Magnetic resonance imaging (MRI) is sensitive to focal multiple sclerosis (MS) lesions. For this reason, conventional MRI measures of the burden of disease derived from dual-echo, fluid-attenuated inversion recovery and postcontrast T1-weighted sequences are regularly used to monitor disease course in patients with confirmed MS and have been included in the diagnostic workup of patients in whom MS is suspected. Other quantitative magnetic resonance (MR)-based techniques with a higher pathological specificity (including magnetization transfer–MRI, diffusion tensor–MRI, and proton MR spectroscopy) have been extensively applied to measure disease burden within focal visible lesions and in the normal-appearing white matter and gray matter of MS patients at different stages of the disease. These methods, combined with functional imaging techniques, are progressively improving our understanding of the factors associated with MS evolution. More recently, the application of new imaging modalities capable of measuring pathological processes related to the disease that have been neglected in the past (eg, iron deposition and perfusion abnormalities) and the advent of high- and ultrahigh-field magnets have provided further insight into the pathobiological features of MS. After a brief summary of the main results obtained from the established and emerging MR methods, this review discusses the steps needed before the latter become suitable for widespread use in the MS research community.
but also in the normal-appearing WM (NAWM) and gray matter (GM). More recently, new imaging methods capable of measuring pathological processes related to the disease that have been neglected in the past (eg, iron deposition and perfusion abnormalities) and the advent of high- and ultrahigh-field magnets have provided further insight into the pathobiological features of MS.

Despite the extensive application of these new techniques in a research setting, their practical value in the assessment of MS in patients in clinical practice has yet to be realized. This review aims to (1) describe the established MR techniques that are currently applied in the evaluation of MS, (2) summarize the results obtained by quantitative MRI techniques that have been used largely in a specialized research setting during the last decade and are now ready to be moved to routine research acquisition, and (3) discuss the potential of new MRI methods (some at a high field) that are currently under investigation by a few well-established research groups.

ESTABLISHED TECHNIQUES

T2-Weighted, Fluid-Attenuated Inversion Recovery and Postcontrast T1-Weighted Sequences

Fluid-attenuated inversion recovery (FLAIR) and T2-weighted sequences are the mainstays in the workup of patients with MS. Together with postcontrast T1-weighted scans, they provide objective information about subclinical disease activity, which occurs at a rate 5 to 10 times higher than that suggested by clinical observation. As a result of rigorous studies on lesion evolution, comparison with other imaging and paraclinical modalities, and assessment of spinal cord imaging in diagnosis and differential diagnosis, neuroimaging researchers have gained confidence in the information provided by MRI.

To reduce acquisition time, conventional spin-echo sequences have largely been replaced by fast spin-echo sequences. Also, by suppressing the signal from the cerebrospinal fluid, FLAIR sequences result in better delineation of juxtacortical and periventricular lesions at the expense of decreased lesion conspicuity in the posterior fossa compared with conventional T2-weighted spin-echo sequences. Intravenous gadolinium (Gd)-based contrast agents show the blood-brain barrier breakdown in acute inflammatory lesions on T1-weighted scans as bright areas.

The T2-hyperintense areas can represent inflammation, edema, abnormal myelination, gliosis, or axonal loss. Gadolinium enhancement indicates fresh lesions with intense inflammatory activity constituting dense perivascular cuffs within lesion centers and parenchymal mononuclear cell infiltration at lesion margins.

Current diagnostic criteria incorporate T2-weighted, FLAIR and post–Gd-enhanced MRI. The identification of MS is helped by the rather characteristic patterns of lesion location and shape. Evaluation of the brain and spinal cord also helps to exclude other possible diagnoses. Typical brain MS lesions are ovoid and large rather than punctate; are located periventricularly, juxtacortically, or infratentorially in a random and asymmetric pattern; and show variable tissue destruction and frequent enhancement. Spinal cord lesions are cigar shaped, extend over less than 2 vertebral bodies and less than half of the spinal cord diameter, lie eccentrically, rarely show mass effect, and preferentially affect the cervical cord and posterior columns.

