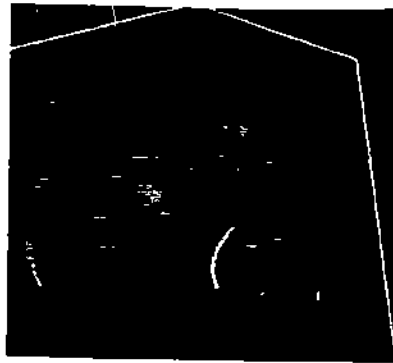




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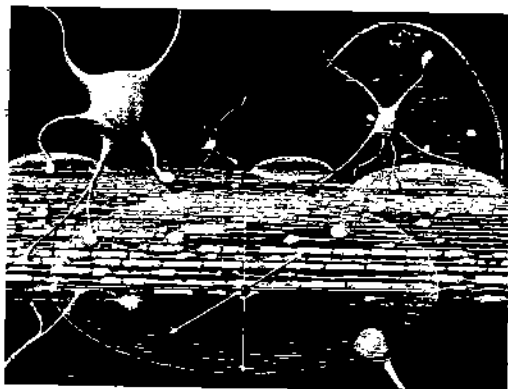
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May 18, 2009

John Harney MD
 375 Municipal Drive
 Suite 222
 Richardson, TX 75080

Dear Dr. Harney,

We would like to share with you an exciting new project starting here at University of Texas Southwestern, and ask for your help in referring patients. In a collaboration between UTSW and Cleveland Clinic, we are working to develop better imaging tools to measure MS tissue injury, recovery, and potentially even long-term restoration. As you know, gadolinium and T2 spots are great at identifying areas of inflammation, but don't measure the *degree* of inflammation and injury. Our goal is to develop a more dynamic imaging metric of tissue injury. This metric will be used in clinical trials of medicines that improve recovery and prevent degeneration. But we need your help.



The National Multiple Sclerosis Society has just awarded us a 3-year grant to use the internuclear ophthalmoplegia (INO) system to validate diffusion tensor imaging as a dynamic measure of tissue injury. Diffusion tensor imaging (or "DTI") is an imaging method that measures the 3-dimensional diffusion of water, which then characterizes tissue injury (see picture on left). A recent review of DTI is enclosed here. In this project, we will study 40 patients with ***new-onset INO*** using both quantitative oculography (which measures eye movements) and DTI of the medial longitudinal fasciculus (MLF). Our pilot study

using this technique is also enclosed here. The primary outcome will be to use DTI at INO onset to predict functional recovery at 1 year.

40 new-onset INO patients is a big group, so we need your help in recruiting them. We are asking you to refer to us patients with new-onset INO. The patients you refer are welcome to continue following in your own practice – they only come to UTSW three times over one year for study visits.

The entry criteria are very simple and are outlined in box. We accept patients on

Acute INO Study - Entry Criteria

Inclusion criteria:

- Acute internuclear ophthalmoplegia
- Relapsing remitting MS or clinically isolated syndrome
- Age 18-50

Exclusion criteria:

- Previous internuclear ophthalmoplegia
- Treatment with corticosteroids for current internuclear ophthalmoplegia

Acute INO Study – Overview

- 1-year, 3-visit observation study:

Baseline	History Oculography DTI MRI
2 Months	Oculography DTI MRI
1 year	Oculography DTI MRI

- Visit Duration: ~ 3 hours each
- Costs: None
- Patient stipends are provided



Brain DTI image, showing fiber tracts. Arrows show disrupted fibers from 2 MS plaques.

or off long-term injectable therapies. All patients would be expected to receive IV steroids as part of their standard treatment for INO. However, we need to perform baseline oculography and imaging ***prior to any steroid administration***. We have made arrangements to enroll patients within 24-48 hours, so we will not significantly delay their steroid treatment.

In advance, we thank you very much for your support of our study. Many of you provided us with a letter of collaboration for our National MS Society application, and for that we are truly grateful. We will be delighted to acknowledge your referral contribution in publications which come from this study.

Please join us as we work to develop better ways to image MS – image its tissue destruction, the later repair, and chronic degeneration. We are relying on you to do this study.

Sincerely,



Elliot Frohman, MD, PhD, FAAN

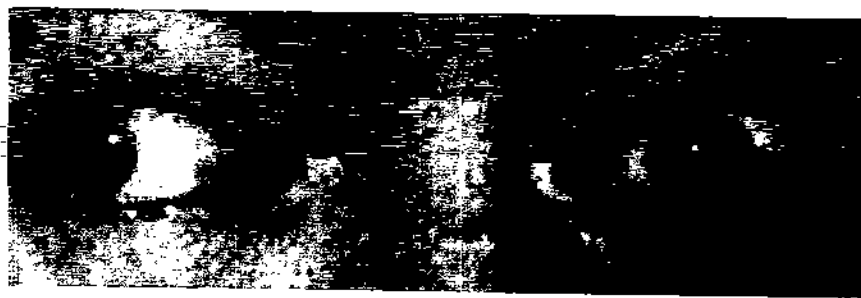


Robert J. Fox, MD, FAAN

Acute INO Study - How to refer patients

- Business Hours: Call/Page Research Nurse:
Gina Remington, RN O: 214-645-0560 P: 214-786-0275
- After 6PM: Elliot Frohman, MD, at 214-502-2079 (Cell)

WANTED



NEW-ONSET INO PATIENTS

FOR 1-YEAR OBSERVATIONAL
MRI & OCULOGRAPHY STUDY

IF FOUND: CALL
DR. ELLIOT FROHMAN
214-645-0555

Picturing Multiple Sclerosis: Conventional and Diffusion Tensor Imaging

Robert J. Fox, M.D.¹

ABSTRACT

Magnetic resonance imaging (MRI) has provided an unparalleled window into understanding multiple sclerosis (MS). Through recognition of relatively specific characteristics of MS, MRI has become an integral part of patient initial evaluation and long-term management. MRI has now been integrated into the formal diagnostic criteria, whereby new lesions can fulfill either dissemination in space or dissemination in time criteria. Long-term MS therapies significantly reduce the development of new lesions as measured by MRI, and clinical trial methodology now routinely uses MRI as the primary outcome in Phase I/II MS trials. Despite the advantages provided by MRI, conventional imaging indicates only the presence of injury to the central nervous system, providing little information on either the severity of injury or its later recovery. Several advanced imaging methodologies such as diffusion tensor imaging (DTI) provide a greater dynamic range for evaluating tissue integrity. DTI has provided useful insights into the pathogenesis of MS, both within lesions as well as within the white matter which appears normal on conventional imaging. Evidence from animal models suggests that DTI may differentiate axonal injury from demyelination and therefore may be useful in the evaluation of neuroprotective therapies.

KEYWORDS: Multiple sclerosis, diffusion tensor imaging, T1 hole, brain atrophy

Multiple sclerosis (MS) is a chronic inflammatory demyelinating disease affecting the brain, spinal cord, and optic nerves. Originally described through pathological studies by Charcot in the nineteenth century, it wasn't until the development of advanced imaging techniques that the full extent and recurrent nature of MS was appreciated, particularly at the early stages of disease. The hallmark of MS is recurrent demyelination of the central nervous system (CNS), disseminated in space and time. The original diagnostic criteria relied exclusively on clinical relapses to fulfill the dissemination requirement, but more recent diagnostic criteria integrate imaging into the formal diag-

nostic process. Multiple sclerosis causes characteristic changes on brain and spine imaging, and these characteristics can be helpful in differentiating MS from other disorders. Many imaging reports of MS will list demyelination in the differential diagnosis of any scan with T2 lesions, regardless of lesion characteristics and location. A greater appreciation of the classic imaging characteristics of MS and their relevance to the disease process can help the clinician in both the accurate diagnosis and successful management of patients with MS. This article will discuss the different imaging characteristics of MS and their relevance to its diagnosis and long-term management.

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Although magnetic resonance imaging (MRI) improves our ability to make an accurate diagnosis of MS and manage the disease over time, advanced imaging methods provide an even greater ability to understand the degree of tissue injury, its recovery over time, and its later degeneration. Diffusion tensor imaging (DTI) is a method to measure the three-dimensional diffusion of water, which is altered in areas of tissue injury. This article will also describe the application of DTI to MS and illustrate its potential utility in the assessment of neuroprotective therapies.

CONVENTIONAL IMAGING FINDINGS IN MULTIPLE SCLEROSIS

Brain Lesions

The most sensitive imaging modality in the evaluation of MS is MRI. Computed tomogram (CT) scans can sometimes show hypodensity within some MS lesions which have severe destruction, but its limited discrimination between healthy and demyelinated tissue renders CT a poor tool in the evaluation for MS.

T2 Lesions

Inflammation and demyelination within the CNS causes prolongation in the T2 relaxation time, which manifests as bright signal on T2-weighted images such as fast spin echo (FSE). In MS, these "T2 lesions" are typically round or ovoid, varying in size from a few millimeters to many centimeters in diameter. Although MS lesions can occur throughout the brain, there is a particular predilection for the periventricular region, particularly the corpus callosum (Fig. 1). In contrast to central corpus callosal involvement of Susac's syndrome,¹ MS lesions within the corpus callosum typically extend radially from the ventricular (or inner) surface of the corpus callosum in a pattern called Dawson's fingers. Subcortical and deep cerebral white matter regions are also commonly involved in MS, although the latter is relatively non-specific for MS (Fig. 1). The brainstem and cerebellum also are commonly involved in MS (Fig. 2). Deep gray matter regions such as the basal ganglia and thalamus can be affected by MS, although this is less common than involvement with white matter regions.

Where recurrent lesions affect the same brain region, confluent lesions develop (Fig. 3). These confluent lesions are particularly common anterior and posterior to the lateral ventricle. In the later stages of MS, this confluence can involve the entire hemisphere in one large confluent T2 lesion. Vasogenic edema is characterized by the indiscrete (or "fuzzy") extension of T2 signal into the surrounding white matter, particularly beyond the border of gadolinium enhancement. Vasogenic edema is uncommon in most MS lesions, but

occasionally can be seen in large lesions and those with avid gadolinium enhancement (Fig. 4).

Dual spin echo imaging techniques yield a proton density image, which provides better contrast between MS lesions and cerebrospinal fluid (CSF). Several other pulse sequences further improve the appreciation of MS lesions, including fast or turbo spin echo, double-inversion recovery (DIR), and fluid-attenuated inversion recovery (FLAIR).² A distinct advantage of FLAIR and DIR images are their suppression of CSF, which allows greater appreciation of periventricular and subcortical lesions (Fig. 1). A disadvantage of FLAIR and DIR is their reduced contrast for lesions in the brainstem and cerebellum, which is best seen on proton density images.

Nonspecific foci of hyperintensities on T2-weighted images are common in the deep white matter, particularly in older patients and those with vascular risk factors and migraine. In a patient with an otherwise negative history for MS, deep white matter T2 lesions which spare the periventricular, subcortical, and posterior fossa regions generally need no further evaluation.

