Advanced imaging tools to investigate multiple sclerosis pathology

Benedetta Bodini 1,2,3,5, Céline Louapre 1,2,3,4, Bruno Stankoff 1,2,3

Available online: 26 March 2015

1. Sorbonne Universités, UPMC Université Paris 06, UMR S 1127, CNRS UMR 7225, ICM, 75013 Paris, France
2. AP–HP, Hôpital Saint-Antoine, 75012 Paris, France
3. AP–HP, Hôpital Pitie-Salpêtrière, 75013 Paris, France
4. Harvard Medical School, Massachusetts General Hospital, Boston, United States
5. Department of Neuroimaging, Institute of Psychiatry, Psychology and Neuroscience, King’s College London, London, United Kingdom

Correspondence:
Bruno Stankoff, AP–HP, Hôpital Saint-Antoine, Neurology Department, University Pierre-et-Marie-Curie, ICM, 75012 Paris, France.
bruno.stankoff@sat.aphp.fr

In this issue
Multiple sclerosis: from new concepts to updates on management
David-Axel Laplaud, Nantes, France
The autoimmune concept of multiple sclerosis
Bryan Nicol et al., Nantes, France
Environmental factors in multiple sclerosis
Vasiliki Pantazou et al., Lausanne, Switzerland
Update on clinically isolated syndrome
Eric Thouvenot, Nîmes, France
Update on treatments in multiple sclerosis
Laure Michel et al., Montréal, Canada
Treatment of multiple sclerosis in children and its challenges
Sona Narula et al., Philadelphia, United States
Advanced imaging tools to investigate multiple sclerosis pathology
Benedetta Bodini et al., Paris, France
Update on rehabilitation in multiple sclerosis
Cécile Donzé, Lille, France

Summary
Conventional MR imaging techniques still lack specificity for the underlying central nervous system tissue damage in multiple sclerosis (MS), impeding a comprehensive investigation of the key mechanisms responsible for neurological disability such as myelin damage and repair, neurodegeneration and neuroinflammation. A range of novel and advanced imaging tools, using quantitative magnetic resonance (MR) or positron emission tomography (PET) technologies are now emerging and open the perspective to obtain unique insights into the disease mechanisms. Both can be employed either in experimental models or in patients with MS, and they have already allowed to obtain imaging metrics that significantly correlate with clinical scores. In this review, we summarize the main evidence supporting the use of quantitative MR and PET as essential investigation tools to explore myelin changes, neuronal damage and compartmentalized inflammation in MS. The clinical translation of these imaging techniques has the potential to improve the design of future clinical trials and to allow the measurement of the effects of new drugs aimed at enhancing myelin repair and reducing neurodegeneration and neuroinflammation.

During the past decades, multiple sclerosis (MS) has been an outstanding example illustrating the considerable contribution provided by imaging techniques, such as magnetic resonance imaging (MRI), to the improvement of clinical diagnosis and the design of clinical trials. Nowadays, the presence of MS white matter lesions on T1- and T2-weighted sequences, together with the pattern of contrast enhancement following gadolinium injection are key criteria to establish the diagnosis of MS [1]. Furthermore, the dynamic evolution of lesions on sequential MRI scans is a strong predictor of the efficacy of disease modifying therapies on relapse rate [2]. However, current immuno-active therapies still fail to prevent long-term disability progression, and imaging metrics derived from classical structural MRI are not yet fully predictive of disease progression.
As a result, a growing interest has developed on investigating the neurodegenerative component of this disease, which could be related to key physiopathological mechanisms such as a failure of myelin repair, grey matter damage, and compartmentalized inflammation. Conventional MRI sequences, while very sensitive for the detection of lesional areas, are not specific for the quantification of myelin content, neurodegeneration or neuroinflammation, as they may reflect equally inflammation, oedema, myelin pathology and axonal degeneration. This has led to the development of many advanced imaging tools with an optimized specificity for the complementary facets of tissue damage in MS.

In this review, we will describe advanced imaging techniques that have been proposed to investigate the myelin compartment, the grey matter damage, the functional brain consequences and the compartmentalized inflammation in MS.

