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Correlation of Multiple Sclerosis Measures Derived from T2-Weighted, T1-Weighted, Magnetization Transfer, and Diffusion Tensor MR Imaging

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BACKGROUND AND PURPOSE: **In multiple sclerosis (MS), the severity of tissue damage can vary from edema and inflammation to irreversible demyelination and axonal loss. Compared with conventional T2-weighted MR imaging, magnetization transfer (MT) and diffusion tensor (DT) MR imaging provide quantitative indices with increased specificity to the most destructive aspects of MS. To increase our understanding of the pathophysiologic processes of MS, we assessed the correlations between MT and DT MR imaging–derived metrics and the correlations between these quantities and measures derived from conventional MR in patients with MS.**

METHODS: **T2-weighted, T1-weighted, MT, and DT MR images of the brain were obtained from 34 patients with relapsing-remitting MS (RRMS) and 15 age-matched control subjects. T2 and T1 lesion volumes (LV) and brain volume were measured. MT ratio (MTR), mean diffusivity ($\bar{D}$), and fractional anisotropy (FA) histograms from the overall brain tissue (BT) and the normal-appearing brain tissue (NABT) were obtained. Average lesion MTR, $\bar{D}$, and FA were also calculated. The correlations between T2 and T1 LV, brain volume, MT-, and DT-derived metrics were assessed with the Spearman rank correlation coefficient.**

RESULTS: **No significant correlations were found between MT and FA histogram–derived metrics and quantities derived from conventional MR scans (T2 and T1 LV and brain volume). On the contrary, T2 and T1 LV (but not brain volume) were significantly correlated with the average $\bar{D}$ values of BT and NABT (r values ranging from 0.52 to 0.78). No significant correlation was found between MT- and DT-derived metrics.**

CONCLUSION: **These results suggest that MT and DT MR imaging provide, at least partially, independent measures of lesion burden in patients with RRMS. This suggests that a multiparametric MR approach has the potential for increasing our ability to monitor MS evolution.**

Multiple sclerosis (MS) is an immune-mediated disorder selectively affecting the central nervous system. The pathologic hallmark of MS is inflammatory demyelination, which can be limited by reparative mechanisms (including remyelination) or can become irreversible and ultimately lead to tissue loss (1). In MS, the progressive accumulation of tissue damage is likely to be one of the major factors contributing to disabling neurologic deficits (2). As a consequence, achieving an accurate in vivo assessment of pathologic signs of MS might be a rewarding exercise for increasing our understanding of the mechanisms leading to irreversible disability and our ability to monitor the efficacy of experimental treatments. In this context, measures derived from conventional MR imaging, such as the load of T2 hyperintense lesions, have several limitations, including the lack of specificity to the heterogeneous pathologic substrates of MS lesions (3, 4) and the inability to detect subtle abnormalities in the normal-appearing white matter (NAWM) (5–8). This can explain why, in patients with MS, the correlation between clinical and conventional MR findings is, at best, moderate (4). Magnetization transfer (MT) and diffusion tensor (DT) MR imaging, may, however, go some way toward overcoming these limitations.

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MT is based on the interactions between protons in a relatively free environment and those wherein motion is restricted. In the brain, these two states correspond to the protons in tissue water, and in the macromolecules of myelin and cell membranes. Off-resonance irradiation is applied that saturates the magnetization of the less mobile protons and is then transferred to the mobile protons, thus reducing the observable signal intensity. Low MT ratio (MTR) indicates a reduced capacity of the macromolecules in brain tissue to exchange magnetization with the surrounding water molecules, reflecting damage to myelin or to the axonal membrane (9). A postmortem study found a correlation between low MTR and the percentage of residual axons in MS lesions (10). Animal studies have also shown that low MTR correlates with histopathologic findings of myelin loss and axonal destruction (11), whereas edematous lesions result in slightly increased MTR values (12). Dramatically reduced MTR is also seen in the ''pure'' demyelinating lesions of patients with progressive multifocal leukoencephalopathy (13) or central pontine myelinolysis (14).

