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Gadolinium-enhancing lesions lead to decreases in white matter tract fractional anisotropy in multiple sclerosis

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Abstract

Purpose—Although MRI identification of new lesions forms the basis for monitoring disease progression in multiple sclerosis patients, how lesion activity relates to longitudinal white matter changes in the brain is unknown. We hypothesized that patients with gadolinium-enhancing lesions would show greater longitudinal decline in fractional anisotropy in major tracts compared to those with stable disease.

Methods—Thirty patients with relapsing-remitting multiple sclerosis were included in this study – 13 had enhancing lesions at baseline and 17 did not. Each patient underwent at least two 3 Tesla contrast-enhanced MRI scans with a DTI sequence and a median interval of 2.1 years between scans. The forceps major and minor of the corpus callosum and the bilateral corticospinal tracts were selected as the major white matter tracts-of-interest. These tracts were reconstructed using region-of-interest placement on standard anatomical landmarks and a fiber assignment by continuous tracking algorithm using TrackVis (version 0.5.2.2) software. Mixed-effects regression models were used to determine the association between enhancing lesions and subsequent longitudinal change in fractional anisotropy.

Results—In patients with enhancing lesions, there was greater decline in fractional anisotropy compared to those with stable disease in the forceps major ($p=0.026$), right corticospinal tract ($p=0.032$), and marginally in the left corticospinal tract ($p=0.050$), but not the forceps minor ($p=0.11$).

Conclusion—Fractional anisotropy of major white matter tracts declined more rapidly in patients with enhancing lesions, suggesting greater diffuse white matter injury with active inflammatory disease. DTI may provide a means of monitoring white matter injury following relapses.

INTRODUCTION

The current clinical paradigm for monitoring disease activity in multiple sclerosis (MS) patients includes MRI evaluation of lesion load, assessed with conventional T2-weighted sequences, and active inflammation, assessed with gadolinium-enhanced T1-weighted sequences. MRI has been found to be 6 to 10 times more sensitive for assessing ongoing disease activity than clinical relapse.^{1,2} In patients with relapsing-remitting MS (RRMS), higher lesion loads on T2-weighted images and faster accumulation of lesions, particularly in the first 5 years of the disease, predict clinical disability up to 14 years after the initial episode.³ Treatment regimens may therefore be modified based on the presence of new lesions on MRI. However, how these lesions relate to underlying changes in the brain and subsequent physical and cognitive disability remains unclear.

One approach to characterizing the longitudinal effects of MS on cerebral white matter is to use diffusion tensor imaging (DTI).⁴ The fractional anisotropy (FA) is a scalar measure that quantifies the directionality of water diffusion, offering a surrogate measure of underlying white matter tract organization. Tractography is a method of tracking the primary eigenvector of the diffusion tensor to create virtual 3-dimensional representations of anatomic white matter tracts.⁵ Cross-sectional studies have shown white matter alterations within specific white matter tracts compared to healthy controls.⁶⁻⁹

The existing literature reporting on longitudinal changes in FA has been contradictory, with a few papers reporting evidence of longitudinal decline¹⁰⁻¹² while others have not.^{13,14} One possibility for the differences could be technical. An alternative hypothesis is that longitudinal FA changes may differ depending on disease activity, with focal active lesions producing greater axonal damage and Wallerian degeneration,¹⁵ or underlying inflammation producing global white matter degeneration beyond MR-visible lesions. Only one paper in a small cohort of patients with secondary progressive MS reported accelerated FA decline in the corpus callosum in patients with enhancing lesions compared to those without,¹² suggesting a link between enhancing disease and white matter degeneration.

Therefore, we aimed to expand upon the existing literature by evaluating the effects of gadolinium-enhancing MS lesions on subsequent longitudinal FA changes in 4 major white matter tracts -- the forceps major and minor of the corpus callosum and the bilateral corticospinal tracts -- in a cohort of individuals with RRMS.

Materials & Methods

Subjects and Power Analysis

Thirty patients with RRMS from an ongoing, prospective clinical cohort of patients treated at the Weill Cornell Judith Jaffe Multiple Sclerosis Center were included in this retrospective analysis. Written informed consent was obtained from all subjects, and approval for the study was obtained from the Institutional Review Board at Weill Cornell Medical Center.

