Published in final edited form as:

J Neuroimaging. 2014 November ; 24(6): 569–576. doi:10.1111/jon.12082.

Identifying the Start of Multiple Sclerosis Injury: A Serial DTI Study

Daniel Ontaneda, MD¹, Ken Sakaie, PhD², Jian Lin, MS², Xiaofeng Wang, PhD³, Mark J. Lowe, PhD², Michael D. Phillips, MD², and Robert J. Fox, MD¹

¹Mellen Center, Department of Neurology, Neurological Institute, Cleveland Clinic Foundation, Cleveland, OH, USA

²Imaging Institute, Cleveland Clinic Foundation, Cleveland, OH, USA

³Department of Quantitative Health Sciences, Cleveland Clinic Foundation, Cleveland, OH, USA

Abstract

Background—The events leading up to the development of new multiple sclerosis (MS) lesions on conventional imaging is unknown. The purpose of this study is to use diffusion tensor imaging (DTI) to investigate pre-lesional changes in MS to better understand the pathological changes that lead to lesion development.

Methods—Twenty-one patients with relapsing MS starting natalizumab therapy underwent serial DTI for 12–18 months. Regions of interest were outlined within normal-appearing white matter and new gadolinium-enhancing lesions that developed over the course of the study. Images from all time points were coregistered and non-parametric regression was used to assess DTI changes prior to lesion appearance.

Results—31 newly-enhancing lesions were identified. Significant changes in transverse diffusivity (TD) ($p < 0.001$), longitudinal diffusivity (LD) ($p = 0.025$), mean diffusivity (MD) ($p < 0.001$) and fractional anisotropy (FA) ($p = 0.04$) were observed prior to gadolinium-enhancement. A progressive increase in TD and LD occurred up to 10 months prior to lesion development. DTI measures in normal appearing white matter remained unchanged over the study period.

Conclusion—A significant change in diffusion measures can be seen prior to gadolinium enhancement. Changes in TD drove changes in FA and MD, providing evidence for impaired myelin integrity prior to gadolinium enhancement. DTI may be a sensitive measure for early detection of inflammatory disease activity in MS.

Keywords

MRI; DTI; T2 lesions; multiple sclerosis; natalizumab; pre-lesional

Introduction

Multiple sclerosis (MS) is a chronic relapsing inflammatory disease affecting the central nervous system. Clinical relapses are often associated with the appearance of new or enlarging lesions within the brain and spinal cord. [1] Gadolinium enhancement, which results from breakdown of the blood brain barrier, heralds the onset of new lesion development on conventional imaging and correlates with relapses.[2] Approximately 80% of untreated patients with active relapsing forms of MS demonstrate areas of gadolinium enhancement on monthly magnetic resonance imaging (MRI) scans in short term studies (3–6 months).[3, 4]

Contrary to the notion that the breakdown of the blood brain barrier marks the beginning of lesion formation, advanced imaging modalities have suggested that tissue changes may actually occur prior to gadolinium enhancement.[5] Magnetization transfer ratio has been found to be decreased in normal appearing brain tissue months prior to gadolinium enhancement.[6–8] Serial MRI studies observed decreased diffusivity weeks to months prior to gadolinium enhancement in brain regions that appeared normal on conventional imaging but manifested lesions on follow-up.[9, 10] Biochemical changes also occur prior to lesion development as evidenced using magnetic resonance spectroscopy. Specifically, the choline to creatine ratio was higher in normal appearing brain tissue voxels that became lesional in time as compared to voxels that remained non-lesional.[11] It has been postulated that lesion formation occurs in phases and that early lesions may represent areas of microglial activation and oligodendrocyte apoptosis without inflammation.[12] On histology, these so called pre-active lesions contain activated microglia without a full-blown inflammatory response and with preservation of the blood brain barrier.[13]

