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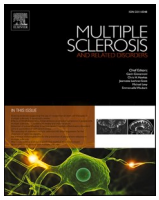

Review article

Mechanisms of myelin repair, MRI techniques and therapeutic opportunities in multiple sclerosis

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ABSTRACT

Multiple sclerosis (MS) is a chronic inflammatory demyelinating disease of the central nervous system (CNS). The remyelination process requires the activation, migration and differentiation of oligodendrocyte progenitor cells (OPC) in demyelinated areas. The metabolic dysfunction in chronic demyelinating lesions impairs the activation of OPCs, the myelin debris clearance by microglia decreases with age, along with diminished secretion of factors promoting OPC differentiation. Conventional magnetic resonance imaging (MRI) sequences have limited ability to differentiate unmyelinated and remyelinated lesions. Advanced MRI sequences based on magnetization transfer ratio (MTR), myelin water fraction (MWF) and diffusion tensor imaging (DTI) have been used to evaluate remyelination in clinical trials. More recently, the q-space myelin map (qMM) has been used on experimental and exploratory clinical studies. The improvement of myelin-specific MRI sequences with high reliability and standardization among centers will allow a more accurate evaluation of new therapies to improve remyelination. These new remyelination promoting treatments alone or in combination with current options may reduce the risk of long-term disability in MS.

1. Introduction

Multiple sclerosis (MS) is a chronic inflammatory demyelinating disease that affects mainly young adults and might cause long-term disability. In recent decades, great progress has been achieved in developing drugs that reduce clinical relapses and control disease activity. However, it was not until recently that central nervous system (CNS) regeneration became a therapeutic target, and the first treatment for primary progressive MS was approved (Montalban et al., 2016) with just moderate effect mainly in active disease cases.

Clinical attacks of the relapsing-remitting MS (RRMS) are caused by waves of central nervous system inflammation (e.g., infiltration of lymphocytes, proinflammatory cytokines) that ultimately generate myelin loss (demyelination). Myelin is responsible for both the conduction of action potentials in saltatory manner and providing neuronal nutrition to axons, via oligodendroglia (Lee et al., 2012; Fünfschilling et al., 2012) contributing to mitochondrial functioning. Therefore, its impairment causes neurological deficits and long-term metabolic dysfunction that may lead to neurodegeneration.

The process of recovery of the myelin sheath is called remyelination. There are evidence suggesting successful remyelination is associated with the prevention of neurodegeneration in both animal models and human studies with Positron Emission Tomography (PET) (Franklin and Ffrench-Constant, 2008; Lubetzki et al., 2020), through guaranteeing neuronal metabolic functioning. The capacity of remyelination varies between patients with MS, and it might explain at least partially the heterogeneity in recovering from attacks in different patients. However, it remains challenging to evaluate remyelination with noninvasive techniques, making difficult to determine the clinical impact of its impairment.

This review summarizes the neuroimaging and neurophysiologic techniques developed for evaluate myelin regeneration *in vivo* as well as published randomized clinical trials with agents that target enhancing remyelination in MS patients.

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1.1. How remyelination works in the CNS?

Remyelination depends on a complex interaction between molecular and cellular mechanisms. It encompasses the recruitment and maturation of oligodendrocyte progenitor cells (OPCs) in demyelinated areas (Baaklini et al., 2019; Franklin and Ffrench-Constant, 2017) along with mature oligodendrocytes capable of ensheath demyelinated axons (Duncan et al., 2018) after surviving inflammatory insult. Important knowledge regarding remyelination dynamics is derived from animal models. Toxic-induced demyelinating models use myelinotoxic agents to generate *in-vivo* demyelinated areas in the CNS with either cuprizone dieting (diffuse demyelination) or local injections with ethidium bromide (EtBr) or lysolecithin (focal demyelination). The cuprizone model can induce microglial response and be administered in different time courses, enabling the assessment of chronic x acute myelin responses. These models are suited for understanding lesions with OPC presence, since they all preserve OPCs. Another typical animal model is the Experimental autoimmune encephalomyelitis (EAE), which is based on the induction of immune response by introduction of myelin agents, simulating lymphocyte-mediated responses. EAE models resample human immunopathology with CD4+ and CD8+ responses, inflammation, and perivascular cell death in acute manner. However, although ongoing remyelination can be visualized, the prominent inflammation hinders proper evaluation of treatment response as improvement can be either due to remyelination or inflammation reduction (Sutiwisesak et al., 2021). Moreover, EAE develop lesions in different anatomical regions, impairing following-up myelin changes in a specific lesion (Plemel et al., 2017). Chronic viral infection can also be used to induce demyelinating animal model, this is mainly done with Theiler's murine encephalomyelitis virus (TMEV). The virus inoculation on the CNS activates myelin-reactive lymphocytes and microglia, generating demyelinated lesions, inflammation, oligodendrocyte death and axonal loss (Gerhauser et al., 2018). In this chronic damage model limited remyelination can be observed along with OPCs death, resampling MS lesions with OPCs depletion and remyelination failure. (Sutiwisesak et al., 2021)

