Interferon beta-1a for brain tissue loss in patients at presentation with syndromes suggestive of multiple sclerosis: a randomised, double-blind, placebo-controlled trial

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Summary

Background In patients who present with clinically isolated syndromes suggestive of multiple sclerosis, interferon beta-1a is effective in delaying evolution to clinically definite disease and in reducing MRI-measured disease activity. We aimed to assess whether this drug can also reduce the rate of brain volume decrease in such patients enrolled in the ETOMS (early treatment of multiple sclerosis) trial.

Methods MRI data for brain volume measurements at baseline, month 12, and month 24 were available from 131, 111, and 112 patients assigned treatment (22 μg interferon beta-1a), and 132, 98, and 99 patients assigned placebo respectively. Normalised brain parenchymal volume (NBV) at baseline and percentage brain volume changes (PBVC) were measured with a fully-automated segmentation technique. The primary endpoint was conversion to clinically definite multiple sclerosis due to clinical relapse. Analysis was by intention to treat.

Findings 41 (31%) of 131 patients on interferon beta-1a and 62 (47%) of 132 on placebo converted to clinically definite multiple sclerosis (odds ratio 0·52 [95% CI 0·31–0·86], p=0·0115). Mean PBVC for patients on placebo was –0·83% during the first year, –0·67% during the second year, and –1·68% during the entire study period. Respective values for treated patients were –0·62%, –0·61%, and –1·18%. The changes in brain volume were significant in both groups at all timepoints. A significant treatment effect was detected for month 24 versus baseline values (p=0·0031). The number of new T2 lesions formed during the first year correlated weakly with PBVC during the second year.

Interpretation Early treatment with interferon beta-1a is effective in reducing conversion to clinically definite multiple sclerosis and in slowing progressive loss of brain tissue in patients with clinically isolated syndromes. The modest correlation between new lesion formation and brain volume decrease suggests that inflammatory and neurodegenerative processes are, at least partly, dissociated from the earliest clinical stage of multiple sclerosis onwards.

Introduction

MRI is widely used to monitor the evolution of multiple sclerosis, mainly because of its high sensitivity for the detection of disease activity, which is much better than that of clinical assessment.[1] Nevertheless, in patients with established multiple sclerosis, the correlation between clinical and MRI findings is modest at best.[1] By contrast, in patients who present with clinically isolated syndromes suggestive of this disease, the burden of T2 lesions is a robust predictor of subsequent evolution to clinically definite multiple sclerosis.[2,3] However, even in these patients, the increase of lesion load in the next few years is only moderately correlated with the long-term accumulation of disability.[4]

There has been much interest in measurement of decrease in brain volume as an adjunctive MRI marker of the most disabling aspects of multiple sclerosis.[5] Although not definitively proven, reduced brain parenchymal volume, which is frequently seen in patients with clinically definite multiple sclerosis, probably indicates demyelination and axonal loss in T2-visible lesions and tissue of normal appearance.[5] The extent of brain tissue loss in multiple sclerosis correlates better with clinical disability than does MRI lesion load,[6–8] and the rate of progression of brain atrophy in the relapsing-remitting phase of the disease is predictive of long-term disability.[9]

Two trials in patients with clinically isolated syndromes[10,11] have shown that interferon beta-1a is effective in reducing the risk of evolution to clinically definite multiple sclerosis and in slowing the accumulation of MRI-detectable disease activity. Since substantial brain parenchymal loss has been described in patients at presentation with clinically isolated syndromes,[12,13] we aimed to investigate whether, in a large sample of such patients enrolled into a phase III, double-blind, randomised, placebo-controlled trial,[11] interferon beta-1a also reduces progression of tissue loss, as measured by brain-volume changes on yearly scans. We also investigated the correlation between these changes and MRI markers of disease activity, with the ultimate aim of improving our understanding of the mechanisms leading to irreversible tissue damage in patients at the earliest clinical stage of multiple sclerosis.

