

# Interferon- $\beta$ -1a in relapsing-remitting multiple sclerosis: effect on hypointense lesion volume on T1 weighted images

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## Abstract

**Objective**—Recently, a strong correlation between the increase in hypointense lesion load on T1 weighted spin echo images, and the increase in disability was reported. Although the effect of interferon- $\beta$  has been demonstrated both in reducing exacerbation rate, frequency of enhancing lesions, and accumulation of disease burden on T2 weighted images, the impact on the accumulation of hypointense lesions has not yet been evaluated. The aims of the present study were: (a) to assess for the first time the effect of interferon- $\beta$ -1a on T1 weighted MRI hypointense lesion volume; and (b) to evaluate the relation between changes on hypointense, hyperintense, and enhancing lesion volume before and during interferon- $\beta$ -1a treatment in relapsing-remitting multiple sclerosis.

**Methods**—After a baseline scan and 6 month pretreatment period, 67 patients with relapsing-remitting multiple sclerosis were treated with interferon- $\beta$ -1a by subcutaneous injection three times a week during a 12 month treatment period. All patients had MRI every month during the 6 month pretreatment period and for the first 9 months of the treatment period. A final MRI was also performed at the end of the 12 month treatment period.

**Results**—There was a significant increase in the mean hyperintense lesion volume during the pretreatment phase (6 months) and a slight decrease during the treatment period (12 months), whereas the hypointense lesion volume increased significantly before treatment and remained substantially stable during treatment. There was a significant correlation between changes in hypointense and hyperintense lesion volume during the observation period, but not during treatment. The monthly mean volume of Gadolinium-DTPA enhancing lesions was significantly higher during the pretreatment than the treatment period, and the enhancing lesion volume correlated with changes of hyperintense and hypointense lesion volumes only during the observation period.

**Conclusion**—These data suggest that interferon- $\beta$ -1a has a stabilising effect on T1 weighted hypointense lesion volume.

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**Keywords:** multiple sclerosis, relapsing remitting, magnetic resonance imaging, disability, interferon- $\beta$ , therapy

Although clinical criteria, usually disability or relapse rate, are the primary outcomes for assessing therapeutic trials in multiple sclerosis, the high sensitivity of MRI to multiple sclerosis disease activity has led to its use as a secondary outcome measure of treatment effect.<sup>1-3</sup>

In this context, the most established MRI indices are serial monthly gadolinium enhanced imaging and yearly T2 weighted imaging with total hyperintense lesion volume quantification. Nevertheless, definitive (phase III) treatment trials rely on clinical assessment as the primary outcome, because there is a relatively weak relation between clinical and MRI measures of disease progression, suggesting that caution is needed when interpreting MRI findings in isolation. The failure to find consistent correlations between lesion volume and disability for different multiple sclerosis patient populations may reflect the inherent lack of specificity of the hyperintense changes on T2 weighted MRI. It is known that a range of diseases cause hyperintensity on T2 weighted imaging in multiple sclerosis, including oedema, demyelination, gliosis, and axonal loss.<sup>4</sup> This non-specificity is a considerable problem when attempting to relate changes on T2 weighted images to disease progression.

Recently, an impressive correlation between increase of hypointense lesion load on T1 weighted imaging and increase in disability has been reported.<sup>5,6</sup> Thus, T1 weighted imaging may be better than T2 weighted imaging in identifying lesions that cause clinical disability, suggesting a role for this index in the monitoring of treatment efficacy.<sup>3</sup>

Although several trials have already shown that interferon- $\beta$  significantly reduces the exacerbation rate,<sup>7-9</sup> its impact on disability has been shown in only a few studies.<sup>9-11</sup> MRI evidence supported the clinical findings, showing a positive effect on disease activity as measured by the frequency of enhancing lesions on T1 weighted imaging and lesion load on T2 weighted imaging.<sup>8-13</sup> However, the effect of interferon- $\beta$ -1a on the accumulation of hypointense lesions has yet to be evaluated.