OPCs are present diffusively in the brain of human and other adult mammals. After demyelination, OPCs enter a reactive state enhancing the transcription of relevant molecular factors (Baaklini et al., 2019). OPCs are then attracted to lesion site (Lubetzki et al., 2020; Boyd et al., 2013), differentiated into mature oligodendrocytes and eventually starts the remyelination process. Moreover, new evidence suggest that mature oligodendrocytes also impact remyelination. Studies with electron microscopy involving rhesus monkey and cat demyelination models found mature oligodendrocytes that survived demyelinating insult were still able to attach to viable internodes (Lubetzki et al., 2020; Duncan et al., 2018) and a postmortem study with MS patients found that mainly old mature oligodendrocytes were present in shadow plaques (remyelinated lesions) suggesting that those partial myelin recovery were carried out by mature cells (Yeung et al., 2019). More detailed description of the molecular basis involving remyelination can be found in other reviews (Lubetzki et al., 2020; Baaklini et al., 2019; Franklin and Ffrench--Constant, 2017).

1.2. Why do remyelination mechanisms fail in MS?

The process of remyelination starts immediately after injury (Kuhlmann et al., 2008) and continues throughout the subacute phase, extending up to 7 months in MS patients (Brown et al., 2014). The extension and frequency of remyelinated lesions vary from patient to patient and even between lesions from the same individual (Patrikios et al., 2006; Goldschmidt et al., 2009). Older age and longer disease duration correlate with a lower remyelination capacity in animal models and human studies (Lubetzki et al., 2020; Al-Temaimi et al., 2017), suggesting that both disease duration and aging exerts complementary negative effects. Some of the possible explanations are that the neuronal metabolic dysfunction in chronic lesions impairs repair because of undermined neuronal electrical activity, OPCs depletion and lesion extension (hindering mature oligodendrocytes to connect to viable existing nodes) (Duncan et al., 2018; Mitew et al., 2018). Aging, on the other hand, is associated with diminished OPC response to factors that induce differentiation and inadequate myelin debris clearance by microglia (Gruchot et al., 2019) impairing repair as myelin debris also inhibit OPCs differentiation (Kotter et al., 2006). Furthermore, histopathological studies suggest anatomy also impacts remyelination effectiveness, as periventricular and infratentorial lesions presented less effective remyelination than subcortical lesions. This may be due to regional factors such as OPC availability or reflect higher neuronal activation in gray matter dense areas which contributes to oligodendrocyte preservation (Goldschmidt et al., 2009; Cunniffe and Coles, 2021).

2. Assessing remyelination *in vivo* with magnetic resonance imaging (MRI)

2.1. Conventional MRI

The most frequently used MR sequences to access demyelinating lesions in MS are T2-weighted (T2WI) and T1-weighted (T1WI) images. T2WI is highly sensitive in identifying new lesions and evaluating total lesion volume ((Franklin and Ffrench-Constant, 2017)). However, T2 hyperintense lesions might represent a wide spectrum of histopathological changes. Both demyelinated and partially remyelinated lesions are hyperintense in T2WI (). This could partially explain the weak correlations between T2 lesion loads and disability (Barkhof, 1999).

T1WI is thought to offer more specific markers of tissue damage. T1 hypointense lesions are considered areas with less remyelination, more axonal loss and degeneration (Barkhof et al., 2003; Bagnato et al., 2003). Nevertheless, T1 relaxation intervals can also be increased in edema, which means that areas of acute inflammation and demyelination might also present as hypointense foci (Thaler et al., 2017). However, lesions with a persistent low signal in T1WI, after the resolution of the acute inflammation, are more likely to become permanent and are denominated "black holes". Longitudinal normalization in the T1-weighted signal is a more direct indication of remyelination (Bagnato et al., 2003). Moreover, T1-weighted signal and T1 relaxation times correlate with the degree of demyelination, remyelination and tissue damage (Thaler et al., 2017).

More recently, the T1W/T2W ratio is being used as a quantitative method for assessing tissue myelin content. Myelination is directly correlated to increase T1W signal and inversely correlated with T2W signal (Hagiwara et al., 2018). The T1W/T2W ratio is a reliable marker of myelin content, when compared with non-conventional MRI modalities such as myelin water fraction (MWF) and magnetization transfer ratio (MTR) (Arshad et al., 2017; Ganzetti et al., 2014). Moreover, T1W/T2W ratio has been correlated with cortical pathology, neurocognitive performance and disability in MS patients (Righart et al., 2017; Granberg et al., 2017).

Therefore, T1W/TW2 ratio has increased sensitivity for myelin when compared to conventional MRI and some of the advanced MRI techniques. Noteworthy, there is no need for prolonged acquisition time, even though postprocessing procedures are needed (Table 1).

2.2. Magnetization transfer ratio (MTR)

Advanced MRI techniques are based on diverse physical-chemical phenomena such as magnetic susceptibility, magnetization transference and diffusion of molecules that allow to determine the different properties of tissues (Table 1).

Magnetization transfer contrast (MTC) is used to observe the contrast of tissues where protons are present in different states: as free water, bound to macromolecules and in cell membrane hydration layers

Table 1
MRI sequences used for myelin assessment.

