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ROLE OF MRI IN MULTIPLE SCLEROSIS I: INFLAMMATION AND LESIONS

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1. ABSTRACT

Conventional magnetic resonance imaging (MRI) can improve accuracy in the diagnosis of multiple sclerosis (MS). Metrics derived from conventional MRI are now routinely used to detect therapeutic effects and extend clinical observations. Hyperintense lesions on T2-weighted MRI scans are related primarily to increased water content and thus cannot distinguish between inflammation, edema, demyelination, Wallerian degeneration, and axonal loss. In addition, T2-weighted and post-contrast images are not sufficiently sensitive to detect occult disease affecting normal appearing gray and white matter. They do not show a reliable correlation with clinical measures of disability and do not provide a complete assessment of therapeutic outcomes. In the past few years a host of advanced MRI techniques and analysis methods have been introduced for the assessment of MS. These MRI techniques appear to have better reliability as surrogate markers for monitoring the pathologic processes that most likely are related to disease activity and clinical progression. They are able to reveal a range of tissue changes that include edema, inflammation, demyelination, axonal loss, and neurodegeneration. Therefore, in a disease with a high degree of longitudinal variability of clinical signs and symptoms within and between patients, and with no current adequate biological markers of disease progression, non-conventional MRI techniques provide a powerful tool to non-invasively study pathological substrates of overt lesions and normal appearing brain tissue. In particular, the use of these techniques is promising in elucidating mechanisms underlying the accumulation of tissue damage, repair and functional reorganization of neural pathways in patients with MS.

2. INTRODUCTION

Multiple Sclerosis (MS) is an inflammatory demyelinating disease of the central nervous system (CNS) of suspected autoimmune origin. It is characterized by demyelination and axonal loss for which the exact immunopathogenic mechanisms underlying disease initiation and progression are unknown.

Since its introduction early in the 1980s, magnetic resonance imaging (MRI) has become the most important laboratory diagnostic and monitoring tool in MS (1). MRI is five to ten times more sensitive than clinical data in the assessment of disease activity (2). Serial T2-weighted images (T2-WI) often detect new lesions that are clinically silent. However, hyperintensity on T2-WI of MS lesions is related primarily to increased water content and thus cannot distinguish inflammation, edema, demyelination, Wallerian degeneration, and axonal loss (3). Hyperintense lesions on T2-WI also show insensitivity to the full extent of pathologic changes in MS, such as areas

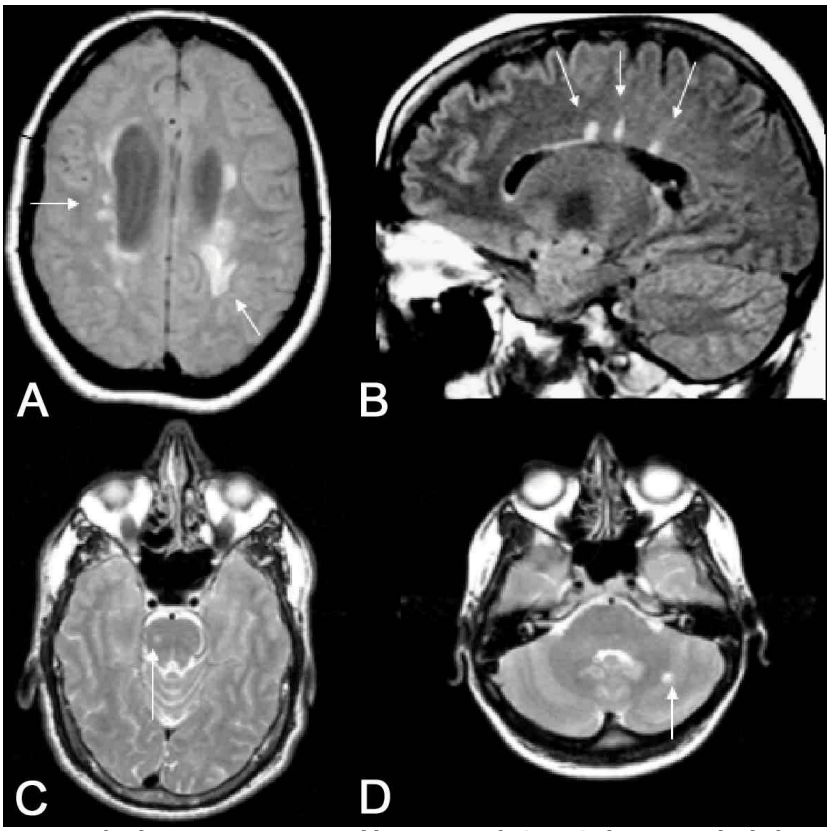

Figure 1. a: Axial T2-PD weighted image in a 44 year-old woman with SP MS showing multiple hyperintense lesions in the periventricular white matter (arrows). The lesion are characteristic for MS including a size of generally >5mm, oval/ovoid morphology and that many directly contact the ventricular ependyma; b: Sagittal FLAIR image in a 44 year-old man with RR MS showing multiple pericallosal lesions (arrows) with a classic perivenular orientation (Dawson's fingers); c/d: Axial T2-WI of a 33 year-old man with RR MS demonstrating typical hyperintense lesions in the pons (c, arrow) and left cerebellum (d, arrow).

that appear normal in the gray and white matter. T2 hyperintense lesions show an unreliable correlation with clinical measures of disability and provide an incomplete assessment of therapeutic outcomes.

Lesions that enhance with gadolinium (Gd) on T1-weighted images (T1-WI) indicate blood-brain barrier (BBB) permeability associated with active inflammatory activity. However, Gd enhancing lesions do not provide sufficient information about the extent and severity of the inflammatory phase, the constitution of its cellular components, or the resultant tissue damage (4).

Conventional MRI is insensitive to tissue damage occurring in so called normal appearing brain tissue (NABT), including normal appearing gray and white matter, which probably contributes to short- and long-term clinical impairments (5-9). However, conventional MRI-derived indices offer several important advantages over clinical outcome measures such as relapse rate and the level of disability. Conventional MRI allows the demonstration of spatial and temporal dissemination of MS lesions earlier than is possible from clinical assessments. In the last decade, metrics derived from conventional MRI have been widely employed in therapeutic clinical trials (10-12). A variety of conventional MRI protocols, in conjunction with clinical assessment, are now routinely used to detect therapeutic effects and extend clinical observations (4).

In the past few years, a host of non-conventional MRI techniques and analysis methods have been introduced for the assessment of MS (13). These MRI techniques appear to be reliable surrogate markers in monitoring the destructive pathologic processes related to disease activity and clinical progression. They are able to reveal a range of tissue changes in MS that include edema, inflammation, demyelination, axonal loss, and neurodegeneration (14). Therefore, in a disease with a high degree of longitudinal variability of clinical signs and symptoms within and between patients, and with no current adequate biological markers of disease progression, non-conventional MRI techniques provide a powerful tool to non-invasively study pathological substrates of overt lesions and NABT (15).

Hypointense lesions on T1-WI ("black holes"), magnetization transfer imaging (MTI), diffusion-weighted imaging (DWI), proton MRI spectroscopy (MRS), functional MRI (fMRI) and ultra high-field MRI are emerging as promising tools for improving our understanding of the pathophysiology of MS.

