# MODERN MR TECHNIQUES TO MONITOR THE EVOLUTION OF MULTIPLE SCLEROSIS
## Multipl skleroz izleminde modern manyetik rezonans teknikleri

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***Abstract:*** *Conventional magnetic resonance imaging (cMRI) is widely used for diagnosing multiple sclerosis (MS) and as a paraclinical tool to monitor disease activity and evolution in natural history studies and clinical trials. However, the correlation between cMRI and clinical findings is far from being strict. Among the reasons for this "clinical-MRI paradox", a major role has been attributed to the limited specificity of cMRI to the heterogeneous pathological substrates of MS and to its inability to quantify the extent of damage in the normal-appearing tissues. Modern quantitative MR techniques have the potential to overcome some of the limitations of cMRI. Metrics derived from magnetization transfer and diffusion-weighted MRI enable us to quantify the extent of structural changes occurring within and outside macroscopic MS lesions with increased pathological specificity over cMRI. MR spectroscopy can add information on the biochemical nature of such changes, with the potential to improve significantly our ability to monitor inflammatory demyelination and axonal injury. Finally, functional MRI might provide new insights into the role of cortical adaptive changes in limiting the clinical consequences of white matter structural damage. Although the application of modern MR techniques is changing dramatically our understanding of how MS causes irreversible disability, their use for clinical trial monitoring is still very limited. Whereas there is increasing perception that modern quantitative MR techniques should be more extensively employed in clinical trials to advance the understanding of MS and derive innovative information, their use in clinical practice should still be regarded as premature.*

***Özet:*** *Konvansiyonel manyetik rezonans görüntüleme (kMRG) Multiple Skleroz (MS) tasınının konmasında, hastalığın aktivitesinin izleminde ve klinik çalışmalarda yaygın olarak kullanılır. Ancak kMRG ile klinik bulgular arasındaki ilişki net değildir. Bunun nedenleri de MS'deki heterojen patolojik maddelere kMRI'nin özgüllüğünün yetersiz olmasıdır. Modern niceliksel MRG teknikleri bu yönlerden kMRG den üstündür. Magnetik transfer ve diffuzyon ağırlıklı manyetik rezonans görüntüleri, makroskopik MS lezyonlarının içindeki ve dışındaki yapısal lezyonların büyüklüğünü anlamamızı sağlar. Ayrıca Manyetik Spektrostopi bu lezyonların biyokimyasal özelliklerini de gösterir. Fonksiyonel MRG ise kortikal adaptif değişiklikler hakkında bilgi verir. Modern MRG tekniklerinin uygulanması, MS'nin nasıl geri dönüşümü olmayan işlev kayıplarına yol açtığını gösterse de, bu tekniklerin klinik çalışmalarda kullanılması henüz çok sınırlıdır.*

***Anahtar Kelimeler:*** *Multipl skleroz*

### Introduction

*Conventional magnetic resonance (MR) imaging (cMRI) is not only the most important paraclinical tool for diagnosing multiple sclerosis (MS), but it also provides several markers of disease activity and evolution [1]. These include the number of gadolinium (Gd)-enhancing and new T2-hyperintense lesions, the overall burden of T2-hyperintense and T1-hypointense lesions [1, 2], and measures of brain and cord atrophy [3-5]. CMRI-derived measures have several advantages over clinical assessment, including their more objective nature and a greater sensitivity to MS-related*

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*changes [1]. As a consequence, cMRI has been extremely useful for understanding the natural history of MS and monitoring the efficacy of experimental treatments. However, the correlation found between cMRI measures of disease activity and burden and the clinical manifestations of the disease are only weak [6-8]. 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.*

*Modern, quantitative MR techniques with increased specificity to the heterogeneous pathological substrates of MS have the potential to improve our understanding of how MS evolves and, as a consequence, to provide better measures for monitoring the efficacy of treatment. Magnetization transfer (MT) MRI [9] and diffusion-weighted (DW) MRI [10] 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 (MRS) can add information on the biochemical nature of such changes, with the potential to improve significantly our ability to monitor inflammatory demyelination and axonal injury [11]. In recent years, these MR-based techniques have largely been applied to the in-vivo assessment of MS pathology and, in the near future, they are likely to enter a new phase of more widespread routine clinical practice and be used (at least as exploratory end-points) in large-scale clinical trials of MS. There are, however, other MR techniques and approaches that are receiving increasing attention and are likely to provide additional insights on the pathophysiology of MS. In this context, functional MRI (fMRI) holds substantial promise to elucidate the mechanisms of cortical adaptive reorganization following MS injury. This review outlines the major contributions given by modern MR-based techniques to the understanding of the mechanisms leading to the accumulation of irreversible disability in MS, and the reasons for supporting the use of modern quantitative MR measures in future MS clinical trials.*