Using a power analysis assuming a Type 1 error of 0.05 and 80% power, we estimated that a sample size of 12 patients in each group would be sufficient to show a 2% annual rate of

change in FA in the enhancing group. Of the thirty patients included in our analysis, 13 had enhancing lesions on the baseline scan and 17 had no enhancing lesions on the baseline or subsequent follow-up scans.

Subjects were included if they had two contrast-enhanced MRI scans with a diffusion tensor imaging (DTI) sequence at least one year apart, with a median interval of 2.1 years (SD 0.7, range 1.5-3.8 years) between scans. All subjects also underwent routine clinical management for their disease, including routine MRI scans between the DTI scans, which demonstrated that subjects with stable disease had no new or enhancing lesions throughout the follow-up period. Demographic information, use of steroids and disease-modifying therapy (i.e. interferon beta, glatiramer acetate, fingolimod, or natalizumab) at the time of or between the MRI scans, disease duration since the patient's first symptom, and clinical scores on the Expanded Disability Status Scale (EDSS) were obtained from the medical record.

MR Imaging

All patients underwent two MRI scans on the same 3 Tesla scanner (Signa HDxt 16.0; GE Healthcare, Milwaukee, Wisconsin) with an 8-channel phased array coil. The imaging protocol included: 3D T2 FLAIR (voxel size $1.2 \times 0.6 \times 0.6$ mm), axial T2-weighted images (voxel size $0.5 \times 0.5 \times 3$ mm), pre- and post-contrast 3D volumetric T1-weighted images (voxel size $1.2 \times 1.2 \times 1.2$ mm), and a 34-direction DTI sequence using TR/TE 10000/98 ms, 60 contiguous 2.5 mm-thick sections, voxel size $0.8 \times 0.8 \times 2.5$ mm, $b=1000$ s/mm².

Postprocessing and Image Analysis

DICOM images of the DTI data were loaded into Diffusion Toolkit (www.trackvis.org, version 0.6.2.2) to generate tensor data and scalar maps for tract reconstruction with TrackVis (version 0.5.2.2).¹⁶ We used a DWI mask threshold and an angular threshold of 35 degrees.

Four major white matter tracts of interest were selected a priori for this analysis: the bilateral corticospinal tracts and the forceps major and minor of the corpus callosum. We selected the corticospinal tracts since motor impairment, usually measured by walking speed, is a common manifestation of multiple sclerosis that is clinically significant and can be used as an outcome measure in clinical trials.¹⁷ Furthermore, the FA of corticospinal tracts has been found to better correlate with clinical disability scales than total lesion load.¹⁸ The corpus callosum was also selected since it is the largest white matter bundle in the brain, affected early in the disease course, and commonly involved in the disease.¹⁹⁻²¹

The tracts of interest were reconstructed using standard anatomical landmarks and a fiber assignment by continuous tracking algorithm. To construct the corticospinal tracts, we used a two region-of-interest (ROI) approach with the cerebral peduncles as the seed regions and the precentral gyri as the targets.²² To construct the forceps minor and major of the corpus callosum, we defined spherical ROIs placed in the genu and splenium of the corpus callosum, which has been shown to have higher inter-observer repeatability than other ROI approaches.^{22,23} In addition, to verify the repeatability of the methods used, all tracts were delineated twice with intraclass correlations²⁴ ranging from substantial to excellent: 0.96 for

the forceps minor, 0.85 for the forceps major, 0.74 for the right corticospinal tract, and 0.89 for the left corticospinal tract. All tracts were confirmed visually for anatomic accuracy by a board-certified neuroradiologist with subspecialty certification (G.C.C.), who was blinded as to whether or not the patient had enhancing lesions during the analysis. The FA across the tract was obtained and included in the subsequent statistical analyses. Posthoc, the three eigenvalues of the diffusion tensor were also measured in each tract: λ_1 (axial diffusivity), as well as λ_2 and λ_3 (the two components of radial diffusivity).

The number of enhancing lesions was enumerated by visual inspection using the gadolinium-enhanced sequence. The volumes of T2-hyperintense nonenhancing lesion load, in total and within the specified tracts, were calculated using FDA-approved software (Olea Sphere 2.3; Olea Medical Solutions, La Ciotat, France). 3D volumes of the white matter lesions were created by selecting a seed voxel at the center of the lesions and expanding the region of interest to include surrounding voxels of similar signal intensities. Manual editing was then performed to include or exclude voxels as necessary. Finally, the locations of the enhancing and nonenhancing lesions were documented, including whether or not they were located within one of the four tracts of interest (Figures 1 and 2).

