Disentangling histological white matter lesion subtypes and characteristics in multiple sclerosis using postmortem quantitative 3T MRI

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Introduction

Multiple sclerosis (MS) is a chronic, immune-mediated and degenerative disease of the central nervous system, which affects almost three million people worldwide and represents the primary cause of non-traumatic disability in young adults. (1)

Neuropathological investigations have provided an invaluable contribution to the understanding of MS pathogenesis (2,3) but also to the discovery of pathologically-meaningful biomarkers for MS diagnosis (4) and to the development of new therapeutic approaches (5,6).

Histology studies were pivotal to reveal that MS lesions are complex and heterogeneous (7): they described focal plaques as (i) *active,* i.e. lesions that contain numerous immune cells (mostly blood-derived monocytes or resident microglia); (ii) *chronic active (or mixed active/inactive or smoldering lesions)*, i.e. lesions that present a rim of activated microglia/macrophages at the lesion edge and loss of myelin/axons in the center; and (iii) *inactive*, when they are hypocellular, strongly demyelinated and depleted of mature oligodendrocytes. Histopathological works also identified a further separate category that is represented by *remyelinating/remyelinated lesions*, which are plaques where new shorter and thinner myelin sheaths replace the destroyed myelin. The repair process often involves only part of the lesion, mostly at its border, but sometimes leads to a complete remyelination leading to the formation of the so called 'shadow plaques' (8) .

To date, only some of the above-mentioned histological lesion types have been associated to qualitative magnetic resonance imaging (MRI) biomarkers. Indeed, active lesions with significant blood-brain-barrier disruption may be identified in their early stage through gadolinium-enhancement in T1 weighted images, and at least a fraction of chronic-active lesions show a rim of increased susceptibility that can be detected with susceptibility-weighted imaging (SWI) (9), phase imaging (10) or quantitative susceptibility mapping (QSM) (11,12). On the other hand, we do not have specific imaging correlates for inactive and remyelinated lesions.

Hence, the goal of this work was to identify quantitative MRI measures which disentangle the histopathological complexity of the MS focal pathology in postmortem human brains, and especially help identifying inactive and remyelinated lesions.

Quantitative MRI (qMRI) techniques such as quantitative T1 (qT1), myelin water imaging (MWI), magnetization transfer (MT), and diffusion imaging provide with measures that are associated to axon and myelin integrity, iron content, and cellularity in the central nervous system tissue (13). Nonetheless, all these metrics are sensitive to more than one tissue component and their relative relationship to specific parts of the CNS is currently

unknown, due to the challenges in comparing studies performed with single qMRI measures in specific experimental settings (14,15).

In this study, our objectives were: (i) to identify the pattern of qMRI measures that distinguish histological lesion types in postmortem brains of MS patients and (ii) to investigate in the same experimental setting the relationship between those qMRI measures and quantitative histological measures of myelin, axons and astrocytes.

Methods

Specimen preparation and experimental setup

This study was approved by the ethical review committee of the University Medical Center Göttingen. Three whole brains from one female and two males deceased MS patients were provided by the German MS Brain Bank of the Competence Network Multiple Sclerosis (KKNMS). The age of the patients was 51, 58 and 66 years. The autopsies were performed 24 hours postmortem at the latest and the brains transferred directly in 4% neutral buffered formaldehyde solution. Two patients had secondary progressive MS (SPMS) and one suffered from relapsing-remitting MS (RRMS). Documents on the clinical course of each patient were obtained from the respective attending neurologist and the family doctor (Table 1).

MRI of the whole brains was performed 3 to 12 months after death. Approximately one week prior to scanning, the brains were placed into a custom-built and MRI-compatible container (16–18) and immersed in Fomblin® perfluoropolyether (Solvay Specialty Polymers USA, LLC, West Deptford, NJ, USA), a liquid-phase proton-free fluorocarbon which lacks any signal in hydrogen-based MRI (19). Since air bubbles produce relevant susceptibility artifacts, the bubbles were removed before imaging by suction using a vacuum pump. After this multi-step preparation process, the whole brains were imaged on a clinical 3T whole-body MR system (Magnetom Prisma, Siemens Healthineers, Erlangen, Germany). We employed the built-in body coil for radiofrequency (RF) transmission; for RF reception we used the standard 20-channel phased-array head and neck coil supplied by the manufacturer.

