

MTR recovery in brain lesions in the BECOME study of glatiramer acetate vs interferon β -1b



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ABSTRACT

Objective: To compare magnetization transfer changes in new brain MRI lesions identified during monthly imaging in patients with multiple sclerosis (MS) randomized to treatment with 250 μ g subcutaneous interferon- β -1b (IFN- β -1b) every other day or daily 20 mg glatiramer acetate (GA) in a post hoc study using data from the Betaseron Versus Copaxone for Relapsing Remitting or CIS Forms of MS Using Triple Dose Gad 3 T MRI (BECOME) trial.

Methods: T1-weighted images acquired with and without fat saturation pulses in the BECOME study were evaluated and found to exhibit magnetization transfer ratio (MTR) effects, and were used to compute MTR images (FSMTR). Forty-three participants who had the required imaging and new lesions, from the 75 originally randomized into the BECOME study, were included in this post hoc analysis and evaluated longitudinally during treatment to determine FSMTR_{Drop}, an experimental measure of the completeness of FSMTR recovery in new lesions. Two sets of new brain MRI lesions were defined, one based on the appearance of gadolinium contrast enhancement (Gd lesions) and the other based on FSMTR decreases (Δ FSMTR lesions).

Results: A total of 887 Gd lesions were identified in 43 participants (19 GA, 24 IFN- β -1b) and 321 Δ FSMTR lesions in 32 participants (16 GA, 16 IFN- β -1b). Participants randomized to GA exhibited greater average postlesion FSMTR recovery than did those randomized to IFN- β -1b in both Gd ($p < 0.0001$) and Δ FSMTR ($p < 0.0001$) lesions.

Conclusions: New brain lesions that developed during treatment with GA exhibited evidence of greater FSMTR recovery than during treatment with IFN- β -1b.

Classification of evidence: This study provides Class III evidence that MTR recovery in patients with MS with new MRI brain lesions is greater with GA than with IFN- β -1b. **Neurology**[®] 2016;87:905-911

GLOSSARY

BDNF = brain-derived neurotrophic factor; **BECOME** = Betaseron Versus Copaxone for Relapsing Remitting or CIS Forms of MS Using Triple Dose Gad 3 T MRI; **CIS** = clinically isolated syndrome; **FS** = fat saturation; **FSMTR** = fat saturation producing a magnetization transfer effect, used to create a fat saturation ratio image; **GA** = glatiramer acetate; **Gd** = gadolinium; **IFN- β -1b** = interferon- β -1b; **LFB** = Luxol fast blue; **MS** = multiple sclerosis; **MTR** = magnetization transfer ratio; **NAWM** = normal-appearing white matter; **PD** = proton density.

Betaseron Versus Copaxone for Relapsing Remitting or CIS Forms of MS Using Triple Dose Gad 3 T MRI (BECOME) (ClinicalTrials.gov NCT00176592)¹ was a randomized head-to-head study of interferon- β -1b (IFN- β -1b) and glatiramer acetate (GA) for the treatment of relapsing-remitting multiple sclerosis (MS) or clinically isolated syndromes (CIS) suggestive of MS. In the study, monthly 3T MRI images with and without a fat saturation (FS) pulse were acquired. It has been shown previously that FS produces a magnetization transfer effect² and this pair of scans can be used to create an FS ratio image (FSMTR), analogous to more standard magnetization transfer ratio (MTR). In MS, changes in CNS MTR have been shown to be more specific to changes in myelin than conventional imaging³; quantitative studies of fresh tissue samples show that MTR in normal-appearing white matter (NAWM) and chronic MS lesions correlates well ($R = -0.84$) with Luxol fast blue (LFB), a standard histologic stain for myelin.⁴⁻⁶

Supplemental data
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Note that the correlation between MTR and LFB is capped by the noise inherent in each measurement; in this study this was 0.81 for MTR.

