Introduction: Traumatic brain injury (TBI) is a leading cause of death and disability in the United States and the world. Hemorrhage is a major radiological marker in clinical diagnosis of TBI. Susceptibility weighed imaging (SWI) has been shown to be 3-6 times more sensitive to microhemorrhages than the current clinical standard T2* GRE sequence [1, 2]. However, very little data has been reported to validate the SWI hemorrhages using histology. Furthermore, the hemorrhagic bleed undergoes several stages of transformation. How SWI shows these changes remains an open question. The objective of this study is to validate the SWI interpretation of hemorrhage in histology and to investigate the blood transformation signature on SWI images in an experimental TBI animal model.

Materials and Methods: Severe traumatic brain injury was induced in 23 male Sprague Dawley rats using the Marmarou impact acceleration model. Seven rats died immediately after injury and 16 rats survived post injury. The surviving rats were sacrificed at different times to validate the SWI blood product with histology. Meanwhile, a series of MRI scans were run at different times prior to sacrifice to detect the temporal signature of SWI measured hemorrhage. The times of sacrifice were 1, 4, and 24 hours, and 3, 4, and 7 days after injury. All of the MRI measurements were performed on a 4.7T Bruker AVANCE scanner. The MRI sequences included SWI and diffusion tensor imaging (DTI) in addition to baseline T1 and T2w sequences. SWI is based on a fully flow compensated, high resolution, 3D gradient echo method. The SWI parameters were TR=83ms, TE=35ms, FA=30°, echo position 25%, FOV=40x40x18 mm³, matrix size=1024x512x18, interpolated to 1024x1024x24, Nacq=2, and total imaging time 45m. SPIN software was used for SWI image post processing. In histology validation, all rat brains were perfused, fixed, and then harvested from their skulls. Prussian blue staining was used to confirm the presence of iron products, which evolved from hemorrhagic blood after injury. Prussian blue staining is a sensitive histochemical test that demonstrates single granules of iron in blood cells [3].

Results: Among the 16 rats that survived the weight drop, eight (50%) rats were identified by SWI to have hemorrhages in the corpus callosum, hippocampus, intraventricular space, and cortex directly underneath the impact site. By using Prussian blue staining, these hemorrhagic locations were confirmed by the presence of iron products, which evolved from hemorrhagic blood after injury. Furthermore, SWI also demonstrated an evolving pattern of hemorrhagic blood transformation at different times. Our data showed that blood products are better detected in the first 24 hours and after 4 days post injury; comparatively, the blood products are not visible between 24 hours and 3 days post injury.

Discussion and conclusions: Our work represents the first reported data regarding the direct histological validation of hemorrhages identified by SWI in traumatic brain injury. It confirmed the previous radiological findings that SWI hemorrhages in trauma [1] represent hemorrhagic blood products. Our work also represents the first reported data on a temporal transformation of hemorrhagic blood shown on SWI images. After hemorrhagic bleed, a series of transformations occur, from the original oxyhemoglobin (diamagnetic), to deoxyhemoglobin (paramagnetic), then intra and extracellular methemoglobin (paramagnetic with high R1), and finally hemosiderin (paramagnetic) left by macrophage cells. An appropriate choice of scan time is critical to detect these blood products for clinical diagnosis.

References: