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MR Spectroscopy Indicates Diffuse Multiple Sclerosis Activity During Remission

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Abstract

Objective—To test the hypothesis that diffuse abnormalities precede axonal damage and atrophy in the MRI normal-appearing tissue of relapsing-remitting (RR) multiple sclerosis (MS) patients, and that these processes continue during clinical remission.

Methods—Twenty-one recently diagnosed mildly disabled (mean disease duration 2.3 years, mean Expanded Disability Status Scale score of 1.4) RR MS patients and 15 healthy matched controls were scanned with MRI and proton MR spectroscopic imaging (¹H-MRSI) at 3 T. Metabolite concentrations: *N*-acetylaspartate (NAA) for neuronal integrity; choline (Cho) for membrane turnover rate; creatine (Cr) and *myo*-inositol (mI) for glial status were obtained in a 360 cm³ volume-of-interest (VOI) with 3D multivoxel ¹H-MRSI. They were converted into absolute amounts using phantom replacement and normalized into absolute concentrations by dividing by the VOI tissue volume fraction obtained from MRI segmentation.

Results—The patients' mean VOI tissue volume fraction, 0.92 and NAA concentration, 9.6 mM, were *not* different from controls' 0.94 and 9.6 mM. In contrast, the patients' mean Cr, Cho and mI levels 7.7, 1.9, 4.1 mM were 9%, 14% and 20%, *higher* than the controls' 7.1, 1.6 and 3.4 mM ($p=0.0097$, 0.003 and 0.0023).

Conclusions—The absence of early tissue atrophy and apparent axonal dysfunction (NAA loss) in these RR MS patients suggests that both are preceded by diffuse glial proliferation (astrogliosis), as well as possible inflammation, *de*- and *re*-myelination reflected by elevated mI, Cho and Cr, even during clinical remission and despite immunomodulatory treatment.

Keywords

Multiple Sclerosis; normal-appearing tissue; MR Spectroscopy

INTRODUCTION

The sensitivity of T_1 - and T_2 -weighted MRI to white matter (WM) lesions and to regional as well as global atrophy has made this modality central to the diagnosis and treatment monitoring of multiple sclerosis (MS).[1-4] Paradoxically, these findings correlate only

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COMPETING INTERESTS

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weakly with clinical disability,[5] an incongruity due MRI's insensitivity to *microscopic* pathology and lack of specificity to distinguish inflammation from demyelination, axonal loss, or gliosis.[1, 6] Since MS is now recognized to be a diffuse disease of both normal-appearing WM (NAWM) and gray matter (GM),[7, 8] these sensitivity and specificity issues limit the potential of clinical MRI to fully assess the total load of the disease and consequently, to monitor treatment response.[6]

Although diffuse pathology that leads to microscopic axonal damage is often MRI-occult, it is nevertheless detectable with quantitative MR methods.[6] For example, proton MR spectroscopy (¹H-MRS) can probe tissue metabolism;[9] whereas magnetization transfer and diffusion tensor imaging can assess myelin and WM integrity.[1] These have shown that NAWM abnormalities may (a) be present in all MS subgroups, (b) correlate with disability and cognitive impairment, (c) occur prior to lesion development, all suggesting they play a role in lesion formation.[6, 7]

¹H-MRS offers the ability to monitor several pathological processes via their specific metabolic markers: *N*-acetylaspartate (NAA) for neuronal integrity, whose decline correlated better with disability than MRI metrics;[10] *myo*-inositol (mI) for glial proliferation,[6] whose elevation has also correlated with impairment;[11] creatine (Cr) for energy status and cell density and choline (Cho) for inflammation, *de*- and *re*-myelination. [10] Unfortunately, most studies to date use single-voxel or 2D ¹H-MRS that sample under 10% of the brain, subjecting them to the implicit assumption that such small volumes are globally representative. In addition, quantification with metabolite ratios may have confounded interpretation since Cr (the frequent denominator) levels are reported to also vary.[12, 13]

Only one study addressed both issues with absolute quantification in a large (~½ liter) WM volume of relapsing-remitting (RR) MS patients and controls.[12] It reported lower NAA and elevated Cho and Cr in the patients but could not ascribe the latter to either astrogliosis or myelin repair since Cr levels are higher in both astrocytes *and* oligodendrocytes;[14] and the long echo-time ¹H-MRS used precluded detection of the astrocyte-specific marker – mI. [6] Furthermore, the NAA loss could not distinguish isolated event(s) from chronic inflammation due to the long, 6+ years, disease duration. Our goal, therefore, is to quantify the glial, *de*- and *re*-myelination markers (mI, Cho and Cr) in NAWM of recently diagnosed RR MS patients with short, *TE*=35 ms, ¹H-MRS to test the hypothesis that these processes precede axonal damage (NAA).

