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ORIGINAL COMMUNICATION

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High field MR imaging and ^{1}H-MR spectroscopy in clinically isolated syndromes suggestive of multiple sclerosis

Correlation between metabolic alterations and diagnostic MR imaging criteria

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Abstract *Purpose* To prospectively investigate metabolic changes in the normal-appearing white matter (NAWM) of patients presenting with clinically isolated syndromes (CIS) suggestive of multiple sclerosis (MS) and to correlate these changes to conventional MR imaging findings in terms of MR imaging criteria. *Materials and methods* Multisequence MR imaging of the brain and ^{1}H-MR spectroscopy of the parietal NAWM were performed in 31 patients presenting with CIS and in 20 controls using a 3.0 T MR system. MR imaging criteria and International Panel criteria were assessed based on imaging, clinical and paraclinical results. Metabolite ratios and absolute concentrations of *N*-acetyl-aspartate (tNAA), myo-inositol (Ins), choline (Cho), and total creatine (tCr) were determined. The metabolite concentrations were correlated with the fulfilled MR imaging criteria. *Results* In comparison to the control group, the CIS group showed significantly decreased mean tNAA concentrations (–8.1 %, p = 0.012). Significant changes could not be detected regarding Ins, tCr and Cho. No significant correlations between absolute metabolite concentrations and MR imaging criteria were observed. Patients with and without a lesion dissemination in space showed no significant differences of their metabolite concentrations. *Conclusion* As assessed by ^{1}H-MRS a significant axonal damage already occurs during the first demyelinating episode in patients with CIS. Conventional MR imaging in terms of diagnostic imaging criteria does not significantly reflect NAWM disease activity in terms of metabolic alterations detected by ^{1}H-MR spectroscopy.

Key words Multiple sclerosis · clinically isolated syndromes · diagnostic criteria · MRI · MR spectroscopy

Introduction

Magnetic resonance (MR) imaging is the most important paraclinical tool for the detection of white and grey matter pathologies in patients with suspected or definite multiple sclerosis (MS) [1, 2]. In patients presenting with clinically isolated syndromes (CIS) suggestive of the first demyelinating event of MS, MR imaging has an important predictive value in terms of clinical disability, brain atrophy and the conversion to definite MS [3–5]. For the latter purpose, several MR imaging criteria have been proposed. Among these criteria the modified Barkhof MR imaging criteria have been incorporated into the International Panel (IP) criteria for multiple sclerosis in order to demonstrate a lesion dissemination in space (DIS) [5–7].

Conventional MR imaging has a limited value in the investigation of grey and white matter pathologies in the so-called normal-appearing white (NAWM) and grey

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matter (NAGM). This has prompted the use of several quantitative in vivo MR methods in MS including proton MR spectroscopy (^{1}H-MRS) [1, 8], magnetization transfer ratio [9, 10], diffusion tensor imaging [11, 12] and T1 relaxation time measurements [13, 14]. ^{1}H-MRS in the NAGM and NAWM of patients with MS has conclusively demonstrated significantly decreased concentrations of total N-acetyl compound (tNAA) indicating a substantial axonal loss even in early disease stages which was highly correlated with clinical disability [8, 15–18]. Increased NAWM concentrations of myo-insitol (Ins), which are highly indicative of an increased glial cell and inflammatory disease activity, are also frequently observed in MS patients and are linked to higher lesion load measurements and functional impairment [8, 19–23].

Besides patients with definite MS, metabolic alterations in patients presenting with CIS are increasingly becoming of important interest. In particular, these metabolic changes in terms of disease activity not visible on conventional MR imaging might be helpful to narrow the differential diagnosis and to identify patients with higher risks for developing definite MS. Recent studies focussing on CIS patients revealed higher Ins levels but showed no evidence for a significant axonal damage in terms of decreased tNAA concentrations when compared to healthy controls [24, 25]. Although NAWM metabolic changes are correlated with lesion load on conventional MR imaging in patients with definite MS, the correlation between metabolic alterations and fulfilled MR imaging criteria in patients presenting with CIS has not been investigated so far.