Statistical Analysis

All statistical analyses were programmed in STATA version 13 (StataCorp, College Station, TX). Baseline differences in age, gender ratios, nonenhancing lesion burden, number of steroid courses, use of disease modifying therapy, EDSS, and disease duration between the enhancing and stable groups were assessed using Wilcoxon rank-sum and Fisher's exact tests, depending on the variable type.

Using a linear mixed-effects regression model using enhancing disease as a dichotomous variable, we tested the primary hypothesis that patients with enhancing MS lesions will show accelerated decline in FA over time in the 4 major tracts of interest, compared to those with stable disease. Age, gender, volume of T2 hyperintense nonenhancing lesion load in total and within each tract, volume of new lesions that developed in the interval between MRI scans, use of disease-modifying therapy (baseline use and change in therapy), number of steroid courses, EDSS (at baseline and change in EDSS between the two MRI scans), and disease duration were each considered as covariates.

Additional exploratory analyses included testing for whether increasing number of enhancing lesions would produce greater longitudinal FA decline, potential effects of nonenhancing lesions on longitudinal FA decline, and testing the effects of lesion location by determining whether the association between enhancing lesions and FA decline persisted after excluding patients with enhancing lesions within the tracts of interest. Finally, the longitudinal changes in the three eigenvalues of the diffusion tensor were assessed.

Results

Baseline patient characteristics are shown in Table 1. The patients with enhancing lesions were slightly younger ($p=0.049$) and were placed on more steroid courses between scans than those with stable disease ($p<0.001$). In addition, the enhancing group had a marginally

longer disease duration ($p=0.07$) compared to the stable group. There were no significant differences between groups in the proportion of women, volume of nonenhancing lesions in total and within tracts of interest, proportion on disease modifying therapy at either time point, proportion on steroids at either time point, or EDSS at baseline and change in EDSS between scans. The distribution of enhancing lesions at baseline is shown in Table 2. The number and volume of new lesions that developed in the interval between MRI scans are shown in Table 3.

The results of the regression analyses are summarized in Figure 3. The presence of at least 1 enhancing lesion was associated with accelerated decline in FA in the forceps major ($p=0.026$), with an annualized rate of change in FA of -0.00875 per year [95% CI: -0.0181 , 0.000627] or a decrease of 1.3% per year, and in the right corticospinal tract ($p=0.032$), with an annualized rate of change in FA of -0.0142 per year [95% CI: -0.0228 , -0.00559] or a decrease of 2.2% per year.

The presence of enhancing lesions was marginally associated with accelerated decline in FA in the left corticospinal tract ($p=0.05$), with an annualized rate of change in FA of -0.0134 per year [95% CI: -0.0214 , -0.00553] or a decrease of 2.2% per year. The presence of enhancing lesions was not associated with longitudinal change in FA in the forceps minor ($p=0.11$).

The presence of enhancing lesions was associated with longitudinal increases in components of radial diffusivity, specifically λ_3 in the forceps major ($p=0.004$) and left corticospinal tract ($p=0.03$) and λ_2 in the right corticospinal tract ($p=0.01$).

Adjusting for covariates, including age, steroid use, disease duration, total volume of T2 hyperintense lesion load at baseline, volume of T2 hyperintense lesions within the tracts at baseline, and number and volume of new lesions that developed in the interval between MRI scans did not change the results. Increasing number of enhancing lesions was not significantly associated with accelerated longitudinal decline in FA in any tract.

After excluding patients who had enhancing lesions within the specified tracts of interest and adjusting for volume of nonenhancing T2 hyperintense lesions within the tracts, enhancing lesions remained associated with greater decline in FA in the forceps major ($p=0.046$) and right corticospinal tract ($p=0.039$). However, the association with greater decline in FA in the left corticospinal tract became less significant ($p=0.13$).

Discussion

The major findings of our analysis were: 1) the presence of enhancing lesions was associated with accelerated longitudinal decline in FA in several large white matter tracts, primarily due to alterations in radial diffusivity, 2) this association persisted after adjusting for nonenhancing lesions within the tracts-of-interest and overall T2 lesion volume, and 3) this association persisted even after excluding enhancing lesions within the tracts of interest from the analysis. Taken together, these findings suggest that the presence of enhancing lesions is associated with accelerated demyelination of major white matter tracts, irrespective of lesion location.

