Longitudinal changes in diffusion tensor–based quantitative MRI in multiple sclerosis

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ABSTRACT

Objective: To estimate longitudinal changes in a quantitative whole-brain and tract-specific MRI study of multiple sclerosis (MS), with the intent of assessing the feasibility of this approach in clinical trials.

Methods: A total of 78 individuals with MS underwent a median of 3 scans over 2 years. Diffusion tensor imaging indices, magnetization transfer ratio, and T2 relaxation time were analyzed in supratentorial brain, corpus callosum, optic radiations, and corticospinal tracts by atlas-based tractography. Linear mixed-effect models estimated annualized rates of change for each index, and sample size estimates for potential clinical trials were determined.

Results: There were significant changes over time in fractional anisotropy and perpendicular diffusivity in the supratentorial brain and corpus callosum, mean diffusivity in the supratentorial brain, and magnetization transfer ratio in all areas studied. Changes were most rapid in the corpus callosum, where fractional anisotropy decreased 1.7% per year, perpendicular diffusivity increased 1.2% per year, and magnetization transfer ratio decreased 0.9% per year. The T2 relaxation time changed more rapidly than diffusion tensor imaging indices and magnetization transfer ratio but had higher within-participant variability. Magnetization transfer ratio in the corpus callosum and supratentorial brain declined at an accelerated rate in progressive MS relative to relapsing-remitting MS. Power analysis yielded reasonable sample sizes (on the order of 40 participants per arm or fewer) for 1- or 2-year trials.

Conclusions: Longitudinal changes in whole-brain and tract-specific diffusion tensor imaging indices and magnetization transfer ratio can be reliably quantified, suggesting that small clinical trials using these outcome measures are feasible. *Neurology*® 2011;76:179-186

GLOSSARY

DTI = diffusion tensor imaging; **FA** = fractional anisotropy; **FLAIR** = fluid-attenuated inversion recovery; **MD** = mean diffusivity; **MNI** = Montreal Neurological Institute; **MPRAGE** = magnetization-prepared rapid gradient echo; **MS** = multiple sclerosis; **MTR** = magnetization transfer ratio; **NAWM** = normal-appearing white matter; **PPMS** = primary progressive multiple sclerosis; **qT2** = quantitative T2; **RRMS** = relapsing-remitting multiple sclerosis; **SPMS** = secondary progressive multiple sclerosis.

Although visualization of inflammatory lesions by MRI is currently the mainstay of diagnosis and treatment effect monitoring in multiple sclerosis (MS), it has become increasingly clear that measuring lesion load captures only part of MS-related pathology. Diffusion tensor imaging (DTI) and magnetization transfer–weighted imaging may provide additional information about tissue damage and treatment response in MS. Quantification of the coherence and magnitude of water diffusion within axonal tracts by DTI, and of tissue macromolecular integrity by means of the magnetization transfer ratio (MTR), may be more specific for certain types of tissue injury (particularly demyelination and axonal degeneration) than measurements of the T2 relaxation time.¹⁻⁴ Indeed, these quantities, when assessed in a tract-specific manner,

Supplemental data at
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are correlated with disability related to that tract's function.⁵⁻⁸ Thus, quantitative DTI and MTR may increase the diagnostic and prognostic power of MRI in MS.⁹

Before integrating these techniques into clinical trials and clinical practice, longitudinal studies are needed to establish expected magnitudes and rates of change over time. Few studies have performed such an analysis, and those that have were limited by small sample sizes.¹⁰ In this article, we present the results of a longitudinal analysis of DTI indices, MTR, and quantitative T2 (qT2). Our primary hypothesis was that quantitative measurement of MRI abnormalities in white matter tracts change at a rate comparable to brain volume loss. Additionally, we aimed to show that with automated and optimized methodologies, alterations of these rates of change could be measured with sample sizes practical for future clinical studies.

METHODS **Standard protocol approvals and patient consents.** Protocols were approved by the Institutional Review Boards at The Johns Hopkins University School of Medicine and the Kennedy Krieger Institute. Written, informed consent was obtained from all participants.

Participants. Participants with MS were recruited from the Johns Hopkins Multiple Sclerosis Center. Individuals with diagnoses of relapsing-remitting (RRMS), secondary progressive (SPMS), and primary progressive (PPMS) MS were enrolled, with the diagnosis being assigned by the treating physician. Data were collected from March 2004 through July 2009. Scans performed within 30 days of a clinical relapse or steroid administration were excluded.

