Magnetic resonance-based techniques for the study and management of multiple sclerosis

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Conventional magnetic resonance imaging (cMRI) is widely used for diagnosing multiple sclerosis (MS) and for monitoring its activity and evolution. However, the correlation between cMRI and clinical findings of MS is limited, possibly due to the low pathological specificity of the abnormalities seen on cMRI scans and to the inability of cMRI to quantify the extent of the damage of the normal-appearing tissues. Magnetization transfer and diffusion-weighted MRI can quantify the extent and pathological severity of structural changes occurring within and outside cMRI-visible MS lesions. Proton MR spectroscopy can add information on the biochemical nature of such changes. Finally, functional MRI can provide new insights into the role of cortical adaptive changes in limiting the clinical consequences of MS structural damage. The application of quantitative MR-based techniques is changing dramatically our understanding of how MS causes irreversible disability and there is increasing perception that these methodologies should be more extensively employed in clinical trials to derive innovative information.

Conventional magnetic resonance imaging (cMRI) is an important paraclinical tool for diagnosing multiple sclerosis (MS) and providing several markers of disease activity and evolution. cMRI-derived measures have clear advantages over clinical assessment, including their more objective nature and an increased sensitivity to MS-related changes. However, the magnitude of the relationship between cMRI measures of disease activity or burden and the clinical manifestations of the disease is weak. This clinical/MRI discrepancy is likely to be the result, at least partially, of the inability of cMRI to quantify the extent and to define the nature of MS tissue damage.

Structural and metabolic MR-based techniques with increased specificity to the heterogeneous pathological substrates of MS have the potential to improve our understanding of how MS evolves. Magnetization transfer (MT) MRI and diffusion-weighted (DW) MRI enable us to quantify the extent of structural changes occurring in T2-visible lesions and in the tissue which appears normal on cMRI images.
Proton MR spectroscopy (1H-MRS) can add information on the biochemical nature of such changes. Functional MRI (fMRI) holds substantial promise to elucidate the mechanisms of cortical adaptive reorganization following MS injury.

This chapter provides an update of the current ‘state-of-the-art’ in the application of structural, metabolic and functional MR-based techniques to the study of MS.

**Conventional MRI**

Unenhanced T2-weighted images are highly sensitive for the detection of MS lesions (Fig. 1A,B). Such a sensitivity makes them very useful for diagnosing MS, monitoring its short-term activity (by counting the number of new or enlarged T2 lesions visible on monthly scans), and assessing the overall disease burden (by measuring the total hyperintense lesion volume on yearly scans). Post-contrast (gadolinium, Gd) T1-weighted scans allow discrimination between active and inactive MS lesions, since Gd enhancement occurs as a result of increased blood–brain barrier (BBB) permeability and corresponds to areas with on-going inflammation (Fig. 1C), but still do not provide information on tissue damage. Chronically hypo-intense lesions on T1-weighted images (known as ‘black holes’; Fig. 1C) have been reported to represent areas where severe demyelination and axonal loss have occurred. However, the definition of what constitutes a ‘black hole’ is arbitrary and highly operator-dependent, T1-hypo-intense lesion volume does not provide graded information about the intrinsic pathology of individual lesions nor about pathology outside MRI-visible lesions. ‘Black holes’ are also difficult to detect in areas critical for the accumulation of MS irreversible disability. Measurements of brain and cervical cord atrophy can be obtained from cMRI scans to assess the extent of tissue loss in MS. However, the pathological basis of MS-related atrophy is still unclear and atrophy is an end-stage phenomenon (in optimal circumstances, it is more desirable to monitor the MS process prior to irreversibility than measuring the end result). Despite these limitations, cMRI has given clues about the pathology associated to MS, as outlined in the following paragraphs.

1. The patterns of MRI activity vary significantly in individual patients over time, from one patient to another, and across the different clinical phenotypes of MS. Disease activity tends to decline with patient age and is very low in the primary progressive (PP) form of MS.

