

Restriction spectrum imaging of white matter and its relation to neurological disability in multiple sclerosis

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ABSTRACT

Background: Restriction spectrum imaging (RSI) is a recently introduced magnetic resonance imaging diffusion technique. The utility of RSI in multiple sclerosis (MS) is unknown.

Objective: To investigate the association between RSI-derived parameters and neurological disability in MS.

Methods: Seventy-seven relapsing-remitting MS patients were scanned with RSI on a 3-Tesla scanner. RSI-derived parameters: fast and slow apparent diffusion coefficient (sADC), fractional anisotropy, restricted fractional anisotropy, neurite density (ND), cellularity, extracellular water fraction and free water fraction were obtained in white matter lesions (WML) and normal appearing white matter (NAWM). Patients were divided into three groups according to their expanded disability status scale (EDSS): with minimal, low and substantial disability (<2.5, 2.5–3 and >3, respectively). Group comparisons and correlation analyses were performed.

Results: All tested RSI-derived parameters differed between WML and NAWM ($p < 0.001$ for all pairwise comparisons). The sADC in WML showed largest difference across disability subgroups (ANOVA: $F = 5.1$, $\eta^2 = 0.12$, $p = 0.008$). ND in NAWM showed strongest correlation with disability ($\rho = -0.39$, $p < 0.001$).

Conclusion: The strongest correlation with EDSS of ND obtained in NAWM indicates that processes outside lesions are important for disability in MS. Our study suggests that RSI-derived parameters may help understand the “clinico-radiological paradox” and improve disease monitoring in MS.

Keywords

Magnetic resonance imaging, Multiple sclerosis, Restriction spectrum imaging, Neurite density, Neurological disability

Abbreviations

ADC = apparent diffusion coefficient; DTI = diffusion tensor imaging; EDSS = expanded disability status scale; DWI = diffusion weighted imaging; EWF = extracellular water fraction; FA = fractional anisotropy; fADC = fast apparent diffusion coefficient; FWF = free water fraction; GM = grey matter; HARDI = high-angular resolution diffusion imaging; MSSS = multiple sclerosis severity score; NAWM = normal appearing white matter; ND = neurite density; NODDI = neurite orientation dispersion and density imaging; rFA = restricted fractional anisotropy; RR = relapsing-remitting; RSI = restriction spectrum imaging; sADC = slow apparent diffusion coefficient; WM = white matter; WML = white matter lesions

1. Introduction

Multiple sclerosis (MS) is a chronic inflammatory disease of the central nervous system primarily affecting young adults and often resulting in severe neurological disability ¹. Even though magnetic resonance imaging (MRI) has become an important tool in the diagnostics and follow-up of MS patients ^{2, 3}, there is a need for new imaging markers to improve the diagnostic and therapeutic precision ². Diffusion MRI is a promising technique in MS. The technique is based on measuring the random Brownian motions of water molecules within tissue. Standard diffusion weighted imaging (DWI) provides the apparent diffusion coefficient (ADC), a measure that helps in early detection of ischemic regions in stroke and can differentiate between various pathological conditions ⁴. More advanced techniques have enabled calculation of ADC for the fast and slow diffusion components: fast ADC (fADC) and slow ADC (sADC), in theory corresponding to the extracellular and intracellular water compartments ⁵. Diffusion tensor imaging (DTI) is a multidirectional diffusion MRI ⁶ that provides fractional anisotropy (FA) which is a scalar value between zero and one, where value of zero means equal diffusion in all directions and value of one means diffusion restricted to one direction only.

In MS, the diffusion MRI has been used to investigate differences between various types of lesions ⁷ or lesions and normal appearing white matter ⁸, between MS patients and healthy controls ^{9, 10}, as well as to study diffusion parameters in relation to clinical measures ¹⁰⁻¹². However, the results of DWI and DTI studies in relation to neurological disability in MS are not consistent. Temel et al. ¹¹ reported no correlation between FA or ADC in lesions or NAWM and neurological disability as measured by the expanded disability status scale (EDSS) ¹³, which was in line with a previous report from 2006 by Phuttharak et al. ¹⁴. Yet

Gratsias et al.¹⁰ and Anik et al.¹⁵ reported a significant correlation between ADC in NAWM and EDSS scores in MS patients. The inconsistent findings can be partly explained by different methodology and different definitions of NAWM across the studies.

The non-specific nature of ADC and known limitations of DTI in describing diffusion in nonhomogenous media¹⁶ have stimulated development of more advanced diffusion techniques like high-angular resolution diffusion imaging (HARDI) and neurite orientation dispersion and density imaging (NODDI)¹⁷. Restriction spectrum imaging (RSI) is a recently introduced MRI technique that is based on measuring water diffusion probed with multiple b-values and various directions. RSI is a straightforward extension of HARDI¹⁸, it enables more specific estimation of tissue microstructure compared to DWI and DTI¹⁹ and has shown promising results in neuroradiology attempting to improve tumor delineation²⁰, recover white matter (WM) tracts in peritumoral regions²¹ and better reflect WM pathology in temporal lobe epilepsy²² as well as in oncologic imaging¹⁹. In addition to the above-mentioned DWI and DTI diffusion parameters, RSI also enables calculation of restricted FA (rFA), neurite density (ND), cellularity, extracellular water fraction (EWF) and free water fraction (FWF), described in more details below. These parameters provide additional information on brain tissue that may be of clinical importance in MS.

The utility of RSI in MS has not been investigated. The purpose of this study was to explore the RSI-derived diffusion parameters in white matter lesions (WML) and NAWM, and to evaluate their association with neurological disability in MS.

2. Materials and methods

2.1 Participants

Seventy-seven relapsing-remitting (RR) MS patients, diagnosed according to the current diagnostic criteria²³ were included. The patients were recruited and referred to MRI by treating neurologists at our institution in the period 2013 – 2014. Mean age of the patients was 39.9 ± 10.3 years (range 20 – 67), whereof 60 females (mean age 39.1 ± 10.4 years, range 20 – 64) and 17 males (mean age 42.6 ± 9.9 years, range 27 – 67). The patients were included at different clinical stages (described in detail in the Results paragraph). The inclusion criteria were: patient age older than 18 years, no prior neurological disease, no contraindication for MRI and no allergy to gadolinium-based contrast media. Since the study was performed in an ambulatory setting we excluded patients completely restricted to bed or wheelchair and unable to move themselves onto the scanner table. Of 94 patients that met the inclusion criteria and were scanned with the RSI sequence, 17 were excluded, mainly due to technical reasons related to image postprocessing. The flow chart for patient inclusion is shown in Supplementary material 1.

