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Value of clinical neurophysiology in early management of neonatal hypoxic-ischaemic encephalopathy

IN *CO-TUTELLE DE THÉSE* WITH UNIVERSITY COLLEGE CORK

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Abstract

Neonatal HIE is a complex neurological condition caused by an intrapartum or perinatal event leading to reduced cerebral perfusion. Therapeutic hypothermia (TH), the cornerstone treatment in HIE, should be instigated within six hours of birth and at present remains only indicated for term or near-term neonates with moderate or severe encephalopathy. TH use in mild encephalopathy is increasing internationally driven by concern that these infants are at risk of injury. The balance of risk against potential benefit is unknown and where best to draw that line in the care of mildly encephalopathic neonates is the subject of active debate.

In Chapter 1 we review some of the different regional and national eligibility guidelines for treatment and highlight the variability that exists between them. Clinical neurological exams often define the severity of encephalopathy differently, with varying number of domains required for determining eligibility and blurred interpretation of findings assigned to different severity grades in different systems. This reflects in different individual care depending on the location in which infants are born and impacts research data. The role of early electrophysiological assessment is also weighted unequally, and in most jurisdictions the role of clinical assessment predominates over electroencephalography. Nonetheless, recent studies demonstrated that clinical grade may underestimate the severity of the disease as compared with EEG assessment.

Existing grading schemes for EEG evaluation in neonatal HIE are focused on background features, while focal abnormalities and recurrent paroxysmal patterns are considered only marginally. In Chapter 2 we characterize the EEG 162 term neonates with HIE within 12 hours of birth to assess severity of encephalopathy and risk for acute provoked seizures. We found that neonates with severe background EEGs had a significantly higher rate of acute provoked seizures. In mildly and moderately altered background EEGs seizure risk was

lower, but spikes and/or rhythmic sequences predicted seizures. Early EEG grading combined with analysis of focal features may therefore be more accurate than background evaluation alone in assessing severity of neonatal encephalopathy and need for treatment.

In addition to EEG, other neurophysiological methods such as SEPs are known to predict outcome after perinatal asphyxia. In Chapter 3 we report the results of a pilot study, in which we demonstrated the feasibility of simultaneous EEG and SEPs assessment together with neurological examination at admission to the NICU. Standardization of timing of evaluation is crucial to the interpretation of findings in an evolving condition such as HIE. To our knowledge, our study is the first testing SEPs in the earliest 12 hours. The one patient with absent cortical responses also showed the worst Total Sarnat score and the worst EEG grade in the cohort, and developed an early abnormal outcome. We found increased latencies for cortical evoked potentials in neonates with HIE compared to an internal group of healthy controls.

Besides refining the care provided in high-income countries, research to support the understanding of the disease in low-income countries should be prioritised to guide appropriate use of available resources. Given the non-availability of TH, prompt detection and adequate treatment of seizures represent the main neuroprotective intervention. In Chapter 4 we performed retrospective characterization of seizure semiology using continuous video-EEG monitoring in neonatal encephalopathy in a Ugandan NE population. Incidence of seizures was high and a relevant proportion of them had a clinical correlate. Clonic, autonomic and automatisms were the more frequently seizure types observed. Respiratory impairment emerged as a prominent concern.

Clinical neurophysiology plays a central role in early management of neonatal HIE, providing useful insights in different settings.

Introduction

Neonatal Hypoxic Ischaemic Encephalopathy (HIE) is a clinical syndrome recognizable in newborns that results from a severe or prolonged hypoxic–ischemic brain insult that occurs in the perinatal period (in utero, at birth or in the immediate postnatal). The complex pathophysiological process leading to brain injury evolves over the course of days, and develops through two phases: hypoxia and/or ischemia represent the primary insult causing an initial energy failure, followed (after a latent period of 6 to 48 hours) by a secondary insult that occurs with reperfusion and produces further damage due to mitochondrial dysfunction (Perlman, 2007; James and Patel, 2014).

This condition is a major global health problem, representing the commonest cause of mortality and long-term morbidity in term newborns worldwide and a primary concern for neonatologist and neonatal neurologist engaged in clinical care and research. The burden of the disease is higher in underdeveloped countries, where the incidence and the rates of mortality and long term morbidity are dramatically higher (Tann et al., 2018). In this setting, the challenges for neonatologists and neonatal neurologists engaged in clinical care are totally different to those in high-income settings. Diagnostic tools are rarely available and treatments options are scarce.

The range of outcomes in HIE correlates with the severity of the initial insult and varies between death and intact survival. Traditionally, neonates with severe encephalopathy are considered to have a very high risk of death and of cerebral palsy, while asphyxiated newborns with a diagnosis of mild encephalopathy are believed to have normal outcome (Robertson and Finer, 1985).

Since the early 2000s, the introduction of early induced therapeutic hypothermia (TH) has changed perspectives for neonatal HIE (Shankaran et al, 2005). TH has shown good efficacy in improving survival and reducing neurological disability, most effectively when initiated within 6 hours of birth (Jacobs et al., 2007). TH has specific indication for moderate and severe encephalopathy, and current recommendations do not mention mild HIE (Perlman et al, 2010).

In the last decade evidence that the outcome for infants not fulfilling cooling criteria is not always normal has emerged: a growing number of studies show that significant proportion of infants with a diagnosis of mild HIE at birth have abnormal outcome at follow up, including moderate and mild impairments that can be recognized only many years later (Murray et al., 2016; Conway et al, 2018). In clinical practice this resulted in an emerging trend towards cooling newborns with mild HIE (Oliveira et al, 2018).

The majority of studies conducted to date investigate HIE and grade its severity at different times after the insult. Given the evolving nature of the pathophysiological mechanisms involved in the disease, this makes any meaningful comparison extremely difficult (Walsh et al., 2011). The lack of a single accepted classification system represents an additional limitation and consensus agreement needs to be reached as to how best classify HIE.

It is of high priority to improve the ability of clinicians to define the severity of injury at the bedside as early as possible, in terms of both demarcating between different grades of encephalopathy and identifying criteria for selecting those babies that might benefit from treatment. Even the RCTs and the national guidelines for selection to TH, assessing the severity of the disease in the narrow window of time available to instigate treatment, are not uniform and show some levels of variability.

The most frequently used methods for early severity grading and selection for treatment are clinical assessment and electroencephalography (EEG). The role of clinical assessment for the selection of therapeutic hypothermia predominates over electroencephalography according to various jurisdictions and thus in clinical practice (Tachenouchi et al., 2012). Nonetheless, recent studies demonstrated that a mismatch between clinical severity grade and the EEG grade is not infrequent. Clinical grade may underestimate the severity of the disease as compared with EEG assessment, and patients with only mildly abnormal findings at neurological examination but altered EEG patterns can develop acute provoked seizures and abnormalities on MRI (Gagne-Loranger et al., 2016).

Clinical neurological exams often define the severity of encephalopathy differently. Existing grading schemes for EEG evaluation in neonatal HIE are mainly focused on background features (voltage, continuity, symmetry or synchrony, sleep wake cycling) (Murray et al., 2006). Focal abnormalities and recurrent paroxysmal patterns, which are frequently observed in this population are not included in most employed EEG grading schemes, or are considered only marginally. Little data are available in terms of the description and significance of these specific patterns in infants with HIE, particularly in the immediate postnatal period.

In addition to EEG, other neurophysiological methods such as somatosensory evoked potentials (SEPs) are known to predict neurodevelopmental outcome after perinatal asphyxia (Swarte et al., 2012). Evidence of feasibility and reliability of this method in the first 6 hours of life dates back to 1990s, but since then applications in clinical practice and research have been scarce (Eken et al., 1995).

Any clue from neurophysiology in the narrow window of time available to instigate treatment is essential in order to support clinical decision making.

As anticipated earlier in the text, challenges regarding neonatal encephalopathy in low-income countries differ from those approached in developed countries. Since the body of knowledge on the disease is largely based on studies conducted in high income-settings, research conducted in underdeveloped countries is tremendously needed to emphasize specific needs and guide interventions and overflow of resources.

There is space for improvement for neurophysiology-based management of neonatal HIE.

Review Article

Regional variability in therapeutic hypothermia eligibility criteria for neonatal hypoxic-ischemic encephalopathy

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Impact

- Variability exists between regional and national therapeutic hypothermia eligibility guidelines for neonates with probable hypoxic-ischemic encephalopathy.
- Differences are common in both criteria indicating perinatal hypoxia-ischemia and criteria defining moderate or severe encephalopathy. The role of early electrophysiological assessment is also weighted unequally.
- This reflects in different individual care and impacts research data. A universally endorsed single severity staging of encephalopathy would be crucial for standardising management.

Abstract

Early induced therapeutic hypothermia represents the cornerstone treatment in neonates with probable hypoxic-ischemic encephalopathy. The selection of patients for treatment usually involves meeting criteria indicating evidence of perinatal hypoxia-ischemia and the presence of moderate or severe encephalopathy. In this review, we highlight the variability that exists between some of the different regional and national eligibility guidelines.

Determining the potential presence of perinatal hypoxia-ischemia may require either one, two or three signs amongst history of acute perinatal event, prolonged resuscitation at delivery, abnormal blood gases and low Apgar score, with a range of cutoff values. Clinical neurological exams often define the severity of encephalopathy differently, with varying number of domains required for determining eligibility and blurred interpretation of findings assigned to different severity grades in different systems. The role of early electrophysiological assessment is weighted differently.

A clinical implication is that infants may receive different care depending on the location in which they are born. This could also impact epidemiological data, as inference of rates of moderate-severe encephalopathy based on therapeutic hypothermia rates are misleading and influenced by different eligibility methods used. We would advocate that a universally endorsed single severity staging of encephalopathy is vital for standardising management and neonatal outcome.

Introduction

Hypoxic-ischemic encephalopathy (HIE) is a subtype of neonatal encephalopathy and a major contributor to global neonatal morbidity and mortality[1]. It is caused by an intrapartum or perinatal event leading to reduced cerebral perfusion with insufficient supply of oxygen and glucose. This primary insult triggers a cascade of events which after a latent phase of approximately six hours, lead to a secondary deterioration characterized by oxidative stress, mitochondrial failure, neuroinflammation and extensive cell death[2,3].

Therapeutic hypothermia (TH) represents the only proven treatment available to attenuate brain injury in infants with probable HIE, and current practice has shown its efficacy in reducing death and improving neurodevelopmental outcomes in survivors[4]. TH should be instigated within six hours of birth, in the latent phase representing the window of opportunity to prevent the secondary programmed cell death. At present, the International Liaison Committee of Resuscitation only recommend it's use for term or near-term neonates with moderate or severe encephalopathy[5], and the neuroprotective effect is obtained through 72 hours of cooling (Whole body or Selective Head)[6].

The selection of patients for treatment usually involves meeting criteria indicating evidence of likely perinatal hypoxia-ischemia (HI) and the presence of significant neonatal encephalopathy on clinical examination. Neurophysiological monitoring with either amplitude integrated or conventional electroencephalography may also be used to assist this assessment. The eligibility criteria for cooling these infants is derived from previous randomised control trials (RCTs)[7]. Several guidelines on TH eligibility have been developed in an attempt to standardise care, however despite these protocols, there remains variability in practice[8]. Even in jurisdictions with a published national TH eligibility protocol, significant between-unit variation in application and adherence to these protocols have been reported, resulting in differences in associated short-term outcomes[9].

While adherence to protocols may be challenging, what is more concerning is differences between eligibility criteria themselves. Each of the TH RCTs differed slightly in their inclusion criteria. These minor differences in study inclusion criteria have resulted in alternate evidence based eligibility criteria being developed and implemented in routine care. Such variability between TH eligibility protocols is concerning and may be associated with risks.

We have previously demonstrated that there are significant differences in TH eligibility depending on which evidence based guideline and exam criteria are used for infant assessment[10,11]. Therefore, differences in TH eligibility guidelines and protocols can result in variation in whether an infant is eligible for neuroprotective therapy according to the location in which they are born, and differences in grade of encephalopathy assigned. Beyond the real impact of this on the individual infant, such variability also impacts the validity of data to assess between unit differences and track national trends of NE severity and TH eligibility.

In this review, we highlight the variability that exists between some of the different regional and national eligibility guidelines for TH, outlining differences in criteria indicating likely perinatal hypoxia-ischemia and criteria defining moderate or severe encephalopathy.

The guidelines that we reference are not an exhaustive list, rather we present a sample of readily available, frequently cited and recently published guidelines that represent a wide range of geographic regions, including national and regional protocols in use in Europe, North America, South America, Asia and Australia (listed in Supplementary table 1)[12,13,14,15,16,17,18,19,20,21,22].

Gestational Age, Birth Weight and Age following delivery

Each of the guidelines reviewed identified a minimum gestational age (GA) for TH eligibility, which ranged between 35 weeks and 36 weeks. Notably, only two of the TH RCTs included infants between 35 and 36 weeks GA, with 7 infants at 35 weeks GA randomised in total[23,24]. In addition to gestational age, a minimum weight threshold of 1800g was consistently identified in the various guidelines reviewed. These criteria were frequently used within the RCTs to limit the potential for confounding variables to impact the outcome, and concerns regarding potential variability in the safety profile. However it should be noted that variation in clinical practice is emerging, with single centres reporting their local practices include offering TH to infants down to 34 weeks PMA[25,26]. However there remains no evidence for efficacy, with preliminary data from the Preemie Hypothermia Trial reporting no benefit in cooling infants between 33 and 35+6 weeks GA[27].

All of the reviewed guidelines recommend that TH is instigated within 6 hours after birth. This is supported by data from the TH RCTs[24,28]. The evidence for the potential benefit of TH started after 6 hours of life is controversial[29,30]. The late hypothermia trial by Laptook et al. reported that if TH was initiated between 6 and 24 hr of age, there was a 76% chance of at least a 1% improvement in death or disability at 18-24 months[30]. While this is statistically significant, the clinical significance is less clear. However, while advocating for initiating TH within the first 6 hours, most guidelines envisage the possibility of initiating treatment later i.e. up to 12 or 24 hours where infants are identified after 6 hours[14,16].

Criteria indicating evidence of acute perinatal hypoxia/ischemia

The criteria indicating evidence of acute perinatal/intrapartum hypoxia-ischemia (HI) differ between published guidelines. Examples are shown in Table 1. This is reflected in the Cochrane review of Therapeutic hypothermia. Among the criteria for studies to be included in the Cochrane review was that the RCT's definition of perinatal asphyxia had to include at least one of the following; Apgar score ≤5 at 10 min, or; cord pH ≤7.1, or an arterial pH ≤7.1 or BD≥12 in first hour of life, or; mechanical ventilation or resuscitation at 10 minutes. However reflecting the variability in studies the actual inclusion criteria of the RCTs entered into the systematic review could be much broader (or narrower) than this, as they only needed to meet one of these criteria. As such the Cochrane review itself does not advocate for any particular defining thresholds of perinatal asphyxia to meet TH eligibility criteria[31].

Depending on individual guidelines, determining the potential presence of perinatal HI may require either one, two or three signs among the following categories: abnormal blood gases, history of acute perinatal event, low Apgar score, prolonged resuscitation at delivery.

All of the reviewed guidelines included a blood gas as a key data point when assessing for evidence of the presence of perinatal HI. In some guidelines[12,14,18] the blood gas is weighted more heavily compared to other criteria of potential perinatal HI, and may be the only criteria required to indicate the presence of HI. In most guidelines, acidosis is defined as the alteration of either pH or BD; in some only pH is considered[15]. A range in cut-off values are used as entry criteria for TH therapy in the published guidelines, from ≤7.0 to ≤7.15 for pH, and >12 to ≥16 mmol/L for BD. The lactate level is seldom included as an indicator for

eligibility, but in those guidelines that did include it, the threshold cut-off for eligibility ranged from 6-10 mmol/L[16,21,22]. The suggested source of blood gas is cord gas or any baby blood gas (arterial, venous, or capillary) within one hour of birth in most guidelines. Therefore, for pragmatic reasons, the differences in acid-base levels between arterial and venous blood samples is not considered when determining threshold values for TH eligibility in the published guidelines.

A history of a clearly recognized perinatal event is sometimes included, although never a mandatory element for the definition of perinatal HI[12,14,15,18]. Fetal heart rate decelerations, cord prolapse or rupture, placental abruption, uterine rupture, maternal trauma or haemorrhage are variably included when defining evidence of potential perinatal HI.

All guidelines include a low Apgar score as potential evidence of an acute perinatal HI event. Despite this, there is less agreement on actual scores or timing of scores. The cut-off for an Apgar score indicating depression at birth varied from <5 to ≤5 in the majority, but was as high as ≤7 in one guideline[22]. Similarly, while most guidelines only included the 10-minute Apgar, one used the 5 minute Apgar score[16], and an alternate included an Apgar score of ≤5 at any time point, 1, 5 or 10 minutes[15]. Continued need for resuscitation and/or ventilation for 10 or more minutes after birth, is also considered as evidence of potential perinatal HI, and is included in most guidelines.

Therefore it is clear that while there is good agreement on what criteria are relevant for screening assessment, there remains tangible differences between the published guidelines in several of the domains that are assessed.

Criteria representing the presence of encephalopathy or defining moderate or severe encephalopthy

The neurological exam has a critical role in determining eligibility for TH. HIE is traditionally classified in stages, which if applied consistently provide useful information about the severity of injury. All guidelines reviewed aimed to identify neonates with moderate or severe encephalopathy. A variety of clinical scoring schemes for HIE have been developed, without universal agreement on which is most accurate. Most guidelines however rely on

neurological scoring systems adapted from the seminal work of Sarnat and Sarnat[32,13,14,18,21]. However there is some variation with the Dutch guidelines being based on the Thompson score[16], and the British association of perinatal medicine (BAPM) guidelines using the neurological abnormality entry criteria from the TOBY trial[17]. The authors are unaware of any TH RCTs using the Thompson score for determining TH eligibility, however it was used In the neo.nEURO.network RCT[33] to evaluate the neurological status at 7 days of age, and the association between Thompson score and the different Sarnat stages has been well documented previously [34].

Regarding the original system proposed by Sarnat and Sarnat it is worth noting that it was meant as a prognostic test based on serial evaluations over the first week, at a time when no early intervention was available. It was never intended to be a single point test for determining the severity of encephalopathy in the first 6 hours after birth. Sarnat et al have recently published a Commentary proposing to update the Sarnat exam, however the detailed protocol to address changes is yet to be published[35]. Therefore it is unclear at this time if any update would impact current definitions for severity of encephalopathy.

In general, the following domains are assessed in newborn neurological examinations assessing TH eligibility (examples in Table 2): level of consciousness, spontaneous activity, posture, tone, primitive reflexes and autonomic activity. Most guidelines reviewed included all of these domains, however there was some variation. Spontaneous activity is not included in the Dutch, British and Japanese guidelines, while posture is not included in the British, Japanese and New Zealand guidelines.

The primitive reflex domain includes single reflexes assessed independently: suck, Moro (not included in the British and Japanese guidelines), oculomotor (only included in the British and Japanese guidelines[19]) and grasp (only included in the Dutch guidelines[16]). In some guidelines each reflex represents a category, with the same importance as every other single main domain[15,16,17,19,20]. In others, individual reflexes represent subdomains, with the worst score providing the global grade for the overall primitive reflex domain[13,14,18,21].

Similarly, the autonomic system domain variably includes the evaluation of pupils, heart rate, respiration and fontanelle. In the SIBEN and Dutch guidelines each of the latter features represents a category, with the same importance as every other single domain[15,16].

In the British, Japanese and New Zealand guidelines, one single autonomic parameter is examined[17,19,22]. In other guidelines they represent subdomains and similar to the primitive reflex domain, the worst score across any autonomic system sub-domain provides the overall domains' score[13,14,18,21].

In many guidelines the neurological evaluation schemes allow the distinction between mild, moderate, and severe encephalopathic features for all the domains examined, and result in a diagnosis of mild, moderate, or severe encephalopathy. However in some, features consistent with either normal or mildly abnormal are grouped together[18], and in others only features consistent with moderate and severe encephalopathy are included[14]. Of note, based upon the TOBY RCT, the British and Japanese guidelines eligibility criteria do not identify a related grade of severity- only if the child is eligible or not[17,19].

When determining if an infant is eligible for TH or not, most guidelines indicate that if 3 or more domains are consistent with moderate or severe encephalopathy then the infant is eligible for TH. The Dutch guidelines use a cut-off of 7 points (on a total of 22) on the Thompson score to determine clinical eligibility, regardless of the severity of the scores given in the single domains[16]. The British and Japanese criteria both give more weight to the level of consciousness over the other exam components, and require an abnormal level of consciousness plus an abnormality in one further domain to determine TH eligibility[17,19].

In addition to these differences, there is variation in the interpretation of specific findings for individual criteria that are shared between guidelines, as summarized in Table 3. Decreased spontaneous activity may be characterized as a mild or moderate grade in the NICHD guidelines[13], while it is invariably classified as moderate in other guidelines[14,15,18,20,21,22]. Regarding tone, both hypotonia and hypertonia are defined as moderate abnormalities in NICHD guidelines whereas in the other systems, only a reduced tone is considered a moderate finding, while having increased tone is often classified as mild[16,18,20,22]. For posture, the interpretation of distal flexion of the limbs is particularly controversial: some guidelines interpret any distal flection as a moderately abnormal posture[14,18], while others distinguish between mild and moderate distal flexion, with each being interpreted as a marker of mild or moderate encephalopathy respectively[13,15,16,21]. For the primitive reflexes, weak suck and Moro are considered moderately abnormal findings

indicating eligibility in some guidelines[14,18,20], while in others they are also classified as mild[16].