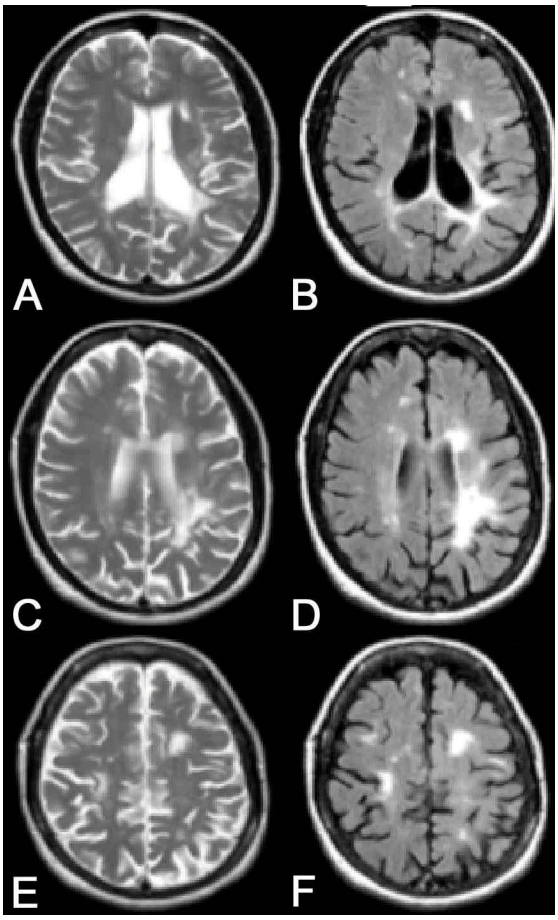

Figure 2. Axial FSE T2-WI (a,c,e) and FLAIR images (b,d,f) in a 27-year old woman with RR MS show typical hyperintense periventricular lesions (a-d) and also involvement of the cortical/juxtacortical region (e,f). FLAIR is superior to T2-WI in the detection of periventricular (b,d) and cortical/juxtacortical lesions (f) because of the heavier T2 weighting combined with the suppression of CSF.

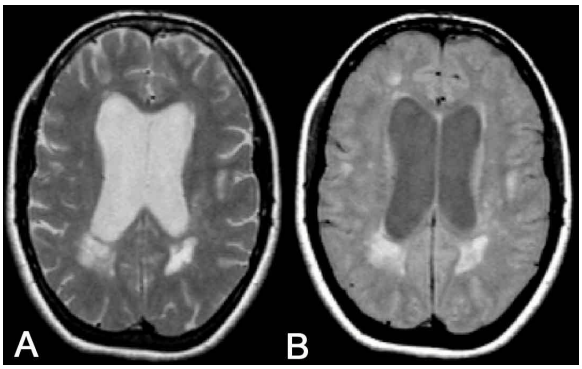

Figure 3. Axial FSE T2-WI (**a**) and PD-WI (**b**) in a 42-year old woman with RR MS show typical hyperintense confluent periventricular lesions. Note the improved lesion contrast achieved with PD-WI because of the relatively lower signal intensity of CSF vs. lesions.

Neurodegenerative aspects of MS, such as brain and spinal cord atrophy and hypointensity on T2-WI (T2 hypointensity), are receiving increased attention as clinically relevant markers of disease progression and are discussed in the review that follows the present article (16). Thus, the present article will outline the major contributions of conventional and non-conventional MRI techniques in detecting inflammation in MS lesions and NABT.

3. ROLE OF UNENHANCED MRI SCANS IN DETECTING INFLAMMATION

3.1. T2-weighted sequences

Conventional spin-echo (CSE) images of the brain are routinely used for the diagnosis and longitudinal monitoring of MS because of their high sensitivity in the detection of lesions (13,15). The MS lesions ("plaques") are widespread throughout the brain, including the white matter or, less commonly, the gray matter. The most typical sites are in white matter of the periventricular region (Figure 1a), corpus callosum (Figure 1b) and posterior fossa (Figure 1c). The periventricular white matter lesions typically make contact with the ependymal surface of the ventricles. The long-axis of periventricular lesions is frequently perpendicular to the long-axis of the lateral ventricles due to perivenular demyelination ("Dawson's fingers", Figure 1b). The lesions in corpus callosum are characteristically seen on the inner surface adjacent to the lateral ventricles. Typical posterior fossa involvement includes lesions in brain stem, middle cerebellar peduncles, and cerebellar white matter (Figures 1c and d). Sagittal images are superior in identifying callosal and pericallosal lesions (Figure 1b), while axial images are more sensitive for posterior fossa lesions (Figure 1c). MS lesions are commonly oval or ovoid (Figure 1), hyperintense on T2-WI and proton density weighted images (PD-WI) (Figures 1, 2a, c, e and 3), and usually $\geq$5 mm in diameter. Small periventricular lesions may be indistinguishable from the adjacent high signal cerebrospinal fluid (CSF) on T2-WI. Better lesion to CSF contrast is achieved with PD-WI because of the relatively lower signal intensity of CSF on this sequence and improved lesion to tissue contrast (Figure 3). T2-WI and PD-WI can be acquired in a single sequence by using a conventional dual spin-echo technique. The main advantages of CSE sequences in multicenter studies are their stability, consistency, and the relative ease of standardization across scanning platforms. Disadvantages include the long acquisition time, lack of adequate CSF suppression, and insensitivity.

In the last several years, continuous technical improvements of MRI hardware and software have led to the development of new pulse sequences with more efficiency and sensitivity. Among them, turbo or fast spin-echo (TSE or FSE) (17) (Figures 1-3) and fast-FLAIR (18) (Figure 1b and 2b, d, f) have already demonstrated their utility in a wide variety of neurologic diseases including MS. FSE showed greater sensitivity than CSE in the detection of areas of T2 prolongation in MS (17). Fast FLAIR sequences are especially helpful in evaluating periventricular and cortical/juxtacortical lesions where CSF

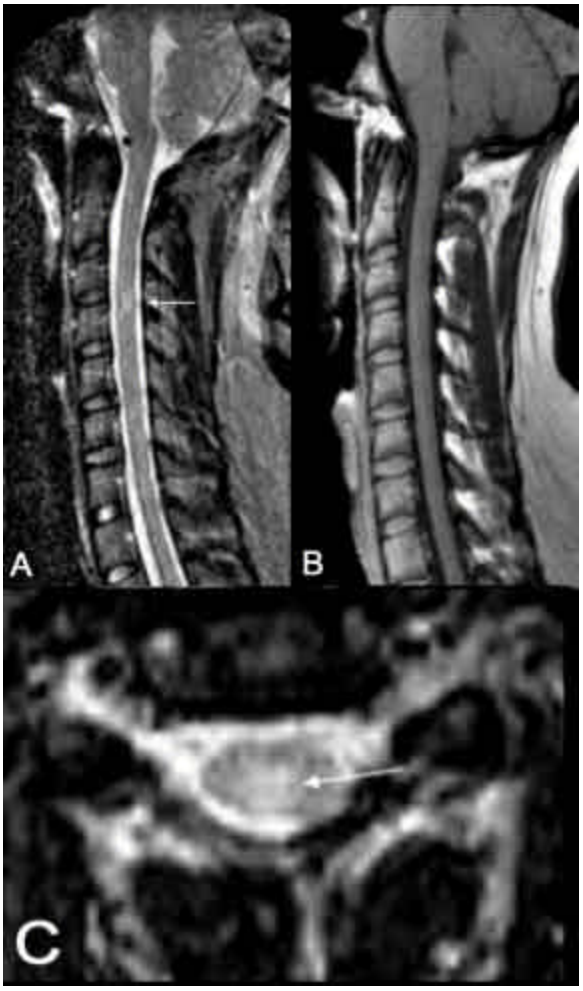

Figure 4. Spinal FSE T2-WI of a 28 year old man with RR MS. Sagittal (a) and axial (c) T2-WI show a hyperintense lesion (arrows) in cervical spinal cord (C3-4 level). The lesion is classic for MS, involving less than one spinal level and less than one-half of the cord diameter. The lesion is isointense on noncontrast T1-WI (b), as are most spinal cord MS lesions.

