

*Afferent Visual Pathway Assessment In An
Exploratory Trial Of Autologous Mesenchymal
Stem Cells In Multiple Sclerosis*

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the degree of Doctor of Philosophy

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Declaration:

I, Madhan Kolappan, confirm that the work presented in this thesis is my own.

I was one of the two full time clinical research fellows who were responsible for undertaking all of the main work of the trial, which took place over a period of three years.

I performed the following: study design, writing of applications for ethics approval, recruitment of patients and healthy controls, development of MRI protocols with help from physicists, arrangement of scan appointments, clinical examination, visual testing, retinal imaging, data acquisition and storage, data analysis, statistical analysis for chapter 5 and writing of the thesis.

Peter Connick (PC) (University of Cambridge, Department of Clinical Neurosciences) was the other research fellow, and he contributed equally to the trial design, writing of applications for ethical approval, recruitment of patients and clinical assessments of patients. He performed and coordinated the bone marrow aspiration and culture and expansion of MSC.

Dr Katherine Miszkiel (KAM) (Consultant Neuroradiologist, Department of Neuroradiology, National Hospital for Neurology and Neurosurgery) looked at the optic nerve images to identify the lesion for lesion length and eligibility criteria and reported on the brain MRI for patients in the study.

Professor Ming Du (University of Cambridge, Dept. of Pathology) performed array-CGH on expanded MSC cultures.

Dr Andrew Michell (AWM) (Dept. of Neurophysiology, Addenbrooke's Hospital) performed blinded analyses of visual evoked responses.

Dr Daniel Altmann (DA) (NMR Research Unit at the Institute of Neurology and also at

the London School of Hygiene & Tropical Medicine) performed the final statistical analysis for MSCIMS trial.

Where information has been derived from other sources, I confirm that this has been indicated in the thesis.

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Abstract

There is a considerable need for treatments in MS for preventing progressive neurological disability. Assessment of the afferent visual pathway shows potential in investigating new therapies in MS. Mesenchymal Stem Cells exhibit properties of potential therapeutic relevance in progressive MS.

A phase I/IIA trial of adult autologous mesenchymal stem cells as a potential therapy for Multiple Sclerosis [MSCIMS] was designed as an open label, pre (up to 20 months) vs. post treatment (up to 10 months) (single intravenous administration of autologous bone marrow derived mesenchymal stem cells) comparison study in ten secondary progressive MS patients. Primary end points were adverse events and secondary end points were efficacy measures.

All 10 patients had previous history of clinical optic neuritis: this was in order to enable longitudinal structural and functional assessments of the disease-affected afferent visual pathway. Piecewise linear mixed models were used to assess the change in gradients over time at the point of intervention.

All 10 patients tolerated the trial assessments and intervention. No significant or serious adverse events were seen. Improvement after treatment was seen in visual acuity and visual evoked response latency, along with an increase in optic nerve cross-sectional area. The results suggest that autologous mesenchymal stem cells are safe and could possibly promote endogenous repair mechanisms such as remyelination, although a definitive conclusion of this cannot be made from this small study.

While MSCIMS was a proof of concept study only, based on the encouraging experience derived from it, there would seem to be potential value in future, larger placebo controlled, double-blinded, randomised therapeutic phase IIb/III trials that could (i) more definitively investigate stem cells as a therapy and (ii) use the visual pathway disease model for investigating the efficacy of potential neuroprotective and reparative therapeutic agents.

Publications related to the thesis

1. Connick P, Kolappan M, Compston DAS, Chandran S. Designing clinical trials to test neuroprotective therapies in multiple sclerosis. Book chapter. Pages (255-276). Editors: Ian D Duncan, Robin J M Franklin. "Myelin Repair and Neuroprotection in Multiple Sclerosis". Springer-Verlag, New York, USA.
2. Connick P*, Kolappan M*, Crawley C, Webber DJ, Patani R, Michell AW, Du MQ, Luan SL, Altmann DR, Thompson AJ, Compston A, Scott MA, Miller DH, Chandran S. Autologous mesenchymal stem cells for the treatment of secondary progressive multiple sclerosis: an open-label phase 2a proof-of- concept study. *Lancet Neurol.* 2012; 11(2): 150-6.
3. Connick P, Kolappan M, Bak TH, Chandran S. Verbal fluency as a rapid screening test for cognitive impairment in progressive multiple sclerosis. *J Neurol Neurosurg Psychiatry.* 2012; 83(3): 346-7.
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6. Samson R S, Kolappan M, Thomas D L, Symms M R, Connick P, Miller D H, Wheeler-Kingshott C A M. Development of a high-resolution fat and CSF-suppressed Optic Nerve DTI protocol at 3T: application in multiple sclerosis. *Functional Neurology.* (In press) 2013

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Abbreviations

9HPT	9 Hole Peg Test
ACE-R	Addenbrooke's Cognitive Examination - Revised
aCGH	array Comparative Genomic Hybridisation
AD	Axial Diffusivity
ADC	Apparent Diffusion Coefficient
AON	Acute Optic Neuritis
APTT	Activated Partial Thromboplastin Time
B-FS	Beck fast screen
BBB	Blood Brain Barrier
BDI	Beck's Depression Inventory
BDNF	Brain Derived Neurotrophic Factor
BET	Brain Extraction Tool
BL	Baseline
BOLD	Blood Oxygen Level Dependent
BRC	Centre for Brain Repair
BRNB	Brief Repeatable Neuropsychological Battery
CAMBS	Cambridge MS basic scale
CCFS	Composite Cerebellar Functional Score
cf	central field
CIS	Clinically Isolated Syndrome
CMDI	Chicago Multiscale Depression Inventory
CNS	Central Nervous System
CSF	Cerebro Spinal Fluid
CSLO	Confocal Scanning Laser Ophthalmoscopy
DIS	Dissemination in space
DIT	Dissemination in time
DMT	Disease modifying treatment
DTI	Diffusion Tensor Imaging
DWI	Diffusion Weighted Imaging
EAE	Experimental Autoimmune Encephalomyelitis
ECG	Electrocardiogram

EDSS	Expanded Disability Status Scale
EEG	Electro encephalograph
EP	Evoked Potentials
EPI	Echo Planar Imaging
ES	Embryonic stem cells
ESR	Erythrocyte sedimentation rate
ETDRS	Early Treatment Diabetic Retinopathy Study
FA	Fractional Anisotropy
FAMS	Functional Assessment of Multiple Sclerosis
ff	full field
fFLAIR	fast Fluid Attenuated Inversion Recovery
FM100hue	Farnsworth-Munsell 100 hue
fMRI	functional Magnetic Resonance Imaging
fsFSE	fat suppressed Fast Spin Echo
FU	Followup
GCL	Ganglion Cell Layer
Gd	Gadolinium-diethylenetriaminepentaacetic acid
GE	Gradient Echo
GM	Grey Matter
HADS	Hospital Anxiety and Depression Scale
HAMA	Hamilton Anxiety scale
HRQOL	Health Related Quality Of Life
HRT	Heidelberg Retinal Tomography
HSC	Haematopoietic Stem Cell
ICARS	International Cooperative Ataxia Rating Scale
INL	Inner Nuclear Layer
ION	Institute of Neurology
IPL	Inner Plexiform Layer
ISCT	International Society of Cellular Therapy
LGN	Lateral Geniculate Nucleus
LHON	Leber's Hereditary Optic Neuropathy
LL	Lesion Length
LOC	Lateral Occipital Complexes
Log MAR	Logarithm of the Minimal Angle of Resolution

LV	Lesion Volume
MACFIMS	Minimal Assessment of Cognitive Function in MS
MarsBaR	MARSeille Boîte À Région d'Intérêt
MD	Mean Diffusivity
MDEFT	Modified Driven Equilibrium Fourier Transform
mfVEP	multifocal Visual Evoked Potentials
MMO	Microcystic Macular Oedema
MMSE	Mini Mental Status Examination
MNI	Montreal Neurological Institute
MRI	Magnetic Resonance Imaging
MRS	Magnetic Resonance Spectroscopy
MS	Multiple Sclerosis
MSC	Mesenchymal Stem Cells
MSCIMS	Mesenchymal Stem Cells In MS
MSFC	Multiple Sclerosis Functional Composite
MSIS	Multiple Sclerosis Impact Scale
MSQOL	Multiple Sclerosis Quality of Life
MT	Magnetization Transfer
MTI	Magnetization Transfer Imaging
MTR	Magnetization Transfer Ratio
MV	Macular Volume
NAA	N-acetyl aspartate
NABT	Normal appearing brain tissue
NAGM	Normal Appearing Grey Matter
NAWM	Normal Appearing White Matter
NGF	Nerve Growth Factor
NPSBMS	Neuropsychological screening battery for MS
NRES	National Research Ethics Service
NSC	Neural Stem Cells
NSF	Nephrogenic Systemic Fibrosis
OCT	Optical Coherence Tomography
ON	Optic Neuritis
ONA	Optic Nerve Area
ONL	Outer Nuclear Layer

ONTT	Optic Neuritis Treatment Trial
OPL	Outer Plexiform Layer
PASAT	Paced Auditory Serial Addition Test
PD	Proton Density
PPMS	Primary Progressive Multiple Sclerosis
PRL	Photoreceptor layer
PT	Prothrombin time
RAPD	Relative Afferent Pupillary Defect
RD	Radial Diffusivity
REC	Research Ethics Committee
RNFL	Retinal Nerve Fibre Layer
ROI	Region of Interest
RRMS	Relapsing Remitting Multiple Sclerosis
SARA	Scale for the Assessment and Rating of Ataxia
SD	Standard Deviation
sd-OCT	spectral domain Optical Coherence Tomography
SE	Spin Echo
SEFCI	Screening Examination For Cognitive Impairment
SIENA	Structural Image Evaluation using Normalisation of Atrophy
SIENAX	cross sectional SIENA
SITA	Swedish Interactive Threshold Algorithm
SLP	Scanning Laser Polarimetry
SPMS	Secondary Progressive Multiple Sclerosis
SPIR	Selective Partial Inversion Recovery
SPM	Statistical Parametric Mapping
sTE	short echo
STIR	Short Tau Inversion Recovery
td-OCT	time domain Optical Coherence Tomography
UKNDS	United Kingdom Neurological Disability Scale
VC	Visual Cortex
VEP	Visual Evoked Potential
VER	Visual Evoked Responses
VOI	Voxel of Interest

VSC	Voxel Scale Connectivity
WM	White Matter
ZOOM-EPI	Zonal Oblique Multislice Echo Planar Imaging

Chapter 1: Testing neuroprotective and repair therapies in

MS

1.1 Introduction

1.1.1 The problem of MS

Multiple sclerosis (MS) is the commonest cause of neurological disability in young adults. It is a cause of significant morbidity and mortality in the industrialised world with prevalence estimates ranging from 110-175 per 100 000 in the UK. MS is a multifocal and multiphasic demyelinating disease of the central nervous system (CNS) with a variable clinical course and pathological manifestations.

In majority of patients, the usual course of MS is characterized by recurrent relapses (relapsing-remitting phase) associated with the eventual onset of progression (secondary progressive phase). Relapses are defined as the first occurrence, recurrence or worsening of symptoms representing neurological dysfunction and marked by sub acute onset and a period of stability followed by partial or complete recovery – the whole process lasting for at least 24 hours. In the relapsing-remitting (RR) phase, relapses alternate with periods of clinical inactivity (remission). The progressive phase of MS is characterized by a steady increase in neurological deficits, either from onset (primary progressive) or after a RR phase (secondary progressive). Patients do not necessarily convert to secondary progressive (SP) phase from RRMS, but when they do, the transition can sometimes be

hard to recognize, especially when the early SP phase is characterised by continuing relapses.

MS is a chronic inflammatory demyelinating disease of the central nervous system and is characterised by destruction of myelin sheaths leading to the formation of plaques of demyelination. Inflammatory cells in an acute MS plaque are dominated by T lymphocytes and activated macrophages or microglia. Axons within the acute lesion are also affected, although to a lesser extent than the myelin sheaths. Demyelination within the plaque results in the impairment of nerve fibre conductivity. However this conductivity can be restored when the inflammatory mediators are cleared, when the sodium channels are redistributed along the demyelinated axons, or when the affected nerve fibres are repaired by remyelination. The symptoms of MS are believed to result from the failure of axonal conduction, and when the conduction is restored through the mechanisms described above, there is recovery of symptoms after a relapse. This recovery is incomplete sometimes resulting in residual neurological deficits or symptoms after a relapse in relapsing remitting MS – this is likely a reflection of axonal loss following acute inflammation. Inflammatory demyelinating focal white matter lesions dominate the pathology in acute MS and RRMS.

In the progressive stage of MS, both in patients with PPMS and SPMS, the pathological picture is somewhat different. Although focal demyelinated lesions are still present, classical active demyelinating plaques are rare. Axonal density is reduced in most chronic MS plaques.(1) In addition the normal appearing white matter (NAWM) is also highly abnormal with diffuse axonal injury and loss and secondary demyelination.(2-4) Axonal loss in multiple sclerosis is a very important pathological feature because it is an

irreversible change that ultimately leads to severe neurological deficit.(5;6) Spinal cord atrophy showed a graded correlation with the Kurtzke EDSS(6) and cerebral atrophy correlated with worsening disability.(7;8) N-acetyl aspartate (NAA) is an amino acid that is virtually confined in the adult to neurons (including axons). Reduction of NAA in proton MR spectroscopy is associated with neuronal loss. Studies in MS have shown a significant correlation of reduction in NAA with clinical disability.(9-11)

In the acutely demyelinated plaque, the axons may be completely transected or there may be structural and functional alterations in them. In the chronic demyelinated plaque, the non transected demyelinated axons are vulnerable and may gradually degenerate as a result of loss of trophic factors from myelin and myelin forming cells.(1;12;13) In addition, abnormal patterns of sodium channel expression in demyelinated axons and their cell bodies may render the axons further vulnerable to degeneration. Furthermore, axons may also be lost secondary to neuronal apoptosis in cortical lesions.(14) PPMS and SPMS MRI studies show limited inflammation but considerable axonal loss in the lesions and also diffusely in the NAWM away from the lesions.(15) Also the immunosuppressant and immunomodulatory drugs have been effective only against early and relapsing stage of the disease, suggesting a relative dissociation between inflammation and neurodegeneration.

There are two explanations to counter the argument that these two processes are dissociated. As explained above, an acute demyelinated plaque consists of axons which are completely transected. These transected axons undergo wallerian degeneration and hence the abnormality may extend beyond the boundary of the radiologically detectable lesion. The other explanation is that there may be a compartmentalised diffuse

inflammation in the central nervous system in progressive MS, but this may be trapped behind an intact or repaired blood brain barrier which is not detectable using conventional MRI methods. The relative dissociation between inflammation and neurodegeneration has also raised the question whether the neurodegenerative component is a primary phenomenon in MS.(16) There is still considerable on-going debate in this, but the evidence for a primary neurodegeneration that precedes inflammation in MS is still largely unconvincing.

MS, due to its complexity and heterogeneity poses a considerable challenge in its management. Clinical manifestations, course and the response to the treatment of MS are unpredictable.

1.1.2 The need for neuroprotective/repair therapy in MS

Specific therapeutic treatments of MS have two aims: (i) to prevent damage (commonly referred to as disease modifying), and (ii) to repair damage that has already occurred. Although advances in treatment to reduce relapse rate have been made in the last decade, little has been achieved in terms of definitive treatments for preventing progressive irreversible disability or achieving repair. The lack of such therapies represents a substantial gap in the treatment of MS. There is a need to develop treatments that prevent axonal loss, the pathological substrate of irreversible clinical disability. The protean manifestations of multiple sclerosis and considerable intra and inter-individual variability make it challenging to tease out the effects of treatment on the underlying pathology in an unselected cohort of MS patients over the relatively short duration of a clinical trial.

1.1.3 The challenge of defining, detecting and measuring neuroprotection/repair

Given that both pathogenesis and host responses in MS are complex and incompletely understood, current methods to detect neuroprotection and repair are limited to crude net assessments of overall response. These composite assessments (eg: EDSS) potentially mask specific effects of therapeutic intervention at multiple points in the disease process and host response(s) that are variously advantageous or deleterious. A major challenge to test neuroprotection and repair is to carefully design a trial with the right patient group and outcomes that are clinically meaningful as well as being able to illuminate potentially multiple effects on disease pathogenesis and host response(s). This twin-aim will likely require careful patient selection and a combination of clinical and paraclinical outcomes: clinical outcomes are essential to determine the net effect of treatment on function, however poor pathogenic specificity limits their utility to accurately measure neuroprotection/repair; paraclinical outcomes give more specific information on pathogenesis, but cannot be used in isolation to establish clinical efficacy.

1.2 Neuroprotective/Repair therapy trial design in MS

1.2.1 Trial design:

Any potential drug or intervention in clinical studies will have to pass through rigorous systematic scrutiny in various phases before it can become established as a treatment. Initial phase (I/IIA) clinical studies will aim to establish safety of the intervention in a small group (10-20) of healthy volunteers (phase I) or patients (phase IIA). Although necessarily underpowered to determine efficacy, it is essential that phase IIA studies

(proof of concept studies) include efficacy outcomes in order to inform the design of subsequent studies in terms of power calculations and outcome relevance. Although a parallel two arm design with the investigational agent arm and a control (placebo or established treatment) arm where the patients are randomly allocated would be ideal to establish efficacy, in view of the primary objective of establishing safety of a potentially high risk investigational agent in phase IIA studies, and also to improve the power and efficiency, a single arm pre versus post intervention comparison design or a two arm crossover design are sometimes preferred. Phase IIB (n ~ 20-300) and phase III (n ~ 300-3000) trials are designed to establish efficacy and parallel, two arm, randomised, double blind, controlled studies are always preferred.

1.2.2 Endpoints:

A trial “endpoint” is the predefined outcome considered to be worth detecting. This can be expressed in terms of clinical measures for safety or efficacy. Decisions on endpoints are required *prior* to trial commencement as they inform power calculations and frame interpretation of the final result. Safety endpoints in phase I/IIA trials are a relatively standard combination of clinical adverse events, laboratory testing and other measurements collected from specific testing (such as electrocardiogram [ECG]). Efficacy endpoints in phase IIB/III trials of neuroprotective/repair therapies are more challenging to define due to the insensitivity of clinical outcomes for measuring disability, variability in the natural history of MS (necessitating large group sizes to achieve adequate statistical power), and the contribution of inflammatory activity (relapses) to *reversible* (although not necessarily brief) changes in disability distinct from the more insidious development of fixed disability reflecting pathological

neurodegeneration.(17) Clinical endpoints may be based on measures of disability such as EDSS (Expanded Disability Status Scale) or on time to progression milestones such as time to onset of progression in relapsing remitting MS cohort. Endpoints based on paraclinical evidence of efficacy may be suitable for proof of principle trials bridging phases IIA & IIB. Endpoints for neuroprotective/repair therapy trials might include changes in brain atrophy measures, tissue specific measures for myelination / axonal integrity *etc.*

1.2.3 Patient selection:

Appropriate patient selection is dependent upon the phase of the trial planned and the potential of the therapeutic intervention. All trials are dominated by the ethical requirement to provide an acceptable balance between potential risks and benefits. Phase I/IIA trials have more emphasis on investigating the former. Part of the aim of the phase IIA trial is also to form the reference upon which future phase IIB and phase III trials can be designed to prove efficacy, once the safety and feasibility are established to a reasonable extent. A challenging setting is the phase IIB trial charged with demonstrating efficacy in a trial limited by practical issues around resources, and an ethical requirement to minimise the number of participants exposed to a therapy-associated-risk that is only partially characterised by preceding (small cohort) phase I/IIA trial data.

In this setting, the aim of eligibility criteria is to provide a phenotypically informative cohort of participants who share pathogenic activity relevant to the intervention. In a multifocal and multiphasic disease like MS, when considering a potential neuroprotective/repair therapeutic agent for which the long term adverse events are

unknown, it may be more ethically acceptable to investigate patients who have more advanced disease and limited therapeutic options in phase I/IIA studies. For phase IIB/III efficacy studies, which require a larger cohort and more sensitive efficacy outcome measures, it may be preferable to opt for a group of patients who are in a less advanced stage of the disease but have a clearly progressive disease process (established clinically by the disease course exhibiting a substantial rate of disability accrual) as a result of high rate of axonal/neuronal loss. Clinical and paraclinical assessments in such a cohort will have greater sensitivity in detecting a potential neuroprotective therapeutic agent. In the case of a potential reparative agent, from a statistical point of view, it may appear to be advantageous to have a cohort with less dynamic progressive disease course in turn with less on-going axonal/neuronal loss to maximise the power of detecting efficacy. However, there is some evidence that endogenous repair in MS is more prominent in early post inflammatory lesions than old burnt out sclerotic lesions, suggesting that some amount of inflammation may be necessary for repair to take place.(18;19) In evaluating a potential reparative therapy which is thought to improve endogenous repair mechanisms and/or to directly cause repair, it may be advantageous to have a cohort with more inflammation and less on-going neuroaxonal loss, such as early RRMS.

1.2.4 Sentinel lesion approach:

Another approach trying to improve detection of efficacy of a neuroprotective/repair therapeutic intervention in a multifocal condition such as MS is selection of patient cohorts with lesions at specific “sentinel” sites and follow them longitudinally with sensitive and site specific outcome measures.

Work in Brain Repair Centre, University of Cambridge by Dr. Brierley and colleagues, tested the feasibility of an approach where patients are selected on the basis of disease affecting “sentinel” CNS sites – optic nerve, spinal cord and cerebellar peduncle using clinical and paraclinical outcomes that are objective, clinically relevant, inform the underlying pathology, and might be extrapolated to the disease as a whole. The optic nerve was the favoured option (of those tested) because clinical and paraclinical assessments were well-tolerated, exhibited low test-retest variability, and there was validity of paraclinical outcomes for pathological demyelination or axonal loss. (20)

1.2.5 Outcome measures:

Outcome measures are parameters used to measure the effect(s) of intervention. This section subdivides clinical and paraclinical outcomes. Within these major divisions, outcomes can be further divided into physician-based (*eg* the EDSS) *vs.* patient-based (*eg* the MSIS-29) and uni-dimensional (where the concept can be measured directly – *eg* relapse rate) *vs.* multi-dimensional (where the concept cannot be measured directly, requiring a series of component measures – *eg* “quality of life” or “disability” scales).

In addition to the general requirement to measure therapeutic effect on disability and adverse events (safety) a further desirable goal in the setting of a neuroprotective or repair therapy clinical trial is to specifically measure effects on pathological processes.

I. Clinical:

- Multi-dimensional measures

In a disease with such wide-ranging phenotypic complexity, multi-dimensional outcomes aim to capture a global representation of function that allows comparison

between individual patients, groups of patients and across trial populations. The challenge facing such scales is self-evident – consequently, all of the currently available instruments have varied strengths and weaknesses.

i) Physician measured:

- Expanded Disability Status Scale (EDSS): Kurtzke’s EDSS is considered the gold standard among most MS clinical investigators for the assessment of disability in MS patients.(21) It is known to have some deficiencies in terms of weighing heavily on the motor system.(22;23) It is still one of the most widely used clinical outcome measure in MS clinical trials.
- Multiple Sclerosis Functional Composite (MSFC): MSFC was developed to overcome the deficiencies of the EDSS.(24) It integrates scores on 25 foot timed walk, nine hole peg test and paced auditory serial addition test (PASAT) to measure the function of the lower limbs, upper limbs and cognitive abilities quantitatively to give one integrated score. MSFC is considered to be a much more sensitive measure of disability than the standard EDSS with potential to detect treatment differences between treated patients and controls in therapeutic trials.(25)
- Others (Disease specific):
Numerous other disease specific, multidimensional, physician-measured scales have been developed aiming to measure global function. These include The Cambridge Multiple Sclerosis Basic Scale (CAMBS), The Scripps Neurological Rating Scale, The UK (previously “Guys”) Neurological Disability Scale (which can also be administered in a patient measured format), and many others. These scales do not enjoy the same high profile as the EDSS and MSFC although their metric properties may

be favourable – particularly the UKNDS.(26) Their relative unfamiliarity to clinicians renders these scales less useful in communicating a result based upon them.

ii) Patient measured:

Patients and doctors differ in their perceptions of what constitutes important determinants to overall quality of life. Physical disabilities are important to clinicians whereas mental health, vitality and general health are more important to patients.(27)

- The Multiple Sclerosis Impact Scale (MSIS-29)

The MSIS-29 is a recently developed multi-dimensional patient-assessed scale. It comprises 29 items with each item assigned an ordinal score of 1-5.(28) Robust methodological development and ease of application are key strengths of the MSIS-29. The MSIS-29 has been shown to achieve an acceptable level of objectivity and perform well in comparison with other instruments. (29;30)

- Others (Disease specific)

The MSIS-29 has several competitors including: the Functional Assessment of MS (FAMS), the Multiple Sclerosis Quality of Life-54 (MSQOL-54), the Leeds MSQoL scale, and the health-related quality of life questionnaire for MS (HRQOL-MS).

- Uni-dimensional (Function specific) measures

The phenotypic heterogeneity of MS (and other multi-focal / diffuse CNS diseases) has resulted in the development of specific tools for the assessment of individual CNS functions. These clinical measures form a natural adjunct to paraclinical “site-specific” measures in trials employing a “sentinel lesion” approach.

- Cognitive function:

Cognitive difficulties is known to occur in MS and even in an early stage.(31;32) Detailed neuropsychological assessment is the gold standard for detection and monitoring of cognitive dysfunction, but may be less practical in the setting of a clinical trial. Numerous instruments have therefore been developed aiming to measure cognition in MS within a more practical framework. The generic Mini Mental State Examination (MMSE) offers advantages of brevity and clinical familiarity, but has poor sensitivity and specificity. This has led to the development of several “brief screening batteries” such as the Neuropsychological Screening Battery for MS (NPSBMS) – which also has a serial version called the Brief Repeatable Neuropsychological Battery (BRNB), the “Basso” battery, and the Screening Examination for Cognitive Impairment (SEFCI). (33) An international conference (of neuropsychologists) aimed at resolving this issue produced a consensus statement recommending a more comprehensive battery as the optimum instrument for cognitive assessment in MS. The Minimal Assessment of Cognitive Function in MS (MACFIMS) takes around 90 minutes to administer and has been partially validated to established psychometric principles (34) This currently represents the best available instrument for valid and comprehensive assessment of cognitive impairment in the setting of a clinical trial. However, administrator training is required, the time taken to administer may be impractical, and the familiarity of neurologists with the MACFIMS is limited.

- Affective Function

Depression is common in MS and it correlates better with the degree of stress perceived by the patient than the extent of lesions on MRI.(35) Lifetime prevalence for major depression in multiple sclerosis is 25-50%.(36) The most widely used assessment scale in MS research is the Beck Depression Inventory (BDI) – recently revised as the BDI-II. BDI-II is a 21 question multiple choice self reported inventory, which has shown good correlation with validated rating scales such as Hamilton depression rating scale.(37) This generic instrument offers strengths of clinical familiarity, the ability to make cross-disease comparisons, and an extensive existing literature. The BDI has also been endorsed by international consensus guidelines for the treatment of depression in MS.(38) However, the challenge of attributing physical symptoms to the syndrome of depression rather than manifestations of MS has led to a debate regarding the specific symptoms of depression in MS. This complicates the use of generic instruments such as the BDI and the Hospital Anxiety and Depression Scale (HADS). Two disease-specific instruments have therefore been developed to address these issues. The comprehensive 42-item Chicago Multiscale Depression Inventory (CMDI) has been psychometrically validated in MS but lacks wide clinical use or familiarity.(39) The rival 7-Item Beck Fast Screen For Medically Ill Patients (B-FS) has also been validated in MS.(40) Comparative analysis of these two instruments, particularly with regards to responsiveness, is not yet available. Those designing a clinical trial at present have a difficult

choice between the attractive generic BDI-II, and the two psychometrically favourable but less familiar disease-specific scales available.

Anxiety is widely neglected as an outcome in MS. There are no validated generic or disease-specific scales available. Generic instruments such as the Hamilton Anxiety Scale (HAMA) and Zung Self Rating Anxiety Scale (SAS) are available without copyright restrictions and have a degree of clinical familiarity.(41;42)

- Visual function

Visual function is commonly affected in MS due to involvement of the afferent visual pathway. Visual acuity, contrast sensitivity, colour vision and field of vision are amenable to detailed quantitative clinical assessment. These are dealt with in detail in chapter 2.

- Cerebellar function:

Cerebellar dysfunction is common in multiple sclerosis.(43) Assessment of tremor and/or dexterity forms the mainstay of available outcomes. The nine-hole peg test and finger-tapping test both provide objective and valid quantitative assessment of upper limb function but lack specificity for cerebellar dysfunction. Observer dependent rating scales such as the *Scale for the Assessment and Rating of Ataxia* (SARA) or the *International Cooperative Ataxia Rating Scale* (ICARS) are not validated for MS and have been shown to exhibit significant metric limitations.(44) The Composite Cerebellar Functional Score (CCFS) comprising a dominant hand “nine-hole peg test” and “click test” (based on kinematic data

regarding optimum assessment for upper limb goal directed multi-joint movement) represents a promising option for quantitative cerebellar assessment.(45) However, validation in MS patients is awaited. A further challenge to cerebellar assessment (in any disease) is the difficulty of a ceiling effect. Severe dysfunction makes assessment impossible with all currently available outcomes. This makes the use of cerebellar disease and dysfunction unattractive to trials adopting a “sentinel lesion” approach.(20)

- Spinal function:

Most commonly used scale for clinical assessment of motor disability in MS is the EDSS. However the EDSS is neither linear nor continuous and patients therefore do not spend equal amounts of time at each level.(46) Prevalent cohorts exhibit bimodal distribution with longer periods spent at levels 1 – 2 (abnormalities on the clinical examination only) and 6.0 – 7 (ambulation difficulties). Limited reliability and responsiveness render the EDSS sub-optimal for the clinical assessment of spinal cord function and it should not be used in isolation for this purpose in clinical trials. Ambulation assessment as a “timed walk” (of fixed distance – most frequently 25 feet) is attractive as a practical, quantitative, and reliable measure. However, it lacks specificity for *spinal cord* function. Indeed, limited site specificity of all currently available clinical outcome measures makes the spinal cord unattractive to trials adopting a “sentinel lesion” approach.(20)

All other (non-ambulatory) spinal cord functions lack standardised objective clinical outcomes: bladder EDSS functional scores show no correlation with objective measures of bladder function.(47)

II. Paraclinical:

Clinical assessments are prone to bias due to the subjective component. It also usually requires a large number of people to be studied over 2-3 years at least in order to demonstrate a treatment effect in a clinical trial using clinical endpoint (eg: relapse rate or disability). Paraclinical outcomes are useful in MS in giving an earlier indication of efficacy in phase IIb trials before larger phase III clinical efficacy trials.(48)

Systemically delivered neuroprotective and repair therapies are likely to have their effects on the whole central nervous system rather than an isolated effect on a single pathway. Although a sentinel lesion approach as described earlier focuses on a single pathway with sensitive clinical and paraclinical outcome measures, brain MRI (which has potential to study many lesions) almost always form an essential part of the outcome measures in any clinical trial. In August 2008, there was a meeting of around 60 international experts in Amsterdam, The Netherlands, on 'Imaging Outcomes for Neuroprotection and Repair in Multiple Sclerosis' which resulted in appraisal of the imaging techniques in five categories of performance: pathological specificity, reproducibility, sensitivity to change, clinical relevance and response to treatment.(49) Quantitative MRI measures used in clinical trials which help in providing insights into potential efficacy of experimental neuroprotective and/or repair therapies are now discussed following which an example of

their application in an on-going experimental neuroprotective and repair therapy (the focus of my thesis) is described.

- MRI Brain:

Brain MRI measures, over the years, have proven to be one of the most useful surrogate outcomes in MS clinical trials. Several quantitative techniques have been developed in order to increase pathological specificity of the measures, albeit to a limited extent. Conventional MRI scanning is helpful in fulfilling the diagnostic criteria(50) for MS and quantitative MRI measures have the potential to be an integral part of all neuroprotective and repair therapeutic trials.

Any repair that occurs in the central nervous system is likely to be helpful at all stages of MS, as there is evidence of damage very early on, even in the normal appearing white matter (NAWM) and normal appearing grey matter (NAGM), both pathologically and radiologically. There is evidence for a limited endogenous repair mechanism(51-54) and any experimental therapeutic measure with a potential to cause repair could act by augmenting the endogenous process or by causing direct repair. In MS, as described earlier, there seems to be a distinction between the two main pathological processes, one which is responsible for relapses and the other which seems to be responsible for accumulating disability. Neuroprotective and repair therapies are aimed at potentially slowing down and/or reversing the latter process respectively and hence the MR measures that are likely to be most useful are the ones which are sensitive for measuring myelination, neurodegeneration and axonal loss.

Conventional MRI techniques like T2-weighted, T1-weighted and T1-weighted with gadolinium enhancement, apart from being mainly used to provide diagnostic and prognostic information in MS, have also been used in clinical trials for monitoring disease activity. More complex MRI techniques such as magnetisation transfer ratio (MTR), diffusion tensor imaging (DTI), magnetic resonance spectroscopy (MRS) are adding to our understanding of the disease pathogenesis as well as serving as biomarkers for evaluation of therapeutic effects including repair.

i) T2W:

T2 weighted scans are sensitive in detecting focal MS white matter lesions although being pathologically nonspecific. In relapsing remitting MS, virtually all-new T2 lesions start as a region of gadolinium enhancement indicating BBB breakdown and an inflammatory phase in their evolution. (55;56) This differs in more advanced disease and primary progressive MS, where there is evidence that some of the T2W abnormalities may develop independently of BBB breakdown. (57) The T2 lesion load in the early stages of relapsing remitting MS provides an indication of the amount of inflammation that has occurred to date. T2 lesion load generally correlates poorly with clinical impairment in cross-sectional studies. (58) The reasons for this poor correlation could be due to low pathological specificity of the T2 abnormalities, limitations of the clinical rating systems like the EDSS and pathological involvement of areas that are normal appearing on conventional MRI.

Even so T2 lesion accumulation early in relapse onset MS has been shown to partly correlate with current and future disability.(59) T2 lesion load at the first clinical event suggesting relapse-onset MS (a clinically isolated syndrome) has a stronger predictive value in anticipating the subsequent short-term clinical course (in particular a further relapse leading to the diagnosis of clinically definite MS).(60) T2 lesion load as measured by T2 lesion volume has been used as a secondary or tertiary outcome measure in many clinical trials of disease modifying treatments in MS, where clinical endpoints have been primary outcome measures. This is because T2 lesion load has only a limited correlation with clinical outcome and has not been regarded as adequate to serve as primary outcome measures in definitive phase 3 clinical trials.(61) T2 lesion load as an outcome measure is likely to be more relevant in clinical trials in relapsing remitting MS and CIS rather than progressive MS.(62)

ii) TIW:

About 20-30% of T2 hyperintense lesions appear hypointense (“black holes”) on T1-weighted spin echo MRI on any single scan. While many of these lesions are chronic and persistent, at least some of the new T1 hypo intense lesions, which start as gadolinium enhancing lesions, will subsequently disappear over a few weeks to months to become iso-intense. Persistent chronic “black holes” have been shown to have greater axonal loss than those lesions which are T1 isointense.(63) The “acute” black holes which appear and resolve reflect initial oedema and demyelination with later resolution of oedema and remyelination.(64) T1 weighted scans are useful to monitor the treatment effect on evolution of Gd enhancing lesions into persistent black holes. If there is a reduction of

such evolution, it might reflect less residual axonal loss due to acute inflammation, possibly as a result of the therapeutic intervention under study.(65;66)

There was significant correlation between T1 hypointense lesion load and clinical disability as measured by EDSS in a cohort of secondary progressive patients in one of the early studies,(67) whereas subsequent studies only showed weaker correlations.(68) Different T1 hypointense lesions may have different degrees of axonal loss depending on the degree of hypointensity, but this is difficult to assess visually. Persistent black holes along with lesion MTR are the most extensively studied measures (among lesion evolution tracking strategies) to infer the neuroprotective capacity of any new agent.(49)

iii) Gadolinium enhancement:

Gadolinium enhancement is often seen in new brain lesions in RRMS and SPMS and typically lasts for 2-6 weeks like a relapse. Post-mortem and biopsy studies have demonstrated that inflammatory features correlate with Gd enhancement in MS lesions.(69) Enhancing lesions are more likely to correlate with clinical relapse if they occur in clinically eloquent sites such as the spinal cord or optic nerves. Gadolinium enhancement correlates poorly with clinical disability.(70) In RRMS, monthly Gd-MRI reveals about 10 new enhancing lesions for every clinical relapse. This finding indicates that subclinical disease activity greatly exceeds clinical measures of activity.(71) The number of gadolinium enhanced lesions that are detected can be increased by more frequent scanning (weekly), use of triple dose Gd, an off resonance magnetisation transfer (MT) pulse, delayed scanning and thinner slices. These approaches do not have much impact on sample size requirements for trials because inter-patient variability also

increases.(72) As the enhancements reflect acute inflammatory activity, it is an attractive MRI measure to test the efficacy of a therapeutic agent in preventing new inflammation. The oft-reported weak correlation with clinical disability has led to Gd enhancement being considered a poor predictor of long-term clinical benefits. However, recent meta-analysis of treatment trials in RRMS has suggested a strong correlation between the treatment effect on relapses and the treatment effect on the MRI lesion activity at the treatment arm/group level.(73) Phase II trial designs based on enhancing lesions as a primary outcome are designed to determine if a therapeutic measure has the potential to proceed to a definitive phase 3 trial with clinical endpoints in relapsing MS. Gd enhancements in clinical trials can be measured as number of enhancements, presence or absence of enhancement or as enhanced tissue volume, with the required sample size increasing in that order.(74) Gd enhancement as an efficacy outcome measure is useful in the patient groups with active relapsing remitting disease and secondary progressive MS patients with superimposed relapses. Gd enhancement as a safety outcome measure is also useful especially in a group of patients who have low enhancement activity at baseline, so that a drug induced increase in disease activity may be detected.

However, since the link between Gd administration in patients with renal disorder and Nephrogenic Systemic Fibrosis (NSF) has been established, there is a need for greater caution in using Gd in human studies and it has become imperative to ensure normal renal function before it is given.(75)

iv) Atrophy:

Atrophy or measurement of tissue loss has been the most robust and widely used imaging measure of the neurodegenerative component of MS in clinical trials. CNS atrophy is a moderate but significant predictor of neurologic impairment that is independent of conventional MRI lesions.⁽⁷⁶⁾ Axons form the bulk of white matter and along with neuronal cell bodies form the bulk of grey matter as well. Neuronal and axonal loss affects both white matter and grey matter. Axonal loss is not the sole cause of atrophy: myelin, tissue water content, variation in glial bulk and inflammation also affect the global and regional tissue volume measures in MS.

Atrophy can be detected in CIS patients even before development of clinically definite MS.⁽⁷⁷⁾ Studies have shown that brain atrophy occurs in relapsing remitting MS even within 3 years of symptom onset in both white and grey matter. Atrophy is seen in both the brain and spinal cord in secondary and progressive MS. The most marked atrophy is seen in SPMS and correlates with disability. Measurable alterations in brain and spinal cord tissue volume has been demonstrated over periods as short as 6-12 months.⁽⁷⁾ Spinal cord atrophy is more evident in patients with early PPMS versus early RRMS.⁽⁷⁸⁾ Spinal cord atrophy is particularly well correlated with motor disability^(6;79) while brain atrophy has been well correlated with neuropsychological impairment.⁽⁸⁰⁻⁸³⁾

Atrophy appears to be progressive from onset and increases with increasing disability; it only correlates modestly with inflammatory lesions and it is considered a more specific marker of neurodegeneration. This makes atrophy measures attractive as an outcome measure in neuroprotective treatment trials. A study of 16 patients with PPMS evaluated riluzole as a neuroprotective agent using change in cord area as a measure of axonal loss. During 1 year pre-treatment, there was a 2% reduction in mean cord area whereas on 1

year treatment, there was only a 0.2% reduction; although the difference was not statistically significant, the findings suggest a potential neuroprotective effect. (84)

Diffuse grey matter atrophy is also evident in MS and may be a valuable endpoint in clinical studies while testing the efficacy of a potential neuroprotective or repair therapeutic agent in MS. GM atrophy occurs at nearly twice the rate of whole brain or white matter atrophy and correlates more strongly with disability in MS.(85) This makes it more potentially sensitive than global atrophy measures in short term studies. However lower sample sizes for showing an effect on atrophy are achieved using registration-based algorithms to detect change in serial images and currently these are applicable in whole brain but not grey matter alone.(86;87)

Global brain atrophy measures remain one of the best studied imaging outcome measures for neuroprotection due to its multicentre applicability, sensitivity to change and relatively small sample sizes to determine treatment effects in a randomized trial setting.(49;86;88)

v) Magnetisation Transfer Imaging:

MTR can be measured globally within the whole brain and within large areas of the brain free from lesions (normal appearing white matter or grey matter) or within lesions and specific regions of interest.

