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ORIGINAL COMMUNICATION

Magnetization transfer ratio in lesions rather than normal-appearing brain relates to disability in patients with multiple sclerosis

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Abstract Magnetization transfer ratio (MTR) is a semi-quantitative measure that seems to correlate with the degree of myelin loss and generally tissue destruction in multiple sclerosis (MS). Our objective was to comprehensively assess the MTR of lesions and normal appearing (NA) tissue separately in the white matter (WM), the cortex, the thalamus and the basal ganglia (BG) and determine their relative contribution to disability. In this cross-sectional study 71 patients were included (59 with relapsing–remitting MS, 12 with secondary progressive MS). We used a three-dimensional MTR sequence with high spatial resolution, based on balanced steady-state free precession. Mean MTR was calculated for lesions and NA tissue separately for each tissue type. Lesional MTR was lower than normal-appearing MTR in WM, cortex and thalamus. In the regression analysis, MTR of cortical lesions ($\beta = -0.23$, $p = 0.05$) and MTR of WML ($\beta = -0.21$, $p = 0.08$) were related by trend to the expanded disability status scale. MTR of WML significantly predicted the paced auditory serial-addition test ($\beta = 0.35$, $p = 0.004$). MTR of normal-appearing tissue did not relate to any outcome. Our results suggest that MTR of lesions in the white matter and cortex rather than of normal-appearing tissue relates to disability in patients with MS.

Keywords Magnetization transfer imaging · Multiple sclerosis

Abbreviations

bSSFP	Balanced steady-state free precession
CL	Cortical lesions
EDSS	Expanded disability status scale
GM	Gray matter
MS	Multiple sclerosis
MTR	Magnetization transfer ratio
NABG	Normal-appearing basal ganglia
NACGM	Normal-appearing cortical gray matter
NAWM	Normal-appearing white matter
PASAT	Paced auditory serial-addition task
WM	White matter
WML	White matter lesions
9HPT	9-hole peg test
25 FTW	25-foot timed walk

Introduction

Magnetization transfer ratio (MTR) is a semi-quantitative measure that seems to correlate well with the degree of myelin loss and generally tissue destruction within lesions

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in multiple sclerosis (MS) [1, 2]. MTR of white matter lesions (WML) correlates with disability in patients with MS, similarly to or even stronger than T2-lesion load [3, 4]. MTR can be also used to assess tissue damage in the whole brain [5, 6], in normal-appearing white matter (NAWM) [7] and in the gray matter (GM) [8–11].

The standard method to measure the magnetization transfer (MT) effect is to scan the respective tissue two times, with and without a specific MT saturation pulse. However, this technique has a long acquisition time, due to the application of the saturation pulses [12, 13]. A faster concept for MT imaging is based on the balanced steady-state free precession (bSSFP) and uses a modification of the duration of the applied radiofrequency pulses to acquire MT-sensitive and non-sensitive images [14, 15]. Thus, this technique allows the acquisition of three-dimensional (3D) MTR maps with high spatial resolution in short acquisition times, minimizing partial volume effects especially in the cortex and small brain structures.

Most previous studies used standard two-dimensional MTR techniques and studied the MTR of the entire GM, without differentiation between cortical and deep gray matter (DGM) [7–9, 16]. Moreover, there is very limited knowledge regarding in vivo MTR changes in GM lesions (GML) compared to NAGM, because of the low sensitivity of conventional magnetic resonance imaging (MRI) sequences for their detection. Thus, there is a need for comprehensive data with assessment of MTR in GML and WML as well as normal-appearing cortex, deep GM and white matter in patients with MS.

We aimed at calculating the MTR of lesions and NA tissue separately in the white matter, cortex and DGM (thalamus and basal ganglia) of patients with MS, using MT-sensitized bSSFP. Our main objective was to study the contribution of lesional and NA-MTR in white matter, cortex and deep gray matter to physical disability and cognitive impairment.

Materials and methods

Patients

A cohort of 71 MS patients was recruited in the Department of Neurology of the University Hospital in Basel over 12 months. All patients were participants in an ongoing study on the phenotype-genotype characterisation of MS [17]. They were treated with disease-modifying immunomodulatory treatments at the discretion of the treating physician. The patients were clinically stable. Patients with an acute relapse were not examined and the MRI scan was postponed at least 30 days after the last dose of steroid treatment. Informed consent was obtained in writing from all participating patients, in accordance with the local ethics committee approval and the declaration of Helsinki.

