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Magnetization transfer imaging of acute black holes in patients on glatiramer acetate

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1. ABSTRACT

The aim of this study was to determine evolution of T1 unenhanced hypointense lesions (acute or chronic black holes (ABHs, CBHs)) by measuring their magnetization transfer ratio (MTR) changes over 12 months. 40 glatiramer acetate (GA)-naive patients with relapsing-remitting MS who presented with 1 or more contrast-enhancing lesions (CELs) at baseline underwent 1.5-T MRI at baseline and after 12 months. Lesions were classified into 4 patterns based on differences in lesion isointensity or hypointensity over 12 months. Of 115 CELs detected at baseline, 64, after 12 months, followed pattern A (isointense-isointense), 6 pattern B (isointense-hypointense), 33 pattern C (hypointense-isointense), and 12 pattern D (hypointense-hypointense). MTR significantly increased for all unenhanced T1 hypointense lesions ($p = 0.02$). Highest MTR increases were observed for patterns C (ABHs +18.2%, p less than 0.001) and D (CBHs +34.2%, $p = 0.023$), but significant improvement was also detected for pattern A (+1.4%, $p = 0.046$); no significant MTR changes were found for pattern B. GA treatment significantly recovered MTR in ABHs and CBHs, possibly indicating a greater potential for remyelination.

2. INTRODUCTION

Even though different conventional and non-conventional imaging techniques can assess neurodegenerative changes in white matter (WM), cortical and deep gray matter, and cortical spinal tracts, no single imaging technique has emerged as a fully reliable predictor of MS-related disease activity or progression (1-3). This paper focuses on the current role of T1 hypointense acute black holes (T1-ABHs) as potential markers of remyelination in patients with MS as it relates to treatment with glatiramer acetate (GA). The study aimed to establish whether the disappearance of hypointense lesions, or the ability of hypointense lesions to revert back to isointensity, may reflect remyelination in MS lesions in patients treated with GA. There has been a paucity of previous longitudinal studies of disease-modifying treatments (DMTs) that have evaluated magnetization transfer ratio (MTR) characteristics of T1 hypointense lesions at the time of enhancement and their evolution over time.

Although current knowledge and conventional wisdom rest with the concept that hypointensity and, in particular, persistent hypointensity (in the form of chronic

black holes (CBHs)), indicate axonal loss and demyelination, (4-7) it was hypothesized that demyelination and remyelination may appear as hypointense ABHs on T1-weighted images (T1-WIs). It is difficult using conventional MRI techniques to distinguish between heterogeneous areas of demyelination and remyelination in lesions. Demyelinated lesions appear as hyperintense on T2-WIs and hypointense on T1-WIs. This is also the case for partially remyelinated lesions (2). On T1-WIs, they may appear as shadow plaques, because the signal may be weaker (8). When compared with histopathologic samples, it was confirmed that remyelinated lesions may appear as slightly hypointense or mostly isointense compared with the strongly hypointense lesions most likely to represent demyelinated tissue (8).

Using magnetization transfer imaging (MTI), a non-conventional MRI technique, this study investigated whether the lesions that appear as ABHs are actually evidence of remyelination and, likewise, whether the conversion of ABHs to isointense areas on subsequent images represented a recovery from demyelination. Decreased MTR values may correspond with demyelination, whereas a return to normal MTR values on follow-up examinations (via serial MRIs) may correspond with remyelination (9). The specific objective of this study was to determine evolution of T1 hypointense lesions on unenhanced T1-WIs in patients with MS treated with GA by measuring their MTR changes over 12 months.

3. METHODS

This study was a phase 4, open-label, single-blinded, post-marketing, MRI observational study conducted over a period of 12 months at The Jacobs Neurological Institute, University at Buffalo, State University of New York, Buffalo, New York.

