Evidence for neuroprotection and remyelination using imaging techniques

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ABSTRACT MRI is used routinely in clinical practice and pharmaceutical trials to measure disease activity and assess the effects of treatment in multiple sclerosis. Conventional MRI techniques sensitively detect inflammation, demyelination, and tissue injury. Less conventional imaging modalities, such as magnetic resonance spectroscopy and magnetization transfer imaging, and advanced image processing to quantify structural changes can provide more specific and inherently quantitative markers of the pathologic processes underlying the accumulation of disease burden and the progression of clinical disability. Together, these techniques can assess both the anti-inflammatory and the neuroprotective effects of immunomodulatory therapies. **NEUROLOGY 2007;68 (Suppl 3):S83–S90**

Multiple sclerosis (MS) is a disease of the CNS characterized by inflammation, demyelination, axon degeneration, and inadequate repair, leading to irreversible neurologic dysfunction and clinical deficits (figure 1).[1] The specific immunopathogenetic mechanisms implicated in the etiology of this disease and its progressive course are poorly understood. However, a growing body of evidence suggests that neuron injury in MS is a consequence of inflammation, which contributes to myelin destruction, axon injury, matrix destruction, and secondary neurodegeneration of compromised central axons and supporting glia.[2,3] In this conceptual framework, a treatment model that disrupts these pathologic cascades by modulating acute neuron injury and limiting axon degeneration might delay progression of clinical disability. Therefore, a therapeutic approach that modulates inflammation, enhances neuron repair (by remyelination and axon recovery), and prevents neuron degeneration would be the most beneficial to patients over the long term.

MRI is used routinely in MS to facilitate diagnosis, assess focal inflammatory disease activity, measure the burden of disease, and monitor the effects of treatment. MRI is more sensitive to the evolution of focal white matter lesions in MS than are clinical measures. Indeed, standard T2-weighted images are particularly sensitive to inflammation, edema, demyelination, and axon loss.[4] Thus, conventional MRI may provide early evidence of acute and chronic, primarily focal pathologic changes,[5] even when these lesions are clinically silent.[6] For these reasons, MRI outcome metrics are often used in clinical trials to assess therapeutic efficacy, at least as far as focal inflammatory activity is concerned.[7–10]

However, neither conventional nor enhanced MRI imaging can distinguish between potentially reversible and irreversible lesion pathology. These techniques are also insensitive to axon injury in normal-appearing brain tissue (NABT), which contributes to clinical disability.[11] As a consequence, these imaging metrics are only moderately correlated with clinical measures of disease activity.[14] Newer MRI techniques, including magnetic resonance spectroscopy (MRS), magnetization transfer ratio (MTR) imaging, and quantitative analysis of changes in brain volume, are more reliable surrogate markers of axon and myelin pathology associated with accumulating burden of disease and progression of disability. They may also show structural changes associated with neurodegeneration, as well as evidence of remyelination and neuroprotection stemming from pharmacotherapy.[12]

This review addresses the potential of MRS, MTR imaging, and measurement of brain volume for assessing injury to axons and myelin as well as neurodegeneration. It also examines the effective-

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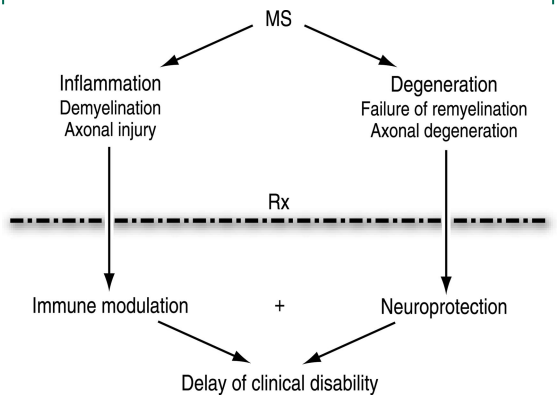
Figure 1