These differences in the interpretation and criteria used to identify the presence of moderate and severe encephalopathy lead to real world differences in the selection of patients for treatment. A clinical research study comparing eligibility using NICHD and British criteria revealed a significant difference in the proportion of infants determined to be eligible for TH depending on which exam is used, with 24% more infants being eligible for the NICHD but ineligible for the British criteria. Interestingly, in that study more than a half of infants in which a discrepancy of eligibility was found demonstrated MRI evidence of cerebral injury, while neither method identified all infants who developed seizures or had cerebral MR injury[11]. Another comparison study between NICHD and SIBEN grading systems, despite a good agreement between methods (92%), highlighted that SIBEN defines significantly more infants as moderate and less as mild, than NICHD. In the same study, numerical scores were also assigned using the same methods, and proved to be superior to standard grades in defining a minimum threshold for cerebral injury[10].

Time of evaluation

Neonatal encephalopathy is a dynamic process, and the severity of neurological findings often change over time. To ensure prompt treatment, it is critical to define the severity of encephalopathy within the narrow window of time available to initiate treatment.

A minimum age at which the neurological examination is reliable in detecting encephalopathy has not been defined. Animal studies suggest that the earlier the treatment is started during the latent phase the better it is in preventing secondary injury in the brain and improving outcomes[36]. In humans, the TOBY trial demonstrated a trend towards better outcomes in infants in which TH was initiated in the first 4 hours after birth[37]; and Thoresen et al described an improved motor outcome in a cohort of newborns cooled within 3 hours[38]. Following this, some regional guidelines advise that the neurological examination should be done as soon as possible after the baby is stabilised and within the first hour of birth[15,22]. However other guidelines recommend assessing the neurological criteria only after 1 hour and before 6 hours after birth[18,20]. There is a lack of evidence about which

approach is more appropriate, as the RCTs did not specify a minimum age of evaluation for study entry[24,28]. Nonetheless, an exam performed immediately post resuscitation is potentially not a true reflection of the neurological status, and waiting for an hour after birth (allowing the baby to recover from the initial resuscitation) appears to be a pragmatic decision.

The majority of guidelines indicate repeated frequent (hourly) assessment of neurological status within the first 6 hours of birth[14,15,16,20,21]. Babies who meet any of the criteria for significant perinatal HI but on initial neurological examination are neurologically normal or mild, should be reviewed several times in order to capture a possible evolution and deterioration of the exam within the first 6 hours. On the other hand, two guidelines[14,16] clearly state that a neonate with a neurological exam that initially meets eligibility criteria, but that rapidly improves within the first few hours may not need TH. This practice is at odds with the concept that the benefit of TH is greater the earlier that it is initiated, and while there is some evidence for early exit from TH at 24 hrs of age in low risk infants[39], there is no evidence to support or refute this practice in the first 6 hours.

Seizures

The presence or absence of seizures is included in all the guidelines examined. In some guidelines, evidence of seizures in the first 6 hours is included similar to each domain of the neuro exam, and therefore it is possible to have seizures but not meet threshold for TH[15,17,19]. Contrary to this, in other guidelines, seizures in the first six hours among infants with evidence of perinatal HI represent an independent indication for TH, even when infants do not have sufficient additional moderate or severe criteria to meet standard TH threshold based on neurological examination[12,14,16,18,20,21]. In other words, if a patient is less than 6 hours old and meets the gestation, weight and blood gas criteria and has a witnessed seizure, the patient is eligible for TH regardless of neurological examination findings.

However, the level of diagnostic certainty of seizures is rarely specified in TH eligibility guidelines. The SIBEN paper refers to seizures assessed clinically, while regional guidelines from Australia refer to seizures witnessed by the medical officer, nurse, or midwife[20,21]. EEG-confirmation is rarely deemed to be necessary. Nevertheless, it is now well known that

diagnosis of neonatal seizures based on clinical exam is difficult and often inaccurate, regardless of the level of experience of the clinician. Seizure like movements are often misinterpreted and only clonic seizures can be reliably diagnosed based on clinical evaluation[40,41]. In the case of suspected seizures in the immediate post-natal period, the clinician should seek EEG/aEEG confirmation when possible.

In addition, in the event of acute provoked seizures emerging within the earliest 6 hours after birth, a subacute injury evolving during the course of labour should be suspected. Literature on the temporal characteristic of seizures in neonatal HIE has flourished in recent years, and the median age at electrographic seizure onset in HIE falls beyond 12 hours of age in most studies. Seizures occurring before 6 hours of age are rare in an acute perinatal HI event[42]. In essence, neonatal acute provoked seizures are a symptom of ongoing encephalopathy, but when detected in the earliest hours after birth they are likely to be an expression of a subacute injury which started and evolved in the hours before birth. If this is the case, even early treatment may partially miss the window of opportunity for successful intervention[43]. Nonetheless, in clinical practice, given that the exact timing of injury will rarely be identified, it is reasonable and appropriate to cool neonates with evidence of perinatal HI and witnessed seizures in the first 6 hours. In fact, these neonates were included in the RCTs and a decision not to cool would represent a deviation from the current evidence base. Furthermore, since there is evidence that TH reduces seizure burden[42,44] which in turn may affect neurodevelopmental outcome, treatment instigated beyond the optimal therapeutic window could still potentially reduce the on-going injury.

Role of aEEG

Use of amplitude integrated EEG to supplement the early assessment is encouraged in some, but not in the majority of guidelines[14,22]. The BAPM guidelines do state that infants who meet exam criteria should then have an aEEG performed, however even then it is stressed that if both perinatal HI and clinical encephalopathy criteria are met that initiation of TH should not be delayed if an aEEG is not promptly available[17]. The recommended duration of the aEEG monitoring varies from 20 to 30 minutes, and the altered aEEG patterns recognised as supporting or determining treatment eligibility are slightly different

between the various guidelines. The definition of these patterns is more detailed in the Dutch guidelines: discontinuous normal voltage (with lower limit equal to or lower than 5 μV), discontinuous low voltage (periods of very low voltage interspersed with peaks of high amplitude), continuous low voltage (constantly around or lower 5 μV) and flat trace (deeply depressed activity near to isoelectric) all indicate TH eligibility[16]. The other guidelines mention the presence of an abnormal baseline or moderately abnormal activity, discontinuity or suppressed activity, but do not further define these criteria. The identification of seizures on the aEEG is invariably described as an indication for TH, and regarding this we refer to the discussion in the previous paragraph.

In most guidelines aEEG findings represent a supporting criterion, and play a subordinate role to clinical assessment in the selection for treatment. Discrepancy between the clinical grade and the aEEG severity may occur. In the CoolCap trial, 8 neonates classified as mildly encephalopathic based on clinical evaluation showed moderately or severely abnormal patterns on the initial aEEG evaluation[45]. In a more recent cohort study, 13 infants were reported to have moderately abnormal aEEG findings despite a mild clinical exam; 31% later displayed an abnormal MRI[46]. Some of the existing guidelines advocate that if there is a discrepancy between findings on aEEG and neurological examination a decision should be made based on physical examination findings[19]. Contrary to this the Dutch guidelines consider the aEEG as non-inferior to the clinical neurological assessment for TH eligibility: an abnormal aEEG can indicate treatment eligibility even in the absence of the clinical criterion of a Thompson score >7[16]. This approach is supported by recent work from the Netherlands which showed; 1) that the aEEG and Thompson score in the first 6 hours were equally predictive of long-term outcome[47]; and 2) some infants who were found to have a low Thompson score, but an abnormal aEEG assessment <6 hours of age, ultimately had a poor long-term outcome[48]. The authors concluded that while they could not determine if one method is superior to the other, using the aEEG helped to identify cases for TH that would not have been offered treatment based on clinical exam alone[47].

Formal EEG was included in the original Sarnat staging system, and the valuable real time information provided by both aEEG and EEG should be thoughtfully considered when available. A recent study focused on neonates with HIE clinically defined as mildly encephalopathic in the first 6 hours after birth, demonstrated a wide spectrum of

electrographic dysfunction on multichannel EEG. One third of infants monitored had moderate to severely abnormal background EEG patterns, which were associated with a higher risk of developing acute provoked seizures. Contrary to this, those infants that were clinically mild and who had a normal or mildly abnormal early EEG background were at lower risk for acute provoked seizures[49]. Therefore it is clear that neurophysiological monitoring (aEEG or EEG), can provide additional information to the clinical exam[48], and if moderate or severely abnormal may assist in determining need for TH. However it must be recognised that access to neurophysiological monitoring and the expertise required to interpret them is frequently not available (e.g. in smaller units and during transport). It is hoped that in the future the development of mobile devices providing real-time aEEG/EEG monitoring with automated or centralized review, will make neurophysiological monitoring more widely available in all health care settings[50].

Milder encephalopathy

For infants with probable HIE, the category of mild encephalopathy is often controversial. There are no consensus recommendations on which of these infants should be monitored with aEEG/EEG, who should receive neuro-imaging, or even how long they should be followed post-discharge. Most controversial of all however is how best to manage them. Many infants with milder encephalopathy are being treated on clinical judgement, without fulfilling eligibility criteria defined in the guidelines. Mehta and collaborators, in a retrospective study on TH infants born between 2007 and 2011 in New South Wales and Australian capital territory, found that 50% did not meet regional eligibility criteria, and 70% did not fulfil the criteria for "evidence of asphyxia"[51]. Data from TH registries set-up in Europe and in the United States after completion of the TH RCTs revealed that around 40% of infants receiving TH lacked clinical features of moderate or severe encephalopathy[52,53]. This data is over a decade old now, and in the interim the use of TH in mild encephalopathy has been increasing internationally[54,55,56]. This practice is driven by concern that these infants, historically considered at minimal risk for adverse outcomes[57], are at risk of injury. The evidence of injury among mild encephalopathy is now well recognised, with recent studies demonstrating significant risk of cerebral injury and adverse neurodevelopmental outcomes in this population[58,59,60,61]. Nonetheless, there is minimal to no data on the risk

profile associated with TH in mild HIE[24]; stress related to the exposure to hypothermia, sedative administration, delay in the initiation of feeds, separation from parents, and longer hospital stay are all elements that deserve to be considered. The balance of risk against potential benefit is unknown and where best to draw that line in the care of mildly encephalopathic neonates is the subject of active debate and research. Additionally, the scoring systems incorporated in currently used treatment guidelines are focused on identification of moderate and severe encephalopathy and there is no consensus on the accurate definition of mild encephalopathy within the first 6 hours after birth[62]. In many centres for children with probable HIE, mild is defined as encephalopathy not meeting local guidelines for TH eligibility, in essence a diagnosis of exclusion. Given the variability in guidelines discussed here, such a definition for mild encephalopathy is fraught with issues. Furthermore, the current method of dividing severity of encephalopathy into three grades is probably an over simplification of the clinical spectrum that exists. Numerical scoring systems based on Sarnat, Thompson and SIBEN scores have been recently proposed[10,13,62]. These systems, which acknowledge the wide spectrum associated with encephalopathy may be better suited to demonstrate and detect the range of clinical variability. Studies prospectively validating such scores in terms of outcomes, identified threshold NICHD Total Sarnat Score of ≥5 or ≥4 (representing infants at the sicker end of mild encephalopathy) as providing the best sensitivity for identifying neonates who would have neurodevelopmental issues, highlighting in particular those at greater risk within the mild encephalopathy group (who fall outside of the classical TH eligibility)[10,13].

Conclusions

Remarkable differences emerge when comparing TH eligibility criteria in different jurisdictions. Most systems require infants to demonstrate evidence of perinatal hypoxia-ischemia plus clinical findings consistent with moderate to severe encephalopathy using a standardised exam. However the criteria indicating evidence of acute perinatal hypoxia-ischemia are not uniform between different guidelines, nor is the clinical neurological examination used to confirm the eligibility. These evidence based exams often define the severity of encephalopathy differently, with varying number of domains required for determining eligibility and blurred interpretation of findings assigned to different severity

grades in different systems. aEEG is commonly used to support clinical decision making, but in many centres it is not available in the first hours after birth and its specific importance varies between guidelines and countries.

Due to the lack of clear agreed definitions for criteria indicating perinatal hypoxia-ischemia and moderate to severe encephalopathy, an individual infant's eligibility status for TH differs between centres and nations. A clinical practice implication is that infants may receive different care depending on the location in which they are born. This could also impact epidemiological data, as inference of rates of moderate-severe encephalopathy based on TH rates are misleading and influenced by different eligibility methods used. We would advocate that a universally endorsed single severity staging of encephalopathy is vital for standardising management and neonatal outcome. The NICHD expanded scoring system, and the associated Total Sarnat Score, are the most frequently referenced for research studies, implying a greater familiarity for clinicians, which would ease adoption across sites and nations. However we would additionally advocate for the incorporation of additional neurophysiological monitoring (aEEG/EEG) into the initial assessment when available, to supplement and support the clinical exam.

Table 1: Examples of criteria indicating evidence of acute perinatal hypoxia-ischemia.

	North America		South America	Europe			Asia	Australia and New Zealand		
	NICHD-PRIME	Canada	SIBEN	Netherlands NVK	UK-BAPM	Ireland	Japan	Queensland	Sydney NETS	New Zealand NZCYCN
Gestational age (weeks)	≥ 36	≥36 (note on ≥35)	>35	≥35	≥36	≥36	≥36	≥35	≥35	≥35
Postnatal age (hours)	≤6	≤6*	Not mentioned	<6	≤6*	≤6*	≤6	<6*	<6	<6*
Minimum weight (kg)	>1,8	Not mentioned	Not mentioned	>1,8	Not mentioned	≥1,8	≥1,8	≥1,8	Not mentioned	≥1,8
Evidence of acute intrapartum/perinatal hypoxia-ischaemia	pH ≤7.00 or BD ≥16 on any cord or baby gas within 1h OR pH 7.01 to 7.15 or BE 10 to 15.9 on any cord or baby gas within 1h plus both the following: - history of acute perinatal event - Apgar ≤5 at 10' or assisted ventilation initiated at birth and continued for at least 10'	Cord pH ≤7.0 or BD ≥16 OR pH 7.01 to 7.15 or BE 10 to 15.9 on any cord or baby gas within 1h plus the following: - history of acute perinatal event - Apgar ≤5 at 10' or requirement for positive-pressure ventilation at 10'	History of acute perinatal event, associated with low Apgar (≤ 5 at 1', 5' or 10') or pH ≤7.1 on cord gas Not mandatory: "If the clinical score demonstrates clear signs of encephalopathy, this should not be ignored even with a pH>7.1"	≥1 of the following: - Apgar ≤ 5 at 5' - resuscitation at birth - requirement for respiratory support for ≥10' - pH <7.0* - BD >16 mmol/L* - lactate >10 mmol/L* *on any cord or baby gas within 1h	≥1 of the following: - Apgar ≤ 5 at 10' - continued need for resuscitation at 10' - pH ≤7.0 or BD ≥16 on any cord or baby gas within 1h	≥1 of the following: - pH <7.0 or BD ≥16 on any cord or baby gas within 1h - Apgar ≤5 at 10' - continued need for PPV or intubated at 10'	≥1 of the following: - Apgar ≤5 at 10' - continued need for resuscitation at 10' - pH <7.0 or BD ≥16 on any baby gas within 1h	≥1 of the following: - Apgar ≤5 at 10' - pH <7.0 or BD ≥12 on any cord or baby gas within 1h -mechanical ventilation or ongoing resuscitation for ≥10'	≥1 of the following: - pH <7.0 or BD ≥12 on any cord or baby gas within 1h - Apgar ≤ 5 at 10' - Mechanical ventilation or ongoing resuscitation for ≥ 10' - pH 7-7.1 or lactate >8mmol/L in the first hour	≥1 of the following: - Apgar ≤ 7 at 10' - mechanical ventilation >5' or ongoing resuscitation for ≥10' - pH <7.1 or BD ≥12 or lactate >6 on any cord or baby gas within 1h

Table 2: Examples of domains assessed in the clinical neurological exam.

		North America		South America	Europe			Asia	Australia and New Zealand		
		NICHD-PRIME	Canada	SIBEN	Netherlands NVK	UK-BAPM	Ireland	Japan	Queensland	Sydney NETS	New Zealand
Level of Consciousness		✓	✓	✓	✓	✓	✓	✓	✓	✓	✓
Spontaneous Activity		✓	✓	✓	X	X	✓	X	✓	✓	✓
Posture		✓	✓	✓	✓	X	✓	X	✓	✓	X
Tone		✓	✓	✓	✓	✓	✓	✓	✓	✓	✓
Primitive Reflexes	Single (S) vs Multiple (M) domain	S	S	M	M	M	S	M	M	S	M
	Suck	✓	✓	✓	✓	✓	✓	✓	✓	✓	✓
	Moro	✓	✓	✓	✓	X	✓	X	✓	✓	✓
	Oculomotor	X	X	X	X	✓	X	✓	X	X	X
	Grasping	X	X	X	✓	X	X	X	X	X	X
Autonomic System	Single (S) vs Multiple (M) domain	S	S	M	M	NA	S	NA	S	S	NA
	Pupils	✓	✓	✓	X	✓	✓	✓	✓	✓	X
	Heart Rate	✓	✓	✓	X	X	✓	X	✓	✓	X
	Respiration	✓	✓	✓	✓	X	✓	X	✓	✓	✓
	Fontanelle	X	X	X	✓	X	X	X	X	X	X
Seizures		✓	✓	✓	✓	✓	✓	✓	✓	✓	✓
Seizures determine automatic eligibility?		Yes	Yes	No	Yes	No	Yes	No	Yes	Yes	Not Specified
Number of domains assessed		6	6	10	9	6	6	6	7	6	6
Number of domains required for eligibility		≥3 or seizure	≥3 or seizure	≥3	TS ≥7/22	LOC + ≥1 other	≥3 or seizure	LOC + ≥1 other	≥3 or seizure	≥3 or seizure	Not defined

Table 3: Examples of severity interpretation of specific findings in neurological clinical exam.

			North America		South America	Europe			Asia	Australia and New Zealand		
			NICHD-PRIME	Canada	SIBEN	Netherlands NVK	UK-BAPM	Ireland	Japan	Queensland	Sydney NETS	New Zealand
Level of Consciousness		Hyperalert	=		=	=		=		=	=	=
		Lethargic	†	†	†	†	●	†	●	†	†	†
		Stupor/Coma	‡	‡	‡	‡	●	‡	●	‡	‡	‡
Spontaneous Activity		Normal	○ | =	○	○ | =			○ | =		○ | =	○ | =	○ | =
		Increased								=		=
		Decreased	= | †	†	†			†		†	†	†
		Absent	‡	‡	‡			‡		‡	‡	‡
Posture		Distal flexion		†				†				
		Mild Distal Flexion	=		=	=				=	=	
		Marked Distal Flexion	†		†	†				†	†	
		Complete Extension	†	†				†		†	†	
		Decerebrate	‡	‡	‡	‡		‡		‡	‡	
Tone		Normal	○ | =	○	○ | =	○	○	○	○	○ | =	○ | =	○ | =
		Increased/Hypertonia	†			=		=		=		=
		Decreased/Hypotonia	†	†	†	†	●	†	●	†	†	†
		Rigid	‡									
		Flaccid	‡	‡	‡	‡	●	‡	●	‡	‡	‡
Primitive Reflexes	Suck	Normal	○	○	○	○	○	○	○	○ | =	○	○ | =
		Incomplete	= | †							=		= | †
		Weak	= | †	†	= | †	=	●	†	●	†	= | †	
		Biting	†			†						
		Absent	‡	‡	† | ‡	†	●	‡	●	‡	‡	‡
	Moro	Strong, low threshold	=		=					=	○	=
		Incomplete/Weak	†	†	†	=		†		†	†	†
		Absent	‡	‡	‡	†		‡		‡	‡	‡
Autonomic System	Pupils	Equal and Reactive	○	○	○		○	○	○	○ | =	○	
		Dilated	=	‡	=		●		●		= | ‡	
		Constricted	†	†	†		●	†	●	†	†	
		Unresponsive, Unequal, deviated	‡	‡	‡		●	‡	●	‡		
	Heart Rate	Normal	○	○	○			○		○	○	
		Tachycardia	=		=					=	=	
		Bradycardia	†	†	†			†		†	†	
		Variable	‡	‡	‡			‡		‡	‡	
	Respiration	Normal	○	○	○ | =	○		○		○ | =	○ | =	○ | =
		Hyperventilate	=			=						
		Periodic/irregular	†	†	†	†		†		†	†	†
		Copious secretions						†				
		Apnoea	‡	‡	‡	‡		‡		‡	‡	‡
		Ventilated	‡			‡		‡				

Legend

○	Normal
=	mild (1pt in TS)
†	moderate (2pts in TS)
‡	severe (3 pts in TS)
●	eligible (grade of severity not assigned)
	not mentioned

Supplementary table 1: List of national and regional protocols used in this review.

