FOUR-YEAR EVOLUTION OF BRAIN TISSUE INTEGRITY USING DIFFUSION TENSOR IMAGING IN MULTIPLE SCLEROSIS

By

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Four-Year Evolution of Brain Tissue Integrity Using Diffusion Tensor Imaging In Multiple Sclerosis

Abstract

by

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Introduction

Diffusion tensor imaging (DTI) is a magnetic resonance imaging (MRI) measure of brain tissue integrity. Little is known regarding the long term longitudinal evolution of lesional and non-lesional tissue using DTI in multiple sclerosis (MS).

Methods

Twenty-one patients with MS starting natalizumab were imaged for 48 months. Gadolinium-enhancing lesions at baseline scan (GAD), chronic T2 lesions, and normal-appearing white matter (NAWM) were followed longitudinally. Within each region of interest, the average value of longitudinal diffusivity (LD) and transverse diffusivity (TD) was derived using Analysis of Functional Neuroimaging Software (AFNI). The longitudinal trend in LD and TD was estimated using a mixed-model regression analysis.

Results

Significant increases over time were observed for TD (p=0.04) and LD (p <0.0001) in GAD tissue. A significant increase in LD (p<0.0001) with stability of TD was seen in chronic T2 lesions. No significant differences were observed in NAWM.

Conclusions

Increases in TD over 4 years suggest ongoing demyelination in GAD lesions. An increase in LD in both GAD and T2 lesions is a new finding and may relate to the complex evolution of brain tissue lesions. NAWM remained stable over 4 years.
**Introduction**

Multiple sclerosis (MS) is a chronic inflammatory demyelinating disease that affects the brain, optic nerves and spinal cord. MS is the leading non-traumatic cause of neurologic disability in young adults. MS is characterized pathologically by areas of demyelination with varying degrees of inflammation. These areas are also known as plaques or MS lesions. The formation of lesions in MS is associated with clinical episodes of neurological dysfunction, known as relapses. Lesions can be detected using standard magnetic resonance imaging (MRI) techniques and the appearance of lesions represents disease activity. New lesion formation is characterized radiologically by the presence of gadolinium (GAD) enhancement on MRI. MRI lesional activity is now commonly used as a primary endpoint in phase II trials of anti-inflammatory disease modifying agents in MS and has also been used to predict the long term course of early MS.

Conventional MRI has limitations as this technique provides little insight into the underlying pathology of MS lesions, is not sensitive to detect tissue changes outside of lesions (i.e. normal appearing white matter, NAWM) and is unable to quantify pathological substrates of disease such as myelin or axonal content. Additionally, conventional MRI is a relatively insensitive technique in progressive forms of MS where there is little overt inflammatory activity. because MRI-detected lesion burden often does not change despite frequent clear-cut clinical progression.

Advanced imaging modalities are now available that may be more sensitive to detect dynamic tissue damage related to MS. Diffusion tensor imaging (DTI) is a quantitative MRI based technique that measures the diffusion of water in brain tissue. By applying diffusion-weighting gradients with different orientations during an MR
acquisition, it is possible to characterize the degree and anisotropy of water motion with a mathematical model known as the diffusion tensor. The diffusion properties are thought to reflect the microstructure of the underlying tissue. From the diffusion tensor three different eigenvectors are obtained including parallel measures ($\lambda_1$) and perpendicular measures ($\lambda_2$ and $\lambda_3$). From the eigenvectors different DTI metrics can be obtained and these include: mean diffusivity (MD), fractional anisotropy (FA), transverse diffusivity (TD) and longitudinal diffusivity (LD). MD is obtained from the average of $\lambda_1$, $\lambda_2$, and $\lambda_3$. TD is the average if the perpendicular eigenvectors ($\lambda_2$ and $\lambda_3$). LD is $\lambda_1$ and FA is the square root of the sum of squares of the diffusivity differences divided by the square root of the sum of squares of the diffusivities. The different DTI metrics carry a pathological specificity as well. MD is considered a measure of the overall integrity of brain tissue, FA is related to the overall alignment of nervous tissues, TD is a measure of myelin integrity and LD is a measure of axonal integrity. However, these metrics are not entirely specific to axonal injury and demyelination.

Despite this caveat, DTI provides a window into the pathological processes in MS and through serial imaging studies, can be used to follow MS lesions in different stages as well as tissue changes over time. Additionally, DTI has the ability to detect changes in brain tissue that is apparently free of lesions (normal appearing brain tissue) under standard MRI techniques. For instance, the evolution of NAWM has been studied in the past using diffusion tensor imaging. Whole brain NAWM studied over 3 years showed slight increases in FA over time without significant changes in MD. Harrison et al studied the evolution of white matter tracts based on tractography and found that there was a significant increase in FA and a decrease in TD over a two year follow-up period.
White matter lesional tissue has been studied in acute phases or over short periods of follow-up where it most consistently has shown increases in MD and TD with a decrease in FA.\textsuperscript{20-25} Diffusion imaging studies have also shown that T1 black holes have higher levels of diffusivity as compared to iso-intense T1 lesions.\textsuperscript{26} There is evidence that radial diffusivity is a strong predictor of T1 black hole conversion.\textsuperscript{27} Alternatively, longitudinal studies of high angular resolution DTI following lesion evolution over time have not been conducted and the long term evolution of lesional tissue is not fully understood. The evolution of DTI metrics over time is of importance if DTI is to be used as a surrogate marker of efficacy in disease modifying agent trials. The dynamic changes in lesional and non lesional tissue overtime need to be fully described in order to make conclusions about how DTI measures relate to tissue pathology. This will allow a better understanding of the effect potential therapies may have on these measures. This is a necessary step to understand how therapies with varying mechanisms of action might differentially affect DTI measures.

DTI may also be useful to detect remyelination. Remyelination is a natural process that occurs in response to demyelinating events in the CNS.\textsuperscript{28} It is believed that remyelination in MS is incomplete, which may explain in part the accumulation of disability in the disease.\textsuperscript{29} Conventional MRI imaging only provides a very crude measure of remyelination. DTI and specifically TD may be used to assess remyelination after lesion formation in a quantitative fashion. Thus, there is a potential role for the use of DTI as a surrogate endpoint in studies of therapeutics that promote remyelination.
Natalizumab is a monoclonal antibody directed at VLA4 receptor on leukocytes. VLA4 is involved in leukocyte adhesion in the brain and other tissues. By blocking VLA4, natalizumab effectively reduces the migration of leukocytes into the central nervous system. Through its effects on leukocytes, natalizumab has profound anti-inflammatory properties in MS and dramatically reduces the number of GAD-enhancing lesions and clinical relapses in MS. Most patients treated with natalizumab enter a relatively inflammation free state, which enables the study of the underlying neurodegenerative and reparative aspects of MS pathology. While standard MRI is considered a good surrogate for relapse activity in relapsing-remitting MS, there still is no validated MRI endpoint in progressive forms of the disease. DTI is a feasible alternative to measure neurodegeneration and to assess the efficacy of potential neuroprotectant medications.

In this study, we aim to study the evolution of lesional and non lesional tissue in patients treated with natalizumab over a four year period. Previous DTI studies have used low angular resolution and have followed patients for only up to 2 years. High angular resolution diffusion imaging acquisition reduces the variability and bias found in lower angular resolution approaches and allows for the more accurate study of DTI metrics over time. This approach is of special interest when studying long term markers of neurodegeneration.

