Seizures and the Neonatal Brain

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Seizures and the Neonatal Brain

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Seizures and the Neonatal Brain

Epileptische aanvallen en het neonatale brein
(met een samenvatting in het Nederlands)

Proefschrift

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Every accomplishment starts with
the decision to try

Gail Devers

Voor jullie, voor de wijze woorden
“zorg dat jij kunt kiezen”

Table of contents

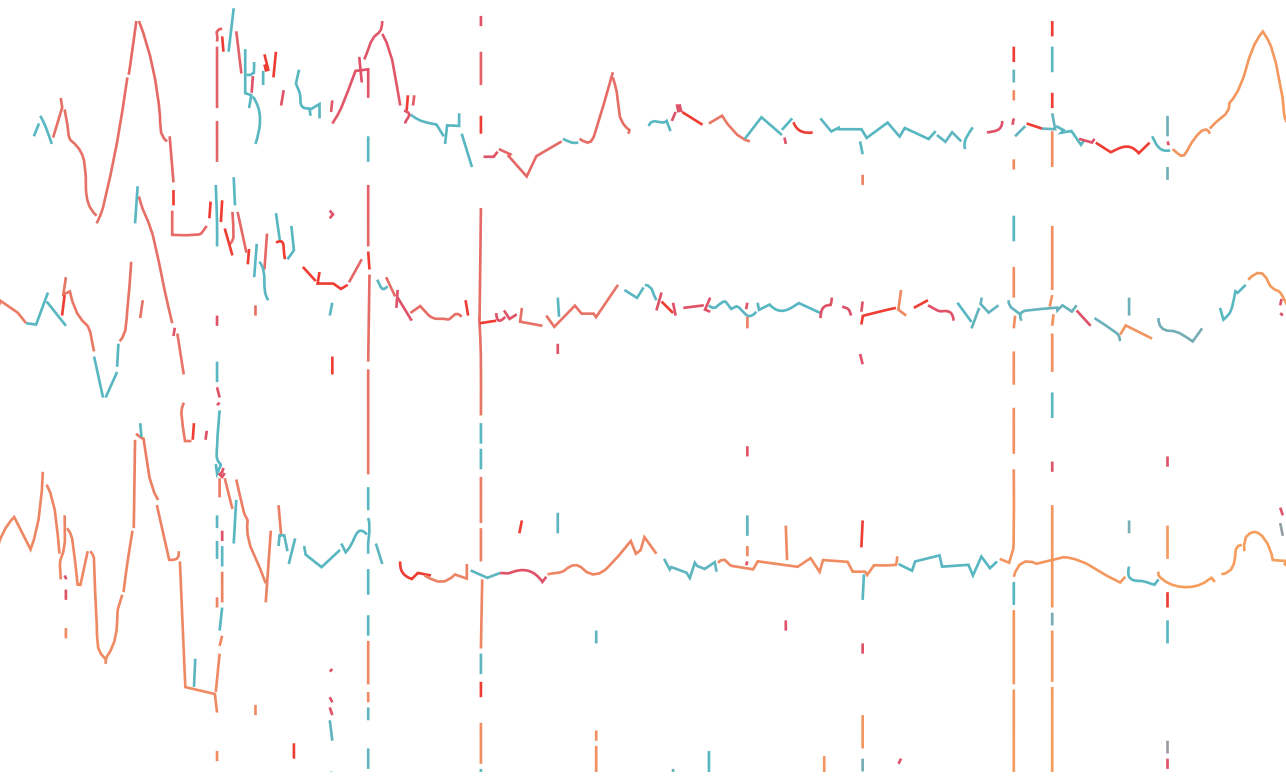
Chapter 1	General introduction	9
PART 1.1 - DIAGNOSIS - ELECTROENCEPHALOGRAPHY		**29**
Chapter 2	Rhythmic EEG patterns in extremely preterm infants: classification and association with brain injury and outcome	31
Chapter 3	Severe hypercapnia causes reversible depression of aEEG background activity in neonates: an observational study	51
PART 1.2 - DIAGNOSIS - NEURO-IMAGING		**67**
Chapter 4	Neuro-imaging in neonatal seizures	69
Chapter 5	The aetiology of neonatal seizures and the diagnostic contribution of neonatal cerebral MRI	91
Chapter 6	Punctate white matter lesions in full-term infants with neonatal seizures associated with *SLC13A5* mutations	117
PART 2 - TREATMENT		**135**
Chapter 7	Lidocaine-associated cardiac events in newborns with seizures: incidence, symptoms and contributing factors	137
Chapter 8	Lidocaine response rate in aEEG-confirmed neonatal seizures: retrospective study of 413 full-term and preterm infants	155

PART 3 - PROGNOSIS **181**

Chapter 9	Role of EEG background activity, seizure burden and MRI in predicting neurodevelopmental outcome in full-term infants with hypoxic-ischaemic encephalopathy in the era of therapeutic hypothermia	183
Chapter 10	Novel MRI score predicts neurodevelopmental outcome in infancy and at school age after perinatal asphyxia and therapeutic hypothermia	207
Chapter 11	Comparison of the Thompson encephalopathy score and amplitude-integrated EEG in infants with perinatal asphyxia and therapeutic hypothermia	229
Chapter 12	Summarising discussion and future directions	241
Chapter 13	Nederlandse samenvatting (summary in Dutch)	261
Chapter 14	List of abbreviations	278
	List of co-authors	282
	List of publications	286
	Curriculum vitae	288
	Dankwoord (Acknowledgements)	290

Chapter 1

General introduction



INTRODUCTION

Neonatal seizures are a common and serious problem in the newborn. Common, because they are often the first and most frequent sign of neurological dysfunction in the newborn infant, and seizures have the highest incidence in the first month of life. [1, 2] Serious, because they usually reflect severe underlying brain injury [2] and are associated with high rates of mortality and morbidity. [3-5] Paediatricians and neurologists are still facing many challenges when it comes to diagnosis, treatment and prognosis of neonatal seizures. No clear evidence based guidelines exist for accurate diagnosis, determining the underlying aetiology and treatment of seizures in newborn infants and no research into neonate-specific antiepileptic drugs (AEDs) has been done. Furthermore, it remains controversial whether seizures themselves are harmful for the neonatal brain and how to best predict neurodevelopmental outcome after neonatal seizures.

DIAGNOSIS

Electroencephalography

Neonatal seizures are difficult to detect and diagnose. They are usually subclinical (66%), especially after a first AED has been administered, [6, 7] which means that clinical signs do not accompany seizure activity on the EEG, while abnormal neonatal movements are often not supported by evidence of seizure activity on the EEG. A study performed by Murray *et al.* using video-EEG, showed that only 34% of all recorded electroencephalographic seizures had overt clinical signs, of which only one-third was correctly recognised by experienced neonatal medical staff. Furthermore, only 27% of clinically suspected seizure episodes had corresponding evidence of electroencephalographic seizure activity. [8] These findings underline the importance of EEG monitoring in infants at risk of developing seizures.

Amplitude-integrated electroencephalography

Multichannel video-EEG is the gold standard for neonatal seizure detection, [9] but it is not always readily available or feasible in the neonatal intensive care unit (NICU). Many NICUs use continuous amplitude-integrated EEG (aEEG) for long-term brain monitoring instead. The aEEG is usually a single- or two-channel EEG recording. The EEG is recorded from two electrodes in the parietal (P4-P3 according to the international 10-20 system for electrode placement) or central (C4-C3) region in case of a single-channel recording and in the fronto-parietal (F4-P4, F3-P3) or centro-parietal (C4-P4, C3-P3) region in case of a two-

channel recording. The aEEG method is based on a time-compressed semilogarithmic (linear 0-10 μV, logarithmic 10-100 μV) display of the peak-to-peak amplitude values of a filtered and rectified EEG signal. The raw EEG signal is filtered through an asymmetric band pass filter that suppresses activity below 2 Hz and above 15 Hz, to minimize artefacts from sweating, movements, muscle activity and electrical interference, and enhances the intermediate EEG frequencies. The signal is then rectified, meaning that negative waves become positive waves, and an amplitude envelope of the signal is computed. This amplitude envelope is then compressed in time and is usually displayed at 6 cm/h on a semilogarithmic scale. [10] Due to the semilogarithmic scale, changes in background activity of very low amplitude (<5 μV) are enhanced. The bandwidth of the aEEG trace reflects the variations in minimum and maximum EEG amplitude and can be used to assess the background brain activity and to screen for seizures. A seizure is visualised as a relatively abrupt and transient rise of the lower border. However, artefacts can also result in a rise of the lower border. Careful annotation of disturbance of the infant is therefore important to distinguish seizures from artefacts on the aEEG. Nowadays, all digital aEEG monitors have the raw EEG signal available as well, which should be used to confirm seizure activity and exclude artefacts *(figure 1)*. The advantages of limited channel aEEG compared to conventional EEG are the easy application and interpretation which can be done by NICU personnel in real-time. This can reduce the time to diagnosis and treatment of seizures significantly. Limited channel aEEG can identify up to 94% [11-14] of patients with seizures and up to 86% of individual seizures, [11-15] depending on the experience of the reviewer, [15, 16] the number of channels used [17] and whether the raw EEG signal is used in combination with the aEEG. [11]

EEG seizure morphology

EEG characteristics of ictal discharges in full-term and preterm infants have been described by a number of studies in terms of amplitude, frequency, morphology and propagation. [18-22] The amplitude of electroencephalographic seizures without clinical signs was usually between 20-64 μV and between 30-124 μV for seizures with clinical signs. [18] The amplitude of neonatal ictal discharges can be as high as 200 μV, and seems to be inversely related to the frequency of the discharge. [21] Also, ictal discharges with an amplitude below 50 μV have been associated with poor background activity and a poor prognosis. [18] The frequency of electroencephalographic seizures with and without clinical signs at onset was usually around 3 Hz, but the maximum frequency was reported to be significantly lower in seizures without clinical signs (4 vs. 5.5 Hz). A frequency below 2 Hz was associated with a poor prognosis in full-term infants. [18] In preterm infants, however, ictal discharges of lower frequency are more common. The maximum frequency showed

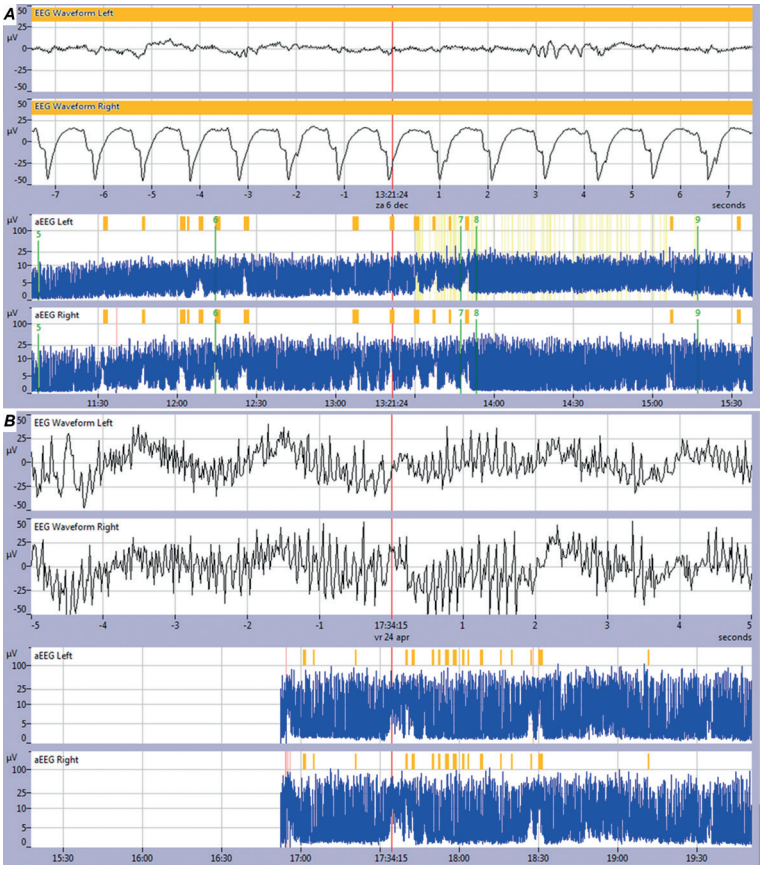


Figure 1. An abrupt, transient rise of the lower border of the aEEG trace (lower panel in figure A and B) can be caused by seizure activity in the raw EEG as seen in the upper panel of figure A or by artefacts as seen in the upper panel of figure B.

a linear association with gestational age in study of 55 preterm and full-term infants. Even ictal discharges with a frequency below 1 Hz were seen in a few preterm patients. [22] Newborn infants can show a wide variety of morphologically different ictal discharges. Rhythmic waves in the beta (12-14 Hz), alpha (8-12 Hz), theta (4-7 Hz) or delta (0.5-3 Hz) frequency, sharp waves and spikes can be found in the EEGs of newborn infants. [19-22] In full-term infants sharp waves or rhythmic delta or theta waves are mostly found. [20, 21] In preterm infants a change from rhythmic alpha or delta to sharp waves or from low voltage spikes to sharp waves to theta and delta has been described. [19, 20] A relation

between seizure morphology on EEG and aetiology has never been established. [20, 21] Propagation and migration is rare in preterm infants. [19, 22] Change in morphology and/or frequency is common in both full-term and preterm infants. [20]

Periodic epileptiform discharges

Periodic epileptiform discharges (PEDs) are a distinct pattern that can be recognised on EEG. PEDs usually repeat every 0.5-4 s and consist of spikes or sharp waves often followed by a slow wave, but they do not show evolution. [23] They have been associated with brain lesions, epilepsy and poor outcome in adults and children. [24-26] In the newborn, they seem to have a similar significance and have been associated with herpes simplex encephalopathy, hypoxic-ischaemic encephalopathy and stroke in particular. [27-29] In preterm infants, however, the phenomenon of PED and possible associations with brain injury and outcome have not been described yet.

Other uses of EEG in the NICU - Background activity

The background activity of the EEG and the background pattern of the aEEG are often used in clinical practice to determine the level of encephalopathy in full-term infants [30] and monitor brain maturation in preterm infants. [31] In full-term infants, five aEEG background patterns can be distinguished: [10] (1) continuous normal voltage (CNV), a continuous trace with a voltage between 10 and 25-50 μV, (2) discontinuous normal voltage (DNV), a discontinuous trace in which the lower border is below 5 μV, (3) discontinuous background pattern (burst suppression, BS), periods of low amplitude activity or inactivity intermixed with bursts of higher amplitude (usually >25 μV), (4) continuous low voltage (CLV), a continuous trace around or below 5 μV, sometimes with bursts of higher (but below 25 μV) amplitude, (5) flat trace (FT), isoelectric tracing (<5 μV) *(figure 2)*.

The background pattern of the aEEG has been shown to correspond well with the background activity on conventional EEG using visual analysis in both full-term and preterm infants. [31, 32] Recently, this association has been confirmed using quantitative analyses. [33, 34] The aEEG background pattern is influenced by a number of different factors. First of all, the background pattern changes between sleep stages and with (gestational) age. In full-term infants the background pattern is predominantly CNV during wakefulness and active sleep, alternated with shorter periods of DNV during quiet sleep. In preterm infants the background activity is more discontinuous and does not show clear sleep stages at very young gestational ages. [35] With increasing age the background pattern becomes more continuous and will start to show clear differences between wakefulness

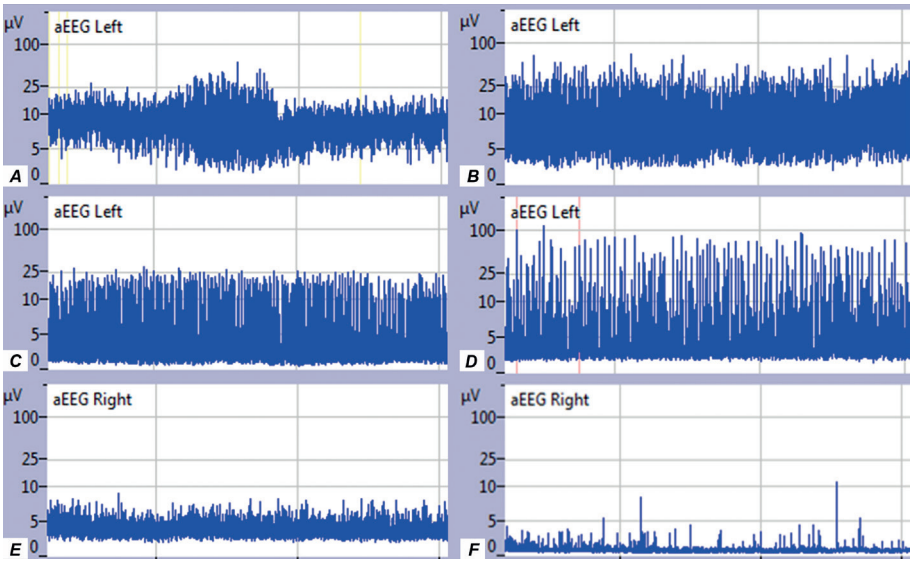


Figure 2. aEEG background patterns. (A) Continuous normal voltage with sleep-wake cycling; (B) discontinuous normal voltage; (C) dense burst suppression; (D) sparse burst suppression; (E) continuous low voltage; (F) flat trace.

and sleeping from 33-35 weeks gestational age.[36,37] The location of the electrodes and the distance between them affects the background pattern. A short inter-electrode spacing will result in a more discontinuous background pattern.[38,39] Common artefacts such as movement and electrocardiogram (ECG) also influence the background pattern, usually resulting in an upward drift of the lower border.[38] Furthermore, any factor that influences brain activity will cause a change in the aEEG background pattern as well. Sedative or antiepileptic drugs, such as morphine and benzodiazepines, suppress brain activity and can subsequently cause a deterioration of the background pattern.[40,41] Brain injury has been shown to impact on the aEEG background pattern as well. In full-term infants, a significant correlation was found between severity of hypoxic-ischaemic injury on MRI and severity of background pattern depression.[42] In preterm infants, intraventricular haemorrhages and cystic periventricular leukomalacia have been reported to be associated with more discontinuous background patterns.[43] Finally, several physiological factors have been related to EEG activity and could potentially influence the aEEG background pattern. Wikström *et al.* showed that carbon dioxide (CO_2) and glucose blood levels were associated with EEG activity in preterm infants. Increasing CO_2 levels had an

increasingly depressant effect on brain activity and in infants with blood glucose levels above or below the normal values the EEG activity was depressed as well. [44] West *et al.* reported a positive linear relation between mean systemic arterial blood pressure and EEG continuity. [45] All these factors should be taken into account when assessing the aEEG background pattern.

Neuro-imaging

Neonatal seizures often reflect severe underlying brain injury. [2] Prognosis is primarily determined by the nature, site and extent of the underlying aetiology, [4, 5, 46] making accurate diagnosis and identification of associated brain lesions essential. Neuro-imaging may help to diagnose an underlying problem, by confirming a diagnosis or by revealing lesions associated with a certain metabolic or genetic disorder, such as polymicrogyria in Zellweger syndrome. Recognition of aetiological factors in neonatal seizures has increased significantly over the years and the aetiologic profile is now better defined due to advances in investigational techniques, in particular in neuro-imaging. [4, 46-48] More accurate diagnoses can be made and the number of infants without identified aetiologies is decreasing. [4, 46, 47, 49]

No evidence based guidelines for the evaluation of neonatal seizures exist. [2] Neuro-imaging has been shown to be one of the most important factors for diagnosis and thereby for outcome prediction in infants with neonatal seizures. [50] Magnetic resonance imaging (MRI), computed tomography (CT) and cranial ultrasonography (cUS) are commonly used modalities for imaging the neonatal brain. Nowadays, the role of CT is limited and its use discouraged because of limited spatial resolution and contrast, and exposure to radiation. [48] cUS will be used in most NICUs as the method of first choice for neuro-imaging. It is readily available and causes minimal disturbance for the infant but is limited in distinguishing different brain injuries or imaging of the deep brain structures. [48] MRI is increasingly used in neonatal seizures. It is likely to provide the most complete and useful information [48, 51, 52] and is now considered the gold standard for diagnosing brain injury and developmental disorders *in vivo* and for determining the prognosis in infants with neonatal seizures. [2]

Over the years, many studies have reported on brain imaging and neonatal seizures, but most studies focused on MRI data in infants with specific underlying problems such as HIE, neonatal stroke or infants with a specific metabolic disorder. Only a few small studies reported on neuro-imaging findings in infants with neonatal seizures in general. [4, 46, 53, 54]

TREATMENT

Animal studies have suggested that seizures can exacerbate brain injury in the developing brain.[55,56] In humans, seizures have been related to more severe brain injury and long-term neurodevelopmental deficits such as cognitive and behavioural problems and epilepsy in full-term [51,57-60] and preterm infants.[61-65] Adequate treatment of neonatal seizures with AEDs can reduce the seizure burden.[66] However, data from randomised controlled trials to support the optimal treatment protocol are limited and no recommendations can be made.[67-69] Phenobarbital remains the most widely used first-line drug [70] despite a suboptimal response rate.[6,71] No consensus exists about the preferred second- and third-line AED.[70,72] Phenytoin, benzodiazepines such as midazolam, and lidocaine are most frequently used.[73] Studies to support the choice of drug are small, have a limited level of evidence and are difficult to compare due to differences in treatment protocols and study design.[69] Phenytoin has been reported to have a response rate of 53% [71,74] and midazolam of 0-100%.[74-79] Lidocaine has only been studied in a few small studies, with a response rate of 60-92%.[75,77,80-85] Growing evidence from experimental data suggests that the currently used AEDs may cause neuronal apoptosis and white matter injury in the developing brain.[86,87] Therefore, newer agents for treatment of neonatal seizures are needed. Levetiracetam is currently being investigated as an alternative first-line drug in several trials (NCT01720667, NCT02229123, NCT02550028).[88] In children and adults it is an effective AED for various seizures and the safety profile in neonates is favourable.[87,89-91] However, the response rate in neonates as second- or third-line AED was only about 30% in a few small studies.[92,93]

Treatment of seizures in preterm infants forms an even bigger challenge, since studies investigating the safety and efficacy of AEDs in preterm infants specifically do not exist. However, the pathophysiological mechanism of seizures in preterm infants may be different from full-term infants.[94] New drugs targeting newborns specifically and randomised controlled trials to support the choice of drug for full-term and preterm infants are therefore still urgently needed.

PROGNOSIS

Neonatal seizures have been associated with substantial long-term morbidity and mortality.[5,57-60] Reliable prognostication in infants with neonatal seizures is important to correctly inform parents about the possible long-term neurodevelopmental

consequences and additional therapeutic options, and ultimately guide decisions on redirection of care. HIE is the most common cause of seizures in full-term infants [4] and has therefore been the focus of many studies investigating predictors of outcome. Identifying infants with HIE at risk of adverse outcome is of special interest because neuroprotective strategies such as therapeutic hypothermia are available. Neurological examination using standardised scores, [95, 96] (a)EEG background activity, [97-99] seizure burden, [58] and MRI [100-105] have mainly been investigated as predictors of long-term outcome in infants with HIE. Early neurological examination and (a)EEG background activity have been related to outcome and are used as selection criteria for therapeutic hypothermia. However, the neuroprotective ability of hypothermia has affected the predictive value of both modalities. [106-108] Hypothermia also significantly reduces seizure burden and brain injury in infants with HIE, [109, 110] but the predictive value of MRI was reported to be unaffected. [110] Attempts have been made to determine the best predictor of outcome, [111, 112] but this proved to be difficult since most predictors were investigated separately in studies with a limited number of infants. MRI and (a)EEG background activity were reported to be the most optimal predictors of outcome, but studies concluded that prediction of outcome could be improved substantially by evaluating multiple factors. [111-113]

AIMS AND OUTLINE OF THE THESIS

The general aim of this thesis is to provide new insights to support evidence based guidelines for diagnosis, treatment and prognosis of neonatal seizures. To ascertain accurate diagnosis of seizures in full-term and preterm infants, determination of the underlying aetiology, and optimal treatment of seizures and to provide tools to predict outcome in infants with neonatal seizures. The second aim of this thesis is to investigate and describe additional uses of EEG and aEEG in the NICU.

PART 1 - DIAGNOSIS

PART 1.1 - ELECTROENCEPHALOGRAPHY

In *Chapter 2* five different rhythmic EEG patterns in extremely preterm infants are described. The association of these patterns with brain injury and cognitive outcome at 2 and 5 years is discussed.

In *Chapter 3* the effect of acute, severe hypercapnia on (amplitude-integrated) EEG and cerebral oxygenation in full-term and preterm infants is described.

PART 1.2 - NEURO-IMAGING

Chapter 4 provides a review of the literature and an overview of the spectrum of neuro-imaging findings in full-term infants with neonatal seizures in the light of different underlying aetiologies.

In *Chapter 5* the aetiologic profile of neonatal seizures is assessed in a cohort of full-term and near-term infants. The additional value of MRI in the diagnostic process compared to cranial ultrasound is discussed.

In *Chapter 6* the neuro-imaging findings of patients with neonatal seizures and mutations in the *SLC13A5* gene are described.

PART 2 - TREATMENT

In *Chapter 7* the incidence of lidocaine-associated cardiac events is assessed in a cohort of full-term and preterm infants receiving lidocaine for seizures. The causal relation between lidocaine and cardiac events is discussed, as well as possible contributing factors. Criteria how to recognise lidocaine-associated cardiac events are provided.

In *Chapter 8* the response rate of seizures to lidocaine is assessed in a cohort of full-term and preterm infants who received lidocaine following standardised regimens for neonatal seizures and compared to the response rate to midazolam. Factors that influence the response to lidocaine are discussed.

PART 3 - PROGNOSIS

In *Chapter 9* the role of EEG background activity, electroencephalographic seizure burden, and MRI in predicting neurodevelopmental outcome at 2 years of age in full-term infants with hypoxic-ischaemic encephalopathy in the era of therapeutic hypothermia is discussed.

In *Chapter 10* a novel MRI brain injury score for predicting outcome in full-term infants with hypoxic-ischaemic encephalopathy who were treated with therapeutic hypothermia is presented. A detailed scoring system with MRI examples for each item of the score is provided.

In *Chapter 11* the Thompson encephalopathy score and amplitude-integrated EEG background patterns and their relation with outcome are compared in full-term infants with hypoxic-ischaemic encephalopathy who were treated with therapeutic hypothermia.

In *Chapter 12* the findings of this thesis are summarised. Possible implications and suggestions for future research are discussed.

In *Chapter 13* a summary in Dutch is provided.

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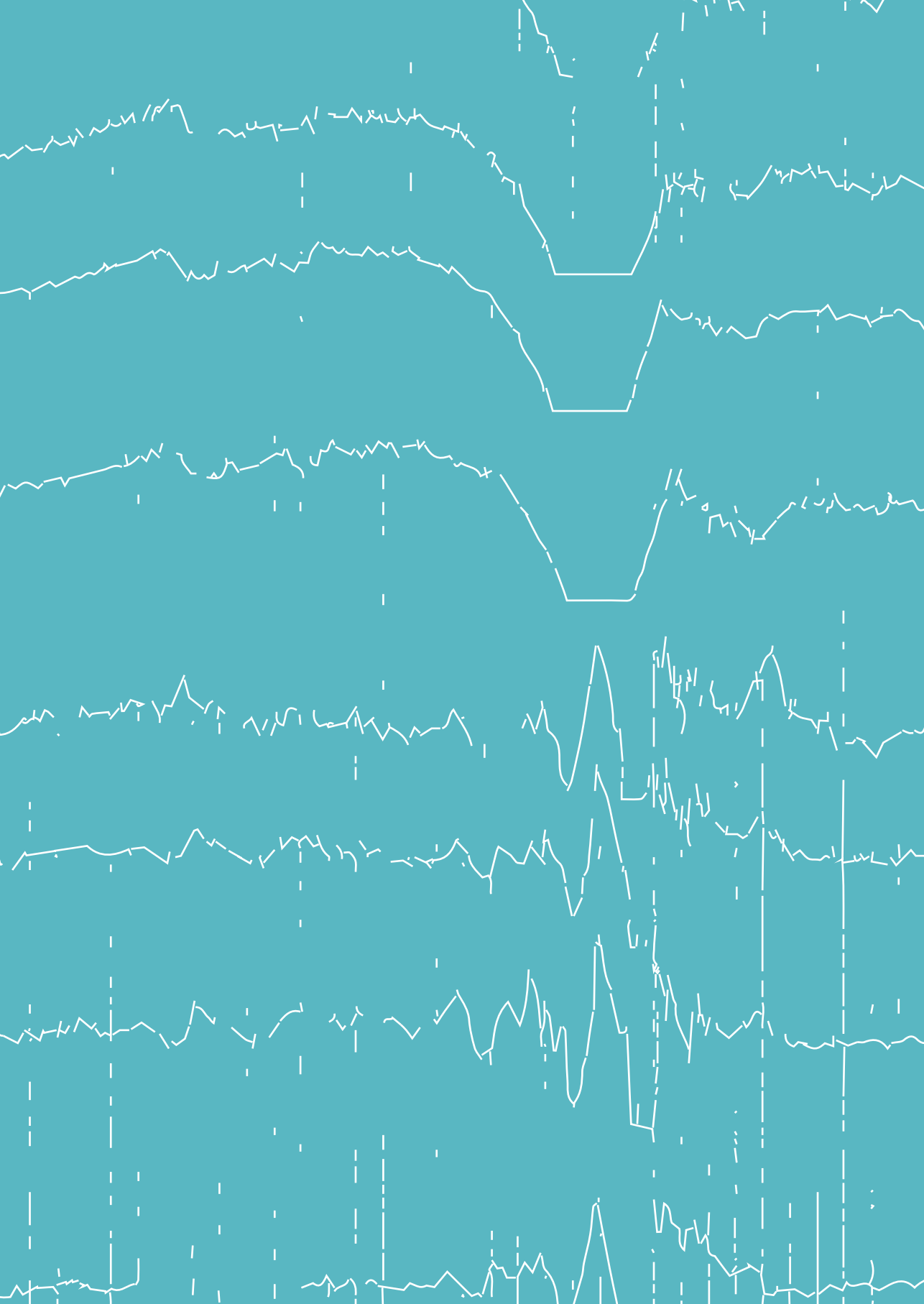

PART 1.1

Diagnosis - Electroencephalography

Chapter 2

Rhythmic EEG patterns in extremely preterm infants: classification and association with brain injury and outcome

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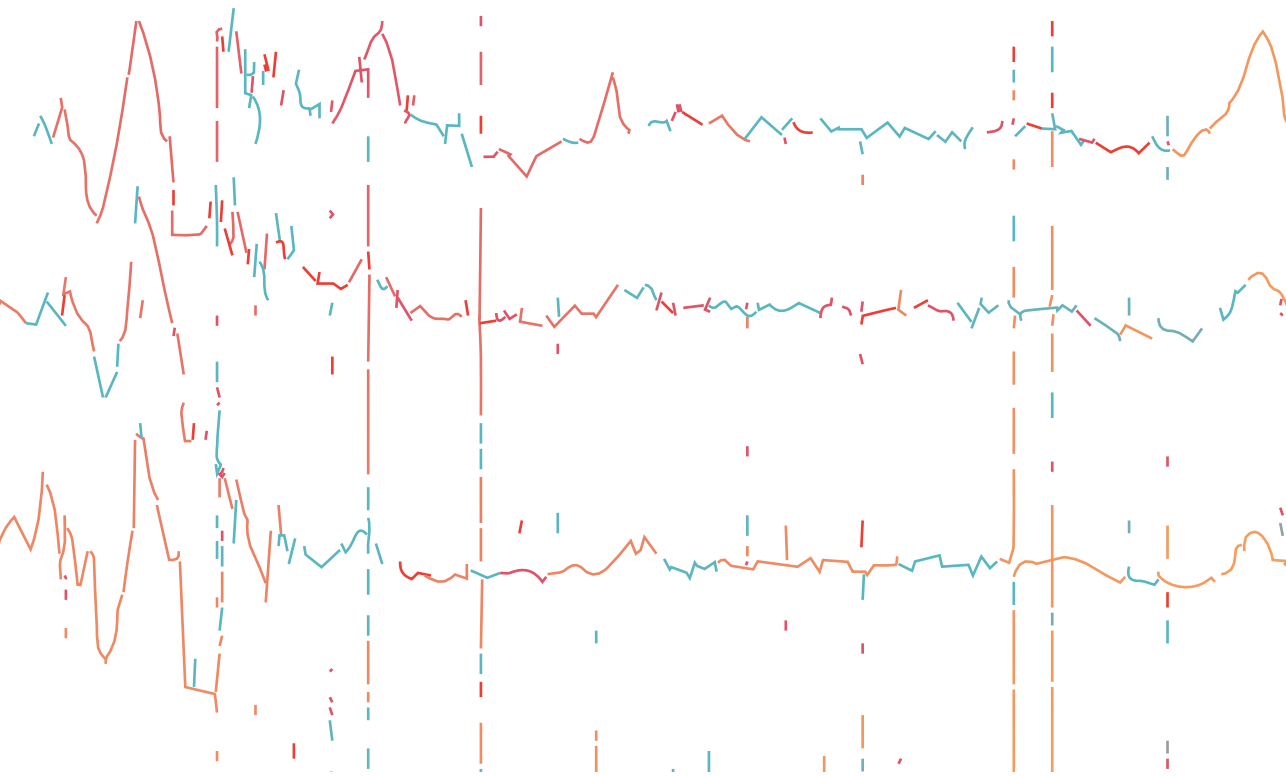

ABSTRACT

Introduction Many rhythmic patterns can be detected in the EEG of extremely preterm infants. Not all comply with the definition of subclinical seizure patterns. They are rhythmic and last for >10 s, but do not show evolution in amplitude and frequency. The aim of this study was to classify these rhythmic EEG patterns in extremely preterm infants and relate these to brain injury and neurodevelopmental outcome.

Methods Retrospective analysis of 77 consecutively born infants <28 weeks gestational age (GA) (median GA 26.6 weeks [range 24.5-27.6]) who had a 2-channel EEG recording during the first 72 h after birth. Rhythmic patterns detected by the BrainZ seizure detection algorithm were categorised into five patterns: ictal discharges, periodic epileptiform discharges (PEDs), PED-like, zeta and sinusoidal waves. Brain injury was assessed with sequential cranial ultrasound (cUS) and MRI at term-equivalent age. Neurodevelopmental outcome was assessed at 2 years with the Bayley-III and at 5 years with the WPPSI-III-NL.