T1 Holes

When tissue injury is severe, MS lesions can cause T1 prolongation, which manifests as dark signal on T1-weighted images, also called "T1 black holes" (Fig. 1). Only a subset of T2 lesions will demonstrate T1 black holes, and this proportion varies between patients. Where tissue destruction is very severe, the inversion recovery pulse on FLAIR can make T1 holes black on even FLAIR images (Fig. 5). T1 holes are rarely seen in the posterior fossa or spinal cord. Pathologic studies have found that T1 hypointensities correlate with demyelination and axonal loss, and this correlation is stronger than that seen with T2 lesions.^{3,4} The evolution of enhancing lesions into T1 black holes is associated with more progressive disease.⁵

Gadolinium-Enhancing Lesions

Gadolinium enhancement within MS lesions indicates areas of very active inflammation, which leads to breakdown of the blood-brain barrier. The pattern of enhancement can be homogeneous, ring, or heterogeneous. Ring enhancement usually indicates reactivation of an old lesion. Almost all T2 lesions will have gadolinium enhancement when they first develop, although its duration may be only a few days or weeks, and so is often missed on routine MRI. In only 5% of patients will enhancement of the same lesion persist for 3 months or more.⁶

Several methods can increase the sensitivity to detect gadolinium enhancement: increased dose of gadolinium, prolonged delay after administration (i.e., 15 minutes or more), and combination with an

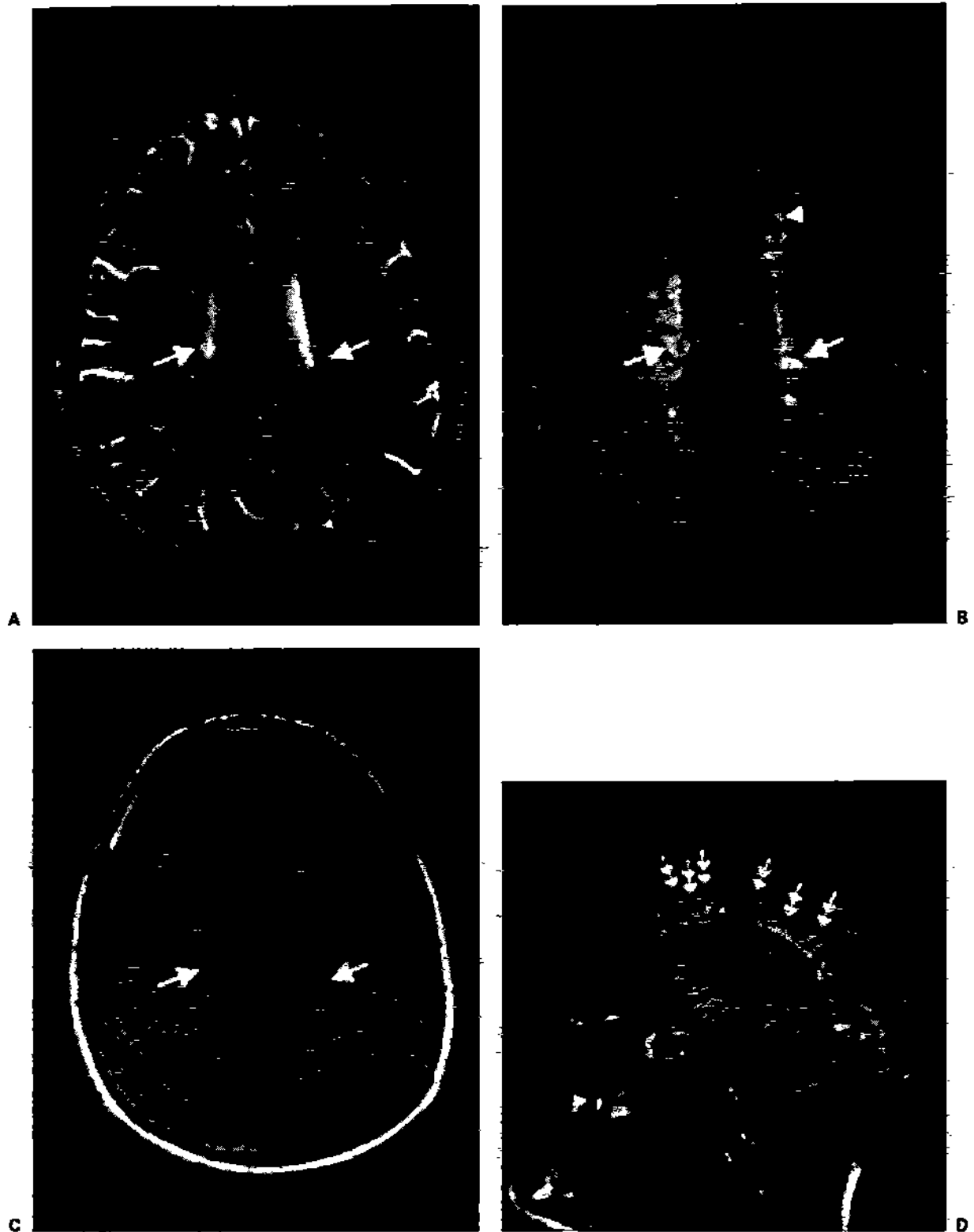


Figure 1 Axial (A) T2, (B) FLAIR, and (C) T1-weighted, and sagittal (D) FLAIR images from a typical multiple sclerosis patient. Many periventricular lesions are seen (arrows), which are better appreciated on FLAIR images. Many lesions (arrows) are also dark on T1 images, but not all (arrowhead). Sagittal FLAIR images provide the best appreciation of Dawson's fingers, in which lesions extend radially from the ventricular surface into the surrounding white matter (D, double arrows). FLAIR, fluid-attenuated inversion recovery.

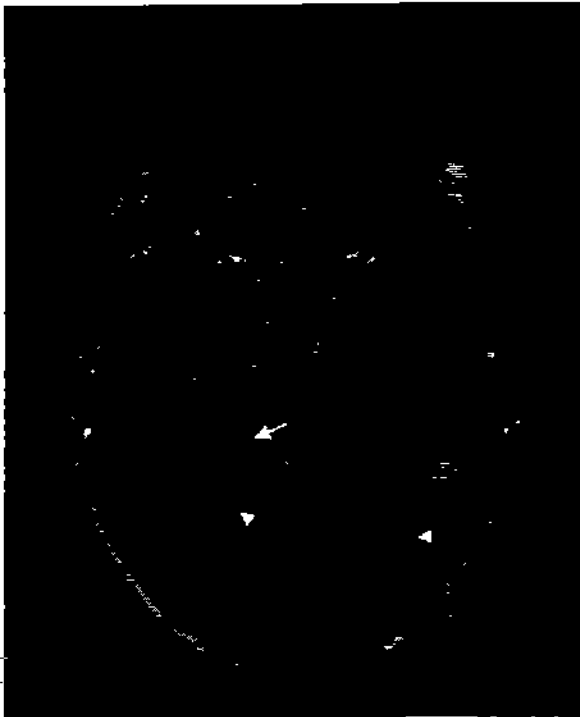


Figure 2 Axial proton density image of the posterior fossa from a patient with multiple sclerosis, showing characteristic lesions in the pons, middle cerebellar peduncle (arrow), and cerebellum (arrowheads).

off-resonance magnetization transfer (MT) sequence.^{7,8} To accurately appreciate the presence of gadolinium-enhancing lesions, a post-gadolinium image that includes MT should have a corresponding pre-gadolinium image that also includes MT. Application of an MT sequence to only the post-gadolinium images can make it difficult to recognize whether bright areas represent gadolinium enhancement or simply increased signal from the added MT sequence.

Spinal Cord Lesions

The spinal cord parenchyma is commonly involved in MS. Similar to the brain, lesions are typically round or

ovoid on T2-weighted images and usually are limited to one to two spinal cord segments (Fig. 6). Eighty percent of cord lesions involve less than half of the cross-sectional area of the cord, and the majority are centered on either the lateral or posterior portion of the cord.⁹ Lesions can be central or bilateral in the cord, but typically are unilateral. Inflammatory edema can lead to short-term cord expansion. Also similar to the brain, gadolinium enhancement indicates areas of active inflammation and secondary breakdown of the blood-brain barrier.

Conventional T2 images provide only limited contrast between MS lesions and normal cord tissue. Proton density imaging increases this contrast, and short tau inversion recovery (STIR) imaging provides even greater contrast (Fig. 7). Spine imaging protocols evaluating for demyelination should include sagittal STIR and fast spin echo T2 and axial fast spin echo T2 pulse sequences to maximize contrast between inflammatory lesions and normal tissue. Pathological studies of the spinal cord have observed significantly greater demyelination than that seen on the corresponding MRI obtained postmortem.¹⁰ This observation contrasts with brain MRI, where demyelination is not seen outside of T2 lesions.³ The decreased sensitivity in MRI detection of demyelinated lesions within the spinal cord emphasizes the importance of using high-field (i.e., 1.5T or greater) magnets and appropriate pulse sequences when imaging the spinal cord. Open MRI systems generally should be avoided when evaluating any part of the CNS for MS, particularly the spinal cord.

Optic Nerve

MS lesions affecting the optic nerve can sometimes be seen on MRI. Robust signal from the surrounding ocular fat overwhelms signal from the small optic nerve on conventional T1-weighted and T2-weighted images such as FSE, proton density, and FLAIR. This problem is solved with STIR and T1-weighted fat suppressed techniques.¹¹

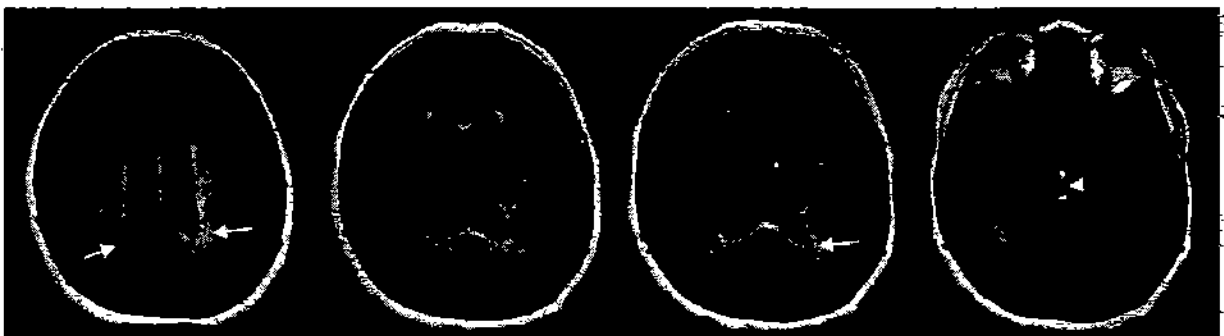


Figure 3 Axial FLAIR images from a 52-year-old patient with multiple sclerosis. The formerly discrete T2 lesions have now become confluent (arrows). Also, significant atrophy can be appreciated through loss of brain parenchyma as well as enlargement of the lateral ventricles, peripheral sulci, and third ventricle (arrowhead). FLAIR, fluid-attenuated inversion recovery.

