

Newborn Liver Functions as an Adjunct Biomarker in Timing Fetal Neurologic Injury

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ABSTRACT

Background: We hypothesized that in the presence of an intrapartum hypoxic ischemic insult, redistribution of cardiac output away from the hepatic circulation will result in unique patterns of hepatic dysfunction dependent on the degree and duration of the hypoxic ischemic insult. We evaluated the rise and clearance of Aspartate Aminotransferase (AST) and Alanine Aminotransferase (ALT) in term newborns with three common patterns of hypoxic ischemic encephalopathy as an adjunct biomarker in timing of fetal neurologic injury.

Methods: We identified 230 term newborns with image proven hypoxic ischemic encephalopathy with profound neurologic impairment over a 30 year period from multiple institutions. Eighty four had liver transaminases in the first 72 hours of life to evaluate patterns of rise and clearance.

Results: A total of 215 AST, 220 ALT and 204 NRBC values were collected. Similar to NRBC's, the general trend was the more chronic asphyxia, the more elevated transaminases are shortly after birth with delayed clearance often beyond 48 hours of life. In acute profound intrapartum injury, liver transaminases demonstrated minimal rise with rapid normalization. There was no difference between groups regarding gender, gestational age and birthweight.

Conclusion: No single proven biomarker is diagnostic of neonatal encephalopathy but newborn AST/ALT measured shortly after birth and daily for three days can provide additional evidence based medicine to confirm or refute allegation of acute intrapartum asphyxia.

Keywords: Hypoxic ischemic encephalopathy; Neonatal encephalopathy; Asphyxia; Biomarkers; Liver functions

INTRODUCTION

Birth asphyxia may result in neonatal encephalopathy, defined as a disturbance of neurologic function evident in the first days after birth in a newborn, characterized by a subnormal level of consciousness and depressed tone and reflexes with or without seizures often with impaired respiration and feeding difficulties among newborns of 35 weeks' gestation or greater [1]. The term asphyxia defined as hypoxic ischemic encephalopathy as a condition of impaired gas exchange in a newborn that leads to progressive hypercarbia, hypoxia, and acidosis depending on the extent and duration of this interruption [2]. Neonatal encephalopathy may be categorized in three stages as described by Sarnat and Sarnat and occurs in approximately 3 per 1000 live births in developed countries. Perinatal brain injury can occur before, during or after delivery [3,4].

With a normal 7000 hours pregnancy, the incidence of cerebral palsy attributed to the last 2 hours is <15%. The majority of cerebral palsy cases occur before the onset of labor [5]. The Apgar score, umbilical cord gas, neuroimaging and multiorgan dysfunction can be used to help determine whether injury is consistent with an intrapartum event [6,7]. As yet, there are no proven biomarkers that are diagnostic for neonatal encephalopathy, or the timing of a potential brain injurious event, or prognostic for long term outcomes following early symptoms [8]. The newborn record in alleged cases of intrapartum asphyxia can provide evidence based medicine to confirm or refute these allegations. [9]

Although not diagnostic for asphyxia, multiple studies have demonstrated distinct patterns of rise and clearance of Nucleated Red Blood Cells (NRBC) with different patterns of asphyxia [10-12] We hypothesized that liver transaminases drawn in newborns

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Received: April 17, 2020, **Accepted:** May 01, 2020, **Published:** May 07, 2020

Citation: Muraskas JK, Dina P, Chiaro BD, Martin BM, Amin SC, Morrison JC, et al. (2020) Newborn Liver Functions as an Adjunct Biomarker in Timing Fetal Neurologic Injury. *J Neonatal Biol* 9:273. doi: [10.35248/2167-0897.20.9.273](https://doi.org/10.35248/2167-0897.20.9.273).

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to assess multiorgan dysfunction would reflect similar trends to NRBCs. The rationale being that a profound cardiovascular response to asphyxia is a redistribution of cardiac output, in part through the primitive diving reflex from less vital negotiable circulation as the liver, kidneys and bone marrow to the non-negotiable circulation of the brain, heart and adrenal glands [13,14].