3.1. Patient Population

The study population included 40 patients with relapsing-remitting MS (RRMS) who satisfied the inclusion and exclusion criteria. All patients were between the ages of 18 and 65 years (mean, 40.9 years) and diagnosed with clinically definite MS based on the Polman criteria (10) with a relapsing-remitting course (11). Their disease duration ranged from 6 months to 30 years (mean, 11.3 years). All patients had to have 1 or more contrast-enhancing lesions (CELs) and a Kurtzke Expanded Disability Status Scale (EDSS) score less than or equal to 5.5 (12).

All patients were GA-naive. Patients were excluded from the study if they presented with an acute relapse 30 days prior to study entry. In addition, patients who had received steroid therapy during the 30 days prior to study entry were excluded. Patients could not participate in the study if they had had previous treatment (60 days prior to study entry) with immunosuppressant agents—mitoxantrone, cyclophosphamide, cladribine, fludarabine, cyclosporine—or total body irradiation. Concomitant immunomodulatory therapies other than GA were not allowed (e.g., azathioprine, methotrexate, intravenous immunoglobulin (IVIG), mycophenolate mofetil, natalizumab).

All patients received monotherapy with GA (20 mg/day subcutaneously) every day beginning at the baseline visit for 12 months; IV methylprednisolone (IVMP) was allowed for relapses (1g MP by IV infusion daily for 3-5 days). Patients were allowed to use additional medications, such as antidepressants, for symptom control.

Study participants were assessed at baseline and after 12 months based on clinical examinations, and via detailed conventional and non-conventional MRI protocols.

The study was approved by the local Institutional Review Board.

3.2. MRI Methods

Patients were scanned at baseline and after 12 months using the same 1.5 Tesla unit 1.5-T General Electric Signa 4x/Lx, Milwaukee, WI). The axial dual spin echo (SE) sequence was acquired with TE 30/90, TR 3000, NEX 1, ETL 14, FOV 24x18, matrix 192 x 256, 3 mm slice thickness (th), with a total of 48 slices, no gap. We also acquired axial 3D spoiled gradient recalled echo (SPGR)-T1 scans with FOV 24x18, matrix 192 x 256, 1.5 mm th, 128 slices, no gap, TE 7, TR 24, NEX 1, FLIP 30, axial FLAIR with FOV 24x24, matrix 192 x 256, 28 slices, 3 mm th, no gap, TE 128, TI 2000, TR 8002, ETL 22, NEX 1, and axial proton density (PD) (conventional SE, FOV 24x24, 192 x 256, 48 slices, 3 mm th, no gap, TE 12, TR 1400, NEX 1) and a similar axial PD with MT contrast (1200 Hz off-water resonance pulse). Axial SE T1-WI was acquired with FOV 24x18, matrix 192 x 256, 48 slices, 3 mm th, no gap, TE 9, TR 600, NEX 2. The gadolinium-enhanced (GdE) SE T1-WI sequence was acquired after injection of a single dose IV bolus of 0.1 mmol/kg gadopentetate dimeglumine 5 minutes after administration of the contrast agent.

A single investigator blinded to the patients' clinical characteristics and clinical status performed the image analysis at baseline and at the end of the study (using a RedHat GNU/Linux Workstation). CELs and T1- and T2-lesion volumes (LVs) were calculated using a highly reproducible semiautomated technique previously described elsewhere (13). Segmentation of the brain into different tissue compartments was performed by a cross-sectional method (SIENAX). (14) To measure MTR, 3D-SPGR-T1, PD, and PD with MT saturation pulse images were used (15). The analysis required several rigid body registration steps performed by a software program, FMRIB's Linear Image Registration Tool (FLIRT) (14). To calculate MTR values, the PD and PD+MT images were algebraically combined to create an MTR map. All scans were visually inspected. All scans and associated masks for each given patient were placed into the same space (defined by the baseline T1 SE scan) via a rigid-body linear coregistration procedure, using a correlation ratio cost function. The coregistered images were: baseline T1 post-contrast, baseline T1 post-contrast mask, follow-up T1 pre-contrast, follow-up T1 pre-contrast mask, follow-up T1