Methods

Subjects

21 relapsing MS subjects starting natalizumab therapy were enrolled into an Institutional Review Board-approved longitudinal observational imaging study. The
subjects were selected from the outpatient MS clinic at the Mellen Center in Cleveland Ohio. Subjects starting natalizumab as recommended by each subject’s clinical neurologist were identified and invited to participate. Previous treatment with natalizumab was allowed, as long as the previous treatment was >6 months previous to the current natalizumab initiation. Inclusion criteria included diagnosis of MS by 2005 Revised McDonald Criteria \(^{34}\), 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, including severe claustrophobia and implanted devices such as neurostimulators and pacemakers.

**MRI Imaging Protocol**

MRI scans of the brain were serially acquired prior to natalizumab initiation at baseline and after 1, 2, 6, 12, 18, 24, 36, and 48 months. Images were obtained on a 3 tesla Siemens Trio (Siemens Medical Systems. Erlangen, Germany). Diffusion-weighted imaging used 71 non-collinear diffusion-weighting gradients (2.5 x 2.5 x 2.5mm voxels, \(b = 2000\text{sec/mm}^2\), 8 \(b = 0\) acquisitions; 260 x 260 mm FOV, 104 x 104 matrix, 48 2.5mm slices, TE = 95 msec, TR = 7300 msec). Anatomical imaging was performed for lesion detection and co-registration: 3D MPRAGE (256 x 256 mm FOV, 128 x 256 matrix, 120 1.2mm slices, TE = 1.71 msec, TR = 1900 msec, T1 = 900 msec, flip angle = 8°); proton density / T2-weighted (230 x 230 mm FOV, 320 x 320 matrix, 48 3-mm slices, TE1 = 20 msec, TE2 = 91 msec and TR = 3600 msec) and T1 post-GAD (230 x 230 mm FOV, 320 x 320 matrix, 48 3mm slices, TE = 2.46 msec, TR 300 msec, flip angle = 75). For each subject, images from each time point were co-registered using Functional MRI of the Brain Software Library (FSL).\(^{35}\) For coregistration the images from the DTI dataset
without diffusion weighting (the b=0 images) were coregistered to the baseline 3DMPRAGE images at baseline with FSL to determine tensor properties. This co-registration was then applied to other maps FA, MD, LD, and TD for all the study time points.

Image Analysis

For each subject, regions of interest (ROIs) were drawn on areas with gadolinium enhancement on T1-post contrast images at the baseline scan and will be referred to as GAD tissue. ROIs were simultaneously observed on FA maps to ensure these did not include cerebrospinal fluid (CSF) or CSF volume averaging artifacts in the baseline scan or subsequent imaging time points. For each subject, 10 ROIs were drawn in selected chronic lesional tissue (T2 lesions and T1 black holes) and these included two lesions in the following locations: periventricular, juxtacortical, corpus callosum, infratentorial, and posterior periatrial (Figure 1). T2 lesions were labeled as black holes (BH) and non black holes (NBH). ROIs were drawn individually on the co-registered T2 maps at each time point with simultaneous observation of the FA and T1 post contrast maps to ensure ROIs did not include T1 GAD-enhancing lesions, CSF, or CSF volume averaging artifact. FA was used given the possibility of image warping in DTI space to ensure ROIs were not in CSF or voxel adjacent to CSF. T1 black holes were differentiated from T2 lesions by a visible decrease in signal intensity on the T2 sequence when compared to normal appearing adjacent white matter tissue.

For each subject, 20 ROIs also were drawn in the normal appearing white matter (NAWM) and grey matter (NAGM) bilaterally. These included 2 ROI’s (ipsilateral and contralateral) in each of the following regions: corticospinal tracts in the pons,
corticospinal tract in the midbrain, anterior limb of the internal capsule, posterior limb of the internal capsule, anterior corpus callosum, posterior corpus callosum, centrum semiovale, deep white matter of the frontal lobe, thalamus, and caudate head (Figure 2). ROIs were drawn individually on the co-registered FA maps at each time point with simultaneous observation of the T2 and T1 post contrast scans to ensure ROIs did not include T2 lesions, T1 gadolinium-enhancing lesions, CSF, or CSF volume averaging artifact as well as voxels adjacent to CSF. Lesions were shifted within the tract when possible to avoid the above changes, but when this was not possible, these ROI’s were eliminated from the analysis.

Statistical Analysis

The data from each ROI from the FA, MD, TD, and LD maps at all the different time points were exported in “.csv” format then imported into R version 2.11.1 statistical software package. For each subject, distribution plots of the data were created to identify extreme outliers (>2 SD from the mean). ROI position of outliers were checked on the different DTI maps and corrected as needed to ensure the ROIs did not include CSF, vascular spaces, or lesional tissue (for NAWM). The data were then re-extracted and re-checked.

A systematic drop in DTI values of LD and MD was observed beyond time points 18 for the first 15 subjects and at month 12 for the last 6 subjects. ROI’s were drawn in air adjacent to the head and were evaluated for noise. This evaluation confirmed the presence of increased noise in all scans dated after February 13th, 2008. This was the approximate time of a software update conducted on the MRI scanner and so the
respective software version was then recorded for every ROI in the database for use as a potential covariate.

Separate linear mixed models were used to estimate the mean difference in DTI metrics (TD, LD, FA, and MD) over time between NAWM and GAD-enhancing tissue, NAWM versus T2 lesions, and BH versus NBH. Each model included fixed effects for: 1) Tissue type (NAWM and GAD-enhancing tissue, NAWM and T2 lesions, or BH and NBH); 2) an interaction between tissue type and follow-up time in months (to separately model the DTI evolution in each group over time); 3) subject age at the time of study initiation (to account for the change observed in DTI metrics with normal aging over time); and 4) software version as a time-varying covariate (to account for the systematic drop in LD, MD and FA values after the MRI software update). The model also accounted for the effects of between-lesion variation over time by adding a normal random effect for each ROI in order to control the heterogeneity over different ROIs. ANOVA tests were used to determine if the rate of change in DTI measures over time differed between tissue types. A significance level of 0.05 was used for all tests. No correction for multiple comparisons was made because the comparisons between different tissue types were independent. A formal sample size calculation was not conducted given the exploratory nature of the study. Sample size of 20 is felt to be sufficient to compare brain regions and lesion types.

**Results**

**Clinical Characteristics**

Twenty-one subjects were recruited into the study. Demographics and baseline characteristics are presented in Table 1. The majority of subjects were female and had
relapsing remitting MS. Mean age was 41.6 years (SD 9.7). Mean disease duration was 11.9 years (SD 7.5). Seventeen (81.0%) subjects completed the 48 month scan, 1 subject died due to an unrelated cardiac event and 3 subjects withdrew from the study prior to completion. Mean duration of natalizumab treatment was 33.9 months (SD 18.7, range 3-48). Eleven (52.4%) subjects completed 48 months of natalizumab treatment. Three subjects (14.2%) completed 36 months of treatment. Two subjects (9.5%) completed 18 months and two subjects (9.5%) completed 6 months of treatment. Two subjects (9.5%) discontinued the medication due to allergic reactions within the first 3 natalizumab treatments. One subject (4.7%) completed only 3 months of treatment and was lost to follow-up. Mean number of relapses during the study period was 1.19 (SD 1.9, range 0-6). Ten (47.6%) subjects remained relapse free during the entire 4 year follow-up and all those subjects were on natalizumab for at least 36 months.