Modality	Advantages	Disadvantages	Remyelination endpoint in clinical trials
T2WI	Available in all scanners	Qualitative measure	Cadavid et al. (2019)
Conventional	Post-processing not needed	Low myelin specificity	Green et al., (2017)
	Sensitive to demyelination		Schwartzbach et al. (2017)
	Low acquisition time		Cree et al. (2020)
T1WI	Available in all scanners	Qualitative measure	Cadavid et al. (2019)
Conventional	Post-processing not needed	Low sensitivity to myelin changes	Green et al., (2017)
	Specific to myelin/ axonal damage		Schwartzbach et al. (2017)
	Low acquisition time		Cree et al. (2020)
T1W/T2W	Available in most scanners	Sensitive to inflammation and myelin debris	–
	Specific and sensitive to myelin damage	Post-processing needed	
	Low acquisition time		
	Quantitative measure		
MTR	Available in most scanners	Sensitive to inflammation and myelin debris	Cadavid et al. (2019)
	Specific and sensitive to myelin changes	Post-processing needed	Green et al., (2017)
	Low-intermediate acquisition time		Schwartzbach et al. (2017)
	Quantitative measure		
DTI	Available in most scanners	Sensitive to structural heterogeneities	Cadavid et al. (2019)
(RD and FA)	Specific and sensitive to myelin changes	Model-based diffusion	Green et al., (2017)
	Low-intermediate acquisition time	Post-processing needed (can be performed directly in scanner workstation depending on commercial software)	
	Quantitative measure		
MWF	Programmable in modern scanners	Long acquisition time	Green et al., (2017)
	Specific and sensitive to myelin changes	Post-processing needed	
	Quantitative measure	Not standardized	
DKI	Programmable in modern scanners (multi-shell diffusion needed)	Post-processing needed	–
	Specific and sensitive to myelin changes		
	Quantitative measure		
	Intermediate acquisition time		
QSI	Programmable in modern scanners (multi-shell diffusion needed)	Long acquisition time	–
	Very specific and sensitive to myelin changes	Post-processing needed	
	Quantitative measure	Not standardized	
		Little experience in patients	
qMM	Programmable in modern scanners (multi-shell diffusion needed)	Post-processing needed	–
	Specific and sensitive to myelin changes	Not standardized	
	Quantitative measure	Little experience in patients	
	Intermediate acquisition time		

DKI=Diffusion Kurtosis Imaging, DTI=Diffusion Tensor Imaging, FA=Fractional Anisotropy, MTR=Magnetization Transfer Ratio, MWF=Myelin Water Fraction, qMM=*q*-Space Myelin Map, QSI=*q*-Space Imaging, RD=Radial Diffusivity, T1WI=T1-weighted imaging, T2WI=T2-weighted imaging.

between macromolecules and free water The MTC is obtained by applying an off-resonance magnetic pulse, saturating the bounded protons. To restore physical equilibrium, the free-water protons start to transfer magnetization to the saturated protons. Thus, the amount of magnetization transferred is directly proportional to the number of macromolecules (i.e. proteins, myelin, cell membranes, etc.) in tissues. It is possible to obtain a quantitative measure from magnetization transfer phenomena by comparing the MR signals in images with and without the off-resonance pulse through an index called magnetization transfer ratio (MTR) (Grossman et al., 1994; Wolff and Balaban, 1989).

Lower MTR is associated with reduced proportion of bound water protons and has shown correlation with the degree of myelin and axonal loss in MS patients (Inglese et al., 2003; Mottershead et al., 2003). Two histopathological studies involving a total of 56 MS patients found that demyelinated lesions have lower MTR values, while remyelinated lesions have significantly higher MTR values (Barkhof et al., 2003; Schmierer et al., 2004). These properties make MTR a promising method to quantify demyelination and remyelination in lesions and in normal-appearing tissues.

Another study showed ongoing changes in demyelination/remyelination rates in MS lesions evaluated by MTR up to three years after lesion appearance. Only subtle changes were observed in T1WI and T2WI (Chen et al., 2008).

Additionally, MS-related tissue damage seems to be heterogeneous within different brain tissues and progress at different rates across subjects. Global and regional changes in MTR are possible predictors accumulation of physical and cognitive dysfunction (Traboulsee et al., 2003; Khaleeli et al., 2008; Deloire et al., 2011), and might help to indicate response to disease modifying treatments (Inglese et al., 2003).

MTR does not require long acquisition times and, to date, is probably the most applied technique for this purpose, allowing lesion analysis, whole-brain measures and voxel-based analysis (Audoin et al., 2004). However, MTR presents some intrinsic limitations. First, it reflects myelin indirectly through restricted proton pool motion, so it is uncapable of differentiating healthy myelin from myelin debris (Hickman et al., 2004).Also, it is influenced by edema and inflammation. A study with cuprizone-induced mice evaluated myelin biomarkers with electronic microscopy (EM) and found that MTR had relatively poor myelin specificity (Jelescu et al., 2016) compared to diffusional kurtosis imaging (DKI).

Together, these limitations may explain why some studies did not

found correlation of MTR with clinical recovery and remyelination in histological analysis (Hickman et al., 2004; Mccreary et al., 2010) especially in acute lesions. Additionally, magnetic transference is influenced by the presence of iron in tissues, by other cellular membranes and by intrinsic heterogeneity of tissues. Furthermore, magnetic field strength, different acquisition techniques and properties of different scanners significantly affect the MTR measurement. These limitations difficult a broader use of MTR as a parameter of MS treatment response, especially in multicentric studies (Wattjes et al., 2015).