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Chalak LF, Adams-Huet B, Sant'Anna G. A Total Sarnat Score in Mild Hypoxic-ischemic Encephalopathy Can Detect Infants at Higher Risk of Disability. J Pediatr. 2019 Nov;214:217-221.e1. doi: 10.1016/j.jpeds.2019.06.026.	PRIME (NICHD expanded scoring system)	Chalak et al., 2019
Lemyre B, Chau V. Hypothermia for newborns with hypoxic-ischemic encephalopathy. Paediatr Child Health. 2018 Jul;23(4):285-291. doi: 10.1093/pch/pxy028.	Canadian	Lemyre and Chau, 2018
Perez JM, Golombek SG, Sola A. Clinical hypoxic-ischemic encephalopathy score of the Iberoamerican Society of Neonatology (Siben): A new proposal for diagnosis and management. Rev Assoc Med Bras (1992). 2017 Jan 1;63(1):64-69. doi: 10.1590/1806-9282.63.01.64.	SIBEN	Perez et al, 2017
Nederlandse Vereniging voor Kindergeneeskunde (NVK). Therapeutische hypothermie na perinatale asfyxie, versie 4.3, maart 2014 Herziene versie 5.11 – 2309 2021. Available at: Over de samenvatting (neonatology.eu)	Dutch	NVK, 2021
British Association of Perinatal Medicine (BAPM). Therapeutic Hypothermia for Neonatal Encephalopathy, a framework for practice. Available at: https://www.bapm.org/resources/237-therapeutic-hypothermia-for-neonatal-encephalopathy *(last modified 16 December 2020, accessed 09 August 2023).*	British	BAPM, 2020
San Lazaro Campillo I, McGinley J, Corcoran P, Meaney S, McKenna P, Filan P, Greene RA, Murphy J on behalf of Neonatal Therapeutic Hypothermia Steering Group. Neonatal Therapeutic Hypothermia in Ireland, Annual Report 2016-2020. Available at: https://www.hse.ie/eng/about/who/acute-hospitals-division/woman-infants/national-reports-on-womens-health/neonatal-therapeutic-hypothermia-in-ireland-annual-report-2020.pdf	Irish	San Lazaro Campillo et al., 2020
Takenouchi T, Iwata O, Nabetani M, Tamura M. Therapeutic hypothermia for neonatal encephalopathy: JSPNM & MHLW Japan Working Group Practice Guidelines Consensus Statement from the Working Group on Therapeutic Hypothermia for Neonatal Encephalopathy, Ministry of Health, Labor and Welfare (MHLW), Japan, and Japan Society for Perinatal and Neonatal Medicine (JSPNM). Brain Dev. 2012 Feb;34(2):165-70. doi: 10.1016/j.braindev.2011.06.009.	Japanese	Takenouchi et al., 2012
Queensland Clinical Guidelines. Hypoxic ischaemic encephalopathy (HIE). Guideline No. MN21.11-V11-R26. Queensland Health. 2021 Available from: http://www.health.qld.gov.au/qcg	Queensland	Queensland Clinical Guidelines, 2021

Hypoxic Ischaemic Encephalopathy in the Newborn. Sydney Children's Hospitals Network, Newborn and Paediatric Emergency Transport Service (NETS). Date of Publishing: 15 March 2022. Accessed: 21 September 2023.	Sydney	NETS, 2022
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Jacopo Proietti selected and analyzed the reviewed guidelines, prepared tables, wrote the first draft of the manuscript.

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Competing interests

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Early multichannel EEG analysis in Neonatal Hypoxic-Ischaemic Encephalopathy: more than just a grade

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Abstract

Objective: EEG evaluation supports the identification of neonates with hypoxic-ischaemic encephalopathy (HIE) who might benefit most from neuroprotective treatment. Current

neonatal EEG grading schemes focus on general background features; specific focal transients are rarely considered. We characterised the EEG in the immediate postnatal period in neonates with HIE to assess severity of encephalopathy and risk for acute provoked seizures.

Methods: EEGs were analysed in 162 term neonates with HIE within 12 hours of birth. Background grades of the first hour of recording were classified, specific EEG transients and patterns were described and correlation with the later occurrence of seizures assessed.

Results: 66 neonates had mild, 30 moderate, and 66 severe background EEGs. Excess sharp waves and spikes were observed in 38% mild, 57% moderate, 26% severe grade EEGs. Rhythmic sequences of sharp-slow wave complexes in 24% mild and 17% moderate grade EEGs.

Neonates with severe background EEGs had a significantly higher rate of acute provoked seizures (59%). In moderate and mild EEG groups, seizure risk was lower (14.6%), but presence of spikes and/or rhythmic sequences predicted seizures (sensitivity 86%, specificity 76%).

Conclusions and significance: Early EEG grading combined with analysis of focal features may be more accurate in assessing severity of neonatal encephalopathy and need for treatment.

Keywords: neonatal HIE, electroencephalography, early assessment, EEG background, EEG transients.

Highlights:

- EEG schemes for neonatal HIE generally assess background features, but a variety of specific transients can also be observed
- A severely abnormal EEG background is strongly associated with worse clinical scores and higher risk of acute provoked seizures
- In mildly and moderately altered background EEGs, spikes and short rhythmic sequences are associated with higher risk of seizures

1 Introduction

Neonatal encephalopathy due to hypoxia-ischaemia (HIE) continues to be the commonest cause of seizures, death and disability in term newborns (Volpe, 2008). Therapeutic hypothermia is currently the only proven treatment available to attenuate injury and reduce seizure burden in neonates with HIE, and is most effective when initiated within 6 hours of birth (Jacobs et al., 2013). According to current protocols, only infants with moderate to severe HIE receive therapeutic hypothermia (Azzopardi et al., 2014), thus the ability to

accurately classify the severity of encephalopathy in the early neonatal period is of critical importance. Neonates with neonatal encephalopathy following perinatal asphyxia not fulfilling the current eligibility criteria and not treated with therapeutic hypothermia have been shown to be at risk of seizures, MRI abnormalities and adverse neurodevelopmental outcomes, highlighting the urgent need for more accurate selection for treatment (Parmentier et al., 2022). Emerging adjuvant neuroprotective agents have shown preclinical efficacy and are being investigated in clinical trials, which can be instituted beyond the first 6 hours, on the first day of life or later (Wang et al., 2019). Infants who develop acute provoked seizures have often experienced a moderate or severe hypoxic-ischemic injury and any clues in the early EEG that can help identify this group would be beneficial. Clinical assessment is the principal method used for encephalopathy classification, but it is often difficult to distinguish between mild and moderate grades in the first hours after birth (DuPont et al., 2013), especially in neonates who are ventilated early after resuscitation and receive intravenous sedation. The most frequently used adjunct parameters, both clinical (Apgar score, need for ventilation) and laboratory (such as pH and lactate), cannot predict the grade of encephalopathy (Murray et al., 2006).

Many neonates fall into a “grey zone” in which the clinical and laboratory parameters do not provide a clear distinction between mild and moderate encephalopathy. Although amplitude-integrated EEG (aEEG) is often used in the NICU, multichannel continuous EEG (cEEG) is considered the gold standard for the EEG assessment of encephalopathic neonates since it provides both temporal and spatial information about real-time brain function and allows for accurate identification of seizures. In addition, using cEEG, focal transients and brief paroxysmal features can be identified which may help supplement EEG grading schemes and provide more detailed information. The challenge of deciding to initiate treatment or not takes place in a narrow window of time, therefore it is crucial that appropriate clinical knowledge of the EEG at this specific time point is available. Similar to clinical assessment, EEG findings are evolving and outcome prediction is much more accurate when performed at later time points e.g. 48 hours (Boylan et al., 2009). In the majority of published studies investigating the correlation between multichannel EEG features and outcomes in HIE populations, the EEG recording was performed at different times (making any meaningful comparison of outcome extremely difficult) and almost never early enough to be useful for clinical intervention decisions (Walsh et al., 2011). Presently, the EEG in full-term neonates with HIE is generally described in terms of its background features (amplitude, continuity, symmetry, synchrony, and sleep-wake state), upon which the existing EEG classification systems are based (Walsh et al., 2011). Additional recurrent transients, such as focal abnormalities and patterns frequently observed in this population, are rarely included in classification schemes and little data are available in terms of description and interpretation of these specific transients.

In this study, we report the visual analysis of the earliest 1-hour EEG epoch recorded in 162 neonates with all grades of HIE, with the aim of providing information not just on general background patterns but also on specific EEG features. We used the most widely implemented scoring system for EEG grading in HIE (Murray et al., 2006), to discriminate different degrees of background severity at this early time point. Then, we provide a

detailed description and illustrations of specific additional features and assess their frequency and distribution in each background EEG group. Their relationship with the later occurrence of acute provoked seizures is also investigated, to assess whether they may help supplement EEG grading schemes and provide more information on those neonates that might benefit most from intervention.

2 Methods

2.1 Study setting and participants

The neonates included in this secondary data analysis derive from a sample of infants with gestational age 36-43 weeks and diagnosis of HIE, recruited for two multi-center cohort studies (ClinicalTrials.gov Identifier: NCT02160171 and NCT02431780) between April 2011 and January 2017, from eight tertiary level Neonatal Intensive Care Units across four European countries (Ireland, United Kingdom, Sweden, The Netherlands) (Pavel et al., 2020; Pavel et al., 2023). The study had ethical approval at each site and informed consent was obtained from parents or legal guardians for all participants. Neonates who did not have at least one hour of EEG monitoring before 12 hours of age and before emergence of electrographic seizures were excluded from this analysis. At each centre, clinical data for each neonate were collected, including delivery details and information on the neonatal course such as Apgar Score, first postnatal pH and first postnatal lactate, need for ventilation at 10 minutes of life and application of therapeutic hypothermia. A Sarnat Score was assigned at 24 hours of age. The diagnosis of HIE was established by clinical teams involved in the care of the newborns at each participating centre, based on signs of perinatal asphyxia and clinical evidence of encephalopathy on neurologic examination.
EEG background and/or magnetic resonance imaging (MRI) changes consistent with HIE injury corroborated the diagnosis, which was confirmed at discharge in all cases.

2.2 EEG monitoring and analysis

EEG was performed using NicoletOne ICU Monitor (Natus, USA), Nihon Kohden EEG (Neurofax EEG-1200, Japan) or XLTek EEG (Natus, USA); the recording methodology was standardised across centres using a standard operating procedure. Electrodes were positioned at F3, F4, C3, C4, Cz, T3, T4, O1/P3 and O2/P4, according to 10:20 EEG electrode placement system adapted for neonates, with a sampling rate of 250 Hz or 256 Hz and a filter bandwidth of 0.5-70 Hz; single channel electrocardiography and respiration monitoring synchronised with the EEG trace were also recorded. EEG recordings commenced as soon as possible after birth and continued up to the end of rewarming following therapeutic hypothermia when possible. After acquisition, EEGs were uploaded to a central EEG review server. The annotation of seizures in each recording was performed by two members of a group of 5 board-certified electroencephalographers, with specific expertise in neonatal EEG (GBB, SRM, KVH, EP, JP); an electrographic seizure was defined as a sudden repetitive, stereotyped discharge of minimum 10 seconds in duration on one or more EEG channels with evolving frequency, amplitude and morphology (Pavel et al., 2020; Clancy and Legido, 1987).
The earliest hour of good quality recording was selected for each EEG, and retrospective visual analysis was performed by two independent electroencephalographers (JP and SRM),

blind to clinical information, including subsequent seizure status. Background EEG features (amplitude continuity, symmetry, synchrony and sleep-wake cycling or SWC) were assessed and a grade was assigned to each trace according to the following EEG grading system: Grade 0 (continuous background with normal amplitude and symmetric activity), Grade 1 (continuous with slightly abnormal activity including mild asymmetry or voltage depression and poorly defined SWC), Grade 2 (discontinuous activity with interburst interval < 10 s, no clear SWC, or clear asymmetry or asynchrony), Grade 3 (discontinuity with interburst 10-60 s, severe attenuation of the background patterns, no sleep-wake patterns) and Grade 4 (background activity <10 μV or severe discontinuity with IBI of >60 s) (Murray et al., 2006). In cases of disagreement in the grade assessment, a third EEG expert (GB) scored the given EEG, and the final score was reached by consensus among the three raters. Based on electro-clinical reasoning and in order to facilitate subsequent statistical analysis, the EEG grades were categorized into three background severity groups: mildly abnormal (scores: 0 and 1), moderately abnormal (score: 2) and severely abnormal EEG background (scores: 3 and 4).

Additional specific transients, not included in the classification, were annotated. We distinguished recognized graphoelements previously described in the literature, and other transient waveforms. Definition of each feature is provided in Supplementary Table 1, and detailed illustration is shown in the results section.

2.3 Statistical Analysis

Statistical analysis was performed using IBM SPSS 25 Statistics V.25 for Windows (SPSS Inc., Chicago, IL, USA). Normally distributed continuous variables were described using the mean and standard deviation (SD) and non-normally distributed continuous variables using the median and inter-quartile range (IQR). Categorical variables were described using number and percentage. Categorical variables were compared between groups using Fisher's exact test. Continuous variables were compared between groups using one-way ANOVA when the data was normally distributed data and the Kruskal-Wallis test otherwise. Pairwise comparisons with Bonferroni correction were performed when the omnibus test was statistically significant. Diagnostic accuracy was assessed using sensitivity, specificity, positive predictive value (PPV), negative predictive value (NPV), and area under the ROC curve and their corresponding 95% confidence intervals (CIs). Multivariable binary logistic regression was used to develop the best prediction model for occurrence of acute provoked seizures and variables with $p<0.05$ in the univariable analyses were eligible for inclusion in the multivariable model. Youden's index (index = sensitivity + specificity-1) was used to find the optimal sensitivity-specificity cut-off point on the ROC curve and the corresponding measures of diagnostic accuracy were estimated. All tests were two-sided and a p-value<0.05 was considered statistically significant.

3 Results

A total of 162 neonates with a diagnosis of HIE and at least one hour of EEG monitoring before 12 hours of age and before the emergence of electrographic seizures were included in the analysis; their demographics are summarized in Table 1.

The median age at the start of the one-hour EEG epoch was 6.4 hours (IQR: 4.2-8.7). The one-hour epoch was completed before 7 hours of age in 76 (46.9%) neonates and of those, 60 (78.9%) neonates were complete before 6 hours of age.

Table 1. Demographic characteristics, n=162

	N	
Gestational age at birth (weeks), mean(SD)	162	40.02 (1.32)
Mode of delivery, n(%)	162	
Unassisted vaginal delivery		46 (28.4)
Assisted vaginal delivery		62 (38.3)
Elective caesarean section		8 (4.9)
Emergency caesarean section		46 (28.4)
Birth weight (g), mean(SD)	162	3508 (607)
Male gender, n(%)	162	103 (63.6)
Apgar at 1 min, median (IQR)	157	2 (1-3)
Apgar at 5 min, median (IQR)	157	4 (2-5)
Assisted ventilation at 10 min (yes), n(%)	160	109 (68.1)
Lowest cord pH, mean(SD)	137	7.01 (0.20)
First postnatal pH, mean(SD)	127	7.02 (0.19)
First postnatal BE, mean(SD)	121	-15.5 (5.8)
First postnatal lactate, mean(SD)	102	11.8 (4.3)
Sarnat Score at 24 hours of age	141	
Mild, n(%)		53 (37.6)
Moderate, n(%)		61 (43.3)
Severe, n(%)		27 (19.1)
HIE grade at discharge	162	
Mild, n(%)		62 (38.3)
Moderate, n(%)		69 (42.6)
Severe, n(%)		31 (19.1)

Therapeutic hypothermia (yes), n(%)	162	133 (82.1)
Age at start of therapeutic hypothermia (hours), median (IQR)	133	2 (1-4)
Age at start of EEG monitoring (hours), median (IQR)	162	4.7 (3.3-7.7)
Age at first EEG epoch (hours), median (IQR)	162	6.4 (4.2-8.7)
Any ASM given before EEG epoch analysed (yes), n(%)	162	31 (19.1)

HIE, hypoxic-ischaemic encephalopathy; EEG, electroencephalography; AED, anti-epileptic drugs

3.1 Background EEG

The analysis of the first hour EEG epoch for each neonate revealed the following distribution in terms of EEG background activity according to our grading system: 9 (5.6%) neonates with Grade 0, 57 (35.2%) Grade 1, 30 (18.5%) Grade 2, 28 (17.3%) Grade 3, and 38 (23.5%) Grade 4. When combined into three groups, 66 (40.7%) were classified as normal or mildly abnormal, 30 (18.5%) moderately abnormal and 66 (40.7%) were severely abnormal. Illustration of these groups is shown in Figure 1.

3.2 Background EEG and sleep wake cycling

Clear evidence of SWC was seen in 20 out of 66 (30.3%) neonates in the mild background EEG group; present in all 9 tracings which were scored as completely normal (EEG grade 0) and in only 11 out of 57 (19.3%) mildly abnormal tracings (EEG grade 1). SWC was always absent in the moderate and severe background EEG groups.

3.3 Background EEG and early clinical and biochemical parameters

Details on the distribution of early clinical findings in the EEG groups are shown in Table 2. The distribution of Apgar scores, first postnatal pH, Base Deficit and Lactate values in the EEG background groups was similar between mildly abnormal and moderately abnormal EEG groups, while severely abnormal group showed the worst values. Overall, 133 out of 162 (82.1%) patients underwent therapeutic hypothermia: 40 (60.6%) in the mildly abnormal, 28 (93.3%) in the moderately abnormal, and 65 (98.5%) in the severely abnormal EEG group.

Table 2. Distribution of early clinical and biochemical parameters by background EEG groups.

		Mildly Abnormal background EEG (n=66)		**Moderately Abnormal background EEG (n=30)**		**Severely Abnormal background EEG (n=66)**			
	n	**median (IQR)**	**n**	**median (IQR)**	**n**	**median (IQR)**	**p-value**	**Pairwise comparisons**	

								Mil vs Mod	Mil vs Sev	Mod vs Sev
Apgar at 5 min	65	5 (4-7)	27	4 (3-5)	65	3 (1-4)	<0.001	0.934	<0.001	0.001
	n	**mean(SD)**	**n**	**mean(SD)**	**n**	**mean(SD)**				
First postnatal pH	57	7.1 (0.2)	22	7.0 (0.2)	48	7.0 (0.2)	0.203			
First postnatal BE	55	-14.8 (4.5)	21	-13.7 (5.9)	45	-17.2 (6.6)	0.035	1	0.114	0.067
First postnatal lactate	50	10.9 (3.1)	18	10.5 (5.6)	34	13.6 (4.6)	0.007	1	0.012	0.036
	n	**n (%)**	**n**	**n (%)**	**n**	**n (%)**				
Assisted ventilation at 10 min (yes)	65	27 (41.5)	30	22 (73.3)	65	60 (92.3)	<0.001	0.012	<0.001	0.066
Therapeutic hypothermia (yes)	66	40 (60.6)	30	28 (93.3)	66	65 (98.5)	<0.001	0.003	<0.001	0.687

p-value from Kruskal-Wallis test for Apgar at 5 min, from one-way ANOVA for first postnalat PH, BE and lactate, from Fisher's exact test for assisted ventilation at 10 min and therapeutic hypothermia.

3.4 Background EEG and early clinical and specific transients

The distributions of focal features in the three background EEG groups are summarized in Table 3.

-Physiological transients

Normal physiological EEG waveforms were frequently seen in normal and mildly abnormal background tracings and never in a severely abnormal background. Anterior dysrhythmia and encoches frontales, often mixed and mainly synchronous and symmetric over the frontal regions, were clearly observed in 15/66 (22.7%) normal or mildly abnormal tracings (6 out of 9 neonates with EEG grade 0). Delta brushes in 9 EEGs (13.6% of mildly abnormal background tracings), in 6 of which coexisted with anterior dysrhythmia and encoches frontales. Sharp waves were present in the central and/or temporal derivations in 11/66 (16.7%) mildly abnormal tracings (in 6 coexisting with anterior dysrhythmia, encoches frontales and/or delta brushes) and in 4/30 (13.3%) moderately abnormal tracings. Figure 2 shows the morphology of physiological transients.

-Modified anterior transients

In both mildly abnormal and moderately abnormal background tracings, modified anterior transients were observed: 7 out of the 66 mildly abnormal background tracings (10.6%) and 5 out of the 30 moderately abnormal background tracings (16.7%). Their appearance is shown in Figure 3.

-Deformed delta brushes

These graphoelements (Figure 4) were identified in a severely abnormal background in 8 neonates, in a moderately abnormal background in 3 neonates and in a mildly abnormal background in 3 neonates. They were located on the centrotemporal and occipital regions, with symmetrical or asymmetrical distribution.

-Focal abnormalities

Individually, pathological sharp waves (p=0.129) or spikes (p=0.094) were not associated with background EEG grade. Taken together, pathological sharp waves and spikes were observed in 59 out of 162 neonates (36.4%), 25/66 (37.9%) in the mildly abnormal background group, 17/30 (56.7%) in the moderately abnormal background group and 17/66 (25.8%) in the severely abnormal background group. Many EEGs in the severe group showed very low voltage, lacking any EEG transients. Focal abnormalities were mainly distributed in the central and temporal regions, but were sometimes seen in locations such as frontal or occipital regions (Figure 5).

-Short rhythmic sequences

Short rhythmic sequences were seen composed of repetitive complexes of positive or negative sharp waves followed by a slower component, short in duration (usually 4 to 8 seconds), arising clearly from the background EEG, more commonly on temporal derivations, and usually involving only one hemisphere and less frequently over both sides asynchronously. The sharp or the delta component could predominate. Their appearance in some tracings almost reached a "*spike-wave*"-like morphology. This pattern was observed in 16 out of 66 (24.2%) mild background tracings and in 5 out of 30 (16.7%) moderate background tracings (Figure 6).

-Central theta rhythm

Medium voltage sinusoidal theta sequences, variable in duration, were frequently seen over the vertex region, specifically in 28/66 (42.4%) mild and 11/30 (36.7%) moderate background traces, occasionally spreading over the paramedian areas of one or both hemispheres (Figure 7).

Table 3. Distribution of focal features by background EEG group.