**Imaging tissue damage 1: myelin imaging**

Several advanced MRI sequences have been proposed to evaluate more specifically the myelin compartment within the CNS, such as magnetisation transfer imaging (MTI), T2 relaxometry, and diffusion tensor imaging (DTI) [3].

**Magnetisation transfer imaging**

Magnetisation transfer imaging (MTI) is based on the interaction and exchange between the unbound protons in free water and the protons bound to macromolecules. Changes in the magnetisation transfer ratio (MTR, which reflects the exchange rate of magnetisation transfer between the two proton pools) of cerebral white matter are thought to be highly influenced by changes in myelin content because of the overwhelming contribution of myelin to the macromolecules involved in the MT phenomenon. Therefore, a reduced MTR is likely to reflect loss of myelin whereas an MTR increase may indicate remyelination [4,5]. MR-histopathology studies performed on postmortem brains from MS patients have further supported an association between MTR measurements and myelin content [6]. The MTR of remyelinated lesions differed from normal-appearing white matter (NAWM) and demyelinated lesions, with a significant correlation between MTR and myelin content in lesions and in NAWM [6]. In optic neuritis, MTR was shown to correlate with neurophysiological function scores, such as visual evoked potential latency and optical coherence tomography, a measure of retinal neuro-axonal loss [7]. Several ways to quantify the MTR signal have been investigated. The most commonly used relies on the measurement of the mean MTR within regions of interest (acute active lesions, T2 lesions). In longitudinal studies, follow-up MRI examinations have to be coregistered with the reference one, and the evolution of the mean MTR can be determined in each ROI. Using such a strategy, it has been shown that MTR decreases slightly before lesion appearance on T2-weighted sequences [8,9], and then drops dramatically at the time of gadolinium enhancement. A partial recovery of MTR values in active lesions, suggestive of remyelination, has been observed from 1 month up to 6 months [10–16], a time frame compatible with what is expected from experimental studies. However, following optic neuritis, a slower decrease of MTR (nadir at 240 days after the clinical episode) has been observed, with only partial recovery after 1 year: a slow clearance of myelin debris in the optic nerve has been suggested to explain this finding, but remains speculative [17].

As heterogeneity in the level and time course of demyelination and remyelination could occur between lesions and within each lesion, a voxel-based quantification method has been developed [18–20]. Recently, MTR has further been shown to be sensitive to cortical pathology and a methodology to assess cortical demyelination at the individual level has been proposed [21]. Overall, while being probably the most available MRI technique to approach the assessment of myelin content, to date the specificity of MTR for myelin is still suboptimal, as water content, inflammation and axonal damage still represent relevant contributors to the signal modifications [22,23].

**Myelin water fraction imaging**

Besides MT imaging, the multicomponent T2 analysis of spin-echo data, by focusing on the short T2 peak between 10 and 50 ms, has been proposed to extract the myelin water fraction (MWF), which likely corresponds to water trapped within the myelin bilayer [24–27]. The T2 decay in the brain presents a few peaks, which have been assigned to compartmentalized spin populations: the short T2 peak (around 20 ms, between 10 ms and 50 ms) represents the water trapped within myelin layers, the peak around 70–90 ms the intra- and extracellular water, and the third peak above 2 s reflects CSF water [25]. Correlations between MWF and MTR measures within MS lesions and between MWF and luxol fast blue staining on histopathologic slices have been reported [28–30]. As the reproducibility of MWF measure has been questioned using a ROI analysis strategy [31,32], a voxel-based analysis has been recommended [32]. Few longitudinal data on lesional MWF are available, indicating that some changes compatible with remyelination could be identified in a 6 months time frame [33], but with a greater variability than using MT imaging [31]. An optimization of these sequences, aimed to result in shorter acquisition times, increased sensitivity and better reproducibility, is required, and several adaptations of the concept have been developed over recent years providing promising results in MS [34].

**Diffusion tensor imaging**

Diffusion tensor imaging (DTI), a measure of water molecules diffusion, has provided details on tissue microstructure and allowed to perform fiber tracking [35]. The directional diffusivity derived from DTI measurements describes microscopic water movements parallel to (axial diffusivity) and perpendicular to (radial diffusivity, RD) axonal tracts. In experimental models of
white matter injury, the water RD has been shown to reflect mainly myelin content, whereas axonal pathology was the main contributing component to the changes in axial diffusivity [36–39]. Measures of RD could predict further demyelination in ex vivo MS spinal cords [40] and were able to discriminate the functional outcome following optic neuritis in humans [41]. The longitudinal monitoring of MS lesions has shown reduction in RD over time that might be compatible with remyelination [42]. However, to date the measure of RD is not yet pathologically specific as it can be significantly influenced by axonal pathology and inflammation [40].

