM MTR

There were significant correlations between grey matter MTR peak height, peak location and histogram mean and MSFC score and PASAT-3. A significant correlation was also found for grey matter MTR histogram mean and peak location with 1/9HPT. Grey matter MTR peak location correlated modestly with 1/TW. Figure 7 shows a scatter plot with regression line of grey matter histogram mean vs. MSFC composite.

Figure 7. Scatter plot of grey matter histogram mean vs. MSFC composite showing regression line at baseline. Regression coefficient =0.446, $p < 0.0001$ (note there was no adjustment for other variables, so the r value differs from that shown in table 6)



MSFC – multiple sclerosis functional composite

Lesion MTR

Lesion MTR peak height, peak location and histogram mean all correlated significantly with MSFC score, PASAT-3 and 1/9HPT, but not with 1/TW.

NBV

There were significant correlations between NBV and MSFC score, PASAT-3 and 1/9HPT.

T2LV

Significant inverse correlations existed between T2LV and MSFC score, PASAT-3 and 1/9HPT. There was no significant correlation between T2LV and 1/TW.

Best predictor analysis (multiple linear regression) (table 7)

Table 7. Multiple linear regression models for predicting the MSFC and its components with MTR peak height, peak location and histogram mean for grey matter, NAWM and lesions; NBV, T2LV disease duration, duration of secondary progressive MS, age and gender as independent variables. Only significant independent predictors are described in the table.

MSFC		PASAT-3		1/9 HPT		1/TW	
Variable	r_p; p	Variable	r_p; p	Variable	r_p; p	Variable	r_p; p
<i>NAGM mean</i>	0.462; <0.0001	<i>NAGM mean</i>	0.537; <0.0001	<i>NBV</i>	0.361; <0.0001	<i>Duration of SPMS</i>	-0.344; <0.0001
<i>Duration of SPMS</i>	-0.178; ; 0.035	<i>Female gender</i>	-0.168; 0.036	<i>Lesion peak location</i>	0.215; 0.019	<i>Female gender</i>	-0.241; 0.006
						<i>NAWM peak location</i>	0.209; 0.014
						<i>NAGM peak location</i>	0.198; 0.021
$R^2=0.248$, $p<0.0001$		$R^2=0.342$, $p<0.0001$		$R^2=0.231$, $p<0.0001$		$R^2=0.245$, $p=0.004$	

MSFC – multiple sclerosis functional composite, PASAT-3 – paced serial addition test, 9HPT – 9 hole peg test, TW – timed 25 foot walk, NAGM – normal appearing grey matter, SPMS – secondary progressive MS, NBV – normalized brain volume, NAWM – normal appearing white matter

MSFC

NAGM histogram mean and duration of SPMS were the only independent predictors of the MSFC score, accounting for approximately 25% of the variance in the score.

PASAT-3

For the PASAT-3 the best independent predictors were NAGM histogram mean and female gender which accounted for approximately 34% of the measure's variance.

1/9HPT.

The best independent predictors of 1/9HPT were NBV and lesion peak location, which accounted for approximately 23% of the variance of the clinical measure.

1/TW

The best model for predicting 1/TW contained NAGM peak location, NAWM peak location, duration of secondary progressive MS and female gender. This model accounted for 25% of the variation in 1/TW.

4.4. Discussion

The aim of this study was to determine the correlation between brain grey matter MTR measures and neurological deficit in people with secondary progressive MS. This is the only large single centre investigation of such MTR findings in secondary

progressive MS and has the advantage of measuring MTR using a single stable acquisition sequence

There were significant correlations between lesion, NAWM and grey matter MTR measures and the overall MSFC score, as well as with component tests of upper limb and cognitive function (1/9HPT and PASAT-3) such that a higher MTR measure was associated with less impairment. The best predictor analysis in this study identified grey matter histogram mean as an independent predictor of clinical impairment in MSFC and PASAT-3 and grey matter peak location as an independent predictor of the timed walk. These findings suggest that brain grey matter pathology contributes to motor or cognitive dysfunction in this study group.

Previous MTR studies of whole brain, normal appearing brain tissue and lesions in MS

Previous studies have demonstrated limited cross-sectional correlation between disability and whole brain MTR mean in both a group of 79 people with MS including 26 subjects with secondary progressive MS (Kalkers et al 2001a) and a group of 82 subjects with exclusively secondary progressive MS (Inglese et al 2003). In the mixed study group, whole brain peak height and peak location also correlated with EDSS (Kalkers et al 2001a). In a similar study, including 16 people with secondary progressive MS in a mixed group of 83 with MS, whole brain MTR peak height correlated with EDSS (Dehmeshki et al 2001). Whole brain MTR mean and peak height have also been shown to correlate with PASAT-3 (Dehmeshki et al 2003)

These correlations may be partly due to MTR detectable pathology in T2 lesions. Lesion mean MTR has been shown to correlate with EDSS in mixed groups including 10 subjects with secondary progressive MS from a mixed group of 43 subjects (Gass et al 1994) and in a larger group of 25 people with secondary progressive MS from a study group of 95 people with different types of MS (Traboulsee et al 2003). However MTR mean and peak height for normal appearing brain tissue, comprising grey matter and NAWM, has also been shown to correlate with EDSS (Traboulsee et al 2003).

MTR and grey matter abnormalities in MS

Previous studies have reported correlation between MSFC and MTR measures from segmented grey matter in MS, though not always with multivariate analysis (Davies et al 2004, Rovaris et al 2001, Vrenken et al 2007). A study of 43 people with primary progressive MS found strong correlations between: grey matter measures and MSFC composite and grey matter histogram mean and the PASAT (Rovaris et al 2001). In a group of 38 people with early relapsing-remitting MS there were borderline correlations between upper limb function and grey matter histogram mean (Davies et al 2004) and deep grey matter histogram mean and the TW. Another study comparing a heterogeneous group of 66 people with MS, including 19 with SPMS, found significant correlations between grey matter peak location and MSFC composite; grey matter peak height and 9HPT; grey matter peak location and TW; but no correlation between any MTR measure and PASAT-3 (Vrenken et al 2007).

In this study grey matter MTR measures also correlated with the measure of whole brain volume, NBV. While this may partly reflect an increase in partial volume pixels

with lower MTR at grey matter tissue edges in the presence of greater atrophy, the erosion of the outer voxel in the grey matter segments and exclusion of pixels with $MTR < 10\mu$ in this study should have minimized this effect.

MRI studies of relapsing remitting MS (Chard et al 2002b, Miller et al 2002) and a group including 10 subjects with secondary progressive MS (Santos et al 2002) have shown a correlation between T2 lesion measures and brain grey matter atrophy. There are also correlations reported between T2 lesion volume and grey matter MTR measures (Cercignani et al 2001, Davies et al 2004, Ge et al 2002) and the results of present study echoed these findings. One possible mechanism linking reduced grey matter MTR with T2 lesion volume is neuronal loss following retrograde degeneration of axons transected or damaged in white matter lesions (Bjartmar and Trapp 2001). An association might also exist if there is a correlation between grey matter demyelinating lesion load and white matter (T2) lesion load. Grey matter in MS may contain a large number of focal demyelinated lesions (Brownell and Hughes 1962, Kutzelnigg et al 2005); particularly in progressive MS (Kutzelnigg et al 2005). Almost all grey matter lesions are invisible on conventional T2 weighted scans at 1.5 Tesla (Geurts et al 2005). MTR is very sensitive to loss of myelin (Barkhof et al 2003, Schmierer et al 2004, Vrenken et al 2007) and a reduction in grey matter MTR would be compatible with an effect of demyelinating lesions. That the best predictor analysis in this study identified the grey matter histogram mean as an independent predictor of clinical impairment suggests that grey matter pathology – whether due to demyelinating lesions and/or neuroaxonal loss - contributes to disability in secondary progressive MS.

Limited correlation between MTR measures and EDSS

The only MRI measure that significantly correlated with EDSS was grey matter MTR PL. Although the EDSS is well validated, widely understood and gives a measure of both impairment and disability, it has been criticized for being heavily weighted towards mobility and relatively insensitive to upper limb and cognitive dysfunction. The findings from this study group of strong correlations between EDSS and 1/TW, a direct measure of mobility, less pronounced correlation between EDSS and 1/9HPT and no significant correlation between EDSS and PASAT-3 would support this.

Much of the clinical impairment in people with MS is thought to be due to pathology of the spinal cord, which is known to be a common site for neuroinflammation and axonal loss (Lovas et al 2000, Oppenheimer 1978). It is possible that cord MRI measures may correlate better with EDSS and measures of mobility than brain measures do (Furby et al 2008).

However, even though the EDSS range was narrow, 3.5 points, the overall range of mobility, as measured using the TW was relatively broad. Comparing the ratio of standard deviation to mean for 1/TW, PASAT-3 and 9HPT, the ratio was highest for 1/TW. This suggests that even as a measure of mobility the EDSS is simply not sensitive enough to differentiate between different subjects in this secondary progressive group.

5. Longitudinal changes in magnetization transfer ratio in secondary progressive multiple sclerosis: data from a randomised placebo controlled trial of lamotrigine.

5.1. Introduction

Higher MTR has been shown to correlate with the higher myelin and axonal content in both MS lesions and NAWM in post-mortem samples (van Waesberghe et al 1999, Scmierer et al 2004) and is therefore of particular interest in progressive MS, where neuroaxonal loss and grey matter damage have been shown to be prominent (Kutzelnigg et al 2005), and is a potentially useful marker of pathology in the evaluation of putative neuroprotective treatments.

One possible neuroprotective agent is lamotrigine, a sodium channel blocker that has been shown to ameliorate neurological dysfunction and prevent neuroaxonal loss in the EAE animal model (Bechtold et al 2006).

This study comprises data from a randomized, double-blinded, placebo-controlled trial of lamotrigine in secondary progressive MS comparing change in MTR measures in brain NAWM, NAGM and T2 hyperintense lesions over two years in a group of subjects with secondary progressive MS. The hypothesis being that, if lamotrigine were neuroprotective, one would see a greater reduction in MTR in the placebo group.

The data from the trial was also used to assess the utility of MTR as a measure of clinically relevant pathology in secondary progressive MS by calculating the responsiveness of MTR measures to change over a 24 month period i.e. the

longitudinal correlation of MTR measures with MSFC and the association of MTR measures with a sustained increase in EDSS.

5.2. Methods

Subjects

This study comprises 117 people with secondary progressive MS, taking part in the lamotrigine trial (see section 2.1).

Clinical data

Clinical data included here are EDSS and MSFC. For details of how these measures were collected see section 2.2.

MRI acquisition and analysis.

Details of the MRI acquisition parameters used in this study are given in table 1. Data presented here are:

- MTR histograms for T2 hyperintense lesions, NAWM and NAGM. The data presented here were generated from tissue segments derived using SPM05 (Ashburner and Friston 2005).

Statistics

Change in MRI and clinical measures

The change in MRI and clinical measures were calculated by subtracting baseline values from the values at 24 months; thus a negative value indicated a fall in that measure over 24 months; a negative value for the MSFC measure indicated neurological deterioration. Paired Student's t-tests were used to determine whether

any changes measured over 24 months were significant. To account for the fact that the change in MTR may not be normally distributed, Wilcoxon signed-rank tests were also used to identify potentially significant changes.

The effect of treatment with lamotrigine

Differences in change in MTR measures between the placebo and verum arms were assessed using independent samples Student's t-tests. Intention to treat analysis was used in this study, with all randomized subjects invited for every scan irrespective of whether they were still taking the investigational medicinal product (IMP). Two additional, post-hoc, per protocol analyses were employed: a comparison of tablet adherent subjects, defined as those who had taken 80% of prescribed tablets and were still being prescribed tablets at 24 months; and a serum adherent comparison, which compared subjects in the verum arm who had detectable serum lamotrigine at 24 months with the entire placebo arm. Mann-Whitney U tests were also used to account for possible skewed distribution of change in MTR measures.

Reliability of the MTR measures

The scan-reposition-rescan reproducibility of the MRI measures was estimated using MRI scans from three healthy controls, each of whom was scanned on five occasions over a four week period. Coefficients of variability were calculated for the MTR measures.

Longitudinal correlation between MTR measures and MSFC

Longitudinal correlation of MTR measures with MSFC and component scores was assessed in the placebo arm using a random intercept mixed effect linear regression

model with age, gender, disease duration and time as covariates. A second model was calculated evaluating the correlation of MSFC with an interaction variable - [MTR measure]x time. The first model shows the correlation of MTR and clinical measures at any given timepoint. The second shows the correlation of the clinical measure with the change in the MTR measure.

The association between MTR measures and EDSS

Subjects in the placebo arm were divided into those who did or did not experience a sustained increase in EDSS over 24 months, defined as an increase of ≥ 0.5 points from baseline observed at two consecutive assessment visits over six months in subjects with a baseline EDSS of ≥ 6.0 or of 1.0 points in subjects with a baseline EDSS of ≤ 5.5 . Differences in baseline MTR values and change in MTR were assessed using binary logistic regression with age, gender and disease duration as covariates.

5.3. Results

Recruitment started in January 2006 and lasted eight months. The final 24 month scans were completed in September 2008. MRI data were collected on all subjects at baseline, 105 subjects (55 placebo, 50 verum) at 12 months and 108 subjects (56 placebo, 52 verum) at 24 months. Figure 8 is a participant flow diagram showing reasons for withdrawal and loss to follow-up. Baseline clinical and MRI measures are shown in table 8; some of the demographic data has previously been published (Kapoor et al 2010). The mean absolute and annualized change in MTR measures are shown in table 9. The change in clinical measures have been published previously (Kapoor et al 2010).

Figure 8. Trial participant flow chart.

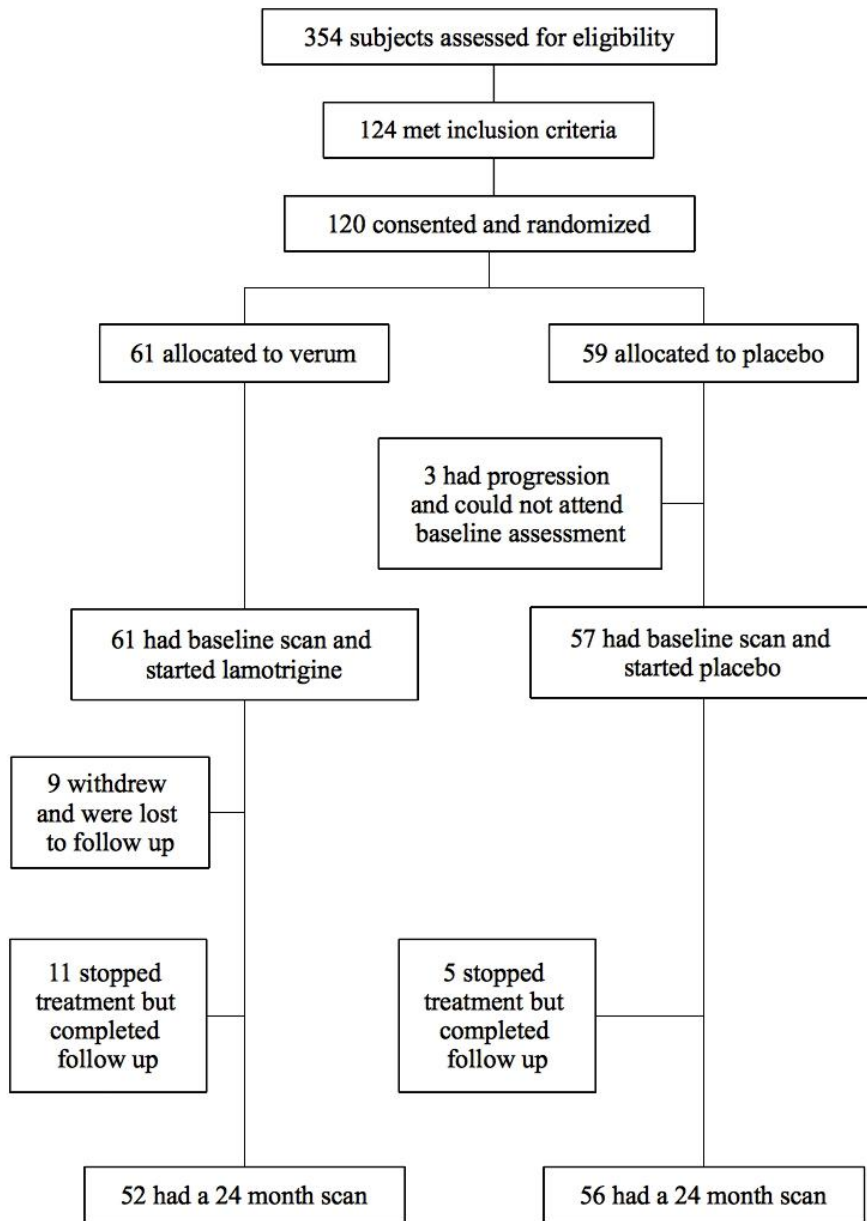


Table 8. Clinical and MRI measures for both verum and placebo arms at baseline.

Data shown are mean values (standard deviation - SD) unless otherwise specified.

Characteristics		Placebo arm, n = 57	Verum arm, n=61	p-value for difference
Sex, F/M (%)		40/17 (70/30)	45/16 (76/24)	0.839*
Age, years - mean (SD; range)		49.6 (6.7; 37 to 60)	51.4 (7.2; 30 to 61)	0.139†
Disease duration, years - mean (SD; range)		19.0 (8.3; 3 to 36)	21.2 (9.2; 5 to 41)	0.135†
EDSS, median (inter quartile range)		6.0 (0.5)	6.0 (0.5)	0.717‡
PASAT-3		44.7 (13.6)	41.7 (14.52)	0.255†
TW, sec		20.5 (21.2)	20.5 (25.7)	0.693†
9HPT, sec		32.3 (15.6)	31.7 (10.6)	0.836†
NAWM	PH - % volume/pu	16.35 (2.57)	16.70 (2.29)	0.452†
	PL - pu	37.88 (0.78)	37.99 (0.68)	0.508†
	Mean - pu	37.22 (1.23)	37.43 (0.78)	0.264†
NAGM	PH - % volume/pu	9.36 (1.08)	9.38 (1.13)	0.398†
	PL - pu	33.79 (0.71)	33.71 (0.87)	0.887†
	Mean - pu	32.08 (1.08)	32.10 (1.02)	0.588†
Lesions	PH - % volume/pu	9.75 (2.41)	10.01 (2.55)	0.217†
	PL - pu	33.82 (1.43)	34.10 (1.60)	0.323†
	Mean - pu	30.58 (2.02)	30.83 (1.94)	0.484†

NAWM - normal appearing white matter, NAGM - normal appearing grey matter, PH - peak height, PL - peak location, EDSS - expanded disability status scale, PASAT-3 - paced auditory serial addition test, TW- timed 25 foot walk, 9HPT - 9 hole peg test, pu - percentage units, *Chi Squared test, †unpaired Student's T-test, ‡Mann-Whitney U test.

Table 9. Change in MTR measures over 24 months. Significant results are highlighted in bold.

		Mean annual change - %	Mean absolute change over 24 months; median (standard error; range) [‡]	Significance of change – t value (p value)*; z value (p value) [~]	Difference between placebo and verum arms - p value for Student's T-test; p value for Mann-Whitney U test
NAWM	PH placebo	-0.61 (1.37)	-0.40; -0.70 (0.29; -6.89 to 3.63)	1.37 (0.177); 2.03 (0.043)	0.104; 0.269
	PH verum	-3.33 (1.23)	-1.15; -0.80 (0.35; -5.86 to 6.08)	3.29 (0.002); 2.75 (0.006)	
	PL placebo	-0.14 (0.14)	-0.12; -0.10 (0.10; -1.70 to 1.10)	1.13 (0.264); 0.97 (0.332)	0.342; 0.199
	PL verum	-0.006 (0.11)	0.01; 0.01 (0.08; -1.70 to 1.10)	0.14 (0.889); 0.67 (0.504)	
	Mean placebo	-0.05 (0.18)	-0.06; -0.05 (0.13; -3.78 to 1.48)	0.486 (0.641); 0.04 (0.969)	0.624; 0.445
	Mean verum	-0.25 (0.20)	-0.15; -0.09 (0.15; -3.69 to 3.00)	1.05 (0.301); 0.96 (0.339)	
NAGM	PH placebo	-0.79 (0.62)	-0.18; -0.40 (0.10; -2.91 to 0.94)	1.76 (0.085); 2.36 (0.018)	0.036 ; 0.078
	PH verum	-2.84 (0.68)	-0.51; -0.30 (0.12; -2.13 to 1.63)	4.25 (<0.0001); 6.10 (<0.0001)	
	PL placebo	-0.20 (0.15)	-0.15; -0.10 (0.09; -1.20 to 2.10)	1.54 (0.123); 0.97 (0.332)	0.456; 0.510
	PL verum	-0.09 (0.14)	-0.05; -0.10 (0.09; -3.00 to 2.20)	0.50 (0.618); 0.83 (0.405)	
	Mean placebo	-0.19 (0.06)	-0.13; -0.10 (0.04; -1.06 to 0.98)	3.54 (0.001); 3.36 (0.001)	0.713; 0.505
	Mean verum	-0.21 (0.07)	-0.11; -0.11 (0.05; -0.76 to 0.63)	3.29 (0.002); 2.12 (0.034)	
Lesions	PH placebo	1.25 (0.60)	0.16; -0.1 (0.12; -4.12 to 1.40)	1.30 (0.200); 1.17 (0.243)	0.004 ; 0.025
	PH verum	-1.48 (0.83)	-0.44; -0.10 (0.17; -2.47 to 2.38)	2.63 (0.011); 2.05 (0.040)	
	PL placebo	0.80 (0.31)	0.49; 0.50 (0.20; -2.39 to 4.29)	2.44 (0.018); 2.62 (0.009)	0.617; 0.515
	PL verum	0.65 (0.28)	0.36; 0.60 (0.18; -4.80 to 3.60)	1.96 (0.055); 1.96 (0.051)	
	Mean placebo	0.70 (0.16)	0.40; 0.30 (0.09; -1.90 to 2.34)	4.21 (<0.0001); 3.84 (<0.0001)	0.418; 0.445
	Mean verum	0.51 (0.19)	0.28; 0.39 (0.11; -1.58 to 2.17)	2.55 (0.011); 2.48 (0.013)	

NAWM - normal appearing white matter, NAGM - normal appearing grey matter, PH - peak height, PL - peak location * paired Student's t-test baseline vs. 24 month measures, ~ Wilcoxon signed-rank test baseline vs. 24 months † Independent samples Student's t-test for change over 24 months; placebo vs. verum. ‡ values for PH are % per pu, values for PL and mean are pu.

Responsiveness and reproducibility

In the placebo arm NAGM mean decreased over 24 months, but lesion PL and mean MTR both increased significantly; none of the other MTR measures changed over time – see table 9. The standardized response means (mean change/standard deviation of the change) were relatively low ranging from 0.05 to 0.56, indicating limited responsiveness and signal to noise ratio.

The scan-reposition-rescan reproducibility of the MTR measures in the control group is shown in table 10. The coefficient of variation was small, ranging from 0.16 to 2.54% with NAWM mean MTR the most reliable parameter. However the variability still exceeded the mean annualized change in the trial study group – see table 10.

Table 10. Scan-reposition-rescan reproducibility of the MTR measures

		Coefficient of variation %
NAWM	<i>Peak height</i>	2.00
	<i>Peak location</i>	0.53
	<i>Mean</i>	0.16
NAGM	<i>Peak height</i>	2.54
	<i>Peak location</i>	0.76
	<i>Mean</i>	0.22

NAWM - normal appearing white matter, NAGM - normal appearing grey matter

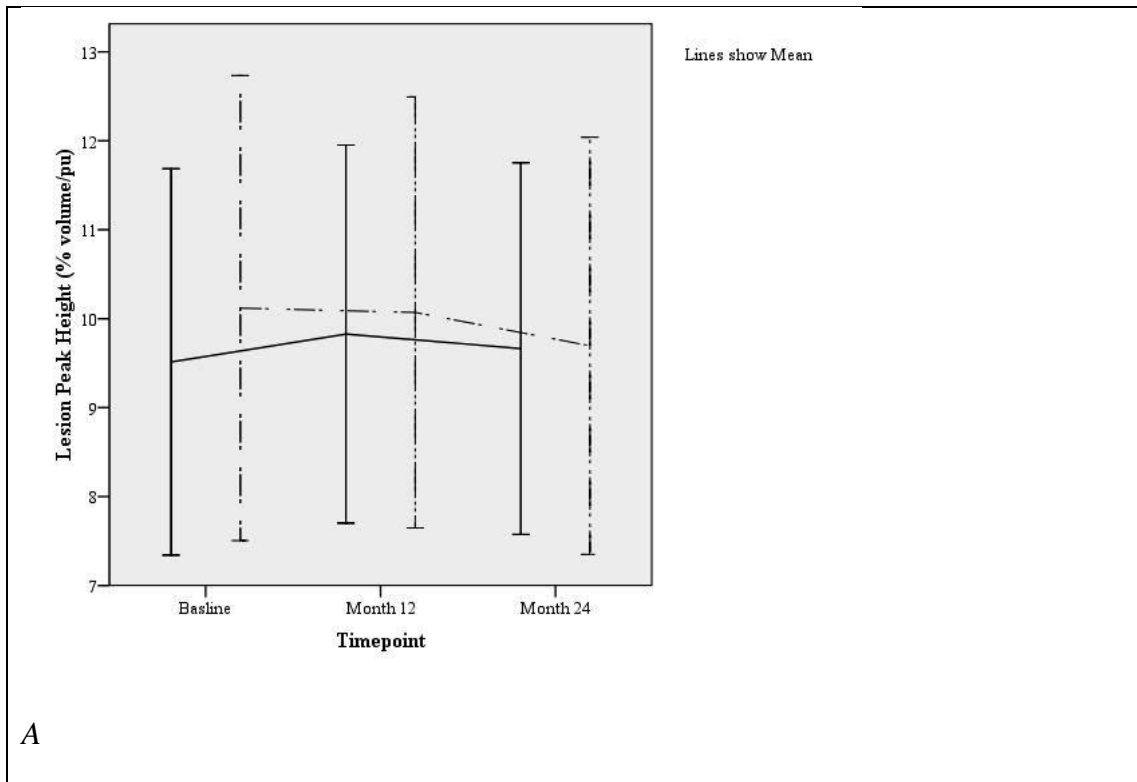
The effect of treatment with lamotrigine: intention to treat analysis.

