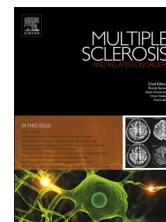




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# Sample sizes for lesion magnetisation transfer ratio outcomes in remyelination trials for multiple sclerosis



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Research design;  
Sample size

## Abstract

**Background:** Enhancing remyelination in MS might improve function and protect axons from future damage. Lesion magnetisation transfer ratio (MTR) is sensitive to myelin content, and may be a useful measure for trials evaluating potential remyelinating agents.

**Objective:** Estimating sample sizes required for a parallel group, placebo-controlled trial in MS using *change in mean MTR of all T<sub>2</sub> lesions* as a primary outcome measure.

**Methods:** The primary sample size calculation was derived from data from a natural history study of relapsing remitting MS ( $n=18$ ). The MTR values observed in demyelinated and remyelinated lesions in an *ex vivo* study were used to estimate the effect of remyelination on lesion MTR. The *ex vivo* data were also used to independently calculate sample sizes in order to inform the robustness of the *in vivo* estimates.

**Results:** Calculations suggest that 30% remyelination of T<sub>2</sub> lesions could be detected with 80% power in 38 (95% confidence interval 12–96) patients per arm based on the *in vivo* data, and in 66 per arm based on the *ex vivo* data.

**Conclusion:** The sample sizes derived are in a range that makes MTR a feasible outcome measure for proof-of-concept trials of putative therapies achieving remyelination in MS lesions.

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## 1. Introduction

Multiple sclerosis (MS) is a chronic demyelinating central nervous system disease with a variable prognosis. Multifocal demyelinating white matter lesions are a cardinal pathological feature. Newly forming white matter lesions exhibit acute inflammation with active myelin breakdown and result in symptomatic relapse when they occur in clinically eloquent locations. The acute features of a relapse are mediated by nerve conduction block due to demyelination, often occurring in the presence of inflammation. Recovery from relapses has multiple mechanisms, including resolution of inflammation, restoration of conduction in demyelinated axons by formation of new sodium channels along the internodal membrane, cortical adaptation and remyelination (Compston and Coles, 2008).

Remyelination is commonly observed in brain tissue of MS patients who have been studied at an early stage with short disease duration (Prineas et al., 1993). It is less commonly observed in pathology studies of patients with longstanding progressive forms of MS (Goldschmidt et al., 2009). Axonal loss in chronic lesions may be marked, and necessarily limits the prospects for remyelination. Axonal loss occurs in the acute inflammatory phase of lesion evolution, and there may be additional mechanisms of axonal loss in longstanding lesions: these include the absence of trophic support from myelin, where demyelination persists, and continuing low grade inflammation.

There are two potential benefits of enhancing remyelination in MS. First, it may restore conduction achieving improved function for patients. Secondly, it may be neuroprotective, and therefore improve longer term prognosis by preventing the accumulation of irreversible disability due to on-going axonal loss. Thus, development of a treatment that enhances remyelination is a current priority of experimental therapeutic research in MS.

Early clinical testing of a potential remyelinating treatment for MS will include safety monitoring and, ideally, a preliminary measure of efficacy. Clinical outcome measures of efficacy alone are unlikely to be sufficient in this setting as it will be difficult to determine the mechanism of a change in clinical function. For example, an improvement in clinical deficit might be due to enhanced axonal conduction through demyelinated segments, cortical adaptation, or remyelination.

*In vivo* imaging measures offer potential means of detecting remyelination. The magnetisation transfer ratio (MTR) of white matter lesions seen on  $T_2$ -weighted magnetic resonance imaging (MRI) is a quantitative measure that reflects tissue macromolecular proton content. In white matter, myelin is a major macromolecule-containing structure and significant differences have been observed in the MTR values of demyelinated (lower) and remyelinated (higher) MS lesions (Schmierer et al., 2004; Barkhof et al., 2003).

We therefore evaluated data obtained from MS white matter lesions, in order to determine the sample sizes that would be required for a proof-of-concept trial of a potential remyelinating treatment using the *change in mean MTR of all lesions visible on  $T_2$ -weighted brain MRI* as the primary outcome measure. Actual MTR values of demyelinated and remyelinated lesions and normal appearing white matter

(obtained from a previous *ex vivo* study) were used to define the extent of remyelinating treatment effects in the *in vivo* data. The *ex vivo* data were also used to independently calculate sample sizes in order to inform the robustness of the *in vivo* estimates.