Table 1. Baseline and 12-month follow-up clinical characteristics

	Baseline (N=40)	**Follow-up (N=40)**
% Female	62.5	62.5
Age in years, mean (SD)	40.9 (8.1)	
Age at onset, mean (SD)	29.6 (8.1)	
Disease duration in years, mean (SD)	11.3 (8.5)	
Relapses in 1 year before study entry or during study,* mean (SD)	0.58 (0.8)	0.4 (0.8)*
RRMS disease course, n (%)	40 (100)	40 (100)
EDSS score, mean (SD)		
Number of relapses in 1 year before study entry or during study* (%)		
0	57.5	75*
1	35.0	17.5*
2	0	7.5*
3	7.5	0*
Number of days from baseline to follow-up, mean (SD)		376 (35.5)

Abbreviations: SD = standard deviation; RRMS = relapsing-remitting multiple sclerosis; EDSS = Expanded Disability Status Scale

Table 2. Baseline and follow-up CEL characteristics

	Baseline (N=40)	**Follow-up (N=40)**	***p* value**
CEL status positivity	100	17	<0.0001
Total CEL, number, sum (min/max)	115 (11-1)	21 (1-5)	< 0.0001
CEL LN, mean (SD)	2.78 (7.1)	0.32 (0.2)	< 0.0001
CEL LV (mm^3), mean (SD)	1414.1 (3196.6)	237.5 (327.6)	<0.012
T1 hypointense-LV in baseline CEL (mm^3), mean (SD	620.9 (1455.9)	60.7 (140.4)	0.011
MTR in baseline CEL	40.6 (9)	45.6 (7.2)	0.02

Abbreviations: CEL = contrast-enhancing lesion; LN = lesion number; LV = lesion volume; SD = standard deviation.

post-contrast and follow-up T1 post-contrast mask. T1 hypointensity was quantified via an atlas-based histogram-matching method. The atlas was nonlinearly warped to the image using FMRIB's Non-linear Image Registration tool (FNIRT) (16). The image was histogram-matched to the warped atlas. The voxel-wise difference in intensity between image and atlas was calculated and scaled by the observed standard deviation (SD) of intensities in the atlas to create a per-voxel hypointensity Z-score. For each baseline CEL for a given patient, a unique lesion number was assigned and the CEL was isolated in a separate mask and, from this point on, was referred to by its assigned number. For each CEL, the following measures were computed: volume of the CEL; baseline mean MTR of the CEL; follow-up mean MTR of the CEL; baseline hypointense volume within the CEL; and follow-up hypointense volume within the CEL.

To determine the proportion of initially hypointense lesions (ABHs) that became isointense, and the proportion of initially hypointense lesions (ABHs) or initially isointense lesions that became hypointense (CBHs) after 12 months in patients treated with GA, MTR changes were measured by assigning a pattern to each lesion according to its baseline and follow-up mean histogram-matched intensity: pattern A — initially isointense lesion remaining isointense; pattern B — initially isointense lesion becoming hypointense; pattern C — initially hypointense lesion becoming isointense; and pattern D — initially hypointense lesion remaining hypointense (Figure 1).

3.3. Statistical Analysis

Statistical analyses were performed using the Statistical Package for the Social Sciences (SPSS, Version 16.0). Absolute and percent changes for lesion and MTR measures were calculated, and the within-patient changes from baseline MRI measures were calculated separately for the 4 lesion patterns and assessed using Wilcoxon rank-sum tests. All p values were based on two-tailed tests and the minimum significance level was p less than 0.05.

4. RESULTS

A total of 40 patients started GA and were included in the study between October 2007 and October 2009. The baseline and follow-up clinical characteristics are provided in Table 1. At baseline, 62.5% of the patients were female, and had a mean age of 40.9 years (SD 8.1 years) and mean disease duration of 11.3 years (SD 8.5 years).

In the year prior to study entry, the mean number of relapses that study patients experienced was 0.58 at baseline and 0.4 during the 12-month follow-up period (25% of the patients presented with 1 or more relapses). EDSS scores were approximately 2.0 at baseline and upon follow-up.

