

Association between pathological and MRI findings in multiple sclerosis

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The identification of pathological processes that could be targeted by therapeutic interventions is a major goal of research into multiple sclerosis (MS). Pathological assessment is the gold standard for such identification, but has intrinsic limitations owing to the limited availability of autopsy and biopsy tissue. MRI has gained a leading role in the assessment of MS because it allows doctors to obtain an ante mortem picture of the degree of CNS involvement. A number of correlative pathological and MRI studies have helped to define in vivo the pathological substrates of MS in focal lesions and normal-appearing white matter, not only in the brain, but also in the spinal cord. These studies have resulted in the identification of aspects of pathophysiology that were previously neglected, including grey matter involvement and vascular pathology. Despite these important achievements, numerous open questions still need to be addressed to resolve controversies about how the pathology of MS results in fixed neurological disability.

Introduction

Multiple sclerosis (MS) is an inflammatory demyelinating disease that affects the CNS by causing focal lesions in white and grey matter and diffuse and unevenly distributed changes in normal-appearing tissue. Pathological studies have helped to define the heterogeneous pathological substrates of this disorder, with the ultimate goal of identifying processes that might be targeted by therapeutic interventions. Although pathological assessment is regarded as the gold standard for such research, it is usually done at late stages of the disease (autopsies) or in selected and partly atypical early cases (biopsies). Additionally, pathological assessment usually provides only one snapshot in time, and therefore does not allow observation of the evolution of pathological changes over time.

Owing to its sensitivity to MS-related abnormalities, non-invasiveness, reproducibility, and repeatability, MRI has become important for the assessment of patients with MS. Additionally, several quantitative magnetic resonance (MR) techniques have been developed and are being used to estimate the degree of focal and diffuse CNS involvement in these patients. However, MRI provides only indirect information about neurological disorders and therefore it is essential to show that MRI findings correspond to pathological findings.

In this Review, we describe regions in which pathological and MRI assessment have provided concordant findings and regions with discrepancies for which additional research is needed, and discuss emerging pathological and MRI findings that might together enhance our understanding of disease pathophysiology and, as a consequence, help us to identify reliable in vivo markers to monitor different pathological aspects of MS. This material is drawn, in part, from presentations and discussions at an international workshop (Correlation between Pathological and MRI findings in MS) held in Milan on June 10–11, 2011.

Focal lesions

The pathological hallmark of MS is focal demyelination in regions called plaques or lesions, which harbour variable degrees of inflammation, demyelination, gliosis, and axonal injury. These are characteristic of the neuropathological diagnosis of the disease. On MRI using proton density and T2-weighted or fluid-attenuated inversion recovery (figure 1) scans, however, MS lesions appear as non-specific focal areas of signal increase and, therefore, resemble many other types of pathology. Characteristic features become more evident when we consider lesion appearance, location, and signal behaviour with additional MRI sequences. MS lesions in the brain are commonly round or ovoid and range from a few mm to more than 1 cm in size. Irregular areas of signal hyperintensity result from confluence of perivascular lesions. The site of lesions is crucial, because both pathological and MRI findings have shown that MS lesions have a high propensity to locate in the brainstem, cerebellum, and periventricular white matter.¹

Around 10–30% of T2 hyperintensities are also seen on mildly T1-weighted spin-echo images as areas of low signal intensity compared with normal white matter. In the acute phase, T1 hypointensity is probably a consequence of marked oedema and demyelination with or without matrix destruction and can completely disappear as inflammation abates. By contrast with these acute T1-hypointense lesions, chronic foci of T1 hypointensity—so-called persisting black holes (figure 1)—indicate areas with pathologically confirmed severe tissue destruction.² Large plaques and ring-enhancing lesions are more likely to evolve to T1 hypointensity in both the acute and chronic stages of MS than smaller plaques.

Pathological insights into MS lesions are mainly based on studies of archival autopsy tissues from individuals with longstanding chronic progressive MS (when relapses and MRI activity are not a prominent feature of the disease) or with early MS (when relapses and MRI inflammatory activity are common) in whom the

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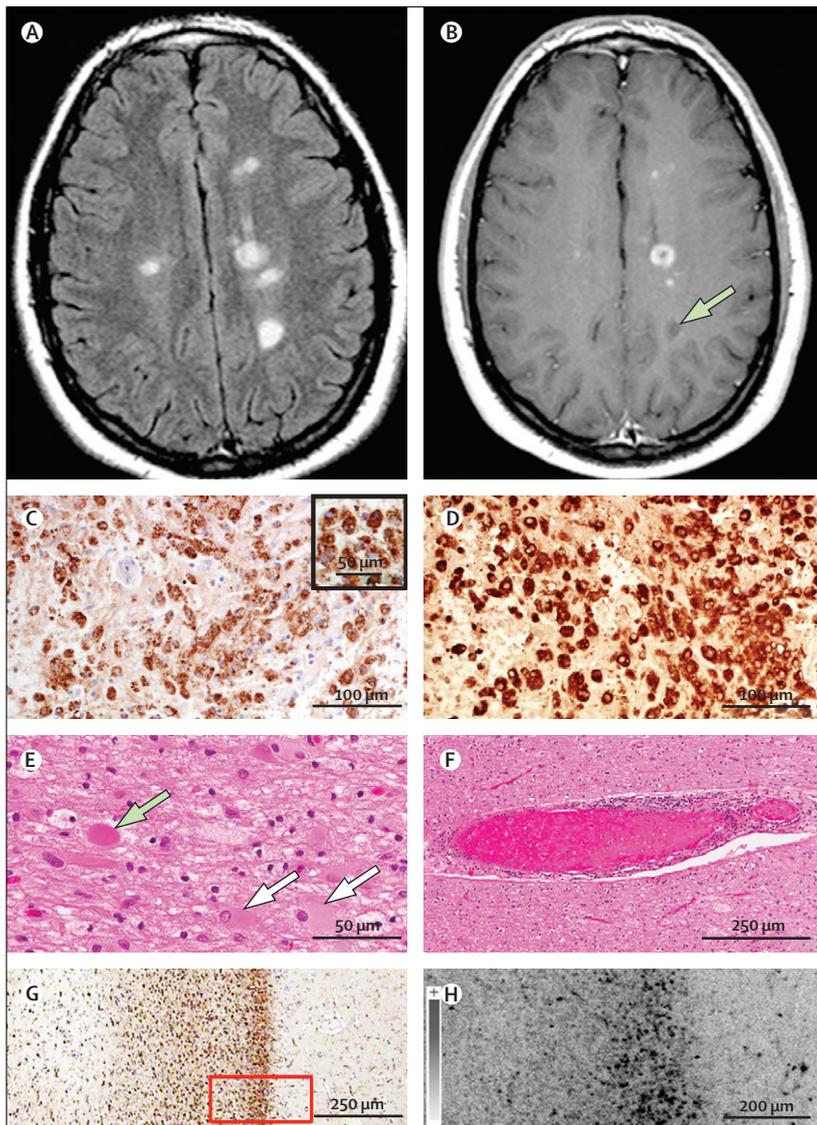