Post-mortem and animal studies have shown that a decrease in MTR reflects demyelination and axonal loss to a certain extent, whereas an increase in MTR from a previous low level is reflective of possible remyelination or resolution of oedema. (89;90)

Studies have shown that, on average, MTR declines slightly for a few months before Gd

enhancing lesions appear and then decrease steeply when the lesion begins to enhance. The magnitude of this decline during the time of enhancement predicts whether the lesion will evolve into a T1 hypointense lesion, suggesting that MTR reduction may be a marker of pathological severity.(91-93) MTR is decreased in the whole brain, normal appearing white matter and grey matter in MS patients compared to controls. Spinal cord and optic nerve have also shown decreased MTR in MS. MTR decrease varies between disease phenotypes and is related to disease course. MTR reduction may occur very early in the disease even at the stage of a clinical isolated syndrome. MTR reduction is greater in patients with progressive MS, but changes are also seen in RRMS.(94)

MT imaging was recommended to be used in large scale MS trials as an adjunctive measure to monitor disease evolution in a consensus by the White Matter Study Group of the International Society of Magnetic Resonance in Medicine in the year 2002.(95) In longitudinal studies, development of progression has been predicted by decrease in whole brain MTR. Whole brain MTR at baseline has shown a high specificity and positive predictive value for EDSS deterioration.(96) Whole brain MTR changes are not pathologically specific but significant changes in MTR have been found in secondary progressive MS over 12 months to be sensitive enough for use in monitoring treatments.(97) Chen et al demonstrated that MTR signal inhomogeneity may be a more useful method in quantifying the potential for demyelination and remyelination in individual lesions and hence may help predict the effect of myelin repair and neuroprotective treatments.(98) Lesion MTR has been studied widely for applicability in multicentre studies, sensitivity to change and treatment effects, and the changes pathologically reflect demyelination with axonal loss and remyelination. It seems to be sensitive to change over time and to treatment effects and can be applied in multiple

centres.(49) However, the changes in MTR are small and achieving stable and precise quantitation and standardization of the MTR sequences across different scanners is challenging.

vi) Diffusion Tensor Imaging:

PPMS patients have been found to have increased ADC in the normal appearing white matter.(99) Areas of NAWM that later developed overt lesions have shown a significant increase in mean diffusivity values beginning 6 weeks before Gd enhancement, suggesting that new inflammatory lesions are preceded by subtle dynamic alterations in diffusion, this being followed by a marked increase at the time of enhancement and a decrease after the resolution of enhancement.(100) Global diffusion abnormalities occur in the NAWM even at the earliest stages of disease (CIS).(101) The demonstration of longitudinal changes in diffusion in MS patients suggests the potential for the use of DTI as a treatment outcome measure in clinical trials.

DTI parameters have limited pathological specificity (like MTI), but are sensitive biomarkers very early in the disease for myelin and axonal structural disruption. DTI is a promising technique for allowing reconstruction of major fibre bundles through tractography. Significant clinical correlations with disability scales have been observed using DTI parameters of single well defined tracts, but less so with global diffusion parameters. This limitation may be because MS causes focal as well as diffuse abnormalities to tissue integrity, and also the correlation will depend on individual tracts (eg: pyramidal tracts) studied and their reflection on the clinical disability scale.(102) DTI has much potential to be applied to study WM fibre bundles with high orientational

coherence and associated functional outcomes (eg: optic nerve DTI and visual function) in the context of neuroprotective and repair therapy trials.

vii) Proton Magnetic Resonance Spectroscopy (MRS):

Proton MR Spectroscopy has the potential to characterise the chemical pathology of brain lesions and normal appearing brain tissue. This information has been used to better define the natural history of the disease process, and to monitor metabolic responses to therapy. In MS, MRS has been particularly informative by providing evidence of neurodegeneration (based on the resonance intensity of N-acetyl aspartate [NAA], an amino acid derivative found almost exclusively in neurons and thought to be a marker of neuronal viability and axonal integrity) in both lesional and non lesional brain tissues from the earliest stages of the disease.(103) A reduction in NAA provides evidence of axonal dysfunction or loss and has been consistently reported in MS lesions and NAWM.(104) These changes have been confirmed pathologically and have led to the appreciation for the substantial role of axonal damage in determining clinical progression in MS.(1;12)

By measuring metabolites such as choline containing compounds [marker of cell membrane integrity], myoinositol [glial cell marker], lipids [products of brain tissue (including myelin) disruption], lactate [product of anaerobic glycolysis], creatine/phospho-creatine [energy metabolism], proton MRS has provided information regarding damage and repair of neuronal and non neuronal brain tissue. Increases in lactate, choline and lipids probably reflect evidence of inflammation and demyelination. Elevated choline indicates increased cell membrane turnover that may be due to

demyelination, remyelination, inflammation or gliosis. Myoinositol is elevated in MS NAWM, (9;105) and may reflect increased glial cell proliferation and activity.

Partly as a result of technical reasons of low signal to noise ratio and modest reproducibility of the measured metabolite concentrations, use of proton MRS has been limited mainly to single centre trials. Whole brain NAA has been applied in trials and reproducible data obtained although it is methodologically challenging and has not been widely taken up.(106) Another limitation to this approach is that changes are not localised to lesions, NAWM or grey matter.

Despite limitations, MR spectroscopy should be investigated further as a potential surrogate outcome in clinical trials of neuroprotection and repair, as the metabolite concentrations – in particular NAA - provide relatively specific information on axonal survival and function.

- Spinal Cord MRI

The potential for spinal cord MRI outcomes to be used in MS treatment trials has gained significant recent interest. Technically, the spinal cord is a more difficult structure to image than brain due to its smaller size, mobility, and proximity to the heart and great vessels. However, these difficulties are largely overcome by technical improvements such as cardiac gating, spatial pre-saturation slabs and the development of phased array coils enabling rapid imaging of the whole spinal cord. Serial assessment of atrophy has potential for use as a surrogate measure for clinical progression, and a marker of axonal loss. MTR and DTI of the cord have been used in exploratory studies, with DTI tractography representing a particularly promising option for trials employing a spinal cord sentinel lesion approach.

- Optic nerve MRI

Clinical involvement of the optic nerve is common in MS (optic neuritis), and clinically silent lesions are also frequently found in the posterior afferent visual pathway. Assessment of the afferent visual pathway has provided insights in to the pathophysiology of the demyelinating lesion, and shows potential in investigating new therapies in both optic neuritis (ON) and MS (See chapter 2).

- Retinal nerve fibre layer (RNFL) imaging

The retinal nerve fibre layer (RNFL) consists of unmyelinated axons within the retina. Consequently, measurements of RNFL thickness in MS are not confounded by loss of myelin. The RNFL is therefore an attractive structure to visualize processes of neurodegeneration, neuroprotection and potentially neural repair. Optical Coherence Tomography (OCT) uses the echo time delay of low coherence light to delineate the RNFL. OCT measurements may therefore be useful in clinical trials to detect axonal loss and monitor neuroprotection.

- Neurophysiological measures: *Evoked Potential (EP)*:

Central demyelination slows down conduction as in the peripheral nerve. This slowing is helpful in distinguishing the underlying pathological process from axonal degeneration. Conventional Visual EP (VEP) measures the cortical response to monocular stimulation in the central 30 degrees of the visual field (known as the P100). In MS the waveform of the P100 is characteristically delayed with a well-preserved amplitude. Response latency can be used as a measure of myelination in the afferent visual pathway (increased with

demyelination), and amplitude can be used as a measure of axonal conduction (reduced with axonal loss or with conduction block due to demyelination)(107). Shortening of the latency of the VEP response with increasing years following optic neuritis is compatible with remyelination.(108) The VEP may therefore be a useful outcome measure when testing a potential remyelination agent in a patient with previous optic neuritis (Chapter 2). Somatosensory, auditory and motor evoked responses have also been used but less frequently than VEPs and mainly in clinical settings in MS.

- Immuno/cytochemical biomarkers:

A number of potential CSF biomarkers of axonal breakdown have been studied in MS. Phosphorylated forms of neurofilament are released during axonal injury and an increase in one such neurofilament has been noted to be related to increasing disability in MS.(109) S110b and 14-3-3 are other biomarkers which have been proposed as potential markers of glial proliferation and axonal damage respectively and are likely to be helpful in demonstrating abnormalities in a number of neurodegenerative disorders in which glial proliferation and axonal loss occur.(110;111)

1.3 Summary

The challenge to detect neuroprotection and repair in MS clinical trials is formidable. Only a comprehensive approach involving consideration of all elements in trial design is likely to achieve success. A traditional, randomised, parallel groups two-arm (control vs experimental treatment) design remains the gold standard although multi-interventional (adaptive and factorial) designs may become increasingly relevant in the near future.(112)

In all trials, patient selection is crucial to maximising statistical power to detect efficacy, with the “sentinel lesion” approach having potential as a novel and sensitive paradigm for phase IIa. Careful selection of outcome measures is also required to achieve the objectives of neuroprotective / repair therapy trials. A “three step” approach represents a useful model in this regard: (i) clinical outcomes to establish clinical efficacy, (ii) structural/imaging measures that directly monitor neuro/axonal degeneration and repair/remyelination, (iii) other biomarkers that are thought to reflect neurodegeneration and repair, eg: VEP, CSF and serum biomarkers.

Chapter 2: Afferent visual pathway assessment in MS and associated ON.

2.1 Introduction

Demyelinating lesions in MS have a predilection to occur in certain sites within the CNS as demonstrated by pathological(113;114) and MRI studies(115). The afferent visual pathway, which extends from the retina to the primary visual cortex, is often affected. Clinical involvement of the optic nerve is common in MS (optic neuritis), and clinically silent lesions are also frequently found in the posterior afferent visual pathway.(116) Assessment of the afferent visual pathway has provided insights in to the pathophysiology of the demyelinating lesion, and shows potential in investigating new therapies in optic neuritis (ON) and MS.

Most patients with ON recover to normal or near normal visual acuity, although they will often continue to report visual symptoms. Imaging, neurophysiological and pathological studies indicate that despite persistent tissue loss, recovery continues long after the acute attack. This discrepancy between structure and function emphasises the need for sensitive methods of assessment of both these aspects in order to better understand the pathophysiology.

This chapter is mainly focussed on imaging measures of structure and function of the afferent visual pathway in MS and associated ON. However, shorter sections will

consider anatomy, pathology, clinical manifestations, visual function and neurophysiological investigations, in order to provide an overall context when considering the imaging techniques and their applications in development of methods and protocol to test neuroprotective and/or repair therapeutic trials in MS.

2.2 Normal structure and function of the afferent visual pathway:

The afferent visual system is made up of four neuronal components: (i) Photoreceptor cells; (ii) Bipolar cells; (iii) Ganglion cells with their axonal processes forming the retinal nerve fibre layer (RNFL), optic nerve, chiasm and the optic tract; (iv) Cell bodies of the lateral geniculate nucleus (LGN) with their axonal processes forming the optic radiation and terminating at the visual cortex.

The RNFL is unmyelinated within the retina in most individuals. As the axons of the ganglion cells form the optic nerve, they are myelinated. The optic nerve is covered by a sheath composed of dura and arachnoid mater with cerebrospinal fluid (CSF) in the subarachnoid space. The nerve itself is invested with pia mater and the three layers are continuous with the meninges of the CNS. The optic nerve axons are myelinated by oligodendrocytes rather than Schwann cells. Thus, the optic nerve is part of the CNS. More than half of the optic nerve axons decussate in the chiasm. The majority of fibres whose ganglion cells are located in the temporal hemi retina, carrying information from the nasal field of the ipsilateral eye, join with nasal fibres, carrying information from the temporal field of the contralateral eye at the chiasm to form the ipsilateral optic tract. This tract terminates in the respective LGN. Neurons from the LGN project to form the optic radiations and terminate in the primary visual cortex. A minority of fibres go into the superior colliculus for pupillary function.

2.3 Pathology:

Thinning of the RNFL and ganglion cell layer occurs in MS.(117) Optic nerve and chiasm lesions exhibit inflammation, demyelination, gliosis, axonal injury and atrophy as do lesions in the brain and spinal cord in MS.(118) Optic nerve lesions are reported in 94-99% of MS autopsy cases.(119;120) Involvement of the retrochiasmatal pathways including the optic radiations is also frequently found in MS.(121;122)

Transection of axons has been demonstrated in acute and to a lesser extent, chronic MS lesions in the brain and may lead to Wallerian degeneration.(123)

Pathology in the LGN and visual cortex may occur as foci of primary demyelination, as a consequence of Wallerian degeneration secondary to lesions of the connecting white matter tracts(124) or as a result of trans-synaptic degeneration due to lesions in an anatomically linked remote region. Evangelou and colleagues investigated the anterior visual pathways of 8 post-mortem cases of MS and found that in addition to significant atrophy and axonal loss in the optic nerve and tract, there was also a relatively size selective atrophy of smaller neurones (parvocellular layer of LGN) consistent with trans-synaptic degeneration and suggesting an increased susceptibility of smaller axons to MS related injury(125) as has also been suggested by studies of achromatic(126) and chromatic(127) contrast sensitivity, visual evoked potentials (VEP)(128), temporal frequency discrimination(129) and the pupil light reflex(130)

2.4 Clinical Features:

Optic Neuritis:

Optic nerve involvement in MS presents most commonly as acute demyelinating ON. The patient with typical demyelinating ON usually has a decline in vision over a 7-10 day period. Progression of visual loss beyond 2 weeks is unusual. Pain on eye movements is typical, reported in about 90% of cases. Some recovery of vision should start to occur within 30 days of onset and most recovery of acuity and field typically occurs within 6 months. Disturbances in colour vision are common and field defects are also sometimes reported by patients. Uhthoff's symptom of visual loss after exercise or a hot bath can occasionally be one of the presenting symptoms of optic nerve demyelination. Patients also occasionally report anomalous perception of motion in depth (Pulfrich's phenomenon).

Clinical features that suggest atypical ON include a markedly swollen nerve, retinal exudates and haemorrhages, absence of pain, severe visual loss to no light perception, and absence of any recovery within 30 days.

Abnormalities commonly found on examination in the North American Optic Neuritis Treatment Trial (ONTT) were reduction in visual acuity (89.5%), visual field defects (97.5%), impairment of contrast sensitivity (98.2%) and colour vision defects (93.8%). Relative afferent pupillary defect (RAPD) is commonly seen and was an inclusion criteria in ONTT. On fundoscopy, optic disc swelling was seen initially (35.3%) followed by pallor and atrophy later.(131) Asymptomatic or subclinical involvement of optic nerves occurs in MS and is inferred by finding abnormal visual evoked responses in clinically unaffected eyes.(132;133)

Chiasm and Retrochiasmal lesions:

Any type of field defect may occur, depending on the location of the lesion. Symptomatic homonymous hemianopic defects are infrequent in MS, occurring in about 1%.(121) Asymptomatic defects are more common. In the ONTT, 13.2% of the patients had evidence of chiasmal (5.1%) and/or retrochiasmal (8.9%) field defects. RAPD in the contralateral eye can be seen in optic tract lesions due to a greater number of nasal fibres decussating to the contralateral side than the number of temporal fibres remaining ipsilateral.(134)

2.5 Visual function assessment:

While the most commonly used measure of visual acuity has been the Snellen chart, log MAR scoring using a retro-illuminated Early Treatment Diabetic Retinopathy Study (ETDRS) chart is now often preferred in research studies. This has an equal number of letters per row, more equal spacing, the letters on each row are balanced for difficulty, and the scoring system provides continuous statistical data. Sloan charts(135) are similar in style to the ETDRS, but consist of several charts with progressively lower contrast. They are reproducible(136) and more sensitive than standard acuity in detecting visual dysfunction in MS.(137) The visual dysfunction in MS measured using these charts has been correlated with Expanded Disability Status Scale (EDSS) and MS functional composite (MSFC) scores,(138) and MRI lesion load.(139)

In research practice, the comprehensive Farnsworth-Munsell 100 hue test is often used in the assessment of colour vision. In this test, the subject places 85 coloured caps in perceived order of hue and an error score is calculated. Dyschromatopsia may be

quantified and the spectral location of deficits determined. No consistent pattern of colour deficit has been found in ON.(140)

The visual field may be measured quantitatively with static targets with a Humphrey perimeter or qualitatively with kinetic targets using the Goldmann method. Both were applied to the ONTT cohort. These patients were shown to have more central than peripheral field abnormalities, and any peripheral deficits recovered more rapidly.(141) Recovery of visual field function was good overall and did not differ between diffuse and localised field defects.(142)

2.6 Neurophysiological measures:

VEP

The conventional VEP measures the cortical response to monocular stimulation in the central 30 degrees of the visual field. In MS it is characteristically delayed, with well-preserved amplitude, although during the acute phase of optic neuritis with visual loss this may be reduced. The observation that the cortically generated VEP (known as P100) was delayed following ON(143), and that this delay persisted after visual recovery(132) was of great importance. The delayed but well formed P100 waveform in ON and MS implying demyelination is a classical feature. The shortening of the latency post acute optic neuritis is considered to be due to remyelination and is known to occur for several months to years independent of functional recovery.(144) Abnormal VEPs in unaffected eyes provided evidence for clinically silent lesions that might help identify dissemination in space (DIS) and hence help with the diagnosis of MS (132) although they are only formally incorporated in the current McDonald criteria for primary progressive MS.(145)

Multifocal VEP

The multifocal VEP (mfVEP) has been developed to examine conduction in the parts of the visual field that the full field VEP does not. The mfVEP uses a paradigm of sectorial stimulation with pseudo-stimulation at other sites, using the fellow eye and normal controls for comparison at each point.(146) The degree of latency is similar to that seen in full-field VEP(147), but mfVEP has the advantage that a particular sector of the visual field can be examined for abnormality, and compared with the results of other retinotopic tests (for example standard automated perimetry). Fraser *et al* found that the average delay of the mfVEP latency predicted progression to MS in a cohort of patients with McDonald criteria “possible MS”, but whether mfVEP would offer an advantage over more conventional VEP was not clear, as conventional VEP was not performed in this study.(148) Grover and colleagues found that mfVEP was more sensitive in detecting abnormality than conventional VEP in patients with MS.(149) mfVEP amplitude and RNFL thickness was shown to have a high functional-topographic relationship in a study of post acute optic neuritis patients. (150)

2.7 Retinal nerve fibre layer imaging:

The retinal nerve fibre layer (RNFL) consists of unmyelinated axons within the retina; therefore, measurements of RNFL thickness in MS are not confounded by loss of myelin *per se*.

Frisen and Hoyt(151) noted retinal axonal loss on red-free photography in eyes both with and without a history of acute optic neuritis (AON). These defects were associated with

corresponding changes in the visual field. In larger surveys of MS patients, even those unaffected by clinically evident ON have visible RNFL defects on retinal photography.(152;153) However, approximately 50% of the RNFL needs to be lost in a sector for it to be visible(154), and gradations in loss cannot be quantified, limiting the use of this technique in assessing retinal structure.

Three different techniques are able to quantify the thickness of the RNFL: optical coherence tomography (OCT), scanning laser polarimetry (SLP), and confocal scanning laser ophthalmoscopy (CSLO). Each uses slightly different techniques to estimate the thickness of the RNFL.

Optical coherence tomography

OCT uses the echo time delay of low coherence light to delineate the RNFL. The layers of the retina have different reflectivity, and can thus be distinguished and measured. The RNFL thickness (giving an estimate of axonal numbers), and the macular volume (giving an estimate of ganglion cell numbers) can be measured.

Time domain OCT (td-OCT) used low coherence interferometry, using a Michelson type interferometer and a low coherence light source. The mirror in the reference arm in the interferometer in the td-OCT is mechanically scanned to measure interference. The development of fourier or spectral domain OCT (sd-OCT) uses a stationary reference arm and a spectrometer which detects all echoes simultaneously thus increasing the speed (50 fold) and the resolution (2-3 fold) significantly.(155;156)

The first observation with td-OCT in MS was made by Parisi et al(157), who found a reduction in RNFL thickness in eyes with a prior history of ON. This finding has been corroborated by Trip et al(158), in a cohort of patients with prior ON selected for a range

of visual loss including those with poor recovery, and Costello et al(159), in a prospectively acquired, unselected cohort of patients with unilateral ON (Fig 2.1). The loss of RNFL is in the range of 5-40 microns for eyes affected with optic neuritis, averaging 10-20 microns.(159)

Several groups have found RNFL loss in the clinically asymptomatic eyes of MS patients.(160) RNFL thinning in eyes not previously affected by an attack of ON may be greater in SPMS than in PPMS, and least evident in RRMS group, particularly when the temporal quadrant of the RNFL is examined separately.(161;162)

Follow up td-OCT measurements of the progressive MS cohort, median of ~2 years from baseline revealed no significant decrease in RNFL thickness. There was a significant decrease in the macular volume in both patient and the control group but no significant changes between the groups. This suggests that td-OCT only has limited ability to detect changes in progressive forms of MS over a 2 year period.(163) Longitudinal follow up of 299 MS (84% RRMS) patients with or without previous optic neuritis for a mean of 18months (range: 6 – 54 months) showed that progressive RNFL thinning occurred as a function of time.(164)

Costello and colleagues presented longitudinal data for RNFL thickness during the first 12 months after optic neuritis, which showed most thinning occurred in 6 months and there was continuing axonal loss in affected eye for at least 12 months.(165) Serial td-OCT assessments of 23 patients with acute unilateral optic neuritis (AON) as CIS presentation showed that RNFL thickness increased significantly in the first few days and there was thinning thereafter. The thinning started to appear at mean of 1.6 months from symptom onset and the rate of thinning decreased thereafter. The initial increase in RNFL

thickness correlated with the baseline visual function (log MAR visual acuity, visual field and colour vision) and VEP latency significantly but was not associated with the 12-month visual outcome. The RNFL thinning at 6 months and 12 months correlated with concurrent visual function suggesting that RNFL loss from 6 months after the AON onset is a suitable outcome measure for proof of concept trials of acute neuroprotection. However some visual function measures (log MAR high and low contrast visual acuity, visual field) and Gadolinium positive lesion length at baseline correlated weakly (univariate association), whereas impairment of colour vision and prolongation of VEP whole field latency at baseline correlated strongly and independently (multivariate association) with eventual RNFL loss. This suggests that visual function measures at the time of AON may help predict future axonal loss and that the extent of demyelination might be more strongly related to eventual axonal loss than the extent of inflammation. This may help select patients with a higher risk for axonal loss for experimental neuroprotection trials.(166;167)

Reductions in RNFL thickness and macular volume are significantly correlated with reductions in visual function(158-162;168-173) Several groups have found a relationship between measures of disability (EDSS and MSSS) and retinal OCT measures(160;174-180), while others have not.(162;181-183) It is interesting to note that the strongest relationships have been found in cohorts of generally low EDSS, where visual impairment per se could make a greater contribution to the EDSS, and in a cohort of progressive patients studied, there was no apparent relationship.(160;162;173)

RNFL thinning post optic neuritis has been found to be associated with reduced VEP amplitude and multifocal (mf) VEP amplitude.(147;158;171;172;184;185) VEP amplitude reduction represents functional axonal loss whereas RNFL thinning represents

structural axonal loss past the acute phase. VEP latency prolongation is representative of demyelination. Several studies have also identified correlation between VEP latencies and RNFL thickness thus suggesting an association of demyelination with axonal loss in the anterior visual pathway.(157;172;184;186)

RNFL thickness correlated with brain global and regional atrophy measures in several studies.(176-178;187) MRI measures that are indicative of axonal integrity such as diffusion tensor and persistent hypointense T1 lesion volume have potential to be related to RNFL thickness.

sd-OCT is increasingly being used to investigate retinal nerve fibre layer and macular thickness in MS and optic neuritis recently.(188-192) The measurements obtained by sd-OCT machines of different manufacturers have been significantly different, although they are reproducible and valid for longitudinal assessments.(193;194) Direct comparison of td-OCT and sd-OCT in a study by Villoslada et al showed the sd-OCT was more sensitive than td-OCT in RNFL measurements.(191) Since several initial studies had been performed using the td-OCT of a single manufacturer, standardisation is required for cross sectional studies using sd-OCT of different manufacturers to be compared. As mentioned earlier, sd-OCT has better axial scan resolution (4-6 microns vs 8-10 microns) and much faster acquisition speeds (50 times faster) in comparison to td-OCT. This enables sd-OCT to segment (manual or automated) and quantitatively assess discrete axonal and neuronal retinal layers (ganglion cell layer (GCL), inner plexiform layer (IPL), outer plexiform layer (OPL), inner nuclear layer (INL), outer nuclear layer (ONL), photoreceptor layer (PRL) and RNFL) based on variability in tissue reflectivity resulting from differing layer compositions. (195-197)

Walter et al found that the GCL+IPL neuronal loss quantified by sdOCT strongly correlated with visual function and vision related quality of life (QOL) in MS eyes both with and without history of ON. This finding suggests that the ganglion cell layer thinning which is a neuronal cell marker analogous to grey matter involvement in the central nervous system may be a better predictor of neurologic disability than just axonal marker such as the peripapillary RNFL. IPL had to be included with GCL due to methodological difficulty in clearly separating the two layers using the current segmentation methods. (192) In a longitudinal study of 164 MS patients and 59 healthy controls, Ratchford and colleagues found that MS patients with clinical and/or radiological non ocular disease activity, particularly early in the disease course, exhibited accelerated GCL+IPL thinning, suggesting that this measure may reflect global CNS process and may be a potential outcome measure for assessing neuroprotective agents in early active MS. (198)

Gelfand et al identified microcystic macular oedema (MMO) of the INL in about 5% of patients with MS. MMO is thought to represent breakdown of the blood retinal barrier and hence retinal inflammation. Presence of MMO was associated with greater disability and visual dysfunction. (199) A further retrospective study, on MS patients and healthy controls over 2 years follow up, by the same group found that MMO does not seem to occur in healthy controls. Baseline increased INL thickness (measured as INL + OPL) was associated with markers of disease activity such as development of contrast enhancing lesions, new T2 lesions and clinical relapses in RRMS patients apart from EDSS progression. This raises the possibility that inflammation in the retina could be directly neuronally targeted without the involvement of myelin and that increase in the INL thickness may be an earlier marker for presence of inflammation before MMO becomes visible. (200)

Scanning laser polarimetry

The axons in the RNFL are birefringent, and retard polarised light. The degree of retardation is proportional to the RNFL thickness, and this characteristic is used by SLP. This approach has some theoretical advantages, because the measures are less affected by optic nerve head swelling, which is common in the early stages of ON. Theoretically this would mean that axonal loss could be detected earlier in the course of acute optic neuritis than with either of the other two retinal imaging methods that are affected by nerve head oedema.(201)

Steel et al demonstrated that RNFL measures obtained by SLP were lower in eyes with a history of ON.(202) Other groups have found similar results.(203-205)

Kupersmith et al assessed a group of 40 patients with acute retrobulbar optic neuritis at the time of presentation, 1 month and 3 months with SLP and td-OCT. OCT showed more thickening of RNFL than SLP at baseline, but SLP also showed some RNFL thickening at baseline which was thought to be due to increased axoplasmic flow of organelles in response to retrobulbar optic neuritis. SLP did not show RNFL thinning earlier than OCT in this study different to Garas et al comparing sd-OCT and SLP derived RNFL measurements in 9 AON eyes, which showed SLP measurements were significantly thinner than OCT at baseline.(188;206) Kupersmith et al tested 3 cohorts with optic head swelling due to papilloedema of raised intracranial pressure, acute optic neuritis and Non arteritic ischemic optic neuropathy (NAION) with OCT (td or sd OCT) and SLP. This study revealed a significant difference between OCT and SLP measures of NAION at baseline and at one month with OCT measures of RNFL significantly thicker than SLP. This reflects the difference in the principles of the two techniques and the

different pathological processes they provide insight into. OCT measurements include the intra and extra-axonal oedema in the RNFL thickness, whereas an SLP measurement reflects the birefringence properties of the parallel structures (axonal membranes and microtubular alignment) and thus reflects axonal integrity. This property of SLP makes it more attractive than OCT as a technique, which could provide useful prognostic information if further studies reveal that SLP is able to detect RNFL thinning earlier than OCT in AON. (207)

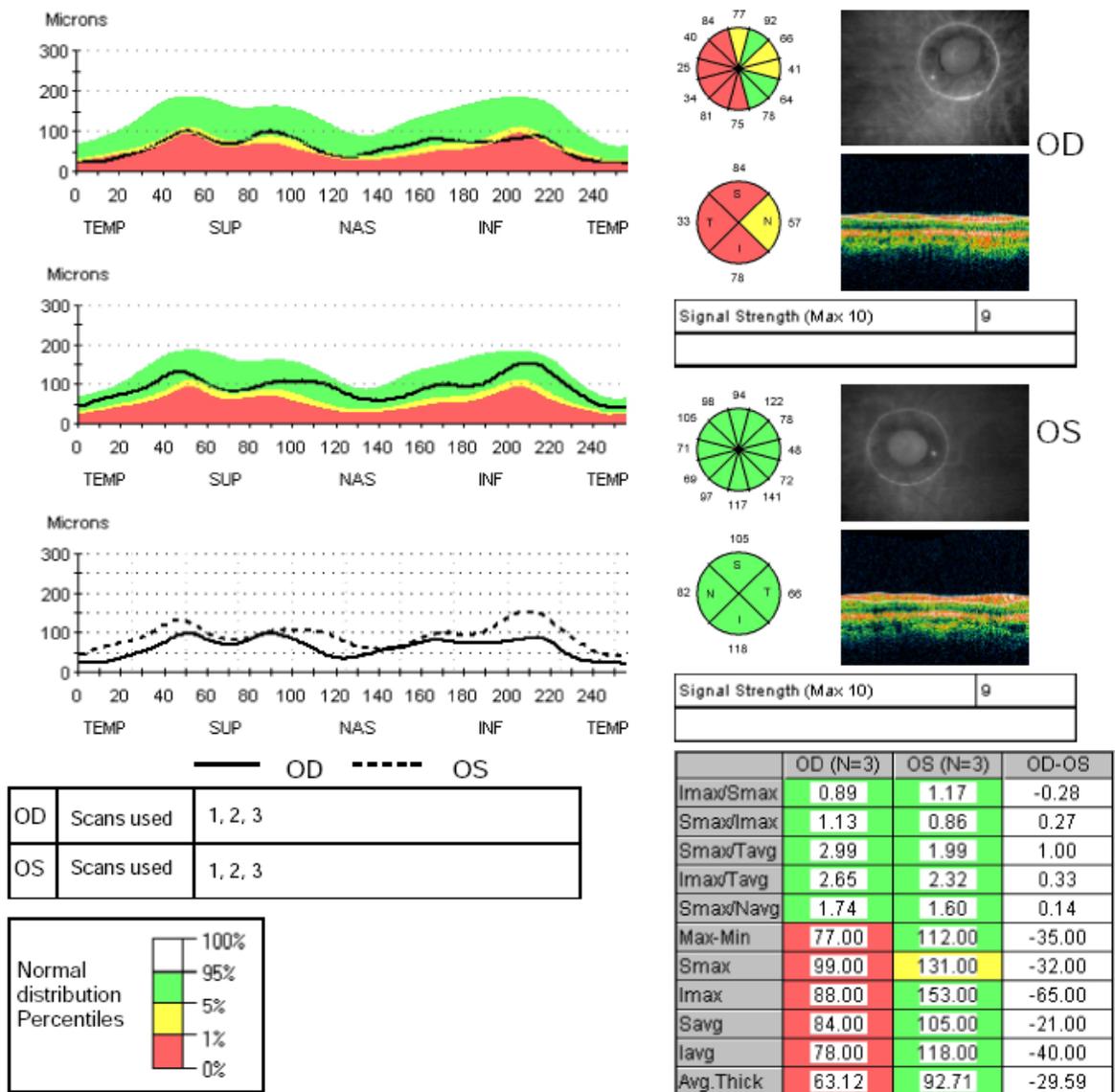
Patients need to fixate well for accurate SLP measures to be obtained, making the reliability of measurements lower in patients with severe central visual loss.

Confocal scanning laser ophthalmoscopy

CSLO also known as Heidelberg Retinal Tomography (HRT) calculates the retinal topography by scanning the retinal surface to construct a three-dimensional map of the retina around the optic nerve head. Placing a reference plane posterior to the temporal portion of the peripapillary retina and calculating the distance from this plane to the retina-vitreous interface infer the RNFL thickness. Trip *et al* found reduction in mean RNFL thickness and neuro-retinal rim volume in their cohort of patients with poor visual recovery after ON.(208) These changes were related to measures of visual function.

OCT measures have been shown to be superior to SLP and HRT in terms of strength of association between structure and function.(209) Incorporation of adaptive optics into retinal imaging has improved the lateral resolution significantly to the possibility of visualising individual nerve bundles in the RNFL, individual cells (single cone receptors) and already is being applied in studying several diseases including optic neuropathies.(210;211)

Fig 2.1: OCT showing thinning of the right RNFL following unilateral acute optic neuritis. OD – Right, OS – Left. (Reproduced from Kolappan et al 2008)



2.8 Optic nerve MRI :

MRI of the optic nerve is not routinely used in the diagnosis of ON, although brain MRI is used to detect clinically silent demyelinating lesions and clarify the risk for MS. Optic nerve MRI may be useful to rule out an alternative diagnosis in doubtful or atypical cases. Serial MRI of the optic nerve has enabled the study of the natural history of the acute ON lesion and, by inference, new MS lesions *in vivo*.

Challenges associated with optic nerve MRI include its small size, mobility, surrounding fat and CSF and the presence of the bony optic canal and nasal cavities. Methods have been developed to overcome these challenges, e.g. suppression of fat(212) and CSF(213) signal, fast sequences, 3D acquisition methods, use of surface coils and high field MR systems to allow high resolution imaging.

One of the advantages of MRI is the ability to acquire images sensitive to different pathological changes, e.g. inflammation, axonal degeneration, blood brain barrier leakage, water molecule displacement, and macromolecular changes. A summary of the main applications of MRI to the study of ON now follows.

Conventional T2 weighted (T2W) imaging:

In ON, there is increased signal intensity within the optic nerve on T2W images. The surrounding fat gives relatively high signal on T2W images making it difficult to detect the optic nerve lesion clearly. To overcome this, several fat suppression sequences have been developed. Among these, the short-tau inversion recovery (STIR) sequences make use of an inversion technique. (214) In a study of 37 patients following an episode of ON, the lesion was detected in 84% of patients with symptomatic ON and in 20% of patients

without clinical symptoms of ON. Slow or poor visual recovery was associated with longer lesions. 73% of patients with involvement of the nerve in the optic canal had poor recovery which was thought to be due to swollen optic nerve being compressed in the narrow bony canal.(215)

Another fat suppression sequence is based on frequency specific selective partial inversion recovery (SPIR).(216) Both STIR and SPIR have long acquisition times. Fat suppressed fast spin echo (fsFSE) T2W sequences have been developed to reduce acquisition times and to acquire high resolution images that minimise partial volume effects (Fig 2.2a). In a comparative study, 18/21 lesions were identified using STIR, whereas 20/21 lesions were identified using fsFSE from symptomatic optic nerves following AON.(212) In these sequences the surrounding CSF was bright and sometimes caused obscuration of the signal from the affected nerve. It is possible to suppress the signal from CSF using a fast fluid attenuated inversion recovery (fFLAIR) sequence in combination with SPIR sequence (SPIR-FLAIR). In a study comparing STIR, SPIR and SPIR-FLAIR sequences, SPIR-FLAIR was shown to be the most sensitive in detecting abnormal optic nerves.(213) Lesion length was also longest in SPIR-FLAIR, although it was not compared with an fsFSE sequence in this study.

The studies mentioned above were all performed using 0.5T/1.5T scanners. Pilot studies using 3 Tesla scanner have been performed on healthy volunteers, whereby higher resolution images of normal optic nerves have been acquired.(217;218) Use of surface coils close to the course of the optic nerve may also improve the resolution.(219)

Gadolinium enhancement:

Many small studies have demonstrated that with gadolinium administration, there is abnormal enhancement of the optic nerve in acute ON on fat suppressed T1W spin echo images.(220;221) The enhancement is thought to be due to disruption of the blood nerve barrier in the acute phase of ON causing leakage of the contrast between the endothelial cells of capillaries into the optic nerve in association with acute inflammation. Abnormal contrast enhancement of the optic nerve is a sensitive finding in acute ON (94%) and is no longer seen in previously affected optic nerves with chronic lesions only.(222) The sensitivity to detect an acute symptomatic lesion may be slightly increased further (96%) by using triple dose gadolinium chelates.(223) In a study by Youl *et al* on 18 patients with AON, contrast leakage (reduced signal on STIR) was associated with decreased visual acuity, colour vision, retroocular pain on eye movement, afferent pupillary defect and reduced P100 amplitude of the VEP. Repeat imaging of these patients a month later, showed leakage had ceased with improvement in vision and an increase in the VEP amplitude, suggesting that acute inflammation is associated with conduction block in the optic nerve and that resolution of inflammation plays a role in recovery.(224) The VEP latency was prolonged at follow up suggesting persistent demyelination: their visual recovery was associated with resolution of inflammation (enhancement) and not with remyelination. Kupersmith *et al* studied 107 patients with AON with contrast MRI and showed that the length of abnormal enhancement on T1W images (Fig 2.2b) within the optic nerve correlated significantly with the severity of visual impairment at baseline. Canalicular involvement was associated with poorer colour vision. However the location and length of the enhancing lesion did not correlate with the degree of visual recovery in the patients after six months follow up. The conclusion was that although enhancing lesions involving the canal or longer segments of optic nerve have worse baseline vision, the location and length of enhancement are not predictive of recovery.(222)

Atrophy:

Atrophy of nervous tissue could potentially result from demyelination or axonal loss. As axons contribute more than myelin to the bulk of the white matter tissue, it is reasonable to deduce that axonal loss contributes more to atrophy than myelin loss.(7) Studies using MRI measures of brain and spinal cord atrophy have demonstrated correlation with disability in MS.(6) MRI techniques have been developed over the last ten years to allow reasonable and reproducible assessment of optic nerve atrophy *in vivo*. Youl *et al* first used draftsman's callipers to show that following acute ON there was initial swelling followed by atrophy(225). Hickman *et al* described a short-echo fast fluid-attenuated inversion-recovery (sTE fFLAIR) sequence, with fat and CSF suppression allowing clear delineation of the optic nerve in its intra orbital course, where the nerve can be visualised almost orthogonal to the coronal plane allowing assessment of the cross sectional area using a semiautomatic threshold based contouring method.(226) In a cross sectional study of 17 patients with previous single episode of unilateral ON using this technique, the cross sectional area was 11.2 mm² in diseased eyes, 12.9 mm² in contralateral eyes and 12.8 mm² in control eyes. The degree of atrophy correlated with disease duration suggesting on-going axonal loss in a previously demyelinated lesion.(226) A subsequent serial study of previously affected ON patients with more residual visual impairment, showed correlation of the degree of atrophy with visual acuity, VEP amplitude and latencies.(227) Inglese *et al* calculated the volume of the optic nerve in 30 MS patients who have had previous optic neuritis. The atrophy was significantly worse for patients with worse visual acuity in comparison to patients with good recovery. The optic nerve volume also correlated moderately with VEP latency in this study.(228)

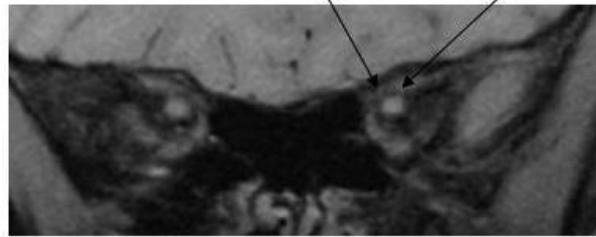
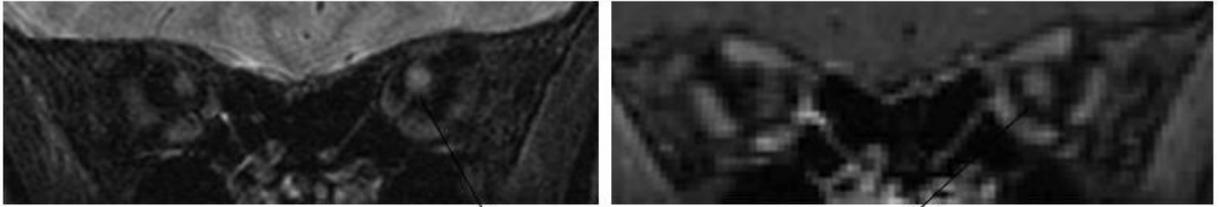
In a serial study of 29 patients with AON, 21 were followed up for a period of 1 year. The mean area of the diseased optic nerves at baseline was 20.1% higher compared with clinically unaffected contralateral nerves and controls. This declined over a year to being 11.7% lower than unaffected contralateral and control nerves (Fig 2.2c). This demonstrates the initial swelling of the nerve is followed by atrophy later. Baseline area of the affected nerve was associated with log MAR visual acuity and visual field mean deviation at baseline, but the one year mean area did not correlate with visual outcome. This recovery of function despite structural loss may reflect that the loss of tissue was small and that a more substantial loss of tissue is required to be clinically significant. Functional recovery could have also been aided by cortical adaptation.(229)

Trip *et al* studied the effect of optic nerve atrophy on a group of patients who had incomplete recovery from a previous unilateral ON [103]. Optic nerve area was found to be reduced by 30% compared to that of controls. This study included OCT to measure thinning of RNFL and loss of macular volume, both of which correlated significantly with optic nerve atrophy. In this group of patients, visual acuity and visual field mean deviation also correlated with optic nerve atrophy. Reduction of whole field VEP amplitude, which probably reflects axonal loss, correlated significantly with optic nerve atrophy, but VEP latency did not. These suggest that optic nerve atrophy on MRI reflects axonal loss.(230)

Fig 2.2: T2W image showing increased signal from the left optic nerve (a) and T1W image showing contrast enhancement (b) following acute optic neuritis followed later by optic nerve atrophy seen in fFLAIR sequence(c).

a)

b)



c)

Magnetization Transfer Imaging (MTI):

Apart from mobile protons, there is another pool of protons in tissues, which are bound to macromolecules such as proteins and lipid membranes (myelin and axonal membranes). Selectively saturating these bound protons, which are in rapid exchange with the free protons, interferes with the normally occurring transfer of magnetisation. The magnitude of this effect is called magnetisation transfer ratio (MTR) and this indirectly measures the amount of macromolecular structure (such as myelin) present in the tissue.(231) MTR is greater in white matter than grey matter and is reduced in MS lesions.(232-234)

Thorpe *et al* measured MTR in a single 3 mm section within the optic nerve of 20 patients with ON. The mean MTR was significantly reduced (42 pu) in affected nerves compared with clinically unaffected nerves (48 pu) and control nerves (49 pu). There was no correlation of MTR with visual acuity in this study, but there was negative correlation with VEP latency suggesting that MTR reduction might be, at least in part, an indicator of myelin loss.(235) Inglese *et al* showed there was a significant reduction in mean MTR values for the affected nerves in a subgroup of patients with poorer visual outcome, compared to a group with good recovery. The MTR values correlated with acuity but not with VEP latency. This may have been because in this study increase in VEP latency was also present in a substantial proportion of affected nerves with good recovery and clinically unaffected nerves whereas MTR in these groups were not significantly reduced in comparison with healthy controls, suggesting that MTR may not be entirely specific for myelin.(228)

Hickman *et al* used a 3D GE sequence to measure optic nerve MTR in AON patients serially for one year. The mean MTR value for the affected nerves during the acute phase was 47.3 pu, compared with 47.9 pu for healthy contralateral nerves. The mean MTR of the affected nerves reduced over 8 months (240 days) to reach a minimum value of 44.2 pu and then started to increase slowly to 45.1 pu at 12 months. This slower reduction of the MTR in comparison with studies of acute MS brain lesions could have been due to slower clearance of the myelin debris from the optic nerve lesion. A slow but insignificant increasing trend of the MTR value after reaching the nadir was possibly due to remyelination. This study further supports MTR as an indicator of myelination in showing that there was a significant inverse correlation between time-linked MTR and VEP latency measures.(236)

Trip *et al* acquired magnetization transfer images on a cohort of 25 patients with incomplete recovery following ON. The mean MTR of the whole nerve on the affected side and the mean MTR of the visible lesion were significantly reduced in comparison to the unaffected nerves and control nerves. The mean MTR of the clinically unaffected nerves in patients was also reduced compared to controls, suggesting subclinical abnormality in these patients. The mean MTR of the affected nerve correlated significantly with central field VEP latency, and the correlation improved with consideration of the lesion MTR rather than the whole nerve. In this study the MTR also correlated with the axonal loss quantified by thinning of the RNFL suggesting that MTR is also reduced due to axonal loss. The relative contributions of demyelination and axonal loss to reduction in MTR was not clear from this study.(237)

Klistorner *et al* examined cross-sectionally 23 patients, who had unilateral acute optic neuritis at least 6 months before and measured MTR, RNFL and performed multifocal

VEP. The average MTR of the affected eye was significantly reduced in comparison to the fellow unaffected eye and the healthy volunteer control eyes. MTR of the affected eye correlated significantly with measures of axonal loss (RNFL thinning and mfVEP amplitude reduction) independent of the level of demyelination. The authors concluded that the reduction of optic nerve MTR after an episode of ON has a strong association with degree of axonal damage, but not with demyelination.(238)

Wang et al performed a 3T coronal 2D gradient echo MTR study longitudinally in 37 acute optic neuritis patients and 11 controls. Patients were scanned at 2 weeks, 1, 3, 6 and 12 months from the time of acute optic neuritis. Patients also underwent multifocal VEP, tdOCT, log MAR high and low contrast visual acuity assessments. The affected optic nerve MTR was significantly reduced compared to controls and unaffected nerves at 3months and further reduced at 6months and stabilised between 6 and 12 months. MTR reductions at 3 months correlated with low contrast and high contrast acuities reduction at 6 months and RNFL thinning and high contrast acuity reduction at 12 months. The authors concluded that these findings suggest that the MTR change early after AON is predictive of axonal degeneration and visual disability outcomes. Multifocal VEP amplitude reduction at 12 months for the subgroup of patients with more axonal loss showed a trend of correlation with the MTR reduction but was not significant. (239)

Pathophysiological basis of MTR reduction is not completely clear. Both demyelination and axonal loss seem to contribute to the reduction in MTR. Further longer longitudinal studies of optic nerve MTR may help in clarifying this issue further.

