High field MRI for lesion characterisation in multiple sclerosis

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Introduction

Multiple sclerosis (MS) not only affects central nervous system white matter (WM) but also grey matter (GM), and pathology in GM may lead to disability and cognitive impairment. At 1.5T MS GM pathology is inferred by detecting volume loss or intrinsic quantitative MR abnormality, e.g. reduced MTR. Unlike the high conspicuity of WM demyelinating lesions (WML), GM – particularly cortical – demyelinating lesions (CL) of MS are rarely detected. In order to better characterise CL, we acquired multimodal quantitative MR data in *post mortem* MS brain samples using a 9.4T system.

Methods

Post mortem brain slices of seven subjects with MS (mean age 50 years; SD 10 years) were fixed in 10% buffered formalin 30-70 hours (mean 39h; SD 16h) after death. Duration of fixation (dur_fix) was 102-1459 days (mean 727d; SD 419d). A tissue block from each case was dissected so as to fit inside a histology cassette, which was then inserted into a universal tube filled with perfluoropolyether. The tube was placed in a quadrature ^{1}H volume MR coil (24mm diameter) and scanned using a 9.4T Varian Inova system. T_1 and T_2 measurements were based on a spin echo (SE) sequence using a field of view of 30x30 mm and matrix size of 256x192, resulting in a pixel size of 117µm². Slices of 1mm thickness were collected (2 averages). For T_1 measurements TE=16 ms and TR=480/600/800/1000/2000/4000ms were employed. For T_2 measurements TR=2000ms and TE= 24/36/48/60ms were used. MTR maps were based on data acquired using a gradient-echo sequence with saturation RF pulses at 100 kHz and 6 kHz offset from water for non-saturated and saturated images, respectively. T_1 and T_2 maps were produced by fitting signal intensities to the equations, $SI=M_0^{(TR/T1)}$ and $SI=M_0^{(TE/T2)}$, respectively, where M_0 is a constant, on a pixel-by-pixel basis. MTR was calculated on a pixel by pixel basis according to the equation, $100(M_0-M_s)/M_0$, where M_s and M_0 are signal intensities with and without saturation, respectively. For DTI single-shot SE (TR/TE: 2000/22ms, matrix size 256x256, 2 averages) was used. Gradient *b* factor was 1000 s/mm². Six diffusion-weighted images were acquired along six non-colinear directions, and one non-diffusion weighted image. A high resolution scan was also performed with parameters TR/TE 3000/60ms, matrix size 256x256 and 16 averages. MR data were processed using ImageJ (NIH, Bethesda, USA). Fractional anisotropy (FA), apparent diffusion coefficient (ADC) and orientation maps were calculated using the ImageJ plug-in JDTI (1). After scanning tissue blocks were re-immersed in formalin prior to processing for embedding in paraffin. Sections were immuno-stained for myelin-basic protein. Regions of interest (ROI) were identified with reference to histology as normal appearing (NA) WM, NA cortex (NAC), WML and CL. When lesions crossed the border between WM and GM they were included as two separate lesions (one WML and one CL). A contrast-to-noise-ratio (CNR) plot was produced for CL vs. NAC and WML vs. NAWM. Student's paired *t*-tests and regression models were applied (SPSS).

Results

In seven blocks 20 histologically confirmed demyelinated MS lesions (11 CL, 9 WML) were detected. Excellent correspondence was achieved between MR maps and histology (figure 1). The highest CNR for both GM and WM was achieved at TE=24 (figure 2). T_1, T_2, MTR, ADC and FA values are summarized in the table. All MR indices differed between (i) NAWM and NAC, (ii) WML and NAWM, and (iii) CL and NAC (except for FA). Analysis of confounders revealed association of dur_fix with T_1 (WM: r= -0.80, p=0.03; GM: r= -0.82, p=0.05) and ADC (WM: r= -0.86, p=0.03), and of time between death and fixation (t_d_fix) with T_2 (GM: r= 0.9, p=0.01), MTR (GM: r= -0.8, p=0.05) and ADC (GM: r= -0.86, p=0.03). Of the following MR indices the *difference* between lesions and their respective NA tissue compartment correlated with dur_fix: T_1 (GM: r= -0.85, p=0.03), ADC (WM: -0.82, p=0.02). The only *difference* affected by t_d_fix was for MTR between WML and NAWM (r= -0.75, p=0.05).