The main objective of the present study was: (a) to assess for the first time the effect of interferon- $\beta$ -1a on hypointense lesion volume on T1 weighted imaging; and (b) to evaluate the relation between changes on hypointense,

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hyperintense, and enhancing lesion volume before and during interferon- $\beta$ -1a treatment in relapsing-remitting multiple sclerosis.

This work completes, by a post hoc analysis, the MRI data analysis of a previously published study on interferon- $\beta$ -1a in relapsing-remitting multiple sclerosis, and extends to 1 year, the results already presented for the first 6 months of treatment.

### Materials and methods

The trial was conducted at the University "La Sapienza" and S Camillo Hospital, Rome. The study was approved by the ethics committee of the two centres, and written informed consent was obtained from all patients.

#### STUDY DESIGN

After a baseline scan and 6 month pretreatment period, 67 patients with relapsing-remitting multiple sclerosis were randomly assigned to receive either 3 MIU (11  $\mu$ g) or 9 MIU (33  $\mu$ g) of interferon- $\beta$ -1a by subcutaneous injection three times a week during a 12 month treatment period. All patients were assessed using proton density (PD), T2, and T1 weighted enhanced MRI every month during the 6 month pretreatment period and for the first 9 months of the treatment period. A final MRI was also performed at the end of the 12 month treatment period.

Inclusion and exclusion criteria, such as disease criteria and concomitant treatment, have already been reported elsewhere.<sup>8</sup>

#### MRI ASSESSMENT

Brain MRI examinations were performed using a superconducting system operating at 0.5 Tesla (Toshiba 50 S). All scans were collected in the axial plane with a 5 mm slice thickness, 1 mm gap, 25 cm field of view and 256 $\times$ 160 matrix. A T1 weighted spin echo sequence (TR 400, TE 18, two measures) was performed 15 minutes after the injection of Gd DTPA (0.1 mmol/kg). To reduce the examination time, a pregadolinium T1 scan was not performed. In addition, PD and T2 weighted imaging were created using a dual echo spin echo sequence (TR 2500, TE 30/90, one measure).

Reproducible axial slice positioning from scan to scan was ensured by performing coronal and midline sagittal scout scans at the beginning of each study. The axial sections were oriented parallel to a line passing through the base of the frontal lobe and the caudal portion of the quadrigeminal plate. All MRI examinations were carried out at the same neuroradiological unit by a well trained technician according to a standardised procedure.

#### MRI ANALYSIS

The images were analysed by the same observer operating for the original study, blinded to the treatment regime of the patients. The observer repeated the lesion volume measurements for the whole set of images used in the previous study and then completed the measurement on the new set of images performed in the extension phase of the present study. Blinding was ensured by replacing the dates on scans

with codes. This procedure was followed for both the pretreatment and the treatment periods. For each patient, all the images were evaluated at the end of the study in random order, according to a list of random numbers generated by statistical software.

The total volume of hyperintense lesions was evaluated using PD weighted images, and the total volume of hypointense lesions was evaluated using T1 weighted imaging. In addition, the volume of enhancing lesions was assessed from the T1 weighted images. A hypointense lesion ("black hole") was defined as any region visible on the T1 weighted images corresponding to a region of high signal intensity on the T2 weighted image, with low signal intensity relative to the surrounding white matter. The quantification of hypointense lesions on post-Gd scans followed standard practice, and is justifiable because many low signal intensity lesions on pre-Gd T1 weighted images are acute lesions that enhance after Gd administration. Some of these then become isointense; therefore such lesions would be counted in two data sets if measured on pre-Gd T1 weighted imaging.

The presence, location, and extent of multiple sclerosis lesions were marked on the films by two radiologists, by consensus. When there was a disagreement, a third senior radiologist also reviewed the films, and a final consensus was reached. The lesion volumes corresponding to the areas marked on the films were evaluated by a technician.