Diffusion tensor imaging (DTI) is a MR-based technique that is a powerful tool for evaluating brain microstructure, including inflammatory demyelination in MS.[14] By applying diffusion-weighting gradients with different orientations during an MR acquisition, it is possible to characterize the degree of anisotropy in water diffusion using a tensor model.[15] The diffusion properties reflect the microstructure of the underlying tissue.[16] For example, the principal eigenvector of the tensor aligns with the direction of myelinated axons in well-organized white matter fascicles while indices calculated from the eigenvalues of the tensor, such as mean diffusivity (MD) and fractional anisotropy (FA), are commonly used as metrics that describe the overall diffusion of water (MD) and anisotropy (FA) in tissue.[15, 17] Longitudinal diffusivity (LD) (also known as axial diffusivity) has been found to correlate with axonal loss, as measured by neurofilament staining (SMI-31), in mouse optic nerve after retinal ischemia.[18] Transverse diffusivity (TD) (also known as radial diffusivity) correlates with myelin content in shiverer mice as measured by toluidine blue staining.[19] LD and TD, however, are not necessarily specific to axonal injury and demyelination. Budde et al [20] found that LD correlated with axonal content, but TD was not associated with changes in luxol fast blue staining both in a model of spinal cord contusion and experimental autoimmune encephalomyelitis (EAE). In a region of interest analysis the authors did however find that TD was increased in areas of low luxol fast blue staining, but not in normal white matter. In rat spinal cord models of demyelination DeBoy et al. found that both longitudinal and transverse diffusivity were correlated with axonal

pathology, but not with demyelination. The authors conclude it may be difficult to separate the effects of demyelination and axonal transection using DTI.[21]

Previous prelesional studies using diffusion *weighted* imaging focused on MD and obviated measurements of tissue anisotropy including LD and TD.[9, 10] We report the first prelesional diffusion *tensor* longitudinal study in MS. Our work, in contrast with prior studies features a high angular resolution diffusion imaging acquisition.[22] This acquisition reduces the variability and bias found in lower angular resolution approaches. Using advanced statistical modeling and including LD and TD, we aimed to expand those preliminary studies to further understand prelesional tissue characteristics as measured by DTI. We studied the changes in DTI measures prior to the appearance of active inflammation on conventional imaging using a cohort of MS patients followed longitudinally over 18 months using serial DTI imaging.

Materials and Methods

Participants

21 relapsing MS patients starting natalizumab therapy were enrolled in an Institutional Review Board-approved longitudinal observational imaging study, as previously described [23]. Inclusion criteria were clinically definite MS by 2005 Revised McDonald Criteria [24], age >18 years and ability to provide informed consent. Exclusion criteria were clinical relapse or steroid treatment in the previous 8 weeks, pregnancy and contraindication to MRI such as severe claustrophobia and implanted devices such as neurostimulators and pacemakers. Details of the patients studied have been previously described [23]. Most patients were females (15/21), mean age was 41.6 years (range 20–62), mean disease duration was 11.9 years (range 2–30), and all patients were started on natalizumab therapy. EDSS was not collected at baseline and hence not available for reporting.

MRI Imaging Protocol

MRI scanning of the brain was serially acquired at time baseline (prior to natalizumab dosing), 1, 2, 6, 12 and 18 months. Images were obtained on a 3 tesla Siemens Trio (Siemens Medical Systems, Erlangen, Germany). A Standard 12-channel head coil was used. Diffusion-weighted imaging used 71 non-collinear diffusion-weighting gradients ($2.5 \times 2.5 \times 2.5$ mm voxels, $b = 2000 \text{ sec/mm}^2$, 8 $b = 0$ acquisitions; 260×260 mm FOV, 104×104 matrix, 48 2.5 mm slices, TE = 95 msec, TR = 7300 msec). Anatomical imaging was performed for lesion detection and co-registration: 3D MPRAGE (256×256 mm FOV, 128×256 matrix, 120 1.2 mm slices, TE = 1.71 msec, TR = 1900 msec, T1 = 900 msec, flip angle = 8°); proton density / T2-weighted (230×230 mm FOV, 320×320 matrix, 48 3-mm slices, TE1 = 20 msec, TE2 = 91 msec and TR = 3600 msec) and T1 post-gadolinium (230×230 mm FOV, 320×320 matrix, 48 3 mm slices, TE = 2.46 msec, TR 300 msec, flip angle = 75°). Iterative motion correction was applied to the HARDI images as previously described [25] followed by coregistration of $b=0$ images to the baseline MPRAGE T1 with FLIRT[26] (12 degree-of-freedom affine transformation, normalized mutual information cost function with trilinear interpolation) from FSL.[27] After motion correction, tensor images were calculated and derived for analysis.

