Correlation of spinal cord axonal density with post mortem NMR measurements in multiple sclerosis and controls.

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Aim. The aim of this study was to correlate axonal density with measurable NMR quantities *post mortem* in spinal cord specimens of healthy controls and patients with multiple sclerosis (MS). This information will help to define the pathological specificity of NMR, with particular reference to axonal loss in multiple sclerosis, since it is a likely pathological substrate of disability in that disease.

Introduction. Knowledge of the relationship between NMR properties and clinically important pathological entities, such as axonal loss, will permit the design of appropriate NMR sequences for the detection of pathology in a sensitive and specific way. It is difficult to obtain pathological material from the CNS in life and animal models are difficult to study and have limitations, therefore the study of fresh post mortem specimens is attractive. NMR contrast between tissues is probably little affected by *post mortem* changes (even though absolute values of parameters do alter) (1,2).

Methods. Fresh specimens of spinal cord, obtained from a MS tissue bank, were kept in wet ice until imaging could begin. The specimens, about 2cm in length, were bathed in normal saline and inserted, in a sealed container, into a 13mm diameter solenoid coil. Imaging was performed in a 7T Bruker Biospec II Spectrometer. Imaging parameters were: Slice thickness 1.5mm, FOV 15mm, matrix size 256x128 zero-filled to 256x256, in-plane resolution 60μm. The following MR parameters were obtained:

T1: Inversion recovery experiment with adiabatic inversion pulse (TR 12s, TE 18ms, TIs 0, 1, 4, 8s, 3-parameter fit).

T2: Spin echo (TR 3000ms, TEs 20, 100ms).

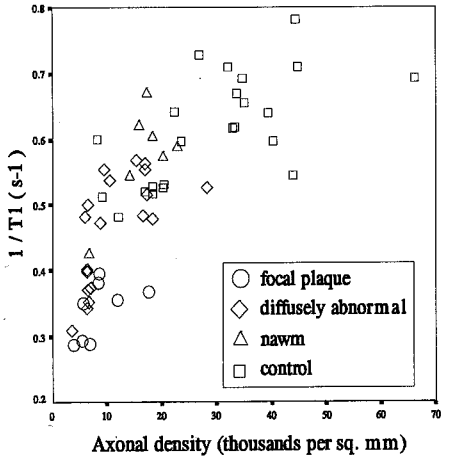
Proton Density (PD): Derived from a PD-weighted image (TR 4000ms, TE 20ms) corrected for T1 and T2.

Apparent Diffusion Coefficient (ADC) and diffusion anisotropy standard deviation index (SDI) were measured using a stimulated echo sequence with 2 diffusion gradient strengths and 3 orthogonal diffusion directions – antero-posterior, medio-lateral and rostrocaudal with respect to the spinal cord specimen. TR 3000ms, TE 24ms, δ=5ms, Δ=200ms, b1=24x10^6, b2=932x10^6 s/m^2.

Magnetisation Transfer Ratio (MTR) was measured using an interleaved spin echo sequence collecting images after 3 seconds of pre-irradiation at 10KHz and 100KHz off-resonance respectively.

After fixation in 10% formalin, the specimens were stained with Bielschowsky's silver stain and axons were counted manually on enlargements of photomicrographs. Regions of interest were first defined on an NMR parameter map (T1) and the same regions were analysed histologically, allowing correlation.

Results. The results show strong correlations between T1, MTR and axonal density. There were also strong relationships between PD, T2, mean ADC, SDI and axonal density in certain sub-groups. Even in normal (control) white matter, strong correlations between T1, MTR and axonal density were seen.



Scatter plot of 1/T1 versus axonal density in white matter

	Mean ADC	SDI	T1	T2	PD	MTR
All	-0.565 p<0.001	0.651 p<0.001	-0.83 p<0.001	-0.415 p<0.001	-0.540 p<0.001	0.851 p<0.001
MS	-0.227 p=0.177	0.550 p<0.001	-0.714 p<0.001	-0.364 p=0.014	-0.770 p<0.001	0.783 p<0.001
Controls	-0.047 p=0.83	0.334 p=0.119	-0.687 p<0.001	-0.54 p=0.008	-0.277 p=0.201	0.650 p<0.001

Spearman Correlation Coefficients of NMR variables versus axonal density.

Mean values in control white matter were: T1 1660 ms, T2 60ms, PD 66%, MTR 58%, mean ADC 0.34x10^{-9}m^{2}s^{-1}, SDI 0.41.

Discussion. The relationship between increasing T1 and decreasing axonal density is in accordance with a recent report of extensive axonal loss in hypointense MS lesions on T1-weighted images (3). A clinical study (4) has shown that increasing hypointense lesion load correlated with increase in disability but T2 lesion load changes did not. Our findings suggest that the reason for these clinical observations may be that T1 is more sensitive than T2 to axonal loss. Our findings also suggest that PD, MTR and SDI may be useful markers of axonal density. Further studies are needed to evaluate the correlation of these NMR parameters with other pathological features found in chronic MS lesions, e.g. demyelination, gliosis.

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References.

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