Diffusion Tensor Imaging (DTI):

Diffusion weighted imaging (DWI) is a technique sensitive to tissue microstructure because of the diffusion properties of water molecules in the tissues. In white matter,

which is composed of packed nerve fibres, the diffusion mechanism is facilitated along the fibre tracts, while it is slower in the direction perpendicular to the main axis of the tract. This restriction and/or hindrance to the diffusion process is disturbed when there is a pathological process like demyelination causing increased diffusivity in the tissues; it also causes a disruption of the normal directional selectivity (anisotropy) of the fibres in allowing diffusion.

As DWI is sensitive to molecular motion, it is also sensitive to macroscopic motion, hence it can be difficult to perform in the optic nerve because of its mobility.(240) Iwasawa *et al* measured the apparent diffusion coefficient (ADC) in patients with ON and found that ADC was increased in chronic ON but not in acute ON. This could have been due to restricted diffusion caused by infiltration of inflammatory cells during the acute phase.(241) A fat and CSF suppressed zonal oblique multi-slice echo planar imaging (ZOOM-EPI) technique was developed to acquire DWI of the optic nerve with better resolution, decreased artefacts and better delineation of the nerve.(242) Hickman *et al* used this technique on 18 patients who had ON a year previously and found that the mean ADC for diseased optic nerves was significantly higher than unaffected contralateral and control optic nerves. ADC was correlated with visual function, VEP whole and central field amplitude and latency. ADC values also correlated modestly with lesion length but not with optic nerve area. The scan acquisition time was 28 minutes. The ADC obtained in this study were only along three orthogonal directions and to calculate anisotropy indices which are rotationally invariant, such as fractional anisotropy (FA), a minimum of six non-collinear diffusion sensitizing directions must be acquired to sample the diffusion tensor (DT).(243)

Trip *et al* applied DTI measurements to a cohort with incomplete recovery following ON. Mean diffusivity (MD) was significantly increased and fractional anisotropy (FA) was significantly reduced in affected nerves compared to unaffected contralateral and healthy control nerves. This probably reflects axonal loss although demyelination and gliosis could have also contributed. There was no association with any of the visual functions. Increase in MD and decrease in FA also correlated significantly with decrease in VEP amplitude, which probably reflects axonal loss in this cohort. This further suggests that diffusion measurements are more indicative of axonal integrity.(244)

Kolbe *et al* measured ON DTI on 16 unilateral optic neuritis patients at a mean of 4 years after the clinical episode. Reduction of FA of the affected nerves and optic nerve atrophy independently correlated significantly with multi focal VEP amplitude reduction but not with prolongation of latency. FA reduction was associated with amplitude reduction more in the periphery compared to atrophy, which was associated with amplitude reduction in the central visual field.(245)

Animal studies has demonstrated that axial and radial diffusivities correlate with axon and myelin pathologies respectively.(246;247) Naismith *et al* measured DTI parameters and visual function in 12 patients with isolated acute optic neuritis at baseline and visual function at 1 and 3 months to find if any of the diffusion parameters at baseline help predict visual outcome. Axial diffusivity at baseline correlated significantly with visual function measured by Snellen's chart visual acuity and Sloan's 5% low contrast acuity at 1 and 3 months. This suggests that reduction of axial diffusivity which occurs acutely may help predict clinical outcome.(248) Further study by the same group on 25 patients with acute isolated optic neuritis demonstrated that AD at baseline correlated with visual acuity, contrast sensitivity, VEP amplitude and latency and RNFL thickness at 6months.(249) Increased radial diffusivity (RD) was associated with poorer visual

outcome in a group with remote optic neuritis at least 6 months previously. RD correlated with visual acuity, contrast sensitivity, VEP latency, amplitude and OCT findings.(250) A future challenge is to develop DTI methods that image the entire optic nerve with high resolution and within an acceptable scan time.

2.9 Optic Radiation MRI:

Although any MRI technique can be used to study the optic radiations, diffusion-based tractography is particularly suitable for assessing the integrity of this white matter tract.

Diffusion Tensor Imaging:

Tractography is a recently developed analysis technique that allows inferences to be made about connectivity between adjacent voxels based on the tissue's DT properties, and therefore anatomical tracts such as the optic radiations can be reconstructed. (Fig 2.3) Quantitative measures reflecting the overall connectivity and integrity of the tract may then be derived, such as voxel scale connectivity (VSC) values, mean FA and MD. Whilst DTI has been applied extensively to other tracts in MS, such as the corpus callosum and pyramidal tract, there are only few studies to have applied DTI to optic radiations in MS and ON.(251;252)

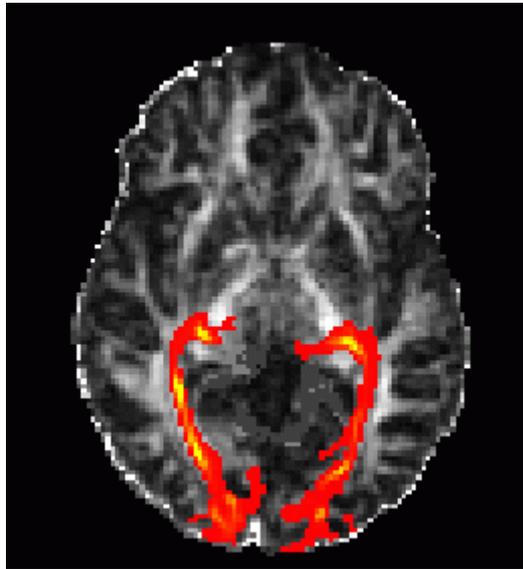
Roosendaal et al applied DTI and used tract based spatial statistics(253) on 30 MS patients with low lesion loads and found that fractional anisotropy was reduced in 12 regions in the brain including the optic radiations in comparison with healthy controls. Radial diffusivities were significantly increased in patients compared to controls whereas

axial diffusivities were not.(252) Ciccarelli *et al*(251) used fast marching tractography(254) in patients 1 year after a clinically isolated attack of optic neuritis. They found the posterior part of the radiations to be located more infero-laterally than in controls. In addition, VSC values were reduced in patients. The authors hypothesized that the VSC changes might reflect trans-synaptic degeneration, reported in pathological studies(125) and showed that they were not correlated with incidental lesions in the optic radiations, which are well recognized in ON.(116) It was suggested that the altered location of the radiations might be related to cortical reorganization, a phenomenon that has been suggested in several other studies of optic neuritis patients using functional MRI.(255-257) A structure-function relationship between optic radiation FA and functional MRI data has been reported in healthy controls, suggesting that occipital functional responses are constrained by the subserving optic radiations. (258)

Reich *et al* investigated the relationship between OR DTI and RNFL as well as visual function in a cohort of MS patients. In this study, it was found that all OR DTI parameters (both lesions and NAWM within the OR) were significantly abnormal than controls. There was a significant but moderate correlation between RNFL and OR fractional anisotropy and radial diffusivity independent of MRI abnormalities in the non-visual pathway, suggesting a possibility of trans-synaptic anterograde or retrograde changes. Low contrast acuity was associated with low FA and higher radial diffusivity in the OR NAWM and OR lesion fraction independent of RNFL thickness suggesting that visual dysfunction in MS may also be contributed by posterior visual pathway damage.(259)

Fig 2.3: Probabilistic tracking of the optic radiations using DTI tractography

Figure 1



2.10 Visual Cortex and association areas MRI:

Visual cortex MTR

Abnormalities in MTR have been found in normal appearing cortical grey matter (GM) and white matter (WM) in MS.(260-264) The relationship between MS lesions in white matter tracts and the specific grey matter areas of the brain they project to have been studied using MTI.(263) Audoin *et al* investigated 80 patients presenting with clinically isolated ON within 6 months and looked specifically for regional abnormality using a voxel based analysis of the grey matter MTR maps. There was a selective and significant reduction in GM MTR in the visual cortex bilaterally in patients compared with controls. This reduction correlated significantly with the patients' baseline and 3 month visual acuity. These findings suggest that the specific MTR reduction in the visual cortex following ON may be due to a mechanism of trans-synaptic morphological changes occurring in the corresponding grey matter specifically due to a remote white matter tract lesion.(265)

Functional MRI

Functional MRI is a method of measuring brain activity *in vivo*, based on the principle of the blood-oxygenation level dependent (BOLD) effect.(266) This takes advantage of the fact that haemoglobin has a different MRI signal depending on whether it is oxygenated or not, and activated brain regions have greater blood flow that outweighs their greater oxygen requirements. (Fig 2.4) Functional MRI may be used to study brain reorganisation, or plasticity, which is thought to be important in minimising the impact of damage to the CNS in various diseases.

Functional MRI has been used extensively to study MS, and evidence for cortical plasticity has been found in the motor, cognitive and visual systems.(267) Less activation of visual cortex is seen in patients with both acute and previous ON than in controls, which probably represents reduced neuronal input due to acute pathological changes in the nerve such as oedema, inflammation, and later demyelination and axonal loss.(255;256;268-272) Higher BOLD signal in the visual cortex correlated significantly with both Snellen's visual acuity and contrast sensitivity measurements.(270)

The first evidence for reorganisation of the visual system after ON came from Werring *et al*, who reported activation in areas outside the visual cortex, normally involved in higher level multi-modal sensory processing.(255) The volume of extra-occipital activation was correlated with VEP latency, suggesting that it might be a response to persistent demyelination in the optic nerve. Toosy *et al* subsequently localised reorganisation to the lateral occipital complexes (LOCs) in a longitudinal study, and found that the changes were correlated with visual outcome, after taking structural factors into account, which suggested that it was a genuine adaptive phenomenon.(257)

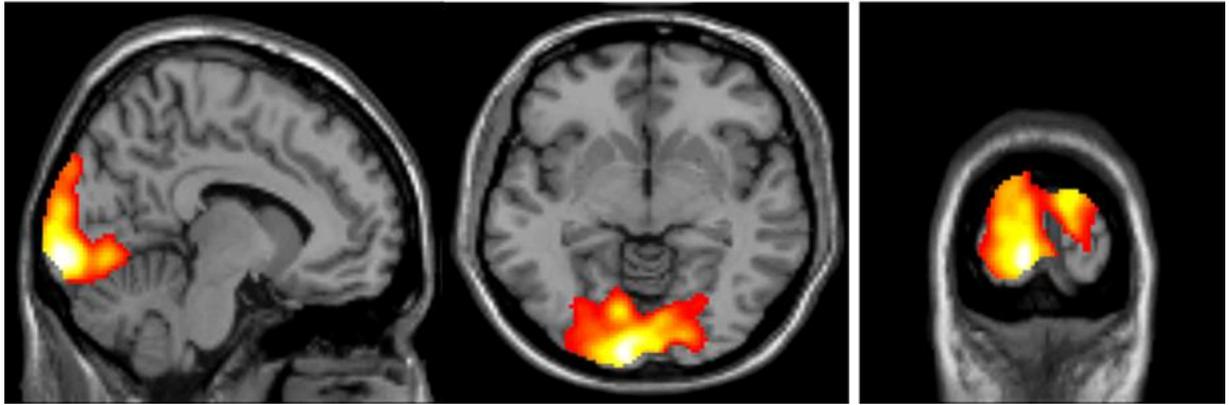
Since then, there have been conflicting results from studies looking at how hierarchical visual areas respond to ON. Some have concluded that the higher visual areas might be robust to disruption of input,(273) whilst others have reported that they are not.(274) In addition, further evidence of brain reorganisation has been found in the LOCs(273) and probably the lateral geniculate nucleus.(274)

Jenkins *et al* performed structural MRI of both the anterior and posterior visual pathway and also visual functional MRI in a group of 28 patients presenting with acute unilateral

optic neuritis as a clinical isolated syndrome, within a month of symptom onset and 10 healthy controls. Visual assessments were also performed in the same day for most of the patients. Severity of acute visual loss in ON was associated with measures of the extent of optic nerve inflammation (lesion length) and conduction block (VEP amplitude). There was an association between increased fMRI activity in the cuneus and better vision in the patients affected eye, when corrected for optic nerve inflammation and conduction block, suggesting a possible adaptive neuroplasticity even in the acute stage. 25 of the 28 patients and 8 controls were studied longitudinally for a period of 12 months. Greater baseline fMRI responses in the LOCs (both affected and unaffected eye stimulation) were associated with better visual outcome at 12 months, independent of tissue damage in the anterior or posterior visual pathway, including neuroaxonal loss (as measured by MRI, VEP amplitude and OCT) and demyelination (as measured by VEP). Cuneus activation at baseline, which was associated with visual outcome in the acute stage, was not associated with the visual outcome at 12 months. This suggests that early neuroplasticity in higher visual areas appears to be an important determinant of recovery from ON and that different regions play a role in adaptive plasticity during different stages of injury.(275;276)

However despite the heterogeneity of the patient cohorts studied and other methodological differences, recent studies agree that there is increasing evidence for compensatory brain plasticity in optic neuritis.(269;270;272;274;276)

Fig 2.4: Statistical parametric map demonstrating activation of the occipital cortex following visual stimulation in a control subject using fMRI



2.11 Applications

Most of the assessment techniques described above are predominantly used in research and have a limited role in clinical practice.

(a) Diagnosis:

The diagnosis of ON is predominantly clinical and orbital MRI has a limited role other than in atypical presentations, when it is especially used to rule out compressive lesions. Conventional brain MRI in ON has an important role in predicting the risk of MS and in making the diagnosis.(277;278) VEP played a role in the diagnosis of primary progressive multiple sclerosis (PPMS) according to the 2005 revised McDonald's criteria, (4-8 brain T2 lesions with positive VEP had the same diagnostic value as ≥ 9 T2 lesions without positive VEP).(145)(This was one of 3 supportive laboratory criteria for PPMS, the other two were positive spinal cord MRI with 2 focal T2W lesions and positive intrathecal oligoclonal bands in CSF) VEP has been excluded from the revised diagnostic criteria by Swanton et al 2010. According to 2010 revision of the criteria, positive brain MRI requires just one or more T2W lesions in atleast 1 of periventricular, juxtacortical, infratentorial or spinal cord regions characteristic for MS. The other two supportive criterions remain the same. This revision simplifies the requirements while maintaining the sensitivity and specificity and allow an earlier diagnosis without the need for VEPs.(279-281) The changes seen on retinal imaging are not specific to MS or ON, and there is wide inter-individual variability, which will probably limit utility in the diagnostic setting.

(b) Understanding pathophysiology:

The optic nerve MRI techniques (Table 2.1) have been used in combination with visual function, visual evoked potential, retinal and retrochiasmal imaging measures to probe pathophysiological mechanisms, particularly the temporal profile of axonal loss and demyelination in the anterior visual pathway, and adaptive cortical responses to tissue injury in the visual pathway. The different MRI techniques and VEPs provide complementary but not fully specific information on the relative contributions of axonal and myelin damage and repair. Measurements of the RNFL provide a direct estimation of axonal quantity, albeit at a restricted anatomical site. Taken together, these imaging and electrophysiological techniques have provided considerable insights into mechanisms of damage and repair involved in MS and associated ON (Table 2.2).

Table 2.1: Optic nerve imaging techniques used in various observational studies with references, number of patients and controls and diagnosis.

S.No	Techniques	Reference	Sequences	Pts	Ctrls (Healthy)	Diagnosis
1	T2W (Lesion detection)	Miller et al 1988 (47)	STIR (Short– Tau Inversion Recovery) 0.5 Tesla	37	ND	ON
		Gass et al 1996 (44)	fsFSE(fat saturated Fast Spin Echo) 1.5T	21	10	ON
		Jackson et al 1996 (45)	SPIR-FLAIR 1.5T (Selective Partial Inversion Recovery-Fluid Attenuated Inversion Recovery)	18	5	ON
2	Gadolinium leakage/enhancement	Youl et al 1991 (56)	STIR sequence followed by single dose Gd admin.; 0.5 Tesla	18	ND	Acute ON
		Kupersmith et al 2002 (54)	Fat suppressed T1W post single dose Gd; 1.5 T	107	ND	AON & MS
		Hickman et al 2004 (55)	Fat saturated T1W spin echo post triple dose (td) Gd; 1.5 T	28	ND	Acute unilateral ON (longitudinal study)
3	Atrophy Measurements	Inglese et al 2002 (62)	T1W spin echo images 15 noncontiguous 3mm slices;	30	18(healthy) 10(LHON)	MS with ON
		Hickman et al 2001 (60)	sTE fFLAIR (short echo fast fluid attenuated inversion recovery)	17	16	Previous unilateral ON
		Hickman et al 2002 (61)	sTE fFLAIR	10	ND	Previous ON and MS; (Serial study for 1 year)
		Hickman et al 2004 (63)	sTE fFLAIR	21	32	First episode unilateral AON (serial study)
		Trip et al 2006 (64)	sTE fFLAIR	25	15	Previous ON with poor recovery
4	Magnetisation Transfer Imaging	Thorpe et al 1995 (69)	2D Gradient Echo (GE) sequence with & without off resonance pulse	20	6	ON
		Inglese et al 2002 (62)	2D GE with & without off resonance pulse	30	18(healthy) 10(LHON)	MS with ON
		Hickman et al 2004 (70)	3D GE with & without off resonance pre-pulse	21	27	First episode unilateral

		Trip et al 2007 (71)	3D GE with & without off resonance pre-pulse	25	15	AON (serial study)
		Klistorner et al 2011 (153)	3T 2D GE	23	10	Previous ON with poor recovery
		Wang et al 2012 (296)	3T 2D GE	37	11	Previous ON
						Unilateral AON
5	Diffusion Weighted Imaging	Iwasawa et al 1997 (73)	Diffusion Weighted SE images with diffusion gradients in 3 directions (X,Y,Z)	8	7	MS with ON (4 acute ON, 9 chronic ON)
		Hickman et al 2005 (75)	Zonal Oblique Multislice Echo Planar Imaging – DWI (ZOOM-DWI)	18	11	Previous single episode ON
		Trip et al 2007 (76)	ZOOM-Diffusion Tensor Imaging (DTI)	25	15	Previous ON with poor recovery
		Kolbe et al 2009 (152)	3T coronal oblique orthogonal fat and csf suppressed EPI-DTI sequence	16	10	Previous single episode of unilateral acute optic neuritis (mean: 4 years)
		Naismith et al 2009 (192)	3T single shot spin echo EPI-DTI trans axial slice	12	12	AON
		Naismith et al 2010 (193)	3T single shot spin echo EPI-DTI trans axial slice	28	12	Previous ON (Atleast 1 year previously)
		Naismith et al 2012 (191)	3T single shot spin echo EPI-DTI trans axial slice	102 nerves (70 pts)		Remote ON, mean: 4years. (MS, CIS, NMO)
				31 nerves (25 pts)		AON

Table 2.2: Imaging & Evoked Potential techniques reflecting pathophysiology in Optic Neuritis.

Clinical status	Pathophysiological mechanism	Imaging & Evoked Potential techniques that reflect mechanism
Acute Optic Neuritis (relapse)	Inflammation Demyelination	Gadolinium enhancing lesion ↑Optic nerve area on T1W FLAIR ↑RNFL thickness ↓MTR, delayed response with well formed waveform in VEP
Recovery	Resolution of inflammation Remyelination Adaptation	Cessation of enhancement ↑MTR, shortening of latency in VEP Functional activation of lateral occipital cortices and possibly other extra-striate regions
Persistent deficits	Persistent demyelination Axonal loss	Low MTR ↑MD, ↓FA ↓optic nerve area on T1W FLAIR ↓RNFL thickness, ↓Macular volume

(c) Therapeutic monitoring:

Acute ON is a good model of the acute demyelinating lesion in MS *in vivo*. Quantification of axonal damage using OCT is likely to be very useful in the evaluation of neuroprotective therapies. The various imaging and electrophysiological techniques available are sensitive to pathophysiological processes, which allows exploration of therapeutic strategies and mechanisms, for example neuroprotection using RNFL thickness, or repair using optic nerve MTR, VEP latency and functional MRI. Proof-of-concept trials may be possible with smaller groups of patients than required with clinical endpoints. Sample size estimates were calculated for RNFL thickness measurements in placebo controlled neuroprotection trials by Henderson et al, which indicated that the numbers needed to show similar treatment effect in 6 months after acute optic neuritis was much less than 3 months follow up and similar to those of 12 months follow up.(167)

An area of interest for potential neuroprotection and repair is stem cell therapeutics. The sentinel lesion approach (ON) was used in an exploratory trial of autologous mesenchymal stem cells in MS (MSCIMS); most of the visual, imaging and electrophysiological assessment techniques described above were used to study the effects that this experimental therapy has on anterior visual pathway structure and function and is described in subsequent chapters. (282)

2.12 Future challenges:

Optic nerve MRI using the parameters mentioned in earlier sections, presents methodological challenges. Future imaging the optic nerve on higher magnetic field scanners and using surface coils should improve resolution and signal-to-noise.

Development of fast acquisition methods will further help minimise movement effects to improve the accuracy of the measures obtained. There have been pilot studies on 3T to obtain reliable measurements of intraorbital optic nerve(218) and diffusion trace analysis of the visual pathways(217). The MSCIMS study uses a 3T scanner to obtain quantitative optic nerve MR measures longitudinally, which will also allow assessment of reproducibility and stability of these measures. MR spectroscopy of the brain has been very helpful in providing cell specific measures of neuronal damage and glial activation or proliferation(283). Further development is required to apply this technique to localised areas within the CNS including the visual pathways, with considerable technical challenges involved in applying it to the optic nerve.

High speed OCT using a “fourier or spectral” detection technique is also a recent advancement. This technique is approximately 50 times faster and has a superior sensitivity compared to the standard time domain OCT. Fourier domain OCT also helps in reducing eye movement artefacts(284).

In summary, imaging the afferent visual pathway provides an attractive approach when investigating potential experimental therapeutic strategies in MS. Further studies will reveal if this becomes a regular part of MS and optic neuritis treatment trials in the near future.

Chapter 3: Stem cells in MS.

3.1 Role of Stem Cells as repair/neuroprotective therapies for MS

3.1.1 Definitions

Stem cells are defined by the property of asymmetric division; specifically the capacity to divide producing identical (self-renewal) and specialised cell progeny. The number of types of specialised progeny able to be produced defines the “potency” of a specific stem cell. Embryonic Stem (ES) Cells derived in early development (inner cell mass of the blastocyst) are pluripotent (able to produce progeny from all 3 germ cell layers), whereas those derived at later stages of development (fetal or adult stem cells) are more restricted in the progeny they produce to recognisable groups of related cell types *eg.* blood cells (erythrocytes, leucocytes, platelets) are progeny of haematopoietic stem cells. This restriction defines multipotency. Stem cells capable of producing only one type of specialised progeny are unipotent and more commonly referred to as “progenitor” cells

3.2 Source of Stem Cell as relevant to rationale for reparative/neuroprotective potential

In humans, stem cells can be obtained at all stages in development. In the adult they are located in tissue specific niches. The stage of development at which they are derived, and for adult stem cells the specific tissue and lineage from which they are derived, defines the potential mechanisms of relevance to potential repair or neuroprotection.

Exogenous repair strategies rely on the stem cell being capable of generating remyelinating cells and neurons. The options in this regard therefore include embryonic

stem (ES) Cells and adult neural stem cells (NSC). Significant practical challenges remain to be met in human ES work regarding reliable and measurable neuronal/glial differentiation in addition to challenges of safety and ethical acceptability. Issues of scale and directed differentiation are equally relevant to adult NSC work, however a more pressing difficulty is the challenge of acquiring adequate material in order to address any such questions. For these reasons, exogenous repair of the CNS is not currently an achievable objective. The recent discovery of a technique to induce pluripotency in adult somatic (non-stem) cells offers the future promise of an alternative source of cells capable of exogenous CNS repair which could avoid several of the current ethical and acquisition problems.(285)

Adult non-neural stem cells are an attractive candidate for clinical translation because they are readily obtained, potentially autologous and therefore ethically acceptable to patients. The rationale for their use as repair or neuroprotective therapies is not necessarily dependent of their ability to generate remyelinating cells or neurons. This may include promotion of endogenous repair or neuroprotection. Evidence that stem cells exhibit properties of relevance to these aims independent of their directed differentiation has been increasing over recent years.(286-288) One such cell of interest is the Mesenchymal Stem Cell (MSC), these are introduced below and the evidence is also reviewed relevant to the hypothesis that they are capable of promoting endogenous repair and/or neuroprotective in progressive MS.

3.3 Mesenchymal Stem Cells

Mesenchymal Stem Cells (MSCs) can be extracted from the bone marrow and other embryonic mesoderm lineage tissues of humans throughout life. The physiological role of

MSCs is to give rise to the supportive stroma of the haematopoietic microenvironment, and facilitate haematopoiesis via interactions with haematopoietic stem cells (HSCs).

Mesenchymal Stem Cells exhibit properties of potential therapeutic relevance to application as a repair and/or neuroprotective therapy in progressive MS.

(i) Exogenous repair through neuronal/glial differentiation

The ability to generate CNS tissue from non-neural adult stem cells is highly contentious. There are no convincing reports of neuronal transdifferentiation *in vivo* however a number of groups have described *in vitro* transdifferentiation.(289;290) Artefacts such as cell fusion, poorly defined initial cell populations containing a mixture of stem and progenitor types, and cytotoxic cell changes are all potentially relevant factors to account for the observation.

(ii) Neuroprotection / facilitation of endogenous repair through mechanisms independent of directed differentiation

Immunomodulatory properties of MSCs are diverse and well established. Effects on all aspects of innate and adaptive immunity have been described, including effects on cell mediated and humoral immune mechanisms relevant in the pathogenesis of MS.(291) None of these properties have been tested in MSCs derived from patients with MS.

Evidence for the production of neurotrophic factors is less well established, however production of BDNF and NGF has been reported. This has not yet been tested in MSCs derived from patients with MS.

(iii) Pathotropism

The ability of MSCs to migrate to the site of pathology may be crucial to their application in a widespread multifocal disease such as MS. There is evidence to support this

property, although the evidence that MSCs can penetrate an intact blood brain barrier is limited.(292)

(iv) Blood-brain-barrier penetrance

The evidence for BBB penetrance is reviewed below for the varying states of BBB integrity. This has implications for the treatment of MS patients who are not experiencing a relapse at the time of treatment (unless the BBB is breached by the chosen delivery method or additional intervention).

- *Intact*

The evidence that MSCs can then penetrate the intact BBB is limited. In rodent models MSCs have been observed in low numbers within the CNS compartment of controls.(293) Specific large animal experiments designed to address this question cannot be interpreted in the context of BBB compromise due to pre-treatment whole body irradiation.(294)

- *Active CNS inflammation*

In EAE models, MSCs are observed in low numbers sub-pially but not within the CNS parenchyma.(286)

- *Between attacks of MS*

The BBB is known to be dysfunctional between attacks of MS and in the progressive phase.(295) However, it is not known if this is permissive to MSC traffic.

(v) Engraftment

In order for therapeutic effects to be sustained, MSC transplants must engraft into host tissue and persist. Proof of principle was established by Liechty transplanting adult human MSCs into a sheep embryo with intra-peritoneal delivery.(296) Widespread mesodermal tissue engraftment with relevant differentiation and functional integration

was observed. Engraftment without differentiation was seen in tissues from all 3 germ cell layers, with MSCs detected sub-pially in the CNS but not within the parenchyma.

(vi) Evidence of effect on functional outcomes in MS Models (proof of principle)

There has been some evidence of functional improvement in EAE mice with intravenously administered MSCs.(297)

(vii) Evidence of effect in human autoimmune disease and MS

The first report of human MSC therapy was published in 1995. More than 150 patients have been treated for a diverse group of clinical conditions. Proof in principle of therapeutic efficacy in human autoimmune disease was demonstrated by case reports in Grade IV graft-versus-host disease – a prototypic T cell mediated autoimmune condition.(291;297-299) (Table: 3.2)

Three reports (Table: 3.1) have been published of MSCs being administered to patients with MS. Mohyeddin et al. report a phase I open-label study to address the feasibility and safety of administering autologous MSCs intrathecally to patients with progressive MS.(300) Ten patients were treated in this study (eight with SPMS, two with PPMS; aged 22 – 40) with a mean of 8.73×10^6 cells/patient (range 2.5×10^6 to 18.0×10^6 ; one patient receiving two injections). Mean follow up was nineteen months. Iatrogenic meningitis was seen in two patients, and headache in nine. Global disability measured by expanded disability status scale (EDSS) was unchanged in four patients, worsened in five patients, and improved in one. No significant change was seen on MRI outcome measures.

Yamout et al. report a phase I open-label study to address the feasibility and safety of administering autologous MSCs intrathecally to patients with progressive MS. Seven

patients were treated in this study (all with SPMS, aged 34 – 56) with a range of 32×10^6 to 100×10^6 cells per patient. Three further patients were not treated due to failure of MSC expansion. Follow up was for twelve months. Transient encephalopathy with seizures was seen in the patient receiving the highest dose of MSCs. Global disability measured by expanded disability status scale (EDSS) was unchanged in one patient, worsened in one, and improved in five. No significant change was seen on MRI outcome measures. Visual function including low contrast acuity was assessed and was found to be improved in 3 out of the 4 patients who had 12 month follow up. Foveal thickness was measured using time domain OCT showed no change from baseline. (301)

Karussis et al. report a phase I open-label study to address the feasibility and safety of administering autologous MSCs intrathecally \pm intravenously to patients with MS not responding to conventional disease modifying therapy. Fifteen patients were treated in this study (MS course not reported, aged 25 – 65) with a mean of 63.2×10^6 cells per patient for intrathecal injection (fifteen patients) and 24.5×10^6 cells per patient for intravenous infusion (five patients). Follow up was for six months. Fever and headache were seen in ten patients and aseptic meningitis in one. Global disability measured by expanded disability status scale (EDSS) showed no change in 4/15 patients and slight improvements in 11/15 patients at 6 months compared to baseline. There was no change in MRI outcome measures.(302)

Table 3.1: Published studies using mesenchymal stem cells in multiple sclerosis

REFERENCE	DESCRIPTION
(Mohyeddin Bonab et al. 2007)	Ten patients with RRMS were administered autologous MSCs intrathecally, and followed for a mean of 19 months by clinical and imaging assessment. No significant changes were seen in clinical or imaging outcomes.
(Yamout et al. 2010)	Ten patients with SPMS were administered autologous MSCs intrathecally, and followed for 12 months by clinical and imaging assessment. Clinical assessment suggested possible improvement but no significant change was seen on imaging outcomes.
(Karussis et al. 2010)	Fifteen patients with MS were administered autologous MSCs both intrathecally and intravenously, and followed for 6 months by clinical and imaging assessments. No significant adverse events, or changes on clinical or imaging outcomes were seen.
(Mohyeddin Bonab et al. 2012)	Twenty-five progressive MS patients were administered autologous MSCs intrathecally, and followed up for 12 months by clinical and imaging assessment. No significant adverse events, with possible stabilisation on clinical and imaging outcomes were seen.

Table 3.2: Trials & case-reports of intravenous MSC therapy in man

Year of Publication	Reference	MSC Source	Underlying disease	Number treated
1995	Bone Marrow Transplantation 16 : 557-64	Autologous	Haematological malignancy	15
2000	Journal of Clinical Oncology 18 : 307-316	Autologous	Breast Cancer (Stage IV)	28
2002	Proceedings of the National Academy of Science USA 99 : 8932-7	Allogeneic	Osteogenesis Imperfecta	6
2002	Bone Marrow Transplantation 30 : 215-22	Allogeneic	Hurlers syndrome (5), Metachromic leucodystrophy (6)	11
2002	British Journal of Haematology 118 : 1128-31	Allogeneic	Acute myeloid leukaemia	1
2003	Leukaemia 17 : 474-6	Allogeneic	Aplastic anaemia	1
2004	Lancet 363 : 1439-41	Allogeneic	Graft versus host disease	1
2005	Annals of Neurology 57 : 874-82	Autologous	Ischaemic stroke	5
2005	Biology of Blood and Marrow Transplantation 11 : 389-98	Allogeneic	Haematological malignancy	46
2005	Transplantation 79 : 1607-14	Allogeneic	Osteogenesis Imperfecta	1
2006	Stem Cells and Development 15 : 349-57	Autologous	Healthy volunteers	12
2006	Transplantation 81 : 1390-7	Allogeneic	Graft versus host disease	12
2007	Leukaemia 21 : 568-70	Allogeneic	Primary haematopoietic stem cell graft failure	1
2007	Leukaemia. 21 : 2271-6	Allogeneic	Haemorrhagic cystitis (7), Pneumomediastinum (2), Colonic perforation (1)	10
2007	Leukaemia 21 : 1733-8	Allogeneic	Adjunct to haematopoietic stem cell graft	7
2008	Clin Pharmacol Ther. 83 : 723-30.	Autologous	Multi-system atrophy	11
2009	J Am Coll Cardiol. 54 : 2277-86.	Allogeneic	Acute myocardial infarction	53
2010	Stem Cells. 28 : 1099-106.	Autologous	Ischaemic stroke	16
2010	Biol Blood Marrow Transplant. 16 : 1293-301.	Allogeneic	Graft versus host disease (paediatric)	11
2010	Ann Intern Med. 153 : 650-4.	Allogeneic	Systemic sclerosis	1
2011	Nucl Med Biol. 38 : 961-7.	Autologous	Liver cirrhosis	4
2011	Brain. 134 : 1790-807.	Autologous	Ischaemic stroke	12
2012	Ann Neurology. 72 (1): 32-40	Autologous	Multi-system atrophy	14
2013	Cytotherapy. 15 (2): 185-91	Umbilical cord	Spinal cord injury	22

Chapter 4: An exploratory trial of Mesenchymal Stem Cells In Multiple Sclerosis (MSCIMS).

4.1 Study design:

4.1.1 Introduction

A phase I/IIA trial of adult autologous mesenchymal stem cells as a potential neuroprotective/repair therapy for Multiple Sclerosis (The Mesenchymal Stem Cells in Multiple Sclerosis [MSCIMS] Trial) was designed as an eighteen-month pre vs post treatment (single intervention of autologous bone marrow derived mesenchymal stem cells) comparison study in a small group of 10 patients. An approach based on detailed assessment of participants with disease involving the anterior visual pathways was chosen.

The MSCIMS trial was conducted in the context of a wider need to develop therapies, which prevent progression, and/or repair existing fixed disability in MS.

4.1.2 Trial objective and purpose

Trial aim:

To establish the methodology, infrastructure and protocols for phase IIB/III trials of cell based and other repair therapies in multiple sclerosis.

Primary objective:

To describe the safety profile over six months of intravenously administered autologous MSCs at a dose of $1 - 2 \times 10^6$ cells / kg in patients with multiple sclerosis.

Secondary objectives:

To explore the potential efficacy over six months of intravenously administered autologous MSCs at a dose of $1 - 2 \times 10^6$ cells / kg by clinical, neurophysiological and imaging assessments in patients with multiple sclerosis.

4.1.3 Trial components

The trial was conducted in five parts. These are described below. All patients recruited followed this path.

- Referral to trial clinic.
- Review of trial eligibility at first visit.
- Pre-treatment assessment phase (12 months).
- Treatment (single administration).
- Post treatment assessment phase (6 months).

4.1.4 Patient selection

MSCIMS Eligibility Criteria

- Clinically definite multiple sclerosis
- Age 18 – 65 inclusive
- Expanded Kurtzke Disability Status Score 2.0 – 6.5 inclusive
- Clinical evidence of optic nerve involvement
- Abnormal visual evoked potential from either eye or both eyes suggestive of demyelination
- Retinal nerve fibre layer not less than 45 microns on optical coherence tomography in either eye.
- Lesion seen on T₂W MRI of optic nerve
- Capacity to give own consent
- No serious underlying bleeding disorder
- Women – Not pregnant at entry, not planning pregnancy during trial.

- Men – Not planning to father a child for 6 months after treatment.
- Not on Beta interferon or Glatiramer acetate within 6 months of trial entry, and not previously on other disease modifying therapies at any point.

4.1.5 Rationale for MSCIMS eligibility criteria:

As an experimental therapy, it was appropriate to consider patients who had established disease with a poor prognosis and few or no established treatment options.

This defines:

- **Clinically definite MS**
(not CIS meeting MacDonald criteria)
- **Established disease progression (fixed disability present)**

The degree of fixed disability at which it is ethically acceptable to invite patients to participate in an experimental (cellular) therapy trial is a question of judgement. Theoretical long-term risks of neoplasia and infection are unquantified.

There is therefore a tension between the drive to limit the exposure of treatment associated risk to those patients with severe disability and a competing drive to treat patients in the early phase of progression based on the likelihood of efficacy from the pre-clinical work.

A lower Expanded Kurtzke Disability Status Score (EDSS of 2) limit was specified to ensure that recruited patients had acquired sufficient disability to merit experimental therapeutic approaches.

An upper Expanded Kurtzke Disability Status Score (EDSS of 6.5) limit was specified to ensure that recruited patients were able to tolerate the trial procedures and assessments.

- **Not currently receiving disease modifying therapy**

The number of patients receiving active therapy in a phase IIA trial of experimental (cellular) therapy is necessarily limited. It was therefore considered to be prudent to maximise the data on safety and efficacy that could be unequivocally be attributable to the intervention.

4.1.6 Methods:

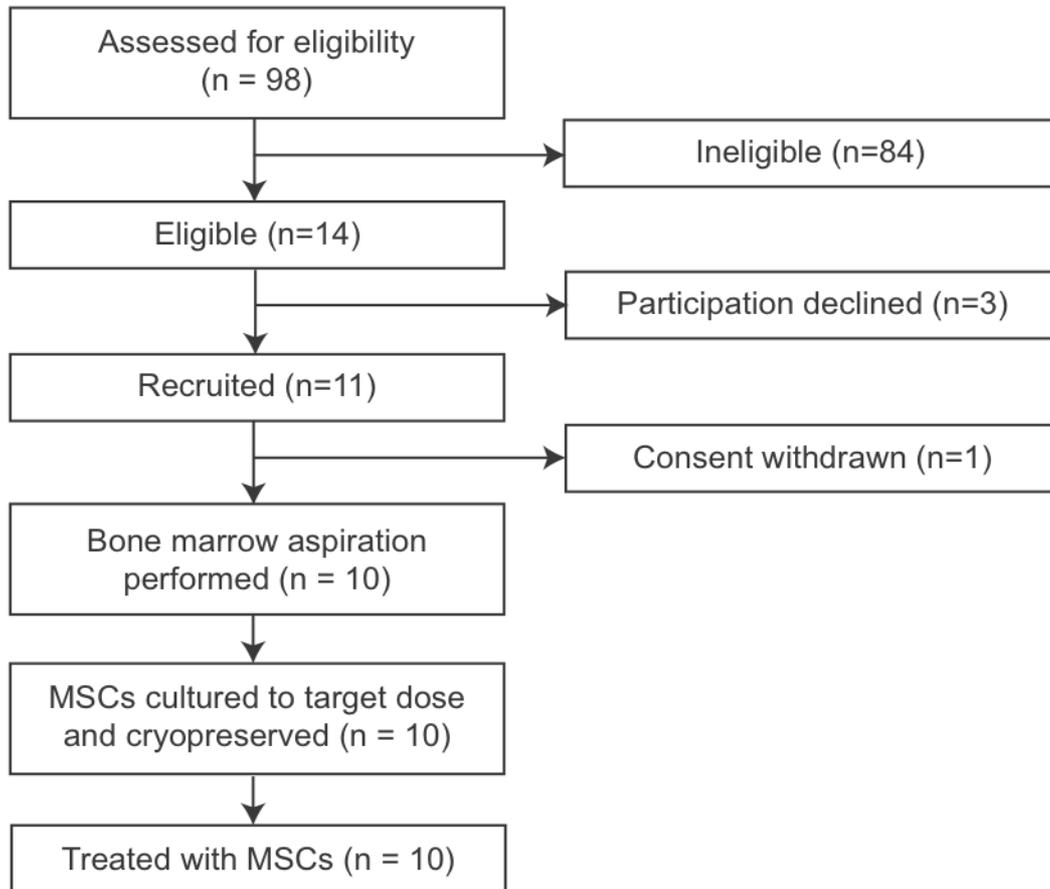
Centres: Cambridge Centre for Brain Repair (BRC) and UCL Institute of Neurology (ION), London were the centres involved. Ethical approval for the trial protocol was obtained from the NRES (National Research Ethics Service) Cambridgeshire 2 REC (Regional Ethics Committee) at Cambridge and approval was also obtained from research and development department at both centres involved.

