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*Understanding the Pathology of Homicidal Pediatric Blunt Neurotrauma through Correlation of
Advanced Magnetic Resonance Images with Histopathology*

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SUMMARY

Traumatic brain injury is the most common neurological condition in the pediatric population in the United States. Approximately 30% of children who are victims of abusive head injury die.^{1,2} Additionally, survivors of abusive head trauma have worse neurological outcome compared to survivors of accidental traumatic brain injury.^{3,4,5} Few areas in forensic pathology are more challenging to the forensic pathologist and have proven to be more controversial in the literature and in courtroom testimony than abusive head trauma, based on the ambiguity of mechanism of injury leading to death. The addition of a biomarker that is sensitive to whole brain localization of injury, as well as injury severity, frequency, and duration would significantly influence our understanding of pediatric homicide, allowing investigators to better understand the last hours, days, weeks and even months of our subjects.

The pathology of abusive head injuries usually includes at least one of the following findings at autopsy: subdural hemorrhage (thin film or space occupying), subarachnoid hemorrhage, retinal hemorrhages, and or diffuse axonal injury.¹⁰ Considering these findings, diffuse axonal injury may not be visualized macroscopically during brain examination, with diagnosis dependent upon microscopic examination, usually with the assistance of an immunohistochemical stain for amyloid precursor protein (APP).

Perhaps the most controversial subject in forensic pathology is the mechanism of injury in the shaken infant with no physical signs of head impact (bruises on the head, subscalpular bleeding). The most common documented findings, as mentioned previously, include diffuse brain swelling, moderate to severe ischemia and traumatic axonal injury.¹⁰ Geddes et al reported statistically significant differences in the neuropathology present between young infants aged 2-3 months and toddlers older than one year who sustained blunt head trauma.¹¹ The younger group analyzed in the Geddes study reported global ischemia, craniocervical axonal injury and thin film (non-space occupying subdural hemorrhage). The craniocervical axonal injury reported by Geddes was the center of the proposed “unified hypothesis,” which surmised that craniocervical injury leads to respiratory disturbances sufficient to cause global hypoxia, which then causes brain swelling and raised central venous pressure, which subsequently causes veins to leak resulting in subdural hemorrhage and retinal hemorrhage. This theory, although widely criticized in the forensic pathology community, has been used extensively during judicial processes to suggest that violent forces may not be necessary to inflict severe neurological damage.^{12,13}

Cases with external evidence of blunt head trauma (bruises on the head or subscalpular hemorrhage) that show only cerebral edema, thin film subdural hemorrhage, retinal hemorrhages and absent axonal injury represent a subset of cases where the exact mechanism of death is unknown and are not supported by Geddes’ hypothesis. The presence of impact injuries on the head demonstrates the precipitating cause of death; however, what happens in the brain between the time of head impact and the time of death is unclear. It may be possible that diffuse axonal injury occurs, which is clinically manifested as concussion symptoms with non-recoverable apnea resulting in death.

Traumatic axonal injury occurs when axonal processes are sheared as a result of blunt head trauma with head impact or by acceleration and deceleration forces of the head (without or without head impact). Traumatic axonal injury has a predilection for the long white matter tracts in the brain, which include the corpus callosum, internal capsule, subcortical white matter, cerebral peduncles, lateral pons, and descending tracts of the cervicomedullary junction. In the medical examiner setting,

the gold standard for the diagnosis of diffuse axonal injury in blunt head trauma is histology, usually with the assistance of the immunostain for amyloid precursor protein (APP), which is a marker of axonal injury.^{12,14} Axonal injury can be seen during microscopic examination by conventional hematoxylin and eosin staining in the form of axonal spheroids, which can only be visualized with a survival period of at least 12-24 hours. With the use of immunohistochemistry for amyloid precursor protein (APP), an axonal fast transport anterograde protein, axonal spheroids are indicative of axonal injury and can be seen with a survival period of approximately two hours, although some literature reports a survival period of 35 minutes.^{15,16}

Postmortem computed tomography (CT) and Magnetic Resonance Imaging (MRI) have been determined to have an expanding spectrum of utility in identifying injuries and the cause of death.¹⁷ CT is currently used in several medical examiner settings in the United States and provides additional diagnostic insights when evaluating the cause of death. While MRI is used less frequently than CT scanning, its utility is most important for purposes in which CT is not clinically useful, such as evaluating cerebral edema, neoplasm, or diffuse axonal injury (DAI). The pathologist's interpretation of APP immunostaining as a marker of axonal injury is often challenging, since other variables (hypoxic-ischemic injury) can show patterns of injury that may be mistakenly interpreted as traumatic injury, such as vascular axonal injury and metabolic axonal injury.¹⁵ Additionally, specific areas of the brain are routinely sampled by the neuropathologist or forensic pathologist for evaluation of diffuse axonal injury, potentially missing other diagnostic areas of injury that are not visible macroscopically as part of brain examination.

