Association between pathological and MRI findings in multiple sclerosis

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Pathological evaluation is the gold standard for identifying processes related to multiple sclerosis that explain disease manifestations, and for guiding the development of new treatments. However, there are limitations to the techniques used, including the small number of donors available, samples often representing uncommon cases, and impossibility of follow-up. Correlative studies have demonstrated that MRI is sensitive to the different pathological substrates of multiple sclerosis (inflammation, demyelination, and neuro-axonal loss). The role of MRI in evaluating other pathological processes, such as leptomeningeal involvement, central vein and rim of lesions, microstructural abnormalities, iron accumulation, and recovery mechanisms, has been investigated. Although techniques used for quantifying pathological processes in different regions of the CNS have advanced diagnosis and monitoring of disease course and treatment of multiple sclerosis, new perspectives and questions have emerged, including how different pathological processes interact over the disease course and when remyelination might occur. Addressing these questions will require longitudinal studies using MRI in large cohorts of patients with different phenotypes.

Introduction

Pathological assessment is the gold standard in multiple sclerosis for understanding the processes involved in the disease and its progression, and for the identification of possible future therapeutic targets. MRI is a particularly suitable technology for in-vivo evaluation of pathology, because of its high sensitivity in revealing abnormalities related to multiple sclerosis and in monitoring disease progression and the effects of treatment. The association between multiple sclerosis pathology and MRI findings needs to be continuously verified to improve the technique's specificity in detecting pathological features in this disorder. MRI or other imaging methods with a high specificity for multiple sclerosis pathology could be applied longitudinally after their specificity has been verified.

In 2012, a review paper[1] based on a workshop held by a group of international experts in neuropathology and neuroimaging in multiple sclerosis summarised the evidence from correlative pathological MRI studies available at that time. The aim was to discuss concordant data, but also to highlight controversies, identify emerging pathological and MRI findings, and open questions for future research. This discussion included the need to improve imaging specificity, the acquisition of high-resolution images, the combination of MRI methods, and the assessment of specific disease processes (eg, remyelination and iron abnormalities). Improvement of MRI technologies and wider availability of MRI scanners since 2012 has allowed better understanding and MRI monitoring of specific pathological processes related to multiple sclerosis, including not only the presence of central vein sign and hypointense rim, the heterogeneous damage in different CNS regions, and iron accumulation, but also mechanisms of tissue recovery.

This Review summarises the advances in MRI techniques and how the measures they provide correlate with the pathological substrates of multiple sclerosis. In particular, white matter and grey matter lesions, normal-appearing brain tissue abnormalities, damage of relevant structures (eg, spinal cord, thalamus, cerebellum, and hippocampus), and iron accumulation were discussed during a second workshop (Nov 23–24, 2017, Milan, Italy), since they represent the main areas of progress in filling the gap between pathology and MRI findings. Emerging pathological and MRI findings that, in combination, might enhance our understanding of disease pathophysiology and help identify reliable in-vivo markers for monitoring different aspects of multiple sclerosis are also discussed.

White matter lesions

Focal white matter lesions, characterised by inflammation and demyelination, are the most obvious hallmarks of multiple sclerosis histopathology. The inflammatory lesion infiltrate mainly consists of blood-derived monocytes and microglia (for simplicity subsequently termed here as phagocytes), T cells, and B cells.[2] In active and demyelinating lesions, commonly found at the beginning of the disease—as documented in biopsy studies in which the onset of symptoms provides an estimate of lesion development—phagocytes with a round and foamy morphology are the dominating inflammatory cell population (figure 1).[3,4] In the first stages of lesion formation, these phagocytes contain myelin debris in their cytoplasm, suggesting ongoing myelin breakdown, as monocytes degrade these myelin proteins within days in vitro.[4,5] During later disease stages, mixed active and inactive lesions with a complete or partial rim of phagocytes and inactive lesions with an almost complete lack of phagocytes, become more prominent (figure 1).[3,6] The percentage of

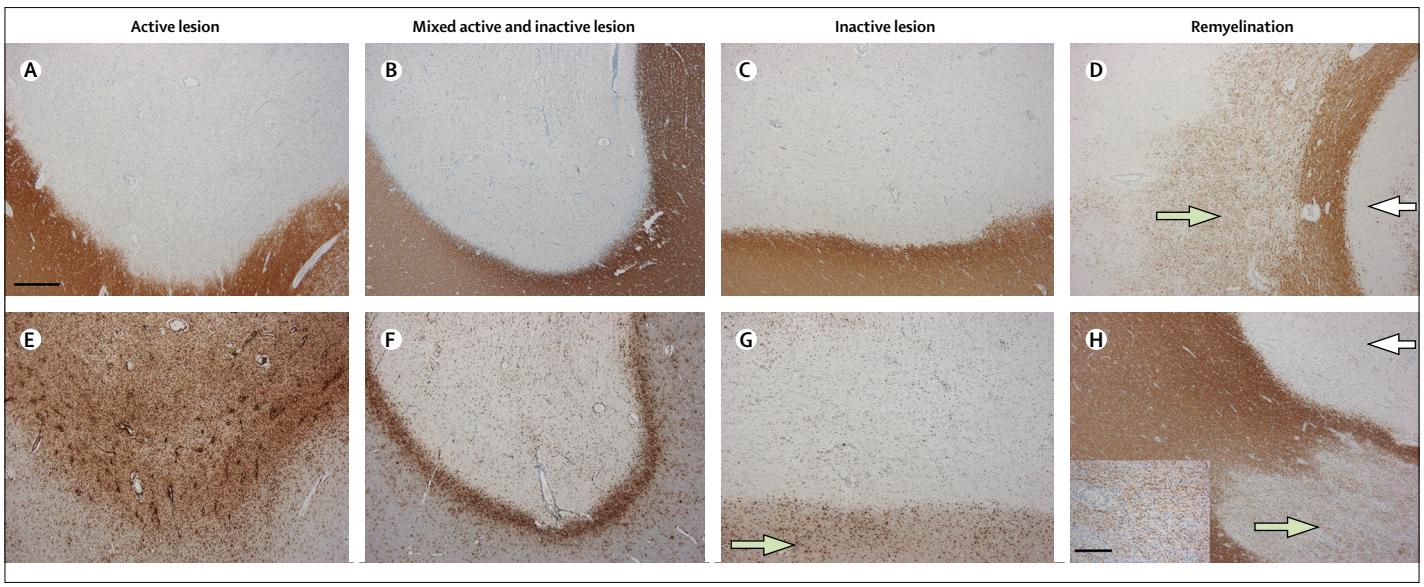

Figure 1: **Pathology of white matter lesions in patients with multiple sclerosis**
Active lesions are characterised by complete demyelination (A) and numerous phagocytes throughout the lesion (E). Mixed active and inactive lesions are completely demyelinated (B) but have a rim of phagocytes at the lesion border (F). In contrast, demyelinated inactive lesions (C) have only a few scattered phagocytes within the lesions (G). Note the presence of concentrated phagocytes in the myelinated normal-appearing white matter (G, green arrow). Remyelination can be limited to the lesion border (D, green arrow) or present throughout the whole lesion (H, green arrow). Note the demyelinated cortex (D, white arrow) and a second demyelinated lesion (H, white arrow). The insert (H) shows thin, irregularly formed remyelinated myelin sheaths at the location of the green arrow. Immunohistochemistry in panels B, D, and F is for CD68, and the slices in the remaining panels were stained for myelin basic protein. Scale bar=500 μm, except in H (insert)=200 μm.

