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VISUALIZING IRON IN MULTIPLE SCLEROSIS

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Abstract

Magnetic resonance imaging (MRI) protocols that are designed to be sensitive to iron typically take advantage of (1) iron effects on the relaxation of water protons and/or (2) iron-induced local magnetic field susceptibility changes. Increasing evidence sustains the notion that imaging iron in brain of patients with multiple sclerosis (MS) may add some specificity toward the identification of the disease pathology. The present review summarizes currently reported *in vivo* and *post mortem* MRI evidence of (1) iron detection in white matter and grey matter of MS brains, (2) pathological and physiological correlates of iron as disclosed by imaging and (3) relations between iron accumulation and disease progression as measured by clinical metrics.

INTRODUCTION

Multiple sclerosis (MS) is a disease of the central nervous system (CNS). It affects young adults and may lead patients to a substantial accretion of physical, cognitive and emotional disability over time (1). The pathogenesis of MS although widely studied remains not fully elucidated. Several factors puzzle the comprehension of MS disease mechanisms. Among these factors, is the challenge in characterizing pathological specificity of MS-induced disease, *in vivo*, using magnetic resonance imaging (MRI) (2). To overcome this limitation, in recent years research has been focused toward the understanding of the role of iron as possible *in vivo* tracer of disease pathology in MS. Several authors have contributed to the notion that tracking iron may add some specificity toward the identification of pathological processes in MS (3, 4).

In the present review, we will appraise the current knowledge on *iron imaging* in MS. We will first briefly elucidate on the current knowledge on iron as possible indicator of different

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physiological and pathological processes in MS as demonstrated by histopathological studies. Thereafter, we will describe the physical mechanisms at the basis of iron detection by MRI. Last, we will appraise on the current *in vivo* and *post mortem* imaging evidence of iron detection in white matter (WM) and grey matter (GM) of MS brains as well as on its relation with measures of MS-induced disability and other imaging measurable disease parameters. By using the term *iron* in the present assay, we refer to the nonheme iron in contrast to the heme-bound iron, which is attached to hemoglobin.

THE PHYSIOLOGICAL AND PATHOLOGICAL ROLE OF IRON IN MS: EVIDENCE FORM HISTOPATHOLOGICAL STUDIES *POST MORTEM*

Non-hemeiron in normal brain tissue

Iron is essential for many cellular functions. Iron is however also potentially detrimental due to its ability to produce toxic oxygen radicals (5). Therefore iron metabolism is tightly regulated. Many neurodegenerative conditions including MS have been linked to excess brain iron and subsequent oxidative injury (6, 7). Iron accumulates with age in the healthy human brain, reaching a plateau after the age of 50 (7). Most of the non-hemeiron found in human brain parenchyma is stored within ferritin, where it is efficiently detoxified. It can be detected histochemically on formalin-fixed, paraffin-embedded archival human brain tissue by the diaminobenzidine-enhanced Turnbull blue staining (8), applied in the figures 1–2 presented in this review. Using this method, highest concentrations of iron may be seen in the basal ganglia (BG), predominantly in the globuspallidus (GP), the red nucleus (RN) and the substantianigra (SN) (figure 3) (9). Of all neocortical regions, the precentralgyrus (i.e., primary motor cortex) is known to store the highest amounts of cortical iron (10, 11). By comparison, less iron is detected in the WM (7). In normal human WM, cortexand deep GM iron is essentially stored within oligodendrocytes and the inner and outer loops of myelinsheats (12). This storageis reflected by the ferritin immunoreactivity of these cells, as illustrated in figure 1. The physiological role of this iron is partially known. Some iron is certainly needed for myelinogenesis (13). This fact alone, however, is insufficient to explain the increase in non-functional storage iron because suchstorage continues long after the completion of myelination. The high expression of ferritin within oligodendrocytes rather suggests an iron-buffering function of ferritin to protect other structures of the CNS from toxic iron overload. The considerable amounts of iron within oligodendrocytes and myelin gain particular relevance in MS, a disease where both oligodendrocytes and myelin are destroyed.