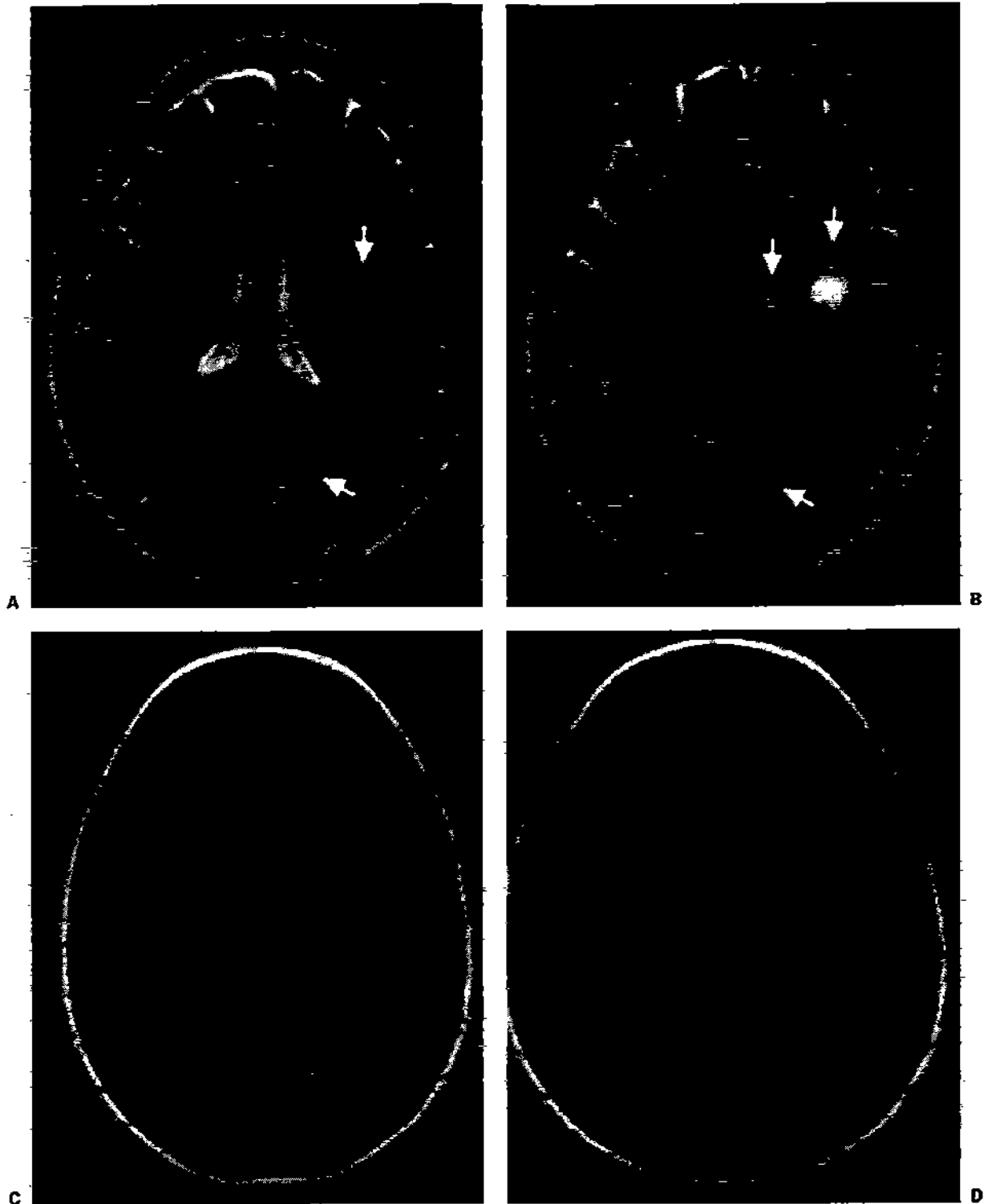


Figure 4 Axial (A, B) T2-weighted and (C, D) post-gadolinium T1-weighted images from a multiple sclerosis patient with vasogenic edema. Note the fuzzy border of T2 signal change (arrows), which extends beyond the limits of gadolinium enhancement.

Brain Atrophy

Pathologic studies have observed an average of 11,000 transected axons/mm³.¹² Therefore, it is no surprise that atrophy of the brain and spinal cord is common in patients with MS.¹³⁻¹⁵ Brain atrophy can be appre-

ciated as enlargement of the lateral and third ventricles as well as widening of the cortical sulci (Fig. 3). Brain atrophy is observed at the earliest stages of MS and is best appreciated with quantitative image analysis software. The relevance of brain atrophy is illustrated by its

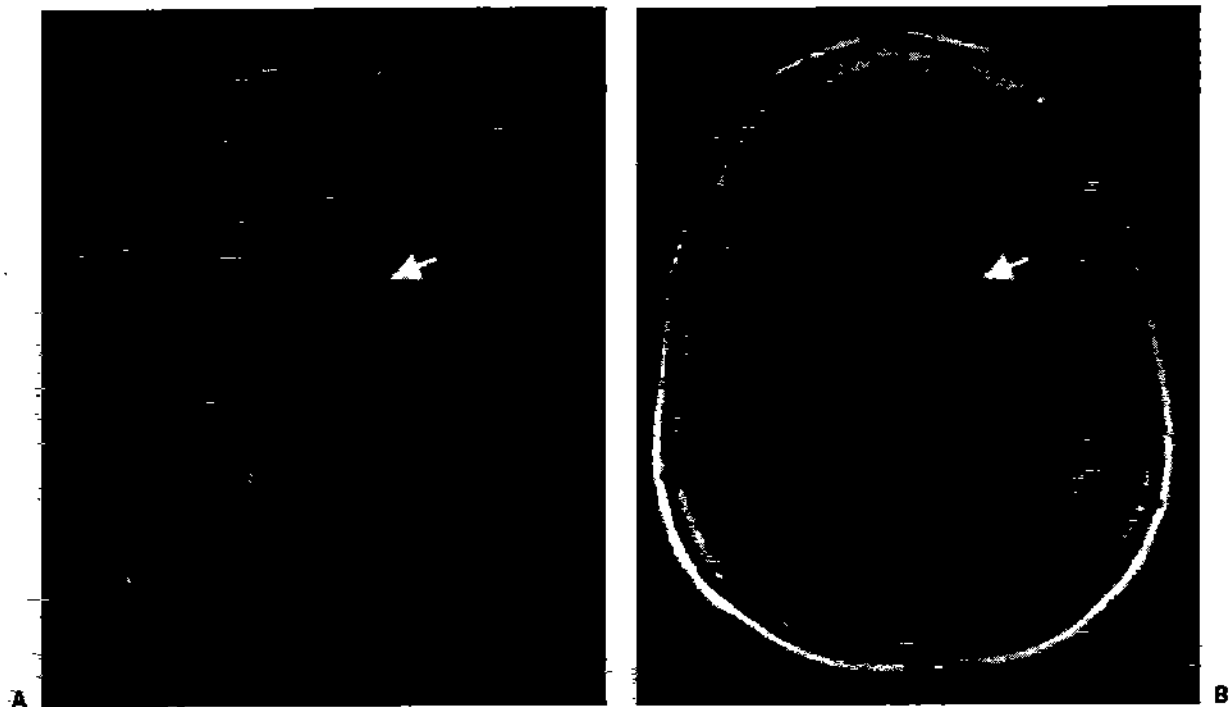


Figure 5 Axial (A) FLAIR and (B) T1-weighted images showing a T1 black hole which is mixed hypo-/hyperintense on FLAIR (A, arrows). FLAIR, fluid-attenuated inversion recovery.

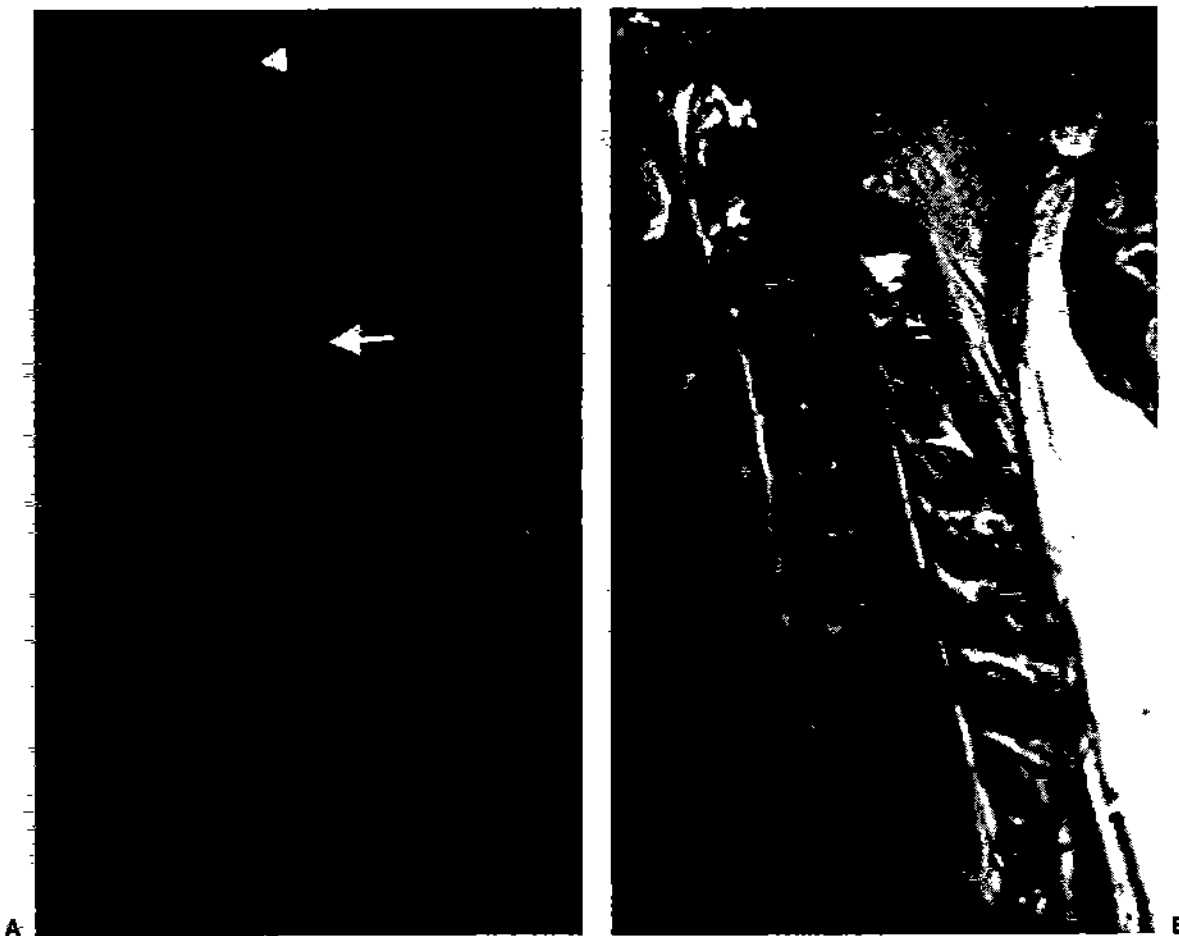


Figure 6 Sagittal (A) T2-weighted and (B) post-gadolinium T1-weighted images from a patient with multiple sclerosis, showing a discrete, enhancing T2 lesion (arrows). In addition, a nonenhancing lesion is seen in the medulla (A, arrowhead).

