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Acute Kidney Injury in Asphyxiated Newborns treated with Therapeutic Hypothermia

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ABSTRACT

Introduction: Perinatal asphyxia represents one of the main causes of multiorgan dysfunction in newborns. According to the most recent literature[22], acute kidney injury (AKI) is common in neonates with hypoxic-ischemic encephalopathy (HIE) – characterized by coma and seizures in the early neonatal period, as a result of a hypoxic-ischemic insult in the neonatal period.

Objectives: This study aims to: 1) Determine the incidence of AKI in asphyxiated neonates with HIE enrolled in therapeutic hypothermia (TH); and 2) Examine the correlation between the severity of short-term neurologic outcome in these neonates and the development of AKI.

Methods: A prospective database of patients submitted to TH, in Centro Hospitalar Universitário Lisboa Norte, EPE, is maintained since the beginning of the program (2010) until 2017. Patients were divided into two groups based on their short-term neurologic outcome (favourable/unfavourable), according to amplitude-integrated electroencephalogram (aEEG) and magnetic resonance imaging (MRI) at the second week of life. Renal parameters of neonates in both groups were monitored and AKI was determined using neonatal modified Kidney Disease: Improving Global Outcomes (KDIGO)[28] criteria. Data analysis was performed using SPSS.

Results: 92 patients were included in this study, 59 (64%) were male and 32 (35%) were female. The mean weight was 3161 ± 615.7 g and the gestational age was 39.06 ± 1.63 weeks. We reported that 44 (48%) of the neonates had AKI, of whom 38 (86%) neonates had AKI stage I, 2 (5%) had AKI stage II and the remaining 4 (9%) AKI stage III. Neonates who developed AKI had, although not statistically significant, higher mortality (17% vs. 10%) and stayed an average of 2.1 days longer in the NICU, compared to those without AKI. Expected short-term neurologic outcome, according to aEEG and MRI, was determined in 61 (66%) neonates, 39/61 (64%) of these had an unfavourable outcome. We reported a higher incidence of AKI in neonates in the unfavourable group (64% vs. 36%)

Conclusions: In this prospective study we found a significant incidence of AKI in HIE undergoing TH and neonates with both HIE and AKI had a longer length of stay and mortality than HIE alone. There is also a correlation, statistically significant, between the severity of the short-term expected neurologic outcome of HIE and AKI. Future research on early detection of AKI, renoprotective management strategies, and understanding of long-term renal sequelae is recommended in this high-risk group of patients.

Key-words: Asphyxia; Hypoxic-Ischemic Encephalopathy; Acute Kidney Injury; Newborn; Therapeutic hypothermia

RESUMO

Introdução: A asfixia perinatal é uma causa major de disfunção multiorgânica em recém-nascidos. De acordo com a literatura atual[22], a lesão renal aguda é comum em recém-nascidos com encefalopatia hipóxico-isquémica, caracterizada por um quadro de coma e convulsões, em consequência de um processo de isquémia e hipoxia no período perinatal.

Objetivos: Este estudo tem como objetivos: 1) Determinar a incidência de lesão renal aguda em recém-nascidos que desenvolveram encefalopatia hipóxico-isquémica, tendo sido submetidos a hipotermia induzida, e 2) Averiguar a correlação entre a gravidade do prognóstico neurológico a curto-prazo destes recém-nascidos com o desenvolvimento de lesão renal aguda.

Métodos: Foi realizado um estudo prospetivo dos recém-nascidos com encefalopatia hipóxico-isquémica, submetidos a hipotermia induzida no Centro Hospitalar Universitário Lisboa Norte, EPE, desde a implantação do programa (2010) até 2017. De acordo, com o seu prognóstico neurológico expectável a curto-prazo, a população em estudo foi dividida em dois grupos (Favorável/Desfavorável), com base no eletroencefalograma de amplitude integrada (aEEG) e na ressonância magnética realizada às 2 semanas de vida. Para a definição de lesão renal aguda, foram utilizados os critérios modificados para o período neonatal, definidos pela Kidney Disease: Improving Global Outcomes (KDIGO)[28]. Os parâmetros renais (creatinina sérica e débito urinário) da população foram monitorizados durante a hospitalização. Após a colheita dos dados, foi realizada a sua análise estatística, recorrendo-se ao programa SPSS, versão 26.

Resultados: Neste estudo foram incluídos 92 pacientes, dos quais 59 (64%) eram do sexo masculino, enquanto 32 (35%) eram do sexo feminino. A média de peso ao nascer foi 3161 ± 615.7g e a idade gestacional média foi 39.06 ± 1.63 semanas. Verificou-se que 44 (48%) dos recém-nascidos estudados desenvolveram lesão renal aguda (LRA), dos quais 38 (86%) tiveram LRA estádio I, 2 (5%) desenvolveram LRA estádio II, enquanto os restantes 4 (9%) recém-nascidos desenvolveram LRA estádio III. No recém-nascidos com

LRA, verificou-se, embora não estatisticamente significativa, uma maior mortalidade (17% vs. 10%) e uma hospitalização cerca de 2.1 dias mais prolongada na Unidade de Cuidados Intensivos Neonatais, quando comparados com o recém-nascidos sem LRA. No que concerne ao prognóstico neurológico expectável a curto-prazo, este foi determinado em 61 (66%) dos recém-nascidos em estudo, sendo que destes 39/61 (6%) apresentavam prognóstico desfavorável. Globalmente, verificou-se uma maior incidência de lesão renal aguda nos recém-nascidos com prognóstico neurológico a curto-prazo desfavorável (64% vs. 36%).

Conclusões: A realização deste estudo permitiu concluir que a lesão renal aguda ocorre frequentemente em recém-nascidos com encefalopatia hipóxico-isquémica, submetidos a hipotermia induzida, bem como quando a associada a lesão renal aguda, os recém-nascidos apresentam maior taxa de mortalidade e tempo de internamento em Unidade de cuidados intensivos. Observou-se uma correlação, estatisticamente significativa, entre a severidade do prognóstico neurológico a curto-prazo na encefalopatia hipóxico-isquémica e o desenvolvimento de lesão renal aguda. A realização de novos estudos, tendo como objetivo a deteção precoce de lesão renal aguda, possíveis estratégias renoprotetoras e o esclarecimento das possíveis sequelas a longo prazo nesta população de elevado risco de atingimento renal é recomendada.