**Conventional Imaging Results**

MRI at baseline showed a mean T2 lesion volume area of 14.8 ml (SD 10.1). Eleven subjects (52.4%) had gadolinium-enhancing lesions at baseline. The mean number of gadolinium-enhancing lesions at baseline was 2.9 (range 0-13) and the mean volume of gadolinium-enhancing lesions was 1.2 ml. Over 4 year follow-up, 8 subjects (38.1%) developed new gadolinium-enhancing lesions. A total of 54 new gadolinium-enhancing lesions developed over time with 2.6 lesions per subject (range 0-23). Only 3 gadolinium-enhancing lesions were identified in subjects who had completed 48 months of natalizumab treatment. A large proportion of lesions (n=23) developed in a single subject who discontinued natalizumab intermittently.

**DTI Results**
DTI metrics at baseline

DTI metrics from NAWM at baseline were as follows: TD: 464.3 x 10^{-6} \text{ mm}^2/\text{sec} (SD 128.3), LD: 1176.0 x 10^{-6}\text{ mm}^2/\text{sec} (SD 254.1), FA: 536.0 (SD 108.21), and MD 701.4 x 10^{-6} \text{ mm}^2/\text{sec} (SD 145.75). ROIs were drawn on the 61 lesions representing GAD tissue at baseline. DTI metrics from GAD tissue at baseline were as follows TD: 666.3 x 10^{-6} \text{ mm}^2/\text{sec} (SD 110.1), LD: 1005.0 x 10^{-6}\text{ mm}^2/\text{sec} (SD 99.48), FA: 274.5 (SD 92.4), and MD 779.3 x 10^{-6} \text{ mm}^2/\text{sec} (SD 91.9). ROIs were drawn on 141 T2 lesions of which 90 were BH and 51 were NBH. DTI metrics from all T2 lesions at baseline were as follows: TD: 664.3 x 10^{-6} \text{ mm}^2/\text{sec} (SD 177.3), LD: 1072.0 x 10^{-6} \text{ mm}^2/\text{sec} (SD 214.8), FA: 311.2 (SD 132.6), and MD 800.2 x 10^{-6} \text{ mm}^2/\text{sec} (SD 164.9). DTI metrics from BH were as follows: TD: 714.2 x 10^{-6} \text{ mm}^2/\text{sec} (SD 170.2), LD: 1104.0 x 10^{-6} \text{ mm}^2/\text{sec} (SD 199.7), FA: 287.7 (SD 106.2), and MD 844 x 10^{-6} \text{ mm}^2/\text{sec} (SD 161.9). DTI metrics from BH were as follows: TD: 576.1 x 10^{-6} \text{ mm}^2/\text{sec} (SD 155.2), LD: 1016.0 x 10^{-6} \text{ mm}^2/\text{sec} (SD 230.6), FA: 352.6 (SD 162.4), and MD 722.7 x 10^{-6} \text{ mm}^2/\text{sec} (SD 141.0).

DTI Changes in GAD Tissue and NAWM

Longitudinal DTI metrics for NAWM and GAD are presented in Figures 2-5 for TD, LD, FA, and MD respectively. For each DTI metric, results are presented in Tables 2-5 and show evidence that age and software version had a statistically significant effect on LD, FA, and MD over time and tissue type (GAD versus NAWM) had a statistically significant effect on LD, FA, and MD at baseline. While age and tissue type also had a statistically significant effect on TD over time and at baseline respectively, this did not hold for software version.
**Longitudinal Changes in NAWM**

No statistically significant changes on the rate of change in any of the DTI metrics were observed in NAWM tissue (all \( p > 0.08 \)). TD and MD showed an increasing trend over time while FA and LD showed a decreasing trend over time. Average annual changes were as follows: TD +0.54%, LD -0.21%, FA -0.56% and MD +0.05%.

**Longitudinal Changes in GAD Tissue**

An increase of \( 0.66 \times 10^{-6} \text{mm}^2/\text{sec} \) change per month in TD was observed over time in GAD tissue (\( p = 0.037 \)). An increase in LD of \( 1.88 \times 10^{-6} \text{mm}^2/\text{sec} \) per month was also observed (\( p < 0.0001 \)), which resulted in an increase of \( 1.28 \times 10^{-6} \text{mm}^2/\text{sec} \) per month (\( p < 0.0001 \)) in MD as well. FA increased over time by \( 0.51 \times 10^{-6} \text{mm}^2/\text{sec} \) per month (\( p = 0.037 \)). Average annual changes were as follows: TD +1.19%, LD +2.25%, FA -2.22% and MD +1.97%.

**Longitudinal Comparison between GAD Tissue and NAWM**

The tests of fixed effects by ANOVA showed that there was a significant difference between GAD and NAWM in the rate of change over time in LD (\( p < 0.0001 \)), MD (\( p < 0.0001 \)), and FA (\( p = 0.004 \)) but not for TD (\( p = 0.111 \)).

**DTI Changes in T2 lesions and NAWM**

DTI metrics for NAWM and T2 lesions are presented in Figures 6-9 for TD, LD, FA, and MD respectively. For each DTI metric, results are presented in Tables 6-9 and show evidence that TD, LD, FA, and MD at baseline were significantly different between tissue types (T2 versus NAWM) (all \( p < 0.0001 \)). Evidence was also found that software version had a significant effect on LD, FA, MD, but not TD over time.
An increase of $0.54 \times 10^{-6}$ mm$^2$/sec per month in LD (p=0.003) with a concomitant increase in MD of $0.27 \times 10^{-6}$ mm$^2$/sec per month (p=0.021) was observed over the 4 year time period in T2 lesions. FA increased by $0.16 \times 10^{-6}$ mm$^2$/sec per month, but was of borderline statistical significance (p=0.048). TD did not significantly change over time in T2 lesions. Changes over time in any of the DTI metrics were not significant in NAWM, similar to the previous analysis with GAD tissue.

**Longitudinal Comparison between T2 lesions and NAWM**

The test of fixed effect by ANOVA showed that a significant difference in lesion evolution existed between T2 lesions and NAWM for LD (p=0.002) and MD (p=0.036). No significant difference was found for FA (p=0.09) or TD (p=0.86).

**DTI Changes in BH and NBH**

DTI metrics for BH and NBH are presented in Figures 10-13 for TD, LD, FA, and MD respectively. For each DTI metric, results are presented in Tables 10-13 and show a statistically significant difference between tissue types (BH versus NBH) for TD (p=0.0006), FA (p=0.02), and MD (p=0.002) at baseline. As expected, TD and MD at baseline were higher and FA at baseline was lower in BH as compared to NBH. No significant difference between BH and NBH was found in LD at baseline (p=0.12).

**Longitudinal Changes in BH and NBH**

No significant changes were seen in the evolution of DTI metrics in either BH or NBH (all p>0.07) with the exception of a slight decrease in FA of $0.21 \times 10^{-6}$ mm$^2$/sec per month in BH (p=0.044). TD increased over time in BH and decreased in NBH, although changes were not significant.