2.3. Myelin water imaging

Myelin water imaging is based on the behavior and size of water molecules in different tissues (e.g., gray matter, white matter, myelin) and measures T2 relaxation curves whose amplitudes are proportional to the content of water in each environment. The myelin water fraction (MWF) is the ratio of an area in T2 distribution due to myelin to the area of entire T2 distribution (Laule et al., 2006).

The capacity of MWF in accurately identifying myelin in MS patients have been investigated through MRI and histopathological analysis. Laule et al. (2006)evaluated 25 MS patients brains and correlated the MWF with the presence of myelin identified by Luxol Fast Blue (LFB) staining [R^2 = 0.67 (0.45–0.92)]. Moreover, more recent study with a 7-Tesla MRI in 10 brain samples of MS patients showed an even higher correlation between MWF and LFB staining [R^2 = 0.78 (0.56–0.95)] (Laule et al., 2008) indicating higher accuracy of MWF in higher field scanners.

*In vivo*human studies with MWF confirmed that MS lesions have decreased MWF values and also observed increasing MWF over time suggesting remyelination (Vargas et al., 2015). Moreover, there is evidence that normal appearing white matter (NAWM) abnormalities may also be assessed by MWF (Faizy et al., 2016), indicating myelin changes that might precede the occurrence of lesions identified by conventional MRI. Until now, myelin water imaging has been used as a secondary endpoint in one phase II clinical trial (Green et al., 2017) to evaluate both MS lesions and NAWM. An important limitation for applying MWF in clinical settings is its time for acquisition and processing. A study that evaluated different MWF protocols suggested that a whole-brain sequence with 5 mm slice thickness can be acquired within 25–30 min (Alonso-Ortiz et al., 2015). Another possible limitation is the difficulty in distinguishing healthy myelin from myelin debris through MWF analysis (Nakahara, 2019). Currently, no standardized procedure or commercial software are available.

2.4. Diffusion techniques

Diffusion-weighted imaging (DWI) is a widely used MRI technique that providing information about diffusion of water molecules. The motion of water molecules depends on orientation and nature of biological barriers such as myelin sheaths and axons. Some examples of diffusion modalities can be visualized in Fig. 1.

2.4.1. Diffusion tensor imaging (DTI)

DTI provides *in vivo* information about the diffusion of water molecules, assuming microstructural tissue differences. The most frequently evaluated DTI parameters are fractional anisotropy (FA), mean diffusivity (MD), axial diffusivity (AD) and radial diffusivity (RD).

In experimental studies with animal models, FA was correlated with myelin and axonal integrity in healthy tissue (Yano et al., 2018).Yet, RD correlated better with the temporal changes of myelin structure in the demyelination-remyelination process (Jelescu et al., 2016; Yano et al., 2018). Likewise, a longitudinal study with 20 MS patients who were receiving Natalizumab found that FA gradually increased after occurrence of new gadolinium enhancing lesions indicating remyelination (Fox et al., 2011).

DTI metrics (particularly RD and FA) may be useful for evaluating myelin repair in MS brain lesions. Additionally, DTI requires feasible acquisition time and it is available in most commercial scanners (requiring manual quality control and postprocessing procedures though).

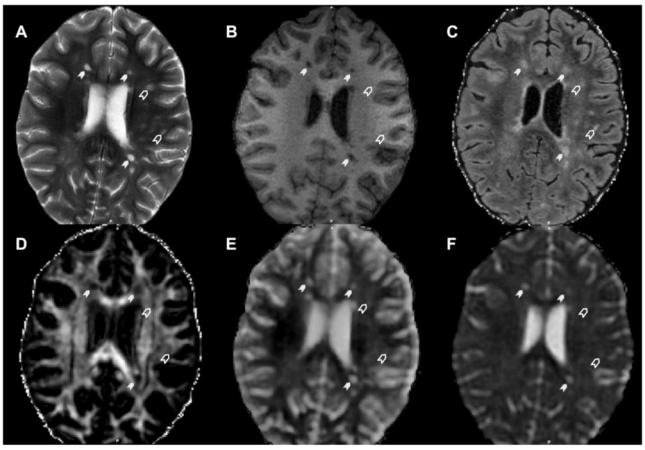

Fig. 1. Conventional and Advanced MRI sequences for MS lesions. (A) T2WI (B) T1WI (C) FLAIR (D) DTI-FA (E) qMM (F) DTI-RD. Images acquired from a RRMS patient showing lesions in different evolution stages. Filled arrows = demyelinating lesions with hypointense T1WI signal. Empty arrows indicate demyelinating lesions with partial remyelination signal.

DTI is limited since it implies that water diffusion has Gaussian distribution. Water diffusion is affected not only by myelin but also by cell membranes, axonal sheaths (Fujiyoshi et al., 2016) and it is influenced by anatomical complexities such as crossing fibers. Other advanced MRI techniques such as diffusional kurtosis imaging (DKI) or q-Space imaging (QSI) (Jelescu et al., 2016; Fujiyoshi et al., 2016) are model-free (non-Gaussian) diffusion reconstruction methods (HORI et al., 2012) that might provide observations more specific to myelin.