mixed active and inactive lesions is significantly higher in patients with primary and secondary progressive multiple sclerosis than in patients with relapsing-remitting multiple sclerosis, and it correlates with disease severity.[3,6] Therefore, MRI correlates that are able to differentiate between these types of lesions might be especially valuable for prognosis. The histopathology in early active and demyelinating lesion stages is heterogeneous with respect to the absence or presence of complement depositions, immunoglobulins, and oligodendroglial loss, suggesting that different pathological mechanisms might trigger the formation of lesions. Whether these different histopathological patterns are specific to the individual patient or to the lesion stage is still a matter of debate.

Axonal damage is closely associated with inflammatory demyelination. In the initial lesion stages, axonal spheroids, indicators of disturbance in transient or permanent axonal transport, and thus axonal damage, are already present. A retrospective study of brain tissue from 39 patients with multiple sclerosis has demonstrated a close correlation between phagocytic infiltration and axonal damage.[7] The formation of new myelin sheaths around demyelinated axons (remyelination) is frequent in active lesions, and demyelination and remyelination might occur in parallel within the same lesion.[8] In a study of 3188 tissue blocks (7562 multiple sclerosis lesions) from the autopsy cohort of the Netherlands Brain Bank, the percentage of remyelinated lesions was higher in patients with relapsing-remitting multiple sclerosis than in those with progressive multiple sclerosis.[6] A prerequisite for remyelination is the presence of oligodendrocyte progenitor cells that mature to myelinating oligodendrocytes upon demyelination. Whether there is ongoing remyelination in long-lasting multiple sclerosis lesions is unclear. However, results from animal and post-mortem studies suggest that remyelination capacity decreases with age and disease duration, and that the high proportion of remyelinated lesions in patients with long disease duration might be due to less severe disease.[6,9,10] Whether the decrease in remyelination capacity is due to decreased activity of phagocytes or to age-associated intrinsic changes in oligodendrocytes has yet to be determined.[8,11] However, remyelination is relatively infrequently reported in patients with multiple sclerosis with a disease duration of more than 10 years. Only about 20% of the lesions are completely remyelinated (so-called shadow plaques), whereas in most lesions, remyelination is either absent or restricted to a small rim at the lesion border (figure 1).[12]

T2-weighted (including fluid-attenuated inversion recovery [FLAIR]) imaging is highly sensitive in detecting focal white matter lesions in multiple sclerosis, enabling a prompt and accurate diagnosis of multiple sclerosis in patients presenting with clinical syndromes typical of demyelination. However, over-reliance on MRI, particularly in the context of atypical clinical symptoms, frequently leads to overdiagnosis of multiple sclerosis, mainly due to a scarcity of a careful exclusion of alternative diagnoses[13] and an inappropriate application of diagnostic criteria to define dissemination of lesions in space and time.[14]

The association between focal white matter lesions and venules in multiple sclerosis can now be visualised

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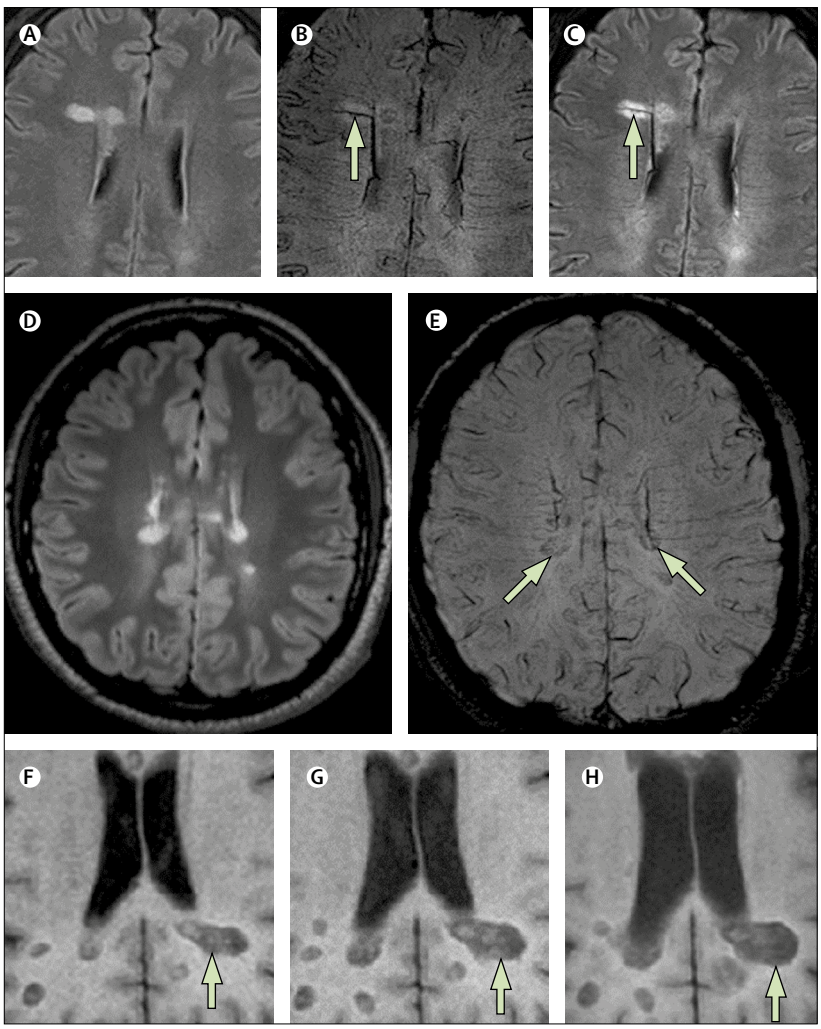

Figure 2: **MRI of white matter lesions in patients with multiple sclerosis**
3T brain MRI scan of a 32-year-old woman presenting with a clinically isolated syndrome suggestive of multiple sclerosis (A–C). A typical demyelinating ovoid lesion of the type seen in multiple sclerosis involving the frontal periventricular white matter was revealed on a T2-FLAIR image. Note the central vein sign on the susceptibility-weighted image (B) and on the combined T2-FLAIR and susceptibility-weighted image (T2-FLAIR*) images (arrows in B and C). 3T brain MRI scan of a 34-year-old woman presenting with a clinically isolated syndrome suggestive of multiple sclerosis (D, E). Typical demyelinating lesions of the type seen in multiple sclerosis involving the periventricular white matter are shown on a T2-FLAIR image (D). Note the hypointense rims that possibly reflect iron deposition (arrows) on the susceptibility-weighted image (E) in several of these lesions. Serial 3T brain MRI scan in a 56-year-old woman with secondary progressive multiple sclerosis acquired at baseline (F), and at 2 years (G), and 4 (H) years later. Unenhanced T1-weighted images demonstrate a slowly expanding lesion (with increases in both size and hypointensity) in the left posterior periventricular white matter (F–G, arrows). FLAIR=fluid-attenuated inversion recovery.

