Department of Veterans Affairs

Journal of Rehabilitation Research and Development
Vol. 39 No. 2, March/April 2002
Pages 243–260

MR-based technology for in vivo detection, characterization, and quantification of pathology of relapsing-remitting multiple sclerosis

Marco Rovaris, MD, and Massimo Filippi, MD
Neuroimaging Research Unit, Department of Neuroscience, Scientific Institute and University Ospedale San Raffaele, Milan, Italy

Abstract—In relapsing-remitting (RR) multiple sclerosis (MS), conventional magnetic resonance (MR) imaging (MRI) has proved to be a valuable tool to assess the lesion burden and activity over time. However, conventional MRI cannot characterize and quantify the tissue damage within and outside such lesions and only can provide some gross measures reflecting the presence of irreversible tissue damage, such as the load of T1 "black holes" and the severity of brain or cord atrophy. Other MR-based techniques, including cell-specific imaging, magnetization transfer (MT) MRI (MT-MRI), diffusion-weighted (DW) MRI (DW-MRI), proton magnetic resonance spectroscopy (^{1}H-MRS), and functional MRI (fMRI), have the potential to overcome this limitation and, consequently, to provide additional information about the nature and the extent of MS tissue damage, which would inevitably remain undetected when only a conventional MRI is obtained. Cell-specific imaging should result in a better definition of the cellular mechanisms associated with MS inflammation. Metrics derived from MT- and DW-MRI can quantify the structural changes occurring within and outside lesions visible on conventional MRI scans. ^{1}H-MRS could add information on the biochemical nature of such changes. fMRI is a promising technique to assess the mechanisms of cortical reorganization, which may limit the consequences of an MS-related injury. The application of these MR techniques to the study of RRMS is likely to provide useful insights into the pathophysiology of this disease and to improve our ability to assess the efficacy of experimental treatments.

Address all correspondence and requests for reprints to Massimo Filippi, Neuroimaging Research Unit, Department of Neuroscience, Scientific Institute and University Ospedale San Raffaele, via Olgettina 60, 20132 Milan, Italy; +39-02-26433032, fax: +39-02-26433054, email: filippi.massimo@hsr.it.

Key words: *cell-specific imaging, diffusion-weighted MRI, functional MRI, magnetic resonance imaging, magnetization transfer MRI, proton magnetic resonance spectroscopy, relapsing-remitting multiple sclerosis.*

INTRODUCTION

In recent years, major advances in the use of magnetic resonance (MR) imaging (MRI) for assessing patients with multiple sclerosis (MS) have been achieved. Conventional MRI (i.e., dual-echo spin-echo and postcontrast T1-weighted spin-echo scans) (**Figure 1**) has become established as the most important paraclinical tool not only for diagnosing MS (1) but also for understanding its natural history and for monitoring the efficacy of experimental treatments (2). This is particularly true for relapsing-remitting (RR) MS (RRMS). In patients with RRMS, measures

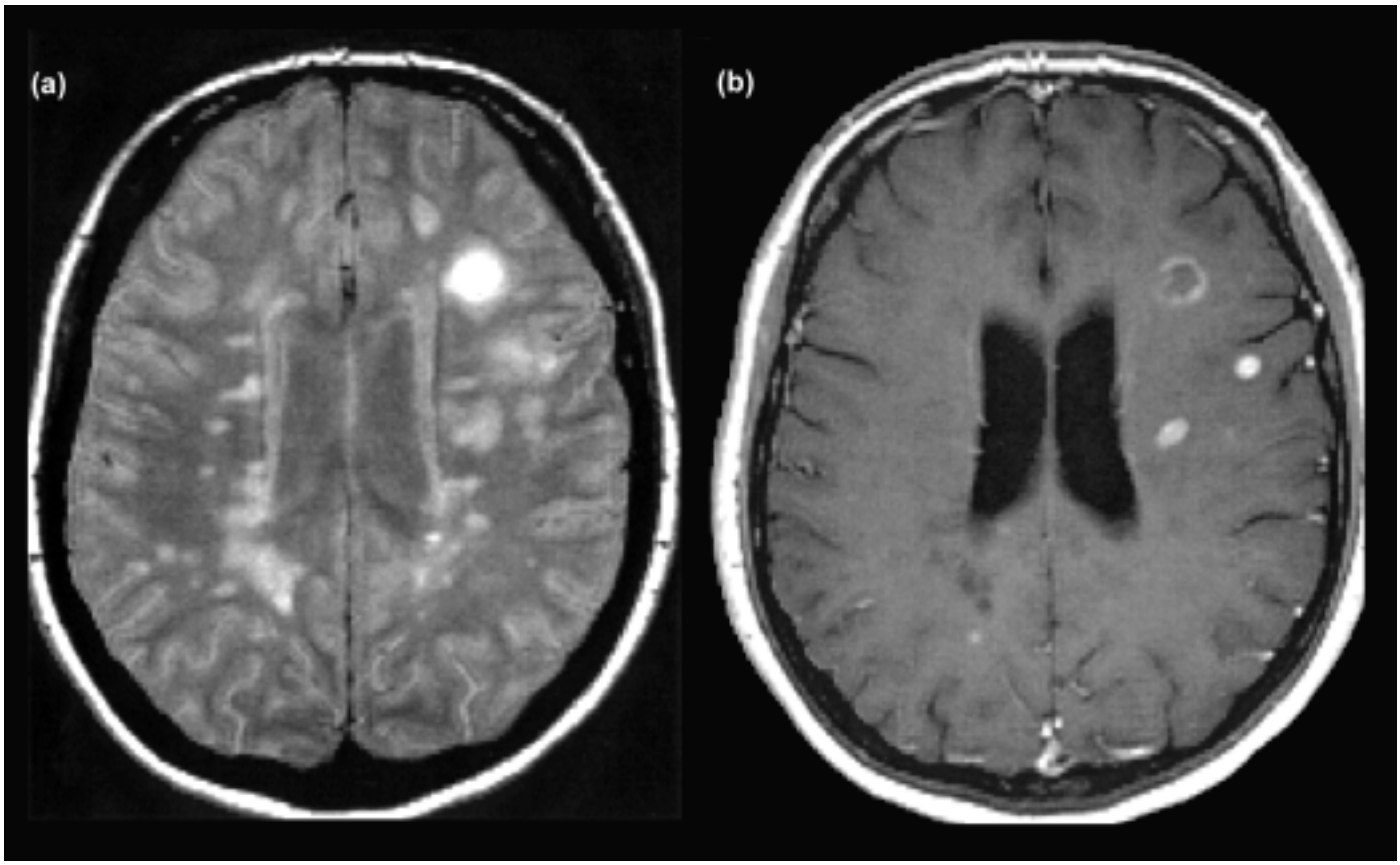

Figure 1.
(a) Axial proton density-weighted spin-echo: multiple hyperintense lesions are visible, suggestive of multifocal white matter pathology, with a predominant involvement of periventricular regions and (b) postcontrast (gadolinium DTPA, 0.1 mmol/kg) T1-weighted spin-echo MRI scans of brain of a patient with clinically definite MS: Some of these lesions are contrast-enhanced, indicating presence of a local blood-brain barrier disruption. This pattern highly suggests MS, which demonstrates a pathological process presence with spatial and temporal dissemination.

derived from conventional MRI scans are much more sensitive than clinical assessments for the detection of disease activity over time (3,4). The application of the measures in large-scale clinical trials has given a fundamental contribution to the approval of disease-modifying treatments for this MS phenotype (5). However, the correlation found between the burden and activity of lesions as seen on conventional MRI scans and the clinical manifestations of RRMS is, at best, moderate (6,7). In addition, conventional MRI findings have a modest predictive value for the subsequent evolution of RRMS patients into a secondary progressive (SP) disease course, which is characterized by the irreversible accumulation of neurological deficits (8).

The discrepancy between conventional MRI and clinical assessment in MS may arise from several factors, including the known limitations of the clinical scoring scales in terms of reliability and responsiveness (9). However, such a discrepancy can be explained largely by the limited capability of a conventional MRI to quantify the extent and to characterize the nature of tissue damage in MS. Other quantitative MR techniques have the potential to overcome these limitations. Among these techniques, cell-specific imaging, magnetization transfer (MT) MRI (MT-MRI), diffusion-weighted (DW) MRI (DW-MRI), proton MR spectroscopy (^{1}H-MRS), and functional MRI (fMRI), are those which have been most extensively applied to the assessment of RRMS.

CONVENTIONAL MRI

Conventional MRI findings support the hypothesis that in RRMS, inflammatory processes play a relevant

role in driving the disease activity. This is indicated by the high frequency of contrast-enhancing lesions visible on T1-weighted scans obtained after the administration of gadolinium (Gd) diethylenetriamine pentaacetic acid (DTPA) in untreated patients (3,4). Hyperintense lesions seen on unenhanced T2-weighted MRI are characterized by a variety of underlying pathological substrates, ranging from edema to demyelination and axonal loss (10). However, studies in animals and in humans with MS have demonstrated that the presence of Gd enhancement is always consistent with histopathological findings of blood-brain barrier (BBB) breakdown (11–13). Perivascular inflammation appears to be a necessary precondition to the development of enhancement, since noninflammatory demyelination is unaccompanied by changes of BBB permeability (14,15). Studies in animals with experimental allergic encephalomyelitis (EAE) have shown that Gd enhancement correlates with the number of inflammatory cells within the lesions and mainly represents macrophage activation (16–18). Because chronic relapsing EAE and MS present similar morphological and functional changes, it is, therefore, likely that enhancement in MS lesions predominantly reflects active inflammation. Although an enhanced MRI with a standard dose (SD) of Gd is sensitive enough to detect numerous active lesions in RRMS, there is evidence that a relevant amount of brain inflammation still goes undetected with the use of this technique. With the use of a triple dose (TD) of Gd, 70 to 80 percent more enhancing lesions can be seen than with the use of an SD (19–22). These findings indicate that enhancing MS lesions form a heterogeneous population and those enhancing only after a TD of Gd are characterized by a milder and shorter opening of the BBB, which is probably most associated with less severe inflammatory changes.