Non-hemeiron in MS brain tissue

Brain tissue of MS patients displays several areas where iron may be found at higher concentration that in non-diseased brains. Such an accumulation is due to several pathological processes, which occur during the course of the disease. Uponmyelin degradation in actively demyelinating lesions, oligodendrocytes release their iron content into the extracellular space. Although this iron might still be bound to ferritin, the acidic environment of inflamed tissues as well as proteases from activated phagocytes may rapidly degrade the protein shells and liberate the iron content from ferritin. Additionally, superoxide from activated microglia mediates the release of iron from ferritin *in vitro* (14).

Microglia and macrophages take up the liberated iron (15) and up-regulate their ferritin, which can be observed at the edges of chronic WM lesions (figure 2A and 2B). In the center of chronic WM lesions, where oligodendrocytes, myelin and even microglia or macrophages have largely vanished, iron and ferritin are often detected within astrocytes and axons (figure 2C and 2D). The centers of inactive MS plaques frequently exhibit less iron than the surrounding normal-appearing WM (NAWM) (figure 3). Similarly, demyelinated cortical lesions frequently show less iron than surrounding normal-appearing GM (NAGM) (figure 3). These findings suggest a removal of iron from WM and cortical GM through phagocytic microglia and macrophages.

Several *in vivo* indirect evidence suggest that BG nuclei of MS patients harbor iron concentrations significantly higher than BG of age-matched non-MS controls (16, 17). However, the results need to be interpreted with caution. To the best of our knowledge there are no histopathological or biochemical reports that have addressed the pathological and physiological significance of this iron in MS.

MRI PROTOCOLS SENSITIVE TO IRON

MRI protocols that are designed to be sensitive to iron typically take advantage of one of two primary effects of iron on the magnetic environment of water molecule protons. First, iron affects the relaxation of water protons including shortening the longitudinal spin-lattice (T_1), transverse spin-spin (T_2) and apparent transverse (T_2^*) relaxation times where $1/T_2^* = 1/T_2 + \gamma B_0$. T_2' characterizes the reversible signal that can be refocused by a spin-echo radio frequency (RF) pulse, γ is the nuclei-specific gyro magnetic ratio in units of radians per second per Tesla, and B_0 is the inhomogeneity in the local static magnetic field in units of Tesla. Second, the local magnetic field susceptibility gradients surrounding iron affect the phase accrual of nearby water protons. Essentially, iron's relaxivity affects the MR signal magnitude while iron's impact on susceptibility changes MR signal phase. Iron-sensitive MR imaging methods include those that quantify or are weighted by T_1 (18), T_2 (18–24), T_2^* (22), T_2' (22), field dependent relaxation rate increase (FDRI) (25, 26), magnetic field correlation (MFC) (27, 28) or susceptibility-induced phase (29–35).

Iron Relaxivity

A magnetic ion's ability to affect proton nuclear magnetic resonance (NMR) relaxation is a function of both its concentration and relaxivity. Additionally, the relaxivity depends on the chemical compound/environment in which the ion is located (36). In general, magnetic ions with concentrations greater than 0.1 mM are expected to affect proton NMR relaxation, and in some brain regions such as the GP, stored iron concentrations may be as high as 3.6 mM (1 mg iron/g wet tissue = 17.9 mM) (37). Non-heme iron within the brain is primarily stored in a mineralized form known as ferrihydrite associated with ferritin or hemosiderin. Tissue T_1 and T_2 relaxation times are reduced when magnetic material is present, but the chemical environment determines the dominant effect. Small clusters of ions with direct exposure to water molecules reduce T_1 via an “inner sphere mechanism” and cause brighter signals on T_1 -weighted MR images (36). Ferritin and hemosiderin contain aggregates of magnetic iron particles shielded from direct contact with water by a protein shell. In ferritin up to 4,000

ferric iron ions are contained within a single ferricoxy hydroxide core encased by a protein shell roughly 25 angstroms in diameter (21). The magnetization of the ferritin core fluctuates and causes inhomogeneities in the local magnetic field (38), and water molecules diffusing through the fluctuating magnetic field will experience decreased transverse relaxation times via an “outer sphere mechanism” (37). The fluctuation in magnetic field is proportional to the square of the static magnetic field strength, and thus the T2 signal dephasing effect of stored iron on water protons increases dramatically with increasing static MR imaging field strength. Though shortened T2 can be a useful indicator of iron accumulation, not all brain tissues with short T2 have iron concentrations.