Molecular imaging of myelin by positron emission tomography

Despite the major advances in MRI techniques, there is no MRI technique specific enough for the assessment of myelin in vivo. Therefore, it would be useful to develop a molecular imaging technique specific for myelin that would quantify CNS remyelination and further validate the MRI-based metrics. This could be achieved using positron emission tomography (PET). Of great interest was the identification of a newly synthesized fluorescent stilbene Congo red derivative, 1,4-bis(p-aminostyryl)-2-methoxy benzene, also named BMB, that selectively binds to myelin ex vivo and in vivo [43]. This compound has allowed the detection of demyelinating lesions in a rodent experimental autoimmune encephalitis model of demyelination. On MS brain samples, BMB staining can differentiate remyelination in shadow plaques from either demyelinated lesions or normal-appearing white matter, suggesting that this biomarker could be used to quantify myelin loss and repair. Reinforcing the hypothesis that the common affinity of BMB towards amyloid plaques and myelin could be explained by a similar protein conformation in both structures [43,44], a specific interaction of BMB with the myelin basic protein has been recently described [45]. Finally, BMB was shown to cross the blood-brain barrier and, when radiolabeled with carbon-11, to allow CNS myelin imaging by PET in non-human primates [43]. Interestingly, a similar affinity for CNS myelin was reported for several other stilbene Congo red derivatives, allowing to follow-up demyelination and remyelination in dysmyelinating models as well as in the cuprizone-induced experimental model, either by using the fluorescent properties of the compounds or by using molecular imaging in vivo [46–48]. On the basis of a common target expressed in amyloid plaques and myelin, we have found that other amyloid markers, related to thioflavinT, could also stain myelin and be used as PET radiotracer for myelin [49]. Using the [(11)C]-2-(4’-methylaminophenyl)-6-hydroxybenzothiazole [(11)C]-PIB, a proof of concept PET imaging study in MS patients has shown that this tracer allowed to visualize demyelinated MS lesions, with a less pronounced reduction of the uptake in gadolinium-enhanced lesions than in non-active lesions. This imaging probe has recently been investigated in a longitudinal study and could allow to classify patients depending on their remyelinating capacity (Bodini et al., submitted for publication). Other families of compounds, either related to coumarin [50] or to the sphingosine-1-phosphate receptor modulator FTY720 [51], are now being developed for myelin imaging with PET. However, for all these compounds, further data are required to gain further information on the specificity toward the myelin target, the binding saturability, and the signal-to-noise ratio on PET imaging.

Imaging tissue damage 2: grey matter pathology and neurodegeneration

Cortical lesions detection

Several cortical lesion subtypes have been identified during MS course [52], which could greatly contribute to neurological disability. Therefore, newer MR sequences with improved contrast for cortical lesions such as DIR, MP-RAGE, or PSIR have been implemented to enhance cortical lesions detection [53,54]. The use of these metrics has been shown to improve the specificity of diagnostic criteria [55], and to better explain cognitive deficits [56]. However, to date, these sequences only detect about one-third of whole cortical lesions when applied on clinical scans (either 1.5 or 3 T), and they usually miss the visualisation of subpial demyelination. This insufficient sensitivity might not yet allow us to perform accurate regional correlations between cortical lesion load and other MRI parameters or clinical symptoms.

Quantitative mapping of T2* in the cortex

One major asset provided by ultra-high-resolution (7 T) MRI is the high spatial resolution needed to quantify cortical degeneration. Due to the thin (~ 3.5 mm) and folded structure of the cortex, developing reliable measures of cortical tissue contrast still remains a technical challenge.