In patients with established MS, the correlation between the abnormalities seen on T2 sequences and disability is weak to moderate depending on the measure and population studied. The T2 and FLAIR lesion load (LL) reflects the accumulation of gross tissue changes. Although newly formed or enlarging T2 lesions indicate new areas of MS-related tissue damage, all T2 hyperintensity is nonspecific with respect to the actual pathological changes within lesions.

T1-Weighted Sequences

A subset of T2 lesions appears dark on T1-weighted spin-echo images. These T1-hypointense lesions, or T1 black holes, range from mildly hypointense, with intensity similar to GM, to severely hypointense, with intensity similar to that of cerebrospinal fluid. The degree of hypointensity is correlated with the degree of pathological severity. When followed up longitudinally, most of the black holes resolve during the course of about 6 months. These are termed acute black holes, and they originate in focal regions of Gd enhancement (Figure 1). The remaining T1-hyperintense lesions, the persistent black holes, constitute only about 36% of all T1-hyperintense lesions and are believed to represent irreversible axonal loss.

There are 2 major types of T1-hypointense lesion measurements commonly applied in MS: T1-hypointense LL and the number of Gd-enhancing lesions that evolve into persistent T1-hypointense lesions. Measurement of the volume of T1 black holes requires identification of regions that are hypointense on the T2-weighted images, are nonenhancing on the postcontrast T1-weighted images, and have an intensity less than that of NAWM. Quantification of T1-hypointense lesions is performed manually or by using semiautomated local thresholding approaches. In relapsing-remitting and secondary progressive MS, the T1-hypointense LL is about 5% to 20% of the total T2 LL, on average. The amount of T1-hypointense LL is low in the early stage of MS and increases during the course of the disease. In some studies, correlations between T1-hypointense LL and disability

Figure 1. Types of T1 hypointensity on magnetic resonance images. A, Postcontrast T1-weighted image at baseline has a gadolinium-enhancing lesion (arrow). B, The lesion (arrow) is hypointense on the baseline precontrast image, and another nonenhancing hypointense lesion (arrowhead) is seen. C, On the follow-up image 1 year later, the gadolinium-enhancing hypointense lesion is isointense and an acute black hole, and the nonenhancing hypointense lesion, still hypointense, is a persistent black hole (arrowhead).
are greater than those seen for T2 lesions. However, in others, the correlations with disability are similar for T1hypointense and T2 lesions, probably owing to the lack of pathological specificity in measures that include acute and persistent black holes.

Tracking the evolution of T1-hypointense lesions in longitudinal studies requires a sufficient frequency of MRI scanning and a study duration long enough to count the number of new Gd-enhancing lesions that evolve into persistent black holes. The resulting count is of particular interest in treatment trials because a reduction in the proportion of new lesions that evolve into persistent black holes may be indicative of neuroprotective effects, particularly when considered in combination with brain atrophy data.

**Atrophy Measurements**

Brain atrophy, which is usually quantified on T1-weighted images, is another marker of MS disease burden.8 The rate of whole-brain atrophy in MS is only 0.5% to 1% per year and, therefore, the techniques used to measure atrophy must be highly reproducible and sensitive to small changes. Analysis methods include segmentation-based approaches that calculate the difference in volumes measured independently at each time point (eg, brain parenchymal fraction), registration-based approaches that measure changes at the edges of the brain between pairs of images, and deformation-based approaches that detect the difference between groups of images after spatial normalization (voxel-based morphometry).9

In MS, tissue loss occurs through various destructive pathological processes, including demyelination and axonal/neuronal loss. Volume loss can also arise from resolution of inflammatory edema and other pathological and physiological reductions in tissue water content. Tissue loss is not confined to specific structures but occurs throughout the WM and GM. Gray matter atrophy may arise from a combination of primary pathological processes and as a secondary effect of damage in the WM. The specific mechanisms that lead to atrophy in MS may change over the course of disease.

