What contributes to disability in progressive MS? A brain-and-cord matched quantitative MRI study

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ABSTRACT (200/200)

Background: We assessed the ability of a brain-and-cord-matched quantitative MRI (qMRI) protocol to differentiate patients with progressive multiple sclerosis (PMS) from controls, in terms of normal-appearing (NA) tissue abnormalities, and explain disability.

Methods: 27 patients and 16 controls were assessed on the expanded disability status scale (EDSS), 25-foot-timed walk (TWT), 9-hole peg (9HPT), and symbol digit modalities (SDMT) tests. All underwent 3T brain and (C2-C3) cord structural imaging and qMRI (relaxometry, quantitative magnetisation transfer, multi-shell diffusion-weighted imaging), using a fast brain-and-cord-matched protocol with brain-and-cord-unified imaging readouts. Lesion and NA-tissue volumes and qMRI metrics reflecting demyelination and axonal loss were obtained. Random forest analyses identified the most relevant volumetric/qMRI measures to clinical outcome. Confounder-adjusted linear regression estimated the actual MRI-clinical associations.

Results: Several qMRI/volumetric differences between patients and controls were observed ($p<0.01$). Higher NA-deep grey matter quantitative-T1 (EDSS: beta=7.96, p=0.006; 9HPT: beta=-0.09, p=0.004), higher NA-white matter orientation dispersion index (TWT: beta=-3.21, p=0.005; SDMT: beta=-847.10, $p<0.001$), lower whole-cord bound pool fraction (9HPT: beta=0.79, p=0.001), and higher NA-cortical grey matter quantitative-T1 (SDMT: -94.31, $p<0.001$) emerged as particularly relevant predictors of greater disability.

Conclusions: Fast brain-and-cord-matched qMRI protocols are feasible and identify demyelination –combined with other mechanisms– as key for disability accumulation in PMS.

INTRODUCTION

In progressive multiple sclerosis (PMS), brain damage affecting both the white matter (WM) and the grey matter (GM) contributes to disability.[1] Damage often goes beyond the presence of visible lesions and affects the normal-appearing neural tissue too, typically in the form of widespread demyelination, chronic inflammation, and axonal loss.[2] Indeed, models based only on brain structural (conventional) magnetic resonance imaging (MRI) measures lack specificity to underlying pathological processes that lead to the development of irreversible disability.[2] The advent of quantitative MRI (qMRI) has enabled the characterisation of disease-specific microstructural abnormalities, by exploiting strong biophysical models which have been histologically validated in healthy populations and several neurological conditions.[2,3]

Importantly, disability in PMS is not entirely explained by brain pathology, and the involvement of the spinal cord has proved crucial too.[4] However, to date, it has been difficult to assess alterations of tissue properties in the brain and spinal cord at the same time because the implementation of qMRI in both structures has been hampered by methodological constraints, including excessively long acquisition times. Several authors have successfully demonstrated that fast imaging protocols in the brain[5–8] or the cord[9] are possible. However, up to now, there has been no proposal of a brain-and-cord matched protocol characterised by a unified MRI signal readout across different qMRI contrasts. This protocol should be able to exploit key MRI physical mechanisms sensitive to inflammation, demyelination and microstructure properties of tissue (such as relaxometry, magnetisation transfer and diffusion MRI). Furthermore, the protocol should be designed to speed up data acquisition, provide means of efficient denoising strategies, and ultimately make biophysical features directly comparable across different anatomical areas of the whole neuroaxis.

In this study we present a bespoke protocol capable of imaging the brain and the spinal cord as similarly as possible, acknowledging the differences in the field-of-view between the brain and the region of the cord assessed, through brain-and-cord-matched acquisitions, and in an acceptable time for patients (<1hour). In this study, we have focused on metrics which have been typically associated with *demyelination*, such as quantitative longitudinal relaxation time (qT1), bound pool fraction, or radial kurtosis; with *inflammation and oedema*, such as isotropic volume fraction or qT1; and with *axonal loss or axonal damage*, such as fractional anisotropy, neurite density index, or, to a lesser extent, axial and radial kurtosis.

Our overall aim was to assess whether pathological processes as detected by qMRI in both the brain and spinal cord explain disability better than only-brain or only-spinal cord approaches in people with MS, while evaluating the feasibility of the protocol too. More specifically, in this cross-sectional study, we aimed to assess whether our qMRI metrics measured in the normal-appearing (non-lesional) tissue were able to differentiate patients from controls. We also aimed to investigate the most relevant brain and cord lesional and non-lesional volumetric and qMRI metrics to concurrent clinical outcome.

METHODS

Participants

For this study, we included patients with a diagnosis of primary or secondary progressive MS according to their neurologist.[10] Furthermore, we included relapse-onset MS patients who showed progression ≥1 point on the EDSS over the last two years in the absence of relapses, even if they had not yet 'officially' been diagnosed with secondary progressive stage. We did not impose any specific inclusion criteria related to time of last relapse, steroid therapy, or disease-modifying treatment (DMT) status. Patients were recruited from the MS clinic at the National Hospital for Neurology and Neurosurgery in London between 2017 and 2020.

Age-matched healthy controls were also recruited. We aimed to include at least 19 patients in our study, to be able to find moderate-strong correlations in patients (rho=0.60) between qMRI metrics and clinical scores, with a risk of type I error (i.e., alpha)=0.05 and a risk of type II error (i.e., beta)=0.20. We assumed that only moderate-strong correlations (rho≥0.60) between clinical and qMRI scores are clinically relevant. We also aimed to include a similarly large cohort of healthy controls.

All participants underwent a 3T MRI scan and were clinically assessed on the 9-hole peg test (9HPT),[11] 25-foot timed-walk test (TWT),[11] and symbol digit modalities test (SDMT).[12] Patients and controls were assessed in exactly the same way. Additionally, patients, unlike controls, were assessed on the expanded disability status scale (EDSS).[13] Each participant was clinically assessed by a single neurologist, on the same day as the MRI scan. We chose these tests to include upper-limb and lower-limb motor function metrics as well as a cognitive test with high specificity and sensitivity for MS. Those patients who were unable to perform the TWT task were automatically assigned a time of 180 seconds. Those unable to perform the 9HPT task were automatically assigned a time of 300 seconds. Please see the Statistical analyses section for information on how clinical variables were obtained and handled in the statistical models.

All patients and controls signed an informed consent to participate in the study (UK MS Society project reference: 1811). This project was approved by the local Ethics Committee at University College London Hospitals (Research Ethics Committee Reference: 19/LO/0649).

MRI acquisition

All participants underwent structural MRI and qMRI of the brain and the spinal cord (C2-C3 vertebral level) using a 32-channel receive-only vendor head coil on a 3T Philips Ingenia CX

scanner (Best, The Netherlands). We used both product and purposely developed sequences (see **Supplementary Table 1** for full details).

Structural imaging was based on product sequences and consisted of:

- Brain 3D Fluid Attenuation Inversion Recovery (FLAIR) ($1\times1\times1mm^3$), acquired in the sagittal plane

- Brain 3D T1 ($1\times1\times1mm^3$), acquired in the sagittal plane

- Spinal cord 3D spoiled gradient-echo ($0.75\times0.75\times2.5mm^3$), acquired in the axial plane

Quantitative imaging was based on a unified single-shot spin-echo echo planar imaging readout across contrasts to harmonise signal-to-noise ratio and image distortions for joint multi-contrast quantitative analysis, using the reduced-field-of-view zonal oblique multislice (ZOOM) technique[14] for spinal cord acquisitions, as this maximises scan time efficiency and mitigates image distortions.

The entire qMRI protocol was acquired in the axial plane and consisted of:

- Relaxometry (inversion recovery):
    - Brain ($2\times2\times2mm^3$, 72 slices)
    - Spinal cord ($0.89\times0.89\times5mm^3$, 16 slices, centred at C2-C3 union)
- Magnetisation transfer imaging (quantitative MT):
    - Brain ($2\times2\times2mm^3$, 72 slices)
    - Spinal cord ($0.89\times0.89\times5mm^3$, 16 slices, centred at C2-C3 union)
- Multi-shell diffusion-weighted imaging:
    - Brain: b=0 (3 volumes), b=1000 s/mm^2 (20 directions), b=2000 s/mm^2 (20 directions), b=2800 s/mm^2 (36 directions); ($2\times2\times2mm^3$, 72 slices)

     o Spinal cord: b=0 (4 volumes), b=1000 s/mm^2 (18 directions), b=2000 s/mm^2 (18 directions), b=2800 s/mm^2 (34 directions); (0.89×0.89×5mm^3, 16 slices, centred at C2-C3 union)

Additional scans were used to correct for B1 and B0 inhomogeneities in quantitative analyses. The total scan time was just below 1 hour.