**Magnetization transfer MRI**

*MT-MRI is based on the interactions between protons in a relatively free environment and those where motion is restricted. In the central nervous system (CNS), these two states correspond to the protons in tissue water, and in the macromolecules of myelin and other cell membranes. 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. The degree of signal loss depends on the density of the macromolecules in a given tissue. Thus, low MT ratio (MTR) indicates a reduced capacity of the macromolecules in the CNS to exchange magnetization with the surrounding water molecules, reflecting damage to myelin or to the axonal membrane. The most compelling among the many evidences indicating that markedly decreased MTR values correspond to areas where severe tissue loss has occurred [9] 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 post-mortem human specimens [12].*

*MT-MRI has substantial advantages over cMRI in the study of MS [9]. First, it provides quantitative information with increased specificity to the more disabling substrates of MS pathology [12]. Secondly, it enables us to quantify the diffuse damage occurring in the normal-appearing brain tissues. Thirdly, with the application of MTR histogram analysis [13], it provides, from a single procedure, multiple parameters influenced by both the cMRI-visible and cMRI-"occult" lesion burdens. In the past few years, several important pieces of information were obtained by the application of MT-MRI to the study of MS. They can be summarized as follows:*

*a) Individual enhancing lesions have different ranges of MTR values according to their size, modality and duration of enhancement [14]. In*

*details, MTR is lower in large than in small enhancing lesions, in those with ring-like than in those with homogeneous enhancement, and in those enhancing on at least two consecutive monthly scans than in those enhancing on a single scan. TD enhancing lesions have significantly higher MTR values than SD lesions [15]. Longitudinal studies of new enhancing lesions [14] show that, on average, MTR drops dramatically when the lesions start to enhance and can show a partial or complete recovery in the subsequent six months. During the three years following their formation [16], new lesions from patients with SPMS were found to have a more severe MTR deterioration than those from patients with mildly-disabling RRMS. These findings confirm the 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.*

*b) MS lesions visible on T2-weighted scans have lower MTR values than the corresponding NAWM [17] and T1-hypointense lesions have lower MTR values than T1-isointense lesions [18].*

*c) The average lesion MTR, a global measure of intrinsic lesion damage, is reduced in patients with RRMS in comparison with patients at presentation with CIS suggestive of MS [19]. Low average lesion MTR values are detected in the two progressive MS phenotypes [20, 21]. Consistent with their clinical evolution, patients with SPMS have a faster decline of their average lesion MTR values than the less disabling phenotypes of the disease [22].*

*d) NAWM changes are invariably detected in all the major phenotypes of MS, using either a region of interest (ROI) [17] or a histogram analysis [23]. NAWM damage seems to be an important pathological aspect particularly in patients with PPMS and SPMS [20]. NAWM changes have been detected also in patients with definite MS and normal cMRI of the brain [24]. 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 [19], in keeping with the demonstration that variable degrees of NAWM changes can precede new lesion formation in MS [25]. However, this finding has not been confirmed by other studies [26, 27].*

*e) Using ROI [28] or histogram [28, 29] analysis, MTR abnormalities can be detected in the grey matter (GM) of MS patients and are significantly correlated with brain T2 lesion volume [28, 29]. This fits with the notion that at least part of GM pathology in MS is secondary to retrograde degeneration of fibers transversing white matter lesions.*

*f) MTR-histogram derived metrics are well correlated with the severity of MS disability [30, 31], particularly in patients with RRMS and SPMS [30]. Whole brain and NAWM-MTR histogram metrics are also correlated with the presence of neuropsychological impairment in MS patients [32, 33]. MTR histogram parameters derived from specific brain regions (i.e., cerebellum, brainstem, frontal lobes) are correlated with the impairment of the corresponding functional systems [30, 33].*

*g) Average cervical cord MTR is significantly lower in MS patients than in healthy subjects [34]. Cervical cord MTR histogram abnormalities are more severe in MS patients with locomotor disability than in those without [34]. No or only moderate correlations have been found between brain T2 lesion load or average brain MTR and cervical cord MTR histogram metrics [35]. This suggests that MS pathology in the cord is not a mere reflection of brain pathology.*