MATERIALS AND METHODS

Human Subjects

Twenty-one (15 women) patients meeting the Poser criteria for RR MS were enrolled.[15] Their mean age was 33 (range 21 – 42) years; mean disease duration was 2.3 (range 0.3 – 5.2) years; and mean Expanded Disability Status Scale (EDSS[16]) score was 1.25 (range 0 – 4), as shown in table 1. All were on immunomodulatory medication and none experienced a relapse in the preceding 3 months. Fifteen age- and gender-matched controls (12 women, mean age 30, range 20 – 44 years) were also enrolled. Their “healthy” status was based on negative answers to a list of disqualifying neurological conditions before the study and unremarkable MRI afterwards. All participants gave written Institutional Review Board-approved informed consent.

MR Acquisition

All measurements were done in a 3 T Trio MRI scanner (Siemens AG, Erlangen Germany) with a TEM3000 transmit-receive head coil (MRInstruments, Minneapolis, MN). For tissue

segmentation, sagittal MP-RAGE (TE/TI/TR=2.6/1800/1360 ms) was acquired at 240×240 mm² field-of-view (FOV), 256×256 matrix and reconstructed in 1 mm thick axial, and coronal slices. To guide the spectroscopic volume-of-interest (VOI), T₂-weighted FLAIR (TE/TI/TR= 88/2500/9000 ms) images were obtained at the same FOV and matrix, but 3.7 mm slice thickness.

Following our chemical-shift imaging (CSI) based shim procedure, a 10 cm anterior-posterior (AP) ×8 cm left-right (LR) ×4.5 cm inferior-superior (IS) =360 cm³ ¹H-MRSI VOI was centered on the corpus callosum (fig. 1) and excited using TE/TR=35/1800 ms PRESS. The VOI was partitioned using Hadamard spectroscopic imaging[17] into six axial slices which were encoded with 16×16 2D-CSI over a 16×16 cm² (LR×AP) FOV. The VOI was defined in the planes of these slices by the two PRESS 11.2 ms long numerically optimized 180° pulses yielding 8×10×6=480 voxels, 1.0×1.0×0.75 cm³ each. The signal was acquired for 256 ms at ±1 kHz bandwidth. At two averages the ¹H-MRSI took 34 minutes and the entire protocol was an hour.

Tissue fraction (T_f) and lesion volume in the VOI

Since the VOI includes ventricles and sulci (*cf.* fig. 1) correction for their cerebro-spinal fluid (CSF) partial volume is needed. To this end the MP-RAGE images were segmented using our MIDAS package.[18] The process starts by automatic detection of the WM signal intensity, I_{WM} , in a periventricular “seed” region. Following selection of all pixels at or above 55, but below 135% (to exclude the fat) of I_{WM} , a tissue-mask is constructed per slice in three steps: morphological erosion, recursive region growth retaining pixels connected to the “seed”; and morphological inflation to reverse the effect of erosion. Pixels whose intensity was under 55% of I_{WM} were defined as CSF. T_f for each subject was the product of the number of “tissue” pixels in the VOI and their volume divided by the nominal VOI-volume. Lesion volumes were obtained from the FLAIR images using the same procedure, but applying a threshold of 120% of I_{WM} .

Absolute metabolite quantification

Processed offline using in-house software, the MRS data was voxel shifted to align the CSI grid with the NAA VOI, Fourier transformed in the time, AP and LR dimensions and Hadamard reconstructed along the IS direction. Each of the VOI's 480 spectra was frequency-aligned and zero-order phased in reference to the NAA peak and then summed. This retained the individual spectra (narrow) linewidth and improved the signal-to-noise-ratio (SNR) by $480^{1/2} \approx 22$. [12]

