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The role of magnetic resonance imaging in the study of multiple sclerosis: diagnosis, prognosis and understanding disease pathophysiology

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Abstract

Magnetic resonance imaging (MRI) has become an established tool to diagnose multiple sclerosis (MS) and to monitor its evolution. In patients at presentation with clinically isolated syndromes suggestive of MS, MRI criteria for MS diagnosis have been proposed and are updated on a regular basis. In addition, MRI "red flags" useful for the differential diagnosis from other neurological conditions which can mimic MS have been identified.

In patients with established MS, the ability of MR measures in explaining patients' clinical status and progression of disability is still suboptimal. This has prompted the extensive application of modern MR-based technologies to estimate the overall disease burden in patients at different stages of the disease. The use of these techniques has allowed to grade in vivo the heterogeneity of MS pathology not only in focal lesions, but also in the normal-appearing white matter and grey matter. Combined with the use of functional MRI, this is ameliorating progressively our understanding of the factors associated to MS evolution.

This review summarizes how MRI has improved our ability to diagnose MS and to predict its course, as well as how it is changing our understanding of the factors associated with the accumulation of irreversible disability in this condition.

Introduction

Magnetic resonance imaging (MRI) has become an established tool to diagnose multiple sclerosis (MS) and to monitor its evolution. MRI has been formally included in the diagnostic work up of patients at presentation with clinically isolated syndromes (CIS) suggestive of MS, and *ad hoc* criteria have been proposed and are updated on a regular basis. On the contrary, in patients with established MS, the ability of MR measures in explaining patients' clinical status and progression of disability is still suboptimal. This has prompted the extensive application of modern MR-based techniques to estimate the overall MS burden in patients at different stages of the disease. This review discusses the main achievements derived from the application of MR-based techniques to diagnose MS, to establish a prognosis early in the course of the disease, and to improve our understanding of the mechanisms leading to irreversible clinical disability.

Diagnosis, differential diagnosis and early prognosis

MRI has a high sensitivity in revealing macroscopic tissue abnormalities in patients with MS. This, combined with its availability, repeatability, and capability of providing objective measures of overall disease activity and burden, has led to the extensive use of MRI for diagnosing MS and monitoring its evolution. In patients who are suspected of having MS, MRI has been formally included in their diagnostic work up through the definition of *ad hoc* sets of criteria to show disease dissemination in space (DIS) and time (DIT). Work has also been performed to identify "red flags" which should alert the clinicians and prompt them to perform non-routine tests to exclude possible alternative conditions.

a) *Typical MRI findings in MS*. A critical feature in the diagnostic evaluation of patients suspected of having MS is the definition of lesion characteristics that are suggestive of the disease. Brain MS lesions are frequently located, asymmetrically, in the periventricular and juxtacortical white matter (WM), the corpus callosum (CC) and infratentorial areas (with the pons and cerebellum more frequently affected than the medulla and midbrain), and are sometimes characterized by oval or elliptical shapes (1). Consensus has also been reached on criteria use-

ful to identify T2-hyperintense (2) and T1-enhancing lesions (3).

Considering the frequent involvement of the spinal cord by MS, MRI features of MS cord lesions have also been identified (4). MS lesions in the cord are more frequently observed in the cervical than in other sections, 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. Acute plaques can cause a swelling of the cord and enhance after gadolinium (Gd) administration.

The optic nerve is also frequently involved by the disease. The sensitivity of MRI for detecting optic nerve lesions in patients with optic neurites (ON) is high (5). The use of new sequences (6-8) has led to an increased sensitivity for detecting lesions in patients with an ON. In MS patients, increased T2 signal can be seen for a long time after an episode of ON, despite the occurrence of an improvement of vision and normalization of visual evoked potentials, and even in the absence of acute attacks of ON (9). T1-hypointense lesions are usually not seen in the optic nerve, whereas Gd-enhancement is a consistent feature of acute ON (10).