**Longitudinal Comparison between BH and NBH**
A significant difference in the evolution of FA was observed between BH and NBH (p=0.012) showing a decrease in FA in BH and an increase in NBH. A significant difference was also observed for TD (p=0.038) with an increase in TD in BH and a decrease in NBH.

**Discussion**

Subjects enrolled into the study who completed natalizumab treatment had good radiological control of disease with only 3 new gadolinium-enhancing lesions among the 11 subjects who completed 48 months of natalizumab. This is similar to the reduction of gadolinium-enhancing lesions observed in clinical trials.\(^{31, 32}\) Several subjects developed gadolinium-enhancing lesions shortly after interruption natalizumab during the study period, which is expected and resulted in a large proportion of gadolinium-enhancing lesions observed.\(^{36, 37}\) Patients who discontinued natalizumab typically switched treatment to other disease modifying agents and some had effective control of inflammatory activity. The total number of gadolinium-enhancing lesions over the entire 4 year study period was lower than the number of gadolinium-enhancing lesions at baseline, indicating that the study population had partial control of inflammatory disease activity.

The effects of partial adherence to natalizumab in our study population posses some challenges to the analysis. The effect of new lesions on subjects not fully treated with natalizumab was mitigated by carefully selecting NAWM ROIs that did not include any new areas of T2 abnormality through the 4 year study period. Also, it is important to note that a large proportion of new lesions (42.5%) came from a single patient. Taking this into account among the remaining 20 patients, there was only 0.38 gadolinium
enhancing lesions per patient per year. The beneficial effects of natalizumab beyond that of new lesion formation (mediated by decreased permeability of the blood brain barrier) have been proposed.\textsuperscript{38} Further sub-analysis according to treatment effect is currently being conducted to study only those patients who remained on natalizumab for 48 months.

**Baseline DTI metrics**

At baseline, DTI metrics showed expected differences with higher TD and MD along with lower FA and LD in gadolinium-enhancing tissue as compared to NAWM. This suggests the presence of more severe demyelination and axon loss in lesional tissue as compared to NAWM based on the findings in animal models showing that TD correlates with myelination and LD correlates with axonal injury.\textsuperscript{14, 39} It is likely edema was an additional contributing factor to an increase in TD at the time of acute demyelination.\textsuperscript{40} Similarly, in chronic T2 lesions (BH and NBH), DTI metrics at baseline showed an increase in TD and MD with lower FA and LD when compared to NAWM. DTI metrics at baseline were similar in GAD and chronic T2 lesions but overall GAD tissues had slightly higher TD and slightly lower FA, MD, and LD at baseline. Similar to diffusion weighted studies conducted in the past \textsuperscript{24, 41}, BH showed greater diffusivity at baseline when compared to NBH, suggesting edema or possibly demyelination. As would be expected, FA, a measure of overall tissue integrity, was significantly lower at baseline in BH than in NBH. Alternatively, LD at baseline was similar in BH and NBH. This would not normally be expected, as axon loss is a clear histological feature of BH lesions.\textsuperscript{42, 43} Our findings illustrate why equating LD with axonal integrity is an oversimplification of a complex measure. Our findings are similar to previous results showing that LD does not predict development of BH formation.\textsuperscript{17, 27}
Evolution of NAWM

No statistically significant changes were observed in the longitudinal evolution of DTI metrics in NAWM. This finding is consistent with the findings of previous groups that have not found longitudinal DTI changes in NAWM\textsuperscript{18}, but is in contrast with previous shorter term studies that have shown tract specific longitudinal changes in NAWM DTI values.\textsuperscript{19} The trends observed in NAWM changes over time in our study were similar to what would be pathophysiologically expected, with an increase in TD and decrease in LD over time indicating progressive demyelination with ongoing axon loss. One explanation for the absence of statistical significance is the relative lack of inflammatory activity seen in our patient population. It is possible that highly effective treatment may mitigate longitudinal changes in DTI. Anti-inflammatory therapies are likely to limit the amount of demyelination and secondary axon loss making differences more difficult to observe.\textsuperscript{44} A second explanation may relate to technical differences between our study and that of Harrison et al.\textsuperscript{19} In our study, ROIs in the NAWM were individually drawn and voxel volume was relatively small when compared to a tractography-based technique. It is possible that a larger sample size of voxels may have shown statistically significant results in the trends described above. To further investigate this possibility, a tractography-based approach was conducted in the bilateral corticospinal tracts (CST). Seed and targets were drawn in the CST at the level of the cerebral peduncle and ipsilateral hand knob respectively. ROIs from the CST were then selected and compared with tractography data and under visual inspection showed no major differences. Analysis of these data is currently ongoing.

Evolution of GAD Tissue
In gadolinium-enhancing tissue, significant changes were observed in TD, LD, FA and MD over the 4 year study period. Changes were as expected in TD and MD with increases in both. This likely represents ongoing demyelination within GAD lesions. This confirms previous findings where MD was shown to increase over time in lesional tissue. The annual changes in TD and MD of 1.19% and 1.75% respectively are likely an under representation given the fact that during acute lesion recovery (months 1 and 2), there was a marked decrease in TD and MD. This change was likely due to a decrease in inflammation and edema associated with resolution of gadolinium-enhancement and possible remyelination. Our study findings indicate that monitoring TD and MD within lesions may be more sensitive to change over time than monitoring in NAWM, which is likely a representation of the ongoing repair process and remyelination in lesional tissue as compared to non lesional tissue.

In gadolinium-enhancing tissues, a progressive increase in FA and LD was observed over the study period. Although similar results for LD have not been previously reported, a progressive increase in FA in NAWM has been described in the past. Naismith and colleagues did not find significant changes in LD at the time of gadolinium-enhancement and in their data it also appears LD increases over 1 year of follow-up. The longitudinal increase in LD and FA may have been partially driven by the increase in LD and FA observed in the first two months following lesion formation, associated with resolution of gadolinium-enhancement. However, given similar results in T2 lesions, this is unlikely. An alternate explanation may relate to the severity of axon loss in acute lesions. Post-mortem analyses have shown that axon loss is maximal at the time of gadolinium-enhancement and that over time axon loss becomes less prevalent. It is
hypothesized that the initial injury causes an acute and severe loss of axons, which is
detected by a marked drop in LD. The subsequent increase in LD might be a reflection of
a secondary process occurring during chronic lesion evolution, which has yet to be
identified. Variability in our measures of GAD tissue may have also been produced as a
result of the heterogeneity between lesions. It is well known that different patterns of
gadolinium-enhancement are associated with different diffusivity values. However, our
data at 1 year suggests those changes are not present longitudinally when comparing T1
black holes with non black hole T2 lesions.

When comparing the longitudinal evolution of DTI in GAD tissue and NAWM,
significant differences were found for LD, FA, and MD, but not TD. Given the
unexpected results in changes in LD and FA observed in lesional tissue, this is not
surprising and may indicate that distinct processes are occurring in lesional tissue that are
being detected by LD but not by TD. Conversely, the changes in TD may represent
ongoing demyelination which has been known to occur both in NAWM and lesional
tissue. The exact changes that may drive the change in LD in lesional tissue are not
clearly understood and will require further study, including an analysis of the different
enhancement patterns within the gadolinium-enhancing lesions.