MRI is a unique evaluation tool for identifying specific regions in the brain that can be targeted for neuropathologic evaluation, increasing the ability to diagnose DAI. DAI most typically appears on MRI as areas of decreased signal/susceptibility on T2* weighted iron-sensitive MRI sequences, such as SWI. SWI is a high-resolution three-dimensional gradient-echo sequence that is sensitive in detecting microbleeds associated with axonal injury in acute injury.¹⁸⁻²¹ DTI measures the diffusion properties of water molecules in tissue and its use in axonal injury evaluation is based on the premise that an injured axon demonstrates decreased anisotropy (directionally asymmetric water diffusion).^{7, 22, 23} More specifically, the diffusion of water molecules in white matter is greatest parallel to the axon. Damaged axons provide physical obstructions, influencing the direction and the amount of diffusion, which can be evaluated using different parameters radiologically.^{7, 23} Axonal injury can also appear as areas of restricted diffusion and if DTI is performed, as areas of decreased fractional anisotropy (FA).^{7,23} DTI and other advanced diffusion techniques can also be used to demonstrate abnormal diffusion parameters along the major white matter tracts as a more delayed effect of TBI and axonal injury.^{7, 23-26} Current literature describes DTI findings in children who survive their head injury, with imaging being performed in subacute and long-term stages of survival.^{7,25,26} There are limitations for DTI when evaluating non-Gaussian water motion, as seen in crossing fibers and within complex structures. Therefore, DKI can be used to probe these more complicated structures and to detect subtle changes in gray and white matter.

Neuropathologic examination of fatal pediatric brain injury, as part of forensic autopsy, usually occurs after a period of whole brain formalin fixation. The approach focuses on specific regions of the brain that are most likely to be affected, using the pathologist's clinical judgment and what is currently known about regions of the brain that are most susceptible to axonal injury. Correlating the specific location of findings in MRI examination would allow pathologist to target sampling in areas not visible

grossly, thus providing a more in-depth examination, and increasing the sensitivity for sampling injured regions, which can provide additional scientific evidence in a criminal justice setting. While the routine practice of MRI in all forensic cases is impractical due to the lack of wide-spread availability in a medical examiner setting, utility demonstrated by this study could possibly identify additional novel regions for standardized histologic sampling or provide support for the selective MR scanning of certain types of fatal pediatric neurotrauma cases. This novel study will provide previously uncharacterized trauma data for a population where the details of injuries have significant implications for the criminal justice system.

The goal of this proposed research project was to correlate magnetic resonance imaging (MRI) biomarkers including advanced diffusion techniques, such as diffusion tensor imaging (DTI) and diffusion kurtosis imaging (DKI) in fatal homicidal pediatric blunt head injuries, with histopathologic findings in the brain. In addition to image-guided sampling, this study aimed to identify unique regions of interest that have previously not been routinely investigated, such as cortical structures. To date, no advanced diffusion imaging studies have been performed on fatal human head injuries in a pediatric population and overall there are few studies of direct histopathologic correlation of MRI biomarkers of traumatic brain injury (TBI).^{6,7} This project utilized MRI, susceptibility weighted imaging (SWI), including DTI and DKI on formalin-fixed whole brains removed at the time of forensic autopsy, with subsequent imaging-focused histologic neuropathologic examination.^{8,9}

Our central questions were:

- Does MRI enable forensic pathologists to characterize inflicted head injuries better and focus histologic evaluations to do so?
- Does advanced imaging identify additional areas of injury that are not typically targeted by routine neuropathologic examination?
- *Hypothesis: The use of MRI imaging in pediatric homicidal blunt traumatic brain injury will supplement the neuropathologic evaluation of traumatic axonal injury and provide additional information useful to the pathologist and criminal justice system.*

To evaluate our hypothesis, we compared standard routine neuropathologic examination (considered the gold standard for forensic evaluation) with the addition of advanced MRI findings. Additionally, as part of our study, we evaluated decedents with various survival intervals, such as hours or less, versus those who survived for days, weeks, months, and possibly even years after their traumatic brain injury, whose demise was ultimately a result of traumatic brain injury. As part of our evaluation, documented regions of the brain that were injured, correlating those findings with known functional impairment affiliated with damage to those regions, demonstrated radiographically and histologically.

The primary objective of our study was to better define the degree and location of fatal traumatic brain injury, comparing those cases with no known survival period (dead upon presentation to the hospital or upon emergency medical services arrival to the scene) versus those with a survival period.

In this proposal, our basic research questions were:

- Can advanced neuroimaging provide additional information about injuries beyond what standard postmortem neuropathological examination can provide?

- What is the difference, neuroanatomically, in injuries that are survivable (based on findings documented in literature) versus those that are non-survivable, if only for a short period of time?
- Based on the imaging findings, do additional immunostains for glial fibrillary acidic protein (GFAP), CD68, and p62 better document the degree of injury mechanistically on a cellular level?
- Is the standard set of sections taken for microscopic examination in fatal pediatric blunt traumatic brain injury adequate? Will MRI provide additional areas of sampling that should be targeted in standard practice?
- Will MRI provide clarification of traumatic axonal injury versus vascular (hypoxic-ischemic axonal injury when the pathologic interpretation of β -APP is inconclusive (cases where vascular axonal injury overpowers the interpretation for traumatic axonal injury)?