	Mildly Abnormal background EEG (n=66)	Moderately Abnormal background EEG (n=30)	Severely Abnormal background EEG (n=66)				
	n (%)	n (%)	n (%)	p – value*	pairwise comparisons		
Physiological transients					**Mil vs Mod**	**Mil vs Sev**	**Mod vs Sev**
Anterior dysrhthmia and encoches frontales	15 (22.7)	0 (0)	0 (0)	<0.001	0.006	<0.001	1
Delta brushes	9 (13.6)	0 (0)	0 (0)	<0.001	0.159	0.009	1
Temporal transients	11 (16.7)	4 (13.3)	0 (0)	<0.001	1	<0.001	0.024
Modified anterior transients	7 (10.6)	5 (16.7)	0 (0)	0.002	1	0.039	0.006
Deformed delta brushes	3 (4.5)	3 (10.0)	8 (12.1)	0.320			
Focal abnormalities							
Sharp waves	18 (27.3)	9 (30.0)	10 (15.2)	0.129			
Spikes	7 (10.6)	8 (26.7)	7 (10.6)	0.094			
Sharp waves / Spikes	25 (37.9)	17 (56.7)	17 (25.8)	0.016	0.360	0.573	0.015
Short rhytmic sequences	16 (24.2)	5 (16.7)	0 (0)	<0.001	1	<0.001	0.006
Central theta rhythm	28 (42.4)	11 (36.7)	0 (0)	<0.001	1	<0.001	<0.001

*from Fisher's exact test

3.5 Correlation of early EEG findings with occurrence of seizures

Overall, 53 out of 162 neonates (32.7%) developed acute provoked seizures after the hour of EEG analysed. The age at first seizure varied from 3.2 to 67.9 hours. The median age at seizure onset was 14.4 hours (IQR: 10.3-19.6). The first seizure occurred within 24 hours of age in 45 (84.9%) neonates and of those, only 3 neonates had their first seizure before 6 hours of age.

3.5.1 Early background EEG, SWC and occurrence of seizures

The distribution of seizures after the initial one hour epoch among different background EEG groups is described in Figure 8. Background EEG was significantly associated with occurrence of acute provoked seizures ($p<0.001$) - seizures were detected in 39 out of 66 (59.1%) neonates in the severely abnormal background group, in 6 out of 30 (20.0%) in the moderately abnormal background group, and in 8 out of 66 (12.1%) neonates in the mildly abnormal background group. Absence of SWC at the beginning of EEG recording was also associated with occurrence of seizures ($p<0.001$) - none of the neonates with evidence of SWC at the beginning of EEG recording developed seizures.

3.5.2 Specific transients and occurrence of seizures: mildly and moderately abnormal early background EEG groups

Given that a severely abnormal EEG background was strongly associated with a higher risk of seizures, we investigated the ability of focal abnormalities and specific patterns in aiding the prediction of seizures within the mild and moderate background groups. Among the 96 neonates showing mild and moderately altered background tracings, the risk of seizures was still present, with an overall incidence of seizures of 14.6% (14/96). For these groups, background EEG grade alone was not significantly associated with occurrence of seizures ($p=0.356$). The distributions of EEG transients among the non-seizure and the seizure subgroups are summarized in Table 4. The presence of spikes and short rhythmic sequences were associated with the occurrence of seizures in mild and moderate EEG groups. Spikes were present in 9 of 82 neonates (11.0%) in the non-seizure group and in 6 of 14 neonates (42.9%) in the seizure group ($p=0.008$). Similarly, short rhythmic sequences were present in 13 of 82 neonates (15.9%) in the non-seizure group and in 8 of 14 neonates (57%) in the seizure group ($p=0.002$).

The accuracy of both background EEG and specific EEG transients to predict the occurrence of acute provoked seizures is shown in Table 5. Individually, the presence of short rhythmic sequences was the strongest predictor of seizure occurrence with an AUC of 0.706, sensitivity of 57%, specificity of 84%, PPV of 38% and NPV of 92%. Using multivariable logistic regression, with spikes and short rhythmic sequences as independent variables in the model, Youden's index identified the presence of spikes and/or short rhythmic sequences to be the optimal predictor of seizure occurrence with a sensitivity of 86% and a specificity of 76%. Finding one or both these features in an early EEG implies a positive predictive value of 38% and a negative predictive value of 97% for later seizures.

Table 4. Distribution of focal features by seizure group within mildly and moderately abnormal background tracings.

	Seizures		
	NO - 82/96 (85.4)	YES - 14/96 (14.6)	
	n (%)	**n (%)**	**p – value***
Physiological transients			
Anterior dysrhythmia and encoches frontales	15 (18.3)	0 (0)	0.117
Delta brushes	9 (11.0)	0 (0)	0.348
Temporal transients	15 (18.3)	0 (0)	0.117
Modified anterior transients	10 (12.2)	2 (14.3)	0.686
Deformed delta brushes	5 (6.1)	1 (7.1)	1
Focal abnormalities			
Spikes	9 (11.0)	6 (42.9)	0.008
Sharp wavs	23 (28.0)	4 (28.6)	1
Spikes / Sharp waves	32 (39.0)	10 (71.4)	0.039
Short rhythmic sequences	13 (15.9)	8 (57.1)	0.002
Central theta rhythm	34 (41.5)	5 (35.7)	0.775

*from Fisher's exact test

Table 5. Sensitivity, specificity, positive predictive value (PPV) and negative predictive value (NPV) calculated for background EEG, spikes and short rhythmic sequences in relation to occurrence of seizures, within mildly and moderately abnormal background tracings.

	AUC (95% CI)	**Sensitivity** (95% CI)	**Specificity** (95% CI)	**PPV** (95% CI)	**NPV** (95% CI)
Background EEG group only	0.568 (0.425-0.711)	43 (18-71)	71 (60-80)	20 (8-39)	88 (78-95)
Spikes only	0.659 (0.521-0.798)	43 (18-71)	89 (80-95)	40 (16-68)	90 (82-96)
Short rhythmic sequences only	0.706 (0.566-0.847)	57 (29-82)	84 (74-91)	38 (18-62)	92 (83-97)
Spikes and/or short rhythmic sequences	0.807 (0.701-0.913)	86 (57-98)	76 (65-84)	38 (21-56)	97 (89-100)

3.5.3 Early EEG findings and occurrence of seizures in neonates who had the one-hour EEG epoch completed before 6 hours of age

In the subgroup of neonates who had the one-hour of recording completed before 6 hours of age, 20 out of 60 (33.3%) had acute provoked seizures (median age at seizure onset 12.4 hours, IQR: 8.1-16.2, within 24 hours of age in all).

A severely abnormal background EEG confirmed to be associated with later occurrence of seizures (p<0.001), which were encountered in 14 out of 20 (70.0%) neonates in the severely abnormal background group, in 1 out of 6 (16.7%) in the moderately abnormal background group, and in 5 out of 34 (14.7%) in the mildly abnormal background group. Absence of SWC was associated with later occurrence of seizures (p=0.003).

Among the 40 neonates showing mild and moderately altered background tracings, the incidence of seizures was 15.0% (6/40). For these groups, background EEG grade alone was not associated with occurrence of seizures (p=1.0). Sharp waves were present in 9 of 34 neonates (26.5%) in the non-seizure group and in 3 of 6 neonates (50%) in the seizure group (p=0.341). Spikes were present in 5 of 34 neonates (14.7%) in the non-seizure group and in 2 of 6 neonates (33.3%) in the seizure group (p=0.279). Short rhythmic sequences were present in 6 of 34 neonates (17.6%) in the non-seizure group and in all neonates (100%) in the seizure group (p<0.001). The presence of short rhythmic sequences was the stronger predictor of seizure occurrence with an AUC of 0.912, sensitivity of 100%, specificity of 82%, PPV of 50% and NPV of 100% for later seizures.

4 Discussion

Electroencephalographic monitoring is often instigated early for assessment of injury severity and to help identify which encephalopathic neonate might benefit most from therapeutic hypothermia. The earlier the intervention starts the better the outcome (Thoresen et al., 2013), therefore any information relating to subsequent outcomes gathered from the EEG at the earliest opportunity has the potential to be significant for clinical management. The presence of acute provoked seizures in infants with encephalopathy, suggests that these infants may be eligible for emerging additional neuroprotective treatments beyond hypothermia (Walsh et al., 2022). Seizures do not generally emerge in the first 6 hours after birth in HIE, except in cases where there is an antenatal chronic or acute on chronic hypoxic-ischaemic injury (Filan et al., 2005). Studies have shown that the median time of seizure onset in HIE is between 14-15 hours after birth (Pavel et al., 2022). Identification of EEG features that are present early and predict the occurrence of later seizures would allow initiation of cooling in a wider population of babies than is currently the case.

In this study, we have shown that in mildly and moderately altered EEGs a lower but still consistent seizure risk is present.

Notably, the occurrence of seizures in the group of neonates with mildly abnormal EEG background is a novel finding and has not been described to date in the literature on HIE. We consider that the reason behind this may be due to different timepoints of evaluation; we evaluated earlier timepoints compared to most previous studies.

Our findings highlight the value of additional specific EEG transients that may intermittently punctuate the background. Consideration of these features is not included in the commonly used classifications. Physiological transients and a clearly recognizable sleep-wake cyclicity

were seen in EEGs with normal or mildly abnormal backgrounds. Their presence in early life seems to predict a good short-term outcome: none of the neonates who showed these features in the first hour of EEG recording in our series later developed acute provoked seizures. Spikes and sharp waves are a widely recognized marker of epileptogenesis when observed on the EEG later in childhood (Blum and Rutkove, 2007), and in patients with structural epilepsy secondary to a perinatal insult (Ko et al., 2022). Their significance in neonatal EEG has rarely been explored. Recent studies on animal HIE models have highlighted that epileptiform transients emerge on EEG 6-7 hours post-insult (Abbassi et al., 2019). In our series, within the group of neonates with mild and moderately altered background EEGs, a careful evaluation of spikes and short sequences of rhythmic activity helped in identifying those babies that would later present with seizures, with good sensitivity and moderate specificity. There remains debate regarding the boundaries that separate physiologic from pathologic sharp waves, and sharp waves located in the centrotemporal regions remain difficult to interpret. Short rhythmic sequences, composed of repetitive complexes of positive or negative sharp waves followed by a slower component, were more frequent in neonates that later developed seizures. This pattern can be described in the broader categories of both BRDs (brief rhythmic discharges) (Tsuchida et al., 2013) and/or LPDs (lateralised periodic discharges) (Hirsch et al., 2021), however these categories are ill-defined and can include sequences with large variability in duration and morphology, thus we kept them separate due to their unique morphology, absence of evolution, and limited duration. Based on clinical experience, it is not specific to the hypoxic-ischaemic aetiology and can be encountered in other neonatal neurological conditions. It is our impression that this pattern represents a continuum ranging from a dysmature feature to an epileptiform discharge. Lastly, given that seizure onset was beyond the sixth hour of life in 49/53 (92.5%) of neonates in our study, it seems obvious that it would be inappropriate to include the presence or absence of seizures in an early EEG scoring system for HIE. In any case, the presence of seizures in the first 6 hours would immediately classify the infant as moderately or severely encephalopathic.

The same patient sample presented in this study was used in a previous study conducted by our group, aimed at investigating, through machine learning techniques, the ability of early clinical parameters and EEG background features to predict those infants with HIE who developed acute provoked seizures. Amongst features included in the clinical model, lower Apgar scores, need for resuscitation at birth, lower base deficit and higher lactate were associated with the occurrence of seizures. In the qualitative EEG analysis, performed visually, background features were assessed individually rather than incorporated in a severity grading system, with low voltage to isoelectric pattern and absence of cyclicity proving the best predictive value. A quantitative analysis of background EEG was also performed and identified specific power, discontinuity and spectral shape features as the most significant for prediction of seizures. The EEG models had similar performance and both outperformed the clinical model alone, and the combined clinical and EEG model showed the best performance (Pavel et al., 2023). The present study highlights the value of focal waveforms and specific patterns in the early EEG, that might add further information to classical background analysis.

The focus of this study is oriented to the earliest time point at which the decision to initiate treatment is taken. An isolated assessment should not be used to predict longer term outcome: the degree of encephalopathy both clinically and electrographically is fluid and can only truly predict outcome when based on repeated recordings over time.

5 Strengths and limitations

This analysis was conducted in a large group of neonates in 8 NICUs across Europe with confirmed diagnosis of HIE, in which cEEG recording was performed according to the same standardised methodology. The evaluated EEG epoch focused on the 12 earliest hours after birth in all cases and was completed before 6 hours of age in 60 out of 162 neonates (37.0%) and before 7 hours of age in 76 (46.9%).

The main limitation of the study is the lack of a clinical grading assigned in close proximity to the time of EEG recording, which makes it impossible to assess the relationship between clinical and electrographic findings and to explore the potential predictive value of a combined score. The first clinical grade available after the Apgar Score was the Sarnat Score assigned at 24 hours. In consideration of the evolving nature of HIE, we did not explore the agreement between electrophysiological and clinical measures collected at different timepoints, which would not have been helpful and potentially confounding. Furthermore, the clinical scores are influenced by the presence of seizures, which is incorporated in the definition of Sarnat grades 2 and 3.

In addition, the specific features that we describe which are predictive of later seizures were manually assessed and are not evident on an aEEG. Therefore, they require interpretation by an expert in neonatal EEG, ideally through internationally shared terminology to maximize reporting standardization and interobserver agreement (Nucera et al., 2023). However quantitative analysis and machine learning is evolving rapidly, and new algorithms are emerging for EEG grading in HIE (Raurale et al., 2021; Moghadam et al., 2022).

We must consider that a large proportion of neonates underwent therapeutic hypothermia, even in those with a mild background EEG pattern: this may have prevented the subsequent occurrence of seizures in these neonates, thus blurring the analysis of the correlation between specific EEG transients and short-term outcome. In addition, decreased body temperature together with concomitant morphine administration may have affected the EEG activity (decreasing amplitude and increasing discontinuity) in those tracings which started when hypothermia was already in place. Nearly 20% of patients had received ASMs before the commencement of the EEG recording for clinically suspected seizures: the analysis of the correlation of early EEG findings with the occurrence of seizures limited to the subgroup of patients who did not receive ASMs before the analyzed EEG-epoch did not show differences compared to the original analysis.

Lastly, a common limitation when approaching qualitative EEG features is the large operator-dependent/subjective ability in “pattern recognition” based on visual inspection of graphoelements (Wusthoff et al., 2017). To minimize this limitation, we supported detailed descriptions with clear illustrations.

6 Conclusions

Background EEG grade in conjunction with the evaluation of focal transients, performed early enough to be useful in the critical treatment window, can objectively aid in determining the severity of hypoxic-ischaemic brain injury in newborn infants and the need for optimising neuroprotective treatment. Some focal features and paroxysmal patterns represent “red flags” for the risk of acute provoked seizures and hence brain injury, particularly when they arise from a mild or moderately abnormal background, and therefore should be considered for inclusion in future EEG grading systems. Evaluation of these features in routine neonatal EEG assessment could help minimize exposure of low-risk infants to interventions and maximize the potential for better outcomes for those at elevated risk.

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Figure captions:

Figure 1. Different groups of background EEG activity: (a) normal/mildly abnormal, (b) moderately abnormal, (c) severely abnormal. The head map on the side shows the location of the electrodes.

Figure 2. Physiological transients detected against normal and mildly abnormal background: (a) encoches frontales, (b) anterior dysrhythmia, (c) delta brushes over the occipital regions, and (d) temporal transients isolated and asynchronous on both hemispheres.

Figure 3. Modified anterior transients arising respectively from a mildly abnormal (a) and from a moderately abnormal (b) background.

Figure 4. Deformed delta brushes against a moderately abnormal (a) and a severely abnormal (b) background tracing; their localisation is medium-posterior in both cases, unilateral in (a), bilateral in (b).

Figure 5. Focal abnormalities in 3 different neonates, recorded at 8, 4 and 5 hours respectively: in (a) right temporal spikes clearly distinguishable from the background activity that is slightly discontinuous and good in voltage, in (b) isolated right occipital sharps against a slightly low voltage background, in (c) multifocal poorly localized paroxysms exclusively found during quite sleep. The latter are included as part of the background EEG pattern, rather than truly distinct EEG transients, and thus were not scored as focal abnormalities.

Figure 6. Short rhythmic sequences observed in three different neonates at 5 (a), 4 (b) and 6 (c) hours of life. Short in duration, predominant on temporal derivations, consist in diphasic theta followed by slow delta complexes. Their appearance is smooth in some tracings (a) and sharp in others (b), in some case reaching a PLED morphology (c).

Figure 7. Medium voltage sinusoidal theta sequences (squares) over the vertex region in two different tracings (a) (b), recorded at 3 and 6 hours respectively, spreading to the left frontal and central areas in (b) (arrows).

Figure 8. Occurrence of acute provoked seizures in the early background EEG groups.

Supplementary Table 1. Definition of focal features annotated on EEG.

Physiological transients		
Anterior dysrhythmia	Monomorphic or polymorphic delta waves of 1—3 Hz, with an amplitude of 50-100 μV; may occur in brief runs for a few seconds over the frontal regions.	André et al, 2010; Clancy et al, 2003
Encoches frontales	Isolated 50-100 μV broad diphasic transients (lasting 0.5 to 0.75 seconds) with a small initial negative deflection and a larger positive deflection; this pattern is intimately related to anterior dysrhythmia and the two are often seen admixed over the frontal regions.	Clancy et al, 2003; Clancy et al, 2011
Delta brushes	Delta slow waves of 50 to 250 μV lasting 0.3 to 1.5 seconds with superimposed fast activity (>8 Hz, 10-60 μV), predominating in the occipital areas during quiet sleep at near-term and term age.	André et al, 2010; Pavlidis et al, 2017
Temporal transients	Mostly single, solitary transients lasting 100-200 milliseconds, appear in greatest abundance in the mid-temporal and centro-temporal regions, synchronous and asynchronous and symmetrically distributed between the hemispheres.	Biagioni et al, 1996; Scher et al, 1994
Modified anterior transients	Graphoelements with morphology and location similar to that of anterior dysrhythmia and encoches frontales, whose modified appearance consists of a sharp shape and higher amplitude (> 100 μV) in comparison to the latter.	
Deformed delta brushes	Complex transients composed of a medium-high volted slow wave in the delta band with superimposed fast component > 22 Hz, showing prominent cogwheel appearance; their appearance is close to that of mechanical brushes, described and widely investigated in preterm infants.	Berger 2007
Focal abnormalities	Mono-, di- or polyphasic waves clearly distinct from the background. Can appear at any electrode location and may be more abundant compared to physiologic sharps and heavily concentrated in one region or hemisphere. Based on duration, two different subgroups can be identified: sharp waves and spikes.	Tsuchida et al, 2013
Sharp waves	Duration of 100 to 200 ms	
Spikes	Duration less than 100 ms	
Short rhytmic sequences	Repetitive complexes of positive or negative sharp waves followed by a slower component, lasting 4 to 8 seconds, arising clearly from the background, in some tracings almost reaching a "*spike-wave*"-like morphology.	

Central theta rhythm	Medium voltage sinusoidal theta rhythm in the vertex region, variable in duration, occasionally spreading over the paramedian areas of one or both hemispheres.

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Figure 1. Different groups of background EEG activity: (a) normal/mildly abnormal, (b) moderately abnormal, (c) severely abnormal. The head map on the side shows the location of the electrodes.

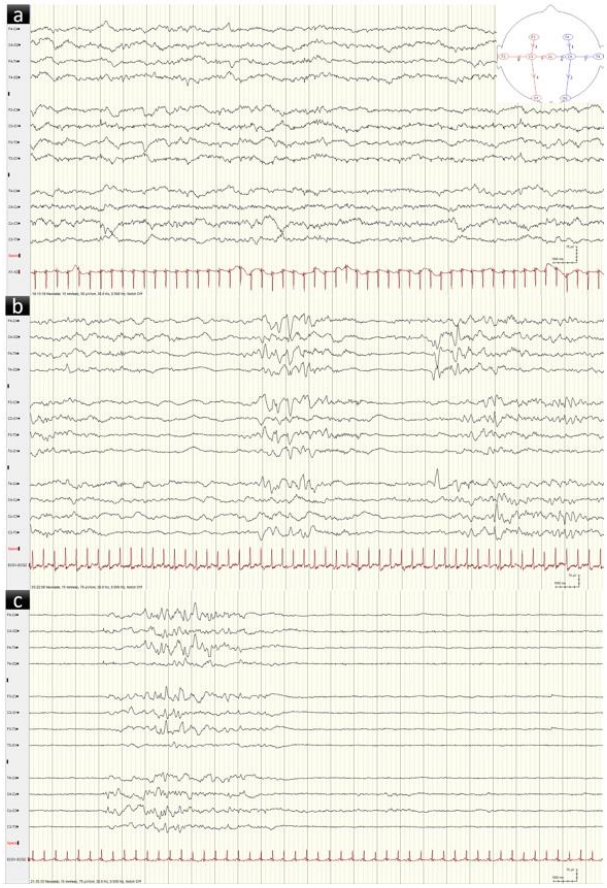


Figure 2. Physiological transients detected against normal and mildly abnormal background: (a) encoches frontales, (b) anterior dysrhythmia, (c) delta brushes over the occipital regions, and (d) temporal transients isolated and asynchronous on both hemispheres.

