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MRI evidence for multiple sclerosis as a diffuse disease of the central nervous system

Abstract The classical view of MS as a chronic inflammatory demyelinating disease leading to the formation of focal central nervous system (CNS) white matter (WM) lesions has been recently challenged by pathological studies and by the extensive application of modern MRI-based techniques. There is now overwhelming evidence supporting the following statements:

- MS causes widespread tissue damage in the normal-appearing white matter (NAWM) of the brain and spinal cord, whose extent and severity is more strictly associated to the clinical manifestations of the disease than the extent of focal pathology. Discrete, macroscopic lesions are just the tip of the iceberg of MS pathology.
- Grey matter (GM) damage is a consistent feature of all MS phenotypes, which is progressive from the start of the relapsing-remitting phase of the disease. As is the case for WM, GM damage is also a mixture of focal lesions and diffuse pathology. High-field strength MR scanners are improving our ability to image focal GM lesions and modern MR-based techniques are enabling us to quantify *in vivo* the extent and severity of GM pathology, which have been shown to correlate only moderately with the amount of WM changes. At least part of GM pathology in MS is not secondary to retrograde degeneration of fibers traversing WM lesions.
- The neurodegenerative component of the disease is not a late phenomenon and it is not completely driven by inflammatory demyelination. In fact, neurodegeneration occurs very early in the course of MS and the correlation between MRI measures of inflammation and neurodegeneration is weak in all disease phases. The interplay of inflammation and neurodegeneration is a complex and still poorly understood phenomenon. At least part of MS-related neurodegeneration is not directly driven by Wallerian degeneration.
- Functional cortical changes can be seen in virtually all MS patients and are likely to play a central role in the ability of the MS brain to respond to tissue injury and, hence, limit the functional consequences of structural damage. MS disability is not just the result of tissue destruction but rather a balance between tissue destruction, tissue repair and adaptive cortical reorganization.

All of this calls for the concept of MS as a focal, inflammatory demyelinating, WM disease to be reexamined and to start viewing MS as a diffuse CNS disease with an important neurodegenerative component. This is central for identifying novel and effective treatment strategies.

Key words multiple sclerosis · normal-appearing white matter · grey matter · neurodegeneration · cortical adaptation

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Introduction

During the last few decades, our ability to diagnose multiple sclerosis (MS) and to monitor its evolution has been notably improved by the application of conventional magnetic resonance imaging (cMRI – dual echo and post-contrast T1-weighted scans). T2-weighted images are highly sensitive for the detection of MS lesions and post-contrast T1-weighted images allow the identification of lesions with an increased blood-brain barrier permeability associated with inflammatory activity, thus allowing the demonstration of dissemination of MS lesions in space and time earlier than with clinical assessment and to detect disease activity with an increased sensitivity with respect to clinical evaluation of relapses [76]. However, the magnitude of the relationship between cMRI measures of disease activity or burden and the clinical manifestations of the disease is weak [79, 107]. This clinical/MRI discrepancy is likely to be the result of the inability of cMRI to quantify the extent and to define the nature of MS-related tissue damage, which is not restricted to T2-visible lesions, but involves diffusely the normal-appearing white and grey matter. In addition, it is now also evident that the neurodegenerative aspects of MS are early events in the course of the disease and that the correlation between MRI measures of MS inflammation and neurodegeneration is weak in all disease phases [60].

Modern quantitative MR techniques have the potential to overcome some of the limitations of cMRI. Metrics derived from magnetization transfer (MT) [35] and diffusion-weighted (DW) [29] MRI enable us to quantify the extent and severity of structural changes occurring within and outside cMRI-visible lesions in patients with MS. Proton MR spectroscopy (^{1}H-MRS) [31] can add information on the biochemical nature of such changes. Functional MRI (fMRI) [30] can provide new insights into the role of cortical adaptive changes in limiting the clinical consequences of MS structural damage.

These pieces of information are changing our picture of the pathology of MS to one of an underlying structural and functional perturbation of the entire central nervous system (CNS). This article reviews briefly the main MRI data that underlie this paradigm shift in our understanding of MS.

MRI evidence for diffuse pathology in the normal-appearing white matter (NAWM)

Post-mortem studies have shown subtle and widespread abnormalities in the NAWM from MS patients, which include diffuse astrocytic hyperplasia, patchy edema, and perivascular cellular infiltration, as well as axonal damage [2, 4, 27]. The application of modern MR-based techniques to the assessment of MS patients has allowed the *in vivo* quantification of the extent of normal-appearing brain tissue (NABT) involvement in this disease, which can be obtained using either a region-of-interest (ROI) analysis or a histogram-based approach. In addition, the recent development of fully automated techniques to segment the various components of the brain has enabled us to obtain histograms of the NAWM in isolation, by excluding from the analysis those pixels belonging to T2-visible lesions and grey matter (GM).