MRI analysis pipeline

All brain and cord structural and quantitative images underwent a generalised pre-processing pipeline (**Figure 1**).

Structural images

In the brain, additional pre-processing steps included: (1) fully automated segmentation of white matter lesions using 3D FLAIR images with the NicMS software;[15] (2) lesion filling of 3D T1-weighted images;[16] (3) subsequent Geodesic Information Flows (GIF) brain region segmentation;[17] (4) computation, for each subject, of whole-brain lesion load; (5) computation of normal-appearing (NA), i.e., lesion-free, white matter (WM) and grey matter (GM) volumes, including cortical and deep GM (i.e., CGM and DGM, respectively) volumes, after subtracting the brain lesion masks from the segmented macroscopic brain areas to obtain the segmented normal appearing tissue.[17] Additionally, we obtained the brain parenchymal fraction, calculated as the ratio between whole-brain tissue volume (i.e., lesions, NA-WM, and NA-GM) and total intracranial volume. NA-GM (including CGM and DGM) and WM volume fractions were also obtained after dividing NA tissue-specific volumes by the total intracranial volume (**Figure 1**).

In the spinal cord, additional pre-processing steps included: (1) segmentation of the cord over the whole field of view using FFE image and then cropped to the area/volume of interest (i.e., C2-C3); (2) computation of whole cord area using Deepseg from Spinal Cord Toolbox (SCT 4.3.); (3)

manual lesion segmentation by an experienced rater, using the 3D spoiled gradient echo sequence, with further review by an MS clinician; (4) cord GM and WM segmentation (of a non-lesion-filled cord image), using the Spinal Cord Toolbox that already includes GM and WM masks of each cord level,[18] with further review by an expert; (5) computation of C2-C3 spinal cord lesion volume, i.e., it only reflects that evaluated at the C2-C3 level; (6) computation of NA cord tissue, i.e., NA-cord area, NA-cord GM area, NA-cord WM area (all measured at the C2-C3 vertebral level).

The structural images in the brain and spinal cord were also used to extract masks of anatomical tissue types used to extract mean values of all the qMRI metrics acquired. In detail, mean and standard deviation values were computed for the following masks:

- In the brain: NA-CGM, NA-DGM, NA-WM, NA-brainstem and cerebellum, and brain lesions;

- In the spinal cord: whole cord, NA-cord, cord NA-GM and NA-WM, and cord lesions.

Quantitative images

After carrying out the general pre-processing pipeline (**Figure 1**) the same MRI signal models were fitted to both brain and spinal cord pre-processed data to derive the same microstructural metrics, thus enabling a direct comparison of the contribution to disability between brain and spinal cord.

The analysis pipeline of qMRI data included the following steps:

- **Relaxometry:**

Quantitative longitudinal relaxation time (qT1) maps were obtained from the inversion recovery data, by fitting a mono-exponential recovery model as previously described.[19,20]

- **Magnetisation transfer imaging:**

Quantitative MT data were analysed using a simplified two-pool model as previously described.[21,22] A measure of the macromolecular proton pool size, i.e., the bound pool fraction, was estimated.

- **Diffusion-weighted imaging:**

We fitted one signal representation and one microstructural model to the diffusion-weighted data: Diffusion Kurtosis Imaging (DKI), and Neurite Orientation Dispersion and Density Imaging (NODDI), respectively.[23] We obtained the following metrics derived from the DKI fitting:[24] Diffusion Tensor Imaging (DTI) metrics: axial diffusivity, radial diffusivity, mean diffusivity, and fractional anisotropy; and kurtosis tensor metrics:[25] axial kurtosis, radial kurtosis, and mean kurtosis. We obtained metrics derived from NODDI:[26] neurite density index, orientation dispersion index, isotropic volume fraction.

Table 1 shows a description of the main qMRI metrics together with their biophysical meaning. **Figure 2** shows examples of the corresponding quantitative maps.

Statistical analyses

Clinical variables (and their *units*) were: EDSS score (*points*); inverse of 9HPT, i.e., mean of the reciprocal value of the mean time of the two right-hand attempts and the reciprocal value of the mean time of the two left-hand attempts (*1/s*);[27,28] inverse of the TWT, i.e., inverse of the mean of two attempts (*1/s*);[27,28] SDMT score (*number of correct answers in 90 seconds*). All clinical variables were considered as continuous.

For all regression models explained below, the assumptions of linear regression were checked whenever possible. All analyses were carried out in Stata/SE 14.2 and RStudio 2021.09.0.

i) Differences between patients and controls

Non-lesional qMRI metrics were tested to see whether they were differentiating PMS patients from healthy controls, through age- and sex-adjusted linear regression models. From these models, we estimated the partial effect size (*Cohen's d*), a standardised measure reflecting the magnitude of the difference (absolute value) in the qMRI metric between the two groups. The significance level was set at 0.01 to reduce the risk of type I error.

ii) Most relevant brain and cord tissue-specific qMRI metrics explaining clinical outcome

First, for each one of the clinical variables (one at a time) and each one of the specific tissue masks in the brain (i.e., NA-CGM, NA-DGM, NA-WM, NA-brainstem-cerebellum, brain lesions) and the spinal cord (i.e., whole cord, NA-cord, cord NA-GM, cord NA-WM, cord lesions), we built univariable regression models to explain the clinical measure (dependent variable) with each one of the tissue-specific qMRI measures. Afterwards, we built four random forest models, one for each clinical measure, to identify the most relevant variables to clinical outcome. To reduce the risk of multiplicity, only those variables which showed an association at 0.05 level with the dependent variable (one at a time) in univariable regression models were entered in the random forest models. Each random forest was made of 1000 trees. We used the default settings specified in the `randomForest` package in R. Variable importance was assessed through '% increase in mean squared error (MSE)'. The greater the value of % increase in MSE, the greater the relevance of the volumetric/qMRI measure.[29] Finally, for each clinical variable, multiple linear regression models assessed the associations between the five most relevant volumetric/qMRI measures (explanatory variable), according to the % increase in MSE, and clincial outcome (dependent variable). Significance level was set at 0.01. For all statistical models, brain lesion load, disease duration, age, and sex were explored as covariates and only retained in the model if significant ($p<0.01$).

RESULTS

Twenty-seven patients with PMS, i.e., 22 with secondary PMS and 5 with primary PMS, and 16 healthy controls were recruited. Of all patients, only one had had a clinical relapse and received steroid treatment over the last years. Ten patients (out of 27) were on DMTs, including intravenous ocrelizumab (2 patients), intravenous natalizumab (1 patient), and platform treatments (7 patients). All participants tolerated the MRI scan without any major problems. The main clinical and demographic characteristics are detailed in **Table 2**.

Differences between patients and controls

After adjusting for age and sex, patients showed significantly smaller brain and cord parenchymal volumes than controls, especially NA-DGM and NA-WM volume fractions (p=0.002 and p=0.001, respectively), whole-cord cross-sectional area (p=0.003), and cord NA-GM cross-sectional area (p<0.001). A number of other significant differences were also found between patients and controls in terms of qMRI measures obtained in NA tissue after adjusting for age and sex (**Table 3**). Considering all MRI metrics (qMRI and volumetric measures), the highest partial effect sizes were observed for qT1, radial diffusivity and mean diffusivity, whose values were greater in patients than controls, especially if obtained in the spinal cord. See **Table 3** for more details.

Most relevant tissue-specific volumetric and qMRI metrics to clinical outcome

For the EDSS, TWT, and SDMT, the random forest regression models were only built with brain volumetric and qMRI measures, since none of the spinal cord measures was significantly associated with the clinical measures in univariable regression models. Instead, for the 9HPT, both brain and cord measures were entered in the random forest regression model (**Figures 3-6,** Supplementary Figure 1).

For the EDSS and the TWT, the most relevant predictors included a combination of volumetric and qMRI measures of damage in NA and lesional brain tissue (**Table 4** and **Figures 3** and **4**). More specifically, for the EDSS, greater qT1 measured in the NA-DGM (beta=7.955, p=0.006) or within lesions (beta=4.346, p=0.001), lower mean kurtosis in the NA-WM (beta=-10.935, p=0.004), and lower bound pool fraction also within lesions (beta=-63.641; p<0.001) were significant predictors of greater EDSS scores. Furthermore, there was some borderline evidence of greater lesion load being also associated with higher EDSS scores (beta=0.031; p=0.010) (**Figure 3, Table 4**).