b) *MRI diagnostic criteria for MS*. In 2001, MRI has been formally included in the diagnostic work up of patients suspected of having MS by an International Panel (IP) of MS experts (11). The definition of MRI criteria for a diagnosis of MS was based, on the one hand, on the demonstration of lesion DIS and DIT, and, on the other, on the exclusion of alternative neurological conditions (12, 13). In 2005, the original IP criteria for MS diagnosis have been revised (14) in an attempt to simplify the approach, while maintaining adequate sensitivity and specificity. The main changes introduced by such a revision pertain to the demonstration of DIT, that can be obtained by the detection of a new T2 lesion, if it appears at any time compared with a reference scan done at least 30 days after the onset of the first clinical event, and the clarification of the use of spinal cord MRI to demonstrate DIS (14). In addition, importance to clinical and imaging (brain or spinal cord) findings has been given for a diagnosis of primary progressive (PP) MS, with less emphasis on cerebrospinal fluid (CSF) assessment. Since 2005, several other proposals have been made to simplify further the revised-IP criteria and to make them easier to be implemented in clinical and research settings. According to the Swanton criteria (15), at least one subclinical T2 lesion in at least two of the four locations defined as characteristic for MS in the revised-IP criteria (i.e., juxtacortical, periventricular, infratentorial, and spinal cord) is required for DIS. When the sensitivity and specificity of these criteria were compared with those of the original 2001 IP criteria (11), and with the 2005 revised criteria (14), the Swanton criteria were slightly more sensitive than the older ones, while maintaining a similarly high specificity (16). The main advantage of the Swanton criteria is that they do not require contrast agent administration, thus saving time and costs. However, this goes at the price of a loss of differential diagnostic information. Subsequently, Rovira *et al*. (17) suggested that a single brain MRI study performed early (i.e., < 3 months) after the onset of a CIS is highly specific for predicting the development of definite MS in the presence of both Gd-enhancing and nonenhancing lesions, which when present are considered a marker of DIT. Both the previous criteria have been included in the recently published criteria proposed by the European Multicenter Collaborative Research Network for MRI in MS (MAGNIMS) (18), as well as in the most recent revision of the 2001 IP criteria (19). One aspect that has not been considered in the available sets of MRI diagnostic criteria is the presence of lesion in the brain grey matter (GM). The introduction of double-inversion recovery (DIR) sequences (20) has contributed to imaging GM lesions, which have been detected in all the main clinical phenotypes of the disease, including patients at presentation with CIS (21). The sensitivity and utility of GM lesions detection in the context of MS diagnosis have been recently considered by Filippi and coworkers (22), who proposed a model for DIS which includes the presence of at least one intracortical lesion (ICL), in addition to the presence of at least one infratentorial and one spinal cord or Gd-enhancing lesion. This new DIS MRI criterion showed to have a higher specificity (93%) and accuracy (86%) than the available sets of criteria while maintaining a relatively high sensitivity (77%).

c) *MRI and differential diagnosis*. Another key requirement in the diagnostic work-up of patients suspected of having MS is the exclusion of alternative neurological conditions that can mimic MS (12, 13). A series of MRI "red flags", derived from evidence-based findings and educated guesses, have been identified in the setting of clinically suspected MS, which should alert the clinicians to prompt the performance of "non-routine" tests and to reconsider differential diagnosis more extensively (12, 13). For example, the presence of symmetric, diffuse T2-weighted hyperintensities in the deep and periventricular WM should be evaluated in the differential diagnosis of MS, because this is a typical MRI finding in leucoencephalopathies (i.e., adrenoleukodystrophy, metachromatic leukodystrophy, Krabbe disease). Neuromyelitis optica (NMO) should be

distinguished from MS for its different course, prognosis and response to immunomodulatory therapy. MRI and NMO-IgG, a serum biomarker, have been formally included in the diagnostic criteria of NMO (23). Most patients with NMO have a normal brain MRI or only a few and non-specific T2-hyperintensities that may have a predilection for regions with a high expression of aquaporin-4, including the hypothalamus, medulla, and other brainstem areas. Myelitis in this condition, unlike that which occurs in MS, is usually accompanied in the acute phase by a T2-weighted spinal cord lesion extending over three or more spinal segments, which may be hypointense on T1-weighted MRI and associated to varying degrees of Gd-enhancement (12, 13).