Recent ex vivo and in vivo imaging studies have explored contrasts underlying myelin and iron concentration in the cortex of healthy subjects and patients with MS. Deistung et al. [57] were able, using susceptibility and R2* (= 1/T2*) mapping, to image ex vivo laminar substructure of the cortex such as the Stria of Gennari in the primary visual cortex. Unlike T2* -weighted signal, T2* relaxation time is a quantitative measure computed from several T2* -weighted images acquired with different echo times, thus independent from imaging parameters such as coil sensitivity or scaling factor. In healthy brain, T2* relaxation time inversely correlates with myelin and iron content [58]. Both in white matter [59] and cortical [60] MS lesions, histopathological-MR correlations demonstrated that increased T2* relaxation time corresponded to areas of demyelination and iron loss, while iron accumulation at the border of the lesions induced shorter T2*.

Imaging subpial demyelination, a major pathological substrate of disease progression in MS, is one of the most important advances offered by quantitative T2* imaging at 7 T. T2* -weighted...
and multi-echo $T_2^*$ imaging has allowed to increase the sensitivity to detect cortical lesions, including the subpial type, with an accuracy of 50% to 90% compared to histological examination [60-62]. Using tools from FreeSurfer, an imaging software designed to identify cortical surfaces, Cohen-Adad et al. [63] detected $T_2^*$ signal abnormalities in the cortex of patients with MS. More recently, Mainero et al. [64] were able to map $T_2^*$ relaxation time in the cortex of subjects with MS, at different depths through the width of the cortical ribbon, from the pial surface to the WM. The authors found a gradient of increased $T_2^*$ relaxation time (underlying myelin and/or iron loss) across all disease stages, correlating with neurological disability, with the most dramatic changes observed in the outer cortical layers, close to the pial surface as described in neuropathological studies [65]. In order to depict the independent contribution of myelin and iron content to cortical degeneration, other MRI contrasts can prove useful to explore subpial pathology. These include magnetization transfer imaging [21,66,67], and $T_1$ mapping [68]. In future, combining different contrasts could help to define more specifically the mechanisms at stake in disease progression.

**Sodium imaging**

In the past decade, experimental studies have highlighted sodium metabolism dysfunction as a mechanism of MS pathogenesis. Increased intracellular sodium accumulation, likely secondary to mitochondrial impairment [69], leads to neuro-axonal dysfunction and death [70]. Imaging sodium concentration in the brain can therefore prove useful as a marker of neuronal dysfunction and loss in vivo in MS. Clinically feasible $^{23}$Na MRI has been developed over recent years, this technique being previously limited by low signal-to-noise ratio and long scan time [71]. Total sodium concentration has been quantified in several brain tissue compartments in patients with MS, and was increased in white matter lesions, normal-appearing white matter, cortical and deep grey matter, throughout all disease stages [72-74]. Moreover, total sodium concentration was higher in secondary progressive patients compared to relapsing-remitting and primary progressive MS, and correlated with clinical disability [73,74]. Future perspectives include the development of novel techniques able to selectively quantify intra- and extracellular sodium concentration independently of each other, and to relate this biomarker to other functional imaging modalities to better understand the impact of sodium accumulation to neuronal dysfunction in vivo.

**Proton MR spectroscopy**

Proton MR spectroscopy (MRS) allows the identification and quantification of brain and spinal cord metabolites, with the N-Acetyl-Aspartate (NAA) being specific for the axonal compartment. A decrease in NAA in patients compared with healthy controls has been reported in all phases of the disease, including in the radiologically isolated syndrome [75], indicating that neuronal dysfunction and/or axonal loss occur since the earliest stage of MS. NAA changes have been correlated with neurological disability and cognitive deficits, attesting that neuro-axonal pathology is a crucial mechanism underlying disability in MS [76]. MRS also allows the identification of other relevant metabolites such as glutamate and GABA that could provide insight into neurodegenerative processes. In particular, glutamate level measured by MRS at 3 T was shown to be increased in NAWM and acute lesions, with no significant elevation in chronic lesions [77]. However, technical limitations for the quantification of MRS peaks as well as a suboptimal spatial resolution still limit a large clinical use of this method.

