Quantitative proton MR spectroscopy of non-enhancing lesions and pre-lesional tissue in early multiple sclerosis

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BACKGROUND AND PURPOSE: Lesions, the radiological hallmark of multiple sclerosis (MS), are focal regions of white matter (WM) hyperintensities in T2-weighted MR images of the brain. Lesion number, volume, contrast enhancement and degree of T1 hypointensity are the gold standard in diagnosis, prognosis, and treatment monitoring. Conventional MRI captures the cumulative effect of a wide range of pathological processes, yielding high sensitivity, but in the case of non-enhancing lesions, no specificity for distinguishing between subtle inflammation, gliosis, de-/re-myelination and neuronal injury. A more direct way to assess the underlying pathophysiology is with proton MR spectroscopy (^{1}H-MRS) through levels of *N*-acetylaspartate (NAA), creatine (Cr), choline (Cho) and *myo*-inositol (mI). Unfortunately, widespread use of ^{1}H-MRS is limited by the fact that most lesions are under its >1 cm^3 spatial resolution, resulting in low signal-noise-ratios (SNR) and voxel partial volume effects from normal-appearing WM (NAWM), gray matter (GM) and cerebro-spinal-fluid (CSF). Moreover, metabolic ratios, often preferred over absolute quantification, mask concomitant changes and halve the specificity by precluding assessment of changes in individual metabolites. We present an absolute quantification workflow with stringent partial volume correction suitable for measuring metabolism of lesions under 1 cm^3. This approach enabled the study of lesions in early relapsing-remitting (RR) MS, *i.e.* the most common form of MS, at a disease stage when therapeutic interventions are started and imaging markers of efficacy are still needed. The specific goal was to compare the metabolism of non-enhancing lesions to pre-lesional tissue and correlate the results with T1 hypointensity.

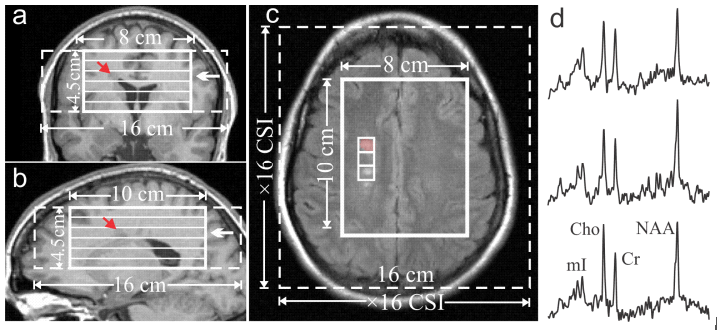
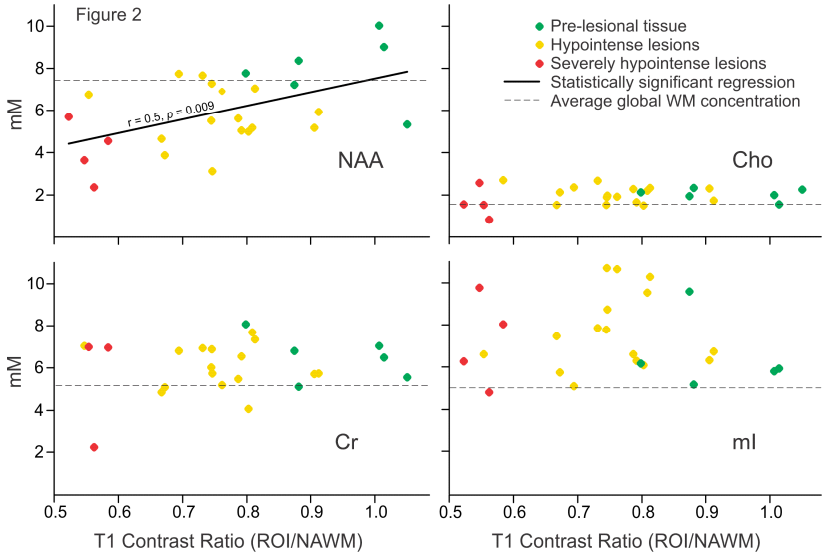


Fig. 1: Coronal (**a**) and sagittal (**b**) MP-RAGE overlaid with the ^{1}H-MRS VOI (white box) and field-of-view (dashed lines). The location of **c** is indicated on **a** and **b** by the white arrow. Spectra (**d**) from the voxels indicated on **c**. Red arrow on **a** and **b** denotes the lesion in **c**, shown overlaid with its mask (transparent red).