Evolution of Chronic T2 Lesions

Similar to what was found in GAD tissue, a progressive increase in LD was
observed over the 4 year study period. This finding is of unclear significance but similar
changes have been previously reported as described above. The lack of any inflammatory
driven changes in these lesions along with the similar changes in GAD tissue suggests the
increase in LD is a real phenomenon and not a spurious result. The pathological
significance of this change is more difficult to determine. The correlation of LD with axonal integrity may be different in brain and optic nerve tissues, where fibers are more or less homogenous in direction. Conversely, brain lesions may contain various different fibers and an increase in LD may be due to selective loss of a certain fibers resulting in counterintuitive changes in DTI measures.

Our study also showed a significant difference in the evolution of TD and FA in BH and NBH. While TD progressively increased in BH, TD progressively decreased in NBH, suggesting ongoing demyelination in BH and possible remyelination in NBH lesions. Remyelination is a well-described phenomenon in NBH and has been demonstrated with magnetization transfer ratio imaging previously. Given these results, one may also hypothesize that TD may be a measure that is sensitive to treatment effects of possible remyelinating therapies.

Study Challenges and Limitations

Significant systematic changes in our data were observed between months 12 and 18 in LD, MD, and FA. Upon further investigation, an MRI software update conducted in February of 2008 was identified as the culprit for this systemic change in the DTI measures and exemplifies the challenges to conducting longitudinal MRI studies. This problem was overcome by including the software version as a covariate in all statistical models. As expected, software version was statistically significant in all models that included LD or summary measures (FA and MD), but not in the model of TD.

Three separate linear mixed models were used to test each of the comparisons (NAWM versus GAD, NAWM versus T2, and BH vs NBH). These were modeled separately as the data became available over time. Because two of the comparisons
involve the same tissue (NAWM) there are advantages to conducting the analysis in a single model and not three separate models. The creation of a single model which incorporates all tissue types together along with tests of the fixed effects of different tissue types (NAWM vs GAD, NAWM vs T2 and BH vs NBH) is planned.

Only 54.2% of the patients remained on natalizumab for the entire 48 month treatment period. The differential treatment effect of natalizumab (due to patient discontinuation of medication) is not accounted for in the current analysis. Several methods can be utilized to mitigate this differential treatment effect. A time varying covariate will be added to the model which indicates if patients had received natalizumab in the 3 months prior to each MRI acquisition time point. Any patient receiving natalizumab within that time period would be considered to be on treatment for that acquisition time point. A three month time period was selected because this is the time at which a return of disease activity is expected after natalizumab treatment discontinuation.

We also plan to conduct a comparison between patients at the extremes of treatment. We will compare those who remained on treatment for 48 months (n=11) and those who were on the medication for only 6 months (1/8 of the entire study period, n=5) to determine if there are differences at the extremes of treatment.

Our study was limited due to the absence of a non-treated control arm. Although valuable information would have been obtained by studying a non-treated comparison group, this posed ethical challenges given the availability of highly effective treatment for MS. Our study was also limited by the presence of patients who dropped out of the study. However, it is likely that these drop-outs were patients who did not tolerate
natalizumab or who stayed on the medication for only a short period of time, thus limiting the overall effect on the study.

There were significant challenges to conducting a 4 year longitudinal MRI study. For example, updated co-registration algorithms became available mid way through the study period and a side by side assessment of the different coregistration methods had to be conducted. Although this revealed that only small coregistration improvements were observed with newer methodologies, significant manual adjustment was still needed. A decision was made to continue to manually adjust ROI’s on the original coregistration method as to not lose the work that was conducted during the first year of the study.

An additional area of concern is the difficulty in delineating lesional tissue over time. Changes in the tissue architecture over time related to gliosis, resolution of edema, and brain atrophy may make the subsequent ROIs include tissue that was not initially delineated in GAD tissue. There is no failsafe way to be certain that this is not occurring in any longitudinal MRI study that investigates lesions. We used the T2 maps as a guide to follow the extent of the lesion on follow-up scans, but this does method did not take into account areas where brain tissue re-myelinates and once again becomes normal appearing or areas which suffer focal atrophy and may even disappear.

**Future Directions and Applicability**

A correction for the systematic change in LD values is being tested and will be applied to the entire dataset and will be re-analyzed. A comparative analysis between patients who remained on treatment and those who discontinued natalizumab will also be conducted. Regions of interest in diffusely appearing white matter have been drawn and will be analyzed in comparison to T2 lesions in the future.
The large amount of manual correction of lesions in our study may limit a similar methodology for a large clinical trial. Currently a tractography algorithm is being implemented to compare with the findings of the ROI analysis from the corticospinal tracts. Tractography will include more voxels of NAWM and will also require significantly less manual correction. Tractography however cannot be used to identify lesional tissue and masking based on T2 intensity or even FA is a possibility. Eliminating software updates during longitudinal studies should be considered, as this may significantly alter the evolution of DTI data.

The use of DTI in clinical trials holds significant promise; however the DTI outcomes to be used should be tailored depending on the therapeutic mechanism of action and MS disease type. The selection of both the DTI metric and the tissue from which these metrics are obtained is of importance when considering outcomes for clinical trials. For trials of agents that promote remyelination TD is a natural choice as it is felt to be a marker of myelin content. Our data shows that TD progressively decreases in tissue that shows recovery (NBH) while it increases in chronically diseased tissue (NBH). Therefore lesional TD may be used as an outcome that measures the effect of remyelinating therapies. In NAWM TD shows a tendency to increase over time. Although in our study this increase was not statistically significant it is possible that therapies that promote remyelination may show a progressive decrease in TD. The study of TD in NAWM is advantageous as the cellular architecture is preserved and there is no confounding inflammatory activity which makes the interpretation of DTI measures more straightforward. These advantages make TD within NAWM a good potential outcome for primary neuroprotection. Although LD has been purported as a measure of axonal
integrity our results suggest that lesional LD is not likely correlated to axonal content, as LD was not significantly different in BH and NBH. If LD is to be used in clinical trials it would appear more feasible to study LD from NAWM, however it is possible that changes in LD are small so it may be difficult to show with either primary or secondary neuroprotective agents. The summary measures FA and MD are somewhat difficult to interpret as stand alone measures, so they are less attractive as outcomes for clinical trials. FA appears to be a good marker of acute lesion formation, however the longitudinal evolution of FA did not show statistically significant changes in NAWM and only marginal changes in lesional tissue. In summary our data suggests that DTI holds the most promise in trials that that promote remyelination and tissue repair within lesions and to a lesser extent in NAWM.