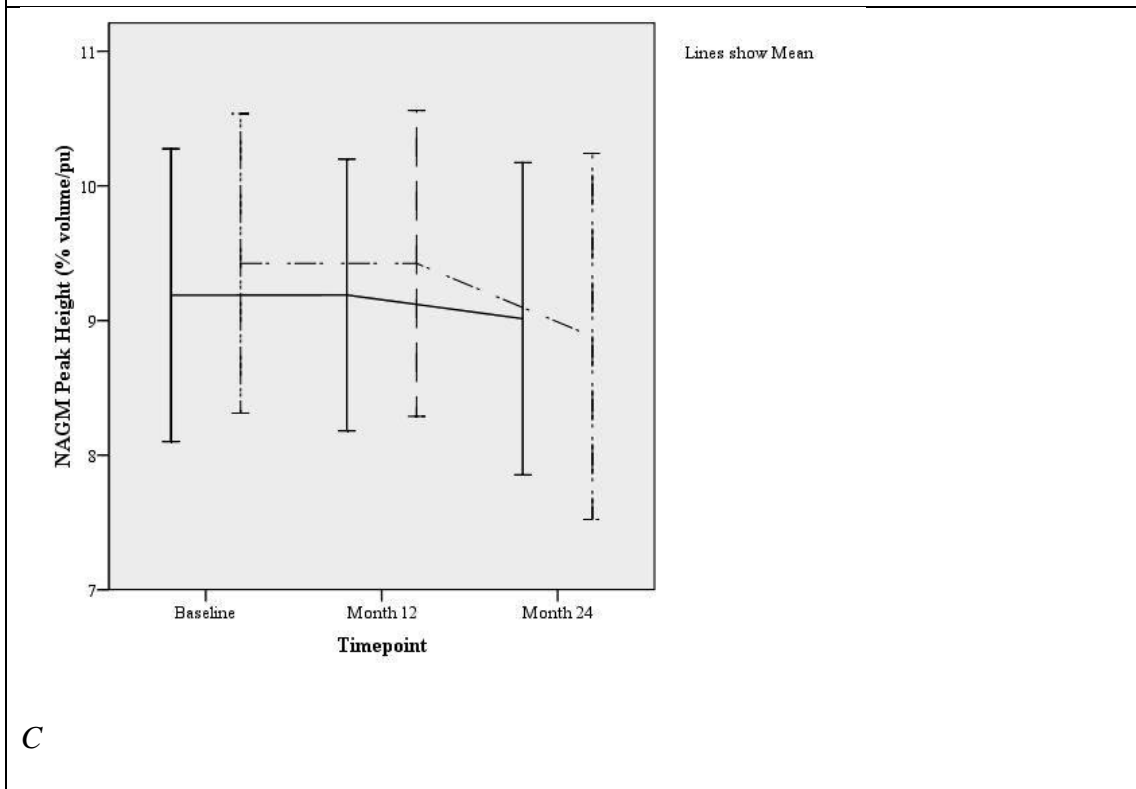
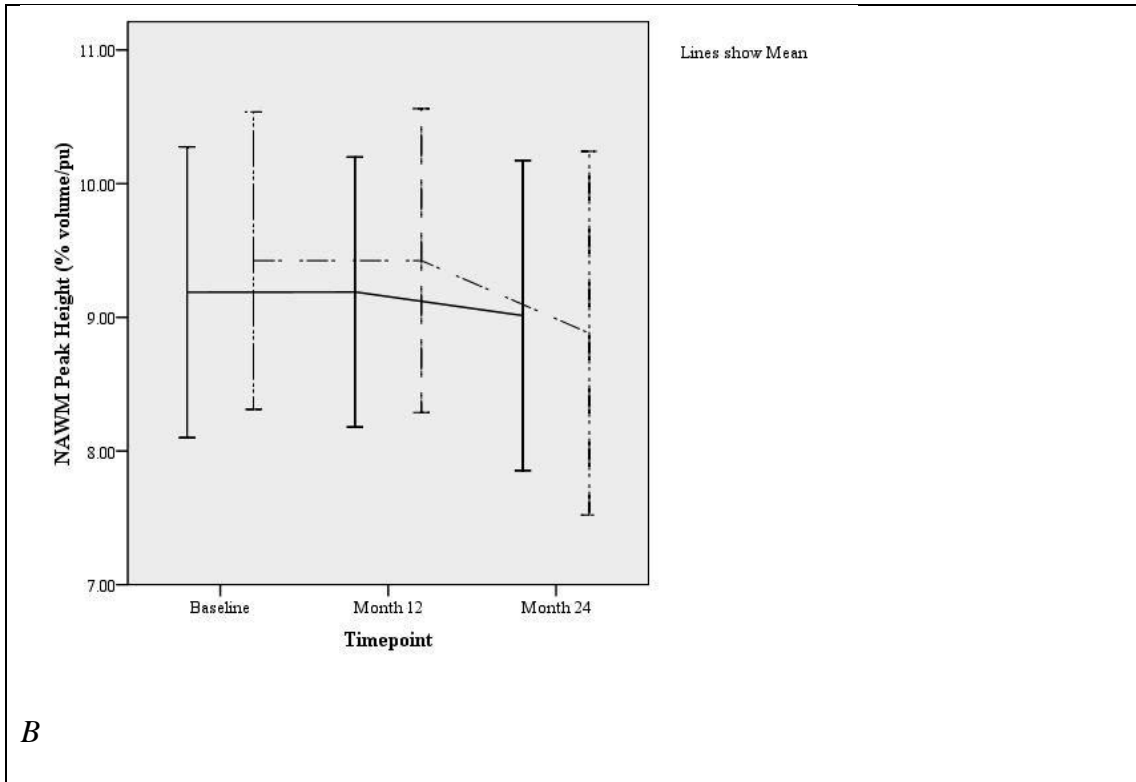
The results of Student’s t-tests comparing change in MTR measures in the placebo and verum arms with intention to treat analysis are shown in table 9. There were significant differences between the two groups in two parameters, NAGM PH and lesion PH. Both measures decreased in the verum arm, but did not change significantly in the placebo arm.

NAWM, NAGM and lesion PH also decreased significantly in the verum arm while no significant change was seen in the placebo arm (See figure 9). However the difference in change between the two groups was not significant for any of the three measures. Finally, there was a significant increase in lesion mean MTR in the verum arm; this was smaller than the corresponding increase seen in the placebo arm, but the difference between the two groups was not significant.

None of these results would support the hypothesis that lamotrigine was neuroprotective.

Figure 9. MTR peak height. Mean values for MTR peak height at baseline, 12 and 24 months for lesions (panel A), NAWM (panel B) and NAGM (panel C). The dashed line is the verum arm, the solid line placebo. Error bars show one standard deviation.





NAWM – normal appearing white matter, NAGM – normal appearing grey matter

The effect of treatment with lamotrigine: per protocol analysis

At 24 months, 32 of 52 subjects in the verum arm were receiving lamotrigine (mean dose 78mg) and 45 of 56 in the placebo arm (mean dose 240mg) were tablet compliant (χ^2 p=0.018). Twenty five of 52 subjects in the verum arm were serum compliant (mean serum concentration 14.1mg/L [standard deviation 8.6]).

The results of the per protocol analysis were similar to the intention to treat analysis. In the 'tablet-adherent' analysis there was a significantly larger reduction of NAGM PH in the verum arm compared to the placebo arm (mean change -0.55 %/pu vs. 0.19 %/pu, p=0.005). There was mean decrease in lesion PH in the verum arm, compared with an increase in the placebo arm (-0.63 %/pu vs. -0.125/pu, p=0.013); but there was a significantly smaller reduction of NAWM PH in the verum arm compared with placebo arm (mean change - 0.17pu vs. -1,44pu, p=0.028).

In the 'serum-adherent' analysis the only significant difference observed was for mean lesion PH, with a reduction in the verum arm (-0.52 %/pu) compared to an increase in the placebo arm (0.16 %/pu, p=0.008).

Longitudinal correlations of MTR and MSFC measures

Longitudinal correlations of MTR measures with MSFC and component scores are shown in table 11. The model used shows the correlation of the MTR measure with the clinical measure at any given timepoint, adjusting for age, sex and disease duration. There were significant correlations of PL and mean for all three tissue types with MSFC, PASAT-3 and 1/9HPT. NAGM mean was the only MTR parameter

which correlated with 1/TW. In all cases the correlations were positive; i.e. the higher the MTR measure, the better the performance in the clinical measure.

Table 11. Longitudinal correlation of MTR measures with MSFC and component measures in the placebo arm. Age, sex and disease duration were covariates in the model. Significant results are shown in bold. B is standardized beta i.e. the number of standard deviations of change in clinical measure for every standard deviation increase in MTR measure.

		Lesion			NAGM			NAWM		
		Peak Height	Peak Location	Mean	Peak Height	Peak Location	Mean	Peak Height	Peak Location	Mean
MSFC	β	-0.002	0.123	0.093	0.178	0.590	0.454	0.022	0.467	0.233
	p	0.912	0.016	0.001	0.044	0.001	<0.0001	0.148	0.001	<0.0001
PASAT-3	β	0.006	0.223	0.125	0.150	0.569	0.469	0.022	0.672	0.315
	p	0.669	<0.0001	<0.0001	0.032	<0.0001	<0.0001	0.062	<0.0001	<0.0001
1/9HPT	β	-0.016	0.143	0.106	0.082	0.568	0.425	0.003	0.459	0.206
	p	0.378	0.002	<0.0001	0.314	<0.0001	<0.0001	0.828	<0.0001	<0.0001
1/TW	β	-0.018	-0.005	0.004	-0.001	0.213	0.134	0.007	0.170	0.080
	p	0.187	0.889	0.824	0.982	0.079	0.022	0.491	0.098	0.060

NAWM - normal appearing white matter, NAGM - normal appearing grey matter, PASAT-3 - paced auditory serial addition test, TW – timed 25 foot walk, 9HPT - 9 hole peg test

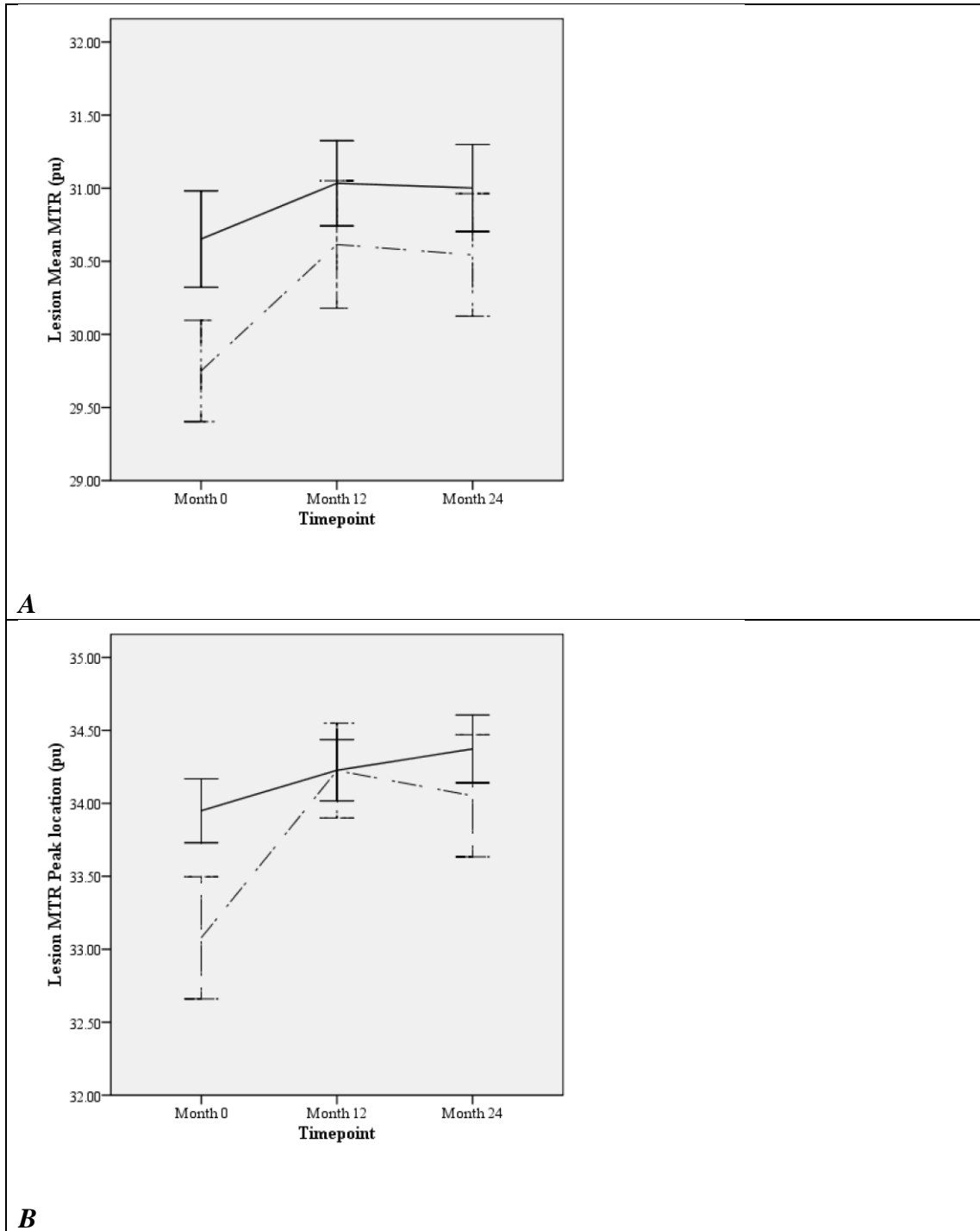
Using the second model significant correlations were seen of change in NAWM PH and mean with PASAT-3 ($\beta= 0.009$, $p=0.035$, $\beta=0.011$, $p=0.044$) and of NAWM mean with 1/9HPT ($\beta=0.009$, $p=0.026$). In all cases the correlations were positive indicating an increase in the MTR measure was associated with better performance in the clinical task and a reduction in MTR with a poorer performance in the clinical task.

Relationship of MTR measures with a sustained increase in EDSS

Eleven subjects in the placebo arm (20%) experienced a sustained increase in EDSS during the 24 month follow up period. Change in two MTR variables was found to significantly predict those subjects who experienced an increase in EDSS: lesion

MTR PL ($p=0.049$, standardized odds ratio (OR)= 0.924) and lesion mean MTR were associated with a sustained increase in EDSS ($p=0.002$, OR= 1.699). In both cases the baseline value was lower, although not significantly so, in the group that experienced a sustained increase in EDSS: PL 33.07pu (standard error [SE] 0.22) vs 33.97pu (SE 0.42, $p=0.07$); mean 29.74pu (SE 0.33) vs 30.71pu (SE 0.35, $p=0.16$); with a larger increase over 24 months in the group who did experience a sustained increase in EDSS: PL 0.97pu (SE 0.42) vs 0.39pu (SE 0.22, $p=0.25$), mean 0.79pu (SE 0.18) vs 0.30 (SE 0.10, $p=0.035$). Graphs illustrating the change in lesion PL and mean in the two groups are shown in figure 10.

Figure 10. Mean lesion MTR measures. Panel A shows MTR mean, panel B shows MTR peak location. The dotted line represents those subjects who experienced a sustained increase in EDSS during the 24 month study period, the solid line represents those who did not. The error bars show one standard error.



5.4. Discussion

The aim of this study was two-fold: firstly to evaluate the neuroprotective potential of lamotrigine in secondary progressive MS and secondly to assess the utility of brain MTR measures (reproducibility, responsiveness to change and the longitudinal correlation of MTR measures with neurological function and disability) as a marker of brain pathology in clinical trials of MS.

Overall the results of the study do not show that lamotrigine has a neuroprotective effect. While the scan-reposition-rescan reproducibility in the non-MS controls was good, the magnitude of the change over time in the MS group was small compared with the variability, perhaps indicating that the responsiveness of MTR measures to change was relatively limited. There were significant correlations of MTR measures with measures of neurological impairment, indicating that MTR does detect clinically relevant brain pathology.

The effect of treatment with lamotrigine

The only measures differing significantly between the two treatment arms were lesion and NAGM MTR PH. In both cases there was a significantly greater decrease in MTR in the verum arm (in fact there was a non significant increase in lesion PH in the placebo group). Since higher MTR is thought to represent higher myelinated axon content (van Waesberghe et al 1999, Schmeirer et al 2004, Barkhof et al 2003, Vrenken et al 2006, Fisher et al 2007) treatment with a neuroprotective drug should have the opposite effect; a greater decrease in the placebo group. It is possible that lamotrigine is neurotoxic rather than neuroprotective and causes increased neuroaxonal damage. However, clinical data from this trial suggests relative

preservation of neurological function in the verum arm (Kapoor et al 2010) which would tend to refute this explanation.

Another possible explanation is that lamotrigine has another physiological effect on brain tissue, which results in a fall in MTR without a reduction in axonal number. For example MTR has been shown to correlate with axonal expression of Na⁺/K⁺ ATPase in MS lesions (Young et al 2008). Reduced axonal sodium content brought about through lamotrigine mediated channel blockade could potentially lead to reduced expression of this enzyme and hence a fall in MTR. It would be interesting to evaluate this possibility in EAE for example.

Responsiveness of MTR measures to change and reliability

The changes in MTR measures seen in this study were small compared to those reported from previous studies (Rocca et al 1999, Inglese et al 2003, Rovaris et al 2003a). To minimize the contribution of partial volume voxels to the MTR histograms in this study, all voxels with an MTR value of <10pu were excluded and an erosion was imposed on NABT masks. The exclusion of this tissue from the histogram analysis may partly explain why MTR changes in this study were smaller than those in previously published reports.

The scan-reposition-rescan variability of MTR measures in the non-MS controls was good; comparable to previously published values for whole brain MTR measures acquired using a similar MRI sequence (Inglese et al 2001). However the period over which over scans were acquired in the MS group was markedly greater than that in the control group and it is known that increasing lesion volume can also lead to changes

in segmentation (Chard et al 2002c) so the variability caused by positioning and segmentation was likely to be somewhat higher in the MS subject group than in the non-MS controls.

Longitudinal association of MTR measures with MSFC and component measures

Despite the relatively limited sensitivity of MTR changes there were still significant longitudinal correlations with clinical measures in the placebo arm.

Greater reduction of MTR in NABT and lesion MTR was associated with poorer performance in the PASAT-3 and the 9HPT. Consistent correlation of NAGM and NAWM MTR with cognitive and arm function was previously seen in the baseline cross-sectional analysis (Hayton et al 2009) and has been observed in other study groups (Barkhof et al 2003, Rovaris et al 2000, Ramio-Torrenta et al 2006, Deoire et al 2005, Davies et al 2004). It may represent neurological impairment due to neuroaxonal loss, particularly since grey and white matter volume and atrophy have also been shown to correlate with deficits in these areas of neurological function (Filippi et al 2000b, Amato et al 2007, Sanfilippo et al 2006, Morgen et al 2006, Sastre-Garriga et al 2009).

Correlation of MTR measures with mobility have also previously been reported (Barkhof et al 2003, Ramio-Torrenta et al 2006, Fisniku et al 2009). Where tissue types have been analyzed in isolation it is primarily grey matter MTR measures which have been found to correlate with and predict mobility (Rocca et al 1999, Fisniku et al 2009, Agosta et al 2006). This study corroborates these findings with only NAGM MTR mean correlating with TW. The consistent correlation of changing NAGM mean

MTR with change in all three components of the MSFC highlights the likely importance of grey matter pathology in determining neurological dysfunction in secondary progressive MS.

Using the second model, looking at the association between change in MTR measures with performance in the MSFC and component measures there were significant correlations observed of change in NAWM MTR mean and PH with performance in the PASAT-3 and 9HPT. These were all positive correlations indicating that an increase in MTR was associated with better performance in the clinical test and a reduction in MTR with a poorer performance. However the β values were very small, indicating that a very large reduction in MTR would be associated with a very small decrease in the clinical measure and of 36 comparisons made, only three were significant, so one should not put too much weight on these findings.

Change in lesion MTR measures is associated with a sustained increase in EDSS

A larger increase in mean lesion MTR over 24 months was associated with an increased probability of a sustained increase in EDSS. This is a surprising result for two reasons: firstly one would not expect lesion MTR to increase in people with secondary progressive MS: secondly it is the opposite of what one would expect if lesion MTR mean is a measure of myelinated axon content; a fall in mean MTR should correspond to an increase in disability.

One possible explanation for the increase in lesion MTR PH and PL in the placebo arm and MTR mean in both arms is that within lesions factors other than just the myelinated axon content, such as resolution of oedema or changes to the structure or

function of axons may influence MTR. In addition to low myelinated axon content low MTR in lesions has been shown to be associated with: larger axonal diameter (Fisher et al 2007) lower expression of the Na⁺/K⁺ ATPase in demyelinated axons (Young et al 2008) and the presence of parenchymal T-cell and chemokine expression in microglia and macrophages (Moll et al 2009). Hence, it is possible that those subjects who experienced a sustained increase in disability had higher levels of inflammation, axonal oedema and axonal dysfunction at baseline and that the increase in MTR represents resolving oedema and attrition of dysfunctional axons. The mean lesion MTR mean and PL were lower at baseline in those patients who experienced a sustained increase in EDSS than in those who did not, which would be consistent with this sort of process. However the differences were not statistically significant so one should not place too much emphasis on this result.

Limitations of the study

The main limitation of this study was the relative insensitivity of the MTR measures. MTR was a secondary endpoint and the power calculations for the study's sample size were based on CCV change which is more reproducible (Losseff et al 1996a) and responsive (Furby et al 2010). Replicating the study with a larger population may generate more reliable results, but the magnitude of the change in MTR measures would probably remain very small. A recent study in relapsing-remitting MS suggests that specifically targeting new lesions may improve the sensitivity of MTR measures (van del Elksamp et al 2010). The lamotrigine trial was not optimized to detect inflammatory activity, one of the eligibility criteria was that subjects should be relatively free of disabling relapses for at least two years prior to recruitment and no Gd was given, making the identification of new lesions somewhat less certain.

However future studies of potentially neuroprotective drugs in relapsing-remitting MS employing MTR as one of the MRI measures, would be advised to consider treating new and chronic lesions separately and in phase 3 trials with larger sample sizes, measurement of grey and white matter MTR changes may also be useful to evaluate putative neuroprotective treatments in secondary progressive MS.

As previously reported (Kapoor et al 2010) non-adherence in the verum arm was higher than expected and disproportionate to the placebo arm. This may have influenced the outcome of the study, but it is difficult to envisage a good way to prevent this.

Finally the design of the study failed to take into account the possible unintended effects of the drug on the outcome measures. This is increasingly recognized as a problem in MS studies (Zivadinov et al 2008). The use of cross-over study design or multiple assessments prior to the introduction of the IMP or after its withdrawal may allow investigators to differentiate the drug effects on neuroaxonal volume or inflammation, which one would expect to occur rapidly, from any effects on neuroaxonal survival, which one would expect to be a much slower process.

6. Clinical and radiological correlates of t1 and t2 lesion volume measures in secondary progressive multiple sclerosis

6.1. Introduction

The detection of T2 hyperintense lesions in specific regions of the central nervous system is useful in the diagnosis of MS (Polman et al 2005) and the number and volume of T2 lesions present in the early stages of the condition can partially predict later disability (Tinotore et al 2006, Rudick et al 2006a, Fisniku et al 2008a). There is a correlation between T2LV and neurological impairment, but the strength of this relationship diminishes with increasing disability, especially in people with secondary progressive MS (Li et al 2006).

A proportion of T2 lesions are hypointense on T1 weighted imaging representing inflammation in the acute phase (van Waesberghe et al 1998a, Rovira et al 1999, Bagnato et al 2003) or, if persistent, a relative reduction in myelinated axon content (Rovira et al 1999, van Waesberghe et al 1999, van Walderveen et al 1999). T1LV may therefore be a relatively specific measure of focal MS pathology and so be a useful tool for monitoring brain pathology in clinical studies.

Clinical status in MS has been shown to correlate with brain and spinal cord atrophy (Losseff et al 1996a, Miller et al 2002, Anderson et al 2006), perhaps indicating that chronic neuroaxonal loss may be partly responsible for progressive disability. One possible mechanism is toxic sodium accumulation following up-regulation of voltage gated sodium channels on demyelinated axons (Smith et al 2001, Craner et al 2003, Moll et al 2009). Sodium channel blockers such as lamotrigine have been found to

ameliorate progressive neurological dysfunction and axonal loss in the animal model EAE (Bechtold et al 2006) and so a recent clinical trial has been conducted to evaluate the neuroprotective potential of lamotrigine in MS (Kapoor et al 2010).