Research Design and Implementation

Our study included only cases from the Office of the Medical Investigator (OMI) in Albuquerque, NM. The OMI is the statewide centralized medical examiner agency for New Mexico that is academically-based at the University of New Mexico School of Medicine. OMI performs approximately 2000 autopsies per year and is staffed by 10 forensic pathologists certified by the American Board of Pathology. OMI has both a CT scanner and an MR scanner in the autopsy laboratory. Imaging at OMI is supported by a Center for Forensic Imaging (CFI).

The study population involved infants, toddlers, and children (ages birth to 15 years) in which forensic autopsy indicated a cause of death due to blunt head trauma and a manner of death was classified as homicide. Included in this study (within the specified age range) were decedents who died from homicidal non-penetrating blunt head injury or injuries, to include autopsy findings with or without an impact site on the head (such as external scalp contusions or subscalpular hemorrhage), with or without subdural hemorrhage, and with or without retinal hemorrhage. Decedents with no known survival period after injury, as well as those with any survival period, were included in this study as long as the cause of death was attributed to the initial traumatic brain injury with or without intervening causes. Cases excluded from this study were decedents with fatal blunt head injury who fell outside the age range, penetrating head injury, any cause of death other than blunt head trauma and any manner of death other than homicide.

Since we compared the number of regions of injury (contusion, axonal injury) that were detected by routine neuropathologic examination, to the number of regions of injury (contusion, axonal injury) that are detected by advanced MR imaging, each case will serve as its own control. Additionally, since there is no known data regarding MR imaging of normal ex vivo pediatric whole brains, a second small negative control population cohort will consist of an age-matched population (ages birth to 15 years) in which forensic autopsy does not indicate a cause of death due to blunt head trauma and investigation does not demonstrate a history of traumatic brain injury. This second control population included deaths where a history of co-sleeping is present, with no findings of blunt head trauma or suspicious circumstance. The second control population likely has normal brains with some manifesting hypoxia-ischemic damage. This population allowed us to further understand any novel findings seen on

MR scans. This control group also allowed us to assure that novel MRI findings don't represent postmortem artifact or changes associated with hypoxic-ischemic injuries. Options for obtaining normal pediatric control brains are limited in forensic pathology practice.

Each autopsy was performed in the jurisdiction in which the death occurred. During autopsy, the brain of the decedent was removed through standard autopsy procedures and underwent fixation in 20% buffered formalin solution for a 2 week period, which is considered standard practice for fatal head injury. The brains were photographed, and external injuries, defects, or other abnormalities were documented. The brain was sectioned sagittally along the midline, separating the brain into right and left halves so that the brain would fit in the MR tube. Ex vivo brain MRI was conducted on a 7 Tesla (Bruker Biospin, 30 cm bore) scanner equipped with 20, 11.2, and 6 cm gradient inserts. The fixed brains were placed in a 3D printed holder. To reduce air signal and imaging artifacts, the brain and holder were placed in a large bag, submerged with Fomblin, and vacuum-sealed. A 3D T1 weighted sequence, inversion recovery prepared spoiled gradient recalled echo was performed for anatomic localization with 1mm in plane resolution. T2 weighted MRI was performed in the coronal plane with 5mm slices. A two-dimensional (2D) single-shot echo planar imaging sequence (repetition time/echo time [TR/TE] = 4500/30 msec; four repetitions) will be used to acquire four unweighted ($b = 0$ s/mm²) images and seven diffusion-weighted images (b-values ranging between 0-5000 s/mm²) using a Stejskal-Tanner diffusion preparation with parameters of $\Delta = 12$ msec, $d = 5$ msec, $\gamma = 14 \cdot 14$ mm², 30 noncollinear diffusion gradient directions. Post-processing of diffusion data will include standard DTI parameters/maps including fractional anisotropy (FA), mean diffusivity (MD), axial diffusivity (AD), radial diffusivity (RD), and color coded fiber tracking maps. DKI data/maps will also be processed including mean kurtosis (MK), axial kurtosis (Ka) and radial kurtosis (Kr).

A neuroradiologist reviewed the MRI and annotated areas of abnormality, with specific attention to areas of low signal suggesting microhemorrhage, and areas of suspected contusion. The location and description were recorded for subsequent correlation with pathological examination. Following imaging, each brain was examined at the OMI by a forensic neuropathologist, who documented abnormalities noted on external examination, followed by sectioning at 1.0 cm intervals. Each coronal section of the cerebral hemispheres was examined and abnormalities documented, as well as each sagittal section of brainstem with attached cerebellum. The routine sections taken during neuropathologic examination included a watershed region (anterior cerebral artery and middle cerebral artery watershed territory), basal ganglia, thalamus, hippocampus, visual cortex, brainstem and cerebellum.²⁷ As part of a pediatric traumatic brain injury histologic workup, additional sections included the body of the corpus callosum with parasagittal white matter, splenium of the corpus callosum, posterior limb of the internal capsule, pons, midbrain, cervicomedullary junction, and all macroscopically abnormal regions.^{27,28} Lastly, any specific areas of the brain demonstrating changes indicative of axonal injury on imaging not included in standard histologic sampling were sampled. The tissue was processed for histology at TriCore Laboratories in Albuquerque, NM.