Relative levels of the i^{th} (NAA, Cr, Cho, mI) metabolite in the j^{th} subject were estimated from their peak area, S_{ij} , using spectral modeling[19] S_{ij} were scaled into concentrations, C_{ij} relative to signals from a 2 L sphere of C_i^{vitro} =12.5, 10.0, 3.0 and 7.5 mM NAA, Cr, Cho and mI in water:

$$C_{ij}=C_i^{vitro} \cdot \frac{S_{ij}}{S_R} \cdot \frac{V_j^{180^\circ}}{V_R^{180^\circ}} \cdot \frac{1}{T_f}, \quad [1]$$

where S_R is the sphere's metabolite signal, $V_j^{180^\circ}$ and $V_R^{180^\circ}$ are the RF voltages needed for a non-selective 1 ms 180° inversion pulse on subject and reference sphere. The C_{ij} s were corrected for relaxation time differences *in vivo* (T_1^{vivo} , T_2^{vivo}) and in the phantom (T_1^{vitro} , T_2^{vitro}) with a factor[12]:

$$f = \frac{\exp(-TE/T_2^{vitro})}{\exp(-TE/T_2^{vivo})} \cdot \frac{1 - \exp(TR/T_1^{vitro})}{1 - \exp(TR/T_1^{vivo})}, \quad (2)$$

using the T_1^{vivo} = 1.4, 1.3 and 1.2 s, T_2^{vivo} = 343, 172 and 248 ms reported at 3 T for NAA, Cr and Cho [20, 21] and T_1^{vitro} = 605, 336 and 235 ms, T_2^{vitro} = 483, 288 and 200 ms in the phantom. Since J -coupling modulation and low voxel SNR for mI prevent obtaining its T_1 and T_2 , we assumed $f=1$ for it in Eq. [2]. Finally, the C_{ij} s were divided by T_f to convert into tissue concentrations.

Statistical Analysis

Analysis of variance (ANOVA) based on ranks was used to compare patients' and controls' NAA, Cho, Cr, mI and T_f . A separate analysis was conducted for each measure. In each case, the observed values were converted to ranks (to better satisfy underlying distributional assumptions) that were then used as the dependent variable. Each model included age and gender as subject level covariates and subject group (patient versus control) as the between subjects factor. Pearson product moment and Spearman rank correlation coefficients were used to evaluate association of disease duration with each measure among patients. When a significant correlation was detected, least squares regression was used to characterize the change in that measure as function of disease duration. SAS version 9.0 (SAS Institute, Cary, NC) was used for statistical calculations.

RESULTS

Our shim procedure adjusted the whole-head water linewidth to 27 ± 4 Hz full-width at half maximum (FWHM) among the 36 subjects, which improved to 21 ± 3 Hz in the VOI. Examples of the size and placement of the VOI and ^1H spectra are shown in figs. 1 and 2. The SNR, estimated as the peak-height divided by the root-mean-square of the noise in 16,800 voxels (35 subjects $\times$ 480 spectra each) were: NAA = 24.0 ± 10.0 , Cr = 13.8 ± 4.8 , Cho = 13.3 ± 4.8 and mI = 3.8 ± 1.0 (mean $\pm$ standard deviation). The metabolites' FWHM linewidth, $\Delta\omega$, was 5.5 ± 1.2 Hz across the 16,800 voxels reflecting a T_2^* ($=1/\pi\Delta\omega$ assuming Lorentzian lines) of 57.8 ± 13.2 ms.

The 480 summed VOI spectra from each of the 21 patients and 15 controls superimposed with their model functions are shown in fig. 3. Their SNRs range from a low of ~ 75 for mI to over 500 for the NAA. The average linewidth in the sums increased only slightly to 6.8 ± 0.6 Hz versus the individual voxels (*vide supra*). These demonstrate the advantages of the aligned summation strategy: preservation of individual voxel linewidth in the sum and substantial SNR boost. [12]

Average absolute metabolic concentrations obtained from the summed spectra of fig. 3 using Eqs. [1] and [2], T_f -s and lesion volumes are given in table 1. Their box plots (fig. 4) show that the patients' medians: T_f = 0.92 and NAA = 9.6 mM, are *not* significantly different from the controls' 0.94 and 9.6 mM. (Based on their standard deviations the smallest difference detectable with 80% power at the 5% significance level is 0.04 for T_f and 1.25 mM for NAA; alternatively, to establish the significance of these observed differences with 80% power would require larger cohorts: 56 subjects in each for T_f and 1100 for NAA). In contrast, the patients' Cr, Cho and mI levels: 7.7, 1.9, and 4.1 mM were significantly (9%, 14% and 20%, p = 0.0097, 0.003 and 0.0023) *higher* than the controls' 7.1, 1.6 and 3.4 mM even after Bonferroni correction. No correlations were found between any metric and time from diagnosis except a trend (p = 0.06) for Cr increase.