Figure 1: Brain lesions from patients with multiple sclerosis
 (A,B) Corresponding axial MRI scans slightly above the lateral ventricles. The fluid-attenuated inversion recovery sequence (A) shows multiple round-to-ovoid lesions in the periventricular and deep white matter. The T1-weighted scan after contrast material administration (B) shows enhancement of most of these lesions with either a nodular or a ring-like pattern; one lesion (arrow) is not enhancing and appears dark—ie, it is probably an old lesion and represents a so-called permanent black hole. (C–F) The pathology of an active lesion. (C) An active demyelinating lesion is evidenced by the presence of particles positive for myelin proteolipid protein within macrophages; inset shows macrophages involved in active demyelination at a higher magnification. (D) Sea of macrophages. (E) Reactive astrocytes (white arrows) and axonal swellings (green arrow). (F) Perivascular inflammation. (G,H) A chronic active lesion. (G) Active macrophages can be seen at plaque edge. (H) An iron map of the area boxed in part G collected with high-resolution x-ray fluorescence microprobe shows that most iron is located within macrophages; black-and-white scale represents the normalised total K α fluorescence counts, proportional to total metal present, from white (lowest) to black (highest).

lesion is heavily infiltrated by macrophages with myelin debris, lymphocytes, and large reactive, sometimes multinucleated, astrocytes called Creutzfeldt-Peters cells (figure 1). Oligodendroglia are often still present in lesions that show signs of remyelination. Despite partial axonal preservation, axonal injury occurs and is shown by axonal swellings (figure 1). The chronic inactive MS plaque is sharply circumscribed and hypocellular with no evidence of active demyelination. Fibrillary gliosis, loss of axons, and oligodendrocytes are prominent. Inflammation might still be present, especially perivascularly.

Conventional MRI can distinguish between acute or active and chronic or non-active lesions, but this separation is based primarily on the evidence of blood–brain barrier breakdown as indicated by contrast enhancement (figure 1). This distinction is somewhat arbitrary because several factors affect enhancement: gadolinium (Gd) dose, the delay and characteristics of image acquisition, and steroid treatment of acute attacks.⁴ Contrast enhancement persists for 2–6 weeks. Monthly MRI might therefore underestimate the degree of disease activity, since weekly MRI scanning suggests that a relatively large proportion of MS lesions have very short-lived enhancement.⁵ The enhancement pattern is variable: nodular or dense, ring-like or arc-like, and usually centripetal. These patterns probably depend on the size and intensity of the inflammatory area and the delay between injection of the contrast agent and scanning. They can change over time in the same lesion within minutes, because of the diffusion of the tracer, and over days, which is indicative of dynamic changes in lesion development.^{6,7}

Both pathological and MRI data suggest that MS lesions evolve differently during early versus chronic disease phases, and, within each phase, different types and stages of demyelinating activity are evident pathologically. A stringent definition of demyelinating activity within a plaque can be obtained by studying the myelin degradation sequence within macrophages: lesions are defined as early active, late active, inactive, early remyelinated, and late remyelinated.⁸ Several plaque subtypes can be distinguished on the basis of the presence and distribution of myelin-laden macrophages within MS lesions: acute active (macrophages contain early and late degradation products throughout the entire lesion, figure 1); chronic active (macrophages accumulate at the plaque edge, diminishing towards its inactive centre, figure 1); smouldering or slowly expanding (few myelin-laden macrophages present at the plaque edge); inactive (demyelinated hypocellular lesions without macrophages); and shadow (remyelinated) plaques. Active plaques predominate in acute and relapsing MS and might be the pathological substrate of clinical attacks. Inactive plaques and smouldering or slowly expanding plaques predominate in chronic progressive MS and slowly expanding plaques

might contribute to progression. Shadow plaques are found in both relapsing and progressive MS.

Whereas active MS lesions generally are assumed to show Gd enhancement, importantly, enhancement is probably absent in slowly expanding lesions in the progressive stages. Pathological studies have shown that in progressive MS inflammation and active tissue injury frequently occur in the absence of blood–brain barrier leakage.⁹ Detailed MRI–pathological correlations regarding these issues do not exist. However, this gap is at least partially filled by MRI studies using ultra-small superparamagnetic particles of iron oxide (USPIO, panel). USPIO enhancement was seen in areas without signal changes on T2-weighted images, sometimes in the absence of Gd enhancement, suggesting prelesional accumulation of monocytes preceding or independent of lesion formation. It often extended for a long time beyond Gd enhancement, which is the present marker for active lesions.¹⁰ These data suggest that infiltration of macrophages (and possibly lymphocytes) into the brain occurs through different mechanisms from blood–brain barrier damage. Also, some lesions that enhance with USPIO tend not to develop into persistent black holes, showing the pluriformity of inflammation and its potential contribution to tissue repair. Whether the use of USPIO provides additional clinically relevant information regarding disease activity in the brain of patients with MS is unclear.