This study aimed to correlate metabolic changes within the NAWM of patients presenting with CIS to conventional MR imaging findings in terms of the classification according to MR imaging and IP criteria for MS.

Materials and methods

Patients and healthy controls

This study was designed as a prospective clinical trail and the complete study protocol was approved by our institutional review board. The study has been performed according to the ethical standards laid down in the 1964 Declaration of Helsinki. A written informed consent was obtained from all patients and healthy controls after the study had been explained to them.

The inclusion criteria were defined as follows: 1) first presentation with a CIS suggestive of a demyelinating episode of the central nervous systems as defined by the International Panel on the diagnosis of MS [7]; 2) age between 18 and 56 years; 3) no evidence for other immunological, malignant or vascular diseases in the present and past medical history, 4) no immunosuppressive and/or immune-modulating treatment during the past month; 5) performance of the MRI and MRS examination within 2 months after the clinical event and baseline MR imaging assessment.

Thirty-one patients (22 female, 9 male, median age 35 years, range 18–55) were included in this study. All patients were recruited from the MS outpatient clinic of our Department of Neurology. Additionally 20 healthy controls (11 female, 9 male; median age 29 years, range 22 to 40 years) were selected.

Clinical assessment

All patients underwent a neurological examination including the assessment of the Expanded Disability Status Scale (EDSS) [26] which was performed by one experienced neurologist prior to the MR examination. Further neurological assessment included the determination of cerebrospinal fluid (CSF) parameters (including the assessment of cellularity, protein level, intrathecal IgG synthesis and oligoclonal bands by isoelectric focussing) and visual evoked potentials. All patients were asked about any neurological events and malignant, vascular, infectious or other immunological diseases in their past medical history. Additionally, all major differential diagnoses of CIS and MS were excluded by appropriate tests.

MR imaging

MR examinations were performed using a 3.0 T whole body MR system (Gyroscan Intera, Philips Medical Systems, Best, the Netherlands) and applying an 8-element phased array SENSE head coil. The MR system was equipped with gradients with a maximum slew rate of 150 mT/m/ms and a maximum strength of 30 mT/m. The MR imaging protocol was based on a multisequence study protocol including 21 contiguous sagittal sections of a T2 Turbo spin echo (T2 TSE) and 24 contiguous axial sections of a T2 TSE, fluid-attenuated inversion recovery (FLAIR) as well as a pre- and postcontrast (gadolinium-diethylene-triaminepentaacetic acid, 0.1 mmol/kg body weight) T1-weighted spin echo (SE). Detailed sequence parameters are given in Table 1. Anatomical scan orientation and repositioning was performed according to the guidelines of the Consortium of MS Centers [27].

The image analysis was performed on a digital workstation by two experienced radiologists in consensus since a low interobserver agreement for the assessment of the MRI and consequently IP criteria has been reported [28]. Both readers were blinded to the clinical presentation and paraclinical data of all participants. Considering the T2 TSE and FLAIR images all high-signal lesions with a size ≥ 3 mm were counted and categorized according to the anatomic locations with special regard to the infratentorial, juxtacortical, periventricular and deep white matter regions. The gadolinium-enhanced T1 SE images were scored for the number of enhancing lesions. Based on the combination of the imaging findings all patients were scored according to the MR imaging criteria. In a second step, all patients were classified as fulfilling or not fulfilling the criterion of DIS according to the IP criteria based on a combination of imaging findings, clinical presentation and CSF parameters. DIS was defined according to the IP criteria as follows: polysymptomatic CIS, ≥ 3 fulfilled MRI criteria, ≥ 2 T2-lesions in combination with chronic inflammatory CSF findings.

^{1}H-MRS

The ^{1}H-MRS was performed directly after the precontrast MR imaging protocol using the quadrature mode of the 8-element phased array SENSE head coil. In the patient and in the control group, a single voxel ^{1}H-MRS was acquired from the NAWM of the centrum semiovale region using a voxel size of 2.5 × 2.0 × 1.6 cm (8 cm^3) in either the right or the left hemisphere since hemispheric differences in white matter metabolite concentration have been excluded [29]. Fig. 1 gives an example of the voxel positioning.