We have proposed a method to quantify demyelination and remyelination using longitudinal analysis of MTR images.^{7,8} We applied this procedure to measure the change in FSMTR between stable postlesion and stable prelesion values (FSMTR_{Drop}) in new brain lesions in participants in the BECOME study. This metric measures the net change of FSMTR in acute lesions, and is expected to be negative, indicating partial recovery. Previous studies have used related techniques to measure MTR recovery in gadolinium (Gd) lesions; we also examined Δ FSMTR lesions,⁷ which are identified based on changes in FSMTR. While Gd lesions are not always precisely co-located with MTR changes, Δ MTR lesions are, and therefore may be better at identifying areas of demyelination.⁷

METHODS Participants and imaging. Detailed inclusion criteria, demographics, and imaging information for the BECOME study have been published previously.¹ Seventy-five participants with MS or a CIS suggestive of MS who provided informed consent were randomized to treatment with either GA or IFN- β -1b. Participants underwent monthly MRI on a single 3T scanner (Allegra; Siemens Medical Solutions, Malvern, PA), including proton density (PD)-weighted, T1-weighted precontrast, T2-weighted, and T1-weighted postcontrast images. Postcontrast images were obtained after administration of triple-dose Gd and delayed imaging, a protocol that had previously shown improved Gd lesion detection rates.⁹ Imaging was mandatory every month for the first year and at the end of the second year and some participants also had optional monthly scans during the second year. Most timepoints also included additional T1-weighted images acquired with identical parameters, except with the addition of a FS preparation pulse, which could be used to construct an MTR-like image. Only T1-weighted and FS-T1-weighted images from before contrast administration were used and only participants who had such imaging at a minimum of 3 timepoints were included in the analysis.

Fat saturation ratio validation. To confirm that FSMTR images computed from scans with and without FS pulses are comparable to conventional MTR images, 2 sets of FSMTR and MTR images were acquired from 5 volunteers in a scan and re-scan experiment. Voxel-wise correlation between MTR and FSMTR was $R = 0.76$. This compares with $R = 0.81$ for MTR with MTR and $R = 0.73$ for FSMTR. This experiment showed a linear relationship and high correlation between the BECOME protocol and conventional MTR, although the FSMTR images demonstrated less MT contrast, translating to lower contrast to noise ratio. Details are included in appendix e-1 and figures e-1 and e-2 at Neurology.org.

Processing. Images from each analyzable timepoint were coregistered into a participant-specific space using a linear 9-parameter transformation calculated using minctracc, part of the MINC toolkit.¹⁰ At each timepoint, using a Bayesian classifier¹¹ and the PD-weighted, T1-weighted, and T2-weighted images, probability maps were constructed for NAWM, normal-appearing gray matter, CSF, T2 lesions, and partial volume tissue. The T2 lesion probability maps were converted into lesion masks by thresholding. The masks from the first timepoint for each participant were reviewed and corrected by trained experts. T2 lesion masks for subsequent timepoints were then refined automatically based on the corrected first-timepoint masks.¹¹ Gd lesions were segmented manually by an expert neuroradiologist.¹ FSMTR images were constructed as the percent difference between the precontrast T1-weighted and T1-weighted + FS images.

Δ FSMTR lesions were segmented following the published procedure.⁷ Since the Δ FSMTR lesion segmentation algorithm depends on the variance of the NAWM, the lower contrast to noise ratio of the FS-derived FSMTR images was automatically accounted for.

For each timepoint, the lesion mask was propagated to all timepoints, and FSMTR values within that mask were measured, producing an average FSMTR timecourse for all the new lesions at each timepoint, for each participant. In previous work we observed that FSMTR changes rapidly during lesion formation, but that average FSMTR shows long-term stability outside a 3- to 6-month window, centered on the time a new lesion is observed.^{7,8} FSMTR measurements outside this acute period are also less likely to be confounded by the effects of acute inflammation.⁷ Therefore, for analysis, samples obtained less than 3 months before or after lesion appearance were excluded. Although not included in the analysis, the sample from the timepoint when the lesion first became evident is included in the figures to better illustrate the pattern of acute FSMTR decrease and recovery.

Analysis. Gd and Δ FSz lesions were analyzed separately, using identical procedures. FSMTR timecourses were produced, and then modeled using a random effects model,¹² with each participant having a random intercept.⁸ The R formula was as follows:

$$\text{MTR} \sim \text{treatment} + \text{postLesion} + \text{treatment:postLesion} + (1|\text{subject})$$

where postLesion is a dummy variable indicating whether the sample is from before (false) or after (true) the lesion formed, treatment was one of GA or IFN- β -1b, and postLesion:treatment is the interaction between treatment and postlesion recovery. The random effect participant accounts for correlations between measurements made in the same participant. The coefficient estimated for postLesion measures the prelesion to postlesion FSMTR difference (FSMTR_{Drop}), which is expected to be negative, indicating incomplete recovery. The postLesion:treatment interaction measures the differential treatment effect on FSMTR_{Drop}.

The explanatory power of the models was evaluated by testing the log-likelihoods achieved by the fully specified model and a null model with no fixed effects, using a χ^2 test. If this gateway test was significant, individual effects were tested using f tests, with the denominator degrees of freedom provided by a Satterthwaite approximation using the MixMod R package.¹³ R^2 was calculated according to the procedure suggested by Nakagawa and Schielzeth.¹⁴ The marginal R^2 is the variance explained by the fixed effects alone, while the conditional R^2 includes the contribution of the random effects. Shaded regions on figures show 95% confidence intervals.

Processing was done with custom software written in Python (Python Software Foundation; python.org) using the MINC

tools (MINC tools; McConnell Brain Imaging Centre, Montreal, Canada) and the Scientific Python package (Scipy; www.scipy.org). Statistical analysis was performed using R¹⁵ via the Python-R bridge RPy2 (RPy2; rpy.sourceforge.net).

All investigators involved with the study were aware of which participants were assigned to each group, but were blind to which group received which treatment. Unblinding occurred only after the statistical analysis was complete.

Standard protocol approvals, registrations, and patient consents. This work was approved by the Research Ethics Board of the Montreal Neurologic Institute and Hospital. The BECOME study and protocol was approved by the University of Medicine and Dentistry of New Jersey (now Rutgers University). Written informed consent was obtained from all patients participating in this study.

RESULTS Baseline characteristics for each subgroup are shown in table 1. Forty-four participants (19 GA, 25 IFN- β -1b) had all the required imaging, as well as Gd lesions; 32 (16 GA, 16 IFN- β -1b) had Δ FSMTR lesions; 16 had Gd lesions but not Δ FSMTR lesions (5 GA, 11 IFN- β -1b); 4 had Δ FSMTR lesions but not Gd lesions (2 GA, 2 IFN- β -1b). A total of 887 Gd and 321 Δ FSMTR lesions were identified with median (interquartile range) volumes of Gd: 62 (160) and Δ FSMTR: 17 (72) mm³. FSMTR timecourses were obtained in each lesion type (figures 1 and 2). The model describing FSMTR timecourses in Gd lesions fit significantly better than the null model ($p < 0.0001$) with a marginal $R^2 = 0.24$ and conditional $R^2 = 0.62$ (table 2). Mean prelesion FSMTR was 25.4 in IFN- β -1b treated participants (intercept) and 0.603 units higher in the GA-treated group (treatment, $p = 0.23$). Both groups had incomplete FSMTR recovery (postLesion, $p < 0.0001$). New Gd lesions in the GA group recovered significantly better (treatment:postLesion, 0.110 FSMTR units, $p < 0.0001$). The model describing FSMTR timecourses in Δ FSMTR lesions

also fit significantly ($p < 0.0001$), with a marginal $R^2 = 0.25$ and conditional $R^2 = 0.53$ (table 3). Again, no significant difference was observed in prelesion FSMTR values (IFN- β -1b 27.2, GA 0.829 units lower, $p = 0.66$), both groups had incomplete recovery ($p < 0.0001$), and the GA group showed better recovery (4.29 FSMTR units, $p < 0.0001$). Although not part of the planned analysis, lesional FSMTR was not significantly different between treatment groups at the time of lesion appearance (Gd: $p = 0.43$; Δ FSMTR: $p = 0.61$).

To examine the possibility of selection bias in the subgroups with Gd and Δ FSMTR lesions, the complete analysis was repeated in the subset of participants who had both lesion types. These results are shown in tables e-1 and e-2 and support the same conclusions as the main analyses, performed in all available participants.

DISCUSSION Both groups exhibited FSMTR timecourses typical of lesional changes in FSMTR that we, and others, have reported previously.^{7,8,16} This consisted of fairly stable FSMTR before the lesion formed, a drop in FSMTR when the lesion became evident, partial recovery over the next few months, and subsequent relatively stable values. This pattern was generally similar in both Gd and Δ FSMTR lesions, and is consistent with acute demyelination and edema, resolution of edema, and partial remyelination.

In contrast to previous findings, Gd lesions were more prevalent than Δ FSMTR. This is likely due to the triple-dose contrast and delayed 3T imaging protocol used in the BECOME study,¹ which provided unusually high sensitivity to Gd enhancement, as well as the high frequency of scanning. However, while the changes in FSMTR were modest and the

Table 1 Baseline characteristics of analysis subgroups

	Gd lesions		Δ MTR lesions		Both lesions		All participants	
	GA	IFN- β -1b	GA	IFN- β -1b	GA	IFN- β -1b	GA	IFN- β -1b
Participants, n	19	25	16	16	14	14	39	36
Gd lesions at screening, n (%)	14 (74)	17 (68)	14 (88)	10 (63)	12 (86)	8 (57)	30 (77)	21 (58)
CIS at screening, n (%)	2 (11)	5 (20)	2 (13)	3 (19)	1 (7)	3 (21)	7 (18)	7 (19)
RRMS at screening, n (%)	17 (89)	20 (80)	14 (88)	13 (81)	13 (93)	11 (79)	32 (82)	29 (81)
Male, n (%)	5 (26)	8 (32)	3 (19)	5 (31)	3 (21)	4 (29)	11 (28)	12 (33)
Female, n (%)	14 (74)	17 (68)	13 (81)	11 (69)	11 (79)	10 (71)	28 (72)	24 (67)
Mean age at screening, y	36.5	34.1	37.2	36.7	37.8	36.6	36.1	35.6

Abbreviations: CIS = clinically isolated syndrome; GA = glatiramer acetate; Gd = gadolinium; IFN- β -1b = interferon- β -1b; MTR = magnetization transfer ratio; RRMS = relapsing-remitting multiple sclerosis.

Subgroups are participants with Gd lesions, Δ MTR lesions, or both types of lesion. Characteristics of the full Betaseron Versus Copaxone for Relapsing Remitting or CIS Forms of MS Using Triple Dose Gad 3 T MRI (BECOME) study are shown in the final column, for comparison.

difference between treatment groups was small in Gd lesions (0.110 units), Δ FSMTR lesions showed both greater changes in FSMTR and a much greater difference between groups (4.29 units). This may be due to previously described variable colocalization between Gd lesions and FSMTR changes.⁷ Gd enhancement is a dynamic phenomenon¹⁷ and the spatial extent of a Gd lesion at any point in time may not represent well the full spatial extent of the demyelinating lesion. However, the observation that Δ FSMTR lesions were also smaller on average suggests that the additional enhancing tissue detected with the high-sensitivity Gd protocol (relative to what would have been detected with a standard, single-dose, 1.5T protocol) may also have exhibited less breakdown of the blood–brain barrier,¹⁸ and less demyelination and remyelination. Overall, despite detecting fewer, smaller, lesions, the Δ FSMTR approach achieved similar power to the analysis in Gd lesions due to detection of much larger changes in FSMTR.

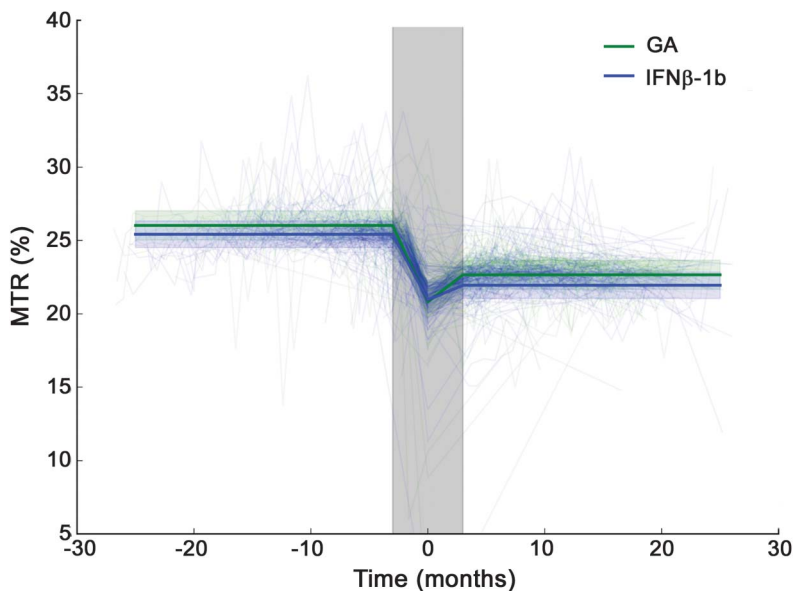
Less prelesion to postlesion FSMTR drop was noted in the GA group in both Gd and Δ FSMTR lesions. This suggests that participants treated with GA, compared to those on IFN- β -1b, experienced less unrepaired lesional damage. Incomplete recovery of FSMTR signal can be the result of a number of processes: (1) the myelin sheaths produced by