Diffusion is the microscopic random translational motion of molecules, and water molecular diffusion can be measured in vivo by use of diffusion-weighted MR imaging (15). Because diffusion is affected by the properties of the medium wherein molecular motion occurs (16), the measurement of diffusion inside biological tissues provides information about tissue structure at a microscopic level (17). The motion of water molecules can be hindered by the presence of structural barriers at a cellular or subcellular level. Pathologic processes that alter tissue organization by decreasing or increasing the number of barriers to water molecular motion or that alter the permeability of the barriers cause abnormal water diffusivity. In addition, diffusion is inherently a three-dimensional process, and in some tissues with an oriented microstructure, such as brain white matter, the molecular mobility is not the same in all directions. This property is called anisotropy, and results in a variation in the measured diffusivity with tissue measurement direction (18, 19). White matter fiber tracts consist of aligned myelinated axons and, therefore, hindrance of water diffusion is much greater across rather than along the major axis of axonal fibers (20). Under these conditions, a full characterization of diffusion can only be found in terms of a tensor (21), a 3×3 matrix where the on-diagonal elements represent the diffusion coefficients along the axes of the reference frame, whereas the off-diagonal elements account for the correlations between molecular displacement along orthogonal directions. From the tensor, it is possible to derive some scalar indices, invariant to the changes in the frame of reference, which reflect the diffusion characteristics of the tissue. These measures include 1) the mean diffusivity (D) (equal to one third of the trace of the diffusion tensor), which is a measure of the average molecular motion independent of any tissue directionality and is affected by cellular size and integrity (22, 23); and 2) the fractional anisotropy (FA), which is one of the most commonly used measures of deviation from isotropy (22) and reflects the degree of alignment of cellular structures within fiber tracts, as well as their structural integrity. The pathologic elements of MS have the potential to alter the permeability or geometry of structural barriers to water molecular diffusion in the brain and, consistent with this, water diffusivity is higher and FA lower in MS lesions than in NAWM and in white matter of healthy volunteers (24–26).

MT and DT characteristics can be analyzed on a region of interest (ROI) basis or on a more global basis by using histogram analysis (8, 27–30), an approach that allows evaluation of all the brain tissue, thus providing an assessment of both macroscopic and microscopic disease burden in MS. Because the relative contributions of conventional, MT, and DT MR imaging to study MS in vivo have never been investigated, aims of this study were to investigate the magnitude of the correlations between various MT and DT MR imaging–derived metrics and between these quantities and measures derived from conventional MR imaging.

Methods

Subjects

We studied 34 patients (21 female and 13 male patients) with clinically definite, relapsing-remitting MS (RRMS) (31). Their mean age was 34.8 years (SD, 7.5), the median duration of the disease was 6.5 years (range, 1–20), and the median Expanded Disability Status Scale (EDSS) score (32) was 1.5 (range, 0.0–4.5). All patients had neither relapses nor steroid treatment during the 3 months preceding study initiation. Fifteen age-matched healthy volunteers (nine female and six male subjects) served as controls. Their mean age was 34.0 years (SD, 9.6). All subjects signed a written informed consent form prior to study entry. The study was approved by the local ethics committee.

Image Acquisition

Brain MR imaging was performed at 1.5 T. During a single session, the following were performed without moving the subject from the unit: 1) dual-echo turbo spin-echo imaging (3300/16–98 [TR/TE]; acquisition, 1; echo train length, 5); 2) T1-weighted conventional spin-echo imaging (768/15 [TR/TE]; acquisitions, 2); 3) 2D gradient-echo (GE) imaging (600/12 [TR/TE]; acquisitions, 2; flip angle, 20°), with and without an off-resonance RF saturation pulse (offset frequency, 1.5 kHz; gaussian envelope duration, 7.68 ms; flip angle, 500°); and 4) a pulsed-gradient spin-echo echo-planar pulse sequence (interecho spacing, 0.8; TE, 123), with diffusion gradients applied in eight noncolinear directions, chosen in order to cover three-dimensional space uniformly. The duration and maximum amplitude of the diffusion gradients were 25 ms and 21 mTm^{-1}, respectively, giving a maximum b factor in each direction of 1044 s mm^{-2}. In order to optimize the measurement of diffusion, only two b factors were used (33) ($b_1 \approx 0$, $b_2 = 1044$ s mm^{-2}). Fat saturation was performed using a 4-RF pulse binomial pulse train to avoid the chemical shift artifact. A bird cage head coil of ~300-mm diameter was used for RF transmission and for signal reception.