(through the central vein sign) using susceptibility-based MRI sequences, taking advantage of the T2*-shortening by deoxyhaemoglobin in venous blood, particularly when using high magnetic field strength MRI scanners (≥3T).[15] The central vein sign has been proposed as a new MRI biomarker to improve diagnostic accuracy of multiple sclerosis, since its presence in white matter lesions can help to differentiate multiple sclerosis from other diseases (figure 2).[16–19] Central vein sign conspicuity can be improved by acquiring a susceptibility-weighted sequence after gadolinium administration, particularly at standard clinical magnetic field strengths (1·5–3T).[20–22]

Using susceptibility-based MRI at ultra-high magnetic field strength (7T), a hypointense rim is seen in some white matter multiple sclerosis lesions (figure 2). This feature is not present in patients with cerebrovascular disorders, CNS inflammatory vasculopathies, or neuromyelitis optica,[17,18,21] and might represent a more specific pathological hallmark of multiple sclerosis. Future studies could demonstrate that incorporating this imaging feature into standard magnetic field strength scanner protocols further improves the specificity of MRI in the diagnosis of multiple sclerosis. This hypointense rim probably reflects iron accumulation within a subset of macrophages and activated microglia at the edge of chronic active white matter lesions (MRI reflection of the pathologically defined mixed active and inactive lesions),[2,22,23] although other relevant factors, such as oxidative stress and disruption of tissue microstructural organisation, might contribute to this signal change in active lesions.[24,25] On MRI at 7T in ten patients with multiple sclerosis (seven in vivo and four scanned before pathology), these chronic active lesions were characterised by progressive lowering of T1 signal intensity, and in some cases by a slow increase in their size (slowly expanding lesions; figure 2).[23] After a period of time (months to years), these lesions could become inactive, with no macrophage and microglia rim at the lesion border, as shown in a retrospective study of 32 patients with multiple sclerosis.[24] Therefore, lesions with hypointense rims could potentially predict ongoing inflammation and tissue injury. This prediction is particularly relevant in patients with progressive multiple sclerosis, in whom detection of inflammatory disease activity could help to identify those most likely to respond to anti-inflammatory treatments and would be highly valuable to improve prognosis, since these lesions correlate with the severity of multiple sclerosis.[3,6] Despite this potential, further longitudinal studies are still necessary to evaluate the natural history of hypointense rims. The appendix describes atypical forms of demyelination (tumefactive demyelinating lesions).

Grey matter lesions

The extent of grey matter demyelination in multiple sclerosis varies considerably between patients and can be widespread, especially during the chronic phase. In the neocortex, the proportion of tissue affected by demyelination ranges between 15% and 40% on histological inspection, whereas an average of 30% demyelination is reported for the deep grey matter, mesencephalon, hippocampus, cerebellar cortex, and spinal cord grey matter, with outliers of nearly complete demyelination of the grey matter also present (appendix).[26–33]

Grey matter lesions are classified according to their location.[34] They include mixed grey matter and white matter lesions, where the inflammatory profile of the white matter portion resembles that in white matter

lesions; and the more common subpial lesions, which can extend over several gyri and are generally non-inflammatory at autopsy, although representative biopsy samples of cortical material from 53 patients with multiple sclerosis with grey matter lesions (a total of 104 lesions) showed extensive involvement of the innate and adaptive immune systems (CD3 T-cell infiltrates and macrophage-associated demyelination).[35] This discrepancy might be because the lesions have been examined in different phases of their evolution. Inflammation is likely to be a key driver of cortical demyelination, since the involvement of C-C chemokine receptor 2 (CCR2)-positive monocytes, as well as B cells and T cells, was shown in the cortical grey matter and overlying meninges,[36,37] together with cortical complement deposition[38] and subtle blood–brain barrier disruption.[39] Rims of activated microglia were reported in autopsy cases of 22 patients with a more aggressive disease course (ie, extensive subpial demyelination) compared with 19 patients with scarce demyelination of the cerebral cortex.[40] In another study of 27 patients with multiple sclerosis compared with 14 healthy controls, both histopathology and PET showed microglia activation throughout the cortex in patients with multiple sclerosis,[41] increasing in severity with disease progression. The way in which patients respond to damage due to inflammation might be determined by their genetic background. In a study of 47 patients with multiple sclerosis, individuals positive for HLA-DRB1*1501 expressed more CD3 and Iba1 microglial reactivity in their cortical lesions.[42]

Using post-contrast FLAIR at 3T in 299 patients with multiple sclerosis, focal leptomeningeal enhancement was found in 74 (25%), with a higher frequency in progressive (39 [33%] of 118) than in relapsing-remitting multiple sclerosis (35 [19%] of 181) phenotypes.[43] Subsequent pathological evaluation of two of the patients showed perivascular lymphocytic, mononuclear infiltration, and subpial demyelination in enhancing areas.[43] However, leptomeningeal enhancement is not specific to multiple sclerosis, and was reported to occur with a high frequency in 18 (35%) of 51 patients with other non-multiple sclerosis inflammatory neurological conditions (eg, neuromyelitis optica spectrum disorder, immune-mediated encephalitis, and systemic inflammatory diseases with white matter MRI abnormalities).[44]

Several other pathogenic mechanisms have been detected in grey matter lesions. Damage to neuropilema (which refers to the complex network of unmyelinated axons, dendrites, and glial cells) has been investigated. Atrophy of the cortical grey matter, measured using MRI, was predominantly determined by neuronal shrinkage and axonal degeneration.[45] However, neuronal loss,[29] damage,[46] and spine loss coupled to dendritic degeneration[47] were also reported. The extent to which these changes are related to, or dependent on, inflammation and their role in explaining clinical symptoms remains a subject of future investigation.[48]