Ex vivo MRI protocol and image processing

Brain images were acquired with the following sequences, adapted to ex vivo conditions and capabilities, since due to natural tissue decomposition but also to chemical fixation the postmortem tissue's MRI properties are to a certain extent different from those found *in vivo* (20): (i) MP2RAGE (670µm isotropic, TR=5s, TE=1.78ms, TI1=194ms and TI2=2500ms) to obtain quantitative T1 maps (qT1); (21) (22) (ii) fast T2prep sequence with spiral readout trajectory (1000µm isotropic, TEprep=[0, 7.5, 17.5, 67.5, 147.5, 307.5]ms, TRreadout=9.3ms) to assess myelin water fraction (MWF)(23); (iii) segmented 3D-EPI (330µm isotropic, TR=65ms, TE=35ms, ETL=13, bandwidth 394Hz/Pixel) to enable QSM(24); (iv) a proton density weighted (TR=25ms, flip angle 5deg; TA: 1:11:54 x4), and MT prepared (TR=25ms, flip angle 5deg) RF-spoiled 3D-GRE of identical geometry (570µm isotropic) to allow MTR map calculation (25); (v) diffusion tensor imaging: Brain 1: resolution 1.4mm isotropic, b-value=0/1400/2000/4000 s/mm2: TE=93.0ms; δ=28.9ms; Δ=42.9ms; Brain 2: resolution 1.5mm isotropic, b-value=0/1650/2350/4650 s/mm2, TE=99.0ms, δ=31.9ms, Δ=45.9ms; Brain 3: resolution 1.3mm isotropic, b-value 0/1350/2650/4000 s/mm2, TE=80.0ms; δ=22.3ms; Δ=36.3ms. Diffusion images were denoised (26) and Fractional anisotropy (FA) and radial diffusivity (RD) maps were computed.

Individualized cutting box and sectioning

In order to ease the registration of the MRI to the histology slices, we designed and 3D-printed an individualized cutting box based on the MRI for each brain, as reported in (16,17)(Figure 1)

Brain slices were then photographed and the 3D EPI images manually registered to the photos by means of ITK-SNAP (27).

After the cutting process, we analyzed the slab-matched 3D EPI images in order to identify regions of interest (focal abnormalities in the white matter), which were then dissected and analyzed histologically.

Histopathological analysis

Tissue blocks were embedded in paraffin and slices of 4 µm thickness were stained for myelin (Luxol Fast Blue/Periodic-Acid Schiff (LFB/PAS)), for axons (Bielschowsky silver

impregnation), for iron (DAB-enhanced Turnbull staining), as well as using haematoxylin/eosin (H&E).

Immunohistochemical staining was performed using an avidin–biotin technique. Primary antibodies comprised anti–myelin basic protein (anti-MBP; Dako, Glostrup, Denmark for myelin), anti-CR3/43 (human HLA-DP, clone CR3/43 for MHC-II expressing microglia/macrophages) and anti-breast carcinoma-amplified sequence 1 (BCAS1, for myelin as well as for pre-myelinating and actively myelinating oligodendrocytes). After incubation with the primary antibody (applied at the dilutions indicated by the supplier and incubated overnight at 4°C), antibody binding was visualized using biotinylated secondary antibodies, peroxidase-conjugated avidin and DAB (Sigma-Aldrich). Double-labelling immunohistochemistry was performed combining DAB and Fast Blue using an alkaline phosphatase-conjugated secondary antibody (Dako, 1:50). Hematoxylin was used as nuclear counterstain. Double immunofluorescence immunohistochemistry was performed using primary antibodies directed against MBP (anti-MBP; Dako, Glostrup, Denmark for myelin) and neurofilament proteins (cocktail of anti-NF200 (Sigma Aldrich, Missouri, USA), SMI31, SMI32, and SMI311 (Sternberger monoclonals incorporated, Maryland, USA) or astrocytes (cocktail of antibodies against glial fibrillary acidic protein; SYSY, Göttingen, Germany, and Aldh1 (aldehyde dehydrogenase 1A1, Merck, Darmstadt, Germany)). Alexa FluorVR488 (Jackson ImmunoResearch Laboratories, Inc.) or CyTM3 (ImmunoResearch Laboratories, Inc.) coupled anti-mouse and anti-rabbit Ig antibodies were used as secondary antibodies. DAPI (4',6-Diamidino-2-phenylindol) staining was used for cell nuclei staining.

After histology/IHC, the sections were scanned automatically by a computer-directed microscope stage (Olympus VS120 Soft Imaging Solutions) under 20x magnification for further investigations. Digital processing of slide images was performed using an open microscopy OMERO server (version 5.6.3).

Staging of MS lesions

Lesion staging was performed using LFB/PAS staining and immunohistochemistry for myelin (myelin basic protein (MBP) and BCAS1) and activated microglia/macrophages (human HLA-DP, clone CR3/43) and was grounded on the recent classification system according to Kuhlmann et. al 2017 (7).

Histological image analysis was carried out by identifying the respective regions of interest (ROI) manually, i.e. white matter lesions, zones of remyelination and normal-appearing white matter (NAWM). NAWM areas were defined as white matter without or with only sparse microglia activation and, if possible, a minimum distance of 0.5 cm from white matter or cortical lesions.