MRI protocol and image analysis. Scans were obtained at baseline and roughly 3 months (RRMS only), 6 months, 1 year, and yearly thereafter. Due to participant dropout and missed visits, some of the planned study scans were not obtained. Our MRI acquisition protocol has been fully described elsewhere, and only the essential details are presented here.^{11,12} On a 3-Tesla Philips scanner, we obtained the following whole-brain sequences at the specified acquired resolution without gaps: DTI (2.2 mm isotropic); magnetization transfer ($1.5 \times 1.5 \times 2.2$ mm); proton density/T2-weighted and fluid-attenuated inversion recovery (FLAIR) ($0.8 \times 0.8 \times 2.2$ mm or 4.4 mm for some of the FLAIR scans); and 3-dimensional magnetization-prepared rapid gradient echo (MPRAGE) (1.1 mm isotropic).

The details of our image-analysis protocol have been described.^{11,12} In brief, MTR and estimated T2-relaxation time (qT2) maps were calculated from the appropriate image sources. The MTR, qT2, and diffusion-weighted images were coregistered, and the diffusion tensor was estimated to generate maps of fractional anisotropy (FA), mean diffusivity (MD), parallel diffusivity ($\lambda_{\parallel}$), and perpendicular diffusivity ($\lambda_{\perp}$). Tract-specific values for each of these MRI indices were calculated across the optic radiations, corticospinal tracts, and corpus callosum in an auto-

mated fashion by linearly registering the data to a DTI atlas containing probabilistic maps of the tract positions,¹¹ removing voxels with low FA (to exclude gray matter and some lesions), and then taking the weighted average over all voxels within the tract of interest. For the corticospinal tracts and optic radiations, data from the right and left sides were averaged.

The DTI data were also used to perform supratentorial brain analyses as described previously.¹² We restricted our analysis to Montreal Neurological Institute (MNI-152) coordinates $54 \leq z \leq 124$, which corresponds to the mammillary bodies inferiorly and the centrum semiovale superiorly. We removed a small section of the anterior frontal lobes ($y \leq 37$) that is particularly susceptible to distortion on the DTI scans, and we also removed CSF by excluding voxels with $MD \geq 1.7 \mu\text{m}^2/\text{ms}$. The resulting brain-parenchymal mask was used to calculate median values of the individual MRI indices and to calculate the cerebral volume fraction ($\text{CVF}_{\text{DTI}} = [\text{brain volume}] / [\text{brain} + \text{CSF volume}]$).

We used the Lesion-TOADS algorithm¹³ (<http://medic.rad.jhmi.edu/download>) for automated segmentation of lesions and for a second measure of CVF (referred to as $\text{CVF}_{\text{TOADS}}$). This segmentation uses the MPRAGE and FLAIR scans and accounts specifically for MS lesions; its performance compares well to that of a trained observer.¹³ The T2 lesion masks obtained by this algorithm were linearly registered to the tractography atlas, allowing measurement of each MRI index within and outside lesions. In addition to the calculation of $\text{CVF}_{\text{TOADS}}$, we also calculated $\text{CVF}_{\text{SIENAX}}$ from the MPRAGE images using SIENAX software,¹⁴ which has been previously studied in the context of sample size measurement for trials using atrophy as a primary outcome.¹⁵

Statistical analysis. All statistical analyses were performed in Stata 10.1 IC (StataCorp, College Station, TX). Comparisons between subgroups for baseline demographic, clinical, and MRI data were performed by Student *t* test (means) and Wilcoxon/Mann-Whitney test (medians). Because of the exploratory nature of this study, adjustment for multiple comparisons was not performed. Subtle data shifts were noted to have occurred due to the inevitable hardware upgrades and repairs to our MRI scanner during the 5-year study period. To adjust for this, a statistical detrending procedure was performed, which is described in appendix e-1 on the *Neurology*[®] Web site at www.neurology.org.

