

Review: Current developments in magnetic resonance imaging for assessing animal models of multiple sclerosis

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Abstract

Magnetic resonance imaging (MRI) is a state-of-the-art radiological imaging technique with a pivotal role in the diagnosis and characterisation of heterogeneous MS lesions in clinical and basic research. Various MRI methodologies such as conventional T_1/T_2 contrast, contrast agent enhancement, diffusion weighted imaging (DWI), magnetization transfer imaging (MTR) and susceptibility weighted imaging (SWI) have been developed to diagnose and investigate the severity of MS pathology, including the processes of vascularization, demyelination/remyelination and damage to regional brain connectivity from permanent axonal loss. The broad spectrum of MS pathology manifests in diverse patient MRI presentations and affects diagnostic accuracy. To study specific aspects of the disease, several mouse models such as Experimental Autoimmune Encephalomyelitis (EAE) and virus and toxin-induced demyelination models have been developed to explore the pathologies that are otherwise difficult to unambiguously determine in patients. This review aims to provide an overview of recent developments in MRI methodology that have been tailored to study specific aspects of MS in animal models and to describe their role in the interpretation of pathology and their contribution to abnormal observations in patient MRI examinations. Key aspects of MS have been investigated in various mouse animal models. For example, the toxin-induced cuprizone model has been used in assessing the MS-related demyelination and remyelination processes. In both animal models and MS patients, DWI parameters such as radial diffusivity and fractional anisotropy are now established as surrogate

biological markers of demyelination. These imaging techniques have all made significant contributions to MS research in both preclinical and clinical environments.

Keywords: Magnetic Resonance Imaging (MRI), Multiple Sclerosis (MS), Diffusion Weighted Imaging (DWI), Susceptibility Weighted Imaging (SWI), Magnetisation Transfer Imaging (MTI), Experimental Autoimmune Encephalomyelitis (EAE), TMEV (Theiler's murine encephalomyelitis virus)

1. Introduction

Multiple sclerosis (MS) can be defined as a chronic inflammatory demyelinating disease [1, 2]. It is the most common central nervous system dysfunction that causes clinical disability in the young adult population with the majority of the MS associated symptoms being either reversible, such as diplopia (double vision), or irreversible, such as blindness [3, 4]. According to the MS Research Australia website (<http://www.msra.org.au/living-ms>), 23,000 people in Australia have been diagnosed with MS and 2.5 million globally in recent years. Three out of every four MS cases are women due to genetic factors. The incidence of the disease increases by 7% annually, with the total cost of health care in Australia for MS patients being approximately \$2 billion dollars [5].

The major cause of MS is not yet resolved, because the pathobiological mechanisms are not fully understood. However, there are several factors that may predominately lead to higher disease susceptibility. These include genetic, gender, ethnic and environmental factors [6]. Genetic factors could play a significant role because the existence of particular tissue type antigens such as HLA (Human Leukocyte antigen) DR2 and/or DW2 in European Caucasian patients increases the risk of developing MS [7].

MS is an autoimmune disease that targets myelin resulting in disruptions of nerve conduction and neurotransmission [1, 8, 9]. The disease initiates with inflammation and destruction of myelin that normally provides protection for the brain and spinal cord nerve fibres [10]. Once myelin is destroyed, it is replaced by hardened sclerotic patches of tissue, known as sclerotic plaques [4, 11]. The damage to the central nervous system is caused by autoimmune reactions, either through T-cells or an antibody-mediated injury. However, MS may result from infectious agents, such as human herpes virus (HHV 6), an endogenous retrovirus and chlamydia pneumonia [7].

Using MRI, MS plaques are commonly observed in several areas of the brain, but the most affected area is the white matter [12, 13]. Highly susceptible areas are the optic nerves, periventricular area, corpus callosum, cerebral peduncle and spinal cord [14, 15]. According to several post-mortem studies [16-19] and recent MR imaging studies [20-23] MS lesions are also present in grey matter. In addition, cortical lesions [24, 25] have been detected by MRI with novel neuroimaging techniques, either imaging at ultra-high field or utilising phase sensitive inversion recovery MRI [18, 20, 24, 26, 27].

Conventional MRI contrast mechanisms such as inversion recovery, T_1 , T_2 and T_2^* weighted-imaging have been used widely to detect MS pathology, however, they are non-specific in describing the progression of neural degeneration [13, 15]. More advanced MR imaging methods have been developed to address these issues: (1) A combination of ultra-high field scanners ≥ 7 tesla (T) for human [19, 28, 29] and ≥ 9.4 T for animals [30-33] and novel contrast agents such as

ultra-small iron oxide particles (USPIOs) enables better detection of early MS changes in the CNS as well as depiction of small lesions within the subcortical layers [34]. (2) Magnetization transfer imaging (MTI) has been developed as a probe for assessment of myelin sheath integrity by measuring the myelin macromolecular structures and myelin water function [35]. (3) The sensitivity of Susceptibility-weighted imaging (SWI) to tissue iron content has been exploited for detecting MS plaques and distinguishing non-symptomatic white matter lesions from MS lesions surrounding the central veins [36]. (4) Diffusion-weighted imaging (DWI) techniques allow early detection of subtle changes in white and grey matter that appear normal in T_1 , T_2 -weighted scans, and DTI fibre-tractography reveals deficits in neuronal connectivity [37]. DWI methodology is becoming increasingly important as diffusion parameters fractional anisotropy (FA) and axial/radial diffusivity measures are central to assessment of MS pathology due to their specificity in detecting the process of demyelination, axonal damage and injury [38].

2. Animal models of Multiple Sclerosis (MS)

Experimental animal models are used to investigate and gain insights into the pathobiology of complex human diseases [39]. These models usually target a specific aspect of the disease rather than attempting to replicate the full spectrum of the corresponding human disease [40]. Although an enhanced understanding of particular aspects of disease can be gained from *in vitro* and *ex vivo* experimentation, *in vivo* experiments in animals are required to gain insight into the inter-relationships of multiple factors that contribute to complex pathobiological changes, such as the relationship between neurodegeneration and MS progression [41]. Research on MS pathobiology has relied upon conventional diagnostic imaging, involving experimental animal sacrifice for histological analysis at varying times throughout the disease course. However, as most pathological processes during the course of MS are dynamic, monitoring of disease progression requires longitudinal studies [31]. MRI has played an increasingly pivotal role as a non-invasive imaging modality allowing longitudinal imaging of MS disease progression [39, 42].

Rodent models have been developed to study the process of inflammation and demyelination associated with MS in the central nervous system. The most commonly used models are experimental autoimmune encephalomyelitis (EAE), Theiler's murine encephalitis virus infection (TMEV) and toxic demyelination models [41]. There is no single animal model that can faithfully reproduce all the pathological aspects of MS in humans. The available models complement each other to enable investigation of various features commonly observed in the human condition [43].

2.1. Experimental Autoimmune Encephalomyelitis (EAE)

Since its introduction by Rivers et al. in 1930 [44] EAE has become a widely used animal model for multiple sclerosis. It can be induced in several mammalian species, including the mouse, rat and primate [45]. Induction of EAE is achieved by an injection of an emulsion comprising an adjuvant and synthetic peptides derived from myelin proteins, such as myelin oligodendrocyte (MOG), myelin basic protein (MBP) or the proteolipid protein (PLP). Alternatively, transgenic EAE can be induced in mice by T cell transfer from EAE donors to native recipients and EAE will be produced without further immunization [46]. In most cases, EAE immunization will result in the activation and expansion of the peripheral antigen specific T-cells, which can penetrate the blood-brain barrier (BBB) and interact with specific myelin antigen inducing MS [47]. In addition to inflammation and demyelination, EAE is used to study acute and chronic axonal injuries. In the EAE

model, macrophages have been suggested to play a vital role in inducing axonal injury, although this has not been definitively confirmed [48].

The course of the EAE model of MS disease varies depending on the immunisation protocols, as well as the choice of animal species and strain. In general, the acute phase of EAE induction is associated with mononuclear cell infiltration into the CNS. This phase is followed by a recovery period, called the remission stage. If the animal has not recovered, the chronic stage is reached (Figure 1) [49].