Results In 62.3% rhythmic patterns were observed (ictal 1.3%, PEDs 44%, PED-like 29%, zeta 17%, sinusoidal 40.3%) with multiple patterns in 36.4% of infants. Ictal discharges were only observed in one infant and therefore excluded from further analyses. The presence of sinusoidal ($p = 0.038$), zeta ($p = 0.001$) and PED-like waves ($p < 0.001$), but not PEDs ($p = 0.238$), was significantly associated with head position. Intraventricular haemorrhage or white matter injury was noted on cUS in 43% of infants and MRI in 47%. No relation was found between the median total duration of each pattern and injury on cUS and MRI or cognition at 2 and 5 years.

Conclusion Ictal discharges were observed in 1.3%. Other rhythmic patterns were more common, but significantly related to head position and therefore more likely to be artefacts. PEDs were common but not related to head position nor to brain injury and poor cognitive outcome.

INTRODUCTION

In many units, neuro-monitoring with electroencephalography (EEG) during the first postnatal days has become standard procedure. Brain protection has become one of the main aims of neonatal intensive care, since the survival rate of extremely preterm infants (born <28 weeks gestational age) has increased due to major advances in perinatal care.[1,2]

Seizures have been related to poor outcome in preterm infants.[3-7] The incidence of seizures in preterm infants has been estimated at 4-48%.[3,8] This is higher than reported in full-term infants,[8] while the response rate to antiepileptic drugs (AEDs) seems to be significantly lower in preterm infants.[9] The majority of seizures are subclinical, requiring continuous EEG for seizure detection.[10] In the neonatal intensive care unit (NICU), detection of subclinical seizure patterns can be aided by automatic seizure detection algorithms.[11]

However, little is known about EEG seizure morphology in extremely preterm infants. Clinicians are faced with a wide range of rhythmic EEG patterns that may be recognised by seizure detection algorithms as subclinical seizures. However, not all patterns seem to comply with the definition of a subclinical electroencephalographic seizure which is rhythmic activity, lasting for at least 10 s, with a clear beginning, middle and end, and showing evolution in amplitude, frequency and/or morphology.[12] This raises the question whether these patterns are subclinical seizures that require treatment and are related to poor outcome or whether they accompany normal brain development.

The aim of the present study was to classify all rhythmic EEG patterns, recognised by the BrainZ seizure detection algorithm, based on morphology and relate these different patterns to brain injury and neurodevelopmental outcome in a cohort of extremely preterm infants who were monitored with 2-channel EEG during the first 72 h after birth as standard of care. Our hypothesis was that ictal discharges and periodic epileptiform discharges (PEDs) would be associated with brain injury and poor cognitive outcome.

METHODS

Patients

All infants born <28 weeks gestational age (GA) between May 2008 and December 2010 who had been admitted to the level III NICU of the Wilhelmina Children's Hospital in Utrecht, the Netherlands, and had a 2-channel EEG recording during the first 72 h after birth were retrospectively analysed (n=77 out of 110). Sixty-three infants were participants of a European multicentre study (Neonatal Estimation of Brain Damage Risk and Identification of Neuroprotectants [NeoBrain]). [13] Clinical data, such as GA, birth weight, Apgar score and head position, was obtained from the electronically available medical records. Permission from the medical ethics committee and parental informed consent was obtained.

EEG monitoring

Patients were monitored using the BrainZ monitor (BRM2 or 3 version, Natus, Seattle, USA). It records a two-channel amplitude-integrated EEG as well as a raw EEG from two needle electrodes over each hemisphere (F4-P4, F3-P3, according to the international 10-20 system of electrode placement modified for neonates) and has a built-in seizure detection algorithm. [11]

EEG patterns

At the markers placed by the seizure detection algorithm of the BrainZ monitor, the raw EEG was visually analysed and categorised by consensus reading of five observers (AH, IO, LV, LH, LW). Rhythmic patterns were categorised into five different categories: ictal discharges, PEDs, PED-like waves, zeta waves and sinusoidal waves. These categories were based on patterns described in older children and adults. [14,15] The patterns that did not fit into these five categories or were clear artefacts caused by high frequency ventilation or electrocardiography were discarded. For each EEG pattern that could be categorised into one of the five categories, the total duration in seconds (burden), the mean wavelength in seconds and the location on the EEG (left, right or bilateral) was determined for each infant.

Ictal discharges: have been described in neonates as rhythmic activity, lasting for at least 10 s, with a clear beginning, middle and end, and showing evolution in amplitude, frequency and/or morphology *(figure 1 A, B)*. [12] This activity can consist of spikes, sharp waves, spike-wave complexes or rhythmic delta or theta waves. [16]

Periodic epileptiform discharges (PEDs): have been described in older infants and adults as sharp waves followed by a pronounced incision and a slow wave. These complexes repeat every 0.5-4 s, but do not show evolution *(figure 1 C, D)*. [17] They have been associated with brain lesions, epilepsy and poor outcome in children and adults. [18-20]

PED-like waves: these complexes were detected in our cohort but could not be related to a category described in the literature. They might be considered as PEDs in which the sharp waves are lacking. They consist of a positive sharp deflection from baseline forming an incision, followed by a periodic slow wave of generally more than one second *(figure 1 E, F)*.

Zeta waves: as described by Magnus and van der Holst [21] consist of a slow wave with a rapid negative first phase, followed by a relatively slower positive second phase crossing the baseline. This wave is followed by a slow negative wave returning to baseline. Thus a Z-shaped complex appears with a duration of more than one second. Such complexes generally occur in trains of several seconds *(figure 1 G, H)*. In adults, zeta waves were associated with brain lesions with acute onset, such as intracranial haemorrhages. [21]

Sinusoidal waves: these waves resemble sine waves. They may occur in different, but generally low delta frequencies *(figure 1 I, J)*. In older children and adults frontal and occipital intermittent rhythmic delta waves have been described and related to deeply localized brain abnormalities. [22, 23] Sine waves were also thought to initiate seizures and metamorphose into other patterns as described by Blume *et al.* [24]

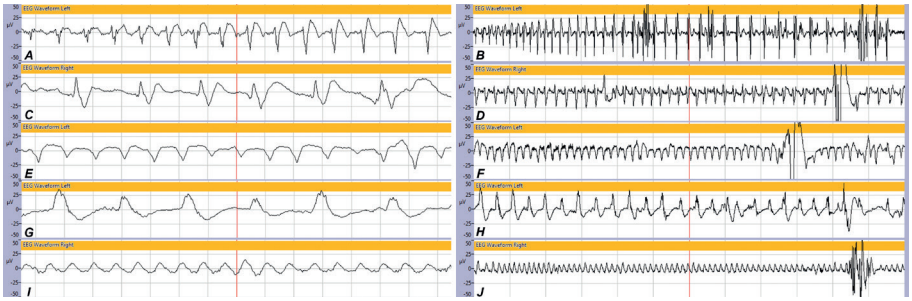

Figure 1. Examples of the different EEG patterns at 15 s (A, C, E, G, I) and 1 min per screen (B, D, F, H, J) that were detected in this study by the seizure detection algorithm of the BrainZ monitor as indicated by the orange markers. Ictal discharges (A, B), periodic epileptiform discharges (PEDs) (C, D), PED-like waves (E, F), zeta waves (G, H) and sinusoidal waves (I, J).

Neuro-imaging

Cranial ultrasonography (cUS) was performed on admission to the NICU and repeated every day for the first 72 h and at weekly intervals thereafter until discharge. Magnetic resonance imaging (MRI) on a 3.0T MR system was performed at term-equivalent age (39-43 weeks). The scanning protocol included T1-, T2-, diffusion- and susceptibility-weighted images. For each infant, it was determined whether abnormalities were seen on cUS or MRI and how severe these abnormalities were. Injury severity was categorised as mild (cUS: intraventricular haemorrhage [IVH] grade 1-2 according to Papile [25]; MRI: IVH grade 1-2, <6 punctate white matter lesions [PWML], <6 punctate cerebellar lesions), moderate (cUS: IVH grade 3; MRI: IVH grade 3, >6 PWML, >6 punctate cerebellar lesions or a large unilateral lesion [<50% of the hemisphere]) or severe (cUS: IVH grade 4, infarction, periventricular haemorrhagic infarction, perforator stroke, cystic periventricular leukomalacia [cPVL], [26] post-haemorrhagic ventricular dilatation [PHVD]; MRI: periventricular haemorrhagic infarction, cPVL, [27] PHVD, large cerebellar haemorrhage [>50% of hemisphere]). The Kidokoro global score was calculated based on the MRI at term-equivalent age as a marker for injury severity and maturation. [28]

Neurodevelopmental outcome

The Bayley Scales of Infant and Toddler Development, third edition (BSITD-III) was used to assess cognitive outcome at 30 months corrected age (corrected for GA at birth), by calculating the cognitive composite score. [29] The Wechsler Preschool and Primary Scale of Intelligence, third edition, Dutch version (WPPSI-III-NL) was used to assess the

intelligence quotient (IQ) at 5 years of age. [30] The mean (standard deviation [SD]) in a normative population is 100 (15) for the cognitive composite score and the IQ.

Statistical analysis

SPSS version 21 (IBM Corp., Armonk, NY, USA) was used to perform statistical analyses. Chi-squared or Fisher's exact test was used to investigate the relation between the location of the EEG patterns (right or left) and the infant's head position (right or left) and compare the proportion of infants with each pattern between those with and without injury on cUS during the first 72 h after birth or on MRI at term-equivalent age and between deceased and surviving infants. Kruskal-Wallis test was used to compare the median PED burden between injury severity groups. Binary logistic regression was performed to investigate the relation between the burden of each pattern and injury on cUS and MRI. Linear regression was performed to investigate the relation between the burden of each pattern, the BSITD-III cognitive composite score (uncorrected), the WPPSI-III-NL total IQ, the PED burden, and the Kidokoro global score.

RESULTS

A total of 77 preterm infants were included in this study. Patient characteristics are shown in *table 1*. In 29 infants (37.7%), no distinctive rhythmic EEG patterns were observed. The distribution and characteristics of the EEG patterns in the rest of the population are shown in *table* 2. Multiple patterns in one infant were observed in 36.4% of the population. Only one infant had ictal discharges during the first 72 h after birth *(figure 2)*. This infant was diagnosed with a *Listeria monocytogenes* meningitis. The cUS on admission to the NICU showed fibrin threads in the lateral ventricles and echogenicity in the white matter. Later on, the infant developed intraventricular and parenchymal haemorrhages. The ictal discharges did not respond to antiepileptic therapy and the infant died at day of life 4. Interestingly, the ictal discharges were recognised on aEEG as a clear rise of the lower border, which was never seen in any of the other rhythmic patterns described in this study. Since ictal discharges were only observed in one infant, they were not included in further analyses.

Location EEG pattern versus head position

The EEG location of the rhythmic pattern was significantly associated with head position for the sinusoidal ($p = 0.038$), zeta ($p = 0.001$), and PED-like waves ($p < 0.001$), but not for PEDs ($p = 0.238$). The influence of head position on these patterns suggests that these are likely artefacts.

EEG patterns versus brain injury

Sinusoidal, zeta and PED-like waves: Of the patterns influenced by head position, the proportion of infants with sinusoidal waves was higher in infants with injury on cUS in the first 72 h after birth (p = 0.027), but not in infants with injury on MRI at term-equivalent age (p = 0.260). The total duration of sinusoidal waves was not related to injury on cUS or MRI. No difference in incidence of zeta or PED-like waves was observed between infants with or without injury on cUS or MRI. The total duration of these patterns was also not related to injury on cUS or MRI *(figure 3)*.

PEDs: The incidence and total duration of PEDs were not different between infants with and without injury on cUS or MRI. The median PED duration was not different between injury severity groups (no, mild, moderate-severe). No positive relation, but a trend towards a negative relation between total PED duration and MRI severity score was observed *(figure 3)*.

Table 1. Patient characteristics

	Cohort n=77
Sex male/female, n	48/29
Gestational age at birth (weeks), median (range)	26.6 (24.5-28.0)
Birth weight (grams), median (range)	924 (178)
Birth weight Z-score, mean (SD)	0.37 (0.92)
Small for gestational age (<p10), n (%)	5 (6.5)
Apgar at 5 min, median (range)	8 (2-10)
Morphine in first 72 h, n (%)	41 (53.2)
Mechanical ventilation, n (%)	70 (90.9)
Neuro-imaging	
cUS performed, n (%)	77 (100)
abnormal	33 (42.9)
IVH grade 1-2	19 (24.7)
IVH grade 3-4	12 (15.6)
WMI	12 (15.6)
CB	0
Other	1 (1.3)
MRI performed, n (%)	68 (88.3)
abnormal	32 (47.1)
IVH grade 1-2	15 (22.1)
IVH grade 3-4	4 (5.9)
WMI	21 (30.9)
CB	12 (17.6)
Other	1 (1.5)
Follow-up	
Died, n (%)	7 (9.1)
Follow-up surviving infants	
- BSITD-III performed, n (%)	70 (100)
- BSITD-III age (months, uncorrected), mean (SD)	33.1 (0.8)
- BSITD-III cognitive composite score (uncorrected), mean (SD)	96 (8)
- WPPSI-III-NL performed, n (%)	53 (75.7)
- WPPSI-III-NL age (years), mean (SD)	5.8 (0.4)
- WPPSI-III-NL TIQ, mean (SD)	99 (12)

BSITD-III: Bayley Scales of Infant and Toddler Development, third edition; CB: cerebellum; cUS: cranial ultrasound; IVH: intraventricular haemorrhage; MRI: magnetic resonance imaging; SD: standard deviation; TIQ: total intelligence quotient; WMI: white matter injury; WPPSI-III-NL: Wechsler Preschool and Primary Scale of Intelligence, third edition, Dutch version.

Table 2. Characteristics of the rhythmic EEG patterns

Pattern	Incidence, n (%)	Total duration (s), median (range)	Wavelength (s), mean (SD)
Ictal discharges	1 (1.3)	696 (NA)	0.76 (NA)
PEDs	34 (44.2)	148 (29-1109)	2.07 (0.58)
PED-like waves	22 (28.6)	528 (25-8109)	1.53 (0.68)
Zeta waves	13 (16.9)	212 (57-1729)	1.90 (0.42)
Sinusoidal waves	31 (40.3)	203 (21-32357)	1.12 (0.58)

NA: not applicable; PEDs: periodic epileptiform discharges; SD: standard deviation.

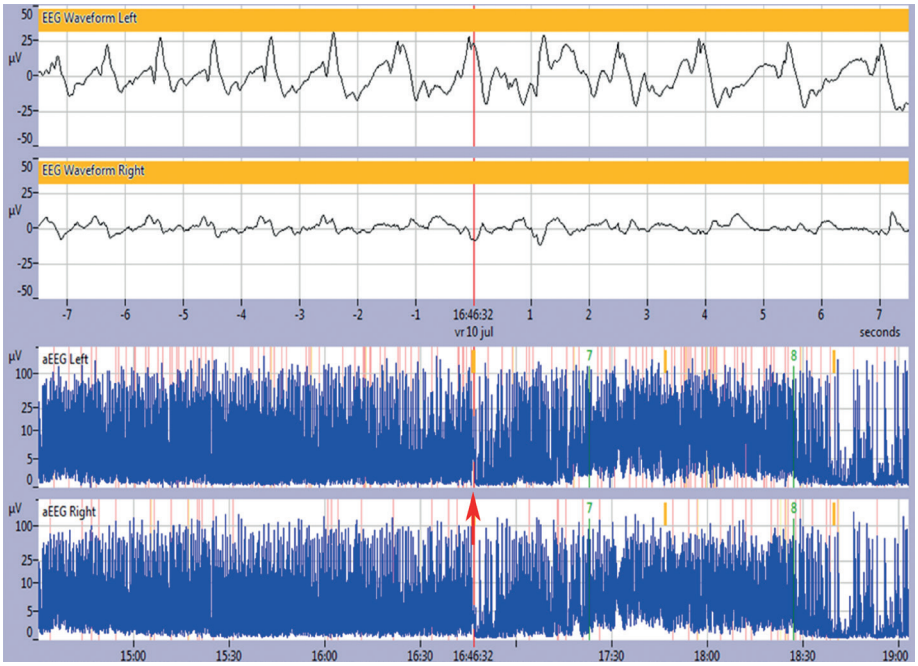

Figure 2. The raw EEG in the upper panel shows an ictal discharge (15 s/screen), with a clear rise of the lower border of the aEEG (arrow) in the lower panel (6 cm/h).

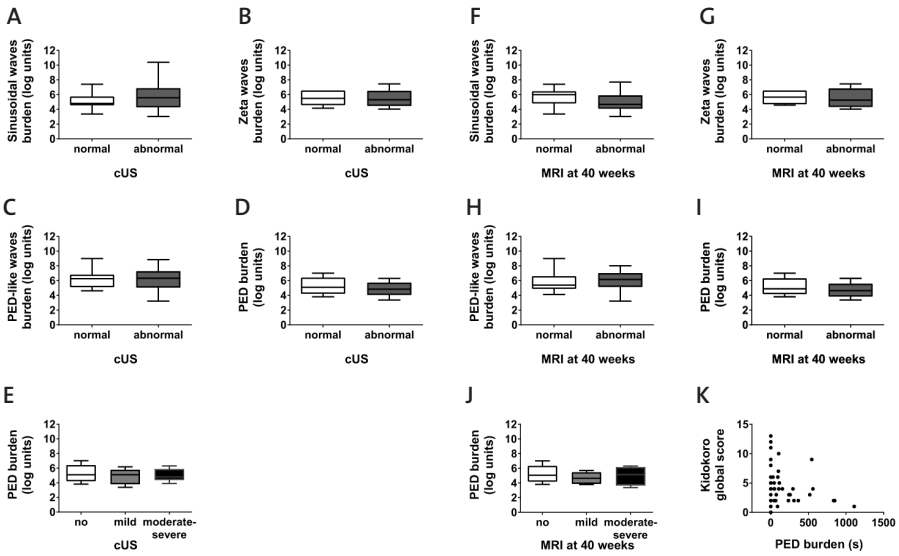

Figure 3. Association between EEG patterns and brain injury on cranial ultrasound (cUS) during the first 72 h (A-E) and MRI at term-equivalent age (F-K). Comparing the log-transformed total duration of each pattern (burden) in those with and without cUS (A-D) or MRI (F-I) abnormalities. (E, J) Comparing the log-transformed PED burden between those with no, mild or moderate-severe injury on cUS (E) and MRI (J). (K) Scatterplot showing the association between the PED burden and the MRI maturation and injury severity score (Kidokoro global score). [28]

EEG patterns versus outcome

Sinusoidal, zeta and PED-like waves: Of the patterns influenced by head position, the proportion of infants with sinusoidal (n = 6 [85.7%], p = 0.015) and PED-like waves (n = 5 [71.4%], p = 0.018) was significantly higher in infants who died, but no relation was found between the total duration of these patterns and the BSITD-III cognitive composite score at 2 years or IQ at 5 years. No associations were found between the incidence or total duration of zeta waves and any of the outcome parameters.

PEDs: The incidence and total duration of PEDs was not associated with death, the BSITD-III cognitive composite score at 2 years or IQ at 5 years *(figure 4).*

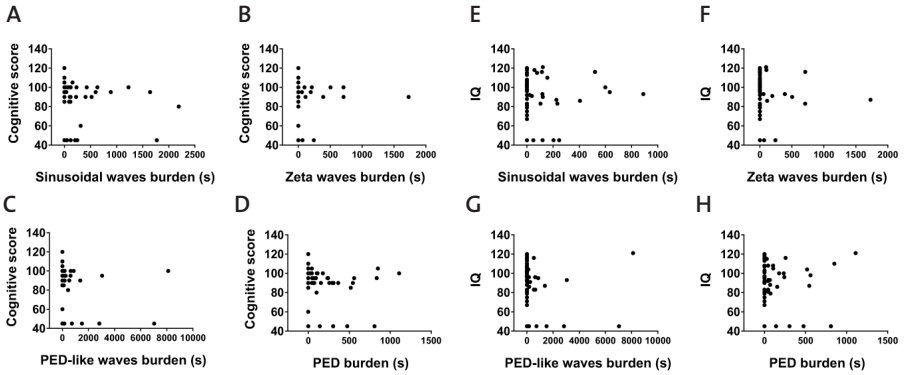

Figure 4. Association between the total duration of the EEG patterns in seconds and the BSITD-III cognitive composite score at 2 years (A-D) and the WPPSI-III-NL total IQ score at 5 years (E-H).

EEG patterns and the effect of morphine and AEDs

The incidence of sinusoidal (+24.3%, p = 0.062), zeta (+16.1%, p = 0.074), and PED-like waves (+17.1%, p = 0.131) increased, while the incidence of PEDs (-11%, p = 0.366) decreased non-significantly when morphine was administered during the first 72 h compared to infants who did not receive morphine. In 11 patients a total of 28 AEDs were administered. In 18 cases the AED was given during a rhythmic pattern, which had a temporary effect on ictal discharges in one, but no effect was seen on any of the other patterns. In 10 cases no rhythmic pattern was observed at the time of AED administration, but in five a rhythmic pattern (sinusoidal, zeta and PED-like waves or PEDs) emerged during administration or shortly thereafter (within 4 h).

DISCUSSION

In a cohort of 77 extremely preterm infants we found that rhythmic EEG patterns are common during the first 72 h after birth (62.3%). However, ictal discharges were only observed in one infant during this period (1.3%). This infant had meningitis and died on day 4 of life. Of the remaining patterns, three (sinusoidal, zeta, and PED-like waves) were significantly related to head position and are likely artefacts. PEDs were not related to head position, nor to brain injury or poor cognitive outcome.

The NICU is an artefact sensitive environment for EEG recording. Appliances like high frequency ventilation or the electrocardiogram can cause rhythmic artefacts on EEG, especially when the EEG background activity is suppressed. Many artefacts have been described by Andre *et al.* and Hagmann *et al.* [16, 31] However, other machines or actions in the NICU could disturb the EEG signal, causing yet unknown artefacts that are more likely to be picked up with long-term monitoring lasting for several days. The significant relation we found between the location of sinusoidal, zeta, and PED-like waves on EEG and the infant's head position is striking and strongly suggests that these patterns are artefacts. Sinusoidal and zeta waves have been described in adults and children and were related to serious brain injury and epilepsy, which is contradictive to our results. [21-23, 32] The frequency of sinusoidal waves was usually around 0.5 to 1 Hz in our cohort. This could correspond to a respiration and pulse artefact, which usually have a frequency of 60 and 120 /min respectively. We found the incidence of sinusoidal waves to be higher in infants with injury on cUS during the first 72 h after birth that subsequently died before an MRI could be made, because this association was no longer seen with MRI at term-equivalent age. We suspect these infants had severe brain injury with a very suppressed EEG making them more susceptible to showing artefacts. This suspicion is strengthened by the fact that the incidence of PED-like waves, which also had a strong relation with head position, was also significantly higher in infants who died. PED-like and zeta waves usually had a frequency of 1 to 2 Hz in our cohort, but we have not been able to relate these to a known artefact. The incidence of sinusoidal, zeta, and PED-like waves increased, although not significantly, when morphine was administered. Morphine has been shown to suppress EEG activity, [33] making it more susceptible to showing artefacts. Combined with the fact that AEDs did not have an effect on sinusoidal, zeta, and PED-like waves, this strengthens our hypothesis that these are artefacts.

PEDs were the only pattern, apart from ictal discharges, that was not related to head position in our cohort. A non-significant decrease in incidence was seen when morphine was administered, which supports our hypothesis that PEDs are brain activity, since morphine suppresses brain activity, and not artefacts. However, PEDs were also not related to brain injury or poor cognitive outcome in extremely preterm infants. These findings are contradictive to results from adult and paediatric studies, where PEDs have been associated with brain lesions, epilepsy, and poor outcome. [18-20] Even in full-term infants they have been associated with herpes simplex encephalopathy, hypoxic-ischaemic encephalopathy and stroke in particular. [34-36] Even more contradictory was the possible negative association observed between PED burden and the global Kidokoro score. This suggests that a higher PED burden could be associated with brain maturation

in extremely preterm infants. Recent studies have shown that increased brain activity during the first 72 h after birth in the form of bursts or spontaneous activity transients results in faster brain growth in preterm infants [37] and that this activity is hyper-synchronised in extremely preterm infants.[37,38] It could be postulated that PEDs are a form of spontaneous, hyper-synchronised activity that is related to normal brain development, but should not persist into infancy and childhood.

The incidence of subclinical electroencephalographic seizures was much lower in our study than previously reported. Scher *et al.* reported an incidence of 3.9% in infants with a postmenstrual age ≤30 weeks. However, EEG was only performed when seizures were suspected clinically and this was not restricted to the first 72 h after birth. [8] Therefore, the a priori chance of seizures was higher. Shah *et al.*, Wikström *et al.* and Vesoulis *et al.* recorded infants with a gestational age <30 weeks prospectively during the first 72 h after birth, but reported an incidence of 22-48%, which is much higher than we report.[3,8,39] A possible explanation for this difference could be that PEDs were considered seizures in those reports, since the incidence of PEDs in our cohort (44%) is strikingly similar to their incidence of seizures. PEDs had a similar morphology as ictal discharges in our study, but had a lower frequency and did not show evolution in amplitude, frequency or morphology. Also, no change in the aEEG trace was observed as opposed to ictal discharges. Ictal discharges in preterm infants have been reported to be lower in frequency and shorter in duration. Different patterns of evolution in frequency such as acceleration and unchanged frequency as opposed to deceleration have been reported as well in preterm infants.[40] In this respect PEDs could be considered as ictal discharges. However, PEDs were not related to brain injury in our cohort and outcome was normal independent of high PED burden. Patterns resembling PEDs that do not show evolution on 2-channel EEG should therefore be considered with caution. They should not immediately be marked as seizures and treatment should be withheld until subclinical electroencephalographic seizures are confirmed on multichannel EEG. Especially, since AEDs did not have an effect on PEDs in our cohort.

It should be noted that this study has some limitations. First, we only assessed the EEG at the markers placed by the seizure detection algorithm of the BrainZ monitor.[11] We chose to do so because in everyday clinic, assessment of long-term EEG recordings is mostly limited to the parts where markers are placed by the seizure detection algorithm and parts with changes in the aEEG trace. However, the BrainZ algorithm has a sensitivity of 83-95% for detecting seizures in full-term infants, and it is likely that the algorithm did not detect all rhythmic EEG patterns and the true burden of these patterns may be

higher than reported in this study.[11] The sensitivity in extremely preterm infants and the sensitivity for non-seizure rhythmic patterns are unknown. Second, the EEG assessments were made with continuous 2-channel EEG without extra polygraphic variables such as electrocardiogram and respiration. Multichannel video-EEG remains the gold standard for detection and quantification of rhythmic patterns and seizures.[41] It can localize the different patterns to certain areas and when polygraphy is used common artefacts such as respiration can easily be ruled out. Furthermore, experienced technicians will detect movement artefacts and artefacts due to inadequate electrode impedance.

Clinicians faced with rhythmic EEG patterns in 2-channel EEG of extremely preterm infants should first place the infant's head in the midline and make sure the electrodes are free from contact with surrounding materials and be placed correctly. This will eliminate several artefacts. If rhythmicity is still observed, only when clear evolution in amplitude, frequency or morphology is visible, treatment with AEDs should be considered.[3-7] The aEEG can aid in distinguishing PEDs from ictal discharges, showing a simultaneous rise in the lower border of the aEEG with ictal discharges. Patterns resembling sinusoidal, zeta or PED-like waves do not need treatment. When PEDs are observed, a multichannel video-EEG should be performed to confirm or rule out ictal discharges. Further research is needed to investigate the significance of PEDs in preterm brain development.

CONCLUSION

Rhythmic EEG patterns are common in extremely preterm infants, but ictal discharges were only observed in 1.3%. Furthermore, three patterns (sinusoidal, zeta and PED-like waves) were significantly related to head position and are likely artefacts. PEDs were common and not related to head position, but were also not associated with brain injury or poor cognitive outcome.

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Chapter 3

Severe hypercapnia causes reversible depression of aEEG background activity in neonates: an observational study

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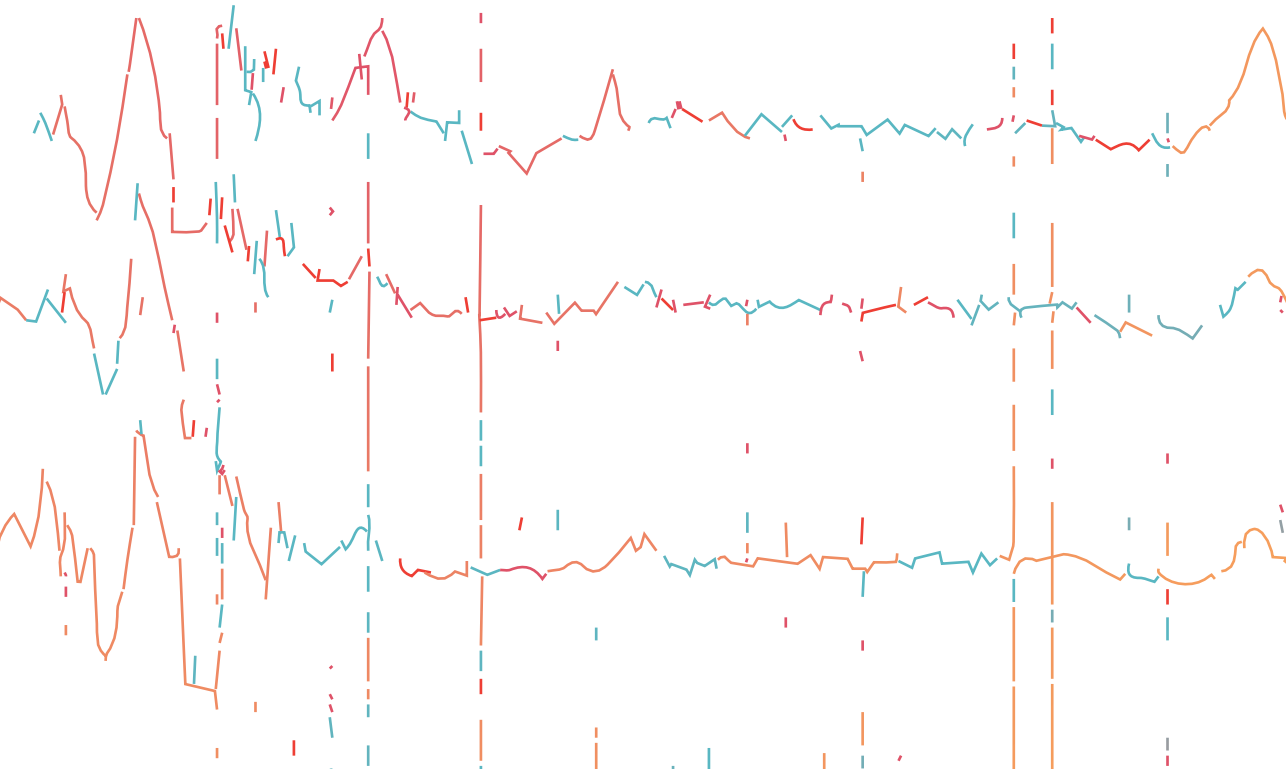

ABSTRACT

Introduction Elevated carbon dioxide (CO_2) blood levels have a depressant effect on the central nervous system and can lead to coma in adults. Less is known about the effect of CO_2 on the neurological function of infants. Our objective was to describe the effect of acute severe hypercapnia ($PaCO_2$ >70 mm Hg) on amplitude-integrated electroencephalography (aEEG) and cerebral oxygenation in newborn infants.

Methods Observational study of full-term and preterm infants with acute severe hypercapnia (identified by arterial blood gas measurements), monitored with aEEG. Visual analysis of the aEEG was performed in all infants. In preterm infants <32 weeks postmenstrual age (PMA), analysis of two-channel EEG was performed. Mean spontaneous activity transients (SAT) rate (SATs/min), interval between SATs (ISI in seconds) and the ISI percentage (ISP) were calculated for 10-min periods before, during and after hypercapnia. Mean regional cerebral oxygen saturation ($rScO_2$) and fractional tissue oxygen extraction (FTOE) measured with near-infrared spectroscopy were also calculated for these periods.

Results Twenty-five infants (21 preterm, 4 full-term) comprising 32 episodes of acute severe hypercapnia were identified. Twenty-seven episodes were accompanied by a transient aEEG depression. Twenty-two episodes in 15 preterm infants <32 weeks PMA were quantitatively analysed. During hypercapnia, SATrate decreased and ISI and ISP increased significantly. No significant change occurred in $rScO_2$ or FTOE during hypercapnia.

Conclusion Profound depression of brain activity due to severe hypercapnia is also seen in infants. It can be recognised by an acute depression of the aEEG, without clinically detectable changes in cerebral oxygenation.

INTRODUCTION

Elevated carbon dioxide (CO_2) blood levels have a depressant effect on the central nervous system and electroencephalography (EEG) activity. [1,2] In adults, hypercapnia can lead to an altered mental state, encephalopathy and coma. [3] CO_2 narcosis has been described in adults with a $PaCO_2$ of 62-269 mm Hg, but the cerebral response to hypercapnia showed a broad inter-individual variability. [2] Less is known about the effect of high CO_2 on the neurological function in infants. High CO_2 levels usually occur in critically ill infants, in whom neurological examination can be difficult, especially when they are preterm and/or sedated. However, EEG and amplitude-integrated EEG (aEEG) are now widely used in the neonatal intensive care unit (NICU) to monitor brain activity. In preterm infants, the depressant effect of CO_2 on EEG has been described. Hypercapnia resulted in longer interburst intervals (IBIs) and decreased total EEG power. [4,5] The phenomenon of CO_2 narcosis as seen in adults has not been described in infants. The aim of this study was to describe the effect of acute, severe hypercapnia on brain activity, cerebral oxygenation and haemodynamics in infants. Our hypothesis was that CO_2-mediated neurological suppression also occurs in the neonatal period and results in profound EEG depression, an increase in cerebral oxygenation due to vasodilatation and a decrease in cerebral oxygen consumption due to reduced brain activity, without significant changes in haemodynamic parameters.

METHODS

Patients

This was an observational study in two Dutch level III NICUs followed by retrospective data analysis. Full-term and preterm infants with acute severe hypercapnia ($PaCO_2$ >70 mm Hg) while being monitored with aEEG between April 2009 and May 2015 were included. To report anonymous data of clinical events, a waiver to obtain ethical approval for the study was received from the ethical committee of both hospitals in compliance with Dutch national regulations.