2. In patients with established MS, the burden of cMRI-visible lesions explains about 10–15% of the observed variance of clinical disability. Conversely, in patients at presentation with clinically isolated syndromes...
In the top row, axial proton density-weighted spin-echo (A), T2-weighted spin-echo (B) and post-contrast (gadolinium DTPA, 0.1 mmol/kg) T1-weighted spin-echo (C) MR images of the brain from a patient with clinically definite MS. In (A) and (B), multiple hyperintense lesions are visible, suggestive of multifocal white matter pathology, with a predominant involvement of the periventricular regions. In (C), some of these lesions are contrast-enhanced, indicating the presence of a local blood–brain barrier disruption, whereas others are hypo-intense, indicating that marked tissue destruction has occurred. This is a pattern highly suggestive of MS, which demonstrates the presence of a pathological process with spatial and temporal dissemination. In the bottom row, the corresponding MTR (D) D (E) and FA (F) maps are shown. MTR is decreased and diffusivity is increased within MS lesions, which appear hypo-intense on MTR (D) and hyperintense on D (E) maps. Some of the lesions are also visible as areas of decreased signal on FA maps (F), indicating a local decrease of anisotropic diffusion.

The harvest of enhancing MS lesions can be markedly increased when administering a triple dose of Gd\(^{14}\). Since those lesions enhancing only after a triple dose are likely to represent areas with only mildly increased BBB permeability\(^{15}\), the simultaneous presence of lesions enhancing at different Gd doses suggests that the severity of MRI-detectable
inflammation is highly variable among lesions from the same MS patients. The use of triple dose-enhanced MRI might be useful in the context of clinical trials to grade the efficacy of treatment on MRI-detectable inflammation and to reduce the sample sizes and follow-up periods needed to run studies powered enough to detect a treatment effect.

Patients with secondary progressive (SP) MS usually have high T1-hypointense lesion load. In these patients, the volume of ‘black holes’ correlates better than the T2 lesion load with disability.

CMRI of the cord (especially, of the cervical region) is very sensitive in detecting lesions in MS patients, including those with no or only a few brain MRI abnormalities. As a consequence, cord CMRI might be helpful in the differential diagnosis between MS and other neurological diseases.

Significant reductions of brain volume and cervical cord size can be observed even in the early phases of MS. The severity of brain and cord atrophy is, however, more pronounced in the progressive forms of MS and can worsen in the absence of MRI-visible disease activity.

Quantitative structural MR-based techniques: MT and DW MRI

MT-MRI is based on the interactions between protons in a relatively free environment and those where motion is restricted. Off-resonance irradiation is applied, which saturates the magnetization of the less mobile protons, but this is transferred to the mobile protons, thus reducing the signal intensity from the observable magnetization. Thus, low MT ratio (MTR) indicates a reduced capacity of the macromolecules in the central nervous system (CNS) to exchange magnetization with the surrounding water molecules, reflecting damage to myelin or to the axonal membrane. The most compelling evidence indicating that markedly decreased MTR values correspond to areas where severe tissue loss has occurred is the strong correlation of MTR values from MS lesions and normal-appearing white matter (NAWM) with the percentage of residual axons and the degree of demyelination found in a post mortem study of patients with MS.

Diffusion is the microscopic random translational motion of molecules. Water molecular diffusion can be measured in vivo using DW-MRI, in terms of an apparent diffusion coefficient (ADC). Although diffusion is inherently a three dimensional process, in some tissues with an oriented microstructure, such as brain white matter, the molecular mobility is not the same in all directions. This property is called anisotropy, and results in a variation of the measured diffusivity with tissue measurement direction. White matter fibre tracts consist of aligned myelinated axons and, therefore, hindrance of water diffusion is...
much greater across rather than along the major axis of axonal fibres. Under these conditions, a full characterization of diffusion can only be found in terms of a tensor, a $3 \times 3$ matrix, where the on-diagonal elements represent the diffusion coefficients along the axes of the reference frame, while the off-diagonal elements account for the correlations between molecular displacement along orthogonal directions. From the tensor, it is possible to derive some scalar indices. These include the mean diffusivity ($D$) – equal to one-third of the trace of the diffusion tensor – which is affected by cellular size and integrity, and the fractional anisotropy (FA), which reflects the degree of alignment of cellular structures within fibre tracts, as well as their structural integrity.