## 2. Methods

In both the *ex vivo* and *in vivo* datasets, 2D dual spin echo images, both echoes obtained with and without a MT weighting prepulse, were acquired on a 1.5T GE Signa Echospeed scanner (General Electric Medical Systems, Milwaukee, Wisconsin, USA) using the following parameters: TR/TE1/TE2: 1720 ms/30 ms/80 ms. The matrix size for the images was  $256 \times 256$ , and the field of view was  $240 \times 240$  mm with 28 5 mm slices. MTR maps were generated from the short echo images using the standard MTR formula:

$$\text{MTR} = [(M_0 - M_s) / M_0] 100$$

where  $M_0$  and  $M_s$  are mean signal intensities without and with presaturation, respectively and the MTR is measured in percentage units (pu).

The demographic data for the *ex vivo* and *in vivo* studies have been published previously; (Davies et al., 2005; Schmierer et al., 2007b) both received ethical approval for the use of human subjects.

### 2.1. *In vivo* data

Longitudinal data were acquired on 18 patients with short duration (<3 years) and low disability (EDSS < 3) relapsing remitting multiple sclerosis (RRMS). They underwent brain imaging on two occasions one year apart (Davies et al., 2005). At each time point, lesions were defined on the short echo non-MT weighted images using a semi-automated method implemented with JIM 5.0\_21 (Xinapse Systems, UK). Mean lesional MTR was derived by applying these lesion maps to the inherently co-registered MTR maps. The same lesion maps were then horizontally inverted along the centre of the image, and checked and edited as necessary in order to apply them to the contra-lateral parenchyma and thus calculate a mean MTR for region specific normal appearing white matter. The relatedness of lesional MTR at the two timepoints was assessed by the Pearson correlation coefficient.

### 2.2. *Ex vivo* data

MTR maps were obtained for unfixed coronal *post mortem* brain slices from 12 patients who died with progressive MS, using stereotactic registration to accurately match regions of interest (ROI) on MRI with homotopic ROI on histology (Schmierer et al., 2003, 2007b). Myelin content was quantified on histological sections using light transmittance, defined as the transmitted light divided by the incident light, on sections stained for Luxol fast blue (LFB) (Schmierer et al., 2004, 2007b).

The mean and standard deviation of MTR were measured in tissue samples from (i) seven patients (six secondary progressive (SP) and one primary progressive) with both

demyelinated and remyelinated lesions and a region of interest in normal appearing white matter (NAWM) in the tissue sample; and (ii) a further five SPMS patients with only remyelinated lesions and a region of interest in NAWM.

### 2.3. Calculation of sample sizes

The sample sizes calculated are based on a placebo-controlled, parallel groups trial design in which it is assumed that *mean MTR of all lesions visible on T<sub>2</sub>-weighted brain MRI does not change in a placebo arm*. This assumption is probably conservative: a number of studies have found no statistically significant change in mean lesion MTR (Davies et al., 2005; Button et al., 2013) while others describe a trend to a reduction (Rocca et al., 1999; Connick et al., 2012) and Mesaros et al. (2010) identified a statistically significant fall. The proposed trial analysis for which we calculate sample sizes is a comparison of mean baseline-to-follow-up MTR changes between trial arms, adjusted for baseline (an ANCOVA analysis (Frison and Pocock, 1992), implemented with a linear regression of the change on a group indicator and baseline). For such analyses power increases with increasing baseline *versus* follow-up Pearson correlation (Frison and Pocock, 1992). It is sufficient, in order to calculate the power for such analyses, to have estimates of the baseline *versus* follow-up correlation, of the cross-sectional MTR standard deviation, and of the magnitude of potential treatment effects. The cross-sectional data are sufficient because a baseline adjusted comparison of cross-sectional follow-up means is mathematically identical to a baseline adjusted comparison of mean changes, and the combination of longitudinal correlation and cross-sectional standard deviation captures the standard deviation of changes. The treatment effects to be detected were inferred from the difference between remyelinated and demyelinated lesions in the *ex vivo* study. The observed correlation of 0.7 in the RRMS sample was used for the calculation for both the *in vivo* and *ex vivo* groups.