Methods

Trial design and participants

Patients with clinical syndromes indicating unifocal or multifocal CNS involvement were included in the study. All patients were aged 18–40 years inclusive, presented with a first neurological episode suggestive of multiple sclerosis in the previous 3 months, had one or more abnormalities at the neurological examination and a positive brain MRI scan. A scan was judged positive when one of the following criteria was met: presence of at least four T2 lesions, or presence of at least three T2 lesions if at least one was infratentorial or enhancing after gadolinium injection. Steroid treatment of the initial attack was allowed. Eligible patients underwent complete physical and neurological examinations at entry, months 12 and 24, and as needed for assessment of acute relapses or safety. Their disability was rated with the expanded disability status scale[14] and the Scripps neurological rating scale.[15] Patients were randomly assigned, in blinded fashion, with a computer generated list at Quintiles (Sydney, Australia), to receive either 22 μg of interferon beta-1a (Rebif, Serono, Geneva, Switzerland) or identical placebo once a week by subcutaneous injection. The randomisation list was generated by the PROC PLAN procedure in SAS with block size four within each centre. Randomisation was stratified by centre, and because centres were small, they were pooled by country (stratification was therefore by country rather than individual centre). The ethics committees of all participating centres approved the study protocol and all patients gave written informed consent before trial entry. Additional information on study design has been reported.[11]

Procedures

Brain MRI was done as part of prestudy screening and at months 12 and 24. All scanners operated at field strengths of 0·5–1·5 Tesla. On every occasion, the following sequences were obtained: (a) dual echo conventional spin-echo (repetition time 2000–2500 ms, echo time 30–60 or 70–120 ms, number of acquisitions=1); (b) precontrast T1-weighted conventional spin-echo (repetition time <800 ms, echo time <25 ms, number of acquisitions ⩾2); and (c) postcontrast T1-weighted conventional spin-echo, with the same acquisition variables and slice locations as precontrast scans, 5–7 minutes after injection of Gd (bolus infusions of 0·2 mmol/kg). For all scans, 24 contiguous interleaved axial slices were acquired with 5-mm slice thickness, a 192–256×256 raw data matrix, and a 220–250 mm square field of view. Sequence acquisition variables were kept constant at each centre for the duration of the study. None of the scanners was changed or underwent a major upgrade during the study. At follow-up, the scan planes were carefully repositioned according to published guidelines.[16]

All scans were sent to the Neuroimaging Research Unit in Milan and reviewed centrally. Unsatisfactory images were rejected and repeated when possible. Identification of T2 lesions on dual echo images and enhancing lesions on postcontrast T1-weighted images were done by consensus of two experienced observers. On the follow-up scans, total and new enhancing lesions and new or enlarging T2 lesions were also counted. T2 and enhancing lesion volumes were measured by trained technicians who were unaware of patient identity and order of scan acquisition, with a semiautomated segmentation technique based on local thresholding.[17] Additional information on lesion counting and volume measurements are reported elsewhere.[11]

Both longitudinal (two timepoints) normalised percentage brain volume change (PBVC) and cross-sectional (single timepoint) normalised brain volume (NBV) were estimated with T1-weighted images. PBVC was estimated with SIENA (structural image evaluation of normalised atrophy)[18,19] and NBV with SIENAX (an adaptation of SIENA for cross-sectional measurement).[19] In each image, SIENA automatically segments brain from extracerebral tissues and estimates the external surface of the skull. Next, it registers the brain images from two timepoints, using the skull images to constrain scaling and skew, allowing correction for changes in imaging geometry over time. Thus, the method is quite insensitive to changes in intensity of tissues from one scan to the next. Brain volume is quantified by taking the mean perpendicular edge motion over all edge points[20] and converting this into PBVC. The normalising factor in the conversion is found by a self-calibration step, as previously described.[18,19] Various validation tests showed the accuracy in measuring PBVC to be around 0·2%.[18]

SIENAX applies a similar registration process, but instead of a second timepoint image, standard space average brain and skull images are used. Thus, the input image is registered to standard space, using the skull for the final scaling. This procedure reduces greatly the variation in brain volume between patients, thus increasing the sensitivity of across-group analyses. The estimate of brain tissue volume for an individual is then multiplied by the normalisation factor to yield NBV.[19] Reproducibility tests have calculated a mean standard error across a group of healthy people of about 1%.[19] SIENA and SIENAX are available freely as part of FMRIB Software Library (www.fmrib.ox.ac.uk/fsl). As an optional processing step of SIENA and SIENAX, upper and lower limits of the brain images can be defined in a standard space and applied to the whole analysis. Although the MRI acquisition protocol of the present study allowed for complete brain coverage, limits were nevertheless applied to increase the accuracy of PBVC estimates, since SIENA needs the same portion of the brain to be imaged at two different timepoints. For this purpose, standard-space based

Z limits of –20 for the bottom slice and 50 for the top slice were used for each analysed MR dataset. Whenever possible, the following measurements were obtained from every patient: baseline NBV, and PBVC between baseline and month 12, month 12 and month 24, and baseline and month 24. Analysis was done blind to treatment assignment.