**Conclusions**

Lesional tissue, both chronic T2 lesions and GAD tissue demonstrated higher values of TD and lower values of LD when compared with NAWM. This finding likely represents demyelination with a component of axon loss in lesional tissue. Although the trends were as expected (increase in TD and decrease in LD), no statistically significant changes were observed over time in NAWM. In GAD tissue, a progressive increase in TD was observed over time, suggesting ongoing demyelination. A progressive increase in LD was also observed in GAD tissues and the significance of this change remains unclear. In chronic T2 lesions, a progressive increase in LD was observed and TD increased in BH while it decreased in NBH. Our findings suggest that TD from lesional tissue may be a more sensitive metric for use in clinical trials than values from NAWM. The use of TD
as a metric for possible remyelinating therapies is also supported by the findings observed in GAD tissue and chronic T2 lesions. LD was not able to differentiate BH from NBH and the use of LD as a surrogate of axon content in MS should be re-visited.
<table>
<thead>
<tr>
<th>Patient Characteristics</th>
<th>Mean (Standard Deviation) or Frequency (Proportion)</th>
</tr>
</thead>
<tbody>
<tr>
<td><strong>DEMOGRAPHICS</strong></td>
<td></td>
</tr>
<tr>
<td>Age (years)</td>
<td>41.6 (9.7)</td>
</tr>
<tr>
<td>Female</td>
<td>15 (71.4%)</td>
</tr>
<tr>
<td>Disease type</td>
<td>Relapsing-remitting: 18 (85.7%)</td>
</tr>
<tr>
<td></td>
<td>Secondary-progressive with relapses: 3 (14.3%)</td>
</tr>
<tr>
<td>Disease duration (years)</td>
<td>11.9 (7.5)</td>
</tr>
<tr>
<td>Time since diagnosis (years)</td>
<td>8.0 (4.6)</td>
</tr>
<tr>
<td>T2 lesion volume (mm$^3$)</td>
<td>14,800 (10,100)</td>
</tr>
<tr>
<td><strong>GADOLINIMUM-ENHANCING LESIONS</strong></td>
<td></td>
</tr>
<tr>
<td>Presence of lesions</td>
<td>11 (52.4%)</td>
</tr>
<tr>
<td>Lesions per patient</td>
<td>2.9</td>
</tr>
<tr>
<td></td>
<td>Median: 5</td>
</tr>
<tr>
<td></td>
<td>Range: 0–13</td>
</tr>
<tr>
<td>Lesion volume (mm$^3$)</td>
<td>1226 (1207)</td>
</tr>
<tr>
<td><strong>NORMAL APPEARING BRAIN TISSUE REGION OF INTEREST</strong></td>
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</tr>
<tr>
<td>Volume (mm$^3$)</td>
<td>502.4 (260.2)</td>
</tr>
<tr>
<td><strong>DTI METRICS FROM NAWM</strong></td>
<td></td>
</tr>
<tr>
<td>TD ($10^{-6}$mm$^2$/sec)</td>
<td>464.3 (128.3)</td>
</tr>
<tr>
<td>LD ($10^{-6}$mm$^2$/sec)</td>
<td>1176.0 (254.1)</td>
</tr>
<tr>
<td>FA</td>
<td>536.0 (108.21)</td>
</tr>
<tr>
<td>MD ($10^{-6}$mm$^2$/sec)</td>
<td>701.4 (145.75)</td>
</tr>
<tr>
<td><strong>DTI METRICS FROM GAD</strong></td>
<td></td>
</tr>
<tr>
<td>TD ($10^{-6}$mm$^2$/sec)</td>
<td>666.3 (110.1)</td>
</tr>
<tr>
<td>LD ($10^{-6}$mm$^2$/sec)</td>
<td>1005.0 (99.48)</td>
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<tr>
<td>FA</td>
<td>274.5 (92.4)</td>
</tr>
<tr>
<td>MD ($10^{-6}$mm$^2$/sec)</td>
<td>779.3 (91.9)</td>
</tr>
<tr>
<td><strong>DTI METRICS FROM T2 LESIONS</strong></td>
<td></td>
</tr>
<tr>
<td>TD ($10^{-6}$mm$^2$/sec)</td>
<td>664.3 (177.3)</td>
</tr>
<tr>
<td>LD ($10^{-6}$mm$^2$/sec)</td>
<td>1072.0 (214.8)</td>
</tr>
<tr>
<td>FA</td>
<td>311.2 (132.6)</td>
</tr>
<tr>
<td>MD ($10^{-6}$mm$^2$/sec)</td>
<td>800.2 (164.9)</td>
</tr>
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</table>
Table 2. Comparison of transverse diffusivity between GAD and NAWM

<table>
<thead>
<tr>
<th>Effect</th>
<th>Estimate</th>
<th>95 % CI</th>
<th>p-value</th>
</tr>
</thead>
<tbody>
<tr>
<td>Mean difference in TD over time per 1 year increase in age</td>
<td>1.1297</td>
<td>0.741, 1.519</td>
<td>&lt;0.0001</td>
</tr>
<tr>
<td>Mean difference in TD over time before and after software change on February 13, 2008</td>
<td>11.4222</td>
<td>-2.373, 25.218</td>
<td>0.1032</td>
</tr>
<tr>
<td>Mean difference in TD at baseline between GAD and NAWM</td>
<td>167.76</td>
<td>112.236, 223.284</td>
<td>&lt;0.0001</td>
</tr>
<tr>
<td>Mean difference in TD per month for GAD</td>
<td>0.6617</td>
<td>0.039, 1.285</td>
<td>0.0374</td>
</tr>
<tr>
<td>Mean difference in TD per month for NAWM</td>
<td>0.2193</td>
<td>-0.187, 0.625</td>
<td>0.2898</td>
</tr>
</tbody>
</table>

Abbreviations: transverse diffusivity (TD), gadolinium enhancing lesions (GAD), normal appearing white matter (NAWM).
Table 3. Comparison of longitudinal diffusivity between GAD and NAWM

<table>
<thead>
<tr>
<th>Effect</th>
<th>Estimate</th>
<th>95% CI</th>
<th>p-value</th>
</tr>
</thead>
<tbody>
<tr>
<td>Mean difference in LD over time per 1 year increase in age</td>
<td>0.6107</td>
<td>0.234, 0.987</td>
<td>0.0015</td>
</tr>
<tr>
<td>Mean difference in LD over time before and after software change on</td>
<td>74.1828</td>
<td>52.245, 96.121</td>
<td>&lt;0.0001</td>
</tr>
<tr>
<td>February 13, 2008</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Mean difference in LD at baseline between GAD and NAWM</td>
<td>-144.49</td>
<td>-217.915, -71.065</td>
<td>0.0001</td>
</tr>
<tr>
<td>Mean difference in LD per month for GAD</td>
<td>1.8831</td>
<td>1.135, 2.631</td>
<td>&lt;0.0001</td>
</tr>
<tr>
<td>Mean difference in LD per month for NAWM</td>
<td>-0.2109</td>
<td>-0.614, 0.192</td>
<td>0.3052</td>
</tr>
</tbody>
</table>

Abbreviations: longitudinal diffusivity (LD), gadolinium enhancing lesions (GAD), normal appearing white matter (NAWM).
**Table 4. Comparison of fractional anisotropy between GAD and NAWM**

<table>
<thead>
<tr>
<th>Effect</th>
<th>Estimate</th>
<th>95% CI</th>
<th>p-value</th>
</tr>
</thead>
<tbody>
<tr>
<td>Mean difference in FA over time per 1 year increase in age</td>
<td>-1.0025</td>
<td>-1.283, -0.722</td>
<td>&lt;0.0001</td>
</tr>
<tr>
<td>Mean difference in FA over time before and after software change on</td>
<td>24.0887</td>
<td>12.582, 35.596</td>
<td>&lt;0.0001</td>
</tr>
<tr>
<td>February 13, 2008</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Mean difference in FA at baseline between GAD and NAWM</td>
<td>-225.08</td>
<td>-273.819, -176.341</td>
<td>&lt;0.0001</td>
</tr>
<tr>
<td>Mean difference in FA per month for GAD</td>
<td>0.5074</td>
<td>0.031, 0.984</td>
<td>0.037</td>
</tr>
<tr>
<td>Mean difference in FA per month for NAWM</td>
<td>-0.2572</td>
<td>-0.551, 0.037</td>
<td>0.0865</td>
</tr>
</tbody>
</table>