The adjusted values were then analyzed for longitudinal change by a linear mixed-model approach (appendix e-1).¹⁶ The final regression model tested for prediction of the natural log of each MRI index by time from baseline, adjusted for age and sex. Annualized rates of change (% per year) in individual MRI indices were derived by exponentiation of the regression slope given by the fixed-effects coefficient for time from baseline. MS-subtype comparisons in both intercept and slope were performed within this regression model via interaction term analysis. The same regression analysis was also performed for the log of the ratio of MRI index values in normal-appearing white matter (NAWM) to each region with lesions included, which indirectly tested for the null hypothesis that the slopes of each are equivalent (meaning there is no difference between changes in NAWM vs NAWM + lesions).

To assess whether these MRI indices can be used as clinical trial outcome measures, sample sizes were calculated. The random effects regression results entered into a sample-size calculation formula for longitudinal studies (appendix e-1).¹⁷ Sample sizes were determined for hypothetical trials of drugs that would cause 25%, 50%, or 75% improvement in the annualized rate of change in each MRI index.

Table 1 Study population characteristics

	All MS (n = 78)	RRMS (n = 40)	SPMS (n = 24)	PPMS (n = 14)
Mean baseline age, y (SD)	43.6 (11.0)	39.9 (9.2)	50.9 (6.6) ^a	51.8 (7.6) ^a
% Female	67	70	54	64
Mean disease duration, y, at baseline (SD)	10.8 (9.3)	7.5 (6.1)	17.6 (9.7) ^a	8.5 (10.5) ^b
Median baseline EDSS (range)	3.5 (0–8)	2.5 (0–6)	6.5 (2–8) ^a	4.0 (2.5–6.5) ^{a,b}
% on disease-modifying therapy at baseline	64	83	50 ^a	36 ^a
% on disease-modifying therapy on exit	63	87	44 ^a	27 ^a
Median no. of scans (range)	3 (2–6)	3 (2–6)	3.5 (2–6)	3.5 (2–5)
Median follow-up time, y (range)	2.0 (0.5–4.1)	2.0 (0.5–3.9)	2.1 (0.5–3.9)	1.8 (0.8–3.3)

Abbreviations: EDSS = Expanded Disability Status Scale; MS = multiple sclerosis; PPMS = primary progressive multiple sclerosis; RRMS = relapsing-remitting multiple sclerosis; SPMS = secondary progressive multiple sclerosis.

^a $p < 0.05$ for comparison to RRMS.

^b $p < 0.05$ for comparison to SPMS.

RESULTS Data from 78 individuals with MS were included (table 1). As expected, participants with SPMS and PPMS were older and had higher baseline EDSS. The number of scans per participant was equivalent across subgroups (median 3, range 2–6). The median length of follow up was 2.0 years (range 0.5–4.1). A total of 64% of subjects were on various disease-modifying drugs at baseline, including interferon- β (58%), glatiramer acetate (26%), natalizumab (6%), and others (10%).

Summary statistics for the tract-specific and cerebral MRI indices are shown in table 2, and sample FA, MTR, and qT2 maps with overlaid tracts for a single individual with RRMS are presented in figure e-1. Values across the whole region of interest (tract or cerebrum) were not significantly different from those in the NAWM alone for either this analysis or for the longitudinal regression modeling described below, so the results reported are those for unsegmented tracts (including both NAWM and lesions).

Annual rates of change (% per year) are reported in table 2. All significant changes were in the expected direction—that is, MD, $\lambda_{\perp}$, and qT2 increased, whereas FA, MTR, and CVF decreased. Of the DTI indices, supratentorial brain values of FA, MD, and $\lambda_{\perp}$ were found to change at comparable rates, whereas $\lambda_{\parallel}$ showed no significant change over time. Tract-specific DTI indices did not change consistently in the corticospinal tracts or optic radiations. However, corpus callosum FA decreased at a rate of 1.7% per year (figure 1), and $\lambda_{\perp}$ increased at a rate of 1.2% per year. Repeated, longitudinal measurements of whole-brain and corpus callosum DTI indices were highly reproducible, and within-participant measurement correlations were ≥ 0.83 .

Estimated qT2 also measurably changed over time in the supratentorial brain analysis and within the corpus callosum and optic radiations. These changes occurred at a faster rate than for DTI indices (for example, 2.0% per year for supratentorial qT2). However, within-participant qT2 had poor reproducibility, such that within-participant correlations were as low as 0.26. Of all indices tested, only MTR had measureable longitudinal changes in all areas studied. When evaluated in supratentorial brain and corpus callosum, this occurred at slightly slower rates than for FA and $\lambda_{\perp}$. There was no longitudinal brain atrophy as assessed by CVF_{SIENAX}, likely due to high within-participant scan-to-scan variability (within-participant correlation value = 0.38). Conversely, CVF_{TOADS} decreased 0.2% per year, and CVF_{DTI} decreased 0.3% per year.