Several studies found that the number of enhancing lesions increases shortly before and during clinical relapses and correlates with the MRI activity in the subsequent months (3,23–26). However, the correlation between enhancement frequency and long-term MS clinical evolution is only modest (6,27). It has been reported that when RRMS patients enter the SP phase of the disease, a decrease of MRI-detectable inflammatory activity can be observed (28,29). Because ring-enhancing lesions may reflect a more destructive pathology, their potential association with disease severity has been recently studied in a small cohort of RRMS patients (12,30). The percentage of ring-enhancing lesions was found to be correlated with patients' clinical disability, T2 lesion load, and duration of disease and to predict the occurrence of relapses during the baseline period of observation, as well as after a 3-year follow-up. This finding suggests that the pathological process reflected by the presence of these lesions may contribute to a more severe clinical evolution of RRMS.

Conventional MRI scans of the spinal cord can demonstrate the presence of hyperintense lesions in 80 to 90 percent of patients with RRMS and in 30 to 40 percent of patients at the onset of the disease (31–34). The latter figure is higher, however, with patients who present with neurological manifestations attributable to myelopathy (34). The demonstration of spinal cord lesions can help (1) in the differentiation between patients with MS and healthy subjects, in whom intrinsic spinal cord lesions are extremely rare and do not seem to occur as a result of aging per se (35), and (2) in the differential diagnosis between RRMS and other neurological conditions with a similar clinical course, such as of equivocal brain MRI findings (36). On the contrary, monitoring an RRMS course with serial cervical cord scans does not significantly increase the harvest of brain MRI-detectable disease activity, even though acute MS symptoms are caused more often by cord lesions than by brain lesions and cord abnormalities are well correlated with the degree of physical disability (31,37–39).

A conventional MRI also can provide measures with increased specificity to the most destructive aspects of MS pathology. These measures include the burden of T1-hypointense lesions and the assessment of brain or cord atrophy. Hypointense lesions on enhanced T1-weighted images (known as "black holes") correspond to areas where chronic severe tissue disruption has occurred (40). Although later studies based on much larger samples of patients have not confirmed the initial strong correlation found between the T1-hypointense lesion volume and disability, a general tendency is to consider assessing the extent of black holes as a valid surrogate measure to monitor RRMS evolution (41–43). However, this approach is not without major limitations, including the arbitrary process underlying black hole identification and the inability to provide any information about the pathology of normal-appearing brain tissue.

The measurement of brain atrophy has also been applied to assess the extent of tissue loss in RRMS (44,45). However, the pathological basis of this process is

still unclear. Although it is intuitive that myelin and axonal loss might contribute to the development of atrophy, the role of other factors is largely unexplored. For instance, reactive gliosis can potentially mask considerable tissue loss. Measurements of brain atrophy are also likely to be biased by fluctuations of tissue water content related to important aspects of MS pathology or management, such as the vasogenic edema associated to active lesions or the administration of "anti-inflammatory" treatment. In addition, atrophy is an end-stage phenomenon. Although detection of atrophy is a hard end point, a series of events conceivably would precede MRI-detectable atrophy. Finally, atrophy is relatively insensitive to disease changes. On average, brain volume decreases by about 1 percent yearly in patients with RRMS and other MS phenotypes, despite evidence of highly variable disease activity and characteristics (45–49). Cross-sectional studies have demonstrated robust correlations between cervical cord atrophy and patients' disability across the different MS subgroups (50–52). A significant increase in cord atrophy over 1 year has been seen in both RRMS and secondary progressive multiple sclerosis (SPMS), with mean reduction in cord area of 2 to 3 percent per annum (51,52). However, no or modest correlations were found between cord area decrease and patients' disability changes (51,52). All these data indicate that MRI-measurable atrophy provides only a limited view on MS heterogeneity and that large patient samples and long follow-up periods might be needed to detect treatment effect on the rate of atrophy development.

The correlation between the occurrence of conventional MRI-measured brain inflammation and the development of permanent tissue damage in RRMS is still not completely elucidated. Longitudinal studies with monthly or weekly MRI scans indicate that only a minority of MS lesions appears without prior Gd enhancement (3,4,53,54). Molyneux et al. noted a correlation between the number of enhancing lesions and changes of T2 hyperintense lesion load in both RRMS and SPMS patients (25). Other longitudinal studies reported that the frequency and extent of enhancement only partially predict the accumulation of T1 hypointense lesions and are only poorly correlated with the rate of development of brain atrophy, which can proceed despite the capability of some treatments to suppress the inflammatory activity (55–57). Patients with RRMS and SPMS have higher MRI activity than patients with benign courses do (58–60). All these findings indicate that brain inflammation does significantly, but not exclusively, contribute to the development of tissue damage in RRMS.

Despite the limitations of the conventional MRI, its sensitivity in revealing RRMS activity makes it a valuable tool to monitor the efficacy of treatments with the potential to modify the clinical course of the disease (5). Since an enhanced brain MRI is 5 to 10 times more sensitive than clinical evaluation, its application allows clinical trials to be performed with reduced sample sizes and follow-up durations (3,4,37,59,61). At present, most large-scale multicenter clinical trials in MS are using enhanced MRI as a primary (phase II) or secondary (phase III) outcome measure (5). Future MS trials, however, will most likely be conducted with the reference arm receiving one of the already available treatments instead of placebo. In a recent study, despite using a powerful measure of outcome, such as the count of new Gd-enhancing lesions, Sormani et al. found that the number of patients needed to detect a significant additional effect of a new treatment compared with those already achievable is relatively high (62). This find suggests the use of outcome measures derived from quantitative MR techniques, based upon their improved sensitivity and specificity to tissue loss, might render comparative MS trials more easily feasible.

CELL-SPECIFIC IMAGING

Gd-enhanced MRI can depict active MS lesions, but it cannot identify the presence of activated inflammatory cells. New methods for cell-specific imaging use markers for tracking various cell components of the immune system (63). A superparamagnetic iron oxide contrast agent, also known as monocrystalline iron oxide nanoparticles (MION), can be used to label lymphocytes in vitro and in vivo for trafficking studies (63–65). This technique has been applied to study relapsing-remitting EAE in Lewis rats (63,64). A MION-enhanced MRI showed a higher sensitivity for the detection of EAE lesions than that of conventional T2-weighted and Gd-enhanced images. The histopathological analysis revealed the presence of macrophages at the sites where MION-enhanced abnormalities were seen (64). Another study has demonstrated that human mononuclear cells labeled with MION can be detected by MRI in vitro, thus suggesting the possibility that the technique could provide new in vivo information on lymphocyte and monocyte trafficking in MS lesions

(65). Preliminary data on patients with RRMS have shown that there is a relatively large group of "active" MS lesions that enhance only after either MION or Gd injection (V. Dousset, personal conversation, June 2001). Understanding this "active" MS lesion heterogeneity might add significantly to our understanding of the disease pathobiology.

MAGNETIZATION TRANSFER MRI

MT-MRI is based on interactions between two predominant pools of water hydrogen protons, bound to macromolecules or free. In the central nervous system (CNS), these two pools correspond to the protons in tissue water and in the macromolecules of myelin and other cell membranes. Off-resonance irradiation is applied, which saturates the magnetization of the less mobile protons, but this is transferred to the mobile protons, thus reducing the signal intensity from the observable magnetization. The degree of signal loss depends on the density of the macromolecules in a given tissue. Thus, low MT ratio (MTR) indicates a reduced capacity of the macromolecules in the CNS to exchange magnetization with the surrounding water molecules, reflecting damage to myelin or to the axonal membrane. Several lines of evidence suggest that a marked reduction of MTR values in MS lesions indicates severe tissue damage (66). The most compelling one comes from a postmortem study showing a strong correlation of MTR values from MS lesions and normal-appearing white matter (NAWM) with the percentage of residual axons and the degree of demyelination (67).

Using MT-MRI and variable frequencies of scanning, several authors have investigated the structural changes of new enhancing MS lesions for time periods ranging from 3 to 36 months (66). The results of all these studies consistently showed that, on average, MTR drops dramatically when the lesions start to enhance and can show a partial or complete recovery in the subsequent 1 to 6 months. The relatively good preservation of axons, which is usual in acute MS lesions, and the rapid and marked increase of the MTR suggest demyelination and remyelination as the most likely pathological mechanisms underlying these short-term MTR changes. Nevertheless, edema and its subsequent resolution can also give rise to the observed pattern of MTR behavior, because of the diluting effect of extracellular water. However, edema alone seems unlikely to be sufficient to explain these findings, since previous studies showed that edema in the absence of demyelination results in only modest MTR reductions (68,69). MT-MRI studies of individual-enhancing lesions also confirmed the perception that the pathological nature of such lesions and the severity of the associated changes in the inflamed tissue may vary considerably (70–72). These changes seem to be related to the severity and duration of the opening of the BBB (20,73).

These results suggest that the balance between damaging and reparative mechanisms is highly variable since the early phases of MS lesion formation. Consequently, different proportions of lesions with different degrees of structural changes might contribute to the evolution of the disease. At present, however, few data support this concept. A 3-year follow-up study showed that newly formed lesions from patients with SPMS have a more severe MTR deterioration than do those from patients with mildly disabling RRMS (74). Established MS lesions have a wide range of MTR values (70,75). Lower MTR has been reported in black holes than in lesions that are isointense to NAWM on T1-weighted scans, and MTR has been found to be inversely correlated with the degree of hypointensity (71,76). In a longitudinal study with monthly MT-MRI and T1-weighted scans, van Waesberghe et al. found that MS lesions that changed from T1-hypointense to T1-isointense when Gd enhancement ceased also had a significant MTR increase (76), whereas a markedly decreased MTR at the time of initial enhancement was predictive of a persistent T1-weighted hypointensity and lower MTR after 6 months. Decreased MTR has also been found for NAWM from RRMS even in the absence of T2-visible lesions (77–79). These changes are more pronounced in NAWM areas adjacent to focal T2-weighted MS lesions, and this is more evident in SPMS than in RRMS patients (77,78). MTR reductions can also be detected in the NAWM before lesion formation (80). Edema, marked astrocytic proliferation, perivascular inflammation, and demyelination may all account for an increased amount of unbound water in the NAWM and, as a consequence, determine MTR changes (81).