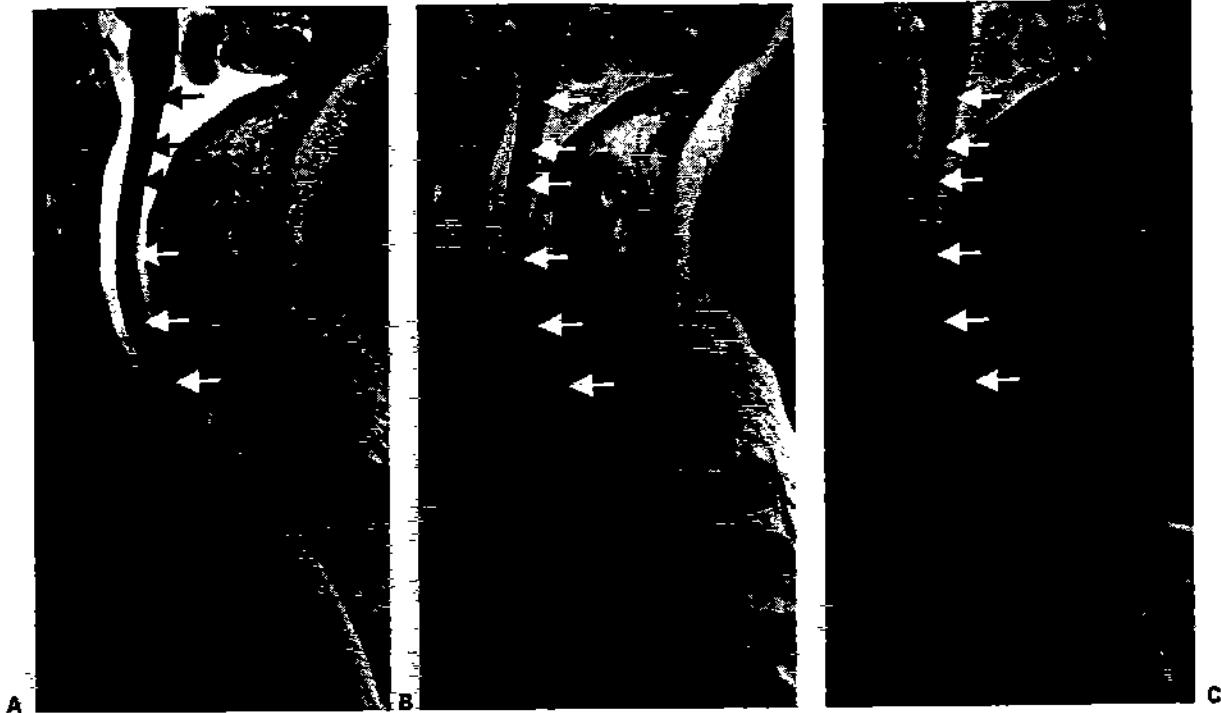


Figure 7 Sagittal (A) T2-weighted, (B) proton density, and (C) short tau inversion recovery (STIR) images from a patient with multiple sclerosis. Increasing contrast between lesions (arrows) and normal tissue is appreciated on the proton density image and STIR, with STIR providing the best contrast.

correlation with the discrete lesions, particularly T1 holes.^{16–19}

The clinical implications of brain atrophy are significant. Brain atrophy correlates with clinical disability, although the magnitude of these correlations is only modest.²⁰ Brain atrophy is predictive of later progressive disability.²¹ Importantly, many standard MS therapies have been shown to slow the progression of brain atrophy over time, which is compelling evidence suggesting a long-term benefit of therapy in altering the course of disease.^{22–24}

CLINICAL DIAGNOSIS

Since the Poser diagnostic criteria²⁵ were developed prior to the widespread availability of MRI, it is no surprise that they contains little place for the use of imaging. MRI was finally integrated into the formal diagnostic algorithm in the 2001 McDonald Criteria, where MRI could fulfill dissemination in both space and time.²⁶ The 2005 revisions to the McDonald Criteria clarified several aspects regarding the role of imaging within these criteria (Table 1).²⁷ These diagnostic criteria require direct review of T2-weighted images, as they require identification of a sufficient number of lesions in specific areas of the brain and spinal cord. Despite the additional benefit provided by MRI, the diagnosis of MS remains a clinical assimilation of patient history, neurological examination, imaging studies, and other appropriate tests, when needed. Multiple

sclerosis should not be diagnosed based upon MRI findings alone.

Occasionally, patients present with classic changes of MS on imaging studies, despite no history of a clinical relapse or progressive neurologic symptoms. This situation is becoming increasingly common as nonspecific neurological symptoms are assessed using MRI. Longitudinal follow-up in these patients often identifies new lesions, thus fulfilling “dissemination in time and space” criteria, despite an absence of an original clinical event. Additional testing, such as evoked

Table 1 MRI Criteria for Dissemination in Space and Time

MRI criteria for dissemination in space require three of the following:

- ≥ 1 gadolinium-enhancing lesion or ≥ 9 T2 hyperintense lesions in the brain or spine
- ≥ 1 infratentorial brain lesion or spine lesion
- ≥ 1 juxtacortical lesion
- ≥ 3 periventricular lesions

MRI criteria for dissemination in time require either:

- gadolinium-enhancing lesion ≥ 3 months after the onset of the initial clinical event and in a different site corresponding to the initial event
- a new T2 lesion compared with a reference scan done at least 30 days after the onset of the initial clinical event

Modified from Polman et al.²⁷
MRI, magnetic resonance imaging.

potentials and CSF studies, can provide further evidence of an ongoing inflammatory disorder. Multiple sclerosis diagnostic criteria do not yet allow for formal diagnosis of MS in this situation, although MS is very likely. There are no longitudinal studies to guide clinical management in these patients. Accumulated neurologic impairment and evidence of ongoing inflammation (i.e., gadolinium enhancement or new T2 lesions) may identify a subset of patients who could benefit from therapy.

Standardized Imaging Protocol

The Consortium of MS Centers has issued consensus guidelines on the standard imaging protocols. Diagnostic brain MRIs should include scout, sagittal fast FLAIR, axial FSE proton density or T2, axial fast FLAIR, and axial post-gadolinium (0.1 mmol/kg followed by a 5-minute delay) T1 sequences.²⁸ Although not part of the formal recommendations, pre-gadolinium T1 images are very useful in the accurate interpretation of gadolinium enhancement. Standard spinal cord pulse sequences include scout, sagittal T1 and FSE proton density/T2, axial FSE proton density/T2, post-contrast sagittal T1, and postcontrast axial T1 through suspected lesions.²⁸ Where available, sagittal STIR could replace or be added to axial FSE proton density/T2. Treatment with corticosteroids leads to rapid closure of the blood-brain barrier and thus decreased gadolinium enhancement. Therefore, routine clinical MRIs usually should be delayed for 1 month following corticosteroids so that the potential return of gadolinium-enhancing lesions can be appreciated.^{29,30}

DIFFERENTIAL DIAGNOSIS OF MULTIPLE SCLEROSIS

When classic recurrent clinical symptoms of MS (i.e., optic neuritis, partial transverse myelitis) present in the context of classic changes on brain and/or spine MRI, the differential diagnosis is extremely limited. Other autoimmune disorders, such as sarcoidosis, Sjögren's syndrome, systemic lupus erythematosus (SLE), and primary CNS vasculitis can present with similar imaging findings to that of MS, but the clinical presentation usually points toward an alternative diagnosis. These clinical features may include an atypical clinical course (i.e., acute onset, prolonged duration of symptoms, or insensitivity to corticosteroids) or additional clinical signs (i.e., pulmonary symptoms in sarcoidosis, or discoid rash and other organ involvement in SLE). For a full review of the differential diagnosis of MS from an imaging perspective, see Charil et al.³¹

Not uncommonly, imaging findings are mild, such as only a few lesions involving the periventricular, subcortical, or posterior fossa regions. In these situations, further diagnostic studies may be useful to confirm

MS, including CSF studies, blood serologies, and evoked potentials.

Acute Disseminated Encephalomyelitis

A common clinical dilemma is the patient with a monophasic clinical course, where the main differential diagnosis is between acute disseminated encephalomyelitis (ADEM) and the first demyelinating event of what will later become MS (also known as a clinically isolated syndrome). Accurate diagnosis is important, since the former requires no long-term treatment, while the latter deserves consideration of long-term MS immunomodulating therapy.³² ADEM cannot always be differentiated from MS based upon imaging alone, but typically involves more numerous enhancing lesions, a greater proportion of enhancing versus nonenhancing lesions, and fewer chronic lesions during the acute clinical phase (Fig. 8). After resolution of ADEM, its differentiation from MS based on imaging alone is usually very difficult.

Neuromyelitis Optica

When inflammatory lesions extend across many spinal cord segments (i.e., ≥ 3), the diagnosis of neuromyelitis optica (NMO, or Devic's disease) is raised. NMO is a disorder in which inflammatory lesions primarily affect the optic nerves and long segments of spinal cord, sometimes involving the entire cord. In contrast to MS, spinal cord lesions in NMO can be hypointense on T1 images (T1 black holes). When NMO involves the brain, it often causes lesions atypical for classic MS: large, expansile lesions, often extending from the midline with heterogeneous morphology.³³ Previously considered a variant of MS, emerging data over the last several years suggest that NMO is separate and distinct from classic MS.³⁴

CLINICAL RELEVANCE TO THE MULTIPLE SCLEROSIS DISEASE COURSE

MRI provides an opportunity to measure the extent and severity of MS injury throughout the CNS. New T2, T1, and gadolinium-enhancing lesions can usually be easily counted, and software tools can accurately quantify overall lesion burden. Even brain atrophy can be measured through a variety of automated and semiautomated software. Initial cross-sectional studies found only modest correlations between MRI measures and clinical disability, and this relationship plateaus with increased disability.³⁵⁻³⁸ This limited correlation likely has several explanations, including heterogeneity of pathology within T2 lesions, variable compensatory capability within tissue, and heterogeneity in tissue importance to neurologic function. For example, a lesion in the deep occipital white matter will likely have vastly less (if any) impact on neurologic function than a similarly sized