signal may mask these plaques on T2-WI (18-20) (Figure 2). In a study of 84 patients with MS, 810 cortical or juxtacortical lesions were identified by fast FLAIR while only 26% of these lesions were seen on T2-WI (18). However, in the posterior fossa (Figure 1**c**) and spinal cord, FLAIR detects significantly fewer lesions than T2-W1 (20). Areas of T2 prolongation can also be detected using short tau inversion recovery (STIR) sequences. This sequence may be superior to T2-WI in detecting spinal cord lesions in MS when using certain scanning platforms (21). Due to fat suppression STIR is an added advantage when imaging the optic nerves because the contrast between lesions and the surrounding retrobulbar fat is increased (22). Recently, a rapid acquisition relaxation enhanced (RARE) dual spin-echo sequence (23) has been developed. The major advantage of RARE over CSE sequences is the substantial saving in acquisition time. The RARE sequence can produce thinner slices than CSE with a comparable signal-to-noise ratio (24). However, RARE and FLAIR sequences are more difficult to standardize across centers than standard CSE sequences. Therefore, their utility in multicenter MS studies remains uncertain (25). The FSE and RARE sequences can be used for two-dimensional (2D) multislice and three-dimensional (3D) acquisition.

The lack of a strong correlation between hyperintense lesions on T2-WI and disability may be in part attributable to the presence of spinal cord disease (26-28). Spinal cord hyperintensities on T2-WI have been detected in 50-90% of patients with MS (26-28) (Figure 4). The presence of characteristic cord lesions may increase the confidence in diagnosing MS (26). Lesions most commonly involve 1-2 contiguous spinal levels or less on sagittal scans and less than one-half of the cord diameter on axial scans (26). In contrast, non-MS myelitis lesions typically involve multiple contiguous spinal levels and more than one-half of the cord diameter (26). This is seen, for example, in Devic's disease. Cord lesions may be seen in approximately 5-15% of patients with a clinical picture suggestive of MS but a normal brain MRI scan (29), and in 30% of those presenting with clinically isolated syndromes (CIS) of the brain suggestive of MS (30). Moreover, spinal cord lesions appear to be symptomatic more often than brain lesions (31) and correlate better with the degree of physical disability (32). Serial studies have revealed a low frequency of new spinal cord lesions, although those are clinically expressed more often than brain lesions (33). Proper sensitivity for the detection of cord involvement requires optimization of MRI hardware and pulse sequences. 1.5 T closed bore systems with FSE sagittal and axial T2-WI and CSE T1-WI with 3 mm slice thickness show excellent sensitivity (Figure 4). On some scanning platforms, PD-WI or STIR may also be useful.

3.2. Strategies to increase sensitivity of unenhanced MRI scans

3.2.1. Slice thickness

Recent consensus guidelines recommend a 3 mm slice thickness on 2D acquisition sequences for the measurement of burden of disease in MS in clinical trials (25,34). However, 5-mm thickness in the brain and 4-mm thickness in the cord are probably sufficient for routine clinical practice. Thinner slices provide increased lesion detection and higher measurement consistency (25,35,36). Recently, 3D acquisition sequences have been developed with as small as a 1 mm slice thickness and an acceptable scan time, improving detectability and reproducibility of lesions (37,38).

3.2.2. Scanner field strength

Recent consensus guidelines recommend that any scanner used in a phase III study should operate at a field strength of at least 1.0 Tesla (T) to 1.5 T. It has been demonstrated that the scanner field strength has a substantial impact on the measured T2 lesion volume (LV), being about 30% higher with standard 1.5 T magnets than for lower field scanners (38). Recently, higher-field MRI scanners have been developed (i.e 3 T or greater). Higher-field MRI increases specificity in the correlation

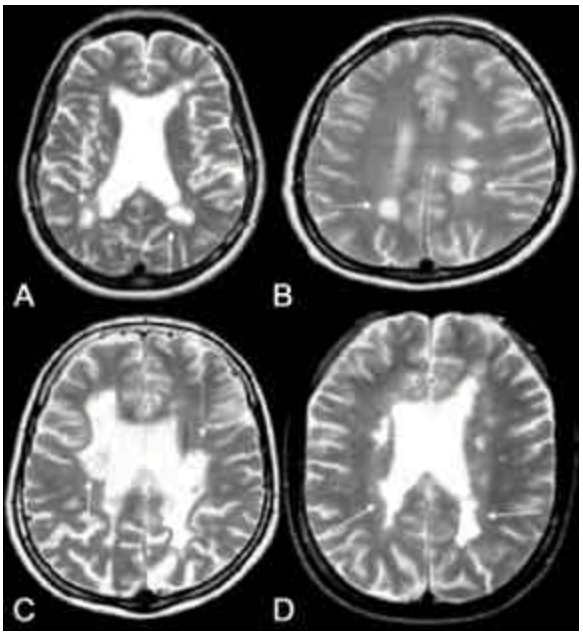

Figure 5. Definition of confluent T2 hyerintense lesions. Figures a and b show single T2 lesions with size larger than 20 mm typically located in the periventricular region and/or on the edges of the anterior and posterior horns of the bodies of ventricles. Figure c and d show areas of white matter abnormalities consisting of two or more T2 lesions interconnected by at least one or more margins.

between detected lesions and clinical disability. The clinically available higher-field that extended up to 4-T has now substantially increased to ultra high-field 7 T and 8 T for human application (39).

Keiper *et al.* (40) compared the detection of white matter abnormalities in MS at 1.5 T and 4 T in 15 patients with clinically definite MS and found that 4 T showed a mean of 6 additional lesions per patients vs. 1.5-T. Twenty-five of the lesions across the cohort were seen on 4 T images but not at 1.5 T. Moreover, 4 T images showed 56 additional consensually identified lesions, which were indistinct and seen only in retrospect on 1.5 T images. All observers also agreed that 4 T images subjectively enhanced the perception of normal perivascular spaces and small perivascular lesions. Another study (41) evaluated the relative sensitivity of MR scanning for MS at 1.5 T and 3 T using identical acquisition conditions, as is typical of multicenter clinical trials. Twenty-five subjects with MS were scanned at 1.5 T and 3 T using FSE, and T1-weighted 3D acquisition sequence with and without Gd contrast injections. The 3 T scans showed a 21% increase in the number of detected Gd-enhancing lesions, a 30% increase in T1 Gd-enhancing LV and a 10% increase in T2LV. Both studies showed that higher-field MRI depicts white matter abnormalities in MS patients not detectable at 1.5 T through higher resolution with comparable signal-to-noise ratio and imaging times. Multicenter trials using higher-field MRI instruments may be affected by these sensitivity differences.