Neuro-monitoring (aEEG and near-infrared spectroscopy)

Continuous neuro-monitoring included aEEG, to assess brain activity and screen for seizures, and near-infrared spectroscopy (NIRS), to assess cerebral oxygenation. neuro-monitoring was performed as standard of care in the Wilhelmina Children's Hospital in Utrecht, in infants following preterm birth <28 weeks of gestation, when at risk for

developing seizures (e.g. sepsis, meningitis, perinatal asphyxia), during surgery and during therapeutic hypothermia in both centres. For single-channel aEEG (P4-P3), the Olympic 6000 cerebral function monitor (Natus, Seattle, Washington, USA) was used. For two-channel aEEG (F4-P4, F3-P3) digital BRM2 and BRM3 BrainZ (Natus) and NicoletOne (Natus) monitors were used. To assess regional cerebral oxygen saturation ($rScO_2$) and fractional tissue oxygen extraction (FTOE), a two-wavelength (730 and 810 nm) near-infrared spectrometer (INVOS 4100-5100, Covidien, Mansfield, Massachusetts, USA) with a fronto-parietal transducer (small adult SomaSensor SAFB-SM, Covidien) was used.

Haemodynamic parameters

Heart rate, mean arterial blood pressure (MABP), measured via an indwelling arterial catheter, and arterial oxygen saturation (SaO_2) were monitored in all infants using a Philips Intellivue MP70 patient monitor. The fraction of inspired oxygen (FiO_2) as measured on the ventilator was documented every minute.

Blood gas analysis

Arterial blood gas measurements (including pH, $PaCO_2$, PaO_2, base excess, bicarbonate and glucose) were performed intermittently, mostly with a four hourly interval, depending on the infant's clinical condition.

Data analysis

The aEEG background activity was visually determined in all infants, both full-term and preterm, by an experienced neonatologist (MCT), who was blinded to the $PaCO_2$ levels, using the background activity classification described by Toet *et al.* [6] The raw EEG signal of infants monitored with a BRM BrainZ monitor and, simultaneous $rScO_2$, FTOE, heart rate, MABP and SaO_2 measurements were analysed using in-house developed software (SignalBase, version 8.5.2, University Medical Center Utrecht, Utrecht, the Netherlands). Artefacts caused by, for example, movement in any of the signals were manually removed. Only infants with a postmenstrual age (PMA) <32 weeks at the time of hypercapnia were included because the quantitative analysis of the raw EEG signal can only be reliably performed on discontinuous EEG backgrounds. After 32 weeks PMA, the EEG becomes more continuous. The raw EEG signal (P4-P3, sample rate 256 Hz) was segmented into spontaneous activity transients (SATs, periods of cortical activity) and interSAT intervals (ISIs, time between SATs, periods of cortical inactivity, comparable to IBI) using a non-linear energy operator in the SignalBase software. [7] This method assumes that the EEG consists of periods of activity alternated with periods of inactivity, which corresponds to a discontinuous background activity. For each infant, a 10-min period was selected

5-10 min before a documented $PaCO_2$ >70 mm Hg. Another 10-min period was selected 5-10 min before the first documented $PaCO_2$ in the normal range (35-45 mm Hg) before and after hypercapnia. In case of permissive hypercapnia, which is the acceptance of hypercapnia in infants in whom ventilation is difficult, the first documented $PaCO_2$ <60 mm Hg before and after hypercapnia was chosen. For each period (before, during and after), the mean number of SATs per minute (SATrate), mean ISI duration in seconds and the percentage of the recording made up by ISI (ISP) were calculated. Mean values of $rScO_2$, FTOE, heart rate, MABP, SaO_2 and FiO_2 were calculated for each period as well.

Statistical analysis

SPSS version 21 (IBM, Armonk, New York, USA) was used to perform t-test or Fisher's exact test to compare the patient characteristics between infants with and without a visual aEEG change. Repeated measures analysis of variance (ANOVA) with Bonferroni correction or Friedman's ANOVA in case of non-normally distributed data was performed to compare periods before, during and after hypercapnia. A p-value <0.05 was considered statistically significant.

RESULTS

Twenty-five infants with a total of 32 episodes (two episodes n = 1, three episodes n = 3) of acute, severe hypercapnia were identified. Four were full-term infants (≥37 weeks gestational age [GA]) and 21 were preterm. Hypercapnia was caused by suboptimal mechanical ventilation (n = 15, 46.9%), tube obstruction (n = 9, 28.1%), pneumothorax (n = 4, 12.5%), tube dislocation (n = 1, 3.1%), pulmonary haemorrhage (n = 1, 3.1%), pulmonary interstitial emphysema (n = 1, 3.1%) or respiratory insufficiency (n = 1, 3.1%). The reasons for EEG monitoring were postoperative monitoring (n = 13, 40.6%), preterm birth <28 weeks gestation (n = 9, 28.1%), being at risk of developing seizures (n = 7, 21.9%) and therapeutic hypothermia (n = 3, 9.4%). Only one infant was not on the ventilator at the time of hypercapnia, this was a preterm born infant, who presented at a PMA of 35.5 weeks with acute, severe hypercapnia five hours after extubation, due to respiratory insufficiency, but without a visible change in the aEEG. Patient characteristics are shown in *table 1*.

Visual analysis aEEG - full-term and preterm infants

Twenty-seven episodes of severe hypercapnia were accompanied by a sudden depression of the aEEG trace *(figure 1)*, from discontinuous normal voltage (DNV) to dense burst suppression (BS+) in six (18.8%), to sparse burst suppression (BS-) in six (18.8%) and to flat trace (FT) in four (12.5%), from BS+ to BS- in six (18.8%) and to FT in one (3.1%), from BS- to FT in two (6.3%), from continuous normal voltage to DNV in one (3.1%) and to FT in one (3.1%). The background activity recovered within one hour of normalisation of the $PaCO_2$ in all patients. During five hypercapnic episodes no change in the aEEG trace was seen. No significant difference in $PaCO_2$ or other clinical parameters was found between those with and those without a visible change in the aEEG trace *(table 1)*. However, infants without a visible aEEG change tended to have a higher GA at birth and were older at the time of hypercapnia ($p = 0.081$).

Quantitative analysis - preterm infants

Data from 15 preterm infants <32 weeks PMA comprising 22 hypercapnic episodes were available for quantitative data analysis. Mean blood gas measurements and EEG, cerebral oxygenation and haemodynamic parameters before, during and after hypercapnia are shown in *table 2*.

Electroencephalography

A significant decrease in SATrate and increase in ISI and ISP was seen during hypercapnia *(table 2, figure 2A, E)*. Of the three episodes not accompanied by a visible depression in the aEEG, two showed a decrease in SATrate and increase in ISI and ISP during hypercapnia, one showed an increase in SATrate and decrease in ISI and ISP during hypercapnia.

Cerebral oxygenation

A non-significant increase in $rScO_2$ and decrease in FTOE was seen during hypercapnia *(table 2, figure 2B, F)*.

Haemodynamic parameters

No changes in heart rate and MABP were seen during hypercapnia *(table 2, figure 2C, G)*. SaO_2 decreased non-significantly during hypercapnia but increased significantly afterwards. FiO_2 increased significantly during hypercapnia *(table 2, figure 2D, H)*.

Table 1. Patient characteristics

Total n = 25 patients, n = 32 episodes	Preterm n = 21	Full-term n = 4	Visible aEEG change n = 27	No visible aEEG change n = 5	p-value
Male, n (%)	12 (57.1)	1 (25)	14 (51.9)	3 (60)	1.000
GA at birth (weeks), median (IQR)	27.3 (25.0-29.5)	38.5 (38.2-40.6)	27.3 (25.1-32.1)	29.2 (25.6-32.6)	0.920
Birth weight (grams), median (IQR)	935 (750-1468)	3218 (2729-3845)	935 (750-1490)	1150 (890-1905)	0.968
GA at hypercapnia (weeks), median (IQR)	28.6 (26.5-31.2)	39.1 (38.5-41.0)	28.4 (26.3-32.3)	30.0 (27.4-37.3)	0.479
Age at hypercapnia (hours), median (IQR)	120 (46.5-441.4)	20.7 (7.2-131)	89.4 (39.0-309.2)	454.4 (112.7-934.5)	0.081
Sedatives, n (%)	18 (85.7)	4 (100)	25 (92.6)	4 (80)	0.410
Duration sedatives before aEEG change (hours), median (IQR)	27.3 (13.9-44.5)	16.6 (4.1-112)	23.4 (14.3-54.8)	61.1 (6.7-477.1)	0.831
IVH, n (%)					0.499
no	4 (19)	4 (100)	9 (34.6)	1 (20)	
IVH before hypercapnia, no progression	8 (38.1)	0	10 (38.5)	3 (60)	
IVH before hypercapnia, progression	3 (14.3)	0	2 (7.7)	1 (20)	
IVH after hypercapnia	5 (23.8)	0	5 (19.2)	0	

aEEG: amplitude-integrated EEG; GA: gestational age; IQR: interquartile range; IVH: intraventricular haemorrhage.

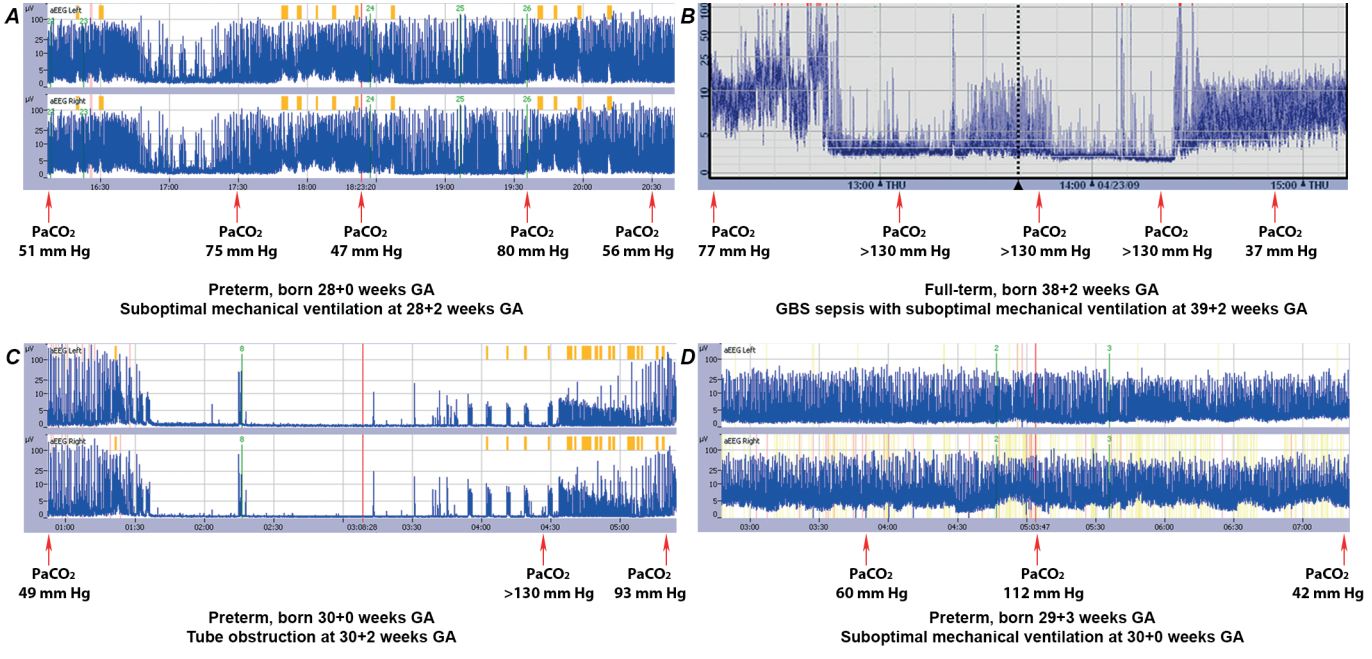

Figure 1. Examples of amplitude-integrated electroencephalography (aEEG) traces showing a sudden, but transient depression during hypercapnia (A-C). In panel D, no change in the aEEG trace was observed during hypercapnia. GA: gestational age; GBS: Group B *Streptococcus*.

Table 2. Blood gas measurements and EEG, cerebral oxygenation and haemodynamic parameters before, during and after hypercapnia in the 22 hypercapnic events that were quantitatively analysed

Blood gas measurements, mean (SD)	Before Median time before 3:06 (hour : min), IQR 2:05-4:03	During	After Median time after 1:25 (hour : min), IQR 0:55-2:46	p-value
pH	7.26 (0.04)	7.02 (0.10)	7.27 (0.04)	<0.001*,†
$PaCO_2$ (mm Hg)	50.70 (5.96)	95.65 (16.45)	49.40 (6.50)	<0.001*,†
PaO_2 (mm Hg)	57.05 (12.74)	57.70 (13.34)	60.15 (31.58)	0.892
Base excess (mmol/l)	-4.39 (1.72)	-5.39 (2.62)	-4.22 (1.99)	0.295*, 1.000†
Bicarbonate (mmol/l)	22.64 (1.66)	25.14 (2.15)	22.61 (1.98)	<0.001*,†
Glucose (mmol/l)	6.64 (1.82)	7.82 (1.79)	6.96 (1.56)	0.086
Quantitative parameters, mean (SD)	**Before**	**During**	**After**	**p-value**
EEG				
SATrate (/min)	4.06 (1.28)	2.42 (1.47)	4.88 (2.34)	<0.001*,†
ISI (sec)	13.00 (5.64)	33.10 (36.17)	12.59 (9.57)	0.004*, <0.001†
ISP (%)	79.24 (9.12)	87.91 (6.84)	76.12 (13.50)	0.001*, <0.001†
Cerebral oxygenation				
$rScO_2$ (%)	66.54 (12.01)	68.36 (11.66)	65.91 (13.22)	1.000
FTOE	0.32 (0.09)	0.23 (0.14)	0.35 (0.14)	0.327*, 0.064†
Haemodynamic parameters				
Heart rate (/min)	153 (16)	153 (17)	157 (20)	0.895
Blood pressure (mm Hg)	33.03 (3.44)	32.98 (8.16)	35.11 (6.07)	0.584
SaO_2 (%)	92.24 (2.11)	89.74 (3.65)	94.29 (2.25)	0.150*, 0.003†
FiO_2 (%)	30.86 (9.26)	44.30 (15.41)	35.19 (18.76)	0.004*, 0.061†

P-value markers: *before vs. during $PaCO_2$ change; †during vs. after $PaCO_2$ change. FTOE: fractional tissue oxygen extraction; ISI: interSAT interval; ISP: interSAT percentage; IQR: interquartile range; SaO_2: arterial oxygen saturation; SATrate: number of spontaneous activity transients per minute; SD: standard deviation.

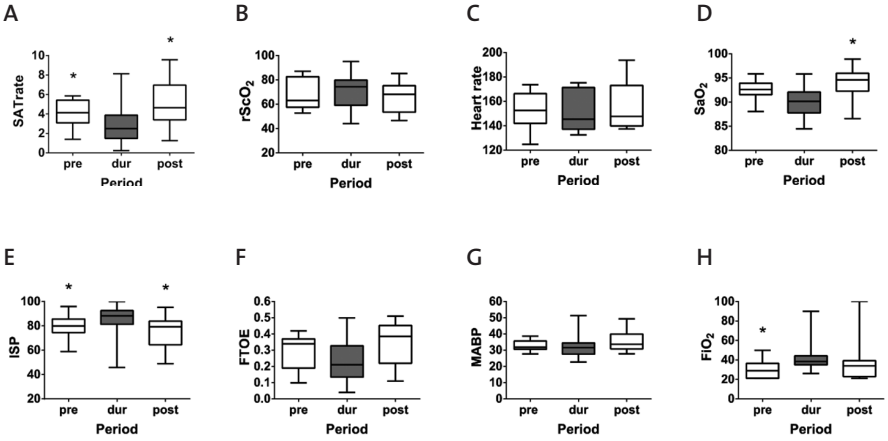

Figure 2. Boxplots comparing mean values of electroencephalography (A, E), cerebral oxygenation (B, F) and haemodynamic (C, G, D, H) parameters before (pre), during (dur) and after (post) hypercapnia. Asterisk (*) indicates a significant change compared with the period during hypercapnia. FTOE: fractional tissue oxygen extraction; ISP: interSAT percentage; MABP: mean arterial blood pressure; SATrate: number of spontaneous activity transients per minute.

DISCUSSION

This study shows that profound depression of brain activity during severe hypercapnia as has been described in adults also occurs in infants. It can be detected by visual inspection of the aEEG trace, showing an acute depression of the background activity, and by quantitative analysis of the EEG, showing a decrease in cortical activity (SATrate) and an increase in cortical inactivity (ISI, ISP). In our cohort, the depression of brain activity was transient. When $PaCO_2$ normalised after hypercapnia, the SATrate, ISI and ISP returned to normal as well. No significant changes were seen in $rScO_2$, FTOE, heart rate and MABP. SaO_2 did not change significantly during hypercapnia, but increased significantly afterwards. Mean values remained within normal ranges mediated through a significant change in FiO_2 from normocapnia to hypercapnia. As seen in adults, [2, 8] a broad interindividual variability in response to hypercapnia was seen in our cohort. Some infants showed severe visible aEEG depression and changes in quantitative EEG parameters, while others showed no visible depression to similar levels of hypercapnia and no or an inverse change in quantitative EEG parameters.

The depressant effect of $PaCO_2$ on EEG has been described in preterm infants. [4, 5] These studies showed that the IBI increased and the total EEG power decreased with increasing $PaCO_2$. However, the $PaCO_2$ levels in these studies were mainly within the normal range with only a few reaching severe hypercapnia levels. Our results support the findings in these studies and show in addition that $PaCO_2$ also affects the brain activity of full-term infants and that severe hypercapnia results in profound depression of brain activity, which can be clearly visible on the aEEG.

The effect of hypercapnia on cerebral oxygenation parameters such as $rScO_2$ and FTOE has been described by a number of studies. [5, 9, 10] Hino *et al.* [10] described an increase in cerebral blood flow and oxygen transport in newborn lambs with increasing $PaCO_2$ levels, but saw no increase in oxygen consumption by the brain, leading to an increase in $rScO_2$. A negative linear association between $PaCO_2$ and FTOE has been described, with $PaCO_2$ ranging from hypocapnic to mild hypercapnic levels. [5, 9] This association can be explained by the reduction of electrical activity in the brain during hypercapnia, leading to decreased oxygen consumption by the brain, since electrical activity is the most energy requiring process in the brain. [11] These observations are supported by our findings as $rScO_2$ increased during hypercapnia and FTOE decreased. However, we did not find these relations to be significant, unlike Vanderhaegen *et al.,* [9] who described a significant positive linear relation between $PaCO_2$ and the tissue oxygenation index, equivalent to

rScO2, in a group of low birth weight preterm infants. This could be explained by the effect of hypoxia on the $rScO_2$. In most of our infants, the SaO_2 decreased during hypercapnia because of an acute respiratory problem. When hypoxia is present, the $rScO_2$ decreases, especially when autoregulation is absent. Therefore, in the presence of hypoxia, the effect of $PaCO_2$ on $rScO_2$ will be attenuated. FTOE corrects for the effect of SaO_2, but was also not significantly affected by the change in $PaCO_2$ from normocapnia to hypercapnia. However, this could be due to the limited number of infants with FTOE values available for analysis. Many studies have tried to unravel the mechanism of action of CO_2 narcosis. Based on animal and human studies, the most likely hypothesis is that the effect of $PaCO_2$ on the central nervous system is mediated through alteration of the intracerebral pH. [12] Both respiratory and metabolic acidosis have been related to EEG depression, but the depression of cerebral function was more consistently correlated with $PaCO_2$ than bicarbonate levels. [13-15] Furthermore, Victor *et al.* [5] did not find a relation between pH and EEG activity. CO_2 seems to have a more profound effect on brain function, because the blood-brain barrier is permeable to CO_2 and relatively impermeable to bicarbonate ions, [16] causing a more rapid crossing of CO_2 over the blood-brain barrier than hydrogen ions.

Hypoxia has only been related to electrocortical depression at extremely low SaO_2 levels (<24%) in newborn piglets, [17] while a study in newborn lambs showed no effect of hypoxia on electrocortical activity. [18] In our cohort, all SaO_2 values remained within the normal range during hypercapnia; it is therefore unlikely that hypoxia played a role in the observed EEG depression.

Wikström *et al.* showed that plasma glucose levels were also associated with EEG activity. [4] In our cohort, plasma glucose concentration did not change significantly during hypercapnia. It is well known that medication such as morphine and benzodiazepines, which are often used in infants admitted to the NICU for sedation or as antiepileptic drugs, can suppress the EEG. [19, 20] The use of sedatives was unlikely to have caused the sudden depression in the aEEG in our cohort because almost all infants were already receiving sedatives for several hours or even days. The fast recovery of the aEEG after normalisation of $PaCO_2$ underlines the profound effect CO_2 has on brain activity, since many of the potential confounding factors, such as the use of sedatives, were constant over this short period of time. It is of interest that during five hypercapnic episodes no EEG depression was visible and that these infants had a higher GA at birth and postnatal age at the time of hypercapnia.

The consequences of CO_2 narcosis in infants are not well known. Extreme fluctuations in $PaCO_2$ and a higher maximum $PaCO_2$ have been associated with worse

neurodevelopmental outcome in very low birth weight preterm infants. [21] This may be mediated through the induction of brain damage such as intracranial haemorrhage and periventricular leukomalacia. [22, 23] Prolonged depression of cortical function has been associated with impaired outcome in preterm infants. [24, 25] However, the EEG depression in CO_2 narcosis is reversible and should therefore be corrected as soon as possible.

This study has some limitations. First, the study design did not allow more thorough investigation of the relation between $PaCO_2$, EEG and cerebral oxygenation, because it was an observational retrospective cohort, without continuous CO_2 measurements. However, in preterm infants reliable end-tidal CO_2 measurements are difficult to obtain. Even though we have no continuous CO_2 data, it is almost certain that hypercapnia was acute in the cases described because $PaCO_2$ was normal during the previous blood gas measurement and the aEEG depression was very sudden. Also, Victor *et al.* [26] showed that chronic hypercapnia does not cause changes in IBI in preterm infants. Second, a comprehensive method for quantitative assessment of EEG in full-term infants is not yet available, limiting quantitative analyses to preterm infants only and reducing the sample size.

CONCLUSION

In conclusion, acute hypercapnia can cause reversible depression of the aEEG background activity, without clinically detectable changes in cerebral oxygenation and other haemodynamic parameters in infants. A sudden depression in the aEEG background activity in these infants should alert clinicians to suspect a high $PaCO_2$ due to a respiratory problem.

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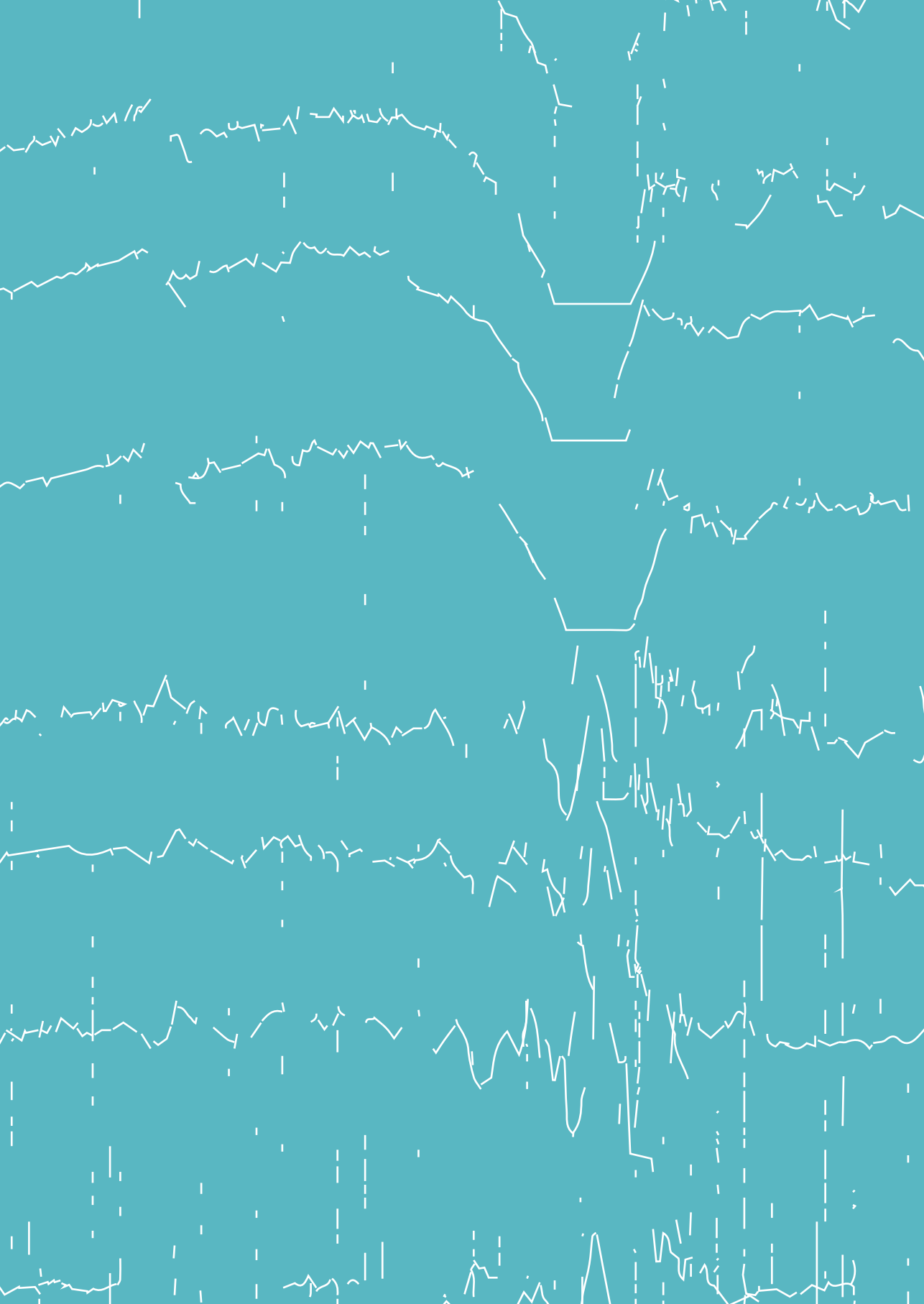

PART 1.2

Diagnosis - Neuro-imaging

Chapter 4

Neuro-imaging in neonatal seizures

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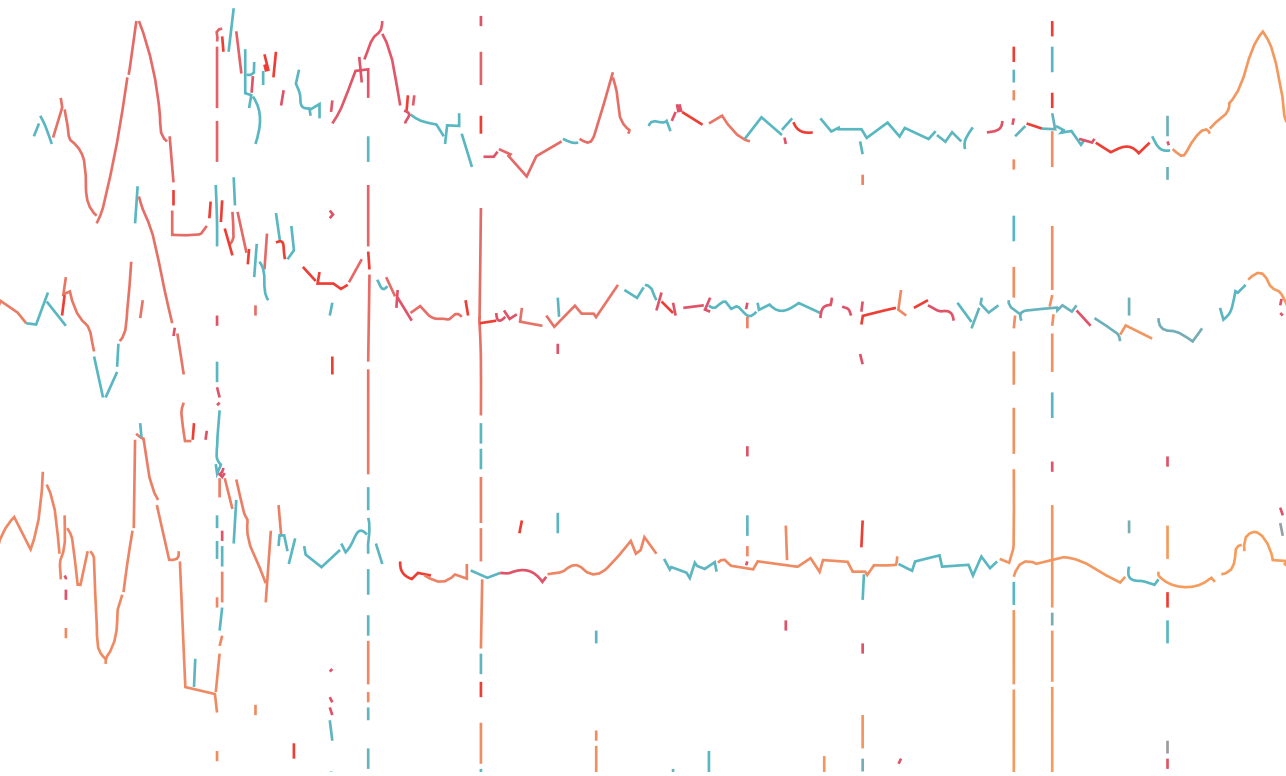

ABSTRACT

Seizures are the most common sign of neurological dysfunction in full-term neonates, with an incidence estimated at 0.15-3.5/1000 live births. Neonatal seizures often reflect severe underlying brain injury and are associated with high rates of mortality and morbidity. Prognosis is primarily determined by the nature, site and extent of the underlying aetiology, making accurate diagnosis and identification of associated brain lesions essential. Data on neuro-imaging in newborns presenting with seizures is limited and most studies report on MRI findings in infants with a specific underlying problem, such as hypoxic-ischaemic encephalopathy, stroke or metabolic disorders. The aim of this review is to discuss the spectrum of neuro-imaging findings in full-term newborns presenting with seizures, divided into subgroups with different underlying aetiologies. A standard neonatal MRI protocol is presented.

INTRODUCTION

Seizures occur more often during the neonatal period than in any other period of life and have a variety of underlying aetiologies. The incidence varies between 0.15 and 3.5 per 1000 live births, with higher rates in preterm infants. It also depends on the threshold for using continuous amplitude-integrated or standard electroencephalography (aEEG) monitoring. [1] In previous studies, the mortality has been reported to be as high as 40%, but in more recent studies the mortality has come down to 21% [2] and even 7%. [3] However, in contrast to this increase in survival, the prevalence of long-term neurodevelopmental sequelae in survivors has been reported to be about 30%. [4] In a more recent study, 70 (66%) of 106 preterm and full-term infants admitted to a neonatal intensive care unit (NICU) had an adverse neurological outcome. Six variables were identified as the most important independent risk factors. Neuro-imaging was one of these six variables, but only cranial ultrasound (cUS) was used. [5] Aetiologies associated with a poor outcome include cerebral dysgenesis, severe hypoxic-ischaemic encephalopathy (HIE), metabolic disorders, and infection of the central nervous system (CNS). Conversely, infants with focal infarction, transient metabolic disturbances, or idiopathic seizures have been reported to have a favourable outcome. Most NICUs around the world use cUS as the method of first choice. Computed tomography (CT) is now less commonly used and should only be performed in an infant who may acutely need neurosurgical intervention. Magnetic resonance imaging (MRI) is increasingly used and recognised as the best imaging modality. The Vermont Oxford neonatal encephalopathy registry showed that 22.7% of infants admitted with neonatal encephalopathy still had a CT scan and almost two thirds (65%) had an MRI during the neonatal period. [6] In this population, admitted between 2006 and 2010, 67% received antiepileptic medication during their stay in the NICU. These imaging data are in agreement with data from Tekgul *et al*. [3] who studied 89 full-term infants with neonatal seizures. In this study, 82% had at least one MRI scan, whereas 18% only had a CT scan. Several studies have shown that in the full-term infant, HIE is by far the most common aetiology, followed by intracranial haemorrhage (ICH) and stroke *(table 1)*. These studies were mostly performed before the introduction of therapeutic hypothermia and recent studies have shown that neonatal seizures are less common and better controlled in infants with moderate HIE, treated with hypothermia. [7, 8] Transient metabolic disturbances or inborn errors of metabolism, infection of the CNS, cerebral dysgenesis, and genetic disorders are less common underlying aetiologies for neonatal seizures. Some of these infants may present with encephalopathy and are referred to as “HIE mimics”. Neuro-imaging may help to diagnose an underlying problem, for instance polymicrogyria in Zellweger syndrome, which is important for

genetic counselling. For those infants who die during the neonatal period and are too unstable to be transported to the MRI unit, a post-mortem MRI should be considered, especially when there is no permission for autopsy. The value of a post-mortem MRI has been shown by several groups. [9, 10] The number of infants with unknown aetiology is decreasing, as lesions that may not be recognised by cUS or CT are identified by MRI, but also because of advances made in genetics. [11, 12] Data on neuro-imaging in newborns presenting with neonatal seizures are scarce and most studies report MRI findings in a specific group of infants, with a diagnosis of either HIE, stroke or metabolic disorders. [3, 13] The aim of this review is to discuss the spectrum of neuro-imaging findings in full-term infants with neonatal seizures with different underlying aetiologies. Examples of cUS and MRI performed during the first week after birth in infants presenting with seizures discussed below are presented in *figures 1* and *2*.

Hypoxic-ischaemic encephalopathy

Several studies have been reported in the literature concerning different patterns of injury in relation to HIE. Two main patterns of injury can be recognised. The first one predominantly affects the central grey nuclei (ventrolateral thalami and posterior putamina) and perirolandic cortex, bilaterally. Associated involvement of the hippocampus and brain stem is not uncommon. This pattern of injury (basal ganglia-thalamic) is most often seen following an acute sentinel event, for instance, a ruptured uterus, placental abruption or a prolapsed cord, and is also referred to as a pattern seen after "acute near-total asphyxia". [14, 15] When MRI is performed during the first week, diffusion-weighted images (DWI) may highlight the abnormalities, which first become apparent by the end of the first week on conventional imaging. [16] The second pattern is referred to as the watershed (WS), a predominant pattern of injury seen following "prolonged partial asphyxia". The vascular WS zones (anterior-middle cerebral artery and posterior-middle cerebral artery) are involved, affecting the white matter and in more severely affected infants, also the overlying cortex. The lesions can be uni- or bilateral, posterior and/or anterior. [17] Although loss of the cortical ribbon and therefore the grey-white matter differentiation can be seen on conventional MRI, DWI highlights the abnormalities and is especially helpful in making an early diagnosis *(table 2)*. More widespread punctate lesions in the white matter (PWML) have also been reported within the HIE population. Li *et al*. found these PWML in 23% of their infants with neonatal encephalopathy and pointed out that infants with this type of injury had a significantly lower gestational age at birth with a milder degree of encephalopathy and fewer clinical seizures relative to other newborns in their cohort, who were diagnosed to have the two more common patterns of injury. [18] This pattern of brain injury is also seen in newborn infants with

congenital heart defects. [18, 19] The pattern of brain injury seen on MRI can predict the severity and type of neurodevelopmental dysfunction in later life, [17, 20, 21] although the degree of encephalopathy and the extent of EEG abnormalities, including seizures, are also predictive of subsequent neurodevelopmental outcome. [22, 23] Basal ganglia-thalamic injury is often associated with a worse neurodevelopmental outcome in general and is primarily associated with motor deficits, in particular dyskinetic cerebral palsy, which can already be detected at 12-18 months of age.