Lesions were classified into four different groups (active, chronic active, inactive and remyelinated) based on detection of focal areas of myelin depletion/repletion as well as presence, density and localization of activated microglia/macrophage and ongoing demyelinating activity. Remyelination was characterized in LFB/PAS staining and BCAS1 immunohistochemistry by subtle myelin pallor when compared to the surrounding normal appearing white matter, and absence of macrophages with early myelin degradation products. Remyelinated lesions were defined as areas with extensive remyelination covering at least 60% of the lesion surface (8). Myelin repletion can be found in inactive, chronic active or active lesion types; in case of classification as remyelinated, these lesions were excluded from the other three categories in our analysis.

All lesions were assessed and classified by two board-certified neuropathologists (EB, CS).

MRI-histopathology analysis

We automatically registered all the images to the slab-matched 3D EPI images through Elastix (28,29) using an affine transformation.
WML accurately selected on histology and areas of NAWM in their vicinity were easily identified on the corresponding 3D EPI images and manually segmented, on two dimensions, on the same spatial plane as the histology section, by using ITK-SNAP 3.6.0 (27) (Figure 3). The segmentation mask was thereafter checked for quality and consistency on 3D FLAIR images. All six qMRI scans were registered to the 3D EPI, average intensity values were then computed for each region of interest.

Quantitative histology

We performed the correlation of MRI data with quantitative histology in a subset of lesions and in their correspondent NAWM regions, in order to minimize batch-related effects in the staining procedure.

Quantification of histochemical (LFB), immunohistochemical (anti-MBP) and immunofluorescence (anti-MBP-, anti-NF- and anti-astrocyte cocktail) stainings was performed through by color deconvolution using an in-house method (JF, RG). Specifically, to analyze MBP immunohistochemical expression, color deconvolution (scikit-image, v0.18.1) of the DAB signal was performed applying the same deconvolution matrix to all images using scikit's predefined RGB to HED deconvolution matrix. LFB color separation was performed by creating an individual color separation matrix for each image manually. The stain separation matrix was defined by annotating the LFB blue stain vector and the hematoxylin blue stain vector for each image. The third vector was defined as the orthogonal vector of the first two. For each ROI, we extracted the mean intensity.

Statistical analysis

We tested two main H0 hypotheses: (H0-1) A combination of qMRI measures sensitive to myelin, axons, cells and tissue structure/anisotropy cannot differentiate histologically defined lesion types and (2) (H0-2) Measures derived from qMRI similarly relate to myelin, axon and cell content as measured histologically.

To confute H0-1, we performed a pairwise logistic regression models using MRI measures as independent variables and histopathological lesion types as dependent variables. Since the different MRI metrics are correlated to one another, we performed a single-model analysis for each MRI parameter.

We used (i) the Akaike's Information Criterion (AIC) to assess the quality of the model fit (the lower the value, the better the data fits the model); and (ii) the c-statistics to assess the agreement between an observed response and a predictor. A c-value of 1 indicates perfect agreement between predicted and observed response. The results were then confirmed assessing all MRI markers together in a LASSO regression model.

In order to visualize the discriminative capabilities of qMRI regarding histological lesion types, we used a t-distributed stochastic neighbor embedding - t-SNE plot-, a statistical algorithm which embeds high dimension data into lower dimensional data enabling their visualization.

To confute H0-2, we firstly used Spearman's rank-order correlation between the data obtained from quantitative histology (quantification of myelin, axon and astrocyte content in selected ROI, see also the paragraph quantitative histology) and the

corresponding qMRI measures (i.e. the average of the signal intensity of the corresponding ROI on each MRI sequence). We further investigated the association between tissue components and MRI through linear mixed effect models with brains as random effect. In a first model, we adjusted for confounding by including the nature of the ROI (lesion vs. NAWM) as a dichotomous variable. In a second model, we also included an interaction term between the MRI and the histological measure in the lesion; thereby, we could analyze the association of MRI and histology quantification in the lesion itself and explore possible differences of this association between lesional areas and NAWM.

Statistical analyses were performed in R (version 3.6.3), α was set at .05, two-tailed.

Data availability

Data supporting the findings of this study are available upon reasonable request.

Results

We identified on MRI and characterized histologically a total number of 65 lesions in three brains: 12 inactive, 35 chronic active, 9 active and 9 (extensively) remyelinated lesions (Figure 2).

Active lesions - a lesion subtype that is uncommon in brain autopsies - were found in only one brain. The other lesion categories were instead represented in all the brains.

Association between histological lesion type and qMRI measures

Pairwise logistic regressions model showed that MTR, MWF, qT1 and RD differentiated between (i) extensive remyelinated and (ii) active, chronic active, and inactive lesions in the order (MTR: AIC = 40.45, c = 0.83, p <0.01, RD: AIC = 42.54, c = 0.83, p<0.01, qT1: AIC = 43.22, c = 0.83, p<0.01, MWF: AIC 43.82, c 0.78, p<0.01). In the LASSO model, MTR and MWF were the strongest predictors (Tables 2 and 3).