MATERIAL AND METHODS

The Loyola University of Chicago Health Sciences Division Institutional Review Board (#209238) approved the observational study that evaluated 84 newborns from a pool of 269 closed cases of alleged intrapartum asphyxia gathered over a 31 year period (1987-2018) reviewed by a neonatal expert (JKM) for causation. Of these, 9 were not reviewed because of conflict of interest with the institution or physician. Thirty cases based on preliminary data were deemed indefensible or non-meritorious by the author resulting in further data becoming unavailable. One hundred and seventy two of 230 (75%) were reviewed for the defense while fifty eight of 230 (25%) were reviewed for the plaintiff. All 230 cases are closed with 16/230 (7%) dismissed, 198/230 (86%) settled and 16/230 (7%) went to trial with 11/16 (69%) received a defense verdict. Ninety four of 230 were cases from Illinois (41%) while 59% of cases came from 34 different states. Of the 84 cases studied, maternal history was significant for: 22/84 (26%) had placental pathology demonstrating chorioamnionitis, 11/84 (13%) had preeclampsia or chronic hypertension, 6/84 (7%) maternal trauma.

Of the 230 cases, 146 were excluded due to absence of liver functions, imaging and/or death prior to imaging. Data points were abstracted and entered into an institutional review board approved de-identified secure electronic database. Demographic data, aspartate aminotransferase (AST), alanine transaminase (ALT), NRBCs, Apgar scores, cord blood gases and initial newborn blood gas were entered into the database. A total of 215 AST, 220 ALT and 204 NRBC values were collected at four different time periods were drawn, 0-12, 13-24, 25-48 and >48 hours of life. Neuroimaging was available to confirm 3 different patterns of hypoxic ischemic injury. Acute profound asphyxia (APA) injury results in a sudden marked or catastrophic decrease in fetal cerebral blood flow and/or oxygenation [6]. It is most often seen in patients with reassuring fetal heart rate tracings that abruptly become a category III, usually with bradycardia and absent variability, secondary to acute placental abruption, uterine rupture and/or cord prolapse [15,16]. Following APA injury, metabolically active deep gray matter in the basal ganglia, thalami, putamen, internal capsule and hippocampus are most often affected. Frequently, dyskinetic cerebral palsy with spastic quadriplegia is seen with this type of injury pattern. In the second pattern (partial prolonged asphyxia-PPA), which can occur silently in utero, the areas between vascular territories shift from the periventricular region towards the cortex and the subcortical white matter in near term and term newborns. Such injuries most frequently are gradual in onset, leading to a progressive but eventually significant reduction in blood flow and oxygenation to tissues at the end of the vessels in the watershed zones. A series of such events usually occurs over several hours demonstrating a gradual change from a category I-II to a category III (persistent late or variable decelerations and absent variability) tracing. Neurologically it results in variable injury to either or both

of the gray and white matter at the site of the watershed zone. It can occur silently within hours before birth or may occur in the last several days before admission for labor. This type of injury is often associated with severe cord compression, oligohydramnios or placental insufficiency. A pregnant woman can also present with a history of decreased fetal movements, abnormal fetal assessment and often a category II or III tracing. The fetus can also recover after a remote insult and present with a category I or II tracing. This represents a fetus who has a preexisting injury with limited reserves that can lead to further insult in the form of a terminal near collapse during labor. The combination of both aforementioned injuries is the third major type of fetal neurologic injury.

A descriptive analysis included subjects' demographic and clinical data stratified by injury type. A Pearson chi-square test was used to compare the breakdown by infant sex using frequencies and proportions. An analysis of variance (ANOVA) then assessed the mean difference by gestational age, birth weight (g), arterial cord pH, and base deficit (BD) as well as newborn gas pH and BD. Kruskal-Wallis tests were employed to examine median 5 and 10-minute Apgar scores due to their non-normal distribution. Linear mixed effects models were then used to estimate patients' ALT, AST, and NRBC levels over time by injury. Random effects were used to account for infants' multiple observations over the analysis period. An alpha error rate of $p \leq 0.05$ was used to determine statistical significance. All analyses were completed using SAS 9.4 (Cary, NC).