Statistical analysis

The primary endpoint was proportion of patients converting to clinically definite multiple sclerosis due to clinical relapse.[11] Assessment of PBVC was a tertiary measure and the primary analysis compared the change from baseline between treatment groups. Analysis was by intention to treat. For comparisons between treatment groups, ANCOVA was calculated on ranked data. For treatment group comparisons of PBVC between baseline and month 12, and baseline and month 24, country and NBV at baseline were included in the ANCOVA models. For treatment group comparisons of PBVC between month 12 and month 24, month 12 NBV was not included in the model because at months 12 and 24, the SIENA method calculates a change on the basis of the previous comparison scan (PBVC) without providing absolute volume measurement with which adjustment could be made. The Wilcoxon signed rank test was used for statistical testing of PBVC within treatment groups. Correlations between variables were assessed with Spearman rank correlation coefficients. No imputation was made for missing data. Because brain volume was a tertiary outcome measure, no correction for multiple analyses has been made and thus probability statements should not be taken at face value and should be interpreted with caution. The study sample size was calculated on the basis of clinical measures and not MRI measures.

Role of the funding source

The study sponsor covered the expenses for statistical analysis, which was run at Quintiles, after authors' specific a-priori requests and under their supervision.

Results

The figure shows the number of patients randomly assigned interferon beta-1a and placebo. The numbers in the study at months 12 and 24 were 151 (98%) and 141 (92%) for interferon beta-1a, and 145 (94%) and 137 (88%) for placebo. However, not all patients had usable MRI data; data loss was the result of loss to follow-up or scans for which digitised images were not suitable for brain volume measurements, mainly because MR images were acquired when many MR sites were still restricted in their ability to provide data digitally.

MRI data for brain volume measurements at baseline were available from 131 patients in the interferon beta-1a group. One patient with month 12 and 24 data had no usable baseline data. During 24 months, nine patients dropped out (figure). An additional 11 patients with baseline volume data did not have usable data at months 12 and 24, leaving 112 patients with usable data at months 12 and 24, but 111 with PBVC data for comparisons between baseline and months 12 and 24 because of the one missing baseline value. Likewise, MRI data were available from 132 patients in the placebo group; 15 dropped out before month 24 and 18 had unusable data

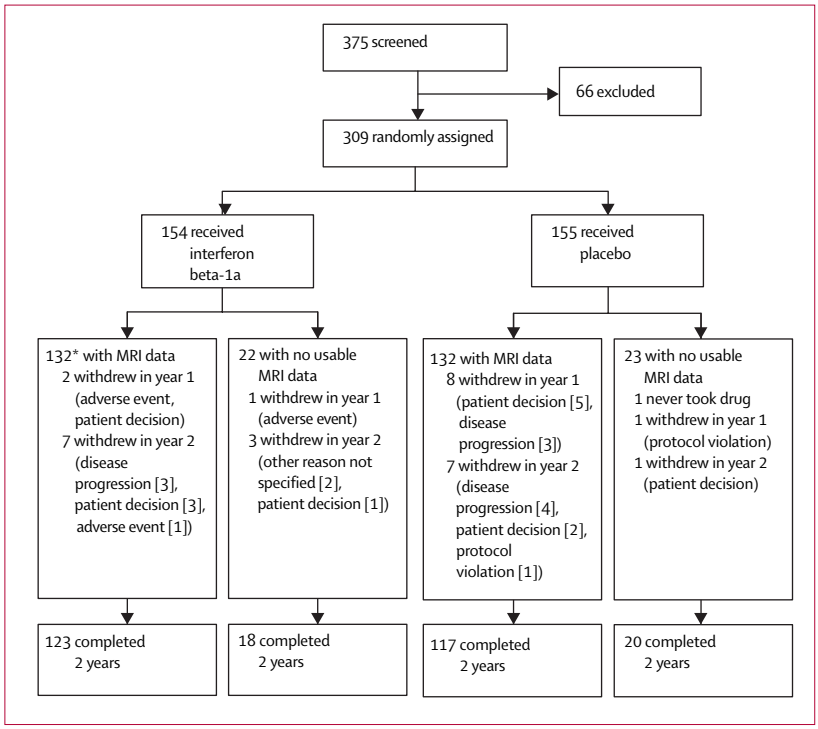

Figure: **Trial profile**
*One patient had no usable baseline data.