d) *MRI and early prognosis*. Several authors have investigated the prognostic role of MR-derived metrics in patients presenting with CIS. The MRI findings at disease onset that showed the strongest predictive value for the subsequent development of clinically definite MS were the number and extent of T2-visible brain lesions (24-27), the presence of infratentorial lesions (27), and the presence of Gd-enhancing lesions (28). For patients with CIS and brain MRI lesions, the chance of developing definite MS was > 80% over the next 14-20 years, in the longest follow up study to date (25, 26). Several studies also showed that the baseline MRI extent of lesion burden is a strong predictor of disability accumulation over time in these patients (26, 29). In a large cohort of patients at presentation with optic neuritis (30), spinal cord, infratentorial, and Gd-enhancing lesions seen on scans obtained within three months of clinical onset and new T2 lesions that appeared after three months predicted disability six years later.

Understanding MS pathophysiology

In patients with definite MS, the strength of the association of conventional MRI findings with the subsequent clinical manifestations of the disease remains modest, at best. This is likely due to the relative lack of specificity of conventional MRI in the evaluation of the heterogeneous pathological substrates of the disease, its inability to provide accurate estimates of damage outside focal lesions, and the fact that it cannot be used to identify the mechanisms through which the central nervous system recovers after tissue injury has occurred. Structural, metabolic and functional MR techniques have provided new markers, more closely linked to the pathological features of the disease, which may in part overcome the aforementioned limitations of conventional MRI.

a) *Heterogeneity of WM lesions*. Variable degrees of magnetization transfer ratio (MTR) reduction have been reported in acute and chronic MS lesions, with the most prominent changes found in T1-hypointense lesions (31). A three-year follow-up MT study has shown that new enhancing lesions of secondary progressive (SP) MS patients have significantly lower MTR values than those of relapsing-remitting (RR) MS patients and that tissue damage after enhancement has ceased is more severe in SPMS (32). Recently, MRI contrast agents composed of iron particles, known as ultrasmall particles of iron oxide (USPIO) or super-paramagnetic iron particles of oxide (SPIO), have been introduced to monitor different aspects of the MS inflammatory process. These particles are taken by cells of the monocyte/macrophage system. As a consequence, USPIO-enhancement reflects cellular infiltration and may complement Gd-enhancement (33). Finally, high-field and ultra-high field MRI scanners are becoming progressively available and they are contributing to detect a greater number and a higher volume of T2 and enhancing brain lesions. This new generation of scanners is also likely to provide a better definition of lesion location in the brain WM and GM, their morphology and their association with vasculature (34-36) at a resolution which resembles that of pathological assessment.

b) *Cortical lesions*. CLs are typically not seen on conventional MRI scans (37) because they are relatively small, have poor contrast with the surrounding normal GM, and because of partial volume effects from the CSF. The recent introduction of DIR sequences, that use two inversion times to suppress the signal from both WM and CSF, has markedly improved the sensitivity of MRI to detect CLs *in vivo* (20). CLs are more frequently seen in patients with SPMS than in those with CIS or RRMS (21), whereas in patients with benign (B) MS they are fewer than in those with early RRMS (38). An association has been found between CL burden and progression of disability over the subsequent two (39) and three (40) years in patients with different disease phenotypes, as well as between CL burden and the severity of cognitive impairment in patients with relapse-onset MS (41, 42). The ability of MRI to visualize CL, however, is still suboptimal (43). As a consequence, a set of new strategies has been proposed to improve the detection and allow a reliable classification of such lesions, including the use of a single-slab 3D DIR sequence (44), and the combination of DIR with other sequences, such as phase-sensitive inversion recovery (45) and 3D magnetization-prepared rapid acquisition with gradient echo (46).

