

# Marker of disease progression in patients with relapsing remitting multiple sclerosis using 2D MRSI

Adriane Gröger<sup>1</sup>, Vinzenz Fleischer<sup>1</sup>, Rupert Kolb<sup>2</sup>, Uwe Klose<sup>2</sup>, and Frauke Zipp<sup>1</sup>

<sup>1</sup>Department of Neurology and Neuroimaging Center (NIC), Mainz, Germany, <sup>2</sup>Department of Diagnostic and Interventional Neuroradiology, Magnetic Resonance Research Group, Tübingen, Germany

## Introduction

Multiple sclerosis (MS) is a heterogeneous inflammatory disease characterized by demyelination, axonal degeneration and neuronal loss. Lack of correlation between disability and T2 lesion load is often due to the inability of MRI to account for microscopic normal-appearing white matter (NAWM) damage. However, MRSI can provide insight into metabolic tissue compositions [1], [2], [3] and may be relevant as a marker for disease progression [4]. Here, metabolic alterations in the brain of relapsing-remitting MS (RRMS) patients with mild and fulminant disease progression were studied to identify reliable markers of disease severity.

## Material and Methods

2D MRSI data were acquired from 33 RRMS patients (19 with baseline therapy, 14 with escalation therapy, i. e. natalizumab) and 20 normal controls (Tab. 1) using a PRESS sequence (TE/TR = 30/1350 ms, nominal voxel volume = 1 cm<sup>3</sup>) with water saturation at 3 T. The volume of interest (VOI) was exactly localized above the corpus callosum in all subjects (Fig. 1). MRSI data were analyzed using LCModel to estimate NAWM and cortical grey matter (GM) metabolite levels. All model spectra were generated based on reported chemical shifts and coupling constants. Macromolecule and lipid spectra were acquired from the same VOI using an inversion recovery sequence and included in basis data set. Metabolite quantifications were corrected for possible differences in the T<sub>2</sub>\* values (tissue myelin and iron contents) [5] by estimating the line width during LCModel analysis. One-way ANOVA with Tukey post hoc tests were used to compare the metabolite concentrations in 4 central NAWM and 4 cortical GM voxels (Fig. 1) between the three groups. Moreover, metabolite concentrations were compared between patients and controls using linear regression models allowing for age, disease duration, and expanded disability status scale (EDSS) scores.

Tab. 1: Demographic data (given in median and range).

Groups	Number	Age (years)	Sex (m/f)	Disease duration (months)	EDSS
Controls	20	30 (24-54)	11/9	-	-
RRMS with baseline therapy	19	27 (15-54)	5/14	19 (2-104)	0 (0-2.0)
RRMS with escalation therapy	14	34.5 (18-50)	5/9	72 (17-284)	1.5 (0-3.5)
p value	-	0.510	0.176	0.004	0.001

were significant for both patient groups compared to controls, whereas the elevations in WM Glx, WM GSH, and WM MM+Lip13 were only significant for patients with escalation therapy. In addition to known age-related changes, correlations of metabolite concentrations with disease duration were significant for GM NAA ( $r=0.343$ ,  $p=0.025$ ), GM MM+Lip13 ( $r=0.498$ ,  $p=0.002$ ), WM Cr ( $r=0.430$ ,  $p=0.006$ ), WM Ins ( $r=0.430$ ,  $p=0.006$ ), and WM MM+Lip13 ( $r=0.394$ ,  $p=0.012$ ), and with EDSS scores for GM Cr ( $r=0.434$ ,  $p=0.006$ ), GM Ins ( $r=0.266$ ,  $p=0.041$ ), WM Cr ( $r=0.430$ ,  $p=0.005$ ), WM Ins ( $r=0.338$ ,  $p=0.027$ ), and WM MM+Lip13 ( $r=0.320$ ,  $p=0.035$ ).

Tab. 2: Metabolite concentrations [arbitrary units] (given in mean and standard deviation) with Cramer-Rao lower bounds estimated in grey and white matter of RRMS patients and controls.

