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Diffusion imaging in multiple sclerosis: research and clinical implications†

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Abstract

Multiple sclerosis (MS) is an inflammatory-demyelinating and neurodegenerative disease of the central nervous system (CNS) and the most frequent cause of non-traumatic disability in young and middle-age adults. Although conventional MRI (including T2-weighted, pre- and post-contrast T1-weighted scans) has had a huge impact on MS by enabling an earlier diagnosis, and by providing surrogate markers for monitoring treatment response, it is limited by the low pathological specificity and the low sensitivity to diffuse damage in normal-appearing white matter and gray matter. Diffusion weighted MRI is a quantitative technique able to overcome these limitations by providing markers more specific to the underlying pathologic substrates and more sensitive to the full extent of ‘occult’ brain tissue damage. This review describes diffusion studies in MS, discusses their pathophysiological implications and emphasizes their clinical relevance.

Keywords

diffusion tensor imaging; diffusion tensor tractography; multiple sclerosis; pathological correlates; clinical outcomes

INTRODUCTION

Multiple Sclerosis (MS) is a chronic inflammatory-demyelinating and neurodegenerative disease of the central nervous system (CNS) and the most common cause of non-traumatic disability in young and middle-age adults (1). MS is estimated to affect more than 400,000 persons in the United States and 2 million people worldwide (2). Although the etiology of the disease is still unknown, several lines of evidence, derived from the experimental autoimmune encephalomyelitis, support the autoimmune pathogenesis of the disease (3). Pathologically, MS is characterized by areas of demyelinated plaques scattered throughout the CNS with a predilection for optic nerves, spinal cord, periventricular white matter (WM), corpus callosum and, as demonstrated by more recent correlative MRI-histological studies, cortical and sub-cortical gray matter (GM) (3). MS lesions reveal a great heterogeneity with respect to the presence and extent of inflammation, demyelination,

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axonal injury, gliosis and remyelination (4). Similar pathological features have been described, albeit to a lesser extent, in normal-appearing brain tissue (NABT) (5–6).

Approximately 85% of MS patients experience a relapsing-remitting (RR) clinical course characterized by the episodic onset of symptoms followed by residual deficits or by a full recovery especially in the early stage of the disease (7). While within 25 years, most of untreated RRMS patients will evolve into a secondary progressive (SP) phase characterized by a chronic and steady increase of disability, 20% of RRMS patients will remain clinically stable for two decades and will be classified as benign MS (BMS). Finally, 10–15% of MS patients experience a primary progressive (PP) course since the onset in absence of relapses (8).

Although the diagnosis of MS is still based on clinical findings, magnetic resonance imaging (MRI) is now integrated in the diagnostic criteria of the disease because of its unique sensitivity in demonstrating dissemination in space and time of demyelinating lesions in the brain and spinal cord (9). Conventional MRI (including T2-weighted, pre- and post-contrast T1-weighted scans) has had a huge impact on MS by enabling an earlier diagnosis, and by providing surrogate markers for monitoring response to current disease-modifying treatments and upcoming experimental agents. Despite its increasing role in the clinical management and scientific investigation of MS, conventional MRI is limited by low pathological specificity and low sensitivity to diffuse damage in normal-appearing white matter (NAWM) and gray matter (NAGM). In addition, conventional MRI shows only limited associations with clinical status.