2.4.2. Q-Space imaging (QSI)

One of the main limitations of conventional DWI methods is the assumption that diffusion of water molecules has normal distribution. In this context, QSI was developed.

QSI is an advanced MRI technique in which multiple high b-(q-) value DWI are acquired to generate a probability density function (PDF) of water diffusion after a Fourier transformation of the signal intensity. QSI has demonstrated to accurately describe CNS microstructure in animal models of myelin deficiency (Biton et al., 2006) and development (Assaf et al., 2000).

In MS patients, QSI was more specific than MTR and more sensitive than DTI to differentiate white matter from gray matter, NAWM and MS lesions (Farrell et al., 2008; Hori et al., 2014). However, despite being probably the most accurate method to evaluate the presence of myelin, it is time-consuming preventing its use in clinical practice (Hori et al., 2012), acquisition times varies depending on the *q*-values sampling and slice number and can range from 25 to 50 min in brain sequences (Lätt et al., 2008; Van et al., 2010). There are only few published studies using this technique in humans, and no standardized software is available yet.

2.4.3. Diffusional kurtosis imaging (DKI)

Kurtosis is a statistical measure of how much the tails of a distribution extend farther than what would be expected in normal distribution. Jensen et al. (2005) described the quantification of MRI signaling using kurtosis-based metrics with lower *b*-values (2000 to 3500 s/mm^2) than what is traditionally used in QSI (~10,000 s/mm^2. DKI avoids the calculation of the full displacement of the PDF using only the excess diffusional kurtosis. This allows a shorter acquisition time.

DKI provides mean diffusion kurtosis, axial kurtosis and radial kurtosis. As expected, mean diffusion kurtosis provides a more accurate

measure of tissue structure than FA and apparent diffusion coefficient (ADC) from DTI and conventional MRI (Jensen et al., 2005).

One study of cuprizone-induced corpus callosum demyelination and DKI revealed that several DKI metrics correlated with histological biomarkers for myelin. DKI had better performance than DTI metrics in regions where higher tissue heterogeneity was expected (Falangola et al., 2014). Another cuprizone-induced demyelination study compared DKI with MTR and DTI. DKI-derived metrics were the most specific for the demyelination-remyelination process, and MTR did not estimate myelin content properly (Jelescu et al., 2016). This was reinforced by other studies in which DKI but not DTI were able to identify spontaneous gray matter remyelination (Guglielmetti et al., 2016).

In clinical settings, DKI was used to detect microstructural changes using tract-based spatial statistics in RRMS patients compared to healthy controls. DKI metrics were sensitive to detect white matter abnormalities in regions with homogenous and heterogeneous fiber arrangements (Li et al., 2018). Another longitudinal study with 40 MS patients revealed that corticospinal tract radial kurtosis was the best predictor of the Expanded Disability Status Scale (EDSS) at 12 months. Indicating an association between DKI metrics and clinical disease progression.

Furthermore, DKI is feasible in clinical practice. However, to date, there are still few human studies that evaluate remyelination using DKI and no standardization is available yet.

2.4.4. Q-space myelin map (qMM)

Recently, a new MRI modality was validated in a translational study (Fujiyoshi et al., 2016) denominated q-space myelin map (qMM). This technique combines the advantages of QSI and DKI. Table 1 A full-scale QSI of reduced steps but preserving the resolution (9 steps with *b*-value up to 10,000) is performed allowing a reduced acquisition time (approximately 10 min with a 3T scanner). The kurtosis is calculated from the QSI-generated PDF.

In animal histological analysis qMM correlated better with the presence of myelin in LFB staining and electron microscopy than DTI and T2WI (Fujiyoshi et al., 2016).

An exploratory study conducted (Tanikawa et al., 2017) with MS patients receiving fingolimod associated treatment response with remyelination identified by qMM. Another observational study with MS patients under Natalizumab treatment suggested remyelination in qMM is correlated with EDSS improvement over time (Kufukihara et al., 2018). These studies demonstrated promising results for further clinical trials (Kira, 2017). Currently, there is no standardized automated procedure to analyze these images.

2.5. Spinal cord assessment

As in the optic nerve, spinal cord lesions tend to present with debilitating neurologic deficits such as motor and autonomic dysfunctions. Therefore, remyelination in spinal cord lesions would potentially implicate significant functional recovery for patients.

However, anatomical and technical issues made the visualization of the spinal cord with the currently available imaging methods challenging. Spinal cord small physical dimensions, CSF flow, respiratory movements and magnetic field inhomogeneity are some of the limitations.

The main instrument for assessing disease progression within the spinal cord is spinal atrophy, which has been used vastly and correlates with clinical disability (Kearney et al., 2015; Casserly et al., 2018; Eshaghi et al., 2018). However, volumetric studies are very limited for inferring mechanisms of repair and microstructure. Therefore, atrophy may be a later finding indicating advanced disease (Kearney et al., 2015).