Microscopic evaluation of brain tissue included analysis of the sampled regions by hematoxylin and eosin (H&E) stains of all paraffin-embedded blocks, as well as a panel of immunostains (below) selected for their ability to indicate neuronal injury and cellular reaction to injury. Standard sections were taken for each brain to include:

- Watershed region (frontoparietal)

- Corpus callosum (body and splenium)
- Basal ganglia
- Thalamus
- Hippocampus
- Occipital lobe (visual cortex)
- Periventricular white matter
- Pons
- Cerebellum with dentate nucleus
- Cervicomedullary junction

Additional regions of the brain demonstrating macroscopic abnormality and regions of brain not included in the standard sections with abnormalities depicted by MR were sampled and documented.

The immunohistochemical panel and its corresponding region of analysis is listed as follows:

- Amyloid precursor protein (APP): corpus callosum (body and splenium), posterior limb of internal capsule, pons, midbrain, cervicomedullary junction; additional regions based on imaging findings
- Glial fibrillary acidic protein (GFAP): Isocortex (subpial glial plate and gray-white matter junction), periventricular region
- CD68: Leptomeninges overlying isocortex and isocortex
- p62: corpus callosum (body and splenium), posterior limb of internal capsule, pons, midbrain, cervicomedullary junction

All tissue processing, histology and immunostains were performed at TriCore Laboratories, a private laboratory that currently handles all OMI material.

Subject Description	Predicted # of Samples	Service Performed
Traumatic brain injury cases (also act as own internal control)	40	Advanced MR and comprehensive neuropathologic examination
Negative control cases (normal brains)	10	Advanced MR and comprehensive neuropathologic examination

In order to address our research questions, the study was divided into two phases, with certain cases meeting criteria to undergo a third phase of study.

Phase 1: Cases were collected that meet the established inclusion criteria, perform advanced CT and MR imaging and comprehensive neuropathologic evaluation.

Phase 1 Methods:

- Brains from infants/children who died from fatal abusive non-penetrating brain trauma were imaged and examined according to previously described methods.
- Control cases were identified based on the established inclusion criteria and underwent imaging and examination.
- The neuropathologist was aware of the advanced imaging findings prior to neuropathologic examination (to be able to guide histologic sampling), with the standard set of microscopic sections collected, in addition to macroscopic abnormalities and target areas of abnormalities detected on imaging.

The specific objectives of Phase 1: 1) Identify abnormalities of the brain detected on imaging and discern whether the documented changes can be seen macroscopically upon brain sectioning. 2) Identify and document any abnormalities seen macroscopically that were not noted upon radiologic examination.

Phase 2 Methods:

- Abnormalities detected on MR and neuropathologic examination were examined histologically by conventional H&E staining and selected immunostains performed on selected regions.
- The microscopic examination provided a histologic correlate for abnormalities visualized by imaging interpretation and by neuropathologic examination.

The specific objective of Phase 2: The following questions were evaluated: 1) How accurate is MR imaging interpretation compared to the current gold standard (histologic evaluation)? 2) If MR imaging provides additional areas of abnormality not visualized by neuropathologic macroscopic examination, is the difference statistically significant?

Data analysis: Eleven negative control (normal) brains were imaged and evaluated by neuropathologic examination, to evaluate any postmortem artifact or hypoxic-ischemic changes that may be interpreted as injury (contusion or axonal injury), followed by neuropathologic examination, which serves as the gold standard of diagnosis. Our statistical power to detect whether the 11 negative controls have no axonal injury findings compared to the alternative of finding at least 1 positive site is over 88%.

This is a proof-of-principle study with 3 brains and 11 negative controls. The wide range of brain trauma severities lead to high variability in findings.

Data management for this study includes the use of Microsoft Word and Microsoft Excel spreadsheet for documentation and for data input of DTI findings (characteristics and location), as well as neuropathologic findings correlating to location, H&E descriptions and interpretation of immunohistochemical findings.

The largest problem in our study was collecting cases that met the inclusion criteria. We were able to collect 3 cases that met the inclusion criteria for the study, as well as 11 control cases.

Other Participants:

Our study was designed as a collaborative effort of the Office of the Medical Investigator (OMI) in Albuquerque, NM and the Office of the Chief Medical Examiner in Oklahoma (Central District). The OMI is the statewide centralized medical examiner agency for New Mexico that is academically-based at the University of New Mexico School of Medicine. OMI performs approximately 2000 autopsies per year and is staffed by 10 forensic pathologists certified by the American Board of Pathology. OMI has both a CT scanner and an MR scanner in the autopsy laboratory. Imaging at OMI is supported by a Center for Forensic Imaging (CFI). The Office of the Chief Medical Examiner (OCME) in Oklahoma City (Central District) is a non-academic office that represents one of two jurisdictions in the state of Oklahoma. The OCME performed 1,824 autopsies in 2015. Access to advanced postmortem imaging (CT and MRI) is not available at the OCME. Both of these agencies are accredited by the National Association of Medical Examiners. Unfortunately, the collaborating office did not respond to the study after multiple attempts to contact, despite having signed a letter of support.