Palavras-chave: Asfixia perinatal; Encefalopatia hipóxico-isquémica; Lesão renal aguda; Recém-nascido; Hipotermia induzida

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LIST OF ABBREVIATIONS

aEEG – Amplitude-integrated electroencephalogram

AKI – Acute kidney injury

CAKUT - Congenital anomalies of kidney and urinary tract

CT – Computerized tomography

CKD – Chronic Kidney Disease

EEG – Electroencephalogram

GFR – Glomerular filtration rate

HIE – Hypoxic-ischemic encephalopathy

KDIGO – Kidney Disease: Improving Global Outcomes

NE – Neonatal encephalopathy

NICU – Neonatal intensive care unit

MRI – Magnetic resonance imaging

SCr – Serum creatinine

TH – Therapeutic hypothermia

INTRODUCTION

Perinatal asphyxia

Despite of perinatal care improvements and fetal monitoring became more sophisticated over the last few decades, asphyxia remains one of the most significant causes of mortality and morbidity. In hypoxic-ischemic insults, organ injury is mainly caused by tissue oxygen deficiency. An oxygen deficit may be the result of either hypoxemia or ischemia, that can occur simultaneously or in sequence. Hypoxemia is defined as diminished oxygen blood content. Ischemia is characterized by reduced blood perfusion in a particular tissue bed.[1] Perinatal asphyxia is a condition characterized by an impairment of gas exchange to organ systems due to hypoxic or ischemic insult that occurs in the period immediately before, during, or after the birth process, which results not only in a deficit of oxygen in blood but also an excess of carbon dioxide, leading to acidosis.[2]

The incidence of perinatal asphyxia varies from one study to another. According to Haan et al.[3], the incidence of perinatal asphyxia is 1 to 6 per 1000 births. The rate is higher in resource-limited countries, where there is limited access to maternal and neonatal care. Moreover, perinatal asphyxia is a major cause of death and acquired brain damage in newborns and infants. According to the World Health Organization, it represents the third major cause of under-five years child mortality worldwide (11%), after preterm birth (17%) and pneumonia (15%).[4]

Asphyxia may develop in utero, during labor and delivery due to an impaired placental gas exchange (*vide* **Table 1**)[5]**.** Although, in several cases, the exact time of asphyxia cannot be determined with certainty.

In 2014, an executive summary published by the American College of Obstetricians and Gynecologists (ACOG) [6] identifies neonatal signs and contributing factors used to determine acute hypoxic-ischemic events in term and late preterm infants (gestational age ≥ 35 weeks) that would likely result in neonatal hypoxic-ischemic encephalopathy (HIE). Therefore, the neonatal signs consistent with an acute perinatal hypoxic-ischemic event include: (1) Apgar score < 5 at 5 minutes and 10 minutes; (2) metabolic acidosis defined by fetal umbilical artery pH < 7.0, or/and base deficit ≥ 12 mmol/L; (3) evidence of brain injury seen on brain magnetic resonance imaging (MRI) or magnetic resonance spectroscopy consistent with acute hypoxia-ischemia; (4) presence of multisystem

organ failure consistent with HIE. In this summary, there is still place for additional criteria, non-specific criteria for asphyxic insult, but suggestive of peri or intrapartum timing: (1) a sentinel hypoxic or ischemic event occurring immediately before or during labor and delivery, such as ruptured uterus or severe abruptio placentae; (2) fetal heart rate monitor patterns consistent with an acute peripartum or intrapartum event, for instance tachycardia with recurrent decelerations and persistent minimal variability with recurrent decelerations; (3) the timing and type of brain injury patterns based on imaging studies that are typical of hypoxic-ischemic injury in the term and late preterm newborn. This includes MRI demonstrating deep nuclear grey matter (basal ganglia or thalamus) or watershed (border zone) cortical injury; (5) no evidence of other proximal or distal factors that could be contributing to encephalopathy, such as abnormal fetal growth, maternal infection, feto-maternal haemorrhage, neonatal sepsis, and chronic placental lesions.

At the national level, the Neonatology Section of the Portuguese Pediatric Society, in its national consensus of Therapeutic Hypothermia in the Treatment of Neonatal Hypoxic-Ischemic Encephalopathy [7], in 2012, defines only clinical criteria suggestive of asphyxia: (1) Apgar scores ≤ 5 at 10 minutes of life; (2) continued need for resuscitation maneuvres at 10 minutes of life; (3) metabolic acidosis with pH < 7 in the first 60 minutes of life; (4) base deficit ≥ 16 mmol/L in the first 60 minutes of life.

Moreover, it is important to note that it has been shown that most infants, i.e., >60% with a cord pH < 7.00 have a normal labor and delivery course, initiate breathing shortly after delivery, are triaged to the regular nursery, and are discharged home within 24 hours.[8,9] In most cases, even those infants with severe fetal acidemia admitted to intensive care units manifest a benign neurologic course. Just a few infants with moderate to severe HIE develop an adverse outcome, either death or subsequent cerebral palsy[9], which highlights the brain's inherent resistance to extreme asphyxia.

Table 1: Risk Factors for Perinatal asphyxia in term newborns

Periconceptional risk factors	Antepartum risk factors	Intrapartum risk factors	Immediate postnatal period risk factors
Maternal age ≥ 35 years; Social factors; Family history of seizures or neurologic disease; Infertility treatment; Previous neonatal death;	Maternal prothrombotic disorders and proinflamatory states; Maternal thyroid disease; Severe preeclampsia; Multiple gestation; Chromosomal/genetic abnormalities; Congenital malformations; Intrauterine growth restriction; Trauma; Breech presentation; Antepartum hemorrahage.	Abnormal fetal heart rate during labor; Chorioamnionitis/maternal fever; Thick meconium; Operative vaginal delivery; General anesthesia; Emergency caesarean delivery; Placental abruption; Umbilical cord prolapse; Uterine rupture; Maternal cardiac arrest; Fetal exsanguination.	Pulmonary, neurological or cardiovascular abnormalities.

In: *Antonucci R, Porcella A, Pilloni MD. Perinatal asphyxia in the term newborn. J Pediatr Neonatal Individ Med. 2014* [5]

In the newborn, the lack of oxygen may lead to multi-organ failure. However HIE is the most studied clinical condition and exhibits the most serious sequelae.

The ACOG describes neonatal encephalopathy (NE) as "a clinically defined syndrome of disturbed neurologic function in the earliest days of life in the infant born at or beyond 35 weeks of gestation manifested by subnormal level of consciousness or seizures, and often accompanied by difficulty with initiating and maintain respiration and depression of tone and reflexes"[6].

