*Imaging biomarkers*

**Imaging biomarkers in multiple sclerosis**

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From the beginning of its application, it appeared evident that MRI might have been a valuable tool for the assessment of patients with multiple sclerosis (MS) in the diagnostic work up and to elucidate the mechanisms underlying disease progression. Conventional MRI (cMRI) (i.e., dual-echo, pre- and post-contrast T1-weighted images) looked very promising for the following reasons: 1) high sensitivity toward MS related central nervous system (CNS) damage, in comparison with other neuroimaging and neurophysiological techniques; 2) ability to provide objective metrics for measuring disease evolution, either natural or modified by treatment; 3) high value in contributing to make a diagnosis of MS and to exclude alternative conditions in patients suspected of having MS (1). A large effort has been devoted to defining the imaging characteristics of "typical" MS lesions, which could ensure a correct diagnosis. From this effort, it was learned that brain MS lesions are frequently located in the periventricular regions, corpus callosum and infratentorial areas, and are characterized by oval or elliptical shapes. Some lesions enhance after the administration of gadolinium (Gd), suggesting that it was feasible to detect *in vivo* different aspects of MS lesion pathology. Enhancement was shown to reflect increased blood-brain barrier permeability associated with inflammatory changes; the observation that the occurrence of enhancing lesions was five to 10 times higher than that of clinical relapses prompted the use of such an approach as a sensitive and reproducible way to assess MS activity. A subset of T2-hyperintense MS lesions appear as hypointense ("black-holes") on T1-weighted scans. If permanent, these T1-hypointense lesions are thought to represent areas of axonal loss and severe matrix destruction. Spinal cord MS lesions are more frequently observed in the cervical than in the other regions; are usually peripheral; limited to two vertebral segments in length or less; occupy less than half the cross-sectional area of the cord; and, are not seen as T1-hypointensities (2). In patients who present with clinically isolated syndromes (CIS) suggestive of MS, the burden of T2 lesions is a robust predictor of subsequent evolution to clinically definite MS (1). Nevertheless, even in these patients, the increase of lesion load in the few years following the onset of the clinical symptoms is only moderately correlated with the long-term accumulation of disability. Several studies have investigated the prognostic role of enhancing MRI on corresponding clinical parameters. The number of enhancing lesions increases shortly before and during clinical relapses and predicts subsequent MRI activity. A moderate correlation has been demonstrated between the degree of clinical disability and the mean frequency of enhancing lesions in patients with relapsing remitting (RR) and secondary progressive (SP) MS. Despite its overall high sensitivity for MS abnormalities, T2-weighted MRI sequences are unable to reveal the actual burden of grey matter (GM) lesions (3). More recently, the application of double-inversion recovery sequences has convincingly demonstrated that cortical lesions (CLs) are a frequent finding in patients with MS, even at the earliest clinical stages. CLs were found to correlate with physical disability, reduced brain parenchymal fraction, and T2 lesion volume.

C-MRI has also been used to measure atrophy of the brain (whole or segmented in white matter [WM] and GM) and, hence, to grade the extent of neurodegeneration related to MS. Atrophy may occur very early in MS, even at the CIS stage (4). Brain atrophy is also present in the early stages of primary progressive (PP) MS, affecting both WM and GM. Moreover, cross-sectional and longitudinal studies have shown a moderate correlation between brain atrophy and the clinical manifestations of MS. Numerous pieces of evidence indicate that GM volume decrease correlates to MS-related impairment. Neuropsychological dysfunction is more closely associated with measures

of brain atrophy than the lesion burden does. In MS, brain atrophy develops in different structures in the different clinical disease phenotypes: ventricular enlargement is predominant in RRMS, whereas cortical atrophy seems to be more important in the progressive forms of the disease. Sophisticated atrophy measurement techniques have also been used to improve the correlations between measures of GM loss and clinical findings. Recently, using voxel-based morphometry, GM loss in the thalamus has been demonstrated in all disease phenotypes (including pediatric MS). Thanks to recent advances in image post-processing techniques, the measurement of atrophy of selected pathways, such as the corpus callosum and the corticospinal tract (CST), may also represent a novel strategy to improve the *in vivo* monitoring of MS damage in clinically eloquent WM regions.

Although cMRI has a great sensitivity in detecting the presence and extent of macroscopic lesions in MS, it lacks specificity towards the heterogeneous pathological substrates of these lesions, which range from edema to demyelination, remyelination, gliosis and axonal loss, as demonstrated by pathological studies (1), as well as it is unable to detect changes in the so-called normal appearing (NA) tissues. In chronic lesions which appear hyperintense on T2-weighted scans, quantitative MRI studies have shown variable degrees of magnetization transfer ratio (MTR) (5), fractional anisotropy (FA) reduction, and mean diffusivity (MD) increase (6). All these values vary dramatically across individual lesions, but are typically more pronounced in lesions that are hypointense on T1-weighted images and in patients with the most disabling courses of the disease.