Iron Sensitivity with Conventional MRI and MR Relaxometry

The relaxivity effect of iron can be captured qualitatively in T1-weighted, T2-weighted or T2*-weighted imaging protocols or quantitatively using maps of T1, T2 or T2*. However, iron's effect on T1 relaxation (20) is much weaker than its influence on T2/T2* relaxation. Data acquisition time for quantitative mapping is typically longer and often includes sacrifices of reduced spatial resolution or limited field of view (FOV) coverage to maintain a reasonable scan time. As early as 1986 (20), comparisons of histology to 1.5 Tesla (1.5 T) brain MRI (considered to be “high field” at the time) in regions known to contain high concentrations of iron appear in the literature. While T1-weighted spin-echo (SE) (repetition time or TR/echo time or TE=400/20 ms) did not yield conspicuous iron contrast, both T2-weighted SE (TR/TE=2000/100 ms) and T2 maps from multiple SE data (TR=1500 or 2000 ms, and TEs=25, 50, 75, 100 ms) revealed decreased signal intensity and reduced T2 values in the GP, reticular SN, RN and dentate nucleus (DN). In 1993 (21), quantitative T2 mapping performed at 1.5 T (TR=1500 ms, TEs=20, 40, 60, 80, 100, 120, 140, 160 ms) revealed age-dependent T2 decreases in the GP and put a men that also correlated with *post mortem* iron-concentration analysis.

Detecting iron using T2*

It is known from iron extraction studies (24) that T2* increases after the removal of iron. However, both global and local field inhomogeneities contribute to T2* signal decay. Sources of global inhomogeneity include interfaces of regions of different magnetic susceptibility such as tissue-to-tissue, e.g. soft-tissue-to-bone, and tissue-to-air boundaries. Macroscopic field inhomogeneity can obscure the T2* variations caused by microscopic sources such as tissue iron content. The effect of global inhomogeneity dominates the longest spatial dimension of the acquired voxel, typically the slice-select direction, and several techniques exist to diminish the T2* signal decay caused by global inhomogeneity including specialized quadratic phase slice selection RF pulses (38) or the collection of a series of images with altered gradient refocusing lobes following the slice-selective RF excitation (22). Ordidge et al. describe a hybrid T2 and multi-echo T2* measurement protocol for the assessment of brain iron concentration. Single-slice scans were performed at 3 T with TR=2000 ms, TE1=29 ms and TE_n=29+n*8 ms. The initial echo was a true SE while the following echoes represent gradient echoes. A second scan was acquired in which the echo spacing remained 8 ms, but the interval between the excitation and refocusing RF pulse was altered to produce the true spin-echo at the 4th echo time (77 ms). The two acquisitions enabled the separate calculation of T2* and T2. T2* was found by fitting an

exponential decay to signals acquired at the different gradient echo times, and T2 was found by fitting to signal intensities derived from the division of images with equal T2'-weighting. High-resolution T2* imaging of brain iron has also been reported using ultra-high field (7T) axial gradient echo acquisitions with (TR/TE=500–1000/28–31 ms), flip angle (FA) = 30–50°, slice thickness=1 mm, FOV=240 mm×180 mm, matrix size=1024×768, sampling bandwidth (BW)=32 Hz/pixel, first-order flow compensation on all imaging gradients, and scan time ranging 6.5 to 13 minutes (31)

Field-Dependent Relaxation Rate Increase (FDRI)