For the (inverse of the) TWT, the most relevant metrics included mainly measures of damage in the NA-WM, i.e., mean kurtosis, radial kurtosis, orientation dispersion index, and qT1, although only higher orientation dispersion index (beta=-3.206, p=0.005) and higher qT1 (beta=-0.616, p=0.003) emerged as statistically significant predictors of greater disability. Higher brain lesion volume (beta=-0.002, p=0.007) also emerged as a relevant predictor of worse outcome (**Table 4, Figure 4**).

For the (inverse of the) 9HPT, the most relevant predictors included measures of damage in the spinal cord and NA brain tissue. In particular, lower values of NA-cord fractional anisotropy (beta=0.078, p=0.006) and whole-cord bound pool fraction (beta=0.792, p=0.001) significantly predicted worse 9HPT performance. Higher NA-DGM qT1 (beta=-0.088, p=0.004) and lower NA-WM radial kurtosis (beta=0.073, p=0.003) also predicted greater disability (**Table 4, Figure 5**).

Finally, for the SDMT, the most relevant predictors included measures of damage to the NA brain tissue, including damage to the NA-CGM, and within lesions (**Table 4, Figure 6**). In particular, higher NA-WM orientation dispersion index (beta=-847.103, p<0.001), higher NA-DGM axial diffusivity (beta=-90629.8, p<0.001), and higher NA-CGM qT1 (beta=-94.306, p<0.001) were

associated with worse SDMT scores. Furthermore, lower mean kurtosis within lesions (beta=119.864, p=0.001) was associated with worse cognitive disability too (**Table 4**).

DISCUSSION

In this study we assessed the importance of the joint evaluation of damage to the brain and the spinal cord in PMS, testing different biophysical properties of tissue (e.g., myelin, neuronal density and morphology, inflammation) thanks to an ultra-fast and rich qMRI protocol. Not only was our protocol feasible, i.e., well tolerated by all patients, but it also provided valuable information about the underlying pathology of PMS across the entire neuroaxis. In general, brain qMRI metrics explained clinical outcome better than cord metrics, except for the upper limb motor function, which was strongly associated with both brain and cord qMRI metrics. Importantly, predictive models of clinical disability included metrics reflecting mainly demyelination but also inflammation/oedema, and axonal loss/neurodegeneration, highlighting the pathological complexity underlying disability accumulation in MS.

In our study, those qMRI metrics which have been associated with demyelination, such as qT1,[20] radial diffusivity, or mean diffusivity,[30] especially if measured in the spinal cord, showed the highest ability to discriminate between patients and controls, implying high partial effect sizes (**Table 3**), advocating for their use in clinical trials. Instead, the effect sizes of brain volumes were relatively small, despite being significant at 0.01 after adjusting for age and sex. This may be explained by the fact that our patient population was quite old at the time of the study. That is, it has been suggested that brain volumes in people with MS who grow older may reflect mainly aging-related changes rather than MS-related pathology,[31] and we could be observing precisely this phenomenon.

Furthermore, when we assessed the most relevant MRI measures to clinical outcome through random forest regression analyses, again those qMRI measures mainly denoting demyelination emerged as the most important ones. Instead, no atrophy measures played such a prominent role. The only volumetric measure that emerged as a relevant predictor was brain lesion load, especially for the prediction of EDSS and (inverse of) TWT. This suggests that qMRI metrics, and especially those reflecting demyelination, might have a greater power to explain clinical outcome than more conventional atrophy measures. Interestingly, though, some of the metrics that in our study best explained disability measures, such as the qT1, could also be reflecting, apart from demyelination, inflammation/oedema and neurodegenerative processes such as axonal loss. On the other hand, a recent study using 7T MRI has shown that long T1 times mainly reflect demyelination rather than axonal loss,[32] which would reinforce the message of demyelination playing a predominant role in disability accumulation in PMS.

Notably, brain qMRI metrics correlated with clinical outcome better than cord metrics, which may be explained at least partly by the higher signal-to-noise ratio obtained with brain metrics. An important exception was observed when explaining the upper limb motor function, which was strongly associated with both brain and cord qMRI metrics. These results suggest that getting granular information on the microstructural alterations present in different tissue types, i.e., cervical cord, brain NA-WM and NA-DGM, may be important to predicting 9HPT performance, while highlighting the leading role of cervical cord pathology in upper limb function.

In relation to the EDSS, the most relevant measures to clinical outcome included those denoting damage to the NA-DGM (qT1) and NA-WM (mean kurtosis). Furthermore, the integrity of the brain tissue underlying visible lesional damage (qT1 and bound protein fraction) and the actual volume of visible brain lesions were also key. For the TWT, instead, measures of damage to the NA-WM were clearly predominant, apart from brain lesion volume. This stresses the pathological

complexity underlying that accumulation of disability so strongly related to the inability to walk, highlighting the role of lesional and non-lesional brain WM integrity, as suggested by other authors.[33]

Finally, the prediction of cognitive performance, as assessed by the SDMT, seemed to depend on the integrity of brain NA-WM (orientation dispersion index), the tissue underlying brain lesions (mean kurtosis), brain NA-DGM (axial diffusivity), and NA-CGM (qT1). Therefore, SDMT was the only clinical measure whose prediction strongly relied on measures of damage to the NA-CGM, in line with the requirement of higher order systems to perform cognitive tasks.[36]

Study considerations and limitations

From the image processing point of view, we should acknowledge that the class tissue segmentation in the spinal cord, unlike that in the brain, was carried out using non-lesion-filled images, using the Spinal Cord Toolbox.[18] Thus, we cannot rule out a possible effect of focal and diffuse lesions on such segmentation, even if all our tissue masks were reviewed and corrected (if needed) by an expert, which is a clear limitation of the study.

A further limitation stems from the fact that NA-cord and, to a lesser extent, brain tissue volumes may have been affected by the actual volume of cord and brain lesions, respectively, and not only by the presence of atrophy in those locations. Whereas this methodological choice allowed us to focus on the effects of the NA tissue, it made it difficult to assess the effects of brain and cord volumes on disability outcomes, which are known to be key in progressive MS.[37,38] So, future studies looking at GM and WM tissue volumes in the spinal cord as potential predictors of concurrent disability are definitely needed. Additionally, since our study focused on a relatively small spinal cord region, we might have underestimated the contribution of spinal cord pathology

to disability accrual. Therefore, further research focused on larger cord regions is needed to better understand the role of cord pathology in disability accumulation in progressive MS.

The sample size of this study is relatively small but was powered to detect moderate-strong correlations between clinical and qMRI variables, which we expected from previous analyses.[39] On the other hand, the very rich imaging protocol, which is extremely innovative *per se*, provided us with uniquely valuable information for each participant, allowing us to generate novel insights reliably. The fact that many qMRI metrics with a similar biophysical meaning but obtained with independent contrasts went in the same direction (e.g., qT1 and F) contributed to strengthening our results as we have independent confirmation of tissue microstructural changes.

Of note, in this study, although a number of statistical tests are reported, a number of separate null hypotheses were examined, rather than one single null hypothesis, whose error rate is affected by every reported test. Therefore, in line with previous recommendations,[40,41] we did not adjust for multiple comparisons, although we set our statistical level at 0.01 to reduce the risk of type I error. Finally, we must acknowledge that some of our clinical measures, especially the EDSS, may have been subject to high levels of inter-rater[34,35] and/or intra-rater[35] variability, implying that the results may be taken with caution and deserving further research.

Conclusions

Our data shows that it is important to study MS progression jointly across the brain and the spinal cord in the same MRI session and with a rich qMRI protocol, since characterising damage beyond focal lesions in both areas is key to explaining disability in terms of different clinical domains. Indeed, the model for explaining cognitive or upper-limb motor function were very different, the latter involving the spinal cord. Our results also reveal the predominant role of qMRI metrics particularly sensitive – although not exclusively – to tissue demyelination measured mainly in non-

lesional areas for prediction of concurrent disability. Of note, metrics sensitive to inflammation, oedema, and axonal loss also played an important role in explaining clinical scores, reflecting the pathological complexity underlying disability accumulation in MS. This study demonstrates that qMRI can be obtained with fast advanced sampling and post-processing strategies like those employed here, supporting their implementation in clinical and research settings.

AVAILABILITY STATEMENT

All the underlying research materials related to our paper (data, qMRI pipelines or statistical models) are available from the corresponding author (CT), upon reasonable request.