In the past, it was assumed that the primary etiology of NE was hypoxia-ischemia. Currently, it is known that the terminology of NE is considered a nonspecific brain reaction to damage that can occur through multiple causal pathways. Hypoxic-ischemia is only one of many possible contributors to NE. Therefore, in contrast with other etiologies of NE (genetic disorders, brain malformations, metabolic defects, etc.), HIE is a potentially modifiable condition, thus it is essential to identify the presence or absence of hypoxia-ischemia.

HIE is the most common neurologic disease of the perinatal period and it is a major cause of chronic disability in childhood. The estimated incidence of HIE is 1,5 to 2,5 per 1000 live births in developed countries.[10]

The diagnosis of neonatal HIE requires careful observation and examination of the term newborns in the context of a detailed history of pregnancy, labor, and delivery. The severity of clinical signs and the time that the signs persist correlate with the severity of the insult[1] (*cf*. **Table 2**). Neonates with moderate and severe HIE may show variable levels of consciousness and behaviour, ranging from hyper-alertness or irritability to lethargy and stupor or coma. There may also be disorders of tone ranging from increased muscle tone to a marked atony, and a spectrum of abnormal movements from tremors to severe seizures. Difficulty in feeding and shrill cry may be observed. The Moro reflex and deep tendon reflexes may be increased, and the decorticate or decerebrate posturing are also included in the EHI clinical manifestations. Occasionally, apnea with bradycardia and oxygen desaturation are present.[1,5,11]

Table 2: Clinical Signs of Hypoxic-Ischemic Cerebral Injury

Mild Encephalopathy
Mild depression or excitation of level of consciousness
Hyperexcitation of sympathetic nervous system
Brisk deep tendon reflexes and vigorous Moro reflexes
Duration of less than 24 hours
Moderate to Severe Encephalopathy
Stupor or coma
Depression of deep tendon reflexes and Moro response
Hypotonia
Seizures and interictal EEG abnormalities
Duration of more than 24 hours.

In*: Rivkin MJ, Volpe JJ. Hypoxic-ischemic brain injury in the newborn. Semin Neurol. 1993; 13:30-9.*

In 1976, Sarnat & Sarnat[11] evaluated 21 neonates with perinatal asphyxia and encephalopathy. In the original Sarnat publication, a classification for HIE based in three stages was defined: mild (stage 1), moderate (stage 2); and severe (stage 3). This classification assesses parameters related to the level of consciousness, neuromuscular control, complex reflexes, autonomic function, presence or absence of seizures, electroencephalogram findings and duration (*cf.* **Appendix 1**).

Despite of the detailed of the classification proposed by Sarnat and Sarnat, Levene's classification[12], for its simplicity and ease of practical application, is

recommended for routine use. (*cf.* **Appendix 2**). According to this assessment tool, mild HIE is characterized by irritability, hypotonia, absence of seizures, and/or a decreased sucking reflex. Moderate HIE is represented by lethargy, marked hypotonia, presence of seizures, and/or an abolished sucking reflex. At last, a severe HIE involves a comatose with severe hypotonia and prolonged seizures, and an inability to sustain spontaneous respiration.[12]

Besides clinical evaluation and laboratory examination, neuroimaging techniques are available in order to confirm the diagnosis of HIE and exclude other differential diagnosis (including acute haemorrhage, depression from maternal anaesthesia or analgesia, infection, cardiac or pulmonary disorders, trauma, neurological disorders and metabolic diseases). Cranial and Doppler ultrasonography, computed tomography (CT) scan and MRI are the most used brain imaging techniques in the newborn with HIE. Since CT scans require dangerous ionizing radiations and transcranial ultrasound exams have low sensitivity, almost all neonatologists deal with MRI.[13]

MRI is the optimal imaging modality to identify structural changes following cerebral insults, to support diagnosis of hypoxic-ischemic injury and for prognosis as well. [14] Despite of the risk and all the monitoring necessary to transport a newborn often hemodynamically unstable for an MRI, this imaging technique is the most sensitive and specific for the evaluation of a newborn with suspected hypoxic-ischemic injury,[5,15] with greater capacity for early detection of injuries and better resolution, when compared to cranial ultrasonography and CT. MRI also has the advantage of not exposing the newborn to ionizing radiation. Moreover, its scanning time is quite long, which frequently requires sedation of newborns.

Relating to the study of brain electrical function, the standard electroencephalogram (EEG) is always recommended in the newborn with HIE, representing a direct and sensitive measurement of neonatal brain function. EEG patterns have a relation with neurological outcomes: it was demonstrated that normal EEG is correlated with a normal outcome, while an EEG with the presence of “burst suppression” on EEG is associated with poor outcome and may even culminate in death.[14]

In order to continuously monitor of cerebral function, amplitude-integrated EEG (aEEG) is increasingly used to assess the occurrence and severity of neonatal

encephalopathy, and according to the Neonatology Section of the Portuguese Paediatric Society, it should be applied as soon as possible after HIE diagnosis is established.[7] In addition, several studies have shown that a single-channel aEEG performed within a few hours after birth can help in assessing the severity of brain injury in neonates with HIE. While a normal aEEG may not necessarily mean that the brain is normal, the presence of an abnormal aEEG with severe or moderately-severe abnormalities may indicate brain injury and poor outcome.[16]

The assessment of the severity of cerebral injury and likely neurological outcome is important to determine the management of newborns with HIE, as well as it is crucial to determine the prognosis, to counsel parents, and to select who may benefit from neuroprotective measures[17].

Therapeutic Hypothermia

In the past, supportive medical therapy to maintain cardiopulmonary function and control seizure activity, was the only treatment option. Currently, several experimental treatments are available to infants with HIE and many others are being evaluated in animal models.[5,10,16]

Nowadays, TH represents the neuroprotective treatment of choice for term neonates with HIE, since is the only treatment which has proven neuroprotective effects in larger clinical studies. The multiple neuroprotective effects of TH, include converting cells programmed for apoptosis, leading to their survival, and also, protecting neurons by reducing cerebral metabolic rate, attenuating the release of excitatory amino acids (glutamate, dopamine), ameliorating the ischaemia-impaired uptake of glutamate and lowering production of toxic NO and free radicals.[18,19]

In 2013, Cochrane review [20] of 11 randomized controlled trials (N= 1050 infants) has demonstrated that TH for term and late preterm newborns with moderate-to-severe HIE reduces mortality, without increasing major neurodevelopmental disability in survivors. Based on these results, they recommended that hypothermia should be instituted in term and late preterm infants with moderate-to-severe HIE before 6 hours of life.