A reduction of MTR values is already detectable in the normal-appearing WM (NAWM) prior to T2-visible lesion appearance, in NAWM areas adjacent to focal T2-weighted lesions, particularly in progressive MS patients, and in patients with MS and no T2-visible WM abnormalities (5). NA brain tissue (NABT) MTR histogram-derived measures are different and evolve at a different pace in the major MS clinical phenotypes (5). A significant prognostic value of NAWM MTR abnormalities for the medium-term evolution of disability in patients with established MS has also been independently reported by longitudinal studies (5). The use of MTR histogram analysis has also allowed to obtain a more global picture of cord pathology in patients with MS. Histograms analysis has demonstrated that cord MTR histogram metrics in patients with CIS, RRMS, and early-onset MS are similar to those of healthy individuals. On the contrary, cord MTR metrics are similarly and markedly reduced in patients with SPMS and PPMS. Only a moderate correlation has been found between average brain MTR and cervical cord MTR, suggesting that cord damage in MS is not a mere reflection of brain pathology.

Similarly to what found using MT MRI, numerous DT MRI studies have consistently shown the presence of diffusion abnormalities in the NAWM of patients with MS, which can precede the development of T2-visible lesions by several weeks (6). DT MRI studies showed that NAWM abnormalities in MS are widespread, but tend to be more severe in sites where MRI-visible lesions are usually located and in periplaque regions. A significant increase of MD and decrease of FA values were reported in CIS patients with paraclinical evidence of disease dissemination in space, although the changes did not predict the short-term occurrence of cMRI disease activity. When the DT MRI characteristics of clinically eloquent NAWM regions were studied, a significant relationship with patients disability was found for the corpus callosum and internal capsule FA values and for the cerebral peduncle MD and FA values. In addition, moderate correlations were found between several NAWM histogram-derived quantities and neuropsychological test scores. The development of a novel DT MRI sequence has made possible to achieve an accurate estimate of the extent of the overall cervical cord damage (6). A reduced average cord FA was found in patients with RR and SPMS. The reduction of cord FA was correlated moderately with the degree of disability in these patients. Altered MD and FA have also been found in the cervical cord of patients with PPMS and neuromyelitis optica. DT MRI tractography also allows the *in vivo* segmentation of the major WM tracts fiber bundles in the brain WM (6). In MS patients, diffusivity and anisotropy along the CST correlate with clinical measures outcomes of locomotor disability, more than T2 lesion burden and the overall brain extent of diffusivity changes (6). Moreover, a DT MRI

tractography study of patients with CIS and motor impairment showed that these patients had increased MD and T2 lesion volume in the CST compared to patients without pyramidal symptoms (6). A DT MRI tractography study in patients with optic neuritis showed reduced connectivity values in both left and right optic radiations compared with controls, suggesting the occurrence of mechanisms of trans-synaptic degeneration secondary to optic nerve damage (6). DT MRI tractography provided also a method to identify NAWM fibers at risk for degeneration because they intersect focal T2-visible lesions (6).

It is likely that GM damage in MS might also be secondary to "diffuse" tissue changes, for instance through retrograde and trans-synaptic degeneration. Several studies have demonstrated reduced MTR values in the brain GM from patients with different MS phenotypes, including those at the earliest clinical stage of MS (3). GM abnormalities increase with disease duration, since they were found to be more pronounced in patients with PPMS or SPMS. In a large, multi-center study of PPMS patients greater MT MRI-detectable GM damage was found in patients who required walking aids than in those who did not. GM MTR changes have been found to correlate with clinical disability and cognitive impairment, whereas no correlation with fatigue emerged (3). GM MTR was also found to be an independent predictor of subsequent accumulation of disability in patients with MS followed up for eight years (3). In line with MT MRI findings, DT MRI confirmed the presence of GM damage in MS and showed that the extent of such damage differs among the various disease phenotypes, being more severe in patients with SPMS. More intriguingly, DT MRI has been shown to be sensitive to the evolution of MS damage over short-term periods of time. Longitudinal studies have indeed demonstrated a worsening of GM damage over time in patients with RRMS, SPMS and PPMS. GM diffusivity was also found to predict accumulation of disability over a five-year period in patients with PPMS.

The extensive application of modern MR-based techniques to the assessment of brain and cord pathology in patients with MS has considerably improved our understanding of MS pathophysiology and has provided new objective metrics that might be useful to monitor disease evolution, either in natural history studies or in treatment trials. From the large body of available literature, it results clear, however, that none of these quantitative techniques, taken in isolation is able to provide a complete picture of the complexity of the MS process. There are several pieces of evidence indicating that a multiparametric approach, combining aggregates of different MR quantities, might improve our ability to monitor the disease.

**References**

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