The ^{1}H-MRS acquisition was performed with a standardized point-resolved spectroscopy (PRESS) sequence using a repetition time of 2000 ms, echo times of 38 and 140 ms. 1024 samples, a band-

Table 1 MR imaging sequence parameters

Parameter	T2 TSE	FLAIR	T2 TSE	T1 SE ± Gd.
Orientation	sagittal	axial	axial	axial
Sections	21	24	24	24
Matrix	256	256	256	256
slice thickness [mm]	2	5	5	5
Turbo factor	16	38	16	–
Repetition time [ms]	3575	12000	4100	500
Echo time [ms]	100	140	100	12
Inversion delay [ms]	–	2850	–	–
Number of signals averaged	1	1	1	1
Measured voxel size [mm]	0.90/0.90/2	0.90/0.90/5	0.90/0.90/5	0.90/0.90/5

TSE Turbo spin echo; *FLAIR* fluid-attenuated inversion recovery; *SE* spin echo; *Gd.* gadolinium-diethylene-triaminepentaacetic acid

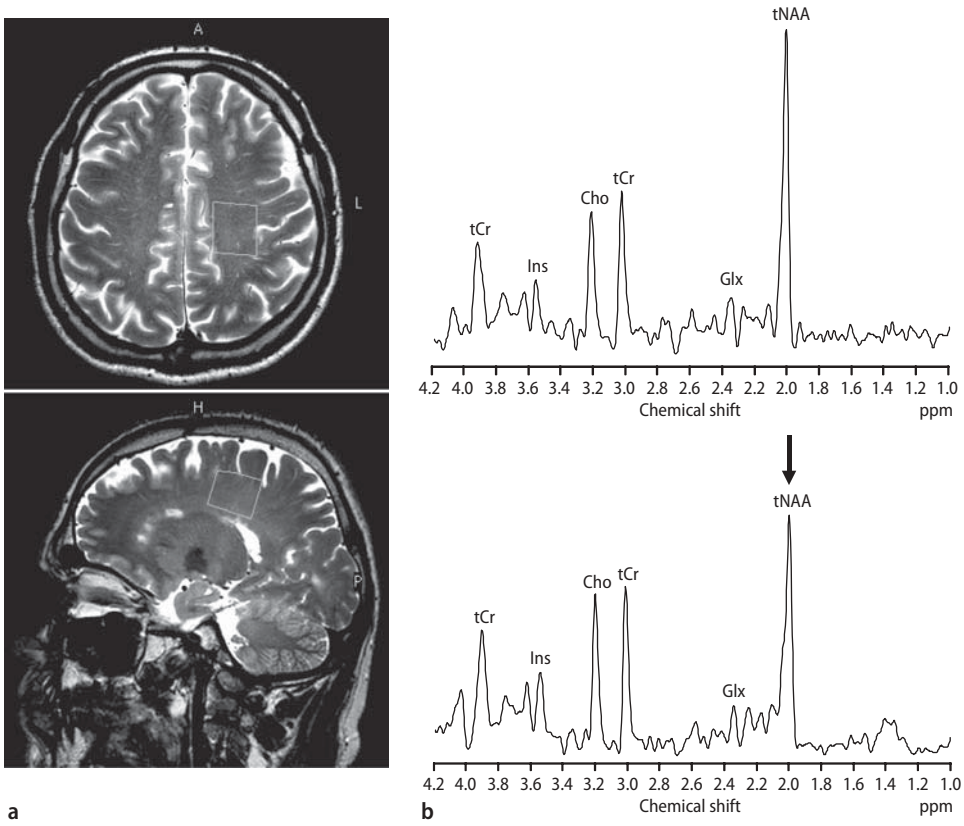

Fig. 1 **a** Axial and sagittal T2-weighted images from a patient presenting with CIS (brainstem syndrome), demonstrating the voxel positioning within the NAWM area. **b** ^{1}H-MR spectra (TR/TE2000/38 ms) obtained from a healthy control (above) and a CIS patient (below) matched for age and gender (vertical scaling normalized to tCr signal). Note the pronounced decrease of tNAA (arrow) when compared to the control subject, whereas all other metabolites show no observable changes