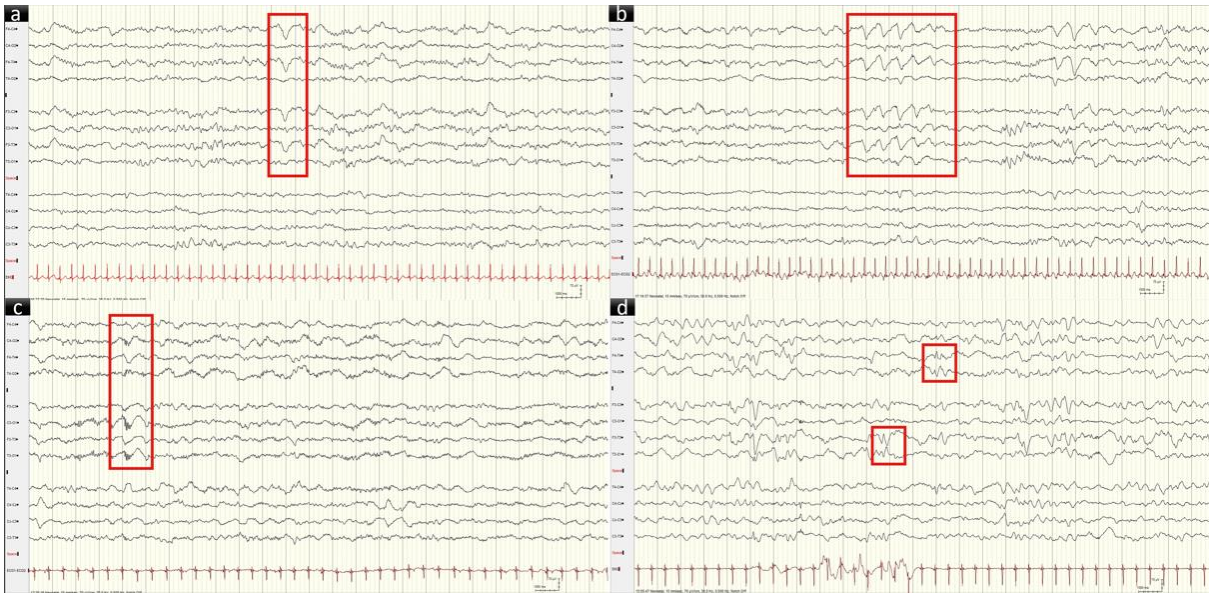


Figure 3. Modified anterior transients arising respectively from a mildly abnormal (a) and from a moderately abnormal (b) background.

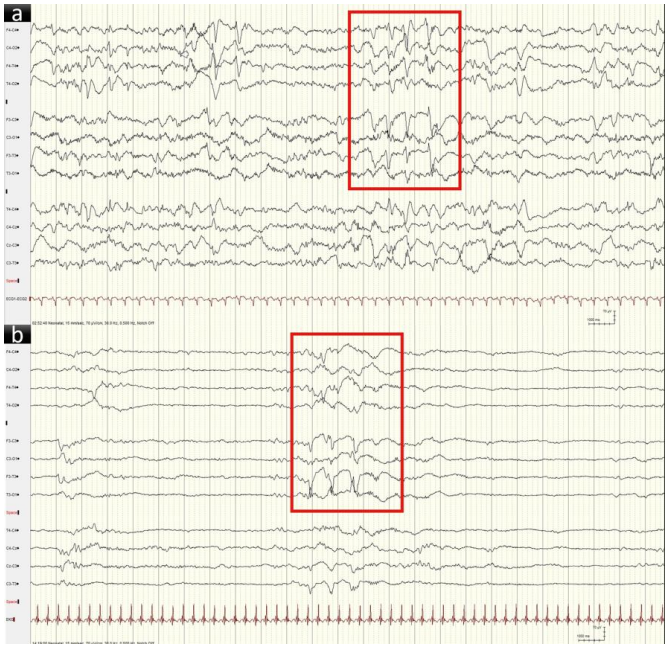


Figure 4. Deformed delta brushes against a moderately abnormal (a) and a severely abnormal (b) background tracing; their localisation is medium-posterior in both cases, unilateral in (a), bilateral in (b).

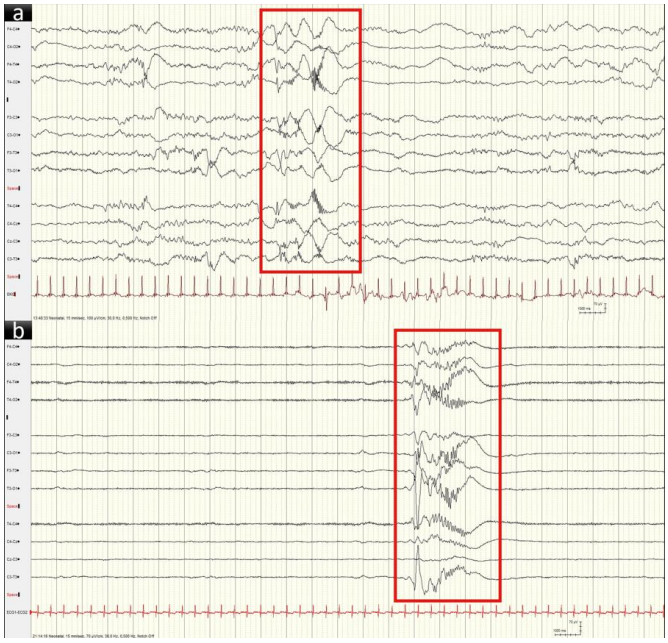


Figure 5. Focal abnormalities in 3 different neonates, recorded at 8, 4 and 5 hours respectively: in (a) right temporal spikes clearly distinguishable from the background activity that is slightly discontinuous and good in voltage, in (b) isolated right occipital sharps against a slightly low voltage background, in (c) multifocal poorly localized paroxysms exclusively found during quite sleep. The latter are included as part of the background EEG pattern, rather than truly distinct EEG transients, and thus were not scored as focal abnormalities.

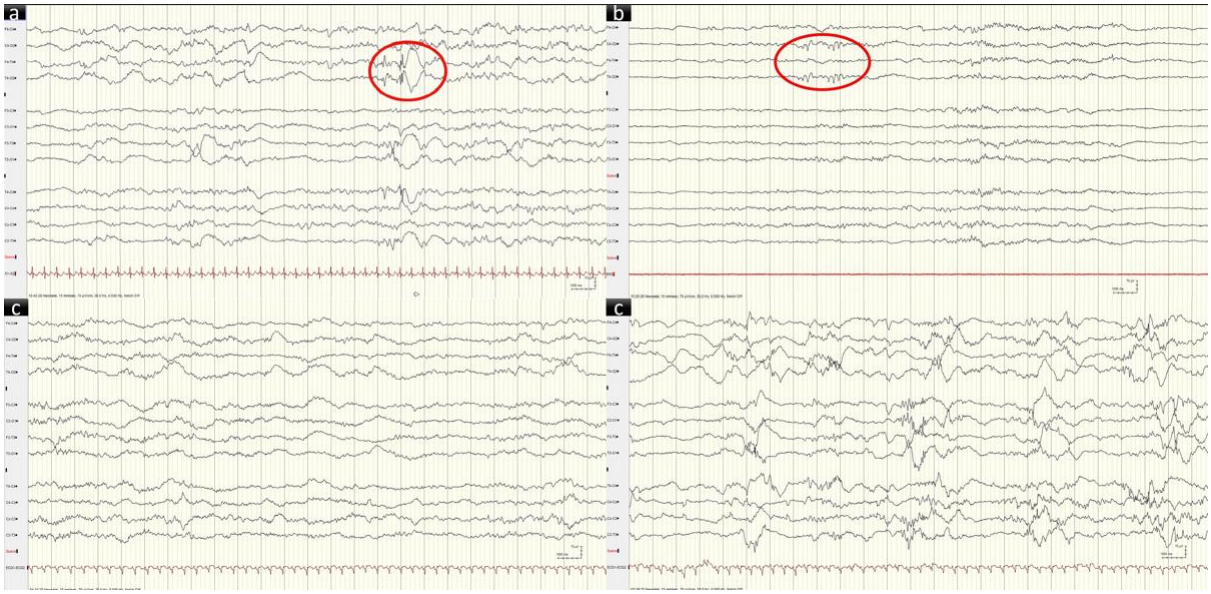


Figure 6. Short rhythmic sequences observed in three different neonates at 5 (a), 4 (b) and 6 (c) hours of life. Short in duration, predominant on temporal derivations, consist in diphasic theta followed by slow delta complexes. Their appearance is smooth in some tracings (a) and sharp in others (b), in some case reaching a PLED morphology (c).

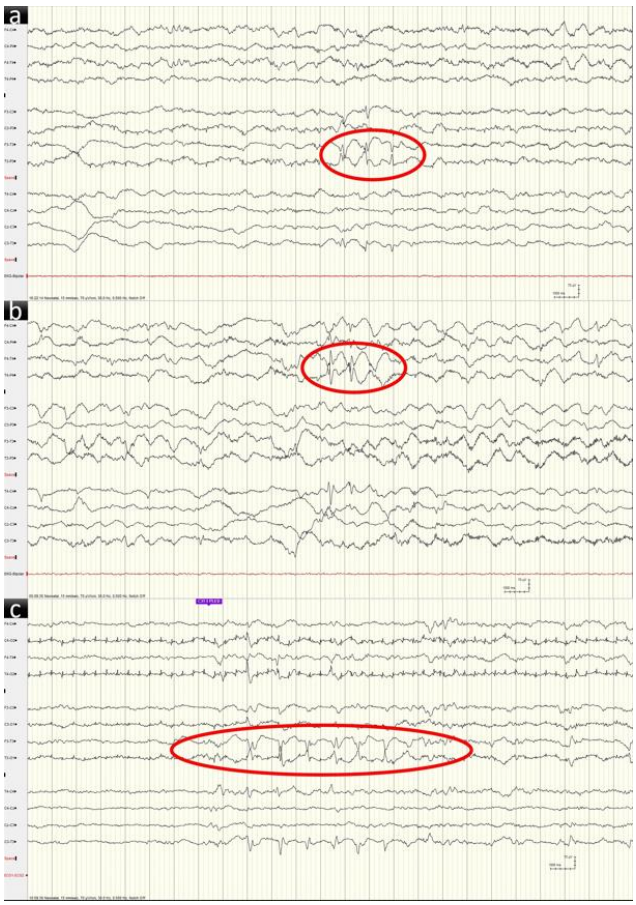


Figure 7. Medium voltage sinusoidal theta sequences (squares) over the vertex region in two different tracings (a) (b), recorded at 3 and 6 hours respectively, spreading to the left frontal and central areas in (b) (arrows).

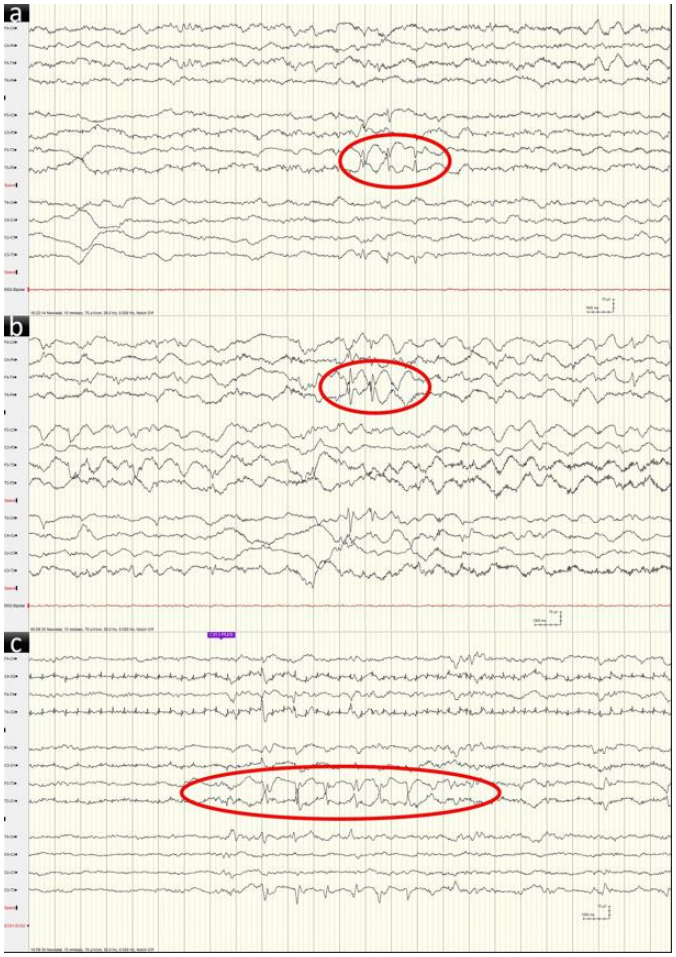
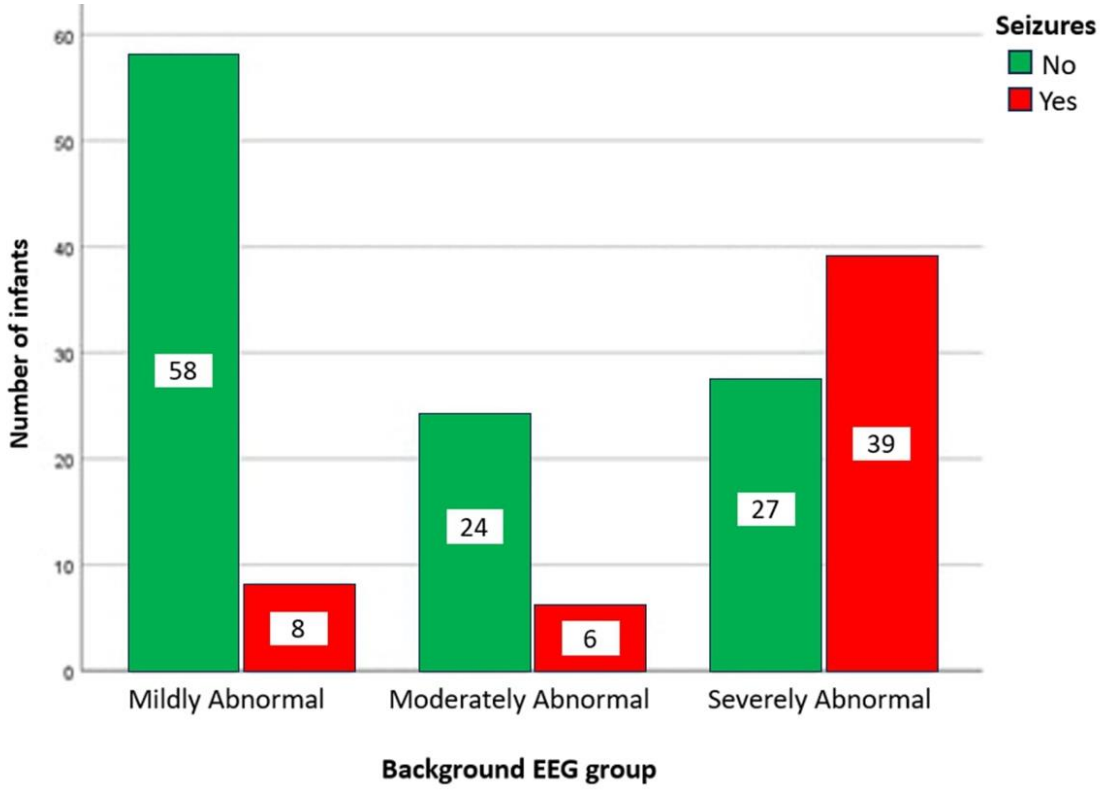


Figure 8. Occurrence of acute provoked seizures in the early background EEG groups.



Early clinical and neurophysiological multimodal assessment with EEG and SEPs in neonatal hypoxic-ischaemic encephalopathy: a pilot study

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Abstract

Early clinical and neurophysiological assessment is crucial to the management of neonates with hypoxic-ischaemic encephalopathy (HIE). In this study we investigate the feasibility of a combined multimodal assessment with continuous video-EEG-polygraphy (EEG) and simultaneously recorded SEPs at NICU admission, complemented by neurological evaluation at the same stage. We describe the early clinical and neurophysiological changes which occur following birth in neonates with HIE, and explore the relationship between the latter and short-term outcome.

In the 12 HIE recruits (mean age at start assessment 5.2 hours (2.8), the Total Sarnat Score ranged between 4-16/18: ≤6 in 17%, between 7 and 12 in 75%, ≥13 in 8%. The background EEG was mildly abnormal (grade 1) in 42%, moderately abnormal (grade 2) in 50%, and severely abnormal (grade 3-4) in 8%. Cortical SEPs were bilaterally normal in 50%, increased latency in 42%, unilaterally absent in 8%. Mean latencies of N1 component and CCT were longer in HIE neonates as a group if compared to a control group. After completion of hypothermia in the HIE group, the Total Sarnat Score was 0 in 60%, between 1 and 4 in 32%, and 8 in 8%. The background EEG was normal or mildly abnormal in 84%, moderately abnormal in 8%, severely abnormal in 8%. Cortical SEPs were bilaterally normal in 80%, increased latency in 10%, bilaterally absent in 10%. The one patient with abnormal short-term outcome (who had seizures, abnormal MRI and abnormal neurological findings at discharge) had total Sarnat score ≥13, severe background EEG alteration and absent SEPs at early assessment.

Introduction

Hypoxic ischemic encephalopathy (HIE) continues to be the commonest cause of mortality and long-term morbidity in term newborns (Kurinczuk et al., 2010). Early induced therapeutic hypothermia (TH) has shown good efficacy in improving survival and reducing neurological disability, most effectively when initiated within 6 hours of birth (Jacobs et al., 2007). The study of adjunct therapies potentially able to further decrease the damage is increasing in clinical trials (Nair and Kumar, 2018). Hence, it is of high priority to improve the ability of clinicians to define the severity of injury at the bedside as early as possible, in terms of both demarcating between different grades of encephalopathy and identifying criteria for selecting those babies that might benefit from treatment.

The main methods currently used for this purpose are clinical assessment and electroencephalography (EEG). In addition to EEG, other neurophysiological methods such as somatosensory evoked potentials (SEPs) (Swarte et al., 2012) are known to predict neurodevelopmental outcome after perinatal asphyxia. They assess the functional integrity of the somatosensory system including key pathways in the deep brain structure (e.g. thalami and brainstem), which are known to be vulnerable to perinatal asphyxia (Gunn and Bennet, 2009). This method is feasible and reliable in the first 6 hours of life (Eken et al., 1995). SEPs have been shown to provide additional value for early outcome prediction when recorded simultaneously with routine EEG (Nevalainen et al., 2017).

To date the majority of published studies investigate clinical and electrophysiological features in HIE populations at different times rather than simultaneously. Given the evolving nature of the pathophysiological mechanisms involved in the disease, this makes any meaningful comparison extremely difficult (Walsh et al., 2011).

The aim of this study was to investigate the feasibility of a combined continuous video-EEG-polygraph (cEEG) and SEPs assessment together with detailed clinical neurological examination at the same stage in the earliest hours after birth, and to document the early clinical and neurophysiological changes which occur following birth in neonates with HIE. In order to achieve that, we simultaneously recorded cEEG and median nerve SEPs as soon as possible after admission to the neonatal intensive care unit (NICU) in term newborns with hypoxic-ischemic encephalopathy, complemented by a detailed clinical neurological evaluation using standardised assessments at the same stage. The same multimodal assessment was obtained from a healthy age-matched control population for comparison.

An additional objective was to evaluate relationships between the above-mentioned parameters and the subsequent development of an abnormal early outcome in the HIE cohort.

The evolution of clinical and neurophysiological parameters over time in neonates with HIE was also assessed, through repetition of the same multimodal assessment between 78 and 96 hours of life (after completion of therapeutic hypothermia).

Participants and methods

Participants

In this prospective longitudinal study, neonates with HIE and a control population were recruited in Verona University Hospital and in Cork University Maternity Hospital between July 2021 and September 2023, after approval from the local ethics committees.

Inclusion criteria for the HIE group were gestational age ≥36, one or more risk factors for asphyxia including Apgar Score ≤ 5 at 5 minutes after birth, need for resuscitation at 10 minutes after birth and pH < 7.1 or base deficit > 16 from any cord or any blood sample within 60 minutes of birth, and clinical evidence of encephalopathy defined as the presence of abnormal neurological findings on the Sarnat Score performed at 1 hour of life. Exclusion criteria were significant congenital

abnormality, suspected or confirmed inborn errors of metabolism, congenital infections, and suspected or confirmed sepsis.

A control cohort of neonates was recruited. For inclusion as a control, the infants had to meet all of the following criteria: gestational age ≥ 36 weeks, Apgar Score > 7 at 1 minute after birth, without dysmorphic features and any suspected or confirmed inborn errors of metabolism or congenital infections.

Written informed consent was obtained by the mother and/or father, or the legal guardian, prior to enrolment for all participants.

Neurological examination

A neurological examination was performed in in all participants after admission to the NICU and cEEG commencement [T1]. A Total Sarnat Score was assigned according to the recently proposed PRIME stratification system (Chalak et al., 2019). In the HIE group a second neurological examination, complete with score assignment using the same method, was performed between 78 and 96 hours of life (after completion of eventual TH) [T2].

Continuous video-EEG-polygraphy monitoring and grading

cEEG data were collected with a Neurogen (Deymed Diagnostic, Czech Republic) or Brain Quick (Micromed, Italy) machine. Electrodes were positioned at F3, F4, C3, C4, Cz, T3, T4, O1 and O2, according to 10:20 EEG electrode placement system adapted for neonates. The EEG was recorded with a sampling frequency of 250-256 Hz and a filter band width of 0.5-70 Hz. Single channel electrocardiography and respiration monitoring synchronised with the EEG recording were also recorded. The recording started as soon as possible after birth and continued for at least two hours for all participants, up to 96 hours of life in neonates with HIE undergoing TH. EEG abnormalities were visually analysed according to one of the most commonly used grading systems for EEG background in neonatal encephalopathy (Murray et al., 2016). A score was assigned to the earliest 2-

hours-epoch in the recording [T1] in all participants, and in the HIE group also to a 2-hour-epoch between 78 and 96 hours of life [T2].