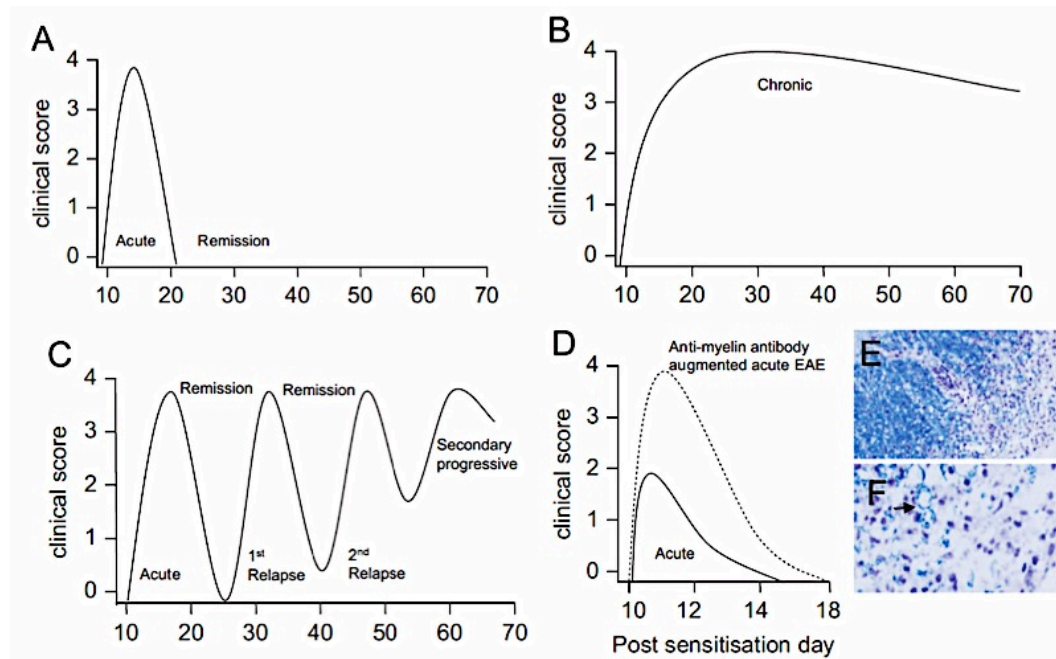


Figure 1. The clinical course spectrum of EAE mouse model Immunisation of animals with myelin antigens produced EAE cycle and could be correlated with clinical scores ranging from 0 to 4, where 0 represents no clinical symptoms and 4 refers to paralysis that is usually observed as a flaccid paralysis of the hind limbs. (A) Acute EAE followed by remission, (B) chronic EAE without recovery, (C) Relapsing-remitting EAE where animals developed relapses and remissions with accumulated neurological deficits. (D) Omission of pertussis toxin will result in a reduction in the clinical score compared with (A), and did not show extensive demyelination in the luxol fast blue (LFB) stain (E). Injection of MOG at the onset of EAE exacerbated the clinical disease presentation, as shown in the LFB stain (F). Arrow points to remnants of myelin [49].

There are three types of adjuvants included in immunization protocols used to induce EAE: Quil Adjuvant (A), complete Freund's adjuvant (CFA) and pertussis toxin, [50, 51]. A mild EAE model with a relapsing remitting (RR) cycle followed by a complete recovery period can be induced using MOG₃₅₋₅₅ (myelin oligodendrocyte glycoprotein peptide sequence 35-55) and Quil A, which acts once at the site of immunisation [52]. CFA is an adjuvant prepared from killed mycobacteria in water and oil emulsion [50]. A chronic EAE model is induced by injection of MOG₃₅₋₅₅ with CFA, which slowly releases the antigen from the injection site [53]. Pertussis toxin is generally required to initiate T cell and B cell immune reactivity to the antigen. As a general rule, adding a high concentration of pertussis toxin in the immunisation protocol increases the disease severity to a hyperacute stage, possibly through a non-selective expansion of T-cells and/or vascular changes (increased BBB permeability) in the CNS [54]. Severe EAE models, however, should be avoided as confounding factors can influence the assessment accuracy of potentially effective treatments [47].

Various EAE rodent models of MS have increased our collective understanding of the immunopathology and neurodegenerative aspects of MS, which led to successful development of three currently marketed therapeutic drugs, namely, glatiramer acetate, mitoxantrone and natalizumab, all which have been approved by the FDA (The US Food and Drug Administration) for clinical treatment [45, 51]. A limitation of EAE-rodent models is that they do not adequately mimic the auto immune factors that contribute to MS disease pathology [47]. In EAE animal models CD4+ T-cell predominate in CNS lesions, which has only been observed in a few patient cases [55] and does not reflect the predominance of CD8+ T-cells and macrophages in MS lesions in humans [56].

2.2. Virus-induced demyelination model

In the 1980s, it was suggested that a combination of a certain genetic background and a viral infection could increase the risk of developing MS [57, 58], although at that time, there was no virus identified to potentially induce MS. More recently [59-61], correlations of EBV (Epstein-Barr Virus) infection with MS suggest EBV as a potential environmental factor. These findings have led to the development of MS animal models using viral induction. The common model is the TMEV (Theiler's murine encephalomyelitis virus), which is induced by an intra-cerebral injection of TMEV into susceptible mouse strains. TMEV pathology is limited to the CNS with an extensive spinal cord lesion [62].

TMEV infection can be highly or less virulent, for example, the immunisation of TMEV GD-VII strain produced fatal encephalitis, whereas the BeAn and Daniel TMEV strains are less virulent [63]. The TMEV model is suggested to be superior to the EAE model for assessment of treatment efficacy due to better correlation with clinical MS [64]. An example of successful application of the TMEV model is in the development of anti-glatiramer acetate antibodies as a remyelination agent [65].

TMEV can be used to produce a mono- or a bi-phasic MS disease course in susceptible mice. During mono-phasic disease, mice experience transient meningo-encephalomyelitis that peaks by approximately seven days with recovery during a three-week period [66]. The mono-phasic disease course is characterized by animal recovery in the absence of persistent neurological impairments. In the case of the bi-phasic disease course, it begins with a mono-phasic stage that subsequently develops into a chronic demyelination stage [67]. MS lesions are commonly observed in the spinal cord, akin to the EAE model [40, 66, 68].

In most cases, TMEV can produce chronic progressive MS, which mirrors chronic progressive MS in humans. The TMEV mouse model offers several advantages; it is potentially able to mimic human MS, in addition to modelling the autoimmune response [50]. It also allows investigation of the role of viral infection in the aetiology of MS and the worsening of MS clinical symptoms [61, 64, 68, 69].

2.3. Toxin-induced demyelination model

Mouse models using toxin-induced demyelination have been developed, not to mimic MS, but to establish and investigate the process of demyelination and remyelination [70]. Two toxins are generally used to induce demyelination, viz lysolecithin and cuprizone. Lysolecithin is an activator of phospholipase and cuprizone is a copper chelator [71]. Lysolecithin acts as a detergent on the myelin sheath, rather than having a secondary effect on oligodendrocytes [47]. On the other hand, a cuprizone-induced copper deficit is thought to be detrimental to mitochondrial function in the

brain. This will disturb the energy metabolism in the oligodendrocyte and cell function and consequently lead to demyelination [70].

The injection of 2 μ l of 1% lysolecithin into the rodent spinal cord will produce focal areas of demyelination [72]. This model has been successfully applied in both rats and mice. During the acute stage, spinal cord lesions are characterized by infiltrating T cells, B cells and macrophages and according to Bieber et al, T cells are essential for the remyelination process [72]. In general, the lysolecithin-induced toxin model can produce a minimum chronic lesion and full remyelination occurs within five to six weeks. If the experimental animals are young, the repair process may occur rapidly [73].

Cuprizone is the most frequently used toxin to study demyelination in mice [70, 74]. Conventionally, the addition of 0.2% cuprizone in the diet of young adult mice for four to five weeks (acute) or 12 weeks (chronic) will produce focal demyelinated lesions within several white matter structures, including the corpus callosum, internal capsule, cerebral peduncles, anterior commissure and thalamic white matter [70, 74]. Once the cuprizone is removed from the diet, the mice will progressively develop remyelination within three to four weeks [68]. The toxin model is potentially reproducible and hence useful for testing novel therapeutic approaches that can suppress demyelination and accelerate remyelination [68, 75]. In Table 1, we have summarized commonly used rodent models of MS and their clinical features [68, 75].

3. MRI studies of animal models of MS

MS clinical studies require recruitment of large numbers of patients with long follow up procedures and the patients must be grouped according to MS stage [76]. However, animal models of MS [77-79] have better accessibility and their use has enhanced our collective understanding of the pathological mechanisms underpinning demyelination and axonal damage in the CNS. Animal studies allow monitoring of the environment as well as validation of experimental results, which is more difficult to achieve in human studies. MS is a heterogeneous disease, and in vivo MRI holds great potential as an assessment tool because it is non-invasive and facilitates the undertaking of longitudinal studies [31] including MRI assessments such as T_1 hypointensity and its correlation with progression of neurological impairment [80]. In conjunction with in vivo MR imaging, ex vivo imaging provides higher resolution without restrictions upon experiment time, allowing detection of more subtle changes [81]. Table 2 summarizes MRI observations in MS patients and mouse models and their correlations with MS pathology. Table 3 summarizes MRI interpretations and their correlations with the pathological expectations.

3.1. Conventional MRI

The MRI protocol for assessing MS patients often includes pre- and post-contrast T_1 -weighted imaging (T_1 WI), T_2 -weighted imaging (T_2 WI), and fluid attenuated inversion recovery (FLAIR) [82]. At higher magnetic field (≥ 3 T), Double Inversion Recovery (DIR) has recently been developed to increase the sensitivity for detecting cortical lesions [15, 20]. These structural MR imaging techniques have played an important role in the study of spatial and temporal characteristics of the disease progression [15, 83, 84].

Table 1. Summary of rodent models of MS and clinical features

MS Model in Mice	Method of induction	Clinical significance, severity	Reference
EAE	MOG ₃₅₋₅₅ + FCA	Chronic relapsing course without recovery	[53]
EAE	MOG ₃₅₋₅₅ +Pertussis Toxin+ Quil Adjuvant	Relapsing remitting (demyelination/remyelination) with complete recovery	[52]
Virus-induced demyelination	TMEV	Either Monophasic stage (relapsing for seven days and recover within three weeks) or bi-phasic (mono-phasic with chronic demyelination)	[64]
Toxin-induced demyelination	Cuprizone	Acute or chronic demyelination during a Cuprizone diet followed by remyelination	[70]
Toxin-induced demyelination	Lysolecithin	Demyelination and remyelination process	[72]

T₁-weighted imaging

In pre- and post-contrast T_1 WI, MS plaques appear hypointense; such observations have been correlated with both demyelination and axonal loss [15]. Post-contrast T_1 WI provides the benefit of measuring blood brain barrier (BBB) permeability, where leakage has been linked to MS lesions and the progression of relapsing symptoms [85]. In MRI studies of MS animal models, similar approaches have been used, including 2D/3D pre- and post-contrast T_1 WI and T_2 WI. These techniques are often used in addition to diffusion tensor imaging (DTI) parameters (explained in Section 3.3), such as fractional anisotropy (FA), relative anisotropy (RA), axial and radial diffusivities (AD, RD) [42, 43].