To distinguish active lesions from all other lesion types, qT1, RD and QSM proved to be the discriminating metrics (qT1 -AIC = 48.08, c = 0.79, p<0.05, RD -AIC = 48.53, c = 0.81,

p<0.05, QSM -AIC = 50.47, c = 0.75, p<0.05). However, when assessing all MRI markers in a LASSO regression model, the effect of qT1 disappeared (Tables 2 and 3).

On the other hand, both the model fit and the concordance seemed to be poor for the separation of inactive from chronic active lesions (Tables 2 and 3).

When experimental conditions were considered (i.e. inclusion of the brain as random effect in the model), obtained results were very similar for all models with the exception of the model differentiating active lesions from other lesions, where only MWF appeared discriminative.

A visualization of the discriminative capabilities of qMRI regarding histological lesion types was performed using a t-SNE-plot in Figure 4.

Correlation of MRI measures with quantitative histology

For the correlation of MRI parameters with quantitative histology we selected a subset of n = 43 lesions (9 extensive-remyelinated, 10 inactive, 19 chronic-active and 4 active) and the corresponding n = 43 regions of NAWM.

Myelin content

Correlation analysis showed that qT1 was most correlated with myelin content quantified with MBP (rho = -0.79, p<0.01), followed by RD (rho = -0.78, p<0.01), MWF (rho = 0.69, p<0.01), MTR (rho = -0.67, p<0.01) and QSM (rho = -0.58, p<0.01) (Figure 5).

On the other hand, linear mixed effect models showed that MTR, MWF and RD were all associated with MBP-myelin content in the lesion, but only MWF and RD showed a similar association with MBP-myelin content in both lesions and NAWM. (Table 4)

For the evaluation of myelin content with LFB staining we could consider 25 lesions (5 remyelinated, 6 inactive, 12 chronic active, 2 active lesions) and 25 NAWM regions. We had to exclude 18 blocks stained with LFB from the analysis because of their insufficient staining quality for color deconvolution. Again, all MRI parameters but FA correlated with LFB-myelin content (in the order: MTR rho = 0.52, qT1 rho = -0.52, RD rho = -0.49, QSM rho = -0.47, MWF rho = 0.42; p <0.01 for all parameters) (figure 6). However, linear mixed effect models showed that only QSM was associated with LFB myelin content when

adjusting for the nature of the ROI (lesion vs. NAWM) and showed a significant association (negative) with LFB-myelin content in the lesion area and in the NAWM. (Table 5)

Axonal damage

Regarding the analysis of axonal damage by means of immunofluorescence for neurofilaments (NF), we could include 39 lesions (6 remyelinated, 10 inactive, 19 chronic active, 4 active lesions- vs. 39 NAWM regions). DTI-FA correlated with neurofilament immunoreactivity (rho = 0.49, $p<0.01$), followed by MWF (rho = 0.27, $p<0.01$) (Figure 7). In both linear mixed effect models, DTI-FA and MWF exhibit an association with this axonal marker, which was similar in lesions and NAWM (table 6).

Astrocyte content

All qMRI measures but DTI-FA showed moderate to small correlations with astrocyte immunoreactivity (in the order: qT1 rho = 0.64, RD rho = 0.63, MWF rho = -0.53, MTR rho = -0.59, QSM rho = 0.34; $p <0.01$ for all parameters), (Figure 8). Nevertheless, MWF was the only parameter associated with astrocyte immunoreactivity in lesions, and this association was independent from experimental conditions and sampling location (table 7).

All lesion types (active, chronic active, inactive and extensive-remyelinated) showed astrocyte immunoreactivity in comparison to NAWM areas, $p<0.001$ (figure 9).

Discussion

This postmortem qMRI-histology study showed that MTR and MWF are the measures that best discriminate extensively-remyelinated lesions from all other lesion categories. Our data also suggest that this might be due to the fact that both these parameters are strongly related to myelin content, in a way that is independent from experimental conditions and sampling location (i.e. lesions vs NAWM). Last, our results showed that FA was the measure most associated to axon content, while MWF was associated with astrocyte immunoreactivity only in MS lesions. (figure 9)

The identification of surrogate markers for remyelination represents one major unmet need for the development of remyelinating and restorative therapies in MS patients. Moreover, the possibility to characterize histological MS-lesion types *in vivo* may help stratifying which patients would most benefit of reparative strategies in clinical studies and in clinical practice.

In this work, we have studied six qMRI measures (MTR, MWF, QSM, qT1, FA and RD) that theoretically exhibit differential sensitivity and specificity to myelin, axon, and cells characteristics (13).