remyelination are generally thinner and more loosely packed than de novo sheaths,^{19,20} (2) some axons may survive but remain chronically demyelinated,²¹ and (3) axons that are transected degenerate and thus are not available for remyelination. Quantitative histopathology studies have found that between 30% and 60% of axons may be lost in postacute MS lesions,^{4,5,22} suggesting that axonal loss may be the dominant process responsible for incomplete postlesion recovery.

Although both IFN- β -1b and GA reduce inflammation, they accomplish this via different mechanisms. IFN- β -1b impairs the ability of peripheral immune cells to cross the blood–brain barrier, reducing peripherally mediated CNS inflammation and demyelination,²³ interferes with antigen presentation,²⁴ and reduces inflammatory B and T cells.^{25,26} IFN- β -1b may influence remyelination by stimulating release of nerve growth factors by astrocytes²⁷ or through a direct neuroprotective effect.²⁸ GA appears to disproportionately promote the production of GA-specific T-helper 2 cells, which produce anti-inflammatory cytokines²³ as well as neurotrophic factors, most prominently brain-derived neurotrophic factor (BDNF).²⁹ GA-specific T-helper cells are cross-reactive to myelin antigens in the CNS, and increase production of both cytokines and BDNF.^{23,30} Our observations suggest that GA may have been more effective at enhancing remyelination or neuroprotection in this study. Enhanced neuroprotection with GA has been observed in vitro, and through imaging evidence in a clinical trial of CIS.^{31,32}

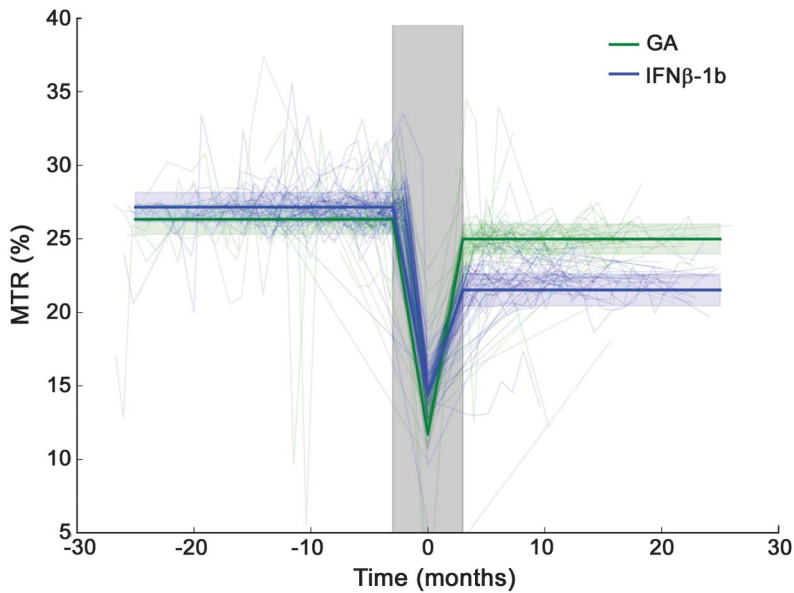
In a previous study³³ we found that IFN- β -1b-treated participants showed a smaller proportion of new brain Gd lesions that remained T1w hypointense (chronic black holes) at 12 months ($p = 0.02$). Quantitative T1 and FSMTR are correlated in MS lesions, but show different information, with FSMTR being a better measure of myelin content.^{5,6} T1 may underestimate the amount of myelin in chronic lesions due to increased extracellular water.³⁴ While our present analysis quantifies the amount of FSMTR signal loss using a continuous scale, the previous work counts the number of lesions that recover to a particular threshold, as determined by visual appearance on T1-weighted imaging. The binary (yes/no) rating of lesions on T1-weighted images may correlate poorly with both quantitative T1 relaxation time and FSMTR³⁴; T1-weighted images are a combination of different contrast mechanisms and can exhibit confounding effects, particularly from concomitant changes in T2. The difference in FSMTR between treatments in Gd lesions was modest in both this and our previous study and these methodologic differences could explain the apparent discrepancy. The