T_1 WI has been shown to be sensitive to chronic progression in the TMEV MS mouse model using normal C57BL6 mice and immune differentiation marker-deficient mice [80]. In this experiment, TMEV was administered to four groups of mice, viz: (1) normal C57BL6 mice, as a reference of T_1 hypointensity lesion load; (2) immuno-deficient C57BL6 mice RAG (Recombination activation gene)-1 deficient; (3) RAG-1 deficient mice with CD4+; and (4) RAG-1 deficient mice with CD8+ T-cells. T_1 WI was performed at 7 T to study MS lesions. In control C57BL6 mice, hypointense regions were mostly located in the periventricular area and hippocampus (Figure 2). In RAG-1 deficient mice, there was a significant reduction in the T_1 hypointensity lesion load compared with C57BL6 TMEV mice, indicating the importance of the innate immune system for disease progression. T_1 hypointense lesions increased in RAG-1 mice administered CD8+ T-cells, but were unaffected in

RAG-1 mice administered CD4+ T-cells [77]. This study highlights the distinctive role of CD4 + T cells (or T helper cells), which play a major role in the EAE model [64, 86]; compared with the role of CD8+ cells in the TMEV mouse model of MS. It has been suggested that these differences may have contributed to the lack of success of treatments targeting CD4+ T cells in the treatment of human MS [80].

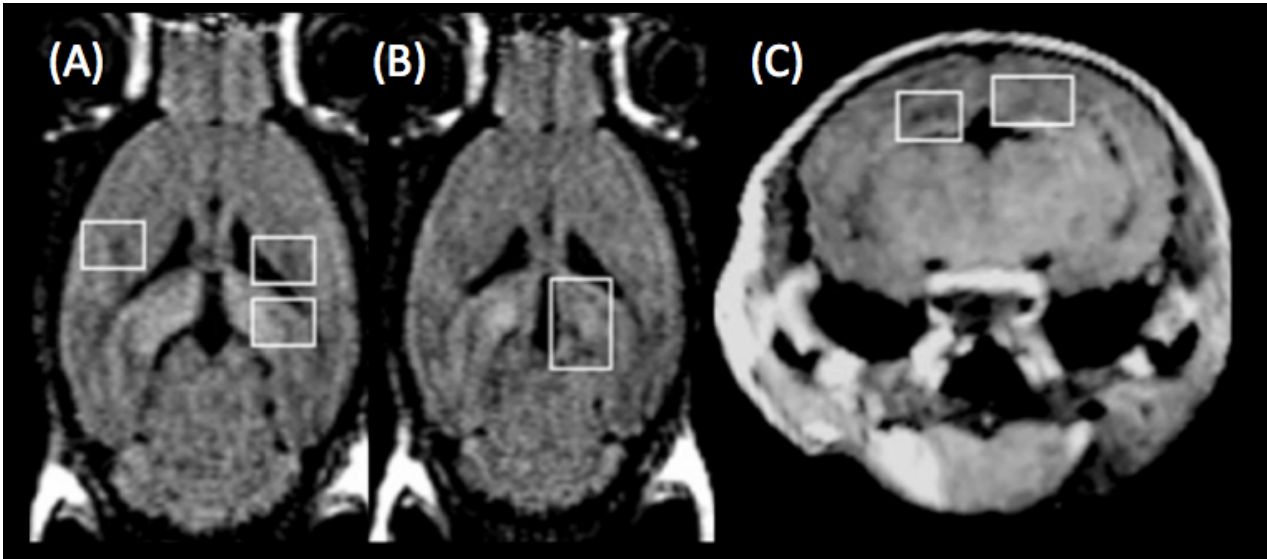


Figure 2. 3D T_1 weighted imaging at 7T of C57BL6 infected with TMEV (A), (B) coronal and (C) axial sections were extracted from 3D T_1 WI. 7 days post infection. T_1 hypointense lesions were more pronounced in the periventricular area and adjacent to the hippocampus as indicated by the white frames. (TR/TE= 200/10 ms, voxel dimensions= 135×200×200 μm) [80].

T_2 -weighted imaging

T_2 WI plays a major role in the detection of an MS lesion. The detection of new T_2 hyperintense lesions after the onset of the first MS attack signifies further progression of the disease, and indicates the beginning of the relapsing remitting disease course [83, 84]. High resolution T_2 WI has also been used to detect brain atrophy in patients with MS [87-89].

High resolution T_2 WI has been shown to be useful for measurement of volumetric changes in EAE mouse brains that were significantly correlated with histopathological analysis [78]. This *ex-vivo* MRI study in EAE-mice found significant reductions in the volume of the cerebellum, cerebellar cortex and molecular layer of the cerebellar cortex. Atrophy in the molecular layer of the cerebellar cortex is related to loss of Purkinje cells [90]. Such detection of grey matter (GM) atrophy was important as it was strongly correlated with progressive development of clinical disability [78, 91].

A more recent T_2 WI *in vivo* study in EAE-mice from the same group [32] re-affirmed the presence of GM atrophy in the cerebral cortex, characterized by a major decrease in the volume of the cerebellum by 80 days post-EAE induction [31]. However, there was no discernable correlation between disease severity and whole brain volume, or volume changes in the cerebral cortices during the course of the disease. Nevertheless, *in vivo* mouse MRI studies are advantageous compared with *ex-vivo* studies, as there is less distortion of the anatomical structures that occurs in *ex vivo* studies due to the brain tissue fixation process [31]. As additionally, *in vivo* longitudinal

studies facilitate measurement of temporal volumetric changes from the data acquired pre- and post induction of the disease and during the disease course [92].

In the TMEV mouse model of MS induced using SJL/J mice, T_2 WI showed a correlation between the presence of deep grey matter hypointense lesions and functional impairments [93]. T_2 WI hypointense signals have been observed in several deep grey matter structures, including the thalamus, caudate, putamen and dentate nuclei (Figure 3), and these have been correlated with cognitive, neuropsychiatric and motor dysfunctions [89]. Additionally, TMEV SJL/J mice experienced severe disability that was correlated with the gradual development of a thalamic T_2 hypointensity [93].

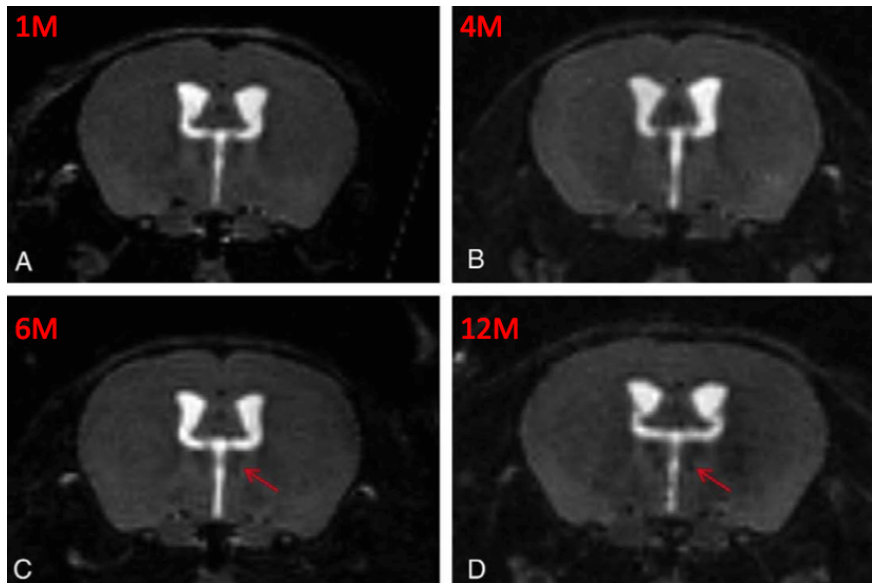


Figure 3. The correlation between increasing hypointensity of the medio-dorsal thalamic nucleus on T_2 -weighted images and disease progression (A) One month post disease induction, (B) 4 months, (C) 6 months and (D) 12 months as indicated by red arrows. 3D volume RARE sequence at 7T with TR=1500 ms, TE=70 ms, ETL=16, FOV=3.20×1.92×1.92 cm, voxel dimensions= 125×150×150 μ m [93].

T_2 hypointensity may be caused by several factors including iron, free radicals, the presence of macrophages and deoxyhemoglobin [94]. T_2 hypointensity has been reported in human studies [95, 96], but as the lesions were located within the deep grey matter, biopsies were difficult to perform to investigate the tissue properties [94]. In a MS study in humans at 3 T [97], there was a correlation between the physical changes assessed using the Expanded Disability Status Scale (EDSS) and alterations of signal intensities in the globus pallidus and caudate nucleus [90].

In other work, an excessive grey iron deposition, assessed by T_2 hypointensity within the deep grey matter structures [98], was correlated with cognitive dysfunction. On the other hand, T_2 hyperintensity may serve as a potential biomarker for detecting active MS lesions [13]. However it exhibits low specificity to acute and chronic MS disease pathology [99-101]. This is due to the presence of inflammation, edema and demyelination in acute lesions, and profound demyelination, axonal loss and gliosis in chronic lesions [13]. These pathologies could result in the elevation of tissue water content and consequently lead to indistinguishable causes of T_2 hyperintensity [102].