Diffusion weighted MRI is a quantitative technique able to overcome these limitations by providing markers more specific to the underlying pathologic substrates of the disease and more sensitive to the full extent of ‘occult’ tissue damage in patients with MS. Diffusion measures the microscopic Brownian motion of water molecules. This motion is hindered by cellular structures such as cell membranes and axonal cytoskeletons (10). The diffusion tensor is a mathematical description of the magnitude and directionality (anisotropy) of water molecules movement in the three-dimensional space (11). Since in brain white matter, the motion of water molecules can be hindered by the presence of highly organized myelin fiber tracts, water molecules move more easily parallel to tracts and are restricted in their movement perpendicular to tracts. The diffusion tensor can be diagonalized to obtain three eigenvalues or diffusivities (λ_1 , λ_2 , and λ_3), which can be combined to define several quantitative scalar indices such as mean diffusivity (MD), equal to the magnitude of diffusion, and fractional anisotropy (FA), which represent a measure of fiber integrity and directionality (11). In the CNS, λ_1 represents the water diffusivity parallel to the axonal fibers and is referred to as $\lambda_{||}$, axial diffusivity. The water diffusivity perpendicular to the axonal fibers, λ_2 , and λ_3 are averaged and referred to as $\lambda_{\perp} = (\lambda_2 + \lambda_3)/2$, radial diffusivity (12). Abnormalities in diffusivity patterns have been seen both in focal MS lesions and in NAWM and NAGM. In this review we summarize the studies that have investigated the diffusion characteristics of tissue damage in patients with MS with the ultimate aim of providing insights into the disease pathophysiology and discussing the clinical implications.

PATHOLOGICAL CORRELATES OF DIFFUSION MRI METRICS IN MS

Histopathologically, MS is characterized by demyelination, axonal injury, inflammation, gliosis and remyelination (4). A few studies have investigated the pathological correlates of diffusion imaging in MS (13–14). In a post-mortem study of patients with progressive MS and chronic lesions Schmierer and colleagues (15) found a strong correlation of MD and FA with myelin content and—to a lesser degree—axonal count and fibrillary gliosis suggesting that, in chronic post-mortem MS brain, MD and FA are mainly affected by demyelination.

Compared to values acquired during *in vivo* study of MS patients, post-mortem studies exhibit reduction in average MD and FA both in lesions and in NAWM. This reduction in diffusivity of brain tissue following death is a well-recognized phenomenon and it is likely due to several factors, including dehydration of post-mortem tissue, the lower temperature during post mortem MRI and the breakdown of energy-dependent ion transport mechanisms resulting in a net influx of water into cells and reduction of the extra-cellular space (16–17).

DIFFUSION MRI AND LESION EVOLUTION

Diffusion tensor imaging (DTI) has proved to be a valuable tool for investigating the variety of pathological features of T2-visible lesions. Increased MD and decreased FA are always more pronounced in lesions than in the NAWM; however their values are highly heterogeneous indicating the variable degrees of tissue damage occurring within MS lesions (18–21). As expected, the more severe MD abnormalities have been found in non-enhancing T1 hypointense lesions which represent areas of irreversible tissue disruption, gliosis, and axonal loss (22) and consequent loss of structural barriers to water diffusion (Fig. 1). Conversely, conflicting results have been obtained when comparing Gd-enhancing lesions vs non-enhancing lesions: one study demonstrated that Gd-enhancing lesions have higher MD values (20) but others did not find any significant difference between the two groups of lesions (21,23–24). This discrepancy might be due to methodological issues or might reflect the concurrent presence of destructive and reparative processes. Along this line, a longitudinal study of Gd-enhancing lesions which were followed up for 1 to 3 months showed that MD values were increased in all lesions at baseline, but continued to increase only in a sub-group of them at follow-up. Furthermore, increased MD values were correlated with a greater degree of T1-hypointensity (25). This suggests that some of the tissue abnormalities in acute lesions are related to neurodegeneration and, therefore, are permanent whereas some other tissue abnormalities are related to edema, demyelination and remyelination. Unlike MD, FA is always lower in Gd-enhancing lesions, especially the ring-enhancing ones (23), than in the non-enhancing lesions reflecting demyelination, axonal injury and accumulation of inflammatory cells (20–21,26). Interestingly, a recent study (27) has reported increased MD and FA values in cortical lesions of MS patients relative to the cortical GM of healthy controls. The increased anisotropy in cortical lesions is opposite to the decrease of FA found in WM lesions and suggests that the reduced dendritic arborization, typical of cortical lesions, might increase coherence, and consequently, diffusion anisotropy.