For the dual-echo, T1-weighted, and GE images, 24 contiguous interleaved axial sections were acquired with a 5-mm section thickness, 256 × 256 matrix, and 250 × 250-mm field of view. The sections were positioned to run parallel to a line that joins the most inferoanterior and inferoposterior parts of the corpus callosum (34). For the DT MR images, 10 axial sections with a 5-mm section thickness, 128 × 128 matrix, and 250 × 250-mm field of view were acquired, with the same orientation as the other images and the second-last caudal section positioned to match exactly the central slices of these sets. This brain portion was chosen because the periventricular area is a common location for MS lesions. In addition, these central sections are less affected by the distortions due to B_0 field inhomogeneity, which can affect image coregistration.

Image Analysis and Postprocessing

An experienced observer examined the hard copies of the proton density (PD)-weighted and T1-weighted scans and marked the PD hyperintense and the T1 hypointense lesions. T2-weighted images were always used to increase confidence in lesion identification. Using the marked hard copies as a reference, a trained technician outlined lesions as ROI on a computer display and measured the T2 and T1 lesion volumes (LV) using a semiautomated technique based on local thresholding (35).

Brain volume was measured from T1-weighted images by using a seed growing technique for brain parenchyma segmentation. This method is based on signal intensity thresholding. A seed point was positioned in any part of the cerebral parenchyma, and from this seed, an ROI was grown. This ROI contained all connected pixels within two given signal intensity values. The upper and lower signal intensity for seed growing could be interactively changed on a section-by-section basis. If the ROI crossed the border of interest, a manual boundary was drawn to limit the seed growing. At the end of the segmentation process, the tissue volume was calculated by multiplying the number of pixels included in the ROI for the voxel size. All brain volume measurements were done by the same observer who identified the MS lesions.

From the two GE images, with and without the saturation pulse, MTR maps were derived pixel-by-pixel according to the following equation: $\text{MTR} = (M_0 - M_S)/M_0 \times 100\%$, in which M_0 is the signal intensity for a given pixel without the saturation pulse and M_S is the signal intensity for the same pixel when the saturation pulse is applied. MTR maps were then coregistered with the dual-echo T2-weighted images by using an algorithm based on mutual information (36).

DT MR images were first corrected for distortion induced by eddy currents by using an algorithm that minimizes mutual information between the diffusion unweighted and weighted images (36). Then, assuming a monoexponential relationship between signal intensity and the product of the b matrix (a 3 × 3 matrix that expresses the relationship between the signal attenuation and the elements of the diffusion tensor matrix) and diffusion tensor matrix components, the diffusion tensor was calculated for each pixel according to the following equation:

$$\frac{M}{M_0} = \exp\left(-\sum_{i=1}^{6}\sum_{j=1}^{6} b_{ij}D_{ij}\right),$$

where M is the measured signal intensity, M_0 is the T2-weighted signal intensity, b_{ij} are the elements of the b matrix, and D_{ij} are the elements of the diffusion tensor matrix. The tensor was estimated by nonlinear regression using the Marquardt-Levenberg method. After diagonalization of the estimated tensor matrix, $\bar{D}$ and FA were derived for every pixel. The diffusion images were interpolated to the same image matrix size as that of the dual-echo images, and then the $b = 0$ step of the echo-planar scans (T2-weighted, but not diffusion-weighted) was coregistered with the dual-echo T2-weighted images by using an algorithm based on mutual information (36). The same transformation parameters were then used to coregister the $\bar{D}$ and FA images to the dual-echo images.

Lesion outlines on PD-weighted images were automatically transferred onto the coregistered MTR, $\bar{D}$, and FA images, and the area, $\bar{D}$, and FA of each lesion measured. Then the average lesion $\bar{D}$ and FA, weighted by lesion area (28), were calculated for each patient.