Computer assisted volume measurements were performed by a single evaluator on a Sparcstation 10 workstation (Sun Microsystems, Mountain View, CA, USA) using a semi-automatic local thresholding technique, which has been shown to have a good reproducibility.<sup>14</sup> The software used was the "/usr/image" library (University of North Carolina, Chapel Hill, NC USA) and Dispimage image display software (Mr D Plummer, Department of Medical Physics, University College, London, UK). The MR scans were displayed on the computer screen and the evaluator indicated the edge of a lesion using a mouse controlled cursor. The computer program first examines the image in a region close to where the mouse was clicked, to find the strongest local intensity gradient, which it considers to be the edge of the lesion. The lesion is then outlined by following a contour of isointensity from this initial edge point, thus defining the lesion as a region of the image where the signal intensity is locally above (or below, for hypointense lesions) the signal intensity at the initial edge position. When this technique occasionally failed (for example, due to low contrast between the lesion and surrounding tissue), the lesions were manually outlined by the evaluator by moving the cursor around the boundary of the lesions. The lesion volume was computed simply as the product of the total area of all lesions in all slices and the slice spacing.

To evaluate the intraobserver variability of the volume measurements, scans from five patients with varying lesion loads were assessed

Table 1 Hyperintense and hypointense lesion volume (ml) (n=67)

	Hyperintense lesion volume on T2 weighted images		Hypointense lesion volume on T1 weighted images	
<i>Observation</i>	Mean (SE)	13.2 (1.4)	Mean (SE)	3.3 (0.6)
Baseline	Range	1.1–48.9	Range	0.03–20.9
	Median	8.9	Median	1.4
<i>End of 6 month</i>	Mean (SE)	15.5 (1.6)	Mean (SE)	4.1 (0.7)
	Range	0.8–58	Range	0.1–23.7
	Median	11.1	Median	1.8
	Mean % change*	+17.4	Mean % change*	+23.6
	Median % change	+24.0	Median % change	+26.5
<i>Treatment</i>	Mean (SE)	15.2 (1.6)	Mean (SE)	4.2 (0.7)
<i>End of 6 month</i>	Range	0.78–54.2	Range	0.07–25.9
	Median	9.9	Median	1.8
	Mean % change	-1.9	Mean % change	+2.2
	Median % change	-10.4	Median % change	0
<i>End of 12 month</i>	Mean (SE)	14.2 (1.6)	Mean (SE)	4.1 (0.7)
	Range	0.9–48.4	Range	0.06–25.8
	Median	9.5	Median	1.8
	Mean % change	-7.0	Mean % change	-0.1
	Median % change	-4.2	Median % change	0

\*Change from previous scan:  $p < 0.001$ , Wilcoxon signed rank test compared with previous scan. SE=Standard error.

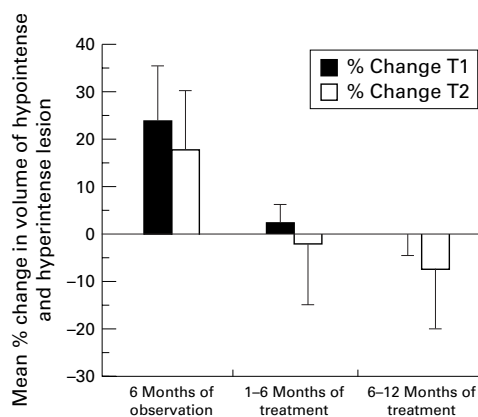


Figure 1 Mean percentage change of hypointense and hyperintense lesion volume. A significant increase (+17.4%,  $p < 0.001$ ) in the mean hyperintense lesion volume during the observation period and a decrease during both treatment periods (-1.9%,  $p = 0.3$  and -7.0%,  $p = 0.04$  respectively) was found. A significant increase (+23.6%,  $p < 0.001$ ) in the mean hypointense lesion volume during the observation period was found, whereas it remained substantially stable during both treatment periods (+2.2,  $p = 0.1$  and -0.1,  $p = 0.9$  respectively). See also table 1.

twice, with a period of 2 weeks between the two evaluations.