**PET with the neuronal specific radiotracer $^{11}$C-flumazenil**

PET with the neuronal specific radiotracer $^{11}$C-flumazenil could also contribute to assess the neuronal component of grey matter pathology in MS. Flumazenil binds to the benzodiazepine site contained within the GABA-A receptor, which is widely expressed by neurons in cortical and deep grey matter regions. A key point is the availability of a robust absolute quantification methodology based on the co-injection of labeled and unlabelled flumazenil, the partial saturation protocol, which provides an absolute quantification of GABA-A receptor concentration at the voxel level [78]. Using a high-resolution research camera it has been possible to quantify neuronal damage, which occurs as early as the relapsing-remitting stage, and to localize the cortical regions where neuronal damage predominates (Freeman et al., submitted for publication).

**Imaging the functional consequences of tissue injury**

Functional MRI (fMRI) is an imaging tool used to evaluate dynamics of cortical function, either during a task (motor, cognitive) or more recently at rest. This technique is based on the blood oxygen level dependent (BOLD) contrast reflecting the hemodynamic response of neurons. Indeed, activation of neurons translates into an increased cerebral blood flow with a delay of one to two seconds, hence a decreased deoxyhemoglobin/oxyhemoglobin ratio, which can be measured as an increased BOLD signal on MRI. In MS, this technique allowed to investigate functional cortical reorganization during a task, which was interpreted as a compensatory mechanism against structural pathology in order to maintain normal performances. Several groups have shown that MS patients with normal cognitive performance exhibit greater brain activation compared to controls during a cognitive task [79-81]. Moreover, subjects with MS recruited additional brain areas, usually not involved in the execution of the task. With the progression of the disease and the appearance of clinical symptoms, this compensatory mechanism tends to fail, and a
decreased activation was observed in MS at later stages in task-related fMRI studies [82,83]. However, one major limitation of task-related fMRI is inherent to the ability of the subjects to perform the task. In subjects with severe cognitive impairment for instance, an overall decrease of activation at the group-level could be explained by the inability of subjects to perform the task. A more recent approach, resting-state fMRI, has risen over the last years to allow the investigation of functional connectivity in the brain while eliminating the constraint of task performance, which proved useful to depict functional reorganization in MS as well as to better understand the relationship between functional connectivity and structural pathology.

One major challenge while studying resting-state fMRI data is the mathematical modelling of the connectivity measures, at the regional level or at the brain level, derived from BOLD time series at each voxel. A simple approach is to start from a specific region, known to be affected in the disease and/or to be a central component of a network, and to use it as a seed. BOLD time series from this seed will then be correlated with the time series of each voxel of the brain. Common seeds used in resting-state fMRI studies in MS include basal ganglia, hippocampi and posterior cingulate. A main finding from these studies is that MS patients exhibit functional abnormalities relative to controls in various regions distant from the seed. For example, Roosendaal et al. [84] found a decreased hippocampal functional connectivity in the anterior cingulate gyrus, thalamus and prefrontal cortex in MS patients with intact memory function. Functional connectivity changes may be more challenging to interpret with the coexistence of clusters of increased and decreased functional connectivity in MS patients relative to controls, as found in thalamic connectivity recently [85]. In this study, increased thalamic connectivity was associated with worse cognitive performance, which was interpreted by the authors as maladaptive brain plasticity.

A limitation of the seed-based approach is that it does not provide insight about the functional organization at the network or whole brain level. Other promising approaches have been developed to overcome this problem. Resting-state networks (RSN) can be individualized without anatomical a priori using a separation source method called independent component analysis (ICA). Computing connectivity measures within and between RSN can provide insights about the functional reorganization of specific networks linked to known neurological function such as attention, memory or motor skills. In clinically isolated syndrome relative to controls and to RRMS, Roosendaal et al. [86] found increased functional connectivity of several RSN, while no atrophy or white matter diffusivity measures were detected. These changes, observed only at very early stages, were interpreted as an early but finite cortical reorganization. Faivre et al. [87] have also demonstrated that in early patients, increase of RSN connectivity was associated with disability, and thus could represent an early marker of disease burden. More recently, Louapre et al. [88] showed that functional connectivity in RSN implied in cognitive processes, such as the default-mode network and the frontoparietal attention networks, was selectively impaired in MS patients with a severe early cognitive deficit. Moreover, the functional disconnection of these networks had the strongest effect size compared to all other structural MRI metrics to predict cognitive impairment, highlighting its robustness as a biomarker for disease severity. Finally, mathematical tools to analyse topological organization of the brain, based on graph theory, are becoming increasingly important and extremely promising. Connectivity measures to quantify the organisation of brain networks in nodes linked by edges can be applied to fMRI resting-state data, and have already been used to better understand network abnormalities in several neurodegenerative diseases, including MS [89–91]. These recent studies have described topological networks abnormalities in patients, related to cognitive impairment. More investigations are needed to understand the link between the network topological characteristics and structural and metabolic pathological changes in MS.