Histograms of MTR, $\bar{D}$, and FA maps were created as previously described (8, 27, 28), after removal of the extracerebral tissue and of CSF, by using the same technique applied to segment lesions (35). Only the brain portion covered by both MT and DT MR images (ie, the central 10 sections) entered the histogram analysis. To correct for the between-subject differences in brain volume, each histogram was normalized by dividing the height of each histogram bin by the total number of pixels contributing to the histogram. MTR and $\bar{D}$ histograms were derived from the overall brain tissue studied (BT) and from the normal-appearing brain tissue (NABT) (ie, the tissue not involved by macroscopic T2 hyperintense lesions). To obtain the MTR and $\bar{D}$ histograms of NABT, MS lesion outlines from T2-weighted scans were automatically transferred onto the coregistered MTR and $\bar{D}$ maps and then nulled out. FA histograms were derived only from the BT. For all the histograms, the average MTR, $\bar{D}$, and FA values were calculated, as well as the heights and locations of the peaks of the histograms.

Statistical Analysis

The univariate correlations between the different MR-derived measures and between the different MR-derived measures and EDSS were assessed using the Spearman rank correlation coefficient. Group comparisons were assessed using a two-tailed Student t test for non-paired data. To reflect the large number of statistical comparisons, only P values $< .001$ were considered statistically significant. Owing to the exploratory nature of this study, we did not apply a more rigorous statistical correction for multiple comparisons in order to minimize the risk of type II errors. Only the correlations that were found to be statistically significant or whose magnitude was at least moderate (ie, with an r value ≥ 0.3) are reported.

Results

In Table 1, the values of the different conventional, MT, and DT MR quantities studied are reported for the whole patient sample. No T2-hyperintense or T1-hypointense lesions were found on the scans of healthy control subjects. Average $\bar{D}$ was significantly lower (0.90×10^{-3} mm^2 s^{-1}, $P = .0001$) and average MTR significantly higher (40.3%, $P = 0.0001$) in control subjects than in patients. All the other MTR and $\bar{D}$ histogram–derived metrics also significantly differed between control subjects and MS patients (P values ranging from .003 to .0001).

No significant correlation was found between any of the MTR and FA histogram–derived metrics and conventional MR measures (T2 and T1 LV and brain volume). Modest, but statistically insignificant correlations were found between T2 and T1 LV and the peak heights of the MTR histograms (r values ranging from -0.31 to -0.45). All the BT and NABT $\bar{D}$ histogram–derived metrics were significantly correlated with T2 and T1 LV (Table 2), whereas they were not significantly correlated with brain volume.

TABLE 1: Measures derived from conventional, MT, and DT MR imaging from 34 RRMS patients

	Mean	Median	Range
T2 LV (mL)	17.1	12.2	0.4–106.9
T1 LV (mL)	3.6	2.4	0.0–17.8
Brain volume (mL)	1136.6	1143.5	916.0–1424.0
Average lesion MTR (%)	36.4	36.3	31.1–42.6
Average lesion $\bar{D}$ ($\times 10^{-3}$ mm^2 s^{-1})	0.95	0.96	0.66–1.11
Average lesion FA	0.25	0.33	0.19–0.34
BT average MTR (%)	38.8	38.7	36.2–42.7
BT MTR peak height	112.2	110.3	92.4–136.6
BT MTR peak position (%)	33.6	33.5	30.0–39.0
NABT average MTR (%)	38.8	38.8	36.3–42.7
NABT MTR peak height	112.9	111.8	92.4–136.8
NABT MTR peak position (%)	33.6	33.5	30.0–39.0
BT average $\bar{D}$ ($\times 10^{-3}$ mm^2 s^{-1})	0.95	0.94	0.87–1.10
BT $\bar{D}$ peak height	93.3	95.1	55.3–117.6
BT $\bar{D}$ peak position ($\times 10^{-3}$ mm^2 s^{-1})	0.78	0.78	0.72–0.90
NABT average $\bar{D}$ ($\times 10^{-3}$ mm^2 s^{-1})	0.95	0.94	0.90–1.09
NABT $\bar{D}$ peak height	93.6	96.0	55.2–118.9
NABT $\bar{D}$ peak position ($\times 10^{-3}$ mm^2 s^{-1})	0.78	0.78	0.72–0.90
BT average FA	0.21	0.21	0.19–0.23
BT FA peak height	45.2	44.6	37.4–53.1
BT FA peak position	9.9	10.0	8.0–12.0

Note.—See text for abbreviations.