Hypothermia treatment is delivered through either selective head or whole-body cooling of the infant. TH involves decreasing the infant's body temperature to 33 -

36.5°C. Infants are generally cooled for 48 to 72 hours and then rewarmed slowly to prevent complications, like hypotension.

In Portugal, the criteria required for the admission of a newborn in the protocol, as well as the hospital care and vital support needed at birth, during transport to a tertiary hospital and during the hypothermia treatment are defined in National Consensus of Therapeutic Hypothermia in the Treatment of Neonatal Hypoxic-Ischemic Encephalopathy defined.[7]

Acute Kidney Injury in Asphyxiated Newborns

Asphyxia can lead to multi-organ dysfunction and a redistribution of cardiac output to maintain cerebral, cardiac, and adrenal perfusion, while potentially can compromising renal, gastrointestinal, and skin perfusion.[21] Only in a few cases (< 15%), the brain is the only organ with dysfunction after asphyxia.[5]

The kidney is one of the most important organs commonly involved in the multiple organ dysfunction caused by perinatal asphyxia. Renal injury can range in severity from minor issues to acute kidney failure. Most patients with mild to moderate AKI are asymptomatic and are identified by laboratory tests. However, patients with severe cases may be symptomatic and present with lethargy, confusion, fatigue, anorexia, nausea, vomiting, weight gain, or edema.[23] Before the advent of TH, the incidence of AKI was high, affecting 47 to 72% of neonates with different degrees of perinatal asphyxia. Nowadays, in the era of TH, AKI remains a significant complication in neonates with HIE, however most recent studies have reported lower AKI rates, ranging from 19% to 40%.[22]

AKI is defined as a sudden decline in kidney function, resulting in derangements in fluid balance, electrolytes, and waste products. The diagnosis of AKI is dependent on a rise of serum creatinine (SCr) and/or decrease in urine output.[23] Unfortunately, SCr is not a good marker of neonatal renal dysfunction. First of all, the SCr reflects the maternal level for up 72 hours after birth. Secondly, as SCr is a marker of kidney function, not damage, significant changes in the glomerular filtration rate (GFR) occur in the absence of a change in SCr. Indeed, there is a considerable delay in the rise of SCr after an insult (48-72 hours) and a significant amount of function has to be lost before SCr rise (> 50% of the GFR). [23,24,25] Additionally, there is a significant variability in neonatal GFR, which fluctuates rapidly in the immediate postnatal period as the infant adapts to

extrauterine life, in term neonates. The GFR improves from 10-20 mL/min/1.73 m^2 during the first days of life to 30-40 mL/min/1.73 m^2 by 2 weeks of life.[23] Moreover, SCr reflects a balance between production from creatinine stores in muscles and renal clearance, so it is suggested that newborns with little muscle mass at birth may have underestimate GFR, due to lower SCr levels. Finally, there is a possibility to overestimate SCr because early SCr levels in newborns reflect maternal values.[26] This suggests that even though SCr is the standard biomarker for the diagnosis of AKI in all populations, any interpretation of SCr alone in the first few days of life should be treated with caution.

On the other hand, although oliguria is another important clinical sign associated with AKI, in neonates, renal failure can occur, in more than 50% of cases, in the absence of oliguria[27], and many neonates maintain a urine output of more than 1mL/kg/h despite significant renal dysfunction[21].

Prior studies documenting AKI in asphyxiated newborns used different definitions of AKI, causing cross-study comparisons difficult. Jetton and Akenazi [28], have proposed a modification of the 2012 Kidney Disease: Improving Global Outcomes (KDIGO) definition of AKI as a standardized definition of AKI for neonates, which is based on the rise in SCr from a previously documented low, rather than absolute, SCr thresholds (*cf*. **Table 3**). Currently, this definition has gained acceptance to define AKI in newborns in most of the research groups.

Table 3: Neonatal AKI KDIGO Classification

Stage	SCr	Urine Output
0	No change in SCr or rise <0.3 mg/dL	≥0.5 mL/kg/h
1	SCr rise ≥ 0.3 mg/dL within 48 h or SCr rise ≥ 0.5-1.9 x reference SCr[a] within 7 days	< 0.5 mL/kg/h for 6 to 12 h
2	SCr rise ≥ 2.0-2.9 x reference SCr[a]	< 0.5 mL/kg/h for ≥ 12 h
3	SCr rise ≥ 3 x reference SCr[a] or SCr ≥2.35 mg/dL[b] or Receipt of dialysis	< 0.3 mL/kg/h for ≥ 12 h or anuria for ≥12h

[a] *Reference SCr will be defined as the lowest previous SCr value*

[b] *SCr value of 2.5 mg/dL represents <10 mL/min/1.73m²*

Therefore, the available short-term outcome data in neonates are similar between them, suggesting that neonates who develop AKI have increased mortality rates and longer hospital stays compared to those without AKI. However, these findings are entirely derived from small retrospective, single-centre studies, which are limited by a small sample size.[22,27,28]

In 2017, the Neonatal Kidney Collaborative worked to develop the largest neonatal AKI study to date, evaluating the incidence and outcomes in neonates with all-causes AKI. The Assessment of Worldwide Acute Kidney injury epidemiology in neonates (AWAKEN)[29] study is a multicentre, multinational, retrospective cohort study of critically ill neonates admitted to twenty-four participating neonatal intensive care units (NICU) in four countries (Australia, Canada, India and United States of America) between January 1 and March 31, 2014. In this study, were enrolled 2022 neonates in the final sample, 605 of whom had AKI. They defined AKI as an increase in SCr of 0.3 mg/dL or more or 50% or more from the previous lowest value, or a urinary output of less than 1mL/kg/h on postnatal days 2-7. This study concluded that even after the authors adjusted for confounders, mortality was higher in infants with SCr-defined or urinary output-defined AKI (10%) than in those no AKI (1%). Also, infants with AKI had longer length of hospital stay than those without AKI.