Patient Recruitment: Referrals were invited from multiple sources in East Anglia and London Regions. These included MS specialist clinics, general neurology clinics, MS Nurse patient lists, and existing databases where available. Recruitment was done through a MS research clinic setup at Wellcome Clinical Research Facility at Addenbrooke's hospital in Cambridge.

Patient Screening: At the initial screening clinic at Addenbrookes, a detailed history was obtained and a clinical examination was performed including assessment of EDSS. If the patients satisfied the first four eligibility criteria (of MS, within the age and EDSS range and evidence of previous optic neuritis as assessed from clinical exam and history), they were given a detailed explanation about the trial and provided with information packs. Further screening tests, including visual evoked potential test at Addenbrookes and MRI scan and OCT at the Institute of Neurology, London, were arranged within the next two weeks. 14 patients were screened at Cambridge and were scanned in London. All were found eligible. After at least a week to consider participation and for longer when required, informed consent was obtained from 11 out of the 14 patients for the trial. The 3 patients declined to participate had milder disability and relapsing remitting MS, this was at least partly because they still had other established treatment options to consider. One

out of the 11 patients who had consented withdrew consent after the first pre-treatment assessment visit due to a change of mind. The remaining 10 Patients progressed through the trial. (Fig: 4.1)

Figure 4.1: Recruitment and retention of trial participants



Patient Assessments: The 10 trial patients underwent 5 visits each to Cambridge (EDSS, MSFC, ACE-R, BDI-II, MSIS, VEP) and London (MRI, OCT and visual function), over a period of 12-15 months with a visit each to the two centres in 3-month intervals.

Isolation, expansion, characterization and administration of MSCs:

During the first 3 months all 10 patients also underwent bone marrow aspiration performed by Dr. Charles Crawley (Dept. of Haematology, Addenbrooke's Hospital, Cambridge, UK) as a day-case procedure using standard aseptic methods. MSCs were successfully isolated and cultured to the target dose from all bone marrow aspirates (mean total cultured dose 2.0×10^6 cells / kg; range 1.1 to 3.7×10^6 cells / kg). Mean

culture duration was 24 days (min–max range 20–30). Clinical-grade MSC preparations were generated under Good Manufacturing Practice conditions by Dr. Mike Scott (Blood & Marrow Transplant Unit, Addenbrooke's Hospital, Cambridge, UK), using standard operating procedures based on those previously described.(298) Briefly, bone-marrow mononuclear cells were separated by density gradient centrifugation in Ficoll-Paque™ PREMIUM (GE Healthcare UK Ltd, UK). Washed cells were re-suspended in PBS/EDTA (MiltenyiBiotec Ltd, UK) and cultured in Dulbecco's modified Eagle's medium–low glucose (Invitrogen, UK) supplemented with 10% foetal bovine serum (Hyclone, Perbio Science, UK) and plated at a density of 1×10^8 cells per cell-factory (Nunc, Thermo Scientific, UK). Near confluent cultures (>80%), were treated with 0.25% trypsin-EDTA (Invitrogen, UK) and re-plated at 3.5×10^6 cells per cell factory. MSCs were harvested and cryopreserved in 4.5% human albumin solution (BPL, UK) with dimethyl sulphoxide (Origen Biomedical Inc.) at a final concentration of 10%. MSCs were then characterised in accordance with International Society for Cellular Therapy (ISCT) minimal criteria for definition.(303). Briefly, this included evidence of tri-lineage differentiation potential (adipocyte, chondrocyte, osteocyte) and flow cytometry assessment confirming expression of CD73, CD90, and CD105 surface molecules (>95%) and absence of CD34, CD45, CD14, and CD3 (<2%). Release criteria for clinical use included absence of contamination by pathogens (as documented by aerobic and anaerobic cultures, and mycoplasma testing), and lack of any genomic copy number changes by 1Mb resolution BAC array comparative genomic hybridization (aCGH) as previously described.(304) This was performed by Dr. Shi-Lu Luan & Professor Ming-Quing Du (Dept. of Pathology, University of Cambridge).

Administration of MSCs was performed as a day-case procedure following pre-medication with chlorpheniramine 10 mg, hydrocortisone 100 mg, and metoclopramide 10 mg. Cryopreserved MSCs were thawed (≤ 4 minutes) and immediately infused over

15 minutes through a peripheral venous cannula. Administration of cell suspensions was followed by infusion of normal saline (500 ml) over 4 hours.

Controls: 8 Healthy volunteers were also recruited to undergo MRI and OCT assessments only, three times over the same period as patients' five assessments to provide normal control measures to compare with patients, and to adjust for normal variation of imaging measures over time.

4.2 Outcome measures

Primary:

Adverse events

Secondary:

(i) Clinical:

MS functional Composite Score
Expanded Disability Status Score
MS Impact Scale-29
Beck's Depression Inventory
Addenbrooke's Cognitive Examination - Revised

(ii) Neuro-ophthalmological:

Visual function (acuity and colour)
Visual fields
VEP latency

(iii) MRI Optic Nerve and Brain:

Optic Atrophy
Optic Nerve MTR
Fractional anisotropy and diffusivity parameters in the optic nerve
Brain atrophy
T2 Lesion volume
T1 Lesion volume
Whole brain and regional MTR
Brain lesion MTR
Functional activation

(iv) Optical Coherence Tomography:

Retinal Nerve Fibre Layer thickness
Macular volume

(v) Immunological:

Common antibody titre
T cell subset counts

4.2.1 Primary: Adverse events

As a phase I/IIA trial, MSCIMS seeks to establish the safety of the intervention and detect relevant outcomes to inform design of phase IIB (and beyond) efficacy trials. The MSCIMS trialists have completed a systematic literature review of the current clinical experience (Table 3.2). However, over 150 patients have undergone MSC based transplantation for other indications (including stroke) with no reports of significant graft related complications. Significantly there is no need for immunosuppression. The procedure is simple, cheap, potentially widely applicable and ethically acceptable to most patients. In MSCIMS, the autologous mesenchymal stem cells that were acquired were characterised (International Stem Cell Society guidelines) and safety checked (bacterial, fungal, viral cultures and karyotyping) pre-administration.

Patients were screened for antibody titres to common viral infections within four weeks before treatment. Informed consent was obtained on the day of the infusion. Pre-infusion bloods were obtained on the day for full blood count, electrolytes, liver and renal function tests. Additional blood was also obtained and sent to immunology for T cell subsets. These blood tests were repeated every week for four weeks following the MSC infusion. Patients were also administered 100 mg of iv Hydrocortisone and 10mg of iv anti-histamine Pheneramine Maleate prior to the infusion as a prophylaxis against any hypersensitivity reactions.

Any adverse symptoms and vital signs were recorded at baseline and every half hour following the infusion for four hours. Any adverse symptoms were also recorded every week for four weeks when they had their bloods taken. Patients were also advised to contact us in the interim if they had noticed any untoward symptoms.

4.2.2 Secondary: Efficacy

Clinical:

EDSS: Kurtzke's EDSS is considered the gold standard among clinicians for the assessment of disability in MS patients.(21) EDSS was assessed and functional system scores recorded on all patients at Addenbrookes hospital, Cambridge by the same observer for all the five visits.

MSFC: Multiple Sclerosis Functional Composite: MSFC integrates scores on 25 foot timed walk, nine hole peg test and paced auditory serial addition test (PASAT) to measure the function of the lower limbs, upper limbs and cognitive abilities quantitatively to give one integrated score. All three components of MSFC was administered to all patients by single observer and a composite z score calculated for all the five visits to Cambridge.

MSIS-29: Multiple Sclerosis Impact Scale – 29: This is a patient based scale whereby patients are asked 29 standard objective questions about their perceptions of the impact of MS on quality of life. (29;30)

ACE: Addenbrooke's Cognitive Examination:

Cognitive difficulties occur in MS even at an early stage.(305;306) ACE is a brief cognitive test battery, which has been validated for dementia, and the individual components of ACE, such as memory recall, verbal fluency, language and visuo-spatial abilities have been validated against standard neuropsychometric tests in controls.(307) ACE was administered to all patients by single observer at all five visits to minimize inter-observer variation.

BDI-II: Beck's Depression Inventory II:

Depression is common in MS and it correlates better with the degree of stress perceived by the patient than the extent of lesions on MRI.(308) BDI-II is a 21 question multiple choice self reported inventory, administered by patients themselves in MSCIMS at 3 monthly intervals during clinical examination sessions to measure the changes in the mood before and after the treatment.

Visual Function:

Visual acuity:

(i) Normal contrast acuity: Visual acuity was tested using a retro-illuminated Bailey-Lovie chart at a distance of 4 m and measured using logmar scores. For patients who could not read any letters at 4 m, the distance was reduced to 1 m and a correction of 0.6 was added to the log mar value at 4 m. If unable to identify any letters correctly at a distance of 1 m, a value of 1.70 was assigned.

(ii) Low contrast acuity: Sloan charts are similar to Bailey-Lovie chart but consists of several charts with progressively lower contrasts.(309) In MSCIMS, three Sloan contrast charts (25%, 5% and 1.25%) were used and the assessments were performed in each eye for all patients. A similar log mar scoring method as described above for normal contrast acuity was used.

Colour vision:

The comprehensive Fansworth-Munsell (F-M) 100 Hue colour vision was used in MSCIMS to assess colour vision. In this test the subject places 85 coloured caps in the perceived order of hue and a square root of error score was used for data analysis. Each eye assessed separately. A score of 36.6 was assigned when vision was too poor to perform the test.

Visual field:

The threshold sensitivity of the central 30° of vision was measured using the full threshold central 30-2 program on a Humphrey visual field analyzer (Carl Zeiss Meditec, Dublin, CA, USA). The visual field mean deviation (MD), a measure of overall field loss was calculated by comparison with a reference field provided by the manufacturer.

Paraclinical:

Electrophysiology:

Visual Evoked Potential: VEP:

Recordings to monocular stimuli comprising of reversal of checkerboard pattern in the whole field and central field were taken using skin surface EEG electrodes attached over the occiput. All ten patients were screened for eligibility and were further assessed during the

other assessment points, as per the protocol, at the clinical neurophysiology department at Addenbrookes hospital, Cambridge.

MRI:

Images were acquired on a Siemens MAGNETOM 3.0T Tim Trio scanner (Siemens, Erlangen, Germany) at UCL Institute of Neurology, using a twelve-element receiver head coil. Total time of acquisition for patients during baseline MRI assessment was approximately 130 minutes. Breaks were given between optic nerve and brain MRI scans and as required. Optic nerve DTI and fMRI was done only once pre and post treatment, this reduced the MRI assessment time during the other three assessment periods to 75 minutes. Controls had 8 minutes less scanning than patients as their optic nerve PD and T2W sequence was not done.

Optic nerve:

(i) Lesion identification:

Patients had a fat saturated turbo spin echo sequence performed with two echoes separately to give two different contrast images to better confirm the presence of the optic nerve lesion that was required for eligibility for the trial participation and also to measure the lesion length. (coronal, TR 2960ms, TE1 71ms, TE2 12ms, 4 averages, matrix size 512 x 384, field of view 24 x 18 cm, in plane resolution 0.5 x 0.5 mm, 16 x 3.0 mm slices for each, acquisition time of 4minutes for each). A neuroradiologist (KAM) blinded to the clinical status, identified and measured lesion length by multiplying the number of slices with abnormal signal by 3 mm.

(ii) Optic nerve area:

The subjects' optic nerves were scanned using fat saturated short echo fast fluid attenuated inversion recovery (sTE fFLAIR) sequence: coronal, TR: 1830ms; TE: 13ms; TI: 800ms; Matrix size: 306 x 384; 22 x 18 cm field of view; 0.60 x 0.60 mm inplane resolution; 16 x 3 mm contiguous slices; 7 averages; acquisition time 13 minutes. The mean cross sectional intra-orbital optic nerve area was calculated on averaging at least 4

sections (mean: 4.85 sections per optic nerve for patients, range: 4 – 7, mean: 5.25 sections per optic nerve for controls, range: 4 – 6) of the intraorbital nerve from the apex of the orbit forwards, which was contoured using a threshold based semi-automatic contouring method and manually edited as required, as previously described.(226) The observer was blinded to subject identity and all 10 patients' and 8 controls' mean intra orbital optic nerve area was calculated.

(iii) Optic nerve MTR:

The subjects' optic nerves were scanned using 3D gradient echo sequence with and without prepulse that saturates the less mobile (bound) macromolecular proton pool. Sequence details: coronal 3D, TR: 36ms, TE: 3.0ms, number of averages: 4, flip angle: 12, Matrix: 256 x 192, field of view: 19 x 14.25 cm, in plane resolution 0.7 x 0.7 mm, 1.5mm x 60 contiguous slices, acquisition time: 16mins.(236) The observer blinded to the subject identity contoured the optic nerves from the chiasm to the globe on each side, using a semiautomatic threshold method with manual correction if required, on the image without the magnetization prepulse. The optic nerve maps were then transferred to the registered MTR maps (generated from the two images on a voxel-by-voxel basis from the expression: $100 \times (M_0 - M_S)/M_0$ percentage units (pu) where M_S and M_0 represent signal intensities with and without saturation pulse respectively) and the MTR was calculated, after manual correction for any mis-registration due to movement between off and on sequences. Only the slices in the MTR map in which the optic nerves could be confidently identified were included for measurement of MTR. All 10 patients' and 8 controls' MTR were measured.

(iv) Optic nerve diffusion tensor imaging:

The subjects optic nerves were scanned with a coronal oblique 3D, fat and fluid attenuated spin echo single shot echo planar sequence: TR: 6seconds, TE: 84 ms, TI: 1.2 seconds, matrix: 128 x 64, field of view: 15cm x 7.5cm, in plane resolution: 1.17mm x 1.17mm, 16 x 4mm contiguous slices, six diffusion directions with $b = 600 \text{ s/mm}^2$ and one

direction with $b = 0$ averaged 40 times, taking approximately 28 mins for acquisition. The diffusion data were then averaged to give 7 diffusion-weighted volumes (one b_0 and 6 $b=600\text{s/mm}^2$), then the data were eddy current corrected using the FSL software library (<http://www.fmrib.ox.ac.uk/fsl>) and the DT was fitted to the eddy-corrected data using the Camino software package (<http://www.cs.ucl.ac.uk/research/medic/camino/guide.htm>).

Square regions of interest (ROIs) of fixed size (2×2 voxels or 5.5mm^2) were placed on the b_0 averaged coronal oblique images using DispImage, and the maximum signal intensity and minimum standard deviation (SD) to guide the positioning of ROIs. The ROIs were then applied to the calculated parameter maps to determine diffusion-related indices. Mean diffusivities (MD), Axial and Radial diffusivities (AD and RD) and fractional anisotropy (FA) was calculated for all optic nerves by averaging at least 3 contiguous slices in each optic nerve.

Brain:

(i) PD-T2:

Axial Proton density (PD)-T2 weighted dual echo, turbo spin echo imaging was acquired on all subjects: Axial, TR: 3seconds; TE1 and 2: 11ms and 101ms; Matrix: 192×256 ; field of view: $24\text{cm} \times 18\text{cm}$; flip angle: 150; In plane resolution $0.9\text{mm} \times 0.9\text{mm}$; $48 \times 3\text{mm}$ contiguous slices for each echo to give 96 slices in 4minutes. Patients' PD-T2W spin echo sequence was displayed in dispimage and hyperintense lesions were contoured on the PD image using a semi-automated threshold based contouring method. This was performed on the PD image, as the contrast between the lesions and CSF is clearer especially for periventricular lesions. The T2W (longer TE) image was referred to for each lesion to confirm its presence. Once all the lesions in a patient had been contoured, the area of the lesions in each slice were added up and multiplied by 3 mm (slice thickness) to give the lesion volume.

(ii) T1 spin echo (SE):

T1 weighted spin echo images were acquired for all subjects and the T1 hypointense lesions were contoured in patients: Axial, TR: 710ms, TE: 8.5ms, 2 averages, Matrix: 233 x 256, field of view: 22cm x 22cm, in plane resolution: 0.9mm x 0.9mm, 48 x 3mm slices acquired in under 5 minutes. A similar approach as described above for T2 lesions was applied to the T1 hypointense lesions in patients to measure the T1 hypointense lesion volume.

(iii) Brain atrophy:

A 3D T1 weighted Modified Driven Equilibrium Fourier Transform (MDEFT) gradient echo sequence(310;311) was acquired on all subjects: Sagittal acquisition; TR: 7.13ms; TE: 2.33ms; Matrix: 224 x 256; field of view: 256mm x 224mm; in plane resolution 1.0mm x 1.0mm; 176 x 1mm slices acquired in 12 minutes. This high spatial resolution image with good tissue contrast is used to quantify brain volume and atrophy. Fully automated method of segmentation is used to measure atrophy (between two time points) known as Structural Image Evaluation using Normalisation of Atrophy (SIENA), and normalized brain volume is measured in a single time point with SIENA cross sectional (SIENAX) which makes use of brain and skull to normalize to standard space and a sub voxel segmentation is carried out to get tissue classification.(312)

(iv) Brain Magnetization Transfer Ratio:

The subjects' brains were scanned using 3D gradient echo sequence to acquire two sets of images of the same volume one with and one without MT-prepulse: coronal 3D, TR: 26ms, TE: 3.0ms, flip angle: 10, Matrix: 256 x 160, field of view: 26cm x 16.25cm, inplane resolution 0.7 x 0.7 mm, 1.0mm x 208 contiguous slices, acquisition time: 20mins in total. In the analysis, the two MT images (with and without MT pulse) and T1 structural image (MDEFT described above) were first oriented to that of the T1SE (spin echo) image. Then the images were resliced and registered to PD-T2 image set.

The brain extraction tool (BET) was used to extract the brain from the registered T1 structural image. The output was manually corrected if necessary using the Jim image analysis software, Version 5.0 (Xinapse Systems Ltd., Northants, UK, www.xinapse.com). MTR maps were produced, and then the T1 structural image was segmented into grey and white matter using SIENAX. The extracted brain was used to mask the MTR maps and a 75% threshold was applied to the segments to create GM and WM segments. These were then thresholded with 10 percentage units and then eroded with 1 voxel for WB (Whole Brain) and GM (Grey Matter) and 2 voxels for WM (White Matter). ROI (region of interest files where the lesions were marked on PD and T1images) files were included for patients to get histograms of WB, GM, WM, NA (normal appearing) GM, NAWM, T2lesions and T1hypointense lesions MTR, and for controls WB, WM and GM MTR histogram values (peak height, peak location and mean) were obtained.

(v) Visual functional MRI (fMRI):

Adaptive cortical plasticity may contribute to functional recovery in MS and optic neuritis.(255;256) Visual functional MRI measures certain aspects of this phenomenon and have been previously applied in MS and optic neuritis patients.(313-315) In MSCIMS, fMRI was performed on all subjects before treatment once and performed again 6 months post treatment. A total of 69 volumes of T2* weighted echo-planar images depicting blood oxygen level dependent (BOLD) contrast were acquired in each 5 minutes experiment with 52 near axial slices of the whole brain (TR: 3940ms, TE: 30ms, field of view: 192mm, Matrix: 64 x 64, slice thickness of 3mm) which was performed 4 times (total ~ 20 minutes acquisition time) with a different pattern of visual stimulation each time.

The visual stimulation paradigm comprised of eight epochs, each of 16 seconds, of flickering checkerboard stimulation, alternated with eight epochs, each of 16 seconds, of grey background, presented on a projection screen. Subjects wore transparent plano

chromatic filter goggles, with one green and one red filter (Haag-Streit, UK). The checkerboard was also green and red, so that the green checkerboard was invisible through the red filter, and vice versa. This was to allow monocular stimulation while testing both eyes within the same run. To facilitate attention and fixation of a central cross, Subjects were instructed to fixate a central ‘ + ’, and asked to press a button when it changed to a ‘ # ’ symbol. Each experiment consisted of 4 sessions, and the orientation of the goggles was reversed in between, to swap the red and green filters. The analysis and results are further described in chapter 8.(316)

Optical Coherence Tomography (OCT)

OCT is a non invasive technique, that allows quantitative cross sectional measurement of the retinal nerve fibre layer (RNFL) thickness.(317) RNFL is predominantly of unmyelinated axons of retinal ganglionic cells, hence OCT provides an in vivo method of measuring axonal loss following optic nerve damage in MS or optic neuritis.(157;158) OCT makes use of the echo time delay of back-scattered infrared light using an interferometer and a low coherence light source. OCT images were acquired with a Stratus OCT 3000 (Carl Zeiss Meditec, Dublin, CA, USA), by a single observer (MK). Images from the OCT device are given a signal strength by the device up to a maximum value of 10. Images were rejected if the signal strength value was < 7 or if the inter eye signal strength difference was greater than 2.

Retinal Nerve Fibre Layer thickness:

RNFL thickness was measured by taking three circular scans of 3.4mm diameter centred on the optic disc for each eye. The mean of the whole 360 degrees was used to express RNFL thickness (fast RNFL scanning protocol). The thickness of each quadrant of the RNFL was calculated by the device automatically.

Macular volume:

Macular thickness maps were acquired by six linear radial scans centred on the fovea (fast macular thickness map scanning protocol). All subjects (10 patients and 8 controls) had their RNFL thickness and macular volume measured.

Chapter 5: MSCIMS: baseline findings.

5.1 Introduction

MSCIMS aims to primarily test safety and secondarily aims to establish the methodology and infrastructure for future larger studies. It is not powered to establish efficacy although sensitive efficacy outcome measures were employed in the trial to test for any hint of potential efficacy. In this chapter, the baseline characteristics and findings are described.

5.2 Subjects:

Patients

All 10 patients fulfilled revised McDonald criteria for the diagnosis of MS(318) and were classified as secondary progressive MS according to Lublin and Reingold criteria(319). There were 7 males and 3 females, mean age: 48.5 (40-54); their demographic and clinical characteristics are provided in table 5.1.

Table 5.1: Patient characteristics at baseline.

	Age (yrs)	Gender	MS subtype	EDSS	Disease duration	ON Side affected	ON duration (years)
Pt01	44	M	SP	6.5	19 yrs	R	19
Pt02	51	M	SP	6	26 yrs	Both	L-26, R-9
Pt03	40	F	SP	6.5	9 yrs	Both	L-6, R-7
Pt04	48	M	SP	6	14 yrs	L	5
Pt05	48	M	SP	6.5	11 yrs	Both	R-11, L-10
Pt06	52	M	SP	6	18 yrs	R	15
Pt07	54	F	SP	6.5	8 yrs	L	7
Pt08	41	M	SP	5.5	6 yrs	Both	R-7, L-6
Pt09	46	F	SP	6.5	11 yrs	Both	R-7, L-6
Pt10	50	M	SP	6.5	6 yrs	Both	R-6, L-5

All ten participants had secondary progressive MS, with clinical and electrophysiological evidence of optic nerve involvement. Nine patients had history of clinical optic neuritis and one of Uhthoff's phenomenon, occurring between two and twenty-six years before recruitment. Two patients described a single clinical relapse event in the pre-treatment phase, neither of which involved the anterior visual pathway. One patient had been previously treated with disease modifying therapy (beta-interferon for one year, with treatment discontinued due to disease progression two years before recruitment).

Mean EDSS of the patients was 6.25 (5.5 – 6.5). Disease duration of patients as calculated from the time of diagnosis ranged between 6 years and 26 years. 6 out of ten patients had optic neuritis clinically in both the eyes; the remaining four had one clinically affected eye. The total number of clinically affected eyes was 16 and of unaffected eyes was 4. Six (Three patients with bilateral and 3 with unilateral involvement; 9 affected eyes and 3 unaffected eyes) out of 10 patients had experienced almost complete recovery of their vision scoring close to 0.0 in log MAR normal contrast acuity (100%) chart which is equivalent to 6/6 vision in Snellen's chart. Four patients out of ten had incomplete visual recovery. Six out of the eight eyes in this cohort had incomplete recovery. Ten eyes out of

16 affected had recovered almost completely. The Log MAR score ranged between 0.20 to 0.66 in the eyes with incomplete recovery.

Controls:

Eight age and sex matched controls (Six males and 2 females, mean age: 43, range: 30 – 55), who were healthy volunteers with no pre-existing history of neurological and ophthalmological problems were recruited. They underwent 3 MRI and OCT assessments, over the same period of 12 months as patients, to serve as control results to adjust for inter assessment session variability for the MRI and OCT measurements.

5.3 Results

Visual assessments were done on all 10 patients and the results are summarized in table 5.2, along with other clinical and electrophysiological measures. Log MAR visual acuity and low contrast acuities with Sloan charts were assessed monocularly. For 5 eyes in 3 patients, a recording was not possible for the 1.25% contrast chart due to the subject being unable to identify any letter correctly even at the distance of 1 metre; these eyes were assigned a standard value of 1.7. Log MAR acuity mean for patients at baseline was 0.138, SD: 0.183; 25% contrast chart, mean: 0.279, SD: 0.230; 5% chart, mean: 0.617, SD: 0.299; 1.25% chart, mean: 1.014, SD: 0.420; FM 100 hue colour vision mean: 15.28, SD: 4.0; Mean deviation for Humphreys automated perimetry was -3.59dB, SD: 1.989. Visual evoked potentials were measured for all 10 patients and an abnormal latency was found in one of the eyes for all 10 patients. There was no recordable waveform for both central and full field measurement for one of the eyes. Central field latency was not measurable in a further 4 eyes. Mean VER full field latency for the 19 eyes measured was 129.52 with SD: 16.50; Mean VER full field amplitude for the 19 eyes was 4.689 with SD: 1.975; Mean full field latency for the affected 15 eyes was 133.2 with SD: 16.41, amplitude was 4.473 with SD: 2.033; Unaffected eyes mean full field latency was 115.75 with SD: 7.5 and amplitude

was 5.5 with SD: 1.732. There was a significant difference between affected and unaffected eyes with p value of 0.0288.

Table 5.2: Clinical outcomes at baseline

	Number	Mean	SD	Range
Age (yrs)	10	48.5	4.22	(40 – 54)
EDSS	10	6.25	0.35	(5.5 – 6.5)
MSFC z score	10	-0.457	1.678	(-4.963 – 0.821)
ACE (%)	10	93.5	6.24	(79 – 100)
BDI-II	10	7.4	9.48	(0 – 25)
MSIS-29	10	76.4	16.48	(51 – 102)
Log MAR acuity	20	0.138	0.183	(-0.14 – 0.66)
Sloan 25%	20	0.279	0.230	(0 – 0.8)
Sloan 5%	20	0.617	.299	(0.3 – 1.66)
Sloan 1.25%	20	1.014	0.42	(0.54-1.7)
F-M 100 hue (error score)	20	15.28	4.007	(9.38 – 25.92)
Visual field (mean deviation)	20	-3.59	1.98	(-6.75 - -0.07)
VEP latency (ms)	19	129.52	16.50	(106 – 164)
VEP amplitude (μ v)	19	4.689	1.975	(0.9 – 9)

Imaging

Imaging measures are summarized in table 5.3 and 5.4. There was no significant difference between the age distribution of the patients and controls ($p = 0.13$).

Mean optic nerve area for patients ($n=20$) was 8.93 sq mm, SD: 1.542; which was significantly different to controls ($n=16$): 10.25 sq mm, SD: 0.764, ($p = 0.003$).

Mean MTR of optic nerve for patients was ($n=20$): 29.29pu, SD: 3.011 which was significantly different to controls optic nerve MTR ($n=16$): mean 32.91pu, SD: 3.357, ($p = 0.001$).

Mean fractional anisotropy (FA) of the patient optic nerves was 0.314 ± 0.091 and was less than the mean for controls which was 0.530 ± 0.099 which was significant at $p < 0.00001$.

Mean diffusivity (MD) for patient optic nerves was $1195 \times 10^{-6} \text{ mm}^2/\text{s} \pm 281$, Mean diffusivity for control optic nerves was $972 \times 10^{-6} \text{ mm}^2/\text{s} \pm 134$, $p=0.006$.

Axial diffusivity (AD) for patients, mean: $1585 \times 10^{-6} \text{ mm}^2/\text{s}$, SD: 396 was not significantly different to controls, mean: $1588 \times 10^{-6} \text{ mm}^2/\text{s}$, SD: 234, $p = 0.97$.

Radial diffusivity (RD) for patients was $1000 \times 10^{-6} \text{ mm}^2/\text{s}$, SD: 246, and was significantly different to controls, mean: $664 \times 10^{-6} \text{ mm}^2/\text{s}$, SD: 126, $p < 0.00001$.

Brain volume of patients (mean: 1485 cc, SD: 74) was significantly lesser than that of controls (mean: 1671 cc, SD: 53), $p < 0.00001$.

Whole brain MTR for patients (mean: 44.51pu, SD: 6.58) was slightly lower than controls (mean: 46pu, SD: 5.04) but the difference was not statistically significant, $p = 0.60$.

GM MTR for patients (mean: 35.19pu, SD: 7.33) was also very slightly lower than controls (mean: 36.90pu, SD: 5.60) but not significant, $p = 0.59$.

WM MTR for patients (mean: 46.37pu, SD: 6.92) was also slightly lower than controls (mean: 47.55pu, SD: 5.14) but not significant, $p = 0.65$.

RNFL thickness (mean: 76.19 microns, SD: 13.4) and macular volume (mean: 6.264 cubic microns, SD: 0.453) for patients were significantly lower than the controls' RNFL thickness (mean: 101.5 microns, SD: 12) and macular volume (mean: 6.857 cubic microns, SD: 0.372). Both $p < 0.00001$.

Spearman rank correlation between structural and functional measures of optic nerve, RNFL and brain revealed, significant correlation between RNFL thickness and 1.25% contrast acuity ($r=0.52$, $p=0.0175$). RNFL did not correlate with any other visual functional measures in the patient cohort. There was a moderate correlation between optic nerve area and Log MAR visual acuity at $r=0.4263$ with a p value at 0.06. There was slightly lesser correlation of this measure with other visual function measures. With regard to brain measures, the brain volume correlated significantly with cognitive function measures such as 3 second PASAT and ACE-R but not so much with global measures such as EDSS and full MSFC 'z' score.

Table 5.3: MRI optic nerve measures for patients and controls at baseline.

	Patients				Controls				P <
	n	Mean	SD	Range	n	Mean	SD	Range	
Optic nerve									
ON LL (mm)	16	20.25	9.262	3.0-39.0					
ON A (mm ²)	20	8.939	1.542	6.66-12.2	16	10.26	0.764	8.49-11.41	<0.003
ON A (a)	16	8.696	1.491	6.66-11.6					
ON A (ua)	4	9.91	1.539	8.9-12.2					
ON MTR	20	29.29	3.011	24.82-34.8	16	32.91	3.357	25.02-38.6	<0.001
ON MTR (a)	16	28.87	2.988	24.82-32.5					
ON MTR (ua)	4	30.99	2.818	27.99-34.8					
<i>ON DTI</i>									
FA	20	314.73	91.794	174.5-466.5	16	530.63	109.3	259.56-665.85	<0.00001
MD	20	1.195	0.2813	0.727-1.571	16	0.9725	0.0557	0.7429-1.272	<0.006
RD	20	1.0003	0.2467	0.535-1.343	16	0.6649	0.0699	0.4307-0.8743	<0.00001
AD	20	1.585	0.3964	0.891-2.186	16	1.5881	0.1819	1.1762-2.067	0.97
<i>Clinically Affected (a)</i>									
FA (a)	16	294.09	87.34	174.5-459.6					
MD (a)	16	1.1784	0.2849	0.715-1.507					
RD (a)	16	0.9999	0.2525	0.535-1.303					
AD (a)	16	1.5334	0.3942	0.891-2.16					
<i>Clinically Unaffected (ua)</i>									
FA (ua)	4	397.27	62.19	329-466.5					
MD (ua)	4	1.265	0.295	0.896-1.571					
RD (ua)	4	1.002	0.258	0.655-1.264					
AD (ua)	4	1.7915	0.3833	1.376-2.186					

SD: standard deviation; ON A: optic nerve area; (a): affected; (ua): unaffected; MTR: magnetisation transfer ratio; DTI: Diffusion transfer imaging; FA: fractional anisotropy; MD: mean diffusivity; RD: radial diffusivity; AD: axial diffusivity.

Table 5.4: MRI brain and OCT measures of patients and controls at baseline.

	Patients				Controls				p <
	n	Mean	SD	Range	n	Mean	SD	Range	
Brain									
Volume	10	1.4856	0.0746	1.361-1.586	8	1.6712	0.0534	1.591-1.735	<0.00001
T2 LV	10	40.532	30.111	3.44-81.10					
T1 LV	10	10.235	9.491	0.63-19.11					
<i>MTR (percentage units; pu)</i>									
WB MTR	10	44.516	6.585	28.82-52.42	8	46.003	5.047	38.5-50.76	0.6
GM MTR	10	35.197	7.335	20.31-48.24	8	36.903	5.608	27.82-42.61	0.59
WM MTR	10	46.372	6.924	30.16-55.75	8	47.558	5.144	40.29-52.56	0.65
T2 L MTR	10	32.566	7.13	21.5-43.64					
T1 L MTR	10	24.187	7.487	17.26-35.15					
Retina									
RNFL (mic)	20	76.195	13.4	49.52-99.49	16	101.5675	12.071	82.48-117.74	<0.00001
RNFL (a)	16	72.814	12.12	49.52-96.16					
RNFL (ua)	4	89.72	9.908	80.94-99.49					
MV (cu. mic)	20	6.264	0.453	5.393-7.049	16	6.857	0.372	6.106-7.349	<0.00001
MV (a)	16	6.181	0.436	5.393-6.608					
MV (ua)	4	6.596	0.405	6.136-7.049					

SD: standard deviation; LV: lesion volume; (a): affected; (ua): unaffected; MTR: magnetisation transfer ratio; WB: whole brain; GM: grey matter; WM: white matter; RNFL: retinal nerve fibre layer; MV: macular volume.

5.4 Discussion:

The sex ratio in the recruited treatment cohort differs from the expected sex ratio for unselected MS. This finding most likely reflects bias in referral patterns to the screening clinic (46 female referrals / 98 total referrals). Potential reasons for biased referral include factors relating to the referring clinicians, and factors relating to the potential participant such as sex-based differences in attitudes to experimental therapy trials.

The treatment cohort in this trial is typical of patients with established progressive MS in terms of: disability levels at recruitment and low relapse frequency (two participants in the treatment group experienced episodic clinical disease activity). While this group is appropriate for safety-assessment of novel therapies, they may be sub-optimal for assessment of therapeutic efficacy in later phase trials. In order to establish efficacy, a group showing dynamic (active) progression and/or relapsing disease may be preferable. Alternatively, in cohorts showing modest rate of progression/neurodegeneration, longer follow up may be required to achieve sufficient power to achieve statistical significant evidence for efficacy.

All 10 patients with secondary progressive MS had abnormal afferent visual pathway and global brain imaging parameters such as brain volume, which were also significantly different to that of age and sex matched controls at baseline. Having comparative control data is important in a longitudinal parallel arm study to adjust for scanner related inter session variations.

The treatment cohort EDSS range was narrow and all had established secondary progression with one or both optic nerves affected previously. In a proof of concept early phase small trial in a clinically heterogeneous condition such as MS, a group with minimal phenotypic differences will be helpful in attributing the changes observed during follow up to be more likely due to the intervention rather than to heterogeneity. However,

other clinical measures such as visual function, MSFC, ACE-R in the treatment cohort had large standard deviations suggesting considerable clinical heterogeneity.

A mean RNFL thickness of 72 microns for clinically affected eyes versus 89 microns for clinically unaffected patient eyes and 101 microns for normal control eyes are in accordance with previous studies in MS patients with previous optic neuritis. This suggests moderate to severe axonal loss in this cohort of patients. A RNFL thickness of at least 45 microns was the inclusion criteria to exclude patients with very severe axonal loss to avoid a floor effect while testing neuro-axonal protection.

Optic nerve MTR for patients (29.29 pu) were significantly lower than healthy controls (32.91 pu). This suggests that there may also be significant demyelination in the optic nerves of patients. Optic nerve DTI findings were interesting in that, the FA of patients' optic nerves were significantly lower than controls. Correspondingly, there was also a higher mean diffusivity for patients than controls. This suggests disruption of structural integrity of the optic nerves in patients. . Animal models suggest that DTI-derived axial and radial diffusivity reflect axonal loss and demyelination respectively. [260] In our study, mean radial diffusivity but not axial diffusivity was increased in nerves affected by previous optic neuritis compared to controls. This finding would thus be consistent with limited axonal loss and predominant demyelination in the affected nerves, and is also consistent with the findings in previous DTI studies of optic neuritis. This is in discrepancy with RNFL findings mentioned above, which suggests moderate to severe axonal loss. This may be because RNFL is a more specific and sensitive measure of axonal loss than axial diffusivity. Other pathophysiological processes such as remyelination, gliosis apart from axonal loss and demyelination may affect DTI parameters. Taken together, prolonged VER latency and reduced amplitude, the optic nerve cross sectional area, optic nerve MTR & DTI measures and RNFL thickness, suggests both significant demyelination and axonal loss in the patient optic nerves at

baseline. However, most patients had good visual functional recovery. This may be explained by the considerable redundancy of the optic nerve fibres or another mechanism such as adaptive plasticity in the brain at baseline as described in chapter 2.

Due to the small number of the patient cohort, most of the structural and functional measures did not show significant correlations. However, this is baseline data of a longitudinal study with pre vs. post intervention comparison method and one is mainly interested in any longitudinal change to patients that could be attributed to the intervention.

Chapter 6: MSCIMS: Clinical and safety results

6.1: Introduction:

MSCIMS is a phase IIa study with safety as the primary outcome. In this chapter the results of the safety measures are described followed by results of clinical outcome measures.

Various non-visual and visual clinical assessments were performed serially on patients as per schedule given in table 6.1. Non-visual clinical measures included EDSS, MSFC, ACE-R, BDI-II, MSIS. Visual clinical measures include high and low contrast acuities using log MAR charts (100%, 25%, 5% and 1.25%), colour vision using Farnsworth-Munsell 100 hue test, visual fields using Humphrey's automated perimetry.

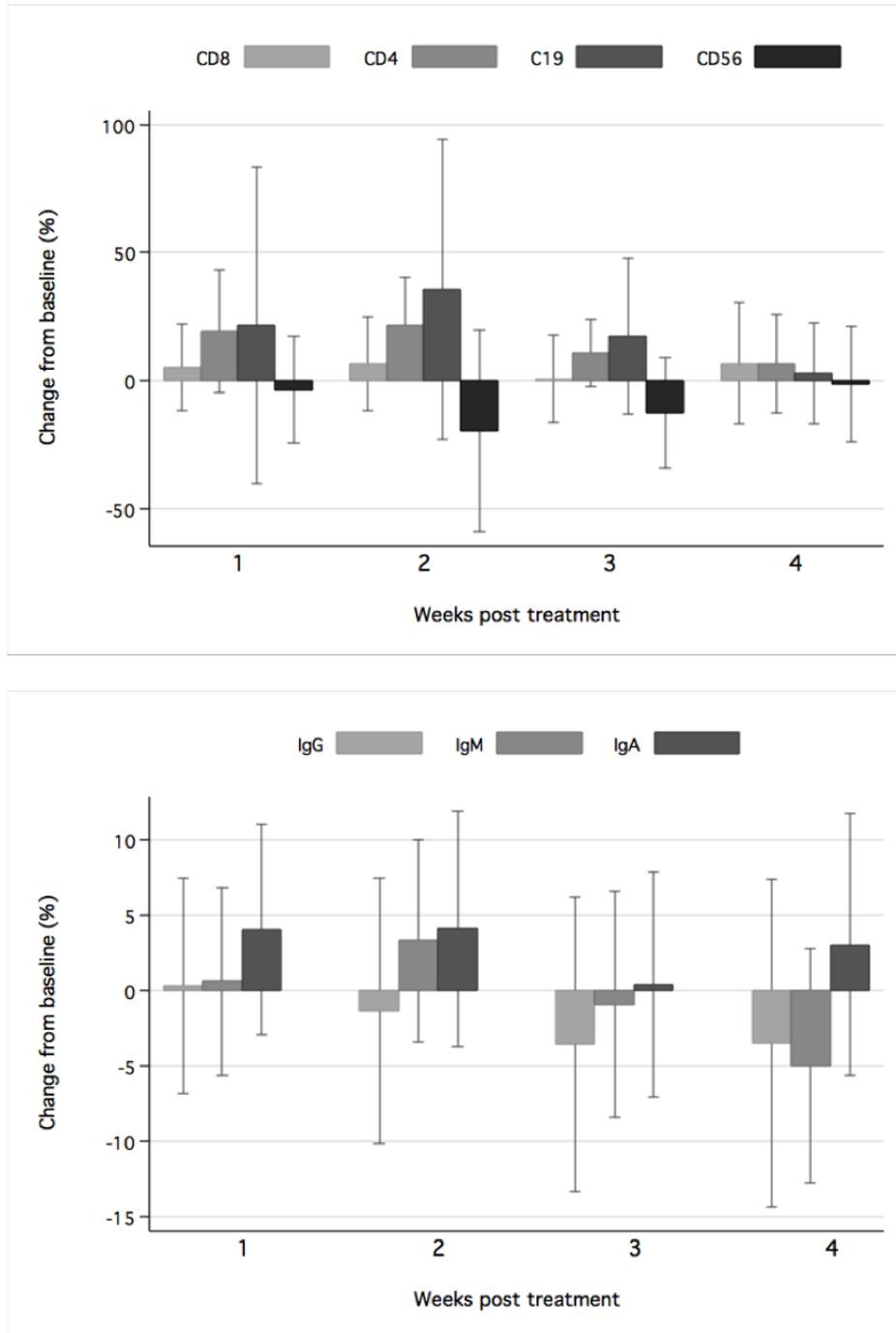
6.2: Safety results:

All patients received a single infusion of autologous MSCs after monitoring during the pre-treatment phase for a mean of 17.3 months (min–max range 14.1–20.9). The mean administered dose was 1.6×10^6 cells per kg bodyweight (min–max range 1.1–2.0). No adverse events were observed during infusion. One patient developed a macular rash over the anterior chest at approximately 3 hours that resolved spontaneously over 12 hours; a further patient described scalp pruritus beginning one week after treatment and resolving spontaneously two weeks later. Two patients had infections: a self-limiting upper respiratory tract infection three weeks after infusion (not requiring treatment); and an *E. Coli* urinary tract infection four weeks after infusion (treated with oral antibiotics).