### 2.4. *In vivo* sample size calculation

The *ex vivo* study indicated that remyelinated lesions have a MTR value that is approximately midway between demyelinated lesions and NAWM (Schmierer et al., 2007b). Therefore, it was assumed for the *in vivo* calculation that a 100% remyelination treatment effect would represent half the difference observed *in vivo* between NAWM and lesion MTR. For this sample size calculation the larger MTR standard deviation (which was for lesions, at baseline) was used. Confidence intervals (95%), incorporating uncertainty around the one-year correlation, were derived for selected sample sizes for the *in vivo* data using a non-parametric bootstrap with 1000 replicates (Carpenter and Bithell, 2000).

### 2.5. *Ex vivo* sample size calculation

For the *ex vivo* study, the difference between the remyelinated and demyelinated MTR means was used to determine the magnitude of a 100% remyelination treatment

effect. The larger MTR SD (which was in demyelinated lesions) was used in the sample size calculation. Confidence intervals were not calculated for the *ex vivo* estimates since correlation data were not directly available.

## 3. Results

In the *in vivo* RRMS data, baseline lesion MTR mean (SD) was 30.26 (1.91) pu and in contralateral NAWM, 36.18 (0.53) pu, a difference of 5.92 pu. Baseline *versus* 1-year lesion MTR Pearson correlation was 0.73 and the (conservative) value of 0.7 was therefore used for the primary sample size calculation.

In the *ex vivo* data MTR mean (SD) was 21.8 (4.8) pu in demyelinated and 27.4 (4.5) pu in remyelinated lesions, and 33.7 (3.0) pu in NAWM (Davies et al., 2005). The *ex vivo* remyelinated mean MTR is approximately mid-way between that for demyelinated lesions and NAWM, and therefore, for the *in vivo* calculation, half of the lesion *versus* NAWM mean MTR difference,  $5.92/2=2.96$ , was used as 100% treatment (remyelination) effect, with the lesion MTR SD of 1.91.

Incorporating uncertainty around the estimated correlation of 0.7 in a baseline adjusted analysis for 30%, 40% and 50% treatment effects with 80% power, the mean (95% confidence interval) sample sizes were 38 (12-96), 21 (7-54) and 14 (6-39) respectively.

Table 1 shows calculated sample sizes (per trial arm) required to detect treatment effects corresponding to different proportions of half the NAWM minus lesion MTR difference based on the *in vivo* data; shown for 80% and 90% power assuming 5% significance.

The difference between demyelinated and remyelinated lesions in the *ex vivo* data set was 5.6 pu. Therefore this was used as 100% treatment effect (with the demyelinated MTR SD 4.8) for the *ex vivo* sample sizes, also shown in Table 1. For 30%, 40% and 50% treatment effects with 80% power, the sample sizes were 66, 37 and 24 respectively.

## 4. Discussion

The *in vivo* sample size calculations are in a range that makes lesion MTR a feasible outcome measure for proof-of-concept trials of putative remyelinating therapies in MS (Table 1). The sample sizes calculated from the *ex vivo* data (Table 1) inform the robustness of the *in vivo* calculation; it is reassuring that they fall within the 95% confidence intervals of the *in vivo* calculations. Thus for example, to detect a 30% treatment effect a sample size of 38 (95% confidence interval 12-96) is estimated from the *in vivo* data and of 66 from the *ex vivo* data.

*In vivo* calculations that include assumptions derived from the *ex vivo* data might not be reliable if there was a significant change in *ex vivo* MTR value from the *in vivo* state. However, the *ex vivo* measures used in this study were obtained from unfixed postmortem brain and they have previously been noted to be similar to *in vivo* MTR measures using the same acquisition sequence (Schmierer et al., 2004).

A number of other factors might affect the accuracy of these estimates, which are summarised in Table 2. This work was performed on a 1.5-T scanner; it has been shown

**Table 1** Sample sizes per trial arm for baseline adjusted analysis.<sup>a</sup>

Power (%):	<i>n</i> (95% CI)			
	From the <i>in vivo</i> data		From the <i>ex vivo</i> data	
	90	80	90	80
Treatment effect <sup>b</sup>				
20%	112 (35, 287)	84 (26, 215)	197	148
30%	50 (15, 128)	38 (12, 96)	88	66
40%	28 (10, 78)	21 (7, 54)	50	37
50%	18 (7, 50)	14 (6, 39)	32	24
60%	13 (5, 35)	10 (4, 27)	22	17

<sup>a</sup>Baseline *versus* follow-up Pearson correlation coefficient of 0.7 assumed; 0.73 was observed in the *in vivo* data. Confidence intervals (95%), incorporating uncertainty around the 1-year correlation, were derived for the *in vivo* data using a non-parametric bootstrap with 1000 replicates. Confidence intervals were not calculated for the *ex vivo* estimates since correlation data were not directly available.

