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Brain damage as detected by magnetization transfer imaging is less pronounced in benign than in early relapsing multiple sclerosis

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The trend to start disease-modifying therapy early in the course of multiple sclerosis makes it important to establish whether the benign form is a real entity. In previous studies, measures of magnetization transfer (MT) ratio (MTr) have been shown to provide good estimates of the amount of tissue damage occurring in multiple sclerosis brains. Thus, with the hypothesis that if benign multiple sclerosis patients were really benign, sensitive measures of subtle tissue damage would be less pronounced in these patients than in very early relapsing–remitting (RR) multiple sclerosis patients. We carried out conventional MRI and MT imaging in 50 patients with benign multiple sclerosis [defined as having Kurtzke Expanded Disability Status Score (EDSS) <3 and disease duration >15 years] and in 50 early RR patients selected to have similar disability (EDSS <3) and short disease duration (<3 years). Data were compared with those of 32 demographically-matched normal controls. We used a fully automated procedure to measure lesional-MTr, perilesional-MTr, normal-appearing white matter (NAWM) MTr and cortical-MTr. We found that, after correction for common effects of age, lesional-MTr and perilesional-MTr of benign patients were significantly ($P < 0.0001$) lower than WM of normal controls, but significantly ($P < 0.0001$) higher than corresponding tissues of RR patients. In NAWM and cortex, MTr values of benign patients were similar to those of normal controls ($P > 0.5$) and significantly higher than those of the RR patients ($P < 0.0001$ and $P < 0.01$, respectively). Similar differences in MTr measures between benign and RR patients were found when patient groups were selected to have no disability (EDSS $\leqslant$ 2) and, for benign multiple sclerosis, very long disease duration (>20 years) or when both groups were matched for high lesion load (T_2-weighted lesion volume >10 cm^3). We conclude that lesional and non-lesional MTr values can be significantly less pronounced in benign multiple sclerosis than in a cohort of RR patients at their earliest disease stages, suggesting that brain tissue damage is milder in benign multiple sclerosis than in early RR disease. This can be due to an extraordinary beneficial response to demyelination of benign patients and may represent the evidence that benign multiple sclerosis truly exists and might be differentiated from other forms of this illness.

Keywords: multiple sclerosis; benign; magnetization transfer; demyelination; remyelination

Abbreviations: EDSS = Kurtzke Expanded Disability Status Score; GM = grey matter; LV = lesion volume; MTr = magnetization transfer ratio; NAWM = normal-appearing white matter; RR = relapsing–remitting; T_1-W = T_1-weighted; T_2-W = T_2-weighted; WM = white matter

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Introduction

Multiple sclerosis is an inflammatory disease characterized by deficits referable to multiple demyelinating lesions disseminated in the CNS. There is a pronounced individual variability in the clinical course of this disease and a significant subgroup of patients may have little disease progression and minimal or no disability many years after the

first clinical manifestation of the disease (Mcalpine, 1961; Hawkins and McDonnell, 1999). The term *benign* is generally used to define this form of the disease (Hawkins and McDonnell, 1999).

There is a general consensus to classify as benign multiple sclerosis patients who are 'fully functional' after 15 years or more from the clinical onset (Hawkins and McDonnell, 1999). As this form may account for about one-fourth of all cases of multiple sclerosis, it is, in principle, not rare (Ramsaransing *et al.*, 2001). However, prospective studies have shown that the classification as 'benign' may sometimes be temporary and, for example, a number of patients who fulfilled the definition of benign multiple sclerosis do show a significant worsening of their disease course in years (Hawkins and McDonnell, 1999; Pittock *et al.*, 2004). With this background, there is a need to best define this form and to identify whether it really exists. In addition, the trend to treat early multiple sclerosis patients with disease-modifying therapies, which would largely be unnecessary for benign patients, provides an additional important reason to attempt to identify markers that may be indicators of the benign course.

Over the past decade, the use of new MR methodologies in clinical studies of multiple sclerosis patients has contributed greatly to the understanding of the clinical–pathological manifestations of this disease. A few MR studies have attempted a comparison between the brain tissue damage occurring in patients with benign multiple sclerosis with that of patients with the relapsing and/or progressive form of the disease (Filippi *et al.*, 1995; Horsfield *et al.*, 1996; Falini *et al.*, 1998; van Waesberghe *et al.*, 1998; Davie *et al.*, 1999; Filippi *et al.*, 2000; Traboulsee *et al.*, 2003; Brass *et al.*, 2004; Minneboo *et al.*, 2005). However, these reports, which were somehow limited by the small number of subjects studied, have shown discrepant results. Thus, whether tissue destruction is less pronounced in patients with benign multiple sclerosis than in other forms of this disease is still mostly unknown.