ACCOMPLISHMENTS:

The major goals of the project consist of three phases of methods, which each contained objectives. Due to loss of a collaborating agency, coupled with challenges during the pandemic, our goal of collecting 40 brains could not be accomplished (target of 40 brains from infants/toddlers who died from fatal abusive head trauma and 11 control cases). We collected 4 brains that served as controls and 3 brains with evidence of head trauma. The brains were imaged using the 7 Tesla magnetic resonance (MR) scanner, utilizing diffusion tensor imaging (DTI) to look for possible axonal injury or other abnormalities. The brains were subsequently examined macroscopically and microscopically according to our research protocol. The macroscopic and microscopic examinations correlated with the imaging interpretations, but immunohistochemical results in cases with axonal injury showed that microscopic examination was more sensitive than the imaging techniques utilized in our study.

A major accomplishment of our project, was the design of a brain holder that adjusts for holding each brain in place, such that scanning can be performed without motion artifact. 3D printing allows for inexpensive rapid prototyping, where designs can be refined quickly with relatively short printing times (usually can be completed overnight). Additionally, given the non-metallic nature of the 3D printing plastic, it also makes a great material for the final product. Details of the prototypes tested and the final prototype being used for current and future imaging sessions is shown in the figure below.

Products:

The concept of the 3D printed brain holder specific for each brain failed when tested due to the inability to place in fomblin and maintain a vacuum seal. We subsequently had to design a holder that could adjust to each brain size and shape, while still keeping the brain submerged in fomblin.

The University of New Mexico Brain and Behavioral Health Institute (BBHI) houses a preclinical scanner with 7T magnet strength being used for brain imaging. Our successful design and testing resulted in a brain holder that was MRI safe, and allows for increased stability of the brain specimen, easier repeatability, and overall better aesthetic of scanning brain specimens. Future work will focus on optimization of advanced acquisition methods, including diffusion and Gadolinium-enhanced imaging.