2.2 Clinical data

The following clinical and laboratory data were collected from the patients' electronic hospital record: age at disease onset, disease duration, disease subtype, neurological disability assessed with EDSS, and type of disease modifying treatment (shown in Supplementary material 2). EDSS from the date closest to the MRI acquisition was collected and MS severity score (MSSS) ²⁴ was determined. Details concerning demographical, clinical and laboratory data of the patient cohort are shown in Table 1.

Table 1 Demographic and clinical characteristics^a, n=77

Age, years	39.9 ± 10.3, range 20 – 67
Females	60 (78%)
Age at disease onset, years	27 (25 – 35), range 16 – 53
Disease duration, years	9 (4 – 13.5), range 1 – 32
Relapsing-remitting phenotype	77 (100%)
EDSS	2.0 (1 – 2.75), range 0 – 6.5
MSSS	2.34 (0.99 – 4.25), range 0.05 – 8.91
Disease modifying treatment ^b (patients n=)	
no treatment	24 (31%)
first line	22 (29%)
second line	30 (39%)
third line	1 (1%)
Oligoclonal bands in CSF (patients n=)	
yes	70 (91%)
no	6 (8%)
unknown	1 (1%)

CSF: cerebrospinal fluid; EDSS: expanded disability status scale; MSSS: multiple sclerosis severity score.

^aData are n (%) for nominal variables, mean ± standard deviation for normally distributed variables, or median (interquartile range) for non-normally distributed variables.

^bDisease modifying treatment is explained in details in Supplementary material 2

2.3 Clinical subgroups

The patients were divided into clinical subgroups based on their neurological disability as measured by EDSS score: group 1 (n=28) had minimal disability and EDSS<2.5; group 2 (n=41) had low disability and EDSS of 2.5–3 and group 3 (n=11) had moderate to substantial disability and EDSS>3 (hereafter referred to as “substantial disability”). Since the patients in general had relatively low disability as reflected in a median EDSS of 2.0 (interquartile range 1 – 2.75, range 0 – 6.5), we used an EDSS threshold of 2.0 and 3.0 to divide the patients into three groups with different disability levels in the analysis. The choice of cut-off of 3.0 resulted in a larger group with higher disability (n=11).

2.4 Image acquisition

The RSI sequence used in our study was available through an inter-institutional collaboration. All MRI scans were acquired on a 3 Tesla scanner (Signa Optima HDxt, General Electric, Fairfield CT, USA). Sixty-nine patients were scanned using an 8-channel head coil and eight patients using a 12-channel head coil, due to different availability of these coils to us during the period of data collection at our site. The distribution of clinical parameters (age, age at

disease onset, disease duration, EDSS and MSSS) was not significantly different between the two groups and all patients were included in the final analysis. The imaging protocol included the following sequences:

(a) Sagittal 3D T1-weighted FSPGR (TE=3-12 ms; TR=7.8 ms; TI=450 ms; FA=12°; FOV=25.6 cm; matrix=256 x 192 mm; slice thickness=1.2 mm);

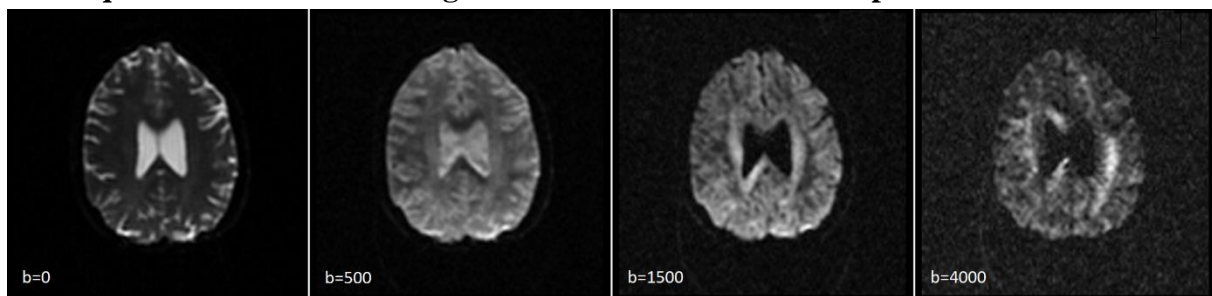
(b) Sagittal 3D T2-weighted FLAIR CUBE (TE/TR=126.5/6000 ms; TI=1861 ms; FOV=25.6 cm, matrix=256 x 256 mm, slice thickness=1 mm);

(c) Axial single-shot spin-echo diffusion-weighted echo-planar multi-shell RSI sequence (TE=96-289 ms; TR=17 s; FA=90°; FOV=24 cm; matrix=96 x 96 mm; slice thickness=2.5 mm, acquired with $b=0, 500, 1500$ and 4000 s/mm² with 6, 6 and 15 unique gradient directions for each nonzero b-value, respectively); Figure 1 shows RSI sequence as raw images acquired with the different b-values in a sample patient;

(d) Post-gadolinium sagittal 3D T1-weighted sequence, with parameters identical to those of pre-gadolinium 3D T1, acquired approximately 5 minutes after i.v. contrast agent injection at a dose of 0.2 ml/kg (Dotarem, Laboratoire Guerbet, Paris, France).

Figure 1

RSI sequence shown as raw images with different b-values in a patient



From the left: images acquired with b-values of 0, 500, 1500 and 4000 s/mm² with 6, 6 and 15 unique gradient directions for each nonzero b-value, respectively. The raw RSI data were used to obtain the RSI parameter maps. RSI: restriction spectrum imaging.