A profound pathological heterogeneity of early active plaques in different patients with MS has been recorded; the injury target and mechanisms of demyelination are different in disease subgroups and at different disease stages with four distinct immunopatterns being described.¹¹ Distinctive corresponding patterns on MRI are not evident. Immunopattern heterogeneity seems to be recorded in the early stages of the disease but how long it persists into the chronic phase is not yet known. With disease chronicity, heterogeneous immunopatterns might converge into a homogeneous pattern that contributes to disease progression in the absence of ongoing relapses.

Diffusion tensor imaging (DTI) and magnetisation transfer imaging (MTI, panel) can quantify and characterise tissue changes on MRI (oedema, demyelination, tissue destruction) before, during, and after the evolution of a new MRI-detected lesion. Unfortunately, these abnormalities cannot yet be linked to specific pathophysiological substrates and can be detected only by analysis on a group level.¹² Substantial evidence, however, suggests that such abnormalities go beyond the presence of oedema. Parallel and supportive information comes from proton MR spectroscopy (¹H-MRS, panel), which has shown lipid peaks in normal-appearing white matter that might be associated with myelin breakdown before the development of an MS lesion with conventional MRI.¹³

Additional insights into the characteristics of MS lesions have been obtained from iron-sensitive MRI

sequences, especially at very high field strengths.¹⁴ These techniques have confirmed the presence of a peripheral ring of iron deposition around many acute, but also chronic MS lesions.¹⁵ Direct correlation between high-field MRI (7.0 Tesla [T]) and pathology showed a close match regarding iron deposition with these different approaches. Iron was present in the normal-appearing white matter predominantly within oligodendrocytes, whereas in active MS lesions it accumulated in macrophages at the edges of lesions. Additionally, perivascular iron deposits were seen, probably indicative of impairment of vascular permeability.¹⁶

Normal-appearing white matter

Normal-appearing white matter has been defined pathologically as macroscopically normal white matter that is microscopically normally myelinated and at least 1 cm away from a plaque's edge.¹⁷ This matter has to be differentiated from diffusely abnormal or dirty-appearing white matter, which includes areas of diffuse myelin pallor with ill-defined borders. Nowadays, diffuse pathology in the MS brain might be a more appropriate definition than focal lesional pathology. The diffuse pathology of normal-appearing white matter was originally described in 1979.¹⁷ Only 27.8% of the specimens of this matter were microscopically normal. The major histological abnormalities included gliosis, demyelination, small round cell infiltration, and the presence of macrophages. Besides these observations, microglial activation, sometimes in clusters of microglial cells with increased expression of MHC class I and II molecules, is also a prominent feature. These foci of microglial activation have also been designated as preactive lesions when they were identified as T2 hyperintense lesions on post-mortem MRI scans.¹⁸ Axonal density is decreased in normal-appearing white matter by 12–42%, depending on the area studied, when analysed in specific tracts of the spinal cord or the corpus callosum.^{19,20} Whether this reduction in axons is attributable to Wallerian degeneration after axonal transections in focal lesions or is an independent process has not been clarified. The same holds true for the prominent astrocyte activation seen in normal-appearing white matter (figure 2). Whether astrocyte activation is a protective or harmful process and whether it is a secondary or an initial primary event preceding lesion development is unclear. However, diffuse pathological abnormalities in the MS brain consisting of inflammation, microglial and astrocyte activation, myelin loss, and axonal damage correlate with the extent of cortical lesions but not with white matter lesion load.²¹ Pathologically, dirty-appearing white matter consists of extensive axonal loss, decreased myelin density, and chronic fibrillary gliosis, all of which are abnormal compared with normal-appearing white matter and different from focal white matter pathology.²² The results of a correlative MRI–pathology study have shown that with MRI, dirty-appearing white matter is a signal intensity on T2-weighted images that is higher than in the

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Panel: Glossary of imaging terms**DIR (double inversion recovery) sequence**

Two inversion times are used to suppress the signal from both white matter and CSF, yielding images that show superior delineation of grey matter; thus, the contrast is attributable to differences in T1 relaxation times between grey matter and CSF, and between grey and white matter.

DTI (diffusion tensor imaging)

Technique that allows in-vivo measurement of the molecular diffusion of water. A full characterisation of diffusion can be found in terms of a tensor, a 3×3 matrix, in which the on-diagonal elements represent the diffusion coefficients along the axes of the reference frame, while the off-diagonal elements account for the correlations between molecular displacement along orthogonal directions.

DT (diffusion tensor) MRI tractography

DT MRI tractography exploits the fact that axonal structures constitute a barrier to water diffusion, making it freer along the axis of a fibre than perpendicular to it. By tracking the principal diffusion direction, the direction of the primary eigenvector of the DT, the fibre bundle pathways can be reconstructed. DT MRI tractography is a promising technique for in vivo segmentation of the major fibre bundles in brain white matter.

FA (fractional anisotropy)

Scalar index derived from DTI that shows the degree of alignment of cellular structures within fibre tracts, and their structural integrity.

¹H-MRS (proton magnetic resonance spectroscopy)

Magnetic resonance (MR) technique that records signals from metabolites that are present in tissues at low concentrations. Resonances in MR spectra are identified primarily by their frequency (ie, position in the spectrum). The signal intensity (or amplitude) of each resonance (or spectral peak) is usually determined by the area under the peak and is a function of the number of nuclei that contribute to the peak in any particular volume element (voxel) of tissue. Thus the resonance intensity is proportional to the concentration (or density) of the metabolite in the voxel.

MD (mean diffusivity)

Scalar index derived from DTI equal to a third of the trace of the diffusion tensor, which is affected by cellular size and integrity.

MTI (magnetisation transfer imaging)

MTI is based on the interactions between protons in a relatively free environment and those where motion is restricted. Off-resonance irradiation is applied, which saturates the magnetisation of the less mobile protons, but this magnetisation is transferred to the mobile protons, thus reducing the signal intensity from the observable magnetisation.

MTR (magnetisation transfer ratio)

Quantitative index derived from MTI. Low MTR indicates a reduced capacity of the macromolecules in the CNS to exchange magnetisation with the surrounding water molecules.