Somatosensory Evoked Potentials recording and measurement

SEPs were recorded during the earlier [T1] cEEG recording. Stimulation was provided at each median nerve at the ventral wrist alternatively on the right and left side, using two disk electrodes and a battery powered portable electrical peripheral nerve stimulator (Micromed Energy Light stimulator; Micromed, Italy), with electrical pulses of 0.2 ms duration at a rate of 1.1 Hz, with intensity (12-20 mA) individually adjusted to produce a small thumb twitch (motor threshold intensity) (Bongers-Schokking et al., 1989). We recorded all the evoked potentials during sleep or quietness. The cervical response was recorded at the 7th cervical vertebra with Fz reference. Cortical responses were recorded at the central location contralateral to stimulation referred to the ipsilateral C3 or C4. C3, C4, ground and reference were the same electrodes in place for the EEG, connected through Y cables to the EP headbox. The skin electrode impedance was usually kept under 5 kΩ. Responses were collected with a sampling frequency 2000 Hz using the TruScan EEG (Deymed Diagnostic, Czech Republic) or Brain Quick EEG (Micromed, Italy) machine, with analysis time 180 ms and band-pass 1-100 Hz. The median number of stimuli was 200 per average. To ensure reproducibility, 3 to 4 recordings were performed for each side. Only the N1 component (early maximal negative deflection, the most typical to be found in the neonatal period (Geneva et al., 2014)) in the presence of a normal cervical potential was considered for analysis and scored as follows: bilaterally normal (0), unilaterally increased latency (1), bilaterally increased latency (2), unilaterally absent (3) and bilaterally absent (4). Mean values and standard deviations (SD) for cervical N13 and cortical N1 component latencies were calculated for left and right sides. The threshold for increased N1 latency (40 ms) was established according to reference values highlighted in a previous study conducted with similar recording methodology on healthy neonates (Lori et al., 2017) and confirmed in our internal control group. Central conduction time (CCT) was calculated as the interpeak latency

between the N13 mean latency and N1 mean latency. A second SEPs recording with the same protocol was performed in the HIE group only, between 78 and 96 hours of life [T2].

Outcome assessment

cEEG monitoring was continued in neonates with HIE undergoing TH up to 96 hours of life. The same subgroup of neonates underwent MRI scan between the 4th and the 6th day of life with a 1.5-T or 3.0-T magnet; standard MRI protocol included axial T1-weighted images or inversion recovery-weighted images, T2-weighted images, DWI including apparent diffusion coefficient mapping. All participants had repeated neurological examination pre-discharge. An abnormal early outcome was defined at discharge from the NICU as development of seizures, abnormal MRI findings, abnormal neurological findings.

Statistical Analysis

Statistical analysis was performed using IBM SPSS Statistics (SPSS Inc., Chicago, IL, USA). Demographic factors, clinical neurological scores, EEG grades and cortical SEPs scores were summarized with counts (percentages) for categorical variables, mean (standard deviation [SD]) for normally distributed continuous variables or median (interquartile range) for other continuous variables. Distributions of categorical variables in the HIE group and in the control group were analysed using Chi-Square or Fisher Exact test, as appropriate. Non-parametric analysis was performed to compare differences among groups of continuous variables (with or without normal distribution), by means of Mann-Whitney U or Kruskal-Wallis tests, as appropriate.

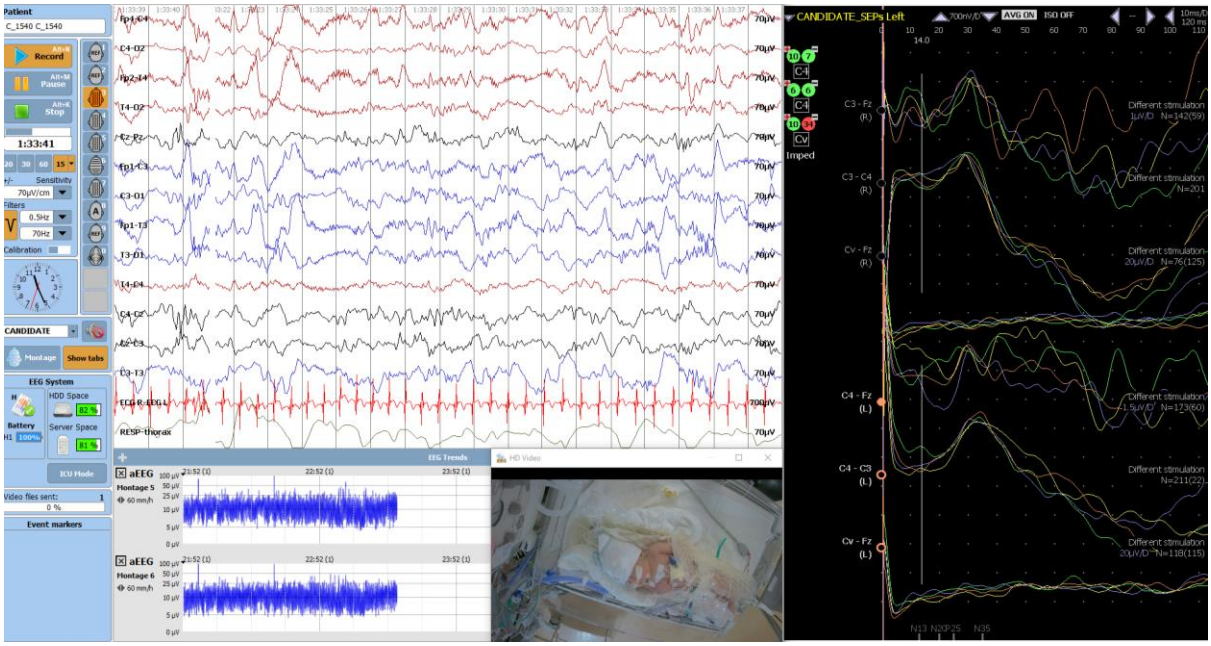
Results

In total, 25 neonates with HIE were recruited. All 13 neonates with HIE recruited in Verona University Hospital were excluded from the analysis after failure in the hospital storage server compromised the files containing the study records.

At Cork University Maternity Hospital 12 neonates with HIE were recruited, 7 males (58%) and 5 females (42%). The mean gestational age in the HIE group was 38.2 weeks (SD 1.3 weeks) and the mean birthweight 3423 g (SD 490 g). The mean values in the first postnatal blood gas were pH 7.07 (SD 0.13), BE -13.7 (SD 4.9), lactate 10.1 (SD 5.6).

Continuous video-EEG-polygraphy monitoring commenced within 12 hours of life in all, with a mean age at cEEG start of 5.2 hours (SD 2.8). A neurological exam followed by SEPs recording was performed in all neonates during the first two hours of cEEG recording. An example of simultaneously recorded cEEG and SEPs in shown in Figure 1.

Figure 1. Multimodal neurophysiological monitoring in the NICU, with video-EEG, aEEG, and simultaneously recorded SEPs.



Ten neonates were enrolled in the control group and underwent the same multimodal assessment at this first-time point [T1]. In the control group, composed of 60% males (6/10) and 40% females (4/12), the mean gestational age was 38.2 weeks (SD 1.3 weeks) and the mean birthweight was 3525 g (SD 291 g). The mean age at cEEG start in the control group was 12.2 hours (SD 3.5).

TH was instigated in 83% (10/12) of HIE neonates. In neonates undergoing TH, cEEG monitoring was continued up to 96 hours of age (24 hours after completion of hypothermia). In the remaining 17% (2/12) the recording was suspended after the first two hours, and restarted between 78 and 96 hours of life, when a second multimodal evaluation was performed in all HIE neonates [T2].

In the HIE group, at the earliest timepoint of evaluation [T1] the neurological exam revealed a Total Sarnat Score (assigned according to the PRIME stratification) of 4 in 17% (2/12), 8 in 17% (2/12), 9 in 8% (1/12), 10 in 42% (5/12), 12 in 8% (1/12) and 16 in 8% (17/2). The EEG grade assigned at the same time point was 1 in 42% (5/12), 2 in 50% (6/12) and 4 in 8% (1/12). 50% of neonates (6/12) showed bilaterally normal cortical SEP components, 8% (1/12) showed unilaterally increased latency of cortical N1 component and CCT, 33% (4/12) showed bilaterally increased latencies, and 8% (1/12) unilaterally absent cortical response (with increased latency in responses recorded in the contralateral hemisphere). In the control group the neurological examination did not show any sign of encephalopathy, and EEG and SEPs were scored normal in all cases (10/10). Mean latencies of N1 component and CCT were longer in HIE neonates as a group if compared to the control group. The mean N1 latency was 37.6 ms (SD 7.7) and 41.6 ms (SD 9.9) on the left and right central cortical electrodes respectively in the HIE group, 32.4 ms (SD 2.2) and 30.3 ms (SD 2.5) in the control group (p=0,002). Mean CCT was 25.6 ms (SD 7.4) and 29.6 ms (SD 9.8) on the left and right central cortical electrodes respectively in the HIE group, 20.7 ms (SD 2.2) and 18.5 ms (SD 2.9) in the control group (p=0,002).

At the later timepoint of evaluation [T2], HIE neonates were assigned a Total Sarnat Score of 0 in 60% (6/12), 1 in 17% (2/12), 2 in 8% (1/12), 3 in 8% (1/12), 4 in 8% (1/12) and 8 in 8% (1/12). The EEG grade was 0 in 50% (6/12), 5 in 42% (5/12), 2 in 8% (1/12) and 3 in 8% (1/12). SEPs were performed in 10 out of 12 neonates in the HIE group at T2, and resulted bilaterally normal in 80% (8/10), bilaterally increased in latency in 10% (1/10) and bilaterally absent in 10% (1/10). The mean N1 latency in the HIE group at T2 was 35.5 ms (SD 4.0) and 33.8 ms (SD 5.6), the mean CCT was 23.9

ms (SD 4.0) and 22.0 ms (SD 5.5) on the left and right central cortical electrodes respectively. The Total Sarnat scores, EEG grades and SEPs scores in the HIE group are summarized in Table 1. N13 and N1 latencies and CCT in the HIE group and in the control group are summarized in Table 2. The distribution of mean N1 latency values is shown in Figure 2.

Table 1: Total Sarnat scores, EEG grades and SEPs scores per single participant in the HIE group at T1 and T2.

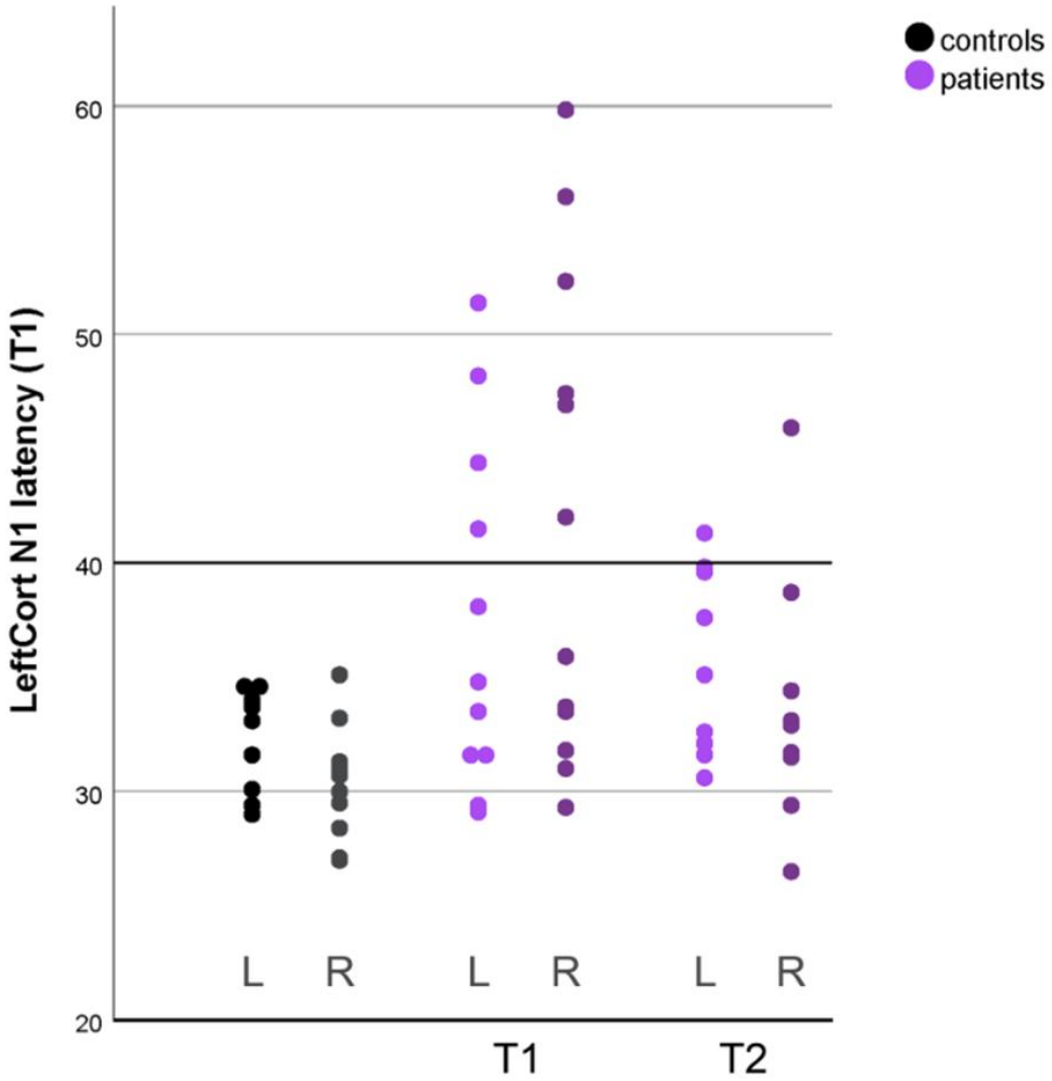
	T1 evaluation			Cooling	T2 evaluation			ST outcome
	PRIME	EEG grade	SEP score		PRIME	EEG grade	SEP score	
P01	10	2	2	Yes	2	2	0	Normal
P02	10	1	2	Yes	0	1	0	Normal
P03	16	4	3	Yes	8	3	4	Abnormal
P04	10	2	0	Yes	4	0	0	Normal
P05	12	2	0	Yes	3	0	0	Normal
P06	10	1	0	Yes	0	0	not done	Normal
P07	4	1	0	No	1	0	0	Normal
P08	8	1	1	Yes	1	1	0	Normal
P09	8	2	0	Yes	0	0	0	Normal
P10	9	2	2	Yes	0	1	0	Normal
P11	10	2	0	Yes	0	1	1	Normal
P12	4	1	2	No	0	0	not done	Normal

Table 2: Latency (ms) of cervical (N13), cortical (N1) and central conduction time (CCT), measured at T1 (for HIE group and control group) and T2 (for HIE group only), reported as mean (SD) values.

	HIE group – T1 [n=12]		Control group – T1 [n=10]		HIE group – T2 [n=10]	
	Right stimulation	*Left stimulation*	*Right stimulation*	*Left stimulation*	*Right stimulation*	*Left stimulation*
N13 mean (SD)	11.9 ms (0,8)	12.0 ms (1.0)	11.8 ms (0.6)	11.8 ms (0.8)	11.7 ms (1.1)	11.8 ms (0.8)

	Left cortical [n=11]	*Right cortical* [n=12]	*Left cortical* [n=10]	*Right cortical* [n=10]	*Left cortical* [n=9]	*Right cortical* [n=9]
N1 mean (SD)	37.6 ms (7.7)	41.6 ms (9.9)	32.4 ms (2.2)	30.3 ms (2.5)	35.5 ms (4.0)	33.8 ms (5.6)
CCT mean (SD)	25.6 ms (7.4)	29.6 ms (9.8)	20.7 ms (2.2)	18.5 ms (2.9)	23.9 ms (4.0)	22.0 ms (5.5)

Figure 2: Distribution of mean N1 latency values in study participants at T1 (HIE group and control group) and T2 (HIE group only).



Discussion

Our study demonstrates that multimodal clinical and neurophysiological assessment is feasible and reliable in neonates with HIE in the earliest hours after birth.

At that time, neurological assessment and aEEG/EEG recording are routinely implemented at the cotside in level three NICUs and aid in diagnosing neonatal encephalopathy and assessing the need for therapeutic interventions. Although providing valuable information, these methods are often still not able to discriminate the exact severity of encephalopathy immediately after delivery. Additional information using a complementary methodology might add to the diagnostic accuracy at the earliest timepoint, when decisions regarding need for therapeutic interventions are being made.

SEPs are difficult to perform in neonates, particularly in the NICU setting (De Vries et al., 1994). Equipment and procedures on the sick neonate add challenges to the complexity of the methodology. Nonetheless, by adapting filter settings and stimulation rate, it is possible to obtain cervical and cortical responses following stimulation of the median nerve. In our sample, the stimulation was well tolerated, heart rate and respiratory rate remained were stable during periods of stimulation compared to values out of stimulation, both in the HIE group and in the control group, and facial grimaces were not detected at the video analysis.

In older children SEP patterns have been proven to be more stable and less affected by drugs then aEEG and EEG, thus providing a more accurate diagnosis of possible cerebral injuries (Carrai et al., 2010). Nonetheless, in the neonatal period, SEPs use and value in prognosis is affected by the lack of reference values for the assessment of responses. In fact, structural and functional immaturity of somatosensory system central evoked responses differ markedly from those of older children and adults, and differences in measurement techniques adopted limit the comparability of published studies. Despite these limitations, there is evidence that in term infants with HIE median nerve SEPs can predict neurodevelopmental outcome (Lori et al., 2011). In the absence of normative data, the most useful and reliable prognostic indicator is the presence or absence of cortical responses while the prognostic implications of altered latency or amplitude values are not fully elucidated. Most

authors scored the cortical evoked potentials in terms of present or absent and found that bilateral loss of responses predicts cerebral palsy (Nevalainen et al., 2017; Suppiej et al., 2018). Others also reported latency values, and presented conflicting results in terms of outcome correlation (Eken et al., 1995; Trollmann et al., 2009). The various studies measured SEPs at different timepoints, ranging from the first to the second week of life (Lori et al., 2017; Suppiej et al., 2018). To our knowledge, our study is the first one testing SEPs in the first day of life and within the earliest 12 hours in HIE neonates in the era of hypothermia.

Only one patient in our study showed absent cortical responses. This patient also showed the worst Total Sarnat score and the worst EEG grade in the cohort, all representing negative prognostic indicators. They were the only infant who developed an early abnormal outcome, namely neonatal seizures and diffusion restriction on the MRI within the basal ganglia, thalami and perirolandic cortex. This is in line with what is known about early clinical and EEG prognostication, and with previously reported evidence of the predictive value of the presence or absence of cortical evoked potentials in the development of neurodevelopmental impairments [14]. Interestingly in this patient, cortical responses were absent unilaterally at the earliest time point of evaluation and bilaterally at repeated assessment in the fourth day of life. This may represent evolution of the injury over time.

An agreed definition of increased latency is not established for neonatal cortical evoked potentials. The presence in our study of an internal group of healthy controls recorded with the same methodology within the first day of life allowed us to detect increased latencies for cortical evoked potentials in neonates with HIE at the earliest measurement after birth. The interpretation of this finding in terms of outcome prediction must be deferred to completion of a long-term follow-up. None of neonates with early detected increased cortical latency values developed seizures in the neonatal period, nor showed abnormalities on the MRI or at neurological examination at discharge. Delayed cortical evoked potentials in neonates with HIE have been previously reported by Trollman and collaborators, who used the same stimulus frequency of 1.1 Hz we used in our study, with

similar mean values to those measured in our HIE group. In their study, also conducted on a small sample size, they found no significant relation between prolonged neonatal cortical latencies and long-term outcome assessed at 6 years of age (Trollman et al., 2009).

The cortical latency values improved at the second assessment performed on the fourth day of life, after rewarming from hypothermia. The improving trend of cortical latencies was accompanied by concomitant improvement of the Total Sarnat scores and of the EEG grades. Several studies on EEG in HIE have highlighted the relevance in prognostication of the time of background EEG normalization after neonatal hypoxic-ischaemic insult. A similar role should be tested for normalization of cortical evoked potentials responses in future studies.

Our data confirm that SEPs are always present in healthy neonates. The latency values recorded in our control group are similar to those published by Lori and collaborators in 2017, who recorded median nerve SEPs in term healthy neonates during the first 2 days of life using the same stimulation frequency, intensity and duration and the same filter settings to those we applied in our study (Lori et al., 2017). The only methodological difference between our study and that of Lori et al, is that in the study of Lori et al a lower number of stimuli was applied. The authors advocated this was necessary to prevent system exhaustion, however in our present study we obtained four traces for each side, each resulting from the average of 200 measurements, and we did not observe exhaustion in responses.

An early combined neurophysiological assessment with video-EEG-polygraphy and simultaneous somatosensory evoked potentials, together with detailed clinical neurological examination, may improve the ability to stratify the spectrum of severity of neonatal hypoxic-ischaemic encephalopathy at the early timepoint when clinical decision-making takes place. An implemented knowledge of the relationships between these parameters and outcome will potentially provide novel insights for the clinician and help identifying those neonates who will benefit from treatment.