TABLE 2: Correlations between $\bar{D}$ histogram–derived metrics and T2 and T1 LV in 34 RRMS patients

	T2 Lesion Volume		T1 Lesion Volume	
	r	*P*	*r*	*P*
BT $\bar{D}$ ($\times 10^{-3}$ mm^2 s^{-1})	0.78	< .001	0.71	< .001
BT $\bar{D}$ peak height	−0.68	< .001	−0.65	< .001
BT $\bar{D}$ peak position ($\times 10^{-3}$ mm^2 s^{-1})	0.58	< .001	0.53	< .001
NABT average $\bar{D}$ ($\times 10^{-3}$ mm^2 s^{-1})	0.77	< .001	0.70	< .001
NABT $\bar{D}$ peak height	−0.65	< .001	−0.63	< .001
NABT $\bar{D}$ peak position ($\times 10^{-3}$ mm^2 s^{-1})	0.57	< .001	0.52	< .001

Note.–See text for abbreviations and statistical analysis.

No significant correlations were found among the corresponding quantities of the MTR, *D*, and FA histograms. Modest, but statistically insignificant correlations were found among the average BT *D* and FA ($r = -0.39$), the peak positions of the MTR histograms, and the average *D* values from both BT and NABT (*r* values ranging from −0.34 to −0.43) and among the average FA and the peak heights of BT or NABT *D* histograms ($r = 0.32$). Average lesion MTR, *D*, and FA were neither significantly correlated nor showed a trend toward significance.

None of the MR quantities we measured were significantly correlated with patient EDSS score. EDSS was modestly, but not significantly, correlated only with T1 LV ($r = 0.36$).

Discussion

Although the assessment of lesion burden from T2-weighted MR scans is widely used as a surrogate marker of disease evolution in MS studies (4), abnormalities seen on T2-weighted images do not provide specific information regarding the heterogeneous pathologic substrates of MS lesions (3, 4), which can range from inflammation and vasogenic edema to irreversible demyelination and axonal loss. Another major limitation of T2-weighted MR imaging is its inability to detect and quantify the extent and severity of the microscopic damage known to occur in NAWM (5–8). The nature and extent of lesions and NAWM pathologic abnormalities are likely to influence MS manifestations and evolution (8, 37, 38). For instance, evidence of tissue loss (1) and widespread NAWM changes (8) can be found in MS patients with severe and irreversible neurologic disability, even when T2 and enhancing lesion loads are relatively low. For all these reasons, quantitative MR techniques, with the potential to provide more pathologically specific and accurate information about MS, have recently been introduced in the assessment of MS patients (2–4). MT and DT MR imaging are two of the most promising techniques for at least two reasons. First, they provide quantitative and objective measures. Second, they allow a large brain coverage,

which is important in multifocal and widespread diseases, such as MS. Previous MS studies used MT or DT MR imaging in isolation and, therefore, did not have the opportunity to investigate the correlation among quantities derived from these techniques. Because different MR techniques necessarily provide overlapped information, defining the magnitude of such a correlation is an important prerequisite to avoid the acquisition of redundant MR data.

In this study, we found that quantities derived from MTR histogram analysis of the BT and NABT are not significantly correlated with the corresponding quantities from *D* and FA histograms. This confirms and extends the results of a previous study that used diffusion-weighted MR imaging and showed a lack of correlation between average MTR and *D* of BT (27). We also did not find significant correlations between lesion MTR, *D*, and FA. These observations suggest that MT and DT MR imaging provide relatively independent measures of MS pathologic abnormalities and that their combined use might result in a gain of relevant information leading to an understanding of the mechanisms underlying the clinical manifestations of the disease. Although correlative studies with histopathology are needed, we believe that the lack of correlation between MTR and DT MR metrics in the brain tissue is the result of the complex relationship between destructive (inflammation, demyelination, and axonal loss) and reparative (remyelination and gliosis) mechanisms occurring within and outside T2-visible lesions and their variable effects on MTR, *D*, and FA values. That MTR and DT MR metrics provide complementary and partially independent information is supported by another finding that DT histogram metrics are strongly correlated with T2 and T1 LV, whereas quantities derived from MTR histograms are modestly, but not significantly, correlated.