The major finding that RRMS patients with enhancing lesions showed accelerated longitudinal FA decline in 3 of the 4 interrogated white matter tracts – forceps major and both corticospinal tracts – is compatible with a prior study in a small cohort of SPMS patients that found accelerated FA decline in the corpus callosum in patients with enhancing lesions.¹² The annualized rates of change in FA in the forceps major and corticospinal tracts of 1.3-2.2% for the enhancing group in our analysis were similar to previously reported rates detected in the corpus callosum and supratentorial white matter of MS patients,¹¹ although this prior report did not assess differential rates related to lesions.

Furthermore, the finding of accelerated longitudinal FA decline persisted, even in white matter tracts that were anatomically distinct from the location of the enhancing lesions. This suggests that the presence of enhancing lesions may serve as a marker for greater underlying inflammatory activity throughout the brain, thereby resulting in greater white matter injury and degeneration. Several papers have described evidence of inflammation, activated microglia, and axonal damage in normal-appearing white matter of multiple sclerosis patients.²⁵⁻²⁸ Axonal damage likely begins focally at the site of the lesion, then spreads antegrade and retrograde through anatomically distinct, but functionally interconnected tracts.²⁹ On a cellular level, widespread white matter injury may result from oligodendrocyte apoptosis in pro-inflammatory state,^{30,31} microglial nodules that promote free radical formation,^{32,33} mitochondrial injury,³⁴⁻³⁷ and T cells that release pro-inflammatory cytokines and kill oligodendrocytes.³⁸⁻⁴⁰ Taken together, the initial inflammatory lesion triggers multiple mechanisms that amplify oxidative tissue injury throughout the brain.

In our analysis, the presence of enhancing lesions was not significantly associated with accelerated longitudinal FA decline in forceps minor, the white matter tract coursing through the genu of the corpus callosum. Tian et al¹² similarly reported longitudinal FA decline in the body and splenium of the corpus callosum, but not the genu. Cross-sectional studies have also found sparing of the genu of the corpus callosum comparing the regional FA in patients with MS or clinically isolated syndrome and healthy controls.⁴¹⁻⁴³ One possible explanation is that the forceps minor and other frontal white matter tracts are better able to compensate through remyelination. Lifespan studies of white matter have suggested that all white matter tracts continue to reorganize and increase in FA to the age of 30, similar to the age group in our study.⁴⁴ Furthermore, Bartzokis et al⁴⁵ reported that the volume of the genu of the corpus callosum continues to increase through the twenties, while the volume of the splenium decreases in this period. Frontal lobe white matter volume reaches a maximum around 44 years. As a result, it could be that the MS patients are better able to compensate for white matter damage in the frontal lobes until that age, resulting in less longitudinal FA decline.

There are a few limitations to our study. First, although powered to show the estimated magnitude of longitudinal FA change, this was a relatively small cohort of patients, with a short duration of follow-up over approximately 2 years. It remains to be seen how longitudinal trajectories of FA may change over longer periods of follow-up. A two-year accelerated decline in FA associated with enhancing lesions in our study may not correlate with disability many years later. Secondly, we used the time-consuming approach of manually-delineated regions-of-interest for the tractography analysis, which would be a

limitation for a larger scale study. Other approaches to analyzing DTI data include probabilistic tractography as well as whole brain voxel-based approaches, such as tract-based spatial statistics¹³ and automated segmentation.¹¹ However, we elected to use this approach to avoid potential errors related to misregistration and decreased sensitivity for FA changes that may be seen in whole brain methods, such as voxel-based approaches and atlas-based automated segmentation. Furthermore, we calculated intraclass correlation coefficients to determine the degree of repeatability of our approach. The deterministic approach to DTI tractography also has limitations, particularly with regard to crossing fibers; the lateral fibers of the corticospinal tracts are not well-visualized. However, this approach is readily usable in the clinical setting should there become a greater role for monitoring FA changes over time in these patients. Third, we only considered four white matter tracts in this analysis due to the small sample size, but other white matter tracts that may have clinical import in multiple sclerosis patients may be interrogated in a larger subsequent study. Finally, we considered many potential confounders in our analysis, but could not control for many other variables that may underlie the heterogeneity of this disease. Further validation in larger cohorts is required.

Conclusion

We found evidence for accelerated longitudinal white matter degeneration in several major white matter tracts in relapsing-remitting multiple sclerosis patients with gadolinium-enhancing lesions. This lends support to the importance of controlling disease activity and the potential utility of DTI as a noninvasive means of monitoring white matter injury following relapses. Further research regarding trajectories of FA changes over longer time courses should be performed.