**Imaging inflammation**

The most available imaging metric to assess the inflammatory component of the disease is based on the injection of gadolinium which allows to identify active recent lesions, associated with disrupted blood–brain barrier (BBB). However, as an indirect measure, it cannot provide a direct quantification of the inflammatory load in the CNS. Ferromagnetic particles named USPIO could be used as contrast agents to identify phagocytic cells infiltrating the CNS. They consist of small nanoparticles of 20–40 nm that display a quite long half-life and can be phagocytized by circulating activated macrophages following systemic infusion. When localized into the CNS, they modify the MR signal and can be detected with a high sensitivity [92]. In both EAE models and MS, pilot clinical trials demonstrated that USPIO-contrasted MRI identifies more inflammatory lesions than gadolinium-injected sequences, and the subgroup of lesions positives with both USPIO and gadolinium were characterized by a more severe evolution, with extended axonal loss and chronic black hole transformation [93–95]. Whether a small amount of USPIO could cross the BBB without being taken up by macrophages remains controversial, but this could contribute to the slight T1 shortening detected in the NAWM of both relapsing and primary progressive patients [96].

PET imaging is a promising tool for a specific investigation of activated microglial cells and macrophages in vivo. The most studied target is the translocator protein, TSPO, which is a macromolecular complex expressed by the outer mitochondrial membrane. The functional role of this molecule remains elusive, but its expression is very low in the rest of the CNS, and becomes
highly up-regulated when microglial cells are activated [97]. The reference TSPO PET compound to date has been the $^{11}$C-PK11195, which has been shown to be sensitive to microglial inflammation in white matter lesions, and to a lesser extent in NAWM [98–100]. However, a larger clinical use of this tracer has been hampered by some weaknesses such as a limited brain entrance and specificity, poor SNR, and labelling with carbon-11. More recently the use of noninvasive quantification methods, based on the extraction of reference regions with no or very low specific signal using supervised clustering algorithms, allowed to detect a significant $^{11}$C-PK11195 binding increase in the cortex of SPMS patients [101]. Beside PK11195, a wide range of second generation TSPO ligands have been designed and synthesized, with up to now more than 40 compounds belonging to seven pharmacological classes [102]. They are characterized by an improved affinity, a better SNR and brain entrance, and some have been labelled with fluorine-18 [103], which allows easier clinical use due to a longer half-life. The results obtained in MS remain so far disappointing as the first pilot studies did not reveal major differences between patients and controls [104]. The recent identification of three individual binding profiles to the receptor (low affinity, high affinity and mixed affinity), related to a single TSPO gene polymorphism, could provide an explanation for this lack of difference and will justify the systematic genotyping of subjects recruited in future studies with TSPO PET [105]. Beside TSPO, new tracers binding to novel targets such as cannabinoid receptors or specific markers for microglial cells oriented towards the M2 phenotype might be applied in clinical studies and provide new insight into the physiopathology of compartmentalized inflammation in MS.

**Conclusions**

Structural MRI is nowadays part of the routine procedures used for MS diagnosis and follow-up in a clinical setting. Besides clinical detection of MS lesions, there are now a wide range of advanced tools that could contribute to understand MS physiopathology and to design future trials, aimed at enhancing myelin repair and reducing neurodegeneration and neuroinflammation. The implementation of specific imaging metrics and their application in MS, together with the development of appropriate post-processing methodologies, therefore represent an outstanding field of research that will contribute to improved knowledge and clinical care in the MS field.

**Disclosure of interest**: B. Bodini and C. Louapre have no conflicts of interest to report. B. Stankoff has received honoraria for consultancy, lecturing, and for taking part in advisory boards from Teva, Biogen, Novartis, Merck, and Sanofi.

**References**


and disease development in relapsing EAE. Neuroimage 2006;32:266-74.