Schematic overview of the inflammatory and degenerative contributions to neural injury in MS that result in accumulation of sustained disability. Optimal therapy should, in the long term, modulate inflammation, enhance remyelination and axon recovery in lesions, and prevent the degeneration that ultimately develops in MS. Adapted from Stys,[2] with permission.

ness of currently approved immunomodulatory therapy to limit and potentially to reverse neurodegeneration and promote axon remyelination, as demonstrated by these advanced MRI techniques. The conclusion is drawn that advanced imaging techniques are capable of quantitatively assessing the temporal evolution of injury and repair of myelin and axons, and that some of the neuroprotective effects of disease-modifying drugs may be fully evidenced only years after injury because of the long time required for degeneration of neurons in the human CNS.

IMAGING FOCAL WHITE MATTER INJURY

Magnetic resonance spectroscopy. MRS allows specific in vivo observation of the spatiotemporal evolution of the two primary pathologic processes of MS: active inflammatory demyelination and neuron/axon injury.[12,13] Elevations of lactate, choline, and lipids are associated with acute inflammatory demyelination. Relative decreases in *N*-acetyl aspartate (NAA) indicate neuroaxon injury or loss.[5] Decreased NAA in the normal-appearing white matter (NAWM) may result from degeneration of axons transected within focal demyelinated lesions (in either the white or gray matter) or from more diffuse axon injury associated with diffuse inflammatory or degenerative processes.[3,14,15]

Longitudinal MRS studies indicate that myelin breakdown occurs during the initial inflammatory stage of lesion development, even before the lesion appears on MRI.[16] In acute demyelinating lesions, large resonances of lactate, choline, and (at short echo times) lipids and myoinositol may be observed.[5,17–19] These resonances are indicative of active inflammation and neuronal metabolic dysfunction. The ratio of NAA to creatine (Cr) is reduced in acute lesions as well as in NAWM. The decrease in NAA is partially reversible, which suggests that reversible axonal mitochondrial injury may be a mechanism of reduction in the density of the resonance intensity of NAA.[20,21]

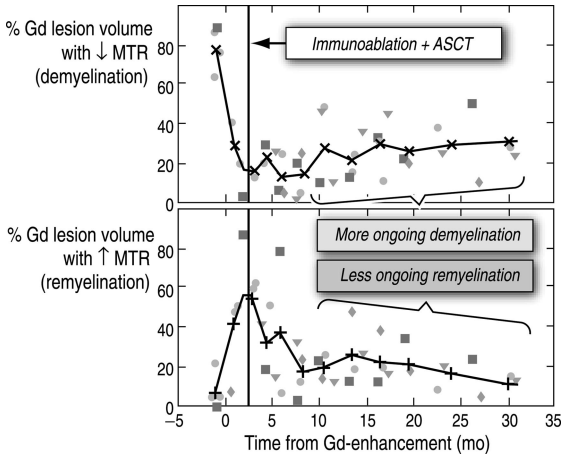
Decreased NAA resonance intensity in NAWM is also associated with functional impairment. Twenty-nine patients with relapsing–remitting MS (RRMS; n = 11) or secondary-progressive MS (SPMS; n = 18) were followed for 30 months.[22] At the end of the study, there was a trend toward decreased central brain NAA:Cr ratio for the 11 RRMS patients and significant correlation between change in NAA:Cr ratio and Expanded Disability Status Scale (EDSS) score (i.e., a lower NAA:Cr ratio related to a higher EDSS score). Moreover, this correlation was even more evident in patients who experienced relapse during follow-up.[23] Therefore, reductions in NAA on MRS suggest that axon injury is an important determinant of long-term disability in MS patients.