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REFERENCES

1.   Thompson AJ, Baranzini SE, Geurts J, Hemmer B, Ciccarelli O. Multiple sclerosis. Lancet
      [online serial]. 2018;391:1622–1636. Accessed at:
      https://linkinghub.elsevier.com/retrieve/pii/S0140673618304811.

2.   Granziera C, Wuerfel J, Barkhof F, et al. Quantitative magnetic resonance imaging
      towards clinical application in multiple sclerosis. Brain [online serial]. 2021;144:1296–
      1311. Accessed at: https://academic.oup.com/brain/article/144/5/1296/6273092.

3.   Grussu F, Schneider T, Tur C, et al. Neurite dispersion: a new marker of multiple
      sclerosis spinal cord pathology? Ann Clin Transl Neurol [online serial]. 2017;4:663–679.
      Accessed at: http://doi.wiley.com/10.1002/acn3.445.

4.   Rocca MA, Valsasina P, Meani A, et al. Spinal cord lesions and brain grey matter atrophy
      independently predict clinical worsening in definite multiple sclerosis: a 5-year,
      multicentre study. J Neurol Neurosurg Psychiatry [online serial]. Epub 2022 Sep 28.:jnnp-
      2022-329854. Accessed at: https://jnnp.bmj.com/lookup/doi/10.1136/jnnp-2022-329854.

5.   Hutter J, Slator PJ, Christiaens D, et al. Integrated and efficient diffusion-relaxometry
      using ZEBRA. Sci Rep [online serial]. 2018;8:15138. Accessed at:
      http://www.nature.com/articles/s41598-018-33463-2.

6.   Kim D, Doyle EK, Wisnowski JL, Kim JH, Haldar JP. Diffusion-relaxation correlation
      spectroscopic imaging: A multidimensional approach for probing microstructure. Magn
      Reson Med [online serial]. 2017;78:2236–2249. Accessed at:
      https://onlinelibrary.wiley.com/doi/10.1002/mrm.26629.

7.   Ning L, Gagoski B, Szczepankiewicz F, Westin C-F, Rathi Y. Joint RElaxation-Diffusion
      Imaging Moments to Probe Neurite Microstructure. IEEE Trans Med Imaging [online
      serial]. 2020;39:668–677. Accessed at: https://ieeexplore.ieee.org/document/8792128/.

8.   Collorone S, Cawley N, Grussu F, et al. Reduced neurite density in the brain and cervical
      spinal cord in relapsing–remitting multiple sclerosis: A NODDI study. Mult Scler J [online

serial]. 2020;26:1647–1657. Accessed at:

http://journals.sagepub.com/doi/10.1177/1352458519885107.

9. Grussu F, Battiston M, Veraart J, et al. Multi-parametric quantitative in vivo spinal cord

MRI with unified signal readout and image denoising. Neuroimage [online serial].

2020;217:116884. Accessed at:

https://linkinghub.elsevier.com/retrieve/pii/S1053811920303700.

10. Lublin FD, Reingold SC, Cohen JA, et al. Defining the clinical course of multiple sclerosis:

The 2013 revisions. Neurology [online serial]. 2014;83:278–286. Accessed at:

https://www.neurology.org/lookup/doi/10.1212/WNL.0000000000000560.

11. Cutter GR. Development of a multiple sclerosis functional composite as a clinical trial

outcome measure. Brain [online serial]. 1999;122:871–882. Accessed at:

https://academic.oup.com/brain/article-lookup/doi/10.1093/brain/122.5.871.

12. Smith A. Symbol Digit Modalities Test: Manual. Los Angeles Western Psychological

Services; 2007.

13. Kurtzke JF. Rating neurologic impairment in multiple sclerosis: An expanded disability

status scale (EDSS). Neurology [online serial]. 1983;33:1444–1444. Accessed at:

http://www.neurology.org/cgi/doi/10.1212/WNL.33.11.1444.

14. Wheeler-Kingshott CAM, Parker GJM, Symms MR, et al. ADC mapping of the human

optic nerve: Increased resolution, coverage, and reliability with CSF-suppressed ZOOM-

EPI. Magn Reson Med [online serial]. 2002;47:24–31. Accessed at:

https://onlinelibrary.wiley.com/doi/10.1002/mrm.10016.

15. Valverde S, Salem M, Cabezas M, et al. One-shot domain adaptation in multiple sclerosis

lesion segmentation using convolutional neural networks. Epub 2018 May 31. Accessed

at: http://arxiv.org/abs/1805.12415.

16. Prados F, Cardoso MJ, Kanber B, et al. A multi-time-point modality-agnostic patch-based

method for lesion filling in multiple sclerosis. Neuroimage [online serial]. 2016;139:376–

384. Accessed at: https://linkinghub.elsevier.com/retrieve/pii/S1053811916303020.

17.   Cardoso MJ, Modat M, Wolz R, et al. Geodesic Information Flows: Spatially-Variant
       Graphs and Their Application to Segmentation and Fusion. IEEE Trans Med Imaging
       [online serial]. 2015;34:1976–1988. Accessed at:
       https://ieeexplore.ieee.org/document/7086081/.

18.   De Leener B, Lévy S, Dupont SM, et al. SCT: Spinal Cord Toolbox, an open-source
       software for processing spinal cord MRI data. Neuroimage. 2017;145.

19.   Battiston M, Schneider T, Prados F, et al. Fast and reproducible in vivo T 1 mapping of
       the human cervical spinal cord. Magn Reson Med [online serial]. 2018;79:2142–2148.
       Accessed at: https://onlinelibrary.wiley.com/doi/10.1002/mrm.26852.

20.   Sereno MI, Lutti A, Weiskopf N, Dick F. Mapping the Human Cortical Surface by
       Combining Quantitative T1 with Retinotopy†. Cereb Cortex [online serial]. 2013;23:2261–
       2268. Accessed at: https://academic.oup.com/cercor/article-
       lookup/doi/10.1093/cercor/bhs213.

21.   Battiston M, Schneider T, Grussu F, et al. Fast bound pool fraction mapping via steady-
       state magnetization transfer saturation using single-shot EPI. Magn Reson Med [online
       serial]. Epub 2019 May 12.:mrm.27792. Accessed at:
       https://onlinelibrary.wiley.com/doi/10.1002/mrm.27792.

22.   Battiston M, Grussu F, Ianus A, et al. An optimized framework for quantitative
       magnetization transfer imaging of the cervical spinal cord in vivo. Magn Reson Med
       [online serial]. 2018;79:2576–2588. Accessed at:
       https://onlinelibrary.wiley.com/doi/10.1002/mrm.26909.

23.   Nørhøj Jespersen S. White matter biomarkers from diffusion MRI. J Magn Reson [online
       serial]. 2018;291:127–140. Accessed at:
       https://linkinghub.elsevier.com/retrieve/pii/S1090780718300764.

24.   Pierpaoli C, Basser PJ. Toward a quantitative assessment of diffusion anisotropy. Magn

Reson Med [online serial]. 1996;36:893–906. Accessed at: https://onlinelibrary.wiley.com/doi/10.1002/mrm.1910360612.

25. Jensen JH, Helpern JA, Ramani A, Lu H, Kaczynski K. Diffusional kurtosis imaging: The quantification of non-gaussian water diffusion by means of magnetic resonance imaging. Magn Reson Med [online serial]. 2005;53:1432–1440. Accessed at: https://onlinelibrary.wiley.com/doi/10.1002/mrm.20508.

26. Zhang H, Schneider T, Wheeler-Kingshott CA, Alexander DC. NODDI: Practical in vivo neurite orientation dispersion and density imaging of the human brain. Neuroimage. 2012;61.

27. Tur C, Grussu F, De Angelis F, et al. Spatial patterns of brain lesions assessed through covariance estimations of lesional voxels in multiple Sclerosis: The SPACE-MS technique. NeuroImage Clin [online serial]. 2022;33:102904. Accessed at: https://linkinghub.elsevier.com/retrieve/pii/S221315822100348X.

28. J. C, F. DA, P. C, et al. MS-SMART Trial: A multi-arm phase 2b randomised double blind, parallel group, placebo-controlled clinical trial comparing the efficacy of three neuroprotective drugs in secondary progressive multiple sclerosis [NCT01910259]. Mult Scler J. 2018;24.

29. Breiman L. Random Forests. Mach Learn. 2001;45:5–32.

30. Seehaus A, Roebroeck A, Bastiani M, et al. Histological validation of high-resolution DTI in human post mortem tissue. Front Neuroanat [online serial]. 2015;9. Accessed at: http://journal.frontiersin.org/Article/10.3389/fnana.2015.00098/abstract.