In previous studies, magnetization transfer (MT) imaging has been demonstrated to be able to provide accurate estimates of tissue damage. The measure provided by MT (i.e. the MT ratio, MTr) has important implications in the clinical and pathological evolution of multiple sclerosis (Filippi *et al.*, 2000) and *ex vivo* MT studies of multiple sclerosis brains have proven that MTr can give a good, quantitative measure of brain damage (van Waesberghe *et al.*, 1999; Barkhof *et al.*, 2003; Schmierer *et al.*, 2004). Thus, in the present study, we used a fully automated method to accurately assess MTr in the white matter (WM) lesions, normal-appearing WM (NAWM) and cortical grey matter (GM) of patients who had either a benign course of the disease or were at a very early stage of the relapsing disease course. The hypothesis is that if benign multiple sclerosis patients were really benign, sensitive measures of subtle tissue damage would be less pronounced in these patients than in very early relapsing–remitting multiple sclerosis patients (RR).

Patients and Methods

Study population

We studied 100 patients with clinically definite multiple sclerosis. They were consecutively selected from the patient population referring at three multiple sclerosis clinics of the University of Siena, University of Florence and Hospital of Empoli. As mentioned above, patients had either a benign disease course ($n = 50$, 34 female and 16 male) or were early RR ($n = 50$; 33 female and 17 male). The benign multiple sclerosis patients were defined as having a Kurtzke Expanded Disability Status Score (EDSS) of $\leqslant 3.0$ after at least 15 years from the clinical onset of the disease. In contrast, the early RR patients were selected to have clinical disability similar to that of the benign group (EDSS $\leqslant 3$), but much shorter disease duration ($\leqslant 3$ years). All multiple sclerosis patients were relapse-free and had not been taking steroids for at least one month before study entry. Eight out of 50 benign patients and 16 out of 50 RR patients were in treatment with β-interferons or glatiramer acetate at the time of study entry. For each patient, neurological evaluation, which included the rating of disability using the EDSS, was performed within 24 h of the performance of the MR examination by an experienced observer who was kept blinded to the MRI results. The latter were compared to the MR results of 32 demographically matched normal controls (*see* Statistical analysis), who were recruited from laboratory and hospital workers and were included in the group if they had normal neurological examination and no history of neurological disorders. The study was approved by the Ethics Committee of the Faculty of Medicine of the University of Siena and informed consent was obtained from all participating subjects.

MR examinations

All subjects were examined using an identical MR protocol. Acquisitions of brain MRIs were obtained in a single session using a Philips Gyroscan operating at 1.5 T (Philips Medical Systems, Best, The Netherlands) at the NMR centre of the University of Siena. A sagittal survey image was used to identify the anterior commissure (AC) and posterior commissure (PC). A dual-echo, turbo spin-echo sequence (TR/TE1/TE2 = 2075/30/90 ms, 256 × 256 matrix, 1 signal average, 250 mm field of view, 50 contiguous 3 mm slices) yielding proton density (PD) weighted and T_2-weighted (T_2-W) images was acquired in the transverse plane parallel to the line connecting the AC and PC. Subsequently, an MT sequence was performed acquiring two transverse T_1-weighted (T_1-W), gradient echo images, one without (No Sat) and one with (Sat) MT saturation pulses (TR/TE = 35 ms/10, 256 × 256 matrix, 1 signal average, 250 mm field of view). This sequence yielded image volumes of 50 slices, 3 mm thick, oriented to exactly match the PD/T_2-W. The MT pulse was a 1.2 ms on-resonance, $1\bar{2}1$ binomial pulse (radio-frequency field strength = 20 μT) placed just before each slice-selective excitation (Pike *et al.*, 1993). The MRI acquisition of all subjects involved in the study was interleaved and was done within 1 year. Monthly quality assurance sessions and no major hardware upgrades were carried out on the scanner during the time of study.

MR data analysis

Lesion volumes

Classification of T_2-W and T_1-W lesion volume (LV) was performed in each patient by a single observer, unaware of subject

identity, employing a segmentation technique based on user-supervised local thresholding. For the T_2-W LV classification, lesion borders were determined primarily on PD weighted images, but information from T_2-W and T_1-W images were also considered as the software used (Jim 3.0, Xinapse System, Leicester, UK) offered the ability to toggle between the PD, T_2-W and T_1-W images, providing the operator with convenient access to the information in both datasets while defining lesions. Hypointense WM T_1-W lesions were defined as those lesions with signal intensity between that of the GM and the CSF on T_1-W scans (van Waesberghe *et al.*, 1998). In both T_2-W and T_1-W images, the value of total brain LV was calculated by multiplying lesion area by slice thickness.