Table 2 shows CEL characteristics at baseline and follow-up. The total number of CELs decreased from 115 at baseline to 21 at the 12-month follow-up for the 40 patients receiving GA treatment (p less than 0.0001). Mean CEL LV also decreased from baseline to follow-up (1414.1ml +/- 3196.6ml to 237.5ml +/- 327.6ml; p less than 0.012).

Table 3 shows CEL pattern characteristics during follow-up. Of the 115 CELs that were detected at baseline and at the 12-month follow-up, 64 of these CELs followed pattern A, 6 pattern B, 33 pattern C and 12 pattern D. During the follow-up period, the MTR significantly

Table 3. Baseline and follow-up CEL pattern[a] characteristics

	Baseline (N=40)	Follow up (N=40)	*p* value
Hypointense pattern total number, sum (%)			
Pattern A		64 (55.7)	
Pattern B		6 (5.2)	
Pattern C		33 (28.7)	
Pattern D		12 (10.4)	
Hypointense pattern total number, mean (SD)			
Pattern A		1.6 (4.4)	
Pattern B		0.15 (0.66)	
Pattern C		0.82 (1.5)	
Pattern D		0.3 (0.85)	
Hypointense pattern MTR, mean (SD)			
Pattern A	45.5 (5.9)	46.2 (6.9)	0.046
Pattern B	40.2 (5.1)	38.7 (12.7)	0.09
Pattern C	37.3 (7)	44.2 (7)	<0.001
Pattern D	32.4 (5.6)	43.6 (6.5)	0.023

[a]An evolution pattern was assigned to each CEL according to its baseline and follow-up mean histogram intensity on unenhanced T1-WI. Pattern A = initially isointense lesion remaining isointense; pattern B = initially isointense lesion becoming hypointense; pattern C = initially hypointense lesion becoming isointense, and pattern D = initially hypointense lesion remaining hypointense. Abbreviations: SD = standard deviation; MTR = magnetization transfer ratio.