Table 1. Aetiologies and incidence of neonatal seizures in full-term infants

	Tekgul *et al.* 2006	**Mastrangelo *et al.* 2005**	**Yildiz *et al.* 2012**	**Ronen *et al.* 1999**	**Weeke *et al.* 2015**
HIE	40%	37.1%	28.6%	40%	46%
ICH	17%	4.8%	17%	18%	12.2%
Stroke	18%	11.3%	NR	1 case	13.5%
Infection	3% (CNS only)	9.7%	7.2% (+sepsis)	20% (+ sepsis)	7.6% (+sepsis)
Cerebral dysgenesis	5%	11.3%	4.5%	10%	2.9%
Metabolic disorders	1%	11.3%	10.7%	19% (including hypoglycaemia)	9% (including hypoglycaemia)
Unknown/ idiopathic	12%	1.6%	8.9%	14%	6.3%

CNS: central nervous system; HIE: hypoxic-ischaemic encephalopathy; ICH: intracranial haemorrhage; NR: not reported.

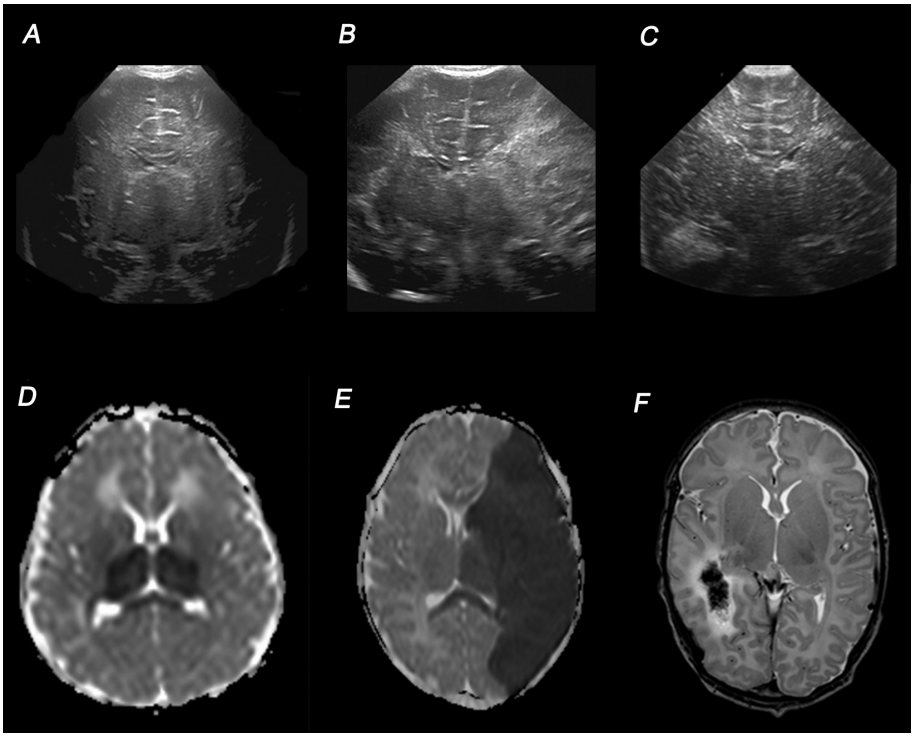

Figure 1. Examples of coronal cUS and axial MR images performed during the first week after birth in infants presenting with neonatal seizures. (A) HIE: hyperechogenicity in both thalami on cUS and low signal intensity on the ADC map in thalami, optic radiation and splenium of the corpus callosum (D); (B) PAIS of main branch of the left-middle cerebral artery: wedge-shaped hyperechogenicity with a linear demarcation line in the left hemisphere on cUS and low signal intensity on the ADC map in territory of the left-middle cerebral artery, as well as optic radiation and splenium of the corpus callosum (E); (C) haemorrhage in the right temporal lobe recognised on cUS as a round area of hyperechogenicity and on T2-weighted image as an area of low signal intensity surrounded by high signal intensity due to oedema (F).

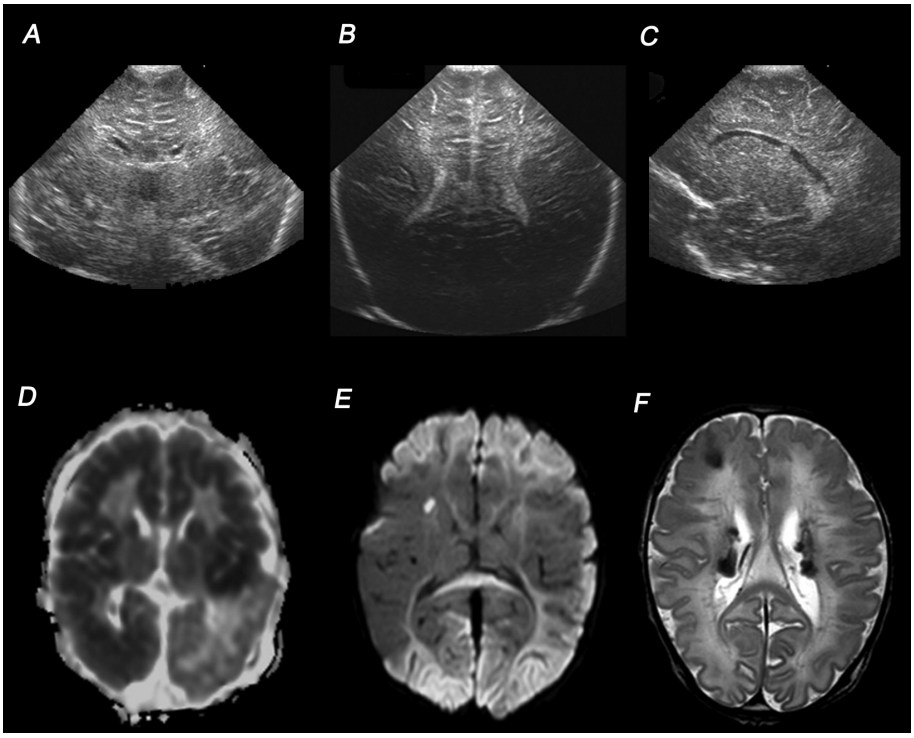

Figure 2. Examples of coronal (A, B) and sagittal (C) cUS and axial MRI performed during the first week after birth in infants presenting with seizures. (A) molybdenum cofactor deficiency with diffuse hyperechogenicity in the white matter on cUS shortly after birth; extensive areas of low signal intensity on the ADC map with sparing of the left parieto-occipital lobe (D); (B) group B *Streptococcus* encephalitis with hyperechogenicity in the periventricular and deep white matter; extensive restricted diffusion of the cortex and subcortical white matter on MRI, also note small infarct in right caudate nucleus (E); (C) antenatal diagnosis of tuberous sclerosis (cardiac rhabdomyoma): subependymal nodule seen on cUS; several periventricular subependymal nodules and right-sided subcortical tuber are seen on T2-weighted image (F).

Involvement of other developmental domains is common as well (cognition, speech and epilepsy). [15, 20, 24, 25] WS injury is most often associated with cognitive impairment [15, 26, 27] which can be overlooked at 12-18 months of age and become more apparent after 30 months of age. [15, 27-29] The prevalence of motor impairment after WS injury is low (6-18%) compared to the prevalence after basal ganglia-thalamic injury (50-75%). [15, 21, 24] An abnormal signal in the posterior limb of the internal capsule (PLIC) is described as an accurate predictor of neurodevelopmental outcome. [15] A normal PLIC is usually seen with normal or mild basal ganglia-thalamic lesions and is predictive of a normal motor outcome. [21] An abnormal PLIC is associated with an unfavourable outcome. [20, 30] However, the abnormal signal in the PLIC is usually first seen beyond 72 hours after birth and myelination can only be assessed in infants with a gestational age beyond 38-40 weeks. Apparent diffusion coefficient (ADC) measurements in the basal ganglia and magnetic resonance spectroscopy (^{1}H-MRS) measurements (lactate/*N*-acetyl aspartate [NAA]) can add significantly to the predictive properties of MRI, although it should be noted that ADC shows pseudonormalisation after the first week, but MR spectroscopy measurements remain abnormal for a prolonged period of time. [31]

Perinatal stroke

Perinatal arterial ischaemic stroke

Neonatal seizures and especially hemiconvulsions often suggest the diagnosis of perinatal arterial ischaemic stroke (PAIS). Compared to those who develop seizures due to HIE, seizures related to PAIS tend to develop significantly later and are more often focal. [32] It is important to use at least a two-channel aEEG or standard EEG recording in infants presenting with hemiconvulsions. [33] It is possible to recognise the larger middle cerebral artery infarcts and lenticulostriate infarcts which are well within the field of view using cUS, but in general it will take 24-72 hours before the increase in echogenicity becomes apparent *(table 2)*. [34] It may not be possible to recognise cortical infarcts or infarcts in the territory of the posterior cerebral artery using cUS, unless the posterior fontanel is used as an acoustic window. [35, 36] MRI and especially DWI enables detection of PAIS within hours after onset and allows prediction of development of subsequent unilateral spastic cerebral palsy by assessing involvement of the corticospinal tracts. [37, 38]

Table 2. Neuro-imaging findings over time in HIE and PAIS

Aetiology		cUS findings			MRI findings	
Time after onset of injury		<24 h	24-48 h	49-72 h	Day 1-7	Day 8-28
HIE						
BGT pattern	BGT hyperechogenicity, oedema	-	+/-	+	DWI changes; BGT	T1WI/T2WI changes; BGT
WM/WS pattern	Subcortical/periventricular hyperechogenicity, oedema	-	+/-	+	DWI changes; cortex, white matter	T1WI/T2WI changes; cortex, white matter
PAIS						
	Focal hyperechogenicity, wedge-shaped, with linear demarcation (MCA infarct)	-	+/-	+	DWI changes; territory of cerebral artery and corticospinal tracts	T1WI/T2WI; loss grey matter-white matter differentiation (MCA infarct)

HIE: hypoxic-ischaemic encephalopathy; BGT: basal ganglia and thalami; DWI: diffusion-weighted images; T1WI: T1-weighted images; T2WI: T2-weighted images; WM/WS: white matter/watershed; PAIS: perinatal arterial ischaemic stroke; MCA: middle cerebral artery.

Cerebral sinovenous thrombosis

Cerebral sinovenous thrombosis (CSVT) may also present with neonatal seizures. The presence of an intraventricular haemorrhage associated with a unilateral thalamic haemorrhage in a full-term infant suggests the presence of a CSVT. [39] Doppler ultrasound may help to diagnose occlusion of the superior sagittal sinus but is less reliable for other sinuses, and an MRI and magnetic resonance venography (MRV) are often required to confirm CSVT. Making the correct diagnosis is important, as anticoagulant therapy is increasingly being used. [40] The presence of a neonatal thalamic haemorrhage is strongly associated with later development of electrical status epilepticus in slow wave sleep. [41]

Intracranial haemorrhage

An intracranial haemorrhage (ICH) in the full-term infant is not as common as in the preterm infant, but does occur. [42] Blood in the *posterior fossa* is common, which can be a chance finding and does not often lead to neonatal seizures. [43] An *intraventricular haemorrhage* can be associated with CSVT, but can also occur without a reasonable explanation. Neonatal seizures in a full-term infant with an intraventricular haemorrhage may be difficult to control. [44] A parenchymal haemorrhage can also be diagnosed in the absence of a complicated delivery. A frontal lobe haemorrhage is most common and, in the presence of a midline shift, neurosurgical intervention may be considered. [45] An infant with a temporal lobe haemorrhage often presents with apnoeic episodes, which turn out to be of epileptic origin when continuous aEEG monitoring is used. [46] Epileptic apnoeic episodes are thought to originate from the limbic system. [47]

Central nervous system infection

Any infection of the CNS can present with seizures. Both bacterial and viral CNS infections can occur in the neonatal period, but severe CNS infections are not so common. Early gram negative bacterial infection, as well as late-onset group B *Streptococcus* infection, may be associated with severe brain injury. [48] The neuro-imaging pattern may be very characteristic *(table 3)*, for instance in *Bacillus cereus* septicaemia, where the white matter may show cystic evolution within hours after the onset of the infection. [49] In infants with an *Escherichia coli* infection, hydrocephalus may only become apparent weeks after the acute illness, and isolated dilatation of the fourth ventricle is often an associated finding. A wide spectrum of viral infections can present with neonatal seizures. Some infants present with a fever and/or rash and PCR in the cerebrospinal fluid (CSF) may confirm the diagnosis of an enterovirus or parechovirus encephalitis. [50, 51] The DWI changes may be extensive, but the outcome may be better than expected on the basis of the DWI findings in the majority of these infants. [52] A similar pattern of diffusion restriction

in the white matter was recently demonstrated in full-term infants presenting with seizures between days 4 and 6 of life with a rotavirus infection. [53] Herpes simplex virus encephalitis is rare and severe lesions in the temporal lobes, but also elsewhere in the neonatal brain, are best seen with MRI, and once again more clearly and earlier with DWI. [54, 55] Other infections, for instance *Toxoplasmosis gondii*, can also present with neonatal seizures, in the presence of hydrocephalus and extensive white matter injury.

Inborn errors of metabolism

Inborn errors of metabolism can be difficult to diagnose, and recognition of characteristic neuro-imaging features is very helpful in the diagnostic process. It is beyond the scope of this review to describe all potential metabolic disorders which may present with neonatal seizures. A useful review was published by Prasad and Hoffmann. [56] A specific imaging pattern can suggest the diagnosis in some disorders and the additional use of ^{1}H-MRS may also aid in making the diagnosis *(table 4)*. [57] Newborn infants with *nonketotic hyperglycinaemia* (NKH) often present with neonatal seizures and/or hiccups which may have been felt by the mother *in utero*. The aEEG typically shows a burst suppression pattern without a history of HIE. The diagnosis may already be suspected using cUS, as the corpus callosum is often dysplastic, associated with a so-called "bull-horn" shape of the ventricles. It is possible to confirm this by MRI, but DWI may also show restricted diffusion of the PLIC and of the dorsal aspect of the midbrain and pons due to vacuolating myelinopathy. [58] Using ^{1}H-MRS, the high glycine peak also suggests NKH. Newborn infants with *molybdenum cofactor deficiency* or sulphite oxidase deficiency are often considered to have HIE, but the history is often not typical and these metabolic disorders should be considered as well. Typically, the onset of seizures is rather early to be due to HIE, seizures are very difficult to control and the background activity tends to deteriorate over time. [59] MRI during the first week may show extensive DWI abnormalities, preceding extensive cystic evolution, which can be seen when the MRI is performed again several weeks later. [60] Those with *peroxisomal biogenesis disorders* do not invariably present with neonatal seizures. Ventricular dilatation, germinolytic cysts, and lenticulostriate vasculopathy may be recognised by cUS. As the fontanel is large in newborn infants with Zellweger syndrome, the Sylvian fissure can often be clearly visualised and polymicrogyria may be suspected with cUS and confirmed with MRI.

Table 3. Neuro-imaging findings in CNS infections

Causative organism	Neuro-imaging findings
Bacterial	
GBS [72]	Ischaemic infarctions, in particular BGT
Escherichia Coli [72]	Empyema, ventriculitis, hydrocephalus, trapped 4th ventricle
Proteus mirabilis [73, 74]	Cerebral abscesses
Streptococcus pneumonia/ haemophilus influenza [75]	Subdural collections/effusions
Klebsiella oxytoca [72]	Frontal lobe infarction
Bacillus cereus [49]	White matter liquefaction
Citrobacter [72]	Ischaemic infarctions, cerebral abscesses
Serratia marcescens [72]	Cerebral abscesses
Viral	
HSV [76]	DWI changes white matter, not restricted to temporal lobes, and brainstem
Parechovirus [50]	DWI changes white matter, PWML, cystic PVL
Enterovirus [50]	DWI changes white matter, PWML, cystic PVL
Rotavirus [53]	DWI changes white matter, PWML, cystic PVL

GBS: group B *Streptococcus*; BGT: basal ganglia and thalami; PVL: periventricular leukomalacia; HSV: herpes simplex virus; DWI: diffusion-weighted images; PWML: punctate white matter lesions.

In addition, MRI may show signal intensity changes in the white matter and delayed myelination of the PLIC. Using ^{1}H-MRS with a short echo time (TE), a reduced NAA peak at 2 parts per million (ppm), a lactate peak at 1.33 ppm, and an abnormal peak at 0.9 ppm can be seen, the latter being due to the resonances of the methyl residues of mobile lipids. [61] *Hypoglycaemia* can be considered as a transient metabolic disturbance and newborn infants may present with seizures due to severe hypoglycaemia, usually with a value below 1 mmol/l. The abnormalities tend to be overlooked with cUS, and are typically located in the occipital white matter, unless the posterior fontanel is also examined. MRI is a better technique to identify the lesions, which are often not restricted to the occipital regions. [62]

Table 4. Neuro-imaging findings in metabolic disorders

Transient metabolic disorders	Neuro-imaging findings
Hypoglycaemia	Predominant occipital abnormalities
Hyperbilirubinaemia (kernicterus)	Increased signal intensity in the globus pallidus on T1WI
Inborn errors of metabolism	
Pyridoxine deficiency	Abnormalities of white matter and corpus callosum; haemorrhage in white matter
Biotinidase deficiency	DWI abnormalities of the PLIC, corpus callosum, corona radiata (vacuolating myelinopathy)
Glutaric aciduria type 1	Widened anterior temporal, Sylvian and frontal CSF spaces and T2WI hyperintensity of the globus pallidus
Nonketotic hyperglycinaemia	Dysplastic corpus callosum, DWI abnormalities and lack of myelination of the PLIC (vacuolating myelinopathy)
Molybdenum cofactor deficiency/ sulphite oxidase deficiency	Extensive DWI changes of white matter with cystic evolution
Zellweger syndrome	Germinolytic cysts, lenticulostriate vasculopathy, polymicrogyria; ^{1}H-MRS: abnormal mobile lipid peak at 0.9 ppm

PLIC: posterior limb of the internal capsule; DWI: diffusion-weighted images; CSF: cerebrospinal fluid; ^{1}H-MRS: proton magnetic resonance spectroscopy; T1WI: T1-weighted images; T2WI: T2-weighted images.

Cerebral dysgenesis/genetic disorders

Infants with migrational disorders can present with neonatal seizures, but this is rather uncommon and most of the infants will develop seizures later during the first year. In most of these infants, there are associated findings leading to a diagnosis, for instance, migrational disorders may be encountered in children with a metabolic disorder (polymicrogyria in Zellweger syndrome) or congenital cytomegalovirus infection. There is considerable overlap with genetic disorders and this overlap is increasing with advances in genetics. [63] Neurocutaneous syndromes (linear naevus syndrome, incontinentia pigmenti and tuberous sclerosis) may also present with neonatal seizures. [64-67] Refractory neonatal seizures can increasingly be explained by an underlying genetic problem. Weckhuysen *et al*. recently showed *mutations in KCNQ2*, which encodes the voltage-gated potassium channel Kv7.2 in children with unexplained neonatal or early-infantile

seizures and associated psychomotor retardation. [11, 12] Early MRI showed characteristic hyperintensity in the basal ganglia and thalamus that later resolved in a subgroup of their patients. *Early infantile epileptic encephalopathy (EIEE or Ohtahara syndrome)* is a diagnosis made when intractable seizures are seen in the neonatal period. Many infants with Ohtahara syndrome have an associated underlying cerebral malformation, for instance, agenesis of the corpus callosum in Aicardi syndrome. EIEE is associated with several gene mutations, including Aristaless-related homeobox *(ARX)*, cyclin-dependent kinase-like 5 *(CDKL5)*, and syntaxin-binding protein 1 *(STXBP1)*. No specific neuro-imaging findings have been reported in these children. [68] *Aicardi-Goutières syndrome* is a genetically determined early-onset encephalopathy with a variable phenotype, including neurological manifestations such as dystonia, spasticity, epileptic seizures, progressive microcephaly, and severe developmental delay. Aicardi-Goutières syndrome is a heterogeneous disorder with five disease-associated genes *(AGS1-5)* accounting for 83% of cases that fulfil the clinical diagnostic criteria. In a recent study, 25% of infants were reported to have seizure onset within the first month after birth. Calcification is more easily detected using cUS than with MRI, but MRI shows delayed myelination, grey and white matter atrophy, ventricular enlargement, and cystic degeneration over time, often quite marked in the temporal lobes and the periventricular white matter. [69]

Unknown

Although not so common anymore, every now and then neonatal seizures may occur without an explanation based on either neuro-imaging or extensive genetic and metabolic investigations. More and more infants, however, can be diagnosed at a later stage. Sometimes, with a second MRI scan, using higher resolution and thinner slices, it may be possible to detect an area of cortical dysplasia. [70] In others, a new mutation may be identified. [71]

Neonatal MRI protocol

A standard neonatal MRI protocol should include sagittal T1-weighted images (T1WI), axial or coronal T2-weighted images (T2WI), and T1WI or inversion recovery-weighted images and DWI, including ADC mapping. MRV, MR angiography (MRA), ^{1}H-MRS, and susceptibility-weighted images (SWI) should preferably be available as well. DWI is especially important in HIE and PAIS and is also useful in CNS infections, since pus shows up as a high signal on DWI. MRV should be added when a CSVT is suspected. MRA can be useful in PAIS and in diagnosing arteriovenous malformations. ^{1}H-MRS can provide additional information in suspected metabolic disorders, and HIE and SWI are useful in diagnosing (small) haemorrhages. Slice thickness should be 2 mm or thinner.

CONCLUSION

Neuro-imaging is very helpful to identify the underlying aetiology of neonatal seizures in full-term infants. cUS should be used in the acute phase and may show severe and centrally-located lesions, as well as calcification, which will not be recognised by MRI. More detailed information may subsequently be obtained with MRI, provided the use of thin (2 mm) slices and sequences suitable for imaging neonates. This renders MRI superior in diagnosing migrational disorders and lesions in the posterior fossa. When ^{1}H-MRS is added to the imaging protocol, metabolic disorders may be identified prior to obtaining results from metabolic investigations. Information on the type and severity of brain lesions may help to give a more accurate prognosis, and in some families, will aid with genetic counselling.

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4

Chapter 5

The aetiology of neonatal seizures and the diagnostic contribution of neonatal cerebral MRI

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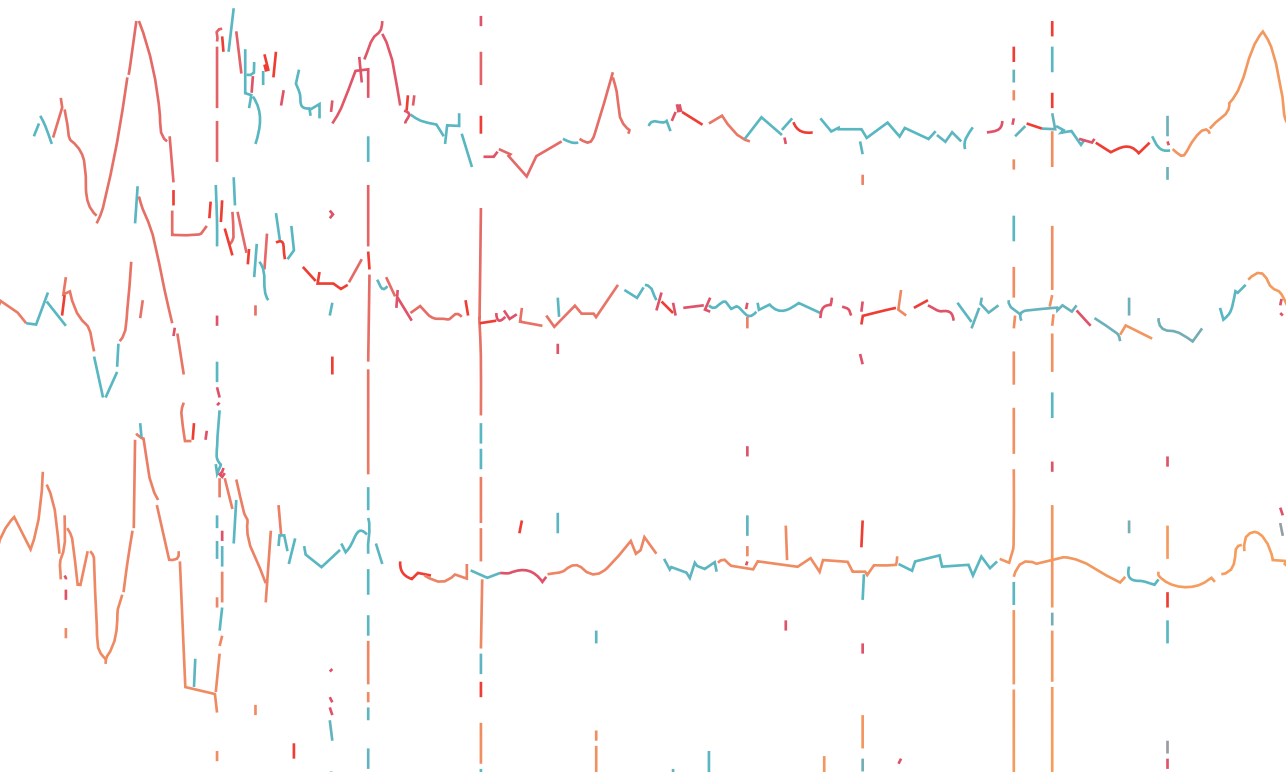

ABSTRACT

Introduction The aim of this study was to delineate aetiologies and explore the diagnostic value of cerebral magnetic resonance imaging (MRI) in addition to cranial ultrasonography (cUS) in infants presenting with neonatal seizures.

Methods This retrospective cohort study comprised infants (gestational age 35.0-42.6 weeks) with seizures, confirmed by either continuous amplitude-integrated electroencephalography (aEEG) or standard EEG and admitted during a 14-year period to a level III neonatal intensive care unit (n = 378; 216 males, 162 females; mean [SD] birth weight 3334 g [594]). All infants underwent cUS and MRI (MRI on median of 5 days after birth [range 0-58]) within the first admission period.

Results An underlying aetiology was identified in 354 infants (93.7%). The most common aetiologies identified were hypoxic-ischaemic encephalopathy (46%), intracranial haemorrhage (12.2%), and perinatal arterial ischaemic stroke (10.6%). When comparing MRI with cUS in these 354 infants MRI showed new findings which did not become apparent on cUS, contributing to a diagnosis in 42 (11.9%) infants and providing additional information to cUS, contributing to a diagnosis in 141 (39.8%). cUS alone would have allowed a diagnosis in only 37.9% of infants (134/354).

Conclusion Cerebral MRI contributed to making a diagnosis in the majority of infants. In 11.9% of infants the diagnosis would have been missed if only cUS were used and cerebral MRI added significantly to the information obtained in 39.8% of infants. These data suggest that cerebral MRI should be performed in all newborn infants presenting with EEG or aEEG-confirmed seizures.

INTRODUCTION

Neonatal seizures are associated with high mortality (21-24%) [1, 2] and morbidity rates (25-35%). [3] No evidence-based guidelines for the evaluation of neonatal seizures exist, [4] but it is likely that magnetic resonance imaging (MRI) would provide the most useful information. [5, 6] MRI is now considered the criterion standard for diagnosing brain injury and developmental disorders and for determining the prognosis in neonates presenting with seizures. [4] Many studies have reported on brain imaging and neonatal seizures, but most focused on MRI data in small groups of infants with a specific underlying problem. Only a few studies have reported on neuro-imaging findings in infants with neonatal seizures. [3, 7-9] The aim of this study was to assess the aetiologies and additional value of MRI compared with cranial ultrasonography (cUS) in a retrospective study of a large cohort of full-term and near-term born infants with neonatal seizures. Our hypothesis was that MRI would make an important contribution to the diagnostic process.

METHODS

Patients

In this retrospective study, infants were included if they had a gestational age of 35 weeks or more and clinical and/or subclinical neonatal seizures, confirmed by amplitude-integrated electroencephalography (aEEG) or standard EEG, cUS, and MRI performed during the first admission period, and had been admitted to the level III neonatal intensive care unit of the Wilhelmina Children's Hospital in Utrecht (March 1999 - October 2013). Infants were excluded if they had clinical seizures without confirmation by either aEEG or standard EEG. Infants were identified using a local database on discharge diagnoses using 'seizures' and 'convulsions' as search terms. These data were compared with a local neuro-imaging database to identify those who underwent cUS and MRI during the same admission period. Subsequently, the medical records and discharge summaries were used to check whether or not seizures had been confirmed by EEG or aEEG. No permission was required from the hospital's medical ethics committee for this retrospective anonymous data analysis.

Amplitude-integrated electroencephalography

Continuous aEEG recordings were routinely used in infants at risk of or with suspected neonatal seizures. Standard EEG was performed when aEEG was inconclusive, to confirm seizures and obtain additional information.

Neuro-imaging studies

Cranial ultrasonography

The first cUS, using a broadband transducer (5-8.5 MHz), was performed on admission and repeated two or three times during the first week following admission. All patients underwent cUS before MRI. When MRI showed additional or new findings, cUS was repeated to assess whether or not these abnormalities could have been seen with cUS as well, for example by using additional acoustic windows.

Magnetic resonance imaging

Magnetic resonance imaging was performed on a 1.5T or 3.0T magnet. Standard MRI protocol included sagittal T1-weighted images (T1WI), axial T2-weighted images (T2WI), inversion recovery-weighted images, and diffusion-weighted images (DWI) including apparent diffusion coefficient (ADC) mapping. Magnetic resonance venography (MRV), magnetic resonance angiography (MRA), proton magnetic resonance spectroscopy (^{1}H-MRS), susceptibility-weighted images (SWI), and contrast (gadolinium)-enhanced imaging were added, depending on the differential diagnosis or findings seen on standard MRI protocol sequences. The MRI was always performed within one week of presentation with seizures.

Neuro-imaging analysis

Cranial ultrasound was performed by the attending neonatologist and discussed with the neonatal neurology team. The magnetic resonance images were independently assessed by two neonatologists and a paediatric radiologist.

Magnetic resonance imaging versus cranial ultrasound

The diagnostic value of MRI compared with cUS was classified into three groups. These were (1) no additional value of MRI (diagnosis made with, or all brain lesions seen on cUS); (2) additional value of MRI (either cUS showed abnormalities and a diagnosis was suspected, but magnetic resonance images contributed significantly to making the diagnosis, or cUS revealed the cause of seizures but MRI showed important additional abnormalities); and (3) MRI diagnosis (cUS showed no or non-specific abnormalities and the brain lesions were first seen on or the diagnosis was made with MR images).

RESULTS

During the study period, 378 neonates (216 males, 162 females; median gestational age 40.0 weeks [range 35.0-42.6]; mean birth weight 3334 g, [standard deviation, SD 594]) admitted to our neonatal intensive care unit had EEG- or aEEG-confirmed seizures and underwent cUS and MRI in the first admission period.

Aetiology

In 354 out of 378 infants (93.7%) an underlying aetiology was found. The aetiologies found were hypoxic-ischaemic encephalopathy (HIE; n = 174, 46%), intracranial haemorrhage (ICH; n = 46, 12.2%), perinatal arterial ischaemic stroke (PAIS; n = 40, 10.6%), cerebral sinovenous thrombosis (CSVT; n = 11, 2.9%), metabolic disorders including hypoglycaemia (n = 34, 9%), central nervous system (CNS) infection (n = 27, 7.1%), cerebral dysgenesis (n = 11, 2.9%), genetic disorders, predominantly benign familial neonatal seizures (n = 8, 2.1%), non-CNS infection (n = 2, 0.5%), and, in one infant, cerebral teratoma (n = 1, 0.3%). Eighty-two (21.7%) infants had more than one diagnosis, and these infants were assigned to the category that was the most likely cause of the seizures *(supplemental table 1)*. MRI was performed a median of 5 days after birth (range 0-58). Overall, 94 out of 378 (24.9%) infants died, 51 (54.3%) due to HIE and 81 (86.2%) following redirection of care. Post-mortem examination was performed in 48 out of 94 (51.1%).

Hypoxic-ischaemic encephalopathy

In total, 174 infants had HIE, all Sarnat grade 2 or 3. Sixteen infants (9.2%) were treated with therapeutic hypothermia. MRI findings were normal in 23 infants (13.2%), while 51 infants (29.3%) had predominant basal ganglia and thalamic (BGT) injury, 51 (29.3%) had predominant white matter/watershed injury, and 21 (12.1%) had severe white matter/watershed injury and BGT involvement (near-total pattern of injury). Twelve (6.9%) infants had PAIS and 16 (9.2%) had an ICH as the predominant finding on MRI. Cranial ultrasonography showed all brain lesions in 78 (44.8%) of the 174 infants with HIE; in one infant (0.6%) lesions became visible on cUS after MRI was performed. MRI revealed important additional information (the exact site and extent of the lesions, abnormalities in the deep brain structures, infra- and supratentorial subdural haemorrhages [SDHs], asymmetry of the posterior limb of the internal capsule, punctate white matter lesions, cerebellar ischaemic and haemorrhagic lesions, occipital infarcts, high lactate, and low *N*-acetyl aspartate/choline ratio on ^{1}H-MRS) in 65 out of 174 (37.4%) infants, and brain lesions were first seen on MRI in seven (4%). In 18 out of the 23 (78.3%) infants in whom MRI findings were normal, cUS showed abnormalities (periventricular or thalamic

hyperechogenicity, cortical highlighting, lenticulostriate vasculopathy [LSV], or choroid plexus cyst).

Intracranial haemorrhage

Of the 46 infants with intracranial haemorrhage, eight (17.4%) had an isolated intraventricular haemorrhage (IVH), 10 (21.7%) had an isolated parenchymal haemorrhage, 11 (23.9%) had an isolated extra-axial haemorrhage, and 17 (37%) had a combination of these. A parenchymal haemorrhage was located in the frontal lobe in two out of 10 (20%) infants, in the temporal lobe in two (20%), and the basal ganglia and thalami were affected in two infants (20%). A combination of parietal-temporal haemorrhage was seen in two out of 10 (20%) infants, parietal-occipital haemorrhage was seen in one (10%), and one (10%) infant had a thalamic-brainstem-cerebellar haemorrhage. Extra-axial haemorrhage was located in the posterior fossa in five out of 11 (45.5%) infants. Other extra-axial haemorrhages included supratentorial SDH or subarachnoid haemorrhages. Cranial ultrasonography revealed an ICH in 27 out of 46 (58.7%), but in one infant with IVH (2.2%) the abnormalities on cUS became visible only after MRI was performed. In 14 out of 46 (30.4%) infants, MRI revealed important additional information (SDH, posterior fossa haemorrhage, arteriovenous malformation, PAIS, subarachnoid cyst, cerebral compression). The ICH was first seen on MRI in four out of 46 (8.7%) infants (four posterior fossa haemorrhages).