Correlation of T_1 and T_2 weighted signal intensity changes with histopathology requires an animal model that can produce a large number of inflammatory lesions [81, 103]. This has been established in the clonal adaptive transfer EAE model using SJL/J mice. 3D high resolution T_1 WI and T_2 WI at 2.35T were acquired to trace the lesion formation [104]. During the acute stage (9 days post adaptive transfer), two independent patterns of lesions were detected: (1) hypointense lesions on both T_1 and T_2 WI (Type A lesions), (2) other lesions, characterized by hypointensity on T_1 WI and hyperintensity on T_2 WI (Type B lesions). The histology studies using Mac-3 immunostaining and haematoxylin staining show that type A lesions contained a higher density of inflammatory cells and myelin loss and were more destructive than type B lesions [104]. On the other hand, for B type lesions, T_2 hyperintensity was positively correlated with density of activated microglia and reactive astrocytes, although, T_1 hypointensity was negatively correlated [104]. Serial MRI was performed during the disease cycle, and the patterns of two lesions were heterogeneous [104]. At the end of the disease cycle (30 days post adaptive transfer), for type A lesions, T_1 hypointensity persisted and T_2 hypointensity diminished. For type B lesions, T_1 hypointensity diminished and T_2 hyperintensity persisted [104].

Inversion recovery

DIR and FLAIR imaging can increase the sensitivity of detecting focal areas of hyperintensity, especially in grey matter and cortical lesions [105, 106]. However, these techniques have low sensitivity for the heterogeneous pathologic substrate of individual lesions. Although a recent study [107] compared T_1 WI, T_2 WI and FLAIR at both 3T and 7T, these techniques did not appear to increase sensitivity for detection of WM lesions. On the other hand, 3D-FLAIR at 7T could be tailored to improve sensitivity in depicting grey matter lesions [107]. This technique combined axial FLAIR (1mm^3 isotropic resolution) and T_2^* WI (0.55mm^3 isotropic resolution), to produce FLAIR* with 0.55mm^3 isotropic resolution [106]. The resulting image showed suppression of cerebrospinal fluid (CSF) and high-resolution visualization of microvascular structures, which enabled investigation of the presence of interlesional veins in WM lesions [106]. Such lesions were detected in the pons and cerebellum and could be related to the presence of iron-containing macrophages [106].

In rodent brain MRI, inversion recovery was used to produce stronger T_1 -weighting. For example, a T_1 weighted 3D Modified Driven Equilibrium Fourier Transformation (MDEFT) sequence at 9.4T [108] has been used to enhance visualisation of cortical areas, the striatum and the ventricular system. However, T_1 WI MDEFT could not detect EAE inflammatory lesions, in contrast to T_2 and T_2^* WI [108]. FLAIR with inversion recovery does not appear to have been applied to the study of rodent models of MS, as the T_1 relaxation time is considerably long in high magnetic fields commonly used in these studies [81].

Limitation of conventional MRI in rodent models of MS

The limitations of conventional MRI techniques to monitor or assess the contents of the MS lesion are caused by the heterogeneity of MS lesions, comprising cellular debris from demyelination and/or remyelination processes, permanent axonal loss and cellular activity [27]. In addition, T_1 - and T_2 -weighted-imaging cannot detect MS lesions within normal-appearing white matter (NAWM) but which were observed in post-mortem studies [109, 110].

Cryogenic radiofrequency coil mouse brain MRI

The recent introduction of cryogenic radio frequency (RF) coils (cryocoil) may improve the role of conventional micro MRI in assessing pathological changes in rodent models. At 9.4T, cryogenic coils boost Signal to Noise Ratio (SNR) by a factor of up to 2.9, which can be exploited for in-vivo imaging to significantly shorten experiment time [111, 112]. For example, a volumetric T_2 WI at $(60\ \mu\text{m})^3$ - isotropic resolution could be acquired within 45 min. The cryocoil has a maximum signal penetration of approximately 3 mm from the brain surface and can reveal intricate cortical and subcortical details of the mouse brain [112]. Despite its high sensitivity, the cryocoil has some disadvantages in terms of its inhomogeneous RF excitation profile. Gradual loss of signal-to-noise ratio for deeper (ventral) brain structures were also observed due to the positioning of the surface array coil on the cortex. However, such artifacts can be minimized with careful adjustment of the RF power and image post-processing [111, 112].

In-vivo conventional micro MRI using the cryogenic RF coil (T_1 , T_2 and T_2^* weighted imaging) has been used to detect MS lesions prior to the appearance of the symptoms in EAE-mice immunized with PLP₁₃₉₋₁₅₁, complete Freund's adjuvant (CFA) and heat-killed mycobacterium [108]. High-resolution images were used to detect MS lesions before the disease manifestations in the cerebellum, cerebral cortex and subcortical region (figure 4) [108].

3.2. Contrast agent enhancement and cellular tracking

Paramagnetic gadolinium (Gd) contrast agents are commonly used to investigate MS. Recently developed contrast agents such as ultra-small and small superparamagnetic iron oxide (USPIO and SPIO particles, respectively) [15] have been used to monitor the cellular mechanisms of MS inflammation. The cells of the monocyte-macrophage system take up the iron oxide particles and their infiltration into the lesion sites could be detected using MRI [113]. The major differences between USPIOs and Gd are their pattern of enhancements. Gadolinium enhancement reflects leakage of the blood brain barrier (BBB) whereas the USPIOs' enhancements represent cellular infiltration [34, 114-116]. Several studies [115, 117, 118] have shown that the USPIO pattern of enhancements is correlated with MS disability, axonal loss and patient's response to treatment.

The biological specificity of the USPIO depends upon the molecular characteristics of the applied particles [34, 114]. For example, USPIO particles SHU555C and Ferumoxtran-10 differ in size (25 nm vs. 30nm), and body circulation half-life (6-8 h vs. 24-30 h). In-vitro, the negative charge on SHU555C particles produced preferential uptake by activated monocytes [119].

There is still controversy as to whether Gd or USPIO provides a better early indication of MS pathology [34, 114, 115]. In some cases, USPIO is superior to Gd, because it can detect lesions a few weeks earlier than Gd enhancement [34]. USPIO lesion enhancement may persist longer after Gd enhancement has ceased and show spatial distribution of the immune-reactive cells post BBB repair [34, 82]. A recent comparison between USPIO and Gd enhancements in 10 patients with RR-MS [120] showed that the same lesions were seen with both contrast agents, but that additional lesions were specifically observed using each of USPIO and Gd [120].

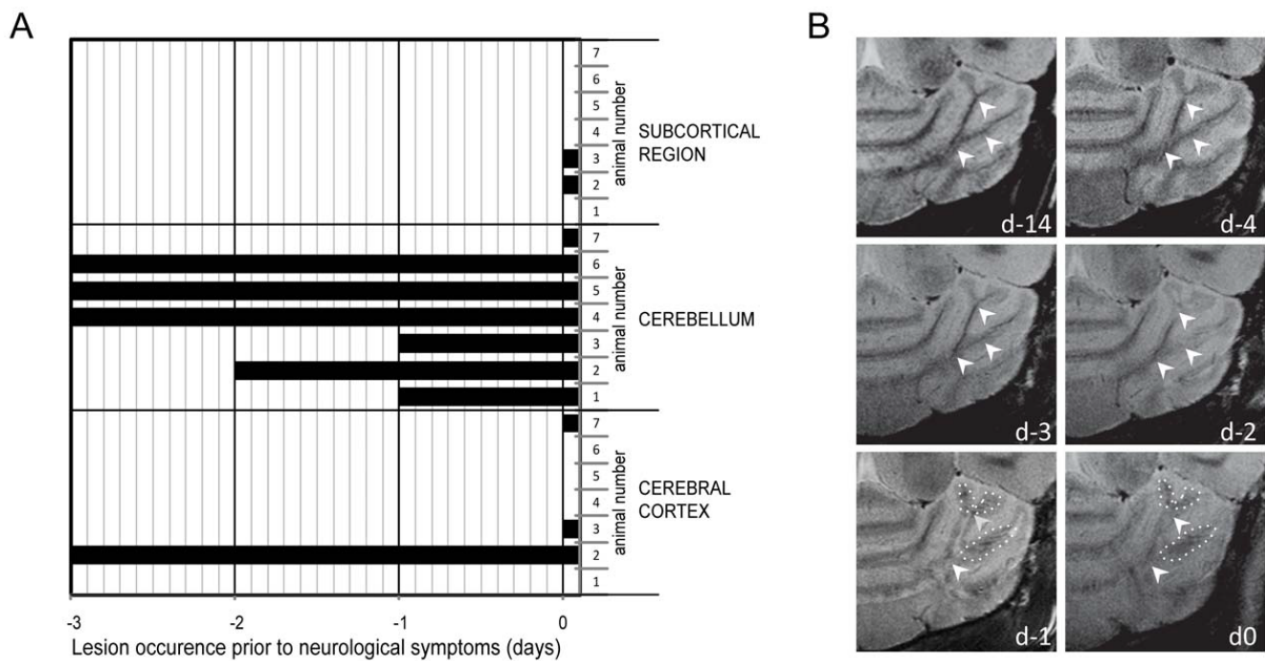


Figure 4. Application of micro MRI T_2 WI in assessing evolution of the lesion prior and during disease onset (A) Detection of MS lesions in subcortical region, cerebellum and cerebral cortex in seven EAE-mice prior to the disease onset until the disease starting time. (B) T_2 weighted images from day 14 (d-14) prior to disease manifestation until the first day when symptoms were initiated (d0)[108].