Brain atrophy begins at the earliest stage of MS and progresses through the whole disease course, probably at a constant rate. It tends to correlate better with disability and cognitive impairment than other conventional MRI measures in cross-sectional and longitudinal studies. Compared with WM, GM atrophy is more strongly associated with disease progression.11 Atrophy in deep GM structures begins very early in the disease, and cortical thinning is detectable soon thereafter. Focal and diffuse damage measured in the WM predict subsequent GM atrophy in relapsing-remitting MS, but predictors of GM atrophy are lacking in secondary progressive MS, when GM atrophy may accelerate.12 The association between spinal cord atrophy and disability progression is also relatively strong. Cord atrophy appears to progress independently from tissue damage in the brain.13

Because of its biological and clinical relevance and its ease of measurement, brain atrophy has been proposed as a marker of neuroprotection in MS clinical trials.14 Most disease-modifying drugs seem to have a delayed effect on the rate of brain atrophy, and the optimal approach for use of atrophy in trials and research studies is still under investigation.

**Summary**

Conventional imaging techniques, including dual-echo, FLAIR, and Gd-enhanced sequences, have a fundamental role in the diagnostic workup of CIS patients, whereas in patients with established MS, they provide poor prognostic information. A lack of standardization of methods for measuring T1 hypointensity among centers and raters hinders its widespread use, whereas atrophy measures are sensitive and relatively easy to standardize.

**TECHNIQUES FOR FUTURE ROUTINE RESEARCH ACQUISITION**

**Double-Inversion Recovery Sequences**

Double-inversion recovery (DIR) sequences, which use 2 inversion pulses to suppress the signal from WM and cerebrospinal fluid, have improved the ability of MRI to detect cortical lesions (CLs) (Figure 2).15 Cortical lesions have been seen in all the major MS clinical phenotypes, including CIS.16 An assessment of CLs contributes to the identification of patients with CIS who are at risk of evolution to definite MS.17 Nevertheless, CLs are more frequently seen in patients with secondary progressive MS than in patients with CIS or relapsing-remitting MS.16 Cortical lesions have also been seen in the hippocampus18 and continue to form over time in patients with different MS clinical phenotypes. An association between CL burden and progression of disability and the severity of cognitive impairment has also been found.16

Several strategies have been proposed to improve the detection of CLs and allow a reliable classification of them, including the use of 3-dimensional DIR sequences19 and the combination of DIR with other sequences.20 However, the high number of false-positive findings remains a concern, as is the limited ability of these sequences to detect a large proportion of CLs, especially subpial lesions, which are seen on histopathologic specimens.

A standard protocol for the acquisition of DIR images has not yet been developed. However, multicenter consensus criteria have recently been proposed for scoring CLs on these images.21
Magnetization Transfer–MRI, Diffusion Tensor–MRI, and Proton MR Spectroscopy

Several advanced MR techniques have been developed during the past couple of decades, providing imaging biomarkers that, compared with conventional MRI measures, are better able to capture the complexity of the pathological processes occurring in the central nervous system of MS patients. Magnetization transfer (MT)–MRI, which is based on the interactions between free-water protons and protons bound to macromolecules, was proved in several studies to be superior to conventional MRI for the detection and quantification of subtle brain tissue changes. In the brain, MT-MRI provides an index of tissue integrity (the MT ratio [MTR]) that may be an expression of the extent of tissue damage.22 The MTR reduction in MS lesions and NAWM has been related to the percentage of residual axons and the degree of demyelination.23 The MT-derived measures are sensitive to MS-related changes in short periods and can provide evidence predicting the accumulation of clinical disability.22 An annual measure of MTR has been incorporated as an exploratory end point to assess treatment efficacy in large-scale multicenter trials.23,24 However, strategies to reduce intersubject and interscanner variations of MTR may be needed in single-center and multicenter studies.30

Diffusion tensor (DT)–MRI has also proved useful in MS.27 Low values of fractional anisotropy and high values of mean diffusivity have been reported in lesions and NAWM. Diffusion tensor–MRI findings in MS lesions appear to relate to different pathological features of tissue damage,28 and longitudinal studies have demonstrated that DT-MRI is sensitive to the evolution of tissue damage within MS lesions.27 Associations between DT-MRI measures in MS brains and clinical disability have also been investigated, although with conflicting findings.27 Overall, DT-MRI appears to be a promising tool for evaluating the integrity of brain structure in MS, but further investigations are warranted to elucidate the correlates with pathological tissue damage.