Neonates with perinatal asphyxia have been recognized as a group that is at high risk of AKI. In 2012, Selewski et al,[22] studied 96 asphyxiated neonates undergoing TH and distinguished some risk factors in this population associated with AKI, including asystole at the time of birth, clinical seizure before cooling, persistent pulmonary hypertension, elevated gentamicin or vancomycin levels, pressor support and transfusions. These investigators also showed that AKI predicted prolonged mechanical ventilation, length of stay, and abnormal brain MRI findings at 7 to 10 days of life, implicating AKI as a potential marker of neurologic outcomes. Furthermore, Kirkley et all,[30] in 2018 performed a retrospective analysis of infants with 34 weeks of gestational age or more with a diagnosis of HIE, from AWAKEN[29] database, and they observed that 41.6% (47/113) of patients with HIE developed AKI. Other studies concluded [21,29] those infants who experience renal failure, as a result of perinatal asphyxia event, especially those with oliguria, have a high mortality risk. Additionally, neonates who had a more severe

asphyxia were more likely to develop renal failure than those who had been milder asphyxiated.

Aim of the study

The aim of this study is to evaluate the incidence of AKI among term neonates with perinatal asphyxia, and to compare incidence of AKI between groups with different disease severity according to the short-term neurologic outcome.

MATERIALS AND METHODS

Study design and population

A prospective study was realized in a cohort of neonates treated for moderate to severe HIE with TH, at NICU in Centro Hospitalar Universitário Lisboa Norte, EPE (CHULN), during a 7-year period (2010-2017). A prospectively collected database was used for the purpose of the study.

Inclusion and exclusion criteria

This study included all the newborns submitted to hypothermia protocol during the period of study. The inclusion criteria were the defined by the National Consensus[7]:

A. Criteria suggestive of asphyxia:

1. Apgar scores ≤5 at 10 minutes of life;

2. Continued need for resuscitation manoeuvres at 10 minutes of life;

3. Metabolic acidosis with pH <7,0 in the cord blood or during the first 60 minutes of life;

4. Base deficit ≥ 16 mmol/L in the cord blood or during the first 60 minutes of life.

B. Moderate to severe encephalopathy, defined by altered state of consciousness, tone, reflexes or respiratory autonomy or evidence of seizures.

For inclusion in the protocol, the neonate must complete 1 criterion A + 1 criterion B.

C. Absence of exclusion criteria for hypothermia, such as:

1. Absolute: major congenital malformations; no passive cooling before 6 hours of life and inability to start treatment before 12 hours of life;

2. Relative: gestational age less than 36 weeks; need for surgery in the first 3 days of life and sudden unexpected postnatal collapse.

Exclusion criteria included a prenatal diagnose of congenital anomalies of kidney and urinary tract (CAKUT).

Group allocation

Newborns were allocated in two groups, according to their short-term neurologic outcome: 1) Favourable group, defined as neonates with mild outcome in aEEG and MRI on the second week of life, and 2) Unfavourable group, defined as neonates with moderate-to-severe short outcome in aEEG and MRI on the second week of life.

Classification of AKI

AKI in enrolled neonates was identified and graded according to the Kidney Disease: Improving Global Outcomes (KDIGO)[28] workgroup AKI definition modified for neonates. It is defined as an increase in serum creatinine of 0.3 mg/dL or more or 50% or more from the previous lowest value, or a urinary output of less than 1 mL/kg/h on postnatal days 1-14. All SCr values were obtained from hospital lab's electronic database.

Perinatal data

Perinatal data was registered prospectively in the database, including: maternal pathologies, delivery (type of labour, cord prolapse and meconium aspiration), resuscitation (gestational age, gender, birth weight, Apgar score at 1, 5 and 10 minutes, need for resuscitation beyond 10 minutes, arterial blood gases within the first hour of life) and clinical data during hypothermia treatment. Recorded events and outcomes during the admission included hypotension, culture proven sepsis and the use of nephrotoxic drugs, natremia, hyperkalaemia, hyperphosphatemia, hypocalcemia, metabolic acidosis, hypoglycemia, length of hospital stay, and mortality before hospital discharge.

Data processing and Analysis

Data figuring on the hypothermia database was anonymously coded and entered into an Excel ® spreadsheet for statistical analysis using the SPSS (Statistical Package for the Social Sciences) software, version 26®.

Ethical considerations

The database used for this study was approved by the CHULN's Ethical Committee.

RESULTS

Characterization of study population

A total of 94 patients underwent therapeutic hypothermia in the NICU of CHULN between 2010 and 2017. Fifty-nine patients were male, while the remaining thirty -two were female. The mean weight was 3161 g (standard deviation= 615.7) and the gestational age was 39.06 weeks (SD = 1.63). Most of the patients (n=90) had been transferred from an outside institution for therapeutic hypothermia. Forty-seven neonates were delivered by the vaginal route (50%), 21 % with vacuum and 4% with forceps delivery, and 43 (45%) via caesarean section. Thirty-two patients had experienced a clinically identifiable sentinel event around the time of birth, the most common of which were placental abruption (n= 14), difficult delivery (n=8), uterine rupture (n= 7), umbilical cord prolapse (n=2). Only 19% of the admitted neonates had a severe 10-minute Apgar score (0-3), while 50 % had a moderate 10-minute Apgar score (4-6).

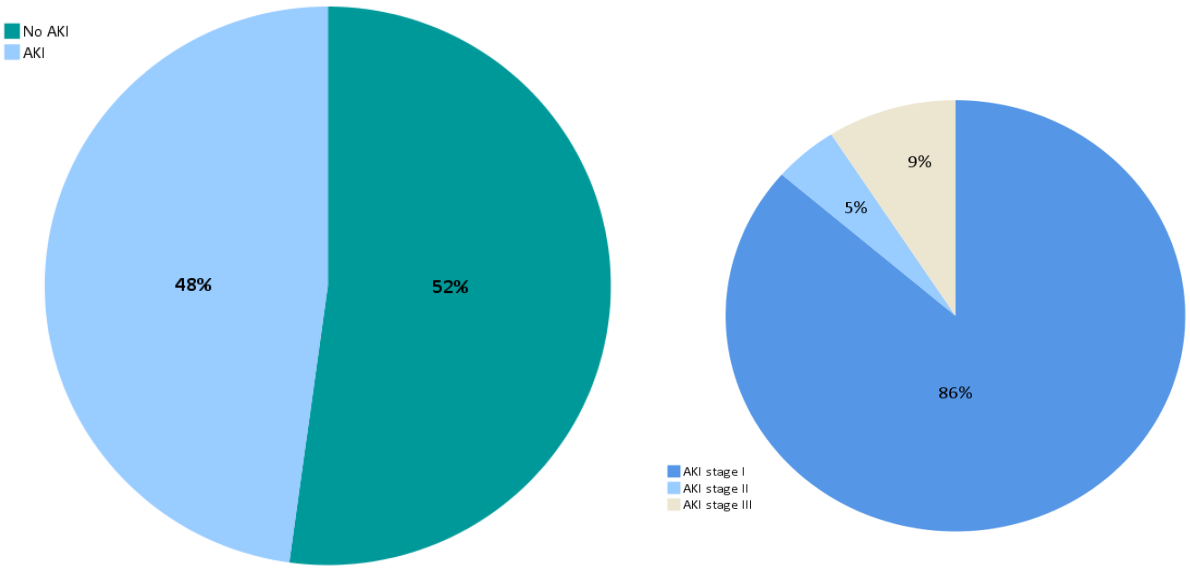
Five (5%) patients had fewer than two serum creatinine measurements and 6 (6%) patients had no quantifiable urinary output data in the first week of life. Of these patients, two (2%) had both fewer than two serum creatinine measurements and insufficient urinary output data, and consequently could not be assigned an AKI classification and were excluded from the analysis.