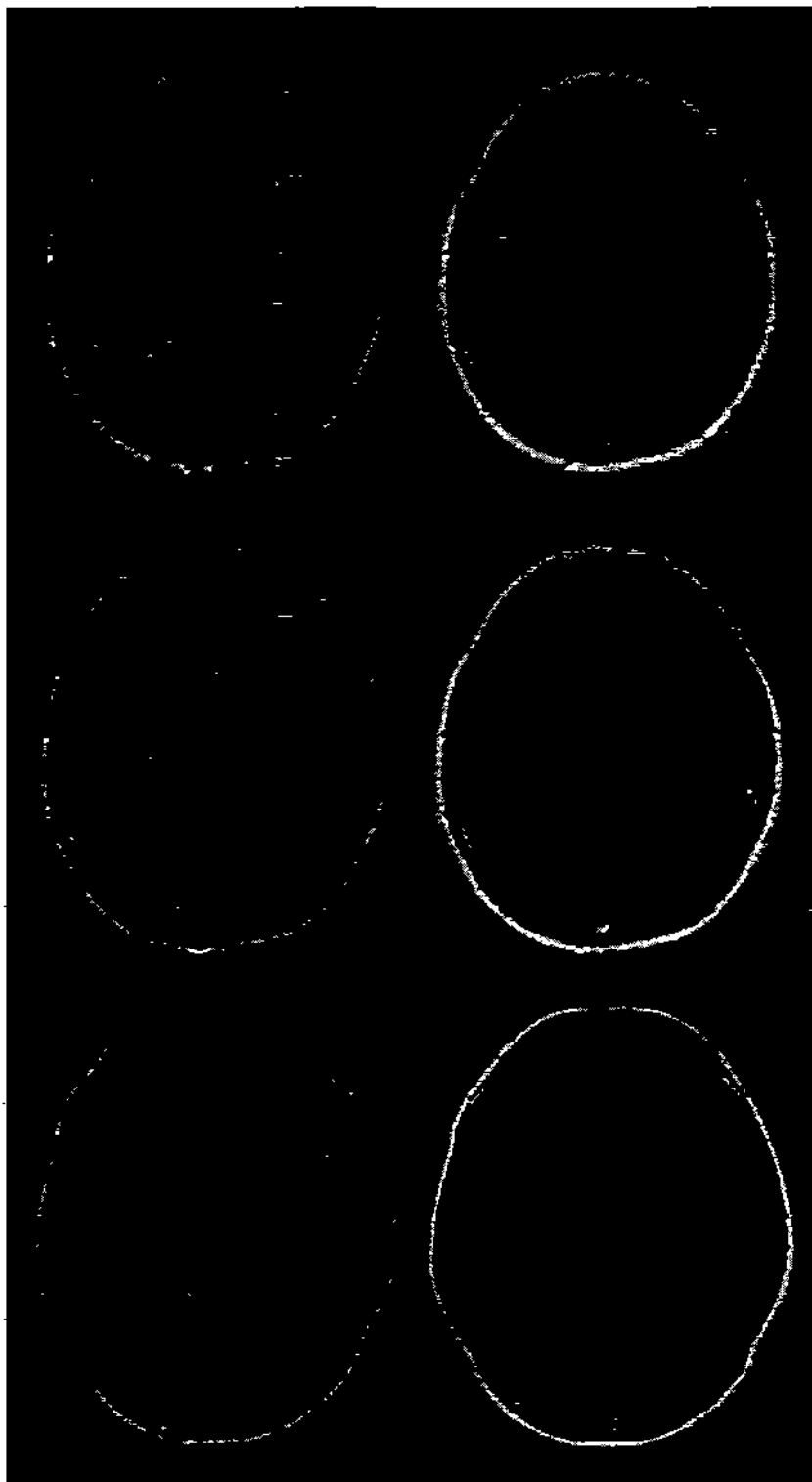


Figure 8 Axial FLAIR (left column) and post-gadolinium T1 (right column) images from a patient with acute disseminated encephalomyelitis. Most lesions demonstrate gadolinium enhancement, suggesting a similar age to the ongoing inflammatory process. FLAIR, fluid-attenuated inversion recovery.

lesion in the pons, cerebellar peduncle, or spinal cord. Active lesions (either enhancing or new T2 lesions) are a much stronger predictor of future disability, most likely due to their overall gauge of ongoing inflammatory disease activity.^{39,40}

The utility of MRI in patient management is controversial, although the results of longitudinal studies suggest that MRI can be used to assess response to therapies. In one study, new T2 or gadolinium lesions over 1 year was a stronger predictor of disability

progression at 2 years than clinical relapses.⁴⁰ Atrophy progression and new lesions over 2 years predict disability progression over 13 years, suggesting that therapies which slow progressive brain atrophy will likely benefit clinical disability over a very long period of time. Indeed, enhancing and new T2 lesions are the conventional primary outcomes in clinical trials of new MS therapies. To date, there has not been a Phase II clinical trial of an MS anti-inflammatory therapy where a therapeutic benefit on MRI lesions was not later confirmed in a Phase III trial by a therapeutic benefit on clinical relapses and, where properly powered, clinical disability. Clearly, newly developing lesions on MRI are relevant to both clinical disease and response to therapy.

DIFFUSION TENSOR IMAGING

Despite significant advantages in studying conventional lesions, these measures have distinct disadvantages. Prolonged T2 relaxation identifies areas of tissue injury, but does not characterize the degree of tissue injury. Similarly, gadolinium enhancement indicates areas of breakdown of the blood-brain barrier, but not the degree of underlying injury. T1 images identify lesions with more significant tissue injury as confirmed in histopathological studies,^{3,4} but methods to standardize T1 hypointensity measurement are still in development. When characterizing brain tissue integrity, the binary classification of each pixel on MRI as either "sick" or "normal" results in a very restrictive dynamic range. This restriction in turn significantly limits how we measure both tissue recovery after injury as well as its later degeneration. Advanced imaging modalities are needed to more accurately characterize the degree of tissue injury and its response to therapies. Several advanced imaging modalities are available to address

this need, including MT imaging, spectroscopy, T2 relaxation, and DTI. The remainder of this article will focus on one of these modalities—DTI.

Diffusion Tensor Imaging Background

Diffusion tensor imaging is a quantitative MRI technique that measures the diffusion of water through the application of multiple diffusion gradients.⁴¹ A single diffusion pulse sequence is standard on many imaging protocols, measuring water diffusion in a single direction. Diffusion tensor imaging applies multiple diffusion gradients on sequential pulse sequences from which the three-dimensional diffusion of water molecules within tissue can be calculated. The power of diffusion techniques comes from its measurement of molecular probing of tissue structures by water molecules at a microscopic scale well beyond the typical MR image resolution. By measuring the interaction of water molecules with cell membranes, myelin sheaths, and macromolecules, tissue integrity can be inferred.

Water diffusion within a nonrestricted volume (such as a glass of water) is equal in all directions (or "isotropic"), and so can be represented as a sphere. However, brain tissue has physical structures that limit diffusion. When diffusion is limited in one direction more than another (i.e., along fiber tracts), diffusion is elongated, or "nonisotropic" (Fig. 9). Diffusion within a nonisotropic volume (i.e., tissue with directional geometry) can be represented as a three-dimensional ellipsoid, and so requires a 3×3 matrix for a full description of its size and orientation. The ellipsoid can be described by its three main axes (Fig. 9). The longest (or primary) axis of the ellipsoid is represented by the largest eigenvector of the diffusion tensor, and is called the primary eigenvector (λ_1). In organized white matter (i.e., fiber tracts),

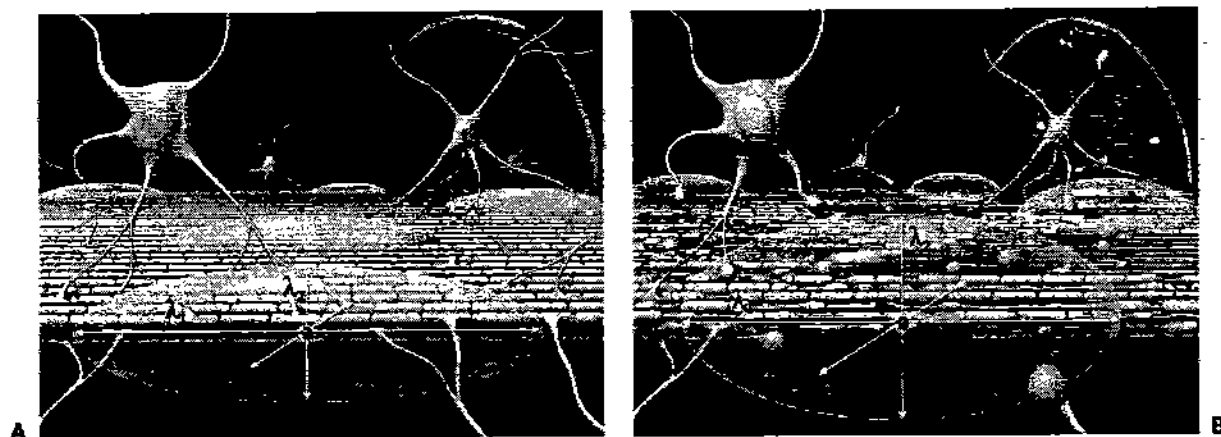


Figure 9 Artist's depiction of water diffusion. (A) Within fiber tracts, where axons and myelin orient diffusion longitudinally, water diffusion is oriented along the fiber tract. (B) In areas of demyelination and axonal injury, water diffusion is greater in overall amount (increased mean diffusivity), and also less elongated (decreased fractional anisotropy). λ_1 indicates the primary, or largest, eigenvector of the diffusion tensor; λ_2 and λ_3 represent the second and third eigenvectors, respectively.

the primary eigenvector is assumed to be oriented parallel to the main fiber tract. The two shorter axes are represented by the second (λ_2) and third (λ_3) eigenvectors of the diffusion tensor.

There are four main measures of interest derived from DTI, as follows.

AXIAL DIFFUSIVITY ($\lambda_{||}$)

The primary eigenvector (λ_1) is thought to represent diffusion along the length of a fiber tract (the left-to-right vector in Fig. 1), and so is referred to as the axial diffusion, or $\lambda_{||}$ ($||$ referring to diffusion parallel to the fiber tracts). Accordingly, $\lambda_{||}$ is thought to best represent axonal integrity.

RADIAL DIFFUSIVITY (λ_{\perp})

The second (λ_2) and third (λ_3) eigenvectors are typically similar in magnitude and are thought to represent diffusion across fibers and myelin (see below). Therefore, these two eigenvectors are averaged together and referred to as the radial diffusivity, or λ_{\perp} (\perp referring to diffusion perpendicular to the fiber tract). Although λ_{\perp} likely represents diffusion across both myelin and axons, λ_{\perp} is thought to best reflect myelin integrity.

MEAN DIFFUSIVITY

An overall measure of water diffusion, mean diffusivity (MD) ignores the anisotropic character of diffusion and simply describes the overall amount of diffusion using a scalar. Mean diffusivity is derived mathematically as an average of the three eigenvectors: $MD = (\lambda_1 + \lambda_2 + \lambda_3)/3$. In general, MD is relatively similar throughout white matter, and is slightly increased in gray matter. Within MS lesions, restriction to diffusion is decreased and MD increases (Fig. 10).

FRACTIONAL ANISOTROPY

An advantage of DTI over standard diffusion pulse sequences (which measure diffusion in only one direction) is its measure of orientation, or "elongatedness," of water diffusion. Tightly packed fiber tracts (i.e., pyramidal tracts and corpus callosum) have highly elongated diffusion, while gray matter has minimally elongated diffusion. A common method to describe the degree of diffusion elongatedness is fractional anisotropy (FA), which measures the fraction of the magnitude of overall diffusion that can be ascribed to anisotropic diffusion. FA varies from 0 to 1, with 0 indicating complete isotropic diffusion (i.e., no contribution of anisotropic diffusion) and 1 indicating complete anisotropy. Lattice index is another measure of anisotropy, which integrates the anisotropy of neighboring voxels together, and so may be a more stable anisotropy measure.⁴² Within MS white matter lesions, radial diffusion is increased more than longitudinal diffusion, which leads to decreased anisotropy, or decreased FA and lattice index.