Using separate acquisitions with constant TR and multiple echo times at two distinct static magnetic field strengths, the field-dependent relaxation rate (R2) increase (FDRI) method (25) estimates the transverse relaxivity increase per unit of magnetic field strength. The difference between the R2 values measured at each field divided by the field strength difference produces FDRI in units of s⁻¹/Tesla. FDRI has been applied successfully in studies of brain iron using field strengths of 0.5T and 1.5T (25) as well as 1.5T and 3T (26). An example 1.5T, 3T FDRI protocol for iron detection (26) utilized multi-shot echo planar SE (EPSE) acquisitions with FA=90°, 256×192 acquired matrix, FOV=24 cm, 4 signal averages, 24 shots with 8 phase-encode lines per shot, and (TR/TE=6000/17 ms) for scan 1 (9:40 m) and (TR/TE=6000/60 ms) for scan 2 (14:20 m). A primary advantage of FDRI demonstrated by studies using clinical scanners with vendor-provided T2 mapping results is that reliable iron quantification is possible with FDRI even if the T2 measurements are not accurate in absolute terms. In FDRI, it is the relative change in relaxivity that matters. Recent work showed that FDRI iron estimates were more highly correlated than susceptibility-weighted imaging (SWI) iron estimates with published *postmortem* values and were more sensitive than SWI to iron concentration differences across basal ganglia structures (26). FDRI appears to have a greater specificity for non-heme iron compared to SWI. Another benefit of FDRI is the positive contrast produced in the FDRI maps in which areas of high iron concentration appear bright. A drawback of FDRI is the requirement for separate acquisitions at different static field strengths. Besides the additional time, cost and effort to scan a subject twice, the images must also be spatially registered.

Magnetic Field Correlation (MFC)

The magnetic field correlation method employs an asymmetric SE acquisition and a detailed theory for the effect of magnetic field in homogeneities on MR signal decay (27). MFC analysis seeks to address the disadvantage of T2 and T2* relaxation measurement approaches in which those relaxation rates are influenced by factors other than the microscopic field in homogeneity caused by iron such as dipole-dipole interactions. The signal model utilized in MFC imaging is not restricted to mono exponential decay. MFC measurements are essentially independent of macroscopic field gradients, and contributions of macroscopic gradients can be suppressed further by reducing voxel dimensions. Compared to corresponding R2 maps, MFC maps of human brain were found to have larger contrast between BG structures and surrounding tissue. Recent work demonstrated that MFC can detect increased iron accumulation in the deep-GM of patients with MS (28). The MFC 3T imaging protocol utilized to scan multiple sclerosis subjects used a single-shot EPI asymmetric echo (ASE) sequence, FOV=256 mm×256 mm, 128×128 acquired matrix, slice

thickness=2 mm, 40 slices, BW=1345 Hz/pixel, 20 signal averages, and TR/TE=2800/59 ms. Images were acquired with refocusing pulse time shifts of 0, -4, -8, -12 and -16 ms, and total scan time for the MFC sequence was 3.5 minutes (28). MFC was found to range from 100 to 1000 s⁻² over the full range of brain iron concentrations while the dynamic range of R2 was limited from approximately 13 to 24 seconds. The MFC effect grows approximately with the square of the static magnetic field strength, which may make high field scanners especially suitable for MFC studies. Because MFC is affected by water diffusion, it may be necessary to acquire diffusion-weighted MRI data in conjunction with MFC measurements to better interpret MFC differences. MFC images are vulnerable to motion artifacts and typically utilize echo-planar imaging (EPI) acquisitions as well as image coregistration.

Susceptibility-induced Phase Imaging

Beyond affecting relaxivity, iron increases the diamagnetic susceptibility of water and influences signal phase in MR gradient echo images in which no refocusing RF pulse is used to form a spin echo. In addition to providing useful stand-alone phase information, especially at high field (31), this effect forms the basis for another well-known method for iron quantification, SWI (30). SWI detects iron content differently than T1, T2, or T2* maps (29) and has been used successfully to map putative iron content in normal and abnormal brains (32). Local field inhomogeneities from paramagnetic iron can be detected by the phase of complex (real and imaginary) components of the MR signal. The amount of accrued phase for a given voxel at a given field strength and echo time is directly related to the iron concentration. In a right-handed phase coordinate system, water protons accrue more negative phase in iron-rich tissue. Low iron concentration can still generate a measurable phase effect while not producing a strong T2* effect. Also, the phase effect is preserved at higher spatial resolution while T2* effects diminish as the intravoxel phase dispersion decreases in a smaller voxel. Previous work has shown that T2* underestimates iron when concentration is low, and phase underestimates iron when concentration is high (39). SWI has been applied to qualitatively and quantitatively characterize iron deposition in multiple sclerosis lesions that were not clearly seen on Fluid Attenuated Inversion Recovery (FLAIR) or T2W images (35), and SWI was found to be sensitive to small lesions only if iron was present. Typically, iron-sensitive phase imaging and SWI utilize a 3D gradient echo protocol with short TR, TE long enough for the desired field-dependent phase evolution, and first-order flow compensation along all gradient directions. A typical 3T SWI protocol is 2 mm slices with no slice gap, FOV=220 mm×220 mm, 512×512 acquired matrix, TR/TE=50/25 ms, FA=20° (35). Complex images are reconstructed to enable calculation of phase images that are high-pass filtered to remove slowly spatially phase caused by macroscopic field inhomogeneity and phase unwrapped in areas of steep phase changes. The post-processed phase images can be analyzed themselves or used to enhance the magnitude image (40), however, it is important to know the phase sign conventions of the employed MR system (41). SWI phase measurements are sensitive to heme and non-heme iron as well as other tissue properties, such as vasculature and junctions between tissue compartments. The non-iron sources of phase effects may confound the use of SWI as a measure of brain iron deposition. Multi-echo SWI is useful for measuring high iron concentrations because the short echo times can guide the phase unwrapping of long echo