DIFFUSION MRI AND ‘OCCULT’ INJURY IN NAWM AND NAGM

Although conventional MRI is very sensitive to macroscopic lesions, it lacks sensitivity to the ‘occult’ microscopic pathology involving NAWM and NAGM (5–6). In contrast, several studies have described abnormalities of diffusion MRI metrics outside T2 lesions in NAWM and in NAGM of MS patients (21,28–32) contributing to improve our understanding of MS pathophysiology. Werring and colleagues demonstrated that a steady and moderate increase of apparent diffusion coefficient (ADC) can precede the development of new plaques (33) and be followed by a rapid and marked increase at the time of Gadolinium enhancement and a slower decay after the cessation of enhancement. Thus, suggesting that new lesions are preceded by progressive, albeit subtle, tissue abnormalities beyond the resolution of conventional MRI. Interestingly, a milder increase of ADC was also detected in regions of NAWM contralateral to the newly appeared lesions supporting the concept that NAWM abnormalities are due the secondary degeneration of axons transected within lesions. However, MD and FA of NAWM and NAGM are only partially correlated with the extent of focal lesions and the severity of intrinsic lesion damage suggesting that diffusivity changes in normal-appearing tissue are not the mere consequence of retrograde degeneration

of axons transected in T2-visible lesions. Indeed, astrocytic hyperplasia, patchy edema, perivascular infiltration, demyelination and axonal loss may also contribute to the diffusion abnormalities of normal-appearing brain tissue (5–6). Moreover, several DTI studies have shown that NAWM injury become more pronounced with increasing disease duration and clinical disability (34–36) suggesting that DTI is likely to be sensitive to more severe pathological processes. Since MS is a diffuse and widespread disease of the CNS, the analysis of DTI changes can be performed on a global basis using histogram analysis (Cercignani, 2000, pathologic; Nusbaum, 2000, histograms; Iannucci, 2001, correlation}. MS patients have significantly higher average MD, lower histogram peak height MD and lower average FA than healthy controls. These DTI changes are significantly more severe in patients with SPMS than in those with RR-MS (37–39) supporting a role for DTI in monitoring advanced stages of the disease (21). Several DTI studies (20–21,34,40–41) have also investigated patients with primary-progressive (PP) MS, in the attempt to clarify the pathophysiology of this MS subgroup characterized by atypical MRI features: few lesions and little Gadolinium enhancement (42). Although MD and FA abnormalities were described in NAWM and NAGM of these patients, no significant correlation was found between lesion load and DTI abnormalities in normal-appearing tissue suggesting that microscopic diffuse brain changes in these patients are independent from T2-visible lesions (34,41). Histogram-derived DTI metrics could be particularly useful for monitoring disease progression and treatment response because they enable the assessment of the global disease burden including macroscopic lesions and microscopic injury in NAWM and NAGM. In other studies, however, the assessment of regional damage using DTI proved to be more sensitive than a global approach to gain insight on the imaging correlates of the clinical status in MS. Using a voxel-based approach, a recent study (43) showed that patients with RR and benign MS differ in topographic distribution of WM damage rather than in the global extent of brain structural changes. Specifically, the less prominent involvement of the frontal lobe WM in benign MS might be associated to the favorable clinical status. Changes of DTI metrics have been described in cortical and subcortical NAGM (30,34,40,44–47). RRMS patients exhibit average NAGM MD higher than that of age and sex matched healthy controls and NAGM MD is higher in patients with SPMS and PPMS than in those with RRMS (30,34,40). NAGM abnormalities might be due to the presence of cortical lesions which are often undetected on T2-weighted images and/or to the retrograde degeneration of cortical neurons secondary to the injury of axons in WM lesions (48–49).