The purpose of this study was to compare T1LV with T2LV and the ratio of T1 to T2 lesion volume in a group of people with secondary progressive MS who were taking part in a clinical trial of neuroprotection with lamotrigine, evaluating the responsiveness of both measures to change; the cross-sectional and longitudinal correlation with clinical status and MRI measures of NABT pathology and global atrophy (suggesting neuro-axonal loss); and the effects of lamotrigine treatment on T1LV and T2LV.

4.2. Methods

Subjects

This study comprises 118 people with secondary progressive MS, taking part in the lamotrigine trial (see section 2.1).

Clinical data

Clinical data included here are EDSS, MSFC and the presence or absence of relapses. For details of how these measures were collected see section 2.2.

MRI acquisition and analysis

Details of the MRI acquisition parameters used in this study are given in table 1. Data presented here are:

- T1LV

- T2LV
- The presence or absence of new and enlarging (active) T1 and T2 lesions
- NBV and percentage brain volume change (PBVC) calculated using SIENA
- Mean MTR for NABT. The data presented here were generated from tissue segments derived using SPM5 (Ashburner and Friston 2005)

Statistics

Change in MRI and clinical measures

The change in MRI and clinical measures were calculated by subtracting baseline values from the values at 24 months; thus a negative value indicated a fall in that measure over 24 months; a negative value for the clinical measure indicated neurological deterioration. Single sample Student's t-tests were used to determine whether any changes measured over 24 months were significant.

The effect of treatment with lamotrigine

Differences in change in MRI measures between the placebo and verum arms were assessed using independent samples Student's t-tests. Intention to treat analysis was used in this study, with all randomized subjects invited for every scan irrespective of whether they were still taking the investigational medicinal product. Two additional, post-hoc, per protocol analyses were employed: a comparison of tablet adherent subjects, defined as those who had taken 80% of prescribed tablets and were still being prescribed tablets at 24 months; and a serum adherent comparison, which compared subjects in the verum arm who had detectable serum lamotrigine at 24 months with the entire placebo arm.

Reliability of the MRI measures

It was not possible to estimate scan-reposition-rescan reproducibility for the lesion measures. Scan-reposition-rescan reproducibility for MTR and NBV was estimated using MRI scans from three healthy controls, each of whom was scanned on five occasions over a four week period. To quantify intra-rater variability for T1 and T2LV and inter-rater variability for T2LV a set of five randomly selected, anonymized test scans taken from the lamotrigine trial library was analyzed on two separate occasions. In all cases coefficients of variability were calculated. The values for T1 and T2LV are shown in table 2.

Longitudinal correlation between lesion volume measures and MSFC

Longitudinal correlation of lesion volume measures with MSFC and component scores was assessed in the placebo arm using a random intercept mixed effect linear regression model with age, gender, disease duration and time as covariates. A second model was calculated evaluating the correlation of MSFC with an interaction variable - [lesion volume measure] x time. The first model shows the correlation of lesion volume and clinical measures at any given timepoint. The second shows the correlation of the clinical measure with the change in the lesion volume measure.

The association between lesion measures and EDSS

Subjects in the placebo arm were divided into those who did or did not experience a sustained increase in EDSS over 24 months, defined as an increase of ≥ 0.5 points from baseline observed at two consecutive assessment visits over six months in subjects with a baseline EDSS of ≥ 6.0 or of 1.0 points in subjects with a baseline

EDSS of ≤ 5.5 . Differences in baseline lesion volume values and change in lesion volume were assessed using binary logistic regression with age, gender and disease duration as covariates.

Treatment effect

Differences in change in lesion volume measures between verum and placebo arms were tested using independent samples Student's t-tests. Intention to treat analysis was employed, so all subjects were invited for scanning at every timepoint, and every subject who attended for scanning at baseline and 24 months was included in the analysis. In addition two per protocol comparisons were also carried out: 1) Tablet Compliant (TC), within the subgroup of participants who consumed at least 80% of prescribed tablets and were still being prescribed tablets at 24 months; 2) Serum Compliant (SC), comparing participants in the active group with detectable serum lamotrigine at 24 months with the whole placebo group.

6.3. Results

Demographic characteristics of the subjects shown in tables 4 and 8. Baseline lesion volume measures are shown in table 12.

Table 12. Lesion volume measures at baseline. Data shown are: mean values (standard deviation - SD) unless otherwise specified.

<i>Characteristics</i>	<i>Placebo arm, n=57</i>	<i>Verum arm n=61</i>	<i>p-value (Student's T-test)</i>
<i>T1LV, ml - median (range)</i>	<i>9.94 (0.18 to 43.67)</i>	<i>9.91 (0.21 to 46.96)</i>	<i>0.526</i>
<i>T2LV, m- median (range)</i>	<i>23.02 (0.82 to 87.97)</i>	<i>25.35 (0.35 to 71.62)</i>	<i>0.706</i>
<i>T1 to T2 lesion volume ratio</i>	<i>0.47 (0.21)</i>	<i>0.44 (0.21)</i>	<i>0.377</i>
<i>Mean NABT MTR, pu</i>	<i>34.26 (1.27)</i>	<i>34.47 (0.92)</i>	<i>0.374</i>
<i>NBV, ml</i>	<i>1480.51 (99.77)</i>	<i>1471.09 (101.09)</i>	<i>0.616</i>

T1LV - T1 hypointense lesion volume, T2LV - T2 hyperintense lesion volume, PBVC - percentage brain volume change, NABT MTR normal appearing brain tissue mean magnetization ratio.

Change in lesion measures and treatment effect

The results of the intention to treat analysis are shown in table 13. There was a significant increase in mean T1LV and T2LV in both verum and placebo arms but no significant difference in the size of this change between the two groups. There was no significant change in T1 to T2 lesion volume ratio over 24 months in either trial arm. The z-score is an indication of the responsiveness of the measures – the higher the z-score the more responsive the measure is to change. In this study the responsiveness of T1 and T2 lesion volumes was similar.

Table 13. Mean change in lesion volume measures. Significant results highlighted in bold

		Mean annual change	Mean absolute change over 24 months (standard error)	Significance of change over 24 months	Significance of difference between verum and placebo arms [‡]
T1LV	Placebo	1.83ml (15.3%)	3.67ml (0.61)	z=5.40* p<0.0001	p=0.46
	Verum	2.01ml (19.9%)	3.96ml (0.54)	z=5.13* p<0.0001	
T2LV	Placebo	2.93ml (18.2%)	5.87ml (1.09)	z=5.47* p<0.0001	p=0.28
	Verum	2.94ml (17.5%)	5.96ml (1.09)	z=5.55* p<0.0001	
T1 to T2 lesion volume ratio	Placebo	-0.003 (-2.67%)	-0.01 (0.18)	t= 0.37 [†] p=0.71	p=0.49
	Verum	0.005 (6.49%)	0.01 (0.18)	t= 0.60 [†] p=0.55	
PBVC	Placebo	-0.59%	-1.19% (0.10)	t=12.00[†] , p<0.0001	p=0.16
	Verum	-0.70%	-1.34% (0.11)	t=12.28 p<0.0001	
NABT mean MTR	Placebo	-0.06pu (-0.16%)	-0.12pu (0.06)	t= 1.92 [†] p=0.60	p=0.77
	Verum	-0.09pu (-0.27%)	-0.15pu (0.08)	t= 1.97 [†] p=0.54	

T1LV T1 hypointense lesion volume, T2LV T2 hyperintense lesion volume, PBVC percentage brain volume change, NABT mean MTR normal appearing brain tissue mean magnetization ratio. *paired sample Wilcoxon sign rank test baseline vs. 24 months; †paired sample Student's t-test; ‡ independent sample student's t-test comparing change over 24 months in the placebo vs. the verum arms.

Similar results were seen with both forms of per protocol analysis with no significant differences between the two groups in the change of any of the lesion measures using either tablet compliance (T1LV p=0.50, T2LV p=0.47, T1 to T2 lesion volume ratio p=0.83); or serum compliance (T1LV p=0.40, T2LV p=0.41, T1 to T2 lesion volume ratio p=0.58).

Reliability of the MRI measures

The scan-reposition-rescan reproducibility for mean NABT MTR and PBVC were very high with coefficients of variability of 0.31% and 0.22% respectively.

Correlation with MSFC: cross-sectional correlation

The correlation of lesion measures with clinical measures at baseline are shown in table 14. There were significant negative correlations of all three lesion volume measures with MSFC, PASAT-3 and 1/9HPT. The best lesion predictor for all three was lnT1LV, explaining approximately 19%, 18% and 9% of the variability of MSFC, PASAT-3 and 1/9HPT respectively. None of the lesion measure correlated significantly with 1/TW.

When NBV and NABT mean MTR were included in the regression models the best predictor model for MSFC comprised NBV ($r_p=0.20$, $p=0.038$) and NABT mean MTR ($r_p=0.26$, $p=0.006$; $R^2=0.21$), while NABT mean MTR was a lone best predictor for PASAT-3 ($r_p=0.53$, $p<0.0001$; $R^2=0.28$) and NBV the lone best predictor for 1/9HPT ($r_p=0.45$, $p<0.0001$; $R^2=0.20$).

Table 14. Cross-sectional correlation of lesion volume measures with other MRI measures, MSFC and component measures. The results of linear regression analysis with NBV, mean NABT MTR, MSFC or component measure as a product of lesion volume measure, age, sex and disease duration. Significant results highlighted in bold.

	<i>MRI measure</i>		<i>Clinical measure</i>			
	<i>NBV</i>	<i>Mean NABT MTR</i>	<i>MSFC</i>	<i>PASAT-3</i>	<i>1/9HPT</i>	<i>1/TW</i>
<i>lnT1LV</i>	<i>$r_p=-0.52$, $p<0.0001$</i>	<i>$r_p=-0.57$, $p<0.0001$</i>	<i>$r_p=-0.31$, $p=0.001$</i>	<i>$r_p=-0.42$, $p<0.0001$</i>	<i>$r_p=-0.31$, $p=0.001$</i>	<i>$r_p=0.076$, $p=0.736$</i>
<i>lnT2LV</i>	<i>$r_p=-0.46$, $p<0.0001$</i>	<i>$r_p=-0.54$, $p<0.0001$</i>	<i>$r_p=-0.26$, $p=0.004$</i>	<i>$r_p=-0.35$, $p<0.0001$</i>	<i>$r_p=-0.28$, $p=0.003$</i>	<i>$r_p=0.01$, $p=0.916$</i>
<i>T1 to T2 ratio</i>	<i>$r_p=-0.41$, $p=0.001$</i>	<i>$r_p=-0.41$, $p=0.002$</i>	<i>$r_p=-0.26$, $p=0.005$</i>	<i>$r_p=-0.31$, $p=0.001$</i>	<i>$r_p=-0.25$, $p=0.009$</i>	<i>$r_p=-0.13$, $p=0.168$</i>
<i>Best predictor model</i>	<i>lnT1LV and disease duration $R^2 = 0.35$</i>	<i>lnT1LV $R^2 = 0.33$</i>	<i>lnT1LV $R^2 = 0.19$</i>	<i>lnT1LV and sex $R^2 = 0.21$</i>	<i>lnT1LV $R^2 = 0.09$</i>	<i>n/a</i>

T1LV T1 hypointense lesion volume, T2LV T2 hyperintense lesion volume, PBVC percentage brain volume change, NABT mean MTR normal appearing brain tissue mean magnetization ratio.

Correlation with MSFC: longitudinal correlation

There were no significant correlations of change in any lesion volume measure with change in MSFC or any of the component measures.

The results of random intercept mixed effect linear regression model are shown in table 15. These models show that at any given timepoint there were significant negative correlations of change in: lnT1LV and T1 to T2 lesion volume ratio with MSFC; lnT1LV and lnT2LV with PASAT-3; and all three lesion measures with 1/9HPT; none of the lesion measures correlated significantly with 1/TW.

There was no significant correlation of any of the lesion measures with an interaction variable [MSFC/component measure]*time.

Table 15. Longitudinal correlation of lesion measures with other MRI measures, MSFC and components. Data shown are standardized β – the number of standard deviations of change in the MRI or clinical measure for every standard deviation increase in lesion measure. Statistically significant results are highlighted in bold.

	<i>Clinical measures</i>			
	<i>MSFC</i>	<i>PASAT-3</i>	<i>1/9HPT</i>	<i>1/TW</i>
<i>lnT1LV</i>	$\beta=-0.20, p=0.013$	$\beta=-0.19, p=0.015$	$\beta=-0.30, p<0.0001$	$\beta=-0.113, p=0.159$
<i>lnT2LV</i>	$\beta=-0.14, p=0.057$	$\beta=-0.19, p=0.011$	$\beta=-0.25, p=0.002$	$\beta=-0.05, p=0.485$
<i>T1 to T2 lesion volume</i>	$\beta=-0.06, p=0.009$	$\beta=-0.05, p=0.054$	$\beta=-0.09, p<0.0001$	$\beta=-0.04, p=0.073$

MSFC – multiple sclerosis functional composite, *PASAT-3* – paced auditory serial addition test, *9HPT* – 9 hole peg test, *TW* – timed 25 foot walk

The association of lesion volume measures with disability or markers of inflammatory activity.

In the placebo arm, 30 subjects (54%) had one or more active T2 lesions, 23 (41%) had one or more active T1 lesions and 26 (46%) experienced one or more relapses during the trial period. In the verum arm 23 subjects (38%) had one or more active T2 lesions, 17 (28%) had one or more active T1 lesions and 25 (41%) experienced one or more relapses. There were no significant differences in any of these measures between the two groups.

In the placebo group, neither change in T1 or T2 lesion volume predicted whether or not a subject was likely to experience a relapse during the 24 month follow up period.

26 subjects in the placebo group were in the higher disability group at baseline none of the baseline lesion measures significantly predicted whether a subject would be in

the higher EDSS group. 11 subjects in the placebo group experienced a sustained increase in EDSS during the trial period. Change in T1, T2LV or T1 to T2 lesion volume ratio did not significantly predict a sustained increase in EDSS.

6.4. Discussion

These data do not show that treatment with lamotrigine has any effect on the rate of change of lesion volume measures. The data confirm cross-sectional correlation of both T1 and T2LV with MSFC and component measures and that these correlations are consistent across all three timepoints. At baseline T1LV emerged as the only independently significant correlate of the clinical measures, perhaps suggesting that it may be the more specific measure of clinically important brain pathology. However even though both T1 and T2LV did change significantly over the 24 months of the study there was no significant correlation of change in lesion volume measures with change in any of the clinical measures, raising the question of whether either measure is sufficiently responsive to be useful in clinical trials.

The effects of lamotrigine

In this study lamotrigine had no significant effect on any lesion measure, using either intention to treat or per-protocol analysis. If lamotrigine does indeed prevent degeneration of chronically demyelinated axons one would hope to see a slower increase in T1 lesion volume in the verum arm compared with the placebo arm, which unfortunately was not the case here.

It is also possible that either lamotrigine is not neuroprotective in MS, or that the neuroprotective effect is too small to be detected using the sample size, follow up time

or assessment measures used in this study. The in vitro and animal studies on which the trial was based do suggest that sodium channel blockade has the potential to be neuroprotective (Garthwaite et al 2002, Lo et al 2002, Kapoor et al 2003, Bechtold et al 2004, Bechtold et al 2006) and it is tempting to suggest longer, larger trials with additional biomarkers – optical coherence tomography (Fisher et al 2006), neurofilament light chain etc. (Semra et al 2002). However when conducting clinical trials one has to consider the welfare of the subjects taking part and the value of effective treatment – is it worth exposing large numbers of patients to prolonged or unproven investigations for a medication that offers a very small benefit?

Responsiveness of T1 and T2LV

In this study the responsiveness of T1 and T2 lesion volumes are very similar (see table 13.). Both compare well to NABT mean MTR (there was no significant change in this measure over the two years) but less well compared to PBVC.

In previously published studies there is a wide range for change in T1 and T2LV (van Walderveen et al 1995, van Walderveen et al 1999a, Truyen et al 1996, Simon et al 2000, Barkhof et al 2001, Wolinsky et al 2001, Zivadinov et al 2001, Rovaris et al 2003a, Sailer et al 2003, Mesaros et al 2008, Korteweg et al 2009) and the absolute values recorded in this study are at the higher end of this range. However the variability of the lesion volume changes in this study was relatively wide, perhaps limiting the responsiveness of these measures. It was not possible to measure the scan-reposition-rescan variability of the lesion measures, but the inter- and intra-rater variability of these measures was quite large (see table 2) highlighting the influence of human error.

The process for deriving PBVC is almost completely automated, substantially improving the reproducibility (Smith et al 2002). The other advantage of SIENA (the process by which PBVC is calculated) is that by coregistering sequential images it very sensitively identifies areas of volume change, which may not be apparent using localized thresholding techniques on individual scans.

Some centres have developed automated techniques for identifying and contouring T1 lesions, which cuts down on the variability in this measure due to human judgment. However this technique still requires reference to a previously identified T2 lesion mask (Fisher et al 2007). To produce a robust comparison of T1 and T2 lesion measures, what is really needed are fully automated techniques for acquiring both measures independently, i.e. the T1LV without reference to the T2 lesion maps. If this also involved interpolation and coregistration of sequential scans, in a similar fashion to SIENA, the sensitivity of both measures would probably also increase.

Correlation with clinical measures

Previously published cross-sectional studies in subjects with secondary progressive MS have shown significant correlations of T1LV with MSFC (Kalkers et al 2001b) and PASAT-3 (Kalkers et al 2001a). In this study there were cross-sectional correlations of both T1LV and T2LV with PASAT-3 and 1/9HPT with T1LV the only independently significant cross-sectional correlate of both measures.

The amount of variance in the clinical measures explained by T1LV (21% and 9% for cognitive and arm function respectively) suggests that other aspects of brain

pathology which may not be directly related to focal lesion burden, such as grey matter damage, may also contribute to neurological dysfunction. Previous MRI studies correlating abnormalities in NABT with neurological impairment and the correlation of grey matter atrophy with neurological impairment would corroborate this (Filippi and Agosta 2007, Fisher et al 2008, Fisniku et al 2008b, Furby et al 2010). Additional corroboration would be the finding from this study that introducing NABT mean MTR into the model correlating MRI values with PASAT-3 scores and NBV into the corresponding model for 1/9HPT rendered lnT1LV association non-significant.

Previous studies have shown correlations of both T1 hypointense and T2 hyperintense lesion measures with EDSS (van Walderveen et al 1995, Truyen et al 1996, Lycklama y Nijeholt et al 1998, van Waesberghe et al 1998b, Ianucci et al 1999, Rovaris et al 1999, van Walderveen et al 1999b Simon et al 2000, Rovaris et al 2003b). In this study, none of the lesion measures were associated with either a higher EDSS at baseline or a sustained increase in EDSS over two years. Furthermore none of the lesion measures correlated with mobility, either cross-sectionally or longitudinally. In this study subjects were selected to have an EDSS of 4.0 to 6.5 at baseline; a range at which variation in EDSS is almost exclusively determined by mobility (Kurtzke et al 1983). It is possible that brain lesions have a relatively limited effect on mobility and so a relatively limited effect on EDSS in this subject group. Previous studies which do show correlations of both T1 hypointense and T2 hyperintense lesion measures with EDSS have subjects with a wider range of EDSS scores, where arm and cognitive function may be more influential (van Walderveen et al 1995, Truyen et al 1996, Lycklama y Nijeholt et al 1998, van Waesberghe et al 1998b, Ianucci et al 1999,

Rovaris et al 1999, van Walderveen et al 1999b Simon et al 2000, Rovaris et al 2003b).

Although the correlations of lesion volume measures with MSFC and component measures were consistent across all three time-points, there was no correlation of change in lesion volume with change in any of the MSFC measures. Nor was a model correlating lesion measures with the interaction variable of [clinical measure]*time statistically significant. It is possible that while the burden of focal lesions accumulated over the whole timecourse of MS does correlate with the degree of neurological dysfunction, new changes in lesion volume in established SPMS do not contribute to concurrent progressive impairment. It is known that there is axonal transection in lesions (Trapp et al 1998) and it has been proposed that this may lead to subsequent brain atrophy and neurological impairment. It would be interesting to follow this study group up after a longer time interval to see if those subjects who had the largest increase in lesion volume over the course of this study subsequently showed a greater change in clinical status.

The effects of inflammatory activity

Change in T1LV may not simply represent axonal degeneration; acute T1 hypointense lesions may represent focal oedema associated with inflammatory demyelination (van Waesberghe et al 1998a, Rovira et al 1999, Bagnato et al 2003). Recent evidence suggests that sodium channel blockers may have some immunomodulatory potential (Black et al 2007) and differences between the two groups in terms of new inflammatory activity is a potentially confounding factor. There was no significant difference between the two groups in terms of the presence of active T1 or T2 lesions.

However the best established marker of new focal neuroinflammation is Gd enhanced lesion load (Tubridy et al 1998) and, because of concerns about the duration of the scanning protocol, we did not acquire this.

7. CLINICAL AND IMAGING CORRELATES OF THE MULTIPLE SCLEROSIS IMPACT SCALE IN SECONDARY PROGRESSIVE MULTIPLE SCLEROSIS.

7.1. Introduction

The correlation of MRI measures of pathology with clinician assessed neurological function is subtotal (Miller et al 2002, Li et al 2006, Filippi and Agosta 2007) but clinicians' assessment of the severity of a patient's MS and patients' perception of the impact of MS on their quality of life (QoL) can also differ (Rothwell et al 1997) and it remains to be established whether MRI detected brain pathology may have an impact on QoL which is independent of clinically detected neurological dysfunction.

In this study we tried to establish whether MRI detectable brain and spinal cord MS pathology and clinical measures of neurological function and disability correlated independently with patient's assessment of the impact MS on their lives and which aspects of both seem to be most important.

7.2. Methods

Subjects

This study comprises 118 people with secondary progressive MS, from the lamotrigine trial (see section 2.1).

Clinical data

Clinical data included here are EDSS, MSFC the presence or absence of relapses and MSIS-29. For details of how these measures were collected see section 2.2. Two scores were derived from the MSIS-29 questionnaire MSIS-phys and MSIS-psych. Higher scores are awarded for greater impact on QoL.

MRI acquisition and analysis

Details of the MRI acquisition parameters used in this study are given in table 1. Data presented here are:

- T2LV
- T1LV
- Active lesions, i.e. new and enlarging T2 and T1 lesions
- NBV
- PBVC derived using SIENA
- CCV
- MTR histograms for T2 lesions, NAGM and NAWM The data presented here were generated from tissue segments derived using SPM05 (Ashburner and Friston 2005)
- GMF and WMF
- SCCA

Statistics

The aim of this study was to identify which measures, clinical and MRI, were independently significant correlates of MSIS-29 measures. In order to keep each regression model as small as possible, while including as many variables as possible a two stage process was used. In the first stage, variables which were thought a priori to

be likely to be intercorrelated were grouped together. The following categories were used: 1. Baseline demographic characteristics – age, sex, disease duration; 2 Tests of neurological function - MSFC and component measures; 3. Disability - EDSS; 4. Lesion volume measures; 5. Brain volume and atrophy measures; 6. MTR measures; 7. SCCA. For the longitudinal analysis two further categories were included: binary variables denoting the presence or absence of clinical relapses and the presence or absence of active lesions.

Because of the limited range of values, EDSS was converted to a binary variable. For cross-sectional analysis subjects were divided into higher ($EDSS \geq 6.5$) and lower ($EDSS \leq 6.0$) disability groups, and for longitudinal analysis divided according to whether or not they experienced an increase in EDSS from baseline observed at two consecutive assessment visits (≥ 1.0 points in subjects with a baseline EDSS of ≤ 5.5 or ≥ 0.5 points from in subjects with a baseline EDSS of ≥ 6.0). The decision to treat the EDSS as a binary variable was made prior to conducting any statistical analysis.

For each group of variables linear regression analysis was used to identify the ‘least significant correlate’ (the one with highest p-value). This variable was ‘discarded’ and the regression analysis repeated to identify the ‘next least significant correlate’. This process was repeated until only independently significant variables were left, then, to make sure no independently significant correlate had been erroneously discarded due to collinearity, each of the discarded variables were tested against the final model. For all binary variables Student’s T-tests, rather than regression analysis, were used to identify whether the MSIS measures were different between the two groups. For

SCCA, which was considered on its own, Pearson correlation coefficient was calculated.

In the second stage of the statistical analysis a ‘multimodal best predictor model’ was established by repeating the stepwise multiple regression analysis including all the independently significant correlates from the first stage in a single model. Again, all previously discarded variables are checked against the final model, including those discarded in the first stage of the analysis.

Cross-sectional analysis

Cross-sectional analysis was done using baseline MSIS-29, clinical and MRI measures. Baseline T1 and T2 lesion volumes were not normally distributed so were log transformed prior to statistical analysis.

Longitudinal analysis

For all continuous variables longitudinal associations were evaluated by comparing the change in a given clinical or MRI value over the 24 months with the change in MSIS-29 values over 24 months. Change in a continuous variable was calculated by subtracting the baseline value from the value at 24 months, so a negative change represented a reduction in that parameter. Single sample Student’s t-tests were used to identify those variables that changed significantly over 24 months. Because of the potentially confounding effects of lamotrigine, only subjects from the placebo group were included in the longitudinal analysis.

7.3. Results

Baseline demographic details are shown in table 16. Two patients experienced an increase in EDSS between screening and the baseline visit meaning that the range of baseline values was wider than would be expected from the trial inclusion criteria.

Table 16. Baseline demographic characteristics for the both the placebo and verum arms combined. (demographic data for the placebo and verum arms is shown in table 9).

<i>Characteristics</i>	<i>Whole group, n= 118</i>
<i>Sex, Female/Male (%)</i>	<i>85/33 (72/28)</i>
<i>Age, years - mean (SD; range)</i>	<i>50.6 (7.0; 30 to 61)</i>
<i>Disease duration, years - mean (SD; range)</i>	<i>20.1 (8.8; 3 to 41)</i>
<i>EDSS, median (inter quartile range; range)</i>	<i>6.0 (0.5; 4 to 7.5)</i>

SD - standard deviation, EDSS – expanded disability status scale.