Figure 1 Magnetization transfer ratio (MTR) in new gadolinium lesions



Light lines indicate timecourses in individual lesions while heavy lines show the model predictions. The shaded gray area indicates the acute period, 3 months before and after lesion formation; this period was not included in the model, but is shown in the figure to illustrate the full MTR timecourse. Prior to lesion formation, MTR was not significantly different in glatiramer acetate (GA) (green)- and interferon- β -1b (IFN- β -1b) (blue)-treated participants. MTR dropped to a minimum around the timepoint the lesion was identified (time 0), then recovered partially. Recovery was slightly better in GA-treated participants. Random intercepts calculated by the model have been subtracted from the individual timecourses in this figure.

Figure 2 Magnetization transfer ratio (MTR) in Δ MTR lesions



As in figure 1, light lines indicate timecourses in individual lesions while heavy lines show the model predictions. The shaded gray area indicates the acute period, 3 months before and after lesion formation; this period was not included in the model, but is shown in the figure to illustrate the full MTR timecourse. The results in MTR lesions were consistent with gadolinium lesions: no significant difference in MTR between glatiramer acetate (GA)- and interferon- β -1b (IFN- β -1b)-treated patients before lesions formed but significantly better subsequent recovery in GA-treated patients. Random intercepts calculated by the model have been subtracted from the individual timecourses in this figure.

previous study did not examine Δ FSMTR lesions, where we detected a much greater effect.

Images with a conventional MT pulse were not available, so FSMTR maps computed from images with a FS pulse were validated and used. Although

these images were highly correlated with FSMTR, they exhibited lower contrast to noise, limiting statistical power. A further limitation is that the relationship between improved FSMTR recovery and clinical measures of disability has not yet been established.

It is important to note that FSMTR recovery quantifies the residual pathologic changes in lesions that developed on therapy. Interventions that act purely by preventing inflammatory demyelination from occurring at all will not necessarily fare well by this metric, although they are, in the broad sense, even more neuroprotective. GA may have advantages over IFN- β -1b in promoting lesion repair; however, a more complete evaluation of relative benefit requires that the ability to prevent lesions be factored in, for example, by comparing the rate of new lesion formation. This was done for the present study¹ and did not identify significant differences between treatment groups. Two large clinical trials contemporary with BECOME also compared IFN and GA. In Rebif vs Glatiramer Acetate in Relapsing MS Disease (REGARD),³⁵ no significant differences were observed in relapses, new T2 lesions, or new T1 lesions; the IFN-treated group had significantly fewer Gd-enhancing lesions. BEYOND³⁶ saw no differences in relapses, Expanded Disability Status Scale progression, or number of T1 or Gd lesions; the IFN group had significantly less T2 lesion volume accrual. A formal meta-analysis from the Cochrane Collaboration, which included data from these 2 studies and BECOME, found no evidence for differences between IFN- β -1b and GA in relapses over 24 months, or number of new

Table 2 Parameter estimates of the statistical model for FSMTR in Gd lesions

Effect	Estimate (FSMTR units)	Standard error	Degrees of freedom	f	p
Intercept	25.4	0.475	—	—	—
Treatment (GA)	0.603	0.705	1/44	1.46	0.234
Postlesion	-3.48 ^a	0.0693 ^a	1/6,734 ^a	1,121	<0.0001 ^a
Postlesion:treatment (GA)	0.110 ^a	0.116 ^a	1/6,734 ^a	40.6	<0.0001 ^a
Random effects	$\chi^2 = 2,006$; $p < 0.00001$ ^a				
Participant	Variance = 4.79				
Residual	Variance = 4.63				

Abbreviations: FSMTR = fat saturation producing a magnetization transfer effect, used to create a fat saturation ratio image; GA = glatiramer acetate; Gd = gadolinium.

The model fit significantly better than the null model ($\chi^2 = 3,020$; $df = 3$; $p > 0.0001$) with marginal $R^2 = 0.236$ and conditional $R^2 = 0.625$. Intercept is the expected FSMTR value of tissue in interferon (IFN)-treated participants before a lesion forms; treatment is the effect on prelesion FSMTR of treatment with GA; postlesion is the estimated change between prelesion and postlesion FSMTR values in the IFN group; and the postlesion: treatment interaction is the estimated effect of treatment with GA instead of IFN on the prelesion and postlesion difference. Individual effects estimate the magnitude and significance of that factor in isolation. To form an estimate of the FSMTR value for a particular scenario, the appropriate effects should be summed: for example, the predicted postlesion FSMTR for a participant treated with GA is intercept + treatment + postlesion + postlesion:treatment. The random effect participant is an estimate of the interparticipant variability.