Abbreviations: fractional anisotropy (FA), gadolinium enhancing lesions (GAD), normal appearing white matter (NAWM).
### Table 5. Comparison of mean diffusivity between GAD and NAWM

<table>
<thead>
<tr>
<th>Effect</th>
<th>Estimate</th>
<th>95% CI</th>
<th>p-value</th>
</tr>
</thead>
<tbody>
<tr>
<td>Mean difference in MD over time per 1 year increase in age</td>
<td>0.9558</td>
<td>0.610, 1.302</td>
<td>&lt;0.0001</td>
</tr>
<tr>
<td>Mean difference in MD over time before and after software change on February 13, 2008</td>
<td>38.3557</td>
<td>24.470, 52.241</td>
<td>&lt;0.0001</td>
</tr>
<tr>
<td>Mean difference in MD at baseline between GAD and NAWM</td>
<td>59.2515</td>
<td>7.046, 111.457</td>
<td>0.0261</td>
</tr>
<tr>
<td>Mean difference in MD per month for GAD</td>
<td>1.2788</td>
<td>0.695, 1.863</td>
<td>&lt;0.0001</td>
</tr>
<tr>
<td>Mean difference in MD per month for NAWM</td>
<td>0.03209</td>
<td>-0.331, 0.396</td>
<td>0.8626</td>
</tr>
</tbody>
</table>

Abbreviations: Mean diffusivity (MD), gadolinium enhancing lesions (GAD), normal appearing white matter (NAWM).
Table 6. Comparison of transverse diffusivity between T2 and NAWM

<table>
<thead>
<tr>
<th>Effect</th>
<th>Estimate</th>
<th>95% CI</th>
<th>p-value</th>
</tr>
</thead>
<tbody>
<tr>
<td>Mean difference in TD over time per 1 year increase in age</td>
<td>1.3406</td>
<td>0.069, 2.613</td>
<td>0.0388</td>
</tr>
<tr>
<td>Mean difference in TD over time before and after software change on February 13, 2008</td>
<td>7.0281</td>
<td>0.070, 13.986</td>
<td>0.0477</td>
</tr>
<tr>
<td>Mean difference in TD at baseline between T2 and NAWM</td>
<td>172.96</td>
<td>166.01, 179.903</td>
<td>&lt;0.0001</td>
</tr>
<tr>
<td>Mean difference in TD per month for T2</td>
<td>0.05683</td>
<td>-0.181, 0.295</td>
<td>0.6401</td>
</tr>
<tr>
<td>Mean difference in TD per month for NAWM</td>
<td>0.03702</td>
<td>-0.156, 0.230</td>
<td>0.7068</td>
</tr>
</tbody>
</table>

Abbreviations: transverse diffusivity (TD), T2 lesions (T2), normal appearing white matter (NAWM).
Table 7. Comparison of longitudinal diffusivity between T2 and NAWM

<table>
<thead>
<tr>
<th>Effect</th>
<th>Estimate</th>
<th>95% CI</th>
<th>p-value</th>
</tr>
</thead>
<tbody>
<tr>
<td>Mean difference in LD over time per 1 year increase in age</td>
<td>0.594</td>
<td>-1.384, 2.572</td>
<td>0.556</td>
</tr>
<tr>
<td>Mean difference in LD over time before and after software change on February 13, 2008</td>
<td>91.9477</td>
<td>82.424, 101.471</td>
<td>&lt;0.0001</td>
</tr>
<tr>
<td>Mean difference in LD at baseline between T2 and NAWM</td>
<td>-92.7151</td>
<td>-132.583, -52.847</td>
<td>&lt;0.0001</td>
</tr>
<tr>
<td>Mean difference in LD per month for T2</td>
<td>0.5411</td>
<td>0.251, 0.831</td>
<td>0.0003</td>
</tr>
<tr>
<td>Mean difference in LD per month for NAWM</td>
<td>-0.1474</td>
<td>-0.378, 0.083</td>
<td>0.2102</td>
</tr>
</tbody>
</table>

Abbreviations: longitudinal diffusivity (LD), T2 lesions (T2), normal appearing white matter (NAWM).
Table 8. Comparison of fractional anisotropy over time between T2 and NAWM

<table>
<thead>
<tr>
<th>Effect</th>
<th>Estimate</th>
<th>95% CI</th>
<th>p-value</th>
</tr>
</thead>
<tbody>
<tr>
<td>Mean difference in FA over time per 1 year increase in age</td>
<td>-1.042</td>
<td>-2.000, -0.084</td>
<td>0.0331</td>
</tr>
<tr>
<td>Mean difference in FA over time before and after software change on February 13, 2008</td>
<td>30.831</td>
<td>25.973, 35.689</td>
<td>&lt;0.0001</td>
</tr>
<tr>
<td>Mean difference in FA at baseline between T2 and NAWM</td>
<td>-199.110</td>
<td>-218.473, -179.747</td>
<td>&lt;0.0001</td>
</tr>
<tr>
<td>Mean difference in FA per month for T2</td>
<td>0.163</td>
<td>0.001, 0.324</td>
<td>0.0482</td>
</tr>
<tr>
<td>Mean difference in FA per month for NAWM</td>
<td>-0.030</td>
<td>-0.160, 0.099</td>
<td>0.6458</td>
</tr>
</tbody>
</table>

Abbreviations: fractional anisotropy (FA), T2 lesions (T2), normal appearing white matter (NAWM).
Table 9. Comparison of mean diffusivity over time between T2 and NAWM

<table>
<thead>
<tr>
<th>Effect</th>
<th>Estimate</th>
<th>95% CI</th>
<th>p-value</th>
</tr>
</thead>
<tbody>
<tr>
<td>Mean difference in MD over time per 1 year increase in age</td>
<td>1.0925</td>
<td>-0.234, 2.419</td>
<td>0.1064</td>
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<tr>
<td>Mean difference in MD over time before and after software change on February 13, 2008</td>
<td>35.261</td>
<td>28.491, 42.031</td>
<td>&lt;0.0001</td>
</tr>
<tr>
<td>Mean difference in MD at baseline between T2 and NAWM</td>
<td>82.9835</td>
<td>56.181, 109.786</td>
<td>&lt;0.0001</td>
</tr>
<tr>
<td>Mean difference in MD per month for T2</td>
<td>0.2695</td>
<td>0.040, 0.499</td>
<td>0.0211</td>
</tr>
<tr>
<td>Mean difference in MD per month for NAWM</td>
<td>-0.05726</td>
<td>-0.242, 0.128</td>
<td>0.5439</td>
</tr>
</tbody>
</table>

Abbreviations: mean diffusivity (MD), T2 lesions (T2), normal appearing white matter (NAWM).
Table 10. Comparison for transverse diffusivity over time between BH and NBH