RESULTS AND DISCUSSION

We studied 84 cases. A total of 215 AST, 220 ALT and 204 NRBC values were collected. All variables were grouped by type of injury. There was no difference between the groups regarding sex, gestational age, and birth weight (Table 1). Newborns with an APA injury tended to report lower 5- and 10-minute Apgar scores compared to newborns with a PPA injury ($p=0.003$ and $p=0.002$, respectively). Newborns with both injuries also recorded lower 10-minute Apgar scores compared to newborns with just a PPA injury ($p=0.002$). Higher newborn blood gas pH and higher newborn blood gas (BD) was observed in newborns with AP ($p=0.02$) and PPA ($p=0.04$) injuries compared to newborns with both injuries. In the 84 cases examined 46/84 (55%) had cord arterial blood gases available for analysis.

Following an APA insult, ALT levels demonstrate mild elevation within the first 48 hours followed by rapid clearance (Figure 1). The levels peak between 25 to 48 hours with a mean of 94.85 (+/- 46.93). In PPA insult, ALT levels rise faster within the first 12 hours and peak between 13 to 24 hours [368.12 (75.08)] with a subsequent delayed clearance. Similar patterns were observed for ALT levels in infants suffering both injuries, rising quickly within the first 12 hours and again peaking at 13 to 24 hours [262.53 (63.37)] with delayed clearance. Infants with APA injuries recorded significantly lower ALT levels compared to infants with PPA and both injury types from 13 to 24 and 25 to 48 hours (both $p=0.01$). For the period of 0 to 12 hours and >48 hours, we observed no difference in mean ALT levels across all three insults ($p=0.26$ and $p=0.10$, respectively).

Following an APA insult, AST levels demonstrate a moderate elevation within the first 12 hours followed by a rapid clearance

Table 1: Baseline demographics.

Variables	Acute Profound (40)	Both 26	Partial Prolonged (18)	Total (84)	p-value	
Sex	Female	44.40%	55.60%	50.00%	48.60%	0.9
	Male	55.60%	44.40%	50.00%	51.40%	
Gestational Age	39.0 (1.7)	39.0 (2.2)	38.8 (1.5)	39.0 (1.8)	0.9	
5 Min APGAR*	2 (1-4)	3 (2-5)	5 (4-7)	3 (1-5)	0	
10 Min APGAR*	4 (2-5)	3 (1-4)	7 (3-8)	4 (2-6)	0	
Birth Weight (g)	3361.12 (472.41)	3301.43 (666.54)	3193.88 (274.75)	3305.66 (507.94)	0.6	
Arterial Cord pH	6.96 (0.22)	6.88 (0.19)	6.96 (0.18)	6.93 (0.20)	0.4	
Arterial Cord BD	-12.23 (6.42)	-18.21 (7.50)	-16.47 (8.12)	-15.80 (7.63)	0.1	
Newborn Gas pH	7.08 (0.22)	6.92 (0.23)	7.06 (0.78)	7.03 (0.22)	0	
Newborn Gas BD	-17.56 (7.09)	-22.84 (7.23)	-18.86 (6.38)	-19.40 (7.22)	0	

Note: Estimates for continuous variables expressed as means and standard deviations, unless otherwise specified. *Median and interquartile range. *Median and interquartile range.