times (39). Figure 4 shows an example of SWI obtained at 7T in a patient with MS. One can appreciate several anatomical and pathological details traceable by the signal changes induced by iron. These details include: (1) the visibility of the vasculature due the susceptibility effect of the heme-iron (figure 4A), (2) the markedly decreased signal intensity noticeable in the BG sustained by the presence of iron (figure 4B) as previously demonstrated (9) and the typical rim of low signal characteristic of iron accumulation around lesions (9) (figure 4C).

CLINICAL EVIDENCE

Iron in deep-GM nuclei

• **Conventional MRI**—Indirect evidence of iron accumulation predominantly in the deep GM is derived from the measurement of signal intensity changes (i.e., reduction) in T2-weighted MRI. To measure signal intensity of the deep-GM nuclei, regions of interest (ROI) are drawn in each nucleus. Thereafter, intensity from an identical sized ROI placed in the ventricular cerebrospinal fluid (CSF) is taken for each subject as a method of intensity normalization. Taking ratios of the deep GM nucleus to the CSF background normalizes signal intensity. The normalization for intensity differences across scans is necessary due to varying scanner calibration between the acquisitions.

In 1987 Drayer and coauthors first demonstrated visible decreased signal intensity in the thalamus, GP and put a men of 47 patients with MS compared to healthy controls. The work of Drayer and coauthors appeared in the literature soon after the reported observations, from the same group, of the relation between iron presence and decrease in signal intensity by T2-weighted images (18). Iron-sensitive T2-weighted SE imaging at 1.5 T (TR/TE=2500/80 ms) showed decreased signal intensity in the thalamus and put a men in MS patients (20). The authors also demonstrated the presence of decreased signal intensity to be associated with a higher number of WM hyper intense lesions by T2-weighted MRI. Several inferences were postulated to explain their findings. First it was speculated that iron accumulation in deep GM could be the result of hypo metabolism and declined use of this metal for oxidative reactions. Second, the authors postulated that the BG regions, normally involved in storing high quantity of iron, would do so more in case of oligodendrocytes impairment and iron release as seen in MS. Third, it appeared conceivable that, due to the frequent leaks of the blood brain barrier in MS patients, higher quantities of iron could cross the BBB and accumulate in the BG.

The results of Drayer and coauthors have been reproduced and expanded by several coauthors over the course of the years by using 1.5T and 3T platforms. In 2000, Bakshi and coauthors referred to signal changes by T2-weighted MRI as T2-black holes (BHs) and reported T2-BHs to be present in thalamus, put a men, caudate and Rolandic cortex in 57%, 42%, 24% and 8% of 114 examined MS patients, respectively (42). The authors also observed that the presence of T2-BHs was significantly related to longer disease duration, advanced neurological disability and worse MS phenotype. Other authors subsequently confirmed the aforementioned results but also added insight into the T2-BHs characterization and relation to MS-induced disability. Ceccarelli and coauthors showed that T2-BHs may be imaging features of any type of MS patients, inclusive of those with benign

MS (43) and early MS stage such as clinically isolated syndrome (CIS) patients (44). Notwithstanding the above demonstrations, as we show in figure 5, T2-BHs remain characteristically present in patients with more advanced disease and also a distinctive signature of MS compared to other CNS immunological conditions (Pawate S *personal communication*). T2-BHs are indeed significantly associated to patient physical (45–47) and cognitive disability (48), brain atrophy (49, 50) and WM lesion load by T2-weighted MRI (49). Altogether the finding raises the question as to whether T2-BHs are main determinants of MS disease or rather an epiphenomenon.