Our data showed that MWF and MTR best differentiated extensive-remyelinated lesions from all the other histological subtypes. On the other hand, none of our imaging markers enabled the distinction of inactive from chronic active lesions. Chronic active lesions may be identified in living patients using susceptibility-based MRI, which enlighten their characteristic rim of microglia/macrophages rich in iron at the lesion edge (9–12). Those lesions exhibit a destructive nature (30) as well as a tendency to slowly grow over time (4). Interestingly, our data provide first evidence that chronic active and inactive lesions have similar extent of qMRI alterations, suggesting that both lesion types share a similar level of tissue destruction. New in vivo biomarkers for inactive lesions are therefore required, in order to establish their contribution to disease progression, as it has been recently performed for chronic active lesions (10).

Concerning the identification of active lesions, RD and QSM proved to be the strongest discriminative measures. Also, our results suggested that active lesions exhibit less microstructural damage than chronic active / inactive lesions but more than the one observed in extensively-remyelinated lesions. These data confirm and extend previous knowledge that active lesions may either efficiently repair or progress and convert to a chronic active and/or to an inactive lesion (31).

Although all previously mentioned qMRI measures exhibit some sensitivity to myelin, none of them is myelin-specific (13). In fact, the MT contrast is by nature also sensitive to macromolecules found in the axonal and cellular membranes and MTR exhibit a certain degree of T1 sensitivity that further decrease its specificity for myelin (32). MWF is thought to be more specific than the MT-based contrast for assessing myelin characteristics (33); however, it has been also shown that some acquisition schemes for

MWF such as the one based on Gradient And Spin Echo (GRASE) are sensitive to iron accumulation (34), although the one applied in this study (FAST-T2) (33) has such a short first echo time that susceptibility-sensitivity (i.e. to paramagnetic substance like iron) is minimal to non-existent. On the other hand, RD - a parameter derived from diffusion tensor imaging experiments that was related to myelin content in animal models of MS and in the human MS spinal cord (35–37) - suffers from low specificity in areas of crossing fibers and low signal to noise (38). Last, qT1 has been shown to correlate with both myelin and axon content in the CNS, but also with iron accumulation (39).

Numerous studies assessed the relationship of each qMRI measure mentioned above to myelin content in the CNS tissue (14,15,34): nevertheless, none of the previous work allow to compare the results obtained applying single or couple of qMRI measures, due to the extreme diversity in experimental settings (14,15,34). Besides, results obtained with these studies are sometimes contradictory (15): for example, qT1 and qT2 were reported to show low correlation values with myelin content in some studies (35) and very high in others (36). Also the reported correlation coefficient between MTR and myelin is variable, ranging from 0.36 (37) to 0.71 (38).

To overcome these challenges, we planned a correlation analysis between multiple qMRI measures and brain measures of myelin, axon and astrocyte density using the same experimental conditions.

Our data showed that all qMRI measures but FA exhibited moderate to high correlation with myelin content ($r = 0.58$-0.79), although only MWF and RD were associated to myelin content in both lesions and NAWM in a way that was independent from the sample type (i.e brain). Moreover, MWF and RD were the only measures showing the same association with myelin content in lesion areas and in the NAWM, hereby confirming previous results obtained with MWF (39) and extending them to RD. The fact that RD exhibits such an independent association with myelin content is quite surprising but also very encouraging, since it is a measure that is easily derived from a clinically compatible MR sequence such as DTI. Nonetheless, as previously mentioned, RD should be used with caution because its relationship with myelin may vary in regions containing crossing fibers (40). Noteworthy is also the observation that, when myelin content was assessed with a fat-staining like LFB instead of a protein staining like MBP, QSM was the measure that mostly related to myelin. Nevertheless, we have to consider that this analysis was

performed in a smaller group of lesions than the one stained with MBP due to the challenges in standardizing the LFB staining across different batches.

Regarding axon content, the intensity of NF-immunofluorescence positively correlated with FA and - to a smaller extent - MWF. While the observed connection of FA with axonal density is in line with previous studies (41,42), the association of MWF with the amount of axons has not been previously described and may underly the strong relationship between the presence of axons and myelin in normal appearing tissue and MS lesions.

Last, we did not observe significant associations between qMRI and astrocyte immunoreactivity, which is in line with some previous observations with single qMRI parameters (36,43). However, when looking exclusively at lesion areas, we observed that MWF was positively correlated with the presence of astrocytes, which may be due to a putative association between this cellular population and the remyelination process (Figure 9). To our knowledge, an extensive astrocytic presence in remyelinated lesions has not previously reported but is in line with recent studies indicating that astrocytes do not impede remyelination as it was long hypothesized (44), but are rather key regulators for the removal of damaged myelin through recruitment of microglia, before remyelination can take place (45). This finding might also explain why a completely remyelinated lesion still exhibit a T2-hyperintensity in conventional MRI images. To note is also that inactive and chronic active lesions showed both a high astrocyte-content, confirming recent knowledge (46,47).