3.2.3. Scanning frequency

The first serial studies on T2-WI were performed at the University of British Columbia, Vancouver (42,43). These studies showed that in patients with relapsing-remitting (RR) MS, new brain lesions occurred at about five to ten times the frequency of clinical relapses, and that they typically show a waxing and waning pattern over a period of one to two months. Many other reports showed that frequent scanning might increase the sensitivity in detecting new or enlarged T2 lesions. Recently, serial 3D fast FLAIR images have been compared with standard 3-mm 2D spin-echo images for the detection of new or enlarging lesions (44). The results of this study demonstrated that the use of serial 3D FLAIR imaging holds great promise for the detection of new or enlarging lesions in MS using registration and subtraction techniques at longer intervals.

Recently, new diagnostic criteria have been proposed by an international panel (McDonald *et al.*) (45). The McDonald criteria reaffirmed the concept of "dissemination in time and space". The panel chose the guidelines modified from Barkhof *et al.* (46) and Tintore *et al.* (47) for the definition of dissemination in space. For dissemination in time the following criteria were adopted: a) Gd-enhancing lesion in a scan done at least three months following the initial clinical attack at an anatomic site separate from the first attack; b) in the absence of Gd-enhancing lesions at the three month scan, a follow-up scan after an additional three months showing a new Gd- or T2-lesion. For the first time the McDonald criteria accepted new Gd-enhancing lesions appearing after a minimum interval of three months as sufficient evidence for dissemination in time in relapsing-remitting (RR) MS. However, the diagnosis of MS in patients with a new lesion on T2-WI that appears at the 3-month follow-up scan is not permitted by the current criteria, although it has been demonstrated that new T2 lesions are detected more often than new Gd-enhancement lesions after 3 months (48). Research studies to validate this minimum interval and determine the optimal interval that provides the maximum yield are beginning to emerge (49-53).

3.2.4. Confluent T2 lesions

Standard T2-weighted images are fairly sensitive in showing changes due to inflammation, edema, demyelination, and axonal loss, but are pathologically nonspecific. Thus, it is not surprising that lesions seen on these sequences only moderately correlate with clinical and MRI parameters and treatment responses (3).

In a post-hoc analysis of MRI measures of disease activity derived from a 5-year phase II clinical trial of intravenous methylprednisolone in RR MS (54), the number, size and confluence of T2 lesions were investigated (55) (Figure 5). While the number (11-13,56,57) and size (58-61) of T2- and Gd-enhancing lesions have been evaluated in several clinical trials in patients with MS, the number of confluent T2 lesions has been assessed only in one study (62). In that study the confluences were defined as lesions with an elongated shape and typically located at the anterior and posterior

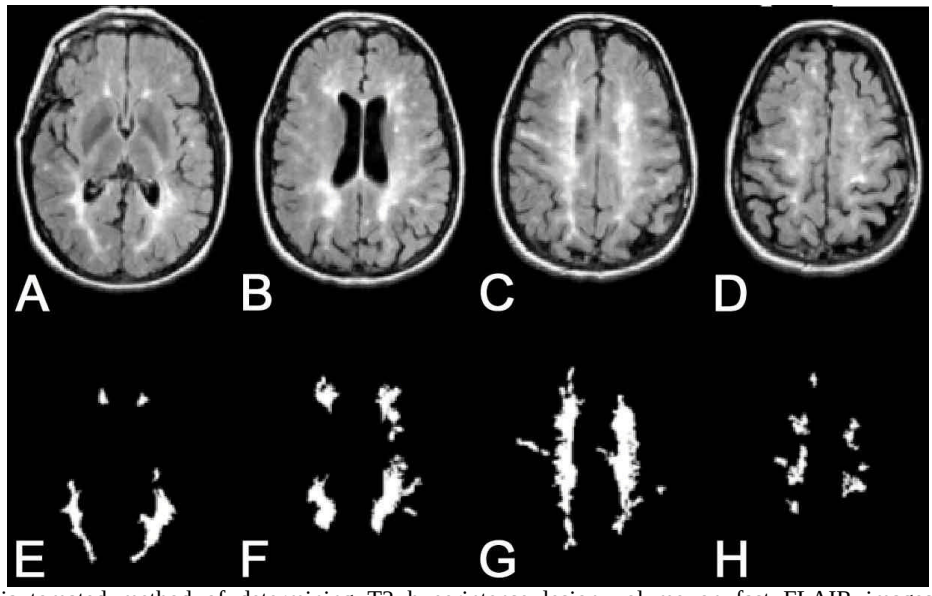

Figure 6. Semiautomated method of determining T2 hyperintense lesion volume on fast FLAIR images at the Buffalo Neuroimaging Analysis Center in a patient with MS (71). Axial fast FLAIR images show hyperintense lesions on raw images (a-d) and images containing lesions only after completing the algorithm (e-h). Extracranial tissue is first removed using a masking tool that involves an automated contour tracing tool. A threshold technique is applied to separate hyperintense lesions from nonlesional tissue. The software automatically calculates the area and volume of the lesions and total brain lesion volume based on the number of voxels retained.

horns of the bodies of ventricles, usually with finger-like spreading in the white matter. Zivadinov *et al.* (55) created more specific criteria for the classification of confluent T2 lesions, which included the presence of typical hyperintense T2 lesions with a size larger than 20 mm (Figure 5**a** and **b**) or at least two interconnected T2 lesions (Figure 5**c** and **d**). On the basis of these criteria we developed a highly reproducible semiautomated local thresholding technique to measure the confluent T2LV (63). We showed that pulsed intravenous methylprednisolone limited the development of confluence and enlargement of T2 lesions, which was associated with prevention of disability progression at 5 years. The changes of confluent T2LV showed remarkably stronger magnitudes of correlation with brain parenchymal volume, T1LV and Expanded Disability Status Scale (EDSS) score than the changes of conventional T2LV. The investigation of the pathologic characteristics of confluent T2 lesions is the subject of current research from our group.

3.3. Quantification

Qualitative MRI assessment of disease activity on serial T2-WI by visual reporting of the number of new and enlarging lesions is a common primary or secondary endpoint in phase II and III clinical trials (10-13,55-60). New lesions on T2-WI are usually defined as rounded or oval lesions arising from an area of previously NABT and/or showing an identifiable increase in size from previously stable-appearing lesions (10-13,55-60). Active T2-weighted scans are defined as scans showing any new, enlarging or recurrent lesions. The calculation of the number of T2 lesions, and of the number and proportion of active scans is typically based on manual identification on hard copies of films. A major difficulty with this approach lies in discriminating new or enlarging lesions on a background of extensive pre-existing T2 lesions. The requirement for extensive training to achieve high intra- and inter-observer reproducibility is a major limit of this approach. Use of image registration techniques (24) and standard guidelines (64,65) may partially overcome these limits. However, even given this low level of reproducibility, there is a strong relationship between the number of new/enlarging T2 lesions and the clinical, pathological, immunological signs of disease activity in MS (25,64). Furthermore, such lesion counts are fairly sensitive to treatment effects in several clinical trials of disease modifying agents (10-13,55-60).

