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A 6-year clinical and MRI follow-up study of patients with relapsing–remitting multiple sclerosis treated with Interferon-beta

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There are few long-term clinical and magnetic resonance imaging (MRI) data on patients treated with interferon-beta (IFN-β) for relapsing–remitting multiple sclerosis (RRMS). The aim of this study was to provide clinical and MRI data on 68 patients with RRMS treated over a 6-year period and to investigate whether a baseline MRI predicts their long-term clinical and MRI outcome. Six MRI scans were performed monthly before treatment and a further 13 scans were performed during treatment with IFN-β, the last of which 6 years after commencement of treatment. The relapse rate, disability as measured by the Expanded Disability Status Scale (EDSS), and MRI parameters, including Gd-enhancing lesion load (Gd-LL), T2 hyperintense lesion load (T2-LL) T1 hypointense lesion load (T1-LL) and supratentorial brain volume (SBV) were measured throughout the study. The mean annual relapse rate over the 6 years was 0.52 (SD 0.67), which is significantly lower (68.6%) than the mean annual relapse rate of 1.6 observed during the 2-year period before the commencement of treatment ($P < 0.01$). The median EDSS score increased from 2 to 2.5, remaining stable in 60% of the patients. From the baseline scan to the final scan, there was a median increase of 7% in the T2-LL and 23.9% in the T1-LL, whilst SBV decreased by 2.7%. The increase in the EDSS over the course of the study was significantly correlated with a reduction in brain volume ($r = 0.46$, $P = 0.001$). Greater brain damage at baseline, as measured by both T2-LL and T1-LL, was significantly associated with an increase in disability over the 6 years ($r = 0.44$, $P = 0.0009$; $r = 0.50$, $P = 0.0007$, respectively). This study shows a sustained effect of IFN-β on the relapse rate, which is lower than during the 2 years before treatment commencement. More than half the patients showed an improvement or stabilization in the EDSS score. The increment in disability was correlated with the development of brain atrophy but not with increases in lesion burden. Finally, the finding that the extent of lesion burden at the baseline was a strong predictor of increasing disability suggests that IFN-β treatment might have a moderate effect in modifying the multiple sclerosis (MS) disease course over 6 years unless preventive treatment is started early.

Introduction

Three large, multicentre, double-blind, placebo-controlled studies have highlighted the beneficial effect of interferon-beta (IFN-β) in modifying the disease course of relapsing–remitting multiple sclerosis (RRMS) patients. These trials have shown that IFN-β can reduce the relapse rate and slow down the progression of disability over a period ranging from 2 to 5 years (IFNB Multiple Sclerosis Study Group, 1993, 1995; Jacobs *et al*., 1996, 2000; PRISMS Study Group, 1998, 2001). Magnetic resonance imaging (MRI) was used in these phase III trials as a secondary end-point, to support the clinical outcome measures of treatment efficacy. The results documented a dramatic effect of treatment in reducing disease activity and lesion burden accumulation in patients when compared with the untreated patient arm (Paty and Li, 1993; Simon *et al*., 1998; Li *et al*., 1999). The MRI parameters used were Gd-enhancement and T2 hyperintense lesion load changes (burden of the disease), which provide outcome measures related to disease activity (acute phase) and progression (chronic phase), respectively (Miller *et al*., 1998). However, the MRI parameters mentioned above

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have not been endorsed as surrogate primary outcome measures for phase III multiple sclerosis (MS) treatment trials because of their weak relationship with clinical changes and their poor ability to predict clinical outcome. More specific MRI-based measures of brain damage, including T1 hypointense lesions (black holes) and brain atrophy, have recently been proposed for MS trials. They are thought to measure the more destructive pathological elements of the disease and appear to correlate better with progressive disability (Truyen *et al.*, 1996; Losseff *et al.*, 1996).

In the present study, we report the clinical and MRI findings from a group of patients treated for a 6-year period with IFN-β. We investigated both conventional MRI parameters and more specific measures of brain damage. The potential role of these MRI parameters in predicting the clinical outcome was also examined.

Materials and methods

Study design and participants

The study was an extension of an open-label short-term study started in 1994 to evaluate the clinical and MRI efficacy of recombinant IFN-β-1a in a group of RRMS patients (Pozzilli *et al.*, 1996). Sixty-eight patients with clinically definite RRMS, a disease duration of less than 10 years, Expanded Disability Status Scale (EDSS) from 1 to 5, were recruited to receive recombinant IFN-β-1a, Rebif® Ares Serono International SA, Geneva, Switzerland (11 or 33 μg) for 2 years. When the study began the drug was not licensed and different doses were tested. At the end of this period, owing to limited availability of the then unlicensed IFN-β-1a, 16 of these patients were selected to continue therapy with IFN-β-1a (33 μg thrice weekly) whilst the remainder were switched to IFN-β-1b (Betaferon®, Schering, Aktiengesellschaft 2000, D-13362 Berlin, Deutschland, 8 MIU subcutaneously, thrice weekly) (Pozzilli and Koudriavtseva, 1997).

Clinical assessment

During the 6-year period, a neurological examination was performed and disability scored on the EDSS (Kurtzke, 1983) every 6 months. Patients who discontinued treatment continued to be visited at the MS outpatient centre as often as when they were being treated. The number of clinical exacerbations, the EDSS scores and other medical events that occurred were recorded at each visit. The exacerbations were defined as the appearance or reappearance of one or more symptoms attributable to MS, accompanied by objective deterioration lasting at least 24 h on neurological examination, in the absence of fever, and preceded by neurological stability for at least 30 days. In case of relapse, each patient was treated with a high dose of steroids (methylprednisolone, 1 g daily for five consecutive days).