MR-histology studies are complex and require targeted expertise in both postmortem MRI and histopathology to achieve an optimal data acquisition and pairing of information obtained from imaging and histological data. In this work, we minimized the challenges of data registration by using the approach proposed by Luciano et al. (17). Secondly, we have analyzed whole MS brain and not brain specimens or slices, providing therefore a comprehensive view of MS pathology. Nevertheless, although the obtained sample size was adequate to explore the primary hypotheses, it was sometimes limited for secondary analyses (i.e. active lesions, LFB). In addition, the histological analysis performed did not allow to assess the presence of myelin structural abnormalities (eg. swelling, blisters)(48), which might have influenced the applied qMRI measures. Future studies will aim at assessing the impact of those abnormalities in the modulation of myelin-axon sensitive qMRI.

In conclusion, we showed that MWF and MTR were the myelin-sensitive qMRI measures that best differentiated extensive remyelinated lesions from all the other histological lesion types. While FA showed the strongest association to axonal density, MWF and RD appeared to be the measures most related to myelin content, suggesting that also DTI-derived measures may well serve the purpose to measure remyelination in MS patients. Last, we also provide evidence of an extensive astrogliosis all lesion types, even in remyelinated ones, suggesting a determining role of astrocytes in repairing processes. Future studies should aim at further characterizing the astrocyte subtypes in the different lesion types and shed light on the ones driving remyelination.

References

1. Koch-Henriksen N, Sørensen PS. The changing demographic pattern of multiple sclerosis epidemiology. Lancet Neurol. 2010 May;9(5):520–32.

2. Adams CW, Poston RN, Buk SJ. Pathology, histochemistry and immunocytochemistry of lesions in acute multiple sclerosis. J Neurol Sci. 1989 Sep;92(2–3):291–306.

3. Lucchinetti C, Brück W, Parisi J, Scheithauer B, Rodriguez M, Lassmann H. Heterogeneity of multiple sclerosis lesions: implications for the pathogenesis of demyelination. Ann Neurol. 2000 Jun;47(6):707–17.

4. Dal-Bianco A, Grabner G, Kronnerwetter C, Weber M, Höftberger R, Berger T, et al. Slow expansion of multiple sclerosis iron rim lesions: pathology and 7 T magnetic resonance imaging. Acta Neuropathol (Berl). 2017 Jan 1;133(1):25–42.

5. Torke S, Pretzsch R, Häusler D, Haselmayer P, Grenningloh R, Boschert U, et al. Inhibition of Bruton's tyrosine kinase interferes with pathogenic B-cell development in inflammatory CNS demyelinating disease. Acta Neuropathol (Berl). 2020 Oct;140(4):535–48.

6. Montalban X, Arnold DL, Weber MS, Staikov I, Piasecka-Stryczynska K, Willmer J, et al. Placebo-Controlled Trial of an Oral BTK Inhibitor in Multiple Sclerosis. N Engl J Med. 2019 Jun 20;380(25):2406–17.

7. Kuhlmann T, Ludwin S, Prat A, Antel J, Brück W, Lassmann H. An updated histological classification system for multiple sclerosis lesions. Acta Neuropathol (Berl). 2017;133(1):13–24.

8. Patrikios P, Stadelmann C, Kutzelnigg A, Rauschka H, Schmidbauer M, Laursen H, et al. Remyelination is extensive in a subset of multiple sclerosis patients. Brain J Neurol. 2006 Dec;129(Pt 12):3165–72.

9. Clarke MA, Pareto D, Pessini-Ferreira L, Arrambide G, Alberich M, Crescenzo F, et al. Value of 3T Susceptibility-Weighted Imaging in the Diagnosis of Multiple Sclerosis. AJNR Am J Neuroradiol. 2020 Jun;41(6):1001–8.

10. Absinta M, Sati P, Masuzzo F, Nair G, Sethi V, Kolb H, et al. Association of Chronic Active Multiple Sclerosis Lesions With Disability In Vivo. JAMA Neurol. 2019 Dec 1;76(12):1474–83.

11. Maggi P, Sati P, Nair G, Cortese ICM, Jacobson S, Smith BR, et al. Paramagnetic Rim Lesions are Specific to Multiple Sclerosis: An International Multicenter 3T MRI Study. Ann Neurol. 2020 Nov;88(5):1034–42.

12. Gillen KM, Mubarak M, Park C, Ponath G, Zhang S, Dimov A, et al. QSM is an imaging biomarker for chronic glial activation in multiple sclerosis lesions. Ann Clin Transl Neurol. 2021 Apr;8(4):877–86.

13. Granziera C, Wuerfel J, Barkhof F, Calabrese M, De Stefano N, Enzinger C, et al. Quantitative magnetic resonance imaging towards clinical application in multiple sclerosis. Brain J Neurol. 2021 Jun 22;144(5):1296–311.