Several techniques are now available to quantify T2LV including manual outlining (10,11), semiautomated threshold-based segmentation (66-68,71) and fully automated approaches (69,72). Semiautomated global thresholding (71) techniques require observer participation to identify the optimal threshold level for each scan, with subsequent manual editing to delete spurious lesions and include undetected lesions (Figure 6). Semiautomated local thresholding techniques such as contour (66,67) and seed growing (73) need manual input for lesion identification and then use an edge finding algorithm to delineate lesion boundaries. Studies using manual, semiautomated or automated techniques reported that T2LV increases consistently and significantly (by approximately 8%-10% per year) in RR MS patients over several years (15,25,34,43,64,69). Changes in lesion size and T2LV are partially related to disease activity in the short term (64) but are not reliably predictive of long-term clinical course and accumulation of disability (74-78).

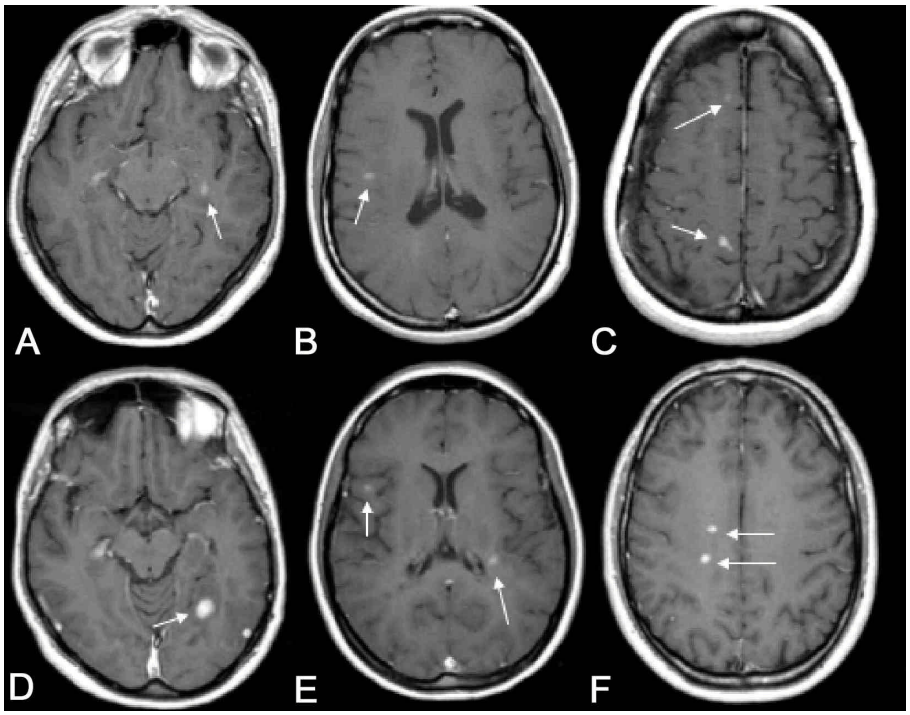

Figure 7. Single dose (0.1 mmol/kg) gadolinium postcontrast axial T1-WI (a-c) show homogeneously enhancing (hyperintense) lesions (arrows) indicating disruption of the blood-brain barrier and acute inflammation in a 34 year-old woman with RR MS. Double dose (0.2 mmol/kg) gadolinium postcontrast axial T1-WI (d-f) more robustly show enhancing lesions in a 28 year-old man with RR MS.

3.4. Prognostic value

MRI plays a role in determining the risk for developing MS in patients who present with CIS. However, the direct relationship between early MRI findings and subsequent long-term disability is not clear. Focal areas of high signal, identical to those seen in patients with established MS, have been found on T2-WI in 50-80% of the patients at presentation with CIS (79-81). Several longitudinal studies using conventional MRI (74-78,82) showed that the number and volume of T2 lesions at presentation with CIS is associated with an increased risk of developing MS and a higher level of disability at long-term follow-up.

In a five-year longitudinal study, 38 of 84 patients presenting with CIS had developed probable or definite MS (75). Patients who developed MS during follow-up had a higher T2LV at presentation than those who did not. At 5 years, there was a strong correlation between baseline T2LV and both the increase in T2LV and the accumulation of disability. Ten year follow-up of this subgroup showed that 45 (83%) developed MS, of whom 11 (20%) had RR MS with at least moderate disability (EDSS > 3), 13 (24%) had secondary progressive (SP) and 21 (39%) had RR MS but mild disability (76). For those with a normal baseline MRI, progression to MS occurred in only three out of 27 (11%), and all had RR MS with mild disability. The number and volume of baseline T2 lesions correlated moderately with the degree of disability 10 years later ($r=0.45$, $p= 0.001$) (77). Forty-four of the 50 patients (88%) with abnormal MRI at presentation and 4 of 21 patients (19%) with normal baseline MRI developed clinically definite MS after 14 years (78). At 14 years, EDSS disability score correlated moderately with T2LV determined on year five ($r=0.60$, $p< 0.001$). There was also significant correlation between EDSS and the increase in T2LV over the first 5 years ($r=0.61$, $p< 0.001$). These data suggest that early MRI has prognostic value in patients with CIS, both in terms of conversion to MS and the long-term development of disability. However, the relationships are only moderate, indicating that other factors are important in predicting long-term outcome.

4. ROLE OF ENHANCED MRI SCANS IN DETECTING INFLAMMATION

4.1. Evidence of blood brain barrier breakdown

Gd-enhancement in MS lesions has been correlated with histopathologic findings of BBB breakdown and active inflammation (83). Gd-enhancing lesions on T1-WI usually correspond to areas of high signal on T2-WI and low signal intensity on unenhanced T1-WI, probably due to edema and demyelination (Figure 7). Gd-enhancement is a transient phenomenon in MS and usually disappears after an average of 3 weeks (84). Approximately 10-20% of lesions on T2-WI will show contrast enhancement at any one time. Serial monthly enhanced MRI scans indicate that enhancement precedes or accompanies almost all of the newly formed lesions on T2-WI in patients with RR or SP MS (83-85). While the majority of enhancing plaques are clinically asymptomatic and correlate poorly with clinical status in cross-sectional

studies (86), the presence of ongoing enhancement carries a high risk of continuous disease activity (85) and likely contributes to cumulative pathophysiology over the long-term (83).

There is an interest in determining whether Gd-enhancing lesions predict clinical status over long-term. In a recently published meta-analysis study, Kappos *et al.* (87) investigated the prognostic value of Gd-enhanced MRI from five natural-course studies and four placebo groups of clinical trials of 307 patients, 237 with RR MS and 70 with SP MS. Neither the initial scan nor monthly scans over six months were predictive of change in the EDSS in the subsequent 12 or 24 months. The mean number of Gd-enhancing-lesions in the first six monthly scans was weakly predictive of EDSS change after 1 year (odds ratio=1.34, p=0.082) and 2 years (odds ratio=1.65, p=0.049). The authors concluded that although Gd-enhancement in MRI is a predictor of subsequent relapses, it is not a strong predictor of the development of cumulative impairment or disability. This discrepancy supports the idea that a variety of factors are operative in the occurrence of relapses and in the development of sustained disability in MS.