	Interferon beta-1a (n=131)	Placebo (n=132)
Male	52 (40%)	43 (33%)
Age (years) (mean, SD)	28·8 (5·8)	27·9 (6·1)
Clinical multifocal presentation	39 (30%)	44 (33%)
Time since first attack (days) (median, range)	82·0 (26–122)	84·0 (26–119)
First attacks treated with steroids,	93 (71%)	93 (70%)
Used within 30 days before MRI	47 (36%)	41 (31%)
Abnormal CSF findings *	68/89 (76%)	74/90 (82%)
EDSS score (median, range)	1·0 (0·0–7·0)	1·0 (0·0–5·0)
SNRS score (median, range)	98·0 (79–100)	98·0 (68–100)
Number of T2 lesions (median, range)	27·0 (3–156)	24·0 (3–134)
Number of enhancing lesions (median, range)	1·0 (0–109)	1·0 (0–50)
Number of patients with enhancing lesions	77 (59%)	81 (61%)
T2 lesion volume (mL) (mean, range)	8·3 (0·4–52·9)	8·9 (0·7–79·0)

Data are number (%) unless otherwise indicated. CSF=cerebrospinal fluid. EDSS=expanded disability status scale. SNRS=Scripps neurological rating scale.
*Information not available for all patients.

Table 1: **Baseline clinical and MRI characteristics**

	Interferon beta-1a	Placebo	p*
NBV at baseline (mL)			
n	131	132	
Mean (SD)	1495·7 (122·5)	1503·0 (89·4)	
Median (range)	1518·0 (1012 to 1854)	1510·0 (1034 to 1664)	
PBVC from baseline to month 12 (%)			
n	111	98	
Mean (SD)	−0·62 (1·40)	−0·83 (1·09)	
Median (range)	−0·60 (−4·76 to 4·27)	−0·73 (−4·15 to 2·41)	0·1668
p†	<0·0001	<0·0001	
PBVC from month 12 to month 24 (%)			
n	112	99	
Mean (SD)	−0·61 (0·99)	−0·67 (1·10)	
Median (range)	−0·45 (−6·09 to 2·15)	−0·67 (−3·19 to 3·55)	0·1723
p†	<0·0001	<0·0001	
PBVC from baseline to month 24 (%)			
n	111	98	
Mean (SD)	−1·18 (1·51)	−1·68 (1·99)	
Median (range)	−0·88 (−9·10 to 4·40)	−1·37 (−6·32 to 2·45)	0·0031
p†	<0·0001	<0·0001	

*Intergroup comparisons were by ANCOVA on ranks, adjusting for country, and NBV (for baseline to month 12, and baseline to month 24). †Intragroup comparisons were by the Wilcoxon signed rank test. 24-month PBVC differs slightly from sum of each year because different patients contributed to each assessment (because of patients lost to follow-up or because T1-weighted images in digitised format were not suitable for brain volume measurements).

Table 2: **Normalised brain volumes and percentage changes of brain volumes**

beyond baseline, leaving 99 with usable volume data at months 12 and 24, and 98 with PBVC data for the comparisons between baseline and months 12 and 24 because of one missing baseline value. The number of patients who could not be assessed because of missing or non-interpretable scans was very close between the two groups. Dropouts were slightly more common in placebo than actively treated patients, consistent with a higher rate of relapse in placebo patients making them eligible for approved disease-modifying therapy for clinically definite multiple sclerosis. The demographic, clinical, and MRI characteristics of this patient cohort at entry (table 1) were similar to those of the original study cohort[11] and there were no significant differences between the two groups at study entry.

Table 2 shows baseline NBV and PBVC during the study period. PBVC fell significantly during follow-up in patients assigned interferon beta-1a and in those assigned placebo. A significant treatment effect was detected for month 24 versus baseline PBVC. 41 (31%) of 131 of patients receiving interferon beta-1a and 62 (47%) of 132 receiving placebo, whose atrophy could be assessed, converted to clinically definite multiple sclerosis consistent with the total cohort,[11] indicating that early treatment with low doses of interferon reduced the risk of evolution to this form of the disease in patients with clinically isolated syndromes (odds ratio=0·52, 95% CI 0·31–0·86, p=0·0115, logistic regression adjusted for country). Median PBVC in patients who did versus did not develop clinically definite multiple sclerosis were –0·92% and –0·56% for month 12 versus baseline (p=0·0495), –0·64% and –0·50% for month 24 versus month 12 (p=0·1445), and –1·63% and –0·97% for month 24 versus baseline (p=0·0461).