Image Analysis

Regions of interest (ROIs) were drawn on each gadolinium enhancing lesion (GAD) that developed after baseline on T1 post-gadolinium images with analysis of functional neuroimaging (AFNI) software.[28] Only new GAD lesions were studied, enlarging or re-enhancing lesions were excluded. As a control, sixteen normal appearing white matter (NAWM) ROIs were drawn on FA maps from each subject over 12 months, as previously described.[23] At the time of ROI placement aligned T2, MPAGE maps were reviewed to ensure adequate anatomic location and detection of lesions in NAWM. Data up to 12 months for NAWM was included as that was complete for all subjects at the time of analysis. These bilateral ROIs included anterior and posterior corpus callosum, centrum semi-ovale, anterior/posterior internal capsule, anterior frontal lobe white matter tracts, corticospinal tract at the level of the midbrain and corticospinal tract at the level of the pons. NAWM ROIs only included regions that showed no abnormal signal on either T1-weighted or T2-weighted imaging at baseline and all follow-up scans. If lesions were present in the pre-defined NAWM regions, that NAWM region was omitted or shifted for that patient. Voxels immediately adjacent to cerebrospinal fluid (CSF) were excluded in both GAD and NAWM ROIs to avoid partial-volume averaging with CSF. All ROIs were visually inspected on subsequent scans to ensure accurate co-registration and adjusted, where necessary.

For GAD ROIs, the time point at which the individual lesion first developed gadolinium enhancement was labeled as time zero for that GAD ROI. DTI measures within each GAD ROI co-registered over time were defined with respect to this time zero. Negative time points referred to months prior to gadolinium enhancement and positive time points to months after onset of gadolinium enhancement for that ROI. Longitudinal analysis was conducted independently for each gadolinium enhancing lesion, and then data was combined together into a single statistical model. Average values for FA, MD, LD and TD were derived for each NAWM and GAD ROI at each time point.

Statistical Analysis

A nonparametric regression model with penalized cubic regression splines [29, 30] was fit to the overall trend of each DTI measure for GAD ROI's followed by F-tests of the variance around the model to test the null hypothesis that the regression function is constant over time.[31] This nonparametric approach models the nonlinear predictor-response relationship without imposing any predetermined form of the regression function, instead constructing it according to information derived from the data. This statistical model was selected because it has the ability to account for the variability in DTI measurements between subjects and also between the different time points occurring in a nonlinear fashion. The standard parametric method for analysis, a linear mixed effect model [32] may miss important trends for a number of reasons. For example, there is considerable longitudinal, between-subject and between-lesion variability. Additionally, measurements were taken at multiple, non-evenly distributed time points. Cubic regression spline analysis is ideal for this type of data analysis because it relaxes the strict linearity assumption of linear regression models, while remaining statistically valid.

A cubic polynomial was fit to evaluate the trend of NAWM ROIs for each DTI measurement over the first 12 months of the study period. Analysis was conducted at 12 months to balance the time pre-lesional tissue was followed (mean 9.7 months). The cubic polynomial was more appropriate than a spline for the NAWM because time points were few in number and were not enough to construct the knots of splines. However, analogous to the spline analysis, the cubic polynomial-based analysis tested the null hypothesis that NAWM DTI values are constant over time.