Using ROI analysis, altered MT and DW MRI metrics as well as metabolic abnormalities have been shown in the NABT and NAWM of MS patients with all the major MS phenotypes [9, 10, 12, 14, 23, 26, 33, 34, 36, 46, 56, 63, 67, 72, 93, 114, 119, 127, 129]. MT ratio (MTR) changes, of a lower magnitude than those observed in T2-visible lesions, have been detected in the dirty-appearing white matter of MS patients [49]. The application of histogram analysis [9, 10, 37, 59, 65, 81, 105, 121, 122] to the study of the NABT and of the NAWM confirmed and extended the previous findings obtained with ROI analysis. Histogram analysis has shown that these abnormalities can be detected even in patients with clinically isolated syndromes (CIS) suggestive of MS [48, 59, 122]. In patients with early onset MS [77], these changes are more pronounced in secondary progressive (SP) MS and primary progressive (PP) MS patients than in patients with the other disease phenotypes [121], although they are similar between patients with SPMS and those with PPMS [105]. Consistent with this is the demonstration that *N*-acetylaspartate (NAA) reduction is more pronounced in the NAWM of SPMS and PPMS patients than in those with relapsing-remitting (RR) MS [46, 119]. The development of an unlocalized ^{1}H-MRS sequence for measuring NAA levels in the whole brain (WBNAA) [53] has allowed previous findings to be extended by showing the presence of marked axonal pathology in patients with clinically definite MS [5, 52, 61], including those with PPMS [110], and in those at the earliest clinical stage of MS [32].

NABT changes tend to worsen over time in all MS phenotypes [38, 46, 59, 103, 113, 116], but these changes seem to be more pronounced in SPMS patients [38]. In patients with CIS suggestive of MS, the extent of NABT changes has been found to be an independent predictor of subsequent evolution to clinically definite MS [59]. In patients with established MS, NAWM-MTR reduction has been shown to predict the accumulation of clinical disability over the subsequent five years [103, 113]. In RRMS patients, the longitudinal decrease over time of NAA/creatine (Cr) in the NAWM correlates strongly with worsening of Expanded Disability Status Scale (EDSS) scores [21, 46], suggesting that progressive axonal damage or loss may be responsible for functional impairment in MS. More recently, it has been demonstrated that brain axonal damage begins in the early stage of MS, develops rapidly in this phase of the disease

and correlates more strongly with disability in patients with mild than in those with more severe disability [22].

NABT MTR, mean diffusivity (MD) and NAA values are only partially correlated with the extent of macroscopic lesions and the severity of intrinsic lesion damage [5, 7, 9–11, 13, 32, 34, 36, 58, 121], thus suggesting that NABT pathology does not only reflect Wallerian degeneration of axons traversing large focal abnormalities, but may also represent small focal abnormalities beyond the resolution of conventional scanning.

The quantification of the extent of NABT and NAWM involvement has strengthened the relationship between MRI metrics and the clinical manifestations of the disease. Several studies have shown moderate to strong correlations between various brain MTR and MD histogram-derived metrics and the severity of physical disability [8, 11, 25, 34, 57, 65, 81, 104, 123] and the presence of neuropsychological impairment [45, 108, 109, 126] in MS patients. The reduction of the NAA/Cr ratio in the NAWM from MS patients has been related to the presence of fatigue [120].

MT MRI, DW MRI and ^{1}H-MRS metrics of specific CNS structures, such as the cerebellum [14, 20, 57], the brainstem [57], the locus coeruleus [47], the pyramidal tracts [70, 83, 130], the spinal cord [125] and the optic nerve [62] of MS patients are significantly associated with impairment of these functional systems.

MRI evidence for pathology in the GM

Several *post-mortem* studies [15, 68, 73, 88] have shown the presence of MS-related damage, including axonal and neuronal loss, in the cortical and deep GM of MS patients. Such abnormalities usually go undetected when using cMRI because of their relatively small size, their relaxation characteristics which result in poor contrast with the surrounding normal GM and because of partial volume effects with the surrounding cerebro spinal fluid (CSF). High-field strength MR scanners are improving our ability to image focal GM lesions, and the application of modern MR-based techniques for the assessment of GM pathology has undoubtedly allowed to be overcome some of the limitations of cMRI.

