Diffusion-weighted spectroscopy of N-acetylaspartate: a novel technique to specifically explore neuroaxonal damage in multiple sclerosis

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Target audience: clinicians interested in multiple sclerosis, and in novel methods to specifically quantify neuroaxonal damage in this disease.

Purpose: Quantitative MRI techniques, such as diffusion tensor imaging (DTI) and magnetization transfer imaging (MTI) have been extensively employed to investigate tissue damage in multiple sclerosis (MS). However, due to the non-specificity of water as a probe of tissue microstructural changes, such techniques do not allow to differentiate between all the pathological processes occurring in the white and grey matter of patients with MS, which include axonal damage, demyelination and microglial activation[1]. In contrast, diffusion-weighted MR spectroscopy (DW-MRS) has been recognised as a powerful tool to investigate brain microstructure in-vivo[2,3], thanks to the specific compartmentation of brain metabolite in different cell types. In particular, N-acetylaspartate (NAA) is exclusively located inside neurons and its diffusivity in white matter (WM) or grey matter (GM) is therefore only influenced by the integrity of intra-neuronal components, thus providing a specific marker to axonal degeneration. Here, we provide our preliminary results on the comparison between the diffusion properties of water, NAA, total creatine (Cr) and choline compounds (Cho) measured in the normal appearing WM (NAWM) of patients with MS and in the WM of aged-matched healthy controls (HC), in order to disentangle the pure intra-axonal damage from other pathological mechanisms.

Methods: Nine healthy controls and three patients with relapsing-remitting MS were scanned on a 3 T whole body Siemens scanner equipped with a 32-channel receive coil. A single VOI diffusion-weighted PRESS[5] sequence was employed to measure diffusion of NAA, Cr, Cho, and water. For both patients and controls, a VOI of dimensions 25(AP)x15(RL)x20(FH)mm^3 was located in the parietal WM, excluding possible lesions in patients (Fig.1a). The acquisition parameters were: diffusion time 60ms, gradient pulse duration 30 ms, TE/TR 120ms/3 cardiac cycles, spectral width 2kHz, sample points 1024, 52 averages, scan time 4 minutes. Diffusion gradients were applied in one direction with a gradient strength of 21mT/m – with positive and negative polarity acquired in an interleaved way to minimize the coupling with the background gradients - resulting in a b value of 3100 s/mm^2. Residual water peak was used to perform phase and frequency corrections on individual scans before summation. Non-water suppressed spectra were acquired under similar conditions for eddy current corrections and to derive water mean diffusivity. Metabolite and water apparent diffusion coefficients (ADCs) were estimated for each subject from mono-exponential fits of the signal decay induced by the diffusion weighting, and then averaged across subjects. The full protocol included conventional sequences, DTI and MTI acquisitions.

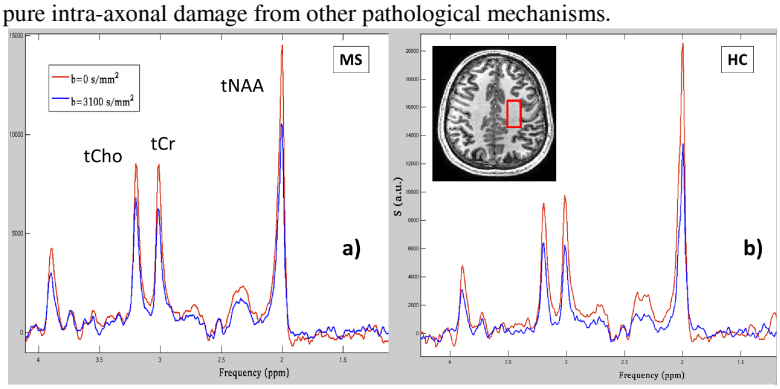

Fig.1: Examples of spectra acquired with and without diffusion-weighting (blue and red line respectively) in one patient with MS (a) and one healthy control (b). Inset: location of the VOI.

