ABSTRACT

Ever-advancing radiologic techniques offer promise to patients with multiple sclerosis, not only as research tools, but also as diagnostic strategies. The following is a brief review of some of the latest advancements, including magnetization transfer and brain atrophy measurements, magnetic resonance spectroscopy, and diffusion tensor imaging.


Selection of a magnetic resonance imaging (MRI) method to study neuroregeneration in multiple sclerosis (MS) depends on how we define neuroregeneration. Neuroregeneration can be defined in broad terms as: 1) “prevention” of demyelination and neuronal damage, or 2) “recovery” from damage (ie, remyelination, restoration of axonal function, and healing of the blood-brain barrier), or 3) “replacement” of damaged brain tissue (by transplantation of stem cells or oligodendrocyte precursor cells). The MRI technique that is used to measure neuroregeneration should possess certain attributes. It should be quantitative; sensitive and pathologically specific; have the capacity to measure reversible changes, such as demyelination and remyelination; and it should be able to measure disease in normal-appearing brain tissue, because it is now known that the disease is not confined to visible white matter lesions. Therefore, the MRI must evaluate normal-appearing gray matter, white matter, as well as disease in the spinal cord. However, the question remains: Which MRI should be used?

Conventional MRI techniques, for example, contrast enhancing lesion number and T2 lesion number/volume, are sensitive quantitative measures of disease activity in MS. However, both are nonspecific markers of tissue damage. T2 lesions encompass a spectrum of pathological changes including edema, demyelination, remyelination, and axonal loss. T1 “black holes,” which evolve from contrast-enhancing lesions and T2 lesions, are thought to represent lesions with severe tissue damage and axonal loss. Recent therapeutic treatment trials have incorporated the MRI measure of T1 black hole number or volume to assess whether treatment will prevent permanent axonal loss. However, the definition of a T1 black hole is subjective, and as a result there is a high degree of interobserver variability. Moreover, quantitation of black holes remains a lesional measure and black hole measures cannot evaluate damage in normal-appearing brain tissue.

Thus, newer, nonconventional MRI methods are now being investigated. These methods include: cerebral atrophy, magnetization transfer imaging (MT), magnetic resonance spectroscopy (MRS), diffusion tensor imaging, and diffusion tractography.

Cerebral Atrophy

Because 70% of brain tissue is composed of axons and myelin, brain tissue loss (or cerebral atrophy) is an
indirect measure of demyelination and axonal loss. Unlike quantitative lesional measures, brain atrophy is a measure of global disease and reflects tissue loss in visible lesions as well as in normal-appearing white matter and normal-appearing gray matter. One limitation is the fact that atrophy is a one-way street and only irreversible tissue damage is measured.

A number of techniques for measuring atrophy have been employed. However, the most reproducible and accurate methods determine the fraction of total brain volume that is represented by brain parenchyma. The brain parenchymal fraction (BPF) or brain fractional volume can be expressed by the equation: BPF = WM + GM/WM + GM + cerebral spinal fluid. Cerebral atrophy has been used in a number of recent therapeutic treatment trials to determine whether these agents can prevent axonal loss and demyelination (Figure 1).1-4

Cerebral atrophy measures have several disadvantages. First, because atrophy is an end-stage measure, it can assess whether a drug will prevent neurodegeneration but it cannot assess remyelination or repair mechanisms. Second, the mechanism of atrophy is not well understood; whether atrophy is initiated by focal lesion damage, whether it results from a diffuse insidious process in the normal-appearing white matter, or whether cortical gray matter lesions initiate atrophy are all questions being investigated in a number of laboratories.

MAGNETIZATION TRANSFER IMAGING

Another measure of tissue structural damage is accomplished by the use of MT. This method takes advantage of the fact that in tissue there are 2 proton pools—a free proton pool and a proton pool that is bound to myelin. These 2 pools are in equilibrium with each other. When a standard MRI T1-weighted (T1W) imaging sequence is performed, the signal intensity of the image results from the protons in the free proton pool. This is shown in Figure 2. For MT, the bound proton fraction is selectively saturated by an off-resonance radiofrequency pulse and these saturated protons undergo equilibrium exchange with the free proton pool. The signal intensity of the MT image is reduced because of the presence of saturated protons in the free pool. In areas of structural damage (eg, myelin or axonal loss), the bound proton fraction is reduced, fewer protons are saturated and undergo exchange, and, therefore, these areas appear bright.