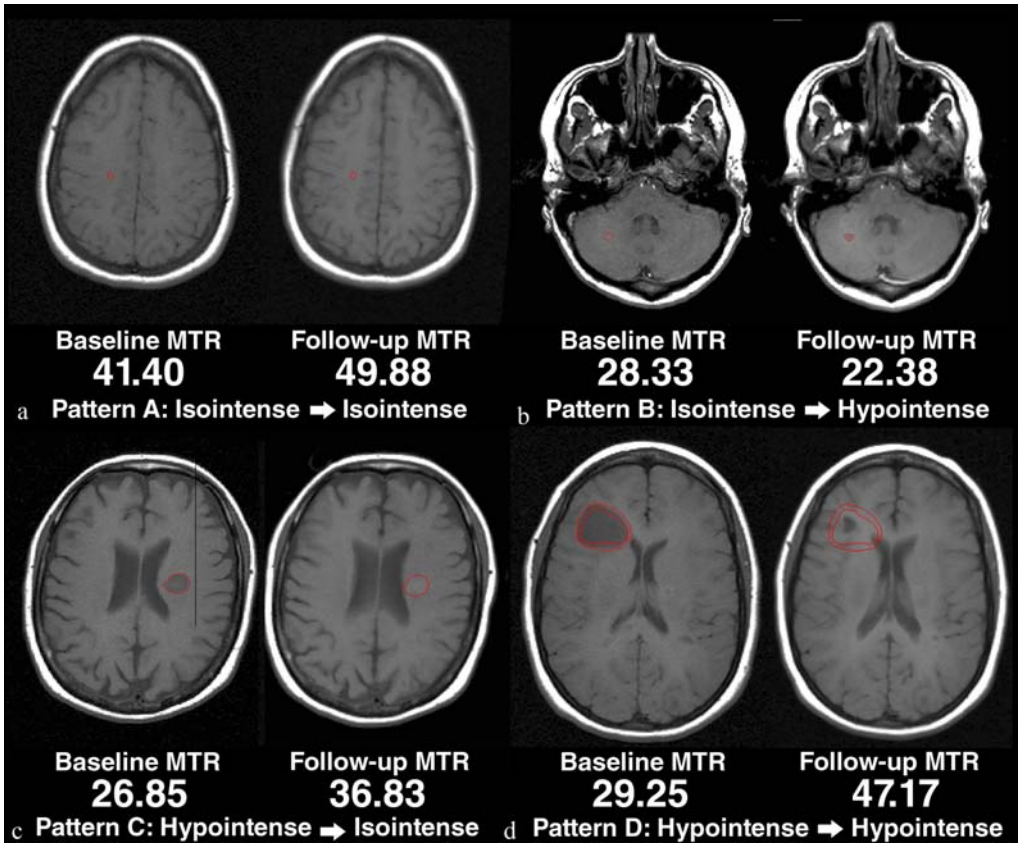

Figure 1. Magnetization transfer ratio values of baseline contrast-enhancing lesions based on histogram matching hypointense lesion evolution pattern over 12 months on unenhanced T1-WI. Pattern A = initially isointense lesion remaining isointense; pattern B = initially isointense lesion becoming hypointense; pattern C = initially hypointense lesion becoming isointense, and pattern D = initially hypointense lesion remaining hypointense

increased in all unenhanced T1 hypointense lesions (p = 0.02). The highest MTR increases were observed for pattern D (+34.2%, p = 0.023) and for pattern C (+18.2%, p less than 0.001), but significant improvement was also detected for pattern A (+1.4%, p = 0.046); no significant MTR change was found for pattern B. All patients included in the study completed the clinical and MRI follow-ups. Patients were exposed safely to GA monotherapy for a mean of 12 months. No serious adverse events (SAEs) were recorded and patients showed the usual AEs as indicated in the package insert for GA; however, AEs were not systematically recorded. No disability progression was noted at the end of the follow-up period.

5. DISCUSSION

This was a study of 40 GA-naive patients with RRMS who had EDSS scores less than or equal to ≤5.5 and 1 or more CELs at baseline and received GA therapy for 12 months. In total, 115 CELs were detected at study inception and this number decreased to 21 at the 12-month follow-up.

Changes on MRI, acquired through either conventional or non-conventional methods, reflect ongoing neurodegenerative activity and may correlate with or predict future disability in patients with MS (2, 5, 6, 17-19). In terms of conventional MRI techniques, T1-WI can detect hypointense T1 ABHs and CBHs. Because they are transient, ABHs more likely represent the resolution of edema and possibly remyelination (20, 21). Once the associated CELs resolve, approximately 40%-80% of ABHs become isointense on T1-WI; however, some remain hypointense (20, 21). Conventional techniques, while useful as surrogate markers, do not have the sensitivity to predict long-term disability. T1 BHs help to reflect underlying neurodegenerative processes, but are limited in the amount of information they can provide about recovery processes (3, 20, 22).

Dividing lesions into various patterns of evolution over time may be helpful in understanding the benefit of DMTs with regard to lesion evolution. In 1998, van Waesberghe et al evaluated patterns of lesion development in MS over time with T1-WI BHs and MTR (providing the model for this study) (9). However, to the best of our knowledge, there have been no subsequent studies that evaluated the MTR lesion pattern evolution of lesions over time in MS-treated patients. Several studies in animal, *in vitro* and *in vivo* models have provided evidence of remyelination as a potential mechanism of action of GA (23, 24). This appears to be the first longitudinal study to evaluate the evolution of ABHs from hypointense to isointense as potential evidence for remyelination (as provided by MTR data) in association with use of GA. Over the 12-month study period, 64 (55.7%) of 115 CELs followed pattern A, meaning that they were isointense at study onset and remained unchanged. In addition, a total of 33 (28.7%) lesions followed pattern C, being initially hypointense and becoming isointense (ABHs), and 12 (10.4%) followed pattern D, being hypointense at study baseline and remaining so after 12 months (CBHs). Only 5.2% of lesions followed pattern B, progressing from isointense, apparently normal tissue, to hypointense.