MT-MRI can also be used to assess global MS lesion burden by means of an MTR histogram analysis (82). This highly automated technique can provide several metrics reflecting both macroscopic and microscopic MS pathology in the whole of the brain or in selected regions.

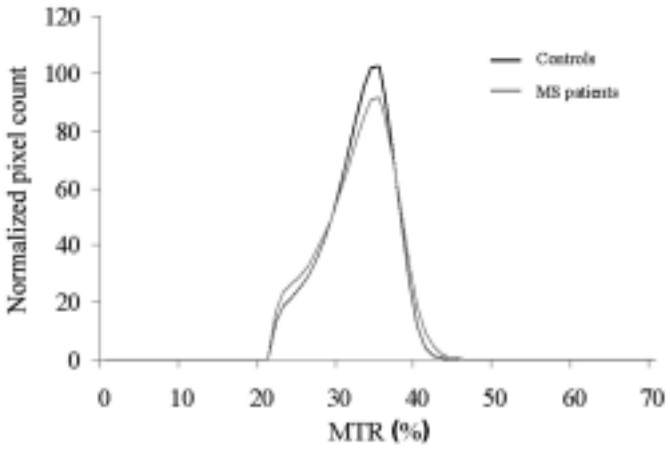

Figure 2.
Histograms of MTR values from whole of brain tissue of 60 patients with MS (gray line) and 40 age- and sex-matched healthy controls (black line). In MS patients, height of MTR histogram peak is reduced when compared to controls, and shape of histogram reflects a shift of pixels toward lower range of MTR values, indicating an increased amount of disrupted brain tissue.

In general, MS patients have lower average MTR, histogram peak height, and position than normal subjects (66,82,83) (**Figure 2**). MTR histogram parameters can be different in the various clinical forms of MS (83). RRMS patients have lower average MTR and peak height than benign MS, whose histograms are similar to those of healthy subjects, while patients with SPMS had the lowest MTR histogram metrics among these three disease subtypes.

Macroscopic lesions segmented on T2-weighted images can be superimposed onto the coregistered MTR maps, and the areas corresponding to the segmented lesions can be masked out, thus obtaining MTR maps of the normal-appearing brain tissue (NABT) in isolation. NABT-MTR histogram measures are different and evolve at a different pace in the major MS clinical phenotypes (49,84). SPMS patients have significantly lower NABT-MTR peak height and position than RRMS patients who, in turn, do not significantly differ from patients with benign MS (84). In addition, NABT changes are only partially correlated with the extent of macroscopic lesions and the severity of intrinsic lesion damage, thus suggesting that NABT pathology does not only reflect Wallerian degeneration of axons transversing large focal abnormalities (84). Reduced NABT-MTR has also been found in patients at presentation with clinically isolated syndromes (CIS) suggestive of MS (85). The extent of NABT changes in these patients is an independent predictor of subsequent evolution to clinically definite RRMS (85). In patients with RRMS, both whole brain and NABT MTR histogram-derived measures were found to be more sensitive than conventional MRI lesion-load assessment in detecting disease-related changes over a 1-year period (49). Both T2 lesion load and NABT histogram changes were significantly less pronounced in RRMS than in SPMS patients but more evident than in benign cases. These findings indicate that lesion accumulation over time and tissue damage within and outside T2-visible lesions might all be important in determining the evolution from RRMS to SPMS. That the amount of truly normal brain tissue is critical in determining the subsequent evolution of RRMS is shown also by the progressive and significant decline of MTR histogram peak heights observed in these patients, since the peak height of the MTR histogram is considered to be a measure of the residual amount of truly normal tissue (82). However, one must be cautious before drawing firm conclusions, since this study was not longitudinal; as a consequence, patients with RRMS were not the same as those who had SPMS some years later (49). The feasibility of such a longitudinal study is debatable, however, considering the large sample of RRMS patients needed, the duration of the follow-up, and the MRI scanner changes and upgrades that would inevitably occur over such a long time period, which would bias the measurements (66).

Using automated techniques for brain tissue segmentation, MTR histograms can be obtained from the gray matter in isolation (86,87). Recent studies have shown that the average values of gray matter MTR from healthy subjects are similar to those of RRMS patients, which are lower than SPMS patients' values (86,87). Again, this suggests that increased tissue damage is a critical footstep in determining the evolution from RRMS to SPMS.

Reliable MTR measurements can also be obtained from the cervical cord (88). Cervical cord MTR histogram-derived quantities are significantly lower in patients with SPMS than with RRMS and in patients with locomotor disability than in those without (89). Interestingly, in patients with MS, cord MTR is only partially correlated with brain MTR, suggesting that MS pathology in the cord is not a mere reflection of brain pathology (90). Consequently, measuring cord pathology might be a rewarding exercise in understanding MS pathophysiology.

Although MTR changes of T2-visible lesions and NAWM are not MS-specific, they may give important

diagnostic information. In patients with RRMS and no or few MRI-visible lesions, whole brain MTR histogram-derived metrics are similar to those from healthy controls, but a region-of-interest analysis revealed the presence of tissue damage in several white matter areas (79). The absence of MTR changes in NAWM from patients with migraine or systemic immune-mediate disorders and multiple T2 lesions reasonably excludes a diagnosis of MS (91,92). The absence of MTR changes in the NAWM of patients with optic neuropathy and myelopathy increases the confidence in diagnosing Devic's disease (93).

DIFFUSION-WEIGHTED MRI

Diffusion is the random translational motion of molecules in a fluid system. In CNS, diffusion is influenced by the microstructural components of tissue, including cell membranes and organelles. The diffusion coefficient of biological tissues (which can be measured in vivo by MRI) is, therefore, lower than the diffusion coefficient in free water and, for this reason, is named apparent diffusion coefficient (ADC) (94). Pathological processes that modify tissue integrity, thus resulting in a loss or increased permeability of "restricting" barriers, can determine an increase of the ADC. Since some cellular structures are aligned on the scale of an image pixel, the measurement of diffusion also depends on the direction in which diffusion is measured. Therefore, diffusion measurements can give information about the size, shape, and orientation of tissues (95). A measure of diffusion that is independent of the orientation of structures is provided by the mean diffusivity, $\overline{D}$, the average of the ADCs measured in three orthogonal directions. A full characterization of diffusion can be obtained in terms of a tensor, a 3×3 matrix that accounts for the correlation existing between molecular displacement along orthogonal directions (96). From the tensor, it is possible to derive $\overline{D}$, equal to one-third of its trace and some other dimensionless indexes of anisotropy. One of the most used is the fractional anisotropy (FA), which is a measure of deviation from isotropy and reflects the degree of alignment of cellular structures within fiber tracts, as well as their structural integrity. Tissue disruption, by removing structural barriers to water molecular motion, typically causes increased $\overline{D}$ and decreased FA values (96,97).

The pathological elements of MS can alter the permeability or geometry of structural barriers to water diffusion in the brain (**Figure 3**). The application of DW-MRI to MS is, therefore, appealing, since it can provide quantitative estimates of the degree of tissue damage and, therefore, might improve the understanding of the mechanisms leading to irreversible disability. The first report of water diffusion in MS showed that MS lesions had increased ADC values compared to NAWM (98). A subsequent study with more stable diffusion measurements confirmed the preliminary results and demonstrated that NAWM of MS patients had higher ADC values than white matter from controls (99). However, these studies suffered from motion artifacts, limited brain coverage, and the application of diffusion gradients in a single direction.

A more recent study used a navigator echo strategy to correct for motion artifacts in a spin-echo diffusion sequence and to cover larger portions of the brain than previous studies (100). Again, previous results were confirmed, and in addition, a significantly increased $\overline{D}$ was found in T1-hypointense compared to T1-isointense lesions and in nonenhancing compared to enhancing lesions (100). In the latter study, the $\overline{D}$ patterns of lesions did not differ between patients with RRMS and those with other MS phenotypes (100).

Many of the problems with the studies just mentioned (98–101) can be addressed by the use of echo-planar imaging (EPI), which is less prone to motion and permits greater brain coverage, with more diffusion gradient directions, in a given time. Recent studies used such an approach and achieved the following results (102–106):

1. $\overline{D}$ values of NAWM from MS patients are diffusely higher than the corresponding values of white matter from controls.
2. $\overline{D}$ values increase in areas of NAWM subsequently involved by MS lesions (105,107).
3. Values continue to increase at the time of enhancement onset and then decrease rapidly in the next few weeks (105,107).
4. T2-visible lesions have higher $\overline{D}$ values than NAWM.
5. T1-hypointense lesions have the highest $\overline{D}$ values.

Conflicting results have been obtained when comparing enhancing verses nonenhancing lesions: one study confirmed that enhancing lesions have higher $\overline{D}$ values, but others, which were based on larger samples of patients and lesions, did not find any significant differ-

ence between the two lesion groups (103,104,106,108). FA has also been found to be reduced within and outside T2-visible lesions (104,106). Among lesions, FA was found lower in enhancing versus nonenhancing lesions (106). $\overline{D}$ and FA also vary significantly among the various types of enhancing lesions (106–110). All these data suggest a diffuse loss of structural barriers to water molecular motion in NAWM from MS patients. As expected, the loss of structural barriers is even greater in macroscopic lesions, and its magnitude seems to be correlated with the intrinsic tissue damage. Since "inflammatory" changes and gliosis could potentially restrict water molecular motion, myelin and axonal loss are the most likely contributors to the increased $\overline{D}$ and decreased FA in MS NAWM and lesions. The correlation between the average $\overline{D}$ values in the lesions and NAWM has been investigated in one study, and it was found not significant (102). This again suggests that subtle NAWM changes are not merely the result of Wallerian degeneration of axons transversing larger lesions. Recent work has also detected $\overline{D}$ changes in the gray matter of MS patients (87), which are likely to be secondary to cortical damage, since $\overline{D}$ and MTR of basal ganglia from patients with MS do not differ from the corresponding quantities from normal controls (111).