2.5 Image analysis

(a) The preprocessing, RSI processing and co-registration of image data were performed using in-house software developed in Matlab (Matlab Works, Natick MA, USA). The RSI diffusion data were corrected offline for spatial distortions, postprocessed in native space and then the derived RSI images were resampled and co-registered to the structural series (also corrected for distortions and registered to each other). The image data from each participant were visually inspected for quality control. FA was calculated from all b-values: $b=0, 500, 1500$ and 4000 s/mm², fADC was calculated from $b=500$ data and sADC from the $b=4000$ data (the RSI-derived fADC and sADC are not equal to DTI-derived ADC which is usually calculated from an intermediate b-value). The rFA was calculated from a tensor fit to the restricted water signal derived from the RSI model, with optimal sensitivity to cylindrically restricted diffusion. Also ND, cellularity, EWF and FWF were calculated. RSI processing is described in details in a method paper with theoretical and histological validation of the RSI sequence

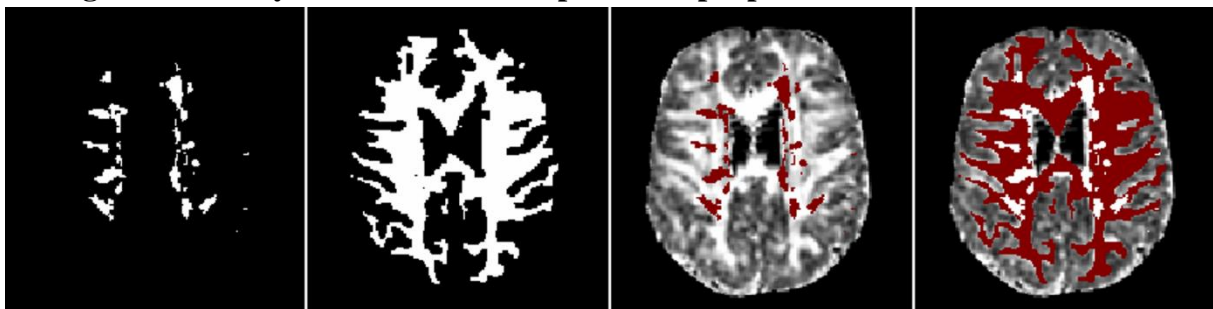
(b) Semi-automated WML segmentation was performed by two radiologists in all 80 subjects using in-house software and MIPAV software (v.7.2.0, Center for Information Technology, National Institutes of Health, Bethesda MD, USA). The first author performed final visual inspection of the WML masks in each patient using MIPAV software. The 3D FLAIR series was used for the WML segmentation. As a result, a WML mask representing all lesions was created for each patient.

(c) Freesurfer software (www.freesurfer.net) was used to segment the 3D T1 series to create masks of WM and grey matter, and to obtain volumetric parameters. The masks were visually inspected and corrected for segmentation errors using the same software. WM volume was calculated using a “lesion filling” approach in nordicICE software (www.nordicneurolab.com) – first the NAWM volume was calculated (using WM mask as inclusion mask and WML mask as exclusion mask) and then the volumes of NAWM and WML were summarized. Nordic Ice was chosen for this purpose because the software was available and well known to us and it was previously used for imaging post-processing in MS ^{25, 26}.

(d) The diffusion parameters were extracted from the whole volume of WML and from the NAWM. For each patient we calculated fADC, sADC, FA, rFA, ND, cellularity, EWF and FWF mean values in WML and in NAWM. Figure 2 shows schematically WML and WM masks, and the rFA map with and without mask overlays in a sample patient. The parameters are described in detail in Supplementary material 3. Briefly, the fADC is more sensitive to changes in extracellular diffusion compared with sADC, but they should not be simply ascribed to specific microcompartments as they are not based on the RSI model. The rFA is the FA derived from the restricted signal from cylindrical structures. ND is the signal fraction of cylindrically restricted water, reflecting the density of neurites in tissue. The EWF represents the signal fraction of water that is hindered due to tortuous geometry of the extracellular space while the FWF is the signal fraction of freely diffusing water.

Figure 2

Co-registered binary masks and rFA map in a sample patient



Here we see from the left: WML mask, WM mask, a sample map (here shown rFA map) with overlaid WML mask (brown) and the same rFA map with overlaid WML and WM masks showing NAWM region (brown). Values of the RSI-derived parameters were obtained from the whole volume of WML and the whole volume of NAWM.

NAWM: normal appearing white matter; rFA: restricted fractional anisotropy; WM: white matter; WML: white matter lesions.

2.6 Statistical analyses

Statistical analyses were performed with the Statistical Package for the Social Sciences software (SPSS v24, IBM, Chicago IL, USA). In addition, the “R” statistical software (v3.1.1, www.r-project.org) was used for the Benjamini-Hochberg false discovery rate method to control for multiple tests. For group comparisons between two groups the parametric independent samples *t*-test was used when the data were normally distributed; otherwise the non-parametric Mann-Whitney *U* test was used. For group comparisons between three or more groups the parametric one-way analysis of variances (ANOVA) test was used (corrected for multiple comparisons with post-hoc Bonferroni test) when the data were normally distributed; otherwise the non-parametric Kruskal-Wallis *H* test was used (with post-hoc Mann-Whitney *U* test and Bonferroni correction for multiple comparisons). Non-parametric Wilcoxon Signed Rank test was used to test differences in diffusion parameters between WML and NAWM. The Spearman’s ρ (rho) was used for assessing correlations between clinical measures and diffusion parameters as the data were non-normally distributed, otherwise the Pearson’s *r* was used for assessing partial correlations (controlled for age). All reported *p*-values are two-sided and $p < 0.05$ was defined as level of significance.

2.7 Ethical approvals

Approval for this study was obtained from the data inspectorate representative at the hospital and from the Regional Committee for Medical and Health Research Ethics. A signed informed consent was obtained from all study participants.

3. Results

3.1 Clinical characteristics

Demographic and clinical characteristics are shown in details in Table 1. The median age of disease onset was 27 years (range 16 – 53), median disease duration at inclusion in the study was nine years (range 1 – 32) and the median EDSS score was 2.0 (range 0 – 6.5). Median difference between EDSS date and MRI acquisition date was two months (range 0 – 8). Fifty-three patients (69 %) were receiving disease modifying treatment.

3.2 Radiological findings

Detailed radiological characteristics are shown in Table 2a and 2b. Confluent lesions were present in 42 patients (54%). In 13 patients (17%) contrast enhancing lesions were observed; most frequently there were one or two enhancing lesions per patient, and more than two enhancing lesions were observed in one patient only. Because of the low number of enhancing lesions no separate analysis of these lesions was performed. The median WML volume was 5.2 ml (range 0.15 – 88.2).

