Correlation of quantitative MRI and histology of surgical specimens in drug-resistant focal epilepsy

Maged Goubran[1], Robert R. Hammond[2], Sandrine de Ribaupierre[3], Terry M. Peters[1], and Ali R. Khan[1]

[1]*Imaging Laboratories, Robarts Research Institute, London, Ontario, Canada,* [2]*Department of Pathology, Western university, London, Ontario, Canada,* [3]*Department of Clinical Neurological Sciences, Western university, London, Ontario, Canada*

Introduction

In the past decade there has been an emerging trend in research towards correlation of pre-operative brain imaging with histology in order to validate both novel and existing imaging techniques. These research protocols provide access to complementary and high resolution anatomical information that can validate, optimize, and inform pre-operative imaging techniques. A clinical example where histological validation can significantly impact patient care is drug resistant temporal lobe epilepsy (TLE). Current clinical MRI protocols used for pre-operative assessment of focal epilepsy lack sensitivity, with greater than 30% of patients diagnosed as MR negative [1]. The histology evaluation of the surgical tissue, nevertheless, often reveals gliosis, malformations of cortical development or focal cortical dysplasia (FCD) undetected pre-operatively. Such data have motivated the need for MRI-histology correlation, to validate improved pre-operative imaging and image analysis techniques for localizing epileptogenic foci, and to understand the pathological correlates of MRI signal. To this end, our objective in this study is to correlate quantitative MRI and histology metrics of surgical specimens from drug-resistant TLE patients.

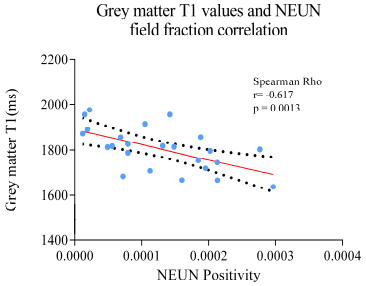
Methods

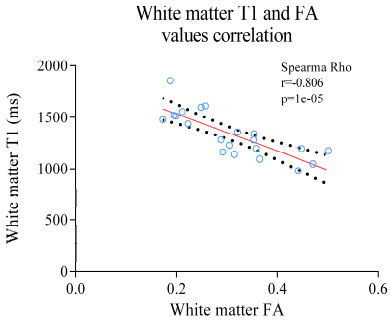
Materials & Imaging: 10 temporal lobe epilepsy patients who were candidates for anterior temporal lobectomy (ATL) surgery were recruited for this study. All patients underwent pre-operative imaging (relaxation mapping and diffusion-tensor imaging) on a 3T Discovery MR750 scanner (General Electric, Milwaukee, WI, USA) with a 32 channel head coil. For T1 mapping the DESPOT1-HIFI approach [2] was used and involved acquisition of two 3D SPGR sagittal T1-weighted images (TR/TE =8.36/3.17ms, flip angles =4° & 18°) with a 1 mm isotropic resolution as well as an additional inversion-prepared SPGR for B1 mapping. For T2 mapping the DESPOT2-FM approach [3], whereby five balanced steady-state free precession (bSSFP) images were acquired with the same resolution and flip angles 5°, 35° and 68° with phase cycling patterns θ_{RF} = 0° and 180° (TR/TE=4.61/2.36ms). Diffusion tensor imaging (DTI) was performed using an axial spin-echo echo-planar imaging (EPI)sequence with 41 diffusion directions, a resolution of 2.5 mm isotropic and a b-value of 1000(TR/TE=1100/63.2ms). Following surgery the excised neocortical specimens were scanned *ex-vivo* on a 9.4T small bore magnet (Varian, Palo Alto, CA, USA), then processed for histological assessment including grossing, sectioning, slicing and digitization in our collaborating pathology department.

Table 1. Summary of patients' demographics & findings. MTS = mesial temporal sclerosis, FCD = focal cortical dysplasia, TS = tuberous sclerosis

Patient	Gender	Age	Onset	Origin	MRI	Path.
1	F	26	20	R	TS	CT
2	F	22	15	R	R. MTS	MTS
3	M	20	3	L	L. MTS	MTS
4	M	19	5	R	Normal	Gliosis
5	M	49	13	L	Normal	Mild MTS
6	F	21	13	R	N-S	Mild FCD
7	F	47	8	R	Normal	Dysplasia
8	M	19	5	L	L. MTS	MTS
9	F	43	3	R	R. MTS	MTS
10	M	34	15	L	L. MTS	MTS