Proton MR spectroscopy (1H-MRS) has the unique ability to provide chemical-pathological characterization of MR-visible lesions and normal-appearing brain tissues.29 By providing evidence of neuroaxonal dysfunction or loss (based on levels of N-acetylaspartate) from the earliest stages of the disease, 1H-MRS studies have led to a reconsideration of the role of axonal damage and, by measuring changes in the levels of metabolites, such as choline and myo-inositol, have highlighted the importance of assessing myelin damage and repair. However, longitudinal studies exploiting these unique properties are rather scant, probably because of the technical challenges, which can be largely overcome by following appropriate guidelines.30

Proton MRS has been used in a few longitudinal, multicenter studies to test whether drug therapies can arrest or reverse the progression of neuroaxonal injury.30 These studies have reported comparable cross-sectional values in healthy subjects from different centers, indicating that 1H-MRS data can be very reproducible between sites when factors such as the method of data acquisition, position and size of the volume of interest, post-MRS processing, and quantification procedures are standardized.31 Because all these MR-derived measures can be routinely obtained from most modern clinical MR scanners, their use in large, multicenter clinical research studies is feasible when the inherent technical complexities are carefully taken into account.30,32,33

Functional MRI

Studies with functional MRI (fMRI) of the visual, cognitive, and motor systems have consistently demonstrated functional cortical changes in all MS phenotypes, with altered activation of regions normally devoted to the performance of a given task and/or the recruitment of additional areas compared with healthy subjects.34 Similar results have been seen with fMRI in the cervical spinal cord.35 Functional MRI abnormalities in MS patients occur relatively early in the course of the disease, even in patients with CIS and pediatric MS,36 and tend to vary over the course of the disease, not only after an acute relapse but also in clinically stable disease.34 Functional and structural MRI abnormalities in MS patients are strictly correlated,34 suggesting that increased recruitment of critical cortical networks helps to limit the functional impact of MS-related damage. However, increased cortical recruitment cannot continue indefinitely, and a lack of, or exhaustion of, the classic adaptive mechanisms has been considered as a possible factor responsible for unfavorable clinical evolution or for accelerated cognitive decline.34

Recently, the potential of fMRI in prospective multicenter studies was explored by the European Magnetic Resonance Imaging in Multiple Sclerosis (MAGNIMS) group in a study of the motor network, which enrolled 56 MS patients and 60 healthy control subjects from 8 European sites.37

Summary

Advanced MR techniques, with a high pathological specificity, have contributed to an improved understanding of different components of MS pathophysiological features. However, they still require careful standardization of acquisition and analysis, monitoring of scanner stability over time, and normative values as a reference. Additional studies are needed to evaluate their applicability in multicenter studies, as well as their sensitivity to disease progression and response to treatment in individual patients.

EMERGING TECHNIQUES AND TECHNOLOGIES

Alternative Contrast Agents

New iron-based MRI contrast agents (ultrasmall particles of iron oxide or super-paramagnetic particles of iron oxide) are useful for tracking peripheral macrophages. In vivo MS studies using ultrasmall particles of iron oxide and Gd demonstrated heterogeneity in contrast enhancement, suggesting that they provide complementary information.30 However, the clearance of these iron
Iron Quantification

In MS patients, GM areas, including the thalamus, dentate nucleus, other basal ganglia nuclei, and rolandic cortex, commonly show hypointensity on T2-weighted images, suggesting iron deposition. Although it remains unclear whether iron deposition contributes to neurotoxic effects in GM or is purely an epiphenomenon, MRI-based studies suggest a link among iron deposition, GM damage, and clinical status. One longitudinal MRI study reported that baseline T2 hypointensity in GM was the best predictor of whole-brain atrophy compared with conventional MRI findings such as lesion number and volume. Furthermore, T2 hypointensity in the GM was more closely associated with neurologic status and cognitive impairment than conventional MRI lesion measures.

Susceptibility-Weighted Imaging

Susceptibility-weighted imaging (SWI) uses a velocity-compensated, high-resolution, 3-dimensional gradient-echo sequence that creates magnitude and filtered-phase information separately and in combination, enhances the effects of local magnetic susceptibility variation, and creates new sources of contrast. Recently, SWI filtered-phase images of MS patients were shown to be useful for detecting increased iron content not only in the basal ganglia but also in lesions. In addition, ring-like hypointensity around some MS lesions visible on SWI, but not on conventional images, has been attributed to iron deposition. However, longitudinal studies are needed to determine the value of SWI. Finally, SWI enables precise in vivo visualization of the venous architecture of the brain and can help improve our understanding of the pathophysiological features of MS lesions.