As for MT-MRI, the analysis of diffusion changes can also be performed more globally with the use of $\overline{D}$ histograms (102,112–114). RRMS patients have a significantly higher average $\overline{D}$ and lower histogram peak height than normals. Histogram broadening and the consequent decrease of peak height show that fewer pixels in patients' brain have normal $\overline{D}$ values. FA histogram-derived metrics were also significantly different from those of normals (112). Conflicting results have been obtained when comparing $\overline{D}$ histogram-derived quantities between MS subtypes, since a preliminary report of higher average $\overline{D}$ values in SPMS than in RRMS patients has not been confirmed by recent studies conducted with larger patient samples (112,114). The magnitude of the correlation between MTR and $\overline{D}$ changes has also been investigated (102). In MS lesions, a strong inverse correlation between average MTR and $\overline{D}$ was found (102). However, this correlation was not found when considering NAWM and the whole of the brain tissue (102). The lack of correlation between MTR and $\overline{D}$ in the brain tissue might be the result of the complex relationship between destructive and reparative mechanisms occurring in the NAWM and their variable effects on MTR and $\overline{D}$ values.

Significant correlations between DW-MRI findings and MS clinical manifestations or disability were not found in some of the earliest studies, perhaps because of the relatively small samples studied, the limited brain coverage, or the narrow range of disabilities that was considered (100–103). With improved DW-MRI technology and increased numbers of patients being studied, correlations between DW-MRI findings and MS clinical manifestations or disability are now emerging (104,112,114,115). Average lesion $\overline{D}$, but not average lesion FA, was found to be significantly correlated, albeit moderately, with clinical disability in a study of 78 patients with MS, including a subgroup with RRMS (104). The lack of correlation between disability and FA indicates that the loss of overall impediment to diffusional motion is more important than the loss of tissue anisotropy in determining patients' clinical status. Interestingly, a significant correlation between disability and T2-lesion volume was found in patients with RRMS and not in those with SPMS, where, in turn, there was a correlation between average lesion $\overline{D}$ or FA and disability. These findings suggest that mechanisms leading to disability are likely to be different in patients with RRMS and SPMS. Although caution must be exercised, one might speculate that new lesion formation is a relevant pathological aspect in RRMS, whereas tissue loss in preexisting lesions is one of the pathological hallmarks of SPMS. Consistently with these observations, water diffusivity in T2-visible lesions has been shown to significantly increase in patients with SPMS when compared to those with RRMS (115). The same authors also found strong correlations between average lesion diffusivity, disability, and disease duration.

PROTON MAGNETIC RESONANCE SPECTROSCOPY

^{1}H-MRS can complement conventional MRI in the assessment of patients with RRMS by defining simultaneously several chemical correlates of the pathological changes occurring within and outside T2-visible lesions. Water-suppressed proton MR spectra of the normal human brain at long echo times reveal four major resonances: one at 3.2 ppm from tetramethylamines (mainly from choline-containing phospholipids (Cho),

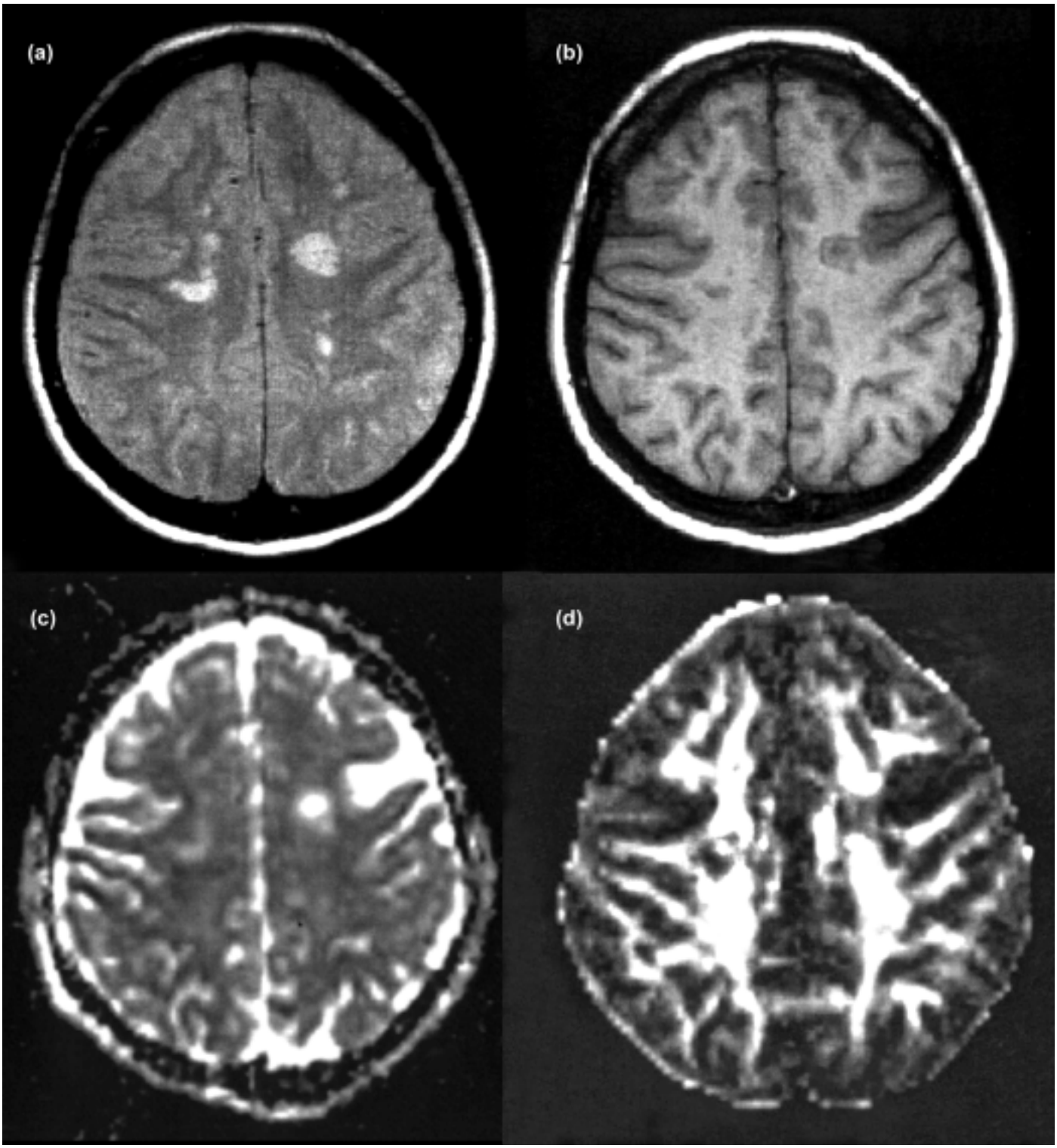

Figure 3.

(a) Axial proton density-weighted spin-echo image: At roof level of lateral ventricles, multiple hyperintense lesions are visible; (b) T1-weighted spin-echo image: Some of these lesions appear T1-hypointense, indicating presence of severe white matter disruption; (c) mean diffusivity ($\overline{D}$) map: Diffusivity is increased within MS lesions, which appear hyperintense on $\overline{D}$ maps; and (d) fractional anisotropy (FA) map from brain of a patient with MS: Conversely, some of these lesions are visible as areas of decreased signal on FA maps, indicating a local decrease of anisotropic diffusion. Both $\overline{D}$ increase and FA decrease are more pronounced for T1-hypointense MS lesions.

one at 3.0 ppm from creatine and phospho-creatine (Cr)), one at 2.0 ppm from N-acetyl groups (mainly N-acetyl-aspartate (NAA), and one at 1.3 ppm from the methyl resonance of lactate (Lac). Although more technically demanding, additional metabolites (including lipids and myoinositol (mI) can be detected with the use of short-echo time measurements.

^{1}H-MRS of acute MS lesions at both short and long echo times reveals increases in Cho and Lac resonance intensities since the early phases of the pathological process (116,117). Changes in the resonance intensity of Cho result mainly from increases in the steady-state levels of phosphocholine and glycerol-phosphocholine, both membrane phospholipids that are released during active myelin breakdown. Increases in Lac are likely to reflect the metabolism of inflammatory cells. In large, acute demyelinating lesion decreases of Cr can also be seen (117). Short echo time spectra can detect transient increases of visible lipids, released during myelin breakdown and mI (116,118). All of these changes are usually followed by a decrease in NAA. Since NAA is a metabolite detected almost exclusively in neurons and their processes of the normal adult brain, the decrease in NAA is considered secondary to axonal dysfunction. After the acute phase and over a period of days to weeks, there is a progressive reduction of raised Lac resonance intensities to normal levels. Resonance intensities of Cr also return to normal within a few days. Cho, lipid, and mI resonance intensities return to normal over months. The signal intensity of NAA may remain decreased or show partial recovery, starting soon after the acute phase and lasting for several months (115,119).

Recovery of NAA may be related to resolution of edema, increases in the diameter of previously shrunk axons secondary to remyelination and clearance of inflammatory factors, and reversible metabolic changes in neurons. Although similar decreases in NAA are found in acute enhancing lesions of patients with benign and SPMS, chronic lesions from patients with benign MS have much higher NAA levels than do chronic lesions from SPMS patients, suggesting a greater recovery of NAA in acute lesions from less disabled MS patients (120). Since in acute MS lesions, Gd enhancement is usually ceased by 2 months, the metabolic changes shown by ^{1}H-MRS can reveal on-going pathology, which would otherwise go undetected. Interestingly, a recent study detected elevated lipid peaks also in NAWM regions. In some of these regions, such ^{1}H-MRS abnormality preceded new MS lesion formation (118).

Since changes of axonal viability may be important determinants of functional impairment in MS, one of the major contributions of ^{1}H-MRS to the understanding of MS is likely to be the quantification of axonal pathology, by measuring NAA levels of lesions and NAWM. The importance of axonal damage in determining clinical deficits in MS has been shown by several authors (117,119–121). The most elegant study is by Davie et al. (122), who found reduced cerebellar NAA levels in patients with MS and cerebellar ataxia similar to that present in those with autosomal dominant spinocerebellar degeneration, whereas the levels of cerebellar NAA were normal in nonataxic MS patients. One can estimate the magnitude of the decrease in brain NAA from large portions of the central brain (119,121–125) or measure it directly using a recently developed technology that enables us to obtain NAA measurements from the whole brain. Decreased NAA levels are found in patients with established MS since the early phases of the disease (120,125). Although the extent of the decrease in NAA/unit lesion volume has been found to be greater in SPMS than in RRMS, the rate of NAA changes with time is faster in RRMS than in SPMS (124). Strong inverse correlations between NAA and disability levels have been found in patients with RRMS (123).