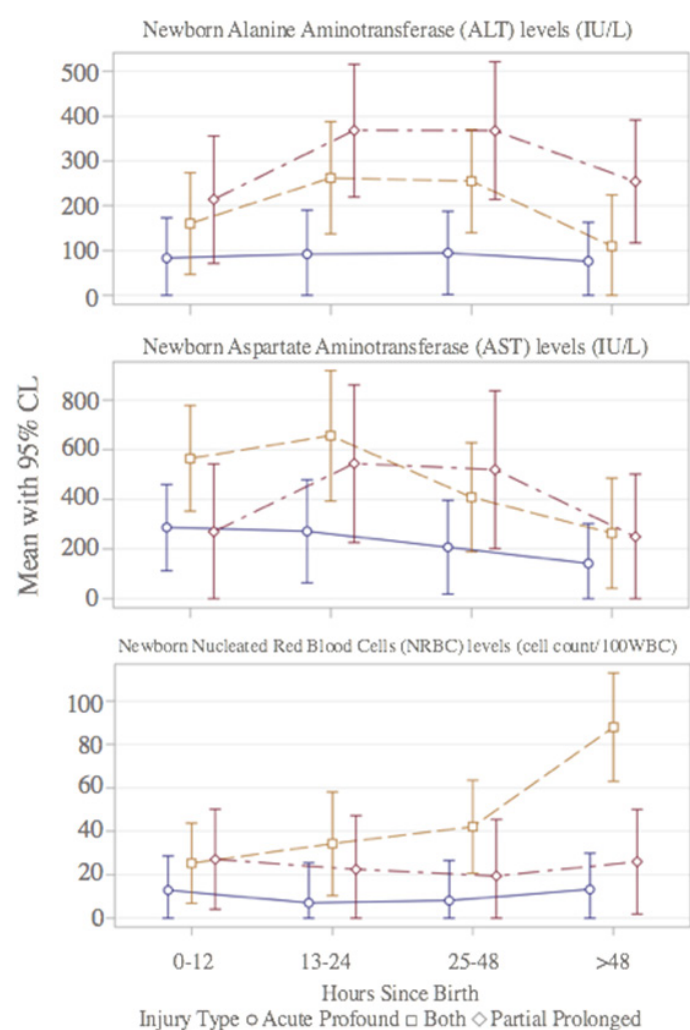


Figure 1: Newborns alanine aminotransferase (ALT), aspartate aminotransferase (AST), and nucleated red blood cell (NRBC) levels by injury type.

of 286.74 (+/-87.69). In a PPA insult, AST levels rise within the first 24 hours and peak between 13 to 24 hours [543.98 (160.06)] with a subsequent delayed clearance. AST levels in infants with both injuries rise fast within the first 12 hours, peaking between 13 to 24 hours [656.03 (132.74)] with delayed clearance. The difference in the mean AST levels between all three insults, taking into consideration every time period, was not statically significant.

Following an APA insult, NRBC levels demonstrate minimal elevation within the first 12 hours followed by a rapid clearance (Figure 1). The levels peak between 0 to 12 hours with a mean of 12.97 (+/-7.92). In a PPA insult, NRBC levels also peak within the first 12 hours followed by delayed clearance [27.16 (11.58)]. In infants with both injuries, the NRBC levels rise in the first 12 hours and keep rising in a steady state to peak at more than 48 hours [87.99 (12.52)]. The difference in mean NRBC levels across all three insults was only statistically significant beyond 48 hours after birth.

ALT (normal value: 10-40 IU/L) and AST (normal value: 20-70 IU/L) are common markers used to evaluate liver function. NRBCs (normal 0-4/100 WBC) are immature erythrocytes that can be elevated in newborns with HIE most likely through a hypoxia-mediated increase in fetal erythropoietin production and release. Its use as a biomarker for fetal hypoxia remains investigational and NRBCs can be elevated with prematurity, maternal diabetes and intrauterine growth restriction. [10,17] ALT is more liver specific and tends to be lower than AST which is an acute phase reactant. Similar to NRBCs, ALT and AST in APA from a sentinel event had minimal elevation shortly after birth with rapid normalization of values consistent with a short diving reflex. In a PPA injury, the ALT was significantly elevated by 12 hours of life rising over the next 24-48 hours with delayed normalization. The AST had mild elevation shortly after birth with moderate elevation over 24-48 hours with delayed normalization. The elevated ALT shortly after birth is consistent with cumulative intermittent remote hepatic hypo perfusion. With both asphyxia insults, the ALT pattern is similar to PPA while the AST is significantly elevated after birth reflecting a double hit (remote and acute) with subsequent delayed clearance. Similar to NRBCs, the general trend is the more chronic the asphyxia, the more elevated transaminases are shortly after birth with delayed clearance often beyond 48 hours of life.

Studies have demonstrated some correlation between the severity of the perinatal asphyxia and the increase in liver transaminase levels [18]. One study looking at serial measurements of ALT and AST found different patterns but did not describe the type or timing of asphyxial insult. They concluded birth asphyxia can induce an enzyme pattern in serum compatible to hypoxic hepatitis and a possible correlation exists between aminotransferases in serum and extent of central nervous system injury. Other studies

have demonstrated hepatic dysfunction in approximately 40% of newborns with birth asphyxia. The definition of asphyxia and timing of blood draws varied in these studies [19,20].

In the last decade, the standard of care for near term and term newborns with moderate to severe neonatal encephalopathy is to initiate therapeutic hypothermia within 6 hours of birth for a 72 hour duration to a depth of 33°C to 34°C. Unfortunately, not all newborns with moderate and most with severe neonatal encephalopathy do not benefit from cooling [21]. Studies have demonstrated that induced therapeutic hypothermia for 72 hours does not appear to affect transaminase levels [22,23]. This is relevant in that not all patients in this study were cooled.