In the placebo arm: 27 subjects (47%) experienced one or more relapses during the study period (range 1 to 4). 30 subjects (53%) in the verum arm had one or more active T2 lesions during follow up period; the median number of active lesions detected was 3 (range 1 to 13 active lesions), with a median annualized active lesion rate for the whole group of 0.75 (range 0 to 6.5). 13 subjects (23%) experienced a sustained increase in EDSS. Other clinical and MRI data are summarized in table 17.

Table 17. Clinical and MRI characteristics at baseline and change over 24 months.

Characteristics	Whole group Baseline values, mean (SD)	Placebo arm Baseline values, mean (SD)	Placebo arm Change over 24 months, mean (SD)	Significance of change over 24 months*, t- value (p-value)
<i>MSIS-phys</i>	60.86 (15.15)	60.98 (15.44)	2.08 (14.38)	1.09 (0.28)
<i>MSIS-psych</i>	24.10 (8.71)	24.07 (8.89)	-0.30 (7.91)	0.29 (0.78)
<i>PASAT-3</i>	43.1 (13.6)	44.7 (13.6)	-2.5 (9.6)	1.96 (0.06)
<i>TW, sec</i>	36.0 (47.9)	20.5 (60.0)	7.8 (18.2)	2.99 (0.004)
<i>9HPT, sec</i>	57.1 (106.5)	32.3(25.0)	13.1 (66.3)	1.47 (0.15)
<i>T2LV, ml</i>	25.96 (17.80)	26.61 (18.39)	5.87 (8.06)	5.40 (<0.0001)
<i>T1LV, ml</i>	13.51 (12.08)	14.25 (12.85)	3.67 (6.19)	4.34 (<0.0001)
<i>T1 to T2 lesion volume ratio</i>	0.46 (0.20)	0.48 (0.21)	-0.01 (0.13)	0.37 (0.71)
<i>CCV, ml</i>	254.89 (23.79)	255.04 (23.62)	-7.30 (5.30)	10.44 (<0.0001)
<i>GMF</i>	0.45 (0.03)	0.46 (0.03)	-0.002 (0.007)	5.19 (<0.0001)
<i>WMF</i>	0.29 (0.02)	0.29 (0.02)	-0.008 (0.01)	2.24 (0.03)
<i>Whole brain atrophy, ml</i>	NBV 1475.6 (SD 100.1)	NBV 1480.5ml (SD 99.8ml)	PBVC -1.18% (SD 0.74%)	12.00[†] (<0.0001)
<i>Lesion MTR, pu</i>	30.70 (1.98)	30.58 (2.02)	0.40 (0.70)	4.21 (<0.0001)
<i>NAGM MTR, pu</i>	32.05 (1.07)	31.99 (1.12)	-0.13 (0.26)	3.54 (0.001)
<i>NAWM MTR, pu</i>	37.37 (1.08)	37.27 (1.33)	-0.06 (0.92)	0.49 (0.64)
<i>SCCA, mm2</i>	63.59 (8.62)	66.29 (9.06)	-2.05 (2.47)	6.20 (<0.0001)

MSIS-phys – *MSIS-29* physical component, *MSIS-psych* – *MSIS-29* psychological component, *PASAT-3* – Paced Auditory Serial Addition Test, *TW* – 25 foot timed walk, *9HPT* – 9 hole peg test, *T1LV* – T1 hypointense lesion volume, *T2LV*, T2 hyperintense lesion volume, *CCV* – central cerebral volume, *GMF* – grey matter fraction, *WMF* – white matter fraction, *MTR* – magnetization transfer, *NAWM* – normal appearing white matter, *NAGM*, normal appearing grey matter, *SCCA* – spinal cord cross sectional area, *NBV* – normalized brain volume, *PBVC* – percentage brain volume change. *two-tailed Student's *t*-test comparing baseline with 24 months. † calculated using a single sample Student's *t*-test against a test variable of 0.

Cross-sectional association: stage 1.

The results of the first stage analysis are shown in table 18.

Table 18. The results of stage 1 cross-sectional analysis. Unless otherwise stated shows the results of multiple linear regression analysis showing the correlation of baseline MSIS-29 with baseline clinical and MRI measures. Only independently significant correlates of are shown.

	MSIS-phys		MSIS-psych	
	Independently variables	significant	Independently variables	significant
Clinical measures				
1. Demographic characteristics*: Age, sex, disease duration	None		Female sex $r_p=0.20, p=0.035$	
2. MSFC and component measures*: MSFC, 1/TW, 1/9HPT, PASAT-3	1/TW $r_p=-0.38, p<0.0001$		PASAT-3 $r_p=-0.31, p=0.001$	
3. EDSS†: EDSS high or low	None		None	
MRI measures				
4. Lesion measures*: lnT1LV, lnT2LV, T1 to T2 ratio	T1 to T2 ratio $r_p=0.24, p=0.009$		T1 to T2 ratio $r_p=0.31, p=0.001$	
5. Brain volume measures: CCV, NBV, GMF, WMF*	CCV $r_p=-0.22, p=0.015$		CCV $r_p=-0.21, p=0.025$	
6. MTR measures*: NAGM, NAWM and lesion (PH, PL, mean)	None		None	
7. SCCA‡	None		None	

*Multiple linear regression analysis. † Two tailed Student's t-test. ‡ Pearson correlation coefficient. MSIS-phys – MSIS-29 physical component, MSIS-psych – MSIS-29 psychological component, PASAT-3 – Paced Auditory Serial Addition Test, TW – 25 foot timed walk, 9HPT – 9 hole peg test, T1LV – T1 hypointense lesion volume, T2LV, T2 hyperintense lesion volume, CCV – central cerebral volume, GMF – grey matter fraction, WMF – white matter fraction, MTR – magnetization transfer ratio, NAWM – normal appearing white matter, NAGM, normal appearing grey matter, SCCA – spinal cord cross sectional area.

1/TW was the only independently significant clinical correlate of MSIS-phys whereas PASAT-3 was the only independently significant correlate of MSIS-psych.

Of the lesion measures, T1/T2 lesion volume ratio was the only independently significant correlate of MSIS phys and MSIS-psych. CCV was the only one of all the brain volume measures to independently correlate with MSIS phys and MSIS-psych. None of the other MRI measures correlated independently with either of the MSIS measures.

Cross-sectional association: stage 2.

The results of stage 2, the multi-modal analysis, are shown in table 19.

Table 19. The results of stage 2 cross-sectional analysis. Shows the results of multiple linear regression analysis correlating baseline MSIS-29 with of all the independently significant correlates from stage 1 analysis.

	MSIS-phys	MSIS-psych
Variables included in the model	1/TW, T1 to T2 ratio, CCV	Female sex, PASAT-3, T1 to T2 ratio, CCV
Independently significant variables	1/TW $r_p=-0.36$, $p<0.0001$	T1 to T2 ratio $r_p=0.24$, $p=0.009$ PASAT-3 $r_p=-0.18$, $p=0.041$
	$R^2=0.13$	$R^2=0.13$

MSIS-phys – MSIS-29 physical component, MSIS-psych – MSIS-29 psychological component, TW – timed 25 foot walk, CCV – central cerebral volume.

1/TW accounted for approximately 13% of the variability in MSIS-phys. T1 to T2 lesion volume ratio was not an independently significant correlate in this model ($r_p = 0.127$, $p=0.051$).

However T1 to T2 lesion volume ratio was also the closest independently significant correlate of MSIS-psych, accounting for approximately 9% of the variability of the latter measure. The inclusion of PASAT-3 increased the proportion of the variability of MSIS-psych accounted for by the model to 13%.

Longitudinal association: Stage 1

Of the clinical measures 1/TW was the only independently significant correlate with MSIS-phys ($r_p=-0.27$, $p=0.047$) and MSIS-psych ($r_p=-0.36$, $p=0.007$)

Change in NAGM MTR peak height ($r_p=0.29$, $p=0.022$), NAGM peak location ($r_p=-0.45$, $p=0.001$) and mean NAWM MTR ($r_p=0.26$, $p=0.041$) all correlated independently with change in MSIS-psych together accounting for 22.2% of the variability of that measure.

Longitudinal association: Stage 2

The results of the multi-modal analysis are shown in table 20.

Table 20. The results of stage 2 longitudinal analysis. Shows the results of multiple linear regression analysis correlating change in MSIS-29 measures with all the independently significant correlates from stage 1 analysis.

	<i>MSIS-phys</i>	<i>MSIS-psych</i>
Variables included in the model	$\Delta 1/TW$	$\Delta 1/TW$, $\Delta NAGM$ peak height, $\Delta NAGM$ peak location, $\Delta NAWM$ mean MTR
Independently significant variables	$\Delta 1/TW$ $r_p=-0.27$, $p=0.047$	$\Delta 1/TW$ $r_p=-0.334$, $p=0.007$
		$\Delta NAWM$ mean $r_p=0.371$, $p=0.003$
		$\Delta NAGM$ peak height $r_p=-0.242$, $p=0.048$
	$R^2=0.09$	$R^2=0.32$

Δ - change in a given variable, *MSIS-phys* – MSIS-29 physical component, *MSIS-psych* – MSIS-29 psychological component, TW – 25 foot timed walk, NAGM – normal appearing grey matter, NAWM – normal appearing white matter, MTR – magnetization transfer ratio, CCV – central cerebral volume.

For change in MSIS-phys change in 1/TW was the only variable included in the model; it accounted for approximately 9% of the variability of change in MSIS-phys. For change in MSIS-psych change in 1/TW, change in NAWM mean and change in NAGM peak height were all independently significant correlates with a multimodal model accounting for approximately 32% of the variability.

7.4. Discussion

The aim of this study was to investigate the relationship between a range of clinical and imaging measurements with the MSIS-29, a measure of the impact of MS on QoL, in secondary progressive MS. Data from this study do show that in people with higher levels of MRI detected brain pathology and poorer clinician assessed neurological function, MS has a greater impact on QoL. However the associations, even from the best models, were modest, indicating that even when using a wide range of different clinical and MRI modalities, there may still be factors influencing QoL in MS, which are not adequately captured.

The contribution of neurological impairment to impact on QoL

There was no association of EDSS with MSIS-29 scores in this subject group. There is some evidence that changes in EDSS scores in the context of clinical trials may partly represent temporary fluctuation in function or variations in measurement rather than sustained disability due to irreversible myelin and neuroaxonal loss (Ebers et al 2008) and it is possible that the EDSS has a limited sensitivity for detecting neurological dysfunction in secondary progressive MS. However a number of previous studies which have shown good correlation between EDSS and the physical component of QoL measures (Rothwell et al 1997, Hoogervorst et al 2004, Benito-Leon et al 2003, Janhardan and Bakshi 2000, Lobentanz et al 2004, Isaksson et al 2005, Simeoni et al 2008, Nordvedt et al 2000, Gold et al 2001, Benito-Leon et al 2002, Benedict et al 2005, Mitchell et al 2005, Ytterberg et al 2008, Mowry et al 2009) and so the lack of a correlation in this study may reflect the limited sensitivity of EDSS in this subject group in particular, in whom the selection criteria ensured a narrow baseline range.

Significant correlation of MSIS-29 measures with components of the MSFC were found. TW was the only independently significant clinical correlate of MSIS-phys in the cross-sectional analysis, change in TW was the only measure of any kind that correlated with change in MSIS-phys over 24 months while PASAT-3 was the single best clinical predictor of MSIS-psych in the cross-sectional analysis. These findings would suggest that physician assessed neurological dysfunction does have an impact on QoL in MS.

Correlation of MRI measures with MSIS measures

It is well recognized that the correlations between T2 lesion volume and disability in MS are modest, particularly in the secondary progressive form of the condition (Miller et al 2002, Li et al 2006, Filippi and Agosta 2007). The pathological heterogeneity of T2 hyperintense lesions (Trapp et al 1998, Kutzelnigg et al 2005) may partly explain this phenomenon, with lesions comprising a higher level of neuroaxonal loss and more severe chronic demyelination contributing more to neurological dysfunction. Chronically hypointense T1 lesions are known to be relatively specific for neuroaxonal loss (van Walderveen et al 1998, van Waesberghe et al 1999, Fisher et al 2007) and so one would expect that, all other things being equal, people with a higher T1 to T2 lesion ratio, would have more severe neurological deficit. In this study cross-sectionally T1 to T2 lesion volume ratio was the most consistent MRI correlate of both MSIS-measures suggesting that a higher burden of lesion-associated neuroaxonal loss may also have an impact on QoL. Previously published studies comparing MRI measures with QoL questionnaires have reported a higher T1 lesion volume being associated with lower QoL would support

this (Janhardan and Bakhi 2000, Mowry et al 2009). There are reports of correlation between T1 lesion volume and depression (Bakshi et al 2000) which may partly explain why T1 to T2 lesion volume ratio was an independently significant correlate in the multimodal model for MSIS-psych.

In the longitudinal analysis there were no correlations of change in MRI measures with change in MSIS-phys, but changes in NAWM mean MTR and NAGM peak height did correlate significantly with change in MSIS-psych. MRI detectable changes, possibly neuroaxonal loss, in normal appearing brain tissue may have an independent role in determining level of neurological impairment (Miller et al 2002, Filippi and Agosta 2007). Data from this study suggest that pathological changes in the normal appearing brain tissue may also have an impact, albeit limited, on psychological QoL. A preliminary study evaluating the link between MTR and fatigue failed to find a significant association (Codella et al 2002) but it would be interesting to revisit this using a larger cohort and longitudinal measurement.

No significant correlations of MSIS-29 measures with measures of inflammatory activity

There was no observed association of either clinical or MRI markers of active neuroinflammation – relapses and active lesions – with change in MSIS-29 measures. Gradual deterioration in neurological function, rather than acute relapse is thought to be the predominant cause of disability in secondary progressive MS (Confavreux et al 2000, Leray et al 2010, Scalfari et al 2010) while MRI measures such as atrophy, that are thought to represent neuroaxonal loss, are more closely correlated with progressive disability than measures focal lesion volume are (the latter thought to be

measures of focal neuroinflammation) (Miller et al 2002). Data from this study would suggest that in progressive forms of the condition new focal inflammatory activity has a limited impact on QoL when compared to measures of neuroaxonal damage and loss.

However one should be cautious about over interpreting this result. Because the data in this study were taken from a trial of a potentially neuroprotective drug the MRI measures used were not optimized to detect neuroinflammation. Inclusion of the MSIS-29 in future trials of immunomodulatory therapies, where Gd enhanced scans of the brain, and T2 weighted images of the cord (allowing an estimation of cord lesion burden) would be a convenient way to correct this omission.

Limitations of the study

Overall the correlations of clinical and MRI measures acquired in this study with MSIS-29 were relatively modest. There are factors such as fatigue and depression which are known to have a substantial impact on QoL in MS (Bakshi et al 2000) that may not be captured well using either the clinical or MRI tools that have been included in this study. Future studies should include appropriate questionnaires quantifying the impact of fatigue and depression.

The statistical methods used in this study assume a linear relationship between continuous variables and the MSIS-29 measures. This may not be the case, which could mean the correlation coefficients derived in this study underestimate the true strength of the associations. Repeating the study with a larger patient cohort may allow the investigation of the possibility of non-linear associations.

Some of the variables used in the models are quite closely linked, for example the descriptors of the MTR histograms, and substantial multicollinearity can also lead to under estimation of correlation coefficients. However the statistical methods employed, grouping the variables for the first stage of the analysis and re-testing all discarded variables against the final model, ensure that the regression models are never so big that the estimate of the regression line becomes unstable and no independently significant correlation should have missed due to collinearity. Repetition of the study with path analysis using a smaller set of variables could give rise to higher correlation coefficients. However this was an exploratory study, and so the inclusion of a large number of variables was felt to be justified.

Conclusion

These data do confirm that there is a correlation between physician assessed measures of neurological dysfunction and the impact of MS on QoL while the correlation of MRI measures with MSIS-29 give new insights into the pathological processes which give rise to impaired QoL. However the correlations are modest, indicating that there are factors that have a substantial impact on a person's experience of MS, which are not captured even using a large number of MRI and clinical measures.

8. Conclusion

The aim of this study was to evaluate two MRI techniques, MTR and T1LV, in secondary progressive MS; specifically:

1. The responsiveness of MTR measures and T1LV to change over 24 months, a timescale that would be typical for treatment trials in MS.
2. The correlation of MTR measures and T1LV with measures of clinical status, including a quality of life measure. This gives some insight into the pathological substrate underlying neurological dysfunction and, along with data regarding sensitivity, gives an indication of the most suitable measures for monitoring clinically important brain pathology.
3. The effects of lamotrigine, a putative neuroprotective agent, on MTR measures and T1LV.

Responsiveness over 24 months

The responsiveness to change of T1LV was very similar to that of T2LV (see table 13). T1 hypointense lesions were defined as regions corresponding to a T2 hyperintense lesion that were hypointense compared to the surrounding brain tissue, so by definition the absolute change in this measure was smaller (see table 13). However the variability in T1LV was slightly smaller and so what was lost in sensitivity may have been gained in reliability. The technique used was largely manual (see section 2.3.) which may have impaired its reproducibility, and inter-rater variability was quite high (see table 2). Other investigators are now employing automated protocols for identifying and contouring T1 hypointense lesions (Fisher et al 2007), which may improve the reproducibility of this measure. Head-to-head

comparison is needed to establish whether this automated technique has a better responsiveness than the semi-automated technique that was employed in this study.

The responsiveness of the MTR measures was limited; in the placebo group only three out of nine measures (NAGM mean, lesion PL and lesion mean) changed significantly over the 24 months. Two of these measures (lesion PL and mean) increased over the follow up period, which would not be consistent with a measure that reflects progressive demyelination or axonal loss. Very strict measures were used to minimize the effects of partial volume voxels, which may have reduced the sensitivity of these measures somewhat but to ensure, as far as possible, that the NABT MTR measures were independent of the effects of atrophy and lesion load, the exclusion of these voxels was necessary.

NAGM MTR mean was the only measure in the placebo group that showed a reliable decrease over the 24 months. This may reflect the relative severity of grey matter pathology in secondary progressive MS (Kutzelnigg et al 2005) or simply a better signal to noise ratio in grey matter measures. Grey matter comprises a greater proportion of brain volume than either white matter or lesions (Furby et al 2010) and so a proportionally smaller change in grey matter measures may be more easily detected. The coefficient of variance for both volume and MTR measures were smaller than those for white matter and for lesion volume measures (Furby et al 2010, table 2, table.) meaning that grey matter may have been more reliably segmented than white matter or lesions. The greater signal to noise ratio of grey matter measures may, therefore, provide part of the explanation for why NAGM mean changed significantly over 24 months, while lesion and white matter MTR did not.

Correlation with clinical status

In both the cross-sectional and longitudinal analysis there were positive correlations of MTR measures in all three tissue types with the MSFC and with the PASAT-3 and 9HPT considered individually. Only NAGM measures, PL in the cross-sectional analysis and mean in the longitudinal analysis, correlated with the timed walk and the strength of the correlation was modest. It is possible to make two inferences from these results:

1. While brain pathology may be important in determining cognitive and upper limb dysfunction in progressive MS, it may have less impact on mobility than cord pathology. This would be consistent with the well recognized clinical-radiological paradox in MS, where lesion burden does not necessarily correlate with disability measured using the EDSS. This phenomenon is most pronounced in progressive forms of the condition (Li et al 2006), particularly primary progressive MS (Thompson et al 1990) where there is a high burden of spinal cord pathology (Bjartmar et al 2000).

2. Using the techniques employed in this study, grey matter measures, are better than white matter or lesion measures for detecting clinically important pathology in secondary progressive MS. In previous studies grey matter atrophy and MTR measures have been consistently found to correlate with and predict neurological dysfunction (Dehmeshki et al 2003, Davies et al 2004, Sanfilipo et al 2005, Tedeschi et al 2005, Agosta et al 2006, Ramio-torrenta et al 2006, Vrenken et al 2007, Fisher et al 2008, Fisniku et al 2008a, Fisniku et al 2008b, Furby et al 2008, Furby et al 2010)

often as the best, or only correlate (Dehmeshki et al 2003, Davies et al 2004, Agosta et al 2006, Fisher et al 2008, Fisniku et al 2008a, Fisniku et al 2008b, Furby et al 2008, Furby et al 2010). This may reflect superior responsiveness of the grey matter measures; that grey matter MRI measures are markers for global brain pathology. On the other hand there is thought to be a dissociation between the burden of grey matter pathology and the severity of pathology in focal white matter lesions (Kutzelnigg et al 2005); an alternative explanation would be that grey matter pathology really does contribute substantially to neurological dysfunction in secondary progressive MS and that this is independent of any effect of focal white matter pathology.

T1LV correlated with arm and cognitive function cross-sectionally and longitudinally. In the cross-sectional analysis T1LV was the only independently significant correlate, 'canceling out' the correlation of T2LV with the same clinical measures. This indicates that T1LV may be a more specific measure for clinically relevant brain pathology than T2LV and might be used in preference in studies of neuroprotective agents in secondary progressive MS.

Across the board the association of brain MRI measures with EDSS was poor. There was a correlation of NAGM PL at baseline and of increasing lesion PL and MTR mean with an increased probability of experiencing a sustained increase in EDSS, but in both cases the association was modest. There was no association of any of the lesion volume measures with EDSS either at baseline or longitudinally; although this finding may partly be due to the fact that the change in EDSS over the course of the trial was very limited.

EDSS is known to be dependent on mobility, especially in the range that was selected for this study (4.0 to 6.5) (Kurtzke et al 1983) and the limited correlation between mobility and brain MRI measures may partly explain why the association between EDSS and MRI measures was so limited. It is possible that if subjects with a wider range of EDSS score had been selected, such that upper limb, visual, cognitive, function and bladder or bowel function contributed more to the overall score, significant correlations would have emerged.

The longitudinal correlation of MRI measures with clinical measures was limited. This may, in part, reflect the relatively low responsiveness of the measures to change over the two-year study period. Previous studies suggest that both white matter lesion volume (Fisniku et al 2008) and grey matter MTR measures (Agosta et al 2006, Fisniku et al 2009) should correlate with or predict late disability. It would be interesting to follow up the study group after a longer interval to see if changes in MTR or lesion volume seen over the two years of the trial period correlate with changes in the level of disability or neurological deficit over the longer term.

Multi-modal correlations of MRI measures with MSFC

The same statistical process used to identify the best MRI and clinical predictors of MSIS-29 (see section 7.2) was applied to identify the best MRI predictors of MSFC and component measures. MRI variables were grouped into: 'brain volume/atrophy measures' (NBV/PBVC, WMF, GMF, CCV); 'lesion volume measures' (lnT1LV, lnT2LV, T1 to T2 lesion volume ratio, active lesion number); 'MTR' (Mean, peak height and peak location for NAGM, NAWM and lesions); and spinal cord cross sectional area. A fifth group of variables was 'baseline demographic data' (Sex, age

and disease duration at baseline). Multiple linear regression analysis was used to identify first the independently significant correlates from each group and then which of these were independently significant when included in a multi-modal model. To ensure that no variables were erroneously omitted from the final model because of multiple colinearity at an earlier stage of the analysis, all discarded variables were tested against the final model. The results for cross-sectional correlations at baseline for the whole cohort are shown in table 21 rows 1 to 4 and the results for longitudinal correlations over 24 months for the placebo group ($\Delta[\text{MRI measure}] * \Delta[\text{clinical measure}]$) is shown in table 21 rows 5 to 8; only the results of the second stage of the analysis are shown.

Table 21: Multimodal ‘Best predictor’ analysis for MSFC and Δ MSFC.

1.	MSFC	PASAT-3	1/9HPT	1/TW
2. Variables included in model	NAGM mean, NBV, GMF, WMF, lnTILV	Female sex, NAGM mean, NBV, lnTILV	NAGM mean, NBV, lnTILV	Female sex, age, NAWM peak location, NAGM peak location, T1 to T2 ratio, NBV
3. Independently significant variables	NAGM mean $r_p=0.22$, $p=0.016$ GMF $r_p=0.20$, $p=0.024$ NBV $r_p=0.184$, $p=0.042$	NAGM mean $r_p=0.54$, $p<0.0001$ Female sex $r_p=-0.17$, $p=0.036$	NBV $r_p=0.45$, $p<0.0001$ Lesion peak location $r_p=0.22$, $p=0.019$	Female sex $r_p=-0.25$, $p=0.009$ NAWM peak location $r_p=0.20$, $p=0.036$ GMF $r_p=0.18$, $p=0.050$
4.	$R^2 =0.24$, $p<0.0001$	$R^2 =0.34$, $p<0.0001$	$R^2 =0.23$, $p<0.0001$	$R^2 =0.25$, $p=0.002$
5.	ΔMSFC	ΔPASAT-3	Δ1/9HPT	Δ1/TW
6. Variables included in final model	PBVC, Δ WMF, Δ NAGM mean, Δ SCCA	Age, Δ NAWM peak height, Δ GMF	Δ GMF, Δ NAWM peak height	PBVC
7. Independently significant variables	PBVC $r_p=0.40$, $p=0.016$ Δ WMF $r_p=-0.27$, $p=0.036$ Δ NAGM mean $r_p=-0.27$, $p=0.036$	Δ NAWM peak height $r_p=0.28$, $p=0.046$	Δ GMF $r_p=-0.31$, $p=0.025$	PBVC $r_p=-0.39$, $p=0.003$
8.	$R^2 =0.24$, $p<0.0001$	$R^2 =0.07$, $p=0.046$	$R^2 =0.09$, $p=0.025$	$R^2 =0.09$, $p=0.048$

MSFC - multiple sclerosis functional composite, PASAT-3 - paced auditory serial addition test, 9HPT - 9 hole peg test, TW - timed 25 foot walk, NAGM - normal appearing grey matter, NBV - normalized brain volume, GMF - grey matter fraction, WMF - white matter fraction, TILV - T1 hypointense lesion volume, NAWM – normal appearing white matter, SCCA – spinal cord cross-sectional area, PBVC percentage brain volume change, Δ change in a variable (value at 24 months – value at baseline).