Advanced MRI techniques have been applied to evaluate the myelin content of the spinal cord. A postmortem study of 4 MS patients showed that spinal cord MTR correlated with the presence of myelin in histological analysis (Mottershead et al., 2003). Another study (Casserly et al., 2018) showed good correlation between spinal cord LFB staining and MWF. MWF was able to distinguish NAWM from gray matter and MS lesions (Laule et al., 2016). DTI also correlated with myelin in the spinal cord in another histological study involving 9 MS patients and 5 controls. RD distinguished various degrees of demyelination (Klawiter et al., 2011) and healthy myelin.

In vivo studies with MTR showed that magnetic transference is reduced in the spinal cord of MS patients and correlates with disability (Hickman et al., 2004; Mallik et al., 2014; Zackowski et al., 2009). Spinal cord FA, RD and MD also demonstrated association with disease progression in MS patients (Naismith et al., 2013; Agosta et al., 2007). Likewise, DKI was able to distinguish gray and white matter from lesions and was associated with clinical disability measured by EDSS (Raz et al., 2013). At last, a case report using qMM showed remyelination of a newly developed spinal cord lesion after treatment (Fujiyoshi et al., 2016).

2.6. Remyelinating therapies in randomized controlled trials

New molecules are being developed to stimulate the intrinsic mechanisms of remyelination. Most of them induce transcriptional factors that promote remyelination or inhibit the inhibitory pathways ((Franklin and Ffrench-Constant, 2017)). Here, we provide a summary of the results of randomized trials already published and ongoing promising trials. Summarized data can be found in Table 2.

3. Anti-LINGO (Opicinumab)

Leucine rich repeat and immunoglobulin containing 1 (LINGO-1) is a glycoprotein selectively expressed in the CNS (oligodendrocytes and neurons) that inhibits OPC differentiation and myelin formation. Experimental studies have shown that LINGO-1 antagonists facilitate remyelination both *in vitro* and in animal models (Mi et al., 2009).

Opicinumab is a human monoclonal antibody that targets the LINGO-1 protein enhancing remyelination and OPC differentiation. The phase I clinical trial (Tran et al., 2014) was designed as two separate blind-placebo-controlled studies. One of them with 72 healthy volunteers and the other with 47 RRMS or secondary progressive MS patients (SPMS). In both studies, the participants were randomized to receive intravenous or subcutaneous opicinumab or placebo. No serious adverse effects (AEs) were reported in the treatment group. MS patients in the opicinumab group had fewer new T2 lesions.

Two phase II clinical trials evaluated efficacy of opicinumab when compared to placebo. The RENEW study (NCT01721161) (Cadavid et al., 2017) selected patients with acute optic neuritis and in the SYNERGY study (NCT01864148) (Cadavid et al., 2019) evaluated RRMS and SPMS patients treated concurrently with intramuscular interferon-beta 1a. The primary endpoint of the RENEW study was the recovery of nerve conduction latency measured with full-field visual evoked potential (FF-VEP) at 24 weeks compared with the unaffected eye at baseline. Intention to treat (ITT) analysis showed that patients enrolled in the Opicinumab group had shortened latency compared to the control group. However, significance was only achieved in the per protocol analysis population within 32 weeks. There were no differences between groups in MRI measures (T2 lesion volume, gadolinium enhancing (Gd+) lesions and new Gd+ lesions) (Cadavid et al., 2017). In the SYNERGY study, the primary endpoint was confirmed improvement of neurophysical and/or cognitive function and/or disability over 72 weeks of treatment in 330 RRMS and 88 SPMS patients. Ninety-three patients received placebo, and the remaining patients were randomized to receive opicinumab in increasing doses: 3 mg/kg (45 patients), 10 mg/kg (95 patients), 30 mg/kg (94 patients) and 100 mg/kg (92 patients).

The primary endpoint was met only in the 30 mg/kg group. Nevertheless, the subgroup analysis showed significant improvement in patients younger than 40 years assigned to receive 10 and 30 mg/kg and those with disease duration of less than 8 years in 3 and 10 mg/kg doses

Table 2
Randomized clinical trials with remyelinating agents and imaging endpoints.

Therapy	Trial name/code (Phase)	Primary Endpoint	MRI Endpoints (secondary)	Primary Endpoint results	Secondary MRI Endpoint results
Anti-LINGO1	RENEW (Phase 2)	FF-VEP latency improvement	Conventional MRI	41% improvement in the PP population (p=.011)	Not met
Anti-LINGO1	SYNERGY (Phase 2)	Clinical improvement	DTI-RD, DTI-FA, MTR, Volumetric	Positive for 30 mg/kg dose group *(See text.)*	Exploratory *(See text.)*
Anti-LINGO1	AFFINITY (Phase 2)	Clinical improvement	–	Not met. (Announcement)	Not met
Clemastine Fumarate	ReBUILD (Phase 2)	VEP P100 latency reduction	DTI-FA, MTR, MWF	−1.7 ms/eye; (p=•0048)	Not met
High dose Biotin (MD1003)	MD1003CT2013–02MS-SPI (Phase 3)	Clinical improvement	Conventional MRI	12.6% vs 0%; p=.005	Not met
High dose Biotin (MD1003)	MD1003CT2013–01MS-ON (Phase 3)	VA improvement	–	−0.061 vs −0.036 logMAR; p=.6	–
High dose Biotin (MD1003)	MD1003CT2013–02MS-SPI2 (Phase 3)	Clinical improvement	Conventional MRI, Volumetric	12% (MD1003 group) vs. 9% (placebo); p=.31	Not met
H3 antagonist (GSK239512)	116,477 (Phase 2)	Newly developed lesions mean MTR changes	Conventional MRI, Volumetric	Positive for lesion-MTR changes (*See text.*)	Not met
RXR-γ agonists (bexarotene)	CCMR One (Phase 2)	Patient-level lesion mean MTR changes	Conventional MRI, Volumetric, Proportion of lesions with MTR increase	bexarotene–placebo difference 0.16 pu; p=•55	Not met