*h) MTR of the optic nerves from MS patients changes accordingly with the degree of functional recovery following an episode of acute optic neuritis [36]. This is a strong evidence in-vivo that, in patients with MS, tissue loss significantly contributes to the incomplete clinical recovery from relapses.*

*Although the optimization and standardization across multiple sites and over time of MT sequences might be challenging and long-term longitudinal studies using MT-MRI are lacking, MT-MRI holds substantial promise to provide good surrogate measures for MS evolution. A recent international consensus conference of the White Matter Study Group of the International Society for MR in Medicine has indeed recommended the use of MT-*

*MRI in the context of MS clinical trials as an adjunctive outcome measure [37]. Several recent MS clinical trials have already incorporated MT-MRI, with a view to assessing the impact of treatment on demyelination and axonal loss. To our knowledge, MT-MRI has been used in phase II and phase III trials for RRMS (injectable and oral interferon beta-1a, interferon beta-1b, and oral glatiramer acetate) and SPMS (interferon beta-1b and immunoglobulins). In these trials, MT-MRI acquisition has been limited to highly-specialized MR centers and only subgroups of patients (about 50-100 per trial) have been studied using MT-MRI. Currently available studies were conducted at single centers with small numbers of patients [38-40] and, as a consequence, they were not confronted with problems of standardization of MT acquisition and post-processing. Two of these studies have shown that treatment with interferon beta-1b [39] or interferon beta-1a [40] favorably modifies the recovery of MTR values which follows the ceasement of Gd enhancement in newly-formed lesions from RRMS patients. These findings suggest that, in addition to its effects in reducing the formation of new lesions, interferon beta might also act by reducing tissue damage and promote remyelination within those lesions which are still formed during therapy. On the contrary, Richert et al. [39] did not find any significant difference in the MTR values of NAWM-ROI before and during interferon beta-1b therapy, as well as in the parameters derived from whole brain MTR histograms [38] in RRMS patients.*

*Clearly, reduced NAWM and lesion MTR values are not MS-specific [14] and, as a consequence, the role of MT-MRI in the diagnostic work-up of individual patients in daily-life practice is modest. Nevertheless, it might be worth noting that the average lesion MTR was found to be the best discriminant between patients with MS and those with CNS symptoms or signs of systemic immune-mediated disorders, independently of the burden of cMRI-visible lesions [41], and that MTR changes of the NAWM have not been found in patients with other conditions [14], such as migraine and multiple T2 lesions, Devic's neuromyelitis optica and acute disseminated encephalomyelitis (ADEM), which can be considered in the differential diagnosis of patients with MS. Recent preliminary work has also suggested a potential role of whole-brain MTR histograms in the diagnostic work-up of individual cases suspected of having MS, expecially in the absence of 'typical' conventional MRI abnormalities [42].*

**Diffusion-weighted MRI**

*Diffusion is the microscopic random translational motion of molecules, and water molecular diffusion can be measured in vivo using DW-MRI, in terms of an apparent diffusion coefficient [10]. Since diffusion is affected by the properties of the medium where molecular motion occurs, the measurement of diffusion inside biological tissues provides information about tissue structure at a microscopic level. The motion of water molecules can be hindered by the presence of structural barriers at a cellular or subcellular level. In addition, diffusion is inherently a three dimensional process, and 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 in the measured diffusivity with tissue measurement direction [10]. White matter fiber tracts consist of aligned myelinated axons and, therefore, hindrance of water diffusion is much greater across rather than along the major axis of axonal fibers. Under these conditions, a full characterization of diffusion can only be found in terms of a tensor [10], a 3x3 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, invariant to the changes in the frame of reference, which reflect the diffusion characteristics of the tissue. These measures include a) the mean diffusivity (equal to one third of the trace of the diffusion tensor) which is a measure of the average molecular motion independent of any tissue directionality, and is affected by cellular size and*

*integrity; and b) the fractional anisotropy (FA) which is one of the most commonly-used measures of deviation from isotropy and reflects the degree of alignment of cellular structures within fiber tracts, as well as their structural integrity.*

*The pathological elements of MS alter the permeability or geometry of structural barriers to water diffusion in the CNS. The application of DW-MRI technology to MS is, therefore, appealing to provide quantitative estimates of the degree of tissue damage and, as a consequence, improve the understanding of the mechanisms leading to irreversible disability. The use of DW-MRI to study MS has provided the following pieces of information:*