	Grey matter			White matter		
	Controls (n = 20)	RRMS with baseline therapy (n = 19)	RRMS with escalation therapy (n = 14)	Controls (n = 20)	RRMS with baseline therapy (n = 19)	RRMS with escalation therapy (n = 14)
SNR	29 $\pm$ 4.0	29 $\pm$ 3.5	29 $\pm$ 4.6	29 $\pm$ 3.5	28 $\pm$ 3.8	28 $\pm$ 3.4
FWHM (ppm)	0.023 $\pm$ 0.007	0.023 $\pm$ 0.006	0.023 $\pm$ 0.004	0.026 $\pm$ 0.005	0.025 $\pm$ 0.005	0.026 $\pm$ 0.006
NAA+NAAG (CRLB)	88.9 $\pm$ 8.3 (2%)	92.2 $\pm$ 8.7 (2%)	88.5 $\pm$ 9.3 (2%)	99.2 $\pm$ 7.4 (2%)	102.2 $\pm$ 9.9 (2%)	99.0 $\pm$ 6.9 (2%)
GPC+PCh (CRLB)	10.0 $\pm$ 1.4 (3%)	10.6 $\pm$ 1.5 (3%)	10.6 $\pm$ 8.9 (3%)	11.5 $\pm$ 1.6 (3%)	12.3 $\pm$ 2.0 (3%)	12.5 $\pm$ 1.4 (3%)
Cr+PCr (CRLB)	61.9 $\pm$ 6.4 (2%)	65.0 $\pm$ 7.4 (2%)	67.0 $\pm$ 4.6 (2%)	56.4 $\pm$ 5.7 (2%)	61.5 $\pm$ 7.5 (2%)	65.2 $\pm$ 5.3 (2%)
Glu+Gln (CRLB)	77.2 $\pm$ 10.0 (8%)	79.3 $\pm$ 9.5 (8%)	80.4 $\pm$ 9.3 (8%)	31.5 $\pm$ 7.3 (33%)	35.6 $\pm$ 7.8 (17%)	41.2 $\pm$ 9.1 (16%)
Ins (CRLB)	52.5 $\pm$ 9.1 (6%)	59.0 $\pm$ 9.0 (6%)	60.9 $\pm$ 6.5 (6%)	44.0 $\pm$ 8.5 (6%)	54.0 $\pm$ 11.1 (6%)	59.1 $\pm$ 9.8 (5%)
GSH (CRLB)	16.4 $\pm$ 1.9 (9%)	17.1 $\pm$ 1.4 (9%)	16.8 $\pm$ 1.0 (9%)	14.7 $\pm$ 1.8 (10%)	15.6 $\pm$ 1.6 (10%)	16.1 $\pm$ 1.5 (9%)
MM09+Lip09 (CRLB)	105.6 $\pm$ 10.3 (14%)	108.8 $\pm$ 18.0 (14%)	107.6 $\pm$ 17.2 (13%)	97.4 $\pm$ 10.1 (14%)	103.7 $\pm$ 14.2 (14%)	107.0 $\pm$ 19.3 (14%)
MM12+Lip13+MM14 (CRLB)	135.6 $\pm$ 27.8 (16%)	140.7 $\pm$ 24.1 (17%)	144.3 $\pm$ 55.1 (17%)	120.5 $\pm$ 16.8 (18%)	136.5 $\pm$ 23.7 (16%)	160.4 $\pm$ 73.3 (15%)
MM20+Lip20 (CRLB)	84.6 $\pm$ 10.9 (12%)	83.2 $\pm$ 10.7 (14%)	85.2 $\pm$ 12.1 (13%)	72.8 $\pm$ 12.2 (15%)	75.1 $\pm$ 9.3 (15%)	76.9 $\pm$ 9.4 (14%)

## Discussion

RRMS patients with baseline therapy as well as with escalation therapy showed significant elevations in Cr and Ins (markers for neuroinflammation and microglia activation) [6], whereas significant increases in Glx (suggesting a role of excitotoxicity), GSH (marker for oxidative stress) and mobile macromolecules and lipids (marker for de- and remyelination processes) [7] were found only in RRMS patients with escalation therapy relative to controls. On the other hand, as in Kirov et al. [8], we did not find a reduction in NAA (putative marker of viable neurons). These findings of increased Cr and Ins levels but not different NAA levels suggest that inflammation processes are more strongly associated with early disease than neuronal loss that is correlated with disease duration. Furthermore, the higher Glx and GSH levels in RRMS patients receiving escalation therapy (no alter metabolites pre to post treatment with natalizumab [9]) can be interpreted as a marker for more fulminant disease progression but not for disease duration.

## References

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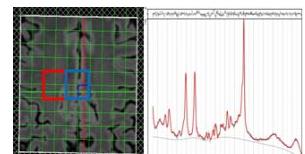


Fig. 1: 2D MRSI voxel localizations for white (red) and grey matter (blue) with analyzed spectrum from one voxel.

## Results

Estimated metabolite concentrations with Cramer-Rao lower bounds (CRLB, estimated error of metabolite quantification) less than 20 % are given in Tab. 2. Significant between-group differences were found for WM creatine (Cr) ( $p=0.001$ ), WM myo-inositol (Ins) ( $p<0.001$ ), WM glutamine + glutamate (Glu+Gln=Glx) ( $p=0.004$ ), WM glutathione (GSH) ( $p=0.042$ ) and WM macromolecules and lipids around 1.3 ppm (MM+Lip13) ( $p=0.028$ ), as well as for GM Ins ( $p=0.011$ ). Furthermore, the increases in GM Ins, WM Ins, and WM Cr