<sup>b</sup>For the *in vivo* data 100% treatment effect equates to 2.96 pu, half the difference between estimated NAWM and lesion MTR means; assumed lesion MTR standard deviation is 1.91 pu. For the *ex vivo* data 100% treatment effect equates to 5.6 pu, the difference between estimated remyelinated and demyelinated lesion MTR means; assumed lesion MTR standard deviation is 4.8 pu.

**Table 2** Factors which might affect the sample size estimates for remyelination of T<sub>2</sub> lesions with examples.

Only a subset of lesions may be capable of remyelination.	<i>If a significant number of lesions are already remyelinated, and mean lesion MTR used, then a given treatment efficacy would result in smaller mean MTR increase, requiring larger sample size. This could be offset by selecting lesions in the subset, e.g., those with lower MTR.</i>
Other processes may affect MTR recovery.	<i>If lesion MTR was significantly increased by a decrease in inflammation/oedema, larger sample sizes would be required to show MTR recovery due to remyelination per se: this is more likely to occur in acute Gd-enhancing lesions.</i>
Treatment might increase quality as well as extent of remyelination	<i>If remyelination proves more effective than that observed in the ex vivo samples (i.e., the new myelin was structurally similar to normal myelin and hence MTR recovered to the level of NAWM), smaller sample sizes would be required.</i>
There may be a small decrease in mean MTR of all T <sub>2</sub> lesions rather than stability in untreated patients (Mesaros et al., 2010)	<i>If the placebo arm has a small decrease in MTR, smaller sample sizes will be required to demonstrate a given remyelinating (i.e. increase in lesion MTR) effect.</i>
Technical limitations	<i>In a multi-centre study power might be lost correcting for inter scanner differences<sup>a</sup>.</i>

<sup>a</sup>This remains a theoretical concern but studies have shown the potential to overcome this (Brown et al., 2013).

that 3-T scanners provide higher contrast to noise ratio and signal to noise ratio in MTR maps than 1.5-T scanners (Cercignani et al., 2006). This means that at 3 T changes in MTR should be more readily detected, reducing the number of subjects required to show a difference. However, given the prevalence of 1.5-T scanners in operation, this study is not overly conservative with regard to field strength. MS subtype, patient age and disease duration might also affect remyelination. In this sample (mean age 40.1 years and mean disease duration 1.7 years) there was no evidence of association between age ( $P=0.281$ ) or

disease duration ( $P=0.895$ ) and the white matter *versus* lesion MTR difference which is the crucial quantity for our calculation. However, we believe that there are good reasons for selecting a relatively homogenous population for any initial trial of a potential remyelinating agent; these reasons are discussed later. Although our study sample is relatively small, we believe it is representative of its patient population, and the confidence intervals around the sample size estimates quantify the uncertainty in our estimates which result from the size of our study sample.



Whether a subset of lesions could be identified that has particular potential for remyelination is an especially important question. There is evidence from experimental studies that spontaneous remyelination occurs mainly in acute lesions and in the presence of inflammation; this may therefore be the condition under which exogenous repair strategies are most likely to succeed (Foote and Blakemore, 2005). Hence there has been interest in using the MTR outcome of *gadolinium enhancing lesions* as a measure of remyelination. Van den Elskamp et al. (2010) reported that approximately 70 patients/arm would be required - to show a treatment effect of 30% of the difference between MTR before enhancement and after the lesion has stabilised - in a treatment *versus* placebo parallel groups study. More recently, for a similar effect size, Brown et al. (2013) estimated a requirement of 12 patients /arm for studies based on *gadolinium enhancing lesions* (these being similar to their estimates for a novel lesion definition  $\Delta MTR$  lesions, for which they have a sophisticated automated method of segmentation). Brown et al. use the novel metric  $MTR_{drop}$  which is based on change in lesion MTR not endpoint lesion MTR. The overlap of the confidence intervals of our estimates (for all  $T_2$  lesions) with both these estimates (for *gadolinium enhancing lesions*) is reassuring. Although the conditions for remyelination may be more favourable in acute lesions and in the presence of inflammation (Foote and Blakemore, 2005) if it is possible to achieve repair in all or most lesions - those with longstanding demyelination as well as those in an acute or post-acute phase - there should be greater clinical benefit. Both approaches for estimating sample size are therefore useful and complementary.