There were no significant differences in baseline MRI indices across MS subtype, nor were there generally differences in rates of change in MRI indices over time. The few exceptions to this are noted in table 3. Of note, for the DTI indices and qT2 measurements with significant changes in the whole study population (as reported in table 2), rates of change did not differ across MS subtype. However, the changes observed in MTR appear to be primarily driven by changes occurring in the progressive subtypes. Specifically, whole-brain MTR had no observable change in RRMS but a 0.7% per year decline in SPMS. Corpus callosum MTR maintained a significant rate of decline in subjects with RRMS (0.6% per year) but declined faster in subjects with PPMS (1.8% per year). Also, whereas no changes were observed in $\lambda_{\parallel}$ in the larger cohort, this index decreased at a rate of 0.9% per year in SPMS but did not change in RRMS. Finally, the changes noted in CVF_{DTI} appear to have been driven mostly by a faster rate of decline in RRMS (0.6% per year).

Sample sizes were calculated for trials of hypothetical drugs that would reduce the observed rates of change. Table 4 shows the number of participants per trial arm needed to reduce the observed rate of change by 25%, 50%, or 75% over the course of a 12- or 24-month trial (each with 3 scans), with 80% power. In addition to the length of study and the magnitude of the rate of change, within-participant variability had a large impact on the sample sizes required for a given effect size. For example, despite the fact that qT2 was found to change at rates 2 to 4 times greater than DTI indices, higher within-participant variability in qT2 resulted in sample sizes that would make it an impractical outcome measure.

Conversely, the lower variability in DTI indices yielded very small sample sizes. For example, a 2-year study with FA and $\lambda_{\perp}$ of the corpus callosum as out-

Table 2 Annualized rate of change in MRI indices

	Supratentorial brain	Corpus callosum	Optic radiations	Corticospinal tracts
FA				
%	-0.5* (-0.7 to -0.2)	-1.7* (-2.1 to -1.3)	-0.3 (-0.7 to 0.1)	0.2 (-0.05 to 0.5)
v_{t0}	0.258 (0.013)	0.510 (0.042)	0.455 (0.038)	0.528 (0.024)
ρ_{μ}	0.83	0.89	0.89	0.78
σ_{ϵ}	0.020	0.029	0.031	0.021
MD				
%	0.3* (0.1 to 0.4)	0.5 (0.0 to 1.0)	0.4 (0.0 to 0.9)	0.2 (-0.2 to 0.5)
v_{t0}	1.928 (0.033)	1.056 (0.097)	0.988 (0.089)	0.830 (0.043)
ρ_{μ}	0.90	0.84	0.88	0.72
σ_{ϵ}	0.011	0.037	0.030	0.026
$\lambda_{\parallel}$				
%	0.1 (0.0 to 0.3)	-0.2 (-0.1 to 0.2)	0.3 (-0.1 to 0.6)	0.2 (-0.1 to 0.5)
v_{t0}	1.167 (0.030)	1.683 (0.115)	1.500 (0.090)	1.367 (0.065)
ρ_{μ}	0.86	0.79	0.85	0.78
σ_{ϵ}	0.010	0.031	0.022	0.022
$\lambda_{\perp}$				
%	0.4* (0.2 to 0.5)	1.2* (0.6 to 1.9)	0.6 (-0.0 to 1.2)	0.1 (-0.4 to 0.5)
v_{t0}	0.808 (0.034)	0.743 (0.097)	0.732 (0.092)	0.561 (0.039)
ρ_{μ}	0.90	0.87	0.89	0.72
σ_{ϵ}	0.010	0.048	0.041	0.035
qT2				
%	2.0* (0.6 to 3.4)	2.7* (0.5 to 4.9)	2.8* (1.3 to 4.4)	0.4 (-0.8 to 1.7)
v_{t0}	109.8 (13.2)	127.1 (25.1)	111.7 (24.6)	97.5 (9.4)
ρ_{μ}	0.26	0.40	0.71	0.38
σ_{ϵ}	0.110	0.169	0.114	0.100
MTR				
%	-0.3* (-0.6 to -0.1)	-0.9* (-1.3 to -0.4)	-0.7* (-1.1 to -0.4)	-0.2* (-0.5 to 0.0)
v_{t0}	0.391 (0.010)	0.421 (0.021)	0.419 (0.025)	0.446 (0.011)
ρ_{μ}	0.47	0.68	0.83	0.64
σ_{ϵ}	0.020	0.030	0.026	0.016
CVF_{SIENAX}				
%	-0.2 (-0.6 to 0.1)			
v_{t0}	0.783 (0.026)			
ρ_{μ}	0.38			
σ_{ϵ}	0.028			
CVF_{TOADS}				
%	-0.2* (-0.5 to 0.0)			
v_{t0}	0.801 (0.028)			
ρ_{μ}	0.89			
σ_{ϵ}	0.011			
CVF_{DTI}				
%	-0.3* (-0.5 to -0.1)			
v_{t0}	0.854 (0.050)			
ρ_{μ}	0.95			
σ_{ϵ}	0.013			