Sustained progression was defined as an increase of at least one point in the EDSS score on two consecutive EDSS measurements at least 6 months apart.

In most cases, the neurologists who conducted the original study continued to examine patients throughout this 6-year observation period.

MRI protocol

Patients were imaged monthly from 6 months before treatment to 9 months after start of treatment. Further MRI scans were performed at month 12 (after first treatment year), month 18, month 24 (second year) and month 72 (sixth year). The annual MRI scan was delayed by 30 days in patients treated with steroids during relapses in order to avoid any influence of steroids on the scans.

The 6-year brain MRI follow-up was performed using the scanner in the initial study (Pozzilli *et al.*, 1996) (superconducting 0.5 T magnet-Toshiba S 50) and the same MRI protocol, which included sagittal T1-weighted images (T1WI) (TR 400, TE 18) and transaxial PD and T2-spin-echo images (TR 2500, TE 30/90). The Gd-enhanced T1WI scans were obtained in the *trans*axial plane, between 5 and 10 min after injection of 0.1 mmol/kg of gadolinium diethylenetriamine penta-acetic acid (Gd-DTPA). All transaxial scans were collected with a 5-mm slice thickness, 1 mm gap, using a 25-cm field of view and 160×256 matrix.

Reproducible slice positioning was maintained throughout the follow-up by using the midline sagittal scout slice obtained on admission to the study and recorded on hardcopies. The transaxial sections were orientated parallel to a line passing through the base of the frontal lobe and the caudal portion of the quadrigeminal plate. The MRI scans were performed in the same neuroradiological unit by a trained technician according to a standardized procedure.

MRI analysis

We measured the Gadolinium-enhancing lesion load (Gd-LL) from month 1 to 6 of the pre-treatment phase (6 months) and then at years 1, 2 and 6 after commencement of treatment. All other assessments from MRI scans were calculated for the baseline and for years 1, 2 and 6 after commencement of treatment. These were: T2 hyperintense lesion load on the PD-WI (T2-LL); T1 hypointense lesion load (black holes) on T1WI (T1-LL);

supratentorial brain volume (SBV); and the cross-section area of the corpus callosum (CC) at the midline.

The Gd-LL, T2-LL and T1-LL were all calculated from the *trans*axial scans using the 'Dispim' display program (DL Plummer, University College London, UK) that incorporates a semiautomated edge-detection and contouring algorithm (Grimaud *et al*., 1996; Miller *et al*., 1998). For these measurements, the images for both the original (baseline, first and second year) and follow-up studies (sixth year) were re-analysed by the same observer (E.G.), who was blinded to the order of the scans. A hypointense lesion was defined as any region with low signal intensity relative to the surrounding white matter, visible on enhanced T1WI and corresponding to a region of high signal intensity on T2WI.

The measurement of cerebral volume was performed from the T1WI by a single blinded observer (A.P.) using an automated algorithm that first extracts the brain from the skull and cerebrospinal fluid (CSF) spaces, then quantifies the volume of the extracted tissue. Details of the algorithm have been fully described elsewhere (Losseff *et al*., 1996). This approach is highly reproducible, with a mean scan–rescan coefficient of variation of less than 1%, regardless of whether a 1.5 or 0.5 T magnet is used (Paolillo *et al*., 1999a, 2000). Supratentorial brain volume measurements were obtained from four selected supratentorial brain slices, the most caudal being at the level of the velum interpositum cerebri, as this was found to be the most reproducible method of covering the region of interest. Next the algorithm was applied slice by slice, with subsequent review of the effectiveness of the extraction process. In a small percentage of slices, in which the extraction was inadequate and resulted in a residual small island of skull, the non-brain regions were manually deleted when necessary.

The CC area was determined by outlining the margins of the structure as seen on the mid-sagittal T1WI. The CC area was calculated from the mean of two repeated measurements performed on two separate occasions.

Statistical analysis

All the data were analysed using SPSS software (SPSS Inc., Chicago, IL). The baseline characteristics of patients who were still being treated and patients who discontinued treatment during the 6-year period were compared by using the Student's unpaired *t*-test. The change in the annual relapse rate from baseline was tested using the Wilcoxon rank sum test.

Correlations between clinical and MRI findings were determined using the Spearman Rank Correlation Coefficient (SRCC). In addition, a stepwise regression analysis was used to identify the clinical or MRI characteristics at baseline that were able to predict subsequent changes in EDSS or brain volume. Age, sex, disease duration, EDSS at the time of the baseline scan, and the number of relapses experienced 2 years before entering the study were defined as the baseline clinical characteristics. The mean Gd-LL averaged over the 6 months before treatment, T2-LL, T1-LL, SBV and CC area on the baseline scan were considered as the baseline MRI characteristics. A *P*-value of 0.15 was required for a variable to be included in the model, whilst a *P*-value of 0.05 was required for the variable to be considered in the final model. We also investigated clinical and MRI changes in the first 2 years of treatment able to predict subsequent changes in EDSS or brain volume from year 2 to 6.