Another subset of demyelinating lesions of particular interest is those in the cortex. These are poorly visualised on conventional MRI and are thus not distinguished from normal appearing grey matter (NAGM). Cortical lesions have been shown to be highly prevalent in both *postmortem* samples and a biopsy study (Bo et al., 2003; Kutzelnigg et al., 2005; Lucchinetti et al., 2011). Their particular importance is in the strength of their association with both neurological and neuropsychological disability and further their predictive value for future disability (Sanfilippo et al., 2005; Fisher et al., 2008; Fisniku et al., 2008). Chen et al. (2013) have recently demonstrated, by comparison of *post-mortem* MRI with histopathology that cortical MTR is a feasible, sensitive, measure of cortical demyelination. We have previously reported MTR evidence for the potential of an experimental treatment (alemtuzumab) to inhibit the development of cortical demyelination (Button et al., 2013).

A key requirement for effective remyelination is good axonal preservation in lesions. Axonal loss is reported to be more extensive and severe in the lesions and normal appearing tissues of progressive than relapsing remitting forms of MS (Kutzelnigg et al., 2005; Tallantyre et al., 2009). Studies with retinal nerve fibre layer imaging in acute optic neuritis show that the severity of irreversible visual dysfunction is correlated with the extent of axonal loss (Trip et al., 2005); by extrapolation, it is plausible that the irreversible deficit occurring elsewhere in the brain and spinal cord, following relapse, will also correlate with the extent of axonal loss in the clinically eloquent lesion. The proportion of lesions with good axonal preservation is

therefore likely to be the greatest in patients with limited disability and without progressive disease. This is why we have derived on our sample size calculations on the serial lesion MTR measures acquired in a group of patients with relapsing remitting MS of short duration (<3 years) and low disability (EDSS<3). Nevertheless, there are other imaging parameters which might be used to infer axonal damage (Van Waesberghe et al., 1999; Schmierer et al., 2007a; Wood et al., 2012). It may be possible in future studies to use registered serial scans to investigate changes in lesion MTR at the level of individual voxels (Chen et al., 2007) along with additional imaging modalities to stratify on the basis of axonal integrity, focusing MTR analysis on areas with most potential for remyelination and thus increasing power.

## 5. Conclusions

Our *in vivo* sample size estimates are based on the novel approach of estimating potential treatment effects from MTR measurements of actual (*ex vivo*) remyelinated lesions. Using baseline adjusted change in lesion MTR corrects for baseline variability, and we demonstrate the feasibility of *change in the mean MTR of all lesions visible on T<sub>2</sub>-weighted brain MRI* as a measure of trials of putative remyelinating agents in multiple sclerosis.

## Contributions

Altmann: Conceptualization of the study; analysis and interpretation of the data; and drafting and revising the manuscript.

Button: Analysis and interpretation of data; drafting and revising the manuscript.

Schmierer: Analysis and interpretation of data; drafting and revising the manuscript.

Hunter: Drafting and revising the manuscript.

Tozer: Drafting and revising the manuscript, and acquisition of data.

Wheeler-Kingshott: Drafting and revising the manuscript.

Coles: Drafting and revising the manuscript.

Miller: Conceptualisation of the study, interpretation of the data and drafting and revising the manuscript.

## Disclosures

Altmann is partially funded by the Multiple Sclerosis Society of Great Britain and Northern Ireland, has received an honorarium for attending a meeting organised by Merck & Co., Inc., and gave expert testimony in the case of Mylan v. Yeda.

Button is funded by a David Walker Fellowship and receives a Sackler Studentship.

Schmierer is a PI on trials sponsored by Novartis and Roche, has received speaking honoraria from, and served on advisory boards for, Novartis, Merck-Serono and Merck & Co., Inc., and is in receipt of a HEFCE Clinical Senior Lectureship, and grant support from Barts and The London Charity and from Novartis.