Most patients were women (50/71). Mean age was 47.9 years (range 23–70 years) and mean disease duration was 17.1 years (range 4–50 years). Fifty-nine patients had a relapsing–remitting (RRMS) and 12 had a secondary progressive disease course (SPMS). The median EDSS was 3.0 (range 0–7.5). The clinical characteristics of the patients are summarized in Table 1.

Image acquisition

All patients underwent a comprehensive MRI examination on a 1.5 T MR system (Magnetom Avanto, Siemens Medical Solutions, Germany) including a double-echo proton density/T2-weighted sequence [repetition time (TR) 3980 ms, echo time (TE) TE1/TE2 = 14 ms/108 ms; spatial resolution: $0.98 \times 0.98 \times 3$ mm^3) and a series of dedicated 3D MR sequences with high spatial resolution in all patients: a T1 weighted (T1w) MPRAGE (TR 2080 ms, inversion time (TI) 1100 ms, TE 3.93 ms, flip angle (α) 15°; spatial resolution: $1 \times 1 \times 1$ mm^3) was acquired for the purpose of tissue segmentation, and double inversion recovery (DIR) images (TR 7500 ms, TI 3000 ms, TE 307 ms, spatial resolution: $1.3 \times 1.3 \times 1.5$ mm^3) were acquired for better delineation of MS lesions, especially cortical lesions [18]. All 3D sequences were acquired in sagittal orientation parallel to the inter-hemispheric fissure.

The 3D MT-sensitized bSSFP sequence had a spatial resolution of $1.3 \times 1.3 \times 1.3$ mm^3 (TE/α = 1.19 ms/45°; ipat = 2). The radiofrequency pulse duration (TRF) of the MT-sensitized acquisition was 0.12 ms (TR = 2.77 ms);

Table 1 Clinical characteristics of the patients included in this study

Patient characteristics	$N = 71$
Age (years)[a]	47.9 (23–70)
Gender (female/male)	50/21
Disease duration (years)[a]	17.1 (4–50)
Disease course (RRMS/SPMS)	59/12
EDSS[b]	3.0 (0–7.5)
9HPT (s)[a]	20.1 (15.6–168.5)
25FTW (s)[a]	7.4 (2.2–36.4)
PASAT[a]	44.8 (11–60)

There was one patient with missing values for 9HPT ($n = 70$), three patients with missing values for 25FTW ($n = 68$) and one patient with missing values for the PASAT ($n = 70$)

RRMS relapsing-remitting multiple sclerosis, *SPMS* secondary progressive multiple sclerosis, *EDSS* expanded disability status scale, *25FTW* 25-foot timed walk, *9HPT* 9-hole peg test, *PASAT* paced auditory serial-addition task

[a] Values are represented as mean (range)

[b] Values are represented as median (range)

TRF of the non-MT-sensitized acquisition was 2 ms (TR = 4.65 ms). Scan time for the bSSFP MTR scans (two acquisitions) was 4:14 min. The entire scanning protocol lasted 26 min.

Image analysis

Post-processing of the MRI data was performed using AFNI (Analysis of Functional NeuroImages, University of Wisconsin, USA) [19] and FSL (FMRIB Software Library, University of Oxford, UK) [20]. In a first step, the MT-sensitized data sets were realigned to the non-MT-sensitized data sets and MTR maps were calculated according to the equation:

$$\mathrm{MTR} = \frac{S_0 - S_{\mathrm{MT}}}{S_0}$$

with S_0 = signal of the non-MT-sensitized image and S_{MT} = signal of the MT-sensitized image.

The 3D T1w volumes were segmented into white matter, cortical gray matter and cerebrospinal fluid (CSF) with FAST [21]. The segmentation of the DGM was performed using FIRST [22]. The segmentation masks of the DGM structures were visually inspected and falsely classified voxels were corrected manually by an experienced rater (MA). Lesions were marked and segmented using the semi-automatic thresholding contour software AMIRA 3.1.1 (Mercury Computer Systems Inc). All lesions were outlined on the DIR images and classified as cortical lesions (CL), deep grey matter lesions (DGML) and white matter lesions (WML).

In more detail, the 3D DIR images were viewed in a multiplanar way by two experienced raters (AP, NML) and the lesions were marked in consensus. For the scoring of CL, the raters followed the criteria proposed by Geurts et al. [23]. Thus, CL had to occupy at least three voxels and involved both strictly intracortical and mixed GM-WM lesions.