Cross-sectional analysis at baseline showed that the majority of the multimodal models included both a brain volume and an MTR measure. One potential criticism of MTR, particularly when used to evaluate grey matter in isolation, is that since it is affected by neuroaxonal density (van Waesberghe et al 1999, Heihle et al 1995, Filippi et al 1999, Fisher et al 2007) and correlates with brain atrophy (Phillips et al 1998, Traboulsee et al 2003, Khaleeli et al 2007, Hayton et al 2009), it may essentially just be another measure of atrophy and not confer any information about brain pathology that is not captured using other, more reproducible atrophy measures. The finding that atrophy and MTR measures are independently significant correlates of the MSFC would suggest that this is not the case and that MTR is measuring some clinically relevant pathological process, such as cortical demyelination (Schemieler et al 2010) or changes in axonal function (Fisher et al 2007, Young et al 2008, Moll et al 2009).

In the longitudinal analysis the picture is not as clear. The ‘best-predictor’ model for MSFC comprised two atrophy measures and one MTR measure (PBVC, change in white matter fraction and change in NAGM mean). However the only independently significant correlates of change in TW or 9HPT were atrophy measures, perhaps reflecting the superior longitudinal responsiveness of atrophy measures (Furby et al 2010). For change in PASAT-3 the only independently significant longitudinal correlate was change in NAWM peak location. Performance in the PASAT-3 is strongly associated with central processing speed (Forn et al 2008), which in turn is associated with white matter tract integrity (Yu et al 2012) so the finding that a putative measure of NAWM myelinated axon content is the best MRI predictor of this particular cognitive test is not totally implausible. However the level of significance is

not high and the amount of variability accounted for by this model is very low, so it would unwise to put too much weight on this finding.

Association of clinical MRI measures with quality of life

The association of MRI and objective clinical measures with quality of life measures was interesting. There was no association of quality of life measures with EDSS, a measure that is thought to be heavily influenced by mobility yet the TW was almost uniformly the only clinical measure that independently correlated with the MSIS-29 scores both cross-sectionally and longitudinally. It seems that impaired and deteriorating mobility is associated with impaired and deteriorating quality of life but that in this quite typical secondary progressive subject group EDSS was not be sensitive enough to detect this change.

Of the MRI measures T1 to T2 lesion volume ratio was the only independent significant correlate of quality of life in the cross-sectional analysis while change in NAGM MTR measures correlated with change in MSIS-29 over the 24 months of the follow up period. These results add further support to the hypothesis that T1 hypointense lesions and NAGM MTR are useful measures of clinically relevant pathology and suggest that the pathological processes denoted by these measures do have an impact on a person's experience of MS.

The amount of variability in the MSIS-29 score explained by even the best combined models was modest, 32%, suggesting that there are other important influences, such as fatigue, depression and social circumstances, which influence quality of life in MS, but are simply not captured using the MRI and clinical tools employed in this study.

Effects of lamotrigine treatment

The effects of lamotrigine treatment were disappointing. There was no significant difference between the two arms of the trial in terms of lesion measures, with a similar increase in both T1LV and T2LV in both groups. Three out of nine of the MTR measures showed a significant difference between the two arms of the trial. However two of these NAGM and lesion PH showed a larger reduction in MTR in the verum arm, which is the opposite of what would be expected if lamotrigine was neuroprotective.

The atrophy data from the trial also gave no support to lamotrigine as a neuroprotective medication. The reduction in whole brain volume was greater in the verum arm than in the placebo arm (Kapoor et al 2010). This reduction was fastest over the first 12 months, plateaued over the second and, in a subgroup who underwent post-treatment scanning, started to reverse when the treatment was withdrawn. Because treatment with lamotrigine was not associated with any greater clinical deterioration in the verum arm it was felt that the more rapid loss of brain volume could be related among other mechanisms to reduction in white matter oedema through possible anti-inflammatory interaction of lamotrigine with sodium channels on microglia or reduction in axonal diameter through inhibition of sodium loading.

There is some recent work which indicates that MTR could potentially change according to the expression of cell surface proteins (Young et al 2008, Moll et al 2009) which could in turn be influenced by lamotrigine. However appropriate studies in EAE would be needed to establish whether these putative mechanisms are biologically plausible. In contrast to the atrophy measures there was no post-treatment

MTR imaging performed, so it is more difficult to argue that the changes in MTR are more likely to represent reversible changes in axonal structure or function rather than axonal loss.

Strengths and limitations of the study and suggested further work

The data presented here are taken from a large, well characterized group of people with secondary progressive MS. MRI data were collected at the same intervals for each subject, using the same protocol on a single MRI scanner. The MRI sequences used were well validated and the MTR measures had good reproducibility. MRI images were anonymized prior to analysis and the same investigator carried the analysis for each subject at each timepoint. For each subject the clinical data were collected by the same investigator at every timepoint, the ‘assessing physician’ who was blinded to recent clinical events.

A large number of MRI measures were obtained, giving information about both focal and global brain pathology and spinal cord atrophy and the measures chosen were appropriate for investigating a condition in which progressive neurodegeneration may be responsible for the much of the associated disability. Both objective and subjective clinical measures were obtained, giving information about disability, neurological dysfunction and quality of life. Subject retention was good, with 108 out of 118 subjects who were scanned at baseline returning at 24 months.

Every reasonable effort was made to ensure that the data acquired were of good quality and that the results presented were reliable. However the interpretation of these results could have been clarified further with some additional data.

T1 weighted images with Gd enhancement and at more frequent intervals would allow better identification of those new, acutely inflamed T1 hypointense lesions and so give a better idea of whether the neurological dysfunction associated with T1LV was due primarily to axonal degeneration and dysfunction in chronic lesions or due to inflammatory demyelination, conduction block and axonal transection in acute lesions. It would also help clarify the pathological substrate underlying the apparent increase in lesion MTR in those subjects who experienced a sustained increase in EDSS. One suspects that those subjects who had a sustained increase in EDSS had more inflammatory lesions at baseline, with a lower MTR and that the increase in MTR was resolution of oedema and/or partial remyelination, but without a reliable measure of acute neuroinflammation this remains pure speculation. Finally it would be interesting to know if lamotrigine had any effect on Gd enhancing lesion number. Recently published data from EAE suggests that sodium channel blockers may have some anti-inflammatory potential (Black et al 2007) and it would be interesting to test this in MS.

Post treatment MTR data would have been valuable in determining whether the apparent differences in MTR measures seen between the two trial arms were temporary and reversible or permanent. As stated in section 2.2, limited post treatment MRI and clinical data was acquired from this subject group, but the principal aim of acquiring these data was to establish safety and to make sure that the validity of the

primary end-point of the trial was not compromised. To maximize uptake, the MRI protocol at the post treatment follow up (27 months) was made as short as possible and comprised just markers of acute inflammation (active T2 lesion number and T2LV) the primary end-point (CCV) and the most sensitive atrophy measure (SIENA).

There are clearly factors which have a substantial impact on quality of life, which are not captured using the clinical and MRI measures used in this study. Some of these, such as the changed social circumstances that come with a chronic disabling medical condition, are likely to be insoluble with medication and largely unrelated to MRI detected brain pathology. However factors such as depression and fatigue may well be related to MRI detectable pathology (Pujol et al 1997, Bakshi et al 2000, Feinstein et al 2004, Sepulcre et al 2009, Andreason et al 2010, Pellicano et al 2010) and are potential targets for medication. It would have been interesting and fairly straightforward to add specific fatigue or depression scales to the clinical assessment and to gather data on social circumstances, co-morbidity or synchronous treatment with anti-depressants etc.

Finally it would be good to re-visit this cohort after a longer interval. Previous studies have shown an association of MTR measures at baseline with progressive neurological impairment up to eight years later (Agosta et al 2006) and it may be informative to see whether baseline MTR or T1LV measures, or change in these measures over one or two years were associated with later brain atrophy or neurological deterioration. In addition, one would like to know if treatment with lamotrigine over 24 months could lead to an improved outcome after a longer interval,

to test the possibility that early neuroprotection could result in long term differences in atrophy, MTR measures or clinical status.

Conclusion

The data presented here do give some insight into the pathological process that give rise to neurological impairment and disability in secondary progressive MS, and should be informative for investigators planning further treatment trials in similar subject groups.

The responsiveness of T1LV was similar to T2LV, but the correlation of the former with clinical measures was closer, perhaps suggesting the two MRI measures have similar sensitivity but the former better specificity for clinically important pathology. These findings would support the hypothesis that T1 hypointense lesions comprise more severe MS pathology and that the pathological processes underlying T1 hypointense lesions – axonal loss and dysfunction (Fisher et al 2007) and acute inflammation (Barkhof et al 2003) – contribute to neurological dysfunction and impact on QoL in secondary progressive MS.

The longitudinal responsiveness of MTR measures was limited, perhaps in part by the stringent exclusion criteria used to minimize the contribution partial volume voxels; only NAGM mean showed a reliable reduction over 24 months. The correlation of MTR measures with clinical measures was good, however, with ‘best predictor’ models of both MSFC (cross-sectionally) and MSIS (longitudinally) comprising at least one MTR measure.

While all three tissue types correlated with MSFC, PASAT-3 and 1/9HPT, only NAGM MTR measures (PL in the cross-sectional analysis and mean in the longitudinal analysis) correlated significantly with TW. These results may reflect the relative importance of grey matter pathology in determining neurological dysfunction or the superior reliability of grey matter MTR when using the analysis techniques employed in this study. Either way these data would support the inclusion of brain grey matter MRI measures when monitoring secondary progressive MS.

Although the EDSS has been criticized for being heavily weighted towards mobility (Cutter et al 1999) the finding that 1/TW does have a significant impact on QoL may indicate that this is not a serious weakness. What is more concerning, however, was the very limited association of any MRI measure with the EDSS, either cross-sectionally or longitudinally, possibly highlighting the relative insensitivity of the latter in this particular subject group over a short study period. The EDSS should be included in future treatment trials in MS, being easily measured, widely understood and the best established measure of disability due to MS, but the data presented here suggest that it should not be used as the only measure of neurological impairment, particularly in short studies in secondary progressive subject groups.

There were significant correlations of both MRI and clinical measures with MSIS both cross-sectionally and longitudinally, demonstrating that the pathological processes and neurological impairment measured using these techniques do have a meaningful impact on people with MS. However even using the best multi-modal predictor models, the association between MRI and clinical measures with the impact of MS on quality of life was limited. This finding demonstrates that a large number of

factors contribute to QoL in MS and the inclusion of a dedicated QoL measure in treatment trials is potentially important.

Finally the effects of treatment with lamotrigine were not as hoped at the start of the trial with no difference in lesion measures and no relative ‘preservation of MTR levels’ in the treatment group. Brain volume data from the trial did suggest that some of the apparent atrophy in the verum arm may be reversible (psuedoatrophy) (Kapoor et al 2010) and it is possible that the same is true for changes in MTR. These findings emphasize the potential value of post-treatment MRI and clinical data and suggest that further trails of putative neuroprotective treatments should include a longer follow up period.

References

Adalsteinsson E, Langer-Gould A, Homer RJ, Rao A, Sullivan EV, Lima CA, Pfefferbaum A, Atlas SW Gray matter N-acetyl aspartate deficits in secondary progressive but not relapsing-remitting multiple sclerosis. *Am J Neuroradiol* (2003) 24, 1941-1945

Agosta F, Rovaris M, Pagani E, Sormani MP, Comi G, Filippi M Magnetization transfer MRI metrics predict the accumulation of disability 8 years later in patients with multiple sclerosis. *Brain* (2006) 129, 2620-2627

Ahern GP, Hsu S-F, Klyachko VA, Jackson MB. Induction of persistent sodium current by exogenous and endogenous nitric oxide. *J Biol Chem* (2000) 275, 28810–28815.

Amato MP, Ponziani G, Rossi F, Leidl CL, Stefanile C, Rossi L. Quality of life in multiple sclerosis: the impact of depression fatigue and disability. *Mult Scler* (2001) 7, 340-344

Amato MP, Bartolozzi ML, Zipoli V, Portaccio E, Mortilla M, Guidi L, Siracusa G, Sorbi S, Federico A, De Stefano N. Neocortical volume decrease in relapsing-remitting MS patients with mild cognitive impairment. *Neurology* (2004) 63, 89-93

Amato MP, Portaccio E, Goretti B, Zipoli V, Battaglini M, Bartolozzi ML, Stromillo ML, Guidi L, Siracusa G, Sorbi S, Federico A, De Stefano N. Association of

neocortical volume changes with cognitive deterioration in relapsing-remitting multiple sclerosis. *Arch Neurol* (2007) 64, 1157-61.

Anderson VM, Fox NC, Miller DH. Magnetic resonance imaging measures of brain atrophy in multiple sclerosis. *J Magn Reson Imaging* (2006) 23, 605-18

Aupperle RL, Beatty WW, Shelton Fde N, Gontovsky ST. Three screening batteries to detect cognitive impairment in multiple sclerosis. *Mult Scler* (2002) 8, 382-389

Ashburner J, Friston K Multimodal image coregistration and partitioning – a unified framework. *NeuroImage* (1997) 6, 209-217

Ashburner J, Friston K. Voxel based morphometry. *NeuroImage* (2000) 11, 805-811

Ashburner J, Friston KJ. Unified segmentation. *NeuroImage* (2005) 26, 839-851.

Bagasra O, Michaels FH, Zheng YM, Bobroski LE, Spitsin SV, Zu ZF, Tawadros R, Koprowski H Activation of the inducible form of nitric oxide synthase in the brains of people with multiple sclerosis. *PNAS* (1995) 92, 12041-12045

Bagnato F, Jeffries N, Richert ND, Stone RD, Ohayon JM, McFarland HF, Frank JA. Evolution of T1 black holes in patients with multiple sclerosis imaged monthly for 4 years. *Brain* (2003) 126, 1782-1789

Barker GJ, Tofts PS, Gass A. An interleaved sequence for accurate and reproducible clinical measurement of magnetization transfer ratio. *Magn Reson Imaging* (1996) 14, 403-411.

Barkhof F, van Waesberghe JHTM, Filippi M, Yousry T, Miller DH, Hahn D, Thompson AJ, Kappos L, Brex P, Pozzilli C, Polman CH for the European Study Group on Interferon beta 1-b in Secondary Progressive Multiple Sclerosis. T1 hypointense lesions in secondary progressive multiple sclerosis : effect of interferon beta 1-b treatment. *Brain* (2001) 124, 1396-1402

Barkhof F, Bruck W De Groot CJA, Bergers E, Hulsof S, Geurts J, Polman CH. Remyelinated lesions in multiple sclerosis. *Arch Neurol* (2003) 60, 1073-1081.

Bakshi R, Czarnecki D, Shaikh ZA, Priore RL, Janardhan V, Kaliszky Z, Kinkel PR. Brain MRI lesions and atrophy are related to depression in multiple sclerosis. *Neuroreport*. (2000) 11, 1153-1158.

Balcer LJ, Frohman EM. Evaluating loss of visual function in multiple sclerosis as measured by low-contrast letter acuity. *Neurology* (2010) 74;S16, S16-23

Barnett MH, Prineas JW. Relapsing and remitting multiple sclerosis: pathology of the newly forming lesion. *Ann Neurol* (2004) 55, 458-68

Bechtold DA, Kapoor R, Smith KJ. Axonal protection using flecainide in experimental autoimmune encephalomyelitis. *Ann Neurol* (2004) 55, 607-16.

Bechtold DA, Smith KJ. Sodium mediated axonal degeneration in inflammatory demyelinating disease J Neurol Sci (2005) 233, 27-35

Bechtold DA, Miller SJ, Dawson AC, Sun Y, Kapoor R, Berry D, Smith KJ. Axonal protection achieved in a model of multiple sclerosis using lamotrigine J Neurol (2006) 253, 1542-1551.

Benedetti B, Rigotti Dj, Liu S, Filippi M, Grossman R.I, Gonen O. Reproducibility of the whole-brain *N*-Acetylaspartate level across institutions, MR scanners, and field strengths AJNR (2007) 28, 72-75

Benedict RH, Wahlig E, Bakshi R, Fishman I, Munschauer F, Zivadinov R, Weinstock-Guttman B. Predicting quality of life in multiple sclerosis: accounting for physical disability, fatigue, cognition, mood disorder, personality, and behavior change. J Neurol Sci (2005) 231, 29– 34

Beniek M, Altmann DR, Davies GR, Ingle GT, Rashid W, Sastre-Garriga A, Thompson AJ, Miller DH Cord atrophy separates early primary progressive and relapsing remitting multiple sclerosis. JNNP (2006) 77, 1036-1039

Benito-Leon J, Morales JM, Riviera-Navarro J. European Journal of Neurology Health-related quality of life and its relationship to cognitive and emotional functioning in multiple sclerosis patients. European J Neurol (2002) 9, 497-502

Benito-Leon JN, Morales JM, Rivera-Navarro JS, Mitchell AJ. A review about the impact of multiple sclerosis on health-related quality of life. *Disabil. Rehabil.* (2003) 25, 1291–1303

Bermel RA, Innus MD, Tjoa CW, Bakshi R. Selective caudate atrophy in multiple sclerosis: a 3D MRI parcellation study. *NeuroReport* (2003) 14, 335-339.

Bhakoo KK, Pearce D In vitro expression of N-acetyl aspartate by oligodendrocytes: implications for proton magnetic resonance spectroscopy signal in vivo. *J Neurochem* (2000) 74, 254-262

Bitsch A, Schuchardt J, Bunkowski S, Kuhlmann T, Brück W. Acute axonal injury in multiple sclerosis Correlation with demyelination and inflammation. *Brain* (2000) 123, 1174-1183.

Bjartmar C, Kidd G, Mork S, Rudick R, Trapp BD. Neurological disability correlates with spinal cord axonal loss and reduced N-acetyl aspartate in chronic multiple sclerosis patients. *Ann Neurol* (2000) 48, 893–901.

Bjartmar C, Trapp BD. Axonal and neuronal degeneration in multiple sclerosis: mechanisms and functional consequences. *Curr Opin Neurol.* (2001) 14, 271-8.

Black JA, Liu S, Carrithers M, Carrithers LM, Waxman SG. Exacerbation of experimental autoimmune encephalomyelitis after withdrawal of phenytoin and carbamazepine. *Ann Neurol* (2007) 62, 21-33

Bø L, Vedeler CA, Nyland H, Trapp BD, Mork SJ. Intracortical multiple sclerosis lesions are not associated with increased lymphocyte infiltration. *Mult Scler* (2003a) 9, 323-331

Bø L, Vedeler CA, Nyland HI, Trapp BD, Mørk SJ. Subpial demyelination in the cerebral cortex of multiple sclerosis patients. *J Neuropathol Exp Neurol* (2003b) 62, 723-32

Bolanos JP, Almeida A, Stewart V, Peuchen S, Land JM, Clark JB, et al. Nitric oxide-mediated mitochondrial damage in the brain: mechanisms and implications for neurodegenerative diseases. *J Neurochem* (1997) 68, 2227–2240

Bostock H, Grafe P. Activity-dependent excitability changes in normal and demyelinated rat spinal root axons. *J Physiol* (1985) 365, 239-257.

Brenner RE, Munro PM, Williams SC, Bell JD, Barker GJ, Hawkins CP, Landon DN, McDonald WI. The proton NMR spectrum in acute EAE: the significance of the change in the Cho:Cr ratio. *Magn Reson Med* (1993) 29, 737-745

Brex PA, Jenkins R, Fox NC, Crum WR, O'Riordan JI, Plant GT, Miller DH. Detection of ventricular enlargement in patients at the earliest clinical stage of MS. *Neurology* (2000) 54, 1689-1691

Brownell B, Hughes J. The distribution of plaques in cerebrum in multiple sclerosis. *JNNP* (1962) 25, 315-320

Bruce JM, Hancock LM, Lynch SG. Objective adherence monitoring in multiple sclerosis: initial validation and association with self-report. *Mult Scler.* (2010) 16, 112-120.

Brück W, Porada P, Poser S, Rieckmann R, Hanefeld F, Kretzschmarch HA, Lassmann H. monocute/macrophage differentiation in early multiple sclerosis. *Annals Neurol* (1995) 38, 788-796.

CAMMS223 Trial Investigators, Coles AJ, Compston DA, Selmaj KW, Lake SL, Moran S, Margolin DH, Norris K, Tandon PK. Alemtuzumab vs. interferon beta-1a in early multiple sclerosis. *N Engl J Med* (2008) 359, 1786-801.

CAST investigators. Preliminary report: effect of encainide and flecainide on mortality in a randomized trial of arrhythmia suppression after myocardial infarction. The Cardiac Arrhythmia Suppression Trial (CAST) Investigators. *N Eng J Med* (1989) 321, 406-412

Cercignani M, Bozzali M, Iannucci G, Comi G and Filippi M Magnetization transfer ratio and mean diffusivity of normal appearing white matter and grey matter from parents with multiple sclerosis. *JNNP* (2001) 70, 311-317

Chard DT, Griffin CM, McLean MA, Kapeller P, Kapoor R, Thompson AJ, Miller DH. Brain metabolite changes in cortical grey and normal-appearing white matter in clinically early relapsing-remitting multiple sclerosis. *Brain* (2002a) 125, 2342-52.

Chard DT, Parker GJ, Griffin CM, Thompson AJ, Miller DH. Brain atrophy in clinically early relapsing-remitting multiple sclerosis. *Brain* (2002b) 125, 327-337

Chard DT, Parker GJM, Griffin CMB, Thompson AJ, Miller DH. The reproducibility and sensitivity of brain tissue volume measurements derived from an SPM-based segmentation methodology. *J MRI* (2002c) 5,259–267

Chard DT, Griffin CM, Rashid W, Davies GR, Altmann DR, Kapoor R, Barker GJ, Thompson AJ, Miller DH. Progressive grey matter atrophy in clinically early relapsing-remitting multiple sclerosis. *Mult Scler* (2004) 10, 387-391.

Cifelli A, Arridge M, Jezzard P, Esiris MM, Palace J, Matthews PM Thalamic neurodegeneration in multiple sclerosis. *Ann Neurol* (2002) 52, 650-653

Codella M, Rocca MA, Colombo B, Rossi P, Comi G, Filippi M. A preliminary study of magnetization transfer and diffusion tensor MRI of multiple sclerosis patients with fatigue. *J Neurol.* (2002) 249, 535-537.

Cohen JA, Fischer JS, Bolibrush DM, Jak AJ, Kniker JE, Mertz LA, Skaramagas TT, Cutter GR. Intrarater and interrater reliability of the MS functional composite outcome measure. *Neurology* (2000) 54, 802-806

Cohen JA Cutter GR, Fischer JS, Goodman AD, Heidenreich FR, Jak AJ, Kniker JE, Kooijmans MF, Cull JM, Sandrock AW, Simon JH, Simonian NA, Whitaker JN for

the IMPACT investigators. Use of the multiple sclerosis as an outcome measure in a phase 3 trial. *Arch Neurol* (2001) 58, 961-967

Cohen JA, Cutter GR, Fischer JS, Goodman AD, Heidenreich FR, Kooijmans MF, Sandrock AW, Rudick RA, Simon JH, Simonian, Tsao EC, Whitaker JN for the IMPACT Investigators. Benefit of interferon β -1a on MSFC progression in secondary progressive MS. *Neurology* (2002) 59, 679-687

Coles AJ, Wing MG, Molyneux PM, Paolillo A, Davie CM, Hale G, Miller D, Waldmann H, Compston A. Monoclonal antibody treatment exposes three mechanisms underlying the clinical course of multiple sclerosis. *Annals Neurol* (1999) 46, 296-304

Coles AJ, Cox A, Le Page E, Jones J, Trip SA, Deans J, Seaman S, Miller DH, Hale G, Waldmann H, Compston DA. The window of therapeutic opportunity in multiple sclerosis: evidence from monoclonal antibody therapy. *J Neurol* (2006) 253, 98-108.

Comi G, Filippi M, Wolinsky JS, and the European/Canadian Glatiramer Acetate Study Group. European/Canadian multicenter, double-blind, randomized, placebo-controlled study of the effects of glatiramer acetate on magnetic resonance imaging-measured disease activity and burden in patients with relapsing multiple sclerosis. *Ann Neurol* (2001a) 49, 290– 297.

Comi G, Filippi M, Barkhof F, Durelli L, Edan G, Fernandez O, Hartung H, Seeldrayers P, Sorensen PS, Rovaris M, Martinelli V, Hommes OR Effect of early

interferon treatment on conversion to definite multiple sclerosis: a randomised study
Lancet (2001b) 357, 1576-1582.

Comui G. Oral laquinimod reduced relapse rate and delayed progression of disability in
allegro, a placebo –controlled phase III trial for relapsing remitting multiple sclerosis.
Neurology (2011) 76(9 suppl 4), 7PP.001.