Post-contrast studies have been used to monitor BBB permeability, which could be disrupted during deposition of new MS lesions [33, 121]. To assess the development dynamics of MS lesions, the TMEV mouse model of MS has been induced in interferon-gamma receptor knockout mice[33]. In TMEV mice, there was acute progressive demyelination without remission resulting in extensive brain and brainstem lesions [33]. This model has allowed a comprehensive understanding of the T_2 lesion load and enhanced lesions. One study [33] has explored four unique patterns of T_2 lesions, including expanding, expanding retracting, fluctuating and stable (Figure 5). As this model produces extensive MS lesions, the T_2 WI detects more MS lesions compared with post-contrast enhanced imaging, and with earlier temporal sensitivity compared with Gd enhancement [33].

Predicting disease severity in MS is a critical issue for planning approaches to treatment and consequently limiting development of neurological disability [13]. USPIO has been used as indicator of disease progression in rats with RR EAE [117]. Rats with positive detection of USPIO particles (+USPIO rats) show significant tissue alteration at the first attack and more severe clinical signs compared to -USPIO rats during the second attack (Figure 6). This study demonstrates the role of macrophage infiltration as an indicator of progressing inflammatory demyelination.

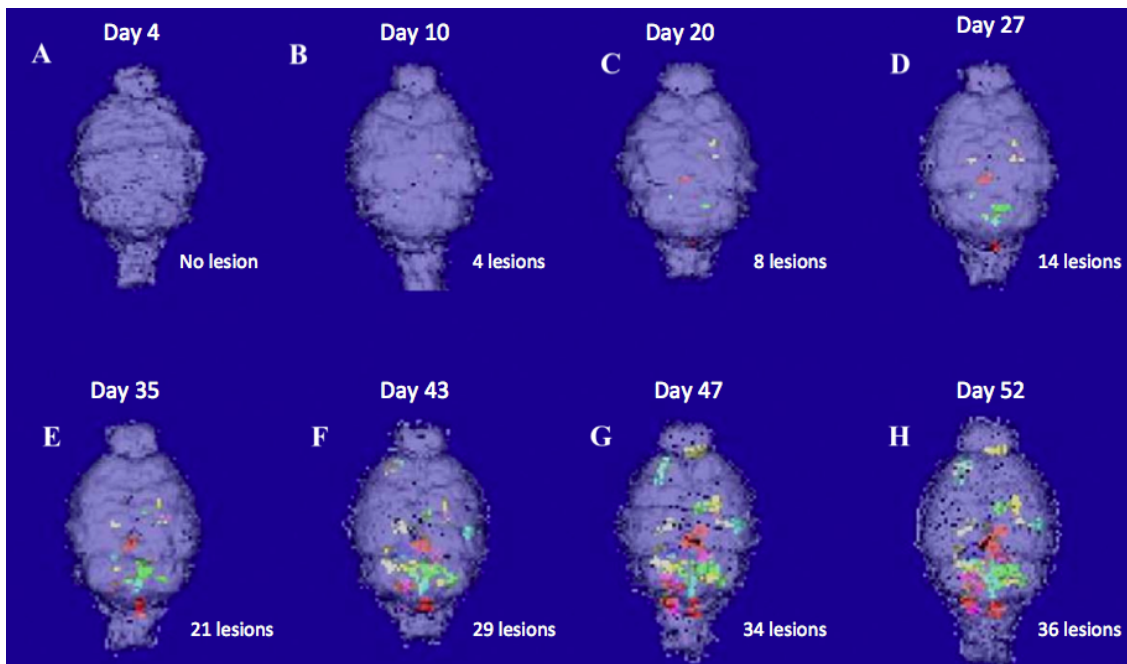


Figure 5. Characterisation of lesion developments in interferon-gamma receptor knockout mice 3D volume rendering resampled from T_2 WI 3D datasets of the examined mice. 3D RARE acquired at 7T ($TR=1500$ ms, $TE=70$ ms, $ETL=16$, $FOV=3.5 \times 3.5 \times 3.5$ cm voxel dimensions $218 \times 218 \times 218 \mu\text{m}$) [33].

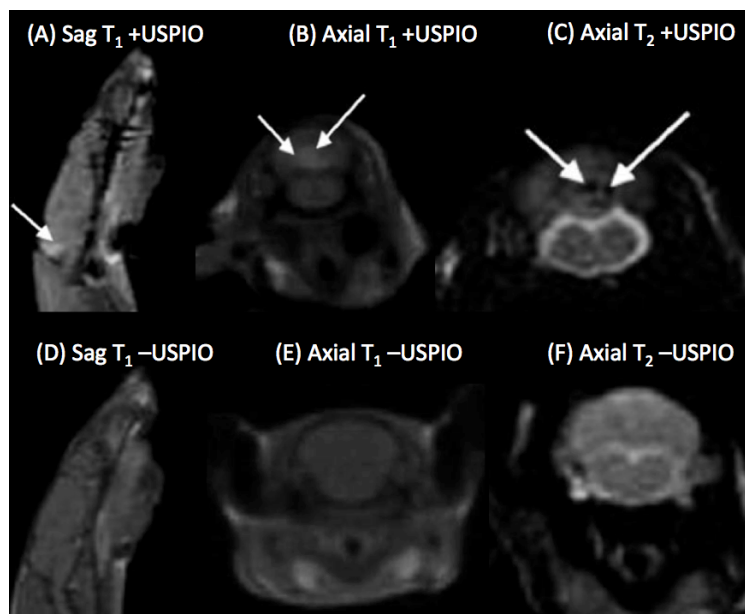


Figure 6. Detection of macrophages using USPIO contrast agent at 1.5T for assessment of their role in the development of secondary progressive EAE in rats (A) Sagittal T_1 WI and (B) axial T_1 WI show hyperintensity and reflects the uptake of USPIO in the CNS. (C) Axial T_2 WI shows hypointensity as in indicated by arrows. These signal alterations have not observed (D), (E) and (F) with other EAE rats in this experiment [117]

3.3. Diffusion Weighted Imaging (DWI) and Tractography

Diffusion tensor imaging

The measurement of tissue molecular diffusion using diffusion weighted imaging (DWI) is widely used in MS research [122-124]. MRI is sensitized to microscopic diffusion of water in tissue by the application of diffusion gradients. Gaussian modelling of tissue water diffusion can broadly be classified as non-restricted (isotropic) and directionally restricted (anisotropic) diffusion. In neuronal tissues, isotropic diffusion can be identified within cerebrospinal fluid (CSF) and to a lesser extent the GM, whereas anisotropic diffusion has often been correlated with the degree of myelination and axonal fibre directionality of the WM [125, 126].

The directionality and strength of anisotropic diffusion can generally be described as an ellipsoid diffusion tensor, which can be measured using magnetic resonance diffusion tensor imaging (DTI) [125, 126]. DTI acquisition requires a minimum of six orthogonally encoded DWI and one unweighted image [127, 128]. From these measurements, three eigenvectors (v_1, v_2, v_3) and their rotational invariant eigenvalues ($\lambda_1, \lambda_2, \lambda_3$) can be derived to describe the diffusion tensor [128]. The largest eigenvector (v_1), the direction of the major diffusion component, and its associated eigenvalue (λ_1) represent the magnitude of axial diffusivity along the length of the axonal fibre bundle [129]. v_2, λ_2 and v_3, λ_3 describe transverse diffusion components orthogonal to the fiber bundle, where the average of λ_2 and λ_3 is known as radial diffusivity (Figure 7) [127, 129, 130].

DTI parameters can be used to derive rotational invariant diffusion metrics: mean diffusivity (MD), Fractional Anisotropy (FA) and Rational Anisotropy (RA). MD describes the average of diffusivity components within each voxel [131]. The diffusion tensor parametric FA is more commonly used than RA in the DTI literature [132]. FA values range from 0 to 1 to describe the degree of the anisotropy of the intra-voxel diffusivity [133]. FA values in WM are generally higher than in GM due to increased diffusion directionality of the myelin axon bundles [128].

DTI parameter changes in MS

DTI derived parameters are powerful and sensitive measures for assessing MS pathological changes. MS pathology is associated with damage to the white matter myelin structure, resulting in disruption of molecular water diffusion and a consequential reduction in diffusion anisotropy [13]. A summary of DTI parametric changes in WM affected by MS is illustrated in Figure 7.

DTI parametric maps provide distinguishing characteristics for careful interpretation of MS lesions, even for those that are readily detectable by the conventional imaging techniques [123]. MS lesions are heterogeneous with transient lesions due to oedema, areas with demyelination and remyelination, and areas with advanced neurodegeneration [82, 134].