Quantitative metrics & Statistical analysis: Field maps of NeuN (representing neuronal density) and GFAP immunohistochemistry stains from digitized histology of surgically resected tissue were automatically computed using the positive pixel algorithm (Aperio Technologies, Vista, CA, USA) and adjusted for differential staining between slides using scripts written in Matlab (MathWorks Inc., Natick, MA, USA). Regions of interest (ROIs) were subsequently delineated on 100 µm downsampled histology slices in histology space: grey matter (GM) regions defined on the cortical crown of the middle temporal gyrus and white matter (WM) regions within the deep white matter of the same gyrus. Using our previously reported histology to MRI registration protocol [4], the histology ROIs were warped to match corresponding regions on *in-vivo* quantitative maps (T1, T2, FA, MD). A final step of manual ROI correction was employed, if needed, to account for registration errors and partial volume effects. Spearman's rank correlation was employed to test for correlation between the quantitative MRI metrics: T1, T2, FA & MD, and field fractions of NeuN & GFAP within both brain tissues (GM & WM). Statistical analyses were performed in Prism 5.04 (GraphPad, San Diego, CA, USA).





Results & Discussion

The registration protocol enabled us to determine correspondences between MR and histology slices, so that metrics from each slice were employed as unique data points in the analysis rather than being averaged across all slices for the patient. A negative correlation between NeuN field fraction and the T1 value in gray matter was found using both tests (r= −0.617, p=0.001). T1 relaxation is related to water content, macromolecule concentration and water binding. Neuronal loss in the grey matter will likely result in the loss of macromolecules, which in turn decreases the amount of bound water and subsequently increases T1 **[5]**. When assessing the relationships between MRI metrics, a negative correlation was found between T1 and FA in white matter (r=−0.806, p=1e-05), as well as a positive correlation between WM T1 and MD (r=0.643, p=0.002) and a negative correlation between WM FA and MD (r=−0.527, p=0.014). Existing studies have also shown reduced FA and increased MD in the ipsilateral white matter in TLE **[6]**; our results reveal that T1 is also affected in these regions. These changes may be due to degeneration of axons, reduced packing, or demyelination **[7]** which may facilitate isotropic diffusion and accumulation of free water.

Conclusion

Our registration and correlation pipeline allows for quantitative assessment of the pathological correlates of MRI by employing information from both modalities, and the potential prediction of pathology from *in-vivo* MRI. This study is the first to relate *in-vivo* T1 values to the proportion of neurons in the grey matter for focal epilepsy. These preliminary findings suggest that T1 in gray matter may act as a predictor of neuronal density and thus *in-vivo* T1 mapping may provide a non-invasive tool for estimating pyramidal cell loss in neurological disorders such as epilepsy and multiple sclerosis, as well as neurodegenerative diseases as Alzheimer's disease.

References

[1] Sylaja et al. Epilepsia (2004) vol. 45 (7) pp. 803-808. **[2]** Deoni. JMRI (2007) vol. 26 pp. 1106-1111. **[3]** Deoni. JMRI (200) vol. 30 (2) pp. 411-417. **[4]** Goubran et al. Neuroimage (2013) vol. 83 pp. 770-781. **[5]** Schmierer et al. Brain (2010) vol. 133 pp. 858-867 **[6]** Focke et al. Neuroimage (2008) vol. 40 pp. 728-737. **[7]** Gross. Epilepsia (2011) vol. 52(Suppl4) pp. 32-34.

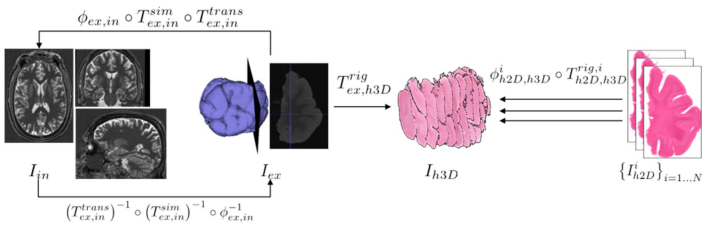


Figure 3. Overview of our histology to *in-vivo* MRI registration protocol.

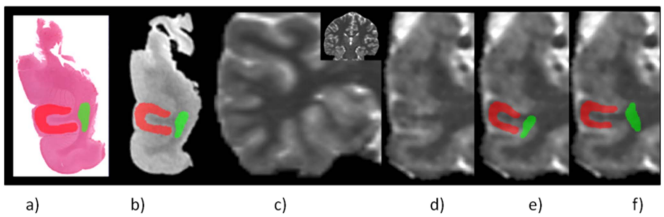


Figure 4. a) Histology ROI, b) ROI on *ex-vivo* scan, c) Unregistered in-vivo T1 map, d) Registered T1 map, e) Mapped ROI to match MRI slice, f) Corrected ROI