Incidence of AKI

Of the remaining sample (n=92), AKI was identified in 44 of 92 patients (48%) during the study period. (*cf*. **Figure 1**). Of these patients, 8 (18%) had serum creatinine-defined AKI and 16 (36%) had urinary output-defined AKI. Twenty (45%) neonates had AKI defined by both measures.

Based on Neonatal KDIGO AKI criteria, 38 (86%) neonates were classified as AKI stage I, two (5%) as AKI stage II, and four (9%) as AKI stage III. Thirty-four patients had been exposed to nephrotoxic drugs, including four to gentamicin, 26 to vancomycin and four to both drugs. Fifty-two (57%) patients required diuretic therapy with a loop diuretic (furosemide) to maintain urine output and two patients received temporary renal replacement therapy with peritoneal dialysis, one due to an extremely elevated SCr value (SCr=7.18) and the other due to persistent oliguria.

Figure 1: AKI incidence and AKI stages incidence



Neonates with AKI and without AKI had similar demographic data, perinatal, and neonatal characteristics (*cf*. **Table 4**). Although, compared to infants without AKI, neonates with AKI were more likely to have hypotension (37 vs. 26) and required treatment with amines. Sixty-three (68%) patients developed hypotension during hospitalization, 37 (58%) of whom were neonates with AKI criteria. Furthermore, 58 (63%) of the neonates who required support with amines during hospitalization, 34 (58%) had AKI versus only 24 (41%) without AKI.

Table 4: Patient demographic data and characteristics. Comparison of patients' baseline characteristics in AKI and non-AKI group

Variable	Overall (n=92)	AKI (n=44)	No AKI (n=48)
Gestational age, weeks	39.06 ± 1.63	39.07±1.63	39.08±1.63
Cesarean delivery, n (%)	43 (45)	18 (41)	25 (52)
Intrapartum event, n (%)	32 (35)	17 (39)	16 (33)
Chest compressions at birth, n (%)	39 (42)	17 (39)	22 (46)
pH cord, mean ± SD	6.93 ± 0.17	6.92±0.17	6.93±0.17
Cord base deficit, mean ± SD	19.12±5.11	19.20 ± 5.17	18.99 ± 5.03
10-minute, Apgar score, mean ± SD	4.73±2.24	4.70±2.26	4.74±2.26
Vasopressor support, n (%)	46 (50)	26 (59)	20 (42)
Resuscitation >10 minutes, n (%)	27 (29)	18 (41)	9 (19)
Hypotension, n (%)	63 (68)	37 (84)	26 (54)

Patient outcomes

Regarding patient outcomes, all-cause mortality before hospital discharge was 14% in patients who underwent TH during the study period (*cf*. **Table 5**). There was a trend for a difference in mortality between neonates with AKI and those without AKI (17% vs. 10%), although not statically significant (p=0.099). Neonates who developed AKI had a longer length of stay in the NICU, staying an average of 2.1 days longer in the NICU compared to those without AKI.

Variable	*Overall*	*AKI*	*No AKI*	*p-value*
Days in NICU, mean± SD	15.13±9.32	16.17±8.42	14.61±9.00	0.013
Mortality before hospital discharge, n (%)	13 (14)	8 (17)	5(10)	0.099

Table 5: Comparison of patients' outcomes in AKI and non-AKI group

Relation between AKI and short-term neurologic outcome

One of the primary aim of this study is to compare renal involvement in neonates with HIE in relation to their short-term neurologic outcome, therefore it is imperative to estimate the prevalence of AKI in each group. Brain MRI was performed in 71 patients at a median age of 10.2 ± 2.9 days. Expected short-term neurologic outcome was determined from aEEG and MRI data and was determined in 61 surviving neonates at discharge. It was found to be favourable in 36% (n=22), intermediate in 26% (n=16), and adverse in 38% (n=23).

We divided patient into two groups, the **Favourable group,** defined as mild short-term neurologic outcome, which included 22 neonates with favourable outcome, and the **Unfavourable group**, defined as moderate-to-severe expected neurologic outcome, which includes 39 neonates with intermediate or adverse expected outcome.

Figure 2, shows the differences in AKI incidence in the two groups. We reported that among 22 neonates with favourable outcome, only 36% (n=8) had AKI, while among neonates belonging to the unfavourable group, 64% (n=25) had AKI (P=0,036).