MAGNETIZATION TRANSFER

Magnetization transfer (MT) is a physical phenomenon that results from interactions and exchanges between magnetized protons in water that are unrestricted in their molecular motion and those that are restricted because of their association with macromolecules. The latter have a much shorter T2 relaxation time and broader resonance, which makes it possible to selectively saturate their magnetization with an appropriate off-resonance pulse. The acquisition of two images, one obtained with the magnetization transfer saturation pulse turned on and the other with it turned off, can be used to generate a calculated MTR image in which the signal intensity of each voxel is determined by the percent magnetization transfer in that voxel. A decrease in the MTR, which reflects a reduction in the exchange of magnetization of protons that are tumbling freely and those that are bound to macromolecules, is evidence of demyelination in cerebral white matter. MTR imaging is sensitive to both microscopic and macroscopic pathology and provides quantitative data on the extent of myelin loss in MS.[24]

Longitudinal MT imaging shows an acute decrease in MTR associated with gadolinium (Gd) enhancement. On average, the MTR recovers partially from this decrease over the following 4 to 6 months,[25] although the evolution of individual lesions and individual lesion voxels is heterogeneous.[26,27] In the case of initial decrease followed by partial recovery, acute demyelination appears to be followed by subsequent repair and partial remyelination. There also is evidence that MT studies are capable of detecting changes over time in white matter areas that appear normal on conventional MRI[28] and that subtle changes in MTR precede lesion appearance on conventional MRI.[29] In patients with

Figure 2 Post-acute phase favors decrease in MTR. ASCT, autologous stem cell transfer; MTR, magnetization transfer ratio. From Chen et al. (unpublished), with permission



clinically isolated syndromes (CIS) suggestive of MS, greater decrease in MTR in NABT correlates with higher risk for subsequent development of clinically definite MS.[30] MTR is also predictive of subsequent disability in patients with RRMS, in whom lower MTR values are associated with poorer long-term outcomes.[31] These studies demonstrate that MT imaging might provide important prognostic information about the progression of disease and degree of disability in MS.

Our group is examining the relation between change in MTR and acute inflammatory demyelination in a clinical trial of immunoablation and autologous stem cell transfer (ASCT) for aggressive MS. Looking at MRI lesion volume over time, we found that, at baseline, 80% of Gd-enhancing lesion volume showed a decrease in MTR consistent with acute demyelination.[32] There was little or no evidence of remyelination at this time. At 2 months after treatment, most of the demyelination (70% to 80%) had ceased and almost half of the Gd-enhancing lesion volume showed increasing MTR consistent with remyelination. This pattern of concurrent demyelination and remyelination continued for at least 30 months after ASCT (figure 2).

The interpretation of these observations of voxel-based changes in MTR as indicating demyelination and remyelination were confirmed by post-mortem histopathology of a patient who died during the trial (figures 3A, B). Overall, these preliminary findings confirm that MTR is a highly sensitive method for measuring both myelin injury and myelin repair after treatment and thus for revealing evidence of this aspect of neurodegeneration and neuroprotection.

BRAIN AND SPINAL CORD ATROPHY Irreversible brain and spinal cord atrophy reflect irreversible tissue loss. Although the precise mechanisms are unknown, CNS atrophy appears to result from the effects of inflammation, leading to demyelination, neuroaxon injury, and Wallerian degeneration.[33] These pathologic substrates are both multifocal (present in MRI-visible lesions) and diffuse throughout the CNS. Imaging research has helped to clarify the relation between these dynamic inflammatory processes and loss of brain tissue.