Figure 1: P*ost mortem* multiple sclerosis tissue block showing a cortical lesion that extends in to subjacent white matter (arrow)

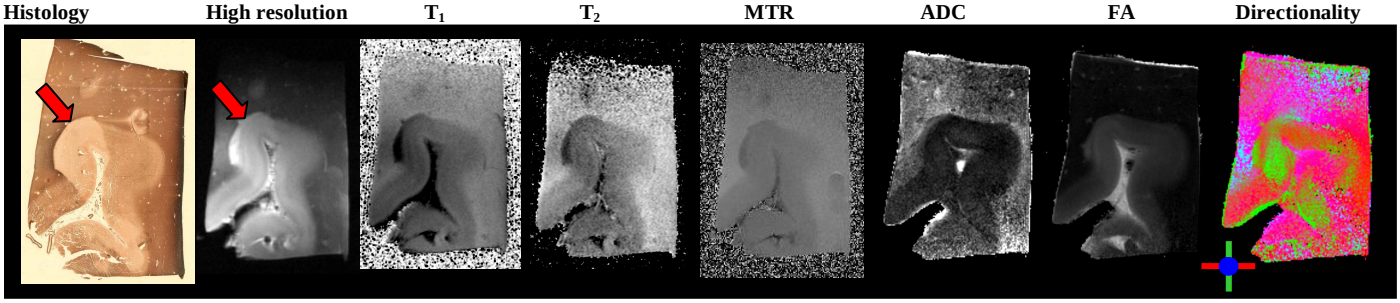


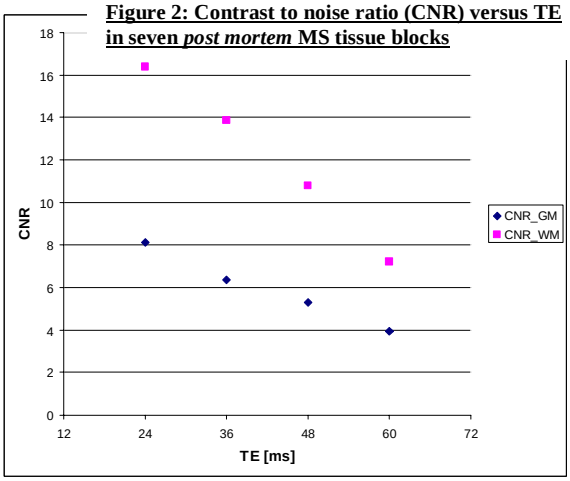
Table: Mean values and standard deviations (SD) of MR indices in seven *post mortem* multiple sclerosis tissue blocks

	NWM	(SD)	WML	(SD)	P	NAC	(SD)	CL	(SD)	P
T_1 [ms]	813	345	1299	455	<0.01	998	433	1080	145	0.01
T_2 [ms]	21	6	30	8	<0.01	22	7	26	7	<0.01
MTR [pu]	39.2	15.6	26.8	9.1	<0.01	38.1	7.3	34.3	9.5	0.03
ADC [*10^{-3} mm²/s]	0.25	0.05	0.57	0.12	<0.01	0.34	0.11	0.38	0.12	0.02
Fractional anisotropy	0.36	0.05	0.28	0.07	<0.01	0.23	0.02	0.26	0.06	0.2

Conclusion

All MR techniques employed in this study at 9.4T provide useful measures to characterise MS WML as well as CL. The lack of a significant difference in FA between CL and NAC in our sample may reflect the overall lower FA in GM (compared to WM). The CNR in both GM and WM was highest at TE=24ms in the multiecho T_2-weighted sequence. The slopes in fig 2 indicate that even shorter TE's may further improve CNR, suggesting that CNR in *post mortem* MS brain is strongly proton density dependent (2). Recent evidence suggested that formalin fixation of brain tissue is complete after approximately 45 days (3). Our tissue sample had been fixed for considerably longer, and our analysis revealed significant association between some of the MR indices and dur_fix. Hence, measurable change appears to occur during formalin fixation long term (4) and needs to be considered when inferring likely *in vivo* changes from *post mortem* studies. Relaxation times, MTR and DTI indices at 1.5T all detect changes in MS cortex but focal lesions are not seen. The resolution and contrast-to-noise achievable using high-field MRI at 9.4T allows CLs to be studied in detail and may identify sequences with potential to detect such lesions *in vivo* at standard field strengths. Histological analysis of the sample used in this study is underway to correlate MR indices with quantitative measures of myelin content, neuron-axonal damage, inflammation and astrogliosis.

Figure 2: Contrast to noise ratio (CNR) versus TE in seven *post mortem* MS tissue blocks



References

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