Results

Six patients discontinued natalizumab therapy during the study period. Imaging was complete up to 18 months for 18 subjects and up to 12 months for 3 subjects. A total of 7 relapses occurred among 5 patients. Relapses were typically treated with courses of intravenous solumedrol. 31 gadolinium lesions were identified among 5 patients during the study period among and a total of 173 discrete observations were made at time points spanning from 18 months prior to gadolinium enhancement to 17 months after gadolinium enhancement. Lesion distribution per subject and time point are presented in Table 1. Gadolinium enhancing lesions occurred both in patients continuing natalizumab and those who discontinued medication.

Nonparametric regression analysis (Figure 1) showed significant changes of TD ($p < 0.001$), LD ($p = 0.025$), MD ($p < 0.001$) and FA ($p = 0.04$) in GAD lesions over the study period. Visual inspection of TD values showed a marked increase during the 10 months prior to GAD lesion development, while LD values showed a modest increase during the same time period. As a result MD also showed a progressive increase 10 months prior to gadolinium enhancement. Visual inspection of FA values showed a decline approximately 3 months prior to gadolinium enhancement. Percent change of DTI metrics at each time point compared to time of enhancement is presented in table 2. Spaghetti plots of all lesions are presented in figure 3. Linear cubic polynomial regression analysis showed no significant change in FA, MD, TD or LD ($p > 0.45$ for all) in NAWM (Figure 2).

Discussion

Serial DTI metrics were used to quantify changes in pre-lesional brain tissue in a cohort of MS patients followed longitudinally over 18 months. Nonparametric regression analysis showed a progressive increase in TD prior to gadolinium enhancement with a concomitant, although more modest, increase in LD. As a result MD values increased prior to gadolinium enhancement. FA values progressively decreased prior to gadolinium enhancement, although these changes were observed closer to the time of gadolinium enhancement. In contrast, no significant changes were observed over time in NAWM. Our analysis observes changes in TD and LD that occurred over the 10 months prior to gadolinium enhancement. This time period would fall within the range that has been described with pre-lesional changes using magnetization transfer ratio imaging.[6, 7, 10] Our data is similar to a previous study reported changes in diffusivity values prior to new lesion formation.[9, 10] We found that changes in TD and LD occur earlier than changes in FA. The early changes in MD occurred with a parallel increase in TD and LD which may indicate uniform swelling in

axons as well as myelin. The decrease in FA values closer to the time of gadolinium enhancement occurred with a concomitant and marked increase in TD, which was greater than the change observed for LD. We hypothesize that this is the result of frank demyelination which occurs at the time of gadolinium enhancement. These findings are in line with pathology studies showing that demyelination is a late change in the cascade of lesions formation.[13] A more recent study examining the role of DTI metrics to predict T1 black hole conversion included some pre-lesional data which showed a similar trend.[33]

Our study utilized the full diffusion tensor to study the physiological substrate of pre-lesional changes. In animal models increase of TD correlates with demyelination and decrease in LD correlates with axonal injury. [19, 34] The correlation between demyelination and an increase in TD, however, was not reproduced in a spinal cord injury model in a study by Budde.[20] The lack of correlation may be in part explained by the fact that spinal cord injury is not a primary demyelinating condition. The authors did find a relationship between TD and demyelination using an ROI based approach in areas of demyelination but not in normal appearing white matter using a EAE model. Because our study is looking at tissue on a path to demyelination we consider our findings using an ROI approach as similar to what was found by Budde. Our conclusion that the progressive increase in TD was a manifestation of demyelination is countered by previous findings which failed to show a correlation between DTI measures and demyelination in animal models of MS.[21] The authors of the afore mentioned paper hypothesize that the lack of correlation between TD and myelin content may be due in part to axonal loss in demyelinating lesions. It is possible that demyelinating lesions in formation have a small contribution of axonal injury, and this may explain the discordant finding. The greater increase in TD as compared to LD prior to gadolinium enhancement therefore suggests myelin disruption with axonal sparing in pre-lesional tissue. Pathology studies of acute demyelinating events have shown that demyelination without frank inflammation can be present early in lesion development.[35] Our observations add additional evidence suggesting that myelin disruption can occur much earlier – up to 10 months earlier - than to breakdown of the blood brain barrier as represented by gadolinium enhancement.