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RETROSPECTIVE CHARACTERISATION OF SEIZURE SEMIOLOGY AND TREATMENT USING CONTINUOUS VIDEO-EEG MONITORING IN NEONATAL ENCEPHALOPATHY IN UGANDA

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J Proietti analysed and interpreted the data and wrote the manuscript; SR Mathieson and S Sadoo analysed and interpreted the data; C Nanyunja, E Duckworth, I Mambule and A Nakimuli collected and verified the data; CJ Tann conceived and designed the study, and collected and verified and interpreted the data; GB Boylan conceived and designed the study and analysed and interpreted the data. All authors edited and approved the final draft of the manuscript.

Abstract

Objective Neonatal encephalopathy (NE) is a leading cause of childhood death and disability particularly in sub-Saharan Africa. Detection of NE-related seizures is challenging. We explored NE seizure semiology and management in Uganda.

Methods Video-EEG was recorded (days 1-4). Seizure semiology was reviewed according to ILAE classification and administration of antiseizure medication (ASM) evaluated. Clinicians treated seizures based on clinical presentation alone.

Results Among 50 participants, 52%(26) had EEG-confirmed seizures; 70%(18) combined electroclinical/electrographic; 4%(1) exclusively electroclinical; 22%(6) electrographic. Of those with electroclinical seizures (19), 42% displayed >1 semiology. Distribution of seizure semiology was; clonic 34%(11); autonomic 24% (8, of which 6 had prolonged ictal apnoea); automatisms 18%(6); behavioural arrest 12%(4); and sequential 12%(4).

ASM was administered to 64% (32/50). Of those with EEG-confirmed seizures, only 62% (16/26) received ASM. In the non-seizure group, 38% (9/24) received ASM during monitoring. ASM was administered 42 times, of which 45% (19) were considered appropriate.

Significance In this Ugandan NE population, incidence of seizures was high and clinical manifestations frequent. Clonic, autonomic and automatisms were most common. Clinical diagnosis was challenging, with both under and overtreatment evident. Respiratory impairment due to autonomic seizures frequently went unrecognised and is a prominent concern, particularly in settings without neonatal intensive care.

Key words: neonatal encephalopathy, Uganda, neonatal seizures, seizure semiology

Key Points

- Intrapartum-related neonatal encephalopathy is a leading cause of early childhood morbidity and mortality, with the highest burden in sub-Saharan Africa.
- Understanding seizure semiology of NE-related seizures is crucial to inform understanding of clinical presentation and prompt seizure management.

- In this Ugandan neonatal encephalopathy cohort, of those with seizures, most presented with clinical manifestations: clonic, autonomic and automatisms were the most common seizure types observed.
- Clinical diagnosis of seizures proved difficult with both under and over treatment evident.
- Respiratory impairment due to autonomic seizures frequently went unrecognised and emerged as a prominent concern in this setting with limited access to neonatal intensive care.

Introduction

Intrapartum-related neonatal encephalopathy (NE) affects over a million term newborns each year globally, and is a substantial contributor to under-5 mortality and long-term morbidity [1,2]. The majority of infants affected by NE are born in low-income countries (LICs) where neonatal intensive care, and neuroprotective interventions such as therapeutic hypothermia, are not available [3,4]. Seizures represent an important risk factor for adverse outcome after NE [5], however, prompt detection and treatment of NE-related seizures can be challenging.

Continuous video-electroencephalographic (cEEG) monitoring remains the gold standard investigation for the early assessment of brain injury severity and detection of seizures. However, the need for specialised equipment, and related technical challenges, mean that it remains largely unavailable in low-resource settings [6]. As a result, acute provoked seizures occurring secondary to brain injury are at risk of being undetected or misdiagnosed, and hence not adequately treated, as shown in high-income countries (HICs) [7]. In the absence of cEEG, identification of seizures relies solely on clinical diagnosis [8,9,10]. For this reason, there is an urgent clinical need to understand how seizures manifest amongst neonates with NE in LIC settings. To date, most studies focusing on the semiology of neonatal seizures in NE have been conducted in HICs, including the recently updated seizure classification for neonates [11,12].

Between 2019-2022, a facility-based NE cohort was established at Kawempe National Referral Hospital in Kampala, Uganda to explore the aetiology, clinical course and early childhood outcomes after NE in a low-resource sub-Saharan African setting. The 'Baby BRAiN' study aims to enhance understanding of NE and inform future research on neurorestorative strategies in settings where therapeutic hypothermia is not routinely practiced [13]. As part of the study, recruited neonates received cEEG to examine electrographic brain activity and seizure burden, providing the opportunity to examine seizure semiology and management amongst neonates with NE in the Ugandan setting. The primary aim of this sub-study was to explore the electroclinical semiology of neonatal seizures amongst NE infants receiving continuous multichannel video-EEG monitoring in Uganda, as per the new ILAE classification of neonatal seizures. In addition, we aimed to understand the relationship between seizures that were clinically suspected and treated at the cotside and retrospective analysis of simultaneous video-EEG annotated seizures.

Methods

Study setting and participants

Kawempe National Referral Hospital is located in Kampala, Uganda's capital city. It receives high-risk referrals from across the city, delivering around 25,000 women each year with around 300-350 neonatal admissions for birth asphyxia each year [unpublished data]. Routine care during the sub-study period included simple continuous positive airway pressure ventilation, intravenous fluids including glucose, antibiotics and first line anti-seizure medication (ASM). Therapeutic hypothermia is not available, and care for NE infants is largely supportive. Between October 2019 and October 2020, infants with NE admitted to the neonatal unit were recruited to the 'Baby BRAiN' study. Inclusion criteria for this observational cohort study were: ≥36 weeks' gestation on Ballard assessment; age <48 hours at time of recruitment; birth weight ≥1.8 kg; need for continued resuscitation at birth and/or Apgar score ≤5 at 5 mins; clinical evidence of NE (defined as Thompson score ≥5) [14]; and parental informed written consent. Neonates with major congenital malformations, and those where death was felt to be imminent, were excluded. Relevant clinical and survival outcome data were collected locally by trained research personnel and included the presence of presumed clinical seizures based on abnormal movements noted by bedside staff. Timing and type of ASM administered was also collected.

EEG recording and seizure analysis

cEEG was applied and maintained by clinical research staff supervised by the research coordinator (CN). Equipment and training regarding EEG application and EEG system function was provided by ED and CT supported by neurophysiology experts from the INFANT research centre, University College Cork, Ireland. Multichannel video-EEG was commenced as soon as possible after birth and was continuously recorded over days 1–4 using the portable Lifelines EEG systems (Lifelines iEEG, UK). Electrodes were positioned at F3, F4, C3, C4, Cz, T3, T4, O1 and O2 according to 10-20 EEG electrode placement system adapted for neonates. Recording and review settings included a bipolar montage (F4-C4, C4-O2, F3-C3, C3-O1, T4-C4, C4-CZ, CZ-C3, C3-T3), high pass filter 0.5Hz, low pass filter 70Hz, notch filter, sensitivity 7-10μV/mm, timebase 15-20mm/sec. Single channel electrocardiography and respiration monitoring synchronised with the EEG trace were also recorded. The video-EEG was uploaded live (subject to network availability) to a cloud-based server with INFANT centre personnel able to provide signal quality assessment and technical feedback to the local team. Feedback regarding the content of the EEG was not given, as per the study protocol, and the video-EEG screen was covered at the cotside.

All recognised seizures in the cohort were treated based on clinical diagnosis, as per local routine practice and according to local protocols for ASM administration. The type, dose and timing of every dose of ASM administered to each neonate were recorded.

The analysis of each recording was performed retrospectively by one member of a group of 3 experienced neonatal neurophysiologists (JP, SRM, GBB), blind to clinical information. All seizures were annotated according to a standard protocol: a seizure was defined as a sudden repetitive, stereotyped discharge of minimum 10 seconds' duration on one or more EEG channel with evolving frequency, amplitude and morphology [15]. Status epilepticus was defined as the presence of more than 30 minutes of seizures in a one hour epoch [16].

For each neonate, all video-EEG segments annotated as seizures were selected and further reviewed for seizure semiology analysis. Seizure types were assigned according to the recently updated ILAE classification of neonatal seizures, based on electroclinical phenotype [17]. Any electrographic seizure seen on EEG that was not associated with evident clinical signs, was defined electrographic-only. An electro-clinical seizure was defined as definite clinical signs simultaneously coupled with an electrographic seizure. Seizure types were described by their predominant clinical features. Automatisms were characterised by non-purposeful, stereotyped, and repetitive motor activity such as orolingual movements, or movements of the limbs like cycling. Clonic seizures were characterised by regularly repetitive jerking, either symmetric or asymmetric, involving the limbs, head or trunk. Tonic seizures showed sustained increase in muscle contraction, focal, unilateral, or bilateral. Autonomic seizures were defined as distinct alteration of autonomic nervous system function (i.e. colour, breathing pattern, heart rate). Behavioural arrest seizures were defined as pause of activities, freezing, immobilisation. Sequential seizures, newly added to ILAE classification of neonatal seizure types, described seizure events with a sequence of signs, symptoms, and EEG changes at different times, in which no predominant feature could be determined. In some cases the video could not be used due to poor lighting in the neonatal unit and/or poor quality images and, in this event, the seizure was reported as unclassified.

Although standardised guidance on how soon seizures should be treated is currently lacking, for the purpose of this study, we anticipated that a seizure episode should be treated within 1 hour of onset if recognised [18]. The time of administration of ASM loading dose was compared with that of seizures retrospectively annotated on the video-EEG recordings. The administration of bolus (20 mg/kg) or hemibolus (10 mg/kg) of either phenobarbital or phenytoin was assessed. Maintenance doses, administered in some cases, were not included in our analysis. Appropriately managed seizures were defined as EEG confirmed seizures treated within 60 minutes of onset. Treatment failure was defined as EEG confirmed seizure not followed by treatment administration. Paroxysmal non-epileptic events occurring in the 60 minutes preceding an ASM load, or concomitant to electrocardiography and/or respiration monitoring alterations, were also examined.

Statistical analysis

Baseline characteristics of the cohort were summarised with counts (percentages) for categorical variables, mean (standard deviation [SD]) for normally distributed continuous variables or median (interquartile range) for other continuous variables. Seizures reported and treated by bedside staff and retrospectively video-EEG recognised seizures were reported as counts and percentages. The burden of each semiology seizure type was reported according to the number and proportion of seizures for each participant.

Results

Baseline characteristics

A total of 51 neonates with NE were recruited, and 50 had video-EEG data of diagnostic quality. The demographics and baseline EEG characteristics of the 50 patients included in the analysis are described in Table 1.

Continuous video-EEG (cEEG) was recorded for a mean duration of 60.9 (SD 19.3) hours. Monitoring commenced within 48 hours of age in all neonates, within 24 hours in 76% (38/50), and continued for at least 24 hours in 92% (46/50). 26 out of 50 neonates (52%) had video-EEG confirmed seizures identified by the expert group. 13 out of 26 (50%) had periods of status epilepticus.

Seizure semiology

On video-EEG analysis, 70% (18/26) of neonates with seizures had a combination of electroclinical and electrographic seizures, 4% (1) had exclusively electroclinical seizures and 22% (6) had only electrographic seizures. 4% (one patient) had exclusively seizures classified as unknown due to a malfunction in video recording. Among patients in which a video-polygraphy clinical correlate was synchronous with the EEG discharge in at least one part of the seizure, 58% (11/19) displayed one seizure type and 42% (8/19) more than one seizure type (2 in three, 3 in four, 4 in one). The distribution of all seizure semiology types was as follows: clonic 34% (11), autonomic 24% (8), automatisms 18% (6), behavioural arrest 12% (4), and in 12% of cases (4) electroclinical manifestations consistent with the definition of sequential seizures. The latter were prolonged in duration and characterised by the succession of distinct ictal patterns on the EEG associated with different clinical manifestations, without a predominance of one or the other being recognisable; clonic, automatisms and autonomic manifestations were more frequently part of sequential seizures. Six of the 8 neonates with autonomic seizures had prolonged ictal apnoea. Among the 18 neonates displaying a combination of electroclinical and electrographic seizures, the mean (SD) percentage of electroclinical seizures was 52% (30) and that of electrographic seizures was 24% (25).

A number of unclassified seizures were also present in 16 out of 18 neonates in this group (mean 18%, SD 17). The distribution of seizure types in the cohort and in single neonates is summarised in Figure 1.

Of 13 neonates with status epilepticus, 84% (11) had a combination of electroclinical and electrographic seizures, 8% (1) had unknown seizures, and 8% (1) had only electrographic seizures (brief repetitive focal discharges never exceeding a low-medium voltage). In the remaining 13 neonates (without status epilepticus), 54% (7) had a combination of electroclinical and electrographic seizures, 8% (1) had only electroclinical seizures and 38% (5) had only electrographic seizures.

Management with anti-seizure medications

Information about seizures and ASM used in the cohort are summarised in Figure 2. Overall, 64% (32/50) of neonates received ASMs either during or prior to EEG starting. All received phenobarbitone as their first-line treatment (dosage between 10 and 20 mg/kg). Of those receiving ASM, 25 (78%) received at least one additional ASM bolus or hemi bolus of either phenobarbital or phenytoin, and 14 (44%) received their first ASM before the video-EEG commenced. Seven neonates received treatment exclusively before the monitoring started: in 4, subsequent EEG recording did not document seizures, while the remaining 3 displayed later seizures.

ASM loading doses were received by 50% (25/50) of neonates during continuous video-EEG recording. Of those with EEG-confirmed seizures, only 16/26 (62%) received treatment (including 5 neonates who also received ASM before the start of EEG monitoring), and 7 out of 26 (27%) neonates with EEG documented seizures never received an ASM at any time. Nine out of 24 (38%) in the non-seizure group were also treated. The administration of ASM loads during the monitoring period in neonates with and without video-EEG recorded seizures is summarised in Figure 3. In total, ASM loading doses were administered 42 times after recording commencement: 15/42 (36%) in the group without video-EEG-documented seizures (thus by definition inappropriate) and 27/42 (64%) in the group with video-EEG documented seizures. Amongst the latter, in relation to the presence or absence of seizures in the 60 minutes prior to the ASM administration, 19 (45%) were appropriate and 8 (19%) non appropriate. Twelve neonates had at least one episode treated appropriately. The number of ASM loads received after EEG commencement ranged from 1 to 4 (median 2, IQR 1–2). In 7 patients the first ASM load was appropriately administered during monitoring, after a median (IQR) time of 8.0 (3.8-8.9) hours from the first video-EEG documented seizure.

In the subgroup of patients with video-EEG documented status epilepticus, 1/13 (8%) received treatment exclusively before the beginning of the recording, 2/13 (15%) were never treated, and 10/13 (77%) patients received ASM while the monitoring was ongoing (including 3 who also received ASM before the start of EEG monitoring). Out of the 15 ASM loads administered during EEG monitoring in this subgroup, 13 were appropriate and 2 inappropriate.

ASM administration, seizure semiology and seizure types for each patient are summarised in Table 2. Video-EEGs of seizures recorded before the administration of any ASM was available in 11 patients, and 8 had a clinical component associated with their seizures prior to treatment. Out of the 19 adequately treated seizures, 8 were clonic, 5 autonomic, 1 automatisms, 2 sequential and 3 had unknown semiology.

The analysis of the video-polygraphy recording in the 60 minutes preceding inappropriate administration of ASM loading doses (23 in total) revealed the presence of generalised tonic extensor posturing events without clear asymmetry in 6 cases, jerky non-rhythmic movements during crying in 4 cases, nonepileptic oral automatisms in 3 cases, paroxysmal nonepileptic tremor in 1 case; these conditions were presumably interpreted as ictal by the local clinical team. In addition, 12 neonates (8 in the group with video-EEG documented seizures and 4 in the non-seizure group) also presented with abnormal repetitive abdominal movements or gasping, sometimes showing pseudo periodic evolution, unrelated to ASM administration; this pattern was never associated with concomitant modifications of the EEG tracings. Illustration of apnoea occurring during autonomic seizures and of the non-epileptic abdominal flutter or gasping is provided in Figure 4.

Discussion

There is an urgent need to further our understanding of NE in LMICs, in order to optimise management and outcomes. With a lack of evidence to date supporting the safety or efficacy of therapeutic hypothermia in LMICs [5] and given increasing evidence that high seizure burden contributes to neurodevelopmental impairment [19], prompt detection and adequate treatment of seizures represent the main neuroprotective intervention available in LMICs to improve morbidity in patients who survive the neonatal period. However, the vast majority of published studies aimed at improving diagnosis and management of neonatal seizures in NE originate from HICs, and most studies from LMICs solely rely on clinical diagnosis for seizure identification due to the limited availability of EEG [20].

This pilot study, one of the first in a LIC in Africa to use continuous video-EEG monitoring, provides evidence of feasibility in this Ugandan research setting. The findings of this study reveal the high

incidence of seizures in this NE cohort in Uganda. Video-EEG documented seizures were present in over 50% of neonates monitored. Overall, a quarter of all neonates, and half of those with seizures, met the criteria for status epilepticus. This is similar to the rate of status epilepticus reported in older NE cohorts in HICs who have not received therapeutic hypothermia, and substantially higher than the rate of status epilepticus reported in NE cohorts in HICs in the era of therapeutic hypothermia [21,22].

Unsurprisingly, clinical diagnosis of seizures in this study proved difficult and both under and overtreatment was evident. This is in line with data on staff identification of seizures in HICs, where both underdiagnosis due to missing discrete seizure manifestations and electrographic only seizures, and overdiagnosis due to misdiagnosing abnormal nonepileptic movements as seizures, have been highlighted [7, 23]. It is now well established that EEG is essential for seizure detection, and clinical diagnosis alone is not reliable [17].

During periods of EEG monitoring, 38% of neonates without any cEEG evidence of seizures were given ASM, and 38% with cEEG evidence of seizures were not treated. Whilst on the one hand, untreated seizures may add to any pre-existing brain injury and alter seizure thresholds in the brain, on the other hand inappropriate ASM administration exposes the newborn to potential ASM-related drug toxicity and complications such as sedation and respiratory depression, which is particularly relevant in low-resource settings where mechanical ventilation is unavailable. A few neonates may have responded to the ASM administered before monitoring started, given that 4 were given an ASM before the commencement of cEEG monitoring and then never had EEG-documented seizures.

The video-EEG analysis of seizure semiology performed according to the updated ILAE classification of seizures in the neonate, has provided novel insights regarding neonatal seizures in an LIC. Indeed, the aim of the ILAE taskforce who developed this classification was to ensure that this scheme was applicable to all healthcare settings. Electroclinical seizures were more common in this Ugandan cohort compared to other similar cohorts in HICs after the advent of therapeutic hypothermia. The ILAE task force who proposed the new classification described 19 electrographic, 5 clonic, 3 sequential and 2 tonic seizures in a sub-cohort of 29 neonates with hypoxic-ischaemic encephalopathy (HIE) and seizures; similarly in a double-cohort study across USA and Belgium, 15/26 HIE neonates never had a clinical correlate to their seizures [18,24,25]. Clonic, autonomic and automatisms were the more frequently encountered seizure types in this Ugandan cohort. Clonic seizures were the more reliably diagnosed on the basis of clinical evaluation only [26]. Neither epileptic spasms nor myoclonic seizures were seen, as expected in neonates with NE [18]. Tonic seizures were not encountered; a focal increase in muscle contraction was only observed in the

context of clonic manifestations. Electrographic only seizures were present in almost every neonate with seizures, but represented the only seizure type in six patients.

Respiratory impairment emerged as a prominent concern, both during seizures and at other times. Seizures manifesting as respiratory impairment only were common (75% of neonates with autonomic seizures and 23% of the total number of patients with seizures had prolonged ictal apnoea), and frequently unrecognised as seizures by clinical staff. Some neonates showed non-ictal repetitive abdominal movements or gasping, sometimes showing pseudo periodic evolution and resembling a diaphragmatic flutter [27].

An increased awareness of the different manifestations of seizures in this setting might help local clinicians recognise seizures. However, the presented semiology analysis was based on video-EEG recordings and conducted by neurophysiologists with specific expertise in neonatal EEG and seizure semiology interpretation. Epileptic and nonepileptic clinical manifestations in critically ill patients will probably remain difficult to identify for healthcare workers without specialised training and without the support of EEG. The development and dissemination of simple and innovative models for neurophysiology monitoring in neonates in low-resource settings should be prioritised.

The presence of seizures in four patients that were consistent with the definition of sequential seizures (seizure presenting with a variety of clinical signs occurring in a sequence without a predominant feature determined) is interesting. According to the existing definition, sequential seizure may be present in some infants with HIE although they are more commonly seen in other aetiologies such as self-limited neonatal epilepsy or neonatal onset epileptic encephalopathy [18]. Seizures in this Ugandan cohort were prolonged and characterised by a sequence of clinical features coinciding with the slow evolution in amplitude and morphology of the discharge, appear to be clearly different compared to the briefer and stereotyped trains of electroclinical manifestations typically seen in genetic aetiologies. Different interpretations are also evident in recently published studies which have applied the new classification to neonatal populations: de Correa et al reported sequential seizures in all aetiology groups in their cohort and mainly in HIE, while Cornet et al identified sequential seizures specifically in 11 out of 18 patients with confirmed channelopathies within a cohort also including a larger number of neonates with seizures due to stroke or HIE [25, 28]. The latter, additionally, recognised tonic features as the most specific for the genetic aetiology, whether representing the initial phase of sequential seizures or constituting “pure” tonic seizures. Of note, we emphasise that none of the neonates with acute provoked seizures in our study had tonic seizures or even a tonic phase in the context of a sequential seizure. Lüders et al suggested the clinical characterisation of seizures in post-neonatal ages [29], Nagarajan and colleagues proposed

an extended semiological classification for electroclinical seizures in the neonate, based on the description of the prominent clinical feature at onset and all features seen during the seizure [30]. This approach provides insights into discharge location and neuronal networks that underpin seizures, and might lead to the recognition of characteristic aetiology-specific ictal electroclinical phenotypes.