*a) T2-visible lesions have higher and lower FA values than NAWM [43] and T1-hypointense lesions have higher and lower FA values [43, 44] than T1-isointense lesions. When comparing enhancing versus non-enhancing lesions, conflicting results have been achieved. Whereas one study [45] found that enhancing lesions have higher values and lower FA values than inactive lesions, another [44], which assessed more MS patients, did not find any significant difference between the two lesion populations. These findings confirm the pathological heterogeneity of MS lesions and, since this heterogeneity is not fully described by cMRI metrics (including measurements of the extent of "black holes"), they also indicate the need to grade the degree of their intrinsic tissue damage using modern quantitative MR technology.*
*b) Values of NAWM regions from MS patients are diffusely higher and FA values are significantly lower than the corresponding and FA values of white matter from healthy controls [45, 46]. Previous MTR data indicating the presence of subtle and progressive NAWM changes in areas subsequently involved by new MS lesions [25] have been confirmed by the application of DW-MRI [47, 48].*
*c) Average lesion , but not average lesion FA, was found to be significantly correlated, albeit moderately, with clinical disability in a large cohort of MS patients [44]. 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. Interestingly, in patients with SPMS a moderate correlation was found between average lesion or FA and disability, whereas no significant correlation was found between disability and T2 lesion volume. On the contrary, a significant correlation between disability and T2 lesion volume was found in patients with RRMS, where, in turn, there was no correlation between average lesion or FA and disability. These findings suggest that mechanisms leading to disability are likely to be different in patients with RRMS and SPMS.*
*d) FA histograms from the whole of the brain tissue and the NABT are different in MS patients in comparison with healthy subjects [43, 44, 46]. However, it is still to be clarified whether and to which extent DW-MRI histograms differ among the various MS phenotypes [10, 44].*
*e) Brain GM from MS patients is higher than that of GM from healthy controls [28]. The severity of DW-MRI abnormalities of the GM is more pronounced in patients with SPMS and PPMS than in those with RRMS [49].*
*f) Quantities derived from DW-MRI are correlated with disability in patients with RRMS and SPMS [46]. In the less disabling phases of the disease, DW-MRI findings are correlated with several cognitive test performances, but not with neurological disability [50].*

*Although DW-MRI has the potential to provide paraclinical measures which can meet some of the requisites needed to be applied in the context of MS trials (e.g., specificity to severe tissue damage and clinical meaningfulness), the inevitable problems associated with standardization of DW-MRI sequences across multiple centers have prevented its use to monitor MS clinical trials. The lack of information about the reproducibility of DW-MRI quantities as well as the lack of longitudinal studies assessing the sensitivity of DW-MRI to disease changes are additional limiting factors. However, the situation is rapidly changing and it is likely that*

*metrics derived from DW-MRI will be implemented in subgroups of patients entering future trials.*

**Magnetic resonance spectroscopy**

*Water suppressed, proton MR spectra of normal human brain at long echo times reveal four major resonances: one at 3.2 ppm from tetramethylamines (mainly from choline-containing phospholipids [Cho]), one at 3.0 ppm from creatine and phosphocreatine (Cr), one at 2.0 ppm from N-acetyl groups (mainly N-acetyl-aspartate [NAA]), and one 1.3 ppm from the methyl resonance of lactate (Lac). NAA is a marker of axonal integrity, while Cho and Lac are considered as chemical correlates of acute inflammatory/demyelinating changes [11]. MRS studies with shorter echo times can detect additional metabolites, such as lipids and myoinositol (mI), which are also regarded as markers of ongoing myelin damage.*

*MRS studies are relatively time-consuming and require, for their acquisition, post-processing and interpretation, knowledgeable and experienced personnel. As a consequence, high-quality MRS technology and operators are still confined to relatively few centers. Although this is perhaps the major factor which is limiting a more extensive use of MRS to assess in-vivo the biochemical correlates of MS pathology, the following are the major insights provided by the application of MRS to the study of patients with MS:*