width of 2 Hz/pixel, and 96 signal averages. Standard automated "high-order" B_0 shimming was performed up to the second order. The tNAA signal was referenced to the internal water signal in unsuppressed spectra using a repetition time of 3500 ms, echo time of 140 ms, and 32 signal averages. A correction for spatial CSF volume within the selected voxel was performed by unsuppressed T2 relaxometry (repetition time 5000 ms, seven different echo times ranging from 40 to 750 ms). The measurement of the T2 relaxation times of tNAA, tCr, and Cho was obtained by a water-suppressed acquisition (repetition time 2500 ms, 6 sets of different echo times ranging from 50 to 425 ms, 16 signal averages). ^{1}H-MRS post-processing included time domain analysis performed by the MRUI software package [30]. Twenty-two spectral components in the metabolite spectra at the shorter echo time were quantified using the AMARES algorithm [31].

Absolute concentrations of tNAA, Ins, choline (Cho) and total creatine (tCr) were determined as well as relative signal ratios of tNAA/tCr, tNAA/Cho, Ins/tNAA, Cho/tCr, Ins/tCr, and Cho/tCr.

Statistical analysis

All statistical analyses were performed by the SPSS software package (SPSS, Inc., Chicago, IL, USA). p values < 0.05 were considered as statistically significant.

The significance of differences in NAWM metabolite concentrations between the CIS patient group and the healthy control subjects was assessed by the Mann-Whitney-U test. The correlation between the metabolite concentrations (median values) and the correspond-

ing MR imaging criteria was calculated using the Spearman's correlation. The comparison between the metabolic changes and the fulfilled criterion of DIS was estimated by a calculation of a relative comparison between the patients who do fulfil the criteria of DIS vs. those patients who do not. The corresponding p value was calculated by the Mann-Whitney-U test.

Results

Clinical presentation

Among the 31 CIS patients, 18 patients presented with unilateral optic neuritis, 6 patients with brainstem syndromes, 5 patients with spinal cord syndromes, and 2 patients with polysymptomatic CIS. At the time of the MRS examination the median EDSS was 1.5 (range 0–3) and the median disease duration was 28 days (range 2–48 days).

Conventional MR imaging findings

No abnormalities on the MR imaging could be observed in the healthy control group.

In 2 patients presenting with CIS, the MR imaging scan showed no acute and/or chronic inflammatory brain lesions. 18 of the remaining 29 patients presenting with inflammatory lesions on MRI fulfilled the criteria for a DIS according to the IP criteria. None of the study patients had lesion dissemination in time (DIT) neither clinically nor on MRI when compared to the baseline assessment which was performed shortly after the clinical event.

The exact distribution of the fulfilled MR imaging criteria among the study group is summarized in Table 2.

Table 2 Characterization of the CIS patients according to the fulfilled MR imaging criteria

Fulfilled MR imaging criteria	Number of patients (n)	Clinical presentation
0	5	Unilateral optic neuritis (n = 3) Brainstem syndrome (n = 2)
1	10	Unilateral optic neuritis (n = 6) Brainstem syndrome (n = 2) Spinal cord syndrome (n = 2)
2	5	Unilateral optic neuritis (n = 2) Brainstem syndrome (n = 2) Spinal cord syndrome (n = 1)
3	5	Unilateral optic neuritis (n = 4) Spinal cord syndrome (n = 1)
4	6	Unilateral optic neuritis (n = 3) Spinal cord syndrome (n = 1) Polysymptomatic CIS (n = 2)

^{1}H-MRS

The NAWM metabolic concentrations of the patients presenting with CIS compared to the healthy controls are presented in Table 3. The concentration values and ratios in our healthy control group have been described in detail elsewhere [32]. Typical spectra from a patient presenting with CIS and from a healthy control are illustrated in Fig. 1.