Both MT- and DW-MRI have substantial advantages over cMRI in the study of MS. First, they may provide quantitative information with increased specificity to the heterogeneous substrates of MS pathology. Secondly, they enable us to quantify the diffuse damage occurring in brain tissues which appear normal on cMRI. Thirdly, with the application of histogram analysis, they can provide multiple parameters influenced by both the cMRI-visible and cMRI-‘occult’ lesion burdens.

Several important pieces of information have been obtained by the application of these MR techniques to the study of MS. They can be summarized as follows.

1. MS lesions visible on T2-weighted scans have lower MTR, higher $D$ and lower FA values than corresponding areas of NAWM. All these values vary dramatically across individual lesions (Fig. 1D–F). T1-hypo-intense lesions have lower MTR, higher $D$ and lower FA values than T1-iso-intense lesions. These findings confirm the pathological heterogeneity of T2-hyperintense and T1-hypo-intense MS lesions and, since this heterogeneity is not fully described by cMRI metrics, they also underpin the need to grade the degree of intrinsic lesion damage using quantitative MR technologies.

2. Individual enhancing lesions have different ranges of MTR values according to their size, modality and duration of enhancement. In detail, MTR is lower in large rather than in small enhancing lesions, in those with ring-like rather than in those with homogeneous enhancement, in those enhancing on at least two consecutive monthly scans than in those enhancing on a single scan, and in standard dose rather than in triple dose enhancing lesions. DW-MRI characteristics of enhancing lesions are less well defined. Whereas one study found that enhancing lesions have higher $D$ values and lower FA values than inactive lesions, another, which assessed more MS patients, did not find any significant difference between the two lesion populations. Longitudinal studies of new enhancing lesions show that, on average, MTR drops dramatically when the lesions start to enhance and can show a partial or complete recovery in the subsequent 6 months. MTR data also indicate the...
presence of subtle and progressive NAWM changes in areas subsequently involved by new MS lesions. These findings have been confirmed by the application of DW-MRI. All of this indicates a significant heterogeneity of intrinsic tissue damage within active, newly-formed MS lesions and the need to quantify it for achieving a more accurate picture of the acute elements of the disease.

The average lesion MTR, a global measure of intrinsic lesion damage, is reduced in patients with relapsing-remitting (RR) MS in comparison with patients at presentation with CIS suggestive of MS. Low average lesion MTR values are detected in the two progressive MS phenotypes and, in patients with SPMS, average lesion MTR values have a faster decline than in the less disabling phenotypes of the disease. Average lesion D, but not average lesion FA, has been found to be significantly correlated, albeit moderately, with clinical disability in a large cohort of MS patients. The lack of the correlation between disability and FA indicates that the loss of overall impediment to diffusional motion might be more important than the loss of tissue anisotropy in determining patients’ clinical status.

Whole brain MTR histogram characteristics are correlated with the severity of MS disability, particularly in patients with RRMS and SPMS, and, when histograms are derived from selected brain regions, their metrics are correlated with the impairment of the corresponding functional systems. Quantities derived from whole brain DW-MRI histograms are also correlated with disability in patients with RRMS and SPMS. In the less disabling phases of the disease, DW-MRI histogram findings are correlated with several cognitive test performances, but not with neurological disability. These findings suggest that the production of MTR, D and FA histograms of the whole brain tissue may enable us to assess and grade overall tissue damage in MS, with the potential to monitor reliably its evolution in patients with the most disabling disease phenotypes.