DISCUSSION

Lack of convincing correlation between disability and T_2 lesion load in MS is often attributed to the inability of MRI to account for microscopic NAWM damage.[5] Since lesions comprise on average less than 3% of the brain volume[5] it is possible that milder, but widespread pathology influences disability. Indeed, quantitative metrics that assess all the tissue in a given volume, *e.g.*, the NAA level, correlate better with clinical impairment.[22] Yet, despite its specificity, ^1H -MRS sensitivity is limited by SNR and WM/GM/CSF partial volume. Furthermore, placement of a single VOI over a lesion or NAMW may miss distal damage propagated by Wallerian and retrograde degeneration.[23-25]

To address both SNR and limited coverage we assessed the metabolite levels in a large (360 cm³) VOI of mostly NAWM. Summing all 480 frequency-aligned spectra in the VOI yielded excellent spectral resolution and sufficient SNR to detect subtle metabolic changes. This approach has previously shown decreased T_f and NAA reflecting axonal loss and dysfunction, as well as elevated Cr and Cho in 11 RR MS patients of 6+ years disease duration.[12] Based on those findings our goals were to: *a*) test for the presence of astrogliosis (via its mI marker[26]); *b*) ascertain the temporal relationship between demyelination, gliosis and NAA decline; in order to *c*) test the hypothesis that widespread pathogenesis precedes axonal damage and atrophy.

The results support the hypothesis and suggest the following sequence of disease evolution: *(i)* astrogliosis, possibly accompanied by inflammation, *de*- and *re*-myelination, preceding *(ii)* axonal damage, that is perhaps followed by *(iii)* atrophy.

Diffuse abnormalities

Since our cohort's average VOI lesion load was under 2% (*cf.* table 1), differences of 9%, 14% and 20% for Cr, Cho and mI from controls must reflect widespread NAWM pathology, (fig. 2). Moreover, the metabolic pattern, seen in the global VOI spectra of fig. 3, is similar to that observed in the lesions (*cf.* figs. 1 and 2), known foci of *ongoing* activity.[9, 27] This similarity may suggest that the same processes may also be widespread in the NAWM, early in the disease course, even during remission and despite immunomodulatory treatment. This conjecture is supported by strong correlations of MRS observed elevated mI and Cho with biopsy findings (astrogliosis and inflammation) in lesions,[28] and the well-documented NAWM abnormalities - astrogliosis and inflammation in autopsied tissue.[7]

Glial pathology

While Cr and Cho are found in all brain cells, their levels are highest in mature and progenitor oligodendrocytes and astrocytes.[14] Their elevation, therefore, may represent glial activity: combinations of inflammation, *de*- and *re*-myelination as well as gliosis. Corroboration and specificity is provided by the mI, an osmolyte and constituent of phosphate- and lipid-containing compounds involved in signal transduction in the brain.[29] Its ^1H -MRS peak is thought to originate from intracellular stores in astrocytes[30] and its increase, therefore, indicate astrogliosis,[6] *i.e.*, cell hypertrophy and hyperplasia.[31]

Evidence of glial abnormalities at the earliest stages is offered by the largest (96 patients) ^1H -MRS study of clinically isolated syndrome suggestive of MS (CIS).[32] It reported elevated mI, Cr and Cho (but no NAA decline) most pronounced in patients who also satisfied the McDonald criteria. Indeed, elevated mI suggestive of astrogliosis is a consistent finding in all disease durations.[11, 33, 34] While astroglial scars are known to form on severed axons, the cause of astrogliosis in the context of (MRS inferred) healthy neurons is unclear. It is known that astrocytes directly contact the endothelial cells comprising the blood-brain barrier (BBB) and enhance its impermeability.[35] Both the

subtle BBB breakdown from microscopic inflammation[36] and hypoperfusion[37] that were proposed as possible pathogenic mechanisms may conceivably cause astrogliosis: the former in response to increased leakage through the BBB and (or) micro plaque formation; the latter due to oxygen deprivation, as shown in a rat model of chronic ischemia.[38]

Axonal sparing

NAA's almost exclusive confinement to neurons renders its ^1H -MRS signal a marker of their integrity.[6] Since axonal loss is thought to proceed mainly by Wallerian or retrograde degeneration from lesions,[24, 39] conflicting reports of normal[33, 34] and low[11-13, 40] NAA may reflect different disease course, duration and subjective VOI placement. The last issue was addressed with whole-brain NAA quantification showing average 22% decline already in CIS.[41] However, since over 60% of that NAA signal originates from cortical GM (known to be affected early in MS[11, 40, 42]), that finding is not necessarily in conflict with the NAA levels reported here which reflect mainly WM.

