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Seizure Severity is Correlated With Severity of Hypoxic Ischemic Injury in Abusive Head Trauma

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Abstract

Objective: to characterize hypoxic ischemic injury (HII) and seizures in abusive head trauma (AHT). **Methods:** We performed a retrospective study over 4 years of 58 children with moderate or severe traumatic brain injury (TBI) due to AHT. Continuous electroencephalograms (cEEGs) and magnetic resonance images (MRIs) were scored. **Results:** Electrographic seizures (51.2%) and HII (77.4%) were common in our cohort. Younger age was associated with electrographic seizures (no seizures: median age 13.5 months, IQR 5-25 months vs seizures: 4.5 months IQR 3-9.5, p=0.001). Severity of HII was also associated with seizures (no seizures: median HII score 1.0 IQR 0-3 vs seizures: 4.5 IQR 3-8, p=0.01), but traumatic injury severity was not associated with seizures (no seizures: mean injury score 3.78 ± 1.68 vs seizures: 3.83 ± 0.95, p=0.89). There was a significant correlation between HII severity and seizure burden when controlling for patient age (r_s=0.61, p<0.001). The ratio of restricted diffusion volume to total brain volume (RD ratio) was smaller on MRIs done early (median RD ratio 0.03, IQR 0-0.23 in MRIs done within 2 days vs median RD ratio 0.13, IQR 0.01-0.43 in MRIs done after 2 days, p=0.03).

Conclusions: Electrographic seizures are common in children with moderate to severe TBI from AHT, and therefore children with suspected AHT should be monitored with cEEG. Severity of hypoxic ischemic brain injury is correlated with severity of seizures, and evidence of HII on MRI may evolve over time. Therefore, children with high seizure burden should be re-imaged to evaluate for evolving HII.

Keywords: Traumatic Brain Injury; Abusive Head Trauma; Acute Seizures; Status Epilepticus; Hypoxic Ischemic Injury.

Introduction

Abusive head trauma (AHT) is an important cause of traumatic brain injury (TBI) in infants, with an annual incidence of approximately 39 cases/100,000 children[1]. Acute symptomatic seizures are defined as seizures occurring within one week of an acquired brain injury[2] and are common in critically ill children with all TBI[3], and with AHT in particular[4]. Several studies have also demonstrated that restricted diffusion on magnetic resonance imaging (MRI), suggesting hypoxic-ischemic injury (HII), is a common finding in children after AHT[5, 6]. Two prior studies including relatively small numbers of AHT patients have noted an association between HII and seizures[4, 6]. We have observed an association between seizures and HII, and that patients with severe post-traumatic seizures often have the most severe HII on MRI. However, to our knowledge, there have not been any studies examining the correlation between seizure severity and severity of HII.

Since August 2009, we have implemented a clinical TBI protocol that includes performing 48 hours of continuous electroencephalogram (EEG) on children with a history of TBI and a Glasgow Coma Scale of less than 12. We performed a retrospective study of children at our institution diagnosed with abusive head trauma who were treated under our TBI protocol and evaluated clinical, MRI and EEG data.

Methods

Subjects

This study was reviewed and approved by the University of Colorado Institutional Review Board. We performed a retrospective chart review of patients admitted to the pediatric intensive care unit (PICU) under the Children's Hospital Colorado TBI protocol between October 2010 and Dec 2014. The TBI protocol covers children with confirmed or suspected traumatic brain injury with Glasgow Coma Scale (GSC) < 12. Patients are treated in the PICU and undergo 48 hours of EEG monitoring. We reviewed the charts of 83 patients under the age of 5 years and included patients diagnosed with AHT by a child abuse pediatrician. Patients who did not have at least 24 hours of continuous EEG available for review were excluded. The clinical care team cared for patients per their clinical practice. No standardized intervention was performed as part of this study.

EEG Analysis

Continuous EEG raw data was reviewed by a single pediatric epileptologist with board certifications in Epilepsy and Clinical Neurophysiology (KEC), blinded to other patient characteristics. An electrographic seizure was defined as a paroxysmal EEG discharge that evolved in frequency and location. The number of seizures in each 24 hour period of recording were counted, and the length of each seizure was noted. For each hour of recording, seizure burden was calculated (% of time in each hour occupied by seizure). Due to challenges in reliably correlating

clinical and EEG findings in the ICU setting, only electrographic seizures were considered in the seizure burden calculation.

MRI analysis

MRI scans were reviewed by a single pediatric neuroradiologist, with board certifications in Pediatric Radiology and Neuroradiology (NVS), blinded to whether patients had seizures. For each MRI in which there was restricted diffusion (defined as hyperintensity on diffusion weighted images and hypointensity on apparent diffusion coefficient images), the volume of tissue with restricted diffusion (vRD) was manually segmented using Aquarius iNtuition (TeraREcon, Forest City, CA). The total brain volume (vTB) was also manually segmented, and the ratio of vRD/vTB (RD ratio) was calculated. In addition, for each study a hypoxic-ischemic injury score (HII) and a brain traumatic injury score (bTS) were determined, as previously described[6]. Briefly, the total HII score was calculated by assigning a severity of 1-4 to 5 different patterns of diffusion weighted injury (vascular borderzone, subcortical gray matter, distribution of focal/unilateral arterial ischemic stroke, cortical laminar necrosis, and diffuse or global) leading to possible total HII score values of 0 to 20. Similarly, the bTS score was derived by assigning a severity of 1-4 to 5 different types of intracranial injury (extra-axial hemorrhages, cortical contusions, white matter shear injury, white matter hemorrhage, and parenchymal hemorrhage).