We did not find any correlation between brain volume and quantities derived from MT and DT MR imaging. The measurement of brain volume has recently been suggested as a marker of MS severity with the potential to monitor the disease evolution accurately (39). Several studies found that MR imaging measures of brain atrophy were correlated with the level of disability or the course of the disease (40–44) and found moderate correlation between brain volume and MTR histogram–derived metrics (45–47). The discrepancy between our results and those of previous studies (45–47) might be due to the clinical characteristics of the patients we studied, who were mildly disabled by RRMS. Although brain volumes vary markedly in healthy individuals and, as a consequence, a normalization of brain volumes would have been desirable (43), this observation suggests that brain atrophy measurement might not be sensitive to the most early and subtle aspects of the MS pathologic abnormalities. It is indeed conceivable that brain atrophy in MS is a late phenomenon, which follows the appearance of other evidences of tissue loss and disorganization, detectable by MT and DT MR imaging. This is consistent with recent MR spectroscopy findings, indicating that the presence of axonal damage can be detected in RRMS patients in the absence of concomitant decreases of brain volume (48).

Conclusion

Although preliminary and warranting further investigation, our results suggest that the application of different MR techniques with variable sensitivity to the heterogeneous pathologic aspects of MS might contribute to a better understanding of MS pathophysiology and call for a multiparametric MR approach in the study of patients with MS.

References

1. Coles AJ, Wing MG, Molyneux P, et al. **Monoclonal antibody treatment exposes three mechanisms underlying the clinical course of multiple sclerosis.** *Ann Neurol* 1999;46:296–304
2. Arnold DL. **Magnetic resonance spectroscopy: imaging axonal damage in MS.** *J Neuroimmunol* 1999;98:2–6
3. Filippi M. **The role of non-conventional magnetic resonance techniques in monitoring evolution of multiple sclerosis.** *J Neurol Neurosurg Psychiatry* 1998;64Suppl 1:S52–S58
4. Miller DH, Grossman RI, Reingold SC, McFarland HF. **The role of magnetic resonance techniques in understanding and managing multiple sclerosis.** *Brain* 1998;121:3–24
5. Filippi M, Campi A, Dousset V, et al. **A magnetization transfer imaging study of normal-appearing white matter in multiple sclerosis.** *Neurology* 1995;45:478–482
6. Filippi M, Tortorella C, Bozzali M. **Normal-appearing-white-matter changes in multiple sclerosis: the contribution of magnetic resonance techniques.** *Mult Scler* 1999;5:273–282
7. Loevner LA, Grossman RI, Cohen JA, Lexa FJ, Kessler D, Kolson DL. **Microscopic disease in normal-appearing white matter on conventional MR images in patients with multiple sclerosis: assessment with magnetization-transfer measurements.** *Radiology* 1995;196:511–515
8. Tortorella C, Viti B, Bozzali M, et al. **A magnetization transfer histogram study of normal-appearing brain tissue in MS.** *Neurology* 2000;54:186–193
9. Grossman RI. **Magnetization transfer in multiple sclerosis.** *Ann Neurol* 1994;36[Suppl]:S97–S99
10. van Waesberghe JH, Kamphorst W, De Groot Cj, et al. **Axonal loss in multiple sclerosis lesions: magnetic resonance imaging insights into substrates of disability.** *Ann Neurol* 1999;46: 747–754
11. Brochet B, Dousset V. **Pathological correlates of magnetization transfer imaging abnormalities in animal models and humans with multiple sclerosis.** *Neurology* 1999;53[Suppl] 3:S12–S17
12. Dousset V, Grossman RI, Ramer KN, et al. **Experimental allergic encephalomyelitis and multiple sclerosis: lesion characterization with magnetization transfer imaging.** *Radiology* 1992;182: 483–491
13. Dousset V, Armand JP, Lacoste D, et al. **Magnetization transfer study of HIV encephalitis and progressive multifocal leukoencephalopathy. Groupe d'Epidemiologie Clinique du SIDA en Aquitaine.** *AJNR Am J Neuroradiol* 1997;18:895–901
14. Silver NC, Barker GJ, MacManus DG, Miller DH, Thorpe JW, Howard RS. **Decreased magnetisation transfer ratio due to demyelination: a case of central pontine myelinosis.** *J Neurol Neurosurg Psychiatry* 1996;61:208–209
15. Le Bihan D, Breton E, Lallemand D, Grenier P, Cabanis E, Laval-Jeantet M. **MR imaging of intravoxel incoherent motions: application to diffusion and perfusion in neurologic disorders.** *Radiology* 1986;161:401–407
16. Tanner JE, Stejskal EO. **Restricted self-diffusion of protons in colloidal systems by the pulsed gradients spin-echo method.** *J Chem Phys* 1968;49:1768–1777