Pathologic Specificity of Diffusion Tensor Imaging

Several studies evaluated the pathologic specificity of $\lambda_{||}$ and λ_{\perp} using animal models of CNS inflammation and provide consistent pathologic evidence that $\lambda_{||}$ and λ_{\perp} reflect the integrity of axons and myelin, respectively. Diffusion tensor imaging of the spinal cord of mice with experimental allergic encephalomyelitis showed decreased $\lambda_{||}$ throughout the spinal cord, which correlated with increased staining of antibody against the axonal-relevant β -amyloid precursor protein.⁴³ In focal regions of myelin loss (as visualized by Luxol fast blue staining), λ_{\perp} was decreased. Retinal ischemia causes wallerian degeneration of the optic nerve, leaving myelin intact for a period of time. Three days after induction of retinal ischemia, DTI of the optic nerve showed a decrease of $\lambda_{||}$ with no change in λ_{\perp} . Subsequent pathology confirmed axonal degeneration without demyelination.⁴⁴ Five days after ischemia, there were changes on DTI in both $\lambda_{||}$ and λ_{\perp} , which correlated with both axonal degeneration and demyelination. Systemic administration of cuprizone causes demyelination, followed by remyelination. Diffusion tensor imaging evaluation in cuprizone-treated mice found that changes in λ_{\perp} paralleled pathologically-confirmed demyelination and remyelination.⁴⁵ Shiverer mice have congenital dysmyelination with intact axons. Diffusion tensor imaging studies of shiverer mice found increased λ_{\perp} , but normal $\lambda_{||}$.⁴⁶

Diffusion Tensor Imaging in Multiple Sclerosis

Diffusion tensor imaging identifies tissue injury in patients with MS. In T2 lesions, MD is increased and FA is decreased compared with normal-appearing white matter (NAWM), or isointense tissues on T2-weighted images.^{47,48} MD is further increased and FA further decreased in T1 holes.⁴⁹ Even NAWM demonstrates abnormalities in MD and FA compared with healthy controls. Gray matter also shows abnormalities on DTI, which is of particular note since T2-weighted images are typically normal in the gray matter.⁵⁰ The clinical relevance of DTI is demonstrated by the significant correlations between expanded disability status scale and for MD and FA.^{48,50} The functional relevance of DTI was shown through a study of the median longitudinal fasciculus of MS patients with internuclear ophthalmoplegia, where DTI measures of injury to the median longitudinal fasciculus correlated with the degree of ocular dysmotility.⁵¹

Longitudinal studies have evaluated DTI changes over time. Lesional FA and MD as well as normal-appearing gray matter MD was observed to worsen in a group of MS patients followed for 15 to 18 months.^{52,53} In fact, progressive worsening on DTI can be detected in the NAWM prior to the appearance of new lesions.⁵⁴ Diffusion tensor imaging has also been found to be a

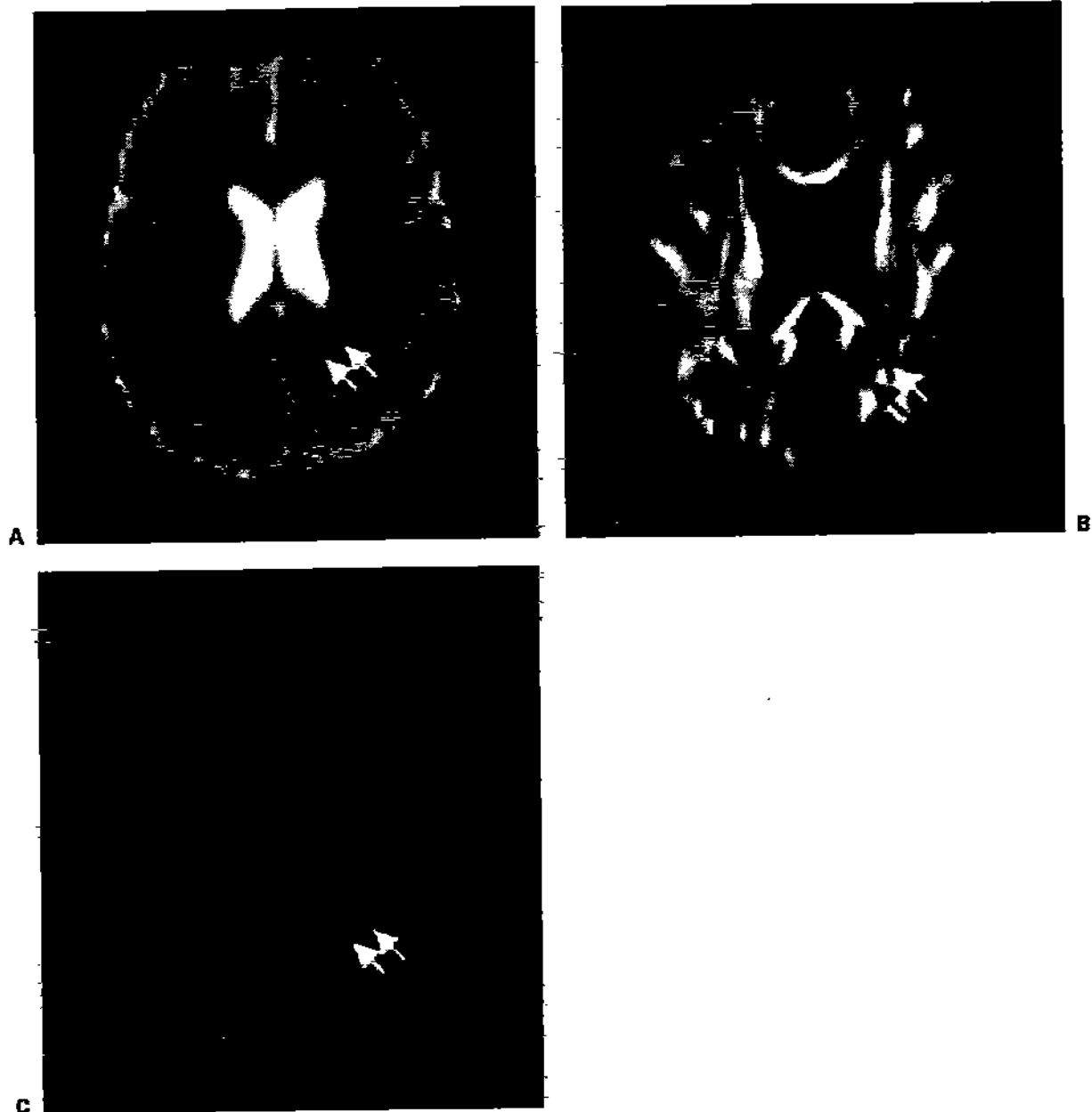


Figure 10 Diffusion tensor images from a patient with multiple sclerosis. (A) Mean diffusivity shows the overall amount of diffusion; (B) fractional anisotropy shows the amount of anisotropy, or "elongatedness," of diffusion; and (C) colorized primary eigenvector maps illustrate different directions of the primary eigenvector, or fiber tract. Red is left-right; green is up-down; blue is in-out of the page. The arrows indicate the effect of two multiple sclerosis lesions: mean diffusivity is increased (bright), fractional anisotropy is decreased (dark), and the primary fiber direction is disrupted (dark).

useful tool for monitoring the progression of upper motor neuron pathology in the neurodegenerative disease amyotrophic lateral sclerosis.⁵⁵

The continuous scale and relative pathologic specificity of DTI makes it an attractive candidate imaging marker of axonal injury, demyelination, and remyelination in MS. Although further studies are needed to fully understand its clinical implications and potential utility, DTI appears to hold potential as a useful marker of tissue integrity in clinical studies of neuroprotective therapies. Potential applications are

many, ranging from acute inflammatory injury in relapsing remitting MS to chronic degeneration in primary and secondary progressive MS.

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NOTES

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A Preliminary Validation Study of Diffusion Tensor Imaging as a Measure of Functional Brain Injury

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Ken Sakaie, PhD; Elliot Frohman, MD, PhD

Background: Diffusion tensor imaging (DTI) characterizes multiple sclerosis (MS) tissue injury, although it has remained unproven whether DTI changes in disease have functional consequences. The medial longitudinal fasciculus (MLF) is a key brainstem pathway for ocular adduction and is commonly injured in patients with MS, typically resulting in internuclear ophthalmoparesis.

Objective: To validate DTI as a physiologically relevant measure of brain tissue integrity.

Design: A correlation study of ocular dysmotility and DTI conducted between January 2004 and September 2004.

Setting: Multiple Sclerosis Center, University of Texas Southwestern Medical Center, Dallas.

Patients: Six patients with chronic, unilateral, or bilateral internuclear ophthalmoparesis and 10 healthy control subjects.

Main Outcome Measure: We used infrared oculography to correlate the velocity versional dysconjugacy index, defined as the ratio of the velocity of the abducting

to adducting eye movements during horizontal saccades, and DTI measures within the MLF as measured through an anatomical overlay. Overall diffusion was measured by mean diffusivity, and anisotropy was measured by the lattice index.

Results: Within the pontine MLF, the mean diffusivity was increased compared with healthy controls ($P < .005$), whereas the pontine lattice index was decreased ($P < .03$). Correlations were observed between the velocity versional dysconjugacy index and the mean diffusivity (left: $r = 0.65$, $P < .01$; right: $r = 0.46$, $P = .07$). Similar correlations were found between the versional dysconjugacy index and the lattice index (left: $r = -0.43$, $P = .09$; right: $r = -0.65$, $P < .01$).

Conclusions: We identified DTI evidence of pathologic disruption of a small brainstem fiber pathway, which is crucial for accurate horizontal eye movements. In this small study, we observed correlations between the DTI changes and oculomotor dysfunction. Our preliminary observations provide criterion validity of DTI as a surrogate marker of brain tissue integrity.

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INTERNUCLEAR OPHTHALMOPARESIS (INO) is a common neurologic finding in patients with multiple sclerosis (MS). As INO manifests diplopia, oscillopsia, or difficulty with reading. Examination shows impaired eye adduction despite intact abduction of the contralateral eye and binocular convergence. An INO is commonly caused by an inflammatory demyelinating lesion that affects the medial longitudinal fasciculus (MLF), which connects the nucleus of the sixth cranial nerve to that of the contralateral medial rectus subnucleus of the oculomotor complex.¹

In patients with MS with an INO, prolonged T2 relaxation (hyperintensities on T2-weighted images) is typically observed in the pons or midbrain.² Although prolonged T2 relaxation indicates areas of tissue injury, it does not

accurately characterize the degree of this injury.^{3,5} Similarly, gadolinium enhancement indicates areas of blood-brain barrier breakdown but not the degree of injury. Other, more advanced imaging modalities may better characterize the degree of tissue injury and may provide greater insight into brain tissue injury and its response to therapies.