Abbreviations: % = percent per year change (95% confidence interval in parentheses); ρ_{μ} = rho value for correlation of measurements within participants; σ_{ϵ} = the natural log of the average within-participant standard deviation of measurement error for the MRI index; $\lambda_{\parallel}$ = parallel diffusivity ($\mu\text{m}^2/\text{ms}$); $\lambda_{\perp}$ = perpendicular diffusivity ($\mu\text{m}^2/\text{ms}$); CVF_{DTI} = cerebral volume fraction calculated from DTI data; CVF_{SIENAX} = cerebral volume fraction by SIENAX methodology; CVF_{TOADS} = cerebral volume fraction by lesion-TOADS methodology; FA = fractional anisotropy; MD = mean diffusivity ($\mu\text{m}^2/\text{ms}$); MTR = magnetization transfer ratio; qT2 = T2 relaxation time (ms); v_{t0} = value of the MRI index at baseline (standard deviation in parentheses).

* $p < 0.05$ for linear rate of change detected by mixed models regression.

Figure 1 Longitudinal change in fractional anisotropy (FA) and magnetization transfer ratio (MTR) in the corpus callosum

Spaghetti plots demonstrate longitudinal changes in FA (A) and MTR (C) in the corpus callosum within individuals. Cross-participant mean FA (B) and MTR (D) values at yearly intervals from baseline demonstrate longitudinal decline in the measured values. Error bars represent 95% confidence intervals. Larger error bars in the year 3 results probably reflect smaller sample sizes.

come measures would only require sample sizes of 7 and 43 per trial arm, respectively, for a 50% reduction in the observed rate of change. Notably, CVF_{DTI} also yielded small sample sizes, with only 14 subjects per trial arm needed to observe a 50% treatment effect in a 2-year study. Treatment effect manifested by reduction in the rate of decrease in MTR in the corpus callosum and optic radiations also demonstrated feasible sample size requirements, whereas supratentorial brain and corticospinal tract MTR did not.

DISCUSSION In order for experimental imaging techniques to be considered as outcome measures for clinical trials, they must be sensitive to change over time. Our results clearly demonstrate that longitudinal changes in supratentorial brain and tract-specific DTI indices, MTR, and qT2 are detectable and yield reasonable sample sizes for potential clinical trials. Although the lack of a matched healthy volunteer group makes it impossible to say whether these

changes are accentuated in MS, the fact that previous work on this cohort has established links between the imaging measures used in this study and disability, as well as differences between people with MS and healthy volunteers, suggests that this is probably the case.^{12,18,19} Further work in our group will be devoted to understanding whether these tract-specific longitudinal changes are linked with longitudinal changes in disability related to the functions of those tracts.