In the original serial MTR van Waesberghe study over 6 months, 15% of the lesions followed pattern A, 5% pattern B, 44% pattern C, and 36% pattern D (9). It seems that fewer patients in our study presented with hypointense T1 lesions at baseline that remained hypointense at follow-up on unenhanced T1-WIs, when compared with previous natural history data (9). These results may indicate that, over time, most lesions in patients treated with GA do not become CBHs. Rather, most remained isointense or changed from being hypointense ABHs (representing abnormal tissue) to isointense (representing normal tissue). This is in line with the recent study that investigated the evolution of CELs into CBHs in MS patients treated with interferon beta-1b (IFNbeta-1b) or GA (25). In that study, the majority of the patients who developed CELs did not develop CBHs over 12 months, although the authors reported that conversion to CBHs was somewhat lower with IFNbeta-1b compared with GA (9.8% *vs* 15.2%, p = 0.02) (25).

In the present study, 15.6% of lesions were hypointense (CBHs) after 12 months in GA-treated patients. Lack of a control group and serial MRI data do not permit further interpretation of our data. However, data from the European/Canadian Glatiramer Acetate Study, a multi-center, double-blind, randomized, placebo-controlled trial of the effects of GA on MRI-measured disease activity and burden in patients with RRMS showed similar results. There were a total of 157 new lesions present in the GA-treated arm of this trial at Month 1 and at Month 8, 21 BHs remained. The percentage of new lesions that evolved into BHs was lower in patients taking GA than in placebo-treated patients and was statistically significant: (18.9% *vs* 26.3%; p = 0.04, at Month 7 and 15.6% *vs* 31.4%; p = 0.002, at Month 8) (26).

In light of weaknesses in the ability of conventional MRI to provide reliable surrogate markers of disease progression, disability, and efficacy of various therapeutic interventions, a number of non-conventional MRI techniques have been used in the research setting to attempt to better correlate the apparent mismatch between conventional MRI monitoring and clinical status. MTI can be used to characterize and chart the evolution of MS lesions and normal-appearing brain tissue and may correlate with the extent of demyelination and conversely remyelination (15, 27). Changes to normal-appearing WM (NAWM) may become evident on MTI prior to the appearance of lesions on conventional MRI scans (28). In unaffected WM, MTR is high because protons are bound within normal myelin. In MS lesions, there is tissue damage that causes a decrease in bound protons (29). Tissue damage, such as demyelination, is shown as a reduced proton exchange, or a decrease in the MTR, whereas an elevated proton exchange, or increased MTR, is potential evidence of remyelination and plasticity in the CNS (29). This has been demonstrated in animal models, on human postmortem analysis, and during ontogenesis (27, 30-34).

Following MTR changes in MS lesions over time has the potential to indicate repair and remyelination of axons, and thus to correlate better with the evolution of pathologic changes and clinical disability compared with conventional MRI. Therefore, lesional MTR recovery may become an important outcome measure for clinical trials attempting to determine the efficacy of various therapies, not on inflammation, but rather on neuroprotection and repair as evidenced by remyelination (29). In the present study, the lesion pattern analysis showed that GA-treated patients significantly recovered MTR in ABH and CBH T1 hypointense lesions, as well as in the isointense areas. This may indicate a greater potential for remyelination in GA-treated patients and a possible neuroprotective effect of

GA. MTR significantly increased for the majority of lesions studied. The highest MTR increases were observed for pattern C and pattern D, indicating potentially that the remyelination may occur in ABHs and CBHs in GA-treated patients. However, a slight improvement was seen for pattern A, lesions initially isointense that remained isointense. This further indicates that GA may aid in the recovery of lesional tissue, as measured by MTR. In a case-controlled study utilizing diffusion-weighted imaging (DWI) and MTI, GA was also shown to recover microscopic tissue damage in 19 patients with RRMS. GA significantly improved microscopic tissue damage in the brain, as measured by DWI over the 1- and 2-year follow-up (p less than 0.001) at Year 2 (35). There was no significant deterioration of MTI measures in this study, (35) again supporting the hypothesis that GA may help inhibit the progression of GdE lesions to CBHs (26).