Table 2 Global imaging characteristics^a, n=77

<i>a. General characteristics by WML</i>		
Distribution of WML (patients n=)		
- periventricular		75 (97%)
- juxtacortical		76 (99%)
- other supratentorial subcortical		75 (97%)
- infratentorial		39 (51%)
Confluent WML (patients n=)		
- no confluent lesions		35 (46%)
- beginning confluence		14 (18%)
- definite confluent lesions		28 (36%)
Patients with enhancing WML		13 (17%)
<i>b. Volumetric data</i>		
IV, ml		1510 ± 146
Brain volume, % of IV		72 ± 4.4
White matter volume, % of IV		31 ± 2.6
Grey matter volume, % of IV		41 ± 2.6
Cortical volume, % of IV		31 ± 2.1
WML volume, ml ^b		5.2 (2 – 17.8), range 0.15 – 88.2
<i>c. RSI-derived diffusion parameters^c</i>		
	<i>in WML</i>	<i>in NAWM</i>
fADC	1.19 ± 0.14	0.92 (0.90 – 0.95)
sADC	0.57 ± 0.06	0.47 (0.46 – 0.48)
FA	0.32 ± 0.04	0.35 (0.33 – 0.36)
rFA	0.70 ± 0.05	0.65 (0.62 – 0.66)
ND	355 ± 52	440 (423 – 449)
Cellularity	106 (88 – 144)	193 (181 – 206)
EWF	695 ± 28	729 (718 – 735)
FWF	563 ± 59	415 (406 – 431)

fADC: fast apparent diffusion coefficient; EWF: extracellular water fraction; FA: fractional anisotropy; FWF: free water fraction; IV: intracranial volume; NAWM: normal appearing white matter; ND: neurite density; RSI: restriction spectrum imaging; sADC: slow apparent diffusion coefficient; rFA: restricted fractional anisotropy; WML: white matter lesions

^aData are n (%) for nominal variables, mean ± standard deviation for normally distributed variables, or median (interquartile range) for non-normally distributed variables.

^bWML volume (lesion load) based on semi-automated segmentation on FLAIR series.

^cAll tested RSI-derived diffusion parameters differed significantly between WML and NAWM (Wilcoxon Signed Rank test, $p < 0.001$ for all paired comparisons). Units for the parameters are given in Supplementary material 3.

3.3 Diffusion parameters in WML and NAWM

All the tested RSI-derived diffusion parameters differed significantly between WML and NAWM ($p < 0.001$ for all pairwise comparisons). The fADC, sADC, rFA, and FWF were higher in WML than in NAWM, while FA, ND, cellularity and EWF were lower in WML than in NAWM (Table 2c).

3.4 Comparison of subgroups defined according to disability measured by EDSS

The clinical and MRI characteristics of each disability subgroup are presented in Table 3 and the differences in RSI-derived diffusion parameters between the subgroups are shown schematically in Figure 3. The diffusion parameter that showed largest difference across disability subgroups was sADC in WML (ANOVA: $F=5.1$, $\eta^2=0.12$, $p=0.008$). Briefly, the fADC, sADC, ND and FWF differed significantly between the disability subgroups when obtained both in WML ($p=0.012$, $p=0.008$, $p=0.024$ and $p=0.036$ respectively) and in NAWM ($p=0.022$, $p=0.036$, $p=0.009$ and $p=0.020$ respectively). Cellularity differed between the disability subgroups only when obtained in WML ($p=0.017$) while FA and rFA differed between the subgroups only when obtained in NAWM ($p=0.017$ and $p=0.019$ respectively). EWF did not differ between the subgroups neither when obtained in WML nor in NAWM. Post-hoc comparisons showed the greatest differences between the subgroups with substantial disability and minimal disability. In WML patients with substantial disability had *higher* fADC ($p=0.010$), sADC ($p=0.007$) and FWF ($p=0.024$), and *lower* ND ($p=0.019$) and cellularity ($p=0.015$). In NAWM patients with substantial disability had *higher* fADC ($p=0.024$), sADC ($p=0.027$) and FWF ($p=0.024$), and *lower* FA ($p=0.018$), rFA ($p=0.024$) and ND ($p=0.0125$) than patients with minimal disability. Since we used two different types of head coil, additional analyses were performed after exclusion of the relatively low number of patients scanned with the 12 channel coil ($n=8$). Performing analyses on the remaining 69 subjects, it was still the sADC parameter in WML that discriminated best between the disability subgroups. The results of these analyses are shown with more details in Supplementary material 4.

Table 3 Comparison of subgroups defined according to disability by EDSS^a, $n=77$

	Neurological disability by EDSS			F, η^2 or H, w^2	p-value
	minimal	low	substantial		
	EDSS < 2.5 $n=27$	EDSS of 2.5 – 3 $n=39$	EDSS > 3 $n=11$		
<i>Characteristics</i>					
Age, years	36.6 ± 8.0	41.0 ± 11.4	43.8 ± 10.2	2.5, 0.06 ^b	0.089 ^b
Age at disease onset, years	27 (25 – 30)	29 (26 – 36)	25 (22 – 35)	3.8, 0.05 ^c	0.147 ^c
Disease duration, years	7.0 (4 – 12)	9 (3 – 15.5)	13 (9 – 27)	7.0, 0.09 ^c	0.030^c
WML volume, ml	2.7 (1.2 – 14.1)	4.5 (2.0 – 16)	19.7 (9.5 – 31.5)	9.9, 0.13 ^c	0.007^c
Brain volume ^d , %	73.4 ± 5.6	71.8 ± 3.1	68.7 ± 3.4	4.7, 0.13 ^b	0.011^b
<i>Diffusion parameters in WML</i>					
fADC	1.15 ± 0.15	1.19 ± 0.13	1.30 ± 0.11	4.7, 0.11 ^b	0.012^{b,e}
sADC	0.56 ± 0.06	0.57 ± 0.05	0.62 ± 0.05	5.1, 0.12 ^b	0.008^{b,f}
FA	0.33 ± 0.05	0.32 ± 0.04	0.30 ± 0.04	2.4, 0.06 ^b	0.100 ^b
rFA	0.70 ± 0.05	0.69 ± 0.05	0.70 ± 0.04	0.1, 0.01 ^b	0.881 ^b
ND	369 ± 56	358 ± 46	319 ± 46	3.9, 0.10 ^b	0.024^{b,g}
Cellularity	120 (96 – 120)	108 (91 – 145)	88 (74 – 102)	8.2, 0.11 ^c	0.017^{c,h}
EWF	700 ± 27	696 ± 28	677 ± 27	2.9, 0.07 ^b	0.061 ^b
FWF	547 ± 62	563 ± 55	601 ± 52	3.5, 0.08 ^b	0.036^{b,i}
<i>Diffusion parameters in NAWM</i>					
fADC	0.91 (0.89 – 0.95)	0.92 (0.90 – 0.95)	0.95 (0.94 – 0.98)	7.7, 0.10 ^c	0.022^{c,j}
sADC	0.47 (0.46 – 0.47)	0.47 (0.46 – 0.48)	0.48 (0.47 – 0.50)	6.6, 0.08 ^c	0.036^{c,k}
FA	0.35 (0.33 – 0.36)	0.35 (0.33 – 0.36)	0.31 (0.29 – 0.35)	8.1, 0.10 ^c	0.017^{c,l}
rFA	0.65 (0.63 – 0.66)	0.65 (0.63 – 0.66)	0.61 (0.58 – 0.65)	7.9, 0.10 ^c	0.019^{c,m}