Hunter reports no disclosures.

Tozer is partially funded by Biogen Idec and Novartis, unconnected to this work.

Wheeler-Kingshott is on the advisory board for BG12 (Biogen) and receives grants (PI and co-applicant) from ISRT, EPSRC, Wings of Life, MS Society, Biogen Idec and Novartis.

Coles is funded in part by the National Institute for Health Research (NIHR) Biomedical Research Centre, Cambridge and has received consulting fees, lecture fees, and grant support from Genzyme.

Miller has received research grants (held by University College London) from Biogen Idec Inc., GlaxoSmithKline, Schering AG, and Novartis to perform MRI analysis in multiple sclerosis trials; and has received honoraria and travel expenses for advisory committee work or as an invited speaker from Biogen Idec Inc, GlaxoSmithKline, Bayer Schering, Novartis and the US National Institutes of Health.

## Conflict of interest statement

None.

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## References

- Barkhof F, Bruck W, De Groot CJA, Bergers E, Hulshof S, Geurts J, et al. Remyelinated lesions in multiple sclerosis—magnetic resonance image appearance. *Archives of Neurology* 2003;60(8):1073-81.
- Buttner T, Altmann D, Tozer D, Dalton C, Hunter K, Compston A, et al. Magnetization transfer imaging in multiple sclerosis treated with alemtuzumab. *Multiple Sclerosis Journal* 2013;19(2):241-4.
- Brown RA, Narayanan S, Arnold DL. Segmentation of magnetization transfer ratio lesions for longitudinal analysis of demyelination and remyelination in multiple sclerosis. *Neuroimage* 2013;66:103-9.
- Bo L, Vedeler CA, Nyland HI, Trapp BD, Mork SJ. Subpial demyelination in the cerebral cortex of multiple sclerosis patients. *Journal of Neuropathology and Experimental Neurology* 2003;62(7):723-32.
- Compston A, Coles A. Multiple sclerosis. *Lancet* 2008;372(9648):1502-17.
- Connick P, Kolappan M, Crawley C, Webber DJ, Patani R, Michell AW, et al. Autologous mesenchymal stem cells for the treatment of secondary progressive multiple sclerosis: an open-label phase 2a proof-of-concept study. *Lancet Neurology* 2012;11(2):150-6.
- Carpenter J, Bithell J. Bootstrap confidence intervals: when, which, what? A practical guide for medical statisticians *Statistics in Medicine* 2000;19(9):1141-64.
- Cercignani M, Symms MR, Ron M, Barker GJ. 3D MTR measurement: from 1.5 T to 3.0 T. *Neuroimage* 2006;31(1):181-6.
- Chen JT, Easley K, Schneider C, Nakamura K, Kidd G, Chang A, et al. Clinically feasible MTR is sensitive to cortical demyelination in MS. *Neurology* 2013;80(3):246-52.
- Chen JT, Kuhlmann T, Jansen GH, Collins DL, Atkins HL, Freedman MS, et al. Voxel-based analysis of the evolution of magnetization transfer ratio to quantify remyelination and demyelination with histopathological validation in a multiple sclerosis lesion. *Neuroimage* 2007;36(4):1152-8.
- Davies GR, Altmann DR, Hadjiprocopis A, Rashid W, Chard DT, Griffin CM, et al. Increasing normal-appearing grey and white matter magnetisation transfer ratio abnormality in early relapsing-remitting multiple sclerosis. *Journal of Neurology* 2005;252(9):1037-44.
- Footo AK, Blakemore WF. Inflammation stimulates remyelination in areas of chronic demyelination. *Brain* 2005;128:528-39.
- Fisher E, Lee JC, Nakamura K, Rudick RA. Gray matter atrophy in multiple sclerosis: a longitudinal study. *Annals of Neurology* 2008;64(3):255-65.
- Fisniku LK, Chard DT, Jackson JS, Anderson VM, Altmann DR, Miszkiel KA, et al. Gray matter atrophy is related to long-term disability in multiple sclerosis. *Annals of Neurology* 2008;64(3):247-54.
