Longitudinal quantitative MRI Provides Quality Assurance Measures in Patients with Ischemic Stroke Treated with Autologous Bone Marrow Derived Mononuclear Cells.

Muhammad E Haque[1], Khader M Hasan[2], Benjamin A Schatz[3], Sarah M Lund[3], Farhaan S Vahidy[4], and Sean I Savitz[4]

[1]Neurology, Unversity of Texas Health Science Center at Houston, Houston, TX, United States, [2]Diagnostic and Interventional Imaging, Unversity of Texas Health Science Center at Houston, Houston, TX, United States, [3]Unversity of Texas Health Science Center at Houston, TX, United States, [4]Neurology, Unversity of Texas Health Science Center at Houston, TX, United States

Introduction: Depending on infarct location and tissue connectivity, stroke affects brain gray and white matter causing functional, metabolic and cognitive impairment. Tissue plasminogen activator (tPA) is the most effective therapy for patients with acute ischemic strokes (AIS) administered within 3.0 to 4.5 hours. Once damage has occurred, rehabilitation is the only option known to promote recovery. Autologous bone marrow derived mononuclear cells (MNCs) is under investigation as an experimental treatment for stroke.[1,2] Clinical trials of MNCs have shown safety and feasibility.[1] In this report, we sought to study longitudinal microstructural changes in remote regions from the injury using quantitative magnetic resonance imaging (qMRI) to provide objective quality assurance measures. Approximately 80% of stroke are ischemic that predominantly occur in the middle cerebral artery (MCA) territory, which supplies blood to the temporal, anterolateral frontal, and parietal lobe with perforating branches to the internal capsule and caudate. However, the corpus callosum (CC), a tightly packed interhemespheric commissure, predominantly receives their blood supply from the anterior cerebral artery (ACA) and posterior cerebral artery (PCA)[3]. We hypothesized that middle cerebral artery MCA-perfused territory stroke will not alter microstructural properties of the CC with cellular intervention. Diffusion Tensor Imaging (DTI) scalar measures such as fractional anisotropy (FA) and mean diffusivity (MD) were used to evaluate anterior and posterior callosal tracts[4] . Our ultimate objective is to use these measures as neuroimaging surrogate markers to quantify post therapeutic microstructural changes. As an additional measure for data stability, we also examined the lateral ventricular cerebrospinal fluid (CSF) from all patients.

Subjects and Methods:

***Human Protection and Enrollment*:** This study was FDA and IRB approved. Twenty five stroke patients were enrolled; here we present a subset of 9 patients (6 females) aged 35-78 years who completed the imaging study and had MCA infarcts.

Bone Marrow Cells Harvesting: The procedural details about MNC isolation and transplantation are described elsewhere[5]. Briefly, a total of two ml/kg of bone marrow was harvested aseptically from the posterior iliac bone; cells were isolated and transplanted through intravenous infusion within 72 hours of stroke onset.

Clinical & Radiological Assessment: The National Institutes of Health Stroke Scale (NIHSS), MRI data were obtained at 1, 3, 6, 12 and 24 months follow-up.

***MRI Data Acquisition Protocol*:** MRI scans were performed on a 3.0 T Philips Achieva. The diffusion-weighted images (DWI) were obtained using a single-shot echo planar imaging (EPI) with 21 icosahderal and uniformly-distributed gradient orientations over the unit hemisphere; b-factor = 1000 s/mm^2, repetition time, TR = 8.0 sec, and echo time TE = 66 ms. the slice thickness was 3 mm with 44 axial slices a square field-of-view = 240 x 240 mm^2. Three-dimensional T1-weighted images were acquired with TR = 8.2sec, TE= 3.66 ms, acquisition matrix = 256 x 256 x 170, slice thickness = 1mm. ***Image Processing*:** The DWI data were preprocessed using FSL (http://www.fmrib.oxford.uk/fsl) for eddy current geometric distortions and motion correction. Scalar metrics such as FA and MD were obtained using both region-of-interest (in CSF) and along the white matter tracts (i.e. anterior and posterior CC). Anterior and posterior callosal fibers (i.e. aCC & pCC) were tracked using DTI studio as detailed elsewhere.[4]

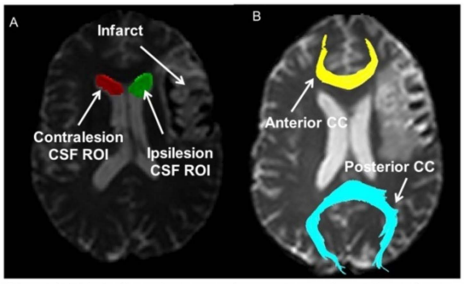

Figure1 illustrate a representive patient with MCA infarct. **A** is showing typical ipsi (green) and contralesion (red) region of interest used for cerebral spinal fluid analysis. **B** is illustrating typical Anterior (yellow) and posterior (cyan) white matter tracts of corpus callosum (CC).

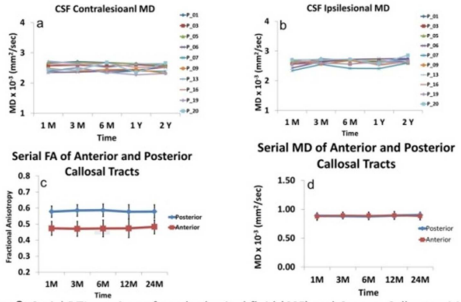

Figure 2: Serial DTI matrices of cerebral spinal fluid (CSF) and Corpus Callosum. Mean diffusivity of CSF were calculated by ROI in contra and ipsilesional ventricle as shown in a and b respectively. The average serial fractional anisotrpy and mean diffusivity in the anterior and posterior regions of corpus callosum were calculated using fiber tracking as shown in c and d respectively.

Results: **Figure 1** illustrates typical ipsi- and contra-lesion ROI and callosal tracts on one patient. The baseline lesion volume ranged 9.7-99.9 mL and NIHSS range was 8-18. **Figure 2** shows serial MD in the ipsi and contralesional side of lateral ventricular CSF, along with FA and MD in anterior and posterior CC tracts. Note longitudinal reproducibility of CSF diffusivity indicating stability. Note also that FA(pCC) > FA(aCC) ($p<0.001$), as expected from studies on healthy adult controls using identical DTI protocol.[4] A repeated measures analysis-of-variance (rANOVA) of the serial FA values in the aCC and pCC provided p=0.92, 0.74, whereas for MD, p=0.27, p=0.28, respectively. The rANOVA for the CSF diffusivity p-values for the stroke and contralateral sides, p=0.65 and p=0.074, respectively. These tests indicate no statistically significant changes over 2 years following cellular intervention.

Discussion: These preliminary results indicate that the adopted longitudinal neuroimaging markers show stability of qMRI measures of non-vulnerable regions in the MCA stroke as evidenced by statistically insignificant microstructural changes in selective anterior and posterior CC white matter tracts, confirming our hypothesis**.** Our results demonstrate the reliability of the methods and analysis strategy to test further more specific clinical and neurobiological hypotheses.

References:

[**1**] Savitz SI, et al. Ann Neurol. 2011;70:59-69. [**2**] Prasad K, et al. Indian J Med Res. 2012;136:221-8. [**3**] Purves D, et al., editors. Neuroscience 2nd edition, 2001. [**4**] Hasan KM, et al. Brain Res. 2009;1249:91-100. [**5**] Misra V, et al. AJNR Am J Neuroradiol. 2011;32:998-100.