Data Collection and Analysis

Clinical data were collected from the medical record, de-identified, and stored in a secure database. Statistical analysis was performed using SPSS software. Mann-Whitney U test was used to compare means of groups with a non-parametric distribution. A Student's t-test was used to compare means of groups with a parametric distribution. The Chi-Squared or Fisher's Exact tests were used to evaluate categorical data. To determine which factors contributed to likelihood of having seizures, variables found be be significant in univariate analysis as well as clinically relevant variables were entered into a logistic regression model. To determine if a correlation between seizure burden and RD ratio existed a Spearman Partial Correlation was used.

Results

Study Population

During the study period 83 patients ≤5 years of age were treated in the PICU under the TBI protocol. Of those, 60 patients were diagnosed with AHT, 58 of whom had continuous EEG available for review (Fig 1) and represent our study population. Of the two patients excluded due to lack of EEG data, one patient had 24 hour EEG performed but the data was not available in archives, and the other patient did not have full EEG due to rapid clinical deterioration and death within 48 hours of presentation. Of the 58 patients in the study population, 53 (91.4%) had MRI during admission. Table 1 details the characteristics of the study population. There was an observed male predominance among children with AHT; 38 patients

(65.5%) were male. The median age of patients with AHT was 8 months (IQR 3-16.5 months).

Medical Care

Ten (17.2%) patients had cardiopulmonary arrest at presentation. Forty patients (69%) required intubation during initial hospitalization and 16(27.6%) were treated with an intracranial pressure (ICP) lowering agent. Hypotension requiring pressors was rare in our population with only 5 patients (8.6%) requiring pressors. There were 17 patients (29.3%) who underwent a neurosurgical procedure, the most common being extra-ventricular drain (EVD) placement (n=11, 19% of patients) (table1). Fifty-two patients (89.7%), were treated with anti-convulsants and 44 (75.9%) of patients remained on anti-convulsants at discharge, regardless of whether they had developed seizures during care.

Seizures

Seizures were common in our cohort with 27 (46.6%) of patients experiencing clinical seizures as a presenting symptom. Thirty patients (51.7%) had electrographic seizures during monitoring. Patients who developed seizures were significantly younger (median age 4.5 months, IQR 3-9.5months), compared to patients who did not (median age 13.5 months, IQR 5-25 months, p=0.001, Mann-Whitney U test, Table 2). Among patients who had seizures, the mean number of days with seizures present was 2.2 (range 1-4). AHT patients who developed seizures tended to have frequent seizures with a median of 52.5 seizures (IQR 17.0-67.5), on the most active day. We defined status epilepticus in two ways: first, as any seizure lasting longer than 30 minutes, and second, as greater than 50% seizure burden (% of any one hour period occupied by seizure). By either measure, status epilepticus was common in our cohort with 20 patients (51.7%) of patients meeting at least one definition of status epilepticus (table1).

Imaging Findings

All 58 patients in our cohort had intracranial hemorrhage (ICH), and the most common type of ICH was subdural hematoma (SDH) (n=56, 98.2%). Few patients had skull fractures identified on imaging (n= 14, 24%, table 1). Most AHT patients (n=53, 91.4%) had MRI performed during initial hospitalization. Of the five patients who did not have MRI done, four died within one week of presentation, and three of those died within two days of presentation. The one patient who survived but did not have an MRI done had no clinical or electrographic seizures and was discharged on hospital day three. A large percentage of patients with MRI imaging had some restricted diffusion on MRI (n= 41, 77.4%, table 1). Restricted diffusion on MRI was not associated with any clinical factors that classically can result in hypoxic ischemic

injury, including cardio-pulmonary arrest, hypotension (as indicated by the need for pressors, or lowest documented systolic blood pressure), respiratory failure (as indicated by the need for intubation), increased intracranial pressure (ICP) (as indicated by the use of an ICP lowering agent), or vascular injury on head and neck imaging (Table 3). There were a total 57 MRIs done within two weeks of presentation for the 53 patients who had MRI performed. For the 53 patients who had MRIs done, the median time to first MRI after presentation was 2.0 days (IQR 1-4 days). The amount of identified ischemic brain tissue, as measured by RD ratio, was greater on MRIs done later after presentation. When MRI's done early (in the first two days after presentation, n=28 MRIs) were compared to MRI's done greater than 2 days after presentation (n=29 MRIs), there was a significantly lower injury size in those MRI's done early (median RD ratio 0.03, IQR 0-0.23 vs median RD ratio 0.13, IQR 0.01-0.43, p=0.03, Mann-Whitney U test). We evaluated factors that may influence whether the initial MRI was done within, or later than, two days after presentation (Table 4). We chose to evaluate the need for a neurosurgical procedure, need for pressors, intubation, as well as lowest systolic blood pressure (SBP) and maximum traumatic brain injury score (bTS) as these are surrogate markers for severity of critical illness. Of the evaluated factors, only need for a neurosurgical procedure was associated with a significantly increased odds of having a later MRI.

There were three patients who had early MRIs without restricted diffusion and who subsequently had follow up MRIs with restricted diffusion (table 5). Of note, two of these three patients had seizures, and their RD ratios were much larger than the patient without seizures. The appearance of restricted diffusion between an initial MRI on hospital day (HD) #1 and HD #6 from a representative patient is shown in figure 2.

Relationship between seizures and hypoxic-ischemic injury

In univariate analysis, the only factors that had a significant association with seizures were younger age and the presence of restricted diffusion on MRI (table 2). A binomial logistic regression was run to determine the effect of age, hypoxic-ischemic injury (as measured by HI injury score), and severity of traumatic injury (as measured by bTS score) on seizures (table 6). The logistic regression model was statically significant, $X^2(3)=32.05$, $p<0.001$(area under the ROC=0.90, 95% CI 0.82-0.98). Patients with higher HI injury scores on MRI and younger age were more likely to have seizures. Increasing traumatic injury (bTS) score did not increase the likelihood of seizures.

A Spearman Partial Correlation was performed to determine if there was a linear relationship between the size of hypoxic ischemic tissue (as represented by the RD ratio) and seizure burden while controlling for the effects of age. There was a positive correlation between hypoxic ischemic injury score and seizure burden while controlling for age, which was statistically significant (r_s=0.61, p<0.001). The correlation between RD ratio and seizure burden remained significant when the timing of MRI (Spearman Correlation, r_s=0.56, p<0.001) was controlled for.

Discussion

In this study, we demonstrate a correlation between severity of hypoxic-ischemic brain injury and seizure severity in patients with abusive head trauma. Electrographic seizures and status epilepticus were very common in our cohort of children with abusive head trauma, a finding that is supported by other studies [3, 4]. In the current study, younger age was significantly associated with seizures, which parallels studies of traumatic brain injury [3], and pediatric stroke[7]. Excitatory neuronal transmission is critical for maturation of neural networks, and studies in rodents suggest that the younger brain has an increased propensity for seizures due to overdevelopment of the excitatory neurotransmitter systems, and underdevelopment of inhibitory systems. There is relative over-expression of excitatory glutamate receptors (NMDA and AMPA) and decreased expression of glutamate re-uptake transporters compared to the mature brain[8], all of which likely contribute to the association between younger age and seizures in our population.

We found that restricted diffusion on MRI, in patterns classically thought to represent hypoxic-ischemic injury to brain parenchyma, was common in our population, which is supported by prior studies[5]. Previous research has suggested that HII is much more common in AHT than in accidental head trauma[6]. In this study, HII was present in 77% of our patients who had MRIs, a much higher percentage than reported in other studies [6, 9, 10]. The reason for brain parenchymal restricted diffusion after AHT is unclear. Previously, encephalopathy after AHT was thought to be due to axonal injury due to rotational forces and axonal shearing[11]. However, more recent pathologic studies examining brain tissue from AHT victims have demonstrated that diffuse axonal injury is rare in AHT [12]. The exception to this is in the brainstem (and particularly in respiratory centers); some studies demonstrate signs of axonal injury in these areas [13], although other pathologic studies dispute this finding[14]. It has been reported that apnea at presentation is associated with hypoxic-ischemic brain injury in AHT [15]. This suggests that HII after AHT may be due to prolonged apnea in the post-injury period. However, in our cohort, cardiopulmonary arrest, or the need for intubation were not significantly associated with HII. Therefore, it is possible that HII is due to un-witnessed apnea that occurs out of the hospital, or that there is another mechanism of HII. Vascular injuries were rare in our cohort, and there was no association between vascular injures, such as arterial dissection or cerebral sino-

venous thrombosis (CSVT), and HII. One possibility is that increased cerebral edema contributes to HII by reducing cerebral perfusion pressure; however, the need for intervention for increased ICP was not associated with HII. Similarly, hypotension during PICU stay was also not associated with HII. This suggests that HII is inherent to the mechanism of injury of AHT rather than secondary to vascular injury or sequelae during PICU care. Although, to our knowledge, we have one of the largest cohorts of patients included in our study of seizures and imaging changes in abusive head trauma, it is possible that this study is not powered to detect contributions of these other variables to developing HII.

