ABSTRACT

Multiple sclerosis (MS) is thought to be an autoimmune disease in which inflammatory demyelination is triggered by T lymphocytes and followed by axonal degeneration. Accumulating evidence suggests that axonal pathology may be a principal early step in the pathophysiology of MS. Axonal degeneration has been observed in normal-appearing white matter from patients with MS. Cortical lesions undergo demyelination and axonal transection in the absence of a classic inflammatory response. These and other observations have led some researchers to suggest that MS may be a primary neurodegenerative disease with superimposed inflammation. A variety of neuroimaging techniques are available for the assessment of MS pathology, each of which has its own advantages and limitations. Two magnetic resonance imaging techniques are especially useful for the assessment of neurodegeneration and neuroregeneration in MS. Magnetization transfer imaging provides a marker of central nervous system myelin and axonal density that can be used to study the effects of disease-modifying therapy in patients with MS. Diffusion-weighted imaging, which measures the random motion of water molecules in tissue, may be used to distinguish changes in myelination and axonal density. Cerebral atrophy is a useful marker of axonal loss that has been used extensively in clinical trials of MS treatment.


THE ROLE OF ADVANCED MAGNETIC RESONANCE IMAGING TECHNIQUES IN MS*

Nancy Richert, MD, PhD†

Multiple sclerosis (MS) is typically viewed as an autoimmune disease in which T lymphocytes are activated in the peripheral circulation, cross the blood-brain barrier into the central nervous system (CNS), and induce inflammatory demyelination.1 Inflammation and demyelination eventually result in axonal loss, which is the substrate of clinical disability.2 According to this view—which has been referred to as the “outside-in” model of MS—demyelination is the primary pathologic event, and axonal loss is a secondary consequence of demyelination.3 More recently, some investigators have proposed that MS may actually reflect an “inside-out” process in which MS lesions develop primarily as the result of a neurodegenerative disease process in normal-appearing white matter (NAWM). According to this model, axonal degeneration triggers an inflammatory response (eg, the activation of microglia), which is followed by demyelination (Figure 1).4

A growing body of evidence supports the view that axonal loss may be the primary pathophysiologic event in MS. For example, Bjartmar et al have demonstrated axonal degeneration and empty myelin sheaths in NAWM in postmortem samples obtained from patients

*Based on a presentation given by Dr Richert at the 5th Annual Johns Hopkins MS Symposium held in Washington, DC, on April 26, 2008.
†Staff Clinician, Neuroimmunology Branch, National Institute of Neurological Disorders and Stroke, National Institutes of Health, Bethesda, Maryland.

Address correspondence to: Nancy Richert, MD, PhD, Staff Clinician, Neuroimmunology Branch, National Institute of Neurological Disorders and Stroke, Building 10 Room B1N 256, National Institutes of Health, Bethesda, MD 20892. E-mail: richertn@ninds.nih.gov.
PROCEEDINGS

with MS. Cortical lesions, which are a prominent feature of MS, can occur despite the absence of an extensive inflammatory infiltrate. In addition, quantitative magnetic resonance imaging (MRI) markers of injury, such as MR spectroscopy (MRS) or magnetization transfer imaging (MTI), have documented changes in NAWM that precede inflammation (indicated by gadolinium-enhancing lesions) by 12 to 24 months. Finally, current treatments primarily target inflammation, but are only modestly effective for the prevention of brain atrophy or disease progression.

Although neurodegeneration is the cause of disability in MS, current neuroimaging techniques possess significant limitations for the evaluation of neurodegeneration. Potential neuroimaging markers of neurodegeneration in MS lesions include T1 hypointense lesions (“black holes”), CNS atrophy, MTI, diffusion tensor imaging, MRS, functional MRI, iron-labeled neural stem cells, and positron emission tomography. Selecting an appropriate imaging technique requires consideration of both imaging specificity (ie, the ability of the technique to measure changes that correspond to histopathologic findings) and feasibility (ie, the ease with which the procedure may be adapted to different imaging systems and standardized for use in clinical trials). Trade-offs between specificity and feasibility are often required in clinical practice. For example, MRS provides a very specific assessment of axonal damage, but is associated with limited feasibility due to considerable variability from site to site.

Two procedures may be especially well suited to the evaluation of neurodegeneration in patients with MS: cerebral atrophy (which is generally feasible but less specific than MRS) and MTI (which possesses moderate specificity but has demonstrated feasibility in clinical trials).

MAGNETIZATION TRANSFER IMAGING

Magnetization transfer imaging has been proposed as a specific marker of CNS myelin. Tissue myelin content is estimated using a value known as the magnetization transfer ratio (MTR), which measures the exchange of magnetization between 2 distinct tissue proton pools: protons that are freely mobile and protons that are bound to macromolecules. The MTR is calculated from a comparison of tissue signal intensity before and after application of a radiofrequency pulse. Higher MTR values indicate greater tissue myelin content. Studies that have examined postmortem pathology in individuals with MS have demonstrated that the MTR is strongly correlated with brain myelin content using multivariate analysis (and to a lesser extent with axonal density), but MTR does not correlate with the extent of gliosis.

Sequential monthly scanning to assess MTR values has been used to study the effects of MS therapies on the rate of remyelination of MS lesions over time. Using a baseline versus treatment trial design, interferon-β-1b was found to accelerate the remyelination of contrast-enhancing lesions (CELs) during the first 3 months after lesion enhancement, compared to the rate of recovery observed in untreated CELs that arose during the baseline period. In contrast, glatiramer acetate (GA) did not significantly affect the mean rate of MTR recovery over a 6-month follow-up period compared to lesion recovery during the baseline period. An analysis of the total extent of recovery during the 12-month treatment trial demonstrated that for patients who received interferon β-1b therapy, 31% of lesions totally recovered (defined as a return of MTR value to within 1 standard deviation of NAWM MTR), 43% of lesions were considered partially recovered, and 26% had evolved to black holes. With GA, 22% of lesions had totally recovered, 73% had partially recovered, and only 5% had evolved to black holes. These data suggest that GA treatment does not accelerate the rate of remyelination, but that it may have a protective
effect in preventing new lesions from evolving into T1 black holes. This pilot study demonstrates the feasibility of using MTI to evaluate neuroprotection strategies in clinical trials of patients with MS.

**DIFFUSION TENSOR MRI**

Diffusion tensor imaging measures the random motion of water molecules in tissue (mean diffusivity [MD]) as well as the directionality of water motion (fractional anisotropy [FA]), providing a second potential method to study alterations in myelin in patients with MS. In white-matter fiber tracts, water tends to move in parallel to the direction of the fibers, whereas in cerebrospinal fluid water tends to move randomly in all directions. In MS lesions and in NAWM, demyelination causes an increase in MD and a decrease in FA due to the loss of structural barriers.

Diffusion tensor imaging has been used to examine changes in MS lesions, NAWM, and in the spinal cord of patients with MS. A loss of FA in a CNS fiber tract may reflect either a decrease in axial diffusion (ie, motion in parallel with the direction of the fiber path) or an increase in radial diffusion (ie, motion that is perpendicular to the direction of the fiber path). Two recent studies—one using a mouse model of spinal demyelination (experimental autoimmune encephalitis) and the other using postmortem brain tissue from individuals with MS—have demonstrated that changes in axial FA correspond to alterations in axonal density, whereas changes in radial FA correspond to altered tissue myelin content. This suggests that it may be possible to use a single MRI technique to provide detailed information about alterations to myelin and axonal density in MS, and to use these outcome measures to evaluate the effects of MS therapy.

**MEASURING AXONAL LOSS**

The most specific MRI method for evaluating axonal loss is MRS, which permits the visualization of specific metabolites in CNS tissue. For the assessment of axonal integrity, the most important of these metabolites is N-acetylaspartate (NAA), which is exclusively contained in neuronal cell bodies and axons. Diminished NAA concentration is apparent in MS lesions and in NAWM in individuals with MS and is associated with neuronal loss or metabolic dysfunction. Studies of patients with MS have also demonstrated that decreased NAA concentration is significantly correlated with higher (ie, worse) Expanded Disability Status Scores (EDSS) scores. The limitations of MRS include low signal-to-noise ratio and limited reproducibility. Several small clinical trials that included MRS as an outcome measure have produced inconsistent findings.

T1 hypointense lesions (black holes), which evolve from CELs and T2 lesions, have also been used as a measure of axonal loss in patients with MS. Initial histopathology studies demonstrated that T1 black holes are characterized by severe axonal loss and CNS matrix destruction (Figure 2). However, a more recent study with postmortem tissue from patients with MS suggests that axonal density did not differ between lesions that were hypointense or isointense using T1 imaging. These investigators suggested that T1 black holes probably represent matrix destruction rather than specific alterations in axonal density. Assessment of black holes is a subjective measure subject to high interobserver variability. In addition, because T1 black holes evolve from and are a subset of T2 lesions, they may provide only minimal

---

**Figure 2. Scatter Plot of MTR and Axonal Density in All Regions**

MTR was strongly correlated with axonal density ($r = 0.83, P < .0001$). In those regions containing NAWM, MTR also correlated with axonal density ($r = 0.76, P = .002$).