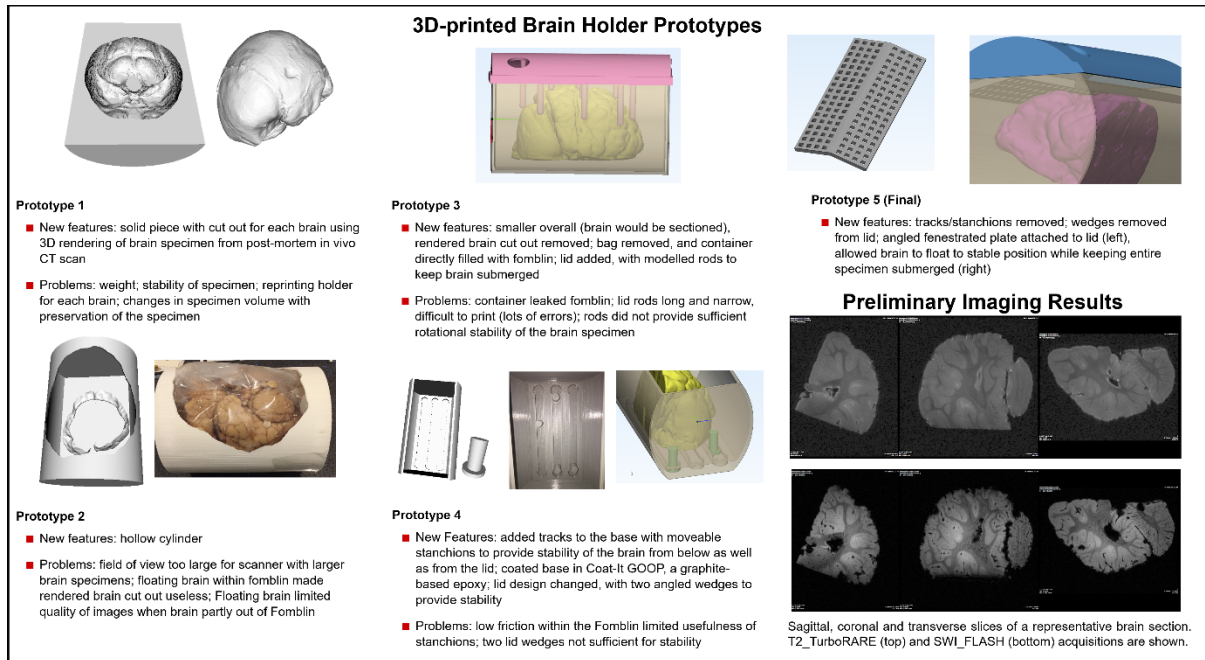
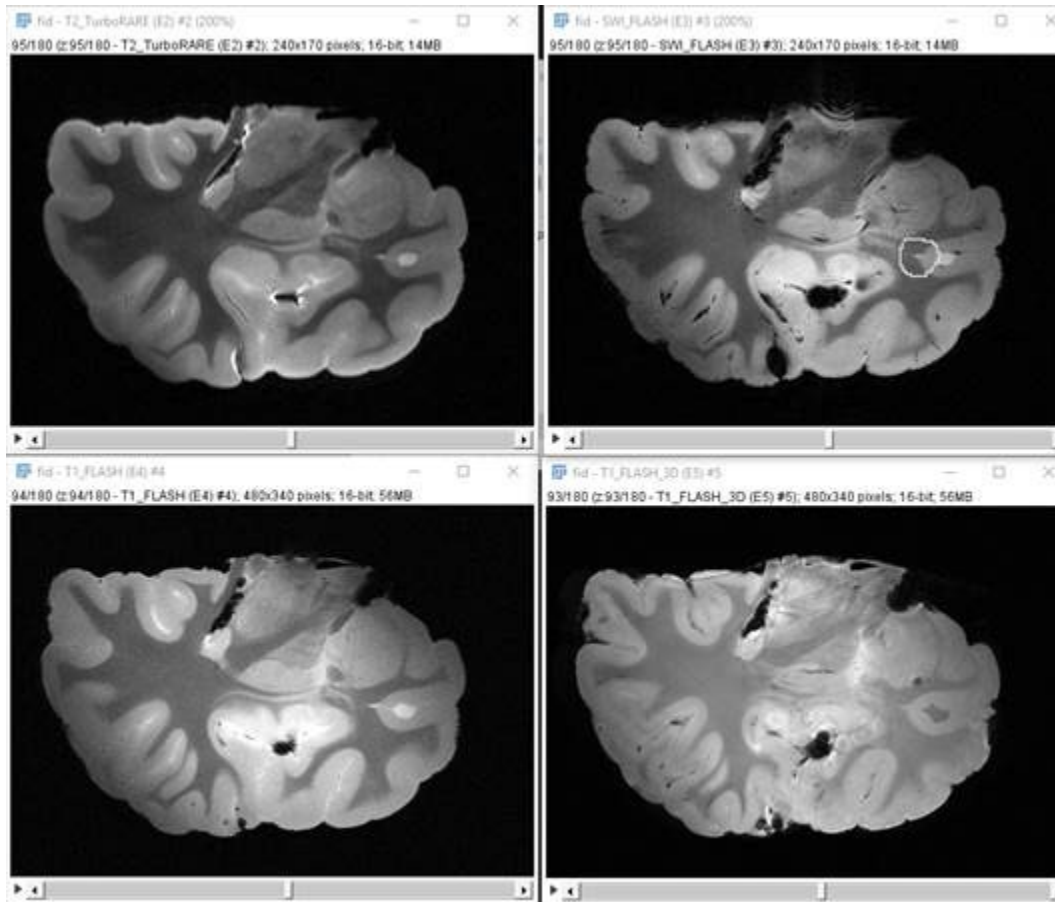


Figure. Details of the prototype brain holder with iterations tested and final prototype selection. The holder was designed to be MRI-compatible and to facilitate reproducible positioning of the pediatric brain specimen in a 7T preclinical scanner. A literature search revealed ex-vivo imaging methods have been developed for clinical scanners at 3-Tesla, but no reported prototypes for ex-vivo 3D-printed pediatric brain holders at higher magnet strength. The holder was designed de-novo to fit on a Bruker guinea pig bed and within a 15-cm volume coil. Prototypes were designed using Mimics Research software version 21.0 (Materialise, Leuven, Belgium) and saved as stereolithography (STL) files. Designs were printed in polylactide (PLA) with a Replicator Z18 fused deposition modelling (FDM) 3D printer (MakerBot, New York City, NY). Post-mortem imaging was performed using sheep and human brain specimens submerged in fomblin. T2, susceptibility, and diffusion-weighted imaging sequences were acquired on a BioSpec 70/30 USR (Bruker, Billerica, MA) 7-Tesla pre-clinical magnet.

Results:

The following images were taken from a brain with known associated blunt head trauma that included a parietal bone fracture, in a 4-year-old child.



The circled region, in the top right corner, is enlarged below.