Figure 1. Brain Atrophy: Brain Parenchymal Fraction

Normalized measure of whole brain atrophy
- Represents ratio of brain parenchymal volume (BPV) to total brain volume (BV) (BPF = BPV/BV)
- Highly reproducible
- More sensitive and consistent than other methods
- Useful in evaluating therapeutic effects in controlled clinical trials

Figure 2. Magnetization Transfer Imaging: a Measure of Structural Damage

MT = magnetic transfer imaging.
Although the areas of tissue damage can be readily identified on MT images, the extent of tissue damage can also be calculated by comparing the signal intensity of the 2 sets of images obtained before and after the saturation pulse. The amount of magnetization transfer is expressed as the magnetization transfer ratio (MTR) and is calculated using the equation: MTR = (SI (T1W) – SI (MT))/SI (T1W) where SI is the signal intensity of the T1W and MT images before and after the saturation pulse respectively. The MTR can be calculated for every voxel in the brain (Figure 3).

In general, white matter has an MTR of approximately 45%, while gray matter has an MTR of 25%, and cerebral spinal fluid, because it has no macromolecular structure to which protons are bound, has an MTR of 0%. MS lesions have variable MTR values depending on the extent of structural damage. Lesions with extensive axonal loss and demyelination have lower MTR values.

Several advantages of MT scanning make it an ideal tool to study neuro regeneration because it is quantitative, sensitive to change, and can measure reversible changes. For example, small decreases in MTR most likely reflect edema without structural damage whereas large decreases in MTR reflect more extensive damage (eg, demyelination and axonal loss). MTR can measure structural changes in individual lesions, as well as changes in normal-appearing brain tissue or spinal cord tissue.

In individual lesions, MTR can be used to follow the evolution of the lesion from the initial event of gadolinium enhancement. Figure 4 demonstrates the diverse MTR patterns of 3 enhancing lesions after their appearance. Lesion #1 demonstrates only a modest decrease in MTR suggestive of edema but no permanent damage. Lesion #2 has a severe decrease in MTR at the time of enhancement and never fully recovers, which is consistent with extensive demyelination and axonal loss. This lesion evolved into a T1 black hole. Lesion #3 shows a decrease in MTR at the time of enhancement but near complete recovery—a pattern that is consistent with demyelination and remyelination. One can use MTR lesional measures to determine whether therapeutic agents can prevent axonal loss and demyelination or promote remyelination and these changes can be measured immediately and over several months (Figure 5).

One can also determine global MTR changes by determining the MTR of each brain voxel and plotting the results as a histogram (Figure 6). By comparison with healthy control subjects, the histogram in MS patients is shifted to the left indicating that an increased number of brain voxels have an abnormally...
low MTR. With disease progression, (as measured by increasing disability by the Kurtzke Expanded Disability Status Scale), the number of diseased voxels increases and the leftward shift becomes more pronounced.

The relatively strong correlation between whole-brain MTR measurements and cerebral atrophy has been noted by a number of investigators. Like brain atrophy, MTR histograms measure change slowly with time and the effect of a putative neuroprotective treatment would require 1 to 2 years to demonstrate an effect whereas lesional MTR measures respond quickly to therapeutic interventions (Figure 5).

Thus, in summary, MTR can be used to follow lesion evolution, and to examine destructive pathology or inflammation, demyelination, and remyelination. It is a quantitative measure of lesion recovery, and lesional MTR can evaluate therapeutic interventions over a shorter period of time than cerebral atrophy measures.

**MAGNETIC RESONANCE SPECTROSCOPY**

MRS is a technique that is able to detect and quantify the presence of brain metabolites other than water molecules, bringing to the field of neuroimaging a higher degree of specificity. It allows access to specific molecules by suppressing water signals during the acquisition. However, because of the rather low concentrations of these proteins relative to water molecules, MRS offers poor resolution compared with other MRI methods. For example, the amino acid N-acetyl-aspartate (NAA) may be used as a neuronal/axonal marker because its presence is believed to be unique to neurons and their processes, and it may also reflect contributions from other compounds containing N-acetyl residues. Produced by mitochondria, NAA is released within the axons and attaches to neurofilaments; its function remains unknown. Fifteen years ago, it was discovered that NAA was reduced in the brains of patients with MS. In addition, myoinositol is a carbohydrate that is currently being studied as a glial marker due to its presence in glial cells. Aside from these 2 markers, researchers also have
been able to isolate the signal from glutamate, allowing for more appropriate quantification of this important excitatory neurotransmitter. MRS is also being utilized in the study of epilepsy to measure yet another neurotransmitter, gamma-aminobutyric acid.