c) *Diffuse NAWM damage*. Outside T2 lesions, quantitative MRI discloses the presence of abnormalities in the NAWM of patients with MS. Several studies with serial MR investigations have shown that, at least in some cases, subtle WM changes can be seen in areas which days to weeks later develop into classical enhancing lesions. These changes consist of a reduction of MTR (47), increase in mean diffusivity (MD) (48), and mild to moderate reduction of N-acetylaspartate (NAA) (49). NAWM MTR histogram-derived measures evolve at different rates in the major MS clinical phenotypes (50). When a multivariate analysis of several conventional MRI- and MT MRI-derived variables was run, it was found that average NAWM MTR is more strongly associated with MS cognitive impairment than the extent of T2-visible lesions and their intrinsic tissue damage (51). NAWM MTR reduction has also been shown to predict the accumulation of clinical disability over the subsequent five years in patients with definite MS (52). DT MRI abnormalities become more pronounced with increasing disease duration and neurological impairment (53), thus supporting the notion that DT MRI is particularly sensitive to the more disabling features of MS pathology. In patients with pediatric MS, the absence or mildness of MT and DT MRI abnormalities in the NAWM and GM has been advocated to explain their favorable short-term clinical evolution (54). Decreases in NAA are not restricted to MS lesions, but also occur in the NAWM adjacent to or distant from them (55). NAA levels of the NAWM tend to decline over time and these changes are more pronounced in patients with SPMS and PPMS (56).

d) *Diffuse GM damage*. Reduced MTR values have been demonstrated in the brain GM from patients with different MS phenotypes (50). Such MTR abnormalities were found to be more pronounced in patients with PPMS or SPMS than in those with RRMS. GM MTR changes correlate with clinical disability and cognitive impairment (50), whereas no correlation with fatigue emerged (50). In patients with BMS, those with cognitive impairment exhibit a more pronounced decrease in neocortical volume and cortical MTR values than the cognitively preserved patients do (57). In patients with RRMS, GM MTR was found to be an independent predictor of the accumulation of disability over the subsequent eight years (58), and GM diffusivity was found to predict accumulation of disability over a five-year period in patients with PPMS (59). Longitudinal studies of GM volume (60) and DT MRI studies (59, 61-63) have demonstrated a worsening of GM damage over time in patients with RRMS (62), SPMS and PPMS (59, 61, 63).

e) *Quantification of regional damage in the NAWM and GM*. Improvements in methods of analysis have allowed assessing the distribution of damage in the NAWM and GM at a regional level. Using a voxel-based approach, a study (64) showed that patients with RRMS and BMS differ in term of the topographical distribution of WM damage, while no between-group differences were found when the overall extent of WM diffusivity changes was assessed using an histogram-based approach. Using tract-based spatial statistics (TBSS), compared to healthy controls, MS patients had reduced fractional anisotropy (FA) values in several WM fibers bundles, which were related to deficits of specific cognitive domains (65, 66). Regional damage can also be assessed by means of DT MRI tractography methods, which allow to segment clinically eloquent WM pathways. A DT MRI tractography study showed that CIS patients with motor impairment have an increased MD in the corticospinal tracts (CST) compared to patients without pyramidal symptoms (67).

Voxel-based morphometry studies have shown consistently that the pattern of regional GM loss differs among patients with the main disease clinical phenotypes (68). In CIS patients, GM atrophy involves the thalamus, hypothalamus, putamen and caudate nucleus (69), whereas in RRMS patients cortical atrophy in the fronto-temporal lobes is typically detected (70). Compared to controls, BMS patients have a reduced GM volume in the subcortical and frontoparietal regions (71). In comparison with BMS patients, those with SPMS have a significant GM loss in the cerebellum (71). More recently, Riccitelli *et al*. (72) showed that the pattern of regional GM atrophy differs among cognitively impaired patients according to their clinical phenotype (Fig. 1). Patients with pediatric MS experience atrophy in the thalamus only, with sparing of the cortex and other deep GM nuclei (73).

Voxel-wise analysis of MT and DT MRI data can also be used to assess intrinsic GM damage. Khaleeli *et al*. (74) showed, in PPMS patients, a significant correlation between decrease of MTR values of cortical motor areas and the Expanded Disability Status Scale scores, as well as between MTR values in cortical areas and the performance at the Paced Auditory Serial Addition Task. Similarly, using a voxel-based analysis of DT MRI maps, Ceccarelli *et al*. (75) showed diffusivity abnormalities of brain areas associated with motor and cognitive functions in PPMS. Bodini *et al*. (76) investigated the relationship between damage occurring in the NAWM and GM in patients with early PPMS and found 11 brain regions with an anatomical correspondence between reduced NAWM FA values and GM atrophy,