Alternatively it is possible that other architectural changes within the white matter are occurring. Microglial accumulation and oligodendrocyte apoptosis have been proposed as early pathologic changes within newly forming lesions and may well produce an increase in mean diffusivity.[12] The effects of microglial accumulation on different diffusion tensor metrics are not well understood, although they may explain some of the changes we observed. It is also possible that the relationship of LD and TD in relation to axon and myelin is obscured in GAD lesions, as these ROIs may not represent a single well differentiated tract but rather several different anatomic structures.

Non parametric regression based upon cubic splines relaxes the usual assumption of linearity and identifies non-linear structures within the data. Using this method of analysis, we found significant changes in DTI metrics prior to gadolinium enhancement. Cubic spline analysis has been shown to be a robust statistical method [30], since it detects non-linear structure in datasets, such as ours, where there is considerable variability between subjects, lesions and over time. As illustrated in figure 3 there was significant heterogeneity between

the different lesions in this study at the different time-points. This was in part due to the fact that lesion arose in brain regions with very different diffusion properties and also due to the fact that lesions may vary in the intensity of tissue destruction, demyelination and inflammation. We consider the use of cubic splines to be ideal for this type of variability. Future studies using a larger sample size and equal interval time points throughout the sample would help mitigate variability observed in our data and may enable the use of more standard, less computationally challenging models.

All patients from this study were treated with natalizumab and this must be considered in the interpretation of the results. Natalizumab decreases the number and frequency of new lesions, but it is unclear if the formation of lesions on the medication would be any different than in untreated patients. This could be tested with a non-treated control group, however this was not available in the present study. Previous studies of NAWM using diffusion tensor imaging demonstrate a progressive decrease in FA and a progressive increase in TD within the corpus callosum.[36] In our study NAWM showed no significant changes over time. The explanation of this discrepancy may be related to the effect of natalizumab treatment on stabilization of tissue injury. Alternatively, a lack of longitudinal change has also been described in earlier studies[37] and it is possible there is little dynamic change in the NAWM of MS subjects. The evolution of DTI measures in NAWM remains an active area of research.

Conclusions

We observed changes in DTI measures of prelesional tissue up to 10 months prior to gadolinium enhancement. One goal of understanding and identifying pre-lesional changes would be the ability to predict new lesion formation in a reliable and accurate manner, which assumes there are biologic changes in brain tissue sometime prior to the development of gadolinium-enhancing T2 lesions. The therapeutic implications of this predictive capability on treatment selection and timing would be substantial. At this time, advanced MR imaging studies are a useful method of studying pre-lesional changes in MS, although they have not yet provided a reliable predictor of future lesion development. DTI is a suitable platform to further study these changes and may provide insight into the underlying physiological processes in MS.

Acknowledgments

We would like to acknowledge the assistance of our research support staff, radiology technicians and information technology staff at the Cleveland Clinic Mellen Center. Funding for this study was provided by K23 NS 47211 (NIH) to RJF, RG 3548A2 (National MS Society) to RJF, FP 1769-A-1 (National MS Society) to DO, and KL2TR000440 (NIH) to DO.

Author disclosures:

Dr Ontaneda is supported by a National Institutes of Health (Clinical and Translational Science Collaborative of Cleveland, (KL2TR000440) KL2 Award and a National Multiple Sclerosis Clinical Fellowship Award FP 1769-A-1. Dr. Ontaneda has received consulting or speaking fees from Acorda Therapeutics, Biogen Idec, and Novartis. Dr. Fox has received consulting fees from Avanir, Allozyne, Biogen Idec, Novartis, Questcor, and Teva Neuroscience, and research support from Novartis.