Interestingly, recent work from Pawate and coauthors (51) has shown that the use of immune modulatory drugs such as Natalizumab may affect T2 hypo intensities with an increase in signal over a 24-month treatment period. The author's explained the findings as possible neuro protective effect of Natalizumab. As stated earlier, the pathological substrate of T2-BHs is unknown. Results from animal models, established that deep GM hypo intensity is present in murine MS model. In this model, thalamic hypo intensity also shows a strong correlation with rotarod detectable disability (52). However, combined histopathological-imaging studies are still on going from the same group to answer the question of the pathological substrate of T2-BHs. *In vivo* studies analyzing the relationship between diffusion tensor imaging-derived metrics such as fractional anisotropy (FA) and mean diffusivity (MD) (43) and T2-BHs, demonstrated a moderate association between MD and T2-hypointensity of the same ROI. The authors interpreted this finding as possible relationship between iron accumulation and axonal and myelin injury as reflected by DTI-MRI.

• **Susceptibility-induced Phase Imaging**—Recent work from Zivadinov and collaborators performed at higher field strength (i.e., 3T magnet) has addressed the question as to whether deep GM nuclei of MS patients present with abnormal phase changes compared to healthy volunteers (figure 6). The study relied upon the hypothesis that phase shift do represent iron accumulation in deep GM nuclei, hence neuro degeneration. The authors examined a relatively large cohort of MS patients (n=233) [inclusive of 169 relapsing-remitting (RR), 64 secondary-progressive (SP)], and 126 age- and sex- matched HVs (53). The results demonstrated that compared to healthy controls, MS patients have significantly increased abnormal mean phase (MPAPT) in the caudate, put a men, GP, thalamus and pulvinar nucleus of the thalamus. Additionally, a trend toward increased MPAPT in the amygdala, accumbens and SN was seen in MS patients. SPMS patients showed significantly increased MPAPT in caudate, put a men and amygdala compared to RRMS ones. In patients with RRMS significant correlations were seen between increased MPAPT of the caudate and GP with WM lesion volume by T2-weighted as well as between increased MPAPT of the caudate and GP with cBHs. Increased MPAPT of the caudate, put a men, GP and thalamus was significantly related to decreased brain volumes with similar results for both the GM and WM. Interestingly none of these relationships was seen in SPMS patients.

Subsequent work pre formed by Hagemeier and coauthors in the same group by focusing on patients with CIS has shown that increased MPAPT up to 12.4% may be found in the put a men, CN and pulvinar nucleus of the thalamus of CIS patients when compared to HVs.

These differences were present even when no differences in normalized volumes of the same regions are seen. The authors interpreted this intriguing finding with the notion that iron accumulation may precede atrophy formation. As a result, atrophy could be one of the possible pathological processes leading to brain atrophy development and disease progression (54).

• **R2* relaxation rates and T2 relaxometry**—The results by Hagemeier and coauthors (55) differ from those reported by Khalili and coauthors who used R2* rates to indirectly measure iron content in the deep-GM nuclei of patients with MS (56). In this work, the authors found R2* relaxation rate to be significantly higher in MS patients than in healthy volunteers and CIS patients in the NP, put a men, caudate and nucleus accumbens. Nevertheless, CIS patients did not present relevant differences with correspondent age- and sex-matched healthy volunteers in the same areas (55, 56). In the hands of these authors, R2* rates of the BG were significantly associated to age (56, 57), disease duration (55, 56), physical (56) and cognitive disability (55, 56). In addition, WM lesions load as well as global, cortical and sub cortical GM atrophy were significantly associated to R2* rates of the BG (55). Stepwise linear regression models showed revealed GM atrophy and age as strong independent predictor of BG R2* levels (54, 55). The authors explain the relationship between GM atrophy and R2* rates as possibly due to axonal transections and iron accumulation in the neurons of the BG. Interestingly they also argue that the correlations seen between WM lesions and BG R2* relaxation rates might be ascribed to the role of BG as “sink” for iron from macrophages from MS lesions. MRI T2 relaxometry was applied in 970 patients with clinically definite MS and 117 controls by Bur get ova and coauthors. Contrary to previous literature, the authors reported an inverse association between T2*relaxation rate of the BG and WM lesion load. That is to say, patients with small lesion volume by T2-weighted images had higher iron accumulation than patients with larger lesions volume (58). Strong associations were seen between T2 relaxometry and brain atrophy. The authors interpreted their results as further evidence of the complex and multi factorial etiology of MS.

