Increased FA in acute TBI rat Marmarau model followed by decreased FA during subacute stage: A TBSS study

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Introduction: Traumatic brain injury (TBI) is common in western societies and is difficult to objectively diagnose in milder cases. The symptoms may be non-specific and cognitive deficits are frequently missed until orthopedic and/or internal injuries resolve. Clinical neuroimaging is surprisingly unrevealing in mild to moderate TBI except when focal hemorrhages are present. Several negative consequences are directly related to the inability to image TBI pathology: 1) uncertainty regarding diagnosis, best management and outcome; 2) underpowered and flawed clinical interventional trials; 3) repeated exposure to trauma, particularly in the military and contact sports arenas. Advanced MR techniques, including susceptibility-weighted imaging (SWI), diffusion tensor imaging (DTI), MR spectroscopy, perfusion and functional MRI, are sensitive to TBI pathology and pathophysiology, being used by a number of research laboratories. The overarching goal of our research lab is to delineate the relationships between clinical/neurobehavioral impairment, TBI pathology and imaging biomarkers of TBI. *With the current study, our objective is to establish an advanced MR imaging-based version of the Marmarou rodent model of diffuse axonal injury*. The Marmarou model is not a perfect model of human TBI, but does approximate the pathological changes and is more feasible and cost effective than large animal studies (pig and dog). A "deliverable" of the current study will be the establishing of imaging surrogates or biomarkers of pathology, such as axonal changes (swelling and degeneration) and vascular/metabolic changes (hemorrhages and ischemia) that can be better interpreted in clinical TBI scans. The focus of the current abstract is to examine DTI measures over the entire rat brain pre and post injury and over time (4 hrs to 7 days) to determine the natural history of fractional anisotropy (FA) at hyperacute, acute and subacute stages. Contradictory findings in the literature regarding FA measures acutely (increase vs. decrease) exist, highlighting the importance of controlled longitudinal studies to clarify this and complex issues. To our knowledge the current study is one of the first few reports utilizing Tract-based Spatial Statistics to analyze FA images pre and post trauma in rats. The motivations for using this method (or similar voxel-based analysis): 1) to develop a bias-free and automated procedure; 2) to examine all of the axonal tracts in the rat brain efficiently. The latter allows for robust comparison of white matter diffusion anisotropy with whole brain SWI and other MR measures acquired in the same session. In addition, we have performed histological analysis on some of the animals after ex vivo imaging. This is a work in progress which will utilize histologic findings, i.e., axonal retraction balls as a "gold standard" to allow for optimization of image parameterization.

Materials and Methods: At total of twenty four adult Sprague-Dawley albino rats (400-450 gm) were studied at a number of time points behaviorally with imaging and finally with histology. There were four groups of 6 rats which differed by when they were sacrificed for histological analysis (4 hr, 24 hr, 4 d, 7d). An impact acceleration model of TBI [(1)] was used with a 450-g weight dropped from 2 m. Rats which did not survive the trauma were not included in analysis. MR imaging included DTI, SWI and T2 acquisitions on a 4.7 T Bruker animal magnet. Single shot spin-echo planar six directional DTI acquisition with a b_0 value of 0 and 800 sec/mm^2 was done with 32X32 mm^2 FOV and a image size of 256X256 pixels acquiring 0.125X0.125 mm^2 in plane and 1mm of slice thickness through plane resolution. Rat brains were fixed with a formalin solution and sectioned for histology with beta-APP and Prussian blues staining. MR imaging was done pre-trauma and again post trauma at 4 hr, 24 hr, 4 d and 7 d depending on when the animal was sacrificed. Ex vivo imaging was done after sacrificing the animal. DTI (FA) image analysis is done using voxel-based (VBA) and tract-based spatial statistic (TBSS)[(2)] approaches utilizing the SPM[(3)] and FSL[(4)] (software platforms). Both methods rely on statistical comparison between subjects' FA maps after spatially transforming images into a common space. An indirect method of transforming FA images to the standard space was implemented; this was done via weighting the b_0 images with the complement of FA (1-FA) to get a maximum contrast between white matter and nonwhite matter tissues. We achieved this non-trivial task using supervised brain extraction (BET) on down sampled b_0 images. All the resultant contrast enhanced b_0 images of every single rat were nonlinearly warped to a single rat's contrast enhanced b_0 image and then shadow transformed the corresponding FA images to the standard space (ICBM_MNI) in SPM8. Nonlinearly warped FA images in the standard space were then fed into the skeletonization algorithm of TBSS thresholded at FA value of 0.2. Voxel based analysis used a weighted nonlinear spatial normalization (SPM) rather than FNIRT and a *Z* statistic while TBSS uses a permutation method and cluster enhancement using general linear model in Randomise tool from FSL.