MT

For the analysis of MT data, we used a fully automated procedure (*see* an example of the output in Fig. 1). Saturated (Sat) images were registered to No-Sat images using a registration method previously described [FLIRT (Smith *et al.*, 2004)]. The brain was extracted from both Sat and No-Sat images using a previously described method [BET (Smith *et al.*, 2004)] and MTr images were then calculated using the formula MTr = 100 × (No-Sat−Sat)/No-Sat (Pike *et al.*, 2000). The extracted No-Sat images were then segmented into different tissue types (GM, WM and CSF) using a previously described segmentation method [FAST (Smith *et al.*, 2004)]. To best avoid partial volume effects in GM and WM, these resulting probabilistic tissue-class images were thresholded to retain voxels with tissue-class probability equal to or greater than $P = 0.75$. This gives fairly conservative GM and WM binary images which were applied to the MTr image to produce GM-MTr and WM-MTr images. To select identical brain regions in each subject, standard space WM and GM masks (made on the MNI152 average normal brain—McConnell Brain Imaging Centre, Montreal Neurological Institute) were automatically applied in native space to the WM-MTr and GM-MTr images using the MNI152-to-native brain space transformation derived during registration. For an even more conservative measurement of the WM-MTr, we thresholded the standard space WM mask to retain voxels with a frequency >60% (thus selecting regions that are more broadly conserved across individuals). This also excludes potential residual voxels from deep GM. For selective MTr measurement of neocortical brain regions, a standard space mask (made on the MNI152 average normal brain and including ventricles, deep GM, cerebellum and brain stem) was used to separate segmented GM-MTr into neocortical and non-neocortical tissues. To further avoid contamination of image noise or potential remaining partial volume with CSF, all voxels of the neocortical regions with values <10% of the GM-MTr mean were excluded. In MS patients, voxels fully inside the lesions and those around the lesions (obtained using non-binary morphological dilatation—of one voxel in x and y dimensions—of the lesion mask) were masked out from the MTr image and assessed separately. Finally, mean values (averaging all voxels contained in the given region) from lesional-MTr, perilesional-MTr, 'non-adjacent-to-lesions' NAWM-MTr and cortical-MTr were evaluated.

Statistical analysis

The non-parametric Mann–Whitney test was used for comparisons of the two groups of multiple sclerosis patients. Values of MTr for RR and benign patients were compared to those of a healthy normal control group. As the group of benign patients were significantly ($P < 0.001$) older than the RR group (benign patients age range = 34–69 years, median = 49 years; RR patients age = 19–45 years, median = 30 years), before statistical comparisons, MR data were corrected for using a Z-score transformation relative to an age-matched normal control group for each patient group (normal control group for comparison with benign patients = 16 subjects, age range: 35–63 years, median = 50; normal control group for comparison with RR patients = 16 subjects, age range: 21–52 years, median = 29). This allowed us to control for potential age-related differences in MTr between patient subgroups. Differences between patient and normal control groups were assessed using analysis of variance (ANOVA) followed by pairwise *post hoc* comparison using Tukey's HSD procedure to account for multiple comparisons. A voxel-based analysis of lesional-MTr for benign and RR multiple sclerosis was done by averaging registered Z-score lesional-MTr images of each subject for each group. The SYSTAT software version 9 running on Windows (copyright SPSS Inc. 1998) was used to perform statistical calculations. Data were considered significant at the 0.05 level.

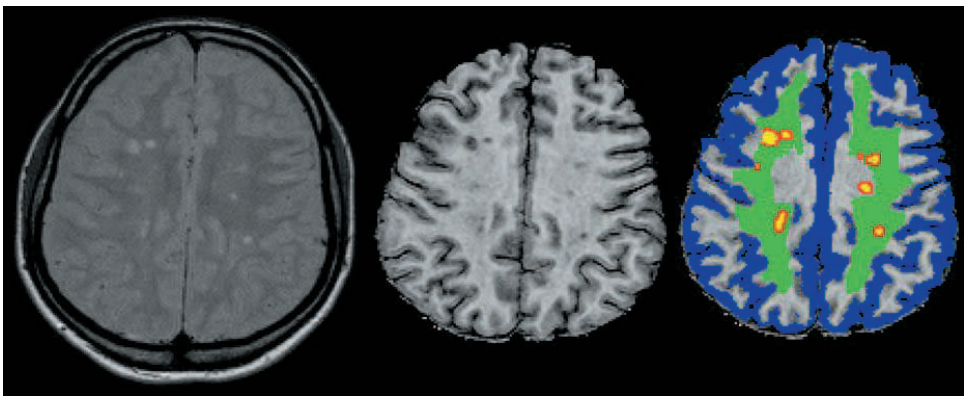

Fig. 1 Typical transverse proton density MR image of a multiple sclerosis patient (*left*), an example of the corresponding MTr image (*centre*) and an illustrative example of the segmented image used for the automatic analysis of the MT data (*right*). In the latter image, note the segmented WM lesions (yellow) and perilesions (red), and the conservative segmentation of the WM non-adjacent to demyelinating lesions (green) and the cortical GM (blue). See Patients and Methods for details.