Perinatal arterial ischaemic stroke

Of the 40 infants with PAIS, the middle cerebral artery was affected in 29 (72.5%), the posterior cerebral artery in four (10%), and the anterior cerebral artery in one (2.5%); in six infants (15%) more than one territory was involved. In 22 infants (55%) the left hemisphere was affected, in 16 (40%) the right side, and in two infants (5%) both hemispheres were involved. PAIS was first seen on MRI in seven (17.5%), mostly cortical and occipital infarctions. In the majority of infants there was some indication of the diagnosis on cUS (n = 33, 82.5%), although this was completely reliable in only 15 (37.5%), and in seven out of these 15 infants (46.7%) it became visible on cUS several days after MRI was performed. MRI, especially DWI, showed important additional information (size of the infarct, second infarct on the side contralateral to the middle cerebral artery, involvement of the corticospinal tracts, punctate haemorrhages in the white matter, and posterior fossa haemorrhage) in 18 out of 40 infants (45%).

Cerebral sinovenous thrombosis

Of the 11 infants with cerebral sinovenous thrombosis, the superior sagittal sinus was

affected in two infants (18.2%), the straight sinus in one (9.1%), multiple sinuses in six (54.5%) (including in one infant, the deep venous system), and the internal cerebral vein in one (9.1%); in one infant (9.1%) the thrombus occurred in a vein of Galen malformation. Associated lesions included thalamic infarct (n = 1), IVH (n = 5), punctate white matter lesions (n = 5), thalamic haemorrhage (n = 6), and periventricular leukomalacia (n = 1). Six infants had cUS abnormalities suggestive of CSVT (IVH, thalamic haemorrhage), but the definite diagnosis was made with MRI, and MRV in particular. Four infants had no or non-specific cUS abnormalities. In one infant Doppler flow imaging was performed and lack of flow over the superior sagittal sinus was seen, but MRI revealed important additional information (thrombosis of the cortical veins and deep venous system and diffuse white matter abnormalities).

Metabolic disorders

Transient metabolic disorder

Of the 18 infants with a transient metabolic disorder, 14 infants (77.8%) had symptomatic hypoglycaemia, two infants (11.1%) had symptomatic hypocalcaemia, and two (11.1%) had kernicterus. Among 14 infants with hypoglycaemia, cUS showed all brain lesions in three (21.4%). MRI showed important additional information (extent of lesions, temporal lobe haemorrhage) in five infants (35.7%). Brain lesions were first seen on MRI in four infants (28.6%; infants with occipital infarction). In two infants, no abnormalities were seen on magnetic resonance images. In the two infants with kernicterus, no abnormalities were seen on cUS, but high signal intensity in the globus pallidus was seen on T1WI and ^{1}H-MRS showed a lactate peak in the basal ganglia. No cUS or MRI abnormalities were observed in the two infants with symptomatic hypocalcaemia.

Inborn error of metabolism

Of the 16 infants with an inborn error of metabolism, a definite diagnosis could be made in eight (50%). In five infants (31.3%) a mitochondrial disorder was proven with muscle biopsy, but could not be defined otherwise. Cranial ultrasonography revealed all brain lesions in 2 out of 16 (12.5%) infants. MRI provided important additional information in 10 (62.5%) (signal intensity changes on DWI involving the corticospinal tracts and peduncles, lack of myelination of the posterior limb of the internal capsule, reduced cortical folding, DWI abnormalities in the brainstem, heterotopia, and a lactate peak on ^{1}H-MRS). The brain lesions were first seen on MRI in three infants (18.8%). MRI findings were normal in one infant. ^{1}H-MRS was performed in all infants and showed abnormalities in 13 (81.3%). Cranial ultrasonography, MRI, and ^{1}H-MRS findings are shown in *supplemental table 2*.

Central nervous system infection

Of the 27 infants with central nervous system infection, meningitis was diagnosed in 11 (40.7%), encephalitis in seven (25.9%) and combined meningo-encephalitis in six (22.2%). There was one infant (3.7%) with ventriculitis following neurosurgery, one infant (3.7%) with multiple cerebral abscesses, and one infant (3.7%) with meningitis-ventriculitis with empyema. A causative organism could be identified in 24 infants (88.9%): group B *Streptococcus* was the most common infecting organism (n = 8, 33.3%), followed by parechovirus (n = 6, 25%), enterovirus (n = 3, 12.5%), and herpes simplex virus type I (n = 3, 12.5%). *Klebsiella oxytoca*, *Escherichia coli*, *Proteus mirabilis*, and coxsackie B1 virus were each identified in one infant. Cranial ultrasonography showed all brain lesions in 5 out of 27 infants (18.5%). MRI showed important additional information in 18 (66.7%) infants (distinction between ischaemic and haemorrhagic lesions, extent of lesions, contrast enhancement, and SDH). MRI revealed abnormalities suggestive of herpes simplex virus encephalitis in two (7.4%; temporal DWI abnormalities). In two infants (7.4%) MRI was normal.

Cerebral dysgenesis

Of the 11 infants with cerebral dysgenesis, a diagnosis was made in five infants (45.5%): in three neurocutaneous syndromes (tuberous sclerosis, Jadassohn syndrome, and incontinentia pigmenti), in one pontocerebellar hypoplasia, and in one cerebral dysgenesis following congenital cytomegalovirus infection. In the remaining infants, the most common malformations were hemimegalencephaly, abnormalities of migration (polymicrogyria, lissencephaly, cortical dysplasia, reduced cortical folding), heterotopia, and corpus callosum agenesis or dysgenesis. Cranial ultrasonography detected all brain lesions in 1 out of 11 infants (9.1%). In nine infants (81.8%) cUS gave some indication of the diagnosis, but MRI was needed to make a definitive diagnosis (hemimegalencephaly, lissencephaly, agenesis of the cerebellar vermis) or provided important additional information (cerebellar cysts, agenesis of pons, polymicrogyria, low *N*-acetyl aspartate/choline ratio, and high lactate on ^{1}H-MRS). The brain lesions were first seen on MRI in one infant (9.1%) (hemimegalencephaly, polymicrogyria, and heterotopia).

Magnetic resonance imaging versus cranial ultrasonography

In those with an identified underlying aetiology, cUS revealed abnormalities in 331 out of 354 (93.5%) infants and MRI in 317 out of 354 (89.5%) infants. In the 37 infants (10.5%) without MRI abnormalities, the diagnoses were HIE (n = 23), transient metabolic disorder (n = 4), inborn error of metabolism (n = 1), benign familial neonatal seizures (n = 6), and (non-)CNS infection (n = 3). In eight of these infants (21.6%) cUS was normal. When

comparing MRI with cUS *(figure 1; supplemental table 3)*, cUS showed all brain lesions and contributed to making the diagnosis in 134 out of 354 (37.9%) infants *(figure 2)*. In 125 (93.3%) of these 134 infants, the lesions were already seen before MRI, and in nine (6.7%) these (PAIS) became apparent several days thereafter. In 39.8%, cUS provided a suspected diagnosis, but MRI/^{1}H-MRS/MRV contributed significantly to confirming the diagnosis (PAIS and metabolic disorders) or MRI provided important additional information (distinction between ischaemic and haemorrhagic lesions, size of lesions, involvement of corticospinal tracts, punctate white matter lesions, extra-axial haemorrhages, arteriovenous malformation, additional cortical PAIS, cerebral compression in ICH, cerebellar lesions, contrast-enhanced lesions, and specific ^{1}H-MRS findings in metabolic disease; *figure 3*).

The imaging abnormalities were first seen on MRI in 42 out of 354 (11.9%) infants (DWI/ADC abnormalities in the BGT or white matter in HIE, DWI/ADC abnormalities in PAIS, lack of flow across one of the sinuses on MRV in CSVT, occipital ischaemic lesions in hypoglycaemia, extra-axial [posterior fossa] haemorrhages, migrational abnormalities, heterotopia, hemimegalencephaly, tuberous lesions, temporal DWI abnormalities in herpes simplex virus encephalitis; *figure 4*). In 33 out of 354 (9.3%) infants, cUS abnormalities were not seen on subsequent MRI. Some were considered of value and suggestive of a genetic or metabolic disorder (LSV, subcortical calcification, or germinolytic cyst), while for other abnormalities (periventricular and thalamic hyperechogenicity) MRI was considered the criterion standard, although it could be that the imaging changes were transient and no longer visible on later MRI.

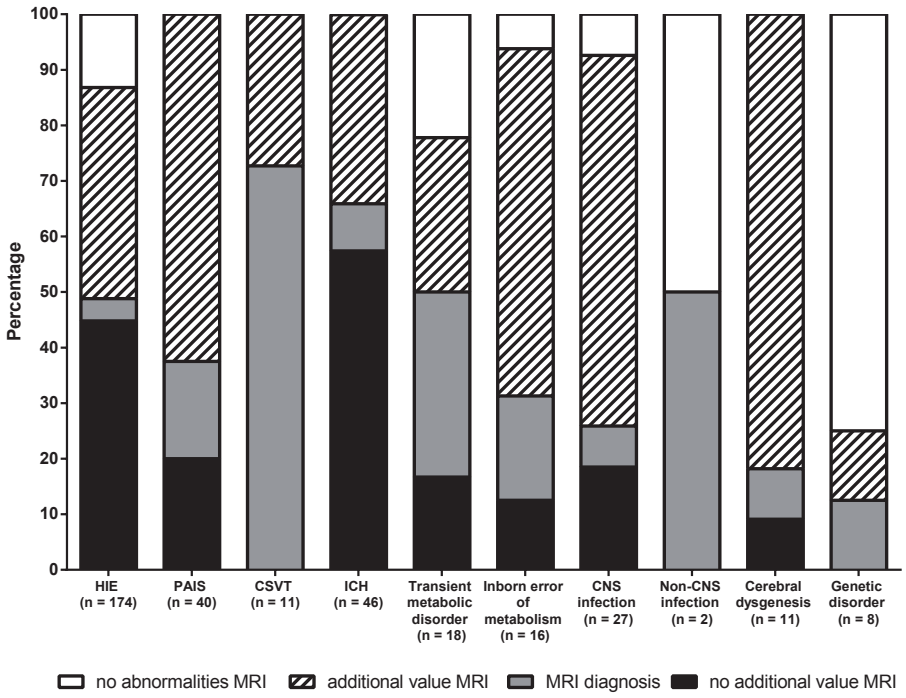

Figure 1. Diagnostic value of magnetic resonance imaging (MRI) compared with cranial ultrasonography in all infants with aEEG and EEG-confirmed seizures in whom a diagnosis could be made. CNS: central nervous system; CSVT: cerebral sinovenous thrombosis; HIE: hypoxic-ischaemic encephalopathy; ICH: intracranial haemorrhage; PAIS: perinatal arterial ischaemic stroke.

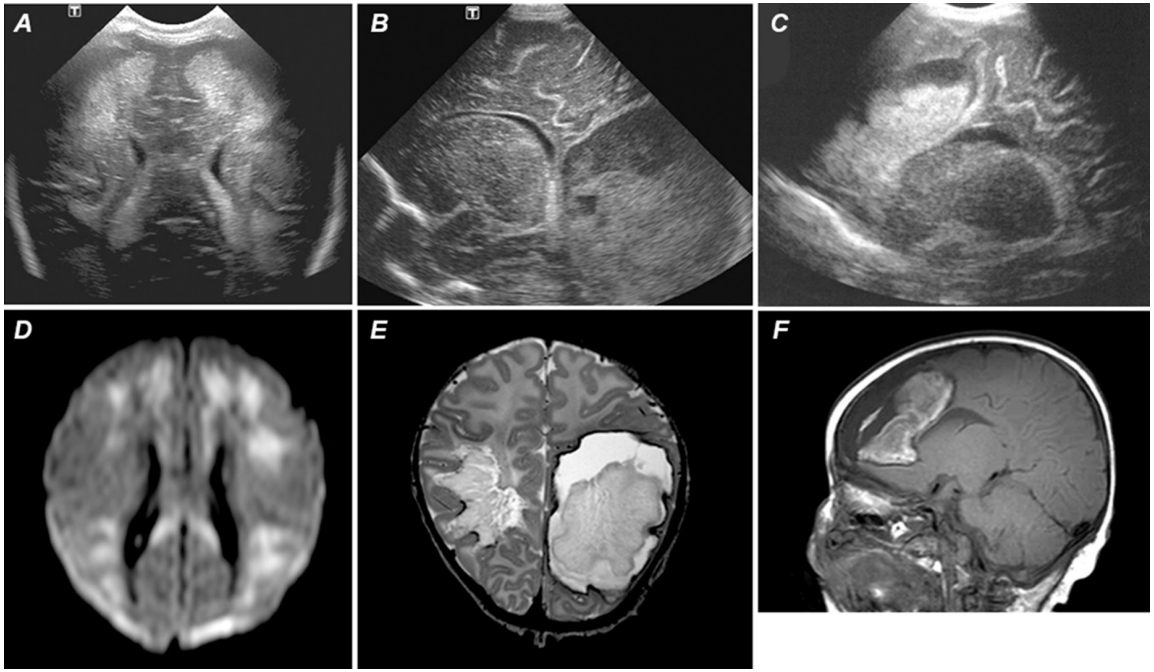

Figure 2. No additional value of magnetic resonance imaging (MRI). Cranial ultrasonography (cUS; A-C) and MRI (D-F): (D) axial diffusion-weighted image (DWI), (E) axial T2-weighted image, and (F) sagittal T1-weighted image. Extensive areas of increased echogenicity were seen on coronal cUS in an infant (gestational age 35 weeks) with hypoxic-ischaemic encephalopathy (A), confirmed by DWI (D). A large abscess was seen on sagittal cUS (B), which was confirmed by axial MRI (E). The contralateral smaller lesion was also seen with cUS (not shown). Frontal lobe haemorrhage was recognised with cUS (C) and MRI (F).

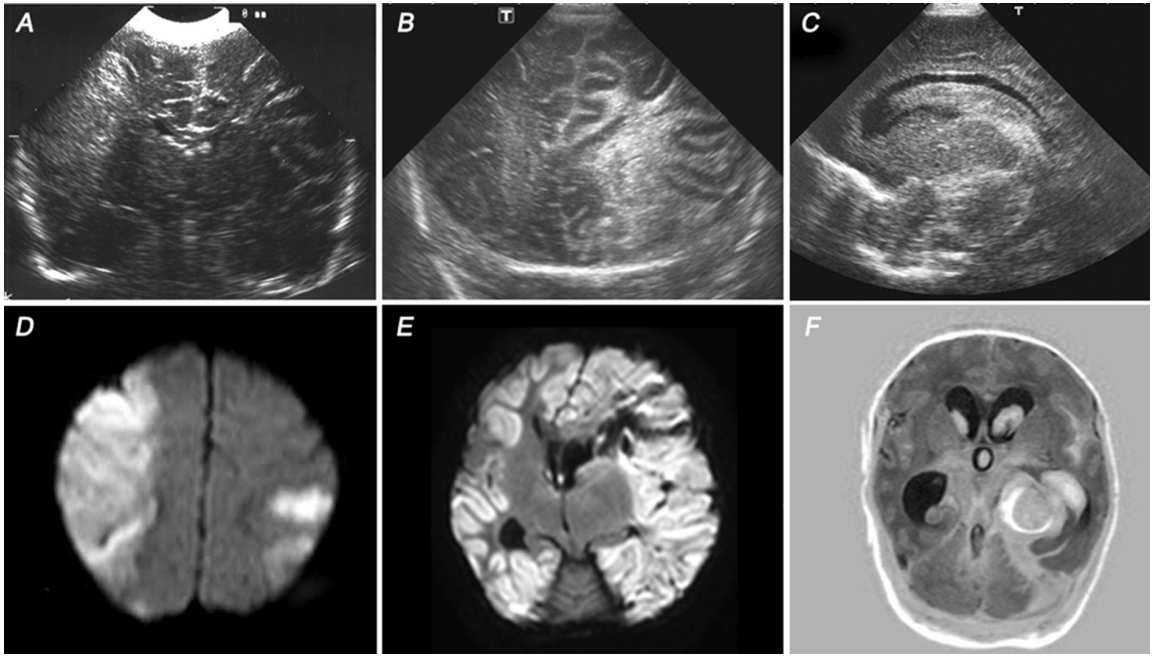

Figure 3. Additional value of magnetic resonance imaging (MRI). Cranial ultrasonography (cUS; A-C) and MRI: (D and E) axial diffusion-weighted image and (F) axial inversion recovery sequence. The infarct in the main branch of the middle cerebral artery in the right hemisphere was seen with cUS (A) and MRI (D), but the smaller cortical infarct in the left hemisphere was seen only with MRI (D). A midline shift and parenchymal echogenicity due to presumed haemorrhage were seen with cUS (B). MRI confirmed the presence of parenchymal haemorrhage and showed extensive restricted diffusion of the entire cortex (E). A large intraventricular haemorrhage and round lesion in the temporal lobe and suspected arteriovenous malformation (C) were confirmed on MRI (F) and an aneurysm was revealed by MR angiography (not shown).

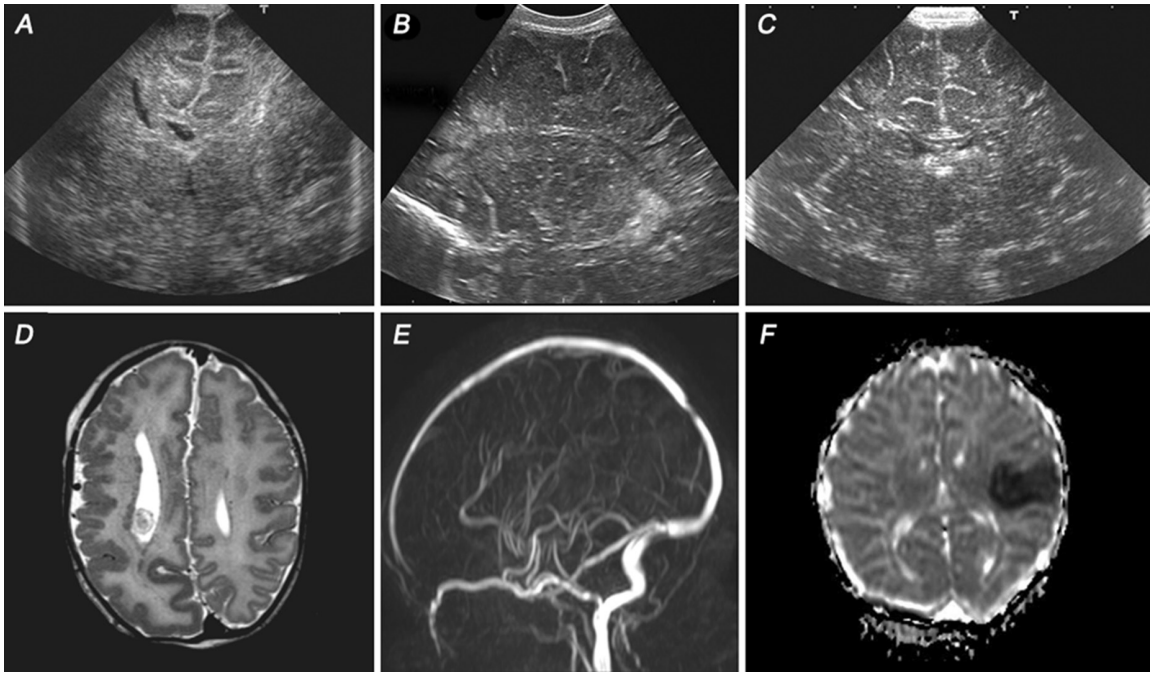

Figure 4. Magnetic resonance image (MRI) diagnosis. Cranial ultrasonography (cUS; A-C) and MRI (axial T2-weighted image, sagittal magnetic resonance venography (MRV), and axial apparent diffusion coefficient (ADC) map; D-F). cUS (A) revealed the odd shape of the right ventricle, but extensive polymicrogyria was diagnosed with MRI (D). Increased echogenicity was seen in the frontal periventricular white matter on cUS (B) with lack of flow in the straight sinus diagnosed with MRV (E). No cUS abnormalities were seen (C), with cortical stroke in the middle cerebral artery territory seen on an ADC map (F).

DISCUSSION

To the best of our knowledge, this is the largest study so far analysing the aetiologies of EEG- or aEEG-confirmed seizures in neonates admitted to a level III neonatal intensive care unit. MRI was performed in all infants, and the diagnostic value of MRI in the context of EEG- or aEEG-confirmed neonatal seizures was evaluated. In all but 6.3% of infants, the underlying aetiology of neonatal seizures could be identified. In agreement with previous studies, HIE, ICH, and PAIS were the most common aetiologies. MRI contributed to making a diagnosis in the majority of the infants. A diagnosis or important imaging abnormalities would have been missed in 11.9% of cases, when only cUS rather than a combination of cUS and MRI would have been used. MRI contributed especially in making a diagnosis of CSVT, metabolic disorders, and cerebral dysgenesis, while cUS was able to make a correct diagnosis or reveal all brain lesions in 46.9% (122/260) of the infants with HIE, ICH and PAIS.

Aetiology

The aetiology of neonatal seizures has been reported in several studies with a limited number of infants. [1-3, 8, 10] Aetiologies were found in 77 to 98.9% of cases, with HIE, ICH, and PAIS being the most common diagnoses. Our percentage of identified aetiologies (93.7%) is at the upper limit of the range, but comparable to the numbers of more recent studies. [1-3, 8, 10]

Magnetic resonance imaging patterns

Hypoxic-ischaemic encephalopathy

Magnetic resonance imaging patterns in HIE (BGT, white matter/watershed, and near-total injury pattern) have been reported in many studies. [11-15] Four studies reported on associated lesions. [12, 14, 16, 17] They found PAIS in approximately 5 to 8% of cases, and only one study reported on the incidence of ICH in HIE. [17] A small number of infants in the present study received therapeutic hypothermia. Although it is unlikely that this affected the patterns of injury, hypothermia may have affected the time of first appearance of the lesions. [15] Many of our infants had EEG or aEEG-confirmed seizures and severe cUS abnormalities but were too unstable to be transferred to the MRI unit and died without MRI confirmation of the cUS abnormalities. The group of infants with HIE as an underlying aetiology would, therefore, have been larger if all infants had undergone MRI.

Intracranial haemorrhage

The existing literature on ICH is a mixture of reports of small studies describing specific types of ICH. [18-20] Several studies have investigated parenchymal haemorrhages and noted that frontal (14.3-27.3%) and temporal lobes (20.8-27.3%) were predominantly affected, [18, 20, 21] which is supported by our findings.

Perinatal arterial ischaemic stroke

Previous studies [22, 23] on PAIS differ in size, inclusion of preterm infants, and in classification of affected cerebral arteries, but in general it was found that the left middle cerebral artery was most often involved, as in our study. PAIS is difficult to diagnose with cUS, since seizures are often present days before abnormalities appear on cUS; therefore, MRI, and especially DWI, are important in diagnosing PAIS early and in more detail, and occasionally with a small PAIS in the contralateral hemisphere. [6]

Cerebral sinovenous thrombosis

The number of infants with CSVT in this study was relatively small, [24] and it is most likely that this was because we included only infants presenting with seizures, while CSVT is a chance finding on neonatal MRI in 5 to 13% of cases. [24] In the majority of infants, multiple sinuses were involved.

Transient metabolic disorder

Burns *et al.* [25] reported an incidence of posterior infarction in hypoglycaemia of only 29%, and a considerable number of infants with BGT involvement. We predominantly found bilateral occipital infarction (64.3%), and identified BGT involvement in only 14.3%. Our findings are more in line with previous reports, [26] although additional lesions were found in 50% of the infants and signal change on early MRI does not necessarily equate to long-term structural damage. This could explain the difference between the results of this review and the results reported by Burns *et al*. [25] as their infants were scanned at a median of 9 days after birth, in contrast to our infants, who were scanned at 5 days after birth. Kernicterus is a difficult neonatal imaging diagnosis and increased signal intensity of the globus pallidus is not always present. [27,28]

Inborn error of metabolism

In general, the findings in our study were comparable to the findings in other studies. [29-35] ^{1}H-MRS is particularly useful in this group of infants. In our study, 81.3% had abnormalities on ^{1}H-MRS and, in the infant with Zellweger syndrome, the ^{1}H-MRS findings were especially useful to suggest the diagnosis along with the cUS (germinolytic cyst and LSV) and MRI findings (polymicrogyria). [36]

Central nervous system infection

Magnetic resonance imaging abnormalities in CNS infections can show a wide variety of patterns, often defined by the organism causing the infection. [37] These findings were supported by our results.

Magnetic resonance imaging versus cranial ultrasonography

Magnetic resonance imaging and cUS are commonly used modalities for neonatal brain imaging. Cranial ultrasonography is regarded as less useful in the full-term infant than in the preterm infant but, more recently, better-quality imaging has been reported using high-quality ultrasound equipment, resulting in a diminished difference in sensitivity between cUS and MRI. [38] In almost half of our study population, MRI showed additional information compared with cUS, which was important for more focused diagnostic testing (e.g. metabolic disorders), more accurate prognosis (e.g. involvement of the corticospinal tracts in PAIS and involvement of the basal ganglia in HIE), and genetic counselling (e.g. polymicrogyria and specific ^{1}H-MRS findings in Zellweger syndrome). In 11.9% of our population, the diagnosis or important brain lesions would have been missed if only cUS had been performed. MRI is essential in certain diagnoses, for example in CSVT, since early treatment is an option and MRI is required for confirmation of CSVT. [6, 13, 39] Both neuro-imaging modalities have advantages and disadvantages. Cranial ultrasonography is readily available and causes minimal disturbance to the infant, but is limited in distinguishing different brain injuries or imaging the deep brain structures, and the quality of the images is user dependent. [5] On the other hand, although MRI is more versatile and sensitive, it is not readily available in all hospitals and unstable patients require specialised care. [5] MRI is generally considered the optimal technique for brain imaging, providing the most complete and useful information. [5, 33] However, these modalities should be used in conjunction with each other. As shown in our study, as well as in others, cUS is good at identifying LSV, germinolytic cysts, and IVH, while MRI provides additional information on white matter abnormalities (signal intensity changes, punctate white matter lesions), myelination, BGT injury, brainstem and cerebellar abnormalities, and migrational disorders. [4, 33, 40] When MRV and ^{1}H-MRS are added to the MRI protocol, this can directly lead to a diagnosis (i.e. CSVT) or more focused diagnostic testing (i.e. metabolic disorders). A combination of cUS and MRI, including MR angiography/MRV/^{1}H-MRS when indicated, provides the most detailed information on neonatal brain abnormalities.

Mortality

The mortality in this cohort (n = 378) was 24.9%, but when also including those infants with EEG- or aEEG-confirmed seizures who died before MRI could be performed, the overall mortality is considerably higher. The former is comparable to other studies. [3,4] It was not the purpose of this study to investigate the prognostic value of MRI in neonatal seizures, since many studies had previously reported on this subject with different aetiologies; therefore, we did not include developmental outcome data in this study. [8] The strength of this study is that it reviewed a large cohort over a long period, as a result of which we could add to the literature an overview of the aetiologies and associated MRI findings in neonates with EEG- or aEEG-confirmed seizures. We also provided support that MRI should be performed in all newborn infants presenting with EEG- or aEEG-confirmed seizures. The retrospective character of the review and long study period were also limitations, since both cUS and MRI quality improved over the years and MRA, MRV, and SWI became available for routine use in neonates. However, all infants in this cohort were scanned on a 1.5T or 3.0T scanner. DWI, MRV, and ^{1}H-MRS have made the biggest contribution to the diagnostic value of MRI. DWI and ^{1}H-MRS were available from the onset of the study period and MRV, mainly important for diagnosing CSVT, became available in 2006. In addition, as stated before, we focused on EEG or aEEG-confirmed seizures in this study to filter out doubtful clinical seizures. Because of this we excluded a number of infants with apparent clinical seizures (e.g. PAIS), who were mostly born at another hospital or at home, had received phenobarbital before transfer to our neonatal intensive care unit and did not show any further clinical or electroencephalographic seizures. We should also take into account that aEEG will only identify 80 to 90% of seizures. [41] If all infants had been monitored with continuous video-EEG recordings, the total number of infants eligible for the study might have been larger. The findings in this study should alert clinicians faced with neonatal seizures, to perform an MRI, preceded by cUS, as part of the diagnostic process, as MRI can either confirm the diagnosis or help with a differential diagnosis. Requirements are, however, MRI with specific neonatal sequences, including DWI, with thin slices, and an option to add MRA, MRV, SWI, and ^{1}H-MRS to the scanning protocol.

CONCLUSION

In conclusion, neonatal seizures are a serious problem and can be caused by a variety of underlying conditions. In our cohort HIE, ICH, and PAIS were the most commonly identified aetiologies. MRI was an important tool in the diagnostic process of neonatal

seizures, since a diagnosis or important imaging abnormalities would have been missed in 11.9% of infants and MRI added significantly to the information obtained in 39.8% of infants.

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Supplemental table 1. Additional diagnoses per aetiologic category in our study population

Aetiology	n*	Additional diagnoses	n (%)
Hypoxic-ischaemic encephalopathy	174	Hypoglycaemia Cerebral sinovenous thrombosis *COL4A1* mutation Congenital heart defect	5 (2.9) 3 (1.7) 1 (0.6) 3 (1.7)
Perinatal arterial ischaemic stroke	40	Non-CNS infection Cerebral sinovenous thrombosis Vein of Galen malformation Inborn error in metabolism of lysine	1 (2.5) 2 (5) 1 (2.5) 1 (2.5)
Cerebral sinovenous thrombosis	11	Intracranial haemorrhage PAIS Vein of Galen malformation	7 (63.6) 1 (9.1) 1 (9.1)
Intracranial haemorrhage	46	Perinatal arterial ischaemic stroke Arteriovenous malformation *COL4A1* mutation Alpha1 antitrypsin deficiency Non-accidental trauma	4 (8.7) 1 (2.2) 1 (2.2) 1 (2.2) 1 (2.2)
Transient metabolic disorder	18	Non-CNS infection Intracranial haemorrhage G6PD deficiency (kernicterus) Inborn error of metabolism	1 (5.6) 3 (16.7) 1 (5.6) 1 (5.6)
Inborn error of metabolism	16	Intracranial haemorrhage Cerebral dysgenesis	1 (6.3) 5 (31.3)
Cerebral dysgenesis	11	Genetic disorder CMV infection	2 (18.2) 1 (9.1)
CNS infection	27	Intracranial haemorrhage (SDH posterior fossa) PAIS Bilateral periventricular haemorrhagic infarction	2 (7.4) 1 (3.7) 1 (3.7)
Non-CNS infection	2	None	0
Familial/genetic	8	Intracranial haemorrhage (SDH posterior fossa)	1 (12.5)

* Single case of cerebral tumour excluded in this table; CMV: cytomegalovirus; CNS: central nervous system; PAIS: perinatal arterial ischaemic stroke; SDH: subdural haemorrhage.

Supplemental table 2. MRI, cUS and ^{1}H-MRS findings per inborn error of metabolism described in our study population

Inborn error of metabolism	cUS findings	MRI findings	^{1}H-MRS findings
Organic acid disorders			
Methylmalonic acidaemia	PV hyperechogenicity	PWML, abnormal occipital cortex, high SI DWI globus pallidus	High lactate, low NAA/Cho ratio
Proprionic acidaemia	PV and thalamic hyperechogenicity	High SI DWI in BG, frontal lobe, brainstem, high SI T1WI globus pallidus	
Sulphite oxidase deficiency			
Isolated sulphite oxidase deficiency	Dysplastic corpus callosum, GLC	Dysplastic corpus callosum, low SI T1WI WM	Normal
Molybdenum cofactor deficiency	WM and thalamic hyperechogenicity	Loss cortical marking T1WI, high SI T1WI BGT, high SI T2WI WM, high SI DWI in cortex, BGT and peduncle, small PLIC	High lactate
Amino acid metabolic disorder			
Nonketotic hyperglycinaemia	Dysplastic corpus callosum	Dysplastic corpus callosum, abnormal myelination WM, reduced cortical folding	Glycine peak
Peroxisomal biogenesis disorder			
Zellweger syndrome	GLC, abnormal Sylvian fissure	GLC, PMG Sylvian fissure and frontal, occipital haemorrhage	Decreased NAA, a lactate peak and an abnormal peak at 0.9 ppm (resonance of methyl residues of mobile lipids)
Other			
Pyruvate dehydrogenase deficiency	Asymmetric ventricles	No abnormalities	Normal
Pyridoxine-dependent epilepsy	GLC, abnormal ventricle shape, abnormal cortical folding occipital, small cerebellum	Abnormal ventricle shape, heterotopia, lack of myelination internal capsule, small cerebellum	High creatinine
Mitochondrial disease	BGT, subcortical and cortical hyperechogenicity	PVL, high SI DWI in BG, WM, germinal matrix haemorrhage, reduced myelination PLIC	High lactate, low NAA/Cho ratio

BG: basal ganglia; BGT: basal ganglia and thalami; Cho: Choline; DWI: diffusion-weighted images; GLC: germinolytic cyst; NAA: *N*-acetyl aspartate; PLIC: posterior limb of the internal capsule; PMG: polymicrogyria; ppm: parts per million; PV: periventricular; PVL: periventricular leukomalacia; PWML: punctate white matter lesions; SI: signal intensity; T1WI: T1-weighted images; T2WI: T2-weighted images; WM: white matter.

Supplemental table 3. Diagnostic value of MRI compared to cUS in all infants with (a)EEG confirmed seizures in whom a diagnosis could be made

	Overall	HIE	PAIS	CSVT	ICH	Transient metabolic disorder	Inborn error of metabolism	CNS infection	Non-CNS infection	Cerebral dysgenesis	Genetic disorder
	n = 354*	**n = 174**	**n = 40**	**n = 11**	**n = 46**	**n = 18**	**n = 16**	**n = 27**	**n = 2**	**n = 11**	**n = 8**
No additional value MRI, n (%)	125 (35.3)	78 (44.8)	8 (20)	0	27 (58.7)	3 (16.7)	2 (12.5)	5 (18.5)	0	1 (9.1)	0
after MRI, n (%)	9 (2.5)	1 (0.6)	7 (17.5)	0	1 (2.2)	0	0	0	0	0	0
MRI diagnosis, n (%)	42 (11.9)	7 (4)	7 (17.5)	10 (90.9)	4 (8.7)	6 (33.3)	3 (18.8)	2 (7.4)	1 (50)	1 (9.1)	1 (12.5)
Additional value MRI, n (%)	141 (39.8)	65 (37.4)	18 (45)	1 (9.1)	14 (30.4)	5 (27.8)	10 (62.5)	18 (66.7)	0	9 (81.8)	1 (12.5)
No abnormalities on MRI, n (%)	37 (10.5)	23 (13.2)	0	0	0	4 (22.2)	1 (6.3)	2 (7.4)	1 (50)	0	6 (75)

* Single case of cerebral tumour excluded in this table. CNS: central nervous system; CSVT: cerebral sinovenous thrombosis; HIE: hypoxic-ischaemic encephalopathy; ICH: intracranial haemorrhage; MRI: magnetic resonance imaging; PAIS: perinatal arterial ischaemic stroke.