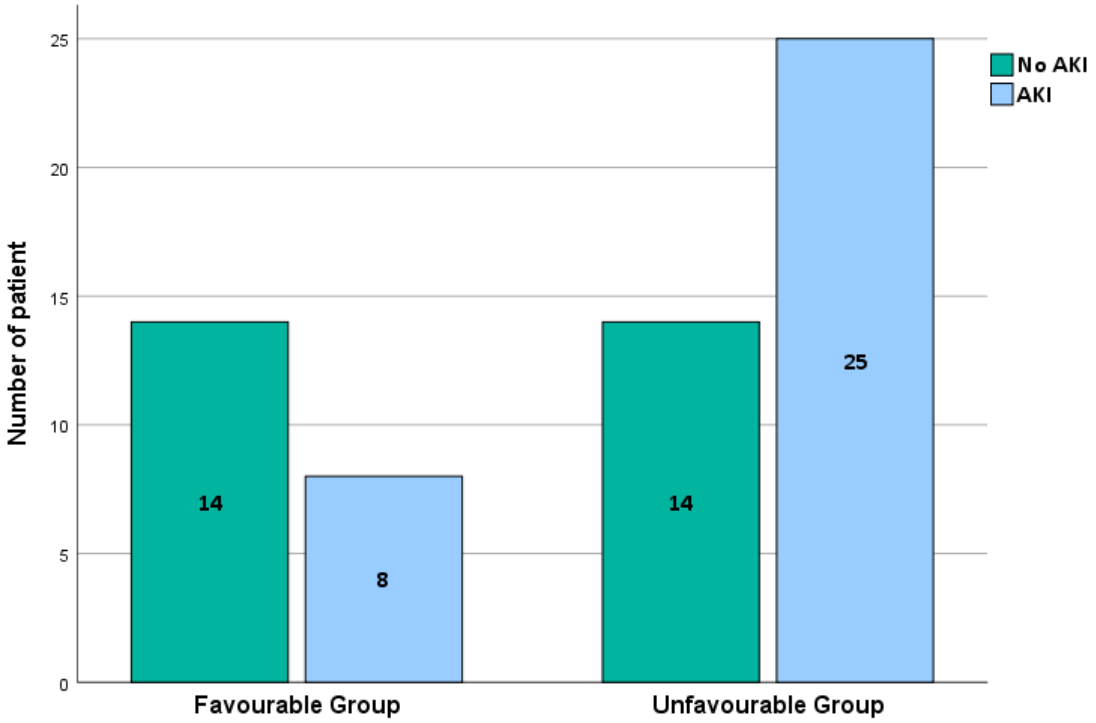
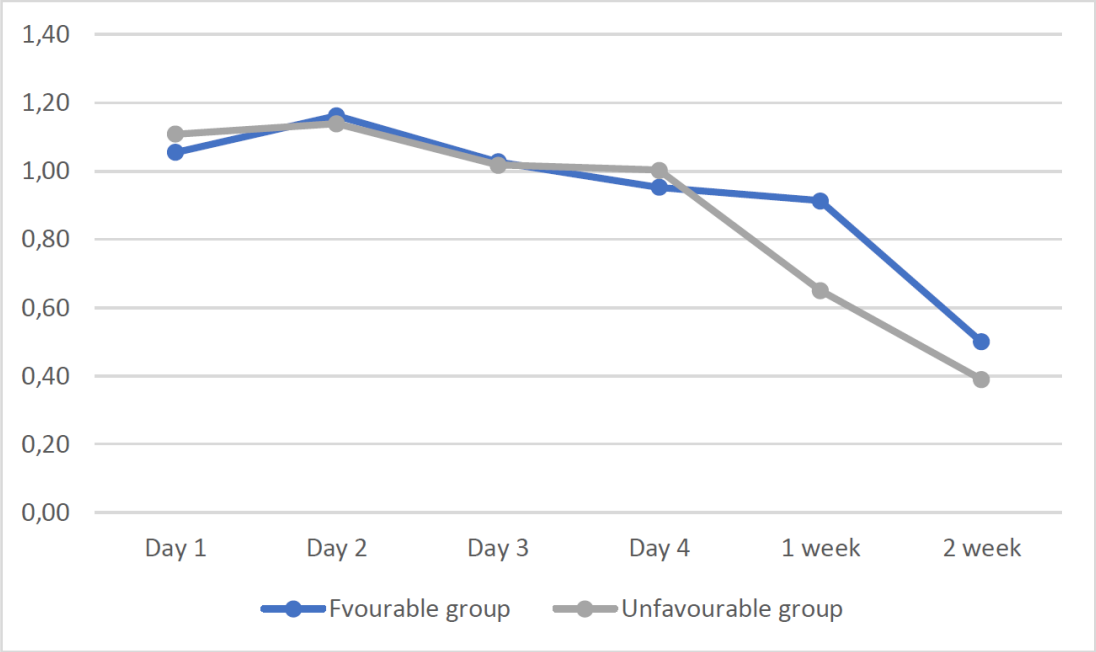


Figure 2: AKI incidence according to short-term neurologic outcome

During the cooling period (6-72 hours of life), SCr levels were higher in both groups, and we can report that AKI events occurred most frequently in the first week after birth. SCr levels improved significantly within one week of birth. Although data of SCr levels in the second week of birth are not widely available, it is shown a normalization of serum creatinine. (*cf*. **Figure 3**)

Although we demonstrate that AKI is most common in neonates with unfavourable short-term neurologic outcome, we note that the increase in SCr level is similar in both groups.

Figure 3: Trends in SCr levels during hospitalization. A comparation between the trend of mean SCr values in the Favourable group and Unfavourable group within the first two weeks of birth.



DISCUSSION

In this prospective study, we evaluated the incidence of AKI and we report an incidence of 48% in this population. In addition, AKI was associated with worse outcomes, including increased length of stay in NICU and increased mortality, compared with those without AKI. Although there are several confounders that contribute to mortality and morbidity, which were not controlled for in this study.

The findings presented here are consistent with previous reports in the literature. Kirkley et al [30], using AWAKEN database, investigated the incidence of AKI in asphyxiated neonates with HIE who underwent therapeutic hypothermia and have shown an incidence of 41.6 %. Considering the same AKI definition was used, AKI rates and outcomes can be compared in both studies.

Comparing AKI incidence with short-term neurological outcome in asphyxiated neonates undergoing TH, we reported a statistically significant increased incidence of AKI in neonates with intermediate or severe expected short-term neurologic outcome, when compared to those with favourable short-term neurologic outcome (64% vs. 36%).

The association between AKI and an unfavourable expected neurologic outcome in asphyxiated neonates may be explained by the pathophysiological mechanisms of the perinatal response to fetal distress. There is a redistribution of blood away from the kidneys to the brain and other vital organs, which, in conjunction with other concomitants signs of asphyxia, such as hypercapnia and acidosis, increases the potential for proximal renal tubule injury. [31] Depending on the severity or duration of fetal distress, insidious injury or necrosis of tubular cells may occur. If the asphyxial insult is severe enough to manifest as persistent oliguria, it is likely that the brain has sustained a more prolonged ischemic injury, leading to the worst outcome in the second week of birth.

Regarding the progression of SCr levels during hospitalization, we can conclude that there is no significant difference between the two groups. Contrary to what would be expected, creatinine is slightly increased at the second week of life in the Favourable group compared to the Unfavourable group. These results highlight the limitations of the SCr-based definition of AKI. First, early SCr levels in newborns reflect maternal values, which are much higher than those found in newborns. Moreover, SCr is a marker of renal function rather than damage, so a delayed increase in SCr of 48-72h is observed

after an insult. From this point of view, quantification of urinary output is an important part of the assessment of neonatal AKI.