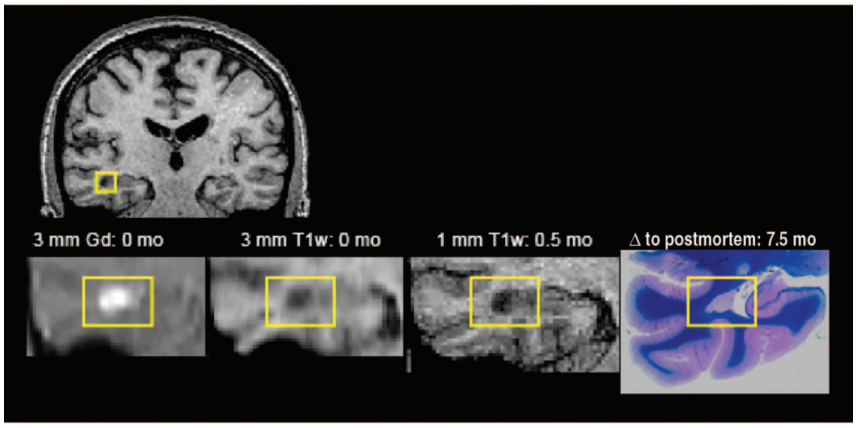
Brain white matter consists largely of axons (46%) and myelin (24%). Progressive atrophy implies loss of these structures; therefore, measurement of CNS atrophy should provide a sensitive indication of neurodegeneration.[34] Chard et al.[35] explored the temporal relation between brain lesion burden and atrophy in MS. They followed 28 patients with CIS, RRMS, or SPMS for 14 years after onset of symptoms. They found that change in lesion burden in the first 5 years correlated more closely with disease-related brain atrophy (as measured by brain parenchymal fraction [BPF]) at 14 years than did later changes in the number of contrast-enhancing lesions. In a related study, Richert et al.[36] found that the rate of CNS atrophy was correlated with the frequency of contrast-enhancing lesions (figure 4). Therefore, brain atrophy is dependent, at least in part, on focal inflammation, demyelination, and axon loss. These findings also suggest that patients who do not respond to treatment with a decrease in the number of contrast-enhancing lesions may be at risk for development of greater whole-brain volume loss over time. It appears that the influence of focal inflammation on atrophy is more important earlier in the course of MS than later in the secondary-progressive phase of the disease.[37]

One mechanism linking focal inflammatory lesion load to brain atrophy is Wallerian degeneration. Axon injury distal to acute demyelinating lesions can be seen with diffusion tensor imaging (DTI),[38] and transected axons are a consistent feature in brain lesions of patients with MS.[39]

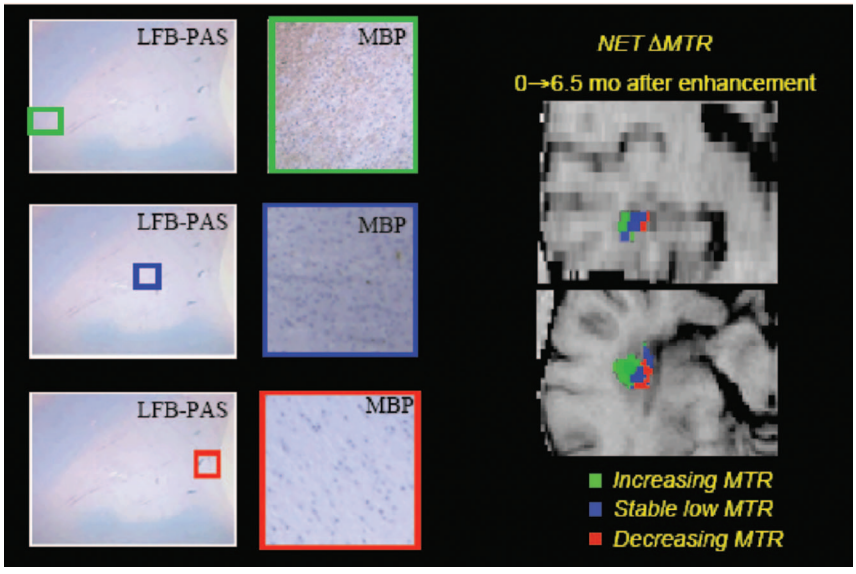
MRS studies also indicate that markers of axon damage, such as the NAA:Cr ratio in deep central brain, as well as brain atrophy, are highly correlated with measures of diffuse occult disease in NABT ($r = 0.67$, $p < 0.001$ for NAA:Cr ratio and $r = -0.58$, $p = 0.007$ for normalized brain volume).[40] NAA declines faster earlier in the course of MS, whereas global brain atrophy accelerates later; this suggests that neuroaxon injury precedes parenchymal loss.[41] Taken together, these observations sug-

Figure 3

A MRI showing the region of the lesion in question and histopathology (LFB stain) of the same region at similar magnification. LFB, Luxol fast blue. From Chen el al. (unpublished), with permission.