The original 3D data sets were interpolated to the same spatial resolution as the MT images and were aligned to the MTR maps. Then, all segmentation masks were co-registered using the transformation parameters of the 3D data sets. For the assessment of MTR in NA tissues, the lesion masks were subtracted from the FAST and FIRST segmentation to create masks for NAWM, NACGM, NA thalamus (NA Th) and NA basal ganglia (NABG: the combination of putamen, pallidum, caudate nucleus, and nucleus accumbens). For each patient, mean MTR of these NA tissues were calculated by fitting a Gaussian distribution onto the central part of the respective MTR histogram. The position of the fitted peak was considered as mean MTR. This approach is less susceptible to effects of minor registration and segmentation errors than calculating the arithmetic mean. For the lesion masks, also a Gaussian distribution fit was performed, except if the respective MTR histogram showed a non-normal distribution, when the arithmetic mean of the MTR was calculated.

Clinical data

On the day of the MRI examination, all patients underwent a comprehensive clinical assessment including a standardized neurological examination by certified physicians (Neurostatus) for the calculation of the expanded disability status scale (EDSS) score. Additionally, three tests of motor and cognitive abilities were performed: the 25-foot timed walk (25FTW), the 9-hole peg test (9HPT) and the paced auditory serial-addition task (PASAT), for the assessment of leg function/ambulation, arm/hand function, and cognitive function, respectively [24].

Statistical analysis

All statistical analyses were performed using IBM SPSS Statistics Version 20 (IBM Corp., Armonk, NY). Differences between the MTR of normal-appearing (NA) tissue in the various brain compartments [white matter (NAWM), cortex (NACGM), thalamus (NA Th) and basal ganglia (NABG)] were analyzed by performing a one-way analysis of variance and Tukey's post hoc test corrected for multiple comparisons. Additionally, paired t tests were calculated between the lesional MTR and NA-MTR in all these brain compartments.

Univariate correlations between MTR measures and clinical outcomes were calculated by using the Spearman's rank correlation (p values Bonferroni corrected).

Moreover, four hierarchical multiple linear regression analyses were performed (one for each dependent variable: EDSS, 25FTW, 9HPT and PASAT) with the first block encompassing clinical/demographic factors (age, gender and disease duration) and the second block including the MTR variables (MTR of white matter lesions, MTR of cortical lesions, NAWM-MTR, NACGM-MTR, NATh-MTR and NABG-MTR) as well as total T2-lesion volume. MTR of thalamic lesions and MTR of BG lesions were not included in these analyses because of the small number of patients having ≥ 1 such lesion. Regarding 9HPT and 25FTW, the reciprocals (1/9HPT and 1/25FTW) were used in the statistical analysis, since they were closer to a normal distribution.

Results

Descriptive results of MTR in lesions and NA tissue

The MTR values of normal-appearing tissue and lesions are summarized in Table 2.

Table 2 Magnetization transfer ratio (MTR) values for different normal-appearing (NA) brain structures and different lesion types

	Normal appearing white matter	Normal appearing cortex	Normal appearing thalamus	Normal appearing basal ganglia	White matter lesions	Cortical lesions	Thalamic lesions	Basal ganglia lesions
Mean MTR (%)	52.90	47.43	50.61	44.87	45.52	43.93	45.92	44.79
SD	0.82	1.11	0.81	0.97	2.21	1.76	1.68	3.45

As expected due to its high myelin context, NAWM had the highest MTR values (mean 52.9 %) and differed significantly (Tukey's post hoc test, corrected $p < 0.05$) from all other structures. The MTR values of the NA thalamus (mean 50.6 %) were also significantly different from all other structures (corrected $p < 0.05$), and were closer to the MTR of NAWM than that of NACGM (mean 47.4 %).

Fifty-two patients had CL, 19 patients had thalamic lesions and 15 patients had lesions in the BG. Lesional MTR was lower than MTR of the corresponding NA tissue in the white matter (MTR of WML 45.5 % vs. MTR of NAWM 52.9 %, $p < 0.001$), the cortex (MTR of CL 43.9 % vs. MTR of NACGM 47.4 %, $p < 0.002$) and the thalamus (MTR of thalamic lesions 45.9 % vs. MTR of NA Th 50.6 %, $p < 0.001$) but not in the basal ganglia (MTR BG lesions 44.8 % vs. MTR NABG 44.9 %, $p = 0.9$).