Although this trial had limitations, such as a small study population, absence of a control group, no randomization or use of serial MRI data, the findings provide evidence to merit additional longitudinal trials, utilizing MTR to track the evolution of isointense and hypointense areas of MRI scans in patients with MS, especially as this relates to treatment options. In 2002, a consensus group recommended the use of MTR to monitor disease progression, stating that this technique can quantify the severity of MS-related tissue damage (36). In 2003, Horsfield *et al* published specific guidelines for using quantitative MTR imaging for monitoring treatment of MS (37). Most recently, quantitative MTI (QMTI) has been shown to be able to track the degree and timing of the partial recovery of CELs. In a study by Levesque *et al*, various key QMTI parameters indicated changes in WM consistent with demyelination and remyelination. Although more complex to perform than MTR, QMTI is also capable of providing a more detailed characterization of the MT effect and can help to shed light on MTR changes, thus elucidating the progression of acute GdE lesions (38).

To date, there have been only a few cross-sectional and longitudinal studies utilizing MTR histogram abnormalities to assess disease progression and response to DMTs. The results have been contradictory (39-43). For example, Inglese *et al* evaluated the effect of IFNbeta-1b in secondary progressive MS in a population of 82 patients. Compared with placebo, there was no significant treatment effect and both arms showed a decrease of average brain MTR values from baseline over 36 months (39). These findings support another earlier, smaller study conducted by Richert *et al* in patients with RRMS, which also did not demonstrate any improvement in MTR following treatment with IFNbeta-1b (40). However, other small studies of both IFNbeta-1b (in combination with IVMP) (41) and IFNbeta-1a (42) revealed a recovery of MTR values in CELs. In the study that combined IFNbeta-1b use with IVMP, the improvement may have been due to the use of steroids. (41) Another study of 70 patients by Filippi *et al* that evaluated IVIG treatment did not show any statistically significant effect of IVIG on MTR results in those patients with secondary progressive MS, although there was a different percentage change of the normal appearing brain tissue MTR histogram peak heights over time between treated and control patients (43).

Although, as previously discussed, MTR is able to identify areas of demyelination prior to their appearance on T2 WIs, (28) the recovery of the mean lesion MTR indicative of remyelination is temporally heterogeneous (44). There may be concurrent myelin destruction and repair taking place (44, 45). These simultaneous and competing areas of demyelination and remyelination when calculated and expressed as a mean change in the area being evaluated, may cancel each other out, resulting in a measurement falsely suggesting a lack of disease (46). A newer method of voxel-wise MTI can separately quantify significant decreases and increases in the MTR of individual voxels of MS lesions as indicators of focal demyelination and remyelination, respectively (44).

In this study, the authors chose to use pattern analysis versus a voxel-wise technique, given that the former may be more effective in detecting ABHs and CBHs and indicating lesion intensities. Furthermore, pattern analysis is relatively new and the voxel-wise technique was unavailable when the study was conducted. However, newer, better means of detecting and analyzing the evolution of MS lesions, such as pattern analysis, continue to be developed and will undoubtedly be used in the future.

In conclusion, although there have been only a few studies employing MTR, this technique may be a useful tool in the context of larger, longitudinal trials to monitor lesion evolution and disease progression. The MTR ABH and CBH T1 hypointense lesion pattern approach utilized in this study is an attractive way for future clinical trials to assess the potential for demyelination and/or remyelination, and may suggest neuroprotective effects beyond the disease-modifying effects of GA.

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Key Words: Acute Black Hole; Chronic Black Hole; Glatiramer Acetate; Magnetization Transfer Imaging, MRI; Multiple Sclerosis; Remyelination; T1 Hypointense Lesion

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