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5

Chapter 6

Punctate white matter lesions in full-term infants with neonatal seizures associated with *SLC13A5* mutations

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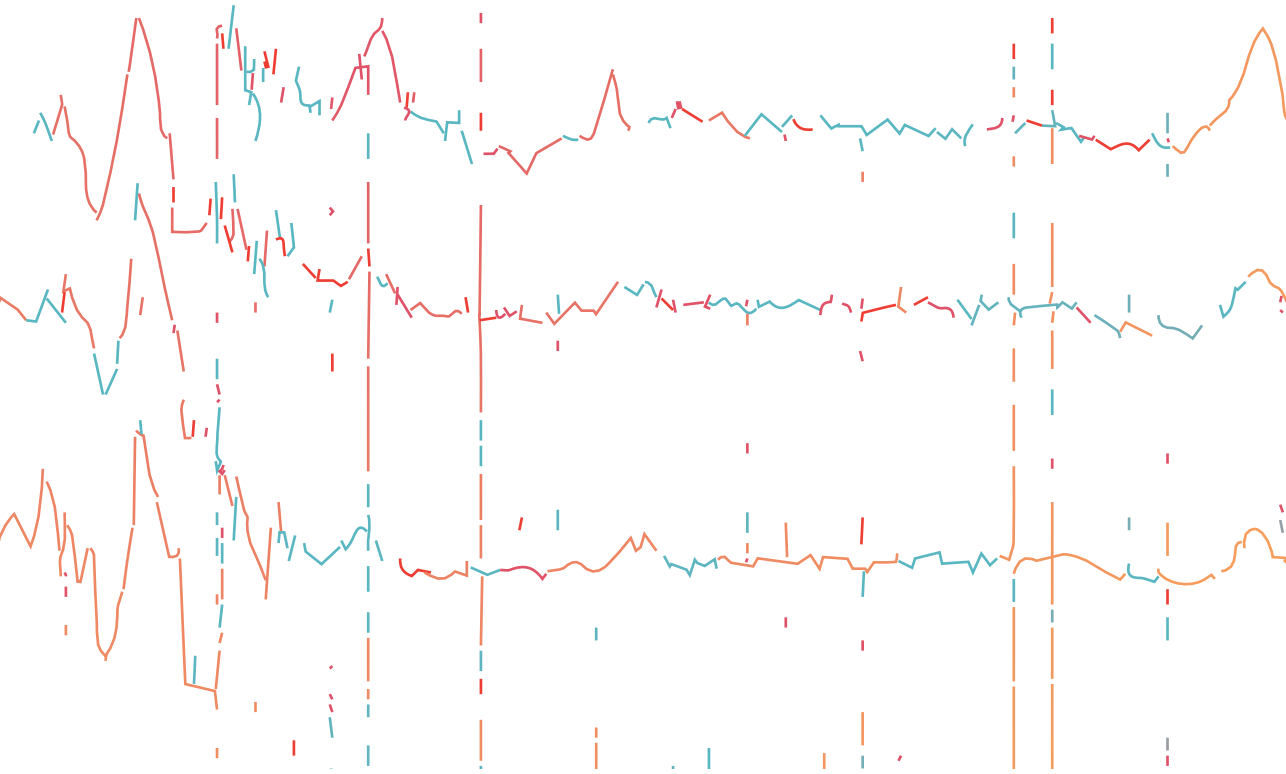

ABSTRACT

Introduction Early-onset epileptic encephalopathy caused by biallelic *SLC13A5* mutations is characterised by seizure onset in the first days of life, refractory epilepsy and developmental delay. Little detailed information about the brain MRI features is available in these patients.

Methods Observational study describing the neuro-imaging findings in eight patients (five families) with mutations in the *SLC13A5* gene. Seven infants had an MRI in the neonatal period; two had a follow-up MRI at the age of 6 and 18 months and one only at 13 months. One patient had follow-up MRIs at 11 and 16 months and 3 and 6 years of age, but no neonatal MRI.

Results All patients presented with refractory neonatal seizures on the first day of life after an uncomplicated pregnancy and term delivery. Six out of seven infants with a neonatal MRI had a characteristic MRI pattern, with punctate white matter lesions (PWML), which were no longer visible at the age of 6 months, but led to gliotic scarring visible on MRI at the age of 18 months. The same pattern of gliotic scarring was seen on the MRIs of the infant without a neonatal scan. One infant had signal abnormalities in the white matter suspected of PWML on T2WI, but these could not be confirmed on other sequences.

Conclusion In infants presenting with therapy resistant seizures in the first days after birth, without a clear history of hypoxic-ischaemic encephalopathy, but with PWML on their neonatal MRI, a diagnosis of *SCL13A5* related epileptic encephalopathy should be considered.

INTRODUCTION

Early-onset epileptic encephalopathies are a clinically and aetiologically heterogeneous group of severe epileptic disorders, characterised by recurrent and pharmacoresistant seizures associated with different degrees of developmental delay. [1] Most epileptic encephalopathies have a genetic cause and due to the increased use of genetic testing, more and more patients with a previously unknown cause of seizures are diagnosed with a specific epileptic encephalopathy. [2] Two recent publications described a distinct neonatal epileptic encephalopathy (MIM 615905) caused by autosomal recessive mutations in the *SLC13A5* gene. [2, 3] This specific epileptic encephalopathy is characterised by seizure onset in the first days of life in otherwise healthy newborns after an uncomplicated pregnancy and delivery, refractory epilepsy with frequent status epilepticus, fever sensitivity, developmental delay and teeth hypoplasia or hypodontia. [2, 3] Little detailed information about the brain MRI features is available in these patients. Thevenon *et al.* described that brain imaging was not conclusive in seven patients from two families and Hardies *et al.* described that two siblings had periventricular leukomalacia-like abnormalities on MRI and a lactate peak on magnetic resonance spectroscopy. [2, 3] We describe the neuro-imaging findings in eight patients from five families with neonatal epilepsy and mutations in the *SLC13A5* gene.

METHODS

Patients

Eight patients from five different families with homozygous or compound heterozygous mutations in the *SLC13A5* gene were included. Seven patients had been admitted to the neonatal intensive care unit (NICU) of the Wilhelmina Children's Hospital in Utrecht, the Netherlands, the Isala Clinics in Zwolle, the Netherlands or the Paediatric Neurology Clinic, Alexandru Obregia Hospital in Bucharest, Romania because of neonatal seizures between 2001 and 2016. One additional patient, an older half-sibling of one of the patients, had not been admitted to a neonatal unit and was already deceased at the time of inclusion for the present study. Medical records were used for information on clinical and demographic features. Parental informed consent was obtained for all patients included.

(Amplitude-integrated) electroencephalography

Continuous amplitude-integrated EEG (aEEG) was used as standard of care in the neonatal intensive care units. In patient 3, a single-channel (P4-P3) analogue aEEG was used (CFM 4640; Lectromed, Devices Ltd, UK), in patients 1, 2, 6 and 7 a two-channel (F4-P4, F3-P3) digital BRM2 or BRM3 BrainZ monitor was used. Patient 5 was enrolled in an EEG-study and was monitored with continuous 8-channel EEG (EEG-1200, Nihon Kohden, Tokyo, Japan). Patient 8 had a 32-channel video-EEG (Nicolet, Natus, Seattle, USA).

Neuro-imaging

Neonatal

Cranial ultrasound (cUS) was performed on admission with a broadband transducer (5-8.5 MHz). MRI was performed as soon as possible after admission on a 1.5T or 3.0T magnet. Standard MRI protocol included sagittal and axial T1-weighted images (T1WI), axial T2-weighted images (T2WI), inversion recovery-weighted images, diffusion-weighted images (DWI) including apparent diffusion coefficient (ADC) mapping and proton MR spectroscopy (^{1}H-MRS).

Follow-up

A follow-up MRI was performed when possible at the age of 6 and 18 months on a 1.5T or 3.0T magnet. Standard MRI protocol included sagittal and axial T1WI, axial T2WI, inversion recovery-weighted images, DWI and coronal T2 fluid attenuated inversion recovery (FLAIR)-weighted images.

Mutation detection

The mutations in *SLC13A5* (NM_177550) in family 1 were detected in a research setting through sequencing of an epilepsy-related gene panel, as described previously.[4] In family 2 and 5 the mutation was detected by exome sequencing in a research setting. In family 3 diagnostic exome sequencing was performed with limited analysis of only the *SLC13A5* and *LAMB3* genes. In family 4 diagnostic direct Sanger sequencing of the complete open reading frame of *SLC13A5* was performed for one of the twins. Subsequently the presence of the mutation was confirmed in DNA of the other twin. All mutations were classified as pathogenic. See *supplemental material* for extensive details on the molecular analysis.

RESULTS

Clinical features at presentation

The clinical features at presentation in the neonatal period are described in *table 1*. All infants were born full-term or near-term following an uncomplicated pregnancy and delivery, with normal birth weights and good Apgar scores, and presented with clonic seizures on the first day of life. Seizures were confirmed on amplitude-integrated EEG (aEEG) or standard EEG and were refractory to most antiepileptic drugs (AEDs). In patients 1, 7 and 8 subclinical seizures did not respond to any AED, in patients 2, 5 and 6 they responded to lidocaine and in patient 3 to midazolam. However, refractory seizures reoccurred within 2-4 weeks. The background activity on the aEEG was normal (continuous normal voltage) to mildly abnormal (discontinuous normal voltage), but recovered to normal background activity with sleep-wake cycling at day of life 3-7. One infant (patient 5) also presented with progressive bullous skin lesions and fragile, long nails and was diagnosed with severe generalised junctional epidermolysis bullosa (type Herlitz) caused by autosomal, recessive mutations in the *LAMB3* gene (c.727C>T p.(Gln243*) and c.1903C>T p.(Arg635*)). Patient 7 had pneumatosis intestinalis on abdominal X-ray without clinical signs of infection or necrotising enterocolitis. Patients 3, 4 and 8 have been described before, but with limited information on neuro-imaging features. [2]

Neuro-imaging

Neonatal

Individual neuro-imaging findings are described in *table 1*. Five infants showed echogenicity in the periventricular and subcortical white matter on cranial ultrasound performed on admission to the NICU. A neonatal MRI was performed in seven infants, all within the first week of life (day 2-7). Six infants had punctate white matter lesions (PWML) seen as high signal intensity (SI) on T1WI *(figure 1E)*, low SI on T2WI *(figure 1A, F)*, high SI on DWI *(figure 1B-D, G)* and low SI on the ADC map *(figure 1H)*. Patient 2 showed bilateral high SI in the frontal and parietal-occipital white matter on T2WI, corresponding to low SI on DWI and high SI on the ADC map, with high (1700 mm^2/s) ADC measurements. T2WI showed diffuse small areas of low SI in the WM which could be the beginning of PWML but no clear punctate white matter lesions were seen on DWI or the ADC map. ^{1}H-MRS measurements in the white matter were performed in five infants showing a decreased *N*-acetyl aspartate/Choline ratio in all and a small lactate peak in two infants (patients 2 and 5).

Table 1. Clinical features at presentation in the neonatal period and neurodevelopmental outcome of the eight patients described in this report

	Family 1		Family 2		Family 3	Family 4		Family 5
Patient	1	2	3	4	5	6	7	8
Mutations	c.655G>A p.(Gly219Arg) and c.1280C>T p.(Ser427Leu)	c.655G>A p.(Gly219Arg) and c.1280C>T p.(Ser427Leu)	c.1280C>T p.(Ser427Leu), homozygous	c.1280C>T p.(Ser427Leu), homozygous	c.680C>T p.(Thr227Met) and c.1280C>T p.(Ser427Leu)	c.655G>A p.(Gly219Arg) homozygous	c.655G>A p.(Gly219Arg) homozygous	c.680C>T p.(Thr227Met) and c.1570G>C p.(Asp524His)
Age at diagnosis	9 years	Post-mortem (5 years after death)	12 years	Post-mortem (16 years after death)	Post-mortem (3 months after death)	15 days	15 days	4 years
Neonatal period								
Sex	Female	Male	Male	Male	Male	Female	Female	Female
Gestational age (weeks)	37.3	40.5	37.4	Term	36.5	38.0	38.0	38.0
Birth weight (grams)	3142	3635	2630	Unknown	3210	3180	3750	3270
Head circumference (cm)	34	36	32	Unknown	32.5	35	34	35
Apgar at 1 and 5 min	9/10	10/10	9/10	Unknown	9/10	9/10	9/10	10/10
Age at presentation of first seizures (day of life)	1	1	1	1	1	1	2	1

Table 1. (continued)

	Family 1		Family 2		Family 3	Family 4		Family 5
Patient	1	2	3	4	5	6	7	8
Seizure type	Status epilepticus: tonic-clonic, cyanosis, lip smacking, subclinical	Repetitive seizures: clonic, cyanosis, subclinical	Status epilepticus: focal clonic seizures right hand and face, subclinical	*"similar to half-sibling"*	Status epilepticus: clonic, lip smacking, subclinical	Status epilepticus: focal clonic seizures hand, mouth, generalised clonic	Status epilepticus: focal tonic-clonic seizures hand, mouth, generalised clonic	Focal clonic seizures face and hand, alternative right or left, or focal-fixed gaze, sucking movements, lip smacking
AEDs administered	PB, MD, LD, CL	PB, PD, MD, LD	PB, LD, MD	Unknown	PB, LD	PB, MD, LD, LV, PT	PB, MD, LD, LV, PT	PB
AED effective	None	LD	MD	Unknown	LD	LD	None	None
Age at reoccurrence of seizures	NA (not seizure free)	NA (died)	4 weeks	Unknown	3 weeks	13 days	NA (not seizure free)	NA (not seizure free)
aEEG background activity	DNV recovering to CNV + SWC at day 3	DNV recovering to CNV + imminent SWC at day 3	CNV-DNV recovering to CNV + SWC at day 7	Unknown	CNV-DNV recovering to CNV + SWC at day 3	DNV recovering to CNV + SWC at day 5	DNV recovering to CNV + SWC at day 3	aEEG not done; video-EEG: spike-waves on discontinuous EEG at day 7
Co-morbidity	None	None	None	None	Epidermolysis bullosa, severe generalised (Herlitz) (c.727C>T p.(Gln243*) and c.1903C>T p.(Arg635*))	None	Pneumatosis intestinalis	None

Table 1. (continued)

	Family 1		Family 2		Family 3	Family 4		Family 5
Patient	1	2	3	4	5	6	7	8
Neonatal imaging								
cUS results	Echogenicity WM	Echogenicity WM	Echogenicity WM, possible haemorrhage WM	Echogenicity WM	Echogenicity and echolucency WM	Echogenicity WM, slit like ventricles	No abnormalities	No abnormalities
Age at MRI (day of life)	4	2	7	NA	3	7	7	2
MRI results	Extensive bilateral PWML, small IVH, low NAA/Cho ratio in WM	High SI WM on T2WI, suspected PWML, small tentorial SDH, low NAA/Cho ratio and small lactate peak in WM	Extensive bilateral PWML, lactate peak	NA	Multiple bilateral PWML, IVH, cerebellar haemorrhage, low NAA/Cho ratio and small lactate peak in WM and left basal ganglia	Extensive bilateral PWML, corpus callosum high SI on DWI, low NAA/Cho ratio in WM	Five small PWML bilateral, delayed gyrification, cortical stroke, corpus callosum high SI on DWI, low NAA/Cho ratio in WM	Three small PWML bilateral
Follow-up imaging								
Age at MRI	5 and 19 months	Died	6 and 14 months	11 and 16 months, 3 and 6 years	Died	Died	Died	13 months
MRI results								
6 months	WM loss (large ECS, wide Sylvian fissures, sulci almost abutting lateral ventricles)	NA	WM loss (large ECS, wide Sylvian fissures, sulci almost abutting lateral ventricles)	NA	NA	NA	NA	NA

Table 1. (continued)

	Family 1		Family 2		Family 3	Family 4		Family 5
Patient	**1**	**2**	**3**	**4**	**5**	**6**	**7**	**8**
18 months	Delayed myelination and gliosis (frontal, parietal, occipital periventricular WM), WM loss (large ECS, wide Sylvian fissures)	NA	Delayed myelination and gliosis (frontal, occipital periventricular WM, centre semiovale), WM loss (large ECS, wide Sylvian fissures)	Delayed myelination and gliosis (frontal, occipital periventricular WM, centre semiovale), WM loss (wide Sylvian fissures, mildly enlarged ventricles)	NA	NA	NA	No abnormalities
Outcome								
Cognitive impairment	Yes	Died	Yes	Yes	Died	Died	Died	Yes
Motor impairment	Yes	Died	Yes	Yes	Died	Died	Died	Yes
Fever sensitivity	Yes	Died	No	Unknown	Died	Died	Died	No
Teeth abnormalities	Yes	Too young (died)	Yes	Yes	Too young (died)	Too young (died)	Too young (died)	Yes

aEEG: amplitude-integrated EEG; Cho: choline; CL: clonazepam; CNV: continuous normal voltage; cUS: cranial ultrasound; DNV: discontinuous normal voltage; ECS: extracerebral space; IVH: intraventricular haemorrhage; LD: lidocaine; LV: levetiracetam; MD: midazolam; NAA: *N*-acetyl aspartate; PB: phenobarbital; PD: pyridoxine; PLIC: posterior limb of the internal capsule; PT: pentothal; PVL: periventricular leukomalacia; PWML: punctate white matter lesions; SWC: sleep-wake cycling; T2WI: T2-weighted images; WM: white matter.

Follow-up

Two infants (patients 1 and 3) had a follow-up MRI around the age of 6 and 18 months. Around 6 months of age the MRI showed white matter loss seen on T1WI and T2WI with an enlarged extracerebral space *(figure 1I-L)*, wide Sylvian fissures *(figure 1K)* and sulci that were almost abutting the lateral ventricles, which were not enlarged. No cystic lesions or gliosis was observed, which should be seen as high SI on a FLAIR sequence *(figure 1L)*, at the site of the PWML seen in the neonatal period. At 18 months of age the MRI showed areas of delayed myelination in the periventricular white matter and the centre semiovale seen as low SI on T1WI *(figure 1M)* and high SI on T2WI *(figure 1N)*, corresponding to areas of gliosis seen as high SI on the FLAIR *(figure 1P)*. An enlarged extracerebral space, with wide Sylvian fissures indicating white matter loss was seen as well, the lateral ventricles were not enlarged *(figure 1O)*. The same MRI pattern was seen on the scans of patient 4 at 11 and 16 months and 3 and 6 years of age. Patient 8 had a follow-up MRI at 13 months of age; this did not show white matter loss or gliosis.

Neurodevelopmental outcome

Five of the eight infants died. Three patients died in the neonatal period (patients 2, 6, 7). Patient 2 died in the neonatal period at the age of 7 days after redirection of care, because of the similarity with the clinical symptoms in an older sibling with known adverse neurological outcome (patient 1). Patients 6 and 7 died at the age of 15 days after redirection of care, because of expected poor prognosis, refractory seizures and ventilator dependency. Two patients (patients 4 and 5) died beyond the neonatal period. Patient 4 developed spastic tetraplegia, severe cognitive impairment, progressive microcephaly, pharmacoresistant epilepsy and died at the age of 7 years as a consequence of status epilepticus. Patient 5 had frequent seizures, exacerbated by fever and died at the age of 6 weeks as a consequence of clinical sepsis. Post-mortem examination in patient 5 showed areas of gliosis mainly in the occipital white matter. Patients 1 and 3 have severe cognitive and motor impairment and suffer from refractory epilepsy, with frequent status epilepticus. Patient 1 was seizure free between the ages of 3 and 6 years but relapsed with yearly episodes of seizures mostly related to (viral) infections and fever. Patient 3 never became seizure free despite several AEDs and a vagal nerve stimulator. Patient 8 has both cognitive and motor impairment, with truncal ataxia and pyramidal signs. This patient is on multiple AEDs and has one to two seizures per year, but never became completely seizure free.

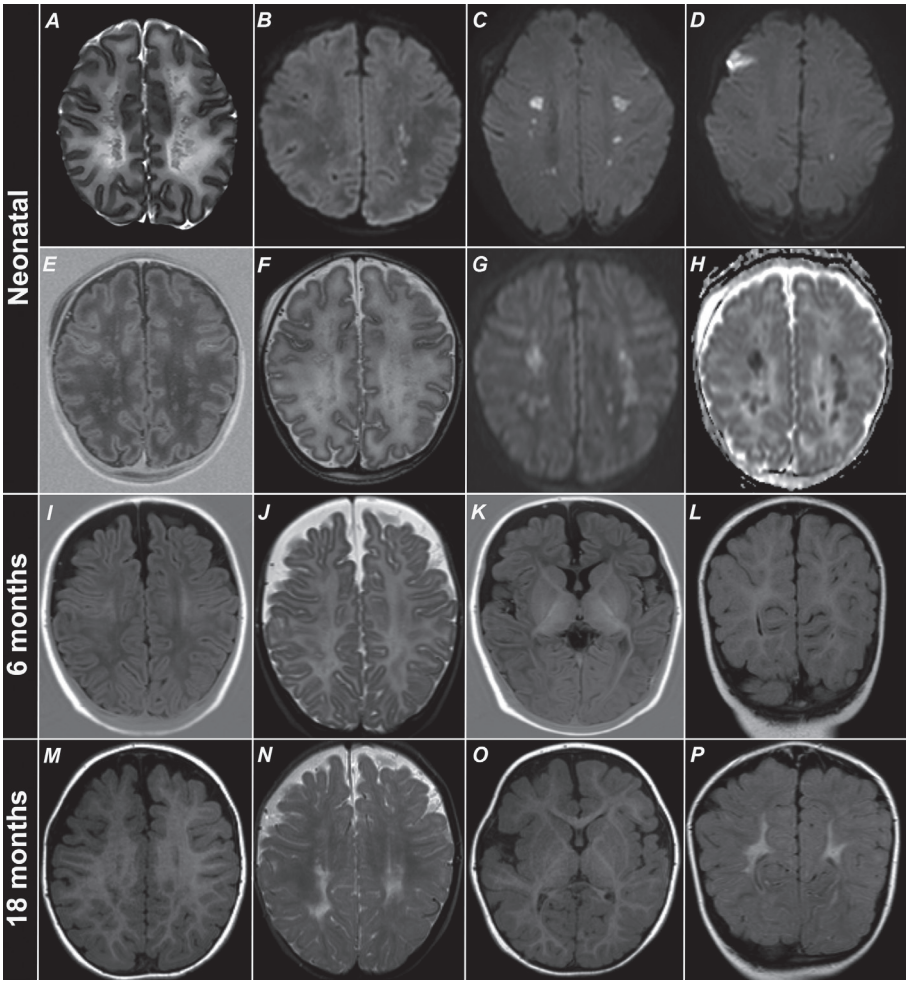

Figure 1. Examples of MRI abnormalities in patients with SLC13A5 mutations. (A-D) Neonatal MRI images showing punctate white matter lesions (PWML) seen as low signal intensity (SI) on T2-weighted image (T2WI) in patient 3 (A) and high SI on diffusion-weighted images (DWI) in patient 5 (B), 6 (C) and 7 (D). Patient 7 (D) had multiple PWML, but only one in the left hemisphere is shown in this image. In this patient, a cortical stroke on the right is visible as well in this image. (E-H) Neonatal MRI images of patient 1, showing PWML seen as high SI on T1-weighted image (T1WI) (E), low SI on T2WI (F), high SI on DWI (G) and low SI on apparent diffusion coefficient (ADC) map (H). (I-L) MRI images of patient 1 at 6 months of age, showing white matter loss on T1WI (I, K) and T2WI (J) with a large extracerebral space, wide Sylvian fissures and sulci that are almost abutting the lateral ventricles, which are not enlarged. No cystic lesions or gliosis is observed on the fluid attenuated inversion recovery (FLAIR) sequence (L). (M-P) MRI images of patient 1 at 18 months of age, showing areas of delayed myelination in the periventricular white matter and the centre semiovale seen as low SI on T1WI (M) and high SI on T2WI (N), corresponding to areas of gliosis seen as high SI on the FLAIR (P). An enlarged extracerebral space, with wide Sylvian fissures indicating white matter loss is seen as well, no enlarged lateral ventricles (O).

DISCUSSION

This is the first study that describes the neuro-imaging features of patients with mutations in the *SLC13A5* gene in detail. Seven out of eight patients included in this study had a characteristic MRI pattern, with PWML on their neonatal MRI scan, which were no longer visible at the age of 6 months, but led to gliotic scarring visible on MRI at the age of 18 months and beyond. One patient did not have PWML, but only showed abnormal signal intensities on T2WI, which could be the onset of PWML, but this could not be confirmed on DWI or the ADC map. Another patient with two small PWML in the neonatal period had a normal MRI at 13 months of age, however no FLAIR-sequence was performed, which is more sensitive for gliosis than T1WI or T2WI. The PWML could also have been too minor to cause significant gliosis in this patient or gliosis was not visible yet, since there appears to be a "silent window" between the neonatal period and approximately 18 months of age where gliosis that is visible on later MRIs cannot be detected. Two studies have mentioned MRI findings in patients with *SLC13A5* mutations, [2, 5] however not as detailed as described here and not including neonatal MRI. Hardies *et al.* described patients 3 and 4 from our study as well, but only mentioning that they had periventricular leukomalacia-like abnormalities on MRI. [2] The study by Schossig *et al.* reported MRI findings in two patients. One patient was scanned at 2 and 10 years and the MRI was normal, however this patient did not have seizures until 7 months of age, which is different from our study population. Another patient was scanned at 11 months and only showed mild atrophy, which is in concordance with our findings. [5] *SLC13A5* associated epileptic encephalopathy is characterised by seizure onset during the first days of life, with tonic-clonic seizures, refractory epilepsy with frequent status epilepticus, prominent convulsive seizures and fever sensitivity later in life, developmental delay with variable cognitive impairment and teeth hypoplasia or hypodontia. [2, 3] *SLC13A5* encodes a high affinity sodium-dependent citrate transporter, which is expressed in the brain and liver. [6] In the brain, citrate plays an important role in cellular metabolism and neurotransmitter synthesis. [3] All *SLC13A5* mutations result in a complete loss of citrate transport. [2] Defected citrate transport in the brain could lead to epilepsy through three possible mechanisms: brain energy failure, an imbalance in glutamate and GABA production, and reduced inhibition of the excitatory *N*-methyl *D*-aspartate (NMDA) receptor. [2] White matter lesions such as PWML and periventricular leukomalacia (PVL) have been associated with epilepsy as well. Twenty-six to 48% of infants with PVL developed epilepsy, [7-10] which was often refractory. [7] However, the onset of epilepsy in these patients was usually beyond the neonatal period. [7, 8] Only 20% had neonatal seizures. [9] It is, therefore, unlikely that the PWML observed in our study are the cause of the early-onset seizures and refractory

epilepsy associated with *SLC13A5* mutations. The cognitive impairment associated with *SLC13A5* mutations can be variable and the pathophysiological mechanism is not clearly understood. [2] Whether this is related to the *SLC13A5* defect, additional genetic factors, severity of seizures or the ischaemic injury to the white matter as seen in our study is not yet known. Cognitive impairment following white matter injury such as cystic lesions in the periventricular or subcortical white matter has been described in preterm infants. [11, 12] Also, recent studies using diffusion tensor imaging have shown that the integrity of the white matter is related to IQ in preterm infants. [13, 14] In full-term infants with hypoxic-ischaemic encephalopathy (HIE), cognitive impairment has also been related to white matter injury specifically. [15] However, the extent of the white matter injury in infants with *SLC13A5* mutations and PWML is less severe than in infants with cystic PVL or HIE. On the neonatal MRI, the extent of the DWI abnormalities is punctate rather than confluent and on follow-up MRI the white matter loss seems to be less severe as well, with enlargement of the extracerebral space and widening of the Sylvian fissures but without irregular enlargement of the lateral ventricles, which is characteristic in cystic PVL. Also, PWML in preterm infants have not been associated with cognitive impairment as severe as observed in patients with *SLC13A5* mutations. [16] How mutations in the *SLC13A5* gene lead to ischaemic lesions in the white matter after birth is not clear either. Hardies *et al.* suggested that patients with *SLC13A5* defects may be more vulnerable to ischaemia, an hypothesis which is supported by our findings of multiple ischaemic PWML shortly after birth in the majority of our patients. PWML, a form of white matter injury, are most often seen in preterm infants and is thought to be related to injury to oligodendrocyte progenitor cells and subplate neurons. [17] PWML have also been reported in some populations of full-term infants. In infants with congenital heart disease, PWML were seen in 54% following cardiopulmonary bypass. [18] In a small study of full-term infants with HIE, PWML were seen in 23%. [19] In these infants younger gestational age, but not birth weight was associated with the severity of white matter injury. This suggests that the state of brain maturation is an important risk factor for the development of PWML in full-term infants. Interestingly, congenital heart disease has also been associated with delayed brain maturation. [20-22] This could be explained by the fact that differentiating oligodendrocytes are sensitive to insults such as hypoxia and ischaemia, while mature oligodendrocytes are relatively resistant to these types of injury, resulting in a maturation-dependent window of vulnerability to white matter injury. [17] In all five infants in our cohort in whom ^{1}H-MRS was performed, this showed a low NAA/Cho ratio in the white matter, which could be a sign of delayed maturation. [20] However, the neonatal MRIs did not show signs of delayed myelination or gyrification on visual analysis. Additional advanced MRI techniques such as diffusion tensor imaging may

be needed to detect more subtle abnormalities in brain development. [20] Therefore, we cannot draw a definite conclusion at the moment on the pathophysiological mechanism of PWML in infants with *SLC13A5* mutations.

CONCLUSION

The association between *SLC13A5* mutations and PWML observed in our study is striking. In otherwise healthy infants presenting with therapy resistant seizures in the first days after birth, without a clear history of HIE but with PWML on their neonatal MRI, a diagnosis of mutations in the *SCL13A5* gene should be considered.

ACKNOWLEDGEMENTS

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SUPPLEMENTAL MATERIAL

Molecular analysis

In families 1, 2 and 3 molecular analysis was performed at the UMC Utrecht Department of Genetics, the Netherlands. In family 1 sequencing of an epilepsy-related gene panel was performed, as described previously (de Kovel *et al.* Molecular Genetics & Genomic Medicine 2016). In brief, probes for enrichment were designed and ordered from Agilent Technologies (Santa Clara, USA) as SureSelect enrichment kit, and sequencing was performed on a SOLiD 5500XL. Alignment, variant calling and annotation were done using an in-house pipeline, which used a BWA-aligner and a GATK-based variant caller versus Human reference GhCr37/hg19. In family 2 exome sequencing was performed as described previously (Hardies *et al.* Brain 2015), using the SureSelect® Human 35 Mb capturing kit (Agilent) and sequencing the resulting library on a SOLiD™ 4 System (Applied Biosystems). Reads were aligned with the Burrows-Wheeler Aligner (Li and Durbin 2009). Variant calling and analysis was done with a custom pipeline. In family 3 exomes of patient and parents were enriched using the SureSelect XT Human All Exon V5 kit (Agilent) and sequenced in rapid run mode on the HiSeq2500 sequencing system (Illumina) at a mean sequencing depth of 100X. Reads were aligned to hg19 using BWA (BWA-MEM v0.7.5a) and variants were called using the GATK haplotype caller (v2.7-2). Detected variants were annotated, filtered and prioritised using Bench Lab NGS software (Cartagenia, Leuven, Belgium). Only the *SLC13A5* and *LAMB3* genes were analysed.

In family 4 molecular analysis was performed at the Metabolic Unit of VU University Medical Center, Amsterdam, the Netherlands. Diagnostic direct Sanger sequencing of the complete open reading frame of *SLC13A5* was performed for one of the twins. Subsequently the presence of the mutation was confirmed in DNA of the twin sister.