B Immunohistochemistry showing evidence of remyelination and demyelination in the same lesion regions detected by voxel-based MTR. LFP-PAS, Luxol fast blue-periodic acid Schiff; MBP, myelin basic protein. From Chen et al. (unpublished), with permission.

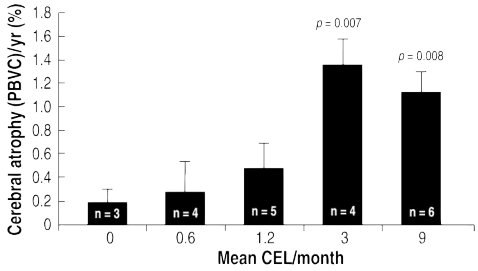


gest that inflammatory damage in NABT is an important factor in global neurodegeneration.

NEUROPROTECTIVE EFFECTS OF IMMUNOMODULATORY THERAPY

Lesion suppression. Contrast enhancement is associated with the acute inflammatory stage of lesion development and is an MRI marker of disease activity.[25] Immunomodulatory therapies, including glatiramer acetate (GA), interferon (IFN)-β, and the humanized anti-adhesion monoclonal antibody natalizumab, are all effective in reducing MRI-measured disease activity (figure 5).[7,25,42–44] For example, patients who received GA showed significant reduction in the total number of Gd-enhancing lesions, the number of new Gd-enhancing lesions, T2-weighted lesion volume, and the number of new T2-weighted lesions

Figure 4 Relation between cumulative contrast-enhancing lesions (CEL) and change in brain volume. From Richert et al.,[36] with permission



seen on MRI, compared with placebo (figure 6).[7] Glatiramer acetate also suppressed the evolution of acute Gd-enhancing lesions into persistent T1-weighted hypointense lesions, often called chronic "black holes," thereby disrupting the progression of tissue destruction in MS lesions after the resolution of enhancement (figure 7).[45] Similar findings were reported in a placebo-controlled study of 213 MS patients who received natalizumab.[46]

Disease-modifying therapies (DMTs) can also enhance recovery within lesions. Richert et al.[25] used MT imaging to compare the effects of glucocorticoids (methylprednisolone IV [MPIV]), IFN-β1b SC, and placebo in patients with RRMS. After enhancement, those lesions treated with IFN-β1b and short-term MPIV showed improved recovery after 12 to 18 months. The extent of lesion recovery as measured by MT imaging was greater with glucocorticoids than with IFN-β1b. The investigators proposed that MPIV, which can penetrate the blood–brain barrier and rapidly achieve high cerebrospinal fluid concentrations, may inhibit demyelination and promote remyelination within MS lesions.

Brain atrophy. By injuring myelin and axons, episodic focal inflammation in brain tissue during the initial stages of MS may result in accumulation of irreversible disability. As MS evolves, degeneration of myelin and axons in NABT also occurs, and this degeneration appears to become the major determinant of progressive disability. Both of these pathologic processes are associated with loss of brain tissue (atrophy).