31. Azevedo CJ, Cen SY, Jaberzadeh A, Zheng L, Hauser SL, Pelletier D. Contribution of normal aging to brain atrophy in MS. Neurol Neuroimmunol Neuroinflammation [online serial]. 2019;6. Accessed at: https://www.neurology.org/doi/10.1212/NXI.0000000000000616.

32. Kolb H, Absinta M, Beck ES, et al. <scp>7T MRI</scp> Differentiates Remyelinated from

Demyelinated Multiple Sclerosis Lesions. Ann Neurol [online serial]. 2021;90:612–626.

Accessed at: https://onlinelibrary.wiley.com/doi/10.1002/ana.26194.

33.   Edwards EM, Stanley JA, Daugherty AM, Lynn J, Borich MR, Fritz NE. Associations

between myelin water imaging and measures of fall risk and functional mobility in multiple

sclerosis. J Neuroimaging [online serial]. 2023;33:94–101. Accessed at:

https://onlinelibrary.wiley.com/doi/10.1111/jon.13064.

34.   Noseworthy JH, Vandervoort MK, Wong CJ, Ebers GC. Interrater variability with the

Expanded Disability Status Scale (EDSS) and Functional Systems (FS) in a multiple

sclerosis clinical trial. Neurology [online serial]. 1990;40:971–971. Accessed at:

https://www.neurology.org/lookup/doi/10.1212/WNL.40.6.971.

35.   Goodkin DE, Cookfair D, Wende K, et al. Inter- and intrarater scoring agreement using

grades 1.0 to 3.5 of the Kurtzke Expanded Disability Status Scale (EDSS). Neurology

[online serial]. 1992;42:859–859. Accessed at:

https://www.neurology.org/lookup/doi/10.1212/WNL.42.4.859.

36.   Palesi F, Tournier J-D, Calamante F, et al. Contralateral cerebello-thalamo-cortical

pathways with prominent involvement of associative areas in humans in vivo. Brain Struct

Funct [online serial]. 2015;220:3369–3384. Accessed at:

http://link.springer.com/10.1007/s00429-014-0861-2.

37.   Cagol A, Schaedelin S, Barakovic M, et al. Association of Brain Atrophy With Disease

Progression Independent of Relapse Activity in Patients With Relapsing Multiple

Sclerosis. JAMA Neurol [online serial]. 2022;79:682. Accessed at:

https://jamanetwork.com/journals/jamaneurology/fullarticle/2792415.

38.   Cagol A, Benkert P, Melie-Garcia L, et al. Association of spinal cord atrophy and brain

paramagnetic rim lesions with progression independent of relapse activity in people with

MS. Neurology. 2023;XX:XX.

39.   Collorone S, Prados F, Kanber B, et al. Brain microstructural and metabolic alterations

detected in vivo at onset of the first demyelinating event. Brain [online serial].

2021;144:1409–1421. Accessed at:

https://academic.oup.com/brain/article/144/5/1409/6246099.

40.   Perneger T V. What's wrong with Bonferroni adjustments. BMJ [online serial].

1998;316:1236–1238. Accessed at:

https://www.bmj.com/lookup/doi/10.1136/bmj.316.7139.1236.

41.   Rothman KJ. No adjustments are needed for multiple comparisons. Epidemiology [online

serial]. 1990;1:43–46. Accessed at: http://www.ncbi.nlm.nih.gov/pubmed/2081237.

FIGURE LEGENDS

Figure 1. MRI protocol pipeline

Figure 1 (legend). Schematic representation of the image analysis pipeline implemented for both brain and spinal cord imaging. The general pre-processing part included the following steps: joint denoising (across MRI modalities, for brain and cord regions separately), through the Marchenko-Pastur Principal Component Analysis (MPPCA) method, which allows a 4D image denoising and noise map estimation by exploiting data redundancy in the Principal Component Analysis (PCA) domain using universal properties of the eigenspectrum of random covariance matrices, i.e. Marchenko-Pastur distribution; joint Gibbs ringing artifact removal or un-ringing (across MRI modalities, for brain and cord regions separately); joint distortion correction (across MRI modalities, for brain MRI data only), carried out with FSL topup; joint Eddy current correction (across MRI modalities, for brain MRI data only), carried out with FSL Eddy; joint motion correction (across MRI modalities, for brain and cord regions separately). Please also note that the rFOV that was used was the ZOOM technique (please see Methods' section for more details). *Abbreviations*: EPI: Echo-Planar Imaging; FSL: FMRIB Software Library; MPPCA: Marchenko-Pastur Principal Component Analysis; NiftyReg: NiftyReg is an open-source software for efficient medical image registration developed by members of the Centre for Medical Image Computing at University College London, UK (http://cmictig.cs.ucl.ac.uk/wiki/index.php/NiftyReg); rFOV: reduced field of view; ZOOM: zonal oblique multislice.

Figure 2. Examples of qMRI maps

Figure 2 (legend). Examples of qMRI metrics in both brain and spinal cord in a representative healthy control and patient with MS, both chosen randomly, alongside tissue segmentations. *Abbreviations:* F: bound pool fraction; ODI: orientation dispersion index; qT1: quantitative T1 relaxation time.

Figure 3. Variable importance for the prediction of EDSS

Figure 3 (legend). Variable importance plot reflecting the relevance of the different MRI measures to the prediction of EDSS. See main text for full details. *Abbreviations:* AD: axial diffusivity; AK: axial kurtosis; BSC: brainstem and cerebellum; CGM: cortical grey matter; DGM: deep grey matter; EDSS: expanded disability status scale; F: bound pool fraction; FA: fractional anisotropy; GM: grey matter; IVF: isotropic volume fraction; MD: mean diffusivity; MK: mean kurtosis; NA: normal-appearing; NDI: neurite density index; ODI: orientation dispersion index; qT1: quantitative T1 relaxation time; RD: radial diffusivity; RK: radial kurtosis; WM: white matter.

Figure 4. Variable importance for the prediction of TWT

Figure 4 (legend). Variable importance plot reflecting the relevance of the different MRI measures to the prediction of TWT. See main text for full details. *Abbreviations:* AD: axial diffusivity; AK: axial kurtosis; BSC: brainstem and cerebellum; CGM: cortical grey matter; DGM: deep grey matter; F: bound pool fraction; FA: fractional anisotropy; GM: grey matter; IVF: isotropic volume fraction; MD: mean diffusivity; MK: mean kurtosis; NA: normal-appearing; NDI: neurite density index; ODI: orientation dispersion index; qT1: quantitative T1 relaxation time; RD: radial diffusivity; RK: radial kurtosis; TWT: 25-foot timed walk test; WM: white matter.

Figure 5. Variable importance for the prediction of 9HPT

Figure 5 (legend). Variable importance plot reflecting the relevance of the different MRI measures to the prediction of 9HPT. See main text for full details. *Abbreviations:* 9HPT: 9-hole peg test; AD: axial diffusivity; AK: axial kurtosis; BSC: brainstem and cerebellum; CGM: cortical grey matter; DGM: deep grey matter; F: bound pool fraction; FA: fractional anisotropy; GM: grey

matter; IVF: isotropic volume fraction; MD: mean diffusivity; MK: mean kurtosis; NA: normal-appearing; NDI: neurite density index; ODI: orientation dispersion index; qT1: quantitative T1 relaxation time; RD: radial diffusivity; RK: radial kurtosis; WM: white matter.

Figure 6. Variable importance for the prediction of SDMT

Figure 6 (legend). Variable importance plot reflecting the relevance of the different MRI measures to the prediction of SDMT. See main text for full details. *Abbreviations:* AD: axial diffusivity; AK: axial kurtosis; BSC: brainstem and cerebellum; CGM: cortical grey matter; DGM: deep grey matter; F: bound pool fraction; FA: fractional anisotropy; IVF: isotropic volume fraction; MD: mean diffusivity; MK: mean kurtosis; NA: normal-appearing; NDI: neurite density index; ODI: orientation dispersion index; qT1: quantitative T1 relaxation time; RD: radial diffusivity; RK: radial kurtosis; SDMT: symbol digit modalities test; WM: white matter.