• **MFCI**—In recent work from Ge and coauthors performed at 3T magnet, MFCI was applied in 17 patients with clinically definite MS to indirectly assess iron content in regions of NAGM and NAWM. In alignment with the results of other authors using different MRI techniques, significantly higher MFC values were found in patients compared to healthy volunteers in GP (24% increase), put a men (39.5% increase) and thalamus (30.6% increase). Conversely, NAWM regions generally had lower values of MFC in patients compared to healthy volunteers although these differences did not reach a statistically significant level (28). In analyzing the associations between MFCI-derived metrics and WM lesion load, the authors found the latter to be significantly associated to MFC value in the BG but not in the NAWM. Moderate associations between MFC value in the thalamus and CI. The authors interpreted their findings as related to iron accumulation in the BG secondary to oligodendrocytes and axons disruption in the WM.

• **Susceptibility-induced Phase Imaging and R2* relaxation rate at 7T; evidence from combined pathological and imaging studies**—In recent years, the

use of ultra-high field MRI at 7T has become increasingly popular. Several gains are expected from the use of ultra-high field MRI at the 7T. Among those is the increase in SNR and CNR while still maintaining clinically acceptable scanning time. In addition, due to its exquisite mechanisms of contrast, the increased magnetic susceptibility effect exploitable at 7T MRI has been shown to be an important factor in characterizing WM-L pathology.

Recent combined *post mortem* imaging and pathological studies aligned to *in vivo* data generated at the NIH demonstrated that in MS brain tissue, low phase signal is not always associated to iron increase as initially postulated. Conversely, if decreased phase signal matches with increases in susceptibility-derived parameter $R2^*$, it corresponds to iron presence (58). The results confirmed the notion that a complex interplay between iron accumulation, myelin loss and WM fibers geometry is at the base of phase shift and $R2^*$ changes (31). Identified-iron in WM of MS brains may be present in several pathological and non-pathological areas and encompasses a heterogeneous biological meaning. Some chronic, non-acutely inflaming WM lesions characteristically present with a rim partly or entirely accentuated by high $R2^*$ and low phase signal surrounding a core with hypo intense signal on $R2^*$. In this type of WM lesions, the high $R2^*$ (and low phase MRI signal) tend to match with iron and ferritin accumulation together with high CD68 immunoreactivity (9). The presence of activated microglia seen around a demyelinated core of WM lesions as by MRI and confirmed by myelin staining resembles the histopathological/MRI pattern of *slowly expanding lesions* (SLE) described in pathology by previous authors (59, 60). $R2^*$ maps also allowed the understanding that iron may be present in tissue outside visible lesions in MS such as preserved oligodendrocytes as well as aggregates of micro bleeding (9). The same finding has been reported in the cortical GM of MS patients (61). The T2*GRE images showed hypo intense rings in some cortical lesions. These rings corresponded with increased density of activated microglia containing intracellular iron.

CONCLUSIONS

Iron-sensitive MR imaging is an attractive modality for the identification of disease in MS as well as for the specific characterization of MS pathology. In recent years, primarily indirect evidence of the sensitivity of some MRI techniques to the iron content of brain tissue in patients with MS has been reported. Pathological characterization of the identified iron in normal appearing-and lesional-WM tissue has been successfully performed. However, questions still remain about the physiological and pathological role of iron in the GM cortical and sub cortical regions that manifest signal changes on T2-weighted and T2*-weighted MRI. Future work is demanded to achieve a more complete understanding of the specificity of these iron-related MRI observations.