Results

Clinical and demographic differences between benign and RR multiple sclerosis patients

The two groups of multiple sclerosis patients had similar EDSS (mean EDSS in benign multiple sclerosis = 1.4 ± 0.9, range = 0–3; mean EDSS in RR multiple sclerosis = 1.4 ± 0.7; range = 0–3, $P = 0.8$). As expected, benign patients had much longer disease duration (mean disease duration in benign patients = 22.5 ± 6 years, range = 15–40 years; mean disease duration in RR patients = 1.2 ± 1 years; range = 0–3 years, $P < 0.0001$) and, as mentioned previously (*see* Statistical analysis), were older than RR patients.

Lesion volumes in benign and RR multiple sclerosis patients

Measures of T_2-W LV, T_1-W LV and the ratio between T_1-W and T_2-W LV were all significantly higher in benign multiple sclerosis than in RR multiple sclerosis (T_2-W LV: benign = 14.2 ± 13 cm^3, RR = 7.3 ± 7 cm^3, $P = 0.003$; T_1-W LV: benign = 5.3 ± 6 cm^3, RR 2.3 ± 2 cm^3, $P = 0.002$; T_1-W LV/T_2-W LV: benign = 0.33 ± 0.1; RR 0.27 ± 0.1, $P = 0.02$; *see* Fig. 2). However, lesion values of the two patient groups were not statistically different after correcting the values for age and disease duration ($P > 0.2$ for both).

MTr values in benign and RR multiple sclerosis patients

This analysis of the raw data showed that lesional and perilesional MTr values were significantly ($P < 0.0001$ for all measures) higher in B-MS than in RR-MS (T_2-W lesional-MTr: B-MS = 27.5 ± 2, RR-MS = 24.1 ± 2; T_2-W perilesional-MTr: B-MS = 33.1 ± 1, RR-MS = 31.9 ± 1; T_1-W lesional-MTr: B-MS = 22.8 ± 3, RR-MS = 19.9 ± 2; T_1-W perilesional-MTr: B-MS = 30.5 ± 3, RR-MS = 28.0 ± 1). The NAWM MTr values were similar between the two patient groups ($P = 0.3$), and, when compared with normal controls, were significantly ($P = 0.005$) lower in RR-MS patients and did not reach significant levels ($P = 0.1$) in B-MS (NAWM MTr: normal controls = 35.3 ± 0.9, B-MS = 34.9 ± 0.9, RR-MS = 34.5 ± 0.8). Finally, in the brain cortex, MTr values of B-MS patients were similar to those of normal controls (cortical-MTr: normal controls = 22.9 ± 0.5, B-MS = 22.7 ± 0.9, $P = 0.5$) and significantly higher than those of the RR-MS group (cortical-MTr = 22.1 ± 0.9, $P = 0.01$).

Age-corrected MTr values in benign and RR multiple sclerosis patients

As benign multiple sclerosis patients were much older than RR patients and, at least in the WM of the normal controls, MTr values decrease with increasing age (WM-MTr versus age = −0.70, $P < 0.0001$; cortical-MTr versus age: $r = -0.20$, $P < 0.2$), we used a *Z*-score transformation relative to an age-matched normal control group for each patient group (*see* Statistical analysis) to control for potentially spurious results because of differences in age between patient subgroups.

After correcting for the effects of age using the *Z*-score transformation, values of both T_2-W and T_1-W lesional-MTr and perilesional-MTr of benign patients were significantly ($P < 0.0001$) lower than those of the WM of normal controls, but also significantly ($P < 0.0001$) higher than the respective lesional values of RR patients (*see* Fig. 3 and Table 1). A voxel-based analysis of T_2-W lesions clearly showed higher MTr values in benign multiple sclerosis than in RR patients (Fig. 4). In NAWM and cortex, MTr values of benign patients were similar to those of normal controls ($P > 0.5$) and significantly higher than those of the RR patients ($P < 0.0001$ and $P < 0.01$, respectively, *see* Fig. 3 and Table 1).

Interestingly, similar results were obtained for all MTr measures when multiple sclerosis patients of both groups were selected to have no disability (EDSS ⩽ 2) and benign patients were also selected for very long disease duration (>20 years, *see* Table 1).

Overlapping results were also found when, to test the importance of the WM T_2 lesion load on lesional and non-lesional MTr values in the two patient groups, only patients with high WM lesion load (T_2-W LV > 10 cm^3) were selected from the whole patient population (*see* Table 1).

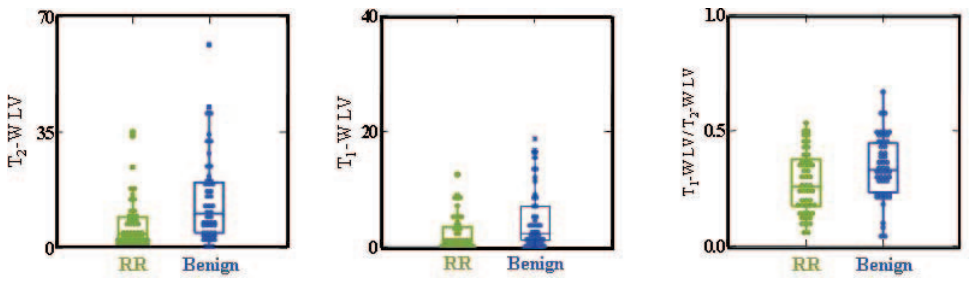

Fig. 2 Box plots comparing measures of T_2-W LV (*left*), T_1-W LV (*centre*) and the ratio between T_1-W and T_2-W LV (*right*) in benign and RR multiple sclerosis. Note that all measures are significantly ($P = 0.003$, $P = 0.002$ and $P = 0.02$, respectively) higher in the benign than in the RR patients. However, all lesional values were not statistically different ($P > 0.2$) after correcting the values for age and disease duration.

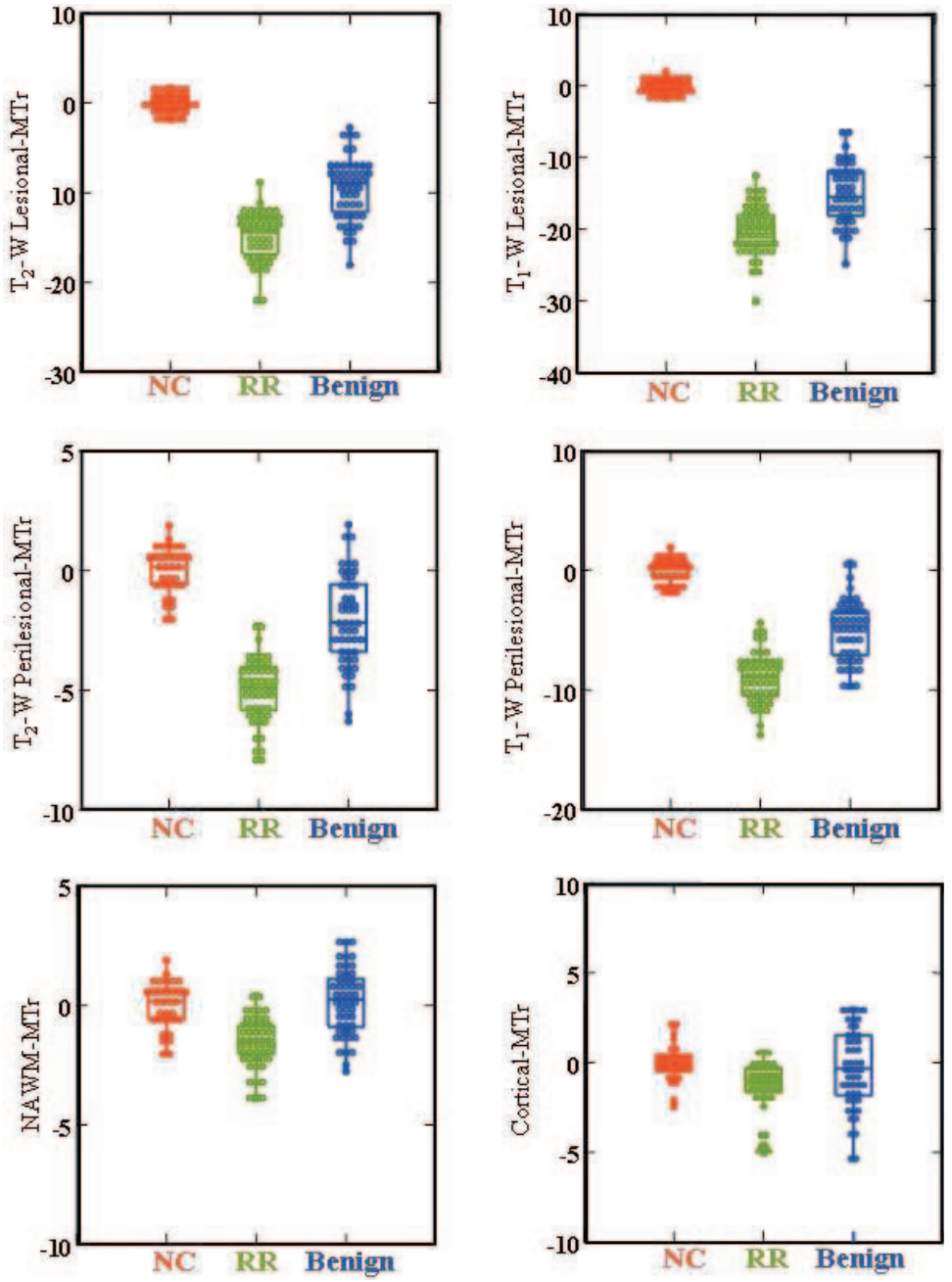

Fig. 3 Box plots comparing the standardized MTr measures of normal controls ($n = 32$, *left box,* NC) RR patients ($n = 50$, *centre box,* RR) and benign patients ($n = 50$, *left box*, Benign). Values are relative to T_2-W and T_1-W lesional-MTr (*top*), T_2-W and T_1-W perilesional-MTr (*centre*), NAWM and cortical-MTr (*bottom*). Data are Z-score transformed to correct for differences in age between the two multiple sclerosis groups. Note the differences between the three groups with generally higher MTr values in benign than in RR multiple sclerosis patients (see Table 1 for details).

Discussion

Owing to the growing consensus to initiate the treatment early in the course of the disease (Rudick, 1999), it has become important to understand whether the benign form of multiple sclerosis is a real entity and whether there might be patients for whom the expensive and often uncomfortable treatment with disease-modifying therapies would be unnecessary. Clinically, it has been recently demonstrated that the longer the duration of multiple sclerosis and the lower the disability, the more a patient is likely to have a good prognosis (Pittock *et al.*, 2004). On these bases, it is conceivable that there should be a percentage of patients with definite multiple sclerosis that along with a very mild disease course should have mild underlying brain pathology. It is also true, however, that all the attempts to identify clinical, immunological and genetic markers able to differentiate benign

Table 1 MTr in relapsing–remitting and benign multiple sclerosis patients. Values are expressed as *Z*-scores relative to an age-matched normal control group for each patient group

	Whole group		MS patients with EDSS ⩽2 and B-MS with disease duration >20 years		MS patients with T_2-W LV > 10 cm^3	
	B-MS	RR MS	B-MS (*n* = 23)	RR MS (*n* = 46)	B-MS (*n* = 24)	RR MS (*n* = 10)
T_2-W lesional-MTr	−9.4 ± 3*†	−14.8 ± 2†	−9.4 ± 3*†	−15.0 ± 2†	−10.0 ± 3*†	−16.9 ± 2†
T_2-W perilesional-MTr	−2.0 ± 2*†	−5.0 ± 1†	−2.2 ± 2*†	−5.0 ± 1†	−2.4 ± 1*†	−5.8 ± 1†
T_1-W lesional-MTr	−14.9 ± 4*†	−20.1 ± 3†	−15.4 ± 4*†	−20.1 ± 3†	−16.0 ± 4*†	−21.0 ± 3†
T_1-W perilesional-MTr	−4.9 ± 2*†	−8.9 ± 2†	−6.1 ± 3*†	−8.9 ± 2†	−6.1 ± 2*†	−9.4 ± 2†
NAWM-MTr	−0.1 ± 1*	−1.6 ± 1†	−0.1 ± 1*	−1.5 ± 1†	−0.4 ± 1*	−2.6 ± 1†
Cortical-MTr	−0.3 ± 2$	−1.4 ± 1‡	−0.8 ± 3$	−1.3 ± 1‡	−1.3 ± 2$,‡	−3.1 ± 2†

B-MS = benign multiple sclerosis; RR = relapsing–remitting; LV = lesion volume; MTr = magnetization transfer ratio; NAWM = normal-appearing white matter; EDSS = Expanded Disability Status Scale.
*Significantly higher ($P < 0.0001$) than RR; †significantly lower ($P < 0.0001$) than normal controls; $significantly higher ($P < 0.01$) than RR; ‡significantly lower ($P < 0.01$) than normal controls.

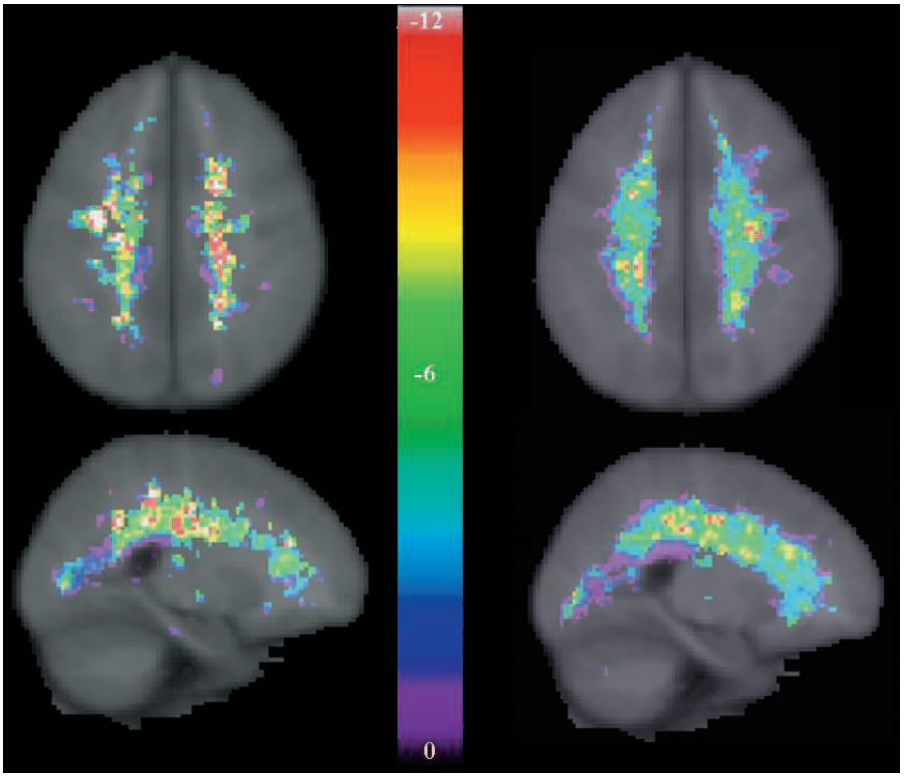

Fig. 4 Illustrative example of the voxel-based analysis of the T_2-W lesional-MTr relative to RR multiple sclerosis (*left*) and benign multiple sclerosis (*right*) patient groups. The colour overlay created on top of the MNI152 standard brain shows, for each voxel containing T_2-W-lesions in each patient group, the mean of the *Z*-score deviations from the WM-MTr values of the normal controls. The colour scale shows lowest *Z*-score values in black-violet and highest *Z*-score values in red-white. Note that *Z*-score values of T_2-W lesional-MTr are diffusely lower in the RR group than in the benign group despite the fact that the latter shows much higher lesion load.

patients from other multiple sclerosis patients have provided inconclusive results (Ramsaransing *et al.*, 2001; Galboiz and Miller, 2002; Schmidt *et al.*, 2002).

Some previous MR studies have explored whether differences in brain tissue damage may be present between patients with benign multiple sclerosis and those with other forms of the disease (Filippi *et al.*, 1995; Horsfield *et al.*, 1996; Falini *et al.*, 1998; van Waesberghe *et al.*, 1998; Davie *et al.*, 1999; Filippi *et al.*, 2000; Traboulsee *et al.*, 2003; Brass *et al.*, 2004; Minneboo *et al.*, 2005). These studies have reported conflicting results, probably owing to the small number of subjects studied as well as the inconsistency in the definition of benign multiple sclerosis and the variability of the disease control group. In some of these studies, however, brain MTr

values were slightly higher in benign patients than in those with other forms of multiple sclerosis (Filippi *et al.*, 2000), suggesting that this MR index could be used to estimate whether or not the mild disease course of benign patients is associated with mild brain tissue damage.

Against this background, we formulated the hypothesis that if a group of clinically classified benign multiple sclerosis patients was 'truly benign' its brain tissue damage should be mild, being less pronounced in this patient group than in a group of RR multiple sclerosis patients at their early disease stage. To investigate this, we made automated assessments of MTr in different brain regions of a normal control group and two relatively large and strictly defined groups of benign and early RR patients. Results of the study showed that (i) MTr values of benign multiple sclerosis were significantly lower than those of normal controls in T_1-W and T_2-W WM lesional and perilesional regions, but they were not different from those of normal controls in substantial regions of NAWM and cortical GM; (ii) despite the fact that total WM lesional load was significantly more pronounced in benign multiple sclerosis than in early RR multiple sclerosis, lesional and perilesional MTr decreases of benign multiple sclerosis were significantly less pronounced than those of RR patients; (iii) MTr values of NAWM and cortical GM regions also were significantly higher in benign multiple sclerosis than in RR multiple sclerosis and (iv) lesional and non-lesional MTr differences between benign and RR multiple sclerosis did not change when MTr measures were obtained in patients who were matched for high cerebral T_2-W lesion load or were selected for absence of clinical disability (EDSS $\leqslant 2$) and, for the benign groups, for very long disease duration (>20 years).