USPIO (ultra-small superparamagnetic particles of iron oxide)

Contrast agents composed of iron particles, known as ultra-small particles of iron oxide.

surrounding normal-appearing white matter, but lower than in focal white matter lesions.²² MTI and DTI values in dirty-appearing white matter similarly show intermediate abnormalities.²³

MRI-defined normal-appearing white matter can be assessed from single or multiple MRI methods (eg, T2-weighted, T1-weighted, MTI, DTI). The sensitivity of

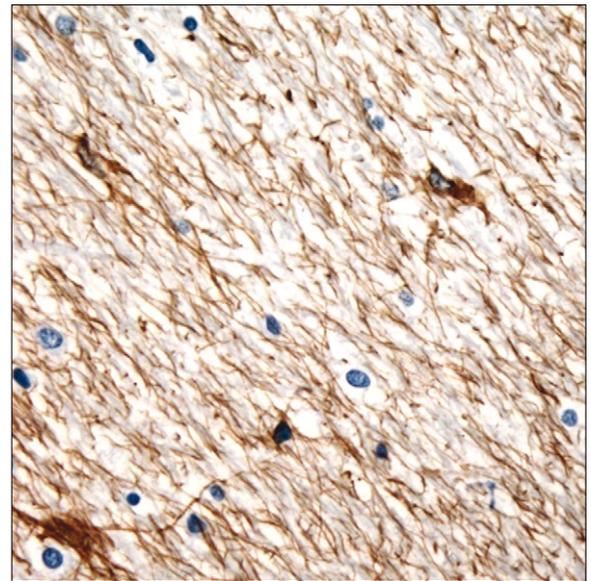


Figure 2: Immunocytochemistry for glial fibrillary acidic protein
Reactive astrocytes and fibre gliosis are present in normal-appearing white matter seen at the autopsy of a patient with multiple sclerosis.

MRI to pathology affecting proton environments results in MRI-defined normal-appearing white matter that does not strictly colocalise with normal-appearing white matter determined by pathology. MRI-guided histopathology of normal-appearing white matter has revealed axonal injury² and shown how it varies with other pathological characteristics depending on magnetisation transfer ratio (MTR, panel) and lesion proximity. In truly normal-appearing white matter (defined as normal on T1-weighted, T2-weighted and MTR scans) microglia were activated despite the absence of axonal pathology or myelin loss.²⁴ In nearly normal-appearing white matter (normal T1-weighted and T2-weighted, slightly reduced MTR), activated microglia were enlarged, no myelin loss was found, and axonal pathology was recorded only close to white matter lesions (suggesting a correspondence to pathologically defined dirty-appearing white matter).²⁴ The association between the slight MTR reduction and the increase in activated microglia, in addition to the association between a substantial MTR reduction and decreased myelin density, support MTR as a marker of tissue damage. MTR reduction in normal-appearing white matter and focal lesions is not related to the extent of gliosis, and it is more pronounced in demyelinated than in remyelinated lesions.²⁵ DTI might complement MTI in the quantification of the severity of tissue damage. In post mortem brains, relatively high correlations (r from -0.81 to 0.70) have been recorded between abnormalities of DTI indexes (ie, fractional anisotropy and mean diffusivity, panel) and myelin content and axonal count in the normal-appearing white matter and white matter lesions, with correlations being higher for anisotropy than for diffusivity indexes.²⁶

Normal-appearing white matter damage quantified using DTI correlates only partly with the extent of focal lesions and the severity of intrinsic lesion damage,^{27–29} which suggests that diffusivity changes in normal-appearing tissues are not entirely dependent on retrograde degeneration of axons that are transected in T2-visible lesions. MTI and DTI are also useful for assessment of the evolution of normal-appearing white matter damage in MS,¹² so their application in monitoring of the evolution of MS-related demyelination and axonal loss over time seems to be promising for possible future trials of neuroprotective therapies. Several approaches have been developed to assess the involvement of normal-appearing white matter at a regional level with MRI techniques, including voxel-wise methods and diffusion tensor tractography (panel). These approaches quantify the structural integrity of selected white matter tracts, and yield improved correlations with clinical manifestations of the disease.^{30–32}

Grey matter

Grey matter demyelination can be very extensive in MS, especially in the chronic phase of the disease.^{21,33} In terms of histopathology, grey matter lesions are different from white matter lesions: they frequently show little T-cell inflammation³⁴ or disruption of the blood–brain barrier³⁵ with leakage of plasma proteins. The description of a patient presenting with a clinically silent, incidentally found, and pathologically confirmed active demyelinating solitary cortical lesion has suggested that the non-inflammatory nature of chronic cortical demyelination might relate to long intervals between lesion formation and autopsy.³⁶ According to their location within the grey matter, different types of lesions have been identified.³⁷ What causes these lesions is poorly understood, and several pathogenetic mechanisms are being investigated.³⁸ Glutamatergic excitotoxic processes in the white matter, disrupted intra-axonal transport, and mitochondrial dysfunction have the potential to lead to axonal damage and a dying-back axonopathy, with secondary effects in the cortex. Alternatively, cortical demyelination might be primarily related to meningeal inflammation,³⁹ although this issue is still debated.⁴⁰ An extensive neuropathological study done with a large number of biopsies has shown that cortical demyelination is present and common early in MS, and that early cortical lesions are inflammatory and topographically associated with meningeal inflammation.⁴¹ The distribution of demyelination throughout the cortex in MS is generally large and random,³⁷ but some regions such as the cingulate gyrus seem to be more often involved than do others.⁴² Also, archicortical structures such as the hippocampus, deep grey matter areas,⁴³ and the cerebellar cortex⁴⁴ are heavily involved.