ND	445 (430 – 454)	440 (429 – 448)	421 (393 – 436)	9.4, 0.12 ^c	0.009^{c,n}
Cellularity	195 (190 – 207)	194 (180 – 207)	182 (162 – 193)	4.7, 0.06 ^c	0.094 ^c
EWF	729 (718 – 734)	728 (714 – 736)	729 (726 – 735)	0.3, <0.01 ^c	0.844 ^c
FWF	411 (398 – 422)	413 (406 – 425)	428 (415 – 453)	7.8, 0.10 ^c	0.020^{e,o}

EDSS: expanded disability status scale; EWF: extracellular water fraction; FA: fractional anisotropy; fADC: fast apparent diffusion coefficient; FWF: free water fraction; ND: neurite density; sADC: slow apparent diffusion coefficient; rFA: restricted fractional anisotropy; WML: white matter lesions

The *p*-values shown are corrected for multiple comparisons with post-hoc Bonferroni test (one-way ANOVA) or with post-hoc Mann-Whitney *U* test and Bonferroni correction (Kruskal-Wallis *H* test) as appropriate.

^aData are mean ± standard deviation for normally distributed variables, or median (interquartile range) for non-normally distributed variables.

^bOne way ANOVA test (normally distributed data), the next to last column shows F and eta-squared

^cKruskal-Wallis *H*-test (non-normally distributed data), the next to last column shows H and w-squared

^dNormalized brain volume (in percent of intracranial volume)

^esignificant difference between group 3 and groups 1 and 2 (*p*=0.010 and *p*=0.047 respectively)

^fsignificant difference between group 3 and groups 1 and 2 (*p*=0.007 and *p*=0.022 respectively)

^gsignificant difference between group 3 and group 1 (*p*=0.019)

^hsignificant difference between group 3 and groups 1 and 2 (*p*=0.015 and *p*=0.039 respectively)

ⁱsignificant difference between group 3 and group 1 (*p*=0.031)

^jsignificant difference between group 3 and group 1 (*p*=0.024)

^ksignificant difference between group 3 and group 1 (*p*=0.027)

^lsignificant difference between group 3 and group 1 (*p*=0.018)

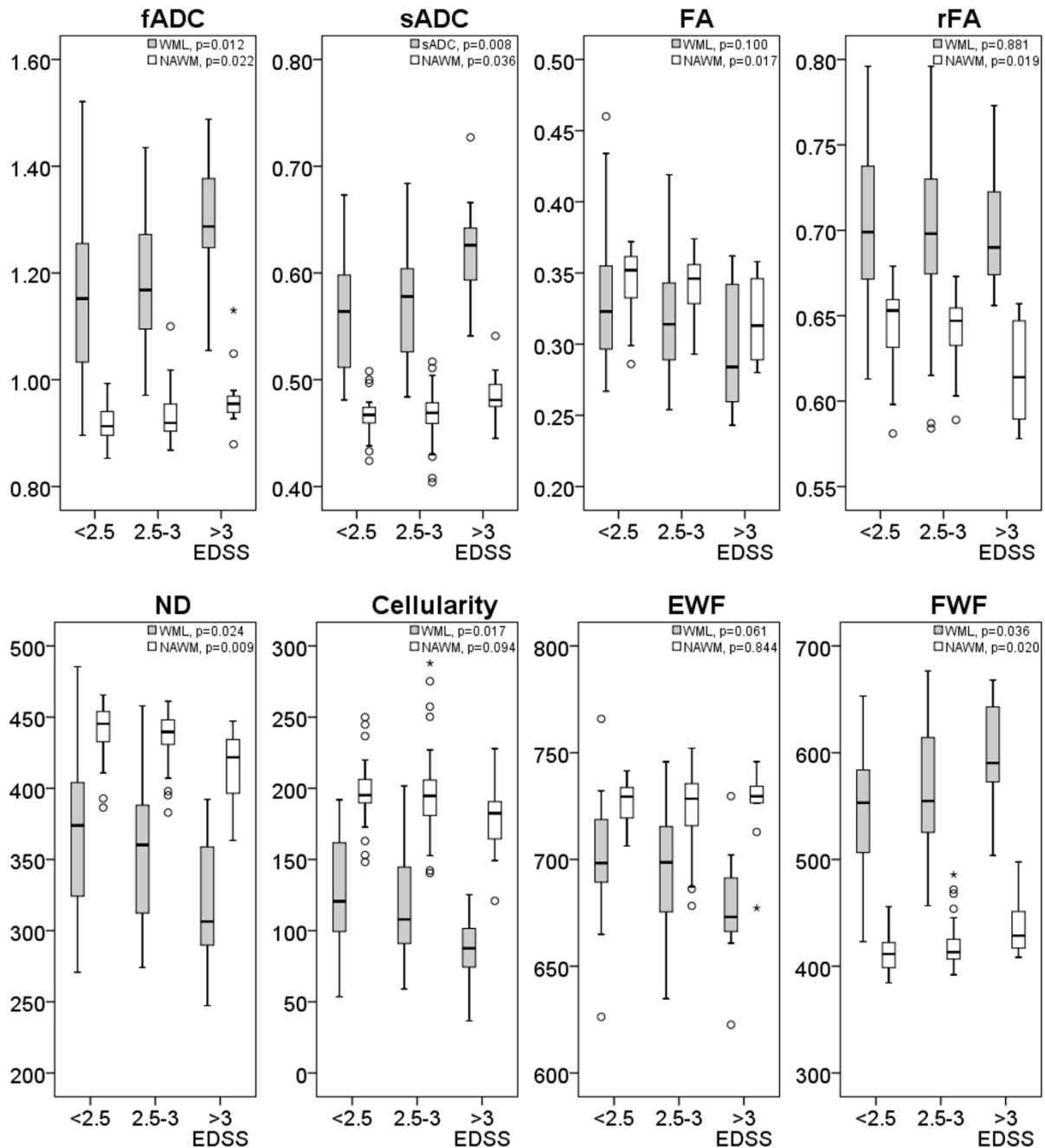
^msignificant difference between group 3 and groups 1 and 2 (*p*=0.024 and *p*=0.033 respectively)