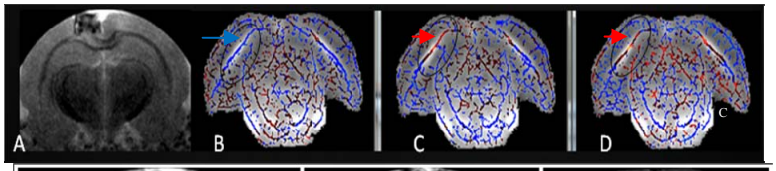

Figure 1. *Right Corpus Callosum (CC) FA*: A) Right cortical hemorrhage at 4h; B) CC with increased FA at 24h (blue in circle with blue arrow); C) FA reduction in CC at 24h and 3d(blue in circle with red arrows) .

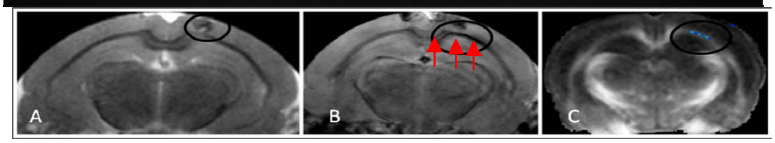

Fig. 2. Ischemia and FA. A) T2 with hemorrhage; B) SWI with dark vein along CC (red arrows); C) TBSS with increased FA (blue)

Results: In vivo DTI Image quality was variable and likely a consequence of respiratory induced motion artifact**.** 16 rats had good quality pre-injury DTI images which were used as the "control" images for TBSS analyses. Hemorrhagic lesions were apparent typically on multiple image types including conventional T1 or T2 and SWI. Vasogenic edema was revealed by T2 and ADC images when present and surrounded hemorrhages. 1) FA generally increased (relative to controls = pre-injury images) at the earliest time point (4 hours) (Fig. 1B). This was apparent in the corpus callosum ipsilateral to the predominant lesion seen on T2, T1 and SWI; 2) FA then decreased at later time points (24h, 3d, 7d) (Fig. 1C, 1D); 3) FA changes were spatially variable across animals but in some FA changes seemed remote from hemorrhagic lesions; 4) SWI images revealed darkened veins which were frequently associated with increases in FA in nearby white matter tracts.

Conclusions: Tract based spatial statistics (Smith et al., NeuroImage 2006) can be used with rodent images to analyze DTI-FA images and reveal FA changes due to trauma using the Marmarou model. FA does appear to increase in the first few hours followed by a decrease. Independently, we see evidence that an FA reduction is associated with deoxyhemoglobin increase suggesting misery perfusion or ischemia. Further analysis is yet to be done including correlation with histology. These results suggest that an image-based injury model with establishment of imaging surrogates of injury is entirely feasible.

References:1.A. Marmarou, M.A.A. Foda, W. van den Brink, J. Campbell, H. Kita and K. Demetriadou, A new model of diffuse brain injury in rats.PartI:Pathophysiology and biomechanics. *J. Neurosurg.* **80** (1994), pp. 291–300; 2. http://www.fil.ion.ucl.ac.uk; 3. http://www.fil.ion.ucl.ac.u 4. http://www.fmrib.ox.ac.uk/fsl/index.html; 5. http://www.fmrib.ox.ac.uk/fsl/tbss/index.html.