^aSignificant.

Table 3 Parameter estimates of the statistical model for FSMTR in Δ FSMTR lesions

Effect	Estimate (FSMTR units)	Standard error	Degrees of freedom	f	p
Intercept	27.2	0.666	—	—	—
Treatment (GA)	-0.829	0.955	1/34	0.19	0.663
Postlesion	-5.64 ^a	0.317 ^a	1/1,388 ^a	122 ^a	<0.0001 ^a
Postlesion:treatment (GA)	4.29 ^a	0.389 ^a	1/1,388 ^a	19.9 ^a	<0.0001 ^a
Random effects	$\chi^2 = 1.97$; $p < 0.00001^a$				
Participant	Variance = 4.47				
Residual	Variance = 7.74				

Abbreviations: FSMTR = fat saturation producing a magnetization transfer effect, used to create a fat saturation ratio image; GA = glatiramer acetate.

The model fit significantly better than the null model ($\chi^2 = 315$; $df = 3$; $p > 0.0001$) with marginal $R^2 = 0.255$ and conditional $R^2 = 0.527$.

^aSignificant.

T1, T2, or Gd lesions over 24 or 36 months. Significantly lower relapse rates were reported in GA-treated participants with 36 months follow-up; T2 lesion volume increased less in the IFN group over 24 months, but not 36; and T1 lesion volume increased less with IFN over 24 months.³⁷

We analyzed FSMTR recovery in newly formed MS brain lesions that developed during treatment with GA or IFN- β -1b in the BECOME study, and demonstrated significantly less residual FSMTR loss with GA than with IFN- β -1b. The greater stable FSMTR value after acute lesion formation and recovery is consistent with greater myelin density in the chronic lesions that persist after focal inflammatory demyelination. Further study is required to determine the relationship between improved FSMTR recovery and clinical disability.

Both overall changes in FSMTR and differences in FSMTR recovery between treatments were modest in Gd lesions and much more pronounced in Δ FSMTR lesions. Although many more Gd lesions were detected by the high-sensitivity contrast protocol used in BECOME, FSMTR analysis in Δ FSMTR lesions achieved similar power to detect treatment effects. This observation is consistent with previous findings that the Δ FSMTR lesion methodology better identifies tissue experiencing acute FSMTR changes.

AUTHOR CONTRIBUTIONS

Robert Brown performed the image processing, MTR lesion segmentation, data and statistical analyses, and prepared the manuscript. Sridar Narayanan conceptualized the validation study, performed data analysis and interpretation, and revised the manuscript for intellectual content. Nikola Stikov performed data analysis for the validation study and critical review of the manuscript. Stuart Cook was involved in the conception and funding of the study and in critical review of this manuscript. Diego Cadavid was co-principal investigator of the BECOME study and participated in conception of the study and critically reviewed the manuscript. Leo Wolansky was one of two principal investigators for the original BECOME study, designing the MRI protocol and analyzing MRI data. He provided input in design and critical manuscript review for the

current study. Douglas Arnold participated in conception and design of study, critical review of manuscript, supervision, and funding of study.

STUDY FUNDING

Funding for the described work was provided by the Rutgers-New Jersey Medical School. R.A.B. received personal funding from the Multiple Sclerosis Society of Canada. The BECOME study was supported by Bayer Schering Pharma, distributors of IFN, but was investigator-initiated and remains the intellectual property of Rutgers-New Jersey Medical School, Newark, NJ.

DISCLOSURE

R. Brown has received personal compensation from NeuroRx Research for consulting services. S. Narayanan has received personal compensation from NeuroRx Research for consulting services. N. Stikov reports no disclosures relevant to the manuscript. S. Cook participated in the original BECOME study. D. Cadavid is presently a full time employee of Biogen, to which the work on the BECOME study is not related, and participated in the original BECOME study. L. Wolansky participated in the original BECOME study and received salary support from Bayer. D. Arnold is the president and CEO of NeuroRx Research. Go to Neurology.org for full disclosures.

Received December 12, 2015. Accepted in final form May 17, 2016.

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