Of the MRI indices we studied, the ones that changed most reliably over the course of the study period were supratentorial and corpus callosum FA and $\lambda_{\perp}$ as well as supratentorial and tract-specific MTR. There were also changes in supratentorial and tract-specific qT2, but these changes were associated with high variability. The variability may be partially due to imperfect registration to the DTI images, a problem less likely to affect MTR because of the echoplanar imaging technique we used for both MTR and DTI scans.¹¹ Most of the DTI and MTR

Table 3 Result of MS subtype analysis for annualized rate of change^a

	RRMS	SPMS	PPMS
FA: Optic radiations	0.0 (−0.6 to 0.6)	−0.2 (−0.9 to 0.6)	−1.2 (−2.1 to −0.3) ^{b,c}
$\lambda_{\parallel}$: Supratentorial brain	0.3 (0.1 to 0.5) ^b	−0.1 (−0.4 to 0.1) ^{b,c}	0.1 (−0.2 to 0.4)
$\lambda_{\parallel}$: Corpus callosum	0.1 (−0.5 to 0.6)	−0.9 (−1.6 to −0.1) ^{b,c}	0.1 (−0.8 to 1.0)
qT2: Corticospinal tracts	−0.3 (−2.0 to 1.4)	−0.1 (−2.4 to 2.2)	3.5 (0.7 to 6.4) ^{b,c,d}
MTR: Supratentorial brain	−0.1 (−0.4 to 0.3)	−0.7 (−1.2 to −0.2) ^{b,c}	−0.6 (−1.2 to 0.0) ^b
MTR: Corpus callosum	−0.6 (−1.1 to 0.0)	−0.9 (−1.6 to −0.2) ^b	−1.8 (−2.7 to −0.9) ^{b,c}
CVF _{DTI}	−0.6 (−0.9 to −0.3) ^b	−0.1 (−0.4 to 0.3) ^c	−0.2 (−0.6 to 0.2)

Abbreviations: $\lambda_{\parallel}$ = parallel diffusivity ($\mu\text{m}^2/\text{ms}$); CVF_{DTI} = cerebral volume fraction calculated from DTI data; FA = fractional anisotropy; MS = multiple sclerosis; MTR = magnetization transfer ratio; PPMS = primary progressive multiple sclerosis; qT2 = T2 relaxation time (ms); RRMS = relapsing-remitting multiple sclerosis; SPMS = secondary progressive multiple sclerosis.

^a Only those differences that attained statistical significance are shown here.

^b $p < 0.05$ for subgroup linear rate of change over time.

^c $p < 0.05$ for comparison to RRMS.

^d $p < 0.05$ for comparison to SPMS.

changes occurred at rates comparable to brain atrophy, but the rate was considerably higher in the corpus callosum.

The DTI findings contrast with the lack of longitudinal change noted in some prior studies^{10,20} but are consistent with a longitudinal trend in a small sample of RRMS and SPMS in at least one study.²¹

Table 4 Proposed sample sizes (n per trial arm) for hypothetical 1- or 2-year clinical trials using the tested MRI indices as outcome measures^a

Measurement	Scan at 0, 6, and 12 months			Scan at 0, 12, and 24 months		
	25% reduction	50% reduction	75% reduction	25% reduction	50% reduction	75% reduction
FA: Supratentorial brain	1,037	259	115	259	65	29
FA: Corpus callosum	106	26	12	26	7	3
MD: Supratentorial brain	631	810	70	810	39	18
$\lambda_{\perp}$: Supratentorial brain	422	105	47	105	26	12
$\lambda_{\perp}$: Corpus callosum	690	172	77	172	43	19
qT2: Supratentorial brain	7,917	1,979	880	1,979	495	220
qT2: Corpus callosum	8,338	2,084	926	2,084	521	232
qT2: Optic radiations	1,635	409	182	409	102	45
MTR: Supratentorial brain	6,374	1,594	708	1,594	398	177
MTR: Corpus callosum	1,307	327	145	327	82	36
MTR: Corticospinal tract	6,088	1,522	676	1,522	380	169
MTR: Optic radiations	722	181	80	181	45	20
CVF _{TOADS}	873	218	97	218	55	24
CVF _{DTI}	227	57	25	57	14	6

Abbreviations: $\lambda_{\perp}$ = perpendicular diffusivity ($\mu\text{m}^2/\text{ms}$); CVF_{DTI} = cerebral volume fraction calculated from DTI data; CVF_{TOADS} = cerebral volume fraction by lesion-TOADS methodology; FA = fractional anisotropy; MD = mean diffusivity ($\mu\text{m}^2/\text{ms}$); MTR = magnetization transfer ratio; qT2 = T2 relaxation time (ms).