The RD ratio was greater in MRI's done later than 2 days after presentation compared to early MRIs. In addition, we report three cases in which the initial MRI did not show any restricted diffusion but a follow-up MRI demonstrated new areas of HII. This evolution of reduced diffusivity after the first few days is similar to that seen in another developmental brain injury mechanism: perinatal hypoxic-ischemic encephalopathy. Perinatal HIE mean diffusivity is lowest 4-5 days after the initial injury [16, 17]. These data suggest that significant parenchymal injury may be missed if MRI is performed within 2 or 3 days of clinical presentation. Our results regarding timing of imaging may be confounded by clinical factors that influence the timing of MRI, and also contribute to the development of restricted diffusion on MRI. Patients who underwent a neurosurgical procedure had a significantly increased odds of having later MRIs, but none of the other surrogate factors for severity of critical illness, including severity of traumatic brain injury, were associated with later MRI. Furthermore, the most common neurosurgical procedures in our cohort overall were extraventricular drain and intracranial pressure monitor placement, which are relatively minor procedures and not necessarily associated with injury severity. Therefore, the increased ratio of ischemic brain tissue on later MRIs is most likely due to temporal evoluation of injury rather than other confounders.

We found that the odds of having seizures were significantly higher in children with hypoxic ischemic brain injury. Furthermore, we found that there was a direct correlation between severity of HII and seizure severity. Several smaller studies have previously demonstrated a relationship between any HII and seizures [4, 6]. To our knowledge, this is the largest study to evaluate this relationship and the only study to demonstrate a direct correlation between the extent of HII and seizure burden. As HII tends to be apparent on later MRI's, often after seizures have developed, an argument could be made that the timing of MRI confounds the relationship between HII and seizure burden, and that perhaps there are other factors that contribute to timing of MRI that are also associated with higher seizure burden. However, when the timing of the MRI is controlled for, the correlation between HII and seizure burden remains significant.

As this is a retrospective study, we are unable to establish a cause and effect relationship between HII and seizures, and therefore cannot rule out that seizures are the cause of restricted diffusion on MRI. Restricted diffusion has been reported

after status epilepticus in adult patients in some studies, but is often reversible[18, 19]. The proposed mechanism of restricted diffusion after status epilepticus is thought to be due to increased cerebral metabolic demands and failure of membrane Na/K-ATPase pumps leading to cyototoxic edema[20, 21]. However, these studies exclusively include adult patients with a variety of underlying etiologies for seizures, including vascular disease. Therefore, it is unclear whether the restricted diffusion noted after seizures in these studies is truly due to the seizures themselves. In contrast, AHT patients are typically previously healthy. If frequent seizures are the cause of restricted diffusion after AHT, there needs to be a plausible etiology for the high seizure burden. Prior studies of all mechanisms of TBI in children have shown an association between skull fracture and intraparenchymal hemorrhage and seizures [3]. However, in our study of AHT specifically, we did not find that skull fracture, ICH or intraparenchymal blood was associated with a higher odds of seizures. Overall traumatic injury score based on imaging was also not associated with a higher odds of seizures. We found that when children with AHT developed seizures, the seizures were frequent, but self limited, which is similar to the pattern of seizures after other known hypoxic ischemic brain injury in early life [22]. Given this clinical similarity in seizure onset with other causes of hypoxic-ischemic injuries, and the lack of another reasonable cause for seizures, it is more likely that the seizures are a result of the original hypoxic ischemic injury, rather than seizures causing restricted diffusion.

Acute post-hypoxic seizures in the developing brain have been well described clinically, and have been demonstrated during hypoxia and reperfusion in mouse models of transent hypoxia[23]. However, the mechanisms of acute post-hypoxic seizures are poorly understood. We found that there was a significant correlation between amount of restricted diffusion and seizure burden in our population, and we hypothesize that seizures are the result of ischemic injury. This could be due to energy failure in the ischemic tissue leading to decreased glutamate reuptake and increased NMDA receptor activation, either in the ischemic tissue or in surrounding tissue (reviewed in [24]). Several MRI imaging studies in adult arterial ischemic stroke have demonstrated that restricted diffusion acutely does not necessarily represent dead tissue, and that at least some tissue with restricted diffusion hs reversible injury, i.e., penumbra[25-27]. It has been shown in a rat model of ischemic stroke that tissue depolarizations occur in the ischemic penumbra [28], suggesting that this region may be the source of acute post-ischemic seizures. However, no clinical studies have evaluated whether ischemic tissue after AHT contains a penumbra-like region, and animal models that accurately mimick AHT are lacking. Further research is needed to determine the underlying mechanicsms of acute seizures in AHT.

Developing frequent seizures after AHT may exacerbate existing ischemic injury by similar mechanisms seen in status epilepticus that cause neuronal injury in the absence of pre-existing acute brain injury. In infants with other types of hypoxic ischemic brain injury, the presence of seizures increased brain lactate as measured by MR spectroscopy in infants with seizures but not infants without seizures[29,

30]. In a mouse model of hypoxic injury, acute post hypoxic seizures result in higher histopathologic injury scores [31]. In addition, brain tissue after hypoxic injury and seizures had more prolonged depletion of high energy phosphate substrates compared to hypoxia or seizurs alone [32]. This suggests that seizures may exacerbate hypoxic injury by further exhausting metabolic reserve.