TABLES

Table 1. Description of the qMRI metrics

MRI sequence	qMRI metric		Biophysical meaning
Inversion recovery	qT1		Spin-lattice relaxation time, providing a measure of the time required by the longitudinal component of the magnetisation (i.e., parallel to the static B0 field) to return to equilibrium following radiofrequency excitation. It is sensitive to the presence of macromolecules, so it increases in demyelinated tissue. It also increases in oedema, inflammation, and atrophy processes.
Magnetisation transfer imaging	Bound pool fraction		Fraction of protons bound to macromolecules, as for example myelin in the brain; it decreases in demyelinated tissue.
Diffusion-weighted imaging	Signal representation: Diffusion Kurtosis Imaging	Axial Diffusivity	Diffusion tensor index parallel to the principal diffusion direction, which, in single white matter bundles, is aligned with the dominant neural fibre orientation. It is sensitive to both myelination and axonal integrity. It can increase or decrease in demyelinated tissue.
		Radial Diffusivity	Diffusion tensor index perpendicular to the principal diffusion direction, which, in single white matter bundles, is aligned with the dominant neural fibre orientation. It is sensitive to myelination and axonal density. Generally, it increases in demyelination.
		Mean Diffusivity	Mean diffusion coefficient from diffusion tensor fitting, averaged across all 3D spatial directions. It is a measure of overall diffusivity; generally, it increases in demyelinated tissue.
		Fractional Anisotropy	Normalised measure of the orientation dependence of the diffusivity from diffusion tensor eigenvalues. It is close to zero where diffusion is isotropic, while approaches 1 in anisotropic areas, where the diffusivity parallel to the principal diffusion direction is much larger than orthogonal to it. It is sensitive to a variety of microstructural properties (e.g., axonal density, axonal orientation), and can exhibit complex patterns of variation in demyelinated tissue.
		Axial Kurtosis	Index quantifying the departure of the water diffusion behaviour from the ideal Gaussian diffusion (i.e., diffusion in pure water) along the principal diffusion direction, which, in single white matter bundles, is aligned with the dominant neural fibre orientation. It is usually reduced in demyelinated tissue and, to a lesser extent, axonal loss.
		Radial Kurtosis	Index quantifying the departure of the water diffusion behaviour from the ideal Gaussian diffusion (i.e., diffusion in pure water) orthogonally to the principal diffusion direction, which, in single white matter bundles, is perpendicular to the dominant neural fibre orientation. It is usually reduced in demyelinated tissue and, to a lesser extent, axonal loss.
		Mean Kurtosis	Average departure from Gaussian diffusion along all 3D spatial directions.
	Microstructural model: Neurite Orientation Dispersion and Density Imaging	Neurite Density Index	Signal fraction of the neurite compartment, modelling diffusion within axons and dendrites. It is sensitive to myelin-weighted axonal density in white matter; it is decreased in axonal loss and demyelination.
		Orientation Dispersion Index	Variability of the orientation of neurites, i.e., amount of neurite dispersion, such that dispersion increases as ODI value increases. It is altered (usually increased) in demyelinated tissue.
		Isotropic Volume Fraction	Free water signal fraction; it is usually increased in heavily demyelinated tissue. It also captures partial volume with the CSF and may be sensitive to inflammation and/or oedema.

Table 1 (footnote). *Abbreviations:* qT1: Quantitative longitudinal relaxation time.

Table 2. Clinical, demographic and basic MRI features of study participants

Measure, in units *Mean (SD)*[&]	Patients N=27	Controls N=16	p-value[#]
Age at study baseline, in years	56.77 (7.58)	56.82 (9.03)	0.278
Sex, number of males (%)	13 (48%)	5 (31%)	0.986
Disease duration, in years	20.68 (11.30)	-	-
Duration of progressive phase, in years	8.41 (6.60)	-	-
EDSS score, median (range)	6 (3.5 to 7.5)	-	-
Inverse of TWT, in 1/s	0.09 (0.06)	0.22 (0.03)	**<0.001**
Inverse of 9HPT, in 1/s	0.03 (0.01)	0.05 (0.004)	**<0.001**
SDMT score, number of correct answers	46.17 (11.39)	52.63 (7.99)	0.053
Brain lesion load, in mL	19.36 (16.14)	0.31 (1.23)Φ	-
NA CGM volume, in mL	585.13 (54.33)	591.88 (52.90)	0.693
NA DGM volume, in mL	34.00 (4.24)	36.02 (3.79)	0.124
NA WM volume, in mL	431.82 (54.72)	451.67 (43.88)	0.224
Cord CSA*, in mm^2	63.67 (8.89)	70.82 (6.05)	**0.009**
Cord NA GM CSA*, in mm^2	11.18 (2.30)	13.95 (0.91)	**<0.001**
Cord NA WM CSA*, in mm^2	52.54 (7.57)	56.89 (5.39)	0.061
Cord lesionsΨ, in mL	0.17 (0.15)	-	-

Table 2 (footnote). &: unless otherwise specified; #: unadjusted p-value; Φ: a few controls had minimal lesion load, which corresponded to chronic vascular disease; *: averaged between C2 and C3; Ψ: computed in C2 and C3. *Abbreviations:* 9HPT: nine-hole peg test; CGM: cortical grey matter; CSA: cross-sectional area (of the cord); DGM: deep grey matter; EDSS: expanded disability status scale; GM: grey matter; GM-CSA: grey matter cross-sectional area averaged between C2 and C3; NA: normal appearing; SD: standard deviation; SDMT: symbol digit modalities test; TWT: 25-foot timed walk test; WM: white matter; WM-CSA: spinal cord white matter cross-sectional area averaged between C2 and C3.

New) Table 3. Description of all MRI metrics and age- and sex-adjusted partial effect sizes

		Mean (SD)			Partial (age- and sex-adjusted) effect size (95%CI)
		Patients	Controls	p	
Volumetric measures, in units indicated below					
Brain	NA GM volume, in mL	619.130 (58.226)	627.899 (55.782)	0.168	0.048 (0; 0.218)
	NA CGM volume, in mL	585.127 (54.328)	591.877 (52.903)	0.209	0.040 (0; 0.205)
	NA DGM volume, in mL	34.002 (4.238)	36.021 (3.789)	**0.008**	**0.169 (0.013; 0.365)**
	NA WM volume, in mL	431.819 (54.721)	451.674 (43.881)	0.033	0.112 (0; 0.303)
	NA GMF	0.433 (0.010)	0.436 (0.008)	0.375	0.020 (0; 0.166)
	NA CGMF	0.409 (0.010)	0.411 (0.008)	0.678	0.004 (0; 0.117)
	NA DGMF	0.024 (0.001)	0.025 (0.002)	**0.002**	**0.213 (0.031; 0.408)**
	NA WMF	0.301 (0.013)	0.313 (0.011)	**0.001**	**0.253 (0.052; 0.445)**
	Brain parenchymal fraction	0.733 (0.016)	0.749 (0.009)	**<0.001**	**0.283 (0.070; 0.713)**
	Brain lesion volume, in mL	19.358 (16.141)	0.308 (1.230)	**<0.001**	**0.357 (0.124; 0.533)**
Spinal cord	CSA, in mm^2	63.666 (8.890)	70.815 (6.053)	**0.003**	**0.229 (0.032; 0.432)**
	NA GM-CSA, in mm^2	11.183 (2.303)	13.948 (0.911)	**<0.001**	**0.344 (0.103; 0.530)**
	NA WM-CSA, in mm^2	52.536 (7.569)	56.887 (5.394)	0.017	0.152 (0.004; 0.357)
	Cord lesions, in mL	0.166 (0.151)	-	-	-
F, in dimensionless units (fraction)					
Brain	NA CGM	0.056 (0.005)	0.058 (0.005)	0.161	0.057 (0; 0.244)
	NA DGM	0.069 (0.007)	0.070 (0.006)	0.512	0.013 (0; 0.160)
	NA WM	0.102 (0.009)	0.106 (0.008)	0.159	0.057 (0; 0.245)
	Brainstem	0.080 (0.006)	0.080 (0.008)	0.876	0.001 (0; 0.082)
	Brain lesions	0.065 (0.012)	-	-	-
Spinal cord	Whole cord	0.055 (0.008)	0.065 (0.006)	**<0.001**	**0.318 (0.081; 0.511)**
	NA cord	0.055 (0.008)	0.065 (0.006)	**<0.001**	**0.314 (0.079; 0.508)**
	NA GM cord	0.063 (0.010)	0.071 (0.005)	0.013	0.168 (0.007; 0.376)
	NA WM cord	0.056 (0.008)	0.067 (0.007)	**<0.001**	**0.313 (0.078; 0.507)**
	Cord lesions	0.047 (0.016)	-	-	-
qT1, in s					
Brain	NA CGM	1.529 (0.085)	1.452 (0.056)	**0.007**	**0.198 (0.017; 0.406)**
	NA DGM	1.306 (0.074)	1.245 (0.043)	0.012	0.172 (0.009; 0.380)
	NA WM	0.993 (0.055)	0.949 (0.034)	0.012	0.173 (0.009; 0.381)
	Brainstem	1.261 (0.067)	1.218 (0.061)	0.059	0.101 (0; 0.303)
	Brain lesions	1.360 (0.157)		-	-
Spinal cord	Whole cord	1.498 (0.114)	1.322 (0.064)	**<0.001**	**0.418 (0.165; 0.588)**
	NA cord	1.488 (0.110)	1.322 (0.064)	**<0.001**	**0.408 (0.156; 0.580)**
	NA GM cord	1.286 (0.101)	1.187 (0.032)	**0.002**	**0.235 (0.035; 0.437)**
	NA WM cord	1.443 (0.122)	1.284 (0.068)	**<0.001**	**0.350 (0.107; 0.535)**
	Cord lesions	1.795 (0.412)		-	-
NDI, in dimensionless units					
Brain	NA CGM	0.352 (0.022)	0.375 (0.019)	**0.006**	**0.196 (0.018; 0.401)**
	NA DGM	0.387 (0.020)	0.396 (0.019)	0.068	0.089 (0; 0.283)