The CIS patient group showed significantly decreased tNAA concentrations (–8.1 %; p = 0.012) as well as ratios tNAA/Cho and tNAA/Cr when compared to the healthy controls. All other absolute and relative metabolite concentrations in the NAWM of the CIS patients did not show any significant changes compared to the controls.

Correlation of NAWM metabolite concentrations and diagnostic MR imaging criteria

The results of the correlation between the absolute metabolite concentrations within the NAWM of the CIS patients and the corresponding number of fulfilled MR imaging criteria obtained by conventional MR imaging are summarized in Table 4.

Table 3 NAWM metabolite concentrations in patients presenting with CIS in comparison to normal controls

NAWM Metabolites	CIS (n = 31)*	Relative Comparison**
tNAA [mM]	**13.33 (± 1.37)** **13.47 (10.55–16.60)**	**–8.1 %** **p = 0.012**
Ins [mM]	3.87 (± 0.90) 3.88 (2.59–6.08)	+ 3.2 % p = 0.735
Cho [mM]	2.16 (± 0.40) 2.20 (1.40–2.90)	+ 4.3 % p = 0.316
tCr [mM]	7.29 (± 1.24) 7.22 (5.13–10.58)	+ 4.3 % p = 0.446
tNAA/Cho	**2.28 (± 0.43)** **2.24 (1.36–3.10)**	**–11.6 %** **p = 0.035**
tNAA/tCr	**2.56 (± 0.33)** **2.59 (3.27–1.97)**	**–8.9 %** **p = 0.010**
Ins/tCr	0.53 (± 0.13) 0.52 (0.37–0.97)	–1.9 % p = 0.482
Ins/tNAA	0.28 (± 0.08) 0.25 (0.18–0.48)	+ 3.2 % p = 0.347
Cho/tCr	1.14 (± 0.16) 1.10 (0.88–1.45)	+ 2.7 % p = 0.623

* Data are expressed as mean values (± standard deviation) and as median values (range). ** Data and p values represent relative comparisons between the control group and the CIS patient group. p values were obtained by the Mann-Whitney U test. Results printed in bold are significantly different ($p < 0.05$) when compared to the control group. *NAWM* normal appearing white matter; *CIS* clinically isolated syndromes; *MS* multiple sclerosis; *tNAA* summation of N-acetyl-aspartate and N-acetyl-aspartyl-glutamate; *Ins* myo-inositol; *Cho* choline; *tCr* summation of creatine and phosphocreatine

Table 4 Correlation between the NAWM metabolic changes and the corresponding fulfilled MR imaging criteria

Metabolite	MR imaging criteria	
	Correlation coefficient*	Significance**
tNAA	–0.263	0.153
Ins	0.311	0.094
Cho	0.007	0.971
tCr	0.339	0.062

* Correlation was estimated using the Spearman correlation coefficient
** Data represent p values regarding the Spearman correlation

No significant correlation was found between any of the measured metabolite concentrations and the number of fulfilled MR imaging criteria. A weak to moderate correlation could be identified concerning tCr, tNAA and Ins (Fig. 2), which however did not reach a significant level.

The results of the comparison between the metabolic concentrations and the IP criteria concerning DIS are given in Table 5. The patient group having DIS showed slightly higher absolute concentrations of Ins, tCr and Cho, as well as lower absolute concentration of tNAA, when compared to those patients without DIS. None of these changes showed a statistical significance.