Strengths and Limitations

This study provides novel data on electroclinical semiology of neonatal seizures amongst infants with NE from the lowest of resource settings, where therapeutic hypothermia is not available.

Evidence of perinatal asphyxia in the eligibility criteria was based clinically on the need for resuscitation after birth and Apgar score, as routine foetal monitoring and blood gas measurement are not routinely performed in Uganda. Therefore, the study cohort may not be fully comparable to NE cohorts in HICs.

The different electroclinical phenotype encountered in this cohort compared to other HIC NE cohorts might be due to other causes of neonatal seizures such as hypoglycaemia and electrolyte imbalance; in our cohort blood glucose and electrolytes levels were infrequently monitored. Furthermore, seizure semiology may have been modified by treatment administration in the patients with video-EEG recorded seizures who received ASM loads before the monitoring started. In the absence of any clear guidelines for the optimal timing of ASM administration, based on evidence that ASM administration within 1 hour of seizure onset potentially reduces the seizure burden [18] we expected that seizures should be treated within 1 hour of onset in order to be considered as having been appropriately treated.

The generalisability of our results on seizure recognition and treatment appropriateness is limited by the fact that a dedicated group of research nurses looked after the study neonates, whilst the ratios of nurses to patients are typically lower in routine practice.

Conclusion

Retrospective review of continuous video-EEG-polygraphy monitoring in a cohort of neonates with NE in Uganda revealed a high incidence of neonatal seizures and status epilepticus. Many seizures were not treated and non-seizure events were often treated with ASM. The majority of patients in the seizure group presented with electroclinical seizures at some point, with clonic, autonomic and automatisms representing the more frequently encountered seizure types. Respiratory impairment emerged as a prominent concern, through both prolonged ictal apnoea during the seizure and non-ictal abdominal flutter or gasping without electrographic seizure. Enhanced knowledge of seizure

semiology in this setting and improved availability of neurophysiology monitoring, would help guide management.

Data availability

Further data that support the findings of this study are available on request from the corresponding author.

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Conflict of interest statement

GB Boylan is founder and shareholder in Kephala Ltd and Cergenx ltd; has received consulting fees and/or honoraria from GW Pharmaceuticals, Nihon Kohden and UCB Pharma. The remaining authors have no conflicts of interest.

Ethics approval statement

Hospital, institutional and national approvals were sought from the ethics committees of Uganda Virus Research Institute (UVRI), London School of Hygiene & Tropical Medicine (LSHTM), Ugandan National Committee of Science & Technology (UNCST), and the Ugandan President's Office.

Participant Consent Statement

Written consent for video recording and data sharing was acquired from parents for all participants.

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Table 1 – Clinical and EEG characteristics of the 50 patients included in the analysis.

Characteristics	**N=50**
Clinical characteristics	
Sex, male	66% (33)
Gestational age, weeks	39 (1.8) [36-42]
Birthweight, grams	3083 (424.8) [2310-4100]
5-min Apgar score*	5.6 (1.9) [0-10]
Age of recruitment, hours	18.6 (10.4) [3.1-46.1]
NE Severity (Sarnat grade): Mild Moderate Severe	 24% (12/50) 30% (15/50) 42% (21/50)
EEG characteristics	
Age at start of monitoring, hours	22.2 (10.7) [5.4-48.8]
Duration of continuous video-EEG, hours	60.9 (19.3) [10.3-75.3]
Confirmed electrographic seizures, % (n)	52% (26/50)
Episodes of status epilepticus, % (n)	50% (13/26)
Neonatal death, % (n)	28% (13/46)

Data are mean (SD) [range] or % (n) *Missing data for 2 participants

Table 2 – ASM administration, seizure semiology and burden of individual seizure type expressed in numbers and percentage in neonates with video-EEG-documented seizures (n=26).

	Status Epilepticus (n=13)	Never received ASM (n=7)	Received ASM before start of cEEG (n=9)	Received ASM after start of cEEG (n=16)	Electroclinical Seizures (n=19)					Electrographic (n=25)	Unclassified* (n=18)
					Clonic (n=11)	Autonomic (n=8)	Automatisms (n=6)	Behavioral Arrest (n=4)	Sequential (n=4)		
P01	No		X	X	-	7 (24%)	-	-	-	19 (66%)	3 (10%)
P04	No			X	-	-	-	-	-	16 (80%)	4 (20%)
P05	Yes		X		35 (33%)	19 (18%)	13 (12%)	-	8 (7%)	25 (23%)	8 (7%)
P07	No	X			12 (60%)	-	-	-	-	3 (15%)	5 (25%)
P08	Yes			X	-	36 (41%)	-	-	-	29 (33%)	23 (26%)
P13	Yes			X	-	67 (44%)	-	-	-	22 (14%)	65 (42%)
P14	No	X			-	-	-	-	-	4 (100%)	-
P15	Yes		X	X	39 (35%)	-	26 (23%)	11 (10%)	-	23 (21%)	12 (11%)
P16	Yes			X	35 (60%)	-	-	5 (9%)	-	13 (22%)	5 (9%)
P18	No		X		10 (29%)	-	-	-	-	2 (6%)	22 (65%)
P21	No		X	X	3 (60%)	-	-	-	-	2 (40%)	-
P23	Yes		X	X	21 (29%)	31 (44%)	-	-	11 (15%)	4 (6%)	4 (6%)
P24	No		X		-	-	18 (33%)	-	-	32 (59%)	4 (8%)
P25	Yes	X			-	-	65 (37%)	-	-	78 (44%)	33 (19%)
P26	Yes			X	11 (31%)	13 (37%)	-	-	7 (20%)	2 (6%)	2 (6%)
P28	No	X			1 (100%)	-	-	-	-	-	-
P29	No	X			-	-	-	-	-	3 (100%)	-
P30	No			X	-	-	-	-	-	19 (100%)	-
P31	Yes	X			-	-	-	-	-	-	147 (100%)
P32	Yes		X	X	96 (29%)	29 (9%)	-	36 (11%)	-	43 (13%)	128 (38%)
P37	Yes		X		-	-	22 (22%)	15 (16%)	-	38 (38%)	24 (24%)
P38	No			X	150 (52%)	-	-	-	-	90 (31%)	49 (17%)
P39	Yes			X	-	-	26 (23%)	-	34 (29%)	29 (25%)	26 (23%)
P41	Yes			X	-	-	-	-	-	442 (100%)	-
P45	No			X	-	93 (61%)	-	-	-	59 (39%)	-
P50	No	X			-	-	-	-	-	4 (100%)	-

*seizures associated with poor quality video

Figure 1 - Distribution of seizure types in the cohort (circle, percentage of the total number of seizures) and in single patients (columns, number of seizures).

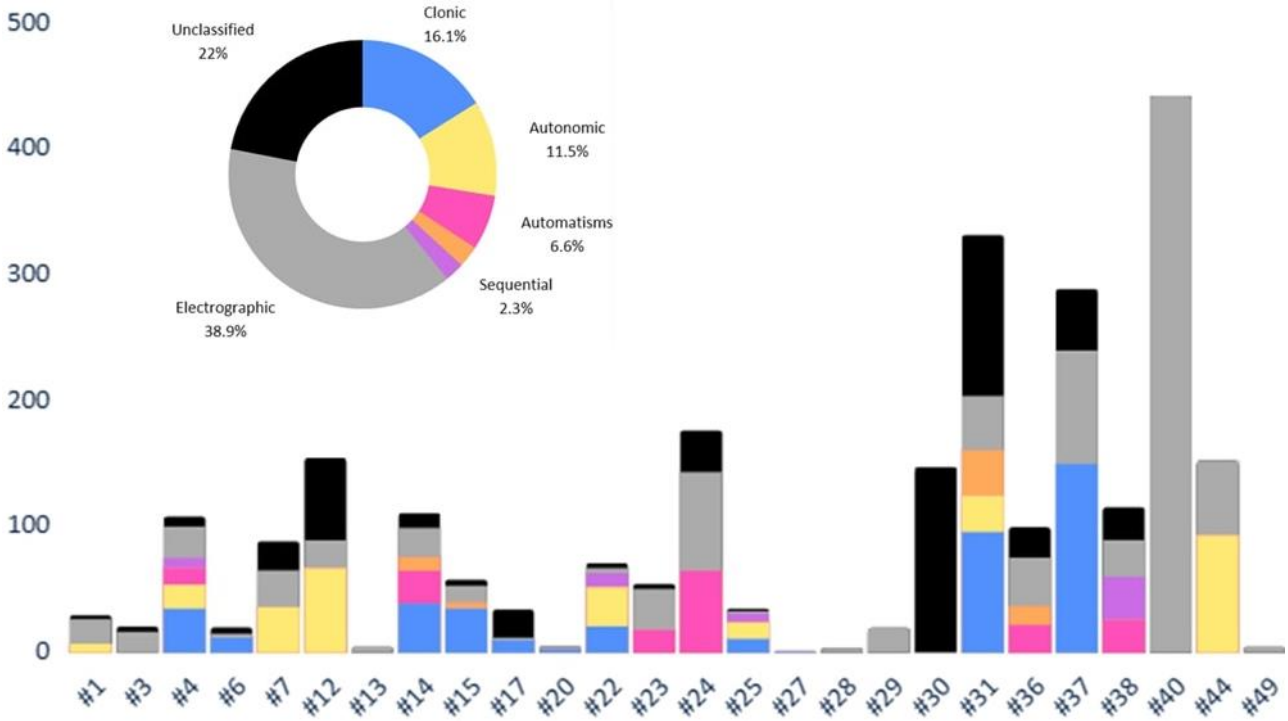


Figure 2- Flow diagram seizures and antiseizure medications (ASM) use in the cohort.

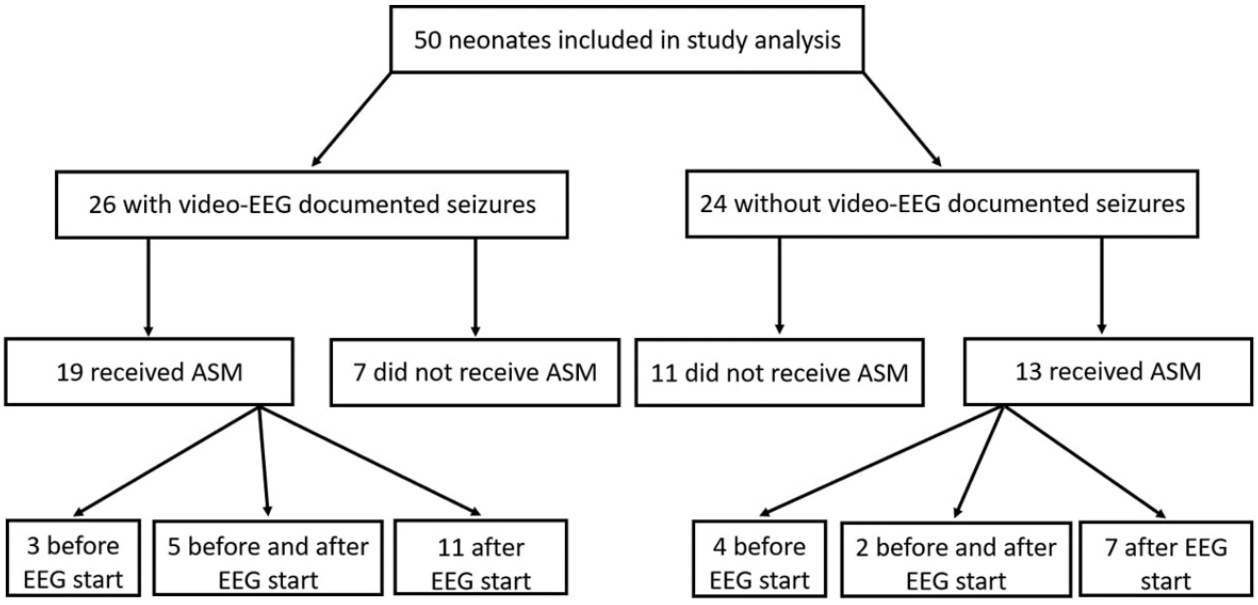


Figure 3 - ASM loads administered during the monitoring period in patients with and without video-EEG recorded seizures.

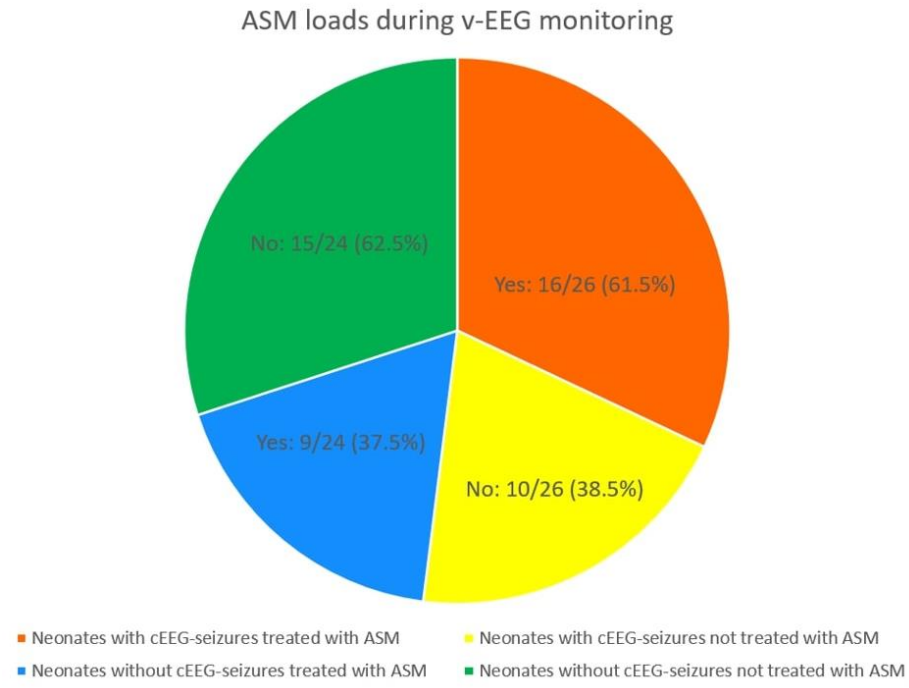
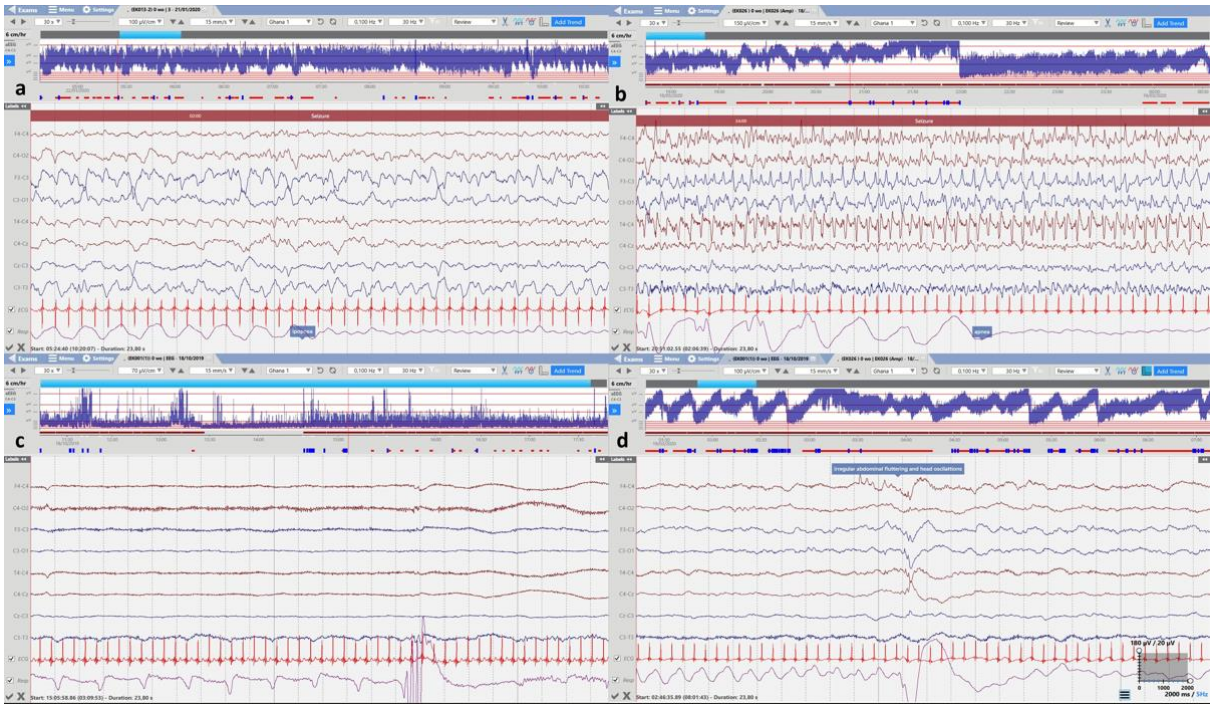


Figure 4 – Apnea occurring during autonomic seizures in patients 13 (a) and 26 (b) and non-epileptic abdominal flutter in patients 1 (c) and 26 (d) evident on the polygraphy channel exploring thoracoabdominal shifts. Position of the electrodes and filters settings are shown in (d).



Discussion

Clinical neurophysiological monitoring plays a central role in early management of neonatal HIE, providing useful insights in different settings.

In developed countries, after the advent of TH and with ongoing research on neonatal neuroprotective therapy, reliable assessment of the severity of developing encephalopathy and its prognosis is of special interest.

The assessment of HIE severity suffers an uncertainty that is method-dependent and timing-dependent. Remarkable variability exists even amongst regional and national therapeutic hypothermia eligibility guidelines for neonatal HIE, reflecting in different individual care depending on the location where infants are born and misleading epidemiological data.

Clinical severity grading is evolving: dividing severity of encephalopathy into three grades is probably an over simplification and recently proposed numerical scoring systems based on Sarnat, which acknowledge the wide spectrum associated with encephalopathy, represent a more accurate measurement of the range of clinical variability (Chalak et al. 2019).

Neurophysiology has shown the potential to add essential information, and ongoing technological efforts are aiming at spreading the availability of aEEG and EEG monitoring.

We present and detail the wide variety of additional EEG transients that may enrich the background activity at the earliest stage in neonatal HIE. A careful evaluation of focal abnormalities and specific patterns in addition to the assessment of the EEG background yields important clues that might be helpful in identifying those babies that will later develop seizures and therefore would benefit from neuroprotective treatment. The evaluation of focal transients should be considered and included in EEG grading systems.

We also provided evidence of the feasibility of a combined continuous video-EEG-polygraphy and SEPs assessment together with detailed clinical neurological examination at the same stage in the earliest hours after birth. Given the peculiarities of the immature neonatal brain and of the complexities added by the busy NICU setting, methodological aspects of our median nerve SEPs recording protocol need to be emphasized. Stimulation applied with pulses at a rate of 1.1 Hz, 0.2 ms in duration and with intensity up to 20 mA was well tolerated by neonates, in both the HIE and the healthy control groups. A high number of responses could be averaged and displayed with analysis time 180 ms and band-pass filtering 1-100 Hz, providing clear reproducibility. Our data, collected prospectively, supports previous work conducted in a small number of neonates (Trollmann et al., 2009). In the future, a metanalysis on published works on this topic, all affected by small sample size, might increase generalizability of the results and set the foundation for reference normative values for the assessment of SEPs responses in the neonatal period.

A combined multimodal neurophysiological assessment with video-EEG-polygraphy and simultaneous somatosensory evoked potentials, together with detailed clinical neurological examination, may improve the ability to stratify the spectrum of severity of neonatal hypoxic-ischaemic encephalopathy. A universally endorsed single severity staging performed at a standardized timepoint will potentially help the clinician identify those neonates who will benefit from treatment, minimizing exposure of low-risk infants to interventions and maximizing benefits to those at high risk.

In addition to refining the care provided in high-income countries, research to support monitoring and assessment of infants in low-income countries should be prioritized. The understanding of the features of the disease which are specific to this setting is the cornerstone to guide appropriate use of available resources. Given the non-availability of TH, and the increasing evidence that high seizure burden contributes to neurodevelopmental

impairment (Kharoshankaya et al., 2022), prompt detection and adequate treatment of seizures represent the main neuroprotective intervention available in low-income settings to improve morbidity in patients who survive the neonatal period.

The study conducted in Uganda provided evidence of feasibility of continuous video-EEG monitoring in sub-Saharan Africa. Our findings reveal a much higher incidence of seizures compared to what is reported in high-income country cohorts in the era of hypothermia (Low et al., 2012; Guidotti et al., 2016). Understanding seizure semiology is crucial to inform understanding of clinical presentation and prompt seizure management. Most neonates presented with clinical manifestations. Respiratory impairment due to autonomic seizures frequently went unrecognised and emerged as a prominent concern in this setting with limited access to neonatal intensive care.

Clinical neurophysiology is a reliable approach for diagnosis, assessment of severity, planning of treatment, seizure monitoring, and determination of prognosis of neonates with HIE.

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