#### STATISTICAL ANALYSIS

A comparison of MRI derived measures during the pretreatment and treatment periods was performed using the Wilcoxon signed rank test. The relation between changes in hypointense, hyperintense, and enhancing lesion volume during the treatment and pretreatment period was investigated by means of Spearman's rank correlation coefficient.

Table 2 Monthly mean volume (ml) of Gd enhancing lesions (n=67)

Scan	Mean	Median	†Mean % change	†Median % change	p Value*
<i>Observation:</i>					
End of 6 month	0.52 (0.78)	0.19			
<i>Treatment:</i>					
End of 6 month	0.18 (0.33)	0.03	-65.3	-84.2	<0.001
End of 12 month	0.16 (0.29)	0.02	-69.2	-89.4	<0.001

†Values relative to the observation period.

\*Wilcoxon signed rank test compared to observation period.

## Results

### PATIENT CHARACTERISTICS

Sixty seven patients with relapsing-remitting multiple sclerosis out of the 68 patients from the sample of the original paper<sup>8</sup> were included in the present analysis. Forty six were women and 21 were men. One patient dropped out after 40 days of treatment due to the development of a cognitive disturbance, and was not considered in this extended protocol. The mean age was 30.5 years (SD 7.3 years); the mean duration of multiple sclerosis before the study was 5.0 years (SD 2.9 years); the EDSS score before entry to the study was 2.2 (SD 0.9); and the exacerbation rate over previous two years was 3.1 (SD 1.3)/year.

### VOLUME MEASUREMENTS

No significant differences between the two doses of interferon-β-1a on lesion volumes were found. The statistical analysis was therefore performed on the two doses pooled together.

Table 1 shows the hyperintense and hypointense lesion volumes at baseline, at the end of the observation period, and at the end of 6 and 12 months of treatment. We found a significant increase in the mean hyperintense lesion volume during the observation phase and a slight decrease during the treatment period (fig 1). The hypointense lesion volumes on the T1 weighted scans showed a significant increase during the observation phase, whereas they remained substantially stable during both treatment periods (fig 1). Table 2 shows the monthly mean volume of Gd enhancing lesions during the observation and treatment periods. The mean volume of Gd enhancing lesions was higher during the observation period than during treatment. This difference was significant, corresponding to reductions of 65% and 69% respectively during the two treatment periods.

A significant correlation between changes in hypointense and hyperintense lesion volume was found during the observation period ( $r = 0.66$ ;  $p < 0.0001$ ) but not during the treatment periods (fig 2). As shown in figure 2, there was a reduction of hypointense T1 lesion volume in 25 patients (35%) during the first 6 month treatment period, and a reduction in 30 patients (45%) during the second treatment period. Furthermore, significant correlations between mean Gd enhancing lesion volume and changes in hyperintense lesion volume ( $r = 0.49$ ;  $p < 0.0001$ ) and changes in hypointense lesion volume ( $r = 0.43$ ;  $p < 0.001$ ) were found during the observation period but not during the treatment periods (data not shown).

### REPRODUCIBILITY

In the sample of five patients, the mean percentage difference between the first and repeat assessments was 5.5% for T1 lesion load and 3% for T2 lesion load. This is comparable with the intraobserver variation found in previous studies.<sup>5 6 15</sup>

## Discussion

In 1996 we reported the results of treating patients with relapsing-remitting multiple scler-