Pivotal clinical trials of IFN-β1a (IM) and GA have shown that these DMTs inhibit brain volume loss over time. In a retrospective analysis of the pivotal 2-year clinical trial of IFN-β1a IM in RRMS, Rudick et al.[47] reported that MS patients in both study groups experienced significant brain atrophy, which worsened during the period of observation. However, active treatment was associated with a 55% reduction in the rate of whole-brain atrophy

Figure 5 Immunomodulatory therapies reduce Gd enhancement. PBO, placebo

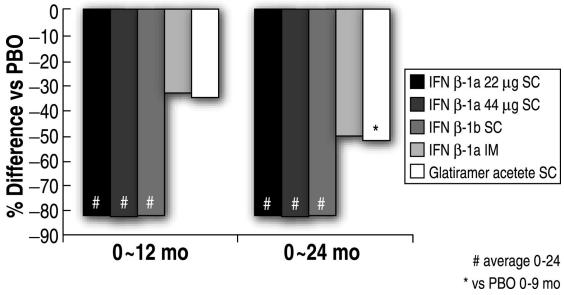
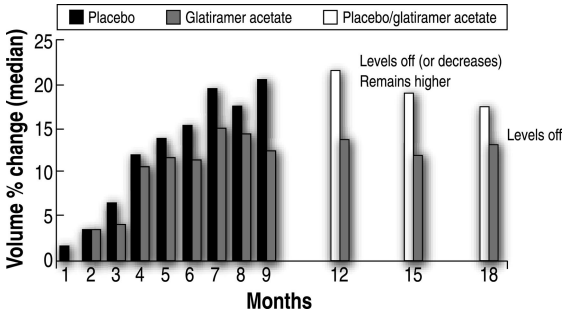


Figure 6 Percentage change in T2 lesion volume



during the second year of treatment (BPF decrease of 0.233% in 68 patients receiving IFN-β1a versus 0.521% in 72 patients receiving placebo). There was no effect of IFN-β1a on atrophy during the first year of this trial. A separate dose-comparison study showed that the rate of atrophy was greatest in the first 4 months after the start of treatment (68%), fell by 50% during year 2, and was then sustained over 3 years.[48] This pattern of atrophy in the months after initiation of immunomodulatory therapy may reflect pseudoatrophy, i.e., atrophy due to the resolution of inflammatory edema.

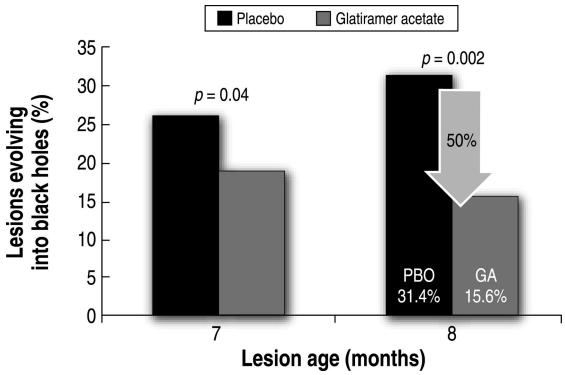
Low-frequency IFN-β1a SC (once weekly) for 2 years also inhibited whole-brain atrophy in patients with CIS.[49] Patients who received active treatment had a 0.62% reduction in brain parenchymal volume during year 1 and 0.61% during year 2, whereas the corresponding changes for subjects receiving placebo were 0.83% and 0.67%, respectively. The difference in the 2-year treatment effect was significant ($p = 0.0031$). However, patients given IFN-β1a SC three times weekly showed no effect of treatment on the level of brain atrophy after 2 years, despite evidence of a strong effect on Gd-enhancing lesion frequency.[50]

Glatiramer acetate also reduced the rate of brain atrophy in RRMS. In an MRI substudy of the original pivotal trial, 27 patients received either active treatment with GA 20 mg (n = 14) or placebo (n = 13).[51] The patients receiving GA had a significantly slower rate of atrophy over 2 years compared with those receiving placebo (−0.6% versus −1.8%; $p = 0.0078$). The changes in enhancing-lesion volume, total T2 and new T2 lesion volume, and T1 hypointense lesion volume also were greater for patients receiving GA (versus placebo) in a 9-month European/Canadian trial.[7] Analysis of brain atrophy in this study, using precise measurements, showed that patients receiving GA for a full 18 months had a slower rate of brain tissue loss compared with those receiving treatment for only the final 9 months.[52]