Confavreux C, Vukusic S, Moreau T, Adelein P. Relapses and progression of
disability in multiple sclerosis. N Eng J Med (2000) 343, 1430-1438

Costelloe L, O'Rourke K, McGuigan C, Walsh C, Tubridy N, Hutchinson M. The
longitudinal relationship between the patient-reported Multiple Sclerosis Impact Scale
and the clinician-assessed Multiple Sclerosis Functional Composite. Mult Scler
(2008) 14, 255-258

Craner MJ, Lo AC, Black JA, Waxman SG. Abnormal sodium channel distribution in
optic nerve axons in a model of inflammatory demyelination. Brain (2003) 126, 1552-
1561

Craner M, Newcombe J, Black JA, Hartle C, Cuzner ML & Waxman SG. Molecular
changes in neurons in multiple sclerosis: Altered axonal expression of Nav 1.2 and
Nav 1.6 sodium channels and Na/Ca exchanger. PNAS (2004a) 101, 8168-73

Craner MJ, Hains BC, Lo AC, Black JA, Waxman SG. Co-localization of sodium channel Nav1.6 and the sodium/calcium exchanger at sites of axonal injury in the spinal cord in EAE Brain (2004b) 127, 294-303

Cucurella MG, Rovira A, Rio J, Pedraza S, Tintore MM, Montalban X, Alonso J, Proton magnetic resonance spectroscopy in primary and secondary progressive multiple sclerosis. NMR Biomed (2000) 13, 57-63

Cutter GR, Baier MS, Rudick RA, Cookfair DL, Fischer JS, Petkau J, Synulko K, Weinshenker BG, Antel JP, Confavreux C, Ellison GW, Lublin F, Miller AE, Rao SM, Reingold S, Thompson A, Willoughby E. Development of a multiple sclerosis functional composite as a clinical trial outcome measure. Brain (1999) 122, 871-882

Dalton CM, Chard DT, Davies GR, Miszkiel KA, Altmann DR, Fernando K et al. Early development of multiple sclerosis is associated with progressive grey matter atrophy in patients presenting with clinically isolated syndromes. Brain (2004) 127, 1101-07

Dalton CM, Miszkiel KA, O'Connor PW, Plant GT, Rice GP, Miller DH Ventricular enlargement in MS: one-year change at various stages of disease. Neurology (2006) 66, 693-698

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

Davie CA, Barker GJ, Thompson AJ, Tofts PS, McDonald WI, Miller DH. IH magnetic resonance spectroscopy of chronic white matter lesions and normal appearing white matter in multiple sclerosis. *JNNP* (1997) 63, 736-742.

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

Davies GR, Ramio-Torrenta L, Hadjiprocopis A, Chard DT, Griffin CMB, Rashid W, Barker GJ, Kapoor R, Thompson AJ, Miller DH. Evidence for grey matter MTR abnormality in minimally disabled patients with early relapsing-remitting multiple sclerosis. *JNNP* (2004) 75, 998-1002

Davies GR, Altmann DR, Hadjiprocopis A, Rashid W, Chard DT, Griffin CM, Tofts PS, Barker GJ, Kapoor R, Thompson AJ, Miller DH. Increasing normal appearing grey and white matter magnetisation transfer ratio abnormality in early relapsing-remitting multiple sclerosis. *J Neurol* (2005a) 252, 1037-1044.

Davies GR, Altmann DR, Rashid W, Chard DT, Griffin CM, Barker GJ, Kapoor R, Thompson AJ, Miller DH. Emergence of thalamic magnetization transfer ratio abnormality in early relapsing-remitting multiple sclerosis. *Mult Scler* (2005b) 11, 276-281

Dehmeshki J, Ruto AC, Arridge S, Silver NC, Miller DH, Tofts PS Analysis of MTR histograms in multiple sclerosis using principle components and multiple discriminant analysis. *MRM* (2001) 46, 600-609.

Dehmeshki J, Chard DT, Leary SM, Watt HC, Silver NC, Tofts PS, Thompson AJ, Miller DH. The normal appearing grey matter in primary progressive multiple sclerosis. A Magnetisation transfer imaging study. *J Neurol* (2003) 250, 67-74

De Stefano N, Matthews PM, Fu L, Narayanan S, Stanley J, Francis GS, Antel JP, Arnold DL. Axonal damage correlates with disability in patients with relapsing remitting multiple sclerosis. Results of a longitudinal magnetic resonance spectroscopy study. *Brain* (1998) 121, 1469-1477

De Stefano N, Narayanan S, Francis GS, Arnaoutelis R, Tartaglia MC, Antel JP, Matthews PM, Arnold DL. Evidence of axonal damage in the early stages of multiple sclerosis and its relevance to disability. *Arch Neurol* (2001) 58, 65-70.

De Stefano N, Matthews PM, Filippi M, Agosta F, De Luca M, Bartolozzi ML, Guidi L, Ghezzi A, Montanari E, Cifelli A, Federico A, Smith SM. Evidence of early cortical atrophy in MS: relevance to white matter changes and disability. *Neurology* (2003) 60, 1157-1162

De Stefano N, Filippi M, Miller Dh, Pouwels PJ, Rovira A, Gass A, Enzinger P, Matthews M, Arnold DL. Guidelines for using proton MR spectroscopy in multicenter clinical MS trials. *Neurology* (2007) 69, 1942-1952

Diem R, Sättler M, Bähr M. Neurodegeneration and –protection in autoimmune CNS inflammation. *J Neuroimmunol* (2007) 184, 27-34

Drake AS, Weinstock-Guttman B, Morrow SA, Hojnacki D, Muschauer FE, Benedict RHB. Psychometrics and normative data for the Multiple Sclerosis Functional Composite: replacing the PASAT with the Symbol Digit Modalities Test. *Mult Scler* (2010) 16, 228-237

Ebers GC, Heigenhauser L, Daumer M, Lederer C, Noseworthy JH. Disability as an outcome in MS clinical trials. *Neurology*. (2008) 71, 624-631.

Edan D, Miller D, Clanet M, Confavreux C, Lyon-Caen O, Lubetzki C, Brochet B, Berry I, Rolland Y, Froment JC, Cabanis E, Iba-Zizen MT, Gandon JM, Lai HM, Moseley I, Sabouraud O. Therapeutic effect of mitoxantrone combined with methylprednisolone in multiple sclerosis: a randomised multicentre study of active disease using MRI and clinical criteria. *JNNP* (1997) 62, 112-118

Evangelou N, Esiri M, Smith S, Palace J, Matthews PM. Quantitative pathological evidence for axonal loss in normal appearing white matter in multiple sclerosis. *Ann Neurol* (2000) 47, 391-395

Evangelou N, De Luca GC, Owens T, Esiri MM. Pathological study of spinal cord atrophy in multiple sclerosis suggests limited role of local lesions. *Brain* (2005) 128, 29-34

Evans AC, Collins DL, Mills SR, Brown ED, Kelly RL, Peters TM. 3D statistical neuroanatomical models from 305 MRI volumes. Proc IEEE- Nuclear Science Symposium and Medical Imaging Conference (1993) 1813-1817

Falini A, calabrese G, Filippi M, Origgi D, Lipari S, Colombo B, Coi G, Scotti G. Benign versus secondary progressive multiple sclerosis: The potential role of proton MR spectroscopy in defining the nature of disability. Am J Neuroradiol (1998) 19, 223-229.

Fatemi A, Smith A, Dubey P, Zackowski KM, Bastian AJ, van Zijl PC, Moser HW, Raymond GV, Golay X. Magnetization transfer MRI demonstrates spinal cord abnormalities in adrenomyeloneuropathy. Neurology (2005) 64, 1739-1745

Feinstein A, Roy P, Lobaugh N, Feinstein K, O'Connor P, Black S. Structural brain abnormalities in multiple sclerosis patients with major depression. Neurology (2004) 62, 586-590.

Ferguson B, Matyszak MK, Esiri MM, Perry VH. Axonal damage in acute multiple sclerosis lesions Brain (1997), 120, 393-399

Filippi M, Barker GJ, Horsfield MA, Sacares PR, MacManus DG, Thompson AJ, Tofts PS, McDonald WI, Miller DH., Benign and secondary progressive multiple sclerosis: a preliminary quantitative MRI study J Neurol (1994) 241, 246-251.

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

Filippi M, Mastronardo G, Bastianello S, Rocca MA, Rovaris M, Gasperini C, Pozzilli C, Comi G. A longitudinal brain MRI study comparing the sensitivities of the conventional and a newer approach for detecting active lesions in multiple sclerosis. *J Neurol Sci* (1998b) 159, 94-101.

Filippi M, Iannucci G, Tortorella G, Minicucci L, Horsfield MA, Colombo B, Sormani MP, Comi G. transfer MRI Comparison of MS clinical phenotypes using conventional and magnetization. *Neurology* (1999) 52, 588- 594.

Filippi M, Rovaris M, Iannucci G, Mennea S, Sormani MP, Comi G Whole brain volume changes in patients with progressive MS treated with cladribine. *Neurology* (2000a) 55, 1714-1718

Filippi M, Inglese M, Rovaris, Sormani MP. Horsfield G, Iannucci G, Colombo B, Comi G. Magnetization transfer imaging to monitor the evolution of MS. A 1 year follow up. *Neurology* (2000b) 55, 940-946.

Filippi M, Dousset V, McFarland HF, Miller DH, Grossman RI. Role of magnetic resonance imaging in the diagnosis and monitoring of multiple sclerosis: consensus report of the White Matter Study Group. *J Magn Res Imaging* (2002) 15, 499-504

Filippi M, Rocca MA, Pagani E European study on intravenous immunoglobulin in multiple sclerosis. Results of Magnetization Transfer Magnetic Resonance Imaging Analysis. *Arch Neurol* (2004) 61, 1409-1412.

Filippi M, Falini A, Arnold DL, Fazekas F, Gonen O, Simon JH, Dousset V, Savoiardo M, Wolinsky JS. Magnetic resonance techniques for the in vivo assessment of multiple sclerosis pathology: consensus report of the White Matter Study Group. *J Magn Res Imaging* (2005) 21, 669-675

Filippi M, Agosta F Magnetization transfer MRI in multiple sclerosis. *J Neuroimaging* (2007) 17, 22S-26S.

Filippini G, Brusafferri F, Sibley WA, Cifferio A, Ciucci G, Midgard R, Cardelise L. Corticosteroids or ACTH for acute exacerbations in multiple sclerosis (Review). *The Cochrane Library* (2008) Issue 1

Fischer JS, Rudick RA, Cutter GR, Reingold SC and the national MS society clinical outcomes assessment task force. The Multiple Sclerosis Functional Composite measure (MSFC): an integrated approach to MS clinical outcome assessment. *Mult Scler* (1999) 5, 244-250

Fischer JS, Jak AJ, Kniker JE, Rudick RA, Cutter G. Multiple sclerosis functional composite (MSFC) Administration and scoring manual. Revised, October 2001. National MS Society/Unitech Communications (2001).

Fisher E, Rudick RA, Cutter G, Baier M, Miller D, Weinstock-Guttman B, Mass MK, Dougherty DS, Simonian NA. Relationship between brain atrophy and disability: an 8-year follow-up study of multiple sclerosis patients. *Mult Scler* (2000) 6, 373-377.

Fisher E, Rudick RA, Simon JH, Cutter G, Baier M, Lee JC, Miller DH, Weinstock-Guttman B, Mass MK, Dougherty DS, Simonian NA. Eight-year follow-up study of brain atrophy in patients with MS. *Neurology* (2002) 59, 1412-1420

Fisher E, Chang A, Fox RJ, Tkach JA, Svarovsky T, Nakamura K, Rudick RA, Trapp BD. Imaging correlates of axonal swelling in chronic multiple sclerosis brains. *Ann Neurol* (2007) 62, 219-228

Fisher E, Lee JC, Nakamura K, Rudick RA. Gray matter atrophy in multiple sclerosis: a longitudinal study. *Ann Neurol*. (2008) 64, 255-265

Fisher JB, Jacobs DA, Markowitz CE, Galetta SL, Volpe NJ, Nano-Schiavi ML, Baier ML, Frohman EM, Winslow H, Frohman TC, Calabresi PA, Maguire MG, Cutter GR, Balcer LJ. Relation of visual function to retinal nerve fiber layer thickness in multiple sclerosis. *Ophthalmology* (2006) 113, 324-332.

Fisniku LK, Brew PA, Altmann DR, Miszkiet KA, Benton CE, Lanyon R, Thompson AJ, Miller DH. Disability and T2 MRI lesions: a 20 year follow-up of patients with relapse-onset of multiple sclerosis. *Brain* (2008a) 131, 808-817.

Fisniku LK, Chard DT, Jackson JS, Anderson VM, Altmann DR, Miszkiet KA, Thompson AJ, Miller DH. Gray matter atrophy is related to long-term disability in multiple sclerosis. *Ann Neurol.* (2008b) 64, 247-254.

Fisniku LK, Cercignani M, Tozer D, Chard D, Jackson J, Miszkiet K, Schmierer K, Thompson A, Miller D. Magnetisation transfer ratio abnormalities reflect clinically relevant grey matter damage in multiple sclerosis. *Mult Scler* (2009) 15, 668-677.

Forn C, Belenguer A, Parcet-Ibars MA, Avila C. Information-processing speed is the primary deficit underlying the poor performance of multiple sclerosis patients in the Paced Auditory Serial Addition Test (PASAT). *J Clin Exp Neuropsychol* (2008) 30, 789-796.

Fox NC, Jenkins R, Leary SM, Stevenson VL, Losseff NA, Crum WR Harvey RJ, Rossor MN, Miller DH, Thompson AJ. Progressive cerebral atrophy in MS: a serial study using registered volumetric MRI. *Neurology* (2000) 54, 807-812

Freeborough PA, Fox NC, Kitney RI. Interactive algorithms for the segmentation and quantification of 3-D MRI brain scans. *Comput Methods Programs Bionmed* (1997) 53, 15-25

Furby J, Hayton T, Anderson V, Altmann D, Brenner R, Chataway J, Hughes RAC, Smith KJ, Miller DH, Kapoor R. Magnetic resonance imaging measures of brain and spinal cord atrophy correlate with clinical impairment in secondary progressive multiple sclerosis. *Mult Scler* (2008) 14, 1068-1075.

Furby J, Hayton T, Altmann D, Brenner R, Chataway J, Hughes RAC, Smith KJ, Miller DH, Kapoor R. Different white matter lesion characteristics correlate with distinct grey matter abnormalities on magnetic resonance imaging in secondary progressive multiple sclerosis. *Mult Scler* (2009) 15, 687-694

Furby J, Hayton T, Altmann D, Brenner R, Chataway J, Hughes RAC, Smith KJ, Miller DH, Kapoor R. A longitudinal study of MRI-detected atrophy in secondary progressive multiple sclerosis. *J Neurol* (2010) 257, 1508-1516

Garthwaite G, Goodwin DA, Batchelor M, Leeming K, Garthwaite J. Nitric Oxide toxicity in CNS white matter: an in vitro study using rat optic nerve *Neurosci* (2002) 109, 145-155

Gasparini C, Paolillo A, Giugni E, Galgani S, Bagnato F, Mainero C, Onesti E, Bastianello S, Pozzilli C. MRI brain volume changes in relapsing-remitting multiple sclerosis patients treated with interferon beta-1a. *Mult Scler* (2002) 8, 119-123.

Gass A, Barker GJ, Kidd D, Thorpe JW, MacManus D, Brennan A, Tofts PS, Thompson AJ, McDonald WI, Miller DH Correlation of magnetization transfer ratio with clinical disability in multiple sclerosis. *Ann Neurol* (1994) 36, 62-67

Ge Y, Grossman RI, Udupa JK, Babb JS, Manon LJ, McGowan JC Magnetization Transfer Ratio Histogram Analysis of Normal-Appearing Gray Matter and Normal-Appearing White Matter in Multiple Sclerosis. *J CAT* (2002) 26, 61-68.

Geurts JJ, Barkhof F, Castelijns JA, Uitdehaag BM, Polman CH, Pouwels PJ Quantitative 1H-MRS of healthy human cortex, hippocampus, and thalamus: metabolite concentrations, quantification precision, and reproducibility. *J Magn Reson Imaging* (2004) 20, 366-371

Geurts JJG, Bo L, Puowels PJW, Castelijns JA, Polman CH, Barkhof F Cortical lesions in multiple sclerosis: Combined postmortem MR imaging and histopathology. *AJNR* (2005) 26, 572-577.

Ghalie RG, Edan G, Laurent M ,Mauch E, Eismann S, Hartung HP, GosetteRE, Butine MD Goodkin PE. Cardiac adverse effects associated with mitoxantrone (Novantrone) therapy in patients with MS. *Neurology* (2002) 59, 909-913.

Giovannoni G, heales SJ, Sivler NC, O’Riordan J, Miller RF, Land JM, Clark JB, Thompson EJ. Raised serum nitrate and nitrite levels in patients with multiple sclerosis *J Neurol Sci* (1997) 145, 77-81

Giovannoni G, Comi G, Cook S, Rammohan K, Rieckmann P, Soelberg Sørensen P, Vermersch P, Chang P, Hamlett A, Musch B, Greenberg SJ;CLARITY Study

Group. A placebo-controlled trial of oral cladribine for relapsing multiple sclerosis. *N Engl J Med.* (2010) 362, 416-426

Gold SM, Heeson C, Schultz H, Guder U, Monch A, Gbadamosi J, Buhmann C, Schultz KH. Disease specific quality of life instruments in multiple sclerosis: validation of the Hamburg Quality of Life Questionnaire in multiple sclerosis (HAQUAMS). *Mult Scler* (2001) 7, 119-130

Grossman RI, Gonzalez-Scarano F, Atlas SW, Galetta S, Silberberg DH Multiple sclerosis: Gd enhancement in MR imaging. *Radiology* (1986) 161, 721-725

Guzman NJ, Fang MZ, Tang SS, Ingelfinger JR, Garg LC. Autocrine inhibition of Na⁺/K⁽⁺⁾-ATPase by nitric oxide in mouse proximal tubule epithelial cells. *J Clin Invest* (1995) 95, 2083–2088.

Hartung HP, Gonsette R, Konig N, Kwiecinski H, Guseo A, Morrissey SP, Krapf H, Zwingers T Mitoxantrone in progressive multiple sclerosis: a placebo-controlled, double-blind, randomised, multicentre trial, *Lancet* (2002) 360, 2018-2025.

Hauser SL, Waubant E, Arnold DL, Vollmer T, Antel J, Fox RJ, Bar-Or A, Panzara M, Sarkar N, Agarwal S, Langer-Gould A, Smith CH; HERMES Trial Group. B-cell depletion with rituximab in relapsing-remitting multiple sclerosis. *N Engl J Med.* (2008) 358, 676-88.

Hawkins CP, Munro PM, MacKenzie F, Kesselring J, Tofts PS, du Boulay EP, Landon DN, McDonald WI. Duration and selectivity of blood-brain barrier

breakdown in chronic relapsing experimental allergic encephalomyelitis studied by Gd-DTPA and protein markers. *Brain* (1990) 113, 365-78.

Hayton T, Furby J, Smith KJ, Altmann DR, Brenner R, Chataway J, Hughes RAC, Hunter K, Tozer D, Miller DH, Kapoor R. Grey matter magnetization transfer ratio independently correlates with neurological deficit in secondary progressive multiple sclerosis *J Neurol* (2009) 256, 427- 435

Hayton T, Furby J, Smith KJ, Altmann DR, Brenner R, Chataway J, Hunter K, Tozer DJ, Miller DH, Kapoor R. Longitudinal changes in magnetisation transfer ratio in secondary progressive multiple sclerosis: data from a randomised placebo controlled trial of lamotrigine. *J Neurol* (2011a) Sep 9. [Epub ahead of print]

Hayton T, Furby J, Smith KJ, Altmann DR, Brenner R, Chataway J, Hunter K, Tozer DJ, Miller DH, Kapoor R. Clinical and imaging correlates of the multiple sclerosis impact scale in secondary progressive multiple sclerosis. *J Neurol* (2011b) Aug 24. [Epub ahead of print]

Helms G, Stawiarz L, Kivisakk P, Link H Regression analysis of metabolite concentrations estimated from localized proton MR spectra of active and chronic multiple sclerosis lesions. *Magn Reson Med* (2000) 43, 102-110

Hobart J, Lamping D, Fitzpatrick R, Riazi A, Thompson A. The multiple sclerosis impact scale: A new patient-based outcome measure. *Brain* (2001) 124, 962-973

Hoogervorst ELJ, Zwemmer JNP, Jelles B, Polman CH, Uitdehaag BMJ. Multiple Sclerosis Impact Scale (MSIS-29): relation to established measures of impairment and disability. *Mult Scler* (2004) 10, 569-574

Houtchens MK, Benedict RH, Killiany R, Sharma J, Jaisani Z, Singh B, Jaisani Z, Singh B, Weinstock-Guttman B, Guttmann CRG, Bakshi R. Thalamic atrophy and cognition in multiple sclerosis. *Neurology* (2007) 69, 1213-23

Howell OW, Reeves CA, Nicholas R, Carassiti D, Radotra B, Gentleman SM, Serafini B, Aloisi F, Roncaroli F, Magliozzi R, Reynolds R. Meningeal inflammation is widespread and linked to cortical pathology in multiple sclerosis. *Brain* (2011) 134, 2755-2771.

Ianucci G, Minicucci L, Rodegher M, Sormani MP, Comi G, Filippi M. Correlations between clinical and MRI involvement in multiple sclerosis: assessment with T1, T2 and MT histograms. *J Neurol Sci* (1999) 171, 121-129

Ingle GT, Stevenson VL, Miller DH, Leary SM, Rovaris M, Barkhof F, Brochet B, Dousset V, Filippi M, Montalban X, Kalkers NF, Polman CH, Rovira A, Thompson AJ. Two-year follow-up study of primary and transitional progressive multiple sclerosis. *Mult Scler* (2002) 8, 108-14

Inglese M, Horsfield A and Filippi M. Scan-rescan variation of measures derived from brain magnetization transfer ratio histograms obtained in healthy volunteers by use of a semi-interleaved magnetization transfer sequence. *AJNR* (2001) 22, 681-684

Inglese M, van Easberghe JHTM, Rovaris M, Beckmann K, Barkhof F, Hahn D, Kappos L, Miller DH, Polman C, Pozilli C, Thompson AJ, Yousry TA, Wagner K, Comi C, Filippi M The effect of interferon β -1b on quantities derived from MT MRI in secondary progressive MS. *Neurology* (2003) 60, 853-860.

Isaksson A-K, Ahlstrom G, Gunnarsson L-G. Quality of life in patients with multiple sclerosis. *JNNP* (2005) 76, 64-69

Jacobs LD, Beck RW, Simon JH, Kinkel RP, Brownschidle CM, Murray TJ, Simonian NA, Slasor PJ, Sandrock AW, for The CHAMPS Study Group. Intramuscular Interferon Beta-1A Therapy Initiated during a First Demyelinating Event in Multiple Sclerosis. *N Eng J Med* (2000) 343, 898-904

Janhardan V, Bakshi R. Quality of life and its relationship to Brain lesions and atrophy on magnetic resonance images in 60 patients with multiple sclerosis. *Arch Neurol* (2000) 57, 1485 -1491

Johnson AW, Land JM, Thompson EJ, Bolanos JP, Clark JB, Heales SJ. Evidence for increased nitric oxide production in multiple sclerosis. *JNNP* (1995a) 58, 107

Johnson KP, Brooks BR, Cohen JA, Ford CC, Goldstein J, Lisak RP, Myers LW, Panitch HS, Rose JW, Sciffer RB, Vollmer T, Weiner LP, Wolinsky JS and the Copolymer 1 Multiple Sclerosis Study Group. Copolymer 1 reduces relapse rate and improves disability in relapsing– remitting multiple sclerosis: results of a phase III

multicenter, double-blind, placebo-controlled trial. *Neurology* (1995b) 45, 1268–1276.

Johnson KP, Brooks BR, Cohen JA, Ford CC, Goldstein J, Lisak RP, Myers LW, Panitch HS, Rose JW, Sciffer RB, Vollmer T, Weiner LP, Wolinsky JS and the Copolymer 1 Multiple Sclerosis Study Group. Extended use of glatiramer acetate (Copaxone) is well tolerated and maintains its clinical effect on multiple sclerosis relapse rate and degree of disability. *Neurology* (1998) 50, 701–708.

Kalkers NF, de Groot V, Lazeron RHC, Killestein J, Ader HJ, Barkhof F, Lankhorst GJ, Polman CH. MS Functional Composite: Relation to disease phenotype and disability strata. *Neurology* (2000) 54, 1233-1239

Kalkers NF, Hintzen RQ, van Waesberghe JHTM, Lazeron RHC, van Schijndel RA, Ader HJ, Polman CH, Barkhof F Magnetization transfer histogram parameters reflect all dimensions of MS pathology including atrophy. *J Neurol Sci* (2001a) 184, 155-162

Kalkers NF, Bergers L, Castelijns JA, van Walderveen MAA, Bot JCJ, Ader HJ, Polman CH, Barkhof F. Optimizing the association between disability and biological markers of MS. *Neurology* (2001b) 57, 1253-1258

Kalkers NF, Ameziane N, Bot JC, Minneboo A, Polman CH, Barkhof F. Longitudinal brain volume measurement in multiple sclerosis: rate of brain atrophy is independent of disease subtype. *Arch Neurol* (2002) 59, 1572-76

Kapoor R, Davies M, Blaker PA, Hall SM & Smith KJ. Blockers of sodium and calcium entry protect axons from nitric oxide- mediated degeneration. *Ann Neurol* (2003) 53, 174-180

Kapoor R, Furby J, Hayton T, Smith KJ, Altmann DA., Brenner R, Chataway J, Hughes RAC, Miller DH. A randomized controlled trial of neuroprotection with lamotrigine in secondary progressive multiple sclerosis. *Lancet Neurology* (2010) 9, 681–88

Kappos L and The European Study Group on Interferon β -1b in Secondary Progressive MS. Placebo-controlled multicentre randomised trial of interferon β -1b in treatment of secondary progressive multiple sclerosis. *Lancet* (1998) 352, 1491-1497.