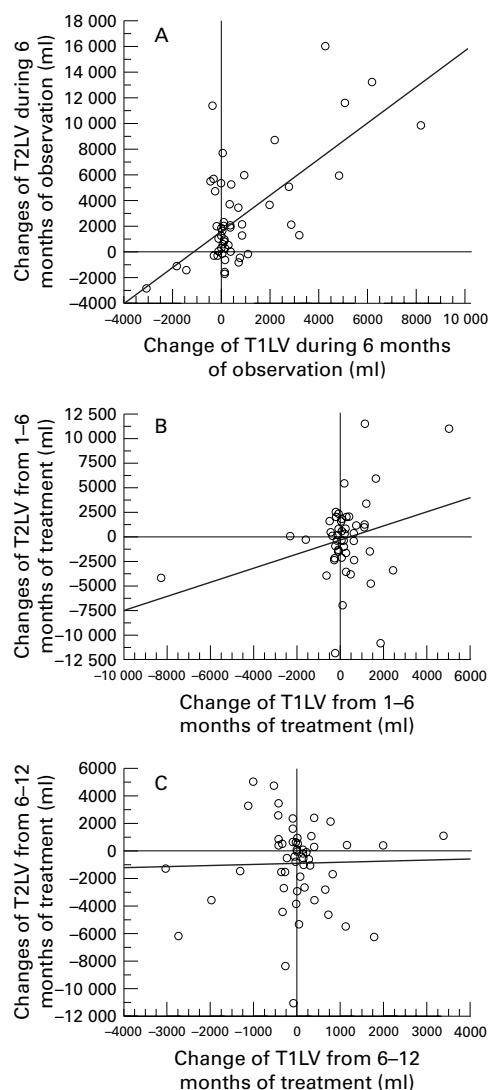


Figure 2 Relation between the change in hyperintense and hypointense lesion volume (LV) on the T2 and T1 weighted images respectively, during the pretreatment and the two treatment periods.

rosis with interferon- $\beta$ -1a (Rebif) for six months. The study showed a decrease of about 65% in the rate of enhancing MRI lesions on T1 weighted images whereas the hyperintense total lesion volume on T2 weighted imaging remained substantially stable during the treatment period.<sup>8</sup> In the current report, we present additional MRI data for this study, and include new MRI findings from a further 6 months of treatment. The findings of the earlier study<sup>8</sup> have been confirmed over a longer treatment period showing both a larger reduction in the volume of enhancing lesions and a slight decrease of hyperintense lesion volume.

However, the predictive value of changes in T2 lesion load and Gd enhancing lesions towards disease progression is still unknown, and only a moderate correlation exists between T2 lesion load and clinical disability.<sup>16-19</sup> This clinical-radiological mismatch may be partly explained by the fact that all pathological processes involved in multiple sclerosis lesions (oedema, inflammation, demyelination, axonal loss, and gliosis) are represented as hyperin-

tense areas on T2 weighted imaging. The origin of persistent deficit, however, probably lies in the occurrence of axonal loss or extensive demyelination. More specific MR measures of tissue changes are needed to resolve this mismatch. At present, studies attempting to measure the degree of tissue disruption using new MR techniques such as MR spectroscopy, magnetisation transfer imaging, and diffusion imaging have shown potential, but are still under development.<sup>18-20, 21</sup>

Recently, on the basis of low magnetisation transfer ratios found in hypointense lesions on T1 weighted SE images, it was speculated that these hypointense lesions represent areas of substantial structural loss.<sup>18, 22</sup> Uhlenbrock *et al* suggested that areas of hypointensity may represent the chronic multiple sclerosis lesion in which astrocyte growth and scarring, and therefore permanent neurological damage, have occurred.<sup>23</sup> This speculation was supported by Hiele *et al*, who found a strong correlation between magnetisation transfer ratio (MTR) reduction and hypointense lesions.<sup>24</sup> Furthermore, van Walderveen *et al*, in a preliminary postmortem study, found that the degree of hypointensity of T1 lesions correlated significantly with matrix destruction and loss of axons.<sup>25</sup> Consequently, hypointense lesions seen on T1 weighted imaging may represent the more disabling lesions. In comparison with T2 lesion volume, T1 lesion volume shows a higher correlation with disability.<sup>5, 6</sup>

We found a significant increase in the mean hypointense lesion volume during the pretreatment phase, whereas it remained substantially stable during the treatment period. Several factors could explain the stabilisation of hypointense T1 weighted imaging lesion volume during interferon- $\beta$ -1a treatment.