Focal cortical lesions are poorly visualised using conventional MRI sequences (including dual-echo T2-weighted and FLAIR) because of their small size, poor contrast with the surrounding normal-appearing grey matter, and partial volume effects with white matter and CSF. Specialised sequences, such as double-inversion recovery,[49] phase-sensitive inversion recovery,[50] and three-dimensional (3D) magnetisation-prepared rapid acquisition with gradient echo,[51] have improved our ability to detect grey matter lesions. However, even a technique such as 3D double-inversion recovery only had a sensitivity of 18% to detect cortical lesions in a post-mortem study of 56 brain samples from 14 patients with multiple sclerosis,[52] with a relatively low inter-observer consensus (19·4% of all cortical lesions detected).[49] The use of ultra-high field scanners has improved the identification of cortical lesions,[53] making it possible to describe lesion patterns that resemble those reported by pathology.[54]

Evaluation of cortical lesions is important in the diagnostic investigation of suspected cases of multiple sclerosis. In patients with a clinically isolated syndrome, the presence of a single cortical lesion identifies those individuals almost certain to have multiple sclerosis confirmed later.[55] Cortical lesions have not been reported so far in other conditions that might mimic multiple sclerosis, including migraine[56] and neuromyelitis optica.[57] Therefore, adding them as diagnostic criteria is likely to improve their specificity as diagnostic criteria.[58] Cortical lesions have been shown in all phenotypes of multiple sclerosis, especially in the progressive forms, and the number and volume of cortical lesions correlated with disability and cognitive impairment, as well as with worsening of disability and clinical phenotype after 2, 5, and 7 years of follow-up (appendix).[59]

Using high field and ultra-high field scanners, a correlation between reduction of magnetisation transfer ratio (MTR) values and the presence of focal cortical demyelination has been reported, suggesting that MTR is sensitive to cortical demyelination and remyelination processes.[60,61] In vivo, using clinical MRI scanners, MTR reduction in the outer cortical surface has been detected in patients with multiple sclerosis who have the main phenotypes (ie, relapsing-remitting multiple sclerosis, secondary progressive multiple sclerosis, and primary progressive multiple sclerosis) and the lowest values reported in secondary progressive multiple sclerosis.[62,63] Abnormal MTR values were most common in the cingulate cortex, insula, and the depths of sulci, in agreement with pathological descriptions of the location of subpial demyelination.[62]

Using diffusion-tensor MRI, it has been shown that, unlike white matter lesions (which usually show decreased fractional anisotropy), cortical lesions have a higher fractional anisotropy than normal-appearing grey matter,[64,65] whereas conflicting results have been reported for mean diffusivity.[64–67] Different pathological substrates have been proposed to explain these abnormalities in diffusivity,

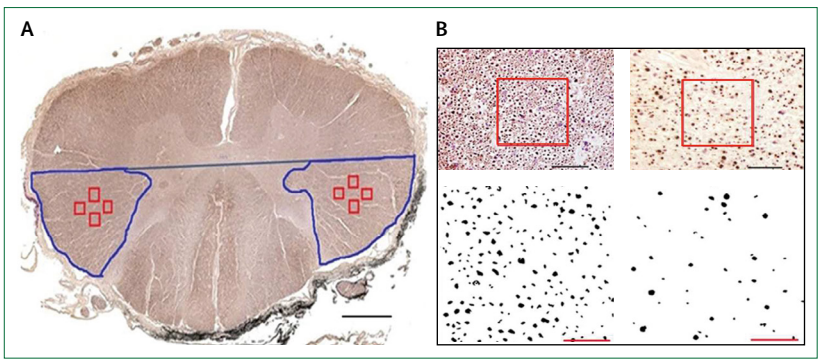

Figure 3: **Cross-sectional analysis of the spinal cord of a patient with multiple sclerosis**
(A) Location of axonal counting fields (red) randomly distributed over the area of the corticospinal tract (outline in blue). Microscopy of 1 counting field (size: 120 × 120 µm) in normal (B, top left) and multiple sclerosis (B, top right) spinal cord. Scale bar=50 µm. Magnified counting frames converted into black and white (8-bit) of control (B, bottom left) and multiple sclerosis (B, bottom right) corticospinal tract. Scale bar=25 µm. All sections were immunostained with SMI31 anti-neurofilament monoclonal antibody. Figure adapted from Petrova et al[30] with permission.

including damage to and loss of synapses, dendrites, and cortical neurons. Neuro-axonal loss and shrinkage have been implicated by a study of 26 patients with multiple sclerosis and 24 healthy controls, which used neurite orientation dispersion and density imaging (NODDI) and showed a decreased intracellular volume fraction.[66] A 7T correlative MRI and pathology study of 14 patients with multiple sclerosis showed higher cell density in cortical lesions than in the normal-appearing grey matter, with few activated microglial cells or increased cell size,[68] suggesting increased cellular density and tissue compaction as a possible mechanism for diffusivity changes in cortical lesions.

The quantification of T1/T2 ratio (the ratio of T1-weighted to T2-weighted image intensities) has been proposed as a possible biomarker of myelin content and cortical integrity. Decreased T1/T2 ratio (which correlated with MTR reduction) was reported in demyelinated cortex, compared with normal-appearing grey matter of six post-mortem brain samples of patients who had multiple sclerosis.[69] The specificity of this technique for cortical demyelination needs further validation, since another post-mortem study of nine brains from patients with multiple sclerosis reported a correlation between decreased cortical T1/T2 ratio and dendrite density, but not myelin content.[70]

Spinal cord

Spinal cord pathology is common in multiple sclerosis and, compared with brain pathology, more likely to be clinically apparent. Acute symptoms and signs at onset and the chronic loss of function, particularly of the lower limbs, correlate to some extent with findings in pathology specimens in which blood–brain barrier damage, inflammation, demyelination, gliosis, and neuro-axonal loss, have been reported.[30,71,72]

A post-mortem study of 13 patients with multiple sclerosis and five healthy controls that examined the extent and location of demyelination across different spinal cord levels showed more extensive demyelination in grey matter (24–48%) than in white matter (11–13%) and in the thoracic level compared with other segments of the spinal cords of patients with multiple sclerosis.[30] Depiction and quantification of spinal cord pathology in vivo using MRI remains a challenge. At high magnetic field strength (4·7T), using post-mortem spinal cord tissue from 11 patients with multiple sclerosis and two healthy controls, 53 (87%) of 61 white matter lesions and 32 (73%) of 44 grey matter lesions were detected.[73] Although replicating this sensitivity in vivo is not yet achievable, the presence of spinal cord lesions is incorporated into current MRI diagnostic criteria for multiple sclerosis.[74] Cervical spinal cord cross-sectional area shrinkage correlated with motor disability in a study of 335 patients with multiple sclerosis or a clinically isolated syndrome compared with 143 healthy controls,[75] and has been used as an exploratory endpoint in a few clinical trials.[76–78]