Additionally, results available thus far suggest that the presence of iron at a cellular level may be associated with several physiological and pathological states of MS, which conventional MRI typically fails to capture. Therefore, iron-related *in vivo* MRI findings should be interpreted cautiously if the findings are not supported by *post mortem* histopathological correlates. Another important limitation of *in vivo* MRI for the characterization of brain tissue iron content is sensitivity to heme-bound iron, which may

reflect normal vascularization. *Post mortem* imaging does not capture the effect of such heme-bound iron.

If properly applied and interpreted, *iron imaging* represents an important step toward improved disease characterization of MS *in vivo* despite the above reservations. Ultimately, iron-sensitive MRI promises to provide a deeper understanding of the pathological iron-related mechanisms in MS.

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Figure 1.

Brain tissue from a 37-year old gentleman who died from cardiovascular failure. Ferritin⁺ (A) and iron⁺ (B) oligodendrocytes in the cortical GM. Ferritin⁺ (C) and iron⁺ (D) oligodendrocytes in the WM. Ferritin immunohistochemistry (A, C), DAB-enhanced Turnbull blue staining (B, D). Note that cortical oligodendrocytes (black arrows) show stronger reactivity to ferritin and iron than their WM counterparts (red arrows). Also note that not all oligodendrocytes are enriched with iron or ferritin (black and red arrowheads). (A, B, C, D: 400×).

Figure 2.

Chronic active lesion edge of a 70-year old gentleman who died from SPMS (A, B). Chronic lesion center of a 67-year old gentleman who died from PPMS (C, D). Ferritin immunohistochemistry (A, C), DAB-enhanced Turnbull blue staining (B, D). At the edge of chronic active lesions, microglia and macrophages are enriched with iron (B) and upregulate their ferritin (A). At the center of chronic lesions, astrocytes (arrows) and axons (arrowheads) often harbor iron (D), and astrocytes upregulate ferritin (C). (A, B: 100×. C, D, insets in A, B: 400×).

Figure 3.

Chronic cortical lesions and WM lesions of a 53-year old lady who died from SPMS. Parts of whole double-hemispheric coronal sections with cortical lesions (red arrows) and a large periventricular chronic WM lesion (blue arrows). The side-by-side comparison of adjacent sections stained for proteolipid protein (A) and iron (B) shows areas of chronic demyelination have lost iron which was originally present within oligodendrocytes and myelin sheaths. Note the strong iron reactivity in the nucleus caudatus as well as the putamen (black arrows), which can be similarly observed in normal brains. Ferritin and protolipid protein (PLP) immunohistochemistry (figures 1–3) were performed as described elsewhere (5, 7).

Figure 4.

0.7 isotropic SWI obtained at 7T in a patient with RRMS. Figure 4A shows the drastic decrease of signal visible in the caudate nuclei and attributed to the presence of iron (white arrows). Figure 2B shows small veins traversing areas of diffuse mild hyperintense signal likely due to MS disease (white rectangle). Figure 4C shows a WM lesion surrounded by a partial rim of decreased signal likely due to the presence of iron surrounding the lesion (white rectangle).

Figure 5.

T2 hypo intensities of DN of representative patient MRIs. All 4 3T MRIs are of female patients. (A) 36 year old with primary progressive MS, EDSS 6.5, dentate T2 hypo intensity 0.182; (B) 34 year old with secondary progressive MS, EDSS 3.5, dentate T2 hypo intensity 0.254; (C) 46 year old with Susac syndrome, EDSS 2.5, dentate T2 hypo intensity 0.446; (D) 36 year old with Devic disease, EDSS 7.0, dentate T2 hypo intensity 0.382. Courtesy of Dr S. Pawate, University of Vanderbilt Neurology Department.

Figure 6.

Mean phase maps (A and C) and images (B and D) of a 33 year old male RRMS patient with EDSS 2.5 (A and B) and age and gender-matched HV (C and D). In the maps, yellow represent higher phases shifts. Abnormal phase values are rendered in red in the phase images. MRIs were obtained at a 3T scanner. Courtesy of Dr R Zivadinov Neuroimaging Center, University at Buffalo.