14. Mancini M, Karakuzu A, Cohen-Adad J, Cercignani M, Nichols TE, Stikov N. An interactive meta-analysis of MRI biomarkers of myelin. eLife. 2020 Oct 21;9.

15. van der Weijden CWJ, García DV, Borra RJH, Thurner P, Meilof JF, van Laar P-J, et al. Myelin quantification with MRI: A systematic review of accuracy and reproducibility. NeuroImage. 2021 Feb 1;226:117561.

16. Absinta M, Nair G, Filippi M, Ray-Chaudhury A, Reyes-Mantilla MI, Pardo CA, et al. Postmortem magnetic resonance imaging to guide the pathologic cut: individualized, 3-dimensionally printed cutting boxes for fixed brains. J Neuropathol Exp Neurol. 2014 Aug;73(8):780–8.

17. Luciano NJ, Sati P, Nair G, Guy JR, Ha S-K, Absinta M, et al. Utilizing 3D Printing Technology to Merge MRI with Histology: A Protocol for Brain Sectioning. J Vis Exp JoVE. 2016 Dec 6;(118).

18. Griffin AD, Turtzo LC, Parikh GY, Tolpygo A, Lodato Z, Moses AD, et al. Traumatic microbleeds suggest vascular injury and predict disability in traumatic brain injury. Brain J Neurol. 2019 Nov 1;142(11):3550–64.

19. Li R, Liu X, Sidabras JW, Paulson ES, Jesmanowicz A, Nencka AS, et al. Restoring susceptibility induced MRI signal loss in rat brain at 9.4 T: A step towards whole brain functional connectivity imaging. PloS One. 2015;10(4):e0119450.

20. Augustinack JC, van der Kouwe AJW. Postmortem imaging and neuropathologic correlations. Handb Clin Neurol. 2016;136:1321–39.

21. Marques JP, Kober T, Krueger G, van der Zwaag W, Van de Moortele P-F, Gruetter R. MP2RAGE, a self bias-field corrected sequence for improved segmentation and T1-mapping at high field. NeuroImage. 2010 Jan 15;49(2):1271–81.

22. Weigel M, Galbusera R, Rahmanzadeh R, Barakovic M, Lu P, Kappos L, Brück W, Kober T, Dechent P, Granziera C. Postmortem Whole-Brain MP2RAGE Optimization at 3T: A New Imaging Window into Multiple Sclerosis Cortical Pathology [Internet]. ISMRM 2020, Poster n. 1759.

23. Nguyen TD, Deh K, Monohan E, Pandya S, Spincemaille P, Raj A, et al. Feasibility and reproducibility of whole brain myelin water mapping in 4 minutes using fast acquisition with spiral trajectory and adiabatic T2prep (FAST-T2) at 3T. Magn Reson Med. 2016;76(2):456–65.

24. Liu T, Xu W, Spincemaille P, Avestimehr AS, Wang Y. Accuracy of the morphology enabled dipole inversion (MEDI) algorithm for quantitative susceptibility mapping in MRI. IEEE Trans Med Imaging. 2012 Mar;31(3):816–24.

25. Tabelow K, Balteau E, Ashburner J, Callaghan MF, Draganski B, Helms G, et al. hMRI - A toolbox for quantitative MRI in neuroscience and clinical research. NeuroImage. 2019 Jul 1;194:191–210.

26. Veraart J, Novikov DS, Christiaens D, Ades-Aron B, Sijbers J, Fieremans E. Denoising of diffusion MRI using random matrix theory. NeuroImage. 2016 Nov 15;142:394–406.

27. Yushkevich PA, Piven J, Hazlett HC, Smith RG, Ho S, Gee JC, et al. User-guided 3D active contour segmentation of anatomical structures: significantly improved efficiency and reliability. NeuroImage. 2006 Jul 1;31(3):1116–28.

28. Klein S, Staring M, Murphy K, Viergever MA, Pluim JPW. elastix: a toolbox for intensity-based medical image registration. IEEE Trans Med Imaging. 2010 Jan;29(1):196–205.

29. Shamonin DP, Bron EE, Lelieveldt BPF, Smits M, Klein S, Staring M, et al. Fast parallel image registration on CPU and GPU for diagnostic classification of Alzheimer's disease. Front Neuroinformatics. 2013;7:50.

30. Dal-Bianco A, Grabner G, Kronnerwetter C, Weber M, Kornek B, Kasprian G, et al. Long-term evolution of multiple sclerosis iron rim lesions in 7 T MRI. Brain. 2021 Mar 1;144(3):833–47.

31. Gaitán MI, Shea CD, Evangelou IE, Stone RD, Fenton KM, Bielekova B, et al. Evolution of the blood-brain barrier in newly forming multiple sclerosis lesions. Ann Neurol. 2011 Jul;70(1):22–9.