There is growing interest in more tissue-specific MRI measures, and a study of 159 patients with multiple sclerosis has shown that it is possible to assess the atrophy of spinal cord grey matter and white matter separately.[79] This methodological advance coincided with pathology data indicating that the size of the spinal cord cross-sectional area does not predict axonal loss of the corticospinal tract,[30] a surprising finding given the long-held belief that axonal loss is the major correlate of chronic disability (figure 3). One possible explanation for this conflicting result might be the loss of synapses, which preliminary data suggest might outweigh the loss of long-tract axons, and indeed seems to correlate with the cross-sectional area of spinal cord grey matter, a measure that has been shown to predict disability better than the total spinal cord cross-sectional area in 113 patients with multiple sclerosis compared with 20 healthy controls.[80]

Techniques that measure the size or volume of segmented tissue compartments might, for the foreseeable future, remain the most commonly used translators between tissue microstructure and clinical indices. However, substantial strides are also being made with techniques that try to quantify microstructure in the spinal cord. These advances include short T2 relaxation, MTR, diffusion-tensor imaging, and new post-processing algorithms, with a shift in focus from measures of myelination[81] to measures of axons and neurites. Two new diffusion-based techniques, diffusion basis spectrum imaging (DBSI)[82,83] and NODDI,[84–86] appear promising. Both DBSI and NODDI have been applied in vivo and to human post-mortem spinal cord, showing strong correlations between the quantitative histology and MRI-derived measures of microstructural complexity.[82–86]

Normal-appearing brain tissue

Pathologically, normal-appearing white matter has been defined as white matter that appears normal macroscopically and shows normal myelination microscopically, is at least 10 mm away from a plaque's edge, and is

differentiated from diffusely abnormal or dirty-appearing white matter, which includes areas of diffuse myelin pallor with ill-defined borders.[87] Normal-appearing grey matter has been defined as a region with no evidence of demyelination using immunohistochemistry.[34] Regarding MRI, the definitions of normal-appearing brain tissue depend on the sensitivity and specificity in the detection of tissue alterations and are prone to changes with technical advances.[88]

A post-mortem MRI study in situ, including magnetisation transfer MRI and diffusion-tensor MRI, assessed the pathological basis of abnormalities in normal-appearing white matter in brain samples of four patients with secondary progressive multiple sclerosis.[89] Magnetisation transfer MRI and diffusion-tensor MRI metrics correlated moderately with myelin density, axonal area, and axonal counts. In truly normal-appearing white matter (defined as white matter tissue without T2-hyperintense, T1-hypointense, and lesions visible through magnetisation transfer MRI), MTR and diffusion-tensor MRI measures only correlated with activated microglia,[89] indicating that pathological changes in brains of patients with multiple sclerosis could even be present in true normal-appearing white matter, as defined by both conventional and non-conventional MRI, such as magnetisation transfer MRI and diffusion-tensor MRI. A 7T MRI-PET imaging study with [^{11}C]-PBR28 (18 kDa translocator protein [TSPO], a marker of activated microglia and macrophages) provided in-vivo evidence of widespread microglial activation in the grey matter of 12 patients with relapsing-remitting multiple sclerosis and 15 patients with secondary progressive multiple sclerosis.[41] Relative to 14 healthy controls, patients exhibited abnormally high [^{11}C]-PBR28 binding, predominantly in cortex and cortical lesions, thalamus, hippocampus, but also in normal-appearing white matter, whereas focal white matter lesions showed only modest increases. Cortical thinning correlated with increased thalamic TSPO concentrations,[41] suggesting widespread neuroinflammation partly linked to neurodegeneration.

The hypothesis that common CSF-mediated factors (eg, soluble antibodies, complement, or both, and cytokines) might contribute to the accumulation of damage in grey and white matter has been tested in 160 patients with multiple sclerosis, via correlations between increased periventricular T2-lesion burden and decreased cortical thickness, indicative of subpial pathology.[90] Of the 160 patients, 91 (57%) with a clinically isolated syndrome and 69 (43%) with relapsing-remitting multiple sclerosis had reduced cortical thickness compared with 58 healthy controls; the reduction was even more pronounced in patients with relapsing-remitting multiple sclerosis than in those with a clinically isolated syndrome. Increased periventricular lesion occupancy correlated with decreased cortical thickness in patients with relapsing-remitting multiple sclerosis, but not in those with a clinically isolated syndrome.[90] Using magnetisation transfer MRI,[91] proximity to the ventricular surfaces was reported to be associated with progressively lower MTR values in 67 patients with relapse-onset multiple sclerosis in supratentorial and cerebellar normal-appearing white matter, brainstem, and deep and cortical grey matter compared with 30 healthy controls. These findings provided evidence for common factors that underlie the spatial distribution of microstructural abnormalities in normal-appearing white matter and grey matter. A gradient in cortical pathology throughout different multiple sclerosis phenotypes has also been observed at 7T using surface-based analysis of T2* relaxation rates.[92] Relative to 17 healthy controls, 41 patients with a clinically isolated syndrome or multiple sclerosis demonstrated increased T2* (consistent with cortical myelin and iron loss), independent from cortical thickness. In early disease, T2* changes were focal and mainly confined to the first 25% of cortical depth and to cortical sulci. In later stages, T2* changes involved deeper cortical laminae and multiple cortical areas and gyri.

Longitudinal studies with advanced MRI to assess changes in normal-appearing brain tissue in patients with multiple sclerosis are scarce. One study in 11 patients with relapsing-remitting multiple sclerosis and four healthy controls looked at measures sensitive to myelin content and intracellular and extracellular water properties in normal-appearing white matter at baseline and at 3·2–5·8 years and reported that progressive changes in myelin integrity occurred diffusely.[93]

Thalamus, cerebellum, and hippocampus

Pathological and MRI studies have consistently demonstrated an early involvement of the thalamus, cerebellum, and hippocampus. Damage to these structures is clinically relevant, since it is strongly associated with locomotor disability and cognitive impairment.[94–96] Other clinically relevant structures are the brainstem and the hypothalamus; however, they have not been discussed during the workshop and are therefore outside the scope of this Review.

The thalamus can show considerable demyelination in progressive disease.[94] In a study of 14 brains from patients with multiple sclerosis and 12 controls (six from healthy controls and six from patients with amyotrophic lateral sclerosis), demyelination solely in grey matter and mixed grey and white matter lesions occurred particularly in the periventricular medial and anterior thalamic nuclei, but not in the ventral thalamic nuclei in patients with multiple sclerosis compared with controls.[27] Thalamic neuronal loss and neuronal atrophy can be substantial, affecting demyelinated and non-demyelinated tissue areas equally.[27,97] In 14 patients with secondary progressive multiple sclerosis compared with 14 healthy controls, a 30–35% reduction in neuronal density was reported in the medial dorsal nucleus, corresponding to a 22% reduction in its volume by MRI, and a 19% reduction in N-acetylaspartate.[97] Microglia activation was not restricted to demyelinated areas, but also occurred in non-lesioned thalamus.