Focal cortical lesions are typically not seen on conventional MRI scans because they are small and have poor contrast with the surrounding normal grey matter,

and because of partial volume effects from the CSF. A correlative MRI–pathology study has shown that many cortical lesions are invisible on MRI.⁴⁵ Therefore, those cortical lesions that are visible are only the “tip of the pathological iceberg” (figure 3).⁴⁵ The use of double inversion recovery sequences (panel) has improved the sensitivity of MRI to detect cortical lesions *in vivo*.⁴⁶ However, at present, inter-observer agreement in the assessment of these sequences is moderate, suggesting a need for increased consistency in acquisition protocols across centres.⁴⁷ With use of double inversion recovery sequences, cortical lesions have been detected in all the major clinical phenotypes of MS, including patients with clinically isolated syndromes suggestive of MS.⁴⁸ In these patients, the quantification of cortical lesions at disease onset could help to identify patients at a high risk of

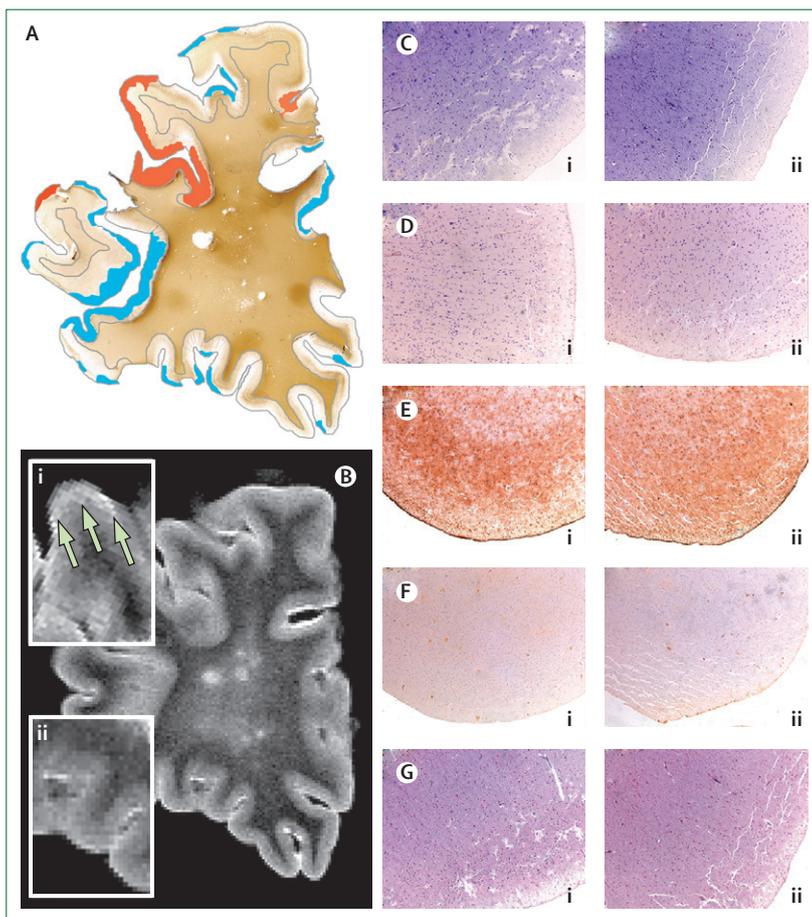


Figure 3: Comparison between MRI-visible and MRI-invisible cortical lesions

(A) Cortical lesions were assessed on proteolipid protein-stained tissue sections (original magnification $\times 0.7$), and after comparison with the corresponding MRI images, marked as visible (red) and invisible (blue).

(B) Corresponding proton density-weighted MR image of the same brain slice. (B1) MRI-visible lesion. Note the subtle signal intensity increase that can be detected after direct comparison with the proteolipid protein-stained tissue section. (B2) MRI invisible lesion. (C–G) MRI visible lesions did not differ from MRI-invisible lesions in terms of histopathology. Left column, (Ci–Gi) histological sections of the MRI-visible lesion shown in B1. Right column, (Cii–Gii) corresponding MRI-invisible lesion of B2. Sections (magnification $\times 200$) stained for neurons (C: Nissl stain; G: Bodian silver), antigen-presenting cells (D: HLA-DR), astroglia (E: glial fibrillary acidic protein stain), blood–brain barrier leakage (F: fibrinogen). Reproduced from Seewann and colleagues,⁴⁵ by permission of SAGE.

evolution to definite MS.⁴⁹ Longitudinal studies have shown that new cortical lesions continue to form in patients with early relapsing-remitting MS and in those with progressive disease phenotypes over 1–2 year periods of follow-up.^{50,51} An association has been recorded between volume of cortical lesions at baseline and progression of disability after 2 and 3 years (r from 0.38 to 0.51).^{50,51} The number and volume of cortical lesions have also been correlated with the severity of cognitive impairment (r from –0.59 to 0.60).^{52,53} The use of ultra-high-field scanners is likely to improve our ability to image cortical lesions. By use of a 7.0 T scanner, Mainero and colleagues⁵⁴ identified the three major lesion patterns described by pathologists (type I: leukocortical; type II: intracortical; and types III–IV: subpial extending partly or completely through the cortical layers). Disseminated subpial increases in T2* signal in patients with MS, which might be indicative of the diffuse subpial pathology described by neuropathologists, have also been described at 7.0 T.⁵⁵

MTI, DTI, and ¹H-MRS can provide accurate estimates of overall grey matter abnormalities and their modifications over time.^{12,56} Clearly, this includes not only diffuse changes, but also (small) focal lesions and, as a consequence, future studies are warranted to disentangle the relative contributions of each of these pathological aspects. MRI studies have shown that the extent and distribution of grey matter damage are only partly related to the presence of focal and diffuse abnormalities in white matter, suggesting that the processes at work in these two brain compartments are at least partly unrelated.⁵⁷ This notion confirms data obtained previously by pathological analysis of MS brains.²¹

The extent of grey matter damage varies substantially among the different clinical phenotypes of the disease, being more severe in patients with the progressive form than in those with the relapsing form of the disease.^{58,59} Assessment of the involvement of strategic grey matter structures could help to explain deficits in selective cognitive domains or specific disease-related symptoms, such as fatigue. Another mechanism that might explain the inter-patient variability of clinical manifestations is the presence and efficiency of functional reorganisation, which can be imaged with functional MRI (fMRI).¹²