ⁿsignificant difference between group 3 and groups 1 and 2 (*p*=0.012 and *p*=0.039 respectively)

^osignificant difference between group 3 and groups 1 and 2 (*p*=0.042 and *p*=0.048 respectively)

Figure 3

Diffusion parameters in WML and NAWM shown by disability subgroups, $n=77$



The gray bars represent WML, the white bars represent NAWM. The units are explained in Supplementary material 3. Upper row: fADC, sADC, FA and rFA in WML (grey bars) and in NAWM (white bars) shown by EDSS subgroups. Lower row: ND, cellularity, EWF and FWF in WML (grey bars) and in NAWM (white bars) shown by EDSS subgroups. Subgroups are defined by neurological disability: no or minimal (EDSS<2.5, $n=27$), low (EDSS of 2.5 or 3.0, $n=39$), and substantial (EDSS>3.0, $n=11$) disability.

EDSS: expanded disability status scale; EWF: extracellular water fraction; fADC: fast apparent diffusion coefficient; FA: fractional anisotropy; FWF: free water fraction; NAWM: normal appearing white matter; ND: neurite density; rFA: restricted fractional anisotropy; sADC: slow apparent diffusion coefficient; WML: white matter lesions.

3.5 Correlations between clinical data, and volumetric and diffusion parameters

Details concerning correlations between the clinical data and volumetric and diffusion parameters are shown in Table 4 (the p -values presented were adjusted to control for multiple tests). ND in NAWM showed strongest correlation with EDSS ($\rho=-0.39$, $p<0.001$) of all the investigated RSI-derived diffusion parameters. Otherwise, EDSS correlated with brain volume ($r=-0.30$, $p=0.017$) and WM volume ($r=-0.32$, $p=0.013$) normalized to intracranial volume, and with WML volume ($r=0.25$, $p=0.048$) normalized to WM volume (correlations controlled for age). For correlations with disease duration see Table 4. MSSS did not correlate with any of the investigated diffusion or volumetric parameters.

Table 4 Correlations between clinical data, and volumetric and diffusion parameters, $n=77$

	EDSS ^a	Disease duration ^a
<i>Volumetric data</i> ^b	r, p	r, p
Whole brain ^c	-0.30, 0.017	-0.11, 0.373
White matter ^c	-0.32, 0.013	-0.05, 0.650
Grey matter ^c	-0.18, 0.129	-0.15, 0.264
Cortex ^c	-0.18, 0.262	-0.06, 0.252
WML volume	0.23, 0.060	0.42, <0.001
- % of white matter volume	0.25, 0.048	0.42, <0.001
- % of intracranial volume	0.24, 0.055	0.42, <0.001
<i>Diffusion parameters in WML</i> ^d	ρ, p	ρ, p
fADC	0.36, 0.011	0.40, <0.001
sADC	0.31, 0.014	0.29, 0.021
FA	-0.22, 0.073	-0.09, 0.431
rFA	0.01, 0.929	0.13, 0.316
ND	-0.30, 0.015	-0.28, 0.025
Cellularity	-0.27, 0.030	-0.22, 0.094
EWF	-0.34, 0.011	-0.46, <0.001
FWF	0.32, 0.011	0.37, 0.002
<i>Diffusion parameters in NAWM</i> ^d	ρ, p	ρ, p
fADC	0.33, 0.011	0.35, 0.004
sADC	0.23, 0.065	0.15, 0.251
FA	-0.35, 0.011	-0.40, <0.001
rFA	-0.32, 0.011	-0.46, <0.001
ND	-0.39, <0.001	-0.38, 0.002
Cellularity	-0.21, 0.081	-0.13, 0.316
EWF	-0.07, 0.590	-0.08, 0.130
FWF	0.33, 0.011	0.35, 0.004

EDSS: expanded disability status scale; EWF: extracellular water fraction; fADC: fast apparent diffusion coefficient; FA: fractional anisotropy; FWF: free water fraction; NAWM: normal appearing white matter; ND: neurite density; rFA: restricted fractional anisotropy; r: partial correlation; ρ (rho): Spearman's correlation; sADC: slow apparent diffusion coefficient; WML: white matter lesions
Multiple sclerosis severity score (MSSS) did not correlate significantly with any diffusion parameters and is not included in the table.

^aThe p -values are controlled for multiple tests using Benjamini-Hochberg false discovery rate method

^bCorrelations with volumetric data are controlled for age (partial correlation).

^cNormalized to intracranial volume.

^dCorrelations with diffusion parameters are Spearman's correlation (non-normally distributed data).

4. Discussion

In the current study we found that sADC in WML showed largest difference across EDSS subgroups, while ND in NAWM showed strongest correlation with disability as measured by EDSS.

The sADC was higher in patients with higher disability score. Increased sADC in WML in these patients may be due to greater exchange of intracellular and extracellular water compartments, possibly caused by demyelination. This is in contrast to the study by Droogan et al. ²⁷, who reported no significant correlation between ADC values in lesions and EDSS. However, Gratsias et al. ¹⁰ reported a correlation between ADC in NAWM and EDSS, in accordance with our results. No correlation between FA in NAWM and EDSS was found in their study. The different results that are reported in these studies may be partly explained by different methodology, since the studies have used different inclusion criteria (different diagnostic criteria over time and differences in study size) as well as different definitions of NAWM (manual defined regions of interest vs. segmentation of the whole NAWM). Also the study by Droogan was published back in 1999, using a scanner with lower magnetic field (1.5 Tesla) thus our study can be of higher imaging quality.