Seizures are an independent risk factor for poorer developmental outcome in this type of injury [33], which may be due to reduced neurogenesis after seizures in the developing brain[34]. In other studies of AHT, early post-traumatic seizures are associated with poorer outcome [35], but it is unclear at this point whether poorer outcome is a function of injury severity, or is independently due to seizures. In addition, seizures associated with early life brain injury significantly increase the risk of developing epilepsy later in life[33, 36]. The relationship between early post-traumatic seizures in AHT and development of later epilepsy is less clear. Although remote symptomatic epilepsy is relatively common in AHT, it is not necessarily correlated with the severity of early post-traumatic seizures[37].

In summary, our data suggests a strong correlation between the severity of early post-traumatic seizures after AHT and severity of hypoxic ischemic injury. In addition, we report that the presence of restricted diffusion on MRI can increase over several days after clinical presentation in AHT, and it may be missed or underestimated if MRI is performed earlier than 3 days after presentation. The early identification and treatment of seizures after AHT may have an impact on outcome and later development of epilepsy. Furthermore, the development of frequent seizures is often a marker of hypoxic-ischemic brain injury. Therefore, MRI should be repeated if frequent seizures or status epilepticus develop in order to evaluate for evolving hypoxic-ischemic brain injury.

References

1. Shanahan, M.E., et al., *National, Regional, and State Abusive Head Trauma: Application of the CDC Algorithm.* PEDIATRICS, 2013. **132**(6).
2. Beghi, E., et al., *Recommendation for a definition of acute symptomatic seizure.* Epilepsia, 2010. **51**(4): p. 671-675.
3. Arndt, D.H., et al., *Subclinical early posttraumatic seizures detected by continuous EEG monitoring in a consecutive pediatric cohort.* Epilepsia, 2013. **54**(10): p. 1780-1788.
4. Hasbani, D.M., et al., *Nonconvulsive Electrographic Seizures are Common in Children With Abusive Head Trauma*.* Pediatric Critical Care Medicine, 2013. **14**(7): p. 709.
5. Kemp, A.M., et al., *Neuroimaging: what neuroradiological features distinguish abusive from non-abusive head trauma? A systematic review.* Archives of Disease in Childhood, 2011. **96**(12): p. 1103-1112.
6. Ichord, R.N., et al., *Hypoxic-Ischemic Injury Complicates Inflicted and Accidental Traumatic Brain Injury in Young Children: The Role of Diffusion-Weighted Imaging.* Journal of Neurotrauma, 2007. **24**(1): p. 106-118.

7. Abend, N.S., et al., *Seizures as a Presenting Symptom of Acute Arterial Ischemic Stroke in Childhood.* The Journal of Pediatrics, 2011. **159**(3): p. 479-483.
8. Sanchez, R.M. and F.E. Jensen, *Maturational Aspects of Epilepsy Mechanisms and Consequences for the Immature Brain.* Epilepsia, 2001. **42**(5): p. 577-585.
9. McKinney, A.M., et al., *Unilateral hypoxic-ischemic injury in young children from abusive head trauma, lacking craniocervical vascular dissection or cord injury.* Pediatric Radiology, 2008. **38**(2): p. 164-174.
10. Kadom, N., et al., *Usefulness of MRI detection of cervical spine and brain injuries in the evaluation of abusive head trauma.* Pediatric radiology, 2014. **44**(7): p. 839-848.
11. Gleckman, A.M., et al., *Diffuse axonal injury in infants with nonaccidental craniocerebral trauma: enhanced detection by beta-amyloid precursor protein immunohistochemical staining.* Archives of pathology & laboratory medicine, 1999. **123**(2): p. 146-151.
12. Geddes, J.F., et al., *Neuropathology of inflicted head injury in children. I. Patterns of brain damage.* Brain : a journal of neurology, 2001. **124**(Pt 7): p. 1290-1298.
13. Matschke, J., et al., *Encephalopathy and death in infants with abusive head trauma is due to hypoxic-ischemic injury following local brain trauma to vital brainstem centers.* International Journal of Legal Medicine, 2015. **129**(1): p. 105-114.
14. Oehmichen, M., et al., *Shaken baby syndrome: re-examination of diffuse axonal injury as cause of death.* Acta neuropathologica, 2008. **116**(3): p. 317-329.
15. Kemp, A.M., et al., *Apnoea and brain swelling in non-accidental head injury.* Archives of Disease in Childhood, 2003. **88**(6): p. 472-476.
16. Barkovich, A.J., et al., *MR imaging, MR spectroscopy, and diffusion tensor imaging of sequential studies in neonates with encephalopathy.* AJNR. American journal of neuroradiology, 2006. **27**(3): p. 533-547.
17. Bednarek, N., et al., *Impact of therapeutic hypothermia on MRI diffusion changes in neonatal encephalopathy.* Neurology, 2012. **78**(18): p. 1420-1427.
18. Canas, N., et al., *The electroclinical-imagiological spectrum and long-term outcome of transient periictal MRI abnormalities.* Epilepsy research, 2010. **91**(2-3): p. 240-252.
19. Huang, Y.-C., et al., *Periictal magnetic resonance imaging in status epilepticus.* Epilepsy Research, 2009. **86**(1): p. 72-81.
20. Wang, Y., et al., *Postictal alteration of sodium content and apparent diffusion coefficient in epileptic rat brain induced by kainic acid.* Epilepsia, 1996. **37**(10): p. 1000-1006.
21. Evans, M., T. Griffiths, and B. Meldrum, *Early changes in the rat hippocampus following seizures induced by bicuculline or L-allylglycine: a light and electron microscope study.* Neuropathology and applied neurobiology, 1983. **9**(1): p. 39-52.
22. Lynch, N.E., et al., *The temporal evolution of electrographic seizure burden in neonatal hypoxic ischemic encephalopathy.* Epilepsia, 2012. **53**(3): p. 549-557.
23. Zanelli, S., et al., *Impact of transient acute hypoxia on the developing mouse EEG.* Neurobiology of Disease, 2014. **68**: p. 37-46.