In our study, among the available patients with expected neurological outcome, only five had AKI defined by creatinine, while most of them had either AKI defined by urine output (n=13) or both measures to define AKI (n=15). The inclusion of urine output in the AKI definition in this study allows us to identify patients with renal impairment who would not have been detected by changes in SCr levels alone. This may explain the difference in AKI incidence observed in the two groups (*cf*. **Figure 2**) and the evolution of creatinine levels during hospitalisation in the same patients (*cf*. **Figure 3**).

We found a normalization of serum creatinine in both groups, indicating improvement in glomerular function by 72-96 hours of life. From this point of view, Chevalier et al.[33] recognized that neonates with AKI show a remarkable recovery of GFR after birth-asphyxia, as evidenced by follow-up serum creatinine. A possible pathophysiological explanatory mechanism is that a decrease in the number of functional nephrons caused by asphyxia, leading to AKI, induces compensatory hypertrophy of the remaining nephrons, which consequently leads to improved glomerular function.

The incidence of AKI in asphyxiated neonates with HIE remains problematic and presents an opportunity to improve care. Earlier detection of AKI will allow caregivers to institute higher blood pressure for improve renal perfusion, eliminate nephrotoxic medications, adjust medications based on renal clearance, prevent fluid overload, and monitor volume status and electrolytes, ideally improving outcomes.

In AWAKEN study, the authors emphasize the importance of measuring urine output in neonatal AKI, and point out that, because of the difficulty of placing urinary catheters in some infants and efforts to reduce catheter-associated urinary tract infections, diaper weighing may be a necessary and an useful measure, however it is not entirely accurate, especially when urine is combined with stool. Contrary to what is claimed, even admitting the risk of catheter-associated urinary tract infections, in the present study we defend the need for a urinary catheter to control urinary output because of the hemodynamic instability associated with these neonates.

To prevent AKI in asphyxiated neonates with HIE, some studies[23] suggest that therapeutic strategies may be useful, such as the use of single-dose theophylline. This

therapeutic strategy is a Level C, although the KDIGO guidelines recommend single-dose theophylline for asphyxiated infants at risk for AKI. Caution is warranted, as theophylline has some potentially harmful neurologic effects and further studies are needed in the context of current clinical practice.

In this study, we did not determine the long-term renal prognosis for asphyxiated infants with AKI in the neonatal period. However, we strongly recommend that these infants must be followed-up indefinitely by a Pediatric Nephrology because it is possible that a significant number of them will develop Chronic Kidney Disease (CKD) later in life. According to the latest KDIGO practice guidelines, it is recommended that all patients who develop AKI should be evaluated for recurrence or worsening CKD, after 3 months.

Limitations

This study has several limitations, including the retrospective nature (despite being based on a prospectively collected database), as well as involves a single centre. Nevertheless, we have a reasonable sample size that allows obtaining important conclusions.

The fact that the prospective database does not include all the relevant variables for the purpose of the study, we had to rely on SCr and urinary output data available in medical records since 2010, so AKI cases may have been missed.

There is also controversy over a standard definition of AKI, particularly in patients undergoing TH for HIE in the first days of life. Although this AKI definition is the best available in neonates with perinatal asphyxia and is the one used in the largest studies conducted to date, it may still be inaccurate and likely misses some neonates with AKI. We chose the KDIGO definition modified for neonates because this definition is the most widely accepted definition of AKI in paediatric and adult cohorts, allowing comparison of our data with other populations, and this definition was explored in an AKI workshop that concluded that this definition is a reasonable starting point for collecting epidemiologic data on neonatal AKI.[32]

CONCLUSIONS

This is the first report, to our knowledge, comparing AKI incidence with short-term neurologic outcome in asphyxiated neonates undergoing TH. This study confirms that AKI occurs frequently in HIE undergoing TH and is associated with worst outcomes, including mortality, predictable neurologic outcome and longer length of stay in NICU. We also showed a correlation between the severity of short-term expected neurologic outcome of HIE and AKI, and we could conclude that infants with unfavourable expected neurologic outcome have higher incidence of AKI.

A future prospective research directed at evaluating long-term renal impaired in infants with neonatal AKI is designed and will be conducted by the NICU and Nephrology Unit at the Department of Pediatrics of the University Hospital Santa Maria.

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APPENDIX

Appendix 1: Classification of HIE - Sarnat and Sarnat

Features	Stage 1 (Mild HIE)	Stage 2 (Moderate HIE)	Stage 3 (Severe HIE)
Level of consciousness	Hyperalert	Lethargic or abounded	Stuporous
Muscle tone	Normal	Mild hypotonia	Flaccid
Posture	Mild distal flexion	Strong distal flexion	Intermittent decerebration
Stretch reflexes	Overactive	Overactive	Decrease or absent
Suck reflex	Weak	Weak or absent	Absent
Moro reflex	Strong; low threshold	Weak; incomplete; high threshold	Absent
Oculovestibular reflex	Normal	Overactive	Weak or absent
Tonic neck	Slight	Strong	Absent
Autonomic function	Generalized sympathetic	Generalized parasympathetic	Both systems depressed
Pupils	Mydriasis	Miosis	Variable; often unequal; poor light reflex
Heart rate	Tachycardia	Bradycardia	Variable
Bronchial and salivary secretions	Sparse	Profuse	Variable
Gastrointestinal motility	Normal or decreased	Increased; diarrhea	Variable
Seizures	None	Common; focal or multifocal	Uncommon (excluding decerebration)
EEG findings	Normal (awake)	Early: low-voltage continuous delta and theta; Later: periodic pattern (awake); Seizures: focal 1-to 1½ Hz spike-and-wave	Early: periodic pattern with isopotential phases Later: totally isopotential
Duration	Less than 24 hr	Two to 14 days	Hours to weeks

Sarnat HB, Sarnat MS. Neonatal Encephalopathy Following Fetal Distress. Arch Neurol. 1976;33(10):696.

Appendix 2: Classification of HIE – Levene

Feature	Mild	Moderate	Severe
Consciousness	Irritable	Lethargy	Comatose
Tone	Hypotonia	Marked hypotonia	Severe Hypotonia
Seizures	No	Yes	Prolonged
Sucking/Respiration	Poor suck	Unable to suck	Unable to sustain spontaneous respiration

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