Results

Demographic and clinical characteristics

The baseline and demographic characteristics of the 68 patients (47 females, 21 males) of the original cohort have been reported in detail elsewhere (Pozzilli *et al*., 1996). Briefly, at study entry the patients' mean age was 30.5 years (SD 7.3, range 18–40), the mean disease duration was 5.0 years (SD 2.9, range 1–10), the mean and median EDSS scores were 2.2 and 2.0 (SD 0.9, range 1–5), respectively and the mean annualized exacerbation rate over the previous 2 years was 1.6 (SD 1.2).

The relapse rate for each year of the 6 years of follow-up period is shown in Fig. 1. The mean annual relapse rate was 0.52 (SD 0.67) per year, which is significantly lower (68,6%) than the mean annual relapse rate of 1.6 observed during the 2-year period before the commencement of treatment ($P < 0.01$). Seventeen of 67 patients (25.4%) remained relapse-free during the study period.

At the end of the 6-year follow-up, the mean and median EDSS scores were, respectively, 3.1 and 2.5 (range 1–6.5). When the EDSS scores at the end of follow-up were compared with the baseline examination using a categorical analysis, 10 of 67 (14.9%) had improved (EDSS $\geq$ 1 step), 30 of 67 (44.8%) were unchanged (EDSS change of $\pm$0.5 step) and 27 of 67 (40.3%) had worsened ($\geq$1 step). During the study eight of 27 patients, who showed an EDSS worsening, developed a secondary progressive MS.

The IFN-β treatment at different doses was well tolerated in the long term and most of the adverse events reported were mild in severity.

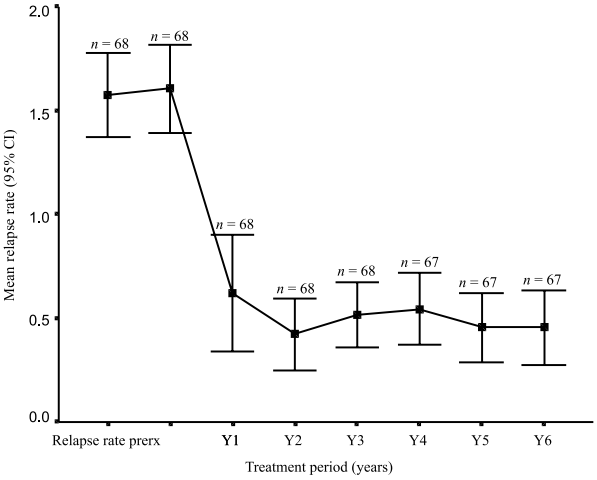

Figure 1 Mean (SD) annual relapse rate during pre-treatment (2 years before entry into the study) and the 6-year open-label treatment phase in RRMS patients. The mean reduction (68.6%) in the annual relapse rate during the 6-year treatment period was significant ($P < 0.01$).

Dropout analysis

Eleven (16.1%) of the 68 patients discontinued treatment during the 6-year follow-up period. The baseline clinical and MRI characteristics of these patients did not differ from those of patients still under high dose IFN-β (Table 1). The reasons for dropping out of the study were: one patient died following a myocardial infarct, an event which was not related to treatment; breast cancer (one patient), severe cognitive dysfunction (one patient), physician's or participant's perception of therapy ineffectiveness (four patients), and dislike of injection (two patients). Two women who remained under IFN-β treatment for 5 years dropped out only after deciding to become pregnant.

Table 2 shows mean annual relapse rate, EDSS categorical changes, treatment exposure and medications taken after IFN-α discontinuation of the 11 patients who dropped out.

The mean annualized relapse rate over the 6 years was 0.68, whilst the mean and median EDSS score at the 6-year follow-up were 3.2 and 3, respectively.

Table 1 Baseline clinical/demographic and MRI characteristics of patients who were or were not consecutively treated with high dose of IFN-β during 6-year follow-up period

	Patients under high dose treatment ($n = 57$)	Dropouts ($n = 11$)
Sex		
Male (%)	17 (30)	4 (36)
Female (%)	40 (70)	7 (64)
Age		
Mean (SD)	30.2 (7.7)	30.7 (5.6)
Range	18–44	18–43
Disease duration		
Mean (SD)	5.2 (2.8)	4.8 (2.9)
Range	1–10	1–10
EDSS score		
Median (SD)	2 (1.2)	2 (1.1)
Range	1–5	1–5
Annualized relapse rate during the previous 2 years		
Mean (SD)	1.6 (1.2)	1.5 (1.2)
Range	1–5	1–5
Baseline Gd-LL[a]		
Mean (SD)	0.49 (0.8)	0.39 (0.5)
Range	0–4.11	0–1.5
Baseline T2-LL[a]		
Mean (SD)	15.7 (11.9)	13.3 (7.5)
Range	0.81–58.1	1.8–47.8
Baseline T1-LL[a]		
Mean (SD)	3.4 (5.6)	3.5 (5.1)
Range	0.12–23.7	0.2–19.1
Baseline SBV[a]		
Mean (SD)	304.1 (47.7)	299.9 (41.8)
Range	229.1–550	230.1–391.6

[a]Gd-LL = Gadolinium lesion load; T2-LL = T2 lesion load; T1-LL = T1 lesion load; SBV = supratentorial brain volume. All MRI values are expressed in cm^3. There were no significant differences between the two groups (Student's unparied *t*-test).