Most spectroscopic brain studies have used hydrogen or proton (1H) MRS using either 1HMRS, a process by which molecules are expressed as a measure for a single voxel that provides a single spectrum, or the imaging technique MRS imaging (1HMRSI), which provides an image of multiple metabolites that can be derived from multiple voxels. In other words, 1HMRS is expressed as a spectrum with no image, while 1HMRSI provides an image associated with metabolites (Figures 7A and B).

Correlating MRS with what is known about NAA in MS, during the acute phase of MS in gadolinium-enhanced lesions, NAA levels seem to be reduced compared with normal white matter. There appears to be an increase following the acute incident in recovery, but not to its baseline level where NAA is often reduced in the chronic lesion. Thus, examining levels of metabolites (such as reduced levels of NAA or peaks of glutamate) on spectroscopy may suggest patterns of illness severity as well, including in normal-appearing white matter, when lesions are not visible on traditional MRI scans.

In the future, scientists would like to determine if these detectable changes in amino acids, neurotransmitters, and other molecules will relate to brain atrophy changes and clinical impairment over time. 1HMRSI images of the corpus callosum, such as that illustrated in Figure 7B, is representative of information that is present for the entire brain, but would be more difficult to represent in its entirety. In single-center and multicenter placebo-controlled clinical trials, this methodology is being used for examining NAA changes within normal-appearing white matter and within lesions, comparing those images with a baseline scan obtained at study onset. This innovation is an important addition to the armamentarium of imaging techniques for MS.
Diffusion Tensor Imaging

Diffusion tensor imaging follows the movement of water molecules within the brain and the boundaries that water molecules may encounter. In healthy white matter, water diffusion as measured by MRI is strongly oriented in the direction of the fibers making up the white matter. However, with injury, water diffusion increases across in (perpendicular to) the direction of the axon, and this can be imaged via diffusion MRI scans. Diffusion is a fundamental property of tissue that is distinct from the other fundamental properties imaged by MRI, such as T1 and T2 relaxation. It provides information about water diffusion magnitude, the various water compartments, directional restrictions on motion of water, and preferential directions of water diffusion. Diffusion MRI also provides information about normal architecture, as well as disturbances in cell and tissue architecture. Again, as previously stated, it can provide information about pathology, perhaps specifically about fiber degeneration, and diffusion tensor MRI is the basis for the diffusion tractography.

Compared with diffusion in a simple liquid, which follows a spherical pattern, diffusion in tissue tends not to be spherical, but rather tends to be organized in a certain direction (Figure 8). Bundles of axons provide a barrier to perpendicular diffusion and a path for parallel diffusion along the orientation of the fibers. This directionality is termed “anisotropic” diffusion and is measurable. Anisotropic diffusion is high in areas of normal, mature axons. By contrast, conditions (such as in demyelination or axonal injury) where the myelin or the cell membranes are disrupted, reduce anisotropy as the barriers are destroyed or disorganized. Figure 9 illustrates how this technique helps to elucidate the sequence of events in MS, including identification of fibers at risk. For example, the “signature” pattern of Wallerian degeneration may be represented as either an increase in or slight decrease in diffusion along the course of the nerve, but most importantly by major increases in diffusion perpendicular to the nerve. In addition, diffusion techniques are being explored to examine how focal lesions affect nerve fibers.

MRI = magnetic resonance imaging.
It is possible to quantitate the change in the fiber characteristics using diffusion and other types of analyses such as magnetization transfer. From a clinical perspective, it is the goal to use these techniques to distinguish relatively benign from relatively aggressive focal or diffuse lesions, and also to use diffusion methods as treatment metrics.

**CONCLUSION**

In the future, clinicians will be able to avail themselves of these cutting-edge approaches to augment traditional MRI throughout the clinical spectrum of MS. These approaches may make it possible to identify the earliest signs of pathology, even in normal-appearing white matter—before patients become symptomatic—as well as to track lesions, especially within the context of how treatments may impact their illness.

**REFERENCES**