Compared with the normally-appearing white matter (NAWM), MS focal lesions showed increased MD which indicated some loss of the structural barrier to water molecular diffusion and a decrease in FA due to disorganisation of WM structures [122]. Co-localisation of T_1 hypointense and DTI lesions may signify areas suffering from irreversible tissue damage [135]. Additionally, a longitudinal progressive MS study [136] showed that worsening lesions could be detected more sensitively as areas with increasing MD, which were otherwise indistinguishable using post-

contrast T_1 WI. On the other hand, lesion areas with increased T_2 relaxation time and increased MD appear to reflect a decrease in the axonal myelin (intracellular) water content and an increase in the extracellular water due to tissue damage [137]

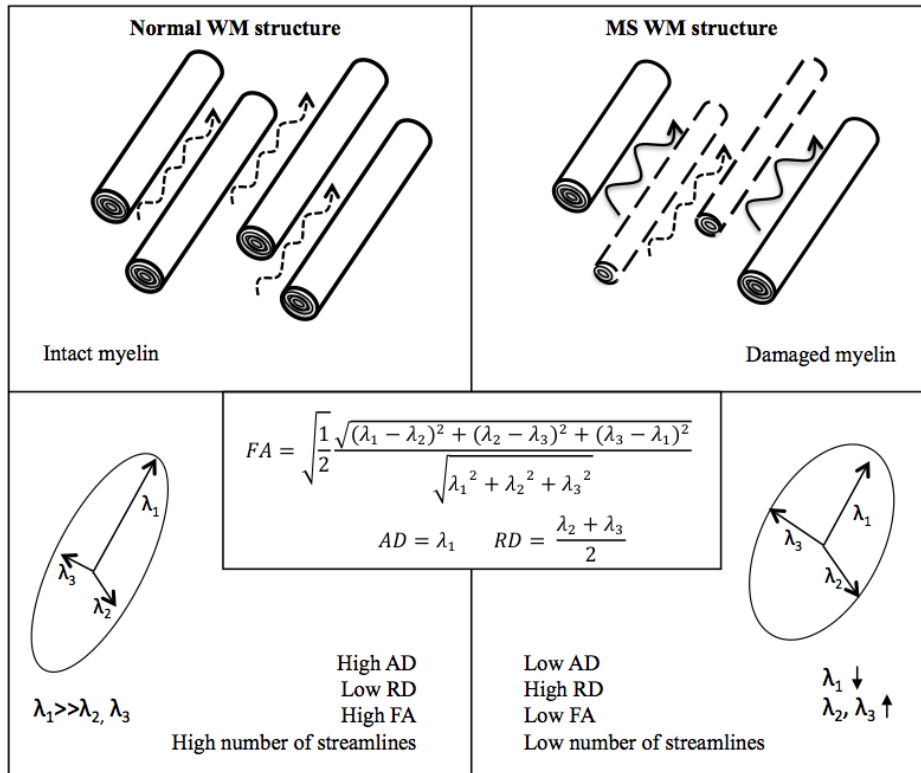


Figure 7. The relationship between the white matter axonal fibres, myelin structure and DTI derived parameters in MS pathology. The demyelination, axonal injury and inflammation processes in MS typically result in a decrease of FA, and AD and an increase in RD.

DTI studies of MS animal models

AD and RD measurements are potentially more sensitive than MD in diagnosis of MS lesions due to characteristic high diffusion anisotropy in the WM [132, 138]. In MS studies using the cuprizone rodent model [139, 140], decreasing AD and increasing RD were robust surrogate markers for axonal damage and demyelination, respectively. These conditions were observed in various DTI MRI animal studies, for example: (i) In a retinal ischemia mouse model, where a decrease of AD correlated with axonal injury [141]. (ii) In a spinal DTI study of a mouse model of chronic EAE [142], histology data confirmed a correlation of intense anti- β -amyloid precursor protein staining with a decrease in AD associated with axonal damage; and an increase in radial diffusivity with diminished luxol fast blue (LFB) staining as a biomarker of demyelination (iii) In a cuprizone-induced demyelination/remyelination mouse model [143], T_2 hyperintensity, a reduction in AD and an increase in RD were specifically observed in the caudal segment of the corpus callosum, and these were correlated with histological observations of demyelination (LFB staining), axonal injury (neurofilaments staining), microglial accumulation and cellular infiltration. (iv) In inflammatory optic neuritis (ON) in an EAE mouse model [144, 145], uniform ON resulted in axonal injury (decreased AD) and demyelination (increased RD), as shown in Figure 8.

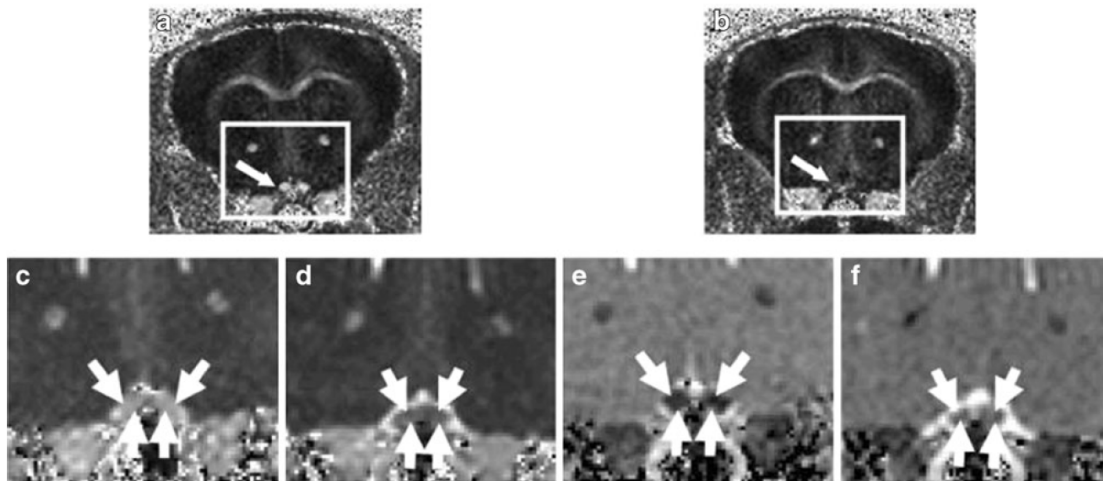


Figure 8. Assessment of optic nerve degeneration in EAE-mice using DTI indices. A reduction in relative anisotropy (RA) value can be observed in the EAE mouse (b) compared with the control subject (a). Axial diffusivity decreases and radial diffusivity increases in the EAE mouse (d, f, respectively), in comparison with the control (c, e, respectively). DTI was acquired using Stejskal-Tanner sequence at 4.7T, TR=1.7 second, TE=50 ms, 6 diffusion directions acquired with b-value=838 s/mm², NEX=4, slice thickness=0.5 mm, FOV=3×3 cm, and resolution=59 × 59 × 500 μm [77].

A recent study [146] has assessed the axial and radial diffusivities within the abnormally low FA areas in the brains of patients with MS. The brain regions examined include fornices, inferior longitudinal fasciculus, optic radiations, and parts of the corpus callosum. There was an increase in RD caused by demyelination but surprisingly, there was only a small increase in AD [146]. A complicated pattern of change in diffusivities may occur due to axonal loss [147] or an increase in axonal diameter. This was supported by previous findings in post-mortem MS spinal cord studies which showed areas of T₂ WI hyperintensities [148]. For areas containing crossing fibers, abnormal changes to the FA, AD and RD must be carefully assessed as MS pathology in these areas can produce unexpected results such as an apparent increase in diffusion anisotropy [133].

Investigation of DTI parametric changes in MS rodent models [77, 142, 143] may not necessarily reflect pathological changes in patients with MS. For example in the mouse model of MS, changes in RD were not observed, although severe demyelination, inflammation and axonal damage were detected by histology [131]. Such negative observations may be due to confounding factors affecting RD sensitivity for detection of demyelination; for example, inflammation due to the presence of activated microglia and macrophages, T cell infiltration, as well as vasogenic oedema affect the apparent diffusion anisotropy in MS lesions [131, 149].

Many DTI studies of human MS [110, 124, 146, 150, 151] and rodent models [77, 143, 152, 153] are focused on the WM structures as they are the anticipated sites of MS pathological changes. High WM anisotropy provides characteristics for sensitive detection of MS pathological changes using DTI measures [110, 124, 146, 150, 151]. MS, however, is a whole brain disease and can affect GM and cortical areas [85]. A three year longitudinal study of relapsing remitting MS patients at 1.5T has reported an increase of FA in normal appearing grey matter (NAGM) and cortical lesion volume, which are correlated with clinical disability [20]. This unexpected finding may be explained by crossing fibers in the grey matter, which appeared to have low FA when measured using DTI [20]. The MS pathology may selectively reduce one fibre population in the crossing fibres, such that this resulted in an apparent increase in FA measures [133].

DTI Tractography studies in MS

DTI tractography reconstruction provides a unique depiction of three-dimensional projections of neural structures, which is useful for studying brain connectivity. DTI tractography can potentially be used to stage MS progression as MS lesions produce local or global disruptions in the WM fibre architecture that affect fibre tractography profiles and streamline numbers [77, 154]. DTI tractography is useful for extracting WM pathways in patients with MS, for example, in the corpus callosum, for investigation of undetected NAWM lesions and consequential correlation between the clinical symptoms and the imaging findings [123, 150].

There are various methods available to process diffusion-weighted imaging data in performing tractography [155-157]. In general, tractography image processing firstly involves the determination of fibre orientation distribution, followed by the propagation of tracts (streamlines) using deterministic [158] and probabilistic fibre tracking methods [133, 159]. Tractography can be performed for the whole brain, or selectively using specific seeding and targeted regions of interest [159, 160].