Inflammatory infiltration was generally lower in regions of grey matter compared with white matter regions.[27]

Cerebellar symptoms, including Charcot's triad of nystagmus, intentional tremor, and dysarthria are frequent in patients with multiple sclerosis. On average, 38% of the cerebellar cortex has been reported to be demyelinated in several studies.[26,31–33,98] In these studies, lesions were often purely cortical, but also mixed grey and white matter lesions were reported. Cerebellar cortical demyelination has also been associated with subarachnoid inflammation.[32] Neuronal pathology has also been reported in the cerebellar grey matter, with a slight reduction in Purkinje cell density, especially in demyelinated areas.[32,98] Neuronal atrophy and loss, as well as synaptic reduction, have also been reported in the deep cerebellar nuclei, irrespective of the presence of focal demyelinated lesions.[33]

Demyelinated hippocampal lesions have been reported in most patients with chronic multiple sclerosis, with mixed hippocampal and parahippocampal lesions being common. In one post-mortem study of brain tissue samples from 19 patients with multiple sclerosis, compared with seven healthy controls,[28] the hilus and cornu ammonis (CA) 2 region were only affected if large areas were demyelinated. In nine of 19 patients, cognitive decline had been noted in the patients' medical files before autopsy.[28] In a study with 45 patients with progressive multiple sclerosis and seven healthy controls, 22 (54%) of 41 lesions found in these patients were partly contained in the subependymal or subpial regions.[99] Lesions were mainly present in the CA1 region, whereas CA4 was only rarely demyelinated.[99] A significant reduction of synaptic density by 46% compared with controls was reported in CA4 of patients with multiple sclerosis.[99] Synaptic loss outweighed neuronal reduction and was independent of focal demyelination.[99] In another post-mortem study of tissue samples from 15 patients with multiple sclerosis compared with ten healthy controls, molecular alterations in hippocampal neurotransmission accompanied neuroaxonal loss.[100]

Using MRI, focal lesions have been consistently reported in the thalamus, cerebellum, and hippocampus of the main multiple sclerosis phenotypes.[94–96] Assessment of the presence and extent of lesions in these critical structures has a clinical role not only for diagnosis (eg, the cerebellum is one of the typical locations for demonstrating disease dissemination in space),[74] but also for improving understanding of some of disease symptoms, including cognitive deficits[101] and motor impairment.[102]

Quantitative MRI techniques have shown microstructural abnormalities beyond focal lesions, which are more severe in patients with progressive multiple sclerosis compared with those with relapsing-remitting multiple sclerosis.[102–104] Such microscopic abnormalities were also reported in patients with a clinically isolated syndrome.[105] Correlative studies have reinforced the clinical relevance of microscopic tissue alterations in these three regions. For example, in a study of 24 patients with multiple sclerosis compared with 24 healthy controls, increased thalamic mean diffusivity correlated with motor dysfunction, particularly in the 11 patients with relapsing-remitting multiple sclerosis.[103] However, in a study of 54 patients with primary progressive multiple sclerosis compared with eight healthy controls, decreased thalamic fractional anisotropy predicted worsening of disability after 5 years.[106] In a study of 37 patients with a clinically isolated syndrome, increased hippocampal mean diffusivity distinguished cognitively impaired from cognitively preserved participants.[105]

Atrophy also occurs in the thalamus, cerebellum, and hippocampus from the very early stages of the disease (ie, clinically isolated syndrome and paediatric multiple sclerosis) and is evident in patients experiencing a short interval between the biological onset of the disease and appearance of clinical symptoms. Consistent with the regional susceptibility to damage of the main hippocampal subfields (CA1 to CA4, dentate gyrus, and subiculum), regional analyses have shown an uneven distribution of atrophy in the hippocampus, with more severe involvement of the CA1 and subiculum than in other hippocampal subfields.[107,108] A study of 95 patients with multiple sclerosis compared with 32 healthy controls reported an association between the functional specialisation of the subregions of the cerebellum and clinical measures, with volume of the posterior cerebellum accounting for variance in cognitive measures, whereas anterior cerebellar volume accounted for variance in motor performance.[109] What drives microstructural and volumetric abnormalities in these structures is unclear. Most studies have reported a correlation between such abnormalities and white matter focal lesion volumes, suggesting secondary degeneration as the cause. A combined diffusion-tensor MRI and atrophy study of 52 patients with multiple sclerosis and 57 healthy controls examined the relative contributions of thalamic damage and its white matter connections in explaining cognitive impairment in patients with multiple sclerosis; in the study, damage to the cortico-thalamic white matter was identified as the most important contributor to cognitive dysfunction.[110] Correlations were also reported between abnormalities in white matter tract and thalamic alterations, suggesting a disconnection syndrome. The hypothesis that some of the clinical symptoms attributable to involvement of the thalamus, cerebellum, and hippocampus are due to disconnection of these grey matter structures from integrated functional and structural systems is supported by findings from functional and diffusion-tensor MRI studies.[111–113]

Iron pathology

The notion of abnormal distribution of brain iron in patients with multiple sclerosis dates back more than 35 years with the observation of iron accumulation around demyelinated multiple sclerosis plaques, using Perls' staining for ferric iron.[114] Subsequent studies showed that

decreased T2 signal intensity in deep grey matter structures correlated with the amount of iron indicated by Perls' staining in healthy post-mortem brains,[115] and that decreased signal intensity on T2-weighted images was seen in the putamen and thalamus of patients with multiple sclerosis.[116] More pronounced T2-hypointensity of the deep grey matter structures due to iron accumulation was confirmed in other multiple sclerosis cohorts, and the degree of T2 signal loss was associated with brain atrophy and predictive of disability and clinical course.[117] Interest in these findings was revived by two avenues of research. New MRI techniques, such as R2* mapping[118] (figure 4) and quantitative susceptibility mapping,[119] have been developed and validated in post-mortem studies.[118–120] Additionally, it was suggested that iron liberated from destroyed cells promotes neurodegeneration by enhancing chronic oxidative injury.[121,122]

Quantitative MRI studies have concentrated primarily on the assessment of iron in the deep grey matter because of the confounding effects of myelin in white matter and signal contamination from adjacent tissues when examining the cortex. Age of the patient also needs to be considered, since iron normally accumulates in the deep grey matter, at least during the first four decades of life. In a cross-sectional multicentre setting of 97 patients with multiple sclerosis compared with 81 healthy controls, R2* values in the basal ganglia, indicative of iron concentration, increased from the stage of clinically isolated syndrome, through relapsing-remitting to progressive multiple sclerosis,[123] whereas a 2-year longitudinal study of 17 patients with multiple sclerosis compared with 17 healthy controls showed an increase in R2* (the inverse of T2*) in the basal ganglia that was related to disability.[124] These findings were confirmed by a 3-year longitudinal study that reported an increase in R2* in the basal ganglia, which was more pronounced in 76 patients with a clinically isolated syndrome than in 68 patients with multiple sclerosis (62 with relapsing-remitting multiple sclerosis and six with secondary progressive multiple sclerosis).[125] A reduction in magnetic susceptibility, presumably related to decreased iron concentration of the thalamus and possibly a consequence of iron depletion from oligodendrocytes, was also reported in a study of 140 patients with a clinically isolated syndrome or multiple sclerosis.[126] Pathological confirmation of all these observations is still needed.