Decreases in NAA are not restricted to T2-visible lesions but also occur in the NAWM adjacent to or distant from them (81). This is consistent with postmortem studies showing axonal loss in the NAWM of MS patients (81). Anterograde and retrograde degeneration of axons transversing large lesions appears to be the most likely pathological substrate, at least in patients with high lesion loads. The role of this factor in determining ^{1}H-MRS changes in NAWM is supported by the recent finding of dramatic but reversible changes of NAA in the NAWM of the hemisphere contralateral to solitary acute MS lesions (127). However, small focal abnormalities independent of larger T2-visible lesions can also contribute to NAA decreases in NAWM. This seems to be the case for patients with primary progressive MS, who have markedly reduced NAA levels in the NAWM despite the paucity of T2 abnormalities (128). Recently, it has been shown that NAWM from SPMS patients has on average 8.2 percent lower NAA levels than NAWM from RRMS (129). However, in RRMS patients, a progressive reduction of NAWM NAA is detectable over time, and this de-

crease correlates strongly with accumulation of disability (129).

FUNCTIONAL MRI

fMRI measures changes of MRI signal that occur during brain activation as a consequence of the changes in the concentration of deoxygenated hemoglobin. Preliminary studies have suggested that fMRI can be used to monitor the recovery after an MS clinical relapse or to study the reorganization of neural pathways in the brain of patients with established MS (130–132) (**Figure 4**). In patients affected by arm paralysis, a correlation was found between fMRI findings and the severity of the functional deficit (131). A case report study has shown that during recovery after MS relapse, dynamic changes in the patterns of cortical activation with hand movements can be detected with fMRI (130). The observed pathologically decreased lateralization of cortical motor activation becomes less marked with progressive clinical recovery and precedes the normalization of NAA levels in the affected area. This suggests that cortical adaptive responses can compensate for MS-related brain injury to maintain normal motor functions despite lesion damage. On the contrary, the cortical activation was found to be reduced after visual stimulation of the affected eye in MS patients with optic neuritis (132).

More recently, the correlations between fMRI findings and other MRI-derived measures of MS disease burden were assessed to define whether and to what extent fMRI changes are adaptive to the underlying MS pathology. In a sample of clinically stable MS patients with varying degrees of upper-limb motor deficit, Lee et al. found that the patterns of cortical activation during a hand motor task were significantly different from those of healthy controls (133). In MS patients, the increase of ipsilateral cortex activation was significantly correlated with increasing T2 hyperintense lesion load in the contralateral hemisphere. In patients with RRMS and no residual motor disability, fMRI reveals an abnormal pattern of recruitment of elements of the cortical motor network, which is correlated with brain T2 lesion load (134). Using MRS imaging, Reddy et al. found that a similar fMRI finding (i.e., an increased activation of the ipsilateral sensorimotor cortex during finger movement) was strongly correlated with decreases in brain NAA (135). Filippi et al. (136) have found significantly different patterns of movement-related cortical/subcortical activations in RRMS patients with and without fatigue. The results of these studies indicate that fMRI can provide estimates of the cortical adaptive changes that follow MS-related tissue damage. The presence of compensatory mechanisms may help to explain why MRI measures of MS lesion burden are only modestly correlated with clinical measures of disability. In addition, fMRI findings suggest that therapies promoting cortical plasticity might be useful to enhance MS recovery.

CONCLUSIONS

Conventional MRI has markedly increased our ability to detect the macroscopic abnormalities associated with RRMS. New quantitative MR approaches with increased sensitivity to subtle NAWM and gray matter changes and increased specificity to the heterogeneous pathological substrates of MS lesions may give complementary information to conventional MRI. Cell-specific imaging should result in a better definition of the cellular mechanisms associated with MS inflammation. MT-MRI and DW-MRI have the potential to provide relevant information on the structural changes occurring inside and outside T2-visible lesions. ^{1}H-MRS could add information on the biochemical nature of such changes. fMRI is a promising technique to assess the mechanisms of cortical reorganization, which may follow MS-related injury. The extensive application of all these MR-based techniques should improve the understanding of the mechanisms leading to a later accumulation of irreversible disability in patients with an RR disease course.

REFERENCES

1. McDonald WI, Compston A, Edan G, Goodkin D, Hartung HP, Lublin FD, et al. Recommended diagnostic criteria for multiple sclerosis: Guidelines from the international panel on the diagnosis of multiple sclerosis. Ann Neurol 2001;50:121–27.
2. Filippi M, Horsfield MA, Ader HJ, Barkhof F, Bruzzi P, Evans A, et al. Guidelines for using quantitative measures of brain magnetic resonance imaging abnormalities in monitoring the treatment of multiple sclerosis. Ann Neurol 1998;43:499–506.
3. McFarland HF, Frank JA, Albert PS, Smith ME, Martin R, Harris JO, et al. Using gadolinium-enhanced magnetic res-

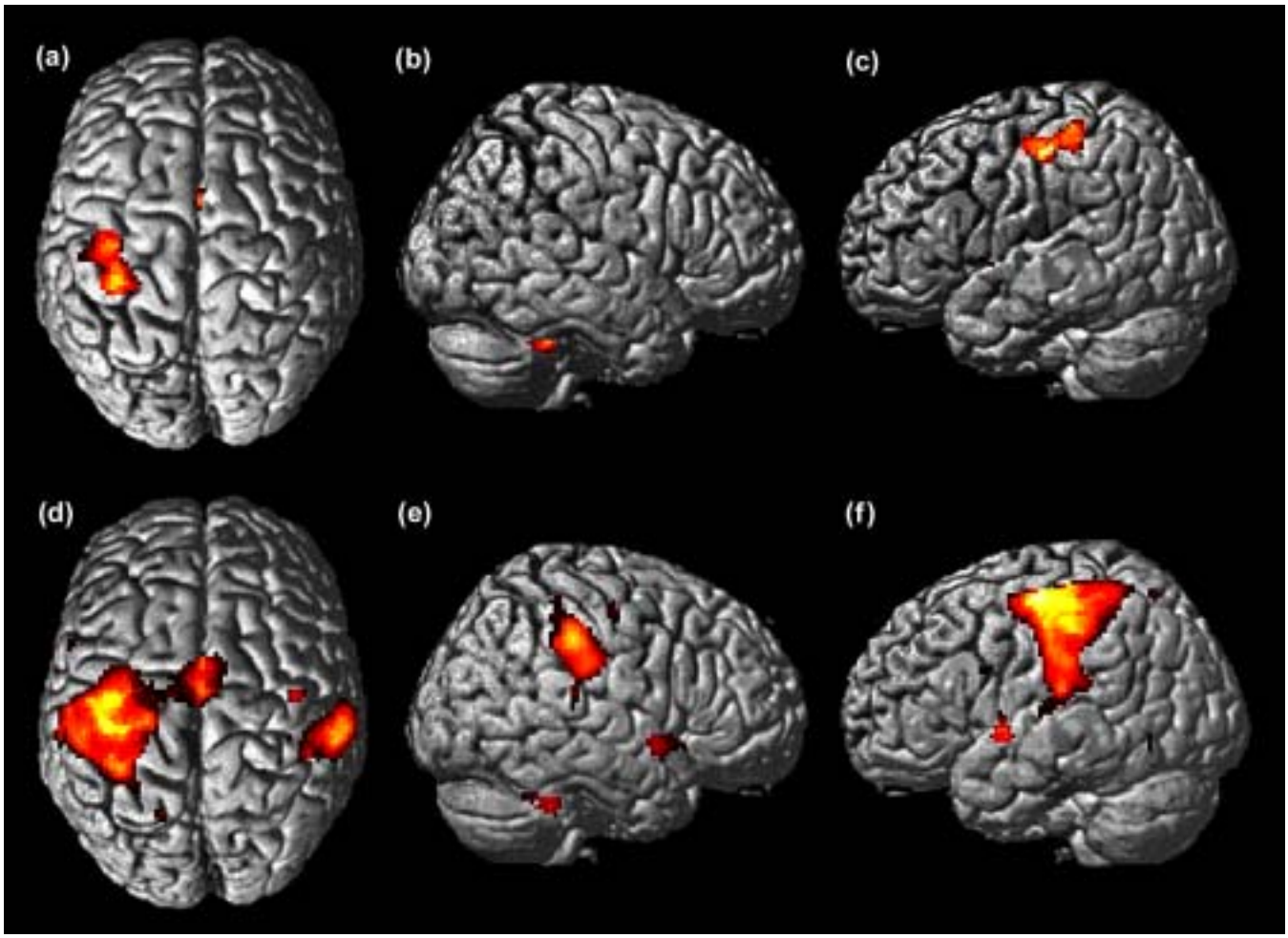

Figure 4.
(a), (b), and (c) are patterns of cortical activations on a rendered brain in 15 right-handed healthy subjects, and (d), (e), and (f) are patterns in 15 right-handed patients with SPMS during performance of a simple motor task with their clinically unimpaired and fully normal-functioning upper-right hands. Compared to controls, a larger and more significant activation of contralateral and ipsilateral primary sensori-motor cortex can be detected in SPMS patients.

onance imaging to monitor disease activity in multiple sclerosis. Ann Neurol 1992;32:758–66.

4. Miller DH, Barkhof F, Nauta JJP. Gadolinium enhancement increased the sensitivity of MRI in detecting disease activity in MS. Brain 1993;116:1077–94.
5. Rovaris M, Filippi M. Magnetic resonance techniques to monitor disease evolution and treatment trial outcomes in multiple sclerosis. Curr Opin Neurol 1999;12:337–44.
6. Kappos L, Moeri D, Radü EW, Schoetzau A, Schweikert K, Barkhof F, et al. Predictive value of gadolinium-enhanced MRI for relapse rate and changes in disability or impairment in multiple sclerosis: a meta-analysis. Lancet 1999;353:964–69.
7. Filippi M. Linking structural, metabolic, and functional changes in multiple sclerosis. Eur J Neurol 2001;8:291–97.
8. Brex PA, Ciccarelli O, O'Riordan JI, Sailer M, Thompson AJ, Miller DH. A longitudinal study of abnormalities on MRI and disability from multiple sclerosis. N Engl J Med 2002;346:158–64.
9. Kurtzke JF. Rating neurologic impairment in multiple sclerosis: An expanded disability status scale (EDSS). Neurology 1983;33:1444–52.
10. McDonald WI, Miller DH, Barnes D. The pathological evolution of multiple sclerosis. Neuropathol App Neurobiol 1992;18:319–34.
11. Hawkins CP, Munro PMG, Mackenzie F, Kesselring J, Tofts PS, duBoulay EPGH, et al. Duration and selectivity of blood brain barrier breakdown in chronic relapsing experimental allergic encephalomyelitis studied by gadolinium-DTPA and protein markers. Brain 1990;113:365–78.