24. McLean, C. and D. Ferriero, *Mechanisms of hypoxic—ischemic injury in the term infant.* Seminars in Perinatology, 2004. **28**(6): p. 425-432.
25. Olivot, J.-M., et al., *Relationships Between Cerebral Perfusion and Reversibility of Acute Diffusion Lesions in DEFUSE.* Stroke, 2009. **40**(5): p. 1692-1697.
26. Labeyrie, M.-A., et al., *Diffusion Lesion Reversal After Thrombolysis A MR Correlate of Early Neurological Improvement.* Stroke, 2012. **43**(11): p. 2986-2991.
27. Kranz, P.G. and J.D. Eastwood, *Does Diffusion-Weighted Imaging Represent the Ischemic Core? An Evidence-Based Systematic Review.* American Journal of Neuroradiology, 2009. **30**(6): p. 1206-1212.
28. Pinard, E., et al., *Penumbral Microcirculatory Changes Associated With Peri-infarct Depolarizations in the Rat.* Stroke, 2002. **33**(2): p. 606-612.
29. Campbell, C., et al., *Seizure-associated brain injury in term newborns with perinatal asphyxia.* Neurology, 2002. **59**(7): p. 1119-1120.
30. Miller, S.P., et al., *Seizure-associated brain injury in term newborns with perinatal asphyxia.* Neurology, 2002. **58**(4): p. 542-548.
31. Wirrell, E.C., et al., *Prolonged seizures exacerbate perinatal hypoxic-ischemic brain damage.* Pediatric research, 2001. **50**(4): p. 445-454.
32. Yager, J.Y., et al., *Prolonged neonatal seizures exacerbate hypoxic-ischemic brain damage: correlation with cerebral energy metabolism and excitatory amino acid release.* Developmental neuroscience, 2002. **24**(5): p. 367-381.
33. Glass, H.C., et al., *Clinical Neonatal Seizures are Independently Associated with Outcome in Infants at Risk for Hypoxic-Ischemic Brain Injury.* The Journal of pediatrics, 2009. **155**(3): p. 318-323.
34. McCabe, B.K., et al., *Reduced neurogenesis after neonatal seizures.* The Journal of neuroscience : the official journal of the Society for Neuroscience, 2001. **21**(6): p. 2094-2103.
35. Bourgeois, M., et al., *Epilepsy associated with shaken baby syndrome.* Child's Nervous System, 2008. **24**(2): p. 169-172.
36. Fox, C.K., et al., *Neonatal seizures triple the risk of a remote seizure after perinatal ischemic stroke.* Neurology, 2016. **86**(23): p. 2179-2186.
37. Barlow, K.M., J.J. Spowart, and R.A. Minns, *Early posttraumatic seizures in non-accidental head injury: relation to outcome.* Developmental medicine and child neurology, 2000. **42**(9): p. 591-594.

Figure 1. Patient Selection

Figure1: Patient Selection

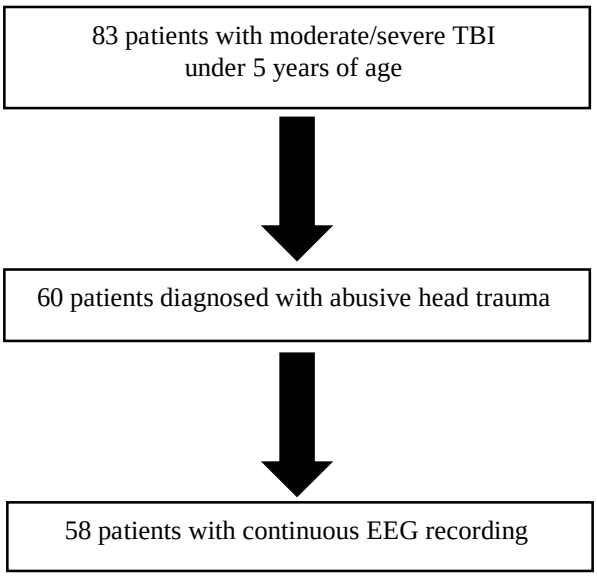

Figure 2: Evolution of Hypoxic-Ischemic Injury Over time: A. Representative patient with initial MRI on hospital day (HD) #1 without restricted diffusion and follow up MRI with restricted diffusion on HD#3. B. Mean ratio of ischemic brain over time.