In a histopathological study of 33 patients with multiple sclerosis and 30 healthy controls, iron concentration in the normal-appearing white matter of patients with multiple sclerosis decreased significantly with disease duration, in contrast to the physiological increase seen in controls with ageing.[121] In active multiple sclerosis lesions, iron is apparently released from dying oligodendrocytes, but accumulates in activated microglia and macrophages at the lesion edges and in astrocytes in inactive centres of a subset of slowly expanding lesions.[23,121] Quantitative correlative assessments in four brains of patients with multiple sclerosis confirmed that black rims around lesions on 7T susceptibility-weighted imaging are a consequence of iron-laden microglia and macrophages.[23] Annual MRI follow-up of seven patients with multiple sclerosis over 3·5 years showed that the size of lesions with a hypointense rim increased over time which, together with the pathological findings,[23] led to the suggestion that iron rings might characterise slowly expanding lesions.[121] Another study of autopsy tissue from 18 patients with multiple sclerosis using synchrotron X-ray fluorescence imaging showed iron accumulation in microglia and macrophages at the edge of a subset of slowly expanding lesions.[123] However, iron accumulation in astrocytes—primarily in inactive centres of slowly expanding lesions—and in a concentric pattern in a single inactive lesion was also detected, suggesting a possible protective role of astrocytes by capturing and neutralising extracellular iron.[127] Iron decreased with patient age in

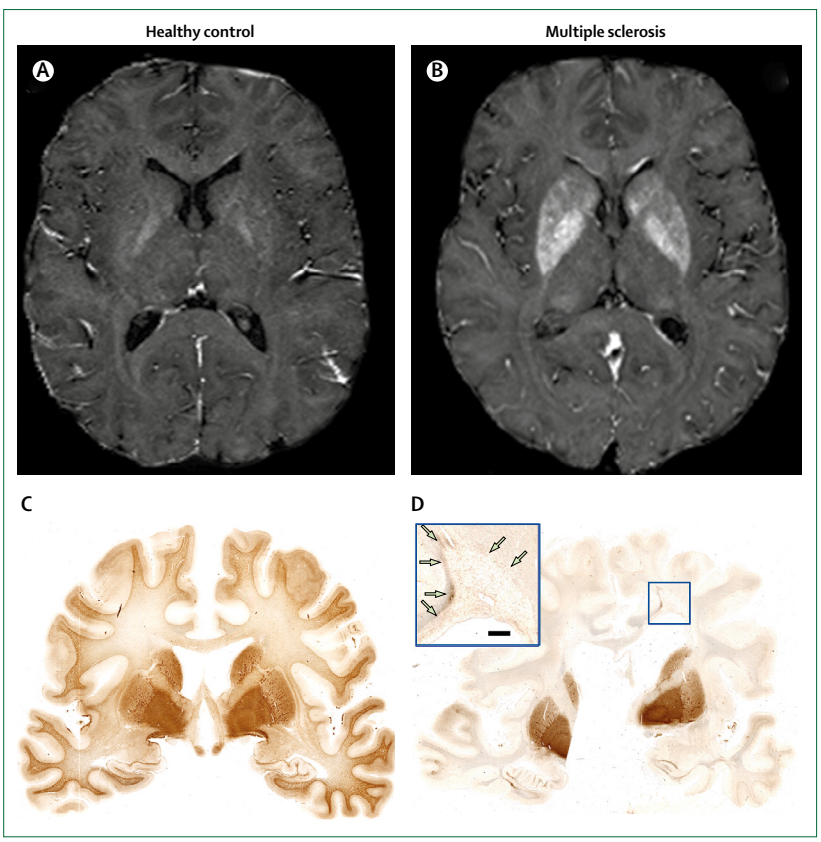

Figure 4: **In-vivo MRI R2* mapping and post-mortem iron staining in patients with multiple sclerosis and healthy controls**
(A) Axial R2* maps of a 37-year-old healthy control and (B) a 33-year-old patient with early multiple sclerosis (Expanded Disability Status Scale score of 3·0). Note the increased R2* relaxation rate in the basal ganglia reflecting increased iron levels, especially of the globus pallidus, of the patient with multiple sclerosis, as indicated by high signal density. (C) Coronal double-hemispheric histological sections of a 63-year-old healthy control and (D) a 74-year-old patient with secondary progressive multiple sclerosis (Expanded Disability Status Scale score of 7·0), stained with the diaminobenzidine-enhanced Turnbull blue iron staining. Note the high iron density in the basal ganglia of the patient with multiple sclerosis, while iron appears depleted across the normal-appearing white matter. A periventricular chronic multiple sclerosis lesion is outlined and magnified in the inset (scale bar=500 μm). The edge of the lesion is partially accentuated with iron accumulation in macrophages, forming an iron rim (arrows).

Search strategy and selection criteria

References for this Review were identified through searches of PubMed with the search terms "autopsy", "histopathology", "multiple sclerosis", "MRI", "pathology", "post-mortem", "lesions", "normal-appearing white matter", "grey matter", "spinal cord", "cerebellum", "hippocampus", "thalamus", and "iron" from Jan 1, 2013, to Oct 31, 2018. Articles were also identified through searches of the authors' own files. Only papers published in English were reviewed. The final reference list was generated on the basis of originality and relevance to the broad scope of this Review.

normal-appearing white matter, cortex, and multiple sclerosis lesions, and iron concentration in the periplaque white matter was significantly lower than in normal-appearing white matter.