NAWM changes are invariably detected using MT- and DW-MRI in all the major MS phenotypes. The severity of NAWM damage may vary between the different patient groups and is associated with increased levels of physical disability and cognitive impairment. One MT-MRI study has shown that the extent of NAWM abnormalities in patients at presentation with CIS is an independent predictor of subsequent disease evolution, but this finding has not been confirmed by other studies. The degree of NAWM changes seems to be only modestly correlated with the burden of cMRI-visible MS lesions, suggesting that NAWM damage does not merely reflect Wallerian degeneration of axons traversing large focal abnormalities.

Using a segmentation technique based on FA thresholds, it has recently been shown that the average brain grey matter MTR is significantly decreased and D significantly increased in patients with MS when compared to healthy volunteers. D values of grey matter and
cortical/subcortical MTR values have been found to correlate with individual neuropsychological test results or overall cognitive decline in MS patients.

7 Average cervical cord MTR is significantly lower in MS patients than in healthy subjects. Cervical cord MTR histogram abnormalities are more severe in MS patients with locomotor disability than in those without. No or only moderate correlations have been found between brain T2 lesion load or average brain MTR and cervical cord MTR histogram metrics. This suggests that MS pathology in the cord is not a mere reflection of brain pathology.

8 MTR of the optic nerves from MS patients changes according to the degree of functional recovery following an episode of acute optic neuritis. This is a solid in vivo evidence that, in patients with MS, tissue loss significantly contributes to the incomplete clinical recovery from relapses.

**Proton MRS**

Water-suppressed, proton MR spectra of the human brain at long echo times reveal four major resonances: one from choline-containing phospholipids (Cho), one from creatine and phosphocreatine (Cr), one from N-acetyl-aspartate (NAA) and one from the methyl resonance of lactate (Lac). NAA is a marker of axonal integrity, whereas Cho and Lac are considered as chemical correlates of acute inflammatory or demyelinating changes. 1H-MRS studies with shorter echo times can detect additional metabolites, such as lipids and myo-inositol, which are also regarded as markers of on-going myelin damage. The following are the major insights provided by the application of proton 1H-MRS to the study of patients with MS.

1 Chronic MS lesions are characterized by markedly reduced NAA/Cr peaks. NAA concentration is lower in severely hypo-intense MS lesions than in iso- or mildly hypo-intense lesions.

2 Acute MS lesions present highly variable metabolic patterns over time. The initial decrease in the signal intensity of NAA may show partial recovery, starting soon after the acute phase and lasting for several months. Although similar decreases in NAA are found in acute enhancing lesions of patients with benign and SPMS, chronic lesions from patients with benign MS have much higher NAA levels than the chronic lesions from SPMS patients, suggesting a greater recovery of NAA in acute lesions from less disabled MS patients. Since, in acute MS lesions, Gd-enhancement is usually ceased by 2 months, the metabolic changes shown by 1H-MRS can reveal on-going pathology which would otherwise go undetected.
Decreases in NAA are not restricted to MS lesions, but also occur in the NAWM adjacent to or distant from them and can precede the appearance of MRI-visible abnormalities. Reversible changes of NAA can be detected in the NAWM of the hemisphere contralateral to solitary acute lesions of the type seen in MS, suggesting that sublethal axonal injury is a contributing factor to acute, potentially reversible MS disability.

NAA levels are significantly correlated with patients’ clinical disability, selective motor impairment and cognitive dysfunction. NAA/Cr values are reduced also in MS patients with short-lasting RRMS and no clinical disability. These data have emphasized the importance of the so-called ‘axonal hypothesis’, i.e. the notion that axonal loss underlies progressive accumulation of irreversible disability in MS cases. A new unlocalized 1H-MRS sequence for measuring NAA levels in the whole brain has been recently developed. This method proved to be reliable and sensitive to MS- and age-related changes of NAA levels and has shown the presence of marked axonal pathology since the earliest phase of MS.