References

1. Smith ME, Stone LA, Albert PS, et al. Clinical worsening in multiple sclerosis is associated with increased frequency and area of gadopentetate dimeglumine-enhancing magnetic resonance imaging lesions. *Ann. Neurol.* 1993; 33(5):480–489. [PubMed: 8498825]
2. Grossman RI, FAU - Gonzalez-Scarano F, Gonzalez-Scarano F, et al. Multiple sclerosis: gadolinium enhancement in MR imaging. *Radiology.* 1986 Dec; 161(3):721–725. (0033-8419 (Print); 0033-8419 (Linking)). [PubMed: 3786722]
3. Stone LA, Smith ME, Albert PS, et al. Blood-brain barrier disruption on contrast-enhanced MRI in patients with mild relapsing-remitting multiple sclerosis: relationship to course, gender, and age. *Neurology.* 1995; 45(6):1122–1126. [PubMed: 7783875]
4. Barkhof F, Scheltens P, Frequin ST, et al. Relapsing-remitting multiple sclerosis: sequential enhanced MR imaging vs clinical findings in determining disease activity. *AJR Am. J. Roentgenol.* 1992; 159(5):1041–1047. [PubMed: 1414773]
5. Fazekas F, Ropele S, Enzinger C, Seifert T, Strasser-Fuchs S. Quantitative magnetization transfer imaging of pre-lesional white-matter changes in multiple sclerosis. *Mult. Scler.* 2002; 8(6):479–484. [PubMed: 12474987]
6. Filippi M, Rocca MA, Martino G, Horsfield MA, Comi G. Magnetization transfer changes in the normal appearing white matter precede the appearance of enhancing lesions in patients with multiple sclerosis. *Ann. Neurol.* 1998; 43(6):809–814. [PubMed: 9629851]
7. Pike GB, De Stefano N, Narayanan S, et al. Multiple sclerosis: magnetization transfer MR imaging of white matter before lesion appearance on T2-weighted images. *Radiology.* 2000; 215(3):824–830. [PubMed: 10831705]
8. Goodkin DE, Rooney WD, Sloan R, et al. A serial study of new MS lesions and the white matter from which they arise. *Neurology.* 1998; 51(6):1689–1697. [PubMed: 9855524]
9. Rocca MA, Cercignani M, Iannucci G, Comi G, Filippi M. Weekly diffusion-weighted imaging of normal-appearing white matter in MS. *Neurology.* 2000; 55(6):882–884. [PubMed: 10994017]
10. Werring DJ, Brassat D, Droogan AG, et al. The pathogenesis of lesions and normal-appearing white matter changes in multiple sclerosis: a serial diffusion MRI study. *Brain.* 2000; 123(Pt 8): 1667–1676. (Pt 8). [PubMed: 10908196]
11. Tartaglia MC, Narayanan S, De Stefano N, et al. Choline is increased in pre-lesional normal appearing white matter in multiple sclerosis. *J. Neurol.* 2002; 249(10):1382–1390. [PubMed: 12382153]
12. Matute C, Perez-Cerda F. Multiple sclerosis: novel perspectives on newly forming lesions. *Trends Neurosci.* 2005; 28(4):173–175. [PubMed: 15808350]
13. van der Valk P, Amor S. Preactive lesions in multiple sclerosis. *Curr. Opin. Neurol.* 2009; 22(3): 207–213. [PubMed: 19417567]
14. Fox RJ. Picturing multiple sclerosis: conventional and diffusion tensor imaging. *Semin. Neurol.* 2008; 28(4):453–466. [PubMed: 18843574]
15. Basser PJ, Pierpaoli C. Microstructural and physiological features of tissues elucidated by quantitative-diffusion-tensor MRI. *J. Magn. Reson. B.* 1996; 111(3):209–219. [PubMed: 8661285]
16. Beaulieu C, Allen PS. Determinants of anisotropic water diffusion in nerves. *Magn. Reson. Med.* 1994; 31(4):394–400. [PubMed: 8208115]
17. Alexander AL, Hasan K, Kindlmann G, Parker DL, Tsuruda JS. A geometric analysis of diffusion tensor measurements of the human brain. *Magn. Reson. Med.* 2000; 44(2):283–291. [PubMed: 10918328]
18. Song SK, Sun SW, Ju WK, Lin SJ, Cross AH, Neufeld AH. Diffusion tensor imaging detects and differentiates axon and myelin degeneration in mouse optic nerve after retinal ischemia. *Neuroimage.* 2003; 20(3):1714–1722. [PubMed: 14642481]
19. Song SK, Sun SW, Ramsbottom MJ, Chang C, Russell J, Cross AH. Dysmyelination revealed through MRI as increased radial (but unchanged axial) diffusion of water. *Neuroimage.* 2002; 17(3):1429–1436. [PubMed: 12414282]
20. Budde MD, Kim JH, Liang HF, et al. Toward accurate diagnosis of white matter pathology using diffusion tensor imaging. *Magn. Reson. Med.* 2007; 57(4):688–695. [PubMed: 17390365]