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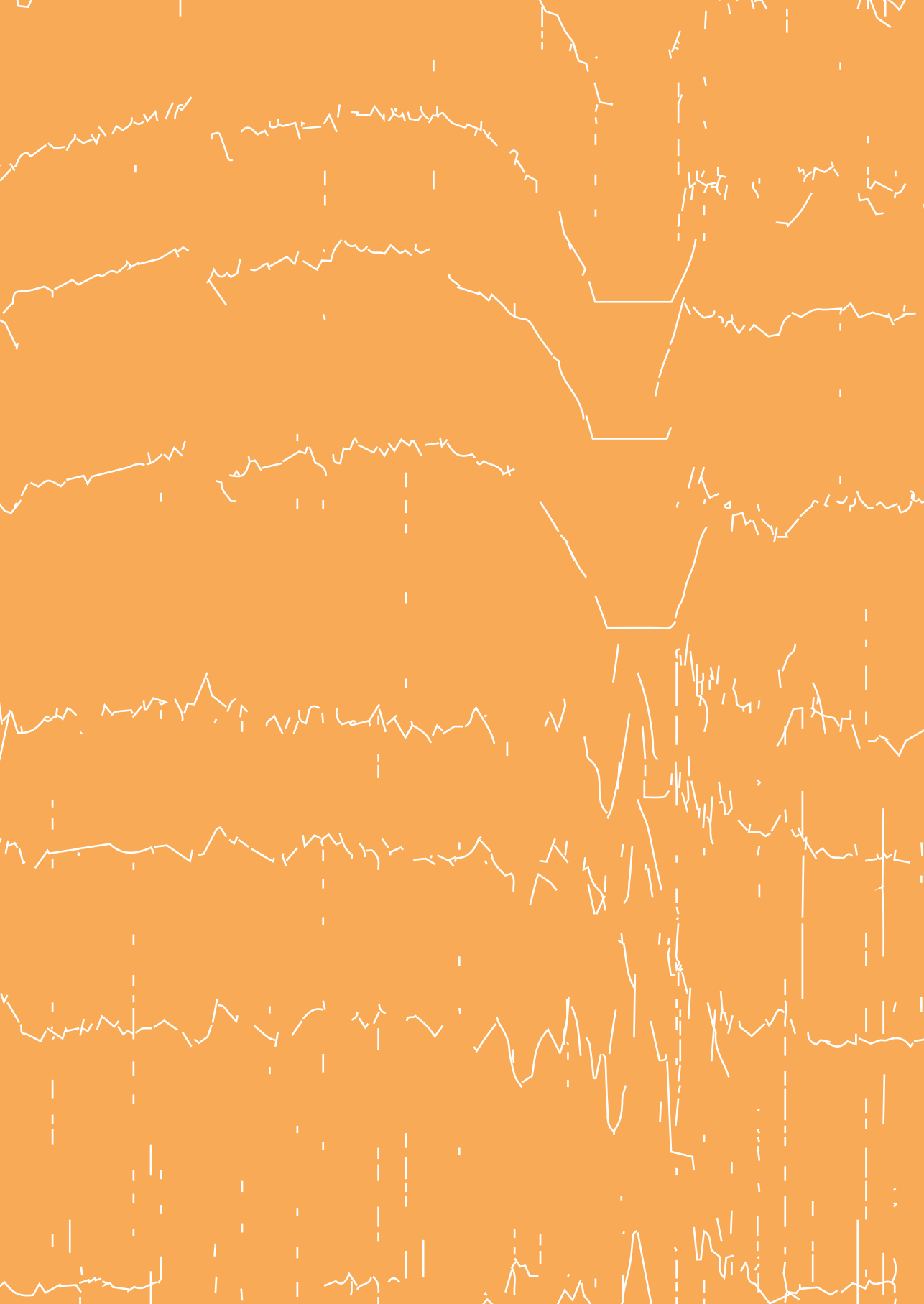

PART 2

Treatment

Chapter 7

Lidocaine-associated cardiac events in newborns with seizures: incidence, symptoms and contributing factors

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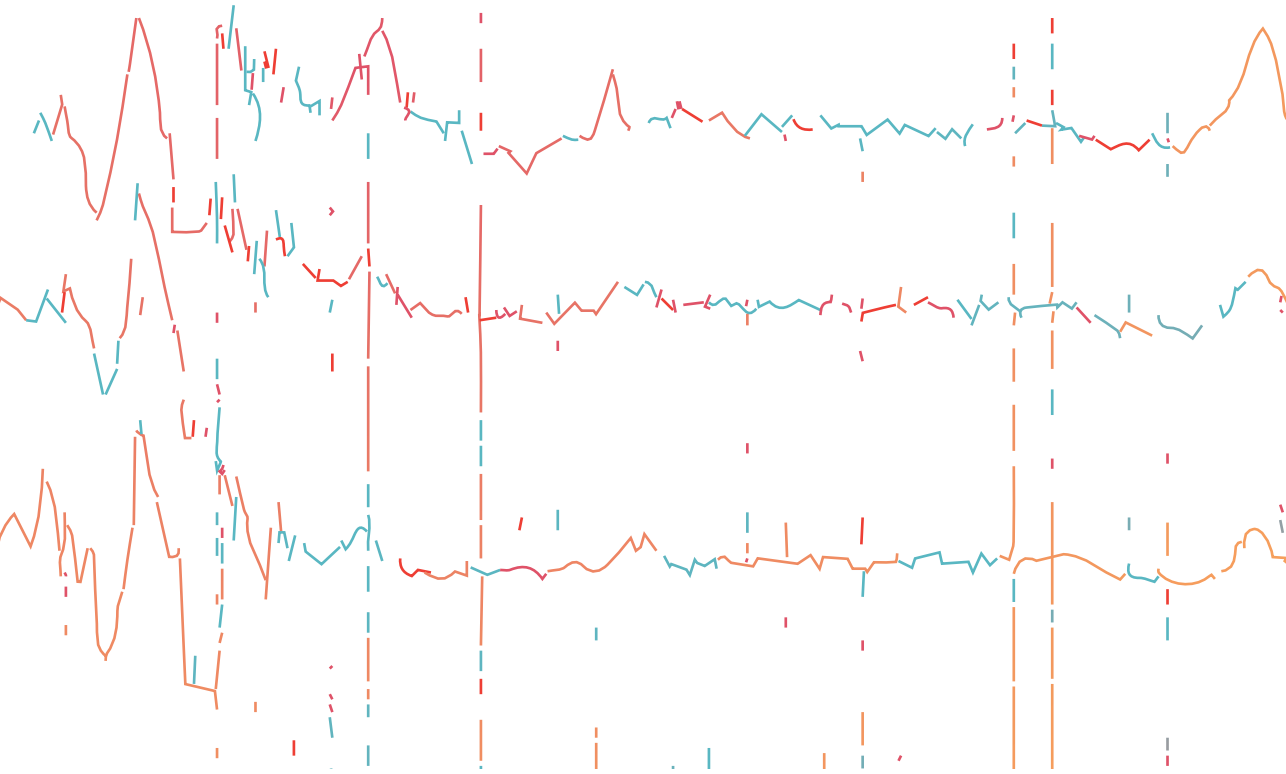

ABSTRACT

Introduction Lidocaine is an effective therapy for neonatal seizures; however, it is not widely used, presumably due to the risk of cardiac events. The objective was to investigate the incidence of cardiac events in full-term and preterm infants receiving lidocaine for seizures.

Methods Full-term (n = 368) and preterm (n = 153) infants, admitted to a level III neonatal intensive care unit from 1992 to 2012, who received lidocaine for seizures were retrospectively studied. The causal relation between reported cardiac events and lidocaine administration was evaluated based on expected plasma concentrations, symptoms and relevant interactions during cardiac events.

Results Cardiac events were reported in 11/521 infants (2.1%; 9 full-term, 2 preterm). In 7/11 infants the causal relation was considered plausible, in 3/11 questionable and in 1/11 implausible. The incidence was calculated to be 1.3-1.9% (n = 7-10/521), but was only 0.4% (n = 1/246, p = 0.02) when using reduced-dose regimens. Important risk factors for cardiac events were unstable potassium, (congenital) cardiac dysfunction and concurrent phenytoin use.

Conclusion Lidocaine-associated cardiac events were rare in our cohort, especially since the introduction of new reduced dose regimens. This indicates that lidocaine is safe to use as an antiepileptic drug in full-term and preterm infants.

INTRODUCTION

Lidocaine promises to be an effective therapy for neonatal seizures persisting despite first- and second-line therapy;[1-4] however, it is not widely used,[5] presumably due to the risk of cardiac events. Cardiac events have been reported in studies with neonates receiving lidocaine for seizures, with incidences ranging from 0 to 4.8%.[1-4, 6-10] Unfortunately, most are small studies and the large study by van Rooij *et al.*[6] dates back to 2004. The aim of the present study was to expand the cohort of van Rooij *et al.* to investigate the incidence of lidocaine-associated cardiac events in a larger cohort of full-term and preterm infants receiving lidocaine for seizures. Secondary objectives were to investigate the association between lidocaine and cardiac events using new criteria, and to determine how to recognise lidocaine-associated cardiac events and which factors contribute.

METHODS

Patients

All infants (full-term and preterm) treated with lidocaine for seizures in our neonatal intensive care unit were studied retrospectively (January 1992 - December 2012). No exclusion criteria were applied. Lidocaine was administered when other antiepileptic drugs had failed *(table 1)*. Infants were identified using a local database. Medical and nursing records were hand searched for cardiac events during lidocaine administration. Cardiac events were defined as non-benign cardiac problems observed during lidocaine administration that were noted by the medical staff (e.g. by electrocardiogram) and were considered to be serious or required intervention. Bradycardia was defined as a heart rate <85 in normothermic and <65 in hypothermic infants that required treatment. Data abstracted from these records included gestational age, birth weight, diagnosis, administered antiepileptic drugs, type of cardiac event, lidocaine-dosing regimen, time of lidocaine infusion, time elapsed between beginning of lidocaine infusion and cardiac event, co-medication, cardiac and clinical status, and treatment outcome. No permission was required from the hospital's medical ethics committee for this retrospective, anonymous data analysis.

Table 1. Changes in lidocaine dosing regimens over the years

	Bolus (mg/kg, 10 min)	Infusion I (mg/kg/h)	Infusion II (mg/kg/h)	Infusion III (mg/kg/h)	Duration of infusion (h)	Total dosage (mg/kg)
Year						
1992-2003	2	6 (24 h)	4.00 (12 h)	2.00 (12 h)	48	218
2004-2005	2	6 (12 h)	4.00 (12 h)	2.00 (12 h)	36	146
2006-2010	2	6 (6 h)	4.00 (12 h)	2.00 (12 h)	30	110
2011-2012						
Weight						
Normothermia						
<2.5 kg	2	6 (4 h)	3.00 (12 h)	1.50 (12 h)	28	80
≥2.5 kg	2	7 (4 h)	3.50 (12 h)	1.75 (12 h)	28	93
Therapeutic hypothermia						
<2.5 kg	2	6 (3.5 h)	3.00 (12 h)	1.50 (12 h)	27.5	77
≥2.5 kg	2	7 (3.5 h)	3.50 (12 h)	1.75 (12 h)	27.5	89.5

Infusions I, II and III are sequential following a loading dose.

Causal relation between lidocaine and cardiac events

The plausibility of a causal relation between reported cardiac events and lidocaine was evaluated by three criteria: (1) time (from 2 h after start and up to 12 h after discontinuation of lidocaine) and dosing (expected plasma concentration >5 mg/l), (2) cardiac symptoms matched expected symptoms based on two underlying mechanisms – impaired electrical conduction (bradycardia, re-entry arrhythmias, atrioventricular (AV) block) or impaired cardiac muscle contractility (hypotension), [11-15] and (3) presence of contributing factors such as relevant pharmacological or physiological interactions, which may explain the observed cardiac events. For each criterion, the relation between lidocaine and the observed cardiac events would be either plausible (1), implausible (-1) or uncertain (0). Overall plausibility was calculated by adding the scores of all three criteria. The relation between lidocaine and cardiac events was considered implausible for cumulative scores <0, plausible for scores >0 and uncertain for scores equal to zero. The incidence of lidocaine-associated cardiac events was calculated as a percentage of all infants treated with lidocaine for seizures. This incidence range was based on the number of plausibility scores >0 (minimum) and the number of plausibility scores ≥0 (maximum).

RESULTS

During the study period, 521 infants (368 full-term, 153 preterm; 291 males, 230 females; median gestational age 39.0 weeks [range: 25.3-42.5]; median birth weight 3160 g [range: 645-5230]) admitted to our neonatal intensive care unit received lidocaine for neonatal seizures. Hypoxic-ischaemic encephalopathy (HIE, 54.3%, n = 283), intracranial haemorrhage (12.3%, n = 64), central nervous system infection (9.6%, n = 50) and perinatal arterial ischaemic stroke (7.1%, n = 37) were the most common aetiologies of seizures. Twenty-eight infants with HIE received therapeutic hypothermia. Cardiac events were observed in 11 infants, nine full-term and two preterm. Symptoms were bradycardia (n = 6) with 2:1 AV block in two infants and QRS prolongation in one infant, irregular heart rate (n = 2), decreased heart rate not fulfilling bradycardia criteria (n = 3) with a prolonged QT interval in one infant, ventricular extrasystoles (n = 2), tachycardia (n = 1), hypotension (n = 1) and asystole following bradycardia (n = 1); more than one symptom could be observed in an individual patient *(table 2)*.

Causal relation between lidocaine and cardiac events

The relation between lidocaine and observed cardiac adverse events was considered plausible in seven infants, implausible in one infant and uncertain in three infants *(table 2)*. This results in an incidence of cardiac events during lidocaine treatment of 1.3-1.9% (n = 7-10/521). In full-term infants the incidence was 1.6-2.2% (n = 6-8/368) and in preterm infants it was 0.7-1.3% (n = 1-2/153). Since 2004 new reduced dose regimens have been implemented in clinical practice. The incidence of lidocaine-associated cardiac events between 1992 and 2003 was 2.2-3.6% (n = 6-9/275) and between 2004 and 2012 it was 0.4% (n = 1/246, p = 0.02).

Table 2. Plausibility score of lidocaine-associated cardiac events

Patient	Year	Gestational age (weeks)	Birth weight (g)	Aetiology of seizures	Dosing regimen and infusion time	Time to start of cardiac event (h)	Cardiac event	Intervention
1	1998	39.2	3900	HIE Sarnat 3	A; stopped after about 21 h	20.5	Untreatable bradycardia and hypotension	Unknown
2	1998	39.6	3415	HIE Sarnat 3	A; stopped after about 28 h	27.25	Bradycardia, asystole	Unknown
3	1999	40.5	3750	HIE Sarnat 2	A; for 48 h	36	Bradycardia (67 bpm) for 3 h (initial heart rate 120-140 bpm), ECG during bradycardia: 2:1 AV block	Spontaneous resolution, LD stopped 9 h after resolution of symptoms

Outcome	Plausibility of lidocaine-associated cardiac event			
	Time and dosing	**Observed cardiac events**	**Interactions**	**Overall**
Died due to cardiac event	+1 High expected plasma concentration	+1 Bradycardia, hypotension	-1 Concurrent PH plasma concentrations (plasma concentration 12 mg/l), changes in blood pressure 6 h after birth observed before LD infusion	1
Died due to cardiac event (possible myocardial infarction, no post-mortem examination)	+1 High expected plasma concentration	+1 Bradycardia, asystole	-1 Concurrent PH plasma concentrations, apnoea and bradycardia observed before LD infusion	1
Survived	+1 High expected plasma concentration	+1 Bradycardia , AV block	-1 Apnoea and bradycardia (72 bpm) observed before LD infusion	1

Table 2. (continued)

Patient	Year	Gestational age (weeks)	Birth weight (g)	Aetiology of seizures	Dosing regimen and infusion time	Time to start of cardiac event (h)	Cardiac event	Intervention
4	1999	30.5	1750	HIE	A; for 48 h (excl. 5.5 h pause after observed toxicity)	30	Irregular, decreased heart rate (100 bpm), ECG: prolonged QT-interval	LD stopped for 5.5 h, then restarted without problems
5	2001	37.4	4700	HIE Sarnat 2	A; stopped after about 24 h	16.75	Bradycardia (70 bpm, initial heart rate 120-140 bpm) after start midazolam, ECG: QRS-complex prolongation	Spontaneous resolution, LD stopped more than 8 h after cardiac event
6	2002	39.6	3470	HIE Sarnat 3	A; stopped after about 11 h	11	Arrhythmia not otherwise classified	LD stopped immediately resulting in normalisation of heart rate
7	2002	41.0	3300	PAIS	A; stopped after about 28 h	27.5	Decreased heart rate (105 bpm, initial heart rate 110-130 bpm), ventricular extrasystoles	Dopamine, LD immediately stopped, resulting in resolution of ventricular extrasystoles

Outcome	Plausibility of lidocaine-associated cardiac event			
	Time and dosing	Observed cardiac events	Interactions	Overall
Survived	+1 High expected plasma concentration	0 Irregular, decreased heart rate (+1) with prolonged QT-interval (-1)	-1 Sporadic apnoea, bradycardia and hypotension (treated with dopamine, dobutamine and hydrocortisone) observed before LD infusion	0
Survived	+1 High expected plasma concentration, toxicity resolved during LD infusion (probably reversed by isoprenaline)	+1 QRS-complex prolongation, bradycardia	-1 Perinatal asphyxia and resuscitation directly after birth	1
Died following redirection of care	+1 High expected plasma concentration, toxicity resolved after discontinuation	+1 Arrhythmia	0	2
Survived	+1 High expected plasma concentration, toxicity resolved after discontinuation	0 Decreased heart rate (+1) Ventricular extrasystole (-1)	-1 Hyperkalaemia (5.9 mmol/l)	0

Table 2. (continued)

Patient	Year	Gestational age (weeks)	Birth weight (g)	Aetiology of seizures	Dosing regimen and infusion time	Time to start of cardiac event (h)	Cardiac event	Intervention
8	2002	33.0	2100	HIE	A; stopped after about 35 h	35	Short bradycardia (30 bpm, initial heart rate 130-160 bpm)	Thorax compressions shortly, LD immediately stopped, after cessation of LD infusion one more short bradycardia to 30 bpm
9	2002	42.3	4296	HIE Sarnat 3	A; stopped after about 11 h	11	Decreased heart rate (102 bpm, initial heart rate 160-180 bpm), ventricular extrasystoles	LD immediately stopped, resulting in resolution of ventricular extrasystoles
10	2003	39.5	3200	HIE Sarnat 3	A; stopped after about 15 min	0.25	Tachycardia 15 and 60 min after start of infusion	LD immediately stopped after first episode of tachycardia, restarted after 13 h without problems
11	2009	38.2	3240	Cerebral dysgenesis	B; stopped after about 25 h	24.75	Bradycardia to 70 bpm (initial heart rate 120-140 bpm) for 12 h, ECG during bradycardia: 2:1 AV block	LD immediately stopped, not resulting in immediate resolution of bradycardia, dopamine

Dosing regimens consisted of: A: bolus of 10 mg/kg in 10 min, followed by 24 h infusion of 6 mg/kg/h, followed by 12 h of 4 mg/kg/h, followed by 12 h of 2 mg/kg/h; B: bolus of 10 mg/kg in 10 min, followed by 6 h of 6 mg/kg/h, followed by 12 h of 4 mg/kg/h, followed by 12 h of 2 mg/kg/h. AV: atrioventricular; bpm: beats per min; ECG: electrocardiogram; HIE: hypoxic-ischaemic encephalopathy; LD: lidocaine; PAIS: perinatal arterial ischaemic stroke; PH: phenytoin.

Outcome	Plausibility of lidocaine-associated cardiac event			
	Time and dosing	**Observed cardiac events**	**Interactions**	**Overall**
Survived	+1 High expected plasma concentration	+1 Bradycardia	-1 Maternal $ß_2$-agonist inhalation, after discontinuation of LD one more bradycardia observed	1
Died following redirection of care	+1 High expected plasma concentration	0 Decreased heart rate (+1) Ventricular extrasystoles (-1)	-1 Hyperkalaemia (7.2 mmol/l), bradycardia observed before LD infusion	0
Died following redirection of care	-1 Low expected plasma concentration Restarted 13 h later without adverse cardiac events	-1 Tachycardia	-1 Perinatal asphyxia, hypotension observed before LD infusion treated with dopamine, dobutamine and hydrocortisone	-3
Died following redirection of care	+1 Plasma concentration at 26 h was 6.1 mg/l	+1 Bradycardia, AV block	-1 Hyperkalaemia (>9 mmol/l)	1

DISCUSSION

Lidocaine-associated cardiac events were rare in our cohort of full-term and preterm infants treated for seizures. We evaluated the causal relation between the cardiac events and lidocaine using new standardised criteria. In only 1.3-1.9% could observed cardiac events be explained by the use of lidocaine; the incidence rate decreased to 0.4% after the introduction of new reduced dose regimens in 2004. The most common cardiac event associated with lidocaine was bradycardia. Regarding the risk of cardiac events, these findings show that lidocaine is safe to use as an antiepileptic drug in both full-term and preterm infants. Lidocaine-associated cardiac events have been reported in several studies, [1-4, 6-10] but most had a limited number of infants. Incidence rates ranged from 0 to 4.8%. In four studies, cardiac events potentially associated with lidocaine were observed. [1-3, 6] Hellström-Westas *et al.* [2] (n = 24) described one infant (4.2%) with severe acidosis who developed bradycardia after 2 h of lidocaine infusion. Another study by this group reported changes in heart rate in about 48% of the study population (n = 46), but this was not directly associated with lidocaine by the authors and they reported that no dangerous cardiac adverse events were observed. [1] Lundqvist *et al.* [3] (n = 30) reported one infant (3.3%) who had bradycardia during lidocaine maintenance infusion, but the heart rate did not completely return to normal after discontinuation of lidocaine. Our study is an extension of the study by van Rooij *et al.* [6] They already studied 207 full-term and preterm infants, also included in the current study, and reported 10 infants with cardiac events. The association between the cardiac event and lidocaine was obvious in eight infants because recovery of cardiac rhythm was observed within minutes after cessation of lidocaine. Two infants did not have another cardiac event after lidocaine rechallenge, indicating that the events may not have been directly related to lidocaine. Our percentage of lidocaine-associated cardiac events is at the lower limit of the range, but it is difficult to compare with other studies since the numbers in other studies are small and because of differences in lidocaine dosing regimens. Furthermore, in previous studies, including the study by van Rooij *et al.*, [6] every cardiac event was ascribed to lidocaine and they did not consider whether the lidocaine plasma concentration was sufficient to cause symptoms, whether symptoms could be explained by the pharmacodynamics of lidocaine or whether other possible causes or contributing factors were present. We anticipated that a lidocaine plasma concentration below 5 mg/l would not be sufficient to cause cardiac symptoms. Neurological effects of lidocaine have been described to occur at concentrations below 5 mg/l. [16] However, cardiac effects occur at much higher plasma concentrations. [17] In the first 2 h after the start of lidocaine infusion, using the dosing regimens described in *table 1*, the lidocaine plasma concentration is unlikely to

reach 5 mg/l. [4,10] Therefore, we do not expect lidocaine-associated cardiac events within 2 h after the start of infusion. Monoethylglycinexylidide, the main metabolite of lidocaine with similar pharmacological effects on the heart, [18] has a longer half-life than lidocaine. [2,9] We calculated that cardiac events due to monoethylglycinexylidide can be expected until 12 h after discontinuation of lidocaine infusion. We have to acknowledge, however, that no correlation between plasma concentrations and cardiac events has been established for lidocaine in infants. [1] A plasma concentration of 9 mg/l is often suggested as a threshold for increased risk of cardiotoxic effects, but this is based on a single study of six adults which only reported an effect on blood pressure and tidal volume. [19] In infants, cardiac events during lidocaine administration has been observed at lower plasma concentrations, while no cardiac adverse events were observed in a study of 24 infants in whom the average plasma concentration exceeded 9 mg/l. [2] A concentration-effect relation seems likely, but this has not been confirmed by clinical studies in infants. Our study does suggest a concentration-effect relation since the incidence of lidocaine-associated cardiac events significantly decreased after the introduction of reduced dose regimens. However, this has to be investigated further in prospective studies. The symptoms of lidocaine-associated cardiac events, based on pharmacodynamics, can be explained by two mechanisms: impaired electrical conduction and impaired cardiac muscle contractility. The electrical conduction in the heart is impaired due to its effect on sodium channels. Lidocaine mainly acts on the conduction in the ventricles, resulting in bradycardia and re-entry arrhythmias. [12,13] In adults, conversion of 2:1 to 1:1 atrial flutter, AV block and even death have been reported. [14] In *in vitro* models, lidocaine has a dose-dependent negative inotropic effect, resulting in hypotension. [11,15] This is probably related to altered physical and functional properties of the Ca-ATPase in the cardiac sarcoplasmic reticulum rather than direct effects on sodium currents. [15,20] Symptoms such as premature ventricular extrasystoles, prolonged QT interval and tachycardia, which were observed in some infants in our study, are difficult to explain pharmacologically. Premature ventricular extrasystoles are not a typical side effect of lidocaine because lidocaine does not promote premature depolarisation in the Purkinje fibre network. [14] Lidocaine has been proven to shorten instead of prolong the QT interval. [13] Tachycardia is also less likely to occur from a pharmacological point of view, unless the myocardium was affected by hypoxia-ischaemia. During myocardial ischaemia, altered sodium channel function causes conduction disturbances and ventricular arrhythmia. [21] In almost all infants with cardiac events in our study, other pharmacological or physiological factors which could have caused or contributed to the cardiac events were present. Contributing factors observed in our study were phenytoin use, hyperkalaemia and compromised cardiac function prior to lidocaine infusion. In two infants with cardiac events in our cohort, phenytoin

concentrations were measured in the plasma when lidocaine was administered. Phenytoin has synergetic pharmacodynamic effects (blocking of sodium channels). Hattori *et al.* [22] observed a 4.9 ($p < 0.001$) times higher risk of cardiac events when lidocaine and phenytoin were combined in 56 children, compared to lidocaine alone. Concurrent or subsequent use of lidocaine and phenytoin should therefore be avoided. Hyperkalaemia inhibits repolarisation and may result in delayed AV conduction, AV block, extrasystoles and bradycardia. [23] These symptoms of hyperkalaemia overlap with symptoms of lidocaine-associated cardiac events, making conclusions about a causal relation of lidocaine and cardiac events in the presence of hyperkalaemia more difficult. Also, both infants with ventricular extrasystoles in our cohort had hyperkalaemia. Since extrasystoles can be explained by hyperkalaemia and not by lidocaine, hyperkalaemia probably caused or enhanced the cardiac events in those infants. Since potassium plasma concentrations affect depolarisation and electrical conduction, attention should be paid to the potassium homeostasis before and during lidocaine administration. A compromised cardiac function, for example due to perinatal asphyxia, resulting in cardiac events such as bradycardia and hypotension already present before initiation of lidocaine infusion or observed long after discontinuation was seen in a number of infants who presented with cardiac events during lidocaine administration in our study. One can argue whether these events were due to lidocaine or whether they would have occurred in the absence of lidocaine as well due to the underlying disease. However, infants with compromised cardiac function may be more susceptible to the development of cardiac events when lidocaine is administered. The safety of lidocaine in infants with HIE deserves special attention. HIE is the most common cause of seizures in full-term infants and is, in severe forms, often accompanied by multi-organ failure resulting in a compromised cardiac function and electrolyte disturbances. Furthermore, nowadays most of these infants will receive therapeutic hypothermia. During therapeutic hypothermia, infants tend to have a lower heart rate and the clearance of lidocaine is decreased, which puts these infants at increased risk of toxicity. [4] However, a customised dosing regimen for therapeutic hypothermia was developed and has proven to be safe. [4] In our cohort, none of the infants with HIE who received the reduced dose regimens, implemented in 2004, had lidocaine-associated cardiac events, including infants with severe HIE and multi-organ failure. Compromised cardiac function and electrolyte disturbances increase the risk of cardiac events during lidocaine administration; therefore, these should be monitored and corrected when possible. The strength of this study is that we were able to add to the literature data on the incidence of lidocaine-associated cardiac events based on the largest cohort of full-term and preterm infants studied so far and provide an overview of symptoms and contributing factors. The

retrospective character was the main limitation of this study. It may have caused underreporting of less severe cardiac events and relevant pharmacological or physiological interactions and lidocaine plasma concentrations were not available. Selection bias may have occurred since patients with increased risk of cardiac events (e.g. congenital cardiac dysfunction) were less likely to be treated with lidocaine after the report by van Rooij *et al.* [6] in 2004. Another limitation was the lack of a control group. However, some studies have addressed the incidence of arrhythmias in the neonatal population as ranging from 1 to 5%. [24, 25] The incidence of lidocaine-associated cardiac adverse events reported in our study is not higher than this background incidence. The findings in this study should reassure clinicians that lidocaine is safe to use in full-term and preterm infants with seizures, with regard to cardiac side effects, when the dosing regimens are customised and attention is paid to electrolyte balance. Administration to infants with congenital cardiac dysfunction should be limited and concurrent or subsequent phenytoin use avoided. Ideally, our results should be confirmed in a prospective study.

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Chapter 8

Lidocaine response rate in aEEG-confirmed neonatal seizures: retrospective study of 413 full-term and preterm infants

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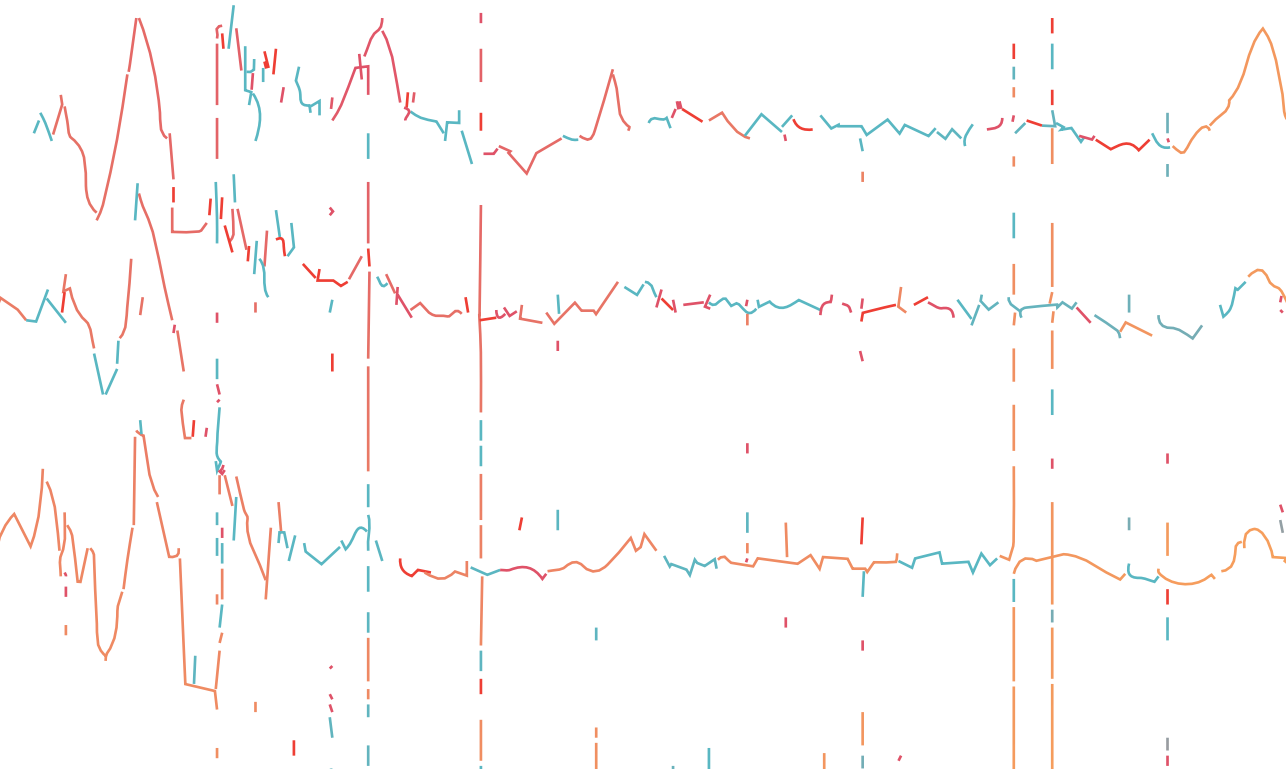


ABSTRACT

Introduction The objective was to investigate the seizure response rate to lidocaine in a large cohort of infants who received lidocaine as second- or third-line antiepileptic drug (AED) for neonatal seizures.

Methods Full-term (n = 319) and preterm (n = 94) infants, who received lidocaine for neonatal seizures confirmed on amplitude-integrated EEG (aEEG), were studied retrospectively (January 1992 - December 2012). Based on aEEG findings, the response was defined as good (>4 h no seizures, no need for rescue medication); intermediate (0-2 h no seizures, but rescue medication needed after 2-4 h); or no clear response (rescue medication needed <2 h).

Results Lidocaine had a good or intermediate effect in 71.4%. The response rate was significantly lower in preterm (55.3%) than in full-term infants (76.1%, $p < 0.001$). In full-term infants the response to lidocaine was significantly better than midazolam as second-line AED (21.4% vs. 12.7%, $p = 0.049$), and there was a trend for a higher response rate as third-line AED (67.6% vs. 57%, $p = 0.086$). Both lidocaine and midazolam had a higher response rate as third-line AED than as second-line AED ($p < 0.001$). Factors associated with a good response to lidocaine were the following: higher gestational age, longer time between start of first seizure and administration of lidocaine, lidocaine as third-line AED, use of new lidocaine dosing regimens, diagnosis of stroke, use of digital aEEG, and hypothermia. The multivariable regression model of seizure response to lidocaine included lidocaine as second- or third-line AED and seizure aetiology.

Conclusion Seizure response to lidocaine was seen in ~70%. The response rate was influenced by gestational age, underlying aetiology, and timing of administration. Lidocaine had a significantly higher response rate than midazolam as second-line AED, and there was a trend for a higher response rate as third-line AED. Both lidocaine and midazolam had a higher response rate as third-line compared to second-line AED, which could be due to a pharmacological synergistic mechanism between the two drugs.

INTRODUCTION

Seizures occur more often during the neonatal period than in any other period of life. Seizures may alter brain development and lead to learning difficulties, behavioural problems, and predispose to epilepsy.[1,2] Rapid, protocol-driven therapy can reduce seizure burden.[3,4] However, data from randomised controlled trials to support the choice of antiepileptic drug (AED) are limited, and no definite recommendations can be made.[5] In Europe lidocaine is often used as third-line AED,[6] whereas internationally it is used by only 1-6% of neurologists and neonatologists.[7] Lidocaine appears to be an effective drug for second- and third-line treatment of neonatal seizures, with seizure response rates ranging from 60 to 92%;[8-16] however, the numbers in these studies are small and the lidocaine treatment regimens used differ greatly. The primary objective of the present study was to retrospectively investigate the response rate to lidocaine in a large cohort of full-term and preterm infants who received lidocaine following standardised regimens for neonatal seizures, and to compare it to the response rate to midazolam. The secondary objective was to study the influence of gestational age, underlying seizure aetiology, administration as second- or third-line drug, timing of drug administration, lidocaine dosing regimen, therapeutic hypothermia, and lidocaine plasma concentration on the response rate. We hypothesised that lidocaine would have a higher response rate than midazolam as second- and third-line AED.

METHODS

Patients

Full-term and preterm infants admitted to the level III neonatal intensive care unit (NICU) of the Wilhelmina Children's Hospital in Utrecht (January 1992 - December 2012), who received lidocaine for clinical and/or subclinical seizures, confirmed by amplitude-integrated electroencephalography (aEEG) or standard EEG, were studied retrospectively. Exclusion criteria were lidocaine for clinical seizures without an electroencephalographic correlate, lidocaine protocol violations, and inability to assess the effect of lidocaine on aEEG due to, for example, artefacts. Infants were identified using a local database on discharge diagnosis and medication received during NICU admission. For comparison, all infants who received midazolam as second- or third-line treatment for seizures during the same study period at our NICU were also studied. Permission was not required from the hospital's medical ethics committee for this retrospective, anonymous data analysis.

(Amplitude-integrated) electroencephalography

Continuous aEEG recordings were used as standard of care since 1992 in our NICU in infants with suspected neonatal seizures and infants at risk of developing seizures. All doctors and nurses are highly experienced in assessing the aEEG. Until 2003, a single-channel analogue aEEG was used (CFM 4640; Lectromed, Devices Ltd, United Kingdom). Since 2003 three digital machines have been used: Olympic 6000 cerebral function monitor (Natus, Seattle, WA, USA) for single-channel aEEG (P4-P3) and BRM2 and BRM3 (including seizure detection algorithm since 2006) BrainZ monitor or Olympic Brainz Monitor (OBM) (Natus) for two-channel aEEG (F4-P4, F3-P3). The digital machines allowed inspection of the raw EEG, which was standard procedure in the evaluation of seizures. A standard EEG (MicroMed machine, 8-12 electrodes, ≥30 min) was performed when aEEG was inconclusive. All aEEG studies, including selected parts of the raw EEG in the digital recordings, were reviewed again for this study by two aEEG experts (MT, LR).