At baseline, NBV was not correlated with number of enhancing lesions, T2 lesion volume, or enhancing lesion volume. A weak, but significant negative correlation was noted between NBV and number of T2 lesions (r=–0·142, p=0·0217). Table 3 shows the univariate correlations between PBVC and MRI-measured disease activity during the same interval. To assess predictive changes, correlation of activity in year 1 with PBVC in year 2 was also measured. The number of enhancing lesions on month 12 scans negatively correlated with PBVC during the subsequent 12 months, in the whole cohort (r=–0·224, p=0·0011) and in those treated with interferon beta-1a (r=–0·231, p=0·0142). The number of new T2 lesions accumulated during the first 12 months was also negatively correlated with PBVC during the subsequent 12 months, in the whole patient cohort (r=–0·286, p<0·0001) and in interferon-treated patients (r=–0·310, p=0·0009).

Discussion

This study shows that treatment with a subcutaneous dose of 22 μg interferon beta-1a per week reduces conversion to clinically definite multiple sclerosis and lessens the rate of brain parenchymal loss in patients who present with clinically isolated syndromes

	All patients (n=211)			Interferon beta-1a (n=112)			Placebo (n=99)		
	Baseline *vs* month 12	Month 12 *vs* month 24	Baseline *vs* month 24	Baseline *vs* month 12	Month 12 *vs* month 24	Baseline *vs* month 24	Baseline *vs* month 12	Month 12 *vs* month 24	Baseline *vs* month 24
Enhancing lesions									
Spearman's r	−0·017	−0·068	−0·188	−0·052	−0·047	−0·162	0·040	−0·099	−0·225
p	0·8054	0·3271	0·0061	0·5883	0·6231	0·0887	0·6972	0·3273	0·0251
New T2 lesions									
Spearman's r	−0·247	−0·173	−0·264	−0·272	−0·188	−0·230	−0·168	−0·107	−0·191
p	0·0003	0·0117	0·0001	0·0037	0·0475	0·0145	0·0975	0·2938	0·0584

All correlations adjusted for baseline values of MRI variable. Values are Spearman's rank correlation coefficients, correlating new lesion values at the end of the interval with PBVC for the same interval (month 12–baseline or month 24–baseline), or correlating new MRI lesion activity at month 12 with PBVC during year 2 (month 24–month 12).

Table 3: **Correlations between percentage change of brain volume and MRI measures of disease activity**

suggestive of multiple sclerosis. These findings strengthen the benefit from early treatment with interferon beta-1a on the occurrence of multiple-sclerosis-enhancing and new T2 lesions.[10,11] Enhancing lesions indicate transiently increased blood-brain barrier permeability and inflammation, and T2-weighted imaging provides non-specific information about the pathological substrate of lesions, whereas brain volume reduction is possibly related to loss of neurons, axons, oligodendrocytes, and myelin sheaths.[5] PBVC over the study period was about 30% less in interferon beta-1a treated patients than in those receiving placebo.

Since 25 (19%) of 132 of patients originally allocated placebo were switched to interferon beta-1a when they converted to clinically definite multiple sclerosis during the 2-year study,[11] the magnitude of treatment effect on prevention of tissue loss at the earliest clinical stage of the disease is probably underestimated. The ability of interferon beta-1a to reduce MRI-detected disease activity in patients who present with clinically isolated syndromes[10,11] may have limited, at least partly, our ability to detect a stronger treatment effect on brain atrophy, because of resolution of oedema by this drug's anti-inflammatory activity. This limitation would result in some degree of pseudoatrophy in treated patients, blunting our ability to detect treatment differences. Patient dropout and unavailability of digitised T1-weighted images for brain volume measurements resulted in data loss of 14–32% at the different timepoints. Nevertheless, we believe that this loss has not substantially influenced our results because baseline clinical and MRI characteristics did not differ between patients whose atrophy could be measured and the original cohort,[11] and baseline NBV was similar between placebo and treated patients, as for other baseline measures. Dropouts were slightly more common in the placebo group (15 *vs* nine), but resulted from worse disease course, a bias that might reduce our ability to detect a difference between groups. Unusable data were also more common in placebo-treated patients (18 *vs* 11), but there is no reason to regard this loss of data as potentially introducing bias.