Using ROI [9] and histogram analysis [6, 9, 24, 50, 51, 104], MT and DW MRI abnormalities have been shown in the GM of MS patients, including those with PPMS [24, 104], whereas no MD abnormalities have been detected in the GM of patients with early RRMS [54]. GM changes are more pronounced in patients with SPMS than in those with RRMS [6, 51], while no difference in the extent and severity of GM involvement were found between patients with SPMS and those with PPMS [104]. In patients with SPMS and PPMS [83], as well as in those with RRMS [82], these changes worsen over time. This suggests a progressive accumulation of GM damage already in the RR phase of the disease, which was previously unrecognized and which might be one of the factors responsible for the development of brain atrophy [78].

Metabolite abnormalities, including a decrease of NAA, choline and glutamate, have also been shown in the cortical GM of MS patients [12, 67, 115, 117], during the early phases of the disease [12], but not in CIS patients [66]. These changes are more pronounced in patients with SPMS than in those with RRMS [1, 115]. ^{1}H-MRS and DW MRI abnormalities have also been demonstrated in the thalamus of SPMS [15, 28] and RRMS patients [28, 131]. As shown for cortical changes, deep GM abnormalities are more pronounced in SPMS than in RRMS patients [28]. More recently, using a voxel-based analysis, MTR abnormalities have also been shown in the cortical and deep GM of patients with CIS [3].

Significant correlations have been reported between MT and DW MRI changes and T2-lesion volume [6, 9, 51, 104]. This fits with the notion that at least part of the GM pathology in MS is secondary to retrograde degeneration of fibers traversing WM lesions.

A precise and accurate quantification of GM damage might help to explain some of the clinical manifestations of MS, such as cognitive impairment, and might strengthen the correlation between clinical and MRI findings. Recent studies have indeed found a correlation between the severity of cognitive impairment and the degree of MTR [109] and MD [112] changes in the GM of MS patients. In addition, GM MTR metrics have been shown to correlate with the severity of clinical disability in patients with RR [50] and PP [24] MS. Disappointingly, no correlation has been demonstrated between the extent of GM pathology, measured using MT and DW MRI, and fatigue [16]. On the contrary, a marked reduction of NAA was found in highly fatigued in comparison with less fatigued patients [120].

MRI evidence for early neurodegeneration and a "mismatch" between MR-measured inflammation and neurodegeneration

Although histopathological studies have shown a significantly increased number of transected axons in MS lesions with large inflammatory infiltrates [124], the magnitude of the correlation between brain tissue loss and enhancement was found to be either absent or poor in virtually all *in vivo* MRI studies [17, 19, 71, 80, 92]. This fits with the results of clinical trials of various therapeutic agents which failed to show a treatment effect on progression of brain atrophy, despite the presence of a dramatic effect on enhancement [19, 43, 64, 71, 75, 86, 92, 106]. Even the complete suppression of enhancement, as measured with monthly scans after the injection of a

triple dose of gadolinium, did not halt the progressive accumulation of brain atrophy in MS patients treated with autologous hematopoietic stem cell transplantation and followed up for two years [64]. Similar results were obtained in MS patients treated with cladribine [43, 92].

Two possible explanations are readily apparent for such a discrepancy. First, the limited ability of measures of brain atrophy to quantify accurately the destructive components of MS pathology. Second, one might argue that, whereas in SPMS patients, the relationship between MRI-detected inflammation and the subsequent development of irreversible tissue damage is weak, this might not be the case earlier in the course of the disease. However, the "inflammation/neurodegeneration mismatch" has also been shown to be present at the earliest clinical stage of MS, when inflammatory demyelination is thought to be the predominant pathological hallmark. In CIS patients, interferon β–1a was shown to be effective in reducing MRI-measured disease activity [18] and brain atrophy [44], but the correlation between brain volume changes and MRI markers of disease activity was again poor [44]. Additional evidence of an early "inflammation/neurodegeneration mismatch" comes from another study based on the assessment of whole brain NAA, where the detected widespread axonal pathology was shown to be largely independent of MRI-visible inflammation [32].

Evidence from fMRI for functional recovery

In MS, several mechanisms have the potential to cause tissue injury and, as a consequence, several mechanisms of recovery can also be advocated. Although our ability to monitor recovery using MR is still limited, it is certain that such a goal would represent a major achievement in our understanding of the disease and the assessment of treatment efficacy. In case of severe and irreversible neuroaxonal damage, cortical reorganization might represent a major contributor in promoting functional recovery.