4.2. Strategies to increase sensitivity of enhanced MRI scans

There are several methods that can be used to increase the sensitivity of Gd-enhanced MRI for the detection of active MS lesions, which lead to increased statistical power of patient samples and a shorter follow-up duration needed to show a treatment effect (67,83,90). These strategies have been reviewed in detail separately (83,90). The first strategy maximizes the information that can be obtained by conventional scanning and includes frequent serial weekly (91) or monthly scanning (92-95), the scanning of the brain and spinal cord instead of only the brain (33) and a delay between Gd injection and scanning of five minutes or more (84,96). At our center, we use a five-minute delay after single dose Gd infusion to increase the sensitivity, which we find to be sufficiently sensitive for clinical practice and cost effective. Several MRI studies demonstrated that the frequency of Gd-enhancing lesions is higher in the spinal cord than in the brain (33,88). The enhancement may become more apparent when scanning the entire cord (88). A second strategy to increase the sensitivity in detecting Gd enhancement uses higher doses of contrast (Figure 7d,e and f) (83,89,96-99) [e.g. a double or triple dose instead of a standard 0.1 mmol/kg dose]. Wolansky *et al.* (98) were the first to show that triple dose is more sensitive than single dose Gd in detecting MS lesions. Sensitivity can also be increased by acquiring thinner tomographic slices (35,100) or co-registration (69). Another approach reduces the signal of background tissue by the application of magnetization transfer pulses to T1-WI (101,102). Other non-conventional MRI techniques [diffusion (103-105) and spectroscopy (106-108)] have been used in detecting a large amount of MS activity that is not recognized when using conventional enhanced T1-WI. The use of these non-conventional MRI strategies suggests that low-grade inflammation can occur independently from overt enhancing lesions.

4.3. Quantification

Gd-enhancing lesions are a primary endpoint used to monitor short-term efficacy of treatment in phase II clinical trials (64). The assessment of the number of Gd-enhancing lesions is usually based on visual identification from images. The number of Gd-enhancing lesions correlates with the onset and severity of clinical relapses and predicts short term and long-term enhancing and nonenhancing lesion activity and brain atrophy (83,85,109-113).

Another outcome measure of Gd-enhancing lesion activity used in clinical trials is to quantitatively measure Gd-LV (10-13). The robust inter-correlation between the number of Gd-enhancing lesions and Gd-LV (r=0.8-0.9) (95), suggests that Gd-LV may add little more information about the overall inflammatory process when compared to lesion counts (111). A negative correlation between Gd-LV and disease duration has been reported and such enhancement is more common in relapsing vs. progressive forms of the disease. These data suggest that BBB abnormalities are less common in later stages of MS for reasons that are not clear (83). Gd-MRI scans should not be interpreted in isolation; MS lesions may show hyperintensity on non-contrast T1-WI (T1 shortening) which may represent paramagnetic effects of free radicals, lipid-containing macrophages, iron deposition, or proteinaceous accumulation (114).

4.4. Evolution of individual lesion intrinsic characteristics

The morphology of Gd-enhanced lesions may provide some specificity of the underlying pathology (Figure 8). Ring enhancement may occur in an incomplete (open) ring pattern that is somewhat more specific for MS than infections or neoplastic diseases (Figure 8) (115). Concentric ring-enhancing lesions with central contrast pallor (Figure 8) arise in previously damaged areas or in areas of accelerated local inflammation (116-121). Their duration is longer than of homogeneously enhancing lesions (116,118). Apparent diffusion coefficients (120) and magnetization transfer ratios (117) are lower in ring-enhancing than in homogeneously enhancing lesions. It has been also shown that ring-enhancing lesions predict weakly the development of persisting hypointense lesions on T1-W1 (122). For all these reasons, ring-enhancing lesions are thought to be related to aggressive disease activity and a high level of tissue damage (115,119,121,122). Several groups investigated the predictive value of homogeneously- and ring-enhancing lesions towards disability progression (113,119,123-126). The findings suggest that the presence and frequency of Gd-enhancement and changes in disability over a short period are predictive of future long-term deterioration. In one study (119), the frequency of ring-enhancement did not predict sustained disability 3 years later. However, in a subgroup of patients treated with interferon beta-1b, ring-enhancing lesions were predictive of EDSS worsening after 3 years. In a recent study (95), we assessed whether the pattern of Gd-enhancement predicted short-term clinical disease activity and progression of disability in RR MS. We showed that the ring-enhancement pattern was most closely associated with the occurrence of

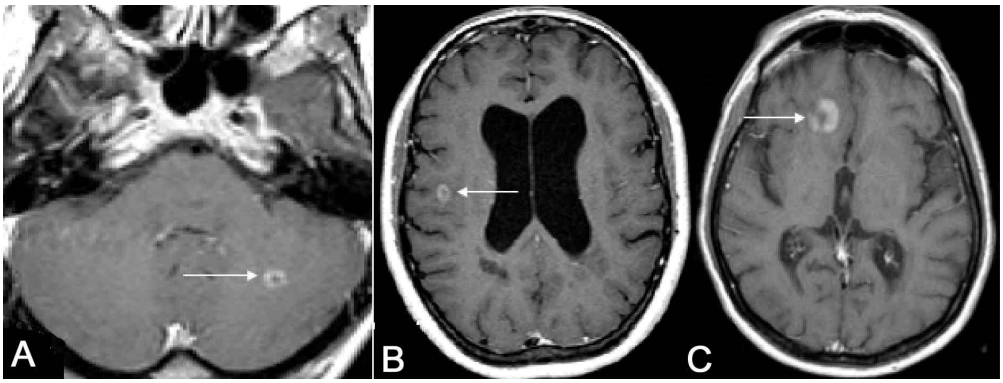

Figure 8. Axial postcontrast T1-WI demonstrates concentric ring-enhancing lesions (arrows) in the infratentorial (a) and periventricular (b) regions in a 37 year-old woman with SP MS. Characteristic open-ring enhancing lesion with increased specificity for MS (arrow) in 46 year-old man with SP MS (c).