Weekly (x4) blood testing of clinical chemistry, haematology, and immunology was unremarkable. Compared to pre-treatment levels, no changes were seen in the post-

treatment period for T-cell subset counts (CD3, CD4, CD8, CD19, and CD56), or humoral immunity assessed by total serum immunoglobulin (IgG, IgM, IgA) levels (figure 6.1) and titres to common antigens (mumps, measles, rubella, varicella zoster, tetanus, haemophilus influenza type B, and pneumococcal antigens 1, 3, 4, 5, 6B, 7F, 8, 14, 18C, 19A, 19F, and 23F). No delayed adverse events were observed during post-treatment phase (mean 7·0 months; min–max range 5·8–10·2)

Figure 6.1: Change in mean lymphocyte subset counts and serum immunoglobulin levels following treatment



Percent change in mean lymphocyte subset counts (upper panel) and serum immunoglobulin levels (lower panel) are shown for the four weeks following infusion of autologous MSCs. Comparison is made to levels at the time of infusion. Vertical lines indicate 95% confidence intervals.

6.3: Clinical assessment results:

6.3.1: Statistical methods:

The MSCIMS trial methodology has been described in detail in chapter 4. Statistical advice from a senior statistician (DA) was obtained from the time of the design of the protocol up to publication of results. Statistical analysis was performed using the software STATA SE (version 9.2 and 11). The assessment schedule is illustrated in Table: 6.1.

For ‘whole-patient’ data, in order to assess the change in gradient over time for a given measure at the point of intervention, piecewise linear mixed models were used with the measure as response variable, and with predictors: time from intervention and a time X after interaction term (where *after* is a binary indicator taking value 1 for data points occurring after the intervention, 0 for those before). The coefficient on time is then interpreted as the estimated gradient before intervention, the coefficient on the interaction term is the estimated change in gradient after minus before; and the sum of the two coefficients estimates the gradient after intervention. Tables: 6.2, 6.3, 7.1 and 7.2.

For the corresponding analyses of optic data involving a separate set of measures over time for each eye, an indicator term identifying the eye was included as a fixed effect in models otherwise as used above. Note that evidence for a change in gradient following intervention must come from a test of this change, not from observing a difference in the separate gradients before and after: comparison of the before and after p-values, for example, although interesting and potentially worth noting, does not provide statistical evidence of a change, which can only come from the single p-value testing of the change.

The design of this study is a before/after treatment comparison, without a randomised parallel arm of patients who do not experience the intervention. It is extremely important to bear in mind that any effect apparently due to intervention in this type of design cannot be attributed causally to the intervention itself; and therefore that any observed before/after changes, however promising, should be treated with caution. With this understood, however, it is worth noting changes which approach but do not reach significance, because of the inherent lack of power in the present context: although the analysis method used above are powerful, and allowed use of all available data points, nevertheless with such a small number of patients there was only power to detect substantial change, and in the less noisy measures.

Table 6.1: Assessment schedule

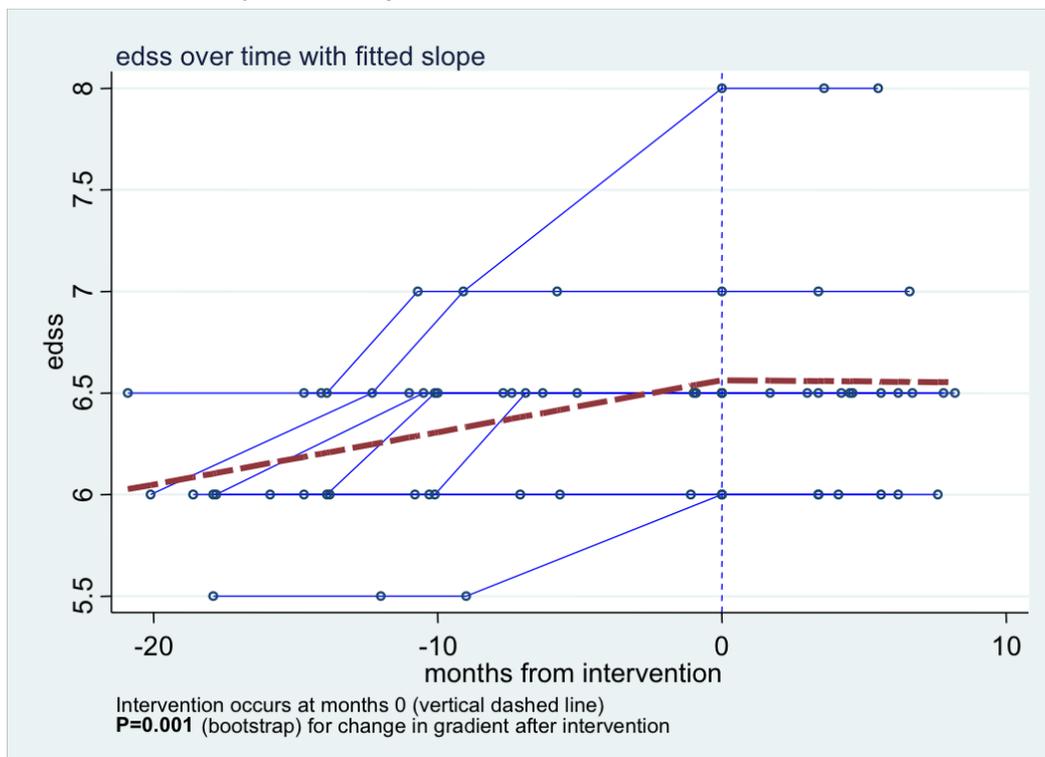
Time-line Visit number	Pre-treatment								Post-treatment							
	-12 months		-9 months		-6 months		0 months		1 week	2 weeks	3 weeks	4 weeks	3 months		6 months	
	1	2	3	4	5	6	7	8	9	10	11	12	13	14	15	16
EDSS	X		X		X		X						X		X	
MSFC	X		X		X		X						X		X	
MSIS-29	X		X		X		X						X		X	
BDI-II	X		X		X		X						X		X	
ACE-R	X		X		X		X						X		X	
VER	X		X		X		X						X		X	
Log MAR visual acuity		X		X		X		X						X		X
FM-100		X		X		X		X						X		X
Visual field		X		X		X		X						X		X
OCT		X		X		X		X						X		X
MRI ON		X		X		X		X						X		X
MRI Brain		X		X		X		X						X		X
MRI Brain (DTI)				X												X
Clinical chemistry	X						X		X	X	X	X				
FBC, ESR	X						X		X	X	X	X				
Immune panel							X		X	X	X	X				
PT & APTT	X						X		X	X	X	X				

EDSS = expanded disability status scale; MSFC = multiple sclerosis functional composite; BDI-II = Beck depression inventory II; ACE-R = Addenbrooke's cognitive examination (revised); FM-100 = Farnsworth Munsell 100 Hue test; VER = visual evoked responses; OCT = optical coherence tomography; MRI ON = MRI of optic nerve; DTI = diffusion tensor imaging; Clinical chemistry = serum urea and electrolytes, liver function tests, serum calcium, glucose, & thyroid function; FBC = full blood count; ESR = erythrocyte sedimentation rate; Immune panel = serum complement, immunoglobulins, common antigen titres, and lymphocyte subset counts; PT = prothrombin time; APTT = accelerated partial thromboplastin time.

6.3.2: Non visual clinical measures:

6.3.2.1: EDSS: EDSS was measured using functional systems scoring objectively by a single observer (MK) for a patient throughout the study to minimise observer bias. There was a significant increase in EDSS in the pre treatment phase, this halted during the post treatment phase and the gradient of change at the point of intervention was statistically significant. Fig: 6.2 & Table: 6.2

Figure: 6.2 EDSS (Patients)



6.3.2.2: MSFC (Multiple Sclerosis Functional Composite):

All three components of MSFC (25ft timed walk, 9 hole peg test and 3second PASAT) were performed by the same observer (PC) for all time points. There was a gradient change especially in the timed walk component of the MSFC. However this did not achieve statistical significance also the result is a bit skewed due to a single subject in whom the pre treatment deterioration was substantial. Figures 6.3 (a-d) & Table: 6.2.

Figure 6.3 (a) MSFC 'z' score (Patients)

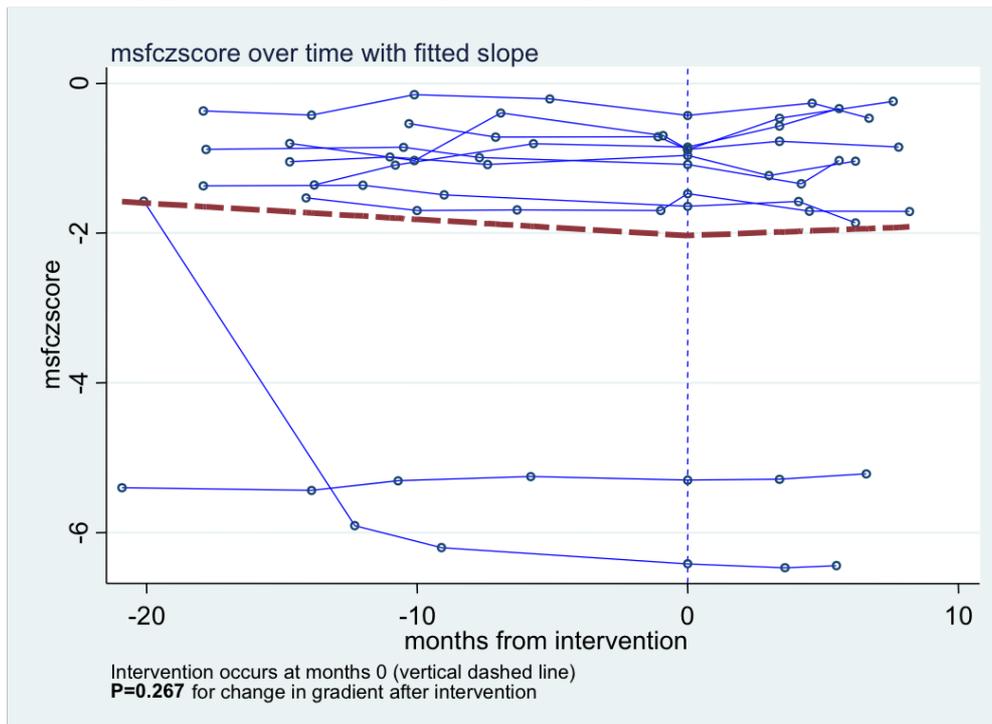


Figure: 6.3 (b) Inverted timed walk (Patients)

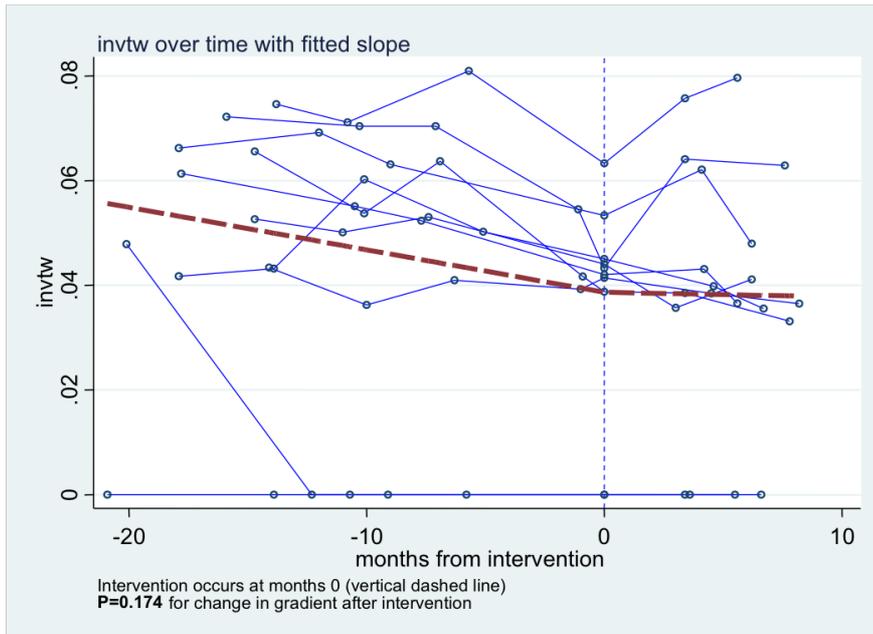


Figure 6.3 (c) Nine hole peg test (z score for arm function) (Patients)

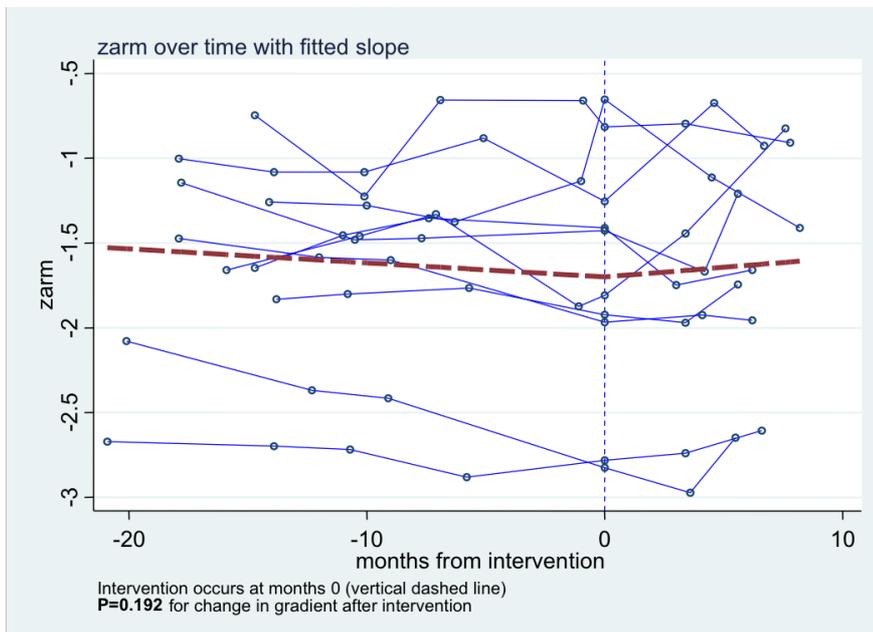
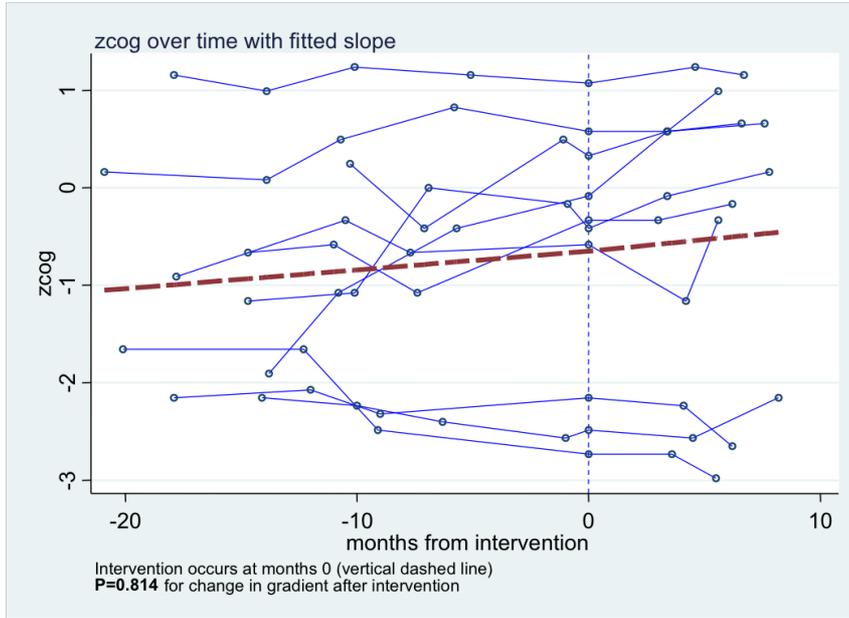


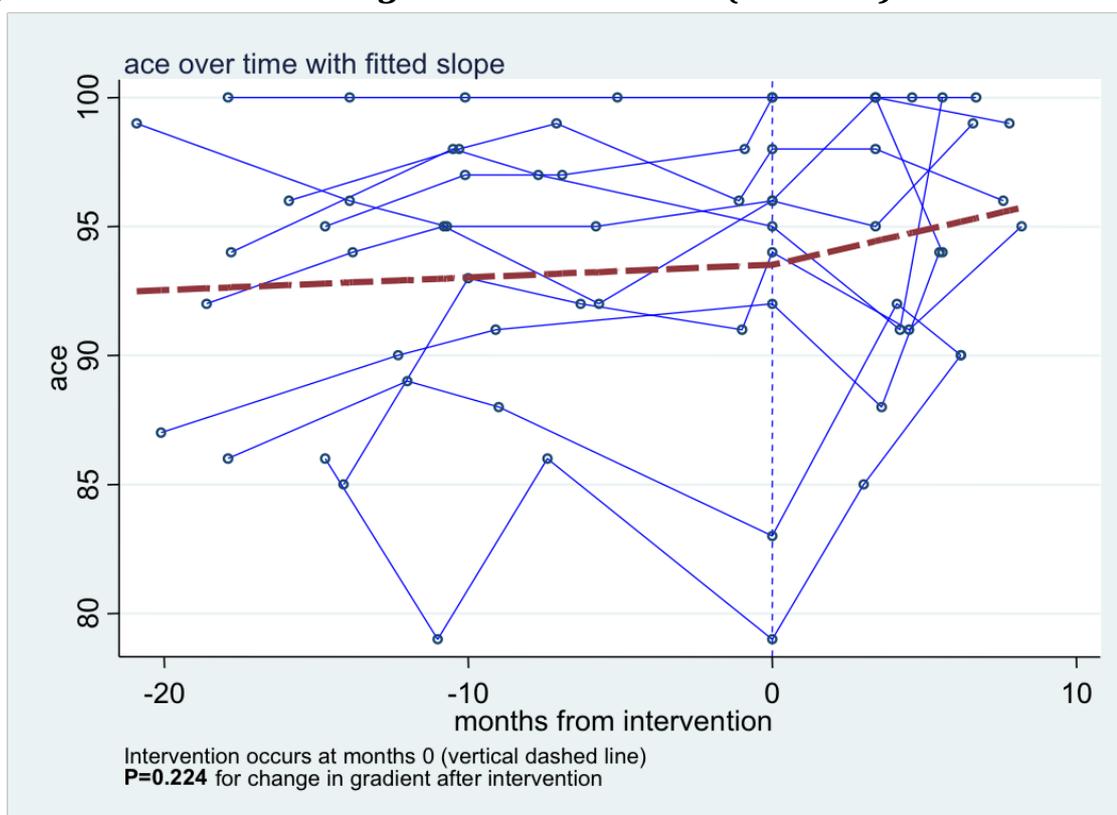
Figure 6.3 (d) PASAT (cognitive function z score) (Patients)



6.3.2.3: ACE-R: Addenbrooke's Cognitive Examination – Revised:

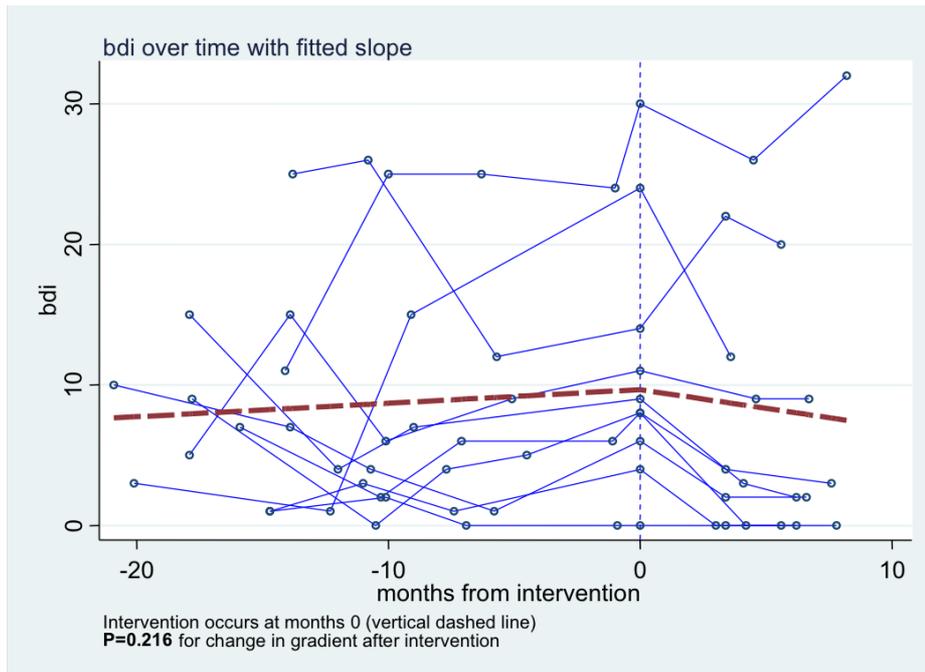
ACE-R was performed by the same observer (PC) for the patients across all time points to minimise observer bias. There was a borderline significant change in the post treatment phase. But there was no significant change in the gradient at the point of intervention. Figure: 6.4 & Table: 6.2

Fig 6.4: Addenbrooke's cognitive examination (Patients)



6.3.2.4: Becks depression inventory II score: BDI-II score was obtained with the self scoring questionnaire. There was no significant change of gradient at the point of intervention. Fig: 6.5 & Table: 6.2.

Figure 6.5: BDI-II (Becks depression inventory)



6.3.2.5: MSIS (Multiple Sclerosis Impact Scale): This is another self reported score which was obtained for all time points pre and post intervention. As described earlier it contains questions about both physical and psychological aspects, which could be affected by MS. There was no change of gradient across the point of intervention for either the physical or the psychological aspects of MSIS scores. Figure 6.6 (a-c) & Table: 6.2.

Figure 6.6 (a): MSIS (physical)

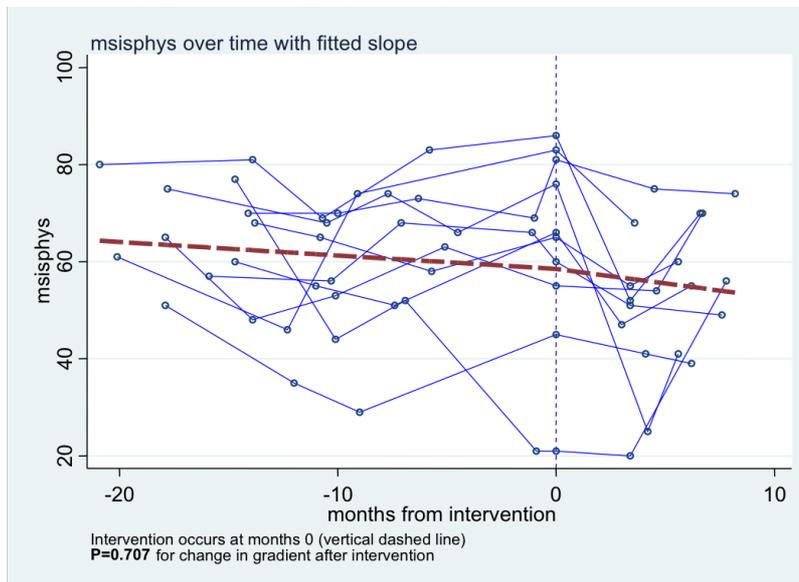


Figure 6.6 (b): MSIS (psychological)

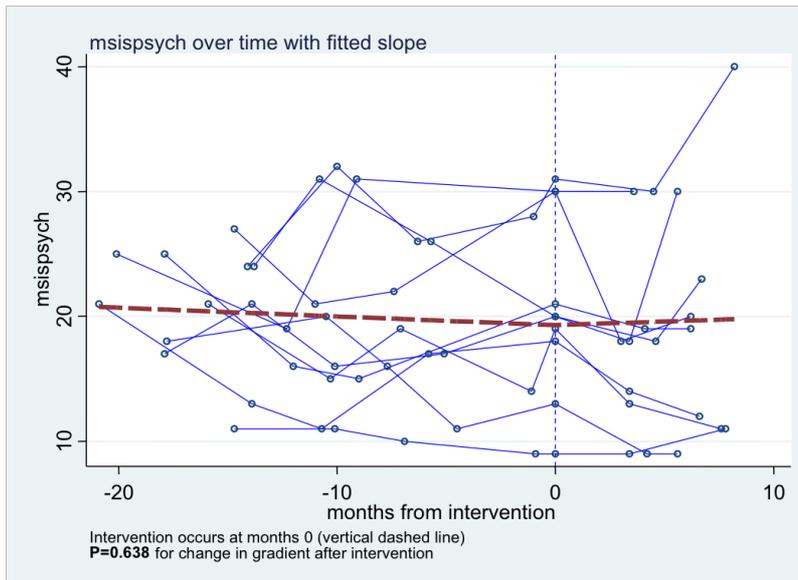


Figure 6.6 (c) MSIS total

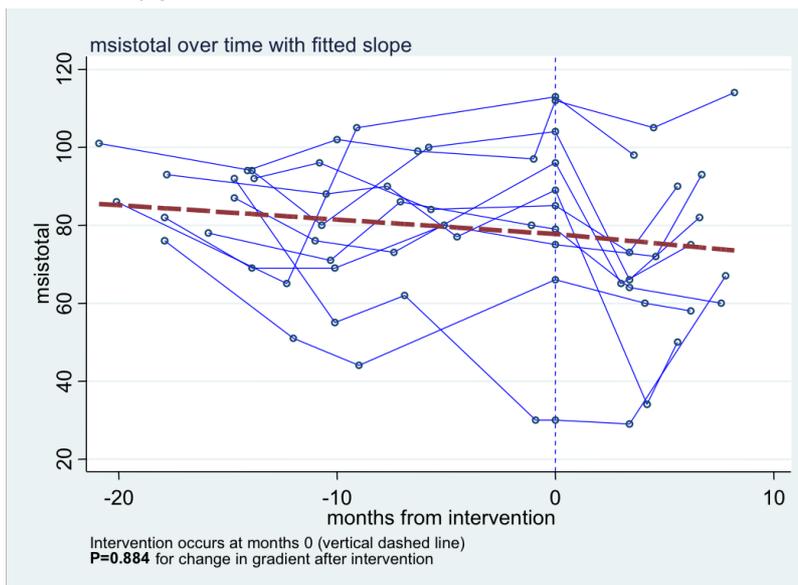


Table 6.2: Change in non-visual clinical outcomes before and after treatment

<u>Outcome Measure</u> (Units)	<u>Rate of change</u> (Units per month)		<u>Difference in rate of change following treatment</u>		<u>p</u>
	Before treatment	After treatment	95% CI		
EDSS	0.0257	-0.0012	-0.0269	-0.0431 to -0.0107	0.001
MSFC (z score)	-0.0217	0.0141	0.0359	-0.0275 to 0.0992	0.267
ACE-R	0.0492	0.2690	0.2198	-0.1343 to 0.5739	0.224
BDI-II	0.0965	-0.2663	-0.3628	-0.9378 to 0.2121	0.216
MSIS-29	-0.3710	-0.5152	-0.1443	-2.0865 to 1.7979	0.884

Piecewise linear mixed model regression is shown for non-visual clinical outcomes, with confidence intervals and significance tests for a change in gradient at the time of treatment. EDSS: Expanded Disability Status Scale; MSFC: Multiple Sclerosis Functional Composite; ACE-R: Addenbrooke's Cognitive Examination – Revised; BDI-II: Becks Depression Inventory – II; MSIS: Multiple Sclerosis Impact Scale - 29

6.3.3: Visual function:

6.3.3.1: Contrast acuity:

High and low contrast acuity: Log MAR visual acuity:

Visual acuity assessments were performed using retro illuminated log MAR charts and Sloan charts for low contrast (25%, 5%, 1.25%) at a distance of 4m by the same observer (MK).

While there was no significant change for all four values pre treatment, there was significant decline (improved vision) post treatment. This was highly significant for the gradient change across the time of intervention. Figure: 6.7 (a-d) & Table: 6.3.

Figure 6.7 (a): Log MAR acuity:

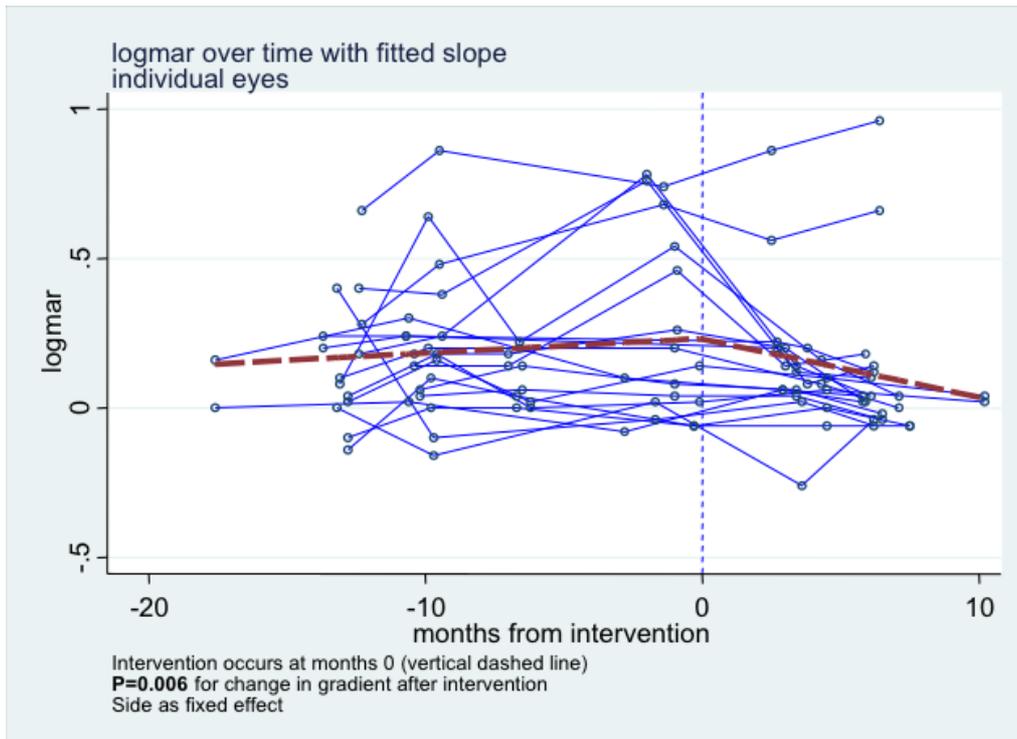


Figure 6.7 (b): Sloan 25% contrast acuity:

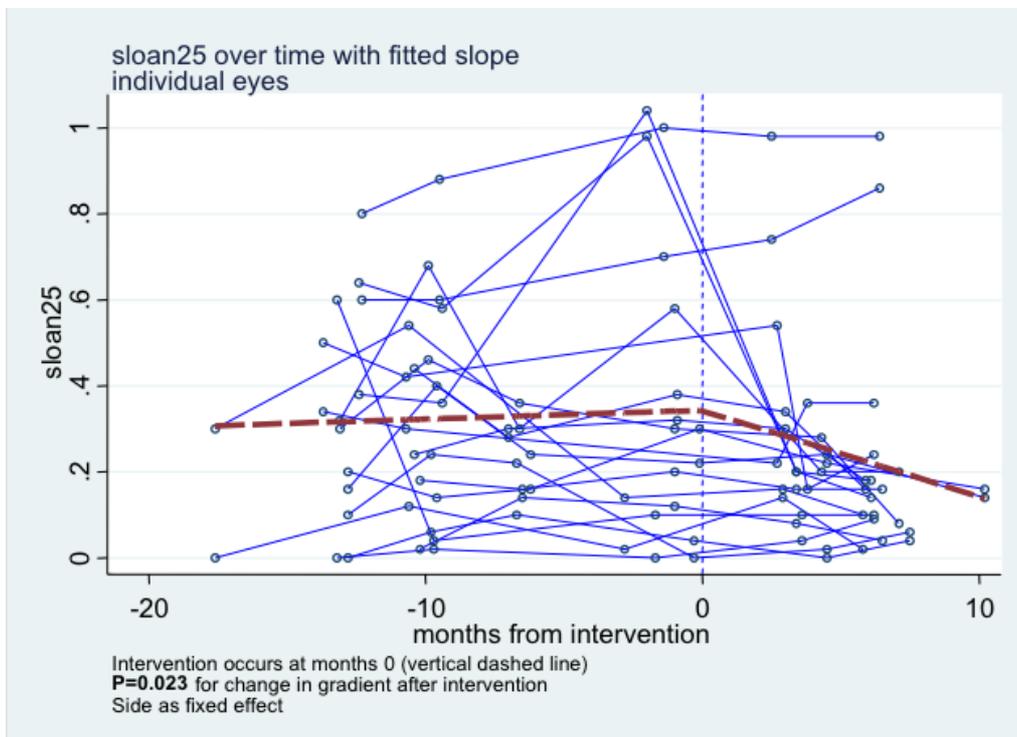


Figure 6.7 (c): Sloan 5% contrast acuity:

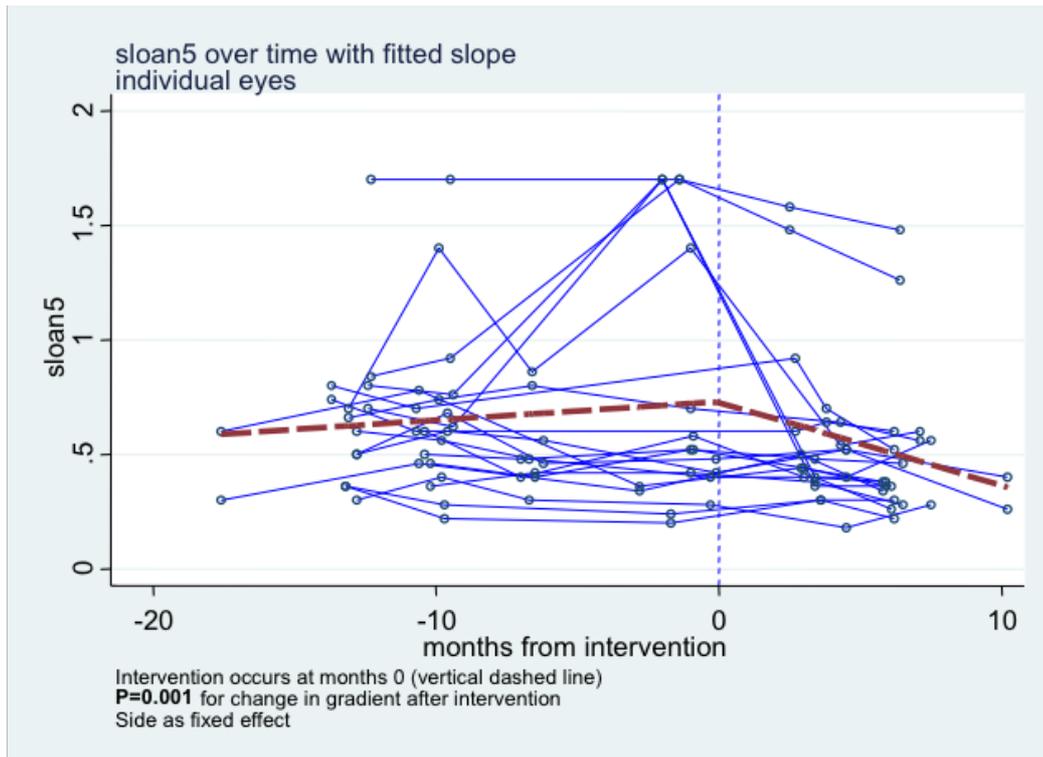
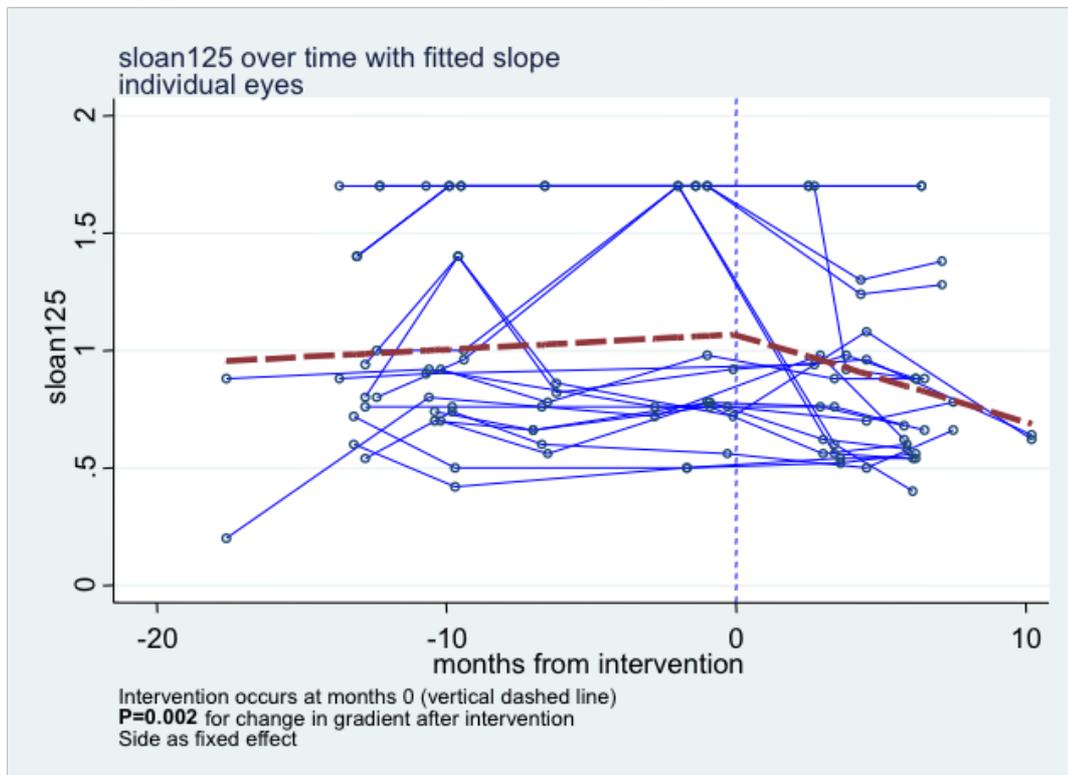


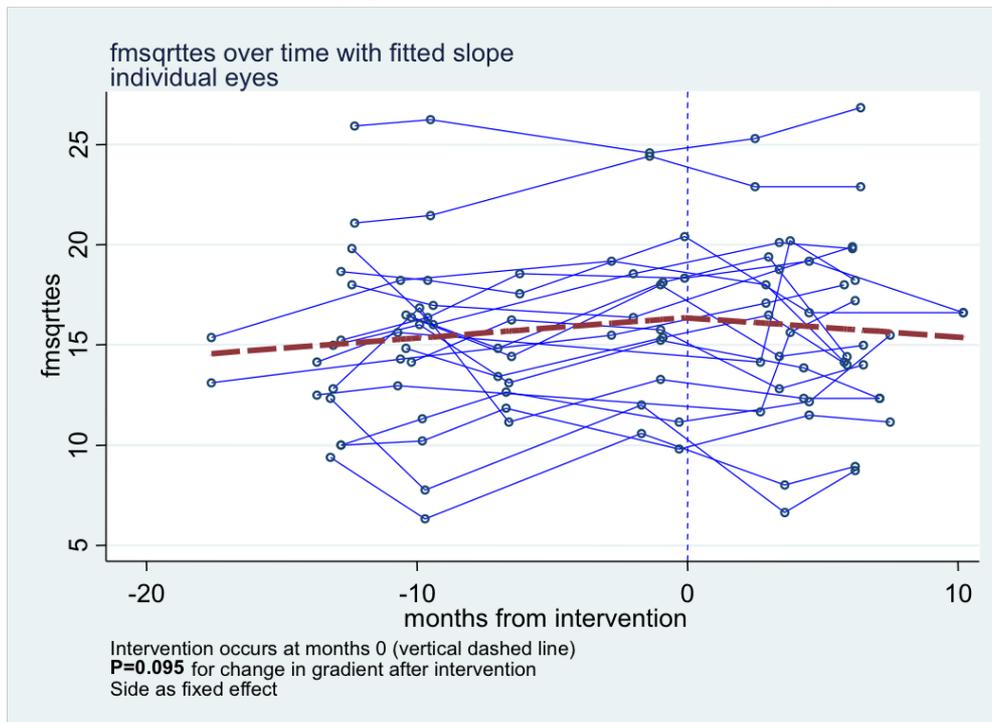
Figure 6.7 (d): Sloan 1.25% contrast acuity:



6.3.3.2: Colour vision: Farnsworth-Munsell 100 Hue colour vision test:

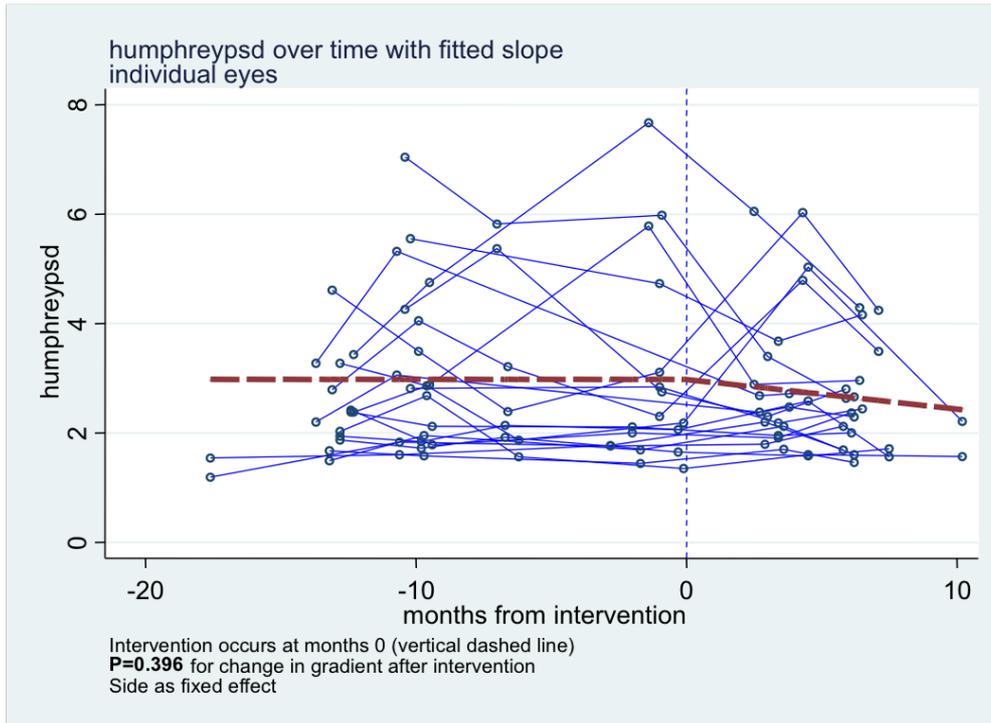
FM 100 hue test was performed at all time points by the same observer (MK). Total error score was calculated using the accompanying software and the square root of the total error score was plotted for each time point. There was a significant increase in the error score pre intervention phase and this was stopped post intervention and the gradient across the point of intervention was borderline significant. Fig 6.8 & Table 6.3.