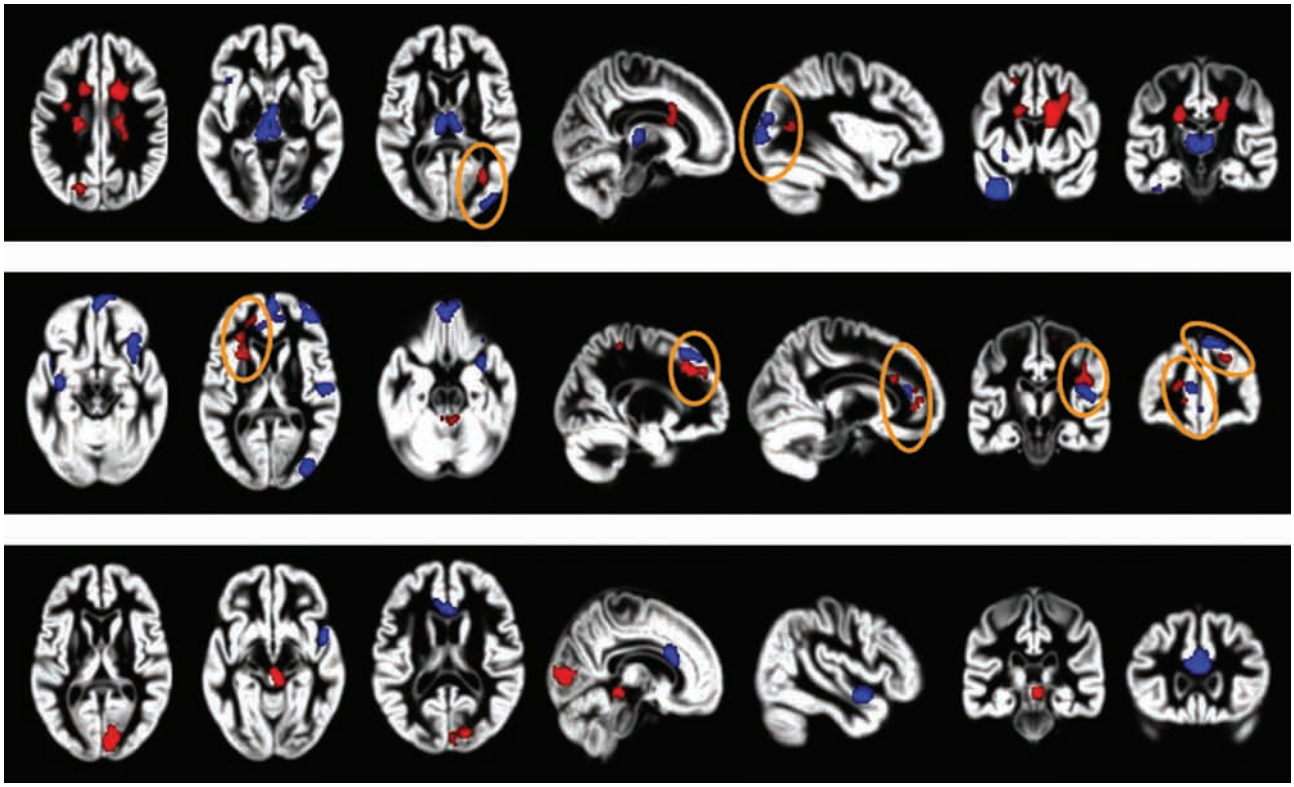

FIG. 1. — Distribution of regions of significant grey matter atrophy ($P < 0.05$, family wise error corrected) (blue) and T2-visible lesions (red) in cognitively impaired (CI) *vs*. cognitively preserved (CP) multiple sclerosis (MS) patients according to the clinical phenotype. Top row: relapsing remitting MS; middle row: secondary progressive MS; bottom row: primary progressive MS. Orange circles identify regions with a correspondence between presence of T2 visible lesions and GM atrophy. Images are in neurological convention. From Riccitelli *et al*. (72) with permission.

supporting a link between the pathologic changes occurring in the NAWM and those of the GM.