DTI-RD=Diffusion Tensor Imaging (Radial Diffusivity), DTI-FA=Diffusion Tensor Imaging (Fractional Anisotropy), FF-VEP=Full-field visual evoked potentials, MRI=Magnetic Resonance Imaging, MTR=Magnetization Transfer Ratio, MWF=Myelin Water Fraction, PP=Per protocol, VA=Visual Acuity, pu = percentage unit. .

(Ruggieri et al., 2017; McCroskery et al., 2017; Petrillo et al., 2018). MRI exploratory analysis showed no significant difference in lesions evaluated by MTR, DTI-RD and DTI-FA in the ITT population across treatment groups. Patients randomized to receive 10 mg/kg who had baseline whole brain DTI-RD showed better response to treatment than placebo. Moreover, post hoc analysis showed improvement in clinical outcomes when stratified patients by pretreatment lesion characteristics in DTI-RD and MTR and shorter disease duration. Additionally, the responder subpopulation showed favorable changes in the mean lesion RD and MTR after treatment (Evans et al., 2017). These exploratory MRI results displayed the potential of MTR and DTI as MRI biomarkers for predicting treatment response. Finally, the AFFINITY (NCT03222973) was a phase II randomized double-blind placebo-controlled trial that enrolled 263 MS patients. The primary outcome was the improvement of disability after 72-weeks. In October 2020, it was announced that AFFINITY did not met neither its primary outcome nor secondary outcomes and opicinumab development program was discontinued.

4. Clemastine fumarate

In 2014, Mei et al. (2014) developed a new model for the *in vitro* evaluation of myelination. They employed micropillars, which are freestanding nanofibers around which membrane wrapping can be visualized. The micropillars were cocultured with oligodendrocytes and exposed to a cluster of antimuscarinic compounds to investigate their ability to improve myelination. They found that Clemastine fumarate promoted OPC differentiation and wrapping in an *in vitro* model and in an *in vivo* adult mouse model after oral administration of the compound (Mei et al., 2014).

A double-blind, randomized, placebo-controlled, crossover trial (ReBUILD study) (Green et al., 2017) was conducted to evaluate the efficacy and safety of Clemastine in RRMS patients with confirmed demyelinating injury in the visual pathway (VEP P100 latency in at least one eye of 118 ms). The primary outcome was shortening of P100 latency on FF-VEP. Nonconventional MRI were evaluated as secondary endpoints (Whole Brain-MTR, White Matter-MTR, White Matter-FA and MWF). Twenty-five patients were randomized per group. Active treatment consisted of 10.72 mg/day of oral Clemastine fumarate. Significant shortening of VEP latency was sustained in the group that initiated the study on clemastine and was also achieved in the delayed-treatment group. None of the secondary MRI endpoints were met or showed tendency to significance (Green et al., 2017). Currently, clemastine is being tested in an ongoing phase 2 randomized, double-blind, placebo-controlled trial (ReCOVER) that targets to evaluate 90 MS patients presenting acute optic neuritis, with the primary outcome of difference in P100 latency and secondary MRI outcomes including change in brain MWF and MTR between from baseline and 9 months (NCT02521311).

5. High-dose biotin (MD1003)

Biotin is a water-soluble vitamin, generally present in human diet, which acts as a coenzyme in important metabolic processes related to fatty acids synthesis. A pilot study involving 23 patients with progressive MS showed high-dose biotin intake was associated with favorable outcomes in visual acuity progression.

The first clinical trial with high-dose biotin (Tourbah et al., 2016) included 154 patients with primary progressive MS or SPMS randomized to receive 100 mg/day of oral biotin or placebo for 12 months. The primary endpoint was the proportion of patients with improvement of MS-related disability (evaluated through EDSS and Timed 25-foot walk test - T25FW) at 9 months and, confirmed in 12 months. Secondary endpoints included conventional and nonconventional MRI in a subgroup of 74 patients. The primary endpoint was achieved for 12.6% of patients in the treatment group and in none of the placebo group. MRI analysis did not show statistically significant results and, the safety profile was similar to the placebo (Tourbah et al., 2016).

The phase 3 randomized, double-blind, placebo-controlled trial (SPI2) allocated 642 MS patients to placebo or MD1003 and failed to achieve its primary outcome which was improvement in disability at month 12 (overall participants with improvement 12% in MD1003 group vs. 9% in placebo; p = .31). MRI volumetric measures were analyzed as an exploratory outcome but there was no significant effect found in whole-brain volume change, thalamic volume and cortical gray matter volume (Cree et al., 2020).