DTI tractography has been used to visualize the effect of MS lesions on the projection, association and commissural fibres [150]. MS patients exhibited an increase in ADC and reduction in FA, fibre density and streamline profiles within MS lesions and NAWM compared with healthy subjects, in which measures were correlated with the pattern of EDSS or clinical score of disability. Region-specific DTI tractography has also demonstrated sensitivity to functional changes: (i) disruption of pyramidal and corticospinal tracts resulting in motor dysfunction [161-163]; (ii) disruption of left/right thalamic connectivity affecting working memory in early MS [151].

Limitations of DWI studies in animal models

DWI studies of the mouse brain, either *ex vivo* [140, 164, 165] or *in vivo* [77, 121, 143] are based upon FA or orientation colour-coded FA maps, rather than fibre tracking [166]. In addition, most of the fibre-tracking algorithms require high diffusion gradient weighting (b-values) and a large number of the applied diffusion-encoding gradients directions (High Angular Resolution Diffusion Imaging, HARDI). This contributes significantly to the experiment time, and can be problematic for *in vivo* DWI involving unhealthy participants.

Ex vivo HARDI could be a reasonable solution, although post-fixation procedures have been shown to reduce the diffusivity measures in comparison to data obtained from *in vivo* imaging [167, 168]. Such affects may be caused from variable tissue temperatures, microstructure properties, cell death and chemical fixation solution [169]. A recent study examined the difference between *in-vivo* and *ex-vivo* DTI in assessing the corpus callosum in both wild type C57BL6 and cuprizone-induced mice. In control subjects, FA measurements were not significantly different between the *in vivo* and *ex vivo* samples; however, the diffusivity measurements (AD and RD) were drastically reduced in the *ex-vivo* experiments [153]. Demyelination has been detected in the corpus callosum of cuprizone mice but there is little agreement between *in-vivo* and *ex-vivo* FA measurements. It is suggested that *ex vivo* RD serves as a potential indicator of demyelination better than *in vivo* RD. On the other hand, *in vivo* AD is more reliable than *ex vivo* AD in detecting axonal injury [153].

The primary technical challenge of performing mouse brain DWI studies is achieving the required spatial resolution while preserving satisfactory signal-to-noise ratio (SNR). This is especially important during the application of strong diffusion-encoding gradients which results in significant signal attenuation and lower SNR [81]. In addition, with high numbers of sampling diffusion-encoding directions, DWI requires a longer experiment time compared with other conventional MR imaging modalities [131]. In most cases, rodent DWI experiments are acquired at high magnetic field (>7T) with small custom coils built to boost SNR. The use of higher magnetic fields brings undesired effects such as higher magnetic susceptibility and chemical shift artefacts, longer T_1 and shorter T_2 relaxation times [42]. DWI is inherently sensitive to motion to measure microscopic water displacement; and therefore, voluntary and involuntary movement can severely affect image quality [129, 131, 154]. The use of respiratory gating and the navigator sequence has been developed to reduce these artefacts [170].

Assessing axonal pathology has become an integral and critical part in the diagnosis of MS. DW-MRI provides unique measurements of the distribution of water molecules in neural tissue, especially along the axons [171, 172]. Recent advances of DWI-MRI involves development of axon density and diameter measurements in the white matter of the living human brain, which potentially bring significant improvement to the detailed characterisation of MR pathology [171, 173]. However, challenges remain before DWI methods can be applied in clinical studies. These include the requirement for high gradient amplitude ($b > 3000 \text{ s/mm}^2$), relatively long imaging times to account for multiple diffusion directions and diffusion weightings, and prior knowledge of the fibre orientation [173].

3.4. Magnetization transfer imaging (MTI)

MTI is a quantitative MRI technique utilising the interaction and exchange between mobile protons in a free water pool and bound protons to macromolecules. The magnetization transfer ratio (MTR) is measured by the incorporation of saturation pulses into the preparative part of a gradient echo or a spin echo sequence [134, 174, 175]. MTR analysis can be done through a whole brain voxel based analysis (VBA) or using regions of interest, in which the MTR values are calculated from two sets of images acquired with and without the magnetisation transfer saturation pulses [134, 174, 175]. MTI allows the depiction of several diffuse occult MS pathologies, for example, demyelination, gliosis and inflammation [82, 176].

In patients [177, 178] and animal models [179], MS pathology was detected by an abnormal reduction in MTR, which was suggested to reflect demyelination and axonal loss. However, MTR determination of MS pathology is somewhat non-specific, because MTR reduction can also be caused by other pathological processes, such as oedema, gliosis and inflammation [134].

Several groups [180-182] have investigated change in MTR and correlated it with lesion genesis and evolution. MTR reduction was observed a few months preceding observable Gd enhancement, and a further reduction in MTR for Gd-enhanced lesions has potential to highlight deteriorating MS lesions [183, 184]. MTR can be combined with conventional MRI methods, providing additional diagnostic value in predicting the evolution of T_1 WI hypointense lesions [183, 184].

A modest reduction in MTR followed by a partial or complete recovery of MTR could indicate remyelination or other repair mechanisms during resolution of inflammation or gliosis [185].

Abnormal changes in MTR were also sensitive to detection of MS lesions within the normal appearing white and grey matter [185]. In other work [35] there were significant fluctuations in the MTR within active gadolinium-enhanced lesions, consistent with the demyelination and remyelination process over a period of three years.

In a comparison study [30] (figure 9) of relapsing remitting MS using the PLP (proteolipid protein) induced mouse model of MS and a chronic MOG-induced EAE mouse model of MS, MTR histogram analysis of the whole brain showed a significant reduction in MTR values in early stage disease (13 days post immunization). Using VBA, MTR reduction was found in multiple brain regions such as the corpus callosum, caudate putamen and hippocampus [30]. These early remarkable changes may reflect widespread changes to the myelin structure [186]. However, as the disease progressed and during the chronic stages of the disease cycle (28 days post immunization), the reduction in MTR values was not significant, which could indicate less structural damage, initiation of the myelin repairing mechanism, or inter-individual variability between mice at the chronic stage [30].

On the other hand, using chronic MOG induced mouse models of MS, there was a reduction in MTR during three stages (11, 17 and 28 days post immunisation) [30]. This emphasises the importance of structural and axonal damage as a primary pathological process in the MOG-induced model. This study confirms changes observed in a previous microscopic and histological study of the spinal cord using the same EAE model [186, 187]. Also, in the same study, hematoxylin and eosin (H &E) staining detected perivascular parenchymal cellular infiltration. LFB additionally demonstrated demyelination in the brain stem, thalamus and corpus callosum[30]. Glatiramer acetate (GA) has been given to both the relapsing remitting PLP and chronic MOG groups where the MTR values showed pseudonormalisation or recovery toward the normal values in both groups. These results support the role of MTR imaging as a marker of remyelination [30].

3.5. Susceptibility Weighted Imaging (SWI)

Susceptibility differences between tissues offers a unique contrast [188]. SWI is based on T_2^* weighted imaging, but consists of magnitude and phase information [189]. At sufficiently long echo time (TE), the signals from white and grey matter, due to their different magnetic susceptibility, become out of phase. Therefore, phase imaging can be used to enrich the contrast between tissue types as well as accentuating iron laden tissues and venous blood vessels [189, 190].

A recent MS imaging study using T_2^* weighted imaging at 7T [191] has explored the relationship between MS lesions and deep veins. The occurrence of plaque in association with deep veins has been established in previous histopathological studies, where the presence of the central vein or venule in the white matter lesion were suggested to be a distinctive marker for differentiating between demyelinated MS lesions and non-MS lesions [191].

However, this technique requires investigation at lower field strength to assess its sensitivity in a clinically relevant environment. Susceptibility weighted angiography at 3T [192] showed that central veins could be correlated with white matter lesions (WML) with or without demyelination. Therefore, distinguishing the origin of central vein WML from MS or other neuropathological changes is problematic [192].

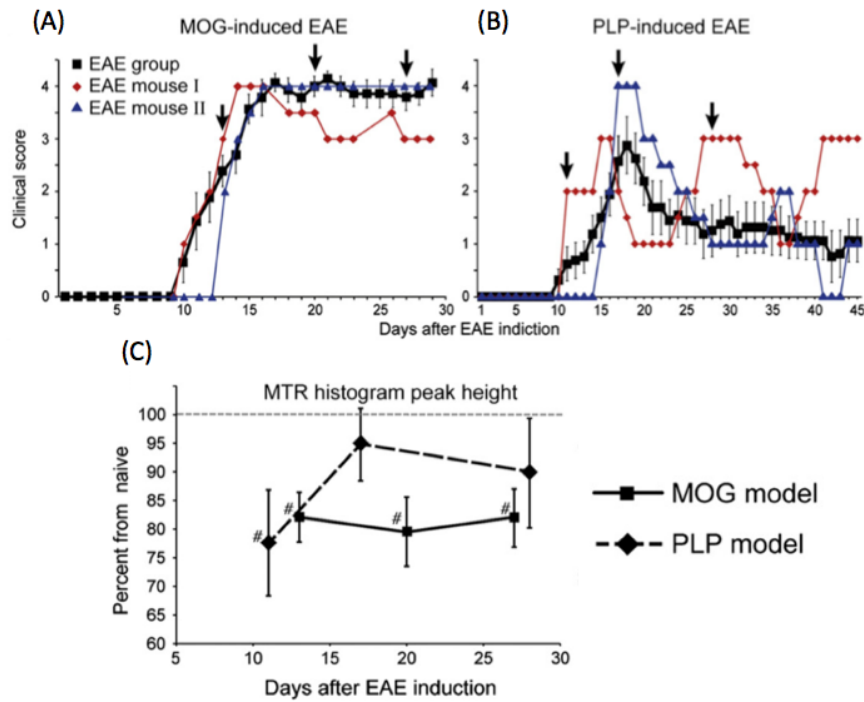


Figure 9. Magnetisation transfer application to study pathological changes in both MOG and PLP models Comparisons between (A) chronic MOG and (B) relapsing remitting (RR) PLP EAE induced models during MS cycle. (C) Whole brain MTR histogram shows reduction of MTR values in both groups but there was variability of the MTR values in the RR-PLP EAE group [30].