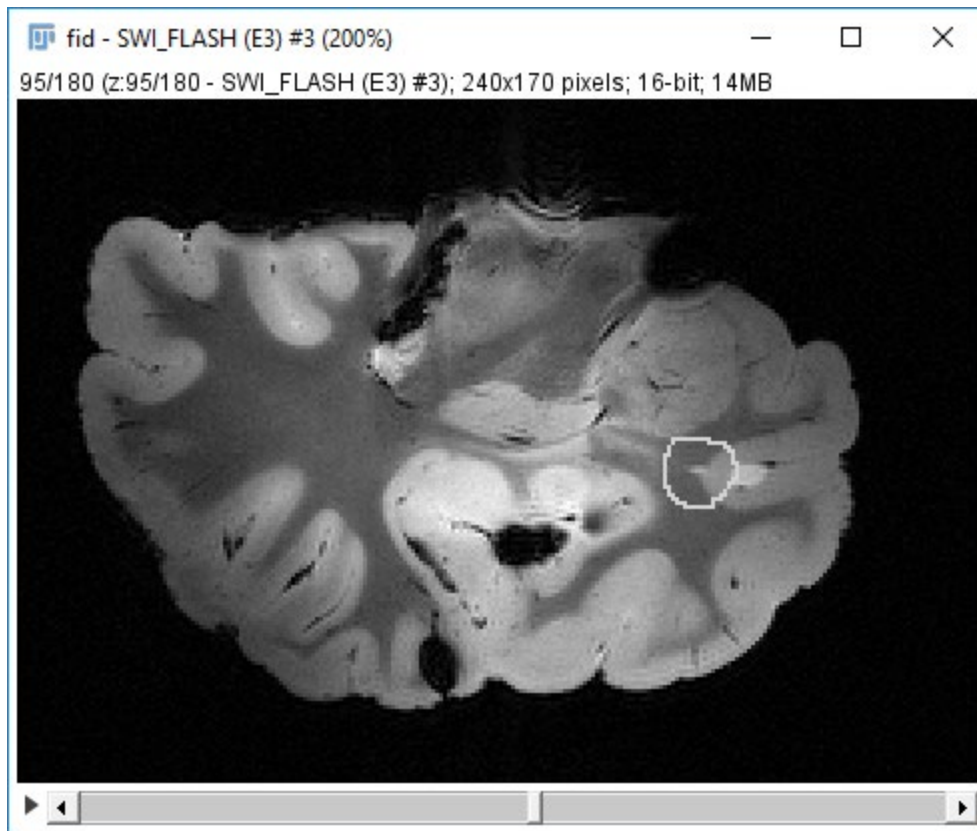


Figure: Radiologic interpretation of this region depicts a small subcortical cystic region of the inferior temporal lobe, with bright signal on T2, consistent with fluid. No microhemorrhages were present.

Macroscopic neuropathologic brain examination showed no evidence of injury. Histologic interpretation demonstrated diffuse traumatic axonal injury by APP immunohistochemistry, with reactive astrocytes by GFAP immunostaining. All other markers were non-contributory.

Per this case, neuropathology examination proved to be more sensitive than advanced imaging.

A second case, with known blunt head trauma from a 5-year-old, demonstrated subdural hemorrhage.

The MRI from this case showed changes associated with decomposition, but no axonal injury.

Microscopic interpretation demonstrated diffuse traumatic axonal injury, which is depicted below within the circled areas.

The third case showed changes associated with hypoxia-ischemia by imaging and by microscopic examination. Axonal injury staining with APP was reflective of the hypoxia-ischemic findings.



Figure: APP immunohistochemistry: Axonal injury is highlighted within the blue circles.

The study overall found that neuropathology was more sensitive for the detection of axonal injury compared to MR. Postmortem changes provided conflicting findings for radiology, that precluded focal evaluation for injury. The regions in question were easily discerned by macroscopic examination of the brain, with axonal injury able to be evaluated by APP immunohistochemistry.

Overall, the additional analyses cannot be conducted due to the small number of cases collected, to determine any statistical significance or draw further conclusions. Unfortunately, compounding factors of the loss of a collaborating agency and the pandemic significantly impacted our ability to collect the target number of cases. Additional collection and studies would need to be performed to draw meaningful conclusions.

Dissemination of findings:

The results of the 3D printed holder were disseminated at the University of New Mexico, Department of Radiology Research Day, which was awarded "Best Poster." (See Appendix A.)

A platform presentation was performed at the National Association of Medical Examiners Annual meeting, which was held by virtual meeting, in October 2020.


We are currently considering options for publication, but the small sample size is a significant limitation. We feel that the study could have produced significantly more valuable data with the collection of more cases, which was out of the investigators' control and was significantly affected by the pandemic.

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Appendix A.



Development of a 3D-Printed Brain Holder for Post-Mortem Pediatric Brain MRI at 7T

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INTRODUCTION

- The purpose of this project was to design, prototype, and test a holder to house a gross pathology brain for post-mortem MRI of pediatric brain specimens.
- This project is part of a larger project correlating post-mortem ex vivo MRI imaging findings with pathology of pediatric non-accidental trauma victims.
- In order to correlate neuroimaging and neuropathology data, we designed a novel brain holder for ex-vivo MR imaging.
- The holder was designed to be MRI-compatible and to facilitate reproducible positioning of the pediatric brain specimen in a 7T preclinical scanner.
- A literature search revealed ex-vivo imaging methods have been developed for clinical scanners at 3-Tesla^{1,2}, but no reported prototypes for ex-vivo 3D-printed pediatric brain holders at higher magnet strength.
- Therefore, our holder was designed de-novo to fit on a Bruker guinea pig bed and within a 15-cm volume coil (Figure 1).