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Abbreviations

RRMS	relapsing-remitting multiple sclerosis
DTI	diffusion tensor imaging
FA	fractional anisotropy
SPMS	secondary progressive multiple sclerosis

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Figure 1.

A new T2 hyperintense, enhancing multiple sclerosis lesion (arrow) seen on axial T2-weighted (A), axial T2 FLAIR (B), and post-gadolinium T1-weighted (C) images. This lesion was considered along the tract of the forceps major of the corpus callosum (D).

Figure 2.

A new T2 hyperintense, enhancing multiple sclerosis lesion (circle) in the left precentral gyrus seen on axial T2-weighted (A), axial T2 FLAIR (B), and post-gadolinium T1-weighted (C) images. This lesion was considered along the tract of the left corticospinal tract (D).

Figure 3.

Scatterplot of all FA values relative to time in each tract, separated by group (solid circle for the group with stable disease, hollow square for the group with enhancing disease). Two data points are visible for each patient - one to represent the baseline FA at timepoint 0 and one to represent the FA value at the follow-up time point. The regression lines showing the longitudinal change in FA in each group are overlaid on the scatterplot (solid line for stable disease, dashed line for enhancing disease). Patients with enhancing multiple sclerosis lesions showed accelerated decline in FA compared to those with stable disease in the forceps major ($p=0.026$) (A), right corticospinal tract ($p=0.032$) (C), and marginally in the left corticospinal tract ($p=0.05$) (D). There was no significant difference in longitudinal change in FA in the forceps minor ($p=0.11$) (B).

Table 1**Baseline characteristics**

	ENHANCING LESIONS (n=13)	STABLE DISEASE (n=17)	P value
Age (years)	31 [9.6]	34 [6.3]	0.049 *
% female	92	76	0.36
Total T2 lesion volume (cubic cm)	10.1 [8.4]	7.1 [8.9]	0.16
Nonenhancing T2 lesion volume (cubic cm) in:			
Forceps minor	0.40 [0.64]	0.33 [0.45]	0.77
Forceps major	0.84 [0.90]	0.89 [1.47]	0.68
Right corticospinal tract	0.35 [0.53]	0.32 [0.41]	0.92
Left corticospinal tract	0.45 [0.47]	0.28 [0.41]	0.28
% on disease modifying therapy on 1st scan	69	88	0.36
% placed on disease modifying therapy between scans	31	12	0.36
% on steroids within 30 days of 1st scan	15	6	0.57
Number of steroid courses between scans	1.5 [1.3]	0.2 [0.5]	<0.001 *
Baseline EDSS	1.5 [1.3]	1.0 [1.2]	0.36
Change in EDSS between scans	0.19 [1.2]	-0.29 [0.97]	0.47
Disease duration from initial symptoms	5.9 [3.1]	4.9 [6.2]	0.07

Data presented are mean [standard deviation].

EDSS = Expanded Disability Status Scale

* significance by Wilcoxon rank-sum test

Table 2
Distribution of enhancing lesions on the baseline MRI scans

Patient	Total number of enhancing lesions	Number of enhancing lesions in:				
		Forceps minor	Forceps major	Right corticospinal tract	Left corticospinal tract	Outside of four interrogated tracts
1	3	0	0	0	1	2
2	1	0	1	0	0	0
3	2	0	0	1	0	1
4	8	0	1	0	0	7
5	3	0	0	0	0	3
6	3	0	0	0	0	3
7	1	0	1	0	0	0
8	2	0	0	0	0	2
9	3	0	0	0	1	2
10	2	0	0	0	0	2
11	2	0	0	0	0	2
12	6	0	0	0	1	5
13	2	0	0	0	0	2

Table 3
Number and volume of new lesions that developed in the interval between the MRI scans, in total and by tract

	Number of new lesions	Volume of new T2 lesions (cubic cm)
Total	4.1 [0-22, 6.3]	0.40 [0-1.58, 0.57]
Forceps minor	0.2 [0-2, 0.60]	0.0062 [0-0.07, 0.019]
Forceps major	0.2 [0-2, 0.60]	0.072 [0-0.8, 0.22]
Right corticospinal tract	0.8 [0-6, 1.74]	0.033 [0-0.25, 0.073]
Left corticospinal tract	0.2 [0-1, 0.38]	0.041 [0-0.45, 0.12]

Data presented are mean [range, standard deviation].