Diffusion tensor imaging (DTI) is a quantitative magnetic resonance imaging (MRI) technique that measures the 3-dimensional diffusion of water molecules within tissue through the application of multiple diffusion gradients well beyond the usual resolution of MRI. In particular, DTI is useful in characterizing white matter.^{6,7} The power of diffusion techniques comes from their measurement of molecular probing of tissue structures by water molecules at a microscopic scale well

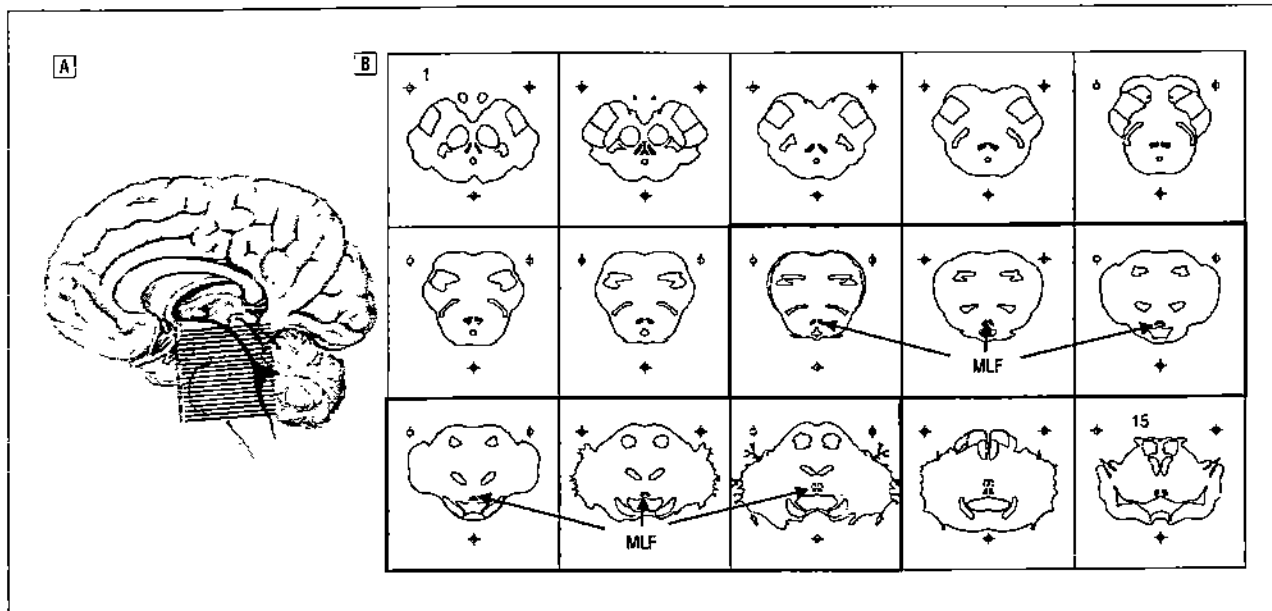


Figure 1. Brainstem sections used to evaluate diffusion tensor imaging measures within the medial longitudinal fasciculus (MLF). A, Illustration of brainstem sections. B, Anatomical overlay maps, identifying the MLF in black from the pons to the midbrain. The 6 black-boxed sections represent the 4 pontine levels evaluated in the primary outcome. The nonboxed midbrain levels were also evaluated in a sensitivity analysis.

beyond the usual MRI resolution. By measuring the interaction of water molecules with cell membranes, myelin sheaths, and macromolecules, tissue integrity and structural injury can be inferred.

Diffusion tensor imaging studies in MS have observed disrupted water diffusion. Within demyelinating lesions, mean diffusivity (MD) (a measure of overall water diffusion) is increased, and anisotropy (the degree of nonspherical diffusion of water, such as along fiber tracts) is decreased.^{8,9} Similar changes are seen in the normal-appearing white matter.^{8,10}

Although a purported advantage to DTI is its quantitative nature, studies correlating DTI with functional impairment have generally used coarse measures, such as overall clinical disability.^{10,11} To date, no studies have correlated DTI changes with electrophysiologic measures of neurologic function, particularly within a discrete tract system. It is important to validate DTI as a functionally relevant measure of neural disruption within the central nervous system. This validation will help lay the foundation for its use to study the pathophysiology of neurologic diseases, as well as corroborate its potential as an imaging metric of neurodegeneration for use in clinical trials. In this preliminary study, we used an anatomical overlay to identify the MLF in patients with MS and correlated DTI measures with functional impairment, as measured with infrared oculographic metrics of horizontal saccadic eye movements.

METHODS

PATIENTS

Between January 2004 and September 2004, we performed DTI and quantitative ocular motility studies on 6 patients with MS (2 with relapsing-remitting disease, 3 with secondary progressive disease, and 1 with primary progressive disease; mean dis-

ease duration, 12.3 years [range, 7-18 years]; mean [SD] age, 51.8 [4.1] years) with varying severity of INO and compared them with 10 healthy controls (mean [SD] age, 36.4 [12.7] years). All patients with MS had long-standing INO documented on previous clinical examinations, often with bilateral involvement.

INFRARED OCULOGRAPHY

Eye movements were evaluated through quantitative infrared oculography. Previous discriminative analyses have identified the ratio of abduction to adduction eye peak velocity during a 20° saccade, called the velocity versional dysconjugacy index (vel-VDI), as the most accurate and reliable measure of the abnormalities of INO.¹² Through use of this within-patient index, the vel-VDI minimizes interindividual and intraindividual (fellow eye) differences that occur with the quantitative analyses of monocular eye movements. The vel-VDI was converted to a z score, using a group of healthy controls as the reference population. Recently, vel-VDI z scores have been used successfully to characterize the effect of heat intolerance on ocular dysfunction.¹³

IMAGING

All imaging was performed on a 1.5-T GE scanner (GE NVI; General Electric, Waukesha, Wisconsin) using a head strap to minimize motion. Pulse sequences included axial fast spin-echo T1-weighted image (echo time [TE], 8.5 milliseconds; repetition time [TR], 600 milliseconds; flip angle, 90°; bandwidth, 244 Hz per pixel; 2 averages; 128 × 128 matrix; 140 × 140-mm field of view; 4-mm section thickness), axial dual echo (first TE, 23 milliseconds; second TE, 118 milliseconds; TR, 4000 milliseconds; flip angle, 90°; bandwidth, 122 Hz per pixel; 1 average; 256 × 192 matrix; 140 × 140-mm field of view; 4-mm section thickness), axial echo planar imaging fluid-attenuated inversion recovery (TE, 90 milliseconds; TR, 6000 milliseconds; flip angle, 90°; bandwidth, 633 Hz per pixel; 8 averages; 128 × 128 matrix; 140 × 140-mm field of view; 4-mm section thickness), and 25-direction, single-shot, diffusion-

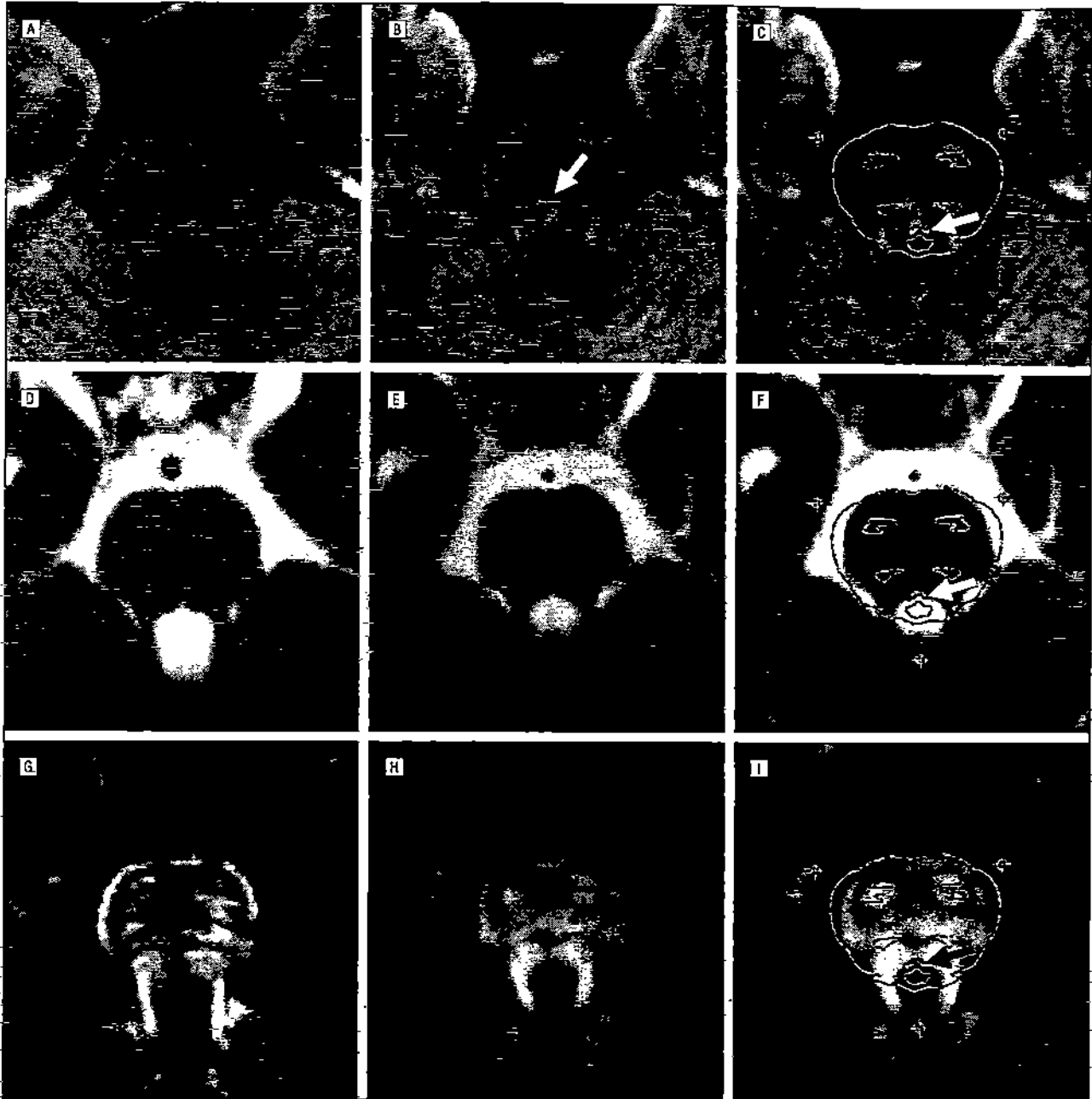


Figure 2. Illustrative diffusion-weighted images. T2-weighted hyperintensities (A-C), mean diffusivity (D-F), and lattice index (G-I) at the level of the pons from a healthy control (first column), a patient with multiple sclerosis (MS) (second column), and a patient with MS with anatomical overlays (third column). In B, the arrow indicates a lesion in the region of the right medial longitudinal fasciculus (MLF); in C, F, and I, the arrows indicate the outlined MLF.

weighted, twice-refocused spin echo with echo planar imaging readout diffusion sequence with 25 diffusion-weighting gradients with $b = 1000$, and 3 with $b = 0$; TE, 89.6 milliseconds; TR, 6200 milliseconds; flip angle, 90° ; bandwidth, 633 Hz per pixel; 128×128 matrix; 140×140 -mm field of view; 4-mm section thickness, with 3 repeats to improve the signal to noise ratio. Singular value decomposition was used to identify its least square error inverse, which was then used to define the tensor matrix. Eigenvalues and eigenvectors were then computed from the independent elements of the tensor matrix.^{14,15}

IMAGE ANALYSIS

The MLF was identified through a brainstem atlas at 7 levels that extended from the tegmentum of the pontomedullary junc-

tion to the rostral extent of the midbrain (**Figure 1**).¹⁶ Advanced Visualization Software (Advanced Visual Systems, Waltham, Massachusetts) was used to apply a 3-*df* affine transform (1 rotation, 2 scale factors) to warp a given atlas section onto the DTIs, as previously described (**Figure 2**).¹⁷⁻¹⁹

Preliminary observations found differences in DTI measures within the MLF of healthy controls along the caudal-rostral extent of the MLF. Therefore, at each brainstem level, the DTI measures of the patients with MS were subtracted from the average of the 10 healthy controls at the corresponding level. We measured the MD (a measure of overall water diffusion) and the lattice index (LI) (a measure of anisotropy that integrates the anisotropy of the surrounding voxels). The LI was used as the primary measure of anisotropy instead of the more commonly reported fractional anisotropy (FA) because our pre-