Table 2 Clinical outcome, treatment exposure of patients who discontinued treatment over 6 years

Dropouts ($n = 11$)	Relapse rate (year/patient)	EDSS change[a]	IFN-β[b] treatment Duration (months)	Other medications
1°	0.33	Worsened	4	–
2°	0.83	Worsened	10	Mithoxantrone–Avonex
3°	0.66	Stable	12	Avonex
4°	1.33	Stable	18	Copaxone–Avonex
5°	1.66	Worsened	22	Mithoxantrone
6°	0.66	Worsened	23	Avonex
7°	0.66	Worsened	38[c]	–
8°	0.33	Stable	50	Avonex
9°	0.17	Improved	60	–
10°	0.33	Stable	62	–
11°	0.5	Stable	64	–

[a]Improved/stable: EDSS: ≤ 0.5 step; worsened: EDSS ≥ 1 step; [b]with high dose of IFN-β; [c]patient died.

No significant differences in term of clinical outcomes (relapse rate and EDSS change) were found between patients who discontinued and patients who were still being treated.

MRI findings

Fifty-five (82%) of 67 patients agreed to undergo the 6-year MRI evaluation. All these patients were still being treated with high dose of IFN-β.

Two patients, albeit on treatment, did not consent to have MRI scan for personal reasons.

The mean (range) Gd-LL, averaged over the 6 months before treatment was 0.49 (0–2.9) cm^3. The mean (range) Gd-LL at years 1, 2 and 6 after treatment was 0.09 (0–1.3), 0.11 (0–1.2), 0.03 (0–0.08) cm^3, respectively. Gd-LL was markedly lower during the treatment phase, corresponding to a reduction of 79% at year 1, 76% at year 2 and 94% at year 6, when compared with the baseline value ($P < 0.01$). When we considered the number of active scans (scans with at least one enhancing lesion), 37 patients (67%) showed Gd-enhancement at baseline, whilst only eight patients (14.5%) showed Gd-enhancement at the 6-year evaluation ($P < 0.01$).

Table 3 shows the mean and median values of T2-LL, T1-LL, SBV and CC area obtained from the baseline and years 1, 2 and 6 follow-up scans. There was a slight reduction, when compared with baseline, in the median T2-LL at year 1 (21%) and a further reduction was seen at year 2 of treatment (28%); conversely, at year 6 there was an increase of 7%.

The T1-LL showed a slight median increase during the first year (3%) and first 2 years (5%), subsequently increasing to 23.9% by year 6. The median SBV decreased by 1% during the first year of treatment, and by 1.7% during the first 2 years of treatment, subsequently accumulating a further decrease of 2.9% at the end of the 6-year follow-up.

The CC areas were comparable with the changes in the supratentorial structures, with decreases of 4 and 8% during the first year and first 2 years of treatment, respectively, and a further decrease of 13% at the end of the 6-year follow-up.

Clinical/MRI correlations

Correlations between the clinical and MRI variables at baseline and at year 6 of follow-up are shown in Table 4. There was a strong correlation between T1-LL

Table 3 MRI abnormalities at each time point (55 patients)

Patients ($n = 55$)	Baseline	First year of treatment	Second year of treatment	Sixth year of treatment
T2-LL (cm^3)[a]	15.74 (13.3)	14.32 (12.9)	13.45 (12.47)	16.52 (12.05)
	11.95 (0.8–58.6)	9.45 (0.9–48.4)	8.55 (0.6–46.9)	12.8 (1.75–54.4)
T1-LL (cm^3)[a]	3.94 (5.6)	4.09 (5.9)	4.16 (5.8)	4.59 (5.3)
	1.82 (0.12–23.74)	1.76 (0.06–25.84)	1.91 (0.08–27.38)	2.25 (0.1–20.91)
SBV (cm^3)[a]	306.08 (46.9)	303.15 (47.6)	300.2 (47.2)	297.73 (45.2)
	295.32 (231.2–550.6)	291.6 (226–550.4)	290.3 (221.2–544.5)	286.9 (211.5–524.9)
CC area (cm^2)[a]	481.8 (104.9)	463.5 (101.3)	453.9 (99)	425.1 (94.5)
	462.5 (266.5–742.1)	451.3 (268.4–738.3)	439.6 (247.5–732)	415.9 (251.5–652.5)

[a]T2-LL = T2 lesion load; T1-LL = T1 lesion load; SBV = supratentorial brain volume; CC = corpus callosum.

All values are expressed as mean (SD) and median (range) (below).

Table 4 Correlations between clinical and MRI parameters at baseline (R*x*0) and at 6-year follow-up

	Baseline		6-year follow-up	
Spearman's Rank Correlation ($n = 55$)	r	P	r	P
T2-LL vs. T1-LL	0.8	< 0.0001	0.85	< 0.0001
CC area vs. T2-LL	−0.35	0.01	−0.59	< 0.0001
CC area vs. T1-LL	−0.39	0.005	−0.56	< 0.001
CC area vs. SBV	0.32	0.01	0.31	0.01
SBV vs. T1-LL	−0.27	0.05	−0.3	0.03
EDSS vs. T2-LL	−0.34	0.01	−0.58	0.0002
EDSS vs. T1-LL	−0.32	0.01	−0.56	< 0.0001

Gd-LL = Gadolinium lesion load; T2-LL, T2 lesion load; T1-LL, T1 lesion load; SBV = supratentorial brain volume; CC = corpus callosum.

and T2-LL both at baseline and at year 6 ($r = 0.8$, $P < 0.001$; $r = 0.85$, $P < 0.001$, respectively). T2 and T1-LL were *weakly* correlated with the CC area ($r = -0.35$, $P = 0.01$; $r = -0.39$, 0.005, respectively) at baseline, whilst they were more strongly correlated at year 6 of follow-up ($r = -0.56$, $P = 0.0001$; $r = -0.58$; $P = 0.0001$, respectively). SBV *weakly* correlated with CC area both at baseline and at 6 years ($r = 0.32$, $P = 0.01$; $r = 0.31$, $P = 0.01$), whilst a modest correlation was also found between SBV and T1-LL ($r = -0.27$, $P = 0.05$; $r = -0.3$, $P = 0.03$). Lastly, there were small but significant correlations between baseline EDSS and T2-LL ($r = 0.34$, $P = 0.01$) and T1-LL ($r = 0.32$, 0.01), which grew slightly stronger at the end of the follow-up period ($r = 0.52$, $P = 0.0002$; $r = 0.56$, $P = 0.0001$, respectively).

No correlations were found between changes in disability and changes in T2-LL, T1-LL or CC area (absolute and percentage change) over the 6-year period. However, a significant correlation was found between EDSS worsening and SBV reduction ($r = 0.46$, $P = 0.001$).

Predictors of disability

Baseline T2-LL and T1-LL were strongly correlated with changes in the EDSS score ($r = 0.44$, $P = 0.0009$; $r = 0.50$, $P = 0.0007$, respectively). These findings are shown in Fig. 2 using a categorical distribution of the EDSS changes from baseline and confirmed using a stepwise regression model which revealed that only baseline T2-LL and T1-LL were inversely correlated with the change in EDSS over the 6 years ($F = 8.75$, $P = 0.01$; $F = 13.75$, $P = 0.001$).

Moreover, when we considered the T1:T2 lesion load ratio, patients in whom EDSS worsened by ≥1 step ($n = 27$) had a baseline mean T1:T2 ratio of 26%, compared with 12% in other patients ($n = 40$) ($P < 0.01$). No correlation was found between changes in clinical or MRI parameters during the first 2-year treatment period and changes in disability from year 2 to 6.

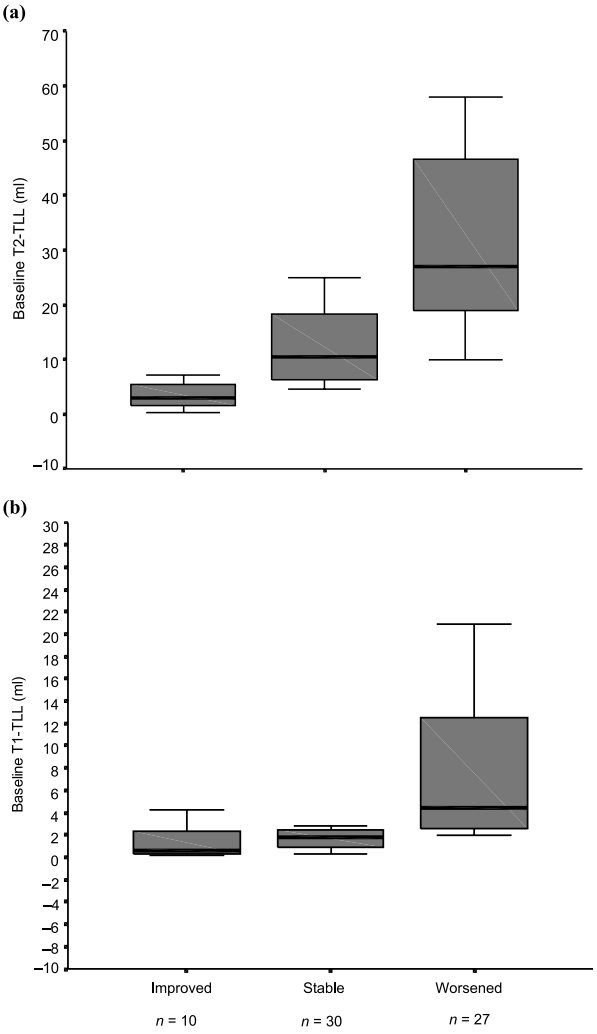

Figure 2 Boxplot showing baseline T2-TLL (a), T1-TLL (b) and categorical distribution of EDSS changes over 6 years. The rectangles represent the 75th, 50th and 25th percentiles, whilst the upper and lower bars represent the 90th and 10th percentiles.

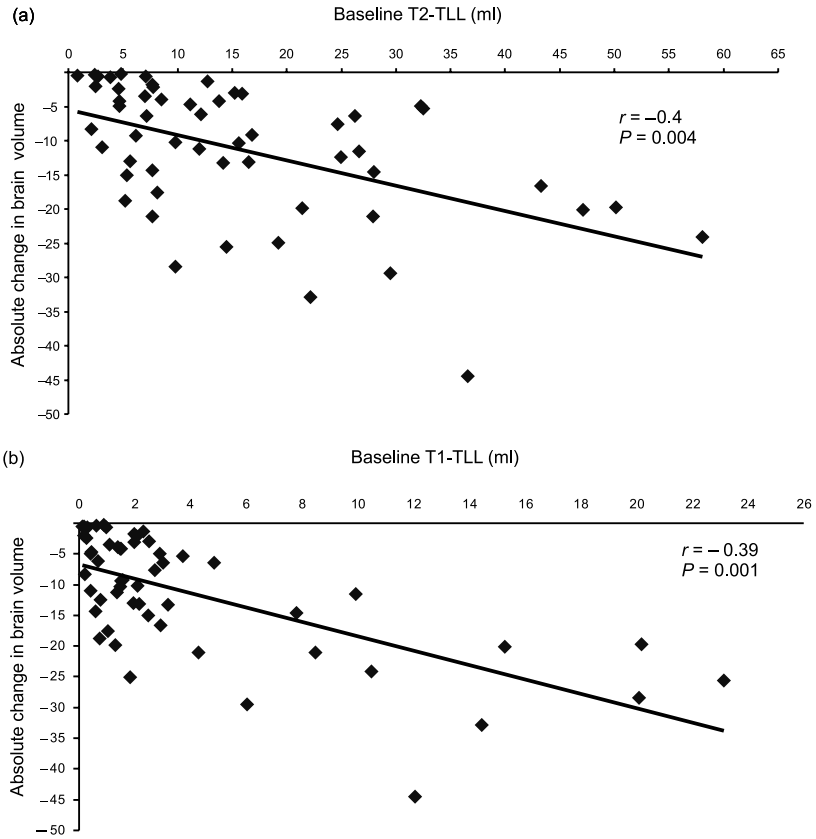

Figure 3 Scatterplot showing correlations between baseline T2-TLL (a) and T1-TLL (b) and absolute change in brain volume over 6 years.

Predictors of brain atrophy

Worse brain atrophy during treatment was associated with higher disease activity (Gd-LL) during the 6 months of the pre-treatment period ($r = -0.36$; $P = 0.01$) and with greater baseline brain tissue damage as measured by T2-LL and T1-LL ($r = -0.4$, $P = 0.004$; $r = -0.39$, $P = 0.001$, respectively) (Fig. 3). These findings were confirmed by using stepwise regression models which showed that only baseline T2-LL and T1-LL were inversely correlated with the absolute change in brain volume over the 6 years ($F = 11.75$, $P = 0.002$; $F = 6.75$, $P = 0.01$). There was also a significant correlation between changes in T1-LL from baseline to year 2, and changes in SBV from year 2 to 6 ($r = 0.35$, $P = 0.01$).

Discussion

This study documents that treatment with IFN-β is safe, well tolerated by most patients and has a prolonged effect in reducing the relapse rate compared with the pre-treatment relapse rate. An improvement in and stabilization of the neurological status, as scored by the EDSS, was found in 60% of patients. With regard to clinical/MRI correlations, the only MRI finding that strongly correlated with EDSS worsening was the development of cerebral atrophy.

Our data also show that a relationship exists between cumulative pre-treatment disease activity and the appearance of long-term atrophy and that baseline lesion burdens (both T2-LL and T1-LL) are long-term predictors of progressive cerebral atrophy as well as of clinical disability.

This study does have some limitations. Given that it is an open-label study, no placebo group is available; moreover, our cohort was given IFN-β-1a at different doses in the first 2 years (11 μg vs. 33 μg), and only a small group continued the treatment with the highest dose (33 μg); many patients switched to IFN-β-1b (8 MIU thrice weekly). The doses of IFN-β-1a used in our trial were different from those utilized in other trials involving the same drug [The Once Weekly Interferon for and Study Group (OWINS), 1999; PRISMS Study Group, 2001], therefore it follows that it is difficult for any comparison to be based on the potential efficacy of the dosage used. Despite these limitations, we believe that our observation is of substantial value because this cohort represents a well-characterized patient group who returned regularly to the same outpatient centre.

All the patients were visited by the same neurological team over the 6-year period and the majority of the patients were on long-term treatment with a high dose of IFN-β (85%). Moreover, the use of the same MRI scanner throughout the study period and the application at each time point of the same protocol further validate our data by limiting possible methodological errors. Finally, we believe that the possibility of identifying, over such a long period of time, MRI parameters which may be used as a surrogate for worsening disability are intriguing.

The results of the present study show that treatment with IFN-β seems to be able to significantly reduce relapse rate throughout the study, when compared with the 2 years before commencement of treatment; moreover, a quarter of the patients had no relapses during the treatment period. However, the results should not be emphasized as the reduction in relapse rate could be a consequence of the natural history of MS. The greater reduction over the first 2 years of our study may be related to regression to the mean effects. The only other MS treatment trial using IFN-β comparable, in duration with ours is the one by the IFN-β Study Group, which assessed the clinical effect of IFN-β-1b (IFNB Study Group, 1995) over 5 years in 166 RRMS patients. In that study, the mean pooled annual relapse rate for the entire study was slightly above that experienced by our cohort (0.78 vs. 0.52). Although our population had a similar mean age, disease duration and comparable relapse rate during the 2 years before treatment, any comparison should be made with some caution for the above-mentioned reasons, i.e. the lack in our study of a placebo group, its open-label fashion and the administration of different drugs (IFN-β-1a and IFN-β-1b).