Functional cortical changes have been demonstrated in all MS phenotypes, using different fMRI paradigms. A study of the visual system [128] in patients who had recovered from a single episode of acute optic neuritis (ON) demonstrated that such patients had an extensive activation of the visual network compared to healthy volunteers. An altered brain pattern of movement-associated cortical activations, characterized by an increased recruitment of the contralateral primary sensorimotor cortex (SMC) during the performance of simple tasks [42, 98] and by the recruitment of additional "classical" and "higher-order" sensorimotor areas during the performance of more complex tasks [42] has been demonstrated in patients at presentation with CIS. More recently, in these patients, it has been shown that such functional changes might contribute to predicting the evolution to definite MS [100]. An increased recruitment of several sensorimotor areas, mainly located in the cerebral hemisphere ipsilateral to the limb which performed the task, has also been shown in patients with early MS and a previous episode of hemiparesis [84]. In patients with similar characteristics, but who presented with an ON, this increased recruitment involved sensorimotor areas which were mainly located in the contralateral cerebral hemisphere [85]. In patients with established MS and a RR course, functional cortical changes have been shown during the performance of visual [102], motor [40, 69, 89, 90, 94], and cognitive [55, 74, 87, 118] tasks. Movement-associated cortical changes, characterized by the activation of highly specialized cortical areas, have also been described in patients with SPMS [95] during the performance of a simple motor task. Finally, two fMRI studies of the motor system [40, 97] of patients with PPMS suggested a lack of "classical" adaptive mechanisms as a potential additional factor contributing to the accumulation of disability. The results of all these studies suggest that there might be a "natural history" of the functional reorganization of the cerebral cortex in MS patients, which may be characterized, at the beginning of the disease, by an increased recruitment of those areas "normally" devoted to the performance of a given task, such as the primary SMC and the supplementary motor area (SMA) in the case of a motor task. At a later stage, bilateral activation of these regions is first seen, followed by a widespread recruitment of additional areas, which are usually recruited in healthy people to perform novel/complex tasks. This notion has been supported by the results of a recent study [41], which has provided a direct demonstration that MS patients, during the performance of a simple motor task, activate cortical regions that are part of a fronto-parietal circuit, whose activation typically occurs in healthy subjects during object manipulation.

Functional and structural changes of the MS brain are strictly correlated. Several moderate to strong correlations have been demonstrated between the activity of cortical and subcortical areas and the extent of brain T2-visible lesions [69, 84, 94, 96, 97], the severity of intrinsic lesion damage [85, 94], the severity of NABT damage, measured using ^{1}H-MRS [89, 98], MT MRI or DW MRI [40, 94, 96], the involvement of specific WM tracts, such as the pyramidal tract [95], the extent of GM damage [97, 101] and, finally, the severity of cervical cord damage [40, 99].

Although the actual role of cortical reorganization on the clinical manifestations of MS remains unclear, there are several pieces of evidence, in addition to the strong correlation found between functional and structural abnormalities, suggesting that cortical adaptive changes are likely to contribute in limiting the clinical conse-

quences of MS-related structural damage. In a patient with an acute hemiparesis following a new, large demyelinating lesion located in the corticospinal tract, dynamic changes of the brain pattern of activations of the "classical" motor areas, ending in a full recovery of function, have been observed [90]. The correlation found between the extent of functional cortical changes and NAA levels suggests that dynamic reorganization of the motor cortex can occur in response to axonal injury associated with MS activity. In patients complaining of fatigue, when compared to matched non-fatigued MS patients [39], a reduced activation of a complex movement-associated cortical/subcortical network has been found. A strong correlation between the reduction of thalamic activity and the clinical severity of fatigue was also found, suggesting that a less marked cortical recruitment might be associated to the appearance of clinical symptomathology in MS. Finally, preliminary work has shown that the pattern of movement-associated cortical activations in MS is determined by both the extent of brain injury and disability and that these changes are distinct [91].

Conclusions

An important paradigm shift has taken place in our understanding of the disease process in MS. An important contribution to this shift has been made by advances in MRI technology, which allow structural damage to be quantified in the brains of living patients with MS and to be followed in time. Our current understanding of the pathophysiology of MS is that this is not only a disease of the WM, characterized by focal inflammatory lesions, but involves more subtle and diffuse damage throughout the WM and GM. The inflammatory and neurodegenerative components of the disease process are present from the earliest observable phases of the disease, but appear to be, at least partially, dissociated. In addition, recovery and repair play an important role in the clinical manifestation of the disease, involving both structural changes and plastic adaptive reorganization of the cortex. This new picture of MS has important implications for treatment, suggesting that agents that protect against neurodegeneration or promote tissue repair may have an important role to play alongside agents acting only on the inflammatory component of the disease.

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