Univariate correlations between the MTR metrics and clinical measures

We found several univariate correlations between the demographic factors and the clinical measures [age: with EDSS, 25FTW, 9HPT and PASAT, gender: with 9HPT (male gender associated with worse performance), disease duration: with EDSS, 25FTW and PASAT], as well as univariate correlations of NAWM-MTR with all clinical measures (EDSS, 25FTW, 9HPT and PASAT). The other MTR parameters did not show any significant univariate correlations with the clinical measures after Bonferroni correction. T2 lesion volume showed a correlation with EDSS and 9HPT. All significant univariate correlations are shown in the Tables 3, 4, 5 and 6.

Multiple linear regression analyses

The regression analysis revealed disease duration and gender as significant predictors of the EDSS (male gender being associated with higher EDSS). The only MRI parameters which were associated by trend to the EDSS were MTR of cortical lesions and MTR of white matter lesions (Table 3). Despite its univariate correlation with the EDSS, NAWM-MTR was not an independent predictor of the EDSS in the regression analysis. MTR parameters in NA cortical and deep GM were also not related to the EDSS.

No MTR-parameter predicted 25FTW (Table 4) or 9HPT (Table 5) in the regression analyses. However, MTR of white matter lesions was a significant predictor of PASAT, next to age, disease duration and T2 lesion volume (Table 6). MTR of CL, NAWM and NACGM did not show significant associations with PASAT in this regression analysis.

Discussion

In this study, we comprehensively examined the clinical associations of MTR of lesions and normal-appearing tissue in the white matter, cortex and deep gray matter in patients with relapse-onset MS (RRMS and SPMS). Our main finding was an association of disability measures with lesional MTR (MTR of cortical lesions and MTR of white matter lesions predicted EDSS by trend, MTR of WML predicted PASAT in the regression analyses), but not with MTR of normal-appearing tissue (cortex, thalamus, basal ganglia, white matter). Of interest, MTR of NAWM showed univariate correlations with all measures of disability (EDSS, 25FTW, 9HPT, PASAT), but was not a significant predictor of any clinical measure in the multiple regression analyses when also the other demographic and MRI variables were included. This indicates that the subtle pathological changes in the NAWM, as measured with MTR may not influence disability independently of other factors, such as tissue destruction in lesions, as assessed by lesional MTR and T2-lesion volume. This is in line with the findings of Vrenken et al., who suggested that MTR changes in NAWM are a secondary result of lesional damage, since they depend on the proximity to white matter lesions [25].

The results of the regression analyses also indicate that MTR changes in NAWM relate to demographic/clinical factors, such as age, disease duration and gender. Indeed, associations between MTR in NA brain tissue and such factors have been reported before: A correlation between whole-brain MTR and NAGM-MTR with age [26, 27] and

Table 3 Associations of MTR measures, T2-lesion volume and demographical characteristics with the EDSS

EDSS		
Parameter	Univariate correlation	Multiple linear regression analysis ($R^2 = 0.311$)
Age	$r = 0.488$	$p =$ ns
	$p < 0.001$	$p =$ ns
Gender	$p =$ ns	$\beta = -0.412$
	$p =$ ns	$p = 0.001$
Disease duration	$r = 0.381$	$\beta = 0.478$
	$p = 0.01$	$p < 0.001$
T2 lesion volume	$r = 0.336$	$p =$ ns
	$p = 0.04$	$p =$ ns
MTR of normal appearing white matter	$r = -0.395$	$p =$ ns
	$p = 0.006$	$p =$ ns
MTR of white matter lesions	$p =$ ns	$\beta = -0.206$
	$p =$ ns	$p = 0.084$
MTR of cortical lesions	$p =$ ns	$\beta = -0.234$
	$p =$ ns	$p = 0.05$

The p values of the univariate correlations are Bonferroni corrected. The other variables (MTR of normal-appearing cortical gray matter, MTR of normal-appearing thalamus and MTR of normal-appearing basal ganglia) did not show any associations with the EDSS

MTR magnetization transfer ratio

Table 4 Associations of MTR measures, T2-lesion volume and demographical characteristics with the 25-foot timed walk

1/25FTW		
Parameter	Univariate correlation	Multiple linear regression analysis ($R^2 = 0.319$)
Age	$r = -0.509$	$p =$ ns
	$p < 0.001$	$p =$ ns
Disease duration	$r = -0.394$	$\beta = -0.392$
	$p = 0.009$	$p = 0.002$
T2 lesion volume	$p =$ ns	$\beta = -0.385$
	$p =$ ns	$p = 0.003$
MTR of normal appearing white matter	$r = 0.451$	$p =$ ns
	$p = 0.001$	$p =$ ns