METHODS: 10 patients with clinically definite RR MS and an average disease duration of 32 months were prospectively recruited. Measurements were done at 3T. The ^{1}H-MRS volume-of-interest (VOI) of 10×8×4.5=360 cm^3 (*TE/TR*=35/1800 ms, 6 slices, 480 voxels, 0.75 cm^3 each) was placed over the corpus callosum, as shown in **Fig. 1a-c**. MP-RAGE, FLAIR and pre- and post contrast T1-weighted sequences were acquired. Binary masks of all lesions within the patient cohort were generated semi-automatically on the FLAIR images, using the FireVoxel software package. Axial MP-RAGE images were segmented into CSF, GM and WM masks using SPM2. FireVoxel was used to co-register the FLAIR image to the MP-RAGE acquired in the same session. An identical transformation matrix was applied to the lesion mask in order to co-register it to the MP-RAGE space. The lesion mask was subtracted from the GM and the CSF masks, eliminating misclassified pixels. Masks were created for any new lesions appearing on FLAIR images obtained at a 6 month follow-up. The FLAIR was then co-registered to the preceding timepoint, and its transformation matrix was applied to the lesion mask, creating a "ghost" mask of the forthcoming lesion. Each region-of-interest (ROI)'s T1 hypointensity contrast ratio (CR) was calculated as its signal intensity divided over that of a similar area of NAWM, outlined on the same slice. Isointense lesions were defined as having a CR within 2 standard deviations of NAWM intensity, while lesions with lower CR were classified as hypointense. A category of "severely hypointense" lesions was defined as having a CR<0.8 of lesion intensity to cortical GM intensity, also calculated from the same slice. To maximize lesion inclusion within the ^{1}H-MRSI voxel(s), *i.e.* ROI, voxel shifting was performed for each lesion. Spectral quality control criteria were 4 Hz< linewidths <13 Hz and CRLB<20%. Partial volume was controlled by including ROIs only with: lesion content >40%; CSF <30% and GM <20%. Pre-lesional tissue was included if it gave rise to a lesion with the same partial volume criteria. The spectra were fitted and the values were normalized for intravoxel lesion and CSF content. Concentrations in millimolar (mM) were obtained using phantom replacement, with correction for T1/T2 relaxation time differences between *in vitro* and *in vivo,* using published MS NAWM and lesion T1/T2 values.



RESULTS: Twenty-six ROIs satisfied the partial volume criteria. Median voxel number in an ROI was 1 (range 1-2). Average lesion volume, ROI lesion fraction, CSF and GM partial volume were: 1.1 cm^3, 56%, 3% and 7%. ROI metabolism and CR is presented in **Fig. 2**: half of the pre-lesional ROIs were of NAWM and half were of 'dirty-appearing WM' with signs of previous lesion activity as revealed by CR of less than 1 (green dots). No lesions fulfilled the criteria of isointensity. Twenty ROIs were classified as hypointense (yellow) with four of them as severely hypointense (red). The Cr and mI spectra of 2 ROIs did not pass the spectral quality control. A statistically significant correlation between CR and metabolism was observed for NAA only. No correlations were observed for any metabolite when pre-lesional tissue was excluded from the analysis. Concentrations are plotted against the average global WM concentration over the entire 360 cm^3 VOI of these patients, reported in ref[1]. Pre-lesional tissue metabolism was significantly different from lesions only in NAA (p=0.002).

DISCUSSION AND CONCLUSION: Controlling for the amount of lesional tissue, CSF and GM inside each ROI minimizes the partial volume effects that arise when studying lesions below the spatial resolution of ^{1}H-MRS. This results in decreased biological "noise", which is especially important in the context of the low SNR inherent of small voxels. Absolute quantification with CSF-correction and lesion-specific relaxation times allows independent assessment of all ^{1}H-MRS markers detectable at small voxel size. Application of this method in early RR MS revealed lesion profiles similar to what is reported for larger lesions and in more advanced MS, *i.e.* evidence of inflammation, gliosis and neuronal injury beyond what is seen in the rest of patients' WM. We report two additional findings. First, a relationship between CR and metabolism was observed for NAA, but it was due to higher NAA levels in pre-lesional tissue. Among lesions only, the degree of T1 hypointensity did not predict levels of any metabolite. Second, pre-lesional tissue exhibited already lesion-like metabolism for the glial markers Cho, Cr and mI, but in contrast, had higher NAA. These findings substantiate the view that inflammation and glial activation are already present prior to lesion formation and do not exacerbate thereafter. Acute disease activity, however, brings about axonal injury, which persists in established lesions, contributing to the accumulating disability seen with advancing disease.

REFERENCES: 1. Kirov II *et al*. Neurology 80:1-8, 2013

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