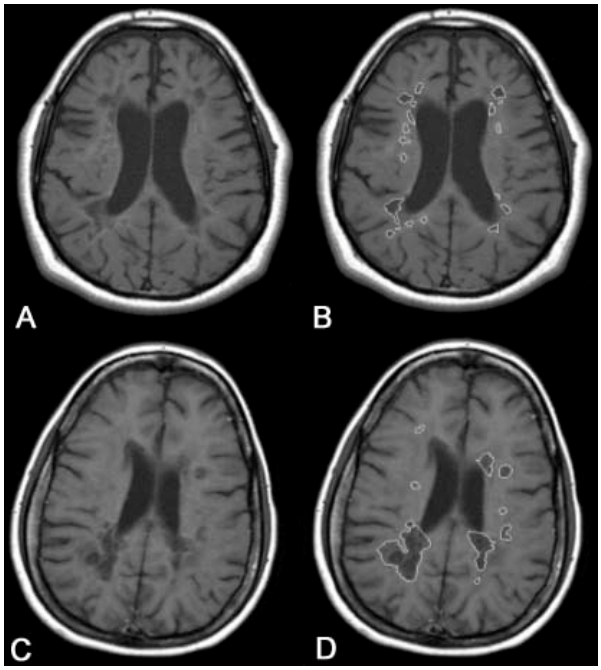

Figure 9. Axial noncontrast T1-WI in a 44 year-old man with SP MS with severe disability (EDSS score 7.0) and multiple hypointense lesions (black holes) in the periventricular white matter on raw images (a and c). Hypointense T1 lesions are segmented for volumetric analysis using an edge finding computerized analysis technique at the Buffalo Neuroimaging Analysis Center (b and d).

relapses and disability progression during three-months of observation.

The role of inflammation towards the development of permanent tissue damage and long-term disability in patients with MS is still not completely clear. On non-contrast T1-WI, most MS lesions are isointense to white matter but some are hypointense [so-called "black holes" (BH)] (16,54,63,71,127-132) (Figure 9). These BH are nonspecific at a given time point as nearly half will revert to normal in a few months (122), most likely due to remyelination and resolution of edema (133). Persistent BH, on the other hand, indicates severe demyelination and axonal loss (127,133) and most likely contributes to Wallerian degeneration. Several longitudinal studies reported that baseline Gd-enhancement only partially predicts the accumulation of persisting BH (128,134,135), because the natural history of Gd-enhancing lesions is variable and unpredictable. Approximately 80% of Gd-enhancing lesions appear simultaneously hypointense on the corresponding noncontrast T1-WI (122). Once Gd-enhancement resolves, 40-44% of BH become isointense on T1-WI and another 40% develop into persistent BH (122,135). In a natural history study, Bagnato *et al.* (135) analyzed BH formation over a 4-year period in nine MS patients monitored with monthly MRI and showed that

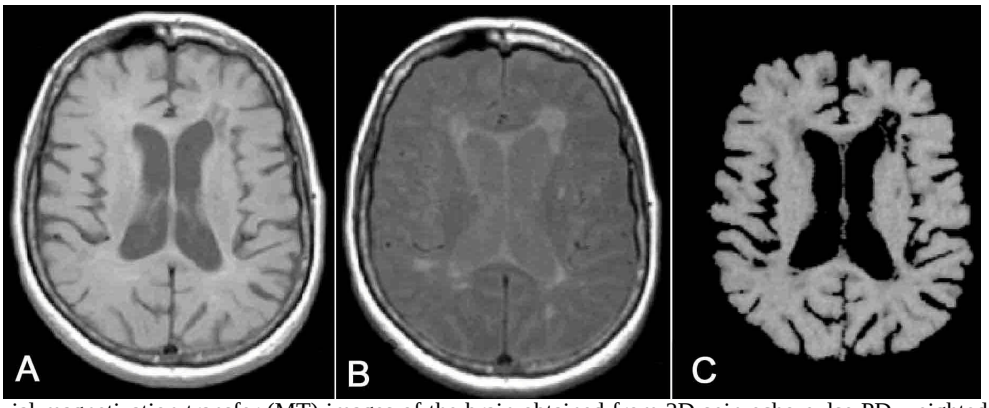

Figure 10. Axial magnetization transfer (MT) images of the brain obtained from 2D spin-echo pulse PD-weighted images (PD-WI) at the Buffalo Neuroimaging Analysis Center in a 35 year-old man with SP MS. The image is shown with (a) and without (b) the on-resonance MT saturation radio frequency pulse. The PD-weighted images are co-registered and MT ratio (MTR) maps are created after nulling the CSF and the lesions (c).

neither progression of time nor the number of Gd-lesions predicted BH persistence. Regarding the clinical relevance of BH, when compared in statistical models, brain atrophy has shown a closer association with neurobehavioral and neurologic impairment in MS (8,131,132,136,137). Thus, the role of BH in the longitudinal monitoring of patients with MS is not clear and requires further validation studies.

5. ROLE OF NON-CONVENTIONAL MRI TECHNIQUES IN DETECTING AND MONITORING INFLAMMATION

Recently, several non-conventional MRI techniques have been used to monitor pathologic characteristics of MS lesions (101-108,122). A more specific understanding of lesion evolution should shed more light on overall disease pathophysiology. Several studies demonstrated that disruption of the BBB is associated with parenchymal tissue changes, which are directly associated with the severity and the duration of the BBB damage (91,103,107,122,135). The results from these studies indicate that the balance between injury and repair may be highly variable during the formation and evolution of lesions. Therefore, pathologic heterogeneity of lesions contributes to variability in disease evolution and may explain why weak correlations have been found between the number of Gd-enhancing lesions and long-term disability progression (87). A variety of non-conventional MRI techniques are capable of detecting inflammatory activity in areas of brain appearing normal on conventional MRI; such activity may be detected weeks or months prior to the formation of overt MS lesions. The importance of these studies will be discussed in the next section of this article.

5.1. Magnetization transfer ratio

Magnetization transfer imaging (MTI) is an advanced MRI technique based on the interactions and exchange between protons that are unbound in a free water pool with those where motion is restricted due to binding with macromolecules (Figure 10) (13). Tissue damage in MS is usually reflected by a reduction in this exchange of protons and thus a decrease in magnetization transfer ratio (MTR). However, decreases in MTR are not specific to MS pathologic substrates and are instead reflect a reduction in the size of the tissue matrix and an increase in the size of the free water pool, regardless of precise cause. Nevertheless, a relationship has been shown between reduced MTR, axonal loss, and the level of demyelination (138). MTR maps can be used to assess tissue injury in the whole brain and in specific brain structures (9).

MTI studies have demonstrated two possible evolutions of new MS lesions: a) moderate decrease of MTR with a subsequent complete recovery of MTR within a few weeks, b) marked reduction of MTR with only partial recovery at follow-up (139-141). While the first pattern might reflect edema and demyelination with subsequent repair and remyelination, the second pattern probably reflects severe pathological destruction and failure of repair (83,90,142).

Several MTI studies (143-145) have revealed clinically relevant pathologic changes in areas of white matter that appear normal on conventional images; such changes in normal appearing white matter (NAWM) occur early in the disease process and provide prognostic information pertaining to the risk of developing MS. Recently, it has been shown that abnormal MTRs may be detected in areas of NABT (white and gray matter) that subsequently show overt lesion formation as shown by Gd-enhancement (146-150).

MTI metrics have been correlated with the degree of disability (9,151) as was investigated in a five year longitudinal study (152). At baseline, there were significant differences in MTR values in NAWM between clinically stable and worsening patients with MS. A strong correlation was found between baseline MTR and the subsequent 5-year change in EDSS disability score. These data support the notion that early MTR abnormalities in NAWM can predict the clinical evolution of MS.