f) *Quantification of cervical cord damage*. The development of sophisticated MR receiver coils and fast imaging techniques has led to a more reliable imaging of the cord, which also includes the use of quantitative MR techniques. Conventional and DT MRI of the cervical cord was obtained from relapse-onset MS patients at baseline and after a mean follow up of 2.4 years (77): baseline cord cross-sectional area and FA correlated with an increased disability at follow up. Using an MT-weighted approach, signal abnormalities in the dorsal and lateral columns of the spinal cord were correlated with vibration sensation and strength, respectively (78). Compared to controls, MS patients with a cervical cord relapse have reduced NAA levels and a lower structural connectivity in the lateral CST and posterior tracts. Such abnormalities were correlated with disability (79).

g) *Cortical reorganization*. fMRI studies with different paradigms have demonstrated consistently functional cortical changes in all MS phenotypes, suggesting that there might be a "natural history" of the functional reorganization of the cerebral cortex in MS patients (80). Such a notion is supported by the results of a cross-sectional study of the motor network in patients with different disease clinical phenotypes (81), which showed, at the beginning of the disease, an increased recruitment of those areas "normally" devoted to the performance of a given task, such as the primary sensorimotor cortex (SMC) and the supplementary motor area. 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 normal people to perform novel/complex tasks. Recently, the preservation of a focused and strictly lateralized movement-associated pattern of cortical activations has been suggested as a possible mechanism to explain the favourable clinical outcome of patients with pediatric MS (82) and BMS (83).

Functional and structural MRI changes of the MS brain are correlated (80). Indeed, several moderate to strong correlations have been demonstrated between the activity of cortical and subcortical areas