12. Katz D, Taubenberger JK, Cannella B, McFarlin DE, Raine CS, McFarland HF. Correlation between magnetic resonance imaging findings and lesion development in chronic, active multiple sclerosis. Ann Neurol 1993;34: 661–69.
13. Nesbit GM, Forbes GS, Scheithauer BW, Okazaki H, Rodriguez M. Multiple sclerosis: histopathological and MR and/or CT correlation in 37 cases at biopsy and 3 cases at autopsy. Radiology 1991;180:467–74.
14. Lusmden CE. The neuropathology of multiple sclerosis. In: Vinken PJ, Bruyn GW, editors. Handbook of Clinical Neurology. Amsterdam: North-Holland Publishing Company; 1970. p. 217–309.
15. Dousset V, Brochet B, Vital A, Gross C, Benazzouz A, Boullerne A, et al. Lysolecithin-induced demyelination in primates: preliminary in vivo study with MR and magnetization transfer. AJNR Am J Neuroradiol 1995;16:225–31.
16. Seeldrayers PA, Syha J, Morrissey SP, Stodal H, Vass K, Jung S, et al. Magnetic resonance imaging investigation of blood-brain barrier damage in adoptive transfer experimental autoimmune encephalomyelitis. J Neuroimmunol 1993;46:199–206.
17. Namer IJ, Steibel J, Piddlesen SJ, Mohr M, Poulet P, Chambron J. Magnetic resonance imaging of antibody-mediated demyelinating experimental allergic encephalomyelitis. J Neuroimmunol 1994;54:41–50.
18. Morrissey SP, Stodal H, Zettl U, Simonis C, Jung S, Kiefer R, et al. In vivo MRI and its histological correlates in acute adoptive transfer experimental allergic encephalomyelitis. Quantification of inflammation and oedema. Brain 1996; 119:239–48.
19. Filippi M, Yousry T, Campi A, Kandziora C, Colombo B, Voltz R, et al. Comparison of triple dose versus standard dose gadolinium-DTPA for detection of MRI-enhancing lesions in patients with MS. Neurology 1996;46:379–84.
20. Filippi M, Rocca MA, Rizzo G, Horsfield MA, Rovaris M, Minicucci L, et al. Magnetization transfer ratios in MS lesions enhancing after different doses of gadolinium. Neurology 1998;50:1289–93.
21. Filippi M, Rovaris M, Capra R, Gasperini C, Yousry TA, Sormani MP, et al. A multi-centre longitudinal study comparing the sensitivity of monthly MRI after standard and triple dose gadolinium-DTPA for monitoring disease activity in multiple sclerosis: implications for clinical trials. Brain 1998;121:2011–20.
22. Silver NC, Good CD, Sormani MP, MacManus DG, Thompson AJ, Filippi M, et al. A modified protocol to improve the detection of enhancing brain and spinal cord lesions in multiple sclerosis. J Neurol 2001;248:215–24.
23. Koudriavtseva T, Thompson AJ, Fiorelli M, Gasperini C, Bastianello S, Bozzao A, et al. Gadolinium enhanced MRI disease activity in relapsing-remitting multiple sclerosis. J Neurol Neurosurg Psychiatry 1997;62:285–87.
24. Smith ME, Stone LA, Albert PS, Frank JA, Martin R, Armstrong M, et al. Clinical worsening in multiple sclerosis is associated with increased frequency and area of gadopentetate dimeglumine-enhancing magnetic resonance imaging lesions. Ann Neurol 1993;33:480–89.
25. Molyneux PD, Filippi M, Barkhof F, Gasperini C, Yousry T, Truyen L, et al. Correlations between monthly enhanced MRI lesion rate and changes in T2 lesion volume in multiple sclerosis. Ann Neurol 1998;43:332–39.
26. Stone LA, Smith E, Albert PS, Bash CN, Maloni H, Frank JA, et al. Blood-brain barrier disruption on contrast-enhanced MRI in patients with mild relapsing-remitting multiple sclerosis: relationship to course, gender and age. Neurology 1995;45:1122–26.
27. Losseff N, Kingsley D, McDonald WI, Miller DH, Thompson AJ. Clinical and magnetic resonance imaging predictors in primary and secondary progressive MS. Mult Scler 1996;1:218–22.
28. Filippi M, Rossi P, Campi A, Colombo B, Pereira C, Comi G. Serial contrast-enhanced MR in patients with multiple sclerosis and varying levels of disability. AJNR Am J Neuroradiol 1997;18:1548–56.
29. Tubridy N, Coles AJ, Molyneux P, Compston DA, Barkhof F, Thompson AJ, et al. Secondary progressive multiple sclerosis: the relationship between short-term MRI activity and clinical features. Brain 1998;121:225–31.
30. Morgen K, Jeffries NO, Stone R, Martin R, Richert ND, Frank JA, et al. Ring-enhancement in multiple sclerosis: marker of disease severity. Mult Scler 2001;7:167–71.
31. Lycklama à Nijeholt GJ, van Walderveen MAA, Castelijns JA, van Waesberghe JHTM, Polman C, Scheltens P, et al. Brain and spinal cord abnormalities in multiple sclerosis. Correlation between MRI parameters, clinical subtypes and symptoms. Brain 1998;121:687–97.
32. Rocca MA, Mastronardo G, Horsfield MA, Pereira C, Iannucci G, Colombo B, et al. Comparison of three MR sequences for the detection of cervical cord lesions in multiple sclerosis. AJNR Am J Neuroradiol 1999;20:1710–16.
33. O'Riordan JI, Losseff NA, Phatouros C, Thompson AJ, Moseley IF, MacManus DG, et al. Asymptomatic spinal cord lesions in clinically isolated optic nerve, brain stem, and spinal cord syndromes suggestive of demyelination. J Neurol Neurosurg Psychiatry 1998;64:353–57.
34. Thorpe JW, Kidd D, Moseley IF, Thompson AJ, MacManus DG, Compston DA, et al. Spinal MRI in patients with suspected multiple sclerosis and negative brain MRI. Brain 1996;119:709–14.
35. Fazekas F, Barkhof F, Filippi M, Grossman RI, Li DK, McDonald WI, et al. The contribution of magnetic reso-

nance imaging to the diagnosis of multiple sclerosis. Neurology 1999;53:448–56.

36. Rovaris M, Viti B, Ciboddo G, Capra R, Filippi M. Cervical cord magnetic resonance imaging findings in systemic immune-mediated diseases. J Neurol Sci 2000;176:128–30.
37. Thorpe JW, Kidd D, Moseley IF, Kendall BE, Thompson AJ, MacManus DG, et al. Serial gadolinium-enhanced MRI of the brain and spinal cord in early relapsing-remitting multiple sclerosis. Neurology 1996;46:373–78.
38. Trop I, Bourgouin PM, Lapierre Y, Duquette P, Wolfson CM, Duong HD, et al. Multiple sclerosis of the spinal cord: diagnosis and follow-up with contrast-enhanced MR and correlation with clinical activity. AJNR Am J Neuroradiol 1998;19:1025–33.
39. Lycklama à Nijeholt GJ, Barkhof F, Scheltens P, Castelijns JA, Ader H, van Waesberghe JH, et al. MR of the spinal cord in multiple sclerosis: relation to clinical subtype and disability. AJNR Am J Neuroradiol 1997;18:1041–48.
40. van Walderveen MAA, Kamphorst W, Scheltens PH, van Waesberghe JHTM, Ravid R, Valk J, et al. Histopathologic correlate of hypointense lesions on T1-weighted spin-echo MRI in multiple sclerosis. Neurology 1998; 50:1282–88.
41. Truyen L, van Waesberghe JHTM, van Walderveen MAA, van Oosten BW, Polman CH, Hommes OR, et al. Accumulation of hypointense lesions ("black holes") on T1 spin-echo MRI correlates with disease progression in multiple sclerosis. Neurology 1996;47:1469–76.
42. Simon JH, Lull J, Jacobs LD, Rudick RA, Cookfair DL, Herndon RM, et al. A longitudinal study of T1 hypointense lesions in relapsing MS: MSCRG trial of interferon beta-1a. Multiple Sclerosis Collaborative Research Group. Neurology 2000;55:185–92.
43. van Walderveen MA, Lycklama A, Nijeholt GJ, Ader HJ, Jongen PJH, Polman CH, Castelijns JA, et al. Hypointense lesions on T1-weighted spin-echo magnetic resonance imaging: relation to clinical characteristics in subgroups of patients with multiple sclerosis. Arch Neurol 2001;58:76–81.
44. Losseff NA, Wang L, Lai HM, Yoo DS, Gawne-Cain ML, McDonald WI, et al. Progressive cerebral atrophy in multiple sclerosis. A serial MRI study. Brain 1996;119:2009–19.
45. 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. Neurology 1999;53: 1698–704.
46. Rovaris M, Comi G, Rocca MA, Wolinsky JS, Filippi M, European/Canadian Glatiramer Acetate Study Group. Short-term brain volume change in relapsing-remitting multiple sclerosis: effect of glatiramer acetate and implications. Brain 2001;124:1803–12.
47. Filippi M, Rovaris M, Iannucci G, Mennea S, Sormani MP, Comi G. Whole brain volume changes in progressive MS patients treated with cladribine. Neurology 2000;55: 1714–18.
48. Thompson AJ, Kermode AG, MacManus DG, Kendall BE, Kingsley DP, Moseley IF, et al. Patterns of disease activity in multiple sclerosis: clinical and magnetic resonance imaging study. Br Med J 1990;300:631–34.
49. Filippi M, Inglese M, Rovaris M, Sormani MP, Horsfield MA, Iannucci G, et al. Magnetization transfer imaging to monitor the evolution of MS: a one-year follow-up study. Neurology 2000;55:940–46.
50. Losseff NA, Webb SL, O'Riordan J, Page R, Wang L, Barker GJ, et al. Spinal cord atrophy and disability in multiple sclerosis. A new reproducible and sensitive MRI method with potential to monitor disease progression. Brain 1996;119:701–8.
51. Filippi M, Colombo B, Rovaris M, Pereira C, Martinelli V, Comi G. A longitudinal magnetic resonance imaging study of the cervical cord in multiple sclerosis. J Neuroimag 1997;7:78–80.
52. Stevenson VL, Leary SM, Losseff NA, Parker GJM, Barker GJ, Husmani Y, et al. Spinal cord atrophy and disability in MS. A longitudinal study. Neurology 1998;51: 234–38.
53. Miller DH, Rudge P, Johnson J, Kendall BE, MacManus DG, Moseley IF, et al. Serial gadolinium-enhanced magnetic resonance imaging in multiple sclerosis. Brain 1988; 111:927–39.
54. Tortorella C, Rocca MA, Codella C, Gasperini C, Capra R, Pozzilli C, et al. Disease activity in multiple sclerosis studied with weekly triple dose magnetic resonance imaging. J Neurol 1999;246:689–92.
55. van Walderveen MAA, Truyen L, van Oosten BW, Castelijns JA, Lycklama A Nijeholt GJ, van Waesberghe JH, et al. Development of hypointense lesions on T1-weighted spin-echo magnetic resonance images in multiple sclerosis. Relation to inflammatory activity. Arch Neurol 1999; 56: 345–51.
56. Ge Y, Grossman RI, Udupa JK, Mannon LJ, Polansky M, Kolson DL. Brain atrophy in relapsing-remitting multiple sclerosis and secondary progressive multiple sclerosis: longitudinal quantitative analysis. Radiology 2000;214: 665–70.
57. Coles AJ, Wing MG, Molyneux P, Paolillo A, Davie CM, Hale G, et al. Monoclonal antibody treatment exposes three mechanisms underlying the clinical course of multiple sclerosis. Ann Neurol 1999;46:296–304.
58. Weiner HL, Guttmann CRG, Khoury SJ, Orav JE, Hohol MJ, Kikinis R, et al. Serial magnetic resonance imaging in multiple sclerosis: correlation with attacks, disability, and disease stage. J Neuroimmunol 2000;104:164–73.
59. Thompson AJ, Miller DH, Youl BD MacManus DG, Moore S, Kingsley D, et al. Serial gadolinium-enhanced