32. Campbell JSW, Leppert IR, Narayanan S, Boudreau M, Duval T, Cohen-Adad J, et al. Promise and pitfalls of g-ratio estimation with MRI. NeuroImage. 2018 Nov 15;182:80–96.

33. Nguyen TD, Deh K, Monohan E, Pandya S, Spincemaille P, Raj A, et al. Feasibility and reproducibility of whole brain myelin water mapping in 4 minutes using fast acquisition with spiral trajectory and adiabatic T2prep (FAST-T2) at 3T. Magn Reson Med. 2016 Aug;76(2):456–65.

34. Lazari A, Lipp I. Can MRI measure myelin? Systematic review, qualitative assessment, and meta-analysis of studies validating microstructural imaging with myelin histology. NeuroImage. 2021 Apr 15;230:117744.

35. Reeves C, Tachrount M, Thomas D, Michalak Z, Liu J, Ellis M, et al. Combined Ex Vivo 9.4T MRI and Quantitative Histopathological Study in Normal and Pathological Neocortical Resections in Focal Epilepsy. Brain Pathol. 2016;26(3):319–33.

36. Schmierer K, Wheeler-Kingshott CA, Tozer DJ, Boulby PA, Parkes HG, Yousry TA, et al. Quantitative Magnetic Resonance of Post Mortem Multiple Sclerosis Brain before and after Fixation. Magn Reson Med Off J Soc Magn Reson Med Soc Magn Reson Med. 2008 Feb;59(2):268–77.

37. Tardif CL, Bedell BJ, Eskildsen SF, Collins DL, Pike GB. Quantitative Magnetic Resonance Imaging of Cortical Multiple Sclerosis Pathology. Mult Scler Int. 2012 Nov 18;2012:e742018.

38. Schmierer K, Tozer DJ, Scaravilli F, Altmann DR, Barker GJ, Tofts PS, et al. Quantitative magnetization transfer imaging in postmortem multiple sclerosis brain. J Magn Reson Imaging JMRI. 2007 Jul;26(1):41–51.

39. Laule C, Kozlowski P, Leung E, Li DKB, Mackay AL, Moore GRW. Myelin water imaging of multiple sclerosis at 7 T: correlations with histopathology. NeuroImage. 2008 May 1;40(4):1575–80.

40. Wheeler-Kingshott CAM, Cercignani M. About "axial" and "radial" diffusivities. Magn Reson Med. 2009;61(5):1255–60.

41. Schmierer K, Wheeler-Kingshott CAM, Boulby PA, Scaravilli F, Altmann DR, Barker GJ, et al. Diffusion tensor imaging of post mortem multiple sclerosis brain. Neuroimage. 2007 Apr 1;35(2):467–77.

42. Preziosa P, Kiljan S, Steenwijk MD, Meani A, van de Berg WDJ, Schenk GJ, et al. Axonal degeneration as substrate of fractional anisotropy abnormalities in multiple sclerosis cortex. Brain. 2019 Jul 1;142(7):1921–37.

43. Schmierer K, Scaravilli F, Altmann DR, Barker GJ, Miller DH. Magnetization transfer ratio and myelin in postmortem multiple sclerosis brain. Ann Neurol. 2004 Sep;56(3):407–15.

44. Haindl MT, Köck U, Zeitelhofer-Adzemovic M, Fazekas F, Hochmeister S. The formation of a glial scar does not prohibit remyelination in an animal model of multiple sclerosis. Glia. 2019 Mar;67(3):467–81.

45. Ponath G, Park C, Pitt D. The Role of Astrocytes in Multiple Sclerosis. Front Immunol. 2018;9:217.

46. Absinta M, Maric D, Gharagozloo M, Garton T, Smith MD, Jin J, et al. A lymphocyte–microglia–astrocyte axis in chronic active multiple sclerosis. Nature. 2021 Sep;597(7878):709–14.

47. Popescu BF, Frischer JM, Webb SM, Tham M, Adiele RC, Robinson CA, et al. Pathogenic implications of distinct patterns of iron and zinc in chronic MS lesions. Acta Neuropathol (Berl). 2017 Jul;134(1):45–64.

48. Luchicchi A, Hart B, Frigerio I, van Dam A-M, Perna L, Offerhaus HL, et al. Axon-Myelin Unit Blistering as Early Event in MS Normal Appearing White Matter. Ann Neurol. 2021 Apr;89(4):711–25.

Table 1

Patients' characteristics

Patient	Age	Sex	Disease course	Disease duration	EDSS
1	51	F	SPMS	8 years	6
2	58	M	SPMS	23 years	8
3	66	M	RRMS	17 years	2,5

F female; M male; PPMS primary progressive MS; RRMS relapsing– remitting MS; SPMS secondary progressive MS; EDSS Expanded Disability Status Scale;