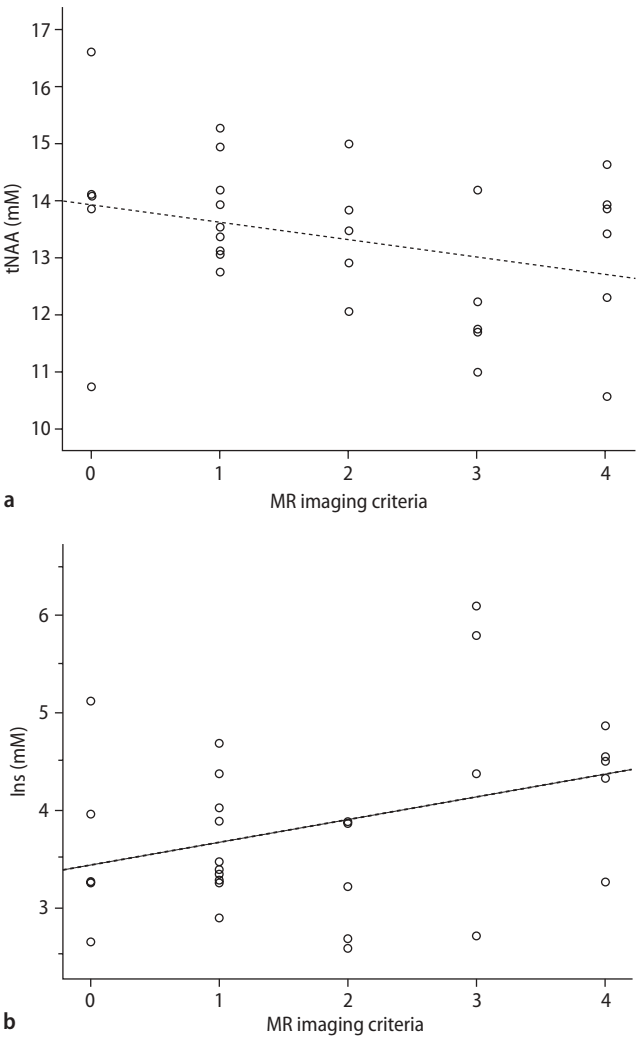

Fig. 2 Plots of the absolute concentration of tNAA (**a**) and Ins (**b**) vs. fulfilled MR imaging criteria indicating no significant correlation between conventional imaging findings in terms of imaging criteria and MR spectroscopy findings regarding the clinically relevant metabolites for axonal damage and increased glial cell activity

Discussion

This is the first study in patients presenting with CIS suggestive of MS which correlates conventional MR imaging findings in terms of MR imaging criteria with metabolic changes in the NAWM using a high field MR system.

High field MR imaging systems are increasingly incorporated into the clinical setting and it has recently been shown that MR imaging at higher magnetic field strengths up to 4 T provides higher lesion load measurements in patients with CIS as well as in patients with definite MS [1, 33, 34]. In patients presenting with CIS, these higher lesion load measurements have substantial consequences for the classification according to current MR imaging criteria [35]. ^{1}H-MRS at field strengths higher than 1.5 T offers a better signal-to noise ratio and improved spectral separation, leading to a more precise quantification and an increased sensitivity and reproducibility [36, 37]. However, experiences regarding the use of high field ^{1}H-MRS in CIS and/or MS patients are limited [23].

Compared to healthy controls, our CIS study population showed significantly decreased absolute concentration of tNAA. Given the fact that axonal damage is constantly detected in patients with definite MS, first evidence for axonal damage already occurring during the first demyelinating episode has been reported in NAWM of CIS patient group with a longer median disease duration and by a whole brain MR spectroscopy analysis incorporating lesions as well as normal appearing brain tissue [32, 38]. In addition to this, the significantly decreased tNAA concentrations in our study are highly indicative for a substantial axonal damage even in the NAWM of CIS patients with disease duration of approximately one month. Interestingly, besides tNAA no other metabolite concentration, including tCr, Cho and Ins, which is frequently altered in the NAWM of patients with definite MS, shows a significant change. Concerning the absolute concentration of Ins, which corresponds well to the glial cell activity, a slight, albeit not significant elevation was observed in our study. In contrast to our results, two recent studies have found signif-

Table 5 Comparison of patients fulfilling (n = 18) and not fulfilling the criterion of DIS (n = 13) regarding the absolute metabolite concentrations