Despite contradictory reports, the concept of eventual NAA decreases,[9] axonal loss[7] and atrophy[2] in NAWM is now generally accepted. We report on glial abnormalities early in the disease course, in the absence of, *i.e.*, prior to overt axonal injury, reflected in patients' NAA level and T_2 not significantly different from the controls'. These findings support the notion that glial processes including astrogliosis precede axonal damage, which in turn precedes tissue atrophy – the final non-specific end point of the various pathologies of this disease.

Based on these findings we conjecture that some axonal damage may arise from astrogliosis and/or inflammation. Indeed, disability's modest correlation with lesion load[7] and histological evidence of axonal loss in the absence of apparent demyelination suggest that not all damage is due to lesions.[43] Indeed, both biopsy and autopsy show that axonal damage correlates better with inflammatory infiltrates than with demyelination.[44, 45] The concept of astrogliosis driven damage is supported by the EAE animal model of MS that shows astrocytic hypertrophy that is spatially and temporally related to axonal damage already in the earliest pre-inflammatory stage.[46]

Caveats

The sensitivity to metabolic changes reported here comes at a price - regional differences, especially between lesions and NAWM, are averaged out. Although the original localized information is still available (see figs. 1 and 2) the proposed strategy reflects a post-processing choice of maximum sensitivity at the expense of localization. Monitoring the global effects of diffuse pathogenesis may make this tradeoff attractive, especially for the much weaker mI signal.

A cautionary note raised by this study underscores the importance of absolute quantification of ^1H -MRS in MS versus use of metabolite ratios to assumed stable Cr levels. This and other reports[34] that show increased Cr and unchanged NAA suggest that lower NAA/Cr (interpreted in the past as "axonal dysfunction") may in fact be due primarily to elevated Cr. Similarly, the 15% Cho increase observed in patients here and in other studies will attenuate to ~5% in a Cho/Cr due to the concomitant Cr increase, reducing the ratio's discriminatory power.

CONCLUSION

Diffuse glial proliferation (elevated mI and Cr) and membrane turnover (high Cho) in the absence of atrophy or axonal injury (normal NAA levels) in RR MS patients suggest ongoing diffuse inflammation, *de*- and (or) *re*-myelination, as well as astrogliosis early in

the disease course, even during clinical remission and despite immunomodulatory treatment. Consequently, mI, Cho and Cr may serve as earlier MRS markers of disease activity than the traditional NAA decline and atrophy that represent in order a later and the final stage of MS pathogenesis.

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Fig. 1.

Left: Sagittal (a) and coronal (b) T₁-weighted and axial T₂-weighted FLAIR (c) MRI of patient #12 in table 1, with the VOI and FOV (solid and dashed white frames) superimposed. Note characteristic periventricular hyperintensities on c (arrows) and little or no atrophy.

Right: Real part of the 8×10 (LR×AP) ¹H spectra matrix from the VOI on c, on common frequency (1.4 to 3.8 ppm) and intensity scales. Note the spectral resolution and SNR in these 0.75 cm³ voxels and elevated Cho and mI at the lesions (black arrows).

Fig. 2.

Axial T_2 -weighted FLAIR image of the patient (#13) with the heaviest lesion load superimposed with the VOI (white frame) and 8×10 (LR $\times$ AP) ^1H spectra on common frequency (1.4 to 3.8 ppm) and intensity scales. Note extensive hyperintensities and visible Cho and mI elevation in voxels within these lesions (arrows) *and* in the NAWM (asterisks), *i.e.*, their overall elevation is truly diffuse *not* merely multifocal.

Fig. 3.

Real part of the ^1H 480 summed spectra from the VOI (black lines) for each of the 36 subjects in table 1: patients (1–21) and controls (22–36 - circled), superimposed with the fitted model functions (gray lines) on common scales. Note: (i) excellent 75 (mI) – 500 (NAA) SNRs and linewidths of the sums compared with the individual voxels in Fig. 1 (benefit of summation and alignment); (ii) the quality of the fit; and (iii) the visual similarity in NAA levels between patients and controls versus elevated Cr, Cho and mI.