ND in NAWM showed the strongest correlation with EDSS of all tested RSI-derived parameters. This finding indicates that pathological processes may occur in WM outside lesions and may have an impact on the development of disability. Therefore, advanced diffusion techniques like RSI may help explain “the clinico-radiological paradox” understood as a poor association between disability and radiological findings on conventional MRI in MS patients ²⁸. Since ND in theory represents the density of intact axons and dendrites in white matter, our results suggest that disability in MS may be associated with diffuse damage of white matter rather than with presence of visible lesions on conventional MRI. Our findings support the results recently published by Brownlee et al. reporting an association between neurological disability and low ND values in NAWM obtained in relapse-onset MS patients with the above mentioned NODDI technique ²⁹. Interestingly, the significance of correlations between FA, rFA and ND in NAWM and EDSS still persists after adjustment for disease duration, but disappears after additional adjustment for multiple testing with false discovery rate method. This may be due to the sample size and should be reexamined in a larger study with a broader range of EDSS scores and disease duration.

RSI enables more specific estimation of tissue microstructure and provides more diffusion parameters compared to traditional DWI and DTI ¹⁹. RSI and NODDI have similar approach (multishell acquisition and multicompartiment diffusion model); however, whereas NODDI characterizes the degree of fiber dispersion, RSI further identifies the geometric pattern of dispersion and has a more efficient acquisition time ^{17,30}. The association we observe between RSI-derived parameters and disability is very interesting, since an MRI-biomarker for disability in MS is much needed. Clinical disability assessment using EDSS has been

criticized, since EDSS mainly reflects motoric disability. Further studies of RSI as a potential biomarker both for physical and cognitive impairment in MS are needed in a larger cohort.

All diffusion parameters investigated in our study differed significantly in pairwise comparisons between WML and NAWM: fADC, sADC, rFA, and FWF measurements were all higher in WML than in NAWM, while values of FA, ND, cellularity and EWF were lower in WML than in NAWM. Thus, our ADC and FA results are in accordance with previous publications^{10, 27} reporting higher ADC and lower FA in WML compared to segmented NAWM regions. Histopathologically, FA correlates with myelin content and axonal count in NAWM and WML³¹. Lower FA values indicate reduced myelin content and lower number of axons. We found that ND was lower and FWF was higher in WML than in NAWM, which can be explained by reduced number of axons in WML compared to NAWM. This has been reported in previous pathological studies^{31, 32}.

Whole brain volume and WM volume normalized to intracranial volume correlated negatively with EDSS (controlled for age) in our study, in line with previous reports: Shiee et al.³³ reported an association between lower WM volume and higher disability in MS patients. WML volume normalized to WM showed a weak positive correlation with disability, and a trend for weak positive correlation while considering the absolute values. These findings are in general in accordance with other reports where disability in MS patients was reported to weakly correlate with absolute WML volume³⁴ and WML volume normalized to WM³⁵.

A strength of this study is the relatively large study group ($n=77$). Furthermore, the RSI technique used in this study is a new diffusion MRI method which has not previously been applied in MS, and we used unbiased methods of analysis. One limitation was the lack of a healthy control group. However, our aim was to study this methodology in MS, while others have reported the use of RSI in other conditions. Two experienced MS neurologists at the university hospital examined all the patients, reducing the heterogeneity in both diagnostics and EDSS scores. Another limitation is a large span in lesion volume across the groups (the WML volume in group 1 was 2.7 ml and in group 3 it was 19.7 ml) which could affect the RSI measures in small lesions due to spatial position effect. No longitudinal data were available from these patients for this study, but further studies of this patient cohort are planned.

In conclusion, RSI-derived sADC in WML showed largest difference across disability subgroups and ND in NAWM showed strongest correlation with disability in MS patients. The strongest correlation with EDSS of a parameter obtained in NAWM indicates that pathological processes outside MS-lesions are of importance for disability. Our findings suggest that imaging biomarkers from advanced diffusion techniques like RSI may help explain the “clinico-radiological paradox” and may improve disease monitoring in MS patients. There is a need to study RSI-derived parameters longitudinally in order to evaluate the usefulness of this technique in follow-up of MS patients.

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Conflicts of interest

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Atle Bjørnerud is a consultant for NordicNeuroLab AS, Bergen, Norway.

Ole A. Andreassen received speaker's fees from Lundbeck.

Anders M. Dale is a Founder of and holds equity in CorTechs Labs, Inc., and serves on its Scientific Advisory Board. He is also a member of the Scientific Advisory Board of Human Longevity, Inc. (HLI), and receives research funding from General Electric Healthcare (GEHC). The terms of these arrangements have been reviewed and approved by the University of California, San Diego in accordance with its conflict of interest policies.

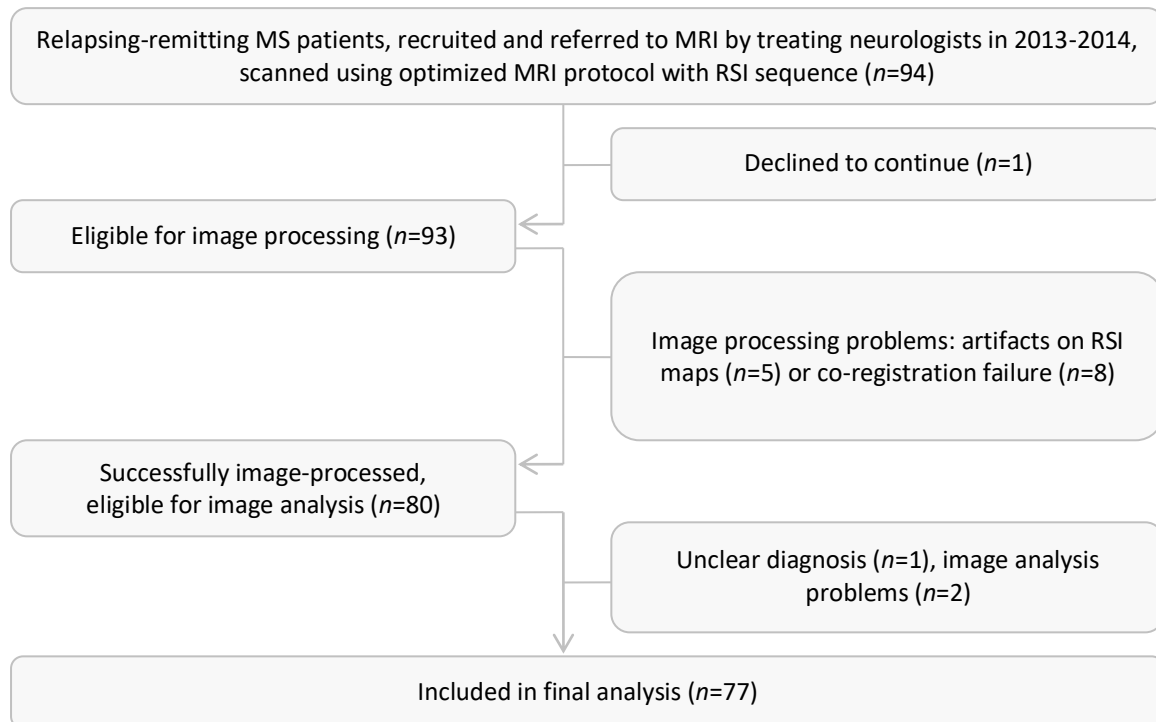
Mona K. Beyer received speaker's fees from Novartis and Biogen Idec.