The reciprocal values of 25-foot timed walk (1/25FTW) were used, as they were closer to a normal distribution than the values of the 25FTW themselves. The p values of the univariate correlations are Bonferroni corrected

The other variables (gender, MTR of normal-appearing cortical gray matter, MTR of normal-appearing thalamus, MTR of normal-appearing basal ganglia, MTR of white matter lesions and MTR of cortical lesions) did not show any associations to the 25FTW

MTR magnetization transfer ratio

a correlation of whole-brain MTR with gender (higher in males) [27] was shown in normative datasets, while a correlation of MTR in NAWM with disease duration was shown in patients with MS [28]. Thus, results exclusively relying on univariate correlations, or the exclusion of demographic/clinical characteristics from multiple regression analyses may overemphasize the impact of MTR in NA tissue on disability in MS.

The presence of cortical lesions in MS and their impact on disability has been increasingly studied in recent years [29]. However, there is limited knowledge regarding in vivo MTR changes in CL compared to normal-appearing cortex, mainly due to technical limitations, such as the low sensitivity of conventional MRI sequences for detection of CL and the use of standard MT sequences, with relatively low spatial resolution. Previous studies assessing MTR in the GM and excluding lesions on conventional MRI sequences [26, 30, 31] probably included a considerable amount of cortical lesions in the normal-appearing GM. In our study, we combined a 3D DIR sequence, which is

Table 5 Associations of MTR measures, T2-lesion volume and demographical characteristics with the 9-hole peg test

1/9HPT		
Parameter	Univariate correlation	Multiple linear regression analysis ($R^2 = 0.371$)
Age	$r = -0.468$	$\beta = -0.254$
	$p < 0.001$	$p = 0.035$
Gender	$r = 0.358$	$\beta = 0.386$
	$p = 0.024$	$p = 0.002$
T2 lesion volume	$r = -0.434$	$\beta = -0.317$
	$p = 0.002$	$p = 0.012$
MTR of normal appearing white matter	$r = 0.437$	$p =$ ns
	$p = 0.002$	$p =$ ns

The reciprocal values of the 9-hole peg test (1/9HPT) were used as they were closer to a normal distribution than the values of the 9HPT themselves. The p values of the univariate correlations are Bonferroni corrected

The other variables (disease duration, MTR of normal-appearing cortical gray matter, MTR of normal-appearing thalamus, MTR of normal-appearing basal ganglia, MTR of white matter lesions, MTR of cortical lesions) did not show any associations to the 9HPT

MTR magnetization transfer ratio

Table 6 Associations of demographical characteristics, T2-lesion volume and MTR measures with the PASAT

PASAT		
Parameter	Univariate correlation	Multiple linear regression analysis ($R^2 = 0.339$
Age	$r = -0.448$	$\beta = -0.436$
	$p = 0.001$	$p = 0.001$
Disease duration	$r = -0.400$	$\beta = -0.291$
	$p = 0.007$	$p = 0.040$
T2 lesion volume	$p =$ ns	$\beta = -0.257$
	$p =$ ns	$p = 0.036$
MTR of normal appearing white matter	$r = 0.404$	$p =$ ns
	$p = 0.005$	$p =$ ns
MTR of white matter lesions	$p =$ ns	$\beta = 0.352$
	$p =$ ns	$p = 0.004$

The p values of the univariate correlations are Bonferroni corrected. The other variables (gender, MTR of normal-appearing cortical gray matter, MTR of normal-appearing thalamus, MTR of normal-appearing basal ganglia, MTR of cortical lesions) did not show any significant associations with the PASAT

PASAT paced auditory serial-addition task, *MTR* magnetization transfer ratio

clearly superior to conventional sequences for the detection of cortical lesions [18] with a 3D bSSFP MT sequence, which has a high spatial resolution, and thus less partial volume effects in the cortex. This allowed studying the MTR of CL separately from NA cortical gray matter. We found that MTR of CL predicted EDSS by trend (borderline significance, $p = 0.05$), in contrast to MTR of NACGM. Hence, previously reported correlations between MTR of the entire GM [8, 10, 32] or of the NAGM [26, 31] with the EDSS may be driven, at least in part, by the presence of cortical lesions. It needs of course to be emphasized that even the 3D DIR sequence visualizes only the "tip of the iceberg" [33] of the total amount of CL and still has a low sensitivity for subpial demyelinating lesions. Hence, even with this improved methodology, the normal-appearing cortex still likely contained lesional tissue in our study [33]. Nevertheless, we believe that our findings emphasize the impact of focal tissue damage in the cortex on disability in MS.