Kappos L, Moeri D, Radue EW, Schoetzau A, Schweikert K, Barkhof F, Miller D, Guttman RC, Weiner HL, Gasperini C, Filippi M. Predictive value of Gd-enhanced magnetic resonance imaging for relapse rate and changes in disability or impairment in multiple sclerosis: a meta-analysis. Gd MRI Meta-analysis Group. *Lancet* (1999) 353, 964-969.

Kappos L, Polman C, Pozzilli C, Thompson A, Beckmann K, Dahlke F; European Study Group in Interferon beta-1b in Secondary-Progressive MS. Final analysis of the European multicenter trial on IFNbeta-1b in secondary-progressive MS. *Neurology* (2001) 57, 1969-1975.

Kappos L, Weinshenker B, Pozzilli C, Thompson AJ, Dahlke F, Beckmann K, Polman C, McFarland H; European (EU-SPMS) Interferon beta-1b in Secondary

Progressive Multiple Sclerosis Trial Steering Committee and Independent Advisory Board; North American (NA-SPMS) Interferon beta-1b in Secondary Progressive Multiple Sclerosis Trial Steering Committee and Independent Advisory Board. Interferon beta-1b in secondary progressive MS: a combined analysis of the two trials. *Neurology* (2004) 63, 1779-87.

Kappos L, Polman CH, Freedman MS, Edan G, Hartung HP, Miller DH, Montalban X, Barkhof F, Bauer L, Jacobs P, Pohl C, Sandbank R and for the BENEFIT study group. Treatment with interferon beta-1b delays conversion to clinically definite and McDonald MS in patients with clinically isolated syndrome. *Neurology* (2006) 67, 1242-1249

Kappos L, Radue EW, O'Connor P, Polman C, Hohlfeld R, Calabresi P, Selmaj K, Agoropoulou C, Leyk M, Zhang-Auberson L, Burtin P; FREEDOMS Study Group. A placebo-controlled trial of oral fingolimod in relapsing multiple sclerosis. *N Engl J Med* (2010) 362, 387-401

Katz D, Taubenberger JK, Cannella B, McFarlin DE, Raine CS, McFarland HF. Correlation between magnetic resonance imaging findings and lesion development in chronic, active multiple sclerosis. *Ann Neurol* (1993) 34,661-669

Khaleeli Z, Cercignani M, Audoin B, Coccarelli O, Miller DH, Thompson AJ. Localised grey matter damage in early primary progressive multiple sclerosis contributes to disability. *NeuroImage* (2007) 37, 253-261

Korteweg T, Rovaris M, Neacsu V, Filippi M, G Comi G, Uitdehaag BM, Knol DL, Polman CH, Barkhof F, Vrenken H, On behalf of the MAGNIMS collaboration Can rate of brain atrophy in multiple sclerosis be explained by clinical and MRI characteristics? *Multiple Sclerosis* (2009) 15, 465–471

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

Kutzelnigg A, Luchinetti CF, Stadelman C, Bruck W, Rauschka H, Bermann M, Schmidbauer M, Parisi JE, Lassman H Cortical demyelination and diffuse white matter injury in multiple sclerosis. *Brain* (2005) 128, 2705-2712

Kurtzke JF. Rating neurologic impairment in multiple sclerosis: An expanded disability status scale (EDSS). *Neurology* (1983) 33, 1444- 1452

Le Bihan D, Mangin JF, Poupon C, Clark CA, Pappata S, Molko N, Chabriat H, Diffusion Tensor Imaging: Concepts and Applications. *J MRI* (2001) 13, 534–546

Leary SM, Davie CA, Parker GJM, Stevenson VL, Wang L, Barker GJ, Miller DH, Thompson AJ. IH magnetic resonance spectroscopy of normal appearing white matter in primary progressive multiple sclerosis. *J Neurol* (1999a) 246, 1023-1026

Leary S, Silver NC, Barker GJ, Miller DH, Thomspson AJ. Magnetisation transfer ratio for normal appearing white matter in primary progressive multiple sclerosis. *Mult Scler* (1999b) 5, 313-316.

Leary SM, Miller DH, Stevenson VL, Brex PA, Chard DT, Thompson AJ. Interferon beta-1a in primary progressive MS: an exploratory, randomized, controlled trial. *Neurology* (2003) 60, 44-51

Leray E, Yaouanq J, Le Page E, Coustans M, Laplaud D, Oger J, Edan G. Evidence for a two-stage disability progression in multiple sclerosis. *Brain* (2010) 133, 1900-1913

Lhermitte JJ. Les formes douloureuses de la commotion de la moelle épinière. *Rev Neurol* (1920) 36, 257-262

Li DKB, Zhao GJ, Paty DW and the University of British Columbia MS/MRI Analysis Research Group and the SPECTRIMS Study Group. Randomized controlled trial of interferon-beta-1a in secondary progressive MS: MRI results. *Neurology* (2001) 56, 1505-1513

Li DKB, Held U, Petkau J, Daumer M, Barkhof F, Fazekas F, Frank JA, Kappos L, Miller DH, Simon JH, Wolinsky JS, Filippi M, and for the Sylvia Lawry Centre for MS Research MRI T2 lesion burden in multiple sclerosis: a plateauing relationship with clinical disability. *Neurology* (2006) 66, 1384-1389.

Lin X, Blumhardt LD, Constantinescu CS. The relationship of brain and cervical cord volume to disability in clinical subtypes of multiple sclerosis: a three-dimensional MRI study. *Acta Neurol Scand.* (2003a)108, 401- 406.

Lin X, Tench CR, Turner B, Blumhardt LD, Constantinescu CS. Spinal cord atrophy and disability in multiple sclerosis over four years: application of a reproducible automated technique in monitoring disease progression in a cohort of the interferon beta-1a (Rebif) treatment trial. *J Neurol Neurosurg Psychiatry* (2003b) 74, 1090-1094

Lo AC, Black JA, Waxman SG. Neuroprotection of axons with phenytoin in experimental allergic encephalomyelitis. *Neuroreport* (2002) 13, 1909-1912

Lo, AC, Saab CY, Black JA, Waxman SG. Phenytoin protects spinal cord axons and preserves axonal conduction and neurological function in a model of neuroinflammation in vivo. *J Neurophysiol* (2003) 90, 3566-3571

Lobentanz IS, Asenbaum S, Vass K, Sauter C, Klosch G, Kollegger H, Kristoferitsch W, Zeitlhofer J, Factors influencing quality of life in multiple sclerosis patients: disability, depressive mood, fatigue and sleep quality. *Act Neurol Scand* (2004) 110, 6-13

Losseff NA, Wang L, Lau HM, Yoo DS, Gawne-Cain ML, McDonald WI, Miller DH, Thompson AJ. Progressive cerebral atrophy in multiple sclerosis. A serial MRI study. *Brain* (1996a) 119, 2009-2019

Losseff NA, Webb SL, O'Riordan JI, Page R, Wang L, Barker GJ, Tofts PS, McDonald WI, Miller DH, Thompson AJ. Spinal cord atrophy and disability in multiple sclerosis. A new reproducible and sensitive MRI method with potential to monitor disease progression. *Brain* (1996b) 119, 701-708

Lovas G, Szilágyi N, Majtényi K, Palkovits M, Komoly S. Axonal changes in chronic demyelinated cervical spinal cord plaques. *Brain*. (2000)123, 308-317

Lovato L, Willis SN, Rodig SJ, Caron T, Almendinger SE, Howell OW, Reynolds R, O'Connor KC, Hafler DA. Related B cell clones populate the meninges and parenchyma of patients with multiple sclerosis. *Brain*. (2011) 134, 5534-41

Lublin FD, Reingold SC and for the National Multiple Sclerosis Society (USA) Advisory Committee on Clinical Trials of New Agents in Multiple Sclerosis Defining the clinical course of multiple sclerosis: Results of an international survey. *Neurology* (1996) 46, 907-911

Lucchinetti C, Brück W, Parisi J, Scheithauer B, Rodriguez M and Lassmann H. Heterogeneity of multiple sclerosis lesions: implications for the pathogenesis of demyelination. *Ann Neurol* (2000) 47, 707-717

Lucchinetti CF, Popescu BF, Bunyan RF, Moll NM, Roemer SF, Lassmann H, Brück W, Parisi JE, Scheithauer BW, Giannini C, Weigand SD, Mandrekar J, Ransohoff RM. Inflammatory cortical demyelination in early multiple sclerosis. *N Engl J Med* (2011) 365, 2188-2197

Lumsden CE (1970) The neuropathology of multiple sclerosis . in Vinken PI, Bruyn GW, eds. *Handbook of clinical neurology* . Elsevier, New York, pp 217-309

Lycklama y Nijeholt, van Walderveen MAA, Castelijns JA, van Waesbergh JHTM, Polman C, Scheltens P, Rosier PFWM, Jongen PJH, Barkhof F. Brain and spinal cord abnormalities in multiple sclerosis. Correlation between MRI parameters, clinical subtypes and symptoms. *Brain* (1998) 121, 687-697

McDonald WI, Compston A, Edan G, Goodkin D, Hartung HP, Lublin FD, McFarland HF, Paty DW, Polman CH, Reingold SC, Sandberg-Wollheim M, Sibley W, Thompson A, van den NS, Weinshenker BY, Wolinsky JS. Recommended diagnostic criteria for multiple sclerosis: guidelines from the International Panel on the diagnosis of multiple sclerosis *Ann Neurol* (2001) 50, 121-127

McGavern DB, Murray PD, Rivera-Quiñones C, Schmelzer JD, Low PA, Rodriguez M. Axonal loss results in spinal cord atrophy, electrophysiological abnormalities and neurological deficits following demyelination in a chronic inflammatory model of multiple sclerosis. *Brain*. (2000)123, 519-531

Magliozzi R, Howell O, Vora A, Serafini B, Nicholas R, Puopolo M, Reynolds R, Aloisi F. Meningeal B-cell follicles in secondary progressive multiple sclerosis associate with early onset of disease and severe cortical pathology. *Brain* (2007) 130, 1089-1104

Magliozzi R, Howell OW, Reeves C, Roncaroli F, Nicholas R, Serafini B, Aloisi F, Reynolds R. A Gradient of neuronal loss and meningeal inflammation in multiple sclerosis. *Ann Neurol* (2010) 68, 477-493

Melhem ER, Breiter SN, Ulug AM, Raymond GV, Moser HW. Improved tissue characterization in adrenoleukodystrophy using magnetization transfer imaging. *Am J Roentgenol* (1996) 166, 689-695

Mesaros S, Rocca MA, Sormani MP, Charil A, Comi G, Filippi M. Clinical and conventional MRI predictors of disability and brain atrophy accumulation in RRMS: A large scale, short-term follow-up study. *J Neurol* (2008) 255, 1378–1383

Miller DH, Rudge P, Johnson G, Kendall BE, MacManus DG, Moseley IF, Barnes D, McDonald WI Serial Gd enhanced magnetic resonance imaging in multiple sclerosis. *Brain* (1988) 111, 927-939

Miller DH, Barkhof F, Berry I, Kappos L, Scotti G, Thompson A J Magnetic resonance imaging in monitoring the treatment of multiple sclerosis: Concerted Action Guidelines. *JNNP* (1991) 54, 683-688

Miller DH, Albert PS, Barkhof F, Francis G, Frank JA, Hodgkinson S, Lublin FD, Paty DW, Reingold SC, Simon J. Guidelines for the use of magnetic resonance techniques in monitoring the treatment of Multiple Sclerosis. *Ann Neurol* (1996) 39,6-16

Miller DM, Rudick RA, Cutter G, Baier M, Fischer JS. Clinical significance of the Multiple Sclerosis Functional Composite: relationship to patient-reported Quality of Life. *Arch Neurol* (2000) 57, 1319-1324

Miller DH, Barkhof F, Frank JA, Parker GJM and Thompson AJ. Measurement of atrophy in multiple sclerosis: pathological basis, methodological aspects and clinical relevance. *Brain* (2002) 125, 1676-1695.

Mitchell AJ, Benito-León J, Morales González J-M, et al. Quality of life and its assessment in multiple sclerosis: integrating physical and psychological components of wellbeing *Lancet Neurol* (2005) 4, 556–566

Moll C, Moure C, Lazdunski M, Ulrich J. Increase of sodium channels in demyelinated lesions of multiple sclerosis. *Brain Res* (1991) 556, 311-316

Moll NM, Cossoy MB, Fisher E, Staugaitis SM, Tucky BH, Rietsch AM, Chang A, Fox RJ, Trapp BD, Ransohoff RM. Imaging correlates of leukocyte accumulation and CXCR4/CXCL12 in multiple sclerosis. *Arch Neurol* (2009) 66, 44-53

Molyneux PD, Miller DH, Filippi M, Yousry TA, Rathi EV, Ader HJ, Barkhof F. Visual analysis of serial T2-weighted MRI in multiple sclerosis: Intra- and interobserver reproducibility. *Neurorad* (1999) 41, 882-888

Molyneux PD, Kappos L, Polman C, Pozzilli C, Barkhof F, Filippi M, Yousry T, Hahn D, wagner K, Ghazi M, Beckmann K, Dahlke F, Losseff N, barker GJ, Thompson AJ, Miller DH and the European study group on interferon beta-1b in secondary progressive MS. The effect of interferon beta-1b treatment on MRI measures of cerebral atrophy in secondary progressive multiple sclerosis. European Study Group on Interferon beta-1b in secondary progressive multiple sclerosis. *Brain* (2000a) 123, 2256-2263

Molyneux PD, Miller DH, Filippi M, Yousry T, Kappos L, Gasperini C, et al. The use of magnetic resonance imaging in multiple sclerosis treatment trials: power calculations for annual lesion load measurement. *J Neurol* (2000b) 247, 34–40

Molyneux PD, Barker GJ, Barkhof F et al. Clinical-MRI correlations in a European trial of interferon beta-1b in secondary progressive MS. *Neurology* (2001) 57, 2191-2197

Montalban X, Sastre-Garriga J, Tintoré M, Brieva L, Aymerich FX, Ríó J, Porcel J, Borràs C, Nos C, Rovira A. A single-center, randomized, double-blind, placebo-controlled study of interferon beta-1b on primary progressive and transitional multiple sclerosis. *Mult Scler* (2009) 15, 1195-1205

Moreau T, Coles A, Wing M, Isaacs J, Hale G, Waldmann H, Compston A. Transient increase in symptoms associated with cytokine release in patients with multiple sclerosis. *Brain* (1996) 119, 225-237

Morgen K, Sammer G, Courtney SM, Wolters T, Melchior H, Blecker CR, Oschmann P, Kaps M, Vaitl D. Evidence for a direct association between cortical atrophy and cognitive impairment in relapsing-remitting MS. *Neuroimage* (2006) 30, 891-898

Mowry EM, Beheshtian A, Waubant E, Goodin DS, Cree BA, Qualley P, Lincoln R, George MF, Gomez R, Hauser SL, Okuda DT, Pelletier D. Quality of life in multiple

sclerosis is associated with lesion burden and brain volume measures. *Neurology* (2009) 72, 1760-1765

Narayanan S, De Stefano N, Francis GS, Arnouetelis R, Caramanous Z, Collins LD et al. Axonal metabolic recovery in multiple sclerosis patients treated with interferon 1b. *J Neurol* (2001) 249, 979–986

Newcombe J, Hawkins CP, Henderson CL, Patel HA, Woodroffe MN, Hayes GM, Cuzner ML, MacManus D, du Boulay EP, McDonald WI. Histopathology of multiple sclerosis lesions detected by magnetic resonance imaging in unfixed postmortem central nervous system tissue *Brain* (1991) 114, 1013-1023.

Nordvedt MW, Riise T, Myhr K-M, Nyland HI. Performance of the SF-36, SF-12 and Rand-36 Summary scales in a multiple sclerosis population. *Medical Care* (2000) 10, 1022-1028

Noseworthy JH, Vandervoort MK, Wong CJ, Ebers GC. Interrater variability with the Expanded Disability Status Scale (EDSS) and Functional Systems (FS) in a multiple sclerosis clinical trial. *Neurology* (1990) 40, 971- 975

Noseworthy JH, Ebers GC, Vandervoort MK, Farquhar RE, Yetisir E, Roberts R. The impact of blinding on the results of a randomized, placebo-controlled multiple sclerosis clinical trial. *Neurology* (1994) 44, 16 – 20

O'Connor P, Wolinsky J, Confavreux C, Comi G, Kappos L, Olsson TP, Benzerdjeb H, Truffinet P, Wang I, Miller A, Freedman MS for the TEMSO Trial Group. Randomized trial of oral teriflunomide for relapsing multiple sclerosis. *N Eng J Med* (2011) 365, 1293-1303

Oppenheimer DR. The cervical cord in multiple sclerosis. *Neuropathol Appl Neurobiol* (1978) 4, 151-62.

Oreja-Guevara C, Charil A, Caputo D, Caravetta R, Sormani MP, Filippi M. Magnetisation transfer magnetic resonance imaging and clinical changes in patients with relapsing remitting multiple sclerosis. *Arch Neurol* (2006) 63, 736-740

Ormerod IE, Miller DH, McDonald IW, du Boulay EP, Rudge P, Kendall BE, Moseley IF, Johnson G, Tofts PS, Halliday AM. The role of NMR imaging in the assessment of multiple sclerosis and isolated neurological lesions. A quantitative study. *Brain* (1987) 110, 1579-1616.

Pagani E, Rocca MA, Gallo A, Rovaris M, Martinelli V, Comi G, Filippi M. Regional brain atrophy evolves differently in patients with multiple sclerosis according to clinical phenotype. *Am J Neuroradiol* (2005) 26, 341-346.

Panitch H and The North American Study Group on Interferon beta-1b in Secondary Progressive MS. Interferon beta-1b in secondary progressive MS: Results from a 3-year controlled study. *Neurology* (2004) 63, 1788-1795

Patani R, Balaratnam M, Vora A, Reynolds R. Remyelination can be extensive in multiple sclerosis despite a long disease course. *Neuropathol Appl Neurobiol* (2007) 33, 277-287

Patrikios P, Stadelmann C, Kutzelnigg A, Rauschka H, Schmidbauer M, Laursen H, Sorensen PS, Brück W, Lucchinetti C, Lassmann H Remyelination is extensive in a subset of multiple sclerosis patients. *Brain* (2006) 129, 3165-3172.

Paty DW, Li DKB and the UBC MS/MRI Study Group and the IFNB Multiple Sclerosis Study Group. Interferon beta-1b is effective in relapsing-remitting multiple sclerosis:II. MRI analysis results of a multicenter, randomized, double-blind, placebo-controlled trial. *Neurology* (1993) 43, 662-667

Pellicano C, Gallo A, Li X, Ikonomidou VN, Evangelou IE, Ohayon JM, Stern SK, Ehrmantraut M, Cantor F, McFarland HF, Bagnato F. Relationship of cortical atrophy to fatigue in patients with multiple sclerosis. *Arch Neurol* (2010) 67, 447-453.

Peterson JW, Bo L, Mork S, Chang A, Trapp BD. Transected neuritis, apoptotic neurons and reduced inflammation in cortical multiple sclerosis lesions. *Annals Neurol* (2001) 50, 389-400

Petroff OA, Pleban LA, Spencer DD Symbiosis between in vivo and in vitro NMR spectroscopy: the creatine, N-acetylaspartate, glutamate, and GABA content of the epileptic human brain. *Magn Reson Imaging* (1995)13, 1197-1211

Phillips MD, Grossman RI, Miki Y, Wei L, Kolson DL, Buchem MA, Polansky M, McGowan JC, Udupa JK. Comparison of T2 Lesion Volume and Magnetization Transfer Ratio Histogram Analysis and of Atrophy and Measures of Lesion Burden in Patients with Multiple Sclerosis. *Am J Neuroradiol* (1998) 19, 1055-1060

Pierpaoli C, Jezzard P, Basser PJ, Barnett A, Di Chiro G. Diffusion tensor MR imaging of the human brain. *Radiology* (1996) 201, 637-648

Plummer DL. Dispimage: a display and analysis tool for medical images. *Revisita Di Neuroradiologica* (1992) 5, 489-95

Polman CH, Reingold SC, Edan G, Filippi M, Hartung HP, Kappos L, Lublin FD, Metz LM, McFarland HF, O'Connor PW, Sandberg-Wollheim M, Thompson AJ, Weinshenker BG, Wolinsky JS, "Diagnostic criteria for multiple sclerosis: 2005 revisions to the "McDonald Criteria" *Ann Neurol* (2005) 58, 840-846.

Polman CH, O'Connor PW, Havrdova E, Hutchinson M, Kappos L, Miller DH, Phillips JT, Lublin FD, Giovannoni G, Wajgt A, Toal M, Lynn F, Panzara MA, Sandrock AW. A Randomized, Placebo-Controlled Trial of Natalizumab for Relapsing Multiple Sclerosis. *N Eng J Med* (2006) 354, 899-910.

Polman CH, Rudick RA. The Multiple Sclerosis Functional Composite: a clinically meaningful measure of disability. *Neurology* (2010) 72: Suppl 3,S8-15

Polman CH, Reingold SC, Banwell B, Clanet M, Cohen JA, Filippi M, Fujihara K, Havrdova E, Hutchinson M, Kappos L, Lublin FD, Montalban X, O'Connor P, Sandberg-Wollheim M, Thompson AJ, Waubant E, Weinshenker B, Wolinsky JS.

Diagnostic criteria for multiple sclerosis: 2010 revisions to the McDonald criteria. *Ann Neurol.* (2011) 69, 292-302

Pujol J, Bello J, Deus J, Martí-Vilalta JL, Capdevila A. Lesions in the left arcuate fasciculus region and depressive symptoms in multiple sclerosis. *Neurology* (1997) 49, 1105-1110

Rademacher J, Engelbrecht V, Burgel U, Freund HJ and Zilles K . Measuring in vivo myelination in human white matter fiber tracts with Magnetization transfer MR. *NeuroImage* (1999) 9, 393-406

Ramio-Torrenta L, Sastre Garriga J, Ingle GT, Davies GR, Ameen V, Miller DH, Thompson AJ. Abnormalities in normal appearing tissues in early primary progressive multiple sclerosis and their relation to disability: a tissue specific magnetisation transfer study. *JNNP* (2006) 77, 40-45

Redford EJ, Kapoor R, Smith KJ. Nitric oxide donors reversibly block axonal conduction: demyelinated axons are especially susceptible. *Brain* (1997) 120, 2149-2157

Redmond IT, Barbosa S, Blumhardt LD, Roberts N Short-term ventricular volume changes on serial MRI in multiple sclerosis. *Acta Neurol Scand* (2000) 102, 99-105

Riazi A, Hobart JC, Lamping DL, Fitzpatrick R, Thompson AJ. Multiple Sclerosis Impact Scale (MSIS-29): reliability and validity in hospital based samples. *JNNP* (2002) 73, 701-704

Ritchie, J.M. and Rogart, R.B., The density of sodium channels in mammalian myelinated nerve fibers and the nature of the axonal membrane under the myelin sheath. *PNAS* (1977) 74, 211-215

Robiner WN. Enhancing adherence in clinical trials. *Contemp Clin Trials*. (2005) 26,59-77.

Rocca MA, Mastronardo G, Rodegher M, Comi G, Filippi M. Long-term changes of magnetization transfer-derived measures from patients with relapsing-remitting and secondary progressive multiple sclerosis. *AJNR Am J Neuroradiol* (1999) 20, 821–827

Rothwell PM, McDowell D, Wong CK, Dornan PJ. Doctors and patients don't agree: cross-sectional study of patients' and doctors' perceptions and assessments of disability in multiple sclerosis. *BMJ* (1997) 314, 1580-1583

Rovaris , Filippi M, Calori G, Rodegher M, Campi A, Colombo B, Comi G. Intra-observer reproducibility in measuring new putative MR markers of demyelination and axonal loss in multiple sclerosis: a comparison with conventional T2-weighted images. *J Neurol* (1997) 244, 266-270

Rovaris M, Comi G, Rocca MA, Cercignani M, Colombo B, Santuccia G, Filippi M. Relevance of hypointense lesions on fast-fluid attenuated inversion recovery MR images as a marker of disease severity in cases of multiple sclerosis. *AJNR* (1999) 20, 813-820.