Brain atrophy

Progressive loss of brain volume is an important feature of MS pathology, leading to massive brain atrophy with widening inner and outer CSF compartments, especially in the progressive stage of the disease. However, reduction of brain volume by tissue degeneration is in part counteracted by increased brain volume owing to inflammation and oedema. Thus, in treatment trials more extensive loss of brain volume is frequently present in early stages, indicative of decreased inflammation and oedema (pseudoatrophy).⁶⁰ Brain atrophy has various potential pathological substrates, although their

individual contributions to overall brain atrophy have not been identified. Shrinkage of lesion volume can be predicted in the course of the maturation of white matter lesions because of loss of myelin, oligodendrocytes and axons,⁶¹ and contraction of astrocyte volume, while maturing from protoplasmic gliosis in active lesions to fibrillary gliosis in inactive lesions. Atrophy of the normal-appearing white matter seems to indicate diffuse axonal loss, in part caused by Wallerian degeneration, which is attributable to focal plaques in the white matter and cortex. As discussed above, diffuse axonal damage and loss also occur in the normal-appearing white matter, independently of focal lesions. Neuronal and glial loss, as shown in cortical and deep grey matter lesions, is one possible substrate of grey matter atrophy.^{62,63} However, thinning of the cortical ribbon has also been recorded in areas devoid of demyelinated lesions and there neuronal loss is sparse or absent. Cortical atrophy was associated with a profound reduction in synaptic density, suggesting additional loss and atrophy of nerve cell processes and an impairment of cortical neuronal connectivity.⁶² In patients with MS with different disease phenotypes, brain volume quantified from T1-weighted images decreases on average by about 0.7–1.0% per year.⁶⁴ Grey matter atrophy occurs in the early stages of the disease,⁶⁵ is associated with clinical disability⁶⁶ and cognitive deterioration,⁶⁷ and tends to worsen over time.⁶⁸ The severity and distribution of atrophy vary in the different brain structures in different phases of the disease.^{69–71}

Spinal cord

The spinal cord is a highly organised and clinically eloquent structure that is often affected in MS. In view of its well characterised anatomy, the spinal cord in patients with MS lends itself to quantitative neuropathology and thus is a useful model to assess the relations between inflammation, demyelination, and axonal pathology. Results of a correlative study with MRI and histopathology showed that demyelination affects both the white and grey matter throughout the spinal cord in MS (figure 4).⁷² Whereas the cervical cord is the site of the most severe burden of white matter pathology, grey matter demyelination is proportionally more extensive and widespread throughout the spinal cord than is white matter demyelination.⁷³ Typically, MS spinal cord lesions are less inflammatory than lesions in the cerebral hemispheres and brainstem.⁷⁴ Remyelination is variable and can depend, to some extent, on the presence of inflammation.⁷⁵ Axonal loss in functionally important tracts, such as the corticospinal and sensory tracts, seems to be diffuse and size-selective (affecting mostly small fibres).⁷⁶ The relation between demyelination and axonal loss is, however, surprisingly weak.⁷⁷ Diffuse inflammation in normal-appearing white matter and meninges could be an important contributor to axonal pathology.⁷⁸ Substantial loss of anterior horn cells occurs throughout the spinal cord in MS and might be linked to neighbouring

inflammation and demyelination. Spinal cord atrophy is most pronounced in the cervical cord, mainly resulting from loss of white matter rather than grey matter volume, and is not affected by local lesion size.⁷⁹ This suggests that spinal cord atrophy only partly indicates axonal loss secondary to their destruction in the lesions, but also reflects diffuse axonal demise in the entire normal-appearing white matter.⁷⁸ This situation seems to be different from neuromyelitis optica, in which lesions are much more destructive and larger.

Most *in vivo* MR studies of the spinal cord have been done with 1.0 or 1.5 T scanners, which might have affected the ability to detect involvement of grey and white matter. Spinal cord MRI is an important diagnostic instrument because focal lesions are common in early MS, and rare in other neurological diseases with non-specific brain lesions, such as small vessel disease.⁸⁰ Involvement of the spinal cord can be asymptomatic and is even recorded in clinically and radiologically isolated syndromes.⁸¹ It varies between patients, ranging from multiple focal lesions to confluent areas of high T2 signal intensity. Intriguingly, results of a correlative MRI–pathological study at 4.7 T showed that MRI measures of spinal cord lesion load do not correlate well with clinical measures of disability and axonal loss.⁸² In some patients, particularly those with primary progressive disease, diffuse areas of increased T2 signal intensity throughout the spinal cord can accompany clinical disability; such abnormalities might be captured better by quantitative MR techniques such as MTI and DTI. The high sensitivity of MRI to MS-related injury in the spinal cord is counterbalanced by an absence of histopathological specificity;⁸⁰ however, post-mortem MRI at 4.7 T has shown an excellent agreement with histopathology in detection of both grey and white matter lesions.⁸³ Spinal cord atrophy correlates well with concurrent measures of disability (r from -0.7 to -0.49).^{84,85} However, suboptimal spatial resolution has limited its sensitivity in detection of clinically relevant longitudinal changes in cord area. In concordance with pathological reports, the extent of cord atrophy on MRI is unrelated to spinal cord lesion load and is independent of brain lesions and brain atrophy.⁸⁶

Optic nerve

At present, no correlative MRI–pathological study of the optic nerve exists. Additionally, few pathological studies of the optic nerve in MS have been done, despite the occurrence of optic nerve lesions in almost all patients with MS.⁸⁷ Pathological studies of the visual pathways show potential in terms of increasing our understanding of MS pathogenesis, owing to the unique features of optic nerve anatomy. For instance, why specific sites, such as the optic nerve, show a predilection to develop MS lesions is still unclear. Additionally, many questions regarding the presence of inflammation and neurodegeneration in the retina, a structure that has no myelin,