Several small studies have used nonconventional imaging techniques, such as MRS and MT, to assess the effects of immunomodulatory therapy on the integrity of myelin and axons. In a 1-year open-label investigation of GA in 15 patients with RRMS, Narayanan et al.[53] reported that the drug preserved the NAA:Cr ratio throughout the study period (from 2.82 at baseline to 2.83 at end of study), whereas untreated controls showed a small (3.5%) but significant decline (from 2.88 to 2.78; $p = 0.015$). Because there is a natural NAA decline over time in MS, and because NAA is a marker of neuroaxon integrity, the authors concluded that maintenance of the NAA:Cr ratio reflected a neuroprotective effect of GA in MS.

In another study, GA acetate also promoted axonal metabolic recovery. Khan et al.[54] performed combined MRI and MRS studies on 18 treatment-naïve patients with RRMS who were beginning GA therapy and followed them for 2 years. Patients receiving active therapy experienced a significant increase in NAA:Cr ratio of 10.7% in the volume of interest (corpus callosum and adjacent white matter) and 7.1% in the NAWM. In contrast, controls showed an NAA:Cr ratio loss in the volume of interest (8.9%) and NAWM (8.2%). Thus, GA therapy led to neuroaxon recovery and protection from sub-

Figure 7 Glatiramer Acetate European-Canadian MRI trial. Evolution of Gd-enhancing lesions to chronic black holes was reduced by 50% in patients treated with GA versus placebo. GA, glatiramer acetate; PBO, placebo. Adapted from Filippi et al.,[45] with permission



lethal axon injury. These findings support the hypothesis that GA provides in situ neuroprotection within the CNS.

Similar results were reported in a 1-year pilot study of 10 patients with RRMS who were beginning treatment with IFN-β1b.[21] Compared with untreated matched controls, who had a slight but insignificant decline in NAA:Cr ratio (4%), patients receiving IFN showed a 5.5% increase in NAA:Cr ratio at 12 months. The difference between the two groups was significant ($p = 0.03$), indicating that chronic axon damage is at least partially reversible with IFN-β1b. However, a subsequent study of the drug in 11 patients with a history of active RRMS found that even though IFN therapy was associated with significant reduction in the relapse rate and white matter water T2 relaxation time, and the T2 lesion load did not increase, the central white matter NAA:Cr ratio decreased by 6% in the year after initiation of therapy.[55] Therefore, a reduction of new inflammatory activity with IFN-β1b may not invariably stop the progression of axon injury in MS.

Only limited data are available on the effect of immunomodulatory therapy on MTR metrics. Richert et al.[56] compared the 12-month change in NAWM in 11 matched patients with RRMS who received either GA (n = 5) or IFN-β1b (n = 6). Both GA (1.30%) and IFN-β1b (0.85%) increased the average MTR relative to baseline. The increase in MTR in the patients receiving GA appeared to be greater than that with IFN-β1b, despite a smaller effect on Gd-enhancing lesion frequency, suggesting dissociation of the effect on MTR from the effect on Gd-enhancing lesion frequency.

CONCLUSIONS The use of conventional MRI to measure disease activity and burden and assess the effects of immunomodulatory therapy is now standard in clinical practice and drug trials. Gd-enhanced and T2-weighted imaging is sensitive to pathology in MS lesions due to inflammation, demyelination, edema, and neuroaxon injury. Advanced MRI techniques, including MRS and MTR imaging, provide more specific markers of pathologic changes involving neurons and myelin. Importantly, these advanced imaging markers can reveal both focal and whole-brain neurodegenerative changes that characterize MS, as well as the neuroprotective effects of treatment.

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