Histopathological studies have convincingly shown that many axons are transected at the sites of inflammatory lesions.[21] Since interferon beta-1a has a striking anti-inflammatory effect,[22] we believe that its neuroprotective effect might have been exerted through its ability to reduce the inflammatory component of multiple sclerosis pathology. Although other mechanisms cannot be excluded, this interpretation is supported by the fact that significant tissue loss was still detectable in treated patients (most of whom continued to have MR evidence of disease activity[11]) and by the significant, albeit modest, correlation between MR-detected disease activity and brain parenchymal loss. The different timing of the treatment effect on brain tissue loss (which needed 2 years to be detectable), compared with that on conversion to clinically definite multiple sclerosis and on MRI-measured disease activity, which was mostly during the first year,[11] is also consistent with our interpretation.

Two studies did not detect a significant treatment effect of higher doses of interferon beta 1-a on the rate of brain volume loss over a similar follow-up period in patients with established relapsing-remitting multiple sclerosis.[23,24] The first assessed the effect of 30 μg interferon beta-1a (Avonex, Biogen, Idec, Cambridge, MA, USA) given intramuscularly once a week on brain parenchymal fraction changes from 172 such patients.[23] Although a modest treatment effect was reported during the second year of the study, no significant effect was evident over the entire study period, in which the rate of change of this fraction was only 18% lower in patients receiving treatment than in those receiving placebo.

The second study assessed the effect of two doses of 22 and 44 μg interferon beta-1a (Rebif) given subcutaneously three times a week on rate of brain tissue loss from 519/560 patients with relapsing-remitting multiple sclerosis enrolled in the PRISMS trial,[25] who were scanned twice yearly for 2 years (yearly scans used for brain volume assessments). Although this post-hoc analysis has not been published, preliminary data did not show any evidence of effectiveness of either drug doses in preventing brain volume reduction.[24] The possibility cannot be excluded that higher doses of interferons might have an overall beneficial effect in preventing brain volume loss over time, through a more striking suppression of the neuroprotective components of the multiple sclerosis inflammatory process.[26] Nevertheless, the finding that low doses of interferon beta-1a offer more pronounced neuroprotection in patients at the earliest clinical stage of multiple sclerosis than do higher doses in patients with established relapsing-remitting multiple sclerosis suggests that inflammatory-related mechanisms of tissue loss could be more important in the initial, rather than more advanced, clinical phase of the disease, and again argues in favour of early treatment of the disease.

Significant reduction in brain volume can be detected over 1–2 years in patients with established multiple sclerosis.[5–8] Only three preliminary studies, however, assessed the dynamics of brain parenchymal loss in patients at presentation with clinically isolated syndromes suggestive of this disease.[12,13,27] Two studies assessed 17 and 55 patients, respectively, and showed substantial ventricular enlargement in those who developed clinically definite multiple sclerosis during 12-months' follow-up compared with those who did not.[12,13] The third study measured loss of whole brain tissue in 31 patients over a mean period of about

5 months, and estimated a yearly loss of brain volume of about –0·7%.[27] Although these studies are difficult to compare because they used different methodologies to measure brain atrophy, it is noteworthy that our 2-year study of about 200 patients reached similar conclusions to these three preliminary studies:[12,13,27] brain tissue loss occurs in patients who present with clinically isolated syndromes, and tends to be higher in patients converting to clinically definite multiple sclerosis during the study period than in those who do not.[13]

The rate of about 0·8% brain tissue loss per year in the placebo patients followed up for 24 months closely matches results from patients with established multiple sclerosis,[28] and is much higher than the annual loss of about 0·02% estimated in healthy volunteers aged between 20–40 years.[29] This finding also fits with the report of widespread axonal damage, detected by non-localised proton MR spectroscopy[1] for assessment of whole brain N-acetylaspartate, at the earliest clinical stage of multiple sclerosis,[27] and with previous magnetisation transfer ratio studies, indicating diffuse normal-appearing brain tissue damage in patients who present with clinically isolated syndromes.[30,31] Although not definitively proven, brain atrophy could be the result of demyelination and axonal loss[5] and, as a consequence, our data confirm that important and progressive structural damage of the brain occurs very early in the course of multiple sclerosis. This argument is even more compelling when considering that all patients by definition had had a recent clinical attack, of whom 70% had been treated with steroids within 3 months of study entry and roughly a third within 30 days of the initial MRI scan, with potential for artificially reduced brain volume associated with this therapy on the first MRI scan.[32] This was not the case when patients were scanned at months 12 and 24, and the degree of measured tissue loss at the earliest clinical stage of disease might have been underestimated.