At post mortem, NAA levels of the spinal cord were found to be significantly reduced in severely disabled patients and to correlate with reduced axonal density within lesions and NAWM.

Functional MRI

fMRI is being widely used to study the neuronal mechanisms of CNS functioning, and to define abnormal patterns of brain activations arising from disease. The signal changes seen during fMRI studies depend on the blood oxygenation level-dependent (BOLD) mechanism, which, in turn, involves changes of the transverse magnetization relaxation time – either T2* in a gradient echo sequence, or T2 in spin echo sequence. These changes are attributable to differences in deoxyhaemoglobin subsequent to variations of neuronal activity.

The application of fMRI in the study of the motor system in patients with MS has provided new insights into the mechanisms leading to progressive clinical worsening in these patients. Preliminary studies have suggested that fMRI can be used to monitor the recovery after an MS clinical relapse or to study the cortical re-organization in patients with established MS. In patients affected by arm paralysis, there was a correlation between fMRI findings and the severity of the functional deficit. During recovery after MS relapse, dynamic changes in the patterns of cortical activation with hand movements were detected using fMRI. The decreased lateralization of cortical motor activation, which can be seen during the acute phase, becomes less marked with progressive clinical recovery, and precedes the normalization of NAA levels in the diseased area. This suggests that cortical adaptive responses...
can compensate for MS-related brain injury to maintain normal motor functions despite the presence of structural damage.

More recently, the correlations between fMRI findings and other MR-derived measures of MS disease burden were assessed to define whether, and to what extent, fMRI changes are adaptive to the underlying MS pathology. In a sample of clinically stable MS patients with varying degrees of upper limb motor deficit, Lee et al. found that the pattern of cortical activations during a hand motor task was significantly different from that of healthy controls. In MS patients, the increase of ipsilateral motor cortex activation was significantly correlated with increasing T2 lesion load in the contralateral hemisphere. In patients with RRMS and no residual motor disability, fMRI revealed an abnormal pattern of recruitment of elements of the cortical motor network, which was correlated with brain T2 lesion load and the severity of MS pathology of lesions and NAWM. Using 1H-MRS imaging, Reddy et al. found that a similar fMRI finding (i.e. an increased activation of the ipsilateral sensorimotor cortex during finger movement) was strongly correlated with decreases in brain NAA. Two fMRI studies of the motor system of patients with PPMS, suggested a lack of these ‘classical’ adaptive mechanisms as a potential additional factor contributing to the accumulation of disability. These studies have also shown that the extent of the activation of a wide-spread sensorimotor network, involving multimodal integration areas, such as the insula and the temporal lobes, strongly correlates with several MR measures of diffuse abnormalities of the brain and cervical cord, again suggesting a potential adaptive role of these functional changes.

Conclusions

Although cMRI has improved our understanding of MS evolution, it provides limited information about MS pathology in terms of both accuracy and specificity. None of the quantitative MR-based techniques taken in isolation is able to provide a complete picture of the complexity of the MS process and this should call for the definition of aggregates of MR quantities, thought to reflect different aspects of MS pathology, to improve our ability to monitor the disease. At present, longitudinal natural history data collected in large samples of MS patients using structural, metabolic and functional MR techniques are needed to gain additional insight into MS pathobiology and on the actual value of modern MR technologies in the management of MS.

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Plate IV. Brain patterns of cortical activations on a three-dimensional rendered brain (A: axial view; B: sagittal view of the left hemisphere) from 15 right-handed patients with relapsing-remitting MS, during the performance of a simple motor task with their clinically-unimpaired and fully normal functioning upper right hands. The activity of the contralateral primary sensorimotor cortex (SMC) was significantly correlated ($r = 0.88, P < 0.001$) with mean diffusivity of the overall lesion population (C).