METHODS

- Prototypes were designed using Mimics Research software version 21.0 (Materialise, Leuven, Belgium) and saved as stereolithography (STL) files.
- Designs were printed in polylactide (PLA) with a Replicator Z18 fused deposition modelling (FDM) 3D printer (MakerBot, New York City, NY) (Figure 2).
- Post-mortem imaging was performed using sheep and human brain specimens submerged in fomblin.
 - T2, susceptibility, and diffusion-weighted imaging sequences were acquired on a BioSpec 70/30 USR (Bruker, Billerica, MA) 7-Tesla pre-clinical magnet (Figure 3).
 - MRI Acquisition Parameters:
 - T2-weighted image had a matrix size of 220x170, field of view of 110 mm x 85 mm, slice thickness of 0.5 mm, with isotropic 0.5 mm voxels, TE/TR = 33/23525 ms, an echo-train length of 8, echo-spacing 11ms, and scan time 17 mins.
 - SWI scans were acquired using FLASH based T2* phase-enhanced sequence with the same geometry as T2, TE/TR = 14/7880 ms, and scan time 23 mins.
 - DTI scans were acquired using a four-shot EPI sequence with the same geometry as T2, TE/TR = 46/40000, 2 averages and scan time was 4 h 14 min. The diffusion preparation consisted of 30 diffusion directions with b = 2,000, 1000, 500 and five b = 0 images, with d/delta = 4/18 ms.




Figure 1. Bruker guinea pig bed (left) and bed inserted into 15-cm volume coil (right).

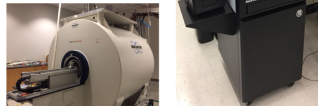
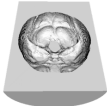
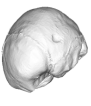


Figure 2. MakerBot Z18 FDM Printer in the UNM BBHI Preclinical Imaging Core.


RESULTS

3D-printed Brain Holder Prototypes

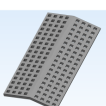
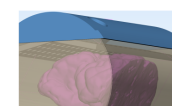
Prototype 1

- New features: solid piece with cut out for each brain using 3D rendering of brain specimen from post-mortem in vivo CT scan
- Problems: weight; stability of specimen; reprinting holder for each brain; changes in specimen volume with preservation of the specimen



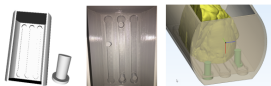
Prototype 2

- New features: hollow cylinder
- Problems: field of view too large for scanner with larger brain specimens; floating brain within fomblin made rendered brain cut out useless; Floating brain limited quality of images when brain partly out of Fomblin

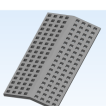
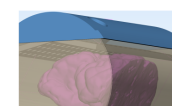
Prototype 3

- New features: smaller overall (brain would be sectioned), rendered brain cut out removed; bag removed, and container directly filled with fomblin; lid added, with modelled rods to keep brain submerged
- Problems: container leaked fomblin; lid rods long and narrow, difficult to print (lots of errors); rods did not provide sufficient rotational stability of the brain specimen



Prototype 4

- New Features: added tracks to the base with moveable stanchions to provide stability of the brain from below as well as from the lid; coated base in Coat-It GOOP, a graphite-based epoxy; lid design changed, with two angled wedges to provide stability
- Problems: low friction within the Fomblin limited usefulness of stanchions; two lid wedges not sufficient for stability

Prototype 5 (Final)

- New features: tracks/stanchions removed; wedges removed from lid; angled fenestrated plate attached to lid (left), allowed brain to float to stable position while keeping entire specimen submerged (right)

Preliminary Imaging Results

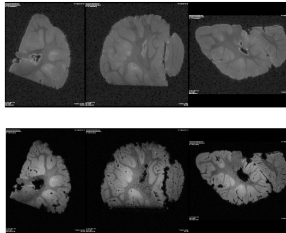


Figure 4. Sagittal, coronal and transverse slices of a representative brain section. T2, TurboRARE (top) and SWI, 15-AMR (bottom) acquisitions are shown.

DISCUSSION & CONCLUSION

- 3D printing allows for inexpensive rapid prototyping, where designs can be refined quickly with relatively short printing times (usually can be completed overnight).
 - Additionally, given the non-metallic nature of the 3D printing plastic, it also makes a great material for the final product.
- The University of New Mexico Brain and Behavioral Health Institute (BBHI) houses a preclinical scanner with higher magnet strength than the 3T scanners used for clinical applications.
 - Acquisition of images in higher magnet strength requires more time, and is therefore more sensitive to patient movement. This limitation is not present in post-mortem imaging.
- Our successful design and testing resulted in a brain holder that was MRI safe, and allows for increased stability of the brain specimen, easier repeatability, and overall better aesthetic of scanning brain specimens.
- Future work will focus on optimization of advanced acquisition methods, including diffusion and Gadolinium-enhanced imaging.

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