MT imaging, which is based on the interactions between the free water protons and protons attached to macromolecules (Grossman *et al.*, 1994), has proven in several studies to be superior to conventional MRI in the detection and quantitation of subtle brain tissue changes (Filippi, 2003; Fazekas *et al.*, 2005; Horsfield, 2005). As low MTr indicates a reduced capacity of the macromolecules in brain tissue to exchange magnetization with the surrounding water molecules, which seems to be strongly associated with the degree of tissue (matrix) damage (Grossman *et al.*, 1994), MTr measures should be able to provide information with considerable pathological specificity, and MTr reductions in lesions and normal-appearing brains of multiple sclerosis patients should reflect damage to cellular structures (Dousset *et al.*, 1992; Grossman *et al.*, 1994). Thus, results of the present study, by showing largely more decreased MTr values in all measured brain regions of early RR multiple sclerosis than in those of benign multiple sclerosis, suggest that brain damage might effectively be milder in patients classified as benign on the basis of their long and mild clinical course than in a cohort of patients with similar disability, but being at the earliest stage of their disease.

Recently, some studies, in the attempt to identify the biological substrate of MT imaging changes, have correlated post-mortem MRI and histopathological findings in multiple sclerosis brains (Barkhof *et al.*, 2003; Schmierer *et al.*, 2004; Chen *et al.*, 2005*b*). All these studies have demonstrated that MTr reductions are the expression of subtle pathology mostly related to myelin damage. The data on MRI visible lesions are particularly relevant: while histological areas of either demyelination or remyelination are all equally hyperintense on T_2-W images, MTr decreases seem to be less marked in remyelinated than in demyelinated areas. Thus, measures of MTr may be able to differentiate demyelination from remyelination in a given lesion, and the finding reported here of significantly less MTr changes in WM lesions of benign patients than in those of early RR multiple sclerosis could be interpreted as being attributed to a more beneficial response to demyelination in patients with benign rather than in RR multiple sclerosis.

Multifocal brain multiple sclerosis lesions are usually associated with various degrees of demyelination and remyelination. Demyelination leads to axonal injury and disability. Remyelination guarantees axonal preservation and favourable clinical evolution. Therefore, a method for an accurate estimation of remyelination and demyelination at the voxel level is desirable as it can provide important insights into disease outcome (Barkhof *et al.*, 2003). A voxel-based analysis of MTr seems to offer this (*see* Fig. 2), and the fully automated method used here adds to other recent methods (Audoin *et al.*, 2004; Chen *et al.*, 2005*a*) in suggesting that the voxel-based analysis of the MTr signals could potentially provide surrogate markers for an accurate assessment of subtle, microscopic pathology occurring in the brains of patients with multiple sclerosis (Chen *et al.*, 2005*b*).

Accurate voxel-based analysis of MTr is, however, difficult (Tanabe *et al.*, 1997; Smith, De Stefano, 2002; Audoin *et al.*, 2004). Because MTr is dependent on tissue type (as well as factors of interest, such as pathology), it is not possible to simply transform MTr images into standard space to carry out a voxel-wise analysis across subjects (Smith, De Stefano, 2002). Here, we presented a way to get around this problem: to split the raw MTr image into very conservative (to prevent partial volume effects) probabilistic tissue-class images of GM and WM and select identical brain regions in each subject by masking these images with standard space WM and GM masks. For multiple sclerosis lesions, voxels fully inside the lesions and those around the lesions can be masked out from the original MTr image and assessed separately. Then, the average of all voxels contained in a given region (i.e. WM, GM or lesions) and a voxel-wise analysis of these regions (*see* example in Fig. 4 for voxel-wise analysis of T2-W lesions) are both possible. This fully automated method can give a more accurate, unbiased assessment than methods based on manually detected region-of-interest and a more detailed analysis than histogram-based methods (Tanabe *et al.*, 1997; Audoin *et al.*, 2004; Horsfield, 2005). Limitation may still arise from the loss of voxels in brain regions with greater partial volume (i.e. WM-GM junction, CSF-GM borders) owing to their exclusion during the segmentation and masking processes,

but this is somehow compensated for by the increased specificity in tissue type classification.

The results of the present study provide indications that lesional and non-lesional tissue damage can be milder in benign multiple sclerosis than in early RR multiple sclerosis, and this is true even when patient groups were selected to have no disability and, for benign multiple sclerosis, very long disease duration (>20 years) or when both groups were matched for high lesion load. This may represent the biological evidence that patients with the benign form of multiple sclerosis truly exist and might explain why this class of patients seem to have a 90% likelihood of remaining clinically stable in the future (Pittock *et al.*, 2004). As a small part of the early RR patients studied here will become benign in the future, a much bigger challenge would be to be able to recognize them in advance. Unfortunately, the design of the present study did not allow us to predict who from the early RR patient group is going to become benign, a question that would require a longitudinal study to be properly answered. In this type of study, MTr measures, which have already been shown to be predictive of clinical and pathological evolution in multiple sclerosis (Santos *et al.*, 2002; Rovaris *et al.*, 2003), can certainly play a role as the surrogate marker of pathology that could best distinguish benign patients from other multiple sclerosis patients.

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