Although a decreasing number of relapses is an attractive feature of the effect of IFN-β in RRMS, the accumulation of disability is the critical indicator of the long-term efficacy of IFN-β therapy. In our study, despite an overall increase in EDSS score from baseline to 6 years (2.2–3.1), a considerable proportion of the patients evaluated at the follow-up had not worsened (60%). Similar clinical findings have been reported in the IFN-β-1b study and in the recently published PRISMS-4 Study Group, in which stable disability was documented in 65 and 56% of treated patients over a 5- and 4-year period, respectively (IFNB Study Group, 1995; PRISMS Study Group, 2001).

Our data showed a prolonged effect of IFN-β in suppressing Gd-enhancing lesion formation. When compared with the baseline scan, Gd-LL was reduced by 76, 79 and 94% after 1, 2 and 6 years, respectively. Similar results have been reported in other studies (Pozzilli *et al.*, 1996; Stone *et al.*, 1997; Simon *et al.*, 1998; Li *et al.*, 1999) over shorter observation periods. This significant decrease in Gd-LL over time may be related to the possible prolonged effect of treatment with IFN-βs on active inflammation. However, these findings may also be influenced by the length of the study. Indeed, it is known that inflammation characterizes the early phase of MS disease and is accompanied and eventually replaced by neurodegenerative aspects, with a gradual loss of Gd-enhancing lesion appearance and the progressive development of atrophy phenomena (Paolillo *et al.*, 1999a; Molyneux *et al.*, 2000).

We observed a median T2-LL increase of 7% over the 6-year period. As regards the T2-LL over the treated period, we found a fluctuating course, with a decrease in the first 2 years and an increase in the subsequent 4 years. The reduction in the first 2 years, which has been reported in other RRMS studies (PRISMS Study Group, 1998; Simon *et al.*, 1998), probably reflects a reduction in the number of transient lesions and a resolution of inflammation and oedema. The slight increase in T2-LL seen at 6 years reflects new lesion accumulation, although Gd-enhancing lesions were even more strongly suppressed towards the end of the follow-up period. As previously reported (Molyneux *et al.*, 1998), T2 lesions can appear or enlarge even in the absence of Gd-enhancement.

Many recent studies have focused their attention on the effect of IFN-β in preventing the accumulation of T1-LL, which is thought to reflect the more severely damaged tissue in MS lesions (Gasperini *et al.*, 1999; Paolillo *et al.*, 1999b; Simon *et al.*, 2000). The median annual increase of T1-LL in the placebo-arm of many MS treatment trials or natural history studies amounts to between 10 and 15% (Truyen *et al.*, 1996; Simon *et al.*, 2000). In the present analysis, we observed a median increase in the T1-LL of 3% in the first year, and 5% in the first 2 years of treatment. The rate of change over the first 2 years is somewhat lower than that reported by Simon *et al.* who saw a median of 11.8% increase in the first 2 years (Simon *et al.*, 2000) in an RRMS patient group treated once weekly with IFN-β-1a (Avonex^{MT}; Biogen Inc., Fouzteen Cambridge Center, Cambridge, MA). The 6-year follow-up showed a median T1-LL increase of 23.9%, which correspond to an annualized rate of about 4%. However, although these data indicate an increased rate of permanent damage as the study progresses, the accumulation of T1-LL appears to be lower than that reported by natural history studies (Truyen *et al.*, 1996; Simon *et al.*, 2000).

The last 5 years have seen the development of a number of methods to measure cerebral atrophy in MS patients, which appears to be one of the most promising approaches for studying disease evolution and is

potentially another surrogate marker with which to evaluate treatment efficacy. Significant changes in brain volume have been seen in previous studies, with an average decrease of approximately 1% per year (range: 0.6–1.5%) in untreated patients with RRMS (Fox *et al*., 2000; Ge *et al*., 2000; Zivadinov *et al*., 2001). In our study, we observed a median decrease of 1% during the first year and 1.7% in the first 2 years, whilst in the subsequent 4 years the net brain volume reduction amounted to 1.2%; a similar trend was seen for the changes in the CC area. Our findings for the first 2 years of treatment are similar to those reported by others (Rudick *et al*., 1999; Simon *et al*., 1999; Ge *et al*., 2000; Zivadinov *et al*., 2001). The 6-year analysis shows, both for the SBV and CC area, a lower rate of atrophy in the subsequent 4 years of treatment when compared with the first 2 years. A number of factors might have contributed to these findings. First, limitations of the method employed, which used only four axial slices, might have influenced the estimated brain volume change. Our scans had a relatively coarse resolution, with a 5-mm slice thickness and 1 mm gap, which would have increased the random sampling errors of the method. Therefore, possible long-term drift in the scanner performance might have slightly changed the image contrast or image dimensions, which may, in turn, have resulted in changes in the measured brain volume.

However, Rovaris *et al*. (2000), when comparing regional (the same region of interest as that taken in our analysis) against global brain volume measurements in RRMS patients concluded that, in the context of clinical trials, brain volume based on regional measurement is both sensitive and reproducible, and requires much less time (Rovaris *et al*., 2000). Secondly, the profound effect of IFN-β-1a in reducing new lesion formation and dramatically facilitating the resolution of inflammation (oedema, acute inflammatory infiltrate) may have resulted in a greater loss of cerebral tissue volume during the first 2 years of treatment than during the last 4 years.