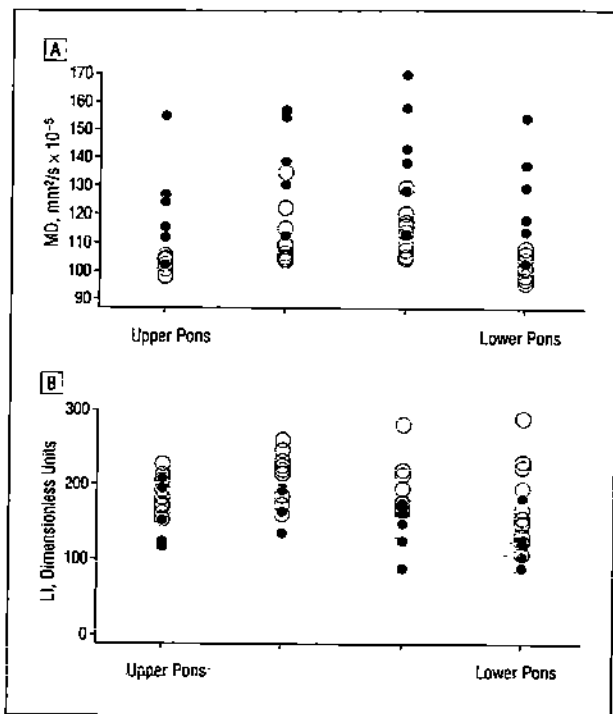


Figure 3. Mean diffusivity (MD) and lattice index (LI) at the level of the pons in patients with multiple sclerosis (MS) (closed circles) and healthy controls (open circles). A, The MD measured at 4 levels of the right pons in patients with MS with right internuclear ophthalmoparesis and in healthy controls. B, The LI measured at 4 levels of the right pons in patients with MS with right internuclear ophthalmoparesis. Compared with healthy controls, patients with MS showed increased MD ($P < .005$) and decreased LI ($P < .03$) in the pons. The MD and LI measures on the left were similar (data not shown).

liminary observations in the healthy controls found it to have decreased variability in the small MLF region of interest. The FA was analyzed as a secondary anisotropy outcome measure.

All 6 patients with MS had T2 hyperintensities in the area of the pontine MLF and sparing of the midbrain tegmentum. Therefore, the DTIs were divided into 2 groups: the caudal 4 sections, which comprised the diseased pontine MLF, and the most rostral 3 sections, which comprised the unaffected midbrain tegmentum MLF. For group comparisons, within-patient DTI measures were averaged across imaging sections for each brainstem region.

STATISTICAL ANALYSIS

The differences in vel-VDI and DTI measures between patient groups were evaluated using a linear mixed model, which accounts for the within-patient correlation between fellow eyes. Pearson correlation coefficients were used to evaluate the relationship between vel-VDI and DTI measures. Because of the expected within-patient correlation between fellow eyes, correlation analyses evaluated the right and left sides separately. Sensitivity analyses included correlations with the unaffected MLF in the midbrain.

RESULTS

As expected from patient selection, oculography evaluations of patients with MS showed an increased vel-VDI compared with controls ($P < .001$; data not shown). Compared with healthy controls, MD within the pontine MLF of patients with MS was increased ($P < .005$; **Figure 3**),

whereas the LI and FA were decreased (LI: $P < .03$, **Figure 3**; FA: $P < .03$, data not shown). The MD, LI, and FA within the unaffected midbrain MLF (ie, where no T2 lesions were seen on conventional MRI) showed less difference between patients with MS and healthy controls (MD: $P < .04$; LI: $P > .10$; FA: $P > .10$).

Significant or trend correlations were observed between the vel-VDI and both the MD and LI within each pontine MLF in the combined group of patients with MS and healthy controls (**Figure 4**). Weaker correlations were observed with FA (left vel-VDI and left FA: $r = -0.24$, $P > .30$; right vel-VDI and right FA: $r = -0.58$, $P = .02$). The same correlations in the midbrain showed consistently weaker correlation coefficients on each side (left vel-VDI and left MD: $r = 0.63$; right vel-VDI and right MD: $r = 0.42$; left vel-VDI and left LI: $r = 0.23$; right vel-VDI and right LI: $r = -0.52$; left vel-VDI and left FA: $r = 0.40$; right vel-VDI and right FA: $r = -0.48$).

COMMENT

We found altered measures of water diffusion within the pontine MLF of patients with MS with a well-characterized neuro-ophthalmologic syndrome. Specifically, we observed increased overall diffusion and decreased anisotropy, which is similar to that reported in cerebral areas of demyelination.⁸⁻¹⁰ In the midbrain tegmentum MLF, no difference was found in any DTI measure between patients with MS and healthy controls, which supports the specificity of DTI measures within the area of pontine injury for the patients we selected.

Despite the small sample size, significant correlations were observed between eye movement function and MRI measures of structural injury. These observations provide direct evidence that the structural injury measured by DTI reflects a physiologically relevant disruption in neural tissues: the greater the alteration in DTI, the greater the resulting physiologic impairment. Our results are particularly conspicuous in that the MLF is an eloquent periventricular tract system at high predilection for inflammatory demyelination.

We observed a stronger correlation with the anisotropy measure LI than the more conventional anisotropy measure FA. The difference is likely due to a relatively low signal to noise ratio when measuring diffusion in a small structure such as the MLF. A low signal to noise ratio has been shown to significantly bias FA estimates more than LI,¹⁵ and the additional noise in the FA measure would likely weaken its correlation with validated physiologic measures. Whereas FA is calculated independently within each voxel, LI includes a spatial average over neighboring voxels.¹⁵ Such averaging tends to reduce the sensitivity of LI to both true physiologic variability and the effects of noise.

These studies are limited by the low signal to noise ratio of DTI performed on a 1.5-T scanner with a 25-direction pulse sequence. Errors in coregistration and warping methods used in the segmentation process may introduce variability. Higher magnetic fields, higher angular resolution of diffusion-weighted vectors, and more advanced coregistration methods should improve the ac-

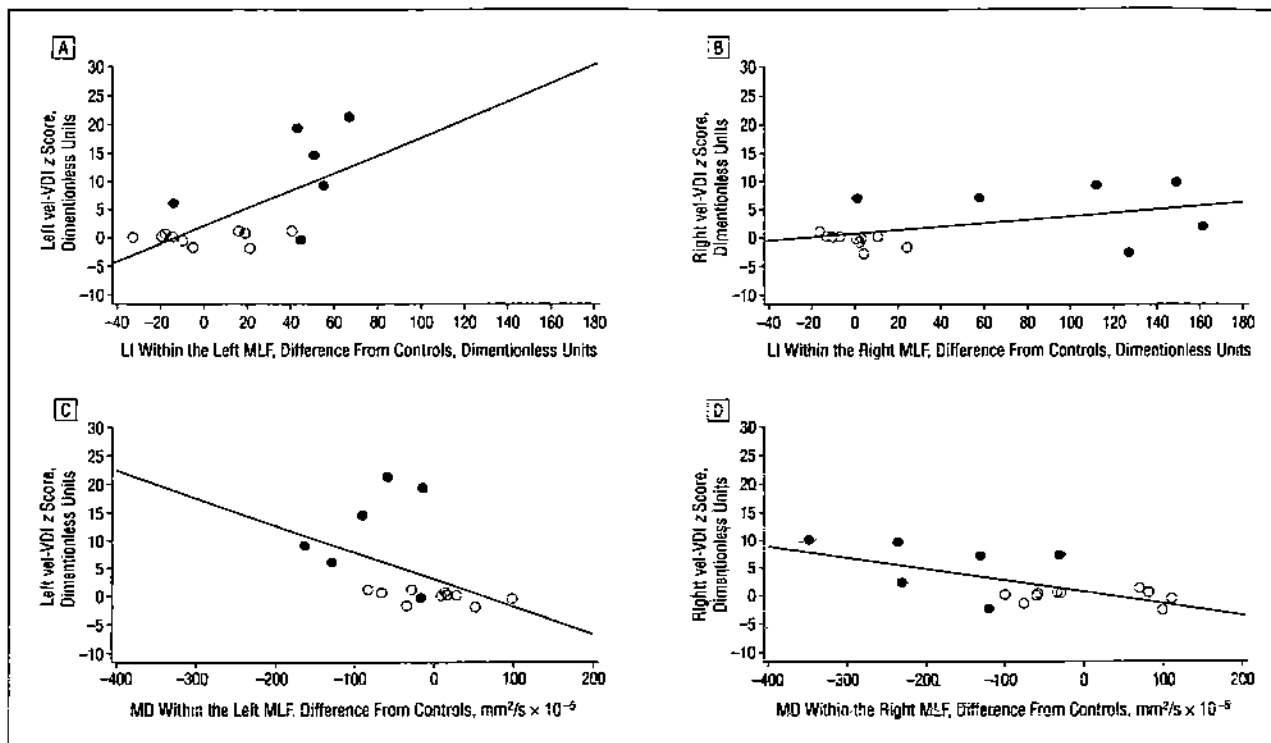


Figure 4. Correlation plots between the velocity versional dysconjugacy index (vel-VDI) and mean diffusivity (MD) and lattice index (LI) for patients with multiple sclerosis (closed circles) and healthy controls (open circles). Each magnetic resonance imaging measure (MD and LI) was subtracted from the mean of 10 healthy controls. A, Correlation between the left vel-VDI and LI within the left medial longitudinal fasciculus (MLF) ($r=0.65$, $P<.01$). B, Correlation between the right vel-VDI and LI within the right MLF ($r=0.46$, $P=.07$). C, Correlation between the left vel-VDI and MD within the left MLF ($r=-0.43$, $P=.09$). D, Correlation between the right vel-VDI and MD within the right MLF ($r=-0.66$, $P<.01$). Statistically significant or trend correlations were observed for each correlation.

accuracy and reproducibility of DTI estimates, thereby improving our ability to evaluate this small fiber tract. Variability was seen in the different correlation coefficients, and this variability is likely secondary to the small sample size, limited accuracy of the pulse sequence, and errors in coregistration.

The goal of many imaging metrics is to capture the relevant characteristics of tissue integrity, although few metrics have been validated as surrogate markers of tissue integrity. Diffusion tensor imaging provides a continuous measure of tissue injury and may even differentiate axonal injury from demyelination.^{20,21} These characteristics make DTI an attractive marker for application to clinical trials of putative neuroprotective therapies in MS. Previous studies have demonstrated DTI's construct validity (ie, alteration in MS lesions compared with normal-appearing white matter), but little is known about its criterion validity (ie, what does it mean functionally?).

We demonstrated DTI's concurrent validity through its increased MD and decreased LI within the MLF of patients with MS compared with controls. The variable correlation between each side is likely secondary to the small sample size and relatively low signal to noise ratio. Convergent validity is supported by its correlation with validated functional and objective measures of ocular dysmotility.

Finally, discriminant validity was demonstrated through the weaker correlations between ocular dysmotility and DTI measures in the contralateral MLF and the unaffected midbrain in our series of patients. As ex-

pected from wallerian degeneration along the MLF, MD was modestly increased within the midbrain MLF of patients with MS compared with healthy controls, although the difference measured by the MD and LI was less than that seen in the pons. Therefore, several significant correlations were still seen between midbrain DTI measures and the vel-VDI, but in every case these correlations were weaker than their pontine equivalent.

Altogether, our observations provide preliminary validation of DTI as a functionally relevant measure of brain tissue integrity and support its implementation into MS clinical trials of neuroprotective therapies. Further studies with a larger number of patients and higher-resolution imaging (ie, using 3-T imaging systems) are planned because they will improve our understanding of the clinical relevance of DTI within this system.

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