Hepatic dysfunction is likely caused by hypoperfusion rather than by hypoxia [24]. The redistribution of cardiac output is the direct result of vasoconstriction in non-vital organs and vasodilation in vital organs as the heart and brain [13,14]. As asphyxia progresses, arterial blood pressure cannot be maintained in spite of peripheral vasoconstriction because cardiac function begins to decline. In a prolonged and cumulative insult, one would expect physiologically more hepatic dysfunction due to extended redistribution of blood flow from the hepatic circulation to more vital organs [25]. Hypothermia can modify imaging parameters, such as afferent diffusion coefficient values and the predicted values of diffusion weight (DWI). Further studies are needed to specify these events [26].

Finally, one should incorporate the intrapartum tracing as it can be helpful in determining the timing of asphyxial insults in the last few days of pregnancy. If the intrapartum strip remains a category I (stable baseline, moderate variability with no late or variable decelerations, and the presence or absence of early decelerations/accelerations) or progresses to a category II tracing (variable or late decelerations, repetitive or not, with moderate or minimal baseline variability) one can most likely rule out any intrapartum insult causing neonatal encephalopathy and long-term neurologic dysfunction. Therefore, given a category I or II tracing and abnormal AST, ALT and NRBCs levels at birth coupled with abnormal imaging studies, one should focus on antepartum events. In these cases, placental study by a perinatal pathologist and a search for anemia or infection (or Fetal Inflammatory Syndrome) may be helpful in elucidating causation [27,28].

Although marked variation in practice has evolved over the years including reducing post term delivery, management of meconium stained amniotic fluid. MRI imaging and neonatal resuscitation, the incidence of HIE in term and near term newborns has not significantly changed. The incidence of cerebral palsy has not been significantly impacted despite advances in fetal heart rate monitoring and high cesarean section rate. Perinatal interventions to improve neonatal outcomes continue to evolve as does the standard of care in the management of central nervous dysfunction from impaired placental gas exchange.

LIMITATIONS

A major limitation of this study is the inability to quantitate the duration and extent of a fetal hypoxic ischemic insult with a partial prolonged pattern of injury. Such injuries most frequently are gradual in onset, leading to progressive reduction of blood flow and oxygenation to the cortical grey and subcortical white matter in the watershed zones. Extended partial prolonged injury can extend

beyond the watershed zones into the adjacent cortices causing massive cerebral edema. There was mild variation in the normal values of AST and ALT in different hospital labs. The majority of newborns in this study had poor outcomes that led to litigation and is likely that newborns with mild asphyxia and good outcomes were not included in this 31 year retrospective study. The strength of this study is the large number of newborns with neuroimaging and outcomes confirming known patterns of hypoxic ischemic encephalopathy with enough liver functions to assess for distinct patterns that would require about 150,000 live births to achieve this amount of cases. There is significant variation on when and if liver functions are drawn by neonatal care givers in depressed newborns.

CONCLUSION

Although there is much overlap between the three patterns of asphyxia, our data supports that longer periods of asphyxia tend to result in more elevated LFT's with delayed return to normal. The majority of newborns with APA delivered within the 30 minute guideline from decision to incision demonstrated minimal rise and rapid normalization of liver functions very similar to NRBCs patterns. No one specific biomarker can time an injury. However, we feel serial evaluations of commonly drawn neonatal labs to evaluate multiorgan failure as NRBCs, ALT and AST can serve as adjunct biomarkers to assist in the timing and duration of injury. Significantly elevated levels of NRBCs and LFT's shortly after birth would be inconsistent with an allegation of acute intrapartum asphyxia and reduce speculation. Similar to NRBCs, LFT's drawn on days 1, 2 and 3 of life can provide useful and meaningful information for the obstetrician, neonatologist, and pediatric neurologist. No one specific biomarker can time an injury and critical analysis of multiple components such as maternal history, cord blood gases, placental pathology, neonatal physical exam and neuroimaging is essential.

DISCLOSURE

There are no conflict of interests.

ACKNOWLEDGMENTS

No disclosures or acknowledgments.

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