Another novel finding of our study was that the correlation between MRI disease activity and PBVC over time was weak, although significant. This result is consistent with previous studies of patients with relapsing-remitting multiple sclerosis, which showed that the correlation between brain tissue loss and MRI enhancement is either absent or weak at best.[33–35] The novelty of this study is to show a mismatch between MR-measured disease activity and tissue loss early in the course of multiple sclerosis, when inflammatory demyelination is thought to be the pathological hallmark of the disease.[36] However, there are several possible explanations for this counterintuitive finding, which are not mutually exclusive. First, tissue loss at a certain time might be the result of inflammation and lesion formation that arose several months earlier.[37] Subclinical activity might arise in otherwise healthy individuals before the occurrence of the first clinical event attributable to multiple sclerosis.[38] Therefore, in this study we might have been measuring the long-term consequences of preclinical occult disease activity on brain tissue integrity. To investigate the contribution of previous undetected disease activity on subsequent irreversible tissue loss in multiple sclerosis, we examined the correlation between new T2 lesion formation during the first year and amount of brain volume loss during the second year of the study. Again, this correlation was only weak, suggesting a marginal role of previous subclinical disease activity on subsequent tissue loss. Nevertheless, we cannot exclude that longer follow-up might result in stronger correlation.

Second, we probably missed a large amount of inflammation that might have played a part in the loss of brain parenchyma in these patients. Although we used a double dose of gadolinium (which, compared with a standard dose, increases the sensitivity of MRI for detection of enhancing lesions[39]), we had only snapshots of disease activity, because patients were scanned yearly. As a consequence, since the expected duration of enhancement in new lesions is typically between 4 and 8 weeks,[1] we probably underestimated MRI disease activity of these patients. Third, evidence from magnetisation transfer ratio[30,31] and non-localised proton MR spectroscopy[27] studies increasingly suggests that normal-appearing brain tissue damage is an important aspect of the disease from the earliest clinical phase. Since occult inflammatory changes might occur in such tissue,[40] and it represents most of the brain tissue in patients at presentation with clinically isolated syndromes, this additional factor might also contribute to the mismatch of the correlation between MRI-detected disease activity and brain parenchymal loss. Fourth, tissue loss could arise at least partly independently of MRI-detectable disease activity as a consequence of chronic demyelination,[41] either through loss of trophic support to the axons,[42] or secondary to altered electrical conduction,[43] which can happen in the absence of inflammation. Finally, the known ability of treatment to reduce enhancing lesions,[11] which in turn might result in pseudo-atrophy on follow-up scans, could also have contributed to the paucity of the correlation between MRI-detected disease activity and brain tissue loss.

This study has confirmed in a large cohort of patients at the earliest clinical stage of multiple sclerosis that brain parenchymal loss takes place rapidly, and has shown that 22 μg interferon beta-1a, given subcutaneously once weekly, can alter this process significantly. Whether higher or more frequent doses would enhance or reduce this effect remains untested. The weak correlation between new lesion formation and brain volume reduction suggests that, even at the earliest clinical phase of the disease, inflammatory demyelination is not enough to fully account for irreversible tissue loss in multiple sclerosis.

Contributors

M Filippi, F Barkhof, and G Comi were responsible for protocol design, and M Filippi, F Barkhof, and G Comi coordinated the study. M Rovaris and M Inglese obtained the data. N De Stefano and S Smith were responsible for independent outcome assessment. M Filippi, M Rovaris, and M Inglese did statistical analysis. M Filippi wrote the report, with contributions from all co-authors.

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Conflict of interest statement

None of the authors is employed by Serono or holds any equity interest or patent rights. During the ETOMS trial, MF, FB, and GC were contracted as consultants for that specific study and therefore reimbursed for their specific services. They also received travel grants from Serono International. However, this study was done without any consultant agreement and corresponding fees from the sponsor. NDS and MR received travel grants from Serono International. MI and SS have no conflict of interest to declare.

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