^a Only those indices showing significant rates of change in longitudinal analysis are shown here. The calculations above are for 80% power and 2-sided $\alpha = 0.05$.

Compared to earlier studies, we enrolled more individuals and scanned them more frequently, which increased our statistical power. Previous evaluations were also limited to histogram-based, whole-brain analyses or limited regions of interest. Although we also used supratentorial brain summary measures, we found larger longitudinal changes when we focused the analysis on specific white matter tracts, notably the corpus callosum. Such tract-specific measures may be better markers of longitudinal white matter change and, because tracts have specific functions, they may also be more predictive of clinical disability. It is not surprising that the greatest changes occurred in the corpus callosum, given its frequent involvement in MS and its highly organized fiber orientation structure, which makes it a good target for DTI.

A possible confound in our analysis was that many of the participants with MS (64% at baseline) were taking disease-modifying drugs, and in some cases drugs were changed during the course of the study. Although we did not include MRI scans performed within 30 days of corticosteroid administration, treatment status might have affected the results by reducing not only the absolute change over time but also the across-participant variability, the key factor in determining sample size. This effect is somewhat mitigated by the fact that a variety of disease-modifying treatments were represented. In addition, as placebo-controlled trials in MS (particularly early MS) are becoming less common, we believe that assessment of typical rates of longitudinal change in a treated population has practical value.

Our analysis was aided by the use of an automated, atlas-based method for defining tracts. In previous work, we showed that this method has significantly lower scan-rescan variability than conventional deterministic tractography but yields similar correlations with disability scores.¹¹ Moreover, the atlas-based method eliminates tractography failures in and around lesions. The low variability of this method was clearly seen in the current study, enabling us to detect small changes over time. These small changes translated into the small sample sizes that we found necessary to demonstrate treatment effects, especially for DTI indices and MTR within the corpus callosum. These sample sizes are similar to, if not smaller than, those previously proposed for brain atrophy as a clinical trial outcome measure.²² Thus, when considering application of DTI-based quantitative analysis on a large scale, such as in a clinical trial, this methodology, with its automated analyses and high reproducibility, has distinct advantages.

Interestingly, comparing various methods of measuring normalized brain volume, we found that CVF_{DTI} yielded the smallest sample sizes. To our knowledge, DTI-based measurement of brain volume has not been previously investigated. This result is particularly surprising because DTI data suffer from distortions related to magnetic susceptibility and eddy currents, which one might expect not only to reduce accuracy but also to increase variability. Although we did not undertake a formal investigation into the reasons for the superior reliability of DTI-based brain volume measurement, we suspect that the reasons may include improved contrast between cerebral and extracerebral structures, such as CSF. Before urging adoption of DTI-based brain volume measurement in longitudinal trials, however, we note that the technique suffers from a limited ability to separate gray from white matter.

Another finding warranting future investigation is the result of the subgroup analyses. While no significant longitudinal changes in $\lambda_{||}$ were found for the group as a whole, this index was found to decrease significantly over time in the corpus callosum in SPMS. The meaning of this is unclear, given that $\lambda_{||}$ has only been shown to decrease in the acute phase of axonal degeneration,^{3,23} but not in the chronic setting. We note, however, that interpretation of directional diffusivity in the face of disease remains complicated.²⁴ The accelerated decline in supratentorial brain and corpus callosum MTR in progressive MS may have the same basis, as MTR is clearly sensitive to axonal loss as well as demyelination.²⁵ The accelerated rate of atrophy measured by CVF_{DTI} in RRMS compared to SPMS is also a curious result, as it contradicts previous findings of similar rates of whole brain and white matter atrophy in RRMS and SPMS.²⁶ However, this could be potentially explained by pseudoatrophy in study participants who went on or changed their disease-modifying therapy during the study period.

The methods used in this study could easily be applied in clinical trials due to the fully automated analysis as well as the small sample sizes that are required to demonstrate treatment effects. Of course, applying such a precise quantitative methodology to a multicenter trial would require that great care be taken to ensure standardization in acquisition and analysis between all sites. We are planning future studies to evaluate combining these indices into composite measures, increasing sensitivity by means of nonlinear registration, and predicting long-term changes in disability.

AUTHOR CONTRIBUTIONS

Statistical analysis was conducted by Dr. Daniel M. Harrison, with guidance by Dr. Brian S. Caffo.

DISCLOSURE

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