Figure 6.8: FM100hue (square root total error score):



6.3.3.3: Humphreys automated perimetry (Visual fields): Objective automated measurement of visual perimetry was obtained for each time point using the SITA 30-2 protocol as described in chapter 4. There was no significant change in the gradient across the point of intervention. Figure 6.9 & Table 6.3.

Figure 6.9: Humphreys automated perimetry



6.3.4: Visual evoked potentials:

Visual evoked potentials were recorded for each time point for patients and were analysed by single observer (AWM) blinded to time points. Both central (cf) and full field (ff) latencies and amplitudes were measured. There was a significant increase in latencies in both the central and full field measurements only during the pre treatment phase with the gradient of change across the period of intervention being significant. Similarly there was also a significant decrease in the amplitudes (both cf and ff) in the pre treatment phase alone with an increase post treatment with significant change in gradient across the intervention. Figure: 6.10 (a-d) & Table: 6.3.

Figure 6.10 (a): VER cf latency:

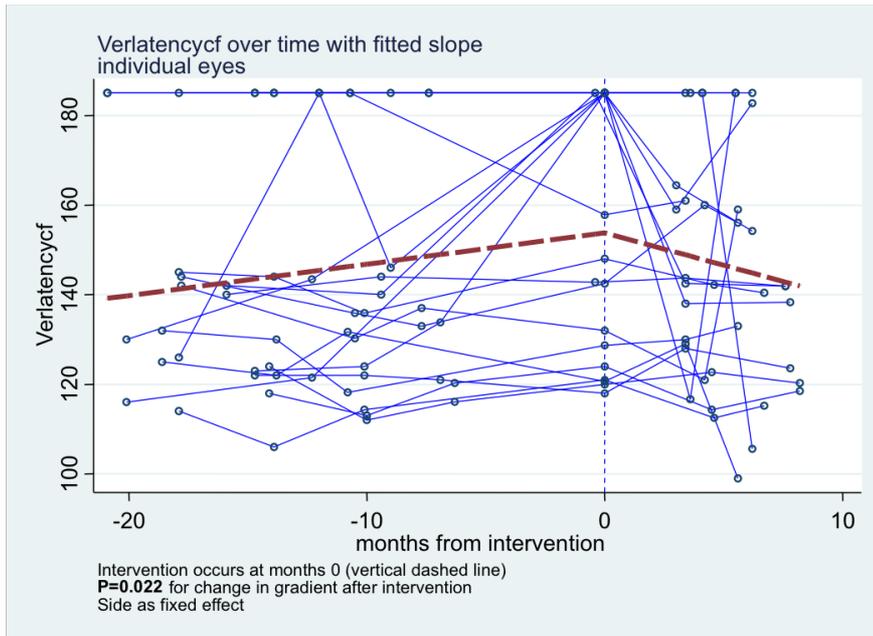


Figure 6.10 (b): VER ff latency:

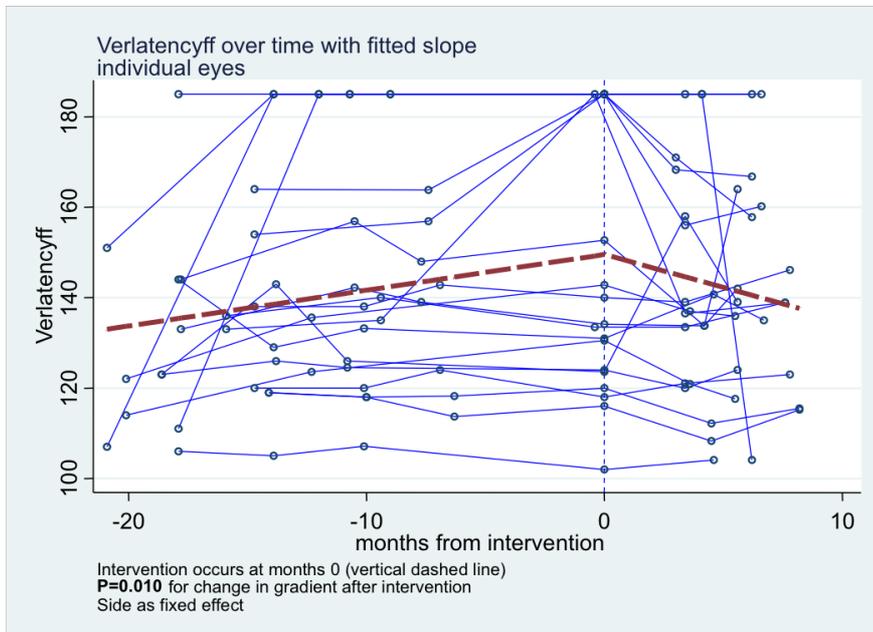


Figure 6.10 (c): VER cf amplitude:

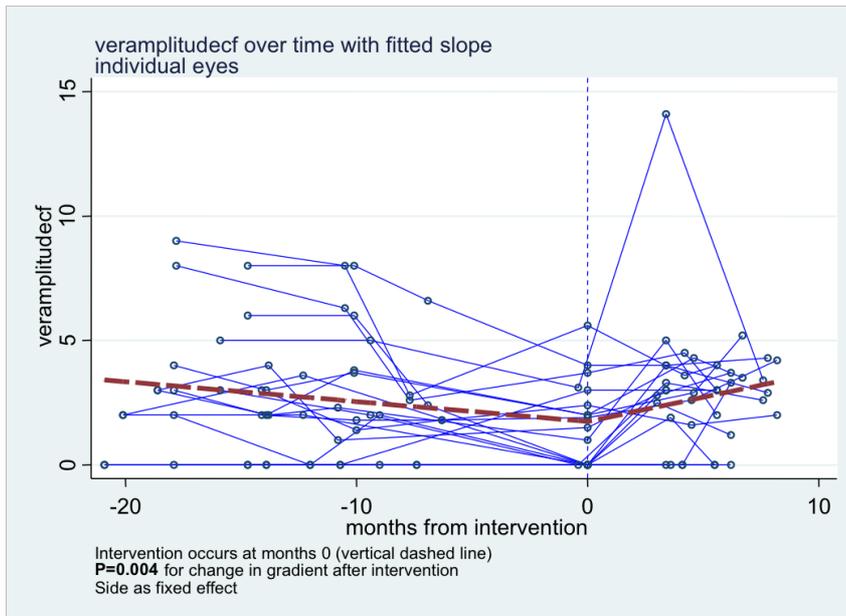


Figure 6.10 (d): VER ff amplitude:

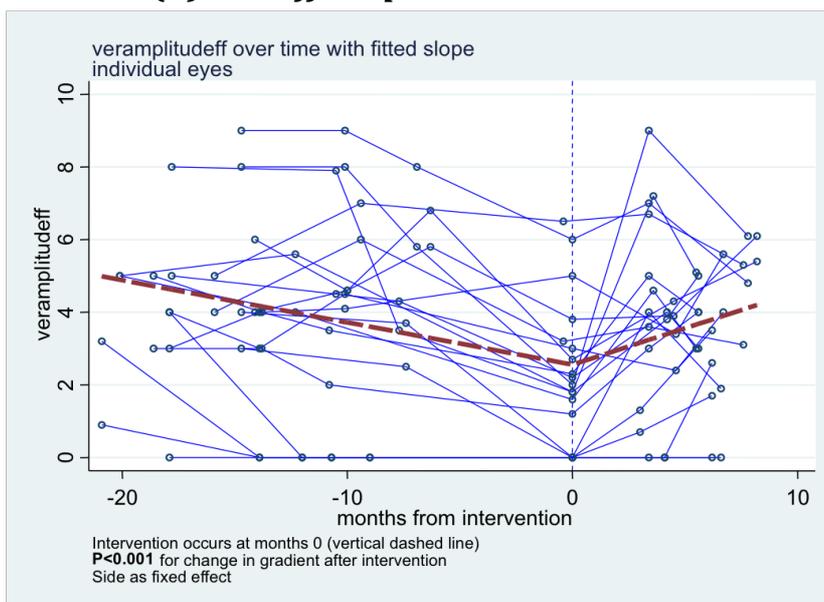


Table 6.3: Change in visual function and VER outcomes before and after treatment

<u>Outcome Measure</u>	<u>Rate of change</u> (Units per month)		<u>Difference in rate of change following treatment</u>	<u>p</u>	
	Before treatment	After treatment			95% CI
Visual acuity (LogMAR)	0.0050	-0.0207	-0.0205	-0.0325 to -0.0085	0.006
25% contrast acuity (logMAR)	0.0022	-0.0207	-0.0202	-0.0330 to -0.0073	0.023
5% contrast acuity (logMAR)	0.0083	-0.0372	-0.0371	-0.0560 to -0.0181	0.001
1.25% contrast acuity (logMAR)	0.0063	-0.0370	-0.0369	-0.0552 to -0.0185	0.002
Colour vision (FM-100 $\sqrt{\text{total error score}}$)	0.1017	-0.0975	-0.1011	-0.2567 to 0.0544	0.095
Visual field (mean deviance)	0.0395	0.00311	0.0192	-0.1062 to 0.1445	0.396
Full field VER latency (ms)	0.4843	-0.8438	-1.3280	-2.4447 to 0.2114	0.010
Full field VER amplitude (μV)	-0.1084	0.1503	0.2587	0.0705 to 0.4469	0.001

Piecewise linear mixed model regression is shown for visual outcomes, with confidence intervals and significance tests for a change in gradient at the time of treatment. FM-100 = Farnsworth Munsell 100 Hue test; VER = visual evoked response.

6.4: Discussion:

The absence of significant adverse events in the short-term follow up suggests that the use of autologous mesenchymal stem cells intravenously in patients with MS is feasible and probably safe. The questions about the safe dose, safety of other routes of administration (eg: intrathecal), whether any adverse events that may arise on repeated administration and long term adverse effects have not been answered in MSCIMS. Studies specifically designed to answer these questions would provide further valuable information in the path towards clinical translation of stem cell therapeutics in multiple sclerosis.

Clinical measurements unlike imaging are influenced by both observer bias and anticipation bias from the subject as they could try harder post intervention. There is also a learning effect for some of these measurements especially cognitive assessments. These biases can be minimised by blinding and randomising. However MSCIMS was an open label trial and blinding can be difficult to obtain in a clinical examination where the tests require direct contact and are not automated. In MSCIMS, a single observer obtained the measurements for the subjects across time points to minimise inter-observer variations.

EDSS is a global clinical scale that is also less sensitive to change. The significant increase in EDSS pre treatment phase was halted post treatment with the change in gradient at the point of intervention was statistically significant. In a small study of only 10 patients, any significant change in one or two patients can significantly affect the outcome. However, the inference with caution that can be made from this finding

is that stem cells treatment may have clinically slowed down accumulation of disability.

MSFC, which has 3 components showed similar trends of improvement without achieving statistical significance. PASAT improvement across the time points was linear suggesting learning and practice could have been the reason.

ACE measurements showed improvement post treatment without significant effect across the gradient. This could again be due to a learning effect.

BDI II and MSIS are self reported scores by the patients themselves. Although such self reported measures are validated, any inference made from changes in these measures has to be taken with lot of caution. Both these measures did not however change significantly across the treatment point.

Visual function outcomes are interesting in that there was a significant improvement across all four contrast acuity measurements post treatment. The colour vision also showed a borderline significant change in gradient across the treatment point. Humphrey's automated perimetry, which is the most objective of the three visual assessments, did not change significantly. Fatigue and false positives and negatives can affect Humphrey's. Despite of no significant change in the perimetry, the visual functional improvement in acuity and colour vision may reflect a treatment effect of mesenchymal stem cells considering the corresponding improvements in objective and blinded measurements such as imaging (an increase in optic nerve area) and VEPs (function).

An examiner blinded to the time point of the subjects objectively examined vERs. There was a significant improvement in both the amplitudes (generally a measure of axonal function) and the latencies (generally a measure of myelin function) at the point of treatment. The potential mechanism could be remyelination or adaptive plasticity. Neuroprotection itself could prevent worsening but cannot be accounted for improvement of conduction and/or function.

As discussed earlier the potential mechanisms through which these mesenchymal stem cells could act are many. With objective measurements of structure, function and physiological measures although helpful in speculating the potential mechanisms, it is difficult to make significant inferences from a small study of ten patients. MSCIMS has safety as the primary outcome measure and any hint of efficacy would be helpful information in designing future larger studies.

Chapter 7: MSCIMS: Imaging results

7.1: Retinal imaging results:

Retinal nerve fibre layer thickness around the peripapillary retina and the macular volumes were measured for both pre vs. post treatment phases as described in chapter 4. There was no change in both the measures gradients across the time of intervention. (Fig: 7.1a and b) (Table: 7.1).

Fig: 7.1 (a) RNFL thickness (microns) patients

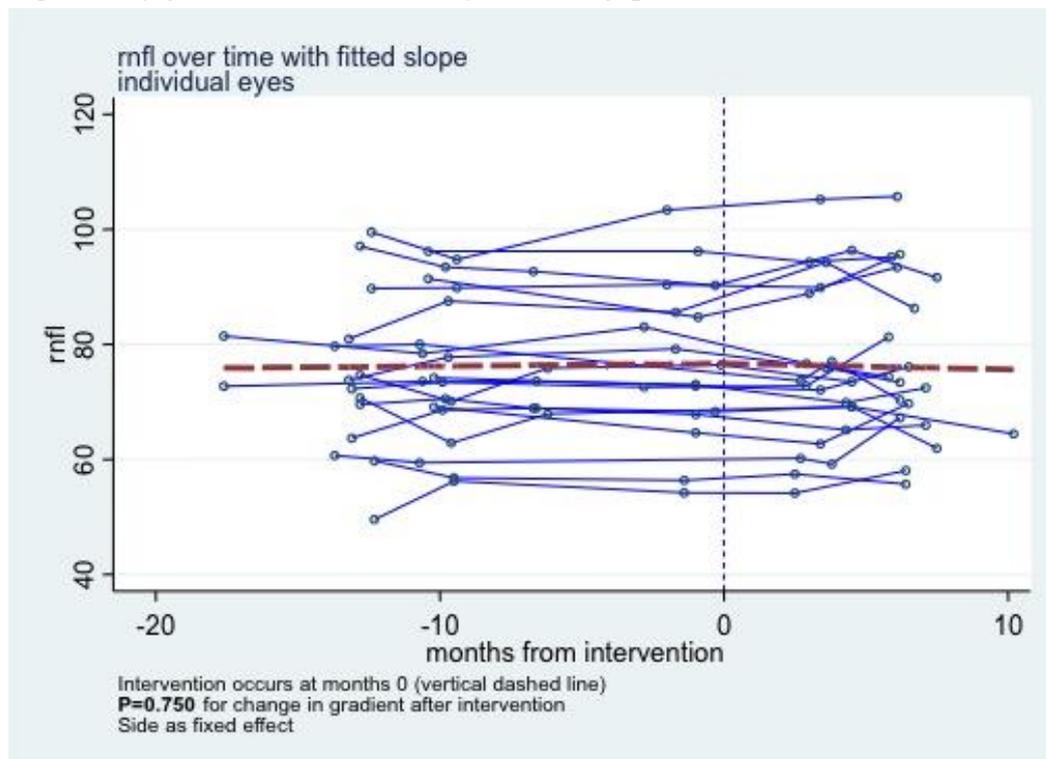
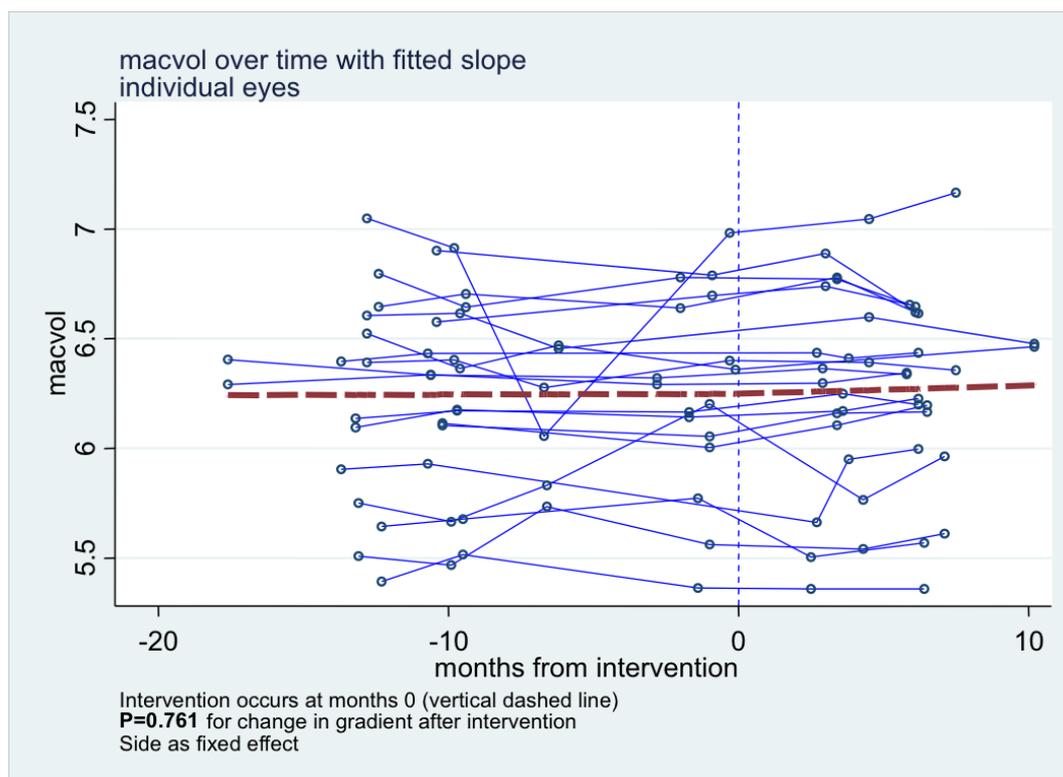


Figure 7.1 (b) Macular volume (mm³) patients



7.2: Optic nerve imaging results:

7.2.1: Optic nerve area:

Optic nerve fat saturated short echo fast FLAIR images (chapter 4) for the patients and controls were analysed and the mean cross-sectional intra-orbital optic nerve area was calculated by a single observer blinded to the subject status and the time points. The FLAIR images were renamed randomly using a five-digit number generated by the computer with the key stored separately. The images of all subjects for all time points were contoured and optic nerve area was calculated. Then they were matched with keys and unblinded.

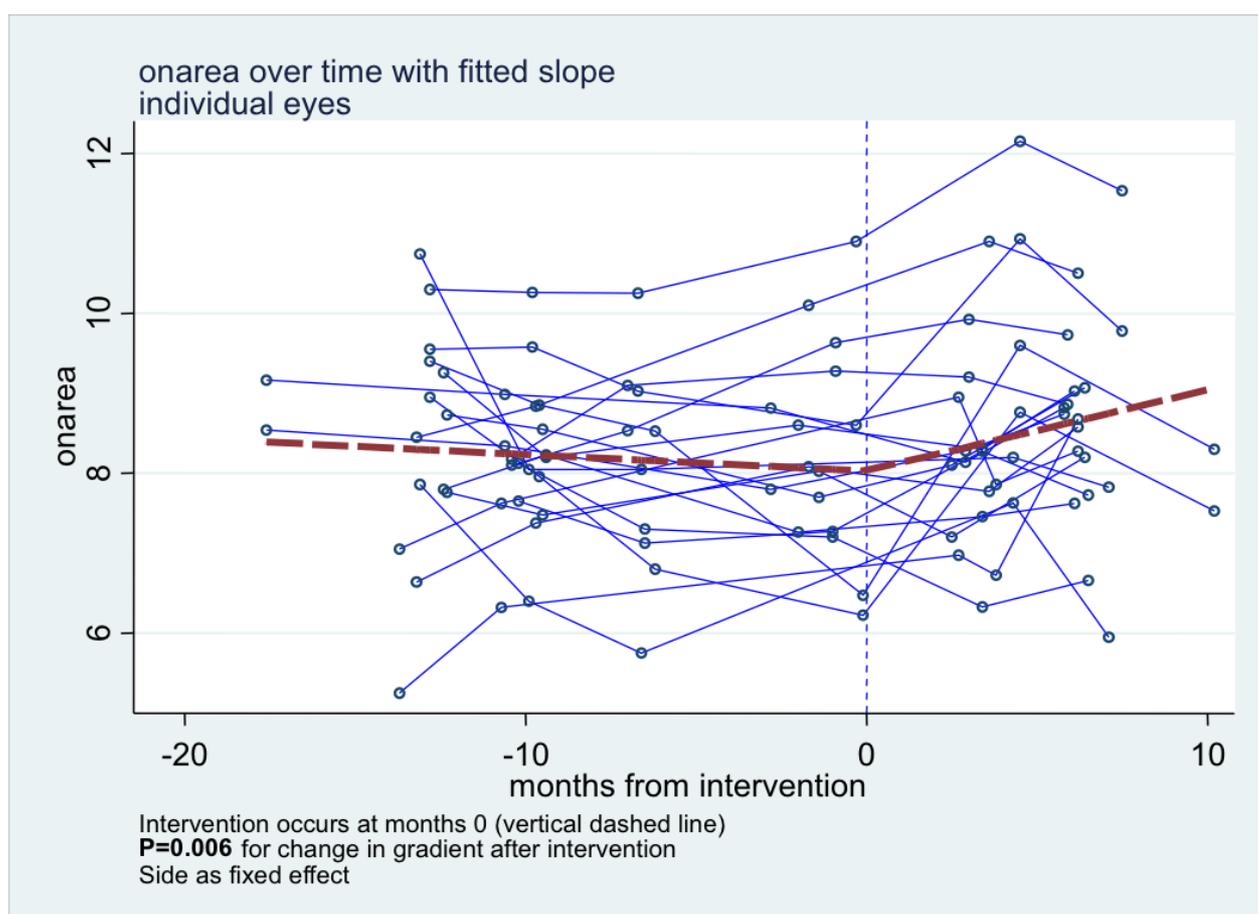
Fig. 7.2 shows the graph representing the change pre vs. post intervention.

There is a slight but non-significant decline over time in this measure during (and only during) the pre-intervention period, followed by a statistically significant increase post intervention, with also a significant change in gradient. There was an increase following

treatment in optic nerve area with difference in monthly rates of change $+0.1262 \text{ mm}^2$ [95% CI 0.0368 to 0.2155], $p = 0.006$. Table: 7.1.

The healthy volunteer controls during the same time did not show any significant change of optic nerve area with intra-individual SD of 0.85 and $p = 0.815$.

Fig: 7.2 Optic nerve area (Patients)



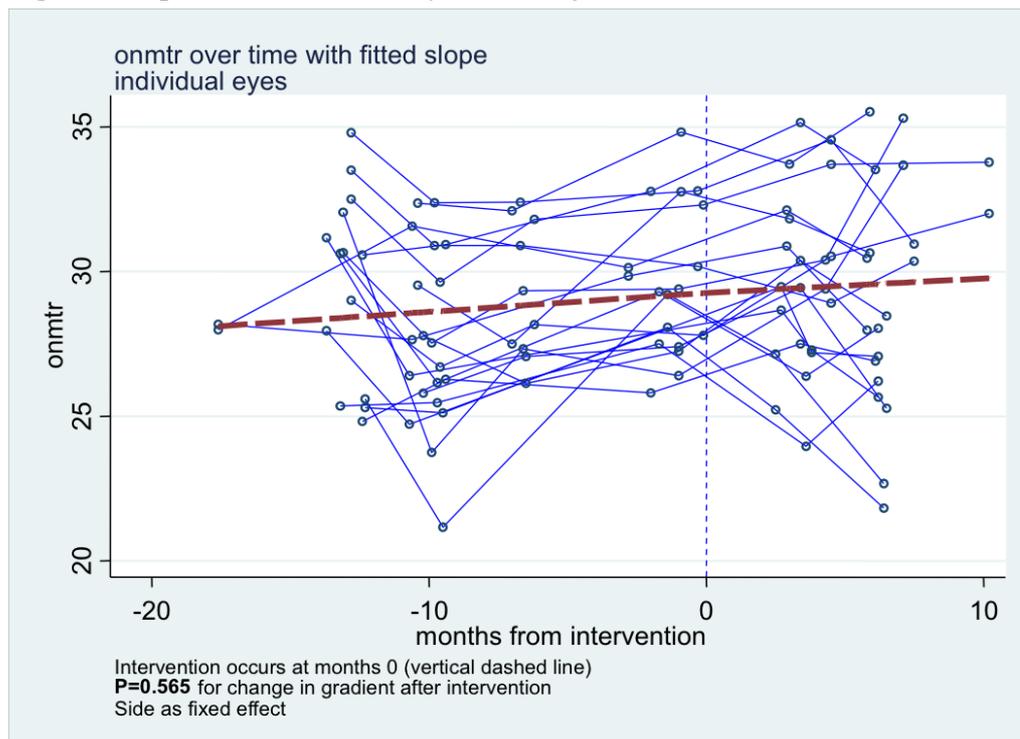
7.2.2: Optic nerve MTR

Optic nerve MTR were calculated as described in chapter 4, over the entire optic nerve (cross-sectional) and chiasm with the observer blinded to the time points of the subjects.

Fig: 7.3 shows the change over time for the patients pre and post intervention. There was a slight increase in the MTR through the study but there was no significant change in the gradient between pre and post intervention measures. The rate of change was 0.0529 pu per month [95% CI -0.1271 to 0.2328], $p = 0.565$.

The healthy volunteer controls during the same time did not show any significant change of optic nerve area with intra-individual SD of 2.66pu and $p = 0.5930$. Table: 7.1.

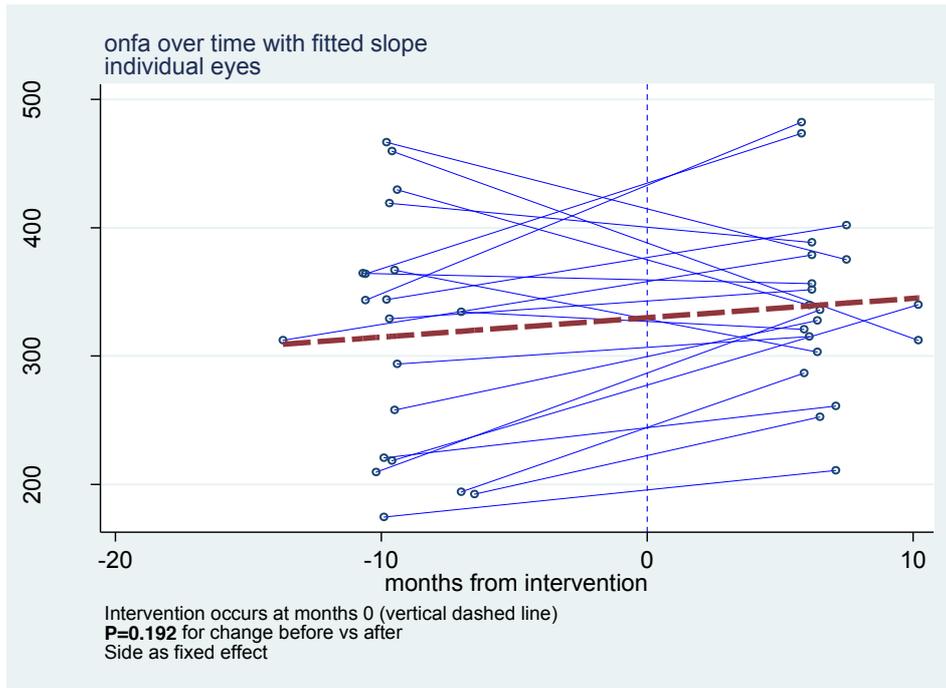
Fig: 7.3 Optic nerve MTR (Patients)



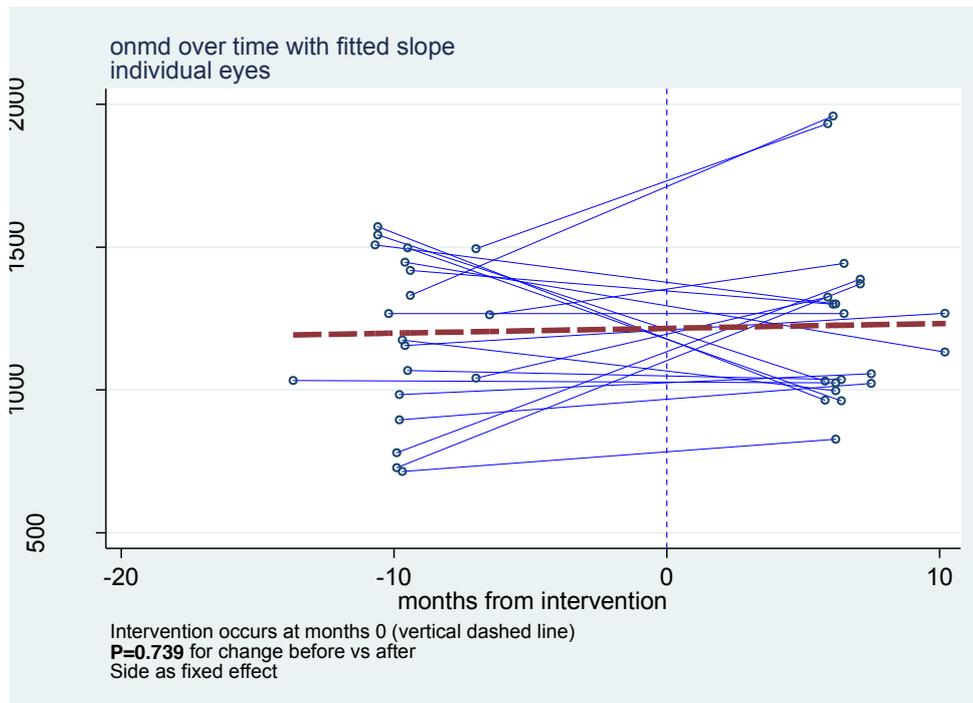
7.2.3: Optic nerve DTI

Optic nerve DTI parameters were calculated for patients and controls twice (once before and once after intervention for patients) during the MSCIMS study. The MR protocol and analysis method has been described in chapter 4. The parameters measured were optic nerve fractional anisotropy (FA), mean diffusivity (MD), axial diffusivity (AD) and radial diffusivity (RD). To take into account of the varying time interval, a fixed effects regression was used with each optic nerve as the unit analysis, regressing the measure on time with side as a fixed effect. This estimates the within optic-nerve gradient of change. The results are shown on the following graphs (Fig 7.4 a-d), giving non-significant P-values of 0.192, 0.739, 0.873 and 0.716 respectively for the four measures, optic nerve FA, MD, RD and AD. Table: 7.1.

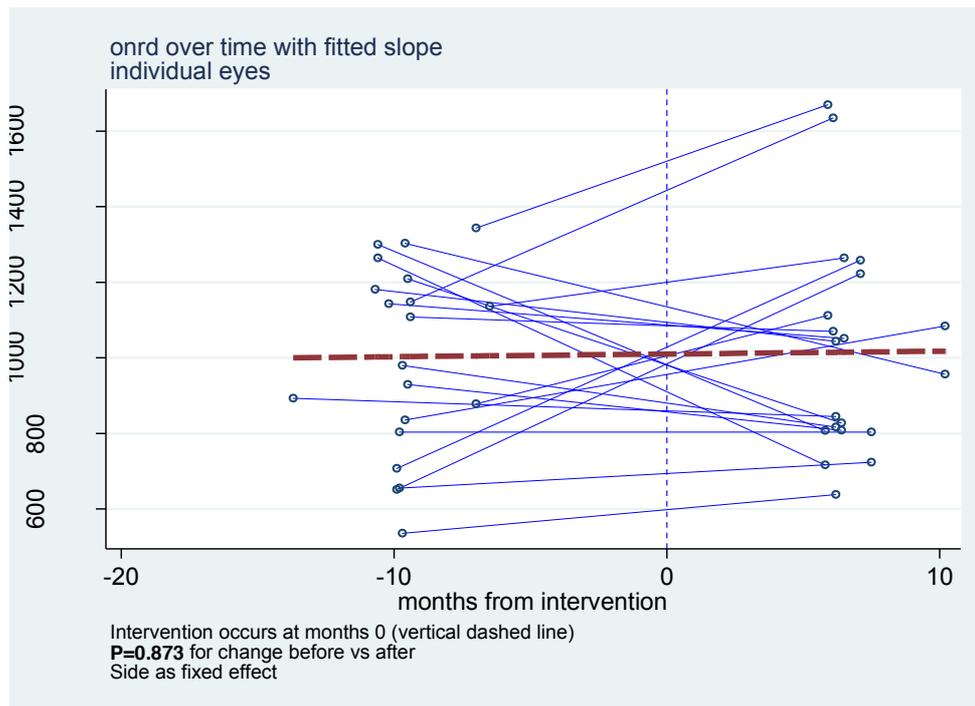
Fig 7.4(a) Fractional anisotropy (patients)



(b) Mean Diffusivity (patients)



(c) Radial diffusivity (patients)



(d) Axial diffusivity (patients)

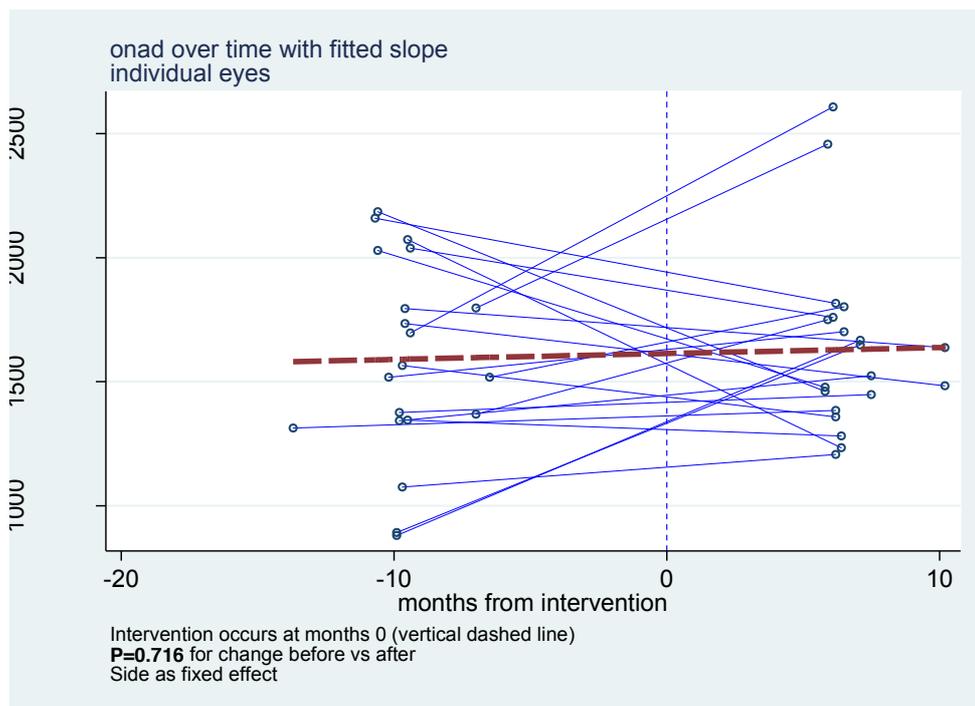


Table 7.1: Change in optic nerve and retinal imaging before and after treatment

<u>Outcome Measure</u> (Units)	<u>Rate of change</u> (Units per month)		<u>Difference in rate of change following treatment</u>		<u>p</u>
	Before treatment	After treatment	95% CI		
Visual pathway imaging measures					
Macular volume (mm ³)	0.0002	0.0041	0.0040	-0.0135 to 0.0214	0.654
RNFL thickness (µm)	-0.0052	0.0474	0.0527	-0.3533 to 0.4586	0.799
Optic nerve area (mm ²)	-0.0216	0.1046	0.1262	0.0368 to 0.2155	0.006
Optic nerve MTR (%)	0.0656	0.0529	-0.0127	-0.1271 to 0.2328	0.565
Optic nerve FA (x 10 ³)	1.6543			-0.4140 to 3.7226	0.192
Optic nerve MD (x 10 ⁻³ mm ² /s)	2.8488			-7.3204 to 13.0180	0.739
Optic nerve AD (x 10 ⁻³ mm ² /s)	2.6089			-10.4445 to 15.6623	0.716
Optic nerve RD (x 10 ⁻³ mm ² /s)	1.6064			-7.1370 to 10.3498	0.873

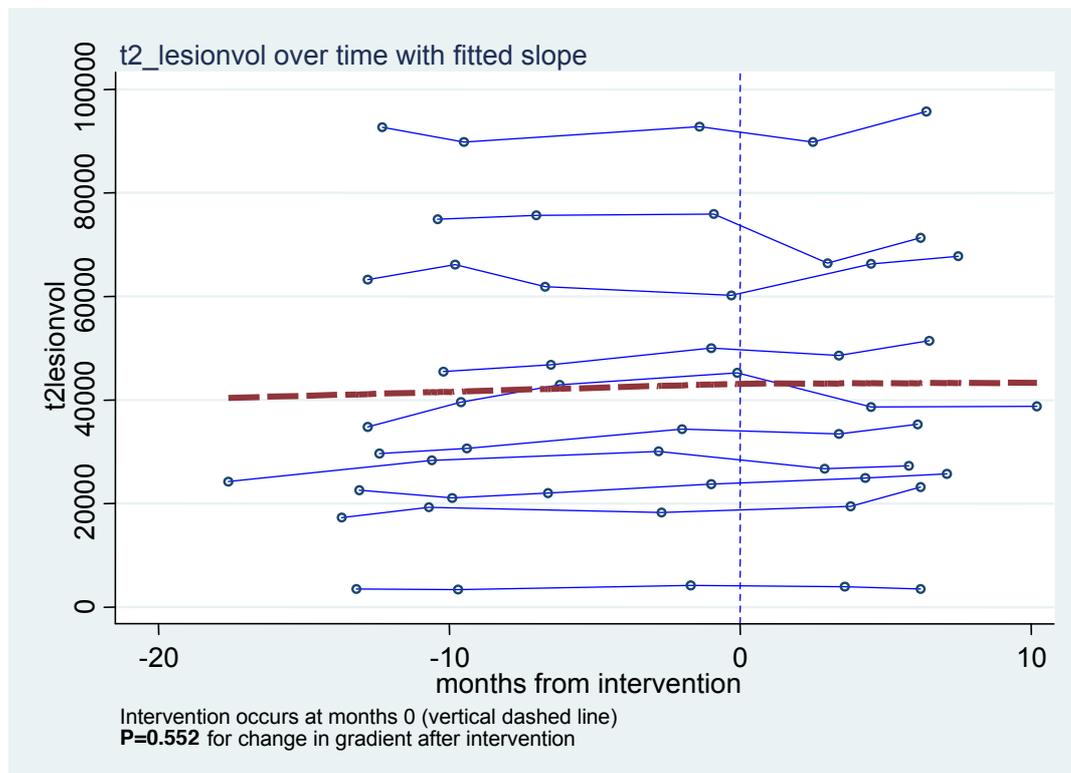
Piecewise linear mixed model regression is shown for optic nerve and retinal imaging outcomes, with confidence intervals and significance tests for a change in gradient at the time of treatment. Optic nerve diffusion tensor imaging (DTI) measures (FA: fractional anisotropy, MD: mean diffusivity, AD: axial diffusivity, RD: radial diffusivity) were measured once before and once after treatment: a single gradient is therefore shown for each with corresponding confidence interval and significance test. RNFL: Retinal nerve fibre layer; MTR: magnetization transfer ratio.