Ultrahigh-Field MRI

Imaging at an ultrahigh field (>3.0 T) affords advantages in signal to noise ratio, image contrast, and resolution. However, these benefits can be realized only when using the appropriate radiofrequency coils and intensity-uniformity correction. Specialized phased-array coils giving improved GM and WM differentiation were used in an effort to improve visualization of MS lesions in vivo at 7.0 T, providing important clues for identifying GM lesions. Imaging at 7.0 T was demonstrated to be safe, was well tolerated, and provided high-resolution anatomical images allowing visualization of structural abnormalities located within or near the cortical layers. Clear involvement of the GM was observed with improved morphological detail compared with imaging at lower-field strength (Figure 3). In particular, SWI is effective at high-field strengths, with greater sensitivity to localized iron deposition, revealing that iron content was strongly correlated with disease duration (Figure 3). The images also showed distinct peripheral rings, which may be consistent with histological data demonstrating iron-rich macrophages at the periphery of lesions. In vivo MRS also benefits from the increased ratio of signal to noise at an ultrahigh field. Additional metabolites relevant to MS, such as glutathione, glutamate, γ-aminobutyric acid, and ascorbic acid, may be more clearly identified at 7.0 T. An effort was made to improve visualization of MS demyelinating lesions in vivo in GM areas, including the thalamus, dentate nucleus, other basal ganglia nuclei, and rolandic cortex, using the appropriate radiofrequency coils and intensity-uniformity correction. Specialized phased-array coils giving improved GM and WM differentiation were used in an effort to improve visualization of MS lesions in vivo at 7.0 T, providing important clues for identifying GM lesions. Imaging at 7.0 T was demonstrated to be safe, was well tolerated, and provided high-resolution anatomical images allowing visualization of structural abnormalities located within or near the cortical layers. Clear involvement of the GM was observed with improved morphological detail compared with imaging at lower-field strength (Figure 3). In particular, SWI is effective at high-field strengths, with greater sensitivity to localized iron deposition, revealing that iron content was strongly correlated with disease duration (Figure 3). The images also showed distinct peripheral rings, which may be consistent with histological data demonstrating iron-rich macrophages at the periphery of lesions. In vivo MRS also benefits from the increased ratio of signal to noise at an ultrahigh field. Additional metabolites relevant to MS, such as glutathione, glutamate, γ-aminobutyric acid, and ascorbic acid, may be more clearly identified at 7.0 T.
bic acid (vitamin C), are under active investigation, along with the macromolecular (background) signal. The quantification of such a broad neurochemical profile by use of a single method should provide insights into the roles of neurodegeneration, tissue repair, antioxidant therapy, and oxidative stress in MS. Preliminary findings suggest that glutathione concentrations in the GM of MS patients could be abnormally reduced relative to healthy controls. 46

Summary

The emerging techniques discussed herein are still in their infancy, and their practical utility remains to be investigated.

CONCLUSIONS

Conventional MRI is well established and widely applied for the diagnosis and evaluation of MS. Standardized acquisition protocols and methods of analysis are currently available and are being applied, relatively homogeneously, by the clinical and research communities. However, these techniques have some intrinsic limitations and lack specificity to the heterogeneous pathological substrates of the disease. Newer MR methods developed during the past decade, such as DIR, are likely to have an important role in the diagnosis of the disease. As a consequence, effort should be devoted to standardizing acquisition between different scanner manufacturers and centers to make them available for the clinical community. For other techniques, such as MT-MRI, DT-MRI, 1H-MRS, and fMRI, guidelines 30,32-34 for acquisition and analysis have been proposed by experts in the field. This should encourage the research community to apply them not only in the research setting but also for treatment monitoring. Nevertheless, many challenges remain. With the increased availability of high-field and ultrahigh-field scanners, these issues are now becoming extremely critical.

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