DIFFUSION MRI OF SPINAL CORD AND OPTIC NERVES

The spinal cord and the optic nerves are common sites of pathology in multiple sclerosis; however, diffusion MRI in these regions presents a considerable technical challenge (50). The majority of cord lesions are limited to one to two spinal cord segments and involve less than half of the cross-sectional area of the cord (51). Similar to the brain, conventional MRI is unable to grade the extent of tissue injury within macroscopic lesions, as well as to detect and quantify the ‘occult’ damage known to occur in the normal-appearing cord of patients with MS (52). Since spinal cord damage is likely to contribute to the accumulation of disability, there is a need to assess spinal cord involvement in MS with new imaging techniques in order to understand better the underlying pathology. Changes in patients with MS have recently been detected in the cervical spinal cord using sagittal DTI and evaluating large regions of interest with the use of histogram analysis (23,53–56) (Valsasina, 2005, histogram; Agosta, 2005, primary; Benedetti, 2009, benign}. Since lesions in primary demyelination have a predilection for the posterior columns of the spinal cord, an analysis of DTI metrics in regions of the spinal cord may be useful to demonstrate spatial differences (57) (Fig. 2). Patients with RRMS, SPMS and PPMS have MD and FA histogram characteristics suggestive of diffuse cord injury (58); moreover, it has been shown that, in these patients, cord FA and brain average MD are independent variables of clinical

disability. When patients with benign MS are compared with patients with SPMS, they present milder cord injury with cord FA and cross-sectional area being independent factors associated with the degree of clinical disability (56). Finally, Ciccarelli and colleagues (59), have demonstrated that the combination of MR spectroscopy and diffusion MRI of the cervical cord not only provide measures that are sensitive to the tissue damage occurring in this area but enable a more comprehensive assessment of spinal cord and clinical disability.

The optic nerves (ONs) provide an ideal structure for study by diffusion MRI (60–63); increases in MD and reductions in FA of ONs have been shown to provide a marker of the structural integrity of axons. Using zonal oblique multislice echoplanar imaging (64) in patients with a single episode of unilateral optic neuritis, Trip and colleagues (62) demonstrated that MD and FA measures of the affected ONs were impaired and increased MD and decreased FA were correlated with the amplitude of the visual evoked potential. Kolbe and colleagues showed that decreased FA and volume of the ON are independent predictors of visual dysfunction 4 years after an episode of unilateral optic neuritis (65). A recent study employed diffusion tensor imaging of the ON and optical coherence tomography to study patients with acute, isolated optic neuritis (66). The decrease in axial diffusivity at onset correlated with visual contrast sensitivity 1 and 3 months later. Since changes in axial diffusivity are thought to reflect axonal injury, the decline of axial diffusivity in the acute setting has potential as a surrogate marker of tissue destruction and outcome. This is further supported by the data collected from three patients who were imaged serially from the acute episode. Axial diffusivity correlated with the thickness of the retinal fiber layer measured by optical coherence tomography, and visual acuity suggesting the potential of axial diffusivity as a predictor of future clinical outcome.

LONGITUDINAL DIFFUSION MRI STUDIES

Few studies have investigated the utility of diffusion imaging in monitoring the temporal evolution of MS damage both in lesions and NABT over time (35,67–70). A longitudinal study of patients in the earliest stage of MS suggested a progressive increase of NAWM diffusivity over 1 year (67). An increase in NAWM diffusivity was also observed in patients with PPMS over the course of 1 year (70). In a study of untreated RRMS patients, a significant decrease of average NAGM FA and increase of average NAGM MD was observed over 18-month follow-up (69). In a large cohort of patients with PPMS, a significant decrease of average NAGM was detected after one-year follow-up (35). Agosta and colleagues (71) used conventional and DT MRI to investigate the temporal evolution of intrinsic tissue injury and atrophy in the cervical cord and their relationship with brain damage and disability. Both cord cross-sectional area and FA decreased during follow-up but they were not strictly correlated suggesting that a multiparametric MRI approach is needed to achieve more accurate estimates of such damage. MS cord pathology was also found to be independent of concurrent brain changes, to develop at a different rate in different MS subgroups and to be associated with medium-term disability accrual. However, the precision and accuracy of DTI in detecting longitudinal, MS-related changes need to be defined and large, prospective studies are warranted. This issue is central for future application of DTI to the monitoring of the disease evolution in MS clinical trials and, eventually, in the assessment of individual patients.