Region	Subregion				
	NA WM	0.492 (0.028)	0.511 (0.018)	0.064	0.094 (0; 0.292)
	Brainstem	0.488 (0.032)	0.516 (0.026)	**0.009**	**0.178 (0.012; 0.384)**
	Brain lesions	0.276 (0.042)	-	**-**	-
Spinal cord	**Whole cord**	0.386 (0.058)	0.445 (0.040)	**0.001**	**0.281 (0.060; 0.478)**
	NA cord	0.390 (0.057)	0.445 (0.040)	**0.001**	**0.259 (0.047; 0.459)**
	NA GM cord	0.428 (0.058)	0.467 (0.036)	0.010	0.173 (0.010; 0.379)
	NA WM cord	0.400 (0.061)	0.459 (0.041)	**0.001**	**0.261 (0.048; 0.460)**
	Cord lesions	0.306 (0.114)	-	-	-
ODI, in dimensionless units					
Brain	**NA CGM**	0.486 (0.008)	0.487 (0.007)	0.779	0.002 (0; 0.108)
	NA DGM	0.391 (0.020)	0.404 (0.017)	0.064	0.095 (0; 0.292)
	NA WM	0.261 (0.010)	0.254 (0.006)	0.021	0.143 (0.002; 0.348)
	Brainstem	0.249 (0.012)	0.250 (0.007)	0.700	0.004 (0; 0.124)
	Brain lesions	0.209 (0.026)	-	-	-
Spinal cord	**Whole cord**	0.117 (0.061)	0.071 (0.018)	0.025	0.136 (0.0002; 0.340)
	NA cord	0.117 (0.062)	0.071 (0.018)	0.027	0.132 (0; 0.336)
	NA GM cord	0.122 (0.059)	0.104 (0.021)	0.457	0.0159 (0; 0.166)
	NA WM cord	0.101 (0.062)	0.052 (0.019)	0.019	0.148 (0.003; 0.353)
	Cord lesions	0.151 (0.091)	-	-	-
IVF, in dimensionless units (fraction)					
Brain	**NA CGM**	0.520 (0.024)	0.526 (0.013)	0.657	0.006 (0; 0.131)
	NA DGM	0.471 (0.037)	0.487 (0.022)	0.530	0.011 (0; 0.153)
	NA WM	0.392 (0.038)	0.365 (0.019)	0.050	0.105 (0; 0.305)
	Brainstem	0.320 (0.024)	0.313 (0.020)	0.187	0.049 (0; 0.230)
	Brain lesions	0.536 (0.052)	-	-	-
Spinal cord	**Whole cord**	0.310 (0.094)	0.310 (0.040)	0.551	0.010 (0; 0.149)
	NA cord	0.307 (0.093)	0.310 (0.040)	0.596	0.008 (0; 0.142)
	NA GM cord	0.425 (0.126)	0.403 (0.048)	0.227	0.041 (0; 0.217)
	NA WM cord	0.307 (0.093)	0.307 (0.044)	0.577	0.009 (0; 0.145)
	Cord lesions	0.306 (0.114)	-	-	-
FA, in dimensionless units					
Brain	**NA CGM**	0.188 (0.013)	0.193 (0.014)	0.219	0.043 (0; 0.219)
	NA DGM	0.285 (0.023)	0.272 (0.016)	0.133	0.063 (0; 0.251)
	NA WM	0.427 (0.021)	0.442 (0.016)	0.034	0.122 (0; 0.325)
	Brainstem	0.468 (0.023)	0.480 (0.024)	0.078	0.086 (0; 0.282)
	Brain lesions	0.325 (0.030)	-	-	-
Spinal cord	**Whole cord**	0.641 (0.067)	0.720 (0.027)	**<0.001**	**0.310 (0.078; 0.502)**
	NA cord	0.643 (0.068)	0.720 (0.027)	**<0.001**	**0.298 (0.070; 0.492)**
	NA GM cord	0.667 (0.056)	0.691 (0.042)	0.127	0.065 (0; 0.254)
	NA WM cord	0.672 (0.075)	0.759 (0.027)	**<0.001**	**0.308 (0.077; 0.500)**
	Cord lesions	0.571 (0.150)	-	-	-
RD, in µm²/ms					
Brain	**NA CGM**	0.00103 (0.00009)	0.00096 (0.00006)	0.013	0.159 (0.007; 0.362)
	NA DGM	0.00095 (0.00006)	0.00091 (0.00005)	0.051	0.101 (0; 0.298)
	NA WM	0.00070 (0.00003)	0.00068 (0.00004)	0.102	0.072 (0; 0.261)
	Brainstem	0.00078 (0.00006)	0.00072 (0.00005)	0.013	0.159 (0.007; 0.362)
	Brain lesions	0.00113 (0.00010)	-	-	-
Spinal cord	**Whole cord**	0.00087 (0.00016)	0.00064 (0.00007)	**<0.001**	**0.398 (0.147; 0.573)**
	NA cord	0.00086 (0.00016)	0.00064 (0.00007)	**<0.001**	**0.384 (0.135; 0.562)**
	NA GM cord	0.00063 (0.00011)	0.00055 (0.00007)	0.030	0.127 (0; 0.330)
	NA WM cord	0.00079 (0.00017)	0.00056 (0.00007)	**<0.001**	**0.376 (0.128; 0.556)**
	Cord lesions	0.00120 (0.00058)	-	-	-
MD, in µm²/ms					
Brain	**NA CGM**	0.00113 (0.00009)	0.00105 (0.00006)	0.013	0.161 (0.007; 0.364)
	NA DGM	0.00111 (0.00007)	0.00106 (0.00005)	0.030	0.124 (0; 0.325)
	NA WM	0.00093 (0.00003)	0.00092 (0.00004)	0.259	0.035 (0; 0.204)
	Brainstem	0.00105 (0.00006)	0.00099 (0.00005)	0.017	0.148 (0.004; 0.350)
	Brain lesions	0.00137 (0.00010)	-	-	-
Spinal cord	**Whole cord**	0.00144 (0.00011)	0.00127 (0.00009)	**<0.001**	**0.383 (0.134; 0.561)**