17. Le Bihan D, Turner R, Moonen CT, Pekar J. **Imaging of diffusion and microcirculation with gradient sensitization: design, strategy, and significance.** *J Magn Reson Imaging* 1991;1:7–28
18. Chenevert TL, Brunberg JA, Pipe JG. **Anisotropic diffusion in human white matter: demonstration with MR techniques in vivo.** *Radiology* 1990;177:401–405
19. Cleveland GG, Chang DC, Hazlewood CF, Rorschach HE. **Nuclear magnetic resonance measurement of skeletal muscle: anisotropy of the diffusion coefficient of the intracellular water.** *Biophys J* 1976;16:1043–1053
20. Beaulieu C, Allen PS. **Determinants of anisotropic water diffusion in nerves.** *Magn Reson Med* 1994;31:394–400
21. Basser PJ, Mattiello J, Le Bihan D. **Estimation of the effective self-diffusion tensor from the NMR spin-echo.** *J Magn Reson B* 1994;103:247–254
22. Basser PJ, Pierpaoli C. **Microstructural and physiological features of tissues elucidated by quantitative-diffusion-tensor MRI.** *J Magn Reson B* 1996;111:209–219
23. Pierpaoli C, Jezzard P, Basser PJ, Blarnett A, Di Chiro G. **Diffusion tensor MR imaging of the human brain.** *Radiology* 1996; 201:637–648
24. Droogan AG, Clark CA, Werring DJ, Barker GJ, McDonald WI, Miller DH. **Comparison of multiple sclerosis clinical subgroups using navigated spin echo diffusion-weighted imaging.** *Magn Reson Imaging* 1999;17:653–661
25. Filippi M, Iannucci G, Cercignani M, Assunta Rocca M, Pratesi A, Comi G. **A quantitative study of water diffusion in multiple sclerosis lesions and normal-appearing white matter using echo-planar imaging.** *Arch Neurol* 2000;57:1017–1021
26. Werring DJ, Clark CA, Barker GJ, Thompson AJ, Miller DH. **Diffusion tensor imaging of lesions and normal-appearing white matter in multiple sclerosis.** *Neurology* 1999;52: 1626–1632
27. Cercignani M, Iannucci G, Rocca MA, Comi G, Horsfield MA, Filippi M. **Pathological damage in MS assessed by diffusion-weighted and magnetization transfer MRI.** *Neurology* 2000;54: 1139–1144
28. Filippi M, Iannucci G, Tortorella C, et al. **Comparison of MS clinical phenotypes using conventional and magnetization transfer MRI.** *Neurology* 1999;52:588–594
29. Nusbaum AO, Tang CY, Wei TC, Buchsbaum MS, Atlas SW. **Whole-brain diffusion MR histograms differ between MS subtypes.** *Neurology* 2000;54:1421–1427
30. van Buchem MA, McGowan JC, Kolson DL, Polansky M, Grossman RI. **Quantitative volumetric magnetization transfer analysis in multiple sclerosis: estimation of macroscopic and microscopic disease burden.** *Magn Reson Med* 1996;36:632–636
31. Lublin FD, Reingold SC. **Defining the clinical course of multiple sclerosis: results of an international survey. National Multiple Sclerosis Society (USA) Advisory Committee on Clinical Trials of New Agents in Multiple Sclerosis.** *Neurology* 1996;46: 907–911
32. Kurtzke JF. **Rating neurological impairment in multiple sclerosis: an expanded disability status scale (EDSS).** *Neurology* 1983;33:1444–1452
33. Bito Y, Hirata S, Yamamoto E. **Optimal gradient factors for ADC measurements [abstract].** *Proc Intl Soc Magn Reson Med* 1995;2:913