6. H3 receptor antagonist (GSK239512)

An experimental study with different compounds to identify possible new targets to enhance remyelination found that histamine receptor 3 (H3) negatively regulates OPC differentiation via cyclic adenosin monophosphate (cAMP) and cAMP-response binding protein (CREB) signaling. The authors administered an H3 receptor antagonist in a cuprizone/rapamycin mouse model of demyelination and observed improvement in remyelination (Chen et al., 2017).

A phase II randomized, placebo-controlled clinical trial with H3 receptor antagonist included 131 MS patients receiving first-line immunomodulatory therapy. They were assigned to receive placebo or oral doses titrated according to tolerance of the H3 antagonist for 48 weeks. Primary endpoints were mean changes in MTR in newly developed lesions. Secondary endpoints included conventional MRI and brain volume measurements, EDSS, cognitive testing (CogState) and MS Quality of Life questionnaire (MSQoL-54). Treatment with H3 receptor antagonist was positively associated with remyelination evaluated by MTR. The overall incidence of AEs was similar between both groups. Although during the titration phase, patients receiving the H3 antagonist presented more adverse events. There were no differences in brain volume measures, EDSS or CogState between groups (Schwartzbach et al., 2017).

7. Retinoid acid X receptor [RXR] gamma agonists (bexarotene)

Bexarotene promoted oligodendrocyte progenitor cell differentiation and remyelination following experimental models of demyelination. A phase II double-blind, placebo-controlled study enrolled 52 MS patients taking dimethyl-fumarate within a 6 months period. The primary efficacy outcome was the mean patient-level lesional change in MTR from baseline values. Other exploratory outcomes included VEP F100 latency change and conventional MRI analysis. The ITT analysis show no significant difference in lesion MTR mean change. Nevertheless, F100 changes trended to a positive biological effect bexarotene-placebo all eyes difference −2.85 ms, CI −5.75 to 0.05; $p = .054$. Yet, there were important safety and tolerability concerns. All patients in bexarotene group developed hypothyroidism, 92% presented with an increase in the triglyceride levels, 50% with rash, and 38% with neutropenia. Betaroxene group had 19% of patients discontinued due to adverse events compared to 8% in the placebo group. These safety concerns limit the use of betaroxene as a potential treatment (Brown et al., 2021).

8. Ongoing clinical trials

At this moment, there are promising ongoing trials for agents that target remyelination promotion.

Nanocrystaline Gold (CNM-Au8) promoted remyelination *in-vitro* and experimental cuprizone mouse model (Robinson et al., 2020), requiring clinical trials in patients.

The VISIONARY-MS (NCT03536559) trial is an ongoing phase 2, placebo-controlled, randomized trial that aims to evaluate efficacy and safety of CNM-Au8 effect in 150 MS patients with chronic optic neuropathy. Primary outcome is the mean change in the best-corrected low contrast letter acuity after 24 weeks. The secondary MRI outcomes include MWF, MTR and DTI measurements.

The REPAIR-MS trial (NCT03993171) is evaluating treatment with CNM-Au8 in an open-label study that targets to enroll 30 MS patients. The primary outcome is 12 weeks changes in metabolic measured with 31P-Magnetic resonance spectroscopy.

The TOTEM RRMS trial aim to evaluate the effect of testosterone in MS patients. Testosterone administration has been shown to promote remyelination in cuprizone model (Hussain et al., 2013). This ongoing trial is phase 2 double-blind placebo-controlled trial will enroll 40 MS patients. Half of the patients will receive testosterone undecanoate 1000 mg/4 ml at baseline, week 6 and then every 12 weeks until 54 weeks. The primary outcome is change in binary criteria of MRI changes defined as thalamic atrophy lower than 0.5% and modification in lesion DTI metric lower than 0.5% per year from baseline to week 66. Other imaging secondary outcomes are conventional MRI, MTR and advanced diffusion model-free imaging.

9. Conclusions

MS has a wide therapeutic arsenal with confirmed efficacy in controlling CNS inflammation. However, the currently available disease modifying treatments have shown limited effects in improving remyelination and functional recovery after demyelinating attacks. In the last years, some experimental models suggested new treatments to promote remyelination, but clinical efficacy is still lacking.

To investigate the efficacy of those medications, we need a reliable non-invasive technique that allows *in vivo* evaluation of myelin. Advanced MRI sequences have emerged as promising tools. For example, DTI and MTR parameters have been used in remyelination clinical trials and they were able to predict individual responses to therapy. In optic neuritis studies, FF-VEP latency is a useful tool to evaluate objectively the evolution of demyelination and remyelination dynamically provided the functional and structural integrity of the retina (which can be assessed with optical coherence tomography) which contrast with myelitis patients presenting with spinal cord lesions, which are still challenging to evaluate *in-vivo* with precision.

One possible explanation for the failure of remyelination therapies in clinical trials is the inability of DTI and MTR in considering the *in vivo* barriers to the diffusion of water molecules. Future clinical trials may benefit from combining MRI methods according to elements such as patients characteristics (spinal cord lesions, optic neuritis, MS clinical phenotype), number of centers involved (availability to standardize parameters and post-processing methods), and acquisition times. Finally, qMM, DKI and MWF showed higher specificity compared to previous methods in experimental and exploratory clinical studies, turning into promising MRI modalities for future trials.

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Declaration of Competing Interest

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