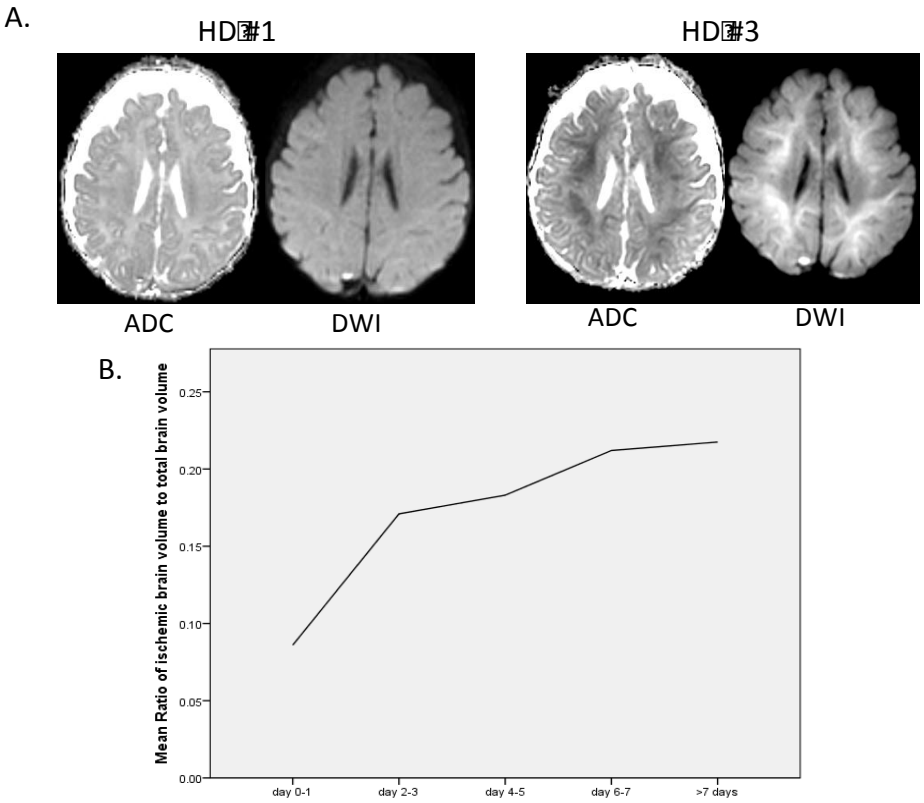

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Table 1: Characteristics of study group, n=58

Male Gender, n(%)	38 (65.5%)
Age at presentation in months, median (IQR)	8 (3-16.5)
Cardiopulmonary arrest, n(%)	10 (17.2%)
Seizures at presentation, n (%)	27 (46.6%)
Intubated during hospitalization, n (%)	40 (69.0%)
Required Pressors, n (%)	5 (8.6%)
Required ICP lowering agent, n (%)	16 (27.6%)
Intracranial hemorrhage, n (%)	58 (100%)
SDH	56 (98.2%)
SAH	33 (56.9%)
IVH	4 (6.9%)
IPH	9 (15.5%)
Vascular injury	7 (12.1%)
Neurosurgical procedure performed, n (%)	17 (29.3%)
Craniotomy and Evacuation	3 (5.1%)
Craniectomy	3 (5.1%)
EVD placement	11 (19.0%)
Intracranial pressure monitor	7 (12.1%)
Retinal hemorrhages, n (%)	44 (75.8%)
Other extra-cranial injuries, n (%)	19 (32.8%)
Skull fracture, n (%)	14 (24.1%)
MRI done, n (%)	53 (91.4%)
Presence of restricted diffusion, n (%)†	41 (77.4%)
Seizures, n (%)	30 (51.7%)
Number of days with seizures, mean (range)	2.2 (1-4)
Maximum number of seizures in one day, mean±SD	55.5 ± 44.0
Maximum seizure burden‡, mean±SD	58.8% ± 25.9%
Status Epilepticus- Traditional definition§, n (%)	10 (33.3%)
Status Epilepticus- seizure burden¶, n (%)	20 (66.7%)
Status epilepticus by any definition, n (%)	20 (66.7%)
Anti-convulsants used, n (%)	52 (89.7%)
Discharge disposition, n (%)	
Died	6 (10.3%)
Home or Foster Care	29 (50%)
Inpatient Rehabilitation	23 (39.7%)

†Out of 53 patients who had MRI done.
‡largest % of any hour occupied by seizure
§Any seizure lasting longer than 30 minutes.
¶ >50% of any hour period occupied by seizure.