7.3: Brain MRI results:

7.3.1: T2 Lesion volume:

T2 lesion volume was contoured on all patients for all time points after the data collection was completed to minimise substantial time gap between measurements to reduce any systematic observer bias. There was a borderline significant increase in the T2 lesion volume in the pre-treatment phase ($p = 0.071$). However there was no statistical evidence of change in the gradient at the point of intervention. ($P = 0.552$). Fig: 7.5 & Table: 7.2

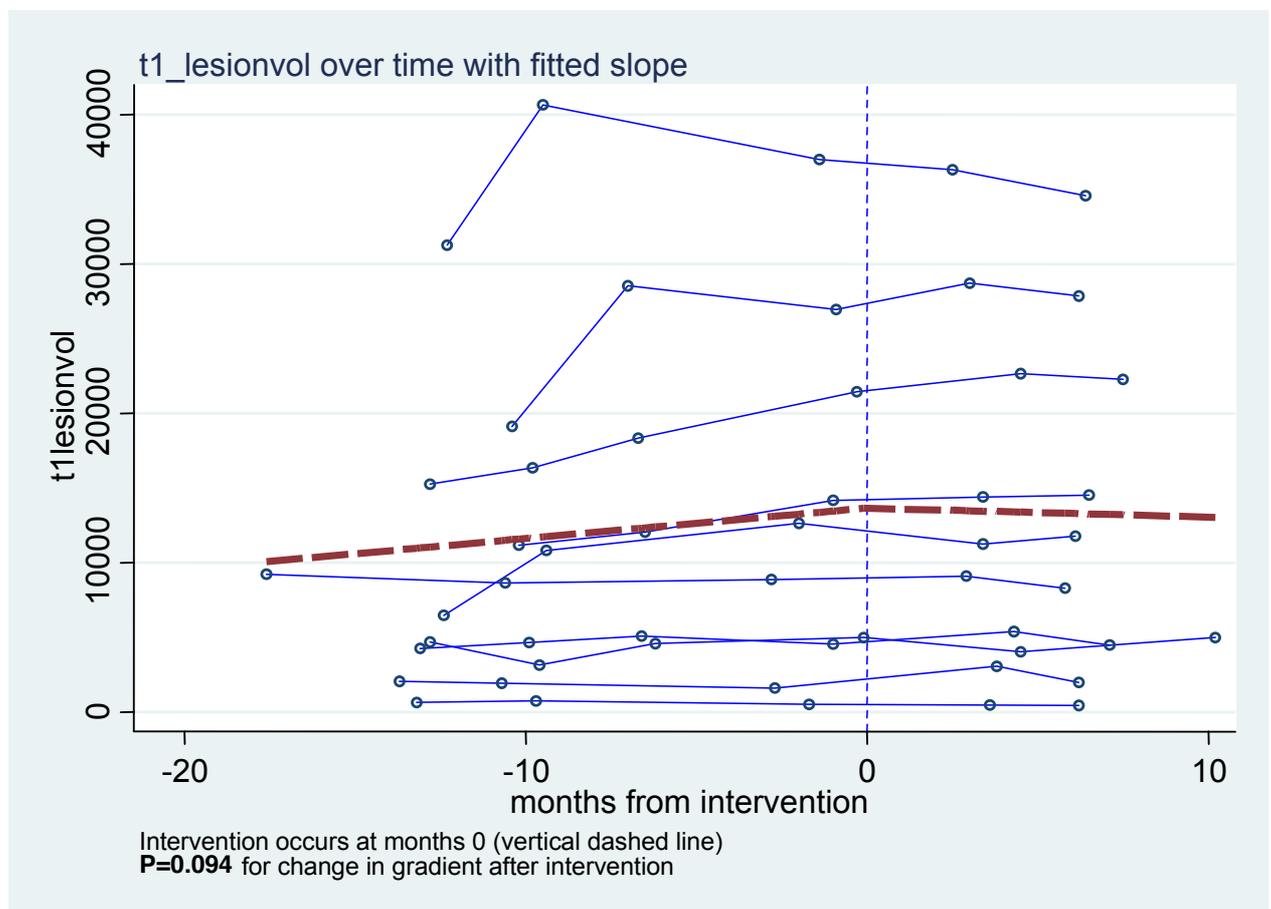
Fig 7.5: T2 lesion volume



7.3.2: T1 hypo-intense lesion volume

T1 hypo-intense lesions were contoured for all time points clustered together at the end similar to T2 hyper-intense lesions. There was a statistically significant increase in the T1 hypo-intense lesion volume pre treatment ($p = 0.005$) and in the post treatment phase, this significance had disappeared ($p = 0.614$). The gradient of change at the point of intervention itself was borderline significant ($p = 0.094$). Fig: 7.6 & Table: 7.2.

Fig 7.6: T1 hypo-intense lesion volume



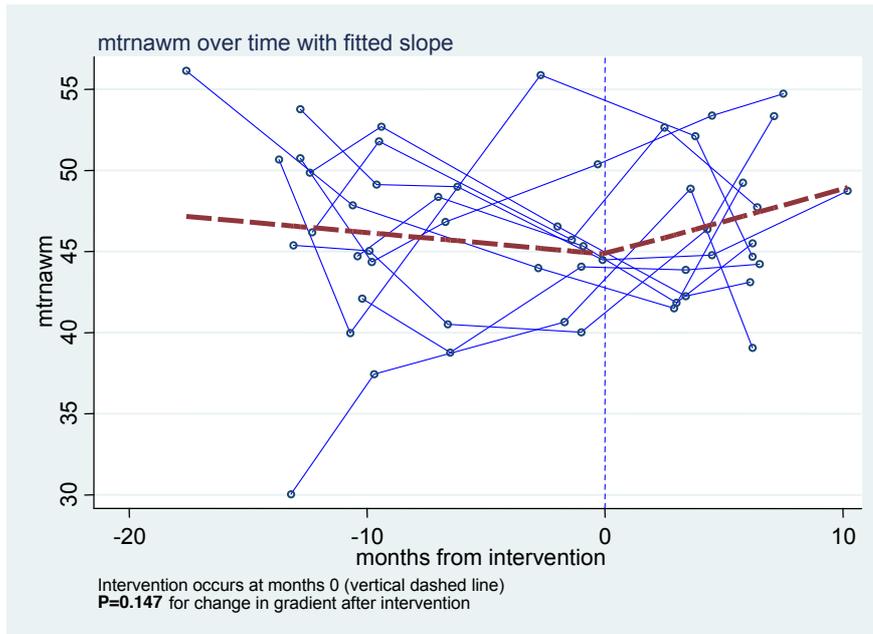
7.3.3: Brain volume

Brain atrophy between time points for patients and controls were measured. For the patient group: the rate of change of brain volume pre-treatment phase was -0.0880% per month and after intervention the rate was -0.1470% per month. This gradient change however was not significant ($p = 0.171$). For the control group: the rate of change between time points corresponding to the pre-treatment phase for patients was -0.0199% per month and for the period corresponding to the post-treatment phase was -0.0634% . The change was not significant ($p = 0.3389$)

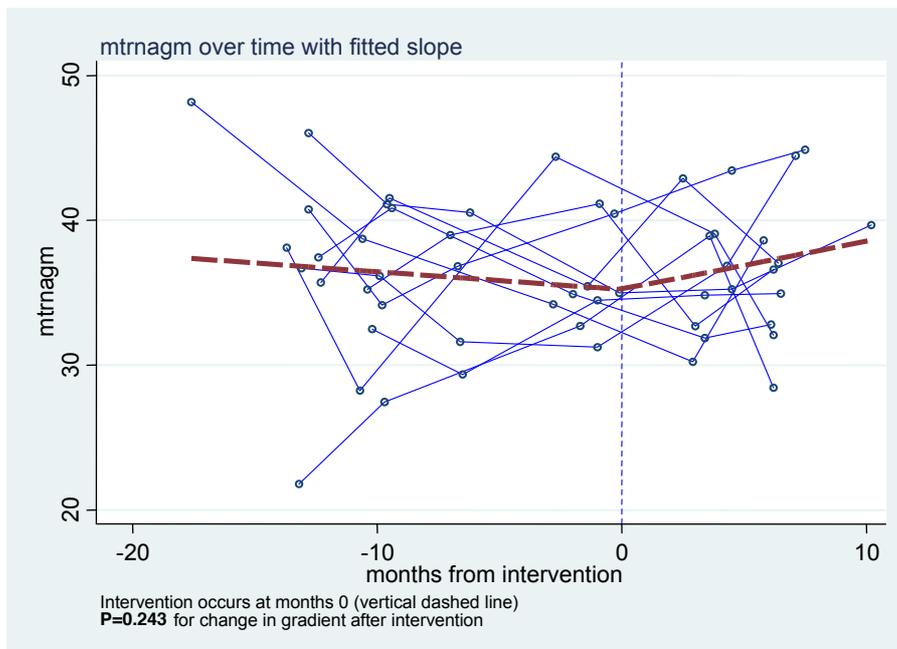
7.3.4: Brain MTR

Magnetisation transfer ratio for the whole brain, normal appearing white matter, normal appearing grey matter, T2 lesions and T1 hypointense lesions were measured for patients and whole brain, grey matter and white matter MTR were measured for controls. For the patients, the pre-intervention MTR for all parameters were decreasing and the post-treatment mtr were increasing. However this change of gradient only achieved borderline significance for T1 hypointense lesion MTR. Fig: 7.7 (a-e)

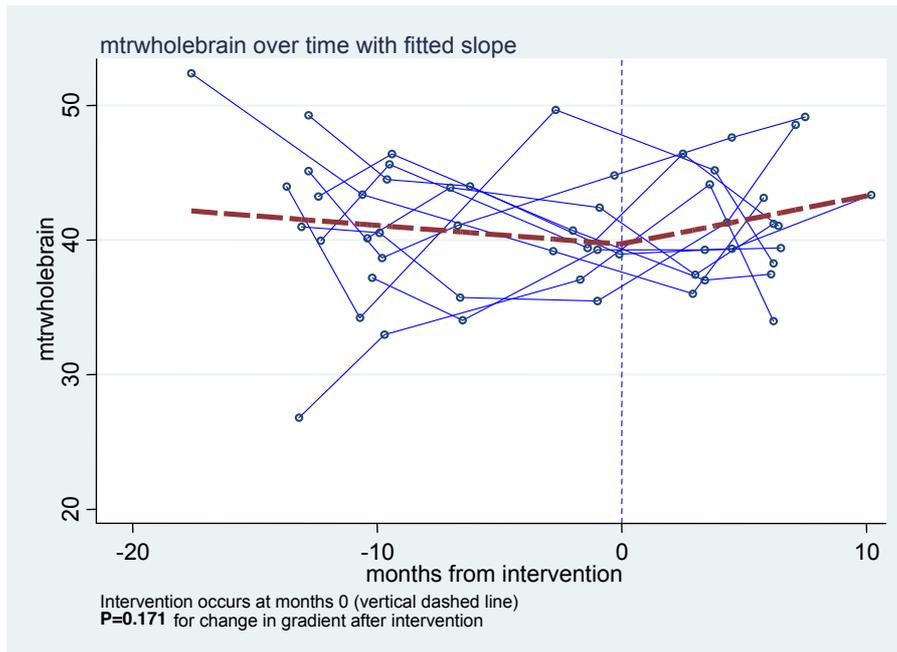
Fig: 7.7 (a) Normal appearing white matter (NAWM) MTR (pu)



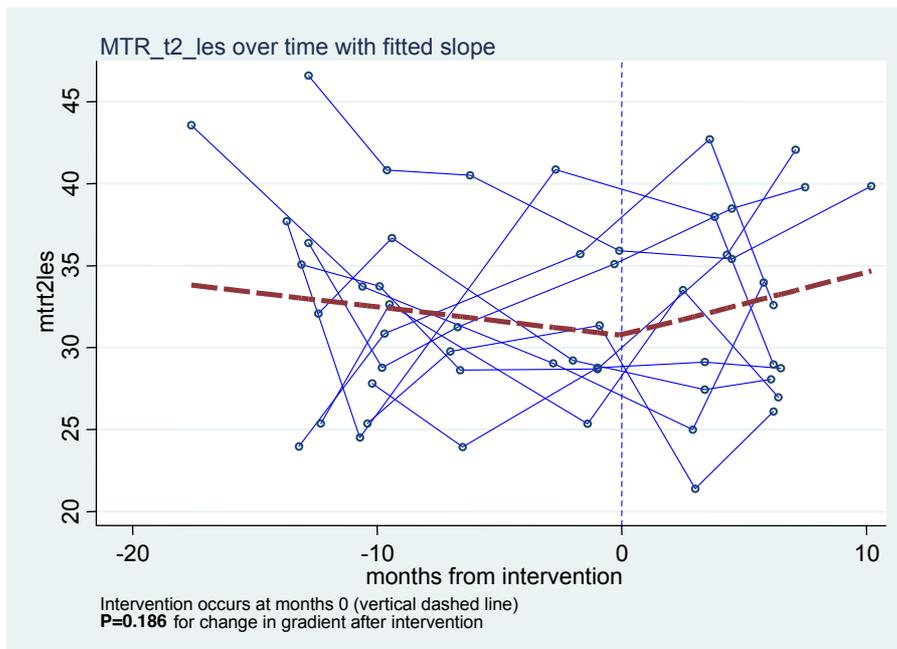
7.7 (b) Normal appearing grey matter (NAGM) MTR (pu)



7.7 (c) Whole brain MTR



7.7 (d) T2W lesion MTR



7.7 (e) T1 hypointense lesion MTR

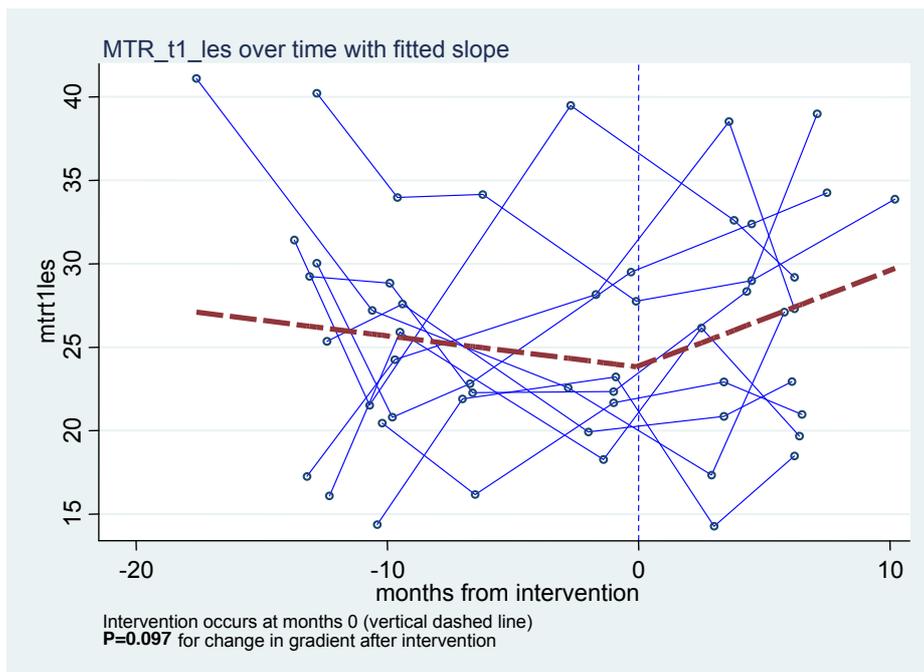


Table 7.2: Change in brain imaging before and after treatment

<u>Outcome Measure</u> (Units)	<u>Rate of change</u> (Units per month)		<u>Difference in rate of change following treatment</u>		<u>p</u>
	Before treatment	After treatment		95% CI	
Brain imaging measures					
T2 Lesion Volume (mm ³)	155.89	20.90	-134.98	-579.64 to 309.67	0.552
T1 Lesion Volume (mm ³)	204.35	-60.73	-265.08	-574.85 to 44.69	0.094
Brain volume (%)	-0.0880	-0.1470	-0.0590	-0.1434 to 0.254	0.171
Whole Brain MTR (%)	-0.1409	0.3605	0.5014	-0.2166 to 1.2195	0.171
Grey Matter MTR (%)	-0.1148	0.3116	0.4264	-0.3362 to 1.1891	0.273
White Matter MTR (%)	-0.1417	0.4093	0.5510	-0.1845 to 1.2865	0.142
NAGM MTR (%)	-0.1222	0.3334	0.4556	-0.03087 to 1.22	0.243
NAWM MTR (%)	-0.1321	0.4023	0.5344	-0.1874 to 1.2562	0.147
T2 Lesion MTR (%)	-0.1738	0.3859	0.5597	-0.2703 to 1.3896	0.186
T1 Lesion MTR (%)	-0.1867	0.5791	0.7659	-0.1389 to 1.6706	0.097

Piecewise linear mixed model regression is shown for brain imaging outcomes, with confidence intervals and significance tests for a change in gradient at the time of treatment. MTR: magnetization transfer ratio; NAGM: Normal Appearing Grey Matter; NAWM: Normal Appearing White Matter.

7.5: Discussion:

Before discussing the imaging results of the study, it is important to re-emphasise that the MSCIMS study is a phase IIa “proof of concept” study with safety as the primary outcome measure. It is also important to realise that the study is only on 10 patients with 20 optic nerves and 16 of them affected.

Optic nerve area (atrophy) was assessed with the operator blinded to both to subject status (patient or control) and to their time points. This is the only imaging measure in the study in which this was possible as the remaining measures had to be done unblinded to the status of the subject. This was because of the various registration and pre-processing steps undertaken before doing the final analysis. Some measures were fully automated during analysis such as baseline brain volume using SIENAX and the brain volume change using SIENA. Blinding was not an issue with such measures.

Optic nerve area showed a significant change in gradient at the time of intervention for the patients. This being the most robust of measures in the study due to the blinding mentioned above, suggests there may be a hint of treatment effect. There was actually an increase in the optic nerve area of the patients at the point of intervention. If it was just neuroprotection, it would have shown just the change in the slope without actual increase. This could have been possibly due to remyelination effect.

Optic nerve MTR did not show any significant change between pre and post intervention time periods. This may be because the sensitivity to detect change using MTR is inadequate in a small number of optic nerves or it may simply be that there was no effect on this measure. It should also be acknowledged that the image quality in 3T sequence was affected due to the noise and several slices of optic nerves had to be excluded during the process of analysis. These missed

segments have included the segments of the nerve affected by optic neuritis and this further reduces the sensitivity. The sequence itself is 20minutes long and when registering two images, the ones with and without the MT pulse, although automated, presumes that there is no significant change in position of the optic nerves. This is a difficulty with imaging an independently mobile structure in life such as the optic nerve. Giving considerations to all these drawbacks, it may be that the number of subjects and the duration of follow up could have been small to detect a change in MTR which is more a marker of myelin than axons. This patient group have had remote optic neuritis and not acute, and the change in myelin would be a much more dynamic factor in the latter than the former.

Optic nerve DTI measures also did not show any significant change in gradient between pre and post intervention. The technical difficulties discussed for MTR also apply for DTI. In addition, there were only two time points of DTI measures and it included only part of the optic nerves (4 slices max). DTI is a measure that is more specific towards neuroaxonal loss and if the technical difficulties are overcome and applied in a larger group of patients with sufficient power to detect change, then it could be very useful potentially.

Brain T2 lesion volume increased significantly in the pre treatment phase and the increase in lesion volume was not significant in the post treatment phase. However the change in gradient at the point of intervention is not significant. It is possible that there is some effect in slowing down the rate of accrual of lesions by the intervention but the finding was not significant. T1 lesion volume showed the same trend as T2W lesion volume, however the p value of the gradient at the point of intervention was borderline significant. As we know that persistent T1 hypointense lesions correlate with axonal loss, it may again hint at the potential neuroprotective effect of the intervention. It may be that these values would reach significance in a bigger cohort and/or a longer follow up.

Brain volume changes in patients were slightly different between pre and post intervention in that there was a slightly increased rate of atrophy post treatment. However the gradient of change was not statistically significant at the point of intervention. Again, this needs further exploration in a larger group of patients and in a longer follow up to draw any definite conclusion. We know from some disease modifying therapy studies that there is an accelerated rate of atrophy in the initial few months after commencing treatment and a reduction of atrophy with longer term follow up. This has been explained to be due to a selection bias of patients having a more active disease receiving DMTs in these studies and also the anti-inflammatory effect of resolution of oedema with DMTs could have contributed to the increased rate of atrophy. Both these explanations may not be applicable to MSCIMS cohort, as most of the patients did not have a clinically active disease and all 10 patients had secondary progressive MS. However, one cannot completely rule out the possibility of an anti-inflammatory effect.

Brain MTR measures of whole brain, NAGM, NAWM, T2 hyperintense lesions and T1 hypointense lesions showed a similar trend of an increase at the point of intervention. However only for the T1lesion MTR did the gradient change approach statistical significance. T1 hypointense lesions have the least MTR values suggesting that although MTR may be more weighted towards myelin, axonal loss also affects MTR. With the gradient of the change in MTR at the point of intervention for the T1 hypointense lesions reaching borderline significance, it is possible that this is a reflection of a potential neuroprotective effect of the stem cells being slightly more obvious in the lesions with the most axonal loss. As the MTR of T1 hypointense lesions tended to **increase** in the post treatment phase ($p=0.093$), there is a hint that remyelination was taking place in the lesions.

Retinal imaging including peripapillary RNFL thickness and macular volume gradients did not change significantly at the point of intervention. The changes in these measures are substantial and dynamic in the first 6-12 months after an acute episode of optic neuritis. It may be that the

changes in the values over time in patients with secondary progressive MS with remote optic neuritis are very minimal and hence it is possible that any axonal protective effect of the intervention is too small in the small group in a limited follow up time. It is also biologically implausible that axonal regeneration would occur: thus it is not surprising that an increase in RNFL thickness was not observed. As mentioned above, the findings of shortening of VER latency together with an increase in optic nerve area with a trend in improvement of T1 lesion volume and MTR indicates that the principle effect of MSC may be due to promotion of myelin repair and this may be why there was no change observed in the RNFL.

In summary, the imaging investigations in MSCIMS have revealed a hint of neuroprotective – and possibly remyelinating - effect as secondary outcome, which needs further exploration using longer and larger studies. Study of a cohort of patients with a dynamic change in both the inflammatory as well as neurodegenerative component in their disease process may be most useful in terms of the ability to detect the potentially multiple effector mechanisms of action of MSC. This means a cohort of patients with an active MS with relapses and at the same time progressing between relapses. However, MSCIMS was not designed to specifically address the effects of intervention on MRI markers of inflammation, accordingly no change was seen following treatment in the rate of T2 lesion accumulation.

Chapter 8: Visual functional MRI results

8.1: Introduction:

As described in chapter 4, visual functional MRI sequences were obtained for 10 patients and 6 controls at two time points during the study, one before and one after the intervention for the patients. Controls were scanned around the corresponding time points for patients as far as possible. Two of the 8 controls could not tolerate the visual stimulation and did not undergo this sequence.

8.2: Method:

8.2.1: MRI protocol: A total of 69 volumes of T2* weighted echo-planar images depicting blood oxygen level dependent (BOLD) contrast were acquired in each 5 minutes experiment with 52 near axial slices of the whole brain (TR: 3940ms, TE: 30ms, field of view: 192mm, Matrix: 64 x 64, slice thickness of 3mm) which was performed 4 times (total ~ 20 minutes acquisition time) for each experiment.

8.2.2: Experiment: The visual stimulation paradigm comprised of eight epochs, each of 16 seconds, of flickering checkerboard stimulation, alternated with eight epochs, each of 16 seconds, of gray background, presented on a projection screen. Subjects wore transparent plano chromatic filter goggles, with one green and one red filter (Haag-Streit, UK). The checker board was also green and red, so that the green checker board was invisible through the red filter, and vice versa. This was to allow monocular stimulation while testing both eyes within the same run. To facilitate attention and fixation of a central cross, subjects were instructed to fixate a central ' + ', and asked to press a button when it changed to a ' # ' symbol. Each experiment consisted of 4 sessions, and the orientation of the goggles was reversed in between, to swap the red and green filters.

8.3: Analysis and Results:

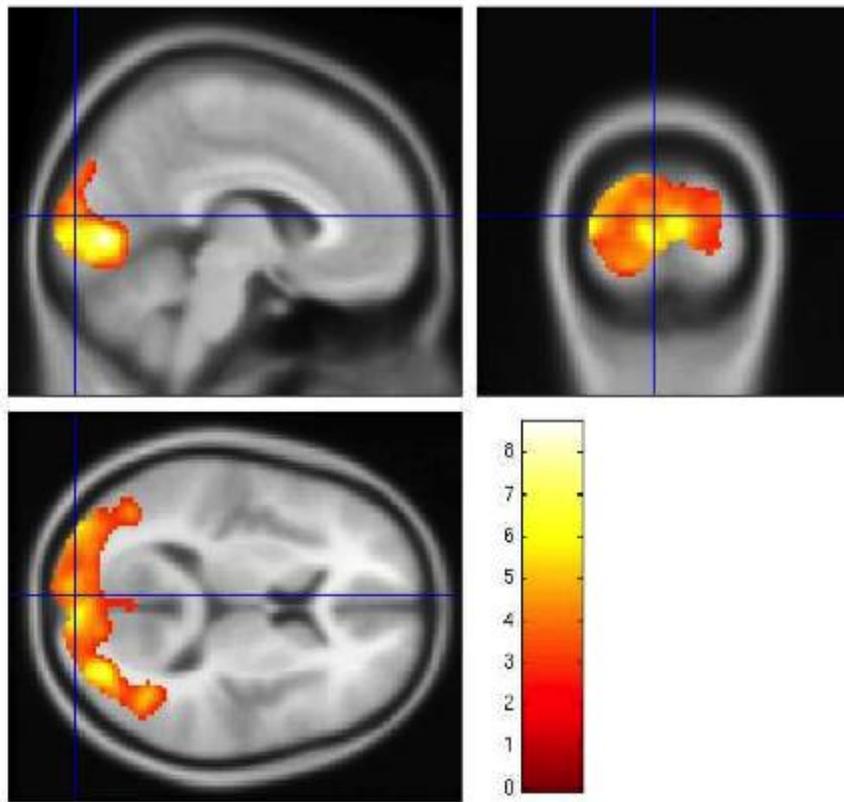
Pre-processing: Statistical Parametric Mapping software (SPM8; Wellcome Trust Centre for Neuroimaging, London, UK) was used for analysis. Each fMRI series was realigned, coregistered and normalized to the T1 volumetric image of the same subject acquired at the same time point. The images were then smoothed, using an 8mm isotropic Gaussian kernel. Realignment parameters and time derivatives were entered as covariates into the general linear model, together with the time-points at which the subjects pressed the button during the task to maintain attention.

First level analysis: For each subject, first level fixed effect contrasts were specified, for right and left eyes individually (1 0 and 0 1, respectively), combining epochs of stimulation through the red and green filters. The subsequent contrast images, representing the main effect of stimulation for each eye, were entered into the second level regression models.

Second level analysis: Fixed effect analysis resulted in two contrasts (one for each eye) for each subject at each time point. These contrasts were then grouped as right eye patients baseline, left eye patients baseline, right eye controls baseline, left eye controls baseline, right eye patients follow up, left eye patients follow up, right eye controls follow up and left eye controls follow up using second level analysis.

Analysis 1: Main Group Effects: One sample 't' tests: In second level group analysis, one sample t tests were performed to obtain group effects for each of the eight groups. The MNI coordinates for the maximum activation cluster, spatial extent (k) in terms of number of voxels, with the voxel level 't' score and voxel & cluster level 'p' values are shown in Figure: 8.1 (a-h).

Fig: 8.1(a) Baseline controls: right eye activation



Significant cluster:

MNI coordinates: (-8 80 0)

Spatial extent in voxels: (K): 15878

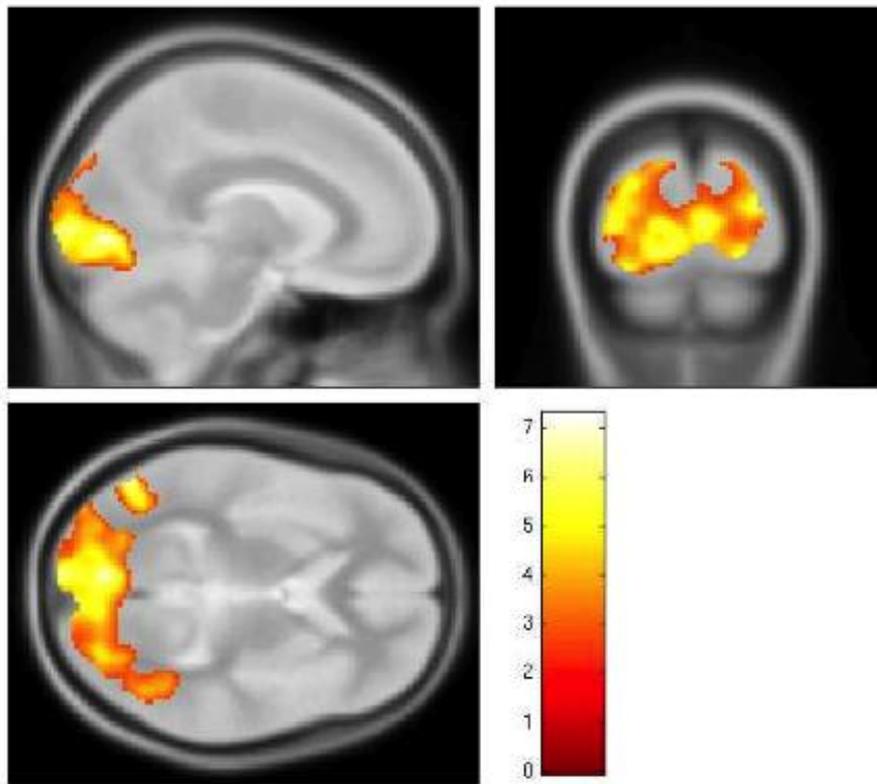
Voxel level T score: 8.71

Voxel level p value: (Pv): <0.001

Cluster level p value: (Pc): <0.001

Analysis: Contrasts obtained from 1st level analysis for the right eye in controls at baseline time point were specified in the second level and one sample t test was performed. There was significant activation in the occipital cortex bilaterally.

(b) Baseline controls: left eye activation



Significant cluster

MNI coordinates: (-44 -66 4)

Spatial extent in voxels: (K): 12118

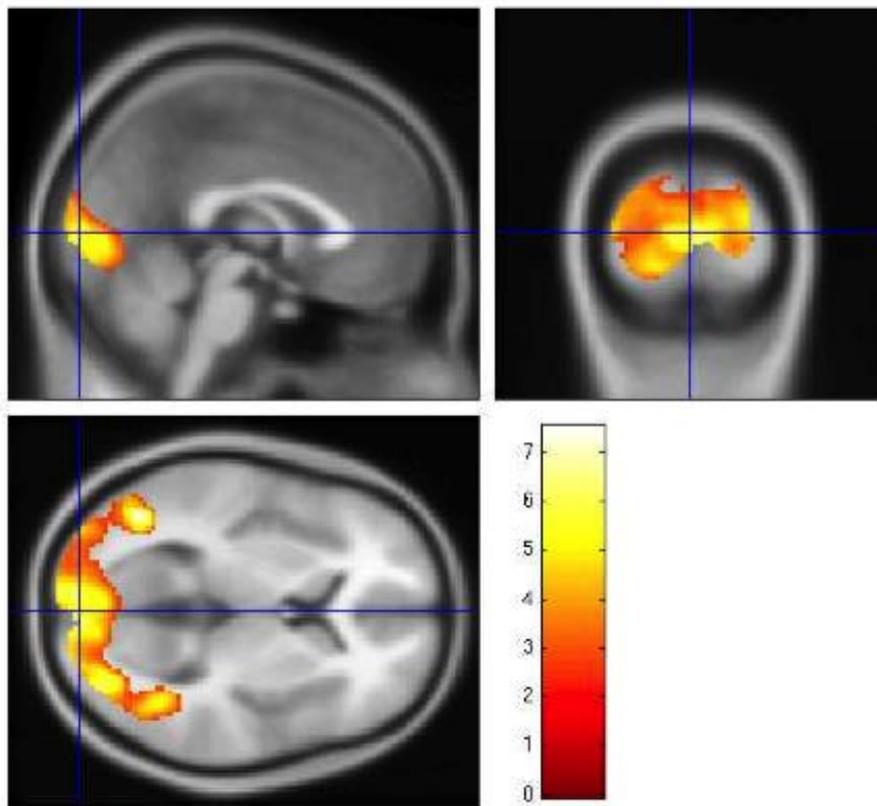
Voxel level T score: 7.3

Voxel level p value: (Pv): 0.003

Cluster level p value: (Pc): <0.001

Analysis: Contrasts obtained from 1st level analysis for the left eye at baseline time point in controls were specified in the second level and one sample t test was performed. There was significant activation in the occipital cortex bilaterally.

(c) Follow up controls: right eye activation



Significant cluster

MNI coordinates: (-44 -64 2)

Spatial extent in voxels: (K): 11015

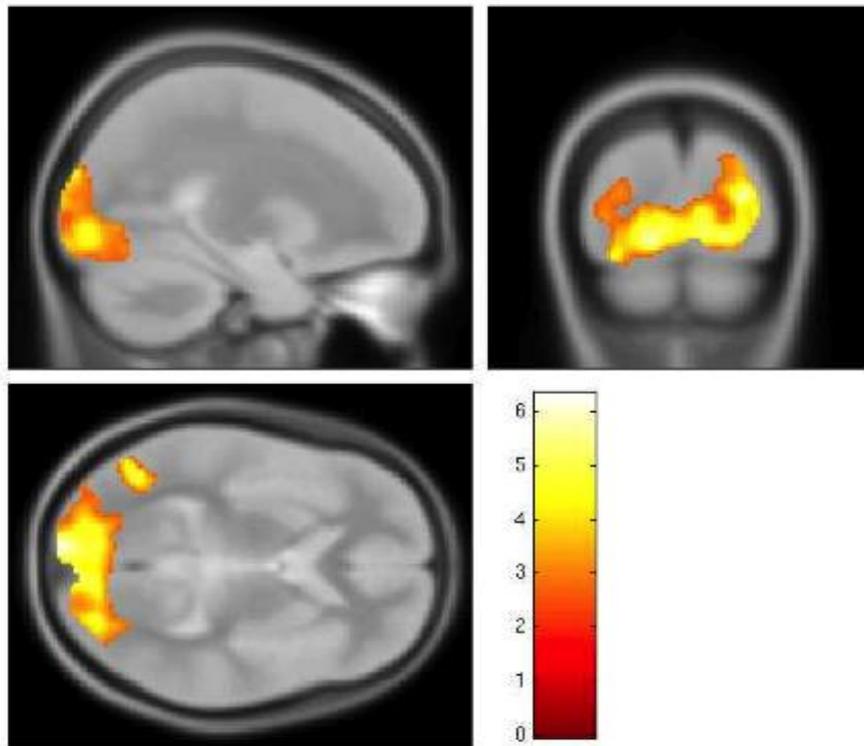
Voxel level T score: 7.54

Voxel level p value: (Pv): 0.002

Cluster level p value: (Pc): <0.001

Analysis: Contrasts obtained from 1st level analysis for the right eye at follow-up time point in controls were specified in the second level and one sample t test was performed. There was significant activation in the occipital cortex bilaterally.

(d) Follow up controls: left eye activation



Significant cluster

MNI coordinates: (-8 -100 2)

Spatial extent in voxels: (K): 6905

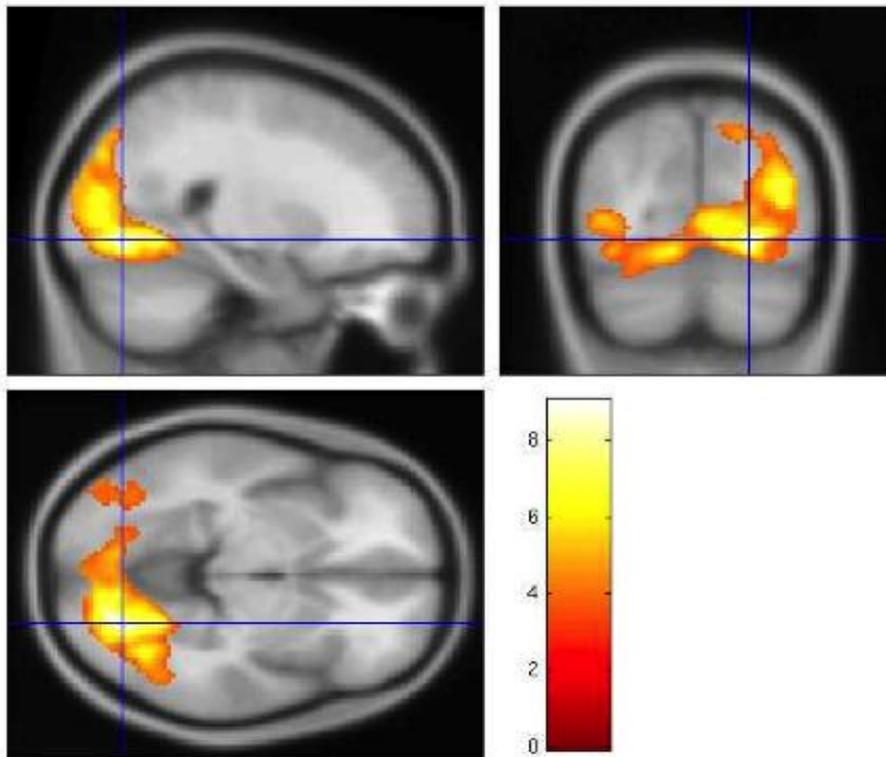
Voxel level T score: 6.34

Voxel level p value: (Pv): 0.023

Cluster level p value: (Pc): <0.001

Analysis: Contrasts obtained from 1st level analysis for the left eye at follow-up time point in controls were specified in the second level and one sample t test was performed. There was significant activation in the occipital cortex bilaterally.

(e) Baseline patients: right eye activation



Significant cluster

MNI coordinates: (24 -74 6)

Spatial extent in voxels: (K): 9543

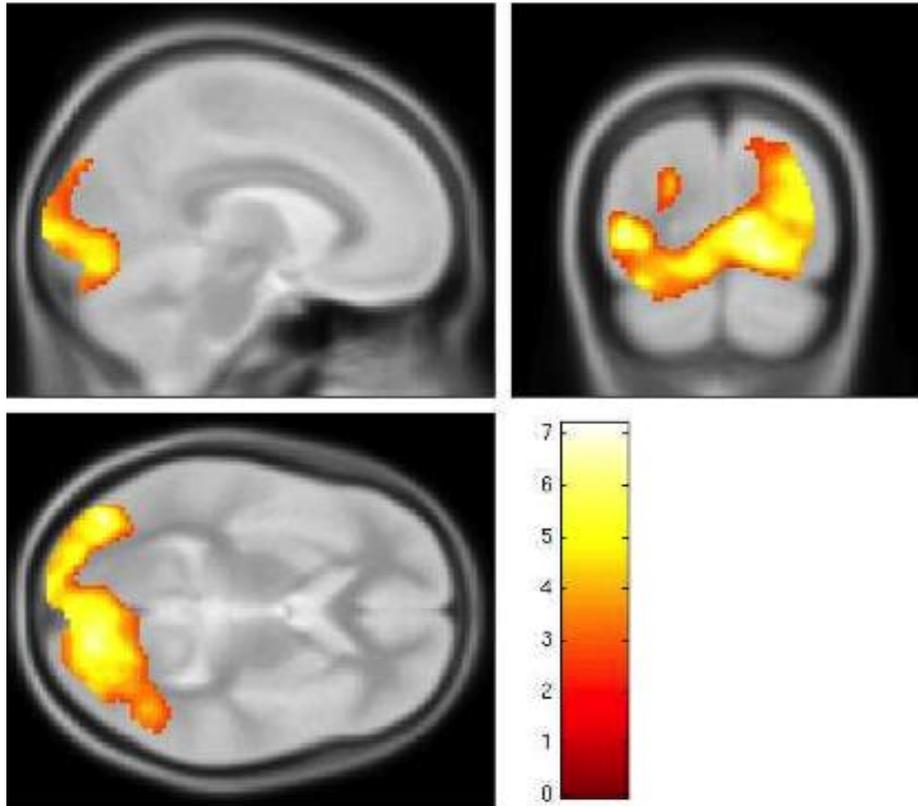
Voxel level T score: 9.06

Voxel level p value: (Pv): <0.001

Cluster level p value: (Pc): <0.001

Analysis: Contrasts obtained from 1st level analysis for the right eye at baseline time point in patients were specified in the second level and one sample t test was performed. There was significant activation in the occipital cortex bilaterally.