*a) Chronic MS lesions are characterized by markedly reduced NAA/Cr peaks [11]. NAA concentration is lower in severely hypointense MS lesions than in iso- or mildly hypo-intense lesions [51].*
*b) Acute MS lesions present highly variable metabolic patterns over time [11, 14]. From the early phases of new lesion formation, acute lesions show increased Cho and Lac resonance intensities. In large acute demyelinating lesions, decreases of Cr can also be seen. Short echo time spectra can detect transient increases in lipids. All these changes are usually followed by a decrease in NAA. After the acute phase and over a period of days to weeks, there is a progressive reduction of raised Lac resonance intensities to normal levels. Resonance intensities of Cr also return to normal within a few days. Cho, lipid and mI resonance intensities return to normal over months. The signal intensity of NAA may remain decreased or 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 [11]. Since in acute MS lesions Gd-enhancement is usually ceased by two months, the metabolic changes shown by MRS can reveal on-going pathology which would otherwise go undetected.*
*c) Decreases in NAA are not restricted to MS lesions, but also occur in the NAWM adjacent to or distant from them [11, 52] and can precede the appearance of MRI-visible abnormalities [53]. Reversible changes of NAA can be detected in the NAWM of the hemisphere contralateral to solitary acute lesions of the type seen in MS [54], suggesting that sublethal axonal injury is a contributing factor to acute, potentially reversible MS disability. No MRS abnormalities seem to involve NAWM of patients at presentation with CIS [11].*
*d) NAA levels are significantly correlated with patients' clinical disability [55], selective motor impairment [56] and cognitive dysfunction [57]. NAA/Cr values are reduced also in MS patients with no clinical disability from the earliest stages of the disease [58]. These data have emphasized the importance of the so-called "axonal hypothesis", based on the notion that axonal loss underlies the progressive accumulation of irreversible disability in MS and have indicated that axonal loss is not just an end-stage phenomenon typical of the most destructive lesions and the most unfortunate cases, but rather a major component of the MS pathology at all phases of the disease.*
*e) 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 [59].*

*To our knowledge, only two studies have been conducted to evaluate the effect of disease-modifying MS treatments on MRS-derived parameters [60, 61]. Using monthly MRS scans, Sarchielli et al. [60] found that treatment with interferon beta-1a has an impact on Cho peaks in spectra of lesions from RRMS patients, suggesting an increase in lesion membrane turnover during the first period of treatment. More recently, Narayanan et al. [61] found an increase of NAA/Cr in a small group of RRMS patients after one year of treatment with interferon beta-1b, suggesting a potential effect of treatment in preventing chronic, sublethal axonal injury.*

*MRS has also been used to assess brain pathology in patients with white matter diseases (WMD) other than MS [14]. Brain WMD are secondary to a variety of pathological processes and are associated with many different myelin abnormalities. This heterogeneity may render the diagnostic work-up of patients with WMD challenging. Although the vast majority of the cMRI and MRS changes detected in WMD are not disease-specific, the complementary information coming from the two techniques can increase the diagnostic confidence, as extensively discussed elsewhere [14].*

**Functional MRI**

*FMRI enables us to map regions of brain activation during motor, sensitive and cognitive tasks, and can define changes of brain activation associated with various diseases. FMRI is based on the blood oxygenation level-dependent effect to detect areas of the brain that have greater local blood flow, reflecting increased neuronal activity during task performance compared with rest. Using a simple motor task, recent studies have shown that functional cortical reorganization does occur in patients with RRMS and SPMS and mainly involves the "classical" motor areas [62, 63]. In patients with PPMS [64], these cortical changes involve a more widespread network, usually active in multisensory integration processes. The correlation between various measures of structural MS damage and the extent of the cortical activation consistently found by these studies [62-64] suggests a role for adaptive cortical changes in mantaining a normal level of functioning in patients with MS. The lack or exhaustion of the ability of the brain to remodel as the subcortical tissue damage increases might be an additional factor responsible for the progressive accumulation of disability in MS.*

**Conclusions**

*Although cMRI has improved our understanding of MS evolution and treatment, it provides limited information about MS pathology in terms of both accuracy and specificity. This suggests that cMRI should not be used to establish long-term prognosis of individual MS patients and that the ability of a given treatment to modify metrics derived from cMRI does not mean necessarily that the treatment will be able to modify favorably the clinical course of the disease. As a consequence, there is an urgent need to define new MRI markers of MS evolution, which should be quantitative and should provide information about the most destructive aspects of MS pathology, derived from (at least) the entire brain. This is particularly compelling now that partially effective treatment for MS is available and the ability to conduct large-scale, placebo-controlled trials is, therefore, limited.*

*None of the MRI 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 MRI quantities, thought to reflect different aspects of MS pathology, to improve our ability to monitor the disease [65]. Moreover, metrics derived from MT-MRI, DW-MRI and MRS should be increasingly used to monitor MS evolution, either natural or modified by treatment. At present, longitudinal natural history data collected in large samples of MS patients using these MR techniques are needed to gain additional insights on disease pathophysiology and on the actual value of modern MR technologies in the assessment of MS.*

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