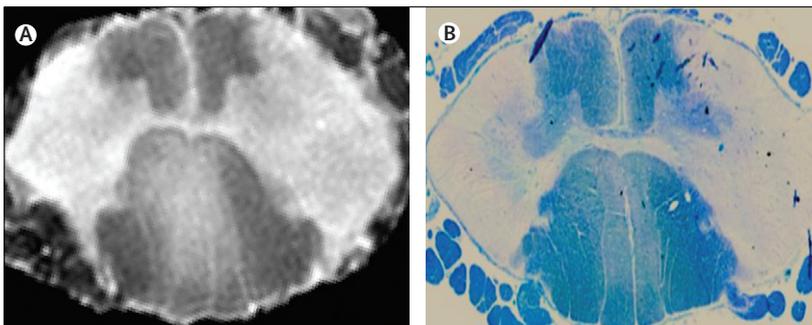


Figure 4: Post-mortem high-resolution proton density MRI

Shows high-signal-intensity abnormality in the lateral white matter columns bilaterally with partial involvement of the central grey matter. A mild increase in signal intensity in the posterior columns is also noted (A). The corresponding histology section stained by Luxol Fast Blue Cresyl Violet for myelin (B) shows excellent agreement with post-mortem MRI in detecting both grey and white matter lesions. Adapted from Nijeholt and colleagues,⁷² by permission of Oxford University Press.

are unanswered. Progressive neurodegeneration, recorded in other CNS tracts, is less well described in the optic pathways, raising questions about the mechanisms of specific tract vulnerabilities.

In vivo imaging methods provide inferential data about optic nerve pathology, and when investigated in combination with clinical (visual function) and electrophysiological (visual evoked potential) measures, pathophysiological mechanisms can be explored. Optic coherence tomography measures retinal nerve fibre layer thickness, and because the axons are unmyelinated, this measure provides evidence for axonal loss. Such studies in optic neuritis show variable axonal loss (mean about 20%), the extent of which is correlated with the degree of persistent visual dysfunction and cerebral atrophy on MRI.⁸⁸ Smaller amounts of loss of retinal nerve fibre layer thickness are also recorded in patients with MS who have not had a previous episode of optic neuritis.⁸⁹

The optic nerve can be imaged using MRI.⁹⁰ Inflammation in acute optic neuritis is inferred by enhancement of the symptomatic lesion and serial studies indicate that inflammation *per se* contributes to the acute visual deficit but has little effect on final visual outcome. Demyelination in the symptomatic optic nerve lesion has been inferred from reductions in MTR and prolongation of visual evoked potentials. Both of these measures have shown a tendency to recover after an episode of optic neuritis, consistent with remyelination.⁹¹

Vascular pathology in the MS brain

As in other chronic inflammatory diseases of the CNS, vascular pathology is profound in the brains of patients with MS, in particular in lesions but also in normal-appearing white matter.⁹² Inflammation in active lesions is associated with upregulation of adhesion molecules and the expression of chemokines and their receptors on endothelial cells and partly in the perivascular space. Dysfunction of the blood–brain barrier is shown by changes in endothelial tight junctions. In chronic lesions,

Search strategy and selection criteria

References for this Review were identified through searches of PubMed with the search terms “multiple sclerosis”, “MRI”, “pathology”, “post mortem”, “lesions”, “normal-appearing white matter”, “grey matter”, “spinal cord”, and “optic nerve” from 1979 until December, 2011. 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.

profound enlargement of the perivascular space and vascular fibrosis, shown by increased perivascular extracellular matrix proteins (vascular fibrosis), is common.^{93,94} However, the relation between inflammation, blood–brain barrier damage, and structural vascular pathology is complex.⁹ In highly inflamed active MS lesions, inflammation in general is associated with serum protein leakage, but damage to the blood–brain barrier is likewise recorded in many vessels that lack inflammatory infiltrates, suggesting increased endothelial permeability induced by proinflammatory cytokines released from inflammatory cells within the plaques. In the progressive stage of MS, serum protein leakage from vessels is much less severe than in active lesions arising at early stages of the disease. Moderate serum protein leakage is, however, frequently recorded in vessels with perivascular fibrosis in the absence of inflammation. Additionally, many vessels in slowly expanding lesions and in the normal-appearing white matter are surrounded by inflammatory infiltrates in the absence of disturbance to the blood–brain barrier. This situation is not only the case in parenchymal lesions but also in inflammatory aggregates in the meninges.⁹⁵ Brain tissue perfusion *in vivo* can be assessed with MRI. Whereas enhancing lesions typically show increased perfusion, chronic MS lesions show decreased perfusion.⁹⁶ Widespread hypoperfusion in normal-appearing white matter and in cortical and deep grey matter has been recorded in patients with relapsing-remitting MS and progressive MS.⁹⁷

Future directions

Issues that should attract more attention in future MRI studies that aim to define *in vivo* the pathological substrates of MS are: improvement of imaging specificity, longitudinal assessment, high-resolution image acquisition, and combination of imaging methods. Advanced neuroimaging techniques that have the potential to resolve the heterogeneity of white matter lesions, normal-appearing white matter damage, and grey matter damage are also warranted. For example, some studies attempted to detect and quantify iron *in vivo*. Such techniques will have to be validated not only by direct histopathological assessment, but also by comparison with non-MRI methods (eg, x-ray

fluorescence, figure 1) that, unlike histopathology, are very sensitive and specific for visualising and quantifying all tissue iron, irrespective of its location, oxidation form, chemical ligands, or paramagnetic properties.⁹⁸

An aspect of focal MS lesions for which future correlative studies between MRI and pathology are urgently needed is remyelination. Pathological evidence suggests that the extent of remyelination between different lesions is very heterogeneous, with some showing nearly complete remyelination and others (most) lacking. Some data provide evidence that remyelination can in principle be visualised by voxelwise MTR analysis,⁹⁹ T2-weighted high-field MRI imaging,¹⁰⁰ or PET.¹⁰¹ How reliable these techniques are in a clinical setting is unclear. However, any clinical trials aimed at improving remyelination depend upon previous identification of lesions that do or do not remyelinate spontaneously.

Another key issue is the need to track accurately changes from truly intact white and grey matter areas to damaged regions. Data for diffuse CNS pathology in patients with early MS are still scarce, and data for the longitudinal evolution of normal-appearing white matter MRI metrics in patients with MS are incomplete. Crucial questions about normal-appearing white matter are how much its pathology contributes to the overall brain damage recorded in MS and how much is primary or secondary. An important advance in relating MRI and pathology in normal-appearing white matter would be the detection of such tissue undergoing inflammation *in vivo*.