DIFFUSION TENSOR IMAGING TRACTOGRAPHY

Diffusion-based tractography is a technique based on the directional movement of water which allows the generation of a non invasive three dimensional representation of white matter fiber tracts (72). The application of DTI tractography in MS is limited by the presence of both focal and diffuse alteration of tissue organization, which results in a

decreased anisotropy and a consequent increase in uncertainty of the primary eigenvector of the DT (73). As a consequence, the low FA can erroneously terminate the tracking algorithm or cause a deviation of the bundles at the level of the lesions (72). A possible approach to overcome this problem is the use of probability maps of tract of interest obtained from healthy subjects to assess DTI metrics from the corresponding patients' tracts (74–75) (Fig. 3). Using this method, A DTI tractography study of patients in the earliest stage of MS showed that those patients who had motor impairment had increased MD and T2 lesion volume in the cortico-spinal tract (CST) compared with those patients who did not have pyramidal symptoms (74). A DTI tractography study of patients who had optic neuritis showed reduced connectivity values both in left and right optic radiations compared with controls, suggesting the occurrence of transynaptic degeneration secondary to optic nerve damage (76). DTI tractography also provided a method to identify NAWM fibers at risk of degeneration when intersecting T2 visible lesions (77). In patients with MS, MD and FA of the CST correlate with clinical measures of locomotor disability, such as the expanded disability status scale (EDSS) score or the pyramidal functional system score at this scale, more than T2 lesion burden and the overall extent of diffusivity changes of the brain (78). The analysis of radial and axial diffusivity of the CST of patients who have RRMS has suggested Wallerian degeneration as the main mechanism underlying diffusion changes in this WM structure. More recently, tract-specific abnormalities of the CST were investigated using a multiparametric approach, which included DTI tractography, magnetization transfer imaging (MTI) and T2 relaxation times in a large cohort of patients with MS (79). On average, tract profiles were different between patients and controls, particularly in subcortical WM and corona radiata for all indices examined except for FA suggesting that a multiparametric approach may provide a better description of the pathological substrates of the disease. A study (80) combining measures of tractography and measures of functional connectivity showed that these measures are correlated in MS patients thus suggesting that injury of WM fibers may induce functional adaptive changes that limit its clinical manifestations (80). Although DTI tractography has the potential to improve our understanding of damage in brain and spinal cord, the interpretation of data requires caution and a priori knowledge.

CLINICAL RELEVANCE OF DIFFUSION MRI STUDIES

Recent studies have reported significant correlation between DTI findings and MS clinical symptoms and disability and suggested a role for DTI as predictive indicator of clinical outcome and as a tool for monitoring response to treatment in clinical trials. The strongest association with clinical disability has been reported with the diffusion characteristics of T2 lesions and GM (81–84). While a study was not able to find correlations between DTI metrics and the presence and severity of fatigue in MS patients(85), other studies have suggested some role for DTI-derived measures in explaining the correlates of cognitive impairment (81,84).

Approximately 50% of MS patients exhibit some degree of neuropsychological (NP) impairment (86). Identifying patients at risk for NP impairment on the basis of MRI would thus enhance quality of care. It is increasingly recognized that early pathological changes in the normal-appearing brain tissue may predict cognitive disorders in MS. Diffusion-derived measures may account for clinical signs independent of variance explained by more conventional, macroscopic MRI measures. Rovaris and colleagues (81) examined the relationship between DTI metrics and cognition in RRMS patients with mild neurological disability. Modest but significant correlations were found between mean brain diffusivity and cognitive tests measuring memory, speed of information processing and verbal fluency. Benedict and colleagues (84) investigated a larger sample of both patient with RRMS and SPMS grouped as either cognitively impaired or intact, and found that cognitive impairment

was associated with more brain injury as measured by ADC, lesion volume and atrophy. The results of a follow-up study in patients with PPMS suggest that the severity of NAGM MD may predict the accumulation of clinical disability 5 years later (87). Few ongoing studies are investigating the utility of DTI in monitoring treatment efficacy (88–89).