additional information about MS pathology. Despite these limitations, the assessment of T1 black holes has been a useful outcome measure in MS clinical trials. For example, a clinical trial that compared GA to placebo in 239 patients with MS demonstrated that treatment with GA reduces the evolution of CELs to T1 black holes, compared with placebo (Figure 3).²⁰,²¹

**Brain Atrophy**

Cerebral atrophy occurs in all MS subtypes and is seen even at the earliest stages of the disease. The rate of brain atrophy is approximately 0.5% to 1% per year in individuals with MS, which is approximately 10-fold greater than in healthy control subjects.²² Measurement of CNS atrophy detects changes in axonal density and myelin, which comprise approximately 70% of the brain volume.²³ Although white-matter and gray-matter atrophy can be detected in patients with early MS, the rate of gray-matter atrophy progresses at a faster rate over an 18-month period of observation,²⁴ and gray-matter atrophy is modestly correlated with clinical disability.²⁵

Measurement of cerebral atrophy is a quantitative and highly reproducible measure for assessment of neurodegeneration in patients with MS. Although it has been incorporated as an outcome measure in all recent clinical trials, the rate of cerebral atrophy is only modestly reduced by current anti-inflammatory therapies.²⁶ Atrophy measurements may be confounded by several factors. Apparent brain volume on MRI may be decreased by dehydration, corticosteroid treatment, or the use of cytotoxic chemotherapy agents, and may be increased by active inflammation and edema.²⁷

Despite these potential pitfalls, atrophy remains a robust measure of tissue damage and is being used as a primary outcome measure for a neuroprotection trial with lamotrigine (a sodium channel blocker) for the treatment of MS.²⁷

**Conclusions**

Neuroprotection is emerging as an important potential strategy for the treatment of MS, and several potentially neuroprotective agents are currently being developed. New technologies permit the specific evaluation of axonal loss, atrophy, demyelination, and remyelination. Ongoing and future clinical trials will continue to define the strengths and limitations of these new techniques in the assessment of MS and the response to treatment.

**Question and Answer Session**

Q: You talked about the fact that measuring brain parenchymal volume is feasible at community radiology sites across the country. Neurologists could get a brain parenchymal fraction and over time see it change. Do you think this is a valid measurement to follow as a clinical marker? Would that affect therapy decisions?

Dr Richert: I think that annual MRIs with that information would be incredibly helpful to a clinician treating a patient. The question is, who is going to develop that program? I understand that Elizabeth Fisher at the Cleveland Clinic is patenting analysis software, and plans to make it available to clinicians. In terms of changing therapy, I think you have to have a significant number of measurements. An apparent increase in atrophy on a single evaluation might be caused by any of several factors. For example, the patient might have been recently treated with steroids causing a transient decrease in brain volume. I think it would be dangerous to make treatment changes based on a single assessment.

Q: Is it true that gray-matter lesions are more likely to correlate with disability? How good are current MRI techniques for assessing gray-matter changes?
**Dr Richert:** One would think that because that is where the neuronal cell bodies are concentrated, gray-matter lesions would correlate better with cognitive deficits and clinical disability. Unfortunately, the ability to quantitate gray-matter lesions has been limited by the lack of an inflammatory response, and these lesions do not enhance with contrast. Double inversion recovery sequences have been developed by Geurts et al., but even this technique only measures a fraction of the total number of cortical lesions present. Some investigators have used higher field strength magnets (eg, 3 T or 7 T), which do increase the number of lesions detected. We need histopathology correlation to say that what we are seeing at higher field strength correlates with a cortical lesion on a pathology specimen, and we are not there yet. I think it is very difficult to conclude that gray-matter lesions can explain all clinical symptoms. We currently have a study at the National Institutes of Health in which we are trying to correlate cortical thickness with cognitive deficits. A very interesting paper recently examined the diffusion tensor properties of cortical lesions. Methods such as these may provide more sensitive assessments of gray-matter changes than we currently have.

**Q:** Do any of the techniques that you discussed predict long-term outcomes?

**Dr Richert:** In clinically isolated syndrome, T2 lesion load and the accumulation of T2 lesions over the first 5 years predict the amount of atrophy and clinical disability 14 to 20 years later. However, in relapsing-remitting MS (RRMS) this does not seem to be the case. In an 8-year follow-up study by Fisher et al in a cohort of patients with RRMS, cerebral atrophy (a lower brain parenchymal fraction) at entry was the strongest predictor of disability, whereas T2 lesion load at baseline did not predict EDSS scores. If T2 lesion number was combined with a measure that detects the amount of damage within those T2 lesions (such as MTR), that measure might be more predictive of future disability.

**REFERENCES**