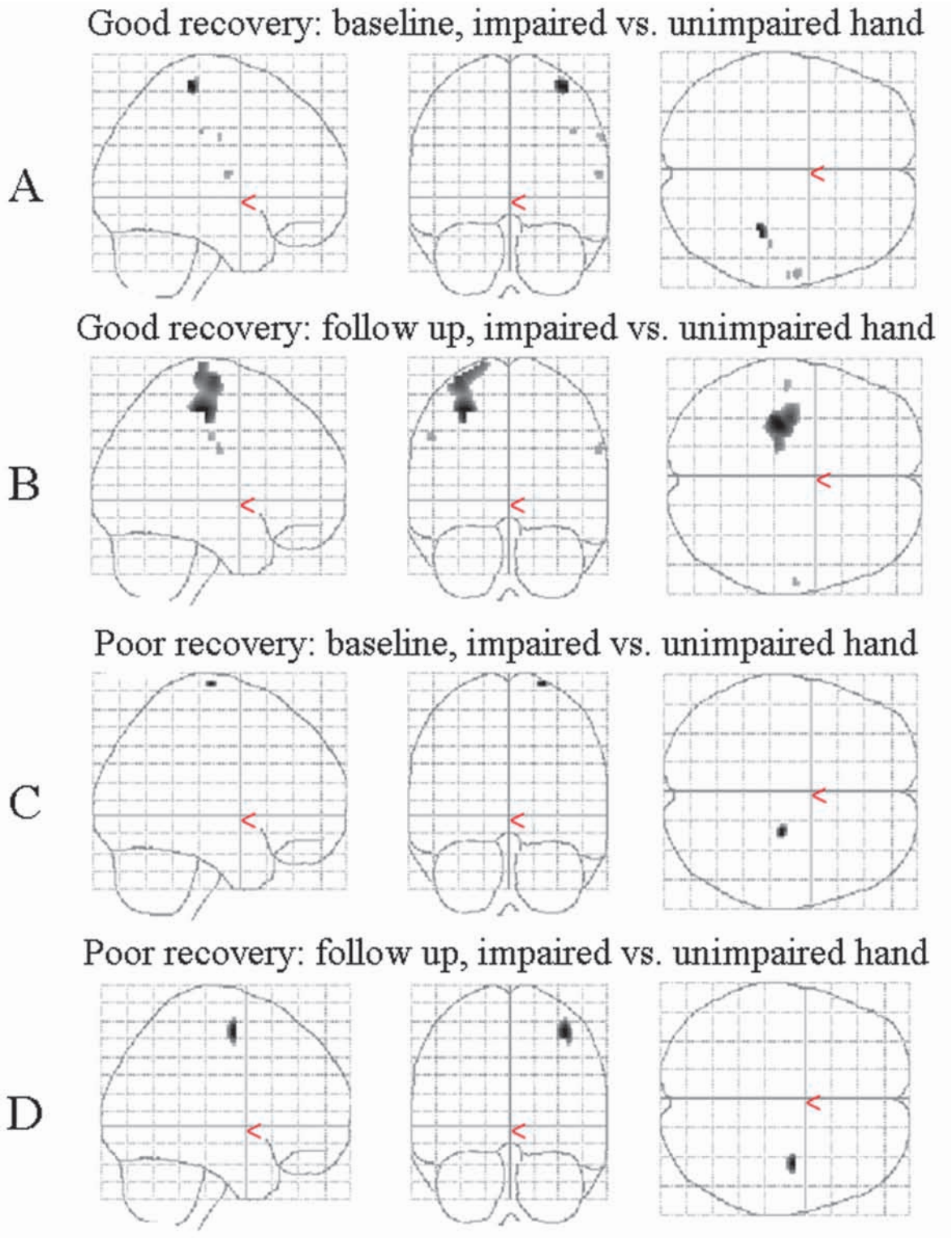

FIG. 2. — Longitudinal evolution of cortical activations in the primary sensorimotor cortex (SMC), bilaterally, during performance of a simple motor task with the impaired hand compared to unimpaired hand in a multiple sclerosis (MS) patient with an acute motor relapse and subsequent complete clinical recovery during follow up (A and B) and in an MS patient with poor clinical recovery (C and D). Scans obtained during the left hand motor task have been flipped in order to keep the left hemisphere contralateral to the movement. Activations have been superimposed on a glass brain. At baseline, both patients showed an increased activation of the primary SMC of the unaffected (ipsilateral) hemisphere (A and C). During follow up, the patient with good clinical recovery showed an increased functionality of the primary SMC of the affected hemisphere (B), while the patient with poor clinical recovery continued to show recruitment of the primary SMC of the unaffected hemisphere (D). From Mezzapesa *et al*. (84) with permission.

of different cerebral networks and the extent of brain T2 lesions, as well as the severity of their intrinsic damage (80). In addition, movement- and cognitive-associated fMRI changes were found to correlate with the amount of NAWM, GM, and cervical cord injury. Such correlations suggest that, at least in some phases of the disease, an increased recruitment of "critical" cortical networks might contribute in limiting the functional impact of MS-related damage. This hypothesis is supported by the results of a study which assessed the early cortical changes following an acute motor relapse secondary to pseudotumoral lesions in MS patients and the evolution over time of cortical reorganization in a subgroup of them (Fig. 2) (84).

Several studies have attempted to develop sophisticated statistical approaches to establish the strengths of activations and synchrony between specific brain areas, through the analysis of functional and effective connectivities (85). The optimization of these methods might help to explain abnormalities of function of specific brain networks and their relationship to clinical symptoms. The combination of measures of functional connectivity with measures of structural damage to specific WM fiber tracts is also likely to improve our understanding of the relationship between structural and functional abnormalities, as suggested by two studies in patients with RRMS (85) and BMS (86). More recently, the analysis of brain function at rest has shown a reduced activity of the anterior regions of the default-mode network in patients with progressive MS and cognitive impairment (87).

Similarly to what happens in the brain, also in the cervical cord, an increased fMRI activation has been demonstrated in all the main MS clinical phenotypes and has been related to the severity of clinical disability and the extent of tissue damage (88).

Conclusions

Conventional and modern MR-based techniques have markedly improved our ability to diagnose MS, to predict its course, and to understand its pathophysiology. From the data available, it is clear that combining different MR modalities, which are sensitive to different aspects of MS pathology, is a promising way to improve further our understanding of the mechanisms accounting for the accumulation of irreversible disability in this disease. Such an approach should include not only the assessment of brain damage, but also that of the spinal cord. Finally, the precision and accuracy of quantitative MRI scans in detecting longitudinal, MS-related changes also need to be defined and *ad hoc* large-scale, prospective studies are warranted. This is a central issue for a future application of quantitative MRI to the monitoring of MS evolution in clinical trials.

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