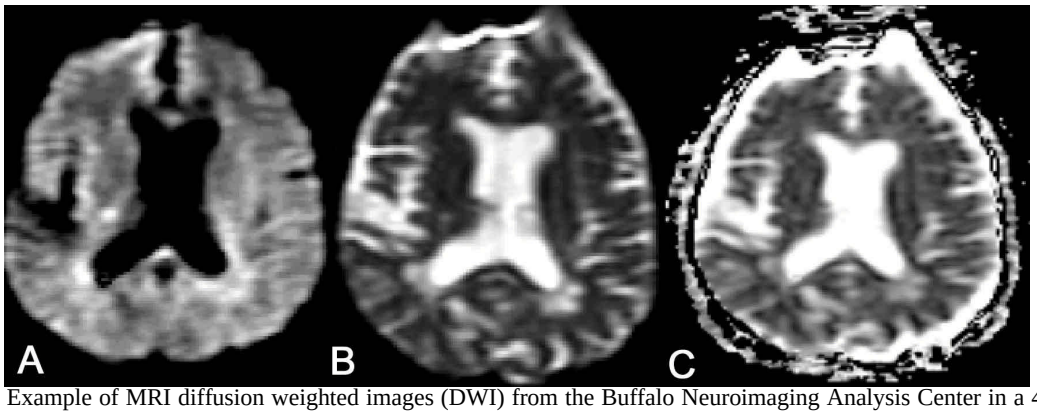

Figure 11. Example of MRI diffusion weighted images (DWI) from the Buffalo Neuroimaging Analysis Center in a 44 year-old man with SP MS. Echoplanar DWI is performed generating trace images with a b-factor of 1000 (a) and 0 (b) s/mm2 applied in three orthogonal directions. On the b-1000 DWI map (a) lesions appear as hyperintense areas compared with the surrounding tissue most likely due to T2 shinethrough rather than restricted diffusion. To calculate apparent diffusion coefficients (ADCs), an ADC map (c) is generated from images a and b. The diffusivity was computed separately in the x, y, and z orthogonal directions, and the results averaged to form the mean ADC map (c). Note the hyperintensity of the lesions on the ADC map (c), consistent with T2 shinethrough.

5.2. Magnetic resonance spectroscopy

MRS provides a quantitative assessment of disease involvement related primarily to two major pathologic aspects of MS, active inflammatory demyelination and axonal/neuronal injury (13,153). While inflammation and demyelination is represented by increases in choline, lactate and lipids, axonal and neuronal injury can be quantified through decreases in N-acetylaspartate (NAA). A decrease of NAA in the white and gray matter of patients with MS most likely reflects changes in density, size or metabolism of axons (153).

Longitudinal MRS studies have demonstrated that the degree of recovery from tissue damage in the post acute phase of lesion evolution is highly variable (154-156). Davie *et al* (154) investigated the evolution of acute MS lesions using MRS in 8 patients with MS. The elevated lactate, choline and other lipid values in some lesions (enhancing for more than one month and remaining elevated for a mean of five months) probably reflected the presence of inflammation, local ischemia and neuronal metabolic dysfunction. The results of this study provided evidence that MRS can detect myelin breakdown occurring in the early inflammatory stage of lesion development. The ratio of NAA to creatine was reduced in acute lesions and in NAWM. In six of the lesions studied there was, however, a subsequent rise in the NAA/creatine ratio indicating that reversible axonal dysfunction is another potential mechanism of reduction in the NAA/creatine ratio.

Recent MRS studies demonstrated that inflammatory activity might be detected in NABT (white and gray matter) (108,157-159). One study suggested that newly Gd-enhancing lesions are preceded by abnormal MRS characteristics several months before the appearance of Gd-enhancement (157).

Decreased NAA levels have been correlated with disability (5,153). MRS has been used by several groups to predict clinical evolution in RR MS (5,160). De Stefano *et al.* (160) assessed NAA values from a large central brain volume to evaluate the relationship between axonal/neuronal integrity and the accumulation of clinical disability in a 30-month longitudinal study of 29 patients with RR MS. Decreased NAA/creatine ratio correlated strongly with clinical disability suggesting that axonal injury contributes to long-term functional impairment of patients with MS.

5.3. Diffusion-weighted imaging

The translational motion of molecules in a fluid system can be measured *in vivo* by using diffusion-weighted MRI (DWI) (13). In the brain, diffusion is influenced by the microscopic architecture of tissue, including axons, cell membranes and organelles. DWI is sensitive to pathologic processes such as infarction that modify tissue integrity and result in changes in the permeability or density of barriers restricting water molecular motion thereby affecting tissue anisotropy (161). Thus, measures derived from DWI reflect changes in the size, shape, geometry, and orientation of tissues (Figure 11) (13). DWI is also sensitive to inflammation and demyelination in MS (4,13,103-105,162-169). MS lesions commonly show hyperintensity on DWI scans due to T2 shinethrough, which can be differentiated from true restriction of diffusion using apparent diffusion coefficient (ADC) maps (Figure 11). Tissue damage related to MS is usually reflected on DWI scans as increased mean diffusivity (MD) and increased ADC (13,162). Another MRI measure derived from DWI is fractional anisotropy (FA), which indicates the structural integrity and degree of structural alignment within fiber tracts.

Several DWI studies demonstrated abnormal ADC, MD or FA values in MS lesions (103-105) that differ between Gd-enhancing vs. non-enhancing lesions. Some studies reported lower ADC and MD in Gd-enhancing lesions comparing with nonenhancing lesions (163-165), whereas the others did not observe significant differences

(104,166,167). On the contrary, the FA studies showed a consistent decrease of FA in Gd-enhancing compared to non-enhancing lesions (164,167).

Significant cross-sectional correlations between DWI and clinical findings in MS are emerging (162,168), indicating that gray matter is not spared by the disease process (162). However, the stage at which DWI can detect abnormalities in NABT is not clear. One study (169) showed that DWI did not detect alterations in NAWM of patients with CIS at baseline but that such abnormalities became apparent one year later. Other DWI studies showed that ADC and MD values are higher in NAWM of patients with MS than in normal control individuals, but lower than in MS lesions (163-167). FA in NAWM is also lower in patients with MS compared to normal controls, but higher than in MS lesions (104,166,167).

5.4. Functional MRI

Functional MRI (fMRI) is a unique MRI technique that can non-invasively detect activation of brain areas during the performance of tasks (13). MS is a disease with widespread distribution of the lesions that may affect many anatomical regions. For that reason and its limited spatial resolution, fMRI has limited use in detecting inflammation and lesions. However, fMRI has a role in studying the response to injury and brain reorganization/plasticity in MS. The correlation of fMRI with other clinical and structural MRI measures of inflammation has been recently assessed. Reddy *et al.* (170) monitored the recovery after a clinical relapse of a single patient with fMRI and MRS. Clinical improvement was associated with normalization of NAA and progressive resolution of a large area of abnormal cortical activation with movement. This study shows the potential of fMRI to monitor functional reorganization of the motor cortex following relapse. Lee *et al.* (171) used fMRI to characterize activation in the motor cortex during finger movements in 12 MS patients and in 12 normal controls. The relative hemispheric lateralization of sensorimotor cortex activation decreased in direct proportion to the T2LV. These observations show disease related cortical recruitment changing both quantitatively and qualitatively in the sensorimotor cortex, suggesting that cortical reorganization or unmasking of latent pathways contributes to functional recovery.

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