T_2 distribution reflects Multiple Sclerosis pathologies: Histology driven regions of interest

T. A. Bjarnason[1], C. Laule[2], E. Leung[2], J. R. Mitchell[1], P. Kozlowski[2], A. L. MacKay[2], and G. W. Moore[2]
[1]University of Calgary, Calgary, AB, Canada, [2]University of British Columbia, Vancouver, BC, Canada

Introduction: Multi-echo T_2 relaxation allows scientists to probe water micro-compartments in vivo [1]. T_2 relaxation is sensitive to myelin content [2] and has uncovered previously undetected micro-compartments in MS and PKU pathological tissue [3]. We aim to characterize how T_2 distributions of fixed brain change with different types of MS pathology, identified using *ex vivo* histological stains, in order to create a model of T_2 distributions specific to stages of MS disease.

Methods: *Tissue Preparation*: Eight brain samples were excised from 3 subjects with pathologically proven MS. The brain samples were fixed for at least two months in 10% formalin. A 1cm thick slab along the coronal or transverse plane was placed into a formalin filled plastic container. *MR Experiments*: All MR experiments were performed on a 7T, 30cm bore Bruker Avance MR scanner. A single slice, multi-echo CPMG imaging sequence [4] was used (256x256 matrix, echo spacing = 6.6673ms, TR = 1500ms, 6 averages, 32 echoes, FOV = 6cm, 1mm slice thickness). *Histological Analysis*: After scanning, the brain slabs were sectioned into 10μm thick slices and stained using Luxol Fast Blue (LFB) for myelin and Bielschowsky for axons. Uncompressed tiff images were created of the slices using a back-lit scanner. One slice of each stain was chosen for further analysis and comparison with the multi-echo MR images. *Data Analysis*: The two histology images (LFB and Bielschowsky) were placed side-by-side to find regions for analysis. Five types of regions were identified on the histology images in white matter: lesions, normal appearing white matter (NAWM), reduced LFB and Bielschowsky stain intensities (rLrB), reduced LFB and normal appearing Bielschowsky stain intensities (rLnB), and normal LFB with reduced Bielschowsky stain intensities (nLrB). These regions were outlined on the TE = 26.7ms image and analysis was performed using AnalyzeNNLS [5]. T_2 distributions were generated using a basis of T_2 times logarithmically spaced between 10 and 427ms and using a smoothing constraint that allowed the χ^2 value to vary between 2 and 2.5% of its nominal value [6]. The area fractions and geometric T_2 times (gmT_2) were determined as follows: 10-20ms for myelin water (MW); 20-100ms for intra/extracellular water (IEW); and 100-427ms for long water (LW). The preceding regions were slightly shifted to ensure T_2 distribution peak separation in rare cases. The fractions and gmT_2s were compared across tissue types using an ANOVA, followed by post-hoc comparisons, with Bonferroni correction, between tissue types.

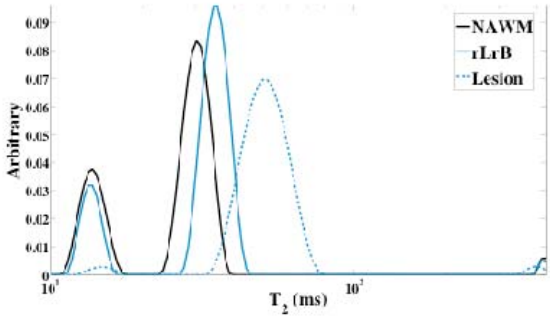

Fig 1: T_2 distributions from histological stain regions of normal appearing white matter (NAWM), reduced LFB and reduced Bielschowsky stain intensities (rLrB), and lesion in fixed brain white matter structures.

Results: Sample T_2 distributions from NAWM, rLrB, and lesion are shown in Fig 1. There were only 2 regions found in the histology stains of rLnB and no regions of nLrB. These two scenarios were excluded from further analysis. More than 20 regions were found in each of NAWM, rLrB, and lesions. A Whisker plot of gmT_2 times for the IE water in fixed brain are shown in Fig 2. All values (row-wise) in Table 1 are significantly different from each other with $p<<0.001$. The LW gmT_2 between NAWM and lesion data approached significance with a corrected p value of 0.067.

	NAWM	rLrB	Lesion
MWF (%)	29.3±1.5	18.0±1.5	2.9±0.9
IEWF (%)	69.4±1.6	80.9±1.6	96.0±0.9
IE gmT_2 (ms)	29.4±0.5	36.0±0.8	46.5±1.7

Table 1: Myelin water fraction (MWF), intra/extracellular water fraction (IEWF), and IE geometric mean T_2 times for NAWM, rLrB, and lesions. Significantly different values reported here (row-wise). Standard errors shown.

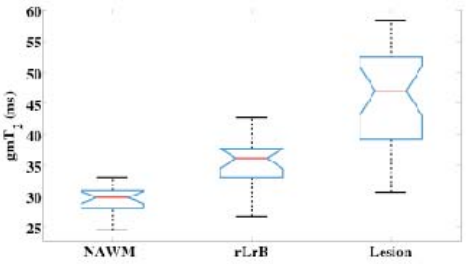

Fig 2: Whisker plot of IE water gmT_2 data for NAWM, rLrB, and lesions.

Discussion: T_2 distributions have been shown to be sensitive to more than myelin content in pathological tissue [3]. In addition to the changes observed in MWF in different MS pathologies, we found changes in IEWF and IE gmT_2 times. Using histology to drive ROI placement, we observed a lengthening of the IEW gmT_2 times from normal appearing white matter, reduced LFB and reduced Bielschowsky stain intensities, to lesions. Histology driven T_2 relaxation analysis in fixed brain may allow better determination of the correspondence between T_2 decay and pathology.

References: [1] MacKay *et al*. MRI 24: 515-24 (2006). [2] Laule *et al*. MS 12: 747-53 (2006). [3] Laule *et al*. JMRI 26: 1117-21 (2007). [4] Poon & Henkelman. JMRI 2:541-53 (1992). [5] www.imaginginformatics.ca/open-source/analyzennls [6] Whittall & MacKay. JMR 84: 64-71 (1989). We acknowledge the MS patients and their families as well as financial support from the MS Society of Canada, AHFMR and iCORE.