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Supplementary material 1
Flow chart for patients' inclusion



Of 94 patients included initially, 77 were included in final analysis.

MRI: magnetic resonance imaging; MS: multiple sclerosis; RSI: Restriction spectrum imaging.

Supplementary material 2
Disease modifying treatment

Type of disease modifying treatment was defined as first line (interferon, glatiramer acetate, teriflunomide, dimethylfumarate), second line (natalizumab, fingolimod, alemtuzumab) or third line (autologous haematopoietic stem cell transplantation). Due to possible prolonged efficacy of disease modifying treatment after withdrawal, patients who were *not* on treatment at the MRI acquisition date were nonetheless classified as on treatment if the MRI acquisition was performed within 3 months after withdrawal ($n=8$), or within one year after stem cell transplantation ($n=1$) or last administration of alemtuzumab ($n=3$).

Supplementary material 3
Description of RSI-derived diffusion parameters

Diffusion parameter	Description
Cellularity	Signal fraction from spherically restricted water compartment, i.e. cell bodies. Lower cellularity values mean decreased number of cell bodies, e.g. glial cell bodies in white matter. Unit: 1000*(signal fraction from restricted isotropic compartment), example: 300 means 30% of the total signal stems from this compartment.
EWF	Extracellular water fraction. Represents the signal fraction of water that is hindered due to tortuous geometry of the extracellular space that may be isotropic or anisotropic. It will be reduced if there is a tissue loss or changes in tissue that reduce geometrical

	complexity and tortuosity. Unit: 1000*(signal fraction from extracellular water compartment).
FA	Fractional anisotropy indicates the degree of anisotropy (or directional dependence); it is given in values between 0 and 1 where 0 means equal diffusion in all directions and 1 means diffusion in one direction only.
fADC	Fast apparent diffusion coefficient. In theory, the fADC measures the effective diffusion coefficient of extracellular water. As such the fADC should be more sensitive to changes in extracellular diffusion (edema or inflammation) compared with sADC. However, the fast and slow ADC components are not based on the RSI model, and should not be ascribed to specific microcompartments. Unit: $10^{-3}\text{mm}^2/\text{s}$.
FWF	Free water fraction. Signal fraction of freely diffusing isotropic water. It will be increased with tissue loss. Unit: 1000*(signal fraction from isotropic free water compartment).
ND	Neurite density. Signal fraction of cylindrically restricted water. Lower ND means reduction of cylindrical structures which in brain tissue is consistent with neurofibers or neurites (axons and dendrites). Unit: 1000*(signal fraction from restricted cylindrical compartment).
rFA	Restricted fractional anisotropy (or tubularity) is the FA derived from the restricted water signal from cylindrical structures, which in theory stems from the intraaxonal and intradendritic (i.e. neurite) compartment. Unit: values between 0 and 1.
sADC	Slow apparent diffusion coefficient. In theory, the sADC measures the effective diffusion coefficient of intracellular water in both cell bodies and neurites. Unit: $10^{-3}\text{mm}^2/\text{s}$.

Supplementary material 4

The effect of the head coil type on the diffusion parameters.

Sixty-nine patients were scanned using an 8-channel head coil and eight patients using a 12-channel head coil, due to different availability of these coils during the period of data collection at our site. In the group with minimal disability, 25 patients were scanned with 8-channel coil and 2 patients with 12-channel coil. In the group with low disability, 35 patients were scanned with 8-channel coil and 4 patients with 12-channel coil. In the group with substantial disability, 9 patients were scanned with 8-channel coil and 2 patients with 12-channel coil.

Due to possible effect of the head coil type on the comparison of diffusion parameters between the disability subgroups, we performed additional analysis with subjects scanned with 8-channel head coil only (n=69), after excluding patients scanned with 12-channel head coil (n=8). In this analysis, it was still the sADC parameter in WML that showed largest difference across the disability subgroups. The p-values of this analysis are presented below.

Comparison of RSI-derived parameters across subgroups defined according to disability by EDSS^a, with patients scanned with 8-channel coil only, n=69.

<i>Diffusion parameters in WML</i>	<i>p-value</i>
fADC	0.031^b
sADC	0.028^b
FA	0.307 ^b
rFA	0.722 ^b
ND	0.094 ^b
Cellularity	0.153 ^c
EWF	0.053 ^b
FWF	0.095 ^b

*Diffusion parameters in
NAWM*

fADC	0.033^c
sADC	0.051 ^c
FA	0.162 ^c
rFA	0.216 ^c
ND	0.060 ^c
Cellularity	0.129 ^c
EWF	0.978 ^c
FWF	0.045^c

EDSS: expanded disability status scale; EWF: extracellular water fraction; FA: fractional anisotropy; fADC: fast apparent diffusion coefficient; FWF: free water fraction; ND: neurite density; sADC: slow apparent diffusion coefficient; rFA: restricted fractional anisotropy; WML: white matter lesions

The *p*-values shown are corrected for multiple comparisons with post-hoc Bonferroni test (one-way ANOVA) or with post-hoc Mann-Whitney *U* test and Bonferroni correction (Kruskal-Wallis *H* test) as appropriate.

^a Patients were divided into three groups according to their expanded disability status scale (EDSS): with minimal, low and substantial disability (<2.5, 2.5–3 and >3, respectively).

^b One way ANOVA test (normally distributed data).

^c Kruskal-Wallis *H*-test (non-normally distributed data).

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