Thirdly, it could be that once treatment with IFN-β has started, the rate of atrophy is genuinely slowed down. One previous report showed an effect on atrophy rate during the second year of treatment with IFN-β-1a (Rudick *et al*., 1999). As possible explanations for this effect, the authors hypothesized, firstly, a delayed therapeutic action and, secondly, that IFN-β-1a had a beneficial effect from the first year of treatment, even if this finding only became apparent in atrophy measurements in the second year; indeed, neurodegenerative phenomenona may evolve from months to years after an injury.

Cross-sectional studies have shown that disability scored by EDSS correlates with lesion burden (both T2-LL and T1-LL). Although this correlation may be weak in the early phases of the disease (IFNB Multiple Sclerosis Study Group, 1995; Li *et al*., 1999; Rudick *et al*., 2000), our findings show that it increases markedly at the 6-year follow-up. A probable explanation for this is that in our patients the EDSS was initially low and narrowly distributed. At the 6-year follow-up, there was a far greater range of disabilities, providing more opportunity to show the EDSS–MRI relationship as already reported (Molyneux *et al*., 1998).

The reduction in brain volume represents the only MRI finding which is linked to the progression of disability in the long term. Previous reports have documented mild or non-significant associations between changes in the EDSS and the rate of atrophy (Fox *et al*., 2000; Ge *et al*., 2000). However, the current study had a much longer follow-up period, and it is possible that tissue loss (brain atrophy) and disability proceed in parallel only in the long term. This hypothesis is supported by a recent report by Fisher *et al*. who measured changes in brain volume in 106 RRMS patients originally enrolled in the Multiple Sclerosis Collaborative Research (MSCR) group phase III clinical trial and re-evaluated after 8 years. The authors found a significant correlation between a decrease in brain volume and a worsening EDSS score from year 2 to 8 (Fisher *et al*., 2000).

Several recent studies have searched for reliable surrogate MRI markers that are predictors of atrophy and progressive disability. In our study, a correlation was observed between enhancing lesion volume during the pre-treatment phase and the severity of brain atrophy; this is in accordance with previous studies (Paolillo *et al*., 1999a; Simon *et al*., 1999; Molyneux *et al*., 2000; Lin and Blhumardt, 2001) conducted over a shorter period. Gd-enhancement is one of the earliest signs of new lesion formation seen at MRI; subsequently, chronic T2-visible lesions form, which may culminate in hypointense areas (black holes) on T1WI that are thought to represent areas of permanent tissue damage. Hence, the enhancing lesions that form prior to commencement of treatment evolve via an inevitable pathological cascade that causes chronic lesions to form and ultimately results in progressive tissue loss expressed by brain atrophy (Fig. 4). In addition, our data indicate that, within our RRMS population, baseline lesion burden (T2-LL and T1 black holes) represents the most important predictor of long-term ongoing disability. These findings are consistent with those observed in patients with a clinically isolated syndrome (Filippi *et al*., 1994; O'Riordan *et al*., 1998; Sailer *et al*., 1999; Brex *et al*., 2002) as well as in those with RRMS (Rudick *et al*., 2000). However, all these studies are based on untreated patient groups, whereas

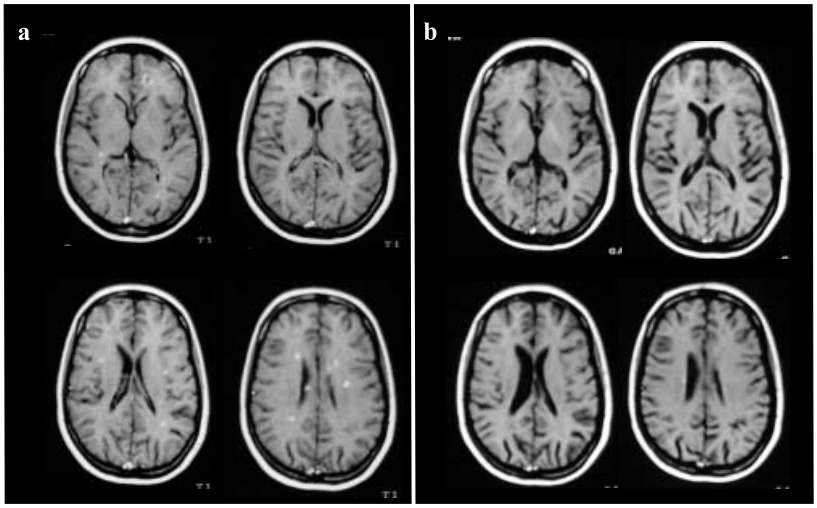

Figure 4 Enhanced MR scan of the same patient at baseline (A) and 6-year later (B). High disease activity is seen at the baseline scan; the 6 years follow-up does not show enhancement but severe cortical-subcortical atrophy is clearly evident.

our analysis, for the first time, has identified predictive MRI parameters in RRMS patients receiving disease-modifying treatments (IFN-β) during a long-term period.

Moreover, we observed a higher T1:T2 lesion load ratio at baseline in patients whose EDSS score was significantly worse 6 years later. These data point to the existence of different lesion patterns, with some patients having more aggressive MRI features associated with an earlier and faster development of clinical disability.

Taken together, these findings imply that IFN-β treatment might have moderate effect in modifying the course of MS over 6 years, unless a preventive treatment is started early in the course of the disease when the extent of lesion burden is modest. On the other hand, the correlation between baseline MRI lesion burden and functional impairment during the course of the study could be suggestive of a more aggressive disease in which the treatment is less effective.

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