The promising results of diffusion imaging studies in MS and other neurological diseases have prompted several interesting technical developments aimed at improving the sensitivity and pathological specificity of the technique. Over the last few years, more complex diffusion models or model-free methods have been suggested which can better describe architectures more complex than a single coherently oriented fiber bundle (i.e. fiber crossing). Technical improvements are aimed both at a better segmentation of fiber bundles and at a better understanding of tissue structure-function relationship (for instance, characterizing distinct anatomical compartments within a voxel). For the first-type technical improvement, important contributions have been provided by methods such as HARDI (90), the deconvolution approach (91) and the Q-ball approach (92). For the second-type technical improvement, q-space and kurtosis imaging have provided promising findings in research applications. High b-value q-space imaging, which emphasizes the diffusion characteristics of the slow-diffusing component, has shown higher sensitivity to the pathophysiological changes in lesions and NAWM of MS patients when compared to DTI (93). In addition, q-space diffusion metrics correlated well with N-acetylaspartate levels suggesting their possible higher specificity for axonal injury (94). Diffusional kurtosis imaging (95) which is related to q-space imaging methods, but is less demanding in terms of imaging time, hardware requirements, and post-processing effort may prove particularly useful in several neurological disorders, including MS (Fig. 4). This method provides a specific measure of tissue structure, such as cellular compartments and membranes and has been shown to be able to characterize and measure age-related diffusion changes not only for white matter but also for gray matter (96).

CONCLUSIONS

The current application of diffusion MRI to patients with MS shows that it has enhanced our understanding of the disease pathophysiology. The studies reviewed here provide evidence that DTI-derived measures are more specific to the disease pathological processes and sensitive to the diffuse microscopic injury in the NAWM and NAGM. From a clinical point of view, DTI-derived measures correlate with physical disability and cognitive impairment and the degree of DTI changes in normal-appearing tissue may have predictive value of the subsequent clinical evolution. Furthermore, DTI-derived measures hold promise as a tool for monitoring treatment response in MS clinical trials. However, the precision and accuracy of DTI in detecting MS-related tissue changes also need to be confirmed in large, prospective studies. Future developments in acquisition and post-processing strategies and clinical application at higher field strength should lead to higher spatial resolution, higher precision of diffusion imaging measures and improve their pathological specificity.

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Abbreviations used

ADC Apparent diffusion coefficient

BMS	benign multiple sclerosis
CNS	central nervous system
CST	cortico-spinal Tract
DTI	diffusion tensor imaging
EDSS	expanded disability status scale
FA	fractional anisotropy
GM	gray matter
HARDI	high-angular resolution diffusion imaging
MD	mean diffusivity
MK	mean kurtosis
MNI	Montréal Neurological Institute
MS	multiple sclerosis
MTI	magnetization transfer imaging
NABT	normal-appearing brain tissue
NAGM	normal-appearing gray matter
NAWM	normal-appearing white matter
NP	neuropsychological
ON	optic nerve
OCT	optical coherence tomography
PPMS	primary progressive
RRMS	relapsing remitting
SPMS	secondary progressive
WM	white matter

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Figure 1. Selected axial and sagittal diffusion tensor maps from a patient with multiple sclerosis. Mean diffusivity (A, B), fractional anisotropy (C, D) and color primary eigenvector map (E, F) illustrating different directions of the primary eigenvector: Red is left-right; green is cranio-caudal and blue is in-out of the page. Note that MS lesions (arrows) show higher mean diffusivity (bright), lower fractional anisotropy (dark) and disruption of the primary fiber direction (dark).

Figure 2. Selected axial T2-weighted image (A) of the cervical spinal cord of a MS patient acquired at C3 level (the arrow indicates a hyperintense lesion). MD (B) and FA (C) maps corresponding to the level of the T2 weighted image.

Figure 3. 3D DTI-tractography of the corpus callosum (CC) (A) in a healthy control. CC (B) and antero-thalamic (AT) (C) tract probability maps in MNI space and in the corresponding single subject space (D) after transformation.

Figure 4. Selected T2-weighted image of a MS patient (A) showing multiple periventricular hyperintense lesions (arrows). Corresponding MD (B), FA (C) and MK (D) maps.