MRI scanners at ultra-high field (≥ 7.0 T), when using the appropriate radio-frequency coils and intensity uniformity correction, afford advantages in signal-to-noise ratio, image contrast, and resolution. *In vivo* imaging at 7.0 T, for example, is safe, well tolerated, and able to provide high-resolution anatomical images allowing visualisation of structural abnormalities located within or near the cortical layers. Furthermore, *in vivo* ¹H-MRS can also benefit from increased signal-to-noise at ultra-high field, allowing accurate measurements of additional metabolites (glutathione, glutamate, GABA, and ascorbic acid) and thus possibly providing important insights into specific biological pathways to investigate the roles of neurodegeneration, tissue repair, and oxidative stress in MS.¹⁰²

Finally, the incorporation of non-conventional MRI techniques into ultra-high-field clinical scanners and their combined use with other advanced neuroimaging techniques (ie, integrated PET-MRI) will probably be the most efficient way to provide a more complete description of the dynamics responsible for pathological changes in this complex disorder.

Conclusions

This workshop, focused on the disciplines of neuropathology and neuroimaging in relation to MS, reached consensus on most of the questions investigated by

recent studies. New methods and techniques must be developed in neuroimaging to come as close as possible to visualisation of pathologically defined structural abnormalities in the brains of patients with MS. This advance is a prerequisite to move into new areas, such as analysing large patient cohorts at different stages of the disease and obtaining information about dynamic changes within lesions over time. These important aspects cannot be addressed by pathological studies. Additionally, many examples of new and unexpected findings, obtained through MRI, have forced pathologists to reinvestigate their material and to describe new entities and interpretations. Important examples are most quantitative studies on lesion load in different brain areas in comparison to clinical aspects and disease development, the detailed studies on neurodegeneration in MS lesions and normal-appearing white matter, the new definition of grey matter pathology in MS brains, and the focus on tissue abnormalities that precede the appearance of new white matter lesions. MRI measures with improved pathological specificity provide an instrument to study in vivo the complete CNS involvement over time, allowing a glimpse of the complexity of the pathological puzzle combining to give clinical impairment over the decades-long trajectory of the disease. As discussed here, numerous open questions that have to be addressed remain, and controversies need to be resolved. The close collaboration and interaction between neuroimaging researchers and neuropathologists, which has developed during the past decade, will undoubtedly provide answers to questions raised by neurologists and lead to new insights relevant for understanding of MS mechanisms and monitoring of treatment in this disease.

Contributors

CL, BFP, FF, and BJE drafted the section on lesions. WB and JC drafted the section on normal-appearing white matter. JJG and MAR drafted the section on grey matter. GDL and FB drafted the section on spinal cord. NE and DHM drafted the section on optic nerve. DP and NDS drafted the section on future directions. MF and HL drafted the introductory and concluding sections and merged the different sections into the complete manuscript, which was commented on, revised, and approved by all the co-authors.

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Conflicts of interest

MF is on scientific advisory boards for Teva Pharmaceutical Industries and Genmab A/S; has received funding for travel from Bayer Schering Pharma, Biogen-Dompè, Genmab A/S, Merck Serono, and Teva Pharmaceutical Industries; is on editorial boards of the *American Journal of Neuroradiology*, *BMC Musculoskeletal Disorders*, *Clinical Neurology and Neurosurgery*, *Erciyes Medical Journal*, *Journal of Neuroimaging*, *Journal of Neurovirology*, *The Lancet Neurology*, *Magnetic Resonance Imaging*, *Multiple Sclerosis*, and *Neurological Sciences*; is as a consultant to Bayer Schering Pharma, Biogen-Dompè, Genmab A/S, Merck Serono, and Teva Pharmaceutical Industries; serves on speakers' bureaus for Bayer Schering Pharma, Biogen-Dompè, Genmab A/S, Merck Serono, and Teva Pharmaceutical Industries; and receives research support from Bayer Schering Pharma, Biogen-Dompè, Genmab A/S, Merck Serono, Teva Pharmaceutical Industries, and Fondazione Italiana Sclerosi Multipla. MAR is as consultant to Bayer Schering Pharma; has received speakers' bureaus for Biogen-Dompè; and receives research support from the Italian Ministry of Health. FB serves on scientific advisory boards for Lundbeck, Bayer Schering Pharma, Sanofi-Aventis, UCB, Novartis, Biogen Idec, BioMS Medical, Merck Serono, and GE Healthcare; is on the editorial boards of *Brain*, *Journal of Neurology*, *Neurosurgery and Psychiatry*, *European Radiology*, *Journal of Neurology*, and *Neuroradiology*; has received speaker honoraria from Novartis, Merck Serono, and BioClinica; is a consultant for Sanofi-Aventis, UCB, Novartis, Biogen Idec, BioMS Medical, Medicinova, and GE Healthcare; and receives research support from the Dutch MS Research Foundation. WB receives research support from Teva Pharmaceutical Industries, BiogenIdec, Novartis, and Bayer Schering Pharma; is a member of scientific advisory boards of Teva Pharmaceutical Industries, Biogen Idec, and Sanofi-Aventis; serves on speakers' bureaus for Bayer Schering Pharma, BiogenIdec, Merck Serono, Teva Pharmaceutical Industries, Sanofi-Aventis, and Novartis; and is on editorial boards of *Acta Neuropathologica*, *Journal of Neurology*, and *Therapeutic Advances in Neurological Disorder*. JTC is as a consultant for NeuroRx Research. GC has received personal compensation for activities with Teva Neuroscience, Merck Serono, Bayer-Schering, Novartis, Sanofi-Aventis Pharmaceuticals, and Biogen-Dompè as a consultant, speaker, or scientific advisory board member. GCDL has received honoraria and travel expenses as an invited speaker for Bayer Schering and Teva Pharmaceutical Industries; and is supported by an AANF/CMSC John F Kurtzke Clinician-Scientist Award and a Goodger Scholarship (University of Oxford). NDS serves on scientific advisory boards for Merck Serono and Novartis; has received funding for travel from Merck Serono, Novartis, and Teva Pharmaceutical Industries; and has received speaker honoraria from Novartis, Teva Pharmaceutical

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