21. DeBoy CA, Zhang J, Dike S, et al. High resolution diffusion tensor imaging of axonal damage in focal inflammatory and demyelinating lesions in rat spinal cord. *Brain*. 2007; 130(Pt 8):2199–2210. [PubMed: 17557778]
22. Tuch DS, Reese TG, Wiegell MR, Makris N, Belliveau JW, Wedeen VJ. High angular resolution diffusion imaging reveals intravoxel white matter fiber heterogeneity. *Magn. Reson. Med*. 2002; 48(4):577–582. [PubMed: 12353272]
23. Fox RJ, Cronin T, Lin J, et al. Measuring myelin repair and axonal loss with diffusion tensor imaging. *AJNR Am. J. Neuroradiol*. 2011; 32(1):85–91. [PubMed: 20947644]
24. Polman CH, Reingold SC, Edan G, et al. Diagnostic criteria for multiple sclerosis: 2005 revisions to the "McDonald Criteria". *Ann. Neurol*. 2005; 58(6):840–846. [PubMed: 16283615]
25. Sakaie KE, Lowe MJ. Quantitative assessment of motion correction for high angular resolution diffusion imaging. *Magn. Reson. Imaging*. 2010; 28(2):290–296. [PubMed: 19695824]
26. Jenkinson M, Bannister P, Brady M, Smith S. Improved optimization for the robust and accurate linear registration and motion correction of brain images. *Neuroimage*. 2002; 17(2):825–841. [PubMed: 12377157]
27. Jenkinson M, Smith S. A global optimisation method for robust affine registration of brain images. *Med. Image Anal*. 2001; 5(2):143–156. [PubMed: 11516708]
28. Cox RW. AFNI: software for analysis and visualization of functional magnetic resonance neuroimages. *Comput. Biomed. Res*. 1996; 29(3):162–173. [PubMed: 8812068]
29. Wang XF, Yang Q, Fan Z, Sun CK, Yue GH. Assessing time-dependent association between scalp EEG and muscle activation: A functional random-effects model approach. *J. Neurosci. Methods*. 2009; 177(1):232–240. [PubMed: 18977246]
30. Eubank, RL. *Nonparametric Regression and Spline Smoothing*. New York: CRC Press; 1999.
31. Wang XF, Ye D. On nonparametric comparison of images and regression surfaces. *J. Stat. Plan. Inference*. 2010; 140(10):2875–2884. [PubMed: 20543891]
32. Brown, H.; Prescott, R. *Applied Mixed Models in Medicine*. New York: John Wiley & Sons; 2006. p. 2006
33. Naismith RT, Xu J, Tutlam NT, et al. Increased diffusivity in acute multiple sclerosis lesions predicts risk of black hole. *Neurology*. 2010; 74(21):1694–1701. [PubMed: 20498437]
34. Wang S, Wu EX, Tam CN, Lau HF, Cheung PT, Khong PL. Characterization of white matter injury in a hypoxic-ischemic neonatal rat model by diffusion tensor MRI. *Stroke*. 2008; 39(8):2348–2353. [PubMed: 18535275]
35. Barnett MH, Prineas JW. Relapsing and remitting multiple sclerosis: pathology of the newly forming lesion. *Ann. Neurol*. 2004; 55(4):458–468. [PubMed: 15048884]
36. Harrison DM, Caffo BS, Shiee N, et al. Longitudinal changes in diffusion tensor-based quantitative MRI in multiple sclerosis. *Neurology*. 2011; 76(2):179–186. [PubMed: 21220722]
37. Rashid W, Hadjiprocopis A, Davies G, et al. Longitudinal evaluation of clinically early relapsing-remitting multiple sclerosis with diffusion tensor imaging. *J. Neurol*. 2008; 255(3):390–397. [PubMed: 18350361]