34. Miller DH, Barkhof F, Berry I, Kappos L, Scotti G, Thompson AJ. **Magnetic resonance imaging in monitoring the treatment of multiple sclerosis: concerted action guidelines.** *J Neurol Neurosurg Psychiatry* 1991;54:683–688
35. Rovaris M, Filippi M, Calori G, et al. **Intra-observer reproducibility in measuring new putative MR markers of demyelination and axonal loss in multiple sclerosis: a comparison with conventional T2-weighted images.** *J Neurol* 1997;244:266–270
36. Studholme C, Hill DL, Hawkes DJ. **Automated three-dimensional registration of magnetic resonance and positron emission tomography brain images by multiresolution optimization of voxel similarity measures.** *Med Phys* 1996;24:25–35
37. Filippi M, Tortorella C, Rovaris M, et al. **Changes in the normal appearing brain tissue and cognitive impairment in multiple sclerosis.** *J Neurol Neurosurg Psychiatry* 2000;68:157–161
38. Iannucci G, Tortorella C, Rovaris M, Sormani MP, Comi G, Filippi M. **Prognostic value of MR and magnetization transfer imaging findings in patients with clinically isolated syndromes suggestive of multiple sclerosis at presentation.** *AJNR Am J Neuroradiol* 2000;21:1034–1038
39. Jagust WJ, Noseworthy JH. **Brain atrophy as a surrogate marker in MS: faster, simpler, better?** *Neurology* 2000;54:782–783
40. Filippi M, Mastronardo G, Rocca MA, Pereira C, Comi G. **Quantitative volumetric analysis of brain magnetic resonance imaging from patients with multiple sclerosis.** *J Neurol Sci* 1998; 158:148–153
41. Fox NC, Jenkins R, Leary SM, et al. **Progressive cerebral atrophy in MS: a serial study using registered, volumetric MRI.** *Neurology* 2000;54:807–812
42. Ge Y, Grossman RI, Udupa JK, et al. **Brain atrophy in relapsing-remitting multiple sclerosis and secondary progressive multiple sclerosis: longitudinal quantitative analysis.** *Radiology* 2000;214:665–670
43. Losseff NA, Wang L, Lai HM, et al. **Progressive cerebral atrophy in multiple sclerosis. A serial MRI study.** *Brain* 1996; 119:2009–2019
44. Rudick RA, Fisher E, Lee JC, Simon J, Jacobs L. **Use of the brain parenchymal fraction to measure whole brain atrophy in relapsing-remitting MS. Multiple Sclerosis Collaborative Research Group.** *Neurology* 1999;53:1698–1704
45. Phillips MD, Grossman RI, Miki Y, et al. **Comparison of T2 lesion volume and magnetization transfer ratio histogram analysis and of atrophy and measures of lesion burden in patients with multiple sclerosis.** *AJNR Am J Neuroradiol* 1998;19: 1055–1060
46. Rovaris M, Bozzali M, Rodegher M, Tortorella C, Comi G, Filippi M. **Brain MRI correlates of magnetization transfer imaging metrics in patients with multiple sclerosis.** *J Neurol Sci* 1999; 166:58–63
47. Miki Y, Grossman RI, Udupa JK, et al. **Differences between relapsing-remitting and chronic progressive multiple sclerosis as determined with quantitative MR imaging.** *Radiology* 1999; 210:769–774
48. Collins DL, Narayanan S, Caramanos Z, De Stefano N, Tartaglia MC, Arnold DL. **Relation of cerebral atrophy in multiple sclerosis to severity of disease and axonal loss [abstract].** *Neurology* 2000;54:A17