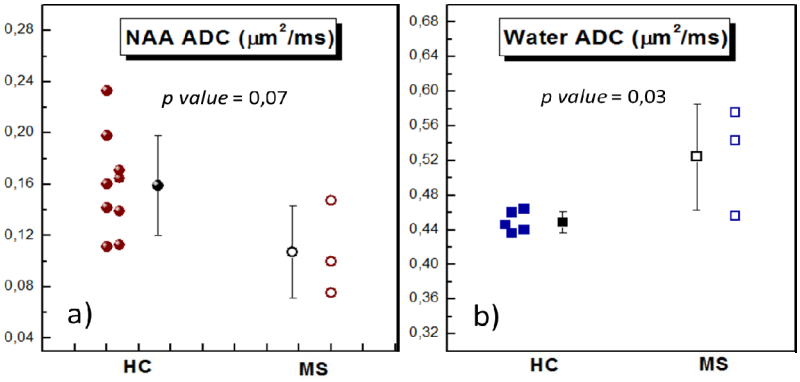

Fig.2: NAA (a) and water (b) ADCs measured in healthy controls (filled symbols) and MS patients (open symbols). The ADC mean values and corresponding standard deviations are

Table 1	*HC (n=9)*	*MS (n=3)*	*P values*
Age range (years)	25-40	23-33	0.3
Gender	5 males, 4 females	2 males, 1 females	0.6
T2W lesion volume	-	9±7 cc	-
EDSS score	-	0, 3, 3	-
Water ADC (μm²/ms)	0.45 ± 0.01	0.53 ± 0.06	0.03
NAA ADC (μm²/ms)	0.16 ± 0.04	0.11 ± 0.04	0.07
Cr ADC (μm²/ms)	0.14 ± 0.04	0.15 ± 0.05	0.5
Cho ADC (μm²/ms)	0.13 ± 0.05	0.15 ± 0.05	0.4

Results: *Fig.1* shows examples of spectra measured in the NAWM of one MS patient (a) and in the WM of one healthy control (b), at the two diffusion-weighting conditions. It is possible to observe that the NAA signal drop at b=3100 s/mm^2 is more pronounced in the HC, indicating faster NAA diffusion in this subject. A difference close to statistical significance (p=0.07) was observed in the ADC(NAA) between MS patients and healthy controls (*Fig.2a*). The estimated average ADC(NAA) was lower in patients then in controls. In contrast, ADC(water) was found significantly greater in MS patients (p = 0.03) with respect to controls (*Fig.2b*). No significant differences were observed in Cr and Cho ADC. The main results and the subject characteristics are summarised in *Tab.1* Interestingly, the only patient showing a value of ADC(NAA) in the range of the HC, has EDSS score = 0, while the ADC(water) is abnormally high (see *Fig2*).

Discussion and conclusion: The decreased values of ADC(NAA) observed in the NAWM of patients reflect the presence of axonopathy outside visible lesions, and are associated with an increased water diffusion, whereas Cr and Cho ADC were not modified. This suggests that the changes in water diffusivity classically described in NAWM of MS patients may be mainly driven by axonal pathology. DW-MRS of NAA could offer the unique opportunity to quantify neuroaxonal damage in both the WM and the GM in MS (ongoing analysis), to explore clinical and prognostic relevance of axonal pathology, and could be used as an outcome measure in clinical trials assessing putative neuroprotective treatments. The complete dataset will include DW-MRS data from both NAWM and deep GM obtained in a larger cohort, that will be combined with data derived from other MRI and molecular imaging measures of microstructural damage and volume changes.

References: [1]Kutzelnigg A et al, Brain,**128**,2705,(2005). [2]K Nicolay et al, NMR Biomed, **14**, 94 (2001). [3] I Ronen *et al*, Brain Struct Funct, **219**, 1773 (2014). [4] Wood et al, J Neurosci, **32**, 6665(2012). [5] Kan et al, MRM, **67**, 1203 (2012).