Similar to these findings regarding the cortex, MTR of WM lesions, but not of NAWM showed associations with measures of disability in the regression analyses (MTR of WML predicted PASAT and EDSS, the latter by trend only). The relative impact of WML vs. changes in NAWM on disability in MS is not fully understood. In contrast to our findings, Santos et al. [7] have reported a stronger

correlation of EDSS changes over five years with MTR values in NAWM ($r = -0.76$, $p < 0.001$) than with MTR in WM lesions ($r = -0.43$, $p = 0.07$). Another study found an association by trend of NAWM-MTR (but not of lesional MTR) with EDSS changes after 18 months [8]. Interestingly, MTR changes in lesions and NAWM may somewhat differ in their pathologic substrates. Although reduced MTR in lesions is clearly associated with myelin and axonal loss, reduced MTR in NAWM in areas remote from lesions seems to rather relate to microglia activation, at least in SPMS [34]. Larger studies, using a combination of quantitative MR measures may further elucidate the associations of focal and more diffuse changes with disability in various MS subtypes.

Regarding cognitive outcomes, Kalkers et al. [35] reported a correlation between whole-brain MTR and PASAT in patients with relapse-onset MS. Whether such correlations are mainly driven by the presence of WM lesions, as our findings suggest, needs to be confirmed.

In this study, the MTR of the deep gray matter structures (thalamus, basal ganglia) did not have an impact on clinical disability. Data from previous studies regarding MTR changes in DGM are very limited and inconsistent. Two studies [36, 37] did not find any significant differences between MS patients and controls regarding MTR of the basal ganglia and thalamus, whereas Davies et al. [38] reported that thalamic MTR was significantly lower in MS patients than in controls and correlated with EDSS. Future work will have to clarify whether quantitative MT measures may be more sensitive to subtle pathological changes in DGM structures than conventional MTR.

This study has several limitations. First, since we focused on the clinical correlations between MTR measures and clinical disability, we did not include a control group. Thus, we cannot say if the MTR values of our MS patients are lower than those of normal individuals, although there is clear evidence in the scientific literature suggesting that in general this is the case [39]. The limited sensitivity of DIR in detecting subpial cortical lesions has been discussed above. Moreover, we were not able to assess the temporal relationship between MTR values and disability accrual, because of the cross-sectional character of the study.

The use of the 3D DIR sequence for lesion segmentation could be considered a limitation of this study, since the DIR sequence is not the "gold standard" for white matter lesion detection and segmentation. However, several previous studies have shown that the detection rates of MS lesions on DIR are very similar to FLAIR and similar to or better than T2-weighted images in other brain compartments than the cortex, including the white matter and the deep gray matter [18, 40–43].

Most patients in our study had relatively low grades of disability (median EDSS = 3.0), and thus the relation of some MTR measures to disability could be underestimated. Finally, due to the small number of patients with SPMS ($n = 12$), we did not perform a SPMS subgroup analysis. Very recent work suggests that cortical MTR, especially in the outer layers, may be lower in SPMS than in RRMS [44]. Future studies with high-resolution MTR are needed to explore potential differences among the different clinical subgroups regarding MTR measures in lesions and normal-appearing tissue and their impact on disability.

The number of patients we included is not very large, but still higher than most previous studies assessing MTR in MS [4, 5, 7–10, 16, 26, 30]. Moreover, the use of a 3D MTR sequence with a high and isotropic spatial resolution is an important methodological advantage of this study. The bSSFP MTR sequence has a short acquisition time [14, 15], and is, therefore, less prone to movement artifacts. Moreover, the acquisition of 3D MTR maps and their combination with other 3D images, such as T1w MPRAGE and DIR images was particularly useful for the accurate calculation of MTR in the cortex and deep gray matter, minimizing CSF partial volume effects.

In conclusion, our findings suggest a limited contribution of MTR measures to outcomes of physical disability and cognitive impairment in patients with relapse-onset MS, mainly driven by lesional MTR rather than MTR of normal-appearing brain structures.

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