- Frison L, Pocock SJ. Repeated measures in clinical-trials - analysis using mean summary statistics and its implications for design. *Statistics in Medicine* 1992;11(13):1685-704.
- Goldschmidt T, Antel J, Koenig FB, Brueck W, Kuhlmann T. Remyelination capacity of the MS brain decreases with disease chronicity. *Neurology* 2009;72(22):1914-21.
- Kutzelnigg A, Lucchinetti CF, Stadelmann C, Bruck W, Rauschka H, Bergmann M, et al. Cortical demyelination and diffuse white matter injury in multiple sclerosis. *Brain* 2005;128:2705-12.
- Lucchinetti CF, Popescu BFG, Bunyan RF, Moll NM, Roemer SF, Lassmann H, et al. Inflammatory cortical demyelination in early multiple sclerosis. *New England Journal of Medicine* 2011;365(23):2188-97.
- Mesaros S, Rocca MA, Sormani MP, Valsasina P, Markowitz C, De Stefano N, et al. Bimonthly assessment of magnetization transfer magnetic resonance imaging parameters in multiple sclerosis: a 14-month, multicentre, follow-up study. *Multiple Sclerosis* 2010;16(3):325-31.
- Prineas JW, Barnard RO, Kwon EE, Sharer LR, Cho ES. Multiple sclerosis—remyelination of nascent lesions. *Annals of Neurology* 1993;33(2):137-51.
- Rocca MA, Mastrorlando G, Rodegher M, Comi G, Filippi M. Long-term changes of magnetization transfer-derived measures from patients with relapsing-remitting and secondary progressive multiple sclerosis. *American Journal of Neuroradiology* 1999;20(5):821-7.
- Schmierer K, Scaravilli F, Altmann DR, Barker GJ, Miller DH. Magnetization transfer ratio and myelin in postmortem multiple sclerosis brain. *Annals of Neurology* 2004;56(3):407-15.
- Schmierer K, Wheeler-Kingshott CAM, Boulby PA, Scaravilli F, Altmann DR, Barker GJ, et al. Diffusion tensor imaging of post mortem multiple sclerosis brain. *Neuroimage* 2007a;35(2):467-77.
- Schmierer K, Tozer DJ, Scaravilli F, Altmann DR, Barker GJ, Tofts PS, et al. Quantitative magnetization transfer imaging in postmortem multiple sclerosis brain. *Journal of Magnetic Resonance Imaging* 2007b;26(1):41-51.
- Schmierer K, Scaravilli F, Barker GJ, Gordon R, MacManus DG, Miller DH. Stereotactic co-registration of magnetic resonance imaging and histopathology in post-mortem multiple sclerosis brain. *Neuropathology and Applied Neurobiology* 2003;29(6):596-601.
- Sanfilippo MP, Benedict RHB, Sharma J, Weinstock-Guttman B, Bakshi R. The relationship between whole brain volume and disability in multiple sclerosis: a comparison of normalized gray vs. white matter with misclassification correction. *Neuroimage* 2005;26(4):1068-77.
- Tallantyre EC, Bo L, Al-Rawashdeh O, Owens T, Polman CH, Lowe J, et al. Greater loss of axons in primary progressive multiple sclerosis plaques compared to secondary progressive disease. *Brain* 2009;132:1190-9.
- Trip SA, Schlottmann PG, Jones SJ, Altmann DR, Garway-Heath DF, Thompson AJ, et al. Retinal nerve fiber layer axonal loss and visual dysfunction in optic neuritis. *Annals of Neurology* 2005;58(3):383-91.

Van den Elskamp IJ, Knol DL, Vrenken H, Karas G, Meijerman A, Filippi M, et al. Lesional magnetization transfer ratio: a feasible outcome for remyelinating treatment trials in multiple sclerosis. *Multiple Sclerosis Journal* 2010;16(6):660-9.

Van Waesberghe J, Kamphorst W, De Groot CJA, van Walderveen MAA, Castelijns JA, Ravid R, et al. Axonal loss in multiple

sclerosis lesions: Magnetic resonance imaging insights into substrates of disability. *Annals of Neurology* 1999;46(5):747-54.

Wood ET, Ronen I, Techawiboonwong A, Jones CK, Barker PB, Calabresi P, et al. Investigating axonal damage in multiple sclerosis by diffusion tensor spectroscopy. *Journal of Neuroscience* 2012;32(19):6665-9.