Firstly, it may result from the strong reduction in the rate of enhancing lesion formation that was shown in the original paper.<sup>8</sup> Indeed, in this study we extend to 1 year's treatment previous findings confirming a significant reduction of enhancing lesion volumes. As Gd enhancement is a marker of disruption of the blood-brain barrier which, in multiple sclerosis, correlates with the presence of inflammation, the decreased frequency of enhancing lesions suggests a less severe inflammatory phase during interferon- $\beta$ -1a treatment. Recently, it has been suggested that the anti-inflammatory effect of interferon- $\beta$  on the development of enhancing lesions is due to a peripheral down regulation of T cell activation, modification of cell adhesion molecules on endothelial cells, or down regulation of class II expression within the CNS, thereby blocking antigen recognition.<sup>26</sup> Moreover, some authors have shown that both steroids and interferon- $\beta$  reduce the activity of the 92 kD matrix metalloproteinase (gelatinase B).<sup>27-29</sup> This protease seems crucially involved in the trafficking of activated T cells across the blood-brain barrier, which is considered an important event underlying changes in the barrier.

Secondly, the stabilisation of hypointense T1 weighted imaging lesion volume may be related to a prevention of new enhancing lesions going

on to become hypointense. Paolillo *et al*<sup>30</sup> recently investigated whether interferon- $\beta$ -1a treatment modifies the short term evolution of new enhancing lesions in relapsing-remitting multiple sclerosis. They reported that during interferon- $\beta$ -1a therapy only 15% of new enhancing lesions evolved to T1 hypointensity, whereas about 50% of those appearing in the pretreatment period did so. Because high inflammatory activity may exhaust repair mechanisms, leading to permanent structural loss with no significant remyelination,<sup>31</sup> it is likely that the anti-inflammatory activity of interferon- $\beta$ -1a prevents the failure of remyelination and consequent axonal loss. Whether the tissue in an individual multiple sclerosis lesion is prevented from becoming permanently damaged by interferon- $\beta$  treatment would therefore depend on the exact point in its history at which treatment is administered. Lesions that have had few inflammatory episodes will be less susceptible to permanent deficit.

Thirdly, a shrinking of existing hypointense T1 weighted imaging lesions during interferon- $\beta$ -1a treatment must be considered a possibility. Although less evident on hypointense T1 weighted imaging lesion volume than on hyperintense T2 weighted imaging volume, figure 2 shows that the volume of hypointense T1 weighted imaging lesions is reduced in about 40% of patients. Thus T1 weighted image hypointensities cannot be completely associated to a definitive axonal loss, as some are at least partially reversible.

We found that changes in T1 weighted imaging volume correlated with changes in T2 weighted imaging lesion volume during pretreatment but not during the treatment period. During treatment, T2 weighted image lesion volume tends to decrease whereas T1 weighted image lesion volume remains substantially stable. As mentioned above, lesions seen on T1 and T2 weighted imaging reflect different pathological substrates and this diversity may help in understanding our findings. The decrease of T2 weighted imaging lesion volume during treatment suggests that interferon- $\beta$ -1a has a major impact on reversible tissue damage such as oedema, inflammation, and demyelination, which contribute substantially to T2 weighted imaging lesion volume. On the other hand, the stability of T1 weighted image lesion volume during treatment may reflect a beneficial effect of interferon- $\beta$ -1a both in slowing the accumulation of new "black holes" and at least partially, in reducing the reversible tissue damage which can be associated with T1 weighted hypointense lesions.

In conclusion, our data suggest that interferon- $\beta$ -1a has a stabilising effect on T1 weighted hypointense lesion volume. However, our findings might be influenced by the short follow up period and by a possible regression towards the mean in a cross over design such as this. These results therefore need to be confirmed in a double blind parallel group study with a longer follow up period. Nevertheless, our initial data suggest that the hypointense lesion volume on T1 weighted

imaging may be a useful secondary outcome measure in monitoring clinical trials.

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