Figure 1.

Gadolinium enhancing lesions nonparametric regression model with penalized cubic regression splines. The solid lines denote the estimated regression functions. The dashed lines denote the 95% point-wise confidence intervals associated with the estimates. For each DTI measure, a statistical test is conducted to test the null hypothesis that the regression function is constant over time: A) Fractional anisotropy (FA) $p = 0.04$, B) Mean diffusivity (MD) $p < 0.001$, C) Longitudinal diffusivity (LD) $p = 0.025$, D) Transverse diffusivity (TD) $p < 0.001$.

Figure 2.

Normal appearing white matter linear cubic polynomial regression model. The solid lines denote the estimated regression functions. The dashed lines denote the 95% point-wise confidence intervals associated with the estimates. For each DTI measure, a statistical test is conducted to test the null hypothesis that the regression function is constant over time: A) Fractional anisotropy (FA) $p = 0.57$, B) Mean diffusivity (MD) $p = 0.56$, C) Longitudinal diffusivity (LD) $p = 0.45$, D) Transverse diffusivity (TD) $p = 0.68$.

Figure 3.

Spaghetti plots of DTI metrics from individual lesions over the study course. A) Fractional anisotropy (FA), B) Mean diffusivity (MD), C) Longitudinal diffusivity (LD), D) Transverse diffusivity (TD).

Table 1

Lesion distribution by subject and time point

Lesion	Subject	Lesion onset
1	A	1
2	B	2
3	B	2
4	B	2
5	C	1
6	C	2
7	C	18
8	C	18
9	C	18
10	D	1
11	D	2
12	D	6
13	D	6
14	D	6
15	D	6
16	D	6
17	D	6
18	D	6
19	D	12
20	E	1
21	E	12
22	E	12
23	E	12
24	E	18
25	E	18
26	E	18
27	E	18
28	E	18
29	E	18
30	E	18
31	E	18

Gadolinium enhancing lesions during study follow-up distributed by subjects(A–E) and study time points in months (1–18).

Table 2

DTI metrics change by time points compared to time of lesion appearance.

Time Point	Mean Percent Change Compared to Baseline			
	TD	LD	FA	MD
-18	-26.5%	-8.9%	24.9%	-18.1%
-17	-26.6%	-6.9%	38.0%	-17.2%
-16	-25.9%	-6.8%	27.4%	-16.7%
-12	-28.3%	-9.7%	28.1%	-19.7%
-11	-25.2%	-3.8%	27.2%	-14.9%
-10	-19.6%	-7.1%	16.9%	-13.7%
-6	-27.2%	-9.7%	26.1%	-19.1%
-5	-22.7%	-7.0%	22.0%	-15.6%
-4	-24.6%	-9.0%	22.1%	-17.6%
-2	-15.2%	-2.7%	17.7%	-9.3%
-1	-16.2%	-3.1%	18.4%	-9.9%

Transverse diffusivity (TD), longitudinal diffusivity (LD), mean diffusivity (MD) and fractional anisotropy (FA) percent changes compared to time of lesion appearance.