Fig. 4. Box plots displaying the 25%, median and 75% (box), 95% (whiskers) and outliers (*) of the NAA, Cr, Cho and ml concentrations as well as tissue-fraction, T_f , distributions in the VOIs of patients and controls. Note the absence of atrophy *and* NAA loss versus significant 9, 14 and 20% elevation of Cr, Cho and ml in the recently diagnosed patients.

Table 1

Demographic, clinical, volumetric and metabolic data for patients (1-21, sorted by disease duration) and controls (22-36).

Subject number	Age/gender	<i>a</i> Disease duration	EDSS	<i>b</i> Lesion volume	<i>c</i> T _r	NAA mM	Cr mM	Cho mM	ml mM
1	38/M	0.3	1	3.7	0.95	10.3	7.4	1.7	4.0
2	39/M	0.3	0	1.6	0.92	8.4	7.0	1.7	4.0
3	26/F	0.9	3.5	10.7	0.97	8.7	7.3	1.7	3.8
4	27/F	0.9	1	5.1	0.92	9.2	7.4	1.6	3.4
5	35/F	1.5	1.5	2.4	0.89	9.3	8.1	1.9	4.2
6	38/F	1.5	0	1.7	0.95	7.7	6.5	1.6	3.5
7	30/M	1.6	0	8.3	0.93	8.8	7.2	1.9	3.4
8	35/F	1.6	1	2.7	0.94	10.9	7.0	2.2	4.0
9	37/F	1.9	0	11.4	0.88	8.4	7.6	1.9	4.6
10	34/M	2.0	2	2.6	0.89	11.8	8.5	1.9	5.5
11	26/F	2.1	*4.0	1.6	0.84	11.8	8.2	2.4	3.1
12	42/F	2.1	0	4.2	0.98	9.0	7.5	1.9	3.6
13	21/F	2.3	1.5	23.2	0.94	9.0	8.3	2.0	5.5
14	28/M	2.3	1	5.7	0.97	8.5	7.3	1.9	4.4
15	32/F	2.7	2	7.3	0.90	8.8	7.9	1.8	4.8
16	29/M	3.2	1	4.9	0.88	11.3	8.5	2.1	3.7
17	29/F	3.4	2.5	16.5	0.85	8.8	6.8	1.7	4.0
18	27/F	4.0	2	11.2	0.94	11.3	9.0	2.1	4.2
19	42/F	4.5	1	1.2	0.90	9.9	8.2	1.8	4.3
20	38/F	4.9	2	2.2	0.94	11.1	8.7	2.0	3.9
21	31/F	5.2	2	2.8	0.94	8.9	7.1	1.6	3.7
22	20/F	-	-	-	0.93	9.5	6.8	1.8	3.5
23	25/F	-	-	-	0.97	11.1	7.8	2.0	2.2
24	26/F	-	-	-	0.93	9.7	7.0	1.7	3.9
25	26/F	-	-	-	0.90	11.3	8.2	1.8	4.2
26	27/M	-	-	-	0.96	9.0	6.5	1.6	3.3
27	28/M	-	-	-	0.94	7.9	5.7	1.4	3.1

Subject number	Age/gender	^a Disease duration	EDSS	^b Lesion volume	^c T _f	NAA mM	Cr mM	Cho mM	ml mM
28	28/F	-	-	-	0.94	10.3	8.2	1.7	3.2
29	28/F	-	-	-	0.87	9.0	7.9	1.7	3.9
30	29/F	-	-	-	0.95	7.9	6.5	1.2	3.1
31	29/F	-	-	-	0.94	9.8	6.9	1.8	3.1
32	31/F	-	-	-	0.91	8.7	6.2	1.4	3.1
33	35/M	-	-	-	0.94	8.3	6.6	1.5	3.8
34	36/F	-	-	-	0.95	11.8	8.1	1.7	4.0
35	37/F	-	-	-	0.99	8.8	6.7	1.6	3.8
36	44/F	-	-	-	0.92	10.4	7.2	1.8	3.0
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Ctrl. avg.	30	-	-	-	0.94±0.03	9.6±1.2	7.1±0.8	1.6±0.2	3.4±0.5
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Pat. avg.	33	2.3	1.38	6.2	0.92±0.04	9.6±1.3	7.7±0.7 ^d	1.9±0.2 ^d	4.1±0.6 ^d

The horizontal line separates patients (above) from controls (below).

^aYears

^binside the VOI, in cm³

^ctissue fraction

^d*p*<0.01.

* EDSS dominated by permanent visual impairment in one eye.