MRI in relapsing/remitting multiple sclerosis of varying disease duration. Neurology 1992;42:60–63.

60. Thompson AJ, Kermode AG, Wicks D, MacManus DG, Kendall BE, Kingsley DPE, et al. Major differences in the dynamics of primary and secondary progressive multiple sclerosis. Ann Neurol 1991;29:53–62.
61. Barkhof F, Scheltens P, Frequin STMF, Nauta JJ, Tas MW, Valk J, et al. Relapsing-remitting multiple sclerosis: sequential enhanced MR imaging vs clinical findings in determining disease activity. Am J Roentgenol 1992;159: 1041–47.
62. Sormani MP, Rovaris M, Bagnato F, Molyneux P, Bruzzi P, Pozzilli C, et al. Sample size estimations for MRI-monitored trials of MS comparing new versus standard treatments. Neurology 2001;57:1883–85.
63. Dousset V, Delalande C, Ballarino L, Queisson B, Selhan D, Coussemacq M, et al. In vivo macrophage activity imaging in the central nervous system detected by magnetic resonance. Magn Reson Med 1999;41:329–33.
64. Dousset V, Ballarino L, Delalande C, Coussemacq M, Canioni P, Petry KG, et al. Comparison of ultrasmall particles of iron oxide (USPIO)-enhanced T2-weighted, conventional T2-weighted and gadolinium-enhanced T1-weighted MR images in rats with experimental autoimmune encephalomyelitis. AJNR Am J Neuroradiol 1999; 20:223–27.
65. Sipe JC, Filippi M, Martino G, Furlan R, Rocca MA, Rovaris M, et al. Method for intracellular magnetic labeling of human mononuclear cells using approved iron contrast agents. Magn Reson Imaging 1999;17:1521–23.
66. Filippi M, Grossman RI, Comi G, editors. Magnetization transfer in multiple sclerosis. Neurology 1999;Suppl 3:53.
67. van Waesberghe JH, Kamphorst W, De Groot CJ, van Walderveen MAA, Casteljins JA, Ravid R, et al. Axonal loss in multiple sclerosis lesions: magnetic resonance imaging insights into substrates of disability. Ann Neurol 1999;46:747–54.
68. Dousset V, Grossman RI, Ramer KN, Schnall MD, Young LH, Gonzalez-Scarano F, et al. Experimental allergic encephalomyelitis and multiple sclerosis: lesion characterization with magnetization transfer imaging. Radiology 1992;182:483–91.
69. Dousset V, Brochet B, Vital A, Gross C, Benazzouz A, Boullerne A, et al. Lysolecithin-induced demyelination in primates: preliminary in vivo study with MR and magnetization transfer. AJNR Am J Neuroradiol 1995;16:225–31.
70. Campi A, Filippi M, Comi G, Scotti G, Gerevini S, Dousset V. Magnetization transfer ratios of contrast-enhancing and nonenhancing lesions in multiple sclerosis. Neuroradiology 1996;38:115–19.
71. Hiehle JF, Grossman RI, Ramer NK, Gonzalez-Scarano F, Cohen JA. Magnetization transfer effects in MR-detected multiple sclerosis lesions: comparison with gadolinium-enhanced spin-echo images and nonenhanced T1-weighted images. AJNR Am J Neuroradiol 1995;16:69–77.
72. Petrella JR, Grossman RI, McGowan JC, Campbell G, Cohen JA. Multiple sclerosis lesions: relationship between MR enhancement pattern and magnetization transfer effect. AJNR Am J Neuroradiol 1996;17:1041–49.
73. Filippi M, Rocca MA, Comi G. Magnetization transfer ratios of multiple sclerosis lesions with variable durations of enhancement. J Neurol Sci 1998;159:162–65.
74. Rocca MA, Mastronardo G, Rodegher M, Comi G, Filippi M. Long term changes of MT-derived measures from patients with relapsing-remitting and secondary-progressive multiple sclerosis. AJNR Am J Neuroradiol 1999;20: 821–27.
75. Gass A, Barker GJ, Kidd D, Thorpe JW, MacManus DG, Brennan A, et al. Correlation of magnetization transfer ratio with disability in multiple sclerosis. Ann Neurol 1994;36:62–67.
76. van Waesberghe JHTM, van Walderveen MA, Castelijns JA, Scheltens P, Lycklama à Nijeholt GJ, Polman CH, et al. Patterns of lesion development in multiple sclerosis: longitudinal observations with T1-weighted spin-echo and magnetization MR. AJNR Am J Neuroradiol 1998;19: 675–83.
77. Filippi M, Campi A, Dousset V, Baratti C, Martinelli V, Canal N, et al. A magnetization transfer imaging study of normal-appearing white matter in multiple sclerosis. Neurology 1995;45:478–82.
78. Loevner LA, Grossman RI, Cohen JA, Lexa FJ, Kessler D, Kolson DL. Microscopic disease in normal-appearing white matter on conventional MR imaging in patients with multiple sclerosis: assessment with magnetization-transfer measurements. Radiology 1995;196:511–15.
79. Filippi M, Rocca MA, Minicucci L, Martinelli V, Ghezzi A, Bergamaschi R, et al. Magnetization transfer imaging of patients with definite MS and negative conventional MRI. Neurology 1999;52:845–48.
80. Filippi M, Rocca MA, Martino G, Horsfield MA, Comi G. Magnetization transfer changes in the normal-appearing white matter precede the appearance of enhancing lesions in patients with multiple sclerosis. Ann Neurol 1998;43: 809–14.
81. Filippi M, Tortorella C, Bozzali M. Normal-appearing white matter changes in multiple sclerosis: the contribution of magnetic resonance techniques. Mult Scler 1999; 5:273–82.
82. 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;36:632–36.