<table>
<thead>
<tr>
<th>Effect</th>
<th>Estimate</th>
<th>95% CI</th>
<th>p-value</th>
</tr>
</thead>
<tbody>
<tr>
<td>Mean difference in TD over time per 1 year increase in age</td>
<td>1.2484</td>
<td>-1.096, 3.593</td>
<td>0.2967</td>
</tr>
<tr>
<td>Mean difference in TD over time before and after software change on February 13, 2008</td>
<td>12.3132</td>
<td>-2.458, 27.084</td>
<td>0.1032</td>
</tr>
<tr>
<td>Mean difference in TD at baseline between BH and NBH</td>
<td>92.2056</td>
<td>39.495, 144.916</td>
<td>0.0006</td>
</tr>
<tr>
<td>Mean difference in TD per month for BH</td>
<td>0.3392</td>
<td>-0.028, 0.706</td>
<td>0.0701</td>
</tr>
<tr>
<td>Mean difference in TD per month for NBH</td>
<td>-0.4137</td>
<td>-0.967, 0.140</td>
<td>0.1432</td>
</tr>
</tbody>
</table>

Abbreviations: transverse diffusivity (TD), black holes (BH), non black holes (NBH)
Table 11. Comparison of longitudinal diffusivity over time between BH and NBH

<table>
<thead>
<tr>
<th>Effect</th>
<th>Estimate</th>
<th>95% CI</th>
<th>p-value</th>
</tr>
</thead>
<tbody>
<tr>
<td>Mean difference in LD over time per 1 year increase in age</td>
<td>0.07411</td>
<td>-2.702, 2.850</td>
<td>0.9583</td>
</tr>
<tr>
<td>Mean difference in LD over time before and after software change on February 13, 2008</td>
<td>48.53</td>
<td>32.486, 64.574</td>
<td>&lt;0.0001</td>
</tr>
<tr>
<td>Mean difference in LD at baseline between BH and NBH</td>
<td>49.0333</td>
<td>-13.198, 111.265</td>
<td>0.1226</td>
</tr>
<tr>
<td>Mean difference in LD per month for BH</td>
<td>0.1967</td>
<td>-0.200, 0.593</td>
<td>0.331</td>
</tr>
<tr>
<td>Mean difference in LD per month for NBH</td>
<td>-0.2392</td>
<td>-0.838, 0.360</td>
<td>0.434</td>
</tr>
</tbody>
</table>

Abbreviations: longitudinal diffusivity (TD), black holes (BH), non black holes (NBH)
Table 12. Comparison of fractional anisotropy over time between BH and NBH

<table>
<thead>
<tr>
<th>Effect</th>
<th>Estimate</th>
<th>95% CI</th>
<th>p-value</th>
</tr>
</thead>
<tbody>
<tr>
<td>Mean difference in FA over time per 1 year increase in age</td>
<td>-0.9386</td>
<td>-2.601, 0.724</td>
<td>0.2682</td>
</tr>
<tr>
<td>Mean difference in FA over time before and after software change on February 13, 2008</td>
<td>12.9557</td>
<td>4.434, 21.477</td>
<td>0.0031</td>
</tr>
<tr>
<td>Mean difference in FA at baseline between BH and NBH</td>
<td>-42.9088</td>
<td>-80.051, -5.767</td>
<td>0.0236</td>
</tr>
<tr>
<td>Mean difference in FA per month for BH</td>
<td>-0.2137</td>
<td>-0.422, -0.005</td>
<td>0.0444</td>
</tr>
<tr>
<td>Mean difference in FA per month for NBH</td>
<td>0.2907</td>
<td>-0.025, 0.606</td>
<td>0.0708</td>
</tr>
</tbody>
</table>

Abbreviations: fractional anisotropy (FA), black holes (BH), non black holes (NBH)
Table 13. Comparison of mean diffusivity over time between BH and NBH

<table>
<thead>
<tr>
<th>Effect</th>
<th>Estimate</th>
<th>95% CI</th>
<th>p-value</th>
</tr>
</thead>
<tbody>
<tr>
<td>Mean difference in MD over time per 1 year increase in age</td>
<td>0.8564</td>
<td>-1.363, 3.076</td>
<td>0.4491</td>
</tr>
<tr>
<td>Mean difference in MD over time before and after software change on February 13, 2008</td>
<td>24.3442</td>
<td>10.194, 38.495</td>
<td>0.0008</td>
</tr>
<tr>
<td>Mean difference in MD at baseline between BH and NBH</td>
<td>77.7949</td>
<td>27.890, 127.700</td>
<td>0.0023</td>
</tr>
<tr>
<td>Mean difference in MD per month for BH</td>
<td>0.2919</td>
<td>-0.059, 0.643</td>
<td>0.1026</td>
</tr>
<tr>
<td>Mean difference in MD per month for NBH</td>
<td>-0.355</td>
<td>-0.174, 0.884</td>
<td>0.1883</td>
</tr>
</tbody>
</table>

Abbreviations: mean diffusivity (MD), black holes (BH), non black holes (NBH)
Figure 1. Regions of interest in normal appearing white matter and gadolinium enhancing tissue

A. Centrum semi-ovale.
B. Deep white matter tract of the frontal lobe.
C. Corpus callosum anterior and posterior, Internal capsule anterior and posterior.
D. Corticospinal tracts midbrain.
E. Corticospinal tracts pons.
F. Gadolinium enhancing lesions.
Figure 2. Transverse diffusivity \((10^{-6}\text{mm}^2\text{/sec})\) in normal appearing white matter and gadolinium enhancing lesions

Abbreviations: gadolinium enhancing lesions (Gad), normal appearing white matter (NAWM).
Figure 3. Longitudinal diffusivity \((10^{-6} \text{mm}^2/\text{sec})\) in normal appearing white matter and gadolinium enhancing lesions.

Abbreviations: gadolinium enhancing lesions (Gad), normal appearing white matter (NAWM).
Figure 4. Fractional anisotropy in normal appearing white matter and gadolinium enhancing lesions

Abbreviations: gadolinium enhancing lesions (Gad), normal appearing white matter (NAWM).
**Figure 5.** Mean diffusivity ($10^{-6} \text{ mm}^2/\text{sec}$) in normal appearing white matter and gadolinium enhancing lesions

Abbreviations: gadolinium enhancing lesions (Gad), normal appearing white matter (NAWM).
Figure 6. Transverse diffusivity ($10^{-6} \text{mm}^2/\text{sec}$) in normal appearing white matter and T2 lesions

Abbreviations: T2 lesions (Lesi), normal appearing white matter (NAWM).
Figure 7. Longitudinal diffusivity \((10^{-6} \text{ mm}^2/\text{sec})\) in normal appearing white matter and T2 lesions

Abbreviations. T2 lesions (Lesi), normal appearing white matter (NAWM).
Figure 8. Fractional anisotropy ($10^{-6}$ mm$^2$/sec) in normal appearing white matter and T2 lesions

Abbreviations. T2 lesions (Lesi), normal appearing white matter (NAWM).
**Figure 9.** Mean diffusivity ($10^{-6} \text{mm}^2/\text{sec}$) in normal appearing white matter and T2 lesions

Abbreviations. T2 lesions (Lesi), normal appearing white matter (NAWM).
References