Metabolite	DIS (n = 31)	Metabolite concentration*	Significance**
tNAA	No DIS	13.54 (10.72–16.60); 13.57 ± 1.29	p = 0.603
	DIS	13.24 (10.55–15.27); 13.15 ± 1.43	
Ins	No DIS	3.87 (2.66–5.12); 3.67 ± 0.66	p = 0.325
	DIS	4.33 (2.59–6.08); 4.03 ± 1.04	
tCr	No DIS	7.12 (4.96–8.93); 7.12 ± 1.07	p = 0.689
	DIS	7.23 (5.13–10.58); 7.46 ± 1.40	
Cho	No DIS	2.12 (1.40–2.67); 2.07 ± 0.37	p = 0.378
	DIS	2.29 (1.51–3.03); 2.24 ± 0.43	

* Data are median values (range) and mean values (± standard deviation).
** p values represent the significance of the relative comparison regarding the metabolite concentrations between patients fulfilling and not fulfilling the criterion of DIS according to the International Panel diagnostic criteria based on a combination of imaging, clinical and CSF findings. p values were obtained by the Mann-Whitney U test

icant metabolic alterations in terms of an elevation of Ins in the NAWM of CIS suggesting increased glial cell activity, however, neither study showed any evidence for significant axonal damage [24, 25]. Both studies were performed at 1.5 T; one study considered relative quantification in terms of metabolite ratios, whereas the other study used a single short echo time acquisition. In contrast to this, our ^{1}H-MRS acquisition was performed at an early and a late echo time which allows a reproducible quantification of both the tNAA and Ins resonance.

Although conventional MR imaging including the measurement of brain atrophy is still the most important MR method for establishing the MS diagnosis and disease monitoring [1, 39], the correlation of visible lesion load with clinical and prognostic features is rather limited [40]. Consequently, MR imaging criteria for MS do not only focus on the amount of T2-lesions, but also include the distribution and anatomic location of lesions, which has a high predictive value concerning the conversion to definite MS and the development of long-term disability [3, 5]. Complementary to this, axonal damage in the NAWM proven by decreased tNAA using MR spectroscopy also highly correlates to clinical disability even in early stages of MS [16–18]. Putting both MR methods together, in patients with definite MS, a correlation between T2-lesion load and NAWM metabolic changes was only observed for Ins [8]. Regarding conventional MR imaging in terms of the number of fulfilled MR imaging criteria which has a prognostic value compared to the pure T2-lesion load, we could not identify any significant correlation between the number of fulfilled MR imaging criteria and the absolute metabolite concentrations in the NAWM of our CIS patient group. The lack of a correlation between NAWM metabolic alterations and current diagnostic imaging criteria is even evident beyond MR imaging. Concerning the IP criteria for MS, which are based on a combination of imaging findings, clinical presentations and paraclinical testing such as CSF parameters, no significant correlation between the fulfilled criterion on DIS and absolute metabolic concentrations could be identified. In other words, MR imaging and consequently diagnostic criteria do not necessarily correspond to the clinical disease activity beyond the visible brain lesions. However, we have to take into account that MR imaging criteria were developed for prognostic purposes in order to predict the conversion to definite MS and not estimate the clinical and subclinical disease activity in the NAWM [5, 6].

Nevertheless, conventional MR imaging and consequently current MR imaging criteria do not sufficiently reflect special aspects of disease activity, in particular grey matter pathology which occurs abundantly in MS [41]. However, grey matter changes in MS can not be sufficiently visualized on conventional MR imaging techniques but may substantially contribute to metabolic changes (e.g. axonal damage) in the normal-appearing brain tissue.

Finally, the lack of correlation between the fulfilled MR imaging and the metabolic alterations in CIS in our study does not necessarily lead to the conclusion that the predictive value of MR spectroscopy findings is poor. Similar to recent approaches in early relapsing-remitting MS [42], long-term follow-up studies including conventional MR imaging and ^{1}H-MRS are necessary in order to investigate the potential value of ^{1}H-MRS in the prediction of the conversion to definite MS and clinical disability in correlation to MR imaging criteria in patients presenting with CIS suggestive of MS.

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