Rovaris M, Filippi M, Minicucci L, Iannucci G, Santuccio G, Possa F, Comi G. Cortical/subcortical disease burden and cognitive impairment in patients with multiple sclerosis. *AJNR Am J Neuroradiol.* (2000) 21, 402-408

Rovaris M, Bozzali M, Santuccio G, Ghezzi A, Caputo D, Montanari E, Bertolotto A, Bergamaschi R, Capra R, Mancardi M, Martinelli V, Comi G, Filippi M. In vivo assessment of the brain and cervical cord pathology of patients with primary progressive multiple sclerosis. *Brain* (2001) 124, 2540-2549

Rovaris M, Agosta F, Sormani MP, Inglesse M, Martinelli V, Comi G, Filippi M. Conventional and magnetization transfer MRI predictors of clinical multiple sclerosis evolution: a medium-term follow-up study. *Brain* (2003a) 126, 2323-2332

Rovaris M, Comi G, Ladkani D, Wolinsky J, Filippi M, and the European/Canadian Glatiramer Acetate Study Group. Short term correlations between clinical and MR imaging findings in relapsing-remitting multiple sclerosis. *Am J Neuroradiol* (2003b) 24, 75-81

Rovaris M, Gass A, Hickman SJ, Ciccarelli O, Miller DH and Filippi M. Diffusion MRI in multiple sclerosis. *Neurology* (2005) 65, 1526-1532

Rovira A, Alonso J, Cucurella G, Nos C, Tintore M, Pedraza S, Rio J, Montalban X. Evolution of multiple sclerosis lesions on serial contrast enhanced T1-weighted and magnetic transfer MR images. *AJNR* (1999) 20, 1939-1945

Rudick RA, Fisher E, Lee J-C, Simon J, Jacobs L. Use of the brain parenchymal fraction to measure whole brain atrophy in relapsing-remitting MS. *Neurology* (1999) 53, 1698-1704

Rudick RA, Cutter G, Baier M, Fisher E, Dougherty D, Weinstock-Guttman B, Mass MK, Miller D, Simonian NA. Use of the Multiple Sclerosis Functional Composite to predict disability in relapsing MS. *Neurology* (2001) 56, 1324-1330

Rudick, RA, Lee J-C, Simon J, Fisher E Significance of T2 Lesions in Multiple Sclerosis: A 13-Year Longitudinal Study. *Ann Neurol* (2006a) 60, 236–242

Rudick RA, Stuart WH, Calabresi PA, Confavreux C, Galetta SL, Radue EW, Lublin FD, Weinstock-Guttman B, Wynn DR, Lynn F, Panzara MA, Sandrock A. Natalizumab plus Interferon Beta-1a for Relapsing Multiple Sclerosis. *N Eng J Med* (2006b) 354, 911-923

Sadovnick AD, Ebers GC. Epidemiology of multiple sclerosis: a critical overview. *Can J Neurol Sci* (1993) 20, 17-29

Sailer M, Fischl B, Salat D, Tampelmann C, Schonfeld MA, Busa E, Bodammer N, Heinze HJ, Dale A. Focal Thinning of the cerebral cortex in multiple sclerosis. *Brain* (2003) 126, 1734-1744

Sanfilippo MP, Benedict RH, Sharma J, Weinstock-Guttman B, Bakshi R. The relationship between whole brain volume and disability in multiple sclerosis: a comparison of normalized grey vs. White matter with misclassification. *Neuroimage* (2005) 26, 1068-1077

Sanfilippo MP, Benedict RH, Weinstock-Guttman B, Bakshi R. Gray and white matter brain atrophy and neuropsychological impairment in multiple sclerosis. *Neurology* (2006) 66, 685-92

Santos AC, Narayanan S, de Stefano N. Magnetization transfer can predict clinical evolution in patients with multiple sclerosis. *J Neurol* (2002) 249, 662-668

Sarchielli P, Presciutti O, Pelliccioli GP, Tarducci R, Gobbi G, Chiarini P, Alberti A, Vicinanza F, Gallai V Absolute quantification of brain metabolites by proton magnetic resonance spectroscopy in normal-appearing white matter of multiple sclerosis patients. *Brain* (1999) 122, 513-521

Sastre-Garriga J, Ingle GT, Chard DT, Ramio-Torrenta L, Miller DH, Thompson AJ. Grey and white matter atrophy in early clinical stages of primary progressive multiple sclerosis. *NeuroImage* (2004) 22, 353-359

Sastre-Garriga J, Arévalo MJ, Renom M, Alonso J, González I, Galán I, Montalban X, Rovira A. Brain volumetry counterparts of cognitive impairment in patients with multiple sclerosis. *J Neurol Sci* (2009) 282, 120-124

Scalfari A, Neuhaus A, Degenhardt A, Rice GP, Muraro A, Daumer M, Ebers GC
The natural history of multiple sclerosis, a geographically based study 10: relapses and long-term disability. *Brain* (2010) 133, 1914–1929

Schmierer K, Scaravelli F Altmann DR, Barker GJ and Miller DH Magnetization transfer ratio and myelin in postmortem multiple sclerosis brain. *Ann Neurol* (2004) 56, 407- 415

Schmierer K, Parkes HG, So PW, An SF, Brandner S, Ordidge RJ, Yousry TA, Miller DH. High field (9.4 Tesla) magnetic resonance imaging of cortical grey matter lesions in multiple sclerosis. *Brain* (2010) 133, 858-867

Schubert F, Seifert F, Elster C, Link A, Walzel M, Mientus S, Haas J, Rinneberg H
Serial 1H-MRS in relapsing-remitting multiple sclerosis: effects of interferon-beta therapy on absolute metabolite concentrations. *MAGMA* (2002) 14, 213-222

Semra YK, Seidi OA, Sharief MK. Heightened intrathecal release of axonal cytoskeletal proteins in multiple sclerosis is associated with progressive disease and clinical disability. *J Neuroimmunol.* (2002) 122, 132-139

Serafini B, Rosicarelia B, Magliozzi R, Stigliano E, Aloisi F. Detection of Ectopic B-cell Follicles with Germinal Centers in the Meninges of Patients with Secondary Progressive Multiple Sclerosis. *Brain Pathol* (2004) 14, 164-174

Serafini B, Rosicarelli B, Franciotta D, Magliozzi R, Reynolds R, Cinque P, Andreoni L, Trivedi P, Salvetti M, Faggioni A, Aloisi F. Dysregulated Epstein-Barr virus infection in the multiple sclerosis brain. *J Exp Med* (2007) 204, 2899-2912

Sepulcre J, Masdeu JC, Goñi J, Arrondo G, Vélez de Mendizábal N, Bejarano B, Villoslada P. Fatigue in multiple sclerosis is associated with the disruption of frontal and parietal pathways. *Mult Scler* 2009 (15) 337-344

Shrager P. Sodium channels in single demyelinated mammalian axons. *Brain Res* (1989) 483, 149-154

Simeoni MC, Auquier P, Fernandez O, Flachenecker P, Stecchi S, Constaninescu C, Idiman E, Boyko A, Beiske AG, Vollmer T, Triantafylliou N, O'Connor P, Barak Y, Beirmann L, Cristiano E, Atweh S, Patrick DL, Robitail S, Ammoury N, Beresniak A, Pelletier J, on behalf of the MusiQol study group. Validation of the multiple sclerosis International quality of life questionnaire. *Mult Scler* (2008) 14, 219-230

Simon JH, Lull J, Jacobs LD, Rudick RA, Cookfair DL, Herndon RM, Richert JR, Salazar AM, Sheeder J, Miller D, McCabe K, Serra A, Champion MK, Fischer JS, Goodkin DE, Simonian N, Lajaunie M, Wende K, Martens-Davidson A, Kinkel RP,

Munschauer FE and the Multiple Sclerosis Collaborative Research Group (MSCRG)*

A longitudinal study of T1 hypointense lesions in relapsing MS: MSCRG trial of interferon b-1a. *Neurology* (2000) 55, 185–192

Smith KJ and McDonald WI. Spontaneous and evoked electrical discharges from a central demyelinating lesion. *J Neurol Sci* (1982) 55, 39-47

Smith KJ, Kapoor R, Hall SM, Davies M. Electrically active axons degenerate when exposed to nitric oxide. *Ann Neurol* (2001) 49, 470-476

Smith SM Fast robust automated brain extraction. *Human Brain Mapp* (2002) 17, 143-155

Smith SM, Zhang Y, Jenkinson M, Chen J, Matthews PM, Federico A, De Stefano N
Accurate, robust and automated longitudinal and cross-sectional brain change analysis. *NeuroImage* (2002) 17, 479-489

Solari A, Radice D, Manneschi L, Motti L, Montanari E. The multiple sclerosis functional composite: different practice effects in the three test components. *J Neurol Sci* (2005) 228, 71-74

Soon D, Altmann DR, Fernando KT, Giovannoni G, Barkhof F, Polman CH, O'Connor P, Gray B, Panzara M, Miller DH, A study of subtle blood brain barrier disruption in a placebo-controlled trial of natalizumab in relapsing remitting multiple sclerosis *J Neurol* (2007) 254, 306-314

Sormani MP, Ianucci G, Rocca A Reproducibility of magnetization transfer ratio histogram derived measures of the brain in healthy volunteers. *AJNR* (2000) 21, 133-136

Sormani MP, Bonzano L, Roccatagliata L, Cutter GR, Mancardi GL, Bruzzi P. Magnetic resonance imaging as a potential surrogate for relapses in multiple sclerosis: a meta-analytic approach. *Ann Neurol* (2009) 65, 268-275

Sormani MP, Li DK, Bruzzi P, Stubinski B, Cornelisse P, Rocak S, De Stefano N. Combined MRI lesions and relapses as a surrogate for disability in multiple sclerosis. *Neurology* (2011) 77, 1684-1690

Secondary Progressive Efficacy Clinical Trial of Recombinant Interferon-beta-1a in MS (SPECTRIMS) Study Group. Randomized controlled trial of interferon-beta-1a in secondary progressive MS: clinical results. *Neurology* (2001) 56, 1496–1504

Stevenson VL, Leary SM, Losseff NA, Parker GJ, Barker GJ, Husmani Y, Miller DH, Thompson AJ. Spinal cord atrophy and disability in MS: a longitudinal study. *Neurology* (1998) 51, 234-238

Stevenson VL, Miller DH, Rovaris M Barkhof F, Brochet B, Douseset V, Dousset V, Filippi M, Montelban X, polman CH, Rovira A, de Sa J, Thompson AJ. Primary and transitional Progressive MS: a Clinical and MRI cross-sectional study. *Neurology* (1999) 52, 839- 848

Stewart WA, Hall LD, Berry K, Paty DW. Correlation between NMR scan and brain slice data in multiple sclerosis. *Lancet* (1984) 18, 412

Stys PK, Waxman SG, Ransom BR. Ionic mechanisms of anoxic injury in mammalian CNS white matter: role of Na⁺ channels and Na⁽⁺⁾-Ca²⁺ exchanger. *J Neurosci* (1992) 12, 430-439

Suhy J, Rooney WD, Goodkin DE, Capizzano AA, Soher BJ, Maudsley AA, Waubant E, Andersson PB, Weiner MW 1H MRSI comparison of white matter and lesions in primary progressive and relapsing-remitting MS. *Mult Scler* (2000) 6, 148-155

Tallan HH, Moore S, Stein WH N-Acetyl-L-aspartic acid in brain. *J Biol Chem* (1956) 219, 257-264

Tedeschi G, Lavorgna L, Russo P, Prnster A, Dinacci D, Salvettieri G Quattrone, Livrea P, Messina C, Reggio A, Bresciamorra V, Orefice G, Paciello M, Brunetti G, Coniglio G, Bonavita S, Di Constanzo A Bellacosa A, Valentino P Quantarelli M, Patti F, Salemi G, Cammarata E, Simone IL, Salvatore M, Bonavita V, Alfano B. Brain atrophy and lesion load in a large population of patients with multiple sclerosis. *Neurology* (2005) 65, 280-285

Thompson AJ, Kermode AG, MacManus DG, Kendall BE, Kingsley DP, Moseley IF, McDonald WI. Patterns of disease activity in multiple sclerosis: clinical and magnetic resonance imaging study *BMJ* (1990) 300, 631-634

Thompson AJ, Montalban X, Barkhof F, Brochet B, Filippi M, Miller DH, Polman CH, Stevenson VL, McDonald WI. Diagnostic criteria for primary progressive multiple sclerosis: a position paper *Ann Neurol* (2000) 47, 831-835

Tiberio M, Chard DT, Altmann DR, Davies G, Griffin CM, Rashid W, Sastre_Garriga J, Thompson AJ, Miler DH. Gray and white matter volume changes in early RRMS: a 2-year longitudinal study. *Neurology* (2005) 64,1001-1007

Tortorella C Viti B, Bozzali M, Sormani P, Rizzo G, Gilardi MF, Comi G, Filippi M. A magnetization transfer histogram study of normal appearing brain tissue in MS. *Neurology* (2000) 54, 186-193

Tofts PS, Davies GR, Dehmeshki J. Histograms: Measuring Subtle Differences. In: Tofts PS editor *Quantitative MRI of the Brain*. London John Wiley and Sons Ltd. 2003: 581-610

Tozer DJ, Tofts PS. Removing spikes caused by quantization noise from high resolution histograms. *Magn. Res. Med* (2003) 50, 649-653

Traboulsee A, Dehmeshki J, Peters KR, Griffin CM, Brex PA, Silver N, Ciccarelli O Chard DT, Barker GJ, Thompson AJ, Miller DH. Disability in multiple sclerosis is related to normal appearing brain tissue MTR histogram analysis. *Mult Scler* (2003) 8, 566-573

Trapp BD, Peterson J, Ransohoff RM, Rudick R, Mork S, Bo L. Axonal transection in lesions of multiple sclerosis. *N Eng J Med* (1998) 338, 278-285

Tremlett H, Paty D, Devonshire V. The natural history of primary progressive MS in British Columbia, Canada. *Neurology* (2005) 65, 1919-1923

Tremlett H, Zhao Y, Devonshire V. Natural history of secondary-progressive multiple sclerosis. *Mult. Scler.* (2008) 14, 314-324

Truyen L, van Waesberghe LHTM, van Walderveen, MAA, van Oosten BA, Polman CH, Hommes OR, Ader HJA, Barkhof F. Accumulation of hypointense lesions (“black holes”) on T 1 spin-echo MRI correlates with disease progression in multiple sclerosis. *Neurology* (1996) 47, 1469-1476

Tubridy N, Coles AJ, Molyneux P, Compston DA, Barkhof F, Thompson AJ, McDonald WI, Miller DH. Secondary progressive multiple sclerosis: the relationship between short-term MRI activity and clinical features *Brain* (1998) 121, 225-231

Turner B, Lin X, Calmon G, Roberts N, Blumhardt LD. Cerebral atrophy and disability in relapsing-remitting and secondary progressive multiple sclerosis over 4 years. *Mult Scler* (2003) 9, 21-27

Uhthoff W. Untersuchungen über die bei der multiplen Herdsklerose vorkommenden Augenstörungen. *Archiv für Psychiatrie und Nervenkrankheiten* (1890) 21, 55-116

Urenjak J, Williams SR, Gadian DG, Noble M Proton nuclear magnetic resonance spectroscopy unambiguously identifies different neural cell types. *J Neurosci* (1993) 13, 981-989

van del Elksamp IJ, Knol DL, Vrenken H, Karas G, Meijerman A, Filippi M, Kappos L, Fazekas F, Wagner K, Pohl C, Sandbrink R, Polman CH, Uitdehaag BMJ, Barkhof F. Lesional magnetization transfer ratio a feasible outcome for remyelinating trials in multiple sclerosis. *Mult Scler* (2010) 16, 660-669

van der Valk P and De Groot CJA. Staging of multiple sclerosis (MS) lesions: pathology of the time frame of MS. *Neuropathol Appl. Neurobiol* (2000) 26, 2-10

van Waesberghe JHTM, van Walderveen MAA, Castelijns JA, Scheltens P, Lycklama y Nijeholt GJ, Polman CH, Barkhof F. Patterns of lesion development in multiple sclerosis: Longitudinal observations with T1-weighted spin-echo and magnetization transfer MR. *AJNR* (1998a) 19, 675-683

van Waesberghe JHTM, van Buchem MA, Filippi M, Castelijns JA, Rocca MA, van der Boom R, Polman CH, Barkhof F. MR Outcome Parameters in Multiple Sclerosis: Comparison of Surface-Based Thresholding Segmentation and Magnetization Transfer Ratio Histogrammic Analysis in Relation to Disability (A Preliminary Note) *AJNR* (1998b) 19, 1857-1862.

van Waesberghe JHTM, Kamphorst W, De Groot CJA, van Walderveen MAA, Castelijns JA, Ravid R Lycklama a Nijeholt GJ, van der Valk P, Polman CH,

Thompson AJ, Barkhof F Axonal loss in multiple sclerosis lesions: Magnetic resonance imaging insights into substrates of disability. *Ann Neurol* (1999) 46, 747-754

van Walderveen MAA, Barkhof F, Hommes OR, Polman CA, Tobi H, Frequin STFM, Valk J. Correlating MRI and clinical disease activity in multiple sclerosis: Relevance of hypointense lesions on short-TW short-echo time (TE) (T1-weighted) spin-echo images. *Neurology* (1995) 45, 1684-1690

van Walderveen MAA, Kamphorst W, Scheltens P, van Waesberghe JHTM, Ravid R, Valk J, Polman CH, Barkhof F. Histopathologic correlate of hypointense spin-echo lesions on T1-weighted MRI in multiple sclerosis. *Neurology* (1998) 50, 1282-1288

van Walderveen MAA, Barkhof F, Pouwels, PJW van Schijndel RA, Polman CH, Castelijns JA. Neuronal damage in T1-hypointense multiple sclerosis lesions demonstrated in vivo using proton magnetic resonance spectroscopy *Ann Neurol* (1999a) 46, 79–87

van Walderveen MA, Truyen L, van Oosten BW, Castelijns JA, Lycklama à Nijeholt GJ, van Waesberghe JH, Polman C, Barkhof F. Development of hypointense lesions on T1-weighted spin-echo magnetic resonance images in multiple sclerosis: relation to inflammatory activity. *Arch Neurol* (1999b) 56, 345-351

van Walderveen MAA, Lycklama a Neijholt GJ, Ader HJ, Jongen PJH, Polman CH, Castelijns JA, Barkhof F. Hypointense lesions on T1 weighted spin echo magnetic resonance imaging. *Arch Neurol* (2001) 58, 76-81

Voltz R, Starck M, Zingler V, Strupp M, Kolb HJ. Mitoxantrone therapy in multiple sclerosis and acute leukaemia: a case report out of 644 treated patients. *Mult Scler* (2004) 10, 472-474

Vrenken H, Barkhof F, Uitdehaag BMJ, Castelijns JA, Polman CH, Pouwels PJW. MR spectroscopic evidence for glial increase but not for neuroaxonal damage in MS normal appearing white matter. *Magn Res Med* (2005) 53, 256-266

Vrenken H, Guerts JJG, Knol DL, Polman CH, Castelijns JS, Pouwels PJW, Barkhof F. Normal appearing white matter changes vary with distance to lesions in multiple sclerosis. *Am J Neuroradiol* (2006) 27, 2005-2011

Vrenken H, Pouwels PJW, Ropele S, Knol DL, Geurts JJG, Polman CH, Barkhof F, Castelijns JA. Magnetization transfer ratio measurement in multiple sclerosis normal appearing brain tissue: limited differences with controls but relationships with clinical and MR measures of disease. *Mult Scler* (2007) 13, 708-716

Waxman SG. Demyelination in the spinal cord. *J Neurol Sci* (1989) 91, 1-14

Weinshenker BG, Bass B, Rice GP, Noseworthy J, Carriere W, Baskerville J, Ebers GC. The natural history of multiple sclerosis: a geographically based study 1. Clinical course and disability. *Brain* (1989) 112, 133-146

Weinshenker BG, Rice GP, Noseworthy J, Carriere W, Baskerville J, Ebers GC. The natural history of multiple sclerosis: a geographically based study 3: Multivariate analysis of predictive and models of outcome. *Brain* (1991a) 114, 1045-1056

Weinshenker BG, Rice GP, Noseworthy J, Carriere W, Baskerville J, Ebers GC. The natural history of multiple sclerosis: a geographically based study 4: Applications to planning and interpretation of clinical therapeutic trials. *Brain* (1991b) 114, 1057-1067

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

Wolff SD and Balaban RS Magnetization transfer contrast (MTC) and tissue water proton relaxation in vivo. *Magn Reson Med* (1989) 10, 135-44

Wolinsky JS, Narayana PA, Johnson KP and the Copolymer 1 Multiple Sclerosis Study Group and the MRI Analysis Center United States open-label glatiramer acetate extension trial for relapsing multiple sclerosis: MRI and clinical correlates *Mult Scler* (2001) 7, 33-41

Yednock TA, Cannon C, Fritz LC, Sanchez-Madrid F, Steinman L, Karin N. Prevention of experimental autoimmune encephalitis by antibodies against $\alpha 4\beta 1$ integrin. *Nature* (1992) 356, 63- 66

Youl BD, Turano G, Miller DH, Towell AD, MacManus DG, Moore SG, Jones SJ, Barrett G, Kendall BE, Moseley IF, Tofts PS, Halliday AM, McDonald WI. The pathophysiology of acute optic neuritis: an association between Gd enhancing leakage with clinical and electrophysiological deficits. *Brain* (1991) 114, 2437- 2450

Young EA, Fowler CD, Kidd GJ, Chang A, Rudick R, Fisher E, Trapp BD. Imaging Correlates of Decreased Axonal Na^+/K^+ ATPase in Chronic Multiple Sclerosis Lesions. *Ann Neurol* (2008) 63, 428-435

Yousry TA, Major EO, Ryschkewitsch C, Fahle G, Fischer S, Hou J, Curfman B, Miszkiel K, Mueller-Lenke N, Sanchez E, Barkhof F, Radue EW, Jager HR, Clifford DB Evaluation of patients treated with natalizumab for progressive multifocal leukoencephalopathy. *N Engl J Med* (2006) 354, 924-933

Ytterberg C, Johansson S, Holmqvist LW, von Koch L. Longitudinal variations and predictors of increased perceived impact of multiple sclerosis, a two-year study. *J Neurol Sci* (2008) 270, 53–59

Yu HJ, Christodoulou C, Bhise V, Greenblatt D, Patel Y, Serafin D, Maletic-Savatic M, Krupp LB, Wagshul ME. Multiple white matter tract abnormalities underlie cognitive impairment in RRMS. *Neuroimage* (2012) 59, 3713-22.

Zivadinov R, Sepcic J, Nasuelli D, De Masi R, Bragadin LM, Tommasi MA, Zambito-Marsala S, Moretti R, Bratina A, Ukmar M, Pozzi-Mucelli RS, Grop A, Cazzato G, Zorzon M. A longitudinal study of brain atrophy and cognitive disturbances in the early phase of relapsing-remitting multiple sclerosis. *JNNP* (2001) 70, 773-780

Zivadinov R, Locatelli L, Cookfair D, Srinivasaraghavan B, Bertolotto A, Ukmar M, Bratina A, Maggiore C, Bosco A, Grop A, Catalan M, Zorzon M. Interferon beta-1a slows progression of brain atrophy in relapsing-remitting multiple sclerosis predominantly by reducing gray matter atrophy. *Mult Scler* (2007) 13, 490-501

Zivadinov R, Reder AT, Filippi M, Minagar A, Stüve O, Lassmann H, Racke MK, Dwyer MG, Frohman EM, Khan O. Mechanisms of action of disease-modifying agents and brain volume changes in multiple sclerosis. *Neurology*. (2008) 71,136-144

Appendix 1: Multiple Sclerosis Impact Scale (MSIS-29)

Please circle or tick the answer you feel in most appropriate.

<i>In the <u>past two weeks</u>, how much has your MS limited your ability to...</i>		<i>Not at all</i>	<i>A little</i>	<i>Moderately</i>	<i>Quite a bit</i>	<i>Extremely</i>
1.	<i>Do physically demanding tasks?</i>	1	2	3	4	5
2.	<i>Grip things tightly (e.g. turning on taps)?</i>	1	2	3	4	5
3.	<i>Carry things?</i>	1	2	3	4	5
<i>In the <u>past two weeks</u>, how much have you been bothered by...</i>		<i>Not at all</i>	<i>A little</i>	<i>Moderately</i>	<i>Quite a bit</i>	<i>Extremely</i>
4.	<i>Problems with your balance?</i>	1	2	3	4	5
5.	<i>Difficulties moving about indoors?</i>	1	2	3	4	5
6.	<i>Being clumsy?</i>	1	2	3	4	5
7.	<i>Stiffness?</i>	1	2	3	4	5
8.	<i>Heavy arms and/or legs?</i>	1	2	3	4	5
9.	<i>Tremor of your arms or legs?</i>	1	2	3	4	5
10.	<i>Spasms in your limbs?</i>	1	2	3	4	5
11.	<i>Your body not doing what you want it to do?</i>	1	2	3	4	5
12.	<i>Having to depend on others to do things for you?</i>	1	2	3	4	5
13.	<i>Limitations in your social and leisure activities at home?</i>	1	2	3	4	5
14.	<i>Being stuck at home more than you would like to be?</i>	1	2	3	4	5
15.	<i>Difficulties using your hands in everyday tasks?</i>	1	2	3	4	5
16.	<i>Having to cut down the amount of time you spent on work or other daily activities?</i>	1	2	3	4	5
17.	<i>Problems using transport (e.g. car, bus, train, taxi, etc.)?</i>	1	2	3	4	5
18.	<i>Taking longer to do things?</i>	1	2	3	4	5
19.	<i>Difficulty doing things spontaneously (e.g. going out on the spur of the moment)?</i>	1	2	3	4	5

<i>In the <u>past two weeks</u>, how much have you been bothered by...</i>		<i>Not at all</i>	<i>A little</i>	<i>Moderately</i>	<i>Quite a bit</i>	<i>Extremely</i>
20.	<i>Needing to go to the toilet urgently?</i>	1	2	3	4	5
21.	<i>Feeling unwell?</i>	1	2	3	4	5
22.	<i>Problems sleeping?</i>	1	2	3	4	5
23.	<i>Feeling mentally fatigued?</i>	1	2	3	4	5
24.	<i>Worries related to your MS?</i>	1	2	3	4	5
25.	<i>Feeling anxious or tense?</i>	1	2	3	4	5
26.	<i>Feeling irritable, impatient, or short tempered?</i>	1	2	3	4	5
27.	<i>Problems concentrating?</i>	1	2	3	4	5
28.	<i>Lack of confidence?</i>	1	2	3	4	5
29.	<i>Feeling depressed?</i>	1	2	3	4	5

Questions 1 to 20 inclusive are summed to give the MSIS-29 physical component (MSIS-phys); questions 21 to 29 inclusive are summed to give the MSIS-29 psychological component (MSIS-Psych)