Table2: Clinical Characteristics of Patients with Seizures vs No Seizures

Of all studied patients (n=58)	No Seizures 28(48.3%)	Seizures 30 (51.7%)	OR (95% CI)	p-value
Age at presentation in months, median (IQR) †	13.5 (5-25)	4.5 (3-9.5)	__	0.001
Any restricted diffusion, n=41‡(out of 53 patients with MRI)	13 (24.5%)	28 (52.8%)	10.0 (1.9-52.0)	0.002
HI injury score, median (IQR)†	1(0-3)	4.5(3-8)	--	0.01
bTS Score, mean±stdev#	3.78 (±1.68)	3.83±0.95	--	0.89
Presence of any ICH, n=58¶	28 (48.3%)	30 (51.7%)	(n/a)*	--
IVH, n=4¶	3 (5.2%)	1 (1.7%)	0.3 (0.0-2.9)	0.34
SDH, n=56¶	26 (44.8%)	30 (51.7%)	--**	0.23
IPH, n= 9¶	5(8.6%)	4 (6.9%)	0.7 (0.2-3.0)	0.73
SAH, n=33‡	14 (24.1)%	19 (32.8%)	1.7 (0.6-4.9)	0.31
Any Neurosurgical procedure, n=17‡	8 (13.8%)	9 (15.1%)	1.1 (0.4-3.3)	0.91
Craniotomy and Evacuation, n=3¶	2 (3.4%)	1 (1.7%)	0.4 (0.0-5.2)	0.61
Craniectomy, n=3¶	1 (1.7%)	2 (3.4%)	1.9 (0.2-22.5)	1.00
EVD, n=11‡	5 (8.6)%)	6 (10.3%)	1.1 (0.3-4.3)	0.84
ICP monitor n=7¶	5 (8.6%)	2 (3.4%)	0.3 (0.1-1.9)	0.25
Skull fracture, n=14‡	6 (10.3%)	8 (13.8%)	1.3 (0.4-4.5)	0.64

†Mann-Whitney U test
‡ Chi- Squared
# t-Test
¶ Fisher's Exact Test
* ICH is a constant as 100% of patients had ICH.
** not calculated as all but 2 patients had SDH.

Table 3: Characteristics of patients with restricted diffusion vs no restricted diffusion.

Of patients with MRI (n=53)	No RD 12 (22.6%)	RD (12) 41 (77.4%)	OR (95% CI)	p-value
Age in months, median (IQR)†	5.0 (3.25-11.5)	8.0 (3.0-17.0)	--	0.36
Cardiopulmonary arrest, n =7‡	0 (0%)	7 (13.2%)	--*	0.33
Need for pressors, n=5‡	0 (0%)	5 (9.4%)	--**	0.58
Lowest documented SBP, median (IQR) †	80.5(64.3-88.8)	70.5 (64.0-78.0)	--	0.20
Intubation, n=37 ‡	6 (11.3%)	31 (58.5%)	2.8 (0.7-10.3)	0.16
Intervention for ICP, n=15 ‡	1 (1.9%)	14 (26.4%)	5.5 (0.6-43.9)	0.15
Vascular injury, n=7‡	1 (1.9%)	6 (11.3%)	1.8 (0.2-17.4)	1.00

†Mann-Whitney U test
‡Fisher's Exact Test
*OR not calculated because all patients requiring pressors had RD on MRI.
** OR not calculated because all patients with CPA had RD on MRI.

Table 4: Characteristics of patients with first MRI within two days of presentation vs patients with first MRI later than two days after presentation.

	Any Neurosurgical Procedure‡	pressors‡	intubation‡	Lowest SBP#	Max bTS#
First MRI ≤2 days n=28	2 (7.1%)	1 (3.6%)	22 (78.6%)	73.1±11.6	3.61±1.45
First MRI >2 days n=25	13 (52.0%)	4 (16.0%)	14 (56.0%)	71.9±10.1	4.08±1.08
Odds Ratio	14.1 (2.7-72.5)	5.1 (0.5-49.5)	0.3 (0.1-1.2)	--	--
p-value	0.001	0.18	0.14	0.7	0.2

‡ Chi- Squared
# t-Test

Table 5: Characteristics of 3 patients with multiple MRIs

Time of 1st MRI	Ratio of RD volume	Time of follow up MRI	Ratio of RD volume	Seizures?	Day seizures started
Day 1	0	Day 6	0.43	Yes*	Day 3
Day 1	0	Day 3	0.27	Yes*	Day 2
Day 0	0	Day 3	0.05	No	--

* Patients had status epilepticus.

Table 6: Logistic regression predicting the likelihood of seizures based on age, hypoxic-ischemic injury (HI) score and traumatic injury score (bTS).

	SE	Wald	p	Exp(B)	95% CI
Age	0.07	8.6	0.003	0.82	0.71-0.93
HI score	0.16	11.5	0.001	1.70	1.25-2.32
bTS score	0.35	3.1	0.08	0.54	0.27-1.08