(f) Baseline patients: left eye activation



Significant cluster

MNI coordinates: (12 -84 6)

Spatial extent in voxels: (K): 13309

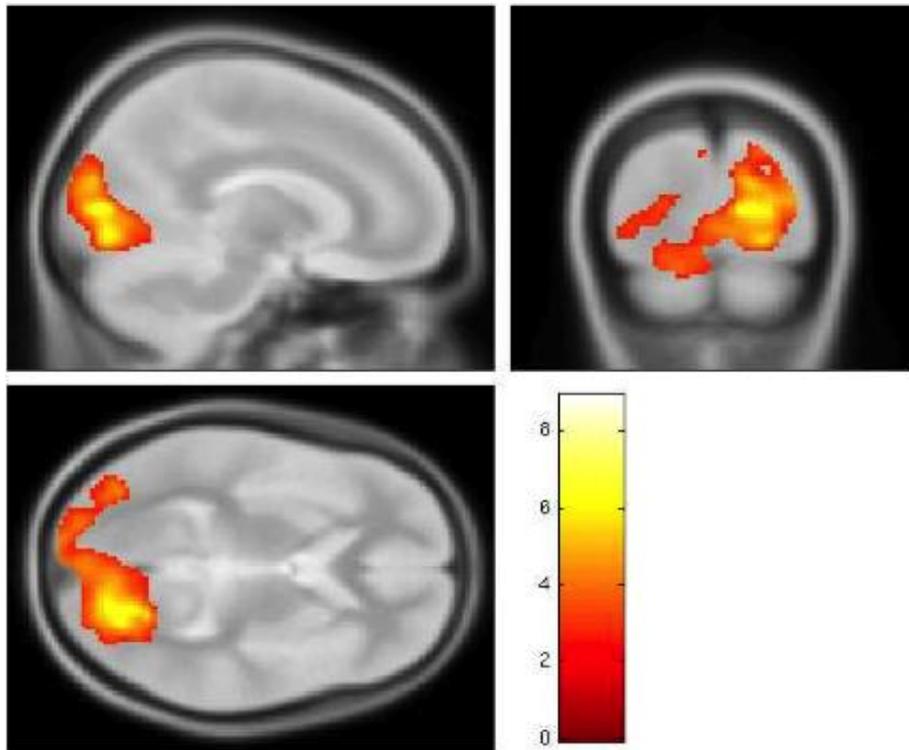
Voxel level T score: 7.20

Voxel level p value: (Pv): 0.004

Cluster level p value: (Pc): <0.001

Analysis: Contrasts obtained from 1st level analysis for the left eye at baseline time point in patients were specified in the second level and one sample t test was performed. There was significant activation in the occipital cortex bilaterally.

(g) Follow up patients: right eye activation



Significant cluster

MNI coordinates: (24 -76 -6)

Spatial extent in voxels: (K): 7256

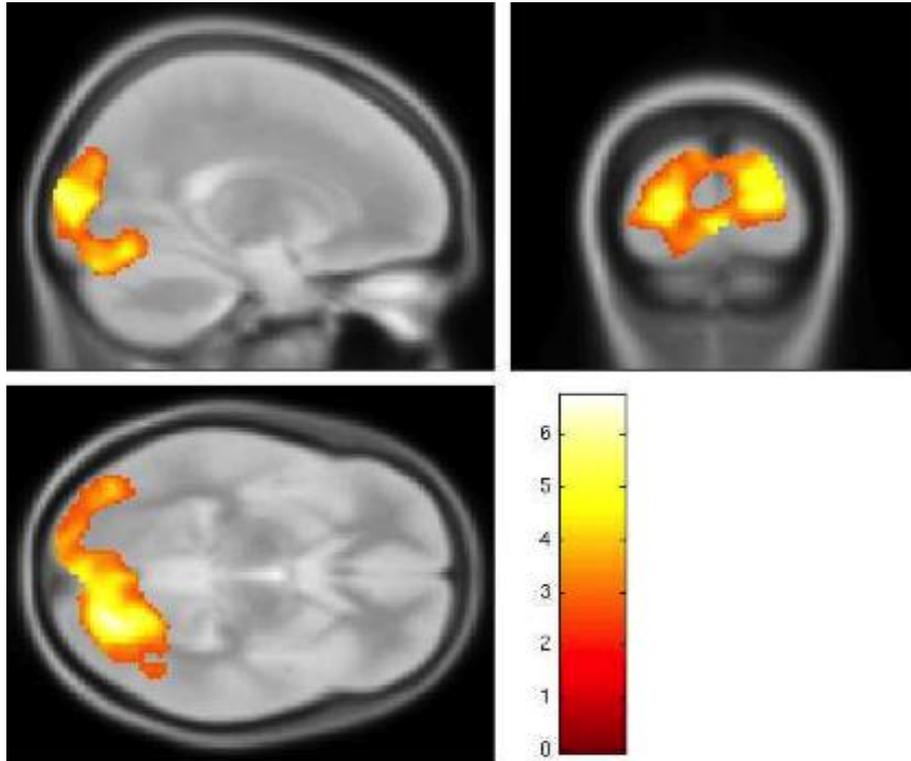
Voxel level T score: 8.93

Voxel level p value: (Pv): <0.001

Cluster level p value: (Pc): <0.001

Analysis: Contrasts obtained from 1st level analysis for the right eye at follow-up time point in patients were specified in the second level and one sample t test was performed. There was significant activation in the occipital cortex bilaterally.

(h) Follow up patients: left eye activation



Significant cluster

MNI coordinates: (16 -84 6)

Spatial extent in voxels: (K): 9871

Voxel level T score: 6.74

Voxel level p value: (Pv): 0.01

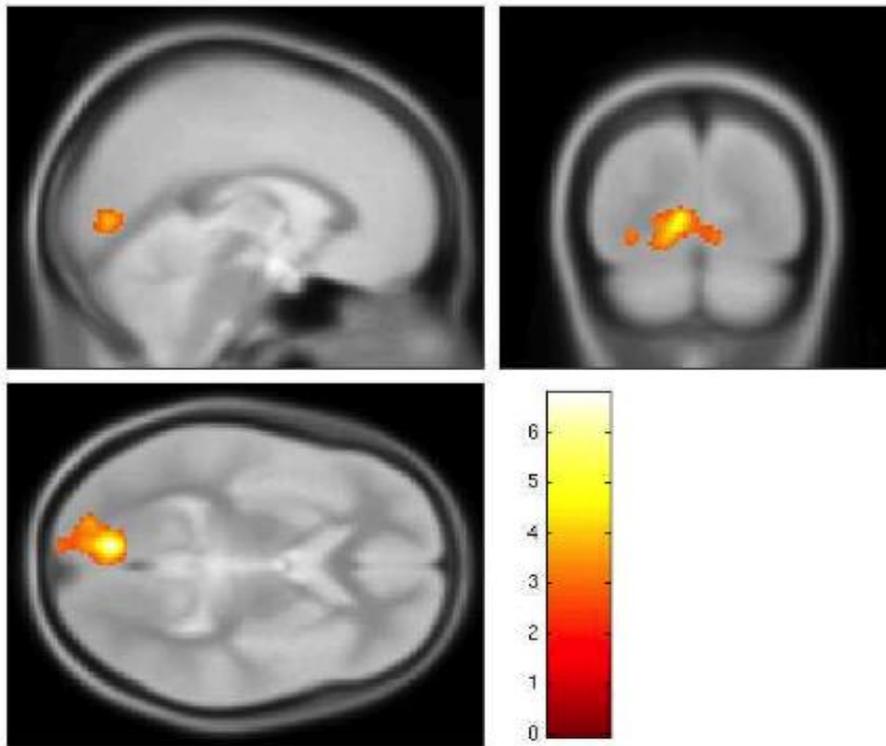
Cluster level p value: (Pc): <0.001

Analysis: Contrasts obtained from 1st level analysis for the left eye at follow-up time point in patients were specified in the second level and one sample t test was performed. There was significant activation in the occipital cortex bilaterally.

Analysis 2: Patients vs Controls: (Two sample 't' tests): A second-level two-sample *t*-test model was used to identify any regions where patients activated more than controls (contrast 1 -1), or vice versa (-1 1). [Fig: 8.2 (a-d)]. Only controls showed significant activation more than patients in the occipital lobes. There was no significant occipital or extra-occipital activation found in patients over that of controls in this study. Only significant activation (controls > patients) are shown below:

Fig: 8.2:

(a) Baseline (right) controls > Patients



Significant cluster

MNI coordinates: (-8 -78 0)

Spatial extent in voxels: (K): 975

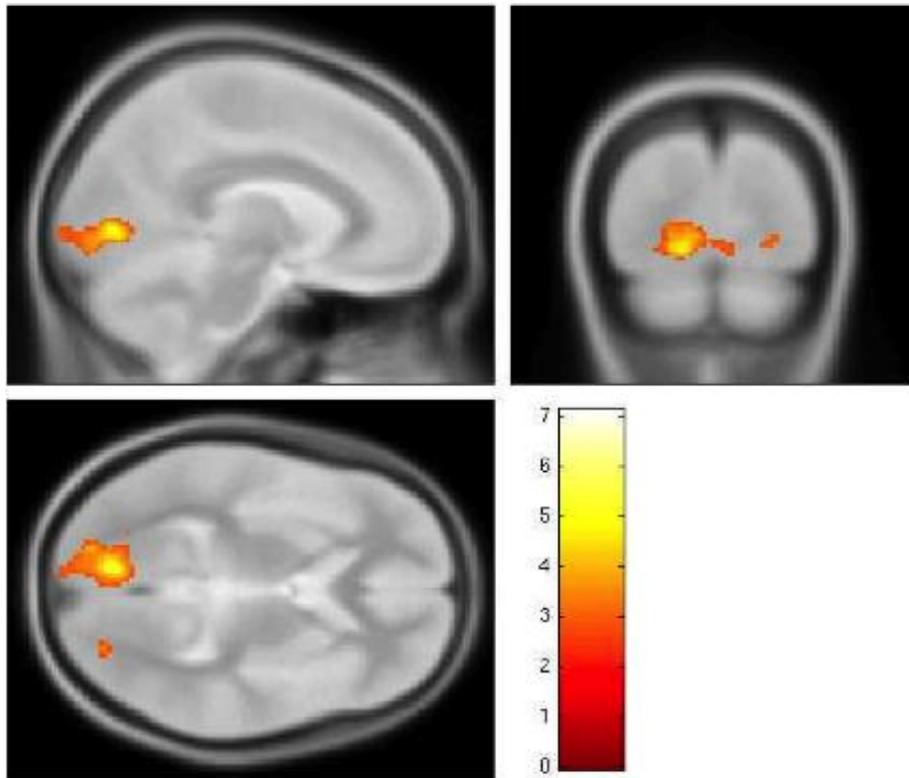
Voxel level T score: 6.78

Voxel level p value: (Pv): 0.193

Cluster level p value: (Pc): 0.025

Analysis: Contrasts obtained from 1st level analysis for the right eye for controls (specified contrast 1) and right eye patients (specified contrast -1) for baseline time points were specified in second level analysis using two sample t test in SPM 8, to get controls > patients. The significant cluster in occipital cortex is shown.

(b) Baseline (left) controls > Patients



Significant cluster

MNI coordinates: (-28 -66 -10)

Spatial extent in voxels: (K): 1906

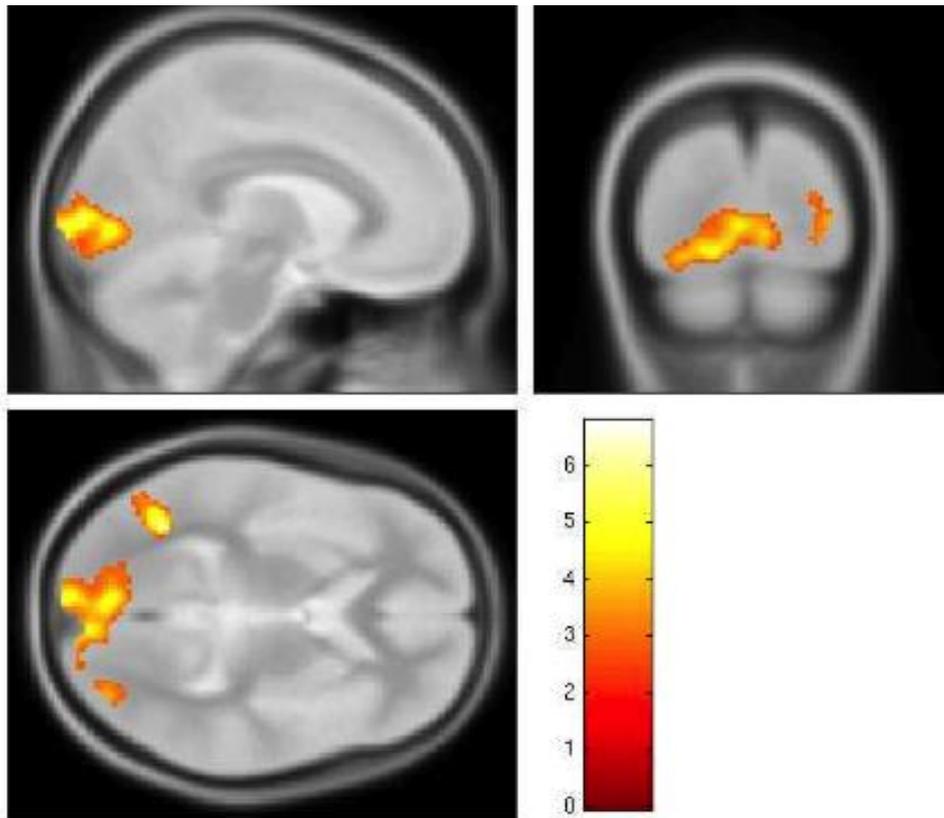
Voxel level T score: 7.11

Voxel level p value: (Pv): 0.143

Cluster level p value: (Pc): <0.001

Analysis: Contrasts obtained from 1st level analysis for the left eye for controls (specified contrast 1) and left eye patients (specified contrast -1) for baseline time points were specified in second level analysis using two sample t test in SPM 8, to get controls > patients. The significant cluster in occipital cortex is shown.

(c) Follow up (right) controls > Patients



Significant cluster(s)

MNI coordinates: (-40 -60 -2); (-38 -32 46)

Spatial extent in voxels: (K): 3262; 741

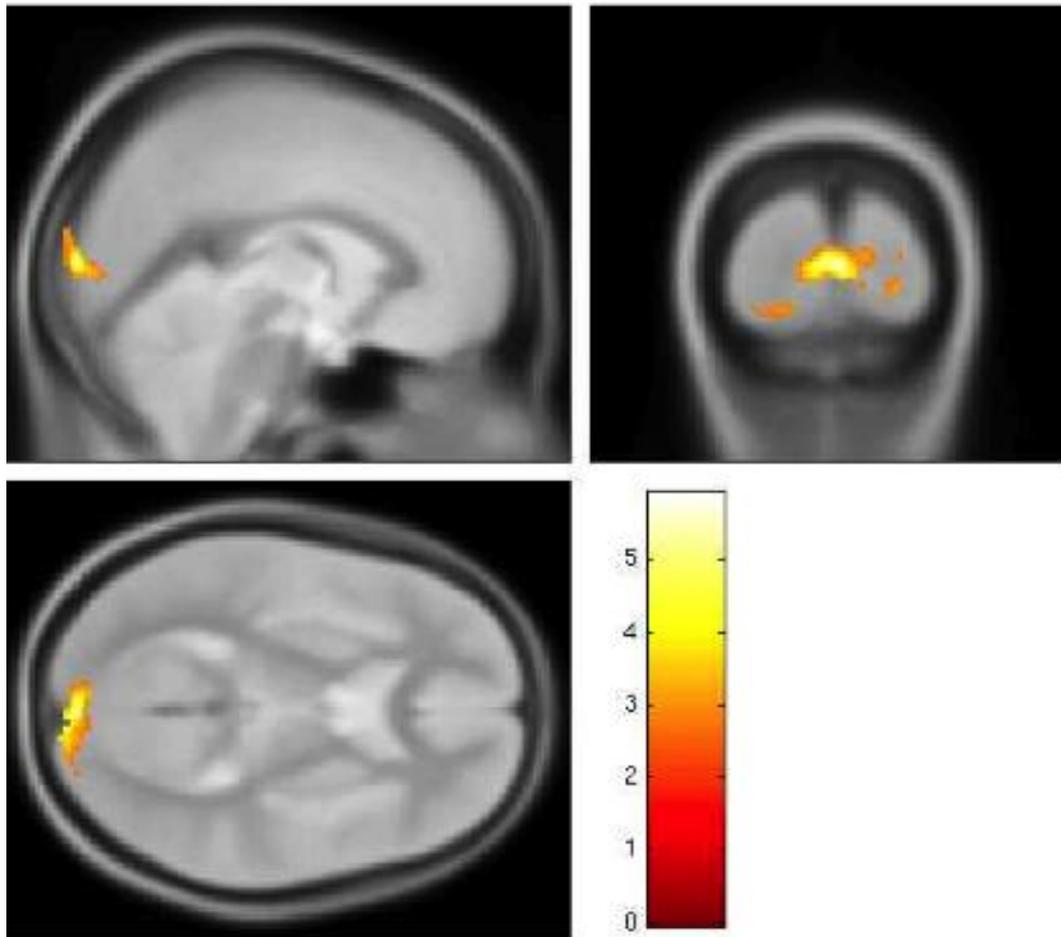
Voxel level T score: 6.78; 5.40

Voxel level p value: (Pv): 0.232; 0.767

Cluster level p value: (Pc): <0.001; 0.039

Analysis: Contrasts obtained from 1st level analysis for the right eye for controls (specified contrast 1) and right eye patients (specified contrast -1) for follow-up time points were specified in second level analysis using two sample t test in SPM 8, to get controls > patients. The significant cluster is shown.

(d) Follow up (left) controls > Patients



Significant cluster

MNI coordinates: (2 -94 6)

Spatial extent in voxels: (K): 1007

Voxel level T score: 5.91

Voxel level p value: (Pv): 0.593

Cluster level p value: (Pc): 0.003

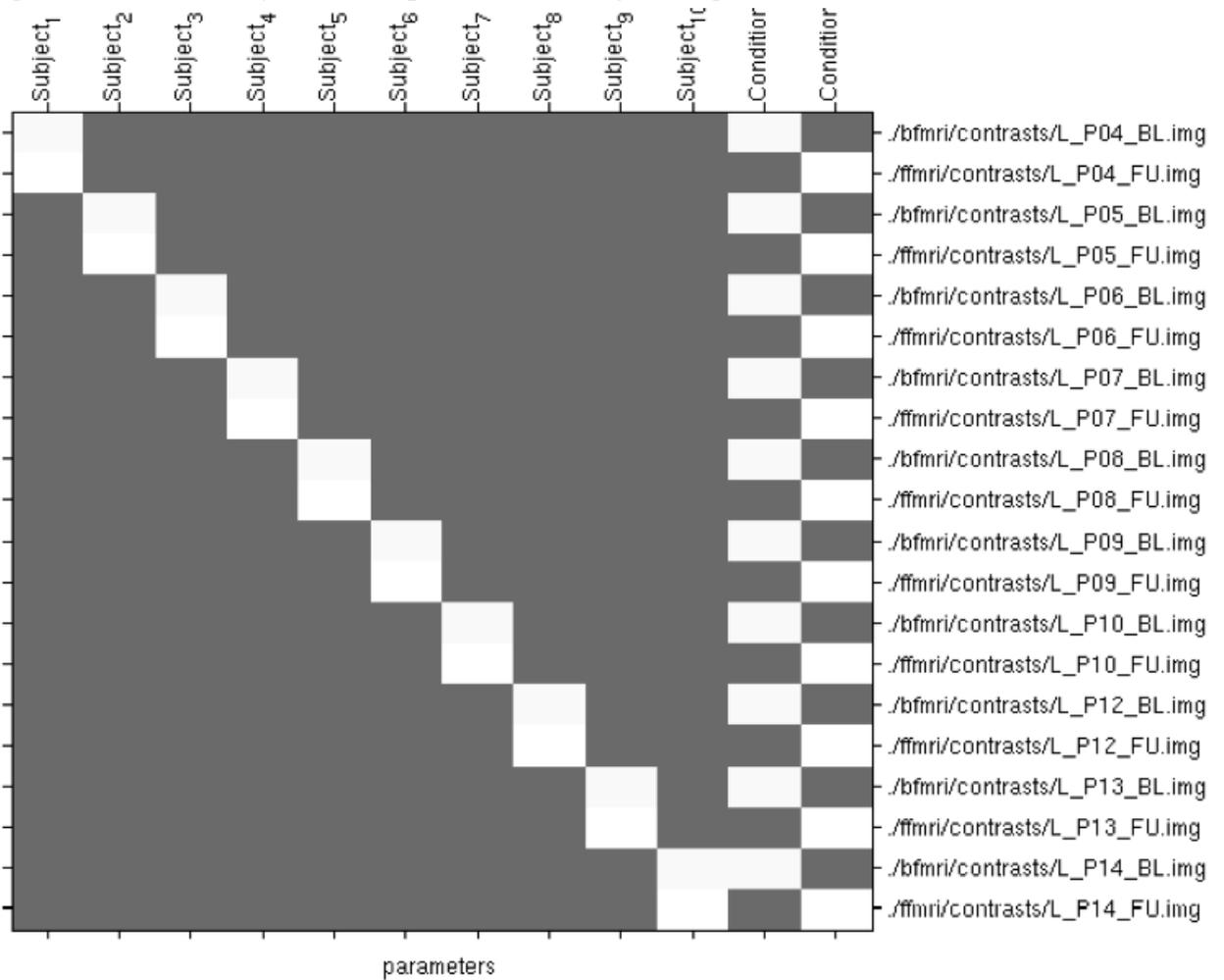
Analysis: Contrasts obtained from 1st level analysis for the left eye for controls (specified contrast 1) and left eye patients (specified contrast -1) for follow-up time points were specified in second level analysis using two sample t test in SPM 8, to get controls > patients. The significant cluster is shown.

Analysis 3: Followup vs Baseline: Paired 't' tests: Then paired t tests were performed to obtain comparison statistics between baseline and follow up for patients and controls for right and left eye groups separately.

Figure 8.3 shows the design matrix for the paired t tests to compare follow up and baseline activation for left eye stimulation for patient group. Each patient's baseline contrast is paired with the follow up contrast as demonstrated by the white rectangular blocks in the first 10 columns. The 11th and 12th columns demonstrate the two conditions which are baseline and follow up respectively. This matrix enables a determination of the group comparison for repeated measures in SPM 8.

There were no significant differences to activation on comparison either way for both patient and controls between baseline and follow up.

Fig: 8.3 Patients (Follow up vs Baseline) design matrix:



Design for paired t tests in SPM8 to compare left follow up vs baseline for the patient group. The 10 initial columns represent the 10 patients with the 11th and 12th column representing conditions 1 and 2, which are time points baseline and follow up respectively. The rows represent the twenty contrast images (obtained from SPM8 using first level analysis as described in chapter 4) for the left eyes of patients with pairing between baseline and follow up for the patients.

Analysis 4: Group interaction: Flexible factorial: The flexible factorial design enables creation of a design matrix one block at a time by specifying which main effects of groups or interactions between groups, one would like to include. [Fig: 8.4]

Three factors were specified: subject, group (patients vs. controls) and time-point (baseline vs. follow-up). Gender and age were entered as covariates of no interest. In Fig 8.4, the first two columns are groups (controls and patients), 3rd and 4th columns are time points (baseline and follow-up), 5th to 8th columns specify interaction between the two factors, group x time points such that 5th column specifies baseline controls, 6th column for follow-up controls, 7th column for baseline patients and 8th column for follow-up patients. 9th and 10th columns specify age and gender as covariates of no interest. The imbalance between number of patients and controls was taken into account in the flexible factorial design by specifying the appropriate contrast, in which the sum of the contrasts in each group was 1, and in the comparison between groups the total sum was 0. For example, for the group interaction, the contrast was specified as: 0 0 0 0 -1/2 1/2 1/2 -1/2 0 0. Again there was no significant interaction effects found.

Fig: 8.4

Group interaction using flexible factorial design matrix:

Group 1 – controls

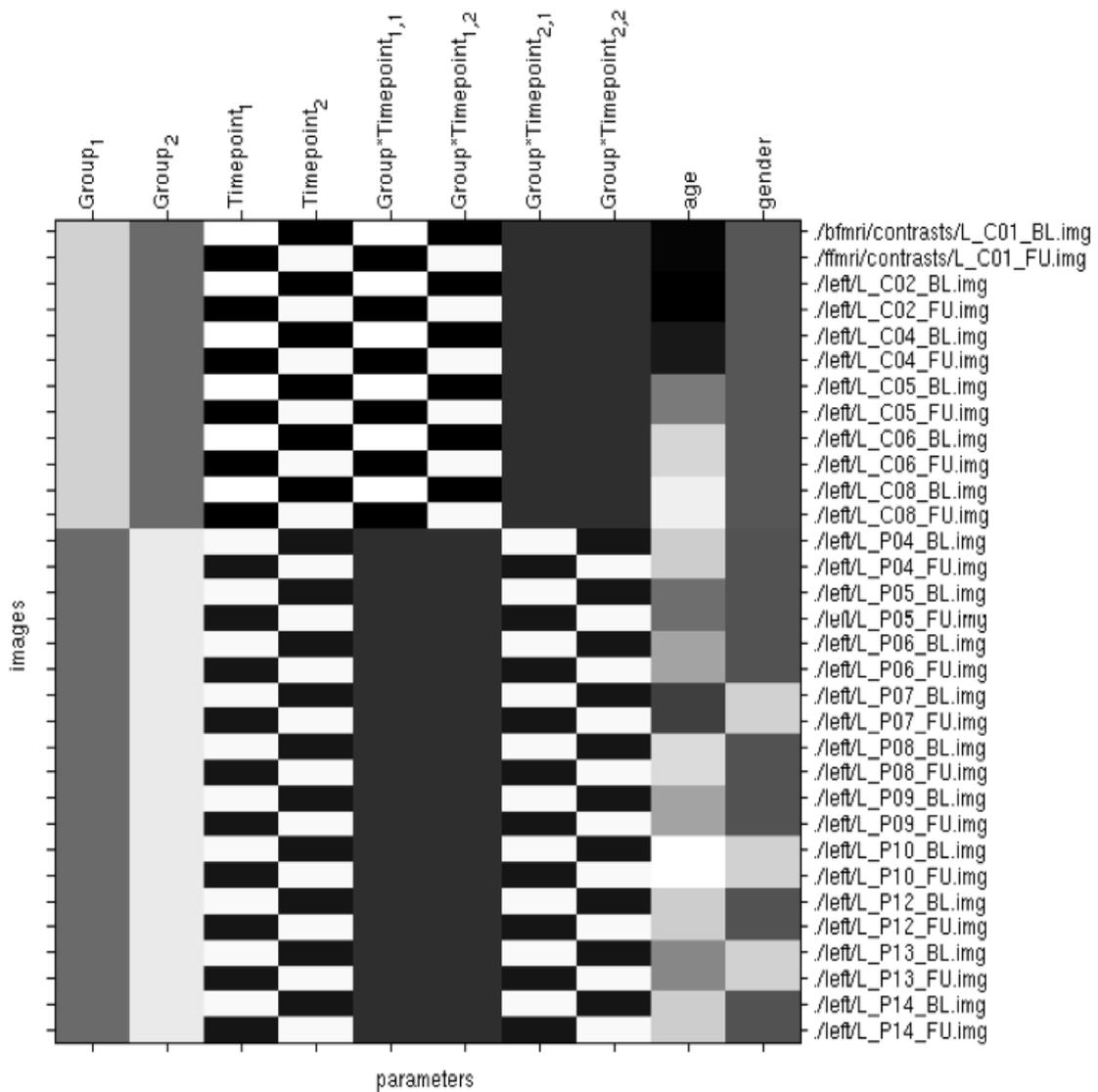
Group 2 – patients

Time point 1 – baseline

Time point 2 – follow up

Columns 9 & 10: Age and Gender are covariates of no interest.

32 rows represent control and patient left eye contrasts for baseline and follow-up time points.



Analysis 5: Voxel of interest: MarsBaR (MARSeille Boîte À Région d'Intérêt), which is a region of interest (ROI) toolbox in SPM 8, was used to extract effect sizes (parameter estimates) from predefined regions/voxels of interest (VOI) for the patient groups mentioned above. The VOIs were defined as right and left visual cortices (VC), lateral occipital complexes (LOC) and cuneus. The MNI (Montreal Neurological Institute) coordinates were used for LOC and cuneus. For the visual cortex (V1), the contrast images for the baseline controls were used to define the coordinates, which were as follows: Left VC: (-20 -88 28), Right VC: (28 -78 2); LOC: (\pm 43 -70 -13); Cuneus: (\pm 10 -84 32). Activation parameters for the four patient groups (right and left X baseline and follow up) in these specified regions of interest were extracted to check the correlation with structural and visual functional data. The extraction parameters from the right and left side of the brain were averaged for the three regions as there is bilateral representation for each eye.

Table: 8.1: Voxel of interest activation parameters extracted using MarsBaR tool in SPM for the 10 patients

Patient	FURPcuneus	FURPLOC	FURPVC	FULPcuneus	FULPLOC	FULPVC	BLRPcuneus	BLRPLOC	BLRPVC	BLLPcuneus	BLLPLOC	BLLPVC
1	1.9889	1.486	7.8301	3.6832	2.2744	7.6338	0.8194	1.5583	8.3513	2.7642	2.8692	7.4403
2	-0.6534	-1.5711	4.9343	0.76	0.269	7.0154	4.8553	1.0786	7.5113	2.7575	2.2759	6.8312
3	1.9752	1.378	6.7099	3.0064	1.192	5.933	1.8838	1.4013	5.9623	0.8761	0.8361	5.8617
4	2.2639	0.1951	3.0823	2.3598	0.0546	2.302	2.5569	1.1562	2.6518	2.6431	0.2153	2.2821
5	-0.9835	0.297	4.5738	-1.0604	0.9854	5.158	0.5295	0.3802	4.3489	-0.5627	0.2067	5.1258
6	0.1849	-0.0656	1.771	0.6372	-0.4582	1.1189	0.852	0.5143	1.2715	0.404	0.4806	1.07
7	-0.3204	0.0713	0.9123	-0.0411	0.6085	1.1836	0.3421	1.1843	1.0977	0.1431	0.6063	1.2091
8	0.5424	0.623	0.6506	-2.1776	-0.1509	0.8188	1.646	0.8867	0.7171	0.738	0.7926	0.7937
9	0.6793	1.4172	5.0284	0.0266	0.3092	4.604	1.0965	1.1082	4.3091	1.1469	1.0915	4.6012
10	0.518	0.3599	6.3418	3.1214	2.6434	7.7825	1.0544	1.3658	8.0912	0.7484	1.0385	7.505

BL is baseline time point

FU is follow-up time point

FURP is follow-up right eye patients

FULP is follow-up left eye patients

BLLP is baseline left eye patients

BLRP is baseline right eye patients

VC is visual cortex

LOC is lateral occipital complexes

cuneus is cuneus

Region of interest analysis: The activation parameters for the three specified region of interests shown in table 8.1 was statistically analysed (by Dr Daniel Altmann, the NMR Unit's statistician) using a bivariate model that models both eyes of the individual patients simultaneously. This model was used because there was no a priori reason to distinguish right from left eye in the study and if one is interested in the change between the follow-up and baseline time points, it is convenient and more robust to model as ten pairs of eyes than 20 separate eyes.

Table 8.2 shows the changes between baseline and follow-up activation parameters for the three specified regions of interest (cuneus, LOC and VC). It gives the change in either side stimulation (right eye and left eye) separately with separate p values and the joint test (which in effect combines both L and R sides to get a single p-value: this would correspond to the single p-value we would get if we made the eye, the unit of the analysis and assessed the change in the 20 eyes, ignoring the side.) for significance using the bivariate analysis. A negative value for change indicates a reduction in activation parameter between baseline and follow-up, and a positive value indicates an increase.

Table 8.2: fMRI activation parameter changes between baseline and follow-up for the three regions of interest

ROI	Right eye		Left eye		Joint
	Change	P value	Change	P value	P value
Cuneus	- 0.944	0.073	-0.134	0.78	0.142
LOC	-0.64	0.012	-0.268	0.38	0.042
VC	-0.247	0.454	0.082	0.006	0.002

LOC – Lateral Occipital Complexes; VC – Visual cortex; ROI – region of interest;

Activation parameter changes for the three ROI between baseline and follow-up for 10 patients’ eyes are shown. The right and left eye changes and their ‘p’ values are represented separately. The joint test ‘p’ values that combine right and left eyes of individual patients are shown. The significant ‘p’ values are shown in red. These results are discussed below.

8.4: Discussion:

Control (healthy volunteer) eyes and patient eyes (both affected and unaffected) were stimulated monocularly, so that the activation patterns for right and left eyes of subjects were obtained separately. Affected and unaffected eyes were not separately analysed, as there were 16 affected and only 4 unaffected eyes. All 8 groups, as shown above (Fig: 8.1a-h), showed significant visual cortex activation.

There was significantly more VC activation for controls when compared with patients corresponding eye and time-point groups. This is shown in Fig 8.2(a-d). This is expected to be the case with the patients' afferent visual pathway being affected with optic neuritis. However, there was no significant extra visual area activation in patients when compared to controls as had been previously noted by various groups as mentioned in chapter 2. [70, 91, 135, 157, 166] This may be because of inadequate power in MSCIMS (i.e., the small sample size). Also, the patients had a remote history of optic neuritis and some of them had poor visual outcome, which could have resulted in poor stimulation due to difficulties in fixation. In a cohort with secondary progressive MS, there may also be significant grey matter atrophy in the cortex including lateral occipital complexes and cuneus where previous studies in acute or recent optic neuritis had demonstrated activation. [134, 135] Such a global structural loss could have contributed to reduction in sensitivity to detect reorganisation in the brain.

There was no significant difference in activation between the global baseline and follow up time points in patients or controls. However, region of interest (ROI) analysis with extraction of activation parameters for visual cortex, lateral occipital complexes and cuneus in MSCIMS showed slightly different results. There were some significant

changes detected between baseline and follow-up activation in these regions for patients. As described above, the joint test, which combines right, and left eye activations of individual patients showed significant reduction in activation (negative change) in LOC and a significant increase (positive change) in visual cortex (Table: 8.2). The cuneus change was negative but did not achieve significance.

The cuneus and LOC are extra visual cortex activations thought to be an adaptive response in a situation where there is reduced input into visual cortex due to pathology in the afferent visual pathway in MS. [134, 135] If the increased activation seen in the visual cortex following treatment was due to improved visual pathway nerve conduction resulting from remyelination or repair in the optic nerve, then the extra-occipital adaptive response (from LOC in particular) might reduce as its contribution is not required by the brain. The ROI findings may therefore support evidence of re-organisation of the brain. However, the changes in the right eye and left eye for the visual cortex were in opposite directions (see Table 8.2) and although the joint eye ROI change was significant as was the positive change in the left eye, the actual value of the positive change is very small compared to the bigger negative change in the right eye. The right eye change did not achieve significance because of the large standard deviation. Overall, these not entirely consistent findings urge caution in the interpretation of the ROI extraction results. Results may possibly be more consistent in a future study of a larger cohort.

It could be speculated that the improvement in the visual function and shortening of latency of the visual evoked potentials were “placebo” effects with the patients trying harder and achieving greater attention during the visual assessment and visual evoked potential tests in the post treatment phase. However, there was a significant increase in optic nerve area, which was analysed blinded to the treatment arm status of subjects and time points. This would be difficult to explain as a “placebo” effect. There was also a trend towards reduction in T1 hypointense lesion volume and increase in T1 lesion MTR,

which would both be compatible with remyelination as a potential mechanism. If indeed the improved vision was due to remyelination, the absence of consistent change in cortical activation may simply mean that there was no change in cortical adaptive plasticity due to the intervention and that the functional MRI measures were not sensitive enough in our small cohort to reflect the functional improvement seen in the visual functional measures and VEP.

Chapter 9: Conclusions:

9.1 Need for repair therapies and trials to detect them:

MS poses a considerable challenge in management due to its clinical and pathological heterogeneity. Clinical, MRI and pathological studies suggest relative dissociation between inflammation and neurodegeneration. While there has been considerable progress made in therapeutic options for the inflammatory component of the disease, very little progress has been achieved in countering neurodegeneration. There is a need to develop treatments that prevent axonal loss, the pathological substrate of irreversible clinical disability. Designing clinical trials to detect neuroprotection and repair in MS is challenging. A “sentinel lesion” approach with longitudinal follow up of lesions at “sentinel” sites such as the optic nerve, using sensitive and site-specific outcome measures is promising in that regard.

The afferent visual pathway is commonly affected in MS. Following an optic nerve lesion or other visual pathway lesions longitudinally -using sensitive structural and functional outcome measures - can be a useful approach in testing potential repair therapies.

9.2 Afferent visual pathway assessment:

Visual function can be assessed using sensitive and quantitative clinical measures such as visual acuity, contrast acuity, colour vision and visual field. Optic nerve, tract, radiation and visual cortex can be specifically assessed using qualitative and

quantitative MRI acquisition sequences and analysis measures, such as are derived from T2W, FLAIR, MTR and DT imaging. Optic nerve lesion length, optic nerve cross-sectional area, optic nerve MTR, optic nerve DTI, optic radiation DTI and visual cortex MTR provide information on the structural integrity of the afferent visual pathway. In addition, significant recent advances in retinal imaging using OCT have helped in studying neuro-axonal loss *in vivo* non-invasively without confounding from loss of myelin. RNFL and macular volume measurements provide insight into neuro-axonal loss in MS, especially the measure of RNFL thickness. Further recent advances have enabled segmentation of different layers of the retina. The recent discovery of microcystic macular oedema in the inner nuclear layer of the retina in patients with MS recently has suggested that inflammation may occur in the central nervous system without the need for myelin. [94, 238] Visual functional MRI helps in assessing the contribution of adaptive plasticity to functional improvement. Visual evoked potential measurements and recently multi focal VEPs further help characterising structure/function relationships in the afferent visual pathway.

When all these measures were applied longitudinally in a repair therapeutic trial cohort of secondary progressive MS patients with a previous history of optic neuritis indicating the presence of a sentinel lesion, the structure-function relationship can be studied in detail. The potential pathophysiological factors and their clinical correlations were analysed and the hypotheses underlying the effect of potential repair therapy in question (stem cells) were investigated.

9.3 Mesenchymal stem cells as potential therapeutic agent in MS:

Stem cells with the potential to replicate and differentiate into various different cell types are very attractive as a potential repair therapy especially in neurodegenerative disorders. Specifically, mesenchymal stem cells have shown immuno-modulatory, anti-inflammatory, remyelinating properties in animal, in vitro and human studies. With the evidence in EAE mice and other autoimmune conditions in humans, the potential of their use as a therapeutic option in MS is worth exploring. As with any therapeutic agent, it is imperative to test the safety before judging its efficacy in MS patients. It means that the initial clinical studies, with the primary outcome measure being safety, could only be done in a small number of patients.

While such studies will not be sufficiently powered to investigate questions about efficacy, they provide a unique opportunity to test the safety and feasibility of the treatment approach, and they can also provide information on the utility of a proof-of-concept trial design, whilst also obtaining preliminary insight in to the *potential* for efficacy (through analysis of exploratory outcome measures). This approach may also provide valuable information to help in designing future larger trials with efficacy measures as the primary outcomes.

9.4 MSCIMS - An exploratory phase IIa trial of autologous mesenchymal stem

cells in MS:

Mesenchymal stem cells were successfully isolated, expanded, and administered intravenously to all trial participants with MS. Karyotypic stability was demonstrated by array Comparative Genomic Hybridisation (CGH). No significant immediate or delayed adverse reactions were observed. Of the visual pathway efficacy outcomes, improvement was seen following treatment in visual acuity and contrast sensitivity, together with a reduction in VER latency, increase in VER amplitude, and an increase in optic nerve cross-sectional area. Although it is not possible to definitively determine the biological mechanism of these effects, taken together they are consistent with the promotion of endogenous remyelination following treatment. A post-treatment cessation of the progressive rise in EDSS seen in the pre-treatment phase, together with the trends observed towards a reduction in brain T1 hypointense lesion volume and an increase in brain T1 hypointense lesion MTR, suggest that the effects of treatment were widely distributed throughout the CNS rather than specific to the anterior visual pathway.

The concept of longitudinal follow up of a single “sentinel” lesion, with objective and quantitative imaging biomarkers which provided insight into the pathophysiological processes, was successfully applied in a small cohort of secondary progressive MS patients in the MSCIMS trial. The structural integrity of the whole afferent visual pathway is responsible for visual function. Any disruption to this structure in MS leads to abnormal visual function. There are endogenous recovery processes that help recover both structure and function to some extent. In MS-associated optic neuritis,

functional recovery is often relatively good, even though there is typically significant permanent structural damage (e.g., retinal nerve fibre layer thinning) after optic neuritis. This discrepancy is analogous to the clinical radiological paradox that is sometimes seen in MS. This may be due to the redundancy of the optic nerve fibres in addition to cortical reorganisation of the brain where extra visual cortical areas may be recruited to help with visual function. Functional MRI helps understanding this concept. However, we did not see clear evidence for such an adaptive response in the MSCIMS trial. Regional analysis for fMRI activation did show differential activation changes between visual cortex and lateral occipital complexes. However, the directions of change were inconsistent between right and left eyes making their interpretation difficult.

Structural imaging, which includes MRI and OCT provides information about various pathological processes in MS such as break down of blood brain barrier, oedema, inflammation, demyelination, axonal loss, gliosis, remyelination. In the MSCIMS trial, these imaging measures provided a unique opportunity to study the effect of a stem cell therapeutic intervention on the structural integrity of a sentinel lesion (in the optic nerve) and also – to some extent hence extrapolate this information on - lesions elsewhere in the brain.

However, there are a few limitations in the MSCIMS study, one has to bear in mind while interpreting the findings. Firstly, it was an open label study with no placebo controls. Also there was no blinding apart from only a few efficacy measures (VEP, Optic nerve area). These factors could mean that, the placebo effect and observer bias have both affected the results. The small sample size and multiple assessments with multiple statistical analyses increases the risk of type II and type I error respectively. MSCIMS is a ‘proof of concept’ study and further larger trials – phase IIb and III – must incorporate essential elements in the study design (e.g., double-blinding, placebo

control, and a sample size that is powered to demonstrate unequivocal efficacy using biologically and/or clinically meaningful outcome measures) that overcome the limitations of our phase IIa trial.

9.5 Summary:

In summary, this thesis discusses the need for neuroprotective and repair therapies in MS and the concept of a “sentinel lesion” approach. Afferent visual pathway assessment in a clinical phase IIa trial of mesenchymal stem cells in multiple sclerosis has been described as a proof of this concept. Isolation, expansion, and infusion of MSC were demonstrated to be feasible and safe. Following treatment, patients improved on clinical, physiological, and structural measures, hinting at the potential for diffuse or multifocal remyelination due to the promotion of endogenous tissue repair.

Although MSCIMS was a small proof of concept study with safety as the primary outcome, the intriguing results that arise from it provide a rationale for larger double-blinded and placebo-controlled trials of this therapeutic approach in the future with measures of efficacy being the primary outcomes.

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