A concomitant loss of myelin and iron in cortical lesions of multiple sclerosis was reported in a correlative histopathology MRI study of two post-mortem multiple sclerosis cases, and this effect was reflected as areas of signal loss in the corresponding 7T R2* images.[128] Another form of focal iron accumulation is in so-called microbleeds, which indicate extravasation of blood through leaking blood vessels. A case-control MRI study of 445 patients with multiple sclerosis found cerebral microbleeds in 41 (20%) of 206 patients aged over 50 years compared with six (7%) in 81 age-matched healthy controls.[129]

Levels of iron in the brains of patients with multiple sclerosis still needs to be explored further. Pathological confirmation of the MRI findings of diffuse iron changes in the basal ganglia and thalamus is also needed. In the white matter and in lesions, the confounding influence of myelin on T2* has been described in detail in a 2018 study.[130] One possibility is temperature-dependent relaxometry, but this method can only be applied to post-mortem tissue.[131] Longitudinal studies are needed to determine whether iron accumulation is a trigger of neurodegeneration and propagates lesion expansion or is just a marker of disease progression.

Conclusions and future directions

Over the past 5 years, there have been many advances in MRI measures that reflect pathological findings in patients with multiple sclerosis. Among them, the evaluation of leptomeningeal enhancement and of the lesional central vein sign have been proposed to improve the diagnostic investigation of patients with suspected multiple sclerosis.[58] The assessment of microstructural abnormalities and iron accumulation has increased our understanding of the mechanisms responsible for the clinical manifestations of multiple sclerosis.[121–128] New methods (eg, NODDI) to assess pathological processes more specifically and to evaluate tissue recovery (eg, remyelination) in vivo by means of MRI have also been used in preliminary studies.[66]

Advances in the field have also given rise to new questions, which will drive further research. Determining timing and duration of the potential for remyelination in different CNS structures is of crucial importance, since promoting remyelination is one of the main objectives of current treatments. Standardisation of the techniques capable of assessing remyelination is also needed. Since low-grade inflammation can be present at the border of some lesions in some patients, there is a need to identify pathological, imaging, and clinical features of these lesions to move towards individualised treatment. It will be a challenge to determine the interplay between the different pathological processes (eg, inflammation, demyelination, and neurodegeneration) over time, which calls for longitudinal evaluation using complementary MRI techniques.

Future research should focus on the early stages of the disease, include cohorts with clinically representative cases of different phenotypes, and seek causative information via repeated imaging. These research strategies would also allow the focus of the field to be switched from studies seeking to correlate MRI measures with clinical variables to those measures that can predict disease progression. Finally, to establish research priorities and surrogate markers of specific pathological processes (eg, inflammation, demyelination, and neurodegeneration), better communication and collaboration between pathologists and MRI researchers is needed, including direct association between pathology and MRI close to autopsy, with the acquisition of MRI sequences of brain and spinal cord in situ and within a short time period post mortem.

Contributors

TK and AR drafted the section on white matter lesions. JJGG and PP drafted the section on grey matter lesions. KS and DC drafted the section on the spinal cord. WB and CE drafted the section on normal-appearing brain tissue. CS and MAR drafted the section on critical structures. SH and FF drafted the section on iron pathology. MF drafted the introductory and concluding sections and merged the different sections into the complete manuscript, which was commented on, revised, and approved by all other coauthors.

Declaration of interests

MF is editor-in-chief of the *Journal of Neurology*; received compensation for consulting services and speaking activities from Biogen Idec, Merck-Serono, Novartis, Teva Pharmaceutical Industries; and receives research support from Biogen Idec, Merck-Serono, Novartis, Teva Pharmaceutical Industries, Roche, the Italian Ministry of Health, Fondazione Italiana Sclerosi Multipla, and ARiSLA (Fondazione Italiana di Ricerca per la SLA). WB served on the scientific advisory board of Genzyme, Novartis, Biogen, and Teva Pharma; received speaker honoraria from Teva Pharmaceutical Industries, Sanofi, Genzyme, Novartis, Merck-Serono, Biogen, Roche, and Bayer Vital; is an editorial board member of *Therapeutic Advances in Neurological Disorders*, *Multiple Sclerosis International*, *Neuropathology*, and *Applied Neurobiology*; and received research support from Teva Pharmaceutical Industries, Novartis, Biogen Idec, Genzyme, MedDay, the German Research Foundation, the German Ministry for Education and Research, the Tschira Foundation, and the German Multiple Sclerosis Foundation. DC has had meeting expenses funded by Merck-Serono and Novartis; and has received research funding from the International Progressive MS Alliance, the MS Society of Great Britain and Northern Ireland, and the National

Institute for Health Research University College London Hospitals Biomedical Research Centre. JJGG is Editor for Europe at *Multiple Sclerosis Journal* and serves on the editorial board of *Neurology*; is President of the Netherlands Organisation of Health Research and Development; is an Executive Board member of the Netherlands Oragnisation of Scientific Research; and has received research funds from Novartis Pharma, Biogen Idec, and Sanofi-Genzyme. CE received funding for travelling and speaker honoraria from Biogen Idec, Bayer Schering Pharma, Merck-Serono, Novartis, Genzyme and Teva Pharmaceutical Industries, and Sanofi-Aventis; received research support from Merck-Serono, Biogen Idec, Teva Pharmaceutical Industries, and Sanofi-Aventis; and serves on scientific advisory boards for Bayer Schering Pharma, Biogen Idec, Merck-Serono, Novartis, Genzyme, Roche, Teva Pharmaceutical Industries, and Sanofi-Aventis. TK received speaker honoraria from Novartis and EXCEMED; received funding from the SFB-TRR 128 (German Research Foundation), European Leukodystrophy Association, International Progressive MS Alliance, and the Interdisciplinary Clinical Research Center Münster. PP received speaker honoraria from Biogen Idec, Novartis, and EXCEMED. ÁR serves on scientific advisory boards for Biogen Idec, Novartis, Sanofi-Genzyme, Icometrix, SyntheticMR, and OLEA Medical, and has received speaker honoraria from Roche, Bayer, Sanofi-Genzyme, Bracco, Merck-Serono, Teva Pharmaceutical Industries, Novartis, and Biogen Idec. KS reports being a principal investigator of trials sponsored by Biogen Idec, Roche, Teva Pharmaceutical Industries, and MedDay; involved in trials sponsored by Sanofi-Genzyme, BIAL, Cytokinetics, and Canbex; and receiving speaking honoraria from Biogen Idec, MSD, Merck-Serono, Novartis, Roche, Sanofi-Genzyme, and Teva Pharmaceutical Industries. CS reports personal fees for lecturing and advisory activity from Novartis, Bayer, Merck, and Roche, and has received grants from Teva Pharmaceutical Industries and research support by the German Research Foundation, the Gemeinnützige Hertie-Stiftung, and the German Multiple Sclerosis Society. MAR received speaker honoraria from Biogen Idec, Novartis, Genzyme, Sanofi-Aventis, Teva Pharmaceutical Industries, Merck-Serono, and Roche, and receives research support from the Italian Ministry of Health and Fondazione Italiana Sclerosi Multipla. FF and SH declare no competing interests.

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