	NA cord	0.00143 (0.00011)	0.00127 (0.00009)	**<0.001**	**0.363 (0.118; 0.546)**
	NA GM cord	0.00113 (0.00009)	0.00106 (0.00009)	0.050	0.105 (0; 0.305)
	NA WM cord	0.00139 (0.00012)	0.00123 (0.00008)	**<0.001**	**0.345 (0.104; 0.531)**
	Cord lesions	0.00173 (0.00050)	-	-	-
AD, in µm²/ms					
Brain	**NA CGM**	0.00132 (0.00009)	0.00124 (0.00007)	0.015	0.153 (0.005; 0.356)
	NA DGM	0.00142 (0.00009)	0.00135 (0.00006)	0.026	0.131 (0; 0.332)
	NA WM	0.00139 (0.00004)	0.00139 (0.00004)	0.867	0.001 (0; 0.082)
	Brainstem	0.00159 (0.00007)	0.00154 (0.00005)	0.040	0.112 (0; 0.311)
	Brain lesions	0.00184 (0.00011)	-	-	-
Spinal cord	**Whole cord**	0.00253 (0.00011)	0.00248 (0.00012)	0.236	0.040 (0; 0.214)
	NA cord	0.00252 (0.00011)	0.00248 (0.00012)	0.355	0.025 (0; 0.185)
	NA GM cord	0.00213 (0.00013)	0.00205 (0.00017)	0.245	0.038 (0; 0.212)
	NA WM cord	0.00256 (0.00013)	0.00255 (0.00012)	0.769	0.002 (0; 0.110)
	Cord lesions	0.00274 (0.00033)	-	-	-
AK, in µm²/ms					
Brain	**NA CGM**	0.648 (0.016)	0.661 (0.017)	0.040	**0.112 (0; 0.310)**
	NA DGM	0.717 (0.026)	0.724 (0.029)	0.169	0.0519 (0; 0.231)
	NA WM	0.706 (0.021)	0.722 (0.014)	0.025	**0.131 (0; 0.333)**
	Brainstem	0.748 (0.031)	0.766 (0.024)	0.021	**0.140 (0.002; 0.342)**
	Brain lesions	0.547 (0.040)	-	-	-
Spinal cord	**Whole cord**	0.601 (0.048)	0.612 (0.025)	0.232	0.041 (0; 0.216)
	NA cord	0.604 (0.048)	0.612 (0.025)	0.326	0.028 (0; 0.192)
	NA GM cord	0.659 (0.051)	0.677 (0.036)	0.117	0.069 (0; 0.259)
	NA WM cord	0.603 (0.049)	0.604 (0.022)	0.602	0.008 (0; 0.141)
	Cord lesions	0.541 (0.089)	-	-	-
MK, in µm²/ms					
Brain	**NA CGM**	0.628 (0.020)	0.642 (0.015)	0.098	0.074 (0; 0.263)
	NA DGM	0.727 (0.032)	0.741 (0.030)	0.041	0.111 (0; 0.310)
	NA WM	0.891 (0.052)	0.939 (0.023)	**0.007**	**0.185 (0.015; 0.388)**
	Brainstem	0.846 (0.045)	0.875 (0.031)	0.024	0.133 (0.0003; 0.335)
	Brain lesions	0.679 (0.059)	-	-	-
Spinal cord	**Whole cord**	0.705 (0.093)	0.785 (0.066)	**0.003**	**0.229 (0.032; 0.432)**
	NA cord	0.708 (0.095)	0.785 (0.066)	**0.004**	**0.214 (0.025; 0.418)**
	NA GM cord	0.701 (0.119)	0.772 (0.072)	0.025	0.136 (0.00005; 0.340)
	NA WM cord	0.719 (0.100)	0.802 (0.077)	**0.004**	**0.209 (0.023; 0.413)**
	Cord lesions	0.641 (0.151)	-	-	-
RK, in µm²/ms					
Brain	**NA CGM**	0.631 (0.028)	0.649 (0.023)	0.151	0.057 (0; 0.238)
	NA DGM	0.744 (0.040)	0.765 (0.033)	0.017	0.149 (0.004; 0.351)
	NA WM	1.139 (0.087)	1.220 (0.039)	**0.006**	**0.192 (0.018; 0.394)**
	Brainstem	1.055 (0.072)	1.102 (0.048)	0.044	0.108 (0; 0.306)
	Brain lesions	0.835 (0.084)	-	-	-
Spinal cord	**Whole cord**	1.004 (0.206)	1.283 (0.199)	**<0.001**	**0.311 (0.079; 0.503)**
	NA cord	1.011 (0.207)	1.283 (0.199)	**<0.001**	**0.299 (0.071; 0.493)**
	NA GM cord	0.950 (0.210)	1.134 (0.189)	0.010	0.176 (0.011; 0.382)
	NA WM cord	1.066 (0.243)	1.375 (0.233)	**0.001**	**0.285 (0.062; 0.481)**
	Cord lesions	0.883 (0.351)	-	-	-

(New) Table 3 (footnote). This table shows partial effect sizes, defined as the proportion of variability of qMRI metric explained by 'patient status', i.e., by the fact of being a patient or a control. Significant results (at 0.01 significance level) are highlighted in bold. *Abbreviations:* AD: axial diffusivity; AK: axial kurtosis; BPF: bound proton fraction; CGM: cortical grey matter; CGMF: cortical grey matter fraction; CSA: cross-sectional area; DGM: deep grey matter; F: bound pool fraction; FA: fractional anisotropy; GM: grey matter; GMF: grey matter fraction; IVF: isotropic volume fraction; MD: mean diffusivity; MK: mean kurtosis; NA: normal-appearing; NDI: neurite density index; NP: not possible; ODI: orientation dispersion index; qT1: quantitative T1 relaxation time; RD: radial diffusivity; RK: radial kurtosis; WM: white matter.

(New) Table 4. Most relevant volumetric and qMRI measures to clinical outcome according to random forest analyses

Rank (a)	EDSS, *in score points*			Inverse of TWT, *in s⁻¹*			Inverse of 9HPT, *in s⁻¹*			SDMT, *in number of correct answers*		
	Metric	Tissue	RC (95%CI), p-value (b)	Metric	Tissue	RC (95%CI), p-value (b)	Metric	Tissue	RC (95%CI), p-value (b)	Metric	Tissue	RC (95%CI), p-value (b)
1	qT1, in s	Brain NA-DGM	**7.96 (2.52; 13.39), p=0.006**	MK, in µm²/ms	Brain NA-WM	0.45 (0.02; 0.88), p=0.040	RK, in µm²/ms	Whole cord	0.02 (0.01; 0.04), p=0.014	ODI, in dimensionless units	Brain NA-WM	**-847.10 (-1197.52; -496.69), p<0.001**
2	MK, in µm²/ms	Brain NA-WM	**-10.94 (-17.94; -3.93), p=0.004**	RK, in µm²/ms	Brain NA-WM	0.29 (0.03; 0.54), p=0.030	qT1	Brain NA-DGM	**-0.09 (-0.15; -0.03), p=0.004**	MK, in µm²/ms	Brain lesions	**119.86 (54.26; 185.47), p=0.001**
3	qT1, in s	Brain lesions	**4.35 (1.97; 6.72), p=0.001**	ODI, in dimensionless units	Brain NA-WM	**-3.21 (-5.37; -1.05), p=0.005**	RK, in µm²/ms	Brain NA-WM	**0.07 (0.03; 0.12), p=0.003**	AD, in µm²/ms	Brain NA-DGM	**-90.63 x10³ (-130.93 x10³; -50.34 x10³), p<0.001**
4	Volume, in mL	Brain lesions	0.031 (0.01; 0.05), p=0.010	Volume, in mL	Brain lesions	**-0.002 (-0.003; -0.001), p=0.007**	FA	NA cord	**0.08 (0.02; 0.13), p=0.006**	MK, in µm²/ms	Brain NA-CGM	129.06 (-67.89; 326.01), p=0.188 (c)
5	F, in dimensionless units (fraction)	Brain lesions	**-63.64 (-91.88; -35.40), p<0.001**	qT1	Brain NA-WM	**-0.62 (-1.00; -0.23), p=0.003**	F, in dimensionless units (fraction)	Whole cord	**0.79 (0.36; 1.23), p=0.001**	qT1, in s	Brain NA-CGM	**-94.31 (-138.78; -49.84), p<0.001**

(New) Table 4 (footnote). (a): Rank according to random forest regression models; (b): multiple linear regression models using as dependent variable the clinical measure and, as explanatory, the qMRI or volumetric measure. Age, sex, disease duration, and brain lesion load are explored as covariates and only included in the models if p<0.01. Only significant results at 0.01 significance level are highlighted in bold. (c): model adjusted for lesion load (in mL), which was significant: beta=-0.396 (-0.643; -0.150), p=0.003. *Abbreviations:* 9HPT: nine-hole peg test; AD: axial diffusivity; AK: axial kurtosis; BPF: bound proton fraction; CGM: cortical grey matter; CGMF: cortical grey matter fraction; CSA: cross-sectional area; DGM: deep grey matter; EDSS: expanded disability status scale; F: bound pool fraction; FA: fractional anisotropy; GM: grey matter; GMF: grey matter fraction; IVF: isotropic volume fraction; MD: mean diffusivity; MK: mean kurtosis; NA: normal-appearing; NDI: neurite density index; NP: not possible; ODI: orientation dispersion index; qT1: quantitative T1 relaxation time; RC: regression coefficient; RD: radial diffusivity; RK: radial kurtosis; SDMT: symbol digit modalities test; TWT: 25-foot-timed walk test; WM: white matter.