83. Filippi M, Iannucci G, Tortorella C, Minicucci L, Horsfield MA, Colombo B, et al. Comparison of MS clinical phenotypes using conventional and magnetization transfer MRI. Neurology 1999;52:588–94.
84. Tortorella C, Viti B, Bozzali M, Sormani MP, Rizzo G, Gilardi MF, et al. A magnetization transfer histogram study of normal-appearing brain tissue in multiple sclerosis. Neurology 2000;54:186–93.
85. Iannucci G, Tortorella C, Rovaris M, Sormani MP, Comi G, Filippi M. Prognostic value of MR and MTI findings at presentation in patients with clinically isolated syndromes suggestive of MS. AJNR Am J Neuroradiol 2000;21: 1034–38.
86. Ge Y, Grossman RI, Udupa JK, Babb JS, Kolson DL, McGowan JC. Magnetization transfer ratio histogram analysis of gray matter in relapsing-remitting multiple sclerosis. AJNR Am J Neuroradiol 2001;22:470–75.
87. Cercignani M, Bozzali M, Iannucci G, Comi G, Filippi M. Magnetisation transfer ratio and mean diffusivity of normal-appearing white and gray matter from patients with multiple sclerosis. J Neurol Neurosurg Psychiatry 2001; 70:311–17.
88. Bozzali M, Rocca MA, Iannucci G, Pereira C, Comi G, Filippi M. Magnetization transfer histogram analysis of the cervical cord in patients with multiple sclerosis. AJNR Am J Neuroradiol 1999;20:1803–8.
89. Filippi M, Bozzali M, Horsfield MA, Rocca MA, Sormani MP, Iannucci G, et al. A conventional and magnetization transfer MRI study of the cervical cord in patients with multiple sclerosis. Neurology 2000;54:207–13.
90. Rovaris M, Bozzali M, Santuccio G, Iannucci G, Sormani MP, Colombo B, et al. Relative contributions of brain and cervical cord pathology to MS disability: a study with MTR histogram analysis. J Neurol Neurosurg Psychiatry 2000;69:723–27.
91. Rocca MA, Colombo B, Pratesi A, Comi G, Filippi M. A magnetization transfer imaging study of the brain in patients with migraine. Neurology 2000;54:507–9.
92. Rovaris M, Viti B, Ciboddo G, Gerevini S, Capra R, Iannucci G, et al. Brain involvement in systemic immune-mediated diseases: a magnetic resonance and magnetization transfer imaging study. J Neurol Neurosurg Psychiatry 1999;68:170–77.
93. Filippi M, Rocca MA, Moiola L, Martinelli V, Ghezzi A, Capra R, et al. MRI and MTI changes in the brain and cervical cord from patients with Devic's neuromyelitis optica. Neurology 1999;53:1705–10.
94. Woessner DE. NMR spin-echo self-diffusion measurement on fluids undergoing restricted diffusion. J Phys Chem 1963;67:1365–67.
95. Le Bihan D, Turner R, Pekar J, Moonen CTW. Diffusion and perfusion imaging by gradient sensitization: design, strategy, and significance. J Magn Reson Imaging 1991; 1:7–8.
96. Basser PJ, Mattiello J, LeBihan D. MR diffusion tensor spectroscopy and imaging. Biophys J 1994;66:259–67.
97. Pierpaoli C, Basser PJ. Toward a quantitative assessment of diffusion anisotropy. Magn Reson Med 1996;36:893–906.
98. Larsson HBW, Thomsen C, Frederiksen J, Stubgaard M, Henriksen O. In vivo magnetic resonance diffusion measurement in the brain of patients with multiple sclerosis. Magn Reson Imaging 1992;10:7–12.
99. Christiansen P, Gideon P, Thomsen, Stubgaard M, Henriksen O, Larsson HB. Increased water self-diffusion in chronic plaques and in apparently normal white matter in patients with multiple sclerosis. Acta Neurol Scand 1993; 87:195–99.
100. 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: 653–61.
101. Horsfield MA, Lai M, Webb SL, Barker GJ, Tofts PS, Turner R, et al. Apparent diffusion coefficients in benign and secondary progressive multiple sclerosis by nuclear magnetic resonance. Magn Reson Med 1996;36:393–400.
102. Cercignani M, Iannucci G, Rocca MA, Comi G, Horsfield MA, Filippi M. Pathologic damage in MS assessed by diffusion-weighted and magnetization transfer MRI. Neurology 2000;54:1139–44.
103. Filippi M, Iannucci G, Cercignani M, Rocca MA, 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: 1017–21.
104. Filippi M, Cercignani M, Inglese M, Horsfield MA, Comi G. Diffusion tensor magnetic resonance imaging in multiple sclerosis. Neurology 2001;56:304–11.
105. Rocca MA, Cercignani M, Iannucci G, Comi G, Filippi M. Weekly diffusion-weighted imaging study of NAWM in MS. Neurology 2000;55:882–84.
106. 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;52:1626–32.
107. Werring DJ, Brassat D, Droogan AG, Clark CA, Symms MR, Barker GJ, et al. The pathogenesis of lesions and normal-appearing white matter changes in multiple sclerosis. Brain 2000;123:1667–76.
108. Bammer R, Augustin M, Strasser-Fuchs S, Seifert T, Kapeller P, Stollberger R, et al. Magnetic resonance diffusion tensor imaging for characterizing diffuse and focal white matter abnormalities in multiple sclerosis. Magn Reson Med 2000;44:583–91.

109. Nusbaum AO, Lu D, Tang CY, Atlas SW. Quantitative diffusion measurements in focal multiple sclerosis lesions: correlations with appearance on T1-weighted MR images. AJR Am J Roentgenol 2000;175:821–25.

110. Roychowdhury S, Maldijan JA, Grossman RI. Multiple sclerosis: comparison of trace apparent diffusion coefficients with MR enhancement pattern of lesions. AJNR Am J Neuroradiol 2000;21:869–74.

111. Filippi M, Bozzali M, Comi G. Magnetization transfer and diffusion tensor MR imaging of basal ganglia from patients with multiple sclerosis. J Neurol Sci 2001;183: 69–72.

112. Cercignani M, Inglese M, Pagani E, Comi G, Filippi M. Mean diffusivity and fractional anisotropy histograms in patients with multiple sclerosis. AJNR Am J Neuroradiol 2001;22:952–58.

113. Iannucci G, Rovaris M, Giacomotti L, Comi G, Filippi M. Correlations of multiple sclerosis measures derived from T2-weighted, T1-weighted, magnetization transfer and diffusion tensor MR imaging. AJNR Am J Neuroradiol 2001;22:1462–67.

114. Nusbaum AO, Tang CY, Wei TC, Buchsbaum MS, Atlas SW. Whole-brain diffusion MR histograms differ between MS subtypes. Neurology 2000;54:1421–26.

115. Castriota Scanderberg A, Tomaiuolo F, Sabatini U, Nocentini U, Grasso MG, Caltagirone C. Demyelinating plaques in relapsing-remitting and secondary-progressive multiple sclerosis: assessment with diffusion MR imaging. AJNR Am J Neuroradiol 2000;21:862–68.

116. Davie CA, Hawkins CP, Barker GJ, Brennan A, Tofts PS, Miller DH, et al. Serial proton magnetic resonance spectroscopy in acute multiple sclerosis lesions. Brain 1994; 117:49–58.

117. De Stefano N, Matthews PM, Antel JP, Preul M, Francis G, Arnold DL. Chemical pathology of acute demyelinating lesions and its correlation with disability. Ann Neurol 1995;38:901–9.

118. Narayana PA, Doyle TJ, Lai D, Wolinsky JS. Serial proton magnetic resonance spectroscopic imaging, contrast-enhanced magnetic resonance imaging, and quantitative lesion volumetry in multiple sclerosis. Ann Neurol 1998; 43:56–71.

119. Arnold DL, Matthews PM, Francis GS, O'Connor J, Antel JP. Proton magnetic resonance spectroscopic imaging for metabolic characterization of demyelinating plaques. Ann Neurol 1992;31:235–41.

120. Falini A, Calabrese G, Filippi M, Origgi D, Lipari S, Colombo B, et al. Benign versus secondary progressive multiple sclerosis: the potential role of 1H MR spectroscopy in defining the nature of disability. AJNR Am J Neuroradiol 1998;19:223–29.

121. Arnold DL, Riess GT, Matthews PM, Francis GS, Collins DL, Wolfson C, et al. Use of proton magnetic resonance spectroscopy for monitoring disease progression in multiple sclerosis. Ann Neurol 1994;36:76–82.

122. Davie CA, Barker GJ, Webb S, Tofts PS, Thompson AJ, Harding AE, et al. Persistent functional deficit in multiple sclerosis and autosomal dominant cerebellar ataxia is associated with axon loss. Brain 1995;118:1583–92.

123. De Stefano N, Matthews PM, Fu L, Narayanan S, Stanley J, Francis GS, et al. Axonal damage correlates with disability in patients with relapsing-remitting multiple sclerosis. Results of a longitudinal magnetic resonance spectroscopy study. Brain 1998;121:1469–77.

124. Matthews PM, Pioro E, Narayanan S, De Stefano N, Fu L, Francis G, et al. Assessment of lesion pathology in multiple sclerosis using quantitative MRI morphometry and magnetic resonance spectroscopy. Brain 1996;119:715–22.

125. De Stefano N, Narayanan S, Francis GS, Arnoutelis R, Tartaglia MC, Antel JP, et al. Evidence of axonal damage in the early stages of multiple sclerosis and its relevance to disability. Arch Neurol 2001;58:65–70.

126. Gonen O, Catalaa I, Babb JS, Ge Y, Mannon LJ, Kolson DL, et al. Total brain N-acetylaspartate A new measure of disease load in MS. Neurology 2000;54:15–19.

127. De Stefano N, Narayanan S, Matthews PM, Francis GS, Antel JP, Arnold DL. In vivo evidence for axonal dysfunction remote from focal cerebral demyelination of the type seen in multiple sclerosis. Brain 1999;122:1933–39.

128. Davie CA, Barker GJ, Thompson AJ, Tofts PS, McDonald WI, Miller DH. 1H magnetic resonance spectroscopy of chronic cerebral white matter lesions and normal-appearing white matter in multiple sclerosis. J Neurol Neurosurg Psychiatry 1997;63:736–42.

129. Fu L, Matthews PM, De Stefano N, Worsley KJ, Narayanan S, Francis GS, et al. Imaging of axonal damage of normal appearing white matter in multiple sclerosis. Brain 1998;121:103–13.

130. Reddy H, Narayanan S, Matthews PM, Hoge RD, Pike GB, Duquette P, et al. Relating axonal injury to functional recovery in MS. Neurology 2000;54:236–39.

131. Clanet M, Berry I, Boulanouar K. Functional imaging in MS. Int Mult Scler J 1997;4:26–32.

132. Rombouts SARB, Lazeron RHC, Scheltens P, et al. Visual activation patterns in patients with optic neuritis: An fMRI pilot study. Neurology 1998;50:1896–99.

133. Lee M, Reddy H, Johansen-Berg H, Pendlebury S, Jenkinson M, Smith S, et al. The motor cortex shows adaptive functional changes to brain injury from multiple sclerosis. Ann Neurol 2000;47:606–13.

134. Rocca MA, Falini A, Colombo B, Scotti G, Comi G, Filippi M. Adaptive functional changes in the cerebral cortex of patients with non-disabling MS correlate with the

extent of brain structural damage. Ann Neurol 2002;51: 330–39.

135. Reddy H, Narayanan S, Arnoutelis R, Jenkinson M, Antel J, Matthews PM, et al. Evidence for adaptive functional changes in the cerebral cortex with axonal injury from multiple sclerosis. Brain 2000;123:2314–20.
136. Filippi M, Rocca MA, Colombo B, Falini A, Codella M, Scotti G, Comi G. Functional magnetic resonance imaging correlates of fatigue in multiple sclerosis. Neuroimage 2002;15:559–67.