The role of perivenous space in developing MS lesion is still controversial [85, 193, 194]. Dynamic contrast enhanced studies may provide a better understanding of the relationship between the central vein and MS lesions. Disruption of the blood brain barrier (BBB) is an early-established MS lesion formation indicator [195]. However, it is not well defined whether the BBB disruption is the primary event that leads to lesion formation, or a secondary event that occurs after diffuse parenchymal tissue damage [85]. A recent study [195] has explored two patterns of enhanced lesions: new lesions tend to enhance centrifugally and established, whereas old lesions tend to enhance centripetally. This observation may be significant in resolving questions between opening and closing of the BBB with the central vein in MS lesion; and also establishing whether vascular permeability could be used as a distinctive surrogate distinctive marker of acute and chronic lesions [194].

SWI hypointense lesions have been demonstrated in EAE mice immunized with MOG₃₅₋₅₅, complete Freud's adjuvant (CFA) and pertussis toxin (PT) [196]. These lesions were more prevalent in the lumbar spinal cord and cerebellum during the peak of disease severity at around days 16-19, as well as during long-term imaging at day 30 up to 6 months (figure 10). In addition, some of the lesions were no longer visible following perfusion; the percentages of the remaining lesions after perfusion were 60.1 % and 46.6% in the spinal cord and cerebellum respectively. This could be an indicator of the role of deoxyhemoglobin in the lumen vessels, in which they would have disappeared in *ex-vivo* imaging [196]. Histopathology analyses of SWI hypointense lesions revealed iron deposition, inflammation and demyelination within the white matter of the lumbar spinal cord, and inflammatory perivascular cuffs within the white matter of the cerebellum [196].

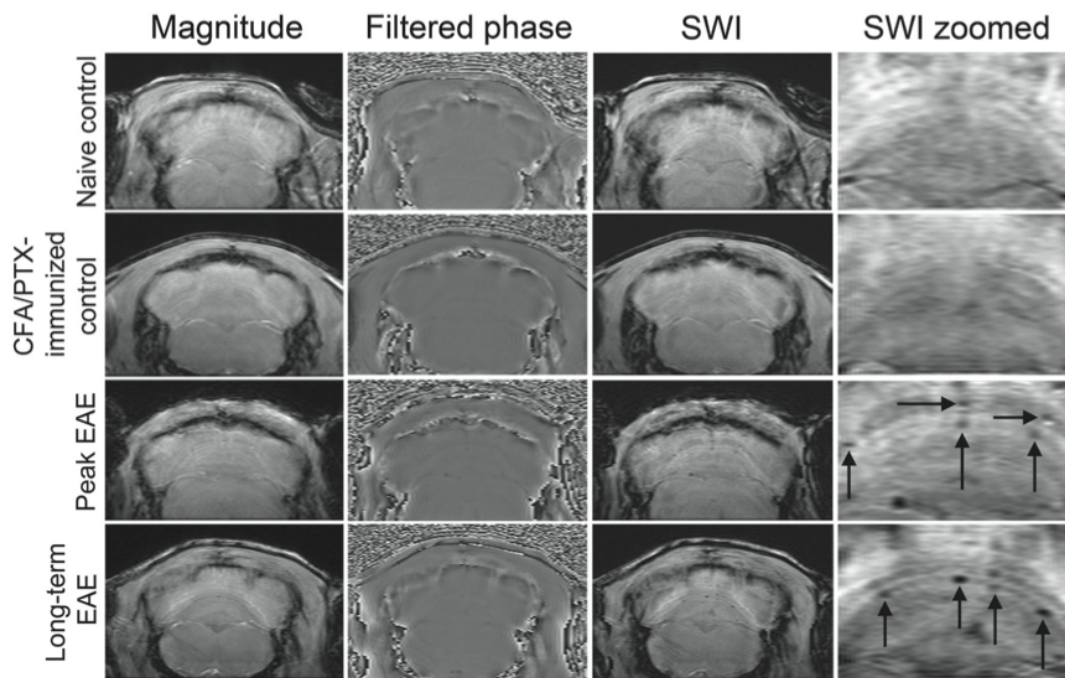


Figure 10. Visualization of SWI hypointense lesions in the cerebellar white matter of EAE mice, MS lesions can be observed in SWI (zoomed column) during the peak EAE (18 days post immunisation) and long-term disease progression (6 months post immunisation). These lesions (black arrows) have not been detected in the naive and control mice immunized with complete Freud adjuvant and pertussis toxin. SWI was acquired using 3D gradient echo flow compensated at 9.4T, imaging parameters: TR/TE= 50/4 ms, FA=15°, NEX=17, FOV=0.92×1.28×1.28, voxel size=48×100×400 μm) [196].

4. Conclusion

This review paper provides an overview of recent developments in MRI modalities tailored to investigation of specific aspects of MS using animal models and to enhance diagnostic accuracy in patients. These recent MRI developments include the introduction of more sensitive cryogenic coils and imaging at higher field strength.

Key aspects of MS that can be monitored by MRI include BBB leakage, immune cell infiltration, inflammation, demyelination, axonal injury, and changes in brain connectivity and structural volumes [1]. In addition to these pathological changes, remyelination, which is observed during the chronic stage, can be a significant biomarker of restoration of nervous system functionality [197]. Also, vascular permeability can be considered as a distinctive marker for distinguishing between acute and chronic MS lesions [194].

In the clinical setting, conventional MRI techniques have been employed to detect established lesions in chronic MS, but are generally insensitive during the initial stages of the disease [15]. Optimised methods such as FLAIR and DIR at high magnetic field strength provide improved sensitivity for detection of cortical lesions [20]. DWI can also detect such changes with additional advantages: (i) sensitivity for detection of early MS especially in the normal-appearing white or grey matter; and (ii) detection of changes in brain connectivity and fibre density with significant correlation with patient disability status [20, 134]. MTR is sensitive to changes in myelin content, and appears useful to differentiate demyelinated from remyelinated lesions [35]. SWI provides unique contrast either based on T2* or susceptibility changes and may become an increasingly

important technique, particularly at higher field strength [198]. There are several controversial imaging markers that have been proposed from SWI studies such as central vein detection but they require lengthy longitudinal studies and standardized imaging protocols [192].

Longitudinal MRI imaging of rodent models of MS facilitate investigation of temporal changes in the brain during progression from the acute to the chronic stages of MS disease. In general, MRI findings observed in patients with MS patients have been similarly observed in rodent models of MS. These include T_2 hypointensity of the deep grey matter lesions [93], USPIO detection of immune cell infiltration [117], reduction in FA due demyelination [77] and brain atrophy [32].

Table 2. Correlation between MRI observations in patients with MS and rodent models

MRI observations	Human MS studies	Rodent MS studies
T_1 WI hypointensity lesions	For detection of advanced MS lesions [15].	Detected in the TMEV mouse model to investigate the role of the adaptive immune system [80].
T_2 WI hypointensity lesions in the deep grey matter	Good correlation with clinical disabilities [95, 96]. Requires detection using high magnetic field, such that it may be difficult to apply in the setting.	Observed in the TMEV mouse model and could be useful for efficacy assessment of novel MS treatments [93].
Changes in brain volumes	Distinct biomarker for advanced disease progression [87-89].	In EAE mouse model, the loss of cortical volume was due to loss of Purkinje cells in the molecular cortex [32, 78].
USPIO enhancement	An indicator of immune cell infiltration [120].	Observed in the relapsing remitting EAE mouse model of MS; linked with presence of macrophages and demyelination [117].
Central veins observed on T_2^* WI and hypointensity on SWI	Distinctive imaging markers in determining MS demyelinated and non-demyelinated lesions [191]	These lesions observed in the lumbar spinal cord and cerebellum of EAE-mice [196]

Table 3. Summary of MRI observations and pathological correlations

Technique	MRI changes in patients with MS	Pathobiological correlations	References
T_1 WI	Hypo-intensity	Severity of tissue damage	[80, 104, 134]
T_2 WI	Hyper-intensity	Active (developing) MS lesions	[13, 82]
T_1 pre/post contrast	Hypo-intensity	Combination of demyelination and axonal loss	[15, 27, 199]
T_1 post contrast	Hyper-intensity	Permeability of the blood brain barrier and activity of MS lesions	[34, 102, 120]
MTR	Decrease	Progressive demyelination over time	[35, 174, 185]
	Increase	Partial remyelination within active MS lesion	
MD	Increase	Extracellular oedema and inflammation	[122]
FA	Decrease	Lower axonal bundle coherence in WM lesions or lesions in NAWM	[123, 200]
Axial diffusivity	Decrease	Axonal damage	[146]
Radial diffusivity	Increase	Demyelination	[139, 140, 146]
MTR	Decrease	Progressive demyelination over time	[150]
	Increase	Partial remyelination within active MS lesions	
SWI	Central veins in the MS lesion on T_2^* WI	Distinctive marker of demyelinated and non demyelinated lesions	[192]
	Hypointense lesions on SWI images	Distinguishing biomarker of active and chronic MS lesions	[196]

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