Lidocaine dosing regimen

Lidocaine was started as second-line AED when epileptiform activity persisted on aEEG despite phenobarbital (20 mg/kg) and as third-line AED after midazolam (loading dose: 0.05 mg/kg in 10 min, infusion: 0.15-0.5 mg/kg/h), clonazepam (loading dose: 0.1 mg/kg, infusion: 0.1-0.5 mg/kg/24 h) or in a few cases phenytoin (20 mg/kg) as second-line AED. Over the years, the lidocaine dosing protocol was subject to some changes *(table 1)*. Lidocaine plasma concentrations were available at t = 4, 6, 8, 12, 36, 48, or 60 h after start of infusion for some infants, taken for monitoring toxicity or research purposes.

Seizure response definition

Seizure definition

On analogue aEEG, no raw EEG was available. A seizure was defined as a relatively abrupt and transient rise of the lower border in the absence of possible artefacts (e.g. annotation of disturbance of the infant). On digital aEEG with raw EEG available, a seizure was defined as evolving rhythmic activity for ≥10 s on the raw EEG in the absence of artefacts. Good response *(figure 1A)*: cessation of seizure activity on aEEG and no recurrence of seizure activity on aEEG for >4 h, after which only single short seizures were observed in some infants, but without the need for rescue AED, in keeping with a previous study on lidocaine responsiveness, [16] after consensus agreement in the NEMO consortium. Intermediate response *(figure 1B)*: cessation of seizure activity on aEEG, but recurrence of repetitive seizures within 2-4 h for which rescue AED was given; cessation of seizure activity on aEEG for >4 h, but recurrence of repetitive seizures within 24 h for which rescue AED was given. No response *(figure 1C)*: seizure activity continued unchanged on aEEG.

Table 1. Changes in lidocaine dosing regimens over the years

	Bolus (mg/kg, 10 min)	Infusion I (mg/kg/h)	Infusion II (mg/kg/h)	Infusion III (mg/kg/h)	Duration of infusion (h)	Total dosage (mg/kg)
Year						
1992-2003	2	6 (24 h)	4.00 (12 h)	2.00 (12 h)	48	218
2004-2005	2	6 (12 h)	4.00 (12 h)	2.00 (12 h)	36	146
2006-2010	2	6 (6 h)	4.00 (12 h)	2.00 (12 h)	30	110
2011-2012						
Weight						
Normothermia						
<2.5 kg	2	6 (4 h)	3.00 (12 h)	1.50 (12 h)	28	80
≥2.5 kg	2	7 (4 h)	3.50 (12 h)	1.75 (12 h)	28	93
Therapeutic hypothermia						
<2.5 kg	2	6 (3.5 h)	3.00 (12 h)	1.50 (12 h)	27.5	77
≥2.5 kg	2	7 (3.5 h)	3.50 (12 h)	1.75 (12 h)	27.5	89.5

Infusions I, II and III are sequential following a loading dose.

Statistical analysis

Data were analysed using SPSS version 20 (IBM Corp., Armonk, NY, USA). The difference in response rate to lidocaine between preterm and full-term infants, second- and third-line AED use, and the difference in response rate per aetiology between lidocaine and midazolam was compared by Pearson chi-squared or Fisher's exact test as appropriate. Logistic regression was used for analysis of influencing factors on response rate and comparing the response to lidocaine and midazolam and stepwise logistic regression for multivariable analysis. The response to lidocaine was dichotomised into those with a response (good response) and those with no response (intermediate or no response). Kruskal-Wallis test with Dunn's post hoc test was used to compare lag time between the three response groups with and without stratification for use as second- or third-line drug. A p-value <0.05 was considered significant.

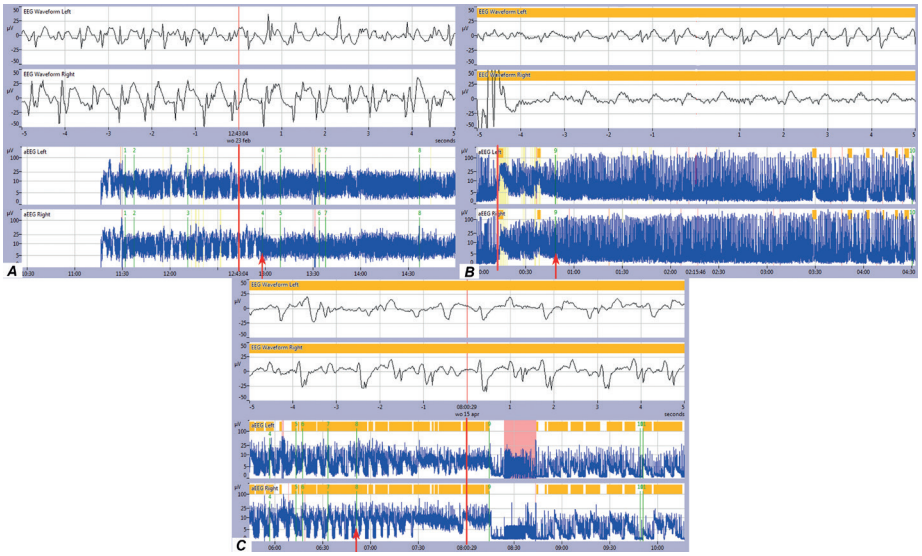


Figure 1. Seizure response definition. (A) Good response. Repetitive seizures are seen in the aEEG as relatively abrupt, transient rises of the lower border, with confirmation on the raw EEG, where an evolving rhythmic pattern is seen lasting >10 s. Lidocaine was given at the red arrow, after which seizures stopped immediately and seizures did not recur. (B) Intermediate response. Two seizures are seen in the aEEG as relatively abrupt and transient rises of the lower border and marked with an orange bar by the seizure detection algorithm; both seizures were confirmed on the raw EEG. Lidocaine was given at the red arrow, after which no seizures were seen until several hours later when repetitive seizures recurred. (C) No response. A status epilepticus is seen as a sawtooth pattern in the aEEG, with confirmation in the raw EEG and marked with the orange bars by the seizure detection algorithm, but the seizure activity continues unchanged after lidocaine was given at the red arrow.

RESULTS

During the study period, 521 infants received lidocaine for neonatal seizures. Of these, 108 (20.7%) were excluded from the study: in 24 no aEEG was available, in 43 lidocaine was given for clinical seizures only, in two there was violation of the lidocaine protocol, and in 39 we were unable to assess the effect of lidocaine on aEEG (no annotations, aEEG recording too short, artefacts, dubious seizures on analogue aEEG). In 413 infants the effect of lidocaine on neonatal seizures was assessed *(table 2)*. In 219, analogue aEEG was used and in 194 digital aEEG. Thirty infants who received midazolam without lidocaine during the study period were added to compare seizure response to lidocaine and midazolam.

Response to lidocaine

The response to lidocaine in the overall study population, full-term infants, and preterm infants is shown in *table 3*. Lidocaine was significantly less effective in preterm infants compared to full-term infants ($p < 0.001$). We therefore excluded preterm infants from further analyses.

Timing of lidocaine administration

In full-term infants, a good response to lidocaine as second-line AED (n = 131) was seen in 21.4%, an intermediate response was seen in 51.1%, and no response was seen in 27.5%. This compared to a good response in 67.6%, intermediate in 12.4%, and no response in 20% when given as third-line AED (n = 145). This shows a significant increase in response rate when given as third-line AED compared to second-line ($p < 0.001$). This pattern is also seen in *supplemental figure 1*. The time between seizure onset and time of lidocaine administration (lag time) was available for 149 infants. The lag time was significantly longer in infants with a good response to lidocaine compared to those with an intermediate ($p = 0.006$) or no response ($p = 0.002$). The median lag time as second-line AED was 6 h (interquartile range [IQR] 4-10.5) and as third-line AED 13 h (IQR 7-22.8). However, when stratified for use of lidocaine as second- or third-line drug, the lag time was not significantly different between the three response groups.

Aetiology

The response rate to lidocaine per underlying aetiology is shown in *table 3*. Infants with a diagnosis of stroke had the highest response rate to lidocaine as second-line AED (45.5% good response), and when lidocaine was given as third-line AED, infants with a diagnosis of ICH or stroke had the highest response rate (84.6% good response).

Table 2. Clinical characteristics

	Total (n = 413)	Full-term infants (≥37 weeks) (n = 319)	Preterm infants (<37 weeks) (n = 94)	Full-term infants receiving lidocaine without midazolam (n = 30)	Full-term infants receiving midazolam without lidocaine (n = 30)
Male/female	227/186	171/148	56/38	17/13	19/11
Gestational age (weeks), median (range)	39.5 (25.3-42.4)	40.1 (37.0-42.5)	33.1 (25.3-36.6)	40.2 (37.3-42.1)	40.1 (37.1-42.1)
Birth weight (grams), median (range)	3240 (645-5230)	3425 (2100-5230)	1800 (645-3725)	3500 (2530-4380)	3450 (2150-5000)
Diagnosis, n (%)	393 (95.2)	303 (95)	90 (95.7)	28 (93.3)	26 (86.7)
	HIE, 228 (55.2) ICH, 45 (10.9) PAIS, 32 (7.7) CNS infection, 40 (9.7) Other, 100 (24.2)	HIE, 192 (60.2) ICH, 25 (7.8) PAIS, 30 (9.4) CNS infection, 19 (6) Other, 72 (22.6)	HIE, 36 (38.3) ICH, 20 (21.3) PAIS, 2 (2.1) CNS infection, 21 (22.3) Other, 17 (18.1)	HIE, 16 (53.3) ICH, 3 (10) PAIS, 5 (16.7) CNS infection, 1 (3.3) Other, 3 (10)	HIE, 20 (66.7) ICH, 0 PAIS, 2 (6.7) CNS infection, 3 (10) Other, 5 (16.7)
Hypothermia, n (%)	26 (6.3)	25 (7.8)	1 (1.1)	1 (3.3)	4 (13.3)
Mortality, n (%)	206 (49.9)	140 (43.9)	66 (70.2)	13 (43.3)	9 (30)
Good + intermediate response, n (%)	295 (71.4)	243 (76.2)	52 (55.3)	28 (93.3)	24 (80)
Total number of AEDs, median (range)	3 (2-8)	3 (2-8)	3 (2-5)	2 (2)	2 (2-3)
Age at onset seizures (days), median (range)	1 (1-96)	1 (1-36)	2 (1-96)	1 (1-8)	1 (1-7)

AED: antiepileptic drug; CNS: central nervous system; HIE: hypoxic-ischaemic encephalopathy; ICH: intracranial haemorrhage; PAIS: perinatal arterial ischaemic stroke.

Table 3. Response rate to lidocaine in the overall study population, full-term, and preterm infants and the comparison in response rate between lidocaine and midazolam in full-term infants and the response rate per aetiology for both lidocaine and midazolam as second- and third-line AED

Lidocaine									
	Overall study population n = 413			**Full-term infants n = 319**			**Preterm infants n = 94**		
Response, n (%)	Good	Intermediate	No	Good	Intermediate	No	Good	Intermediate	No
Second-line	37 (19.9)	90 (48.4)	59 (31.7)	28 (21.4)	67 (51.1)	36 (27.5)	9 (16.4)	23 (41.8)	23 (21.8)
Third-line	109 (63.4)	22 (12.8)	41 (23.8)	98 (67.6)	18 (12.4)	29 (20)	11 (40.7)	4 (14.8)	12 (44.4)

Lidocaine vs. midazolam				
	Lidocaine second-line n = 131 Good effect, n (%)	**Lidocaine third-line n = 145** Good effect, n (%)	**Midazolam second-line n = 165** Good effect, n (%)	**Midazolam third-line n = 107** Good effect, n (%)
Full-term infants (n = 296)	28 (21.4)	98 (67.6)	21 (12.7)	61 (57)
Aetiology				
HIE	17 (20)	50 (65.8)	12 (14)	42 (57.5)
ICH	2 (22.2)	11 (84.6)	0 (0)	4 (57.1)
Stroke	5 (45.5)	22 (84.6)	2 (11.1)	3 (60)
CNS infection	1 (20)	8 (72.7)	3 (21.4)	3 (60)
Metabolic disorder	0 (0)	2 (22.2)	0 (0)	4 (50)
Other	4 (33.3)	5 (41.7)	3 (18.8)	4 (44.4)

CNS: central nervous system; HIE: hypoxic-ischaemic encephalopathy; ICH: intracranial haemorrhage.

Factors influencing response to lidocaine

Features significantly associated with a good response to lidocaine in full-term infants *(table 4)* included lidocaine as third-line AED, use of new lidocaine treatment regimens, longer lag time, a diagnosis of stroke, including perinatal arterial ischaemic stroke and cerebral sinovenous thrombosis, use of digital aEEG, and hypothermia. The multivariable regression model using stepwise logistic regression included lidocaine as second- or third-line AED and diagnosis. Differences in lidocaine dosing regimen, hypothermia, and type of aEEG used were not included in the model to avoid multicollinearity with use of lidocaine as second- or third-line AED; including either of those parameters instead of use of lidocaine as second- or third-line AED did not produce a stable model. The use of lidocaine mainly as third-line drug in combination with midazolam was initiated around the same time as the introduction of the digital aEEG. When stratifying for type of aEEG used, use of lidocaine as third-line AED was associated with a better response rate in both the analogue and digital aEEG groups. Only a few infants received hypothermia in this cohort in a period where lidocaine was given mainly as third-line AED after midazolam. This explains the multicollinearity and lack of a stable model when including these parameters. The lidocaine concentrations did not influence the response.

Lidocaine versus midazolam

In 296 full-term infants, the response to lidocaine and midazolam could be compared; 236 infants received a combination of lidocaine and midazolam, 30 received lidocaine only, and 30 received midazolam only. *Table 3* shows the response rates to lidocaine and midazolam as well as the response rates for both drugs per underlying aetiology. Lidocaine had a significantly higher response rate than midazolam as second-line AED ($p = 0.049$), and there was a trend for a higher response rate as third-line AED ($p = 0.086$). Both lidocaine and midazolam had a significantly higher response rate as third-line AED compared to second-line (both $p < 0.001$). Midazolam had lower response rates for all aetiologies as second-line AED than lidocaine, which was significant in infants with stroke. Midazolam had lower response rates as third-line AED than lidocaine for all aetiologies except for metabolic disorders (50% vs. 22.2%), but this was not a significant difference.

Table 4. Factors associated with a good response in bivariate analysis and multivariable regression model for the prediction of a good response to lidocaine in full-term infants

Full-term infants n = 319	Odds ratio	95% confidence interval
Bivariate analysis		
Lidocaine as second- or third-line AED		
Second-line (n = 131)	reference	
Third-line (n = 145)	7.7	4.5-13.2
Lag time		
<5 h (n = 33)	reference	
5-9 h (n = 42)	4.5	1.3-15.1
10-23 h (n = 45)	5.8	1.7-19.2
≥24 h (n = 29)	10.3	2.9-36.9
Lidocaine dosing regimen		
1992-2003 (n = 163)	reference	
2004-2005 (n = 45)	0.9	0.4-1.8
2006-2010 (n = 96)	4.1	2.4-7.0
2011-2012 (n = 15)	4.8	1.5-15.9
Diagnosis		
Other (n = 283)	reference	
Stroke (n = 36)	4.5	2.0-10.2
Type aEEG		
Analogue aEEG (n = 167)	reference	
Digital aEEG (n = 152)	2.6	1.6-4.1
Hypothermia		
No (n = 259)	reference	
Yes (n = 25)	2.6	1.1-6.0
Multivariable analysis		
Lidocaine as second- or third-line AED	77.9	14.6-415.0
Diagnosis	13.9	2.5-77.3

AED: antiepileptic drug; aEEG: amplitude-integrated EEG.

DISCUSSION

We report the response rate to lidocaine in the largest cohort of full-term and preterm infants studied to date. A good or intermediate response was seen in 68.3-76.2% of infants overall, with cessation of seizures and no need for rescue AED in 19.9-63.4%, depending on whether lidocaine was administered as second- or third-line AED. In full-term infants, a response to lidocaine was seen in 72.5-80%, with cessation of seizures and no need for rescue AED in 21.4-67.6%. Lidocaine had a significantly lower response rate in preterm infants, in whom a response was seen in 55.5-58.2%, with cessation of seizures and no need for rescue AED in only 16.4-40.7%. Infants with perinatal arterial ischaemic stroke or cerebral sinovenous thrombosis were more likely to have a good response to lidocaine than infants with another aetiology of seizures. The response rate to lidocaine was significantly higher compared to midazolam as second-line AED and slightly higher as third-line AED. Both lidocaine and midazolam had a higher response rate as third-line AED.

Lidocaine has been investigated in a few studies with a limited number of infants, with response rates ranging from 60 to 92%. [8-17] Our response rate is within this range. It is, however, difficult to compare the studies because most studies included preterm infants, but did not report separate response rates for preterm infants. Our results show that the seizure response is significantly lower in preterm infants, and including preterm infants will influence the overall response rate. In addition, some studies included clinical as well as electroencephalographic seizures. Murray *et al.* [18] showed that only 27% of clinical seizures have corresponding electroencephalographic seizures and only 9% of electroencephalographic seizures are accompanied by clinical signs. Therefore, including clinical seizures as an outcome measure can overestimate as well as underestimate the effect of an AED. Finally, the definition of a good response differed greatly among the studies as did the treatment regimens used. Hellström-Westas *et al.* [9] showed that without a loading dose or with only a low infusion rate, the response to lidocaine was poorer. Boylan *et al.* and Lundqvist *et al.* [8,11] reported a slightly lower overall response rate (60 and 63%, respectively) than our study. A possible explanation for this discrepancy is that in both studies lidocaine was given as second-line AED. As we have shown in the current study, the response rate is significantly lower when given as second-line AED. In addition, the study population in Boylan *et al.* was very small, consisting of only five infants. The lidocaine treatment regimens in both studies were comparable to ours, except that Lundqvist *et al.* did not use a loading dose, which was previously shown to result in poorer response to treatment. [9]

Three studies had a very high response rate (91-92%) compared to ours. [9, 10, 16] In the

study by Hellström-Westas *et al.*, [9] only half of the study population received lidocaine as third-line AED, and 89.7% had seizure control within 30 minutes, but they did not take recurrence of seizures into account and they had access to only analogue aEEG. Combining the results of this study with our results it shows that lidocaine is a fast-acting AED and can be used for quick cessation of seizures, but seizures tend to reappear after several hours, which could be due to the decrease in infusion as is the design of the lidocaine treatment regimen. In a second study by the same group [10] and the study by van den Broek *et al.*, [16] all infants received lidocaine as third-line AED, possibly explaining the higher response rate. Furthermore, all infants in the study by van den Broek *et al.* [19] received lidocaine while being treated with hypothermia, which may enhance the efficacy of AEDs. The treatment regimens in these three studies were comparable to ours. The second study by Hellström-Westas *et al.* [9] also found no relation between lidocaine plasma concentrations and seizure response, which is in agreement with the current study. Several AEDs for neonatal seizures have been investigated, but all in very limited number of infants (n = 3-46, *table 5*). [8-17, 19-25] Phenobarbital is still the most widely used first-line drug, [7] with a response rate of 21.4-50%. [8, 21, 24] For refractory neonatal seizures, phenytoin (response rate 26.7-53.1%), [22, 24] midazolam (response rate 0-100%), [8, 15, 22, 26, 27] lidocaine (response rate 40-53.3%), [8, 11, 15] clonazepam (response rate 0%), [8] and levetiracetam (response rate 28.6-32%) [20, 23] have been studied as second-line AEDs and midazolam (response rate 50-73.3%) [15, 25, 26] and lidocaine (response rate 62.5-91%) [10, 12-14, 16, 17] as third-line AEDs. Phenytoin, midazolam, and lidocaine show the most promising results. Phenytoin reached a response rate of 53.1% as second-line AED, [22] which is much higher than our results for both lidocaine (20%) and midazolam (12%). However, the response rate in this study was measured using one 4-h EEG per day instead of continuous EEG monitoring. It is possible that recurrence of seizures was missed and the response rate was overrated. For midazolam as second-line AED, two studies reported a response rate of 0%, [8, 15] one of 66.7%, [26] and one of 100%. [22] Both studies with high response rates used intermittent EEG instead of continuous EEG monitoring. Both were very small studies (n = 3 and 13, respectively), as were the two studies that found no effect of midazolam (n = 3 and 8). In addition, the dose of midazolam might be of importance, since Castro-Conde *et al.* [22] reached a response rate of 100% with a dose up to 1 mg/kg/h. However, the risk of hypotension and the probable extreme suppression of brain activity with high doses of midazolam should be kept in mind. All response rates to lidocaine as second-line AED [8, 11, 15] were much higher than our results, which could again be explained by the small numbers in those studies and by the fact that some studies used clinical seizures as an outcome measure and studied the immediate effect of lidocaine, without taking into account a recurrence of seizures. Another possible explanation is that in two previous studies,

[11, 15] a benzodiazepine was used as first-line AED. Based on molecular pharmacology, a synergistic mechanism can be proposed for midazolam, a benzodiazepine, and lidocaine. When midazolam activates the γ-aminobutyric acid A ($GABA_A$) receptor, chloride ions are selectively conducted through the pore. This results in hyperpolarisation of the neuron, thereby reducing the chance of initiating a new action potential. If a sodium channel blocker, such as lidocaine, is present in the neuron at the same time, the refractory period will be prolonged even further and the subsequent depolarisation phase will be less steep. The overall effect will be a prolonged effective refractory period, resulting in a decreased neuronal firing rate. This could also explain the increase in response rate we observed for both lidocaine and midazolam when given as third-line/add on therapy to each other compared to the response rate as second-line/single therapy. Other possible explanations for this observation could be the additive effect of AEDs irrespective of a synergistic relation between AEDs or the timing of administration of third-line AEDs. Temporal evolution of seizure burden in infants shows an evolving increase–decrease pattern in most infants. [28] Third-line AEDs are given at a later point in time, and our results have shown that the chance of a good effect of lidocaine increases when the time between start of seizures and administration of lidocaine (lag time) was longer. It is possible that lidocaine was administered when the seizure burden was already declining. However, the multivariable analysis using stepwise logistic regression included lidocaine as second- or third-line AED, but not lag time, which would imply that an additive effect of or a synergistic relation between AEDs is the best explanation for the increase in response rate as third-line AED. Obviously, these two variables are correlated, making it difficult to draw definite conclusions on which has the strongest effect on response rate. The strength of this study is that we evaluated a large cohort of full-term and preterm infants who received lidocaine for aEEG-confirmed seizures due to different underlying aetiologies, following standardised treatment regimens. The retrospective character and the use of analogue aEEG in a subgroup of our infants were the main limitations of this study.

Table 5. Studies investigating the seizure response to second- and third-line AEDs

	n	Preterm (n)	First-line AED	Second-line AED	Treatment regimen	Seizure response definition	Method of seizure assessment	Response rate (%)	Response rate preterm (%)
First-line AED									
Phenobarbital									
Painter 1999 [24]	30	11		NA	NA	Titrated upon plasma concentration	Cessation of seizures, no need for rescue AED	EEG	43.3	NR
Boylan 2002 [21]	14	7		NA	NA	20 mg/kg up to 40 mg/kg total dose	Cessation of seizures within 1 h, only single seizures allowed to recur	Video-EEG	21.4	28.6
Boylan 2004 [8]	22	8		NA	NA	Up to 40 mg/kg	Cessation of seizures Intermediate: ≥80% reduction in seizure burden	Video-EEG	50	62.5
Phenytoin									
Painter 1999 [24]	29	10		NA	NA	Titrated upon plasma concentration	Cessation of seizures, no need for rescue AED	EEG	44.8	NR
Levetiracetam									
Abend 2011 [20]	4	0		NA	NA	Loading dose 5-20 mg/kg to max 10-61 mg/kg	Cessation of seizures or 50% reduction in seizure burden	Video-EEG	25	NA

Table 5. (continued)

	n	Preterm (n)	First-line AED	Second-line AED	Treatment regimen	Seizure response definition	Method of seizure assessment	Response rate (%)	Response rate preterm (%)
Second-line AED									
Phenytoin									
Painter 1999 [24]	15	unknown	PB	PH	Titrated upon plasma concentration	Cessation of seizures, no need for rescue AED	EEG	26.7	NA
Castro-Conde 2005 [22]	32	30	PB	PH	20 mg/kg	NR	Clinical seizures + intermittent EEG (one per 24 h)	53.1	NA
Midazolam									
Sheth 1996 [26]	3	2	PB	MD	Loading dose 0.15 mg/kg, infusion 0.12-0.4 mg/kg/h	NR	Intermittent EEG	66.7	50
Boylan 2004 [8]	3	0	PB	MD	Loading dose 0.06 mg/kg, infusion 0.15 mg/kg/h (48 h), increase to max 0.3 mg/kg/h after 12 h	Cessation of seizures or >80% reduction of seizure burden	Video-EEG	0	NA

Table 5. (continued)

	n	Preterm (n)	First-line AED	Second-line AED	Treatment regimen	Seizure response definition	Method of seizure assessment	Response rate (%)	Response rate preterm (%)
Castro-Conde 2005 [22]	9	0	PB	MD	Loading dose 0.15 mg/kg, infusion 1 µg/kg/min, increased by 0.5-1 µg/kg/min every 2 min to 18 µg/kg/min	NR	Clinical seizures + intermittent EEG (one per 24 h)	100	NA
Shany 2007 [15]	8	0	PB/DZ/LZ	MD	60-200 µg/kg/h	Cessation of seizures for ≥6 h	Clinical seizures + aEEG	50	NA
van den Broek 2015 [27]	22	0	PB	MD	Loading dose 0.05 mg/kg, infusion 0.15 mg/kg/h or higher	>80% reduction in seizure burden and no need for rescue AED	aEEG	23	NA
Lidocaine									
Boylan 2004 [8]	5	1	PB	LD	Loading dose 4 mg/kg, infusion 2 mg/kg (48 h), increased to 4 mg/kg/h after 12 h	Cessation of seizures Intermediate: ≥80% reduction in seizure burden	Video-EEG	40	100

Table 5. (continued)

	n	Preterm (n)	First-line AED	Second-line AED	Treatment regimen	Seizure response definition	Method of seizure assessment	Response rate (%)	Response rate preterm (%)
Shany 2007 [15]	22	0	PB/ DZ/ LZ	LD	Loading dose 2 mg/kg, infusion 4-6 mg/kg/h	Cessation of seizures for ≥6 h Intermediate: diminished seizure burden or recurrence <6 h	Clinical seizures + aEEG	50	NA
Lundqvist 2013 [11]	30	0	DZ/ MD	LD	Infusion 4-6 mg/kg/h (start), 4-8 mg/kg/h (maintenance, 11-60 h)	Cessation of seizures on aEEG or improvement on sequential EEGs and no need for rescue AED	aEEG	53.3	NA
Clonazepam									
Boylan 2004 [8]	3	2	PB	CL	NR	Cessation of seizures Intermediate: ≥80% reduction in seizure burden	Video-EEG	0	0
Levetiracetam									
Abend 2011 [20]	14	0	PB	LV	Loading dose 5-20 mg/kg, increased to max 10-61 mg/kg	Cessation of seizures Intermediate: >50% reduction or cessation within 24 h	Video EEG	28.6	NA

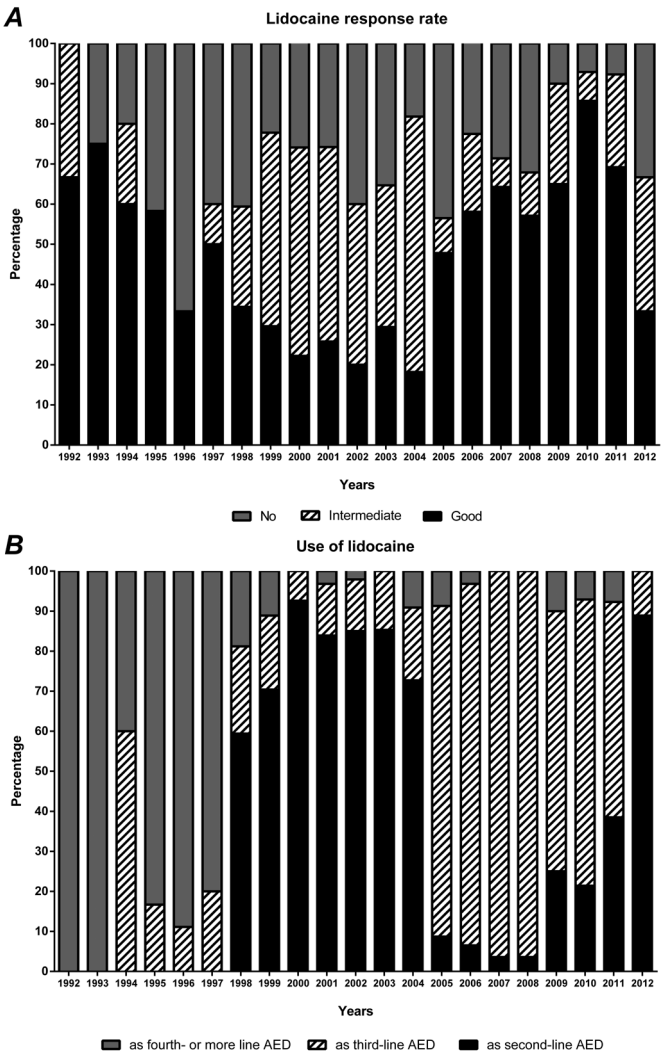
Table 5. (continued)

	n	Preterm (n)	First-line AED	Second-line AED	Treatment regimen	Seizure response definition	Method of seizure assessment	Response rate (%)	Response rate preterm (%)
Khan 2011 [23]	22	0	PB	LV	50 mg/kg	Cessation of seizures within 1 h Intermediate: cessation of seizures within 24 h	Clinical seizures + EEG	32	NA
Third-line AED									
Midazolam									
Sheth 1996 [26]	3	0	PB	PH	Loading dose 0.15 mg/kg, infusion 0.12-0.4 mg/kg/h	NR	Intermittent EEG	66.7	NA
van Leuven 2004 [25]	15	0	PB	LD	Loading dose 0.05 mg/kg, infusion 0.15 mg/kg/h	Cessation of seizures <8 h	Clinical seizures + aEEG	73.3	NA
Shany 2007 [15]	4	0	PB	LD	60-200 µg/kg/h	Cessation for ≥6 h Intermediate: diminished or recurrence <6 h	Clinical seizures + aEEG	50	NA
Lidocaine									
Rey 1990 [14]	13	5	PB	DZ	Infusion 4 (24 h) - 3 (24 h) - 2 (24 h) - 1 (24 h) mg/kg/h	Cessation of seizures for ≥13 h	Clinical seizures + EEG	85	100

Table 5. (continued)

	n	Preterm (n)	First-line AED	Second-line AED	Treatment regimen	Seizure response definition	Method of seizure assessment	Response rate (%)	Response rate preterm (%)
Radvanyi-Bouvet 1990 [13]	26	9	PB	DZ	Infusion 4 (24 h) - 3 (24 h) - 2 (24 h) - 1 (24 h) mg/kg/h	Cessation of seizures for ≥13 h	Clinical seizures + EEG	76.9	88.9
Hellström-Westas 1992 [10]	24	10	PB	DZ/PH	Loading dose 1.6-2.2 mg/kg, infusion 4-6 mg/kg/h (74.3 - 105.2 h)	Cessation of seizures Intermediate: diminished seizure burden or later recurrence of seizures	aEEG	62.5	80
Malingré 2006 [12]	20	5	PB	MD (term)/CL (preterm)	Loading dose 2 mg/kg, infusion 6 (12 h) - 4 (12 h) - 2 (12 h) mg/kg/h	Cessation of seizures Intermediate: diminished seizure burden or later recurrence of seizures	Clinical seizures + aEEG	64	25
van den Broek 2013 [16]	22	0	PB	MD	Loading dose 2 mg/kg, infusion 4 (6 h) - 2 (12 h) mg/kg/h	>80% reduction in seizure burden within 4 h	aEEG	91	NA

AED: antiepileptic drug; aEEG: amplitude-integrated EEG; CL: clonazepam; DZ: diazepam; LD: lidocaine; LV: levetiracetam; LZ: lorazepam; MD: midazolam; NA: not applicable; NR: not reported; PB: phenobarbital; PH: phenytoin.



Supplemental figure 1. Association between timing of administration of lidocaine and response rate. (A) A clear increase in response rate was seen after 2005; (B) after 2005 lidocaine was most often given as third-line AED, compared to the years before 2005, when lidocaine was given mainly as second-line AED.

We may have missed seizures, as we did not have continuous video-EEG monitoring and only had access to an automated seizure detection algorithm since 2006 (BrainZ BRM2 and BRM3 monitor). The changes, although minor, in the treatment regimen were a limitation as well. Finally, due to the use of aEEG rather than EEG, we did not have detailed data on seizure burden, which may influence the reported seizure response rate of these AEDs. Nevertheless, aEEG is used most often in the NICU to assess seizure activity and treatment effect making these results clinically relevant. The findings in this study show that there is an urgent need to continue investigating treatments for seizures in neonates. The response rates to both lidocaine and midazolam should ideally be confirmed in a prospective multicentre randomised controlled study. The possible synergistic or additive effect of AEDs as well as the effect of timing of AED administration clearly needs further investigation. In addition, the pathophysiological mechanisms of seizures in preterm infants need further investigation in order to develop better treatment strategies for these infants. Based on our results, a combination of lidocaine and midazolam may be a good treatment option for neonatal seizures refractory to phenobarbital. We would advise starting with lidocaine as second-line AED, as this AED does not affect the background activity as much as midazolam, and in the spontaneously breathing infant this AED is less likely to have an effect on respiratory drive. Lidocaine-associated cardiac events, the main concern of lidocaine treatment, proved to be rare in full-term and preterm infants treated with lidocaine for seizures, a study based on the same cohort as the present study. [29] Midazolam should be given if seizures persist.

CONCLUSION

In conclusion, treatment of neonatal seizures remains challenging. Lidocaine can reach a response rate of about 70%, but gestational age, the underlying aetiology, and timing of administration influence the seizure response to treatment. Lidocaine had a significantly higher response rate than midazolam as second-line AED after phenobarbital, and there was a trend for a higher response rate as third-line AED. Both lidocaine and midazolam were more effective as third-line compared to second-line AED, which could be due to a pharmacological synergistic mechanism between the two drugs.

ACKNOWLEDGEMENTS

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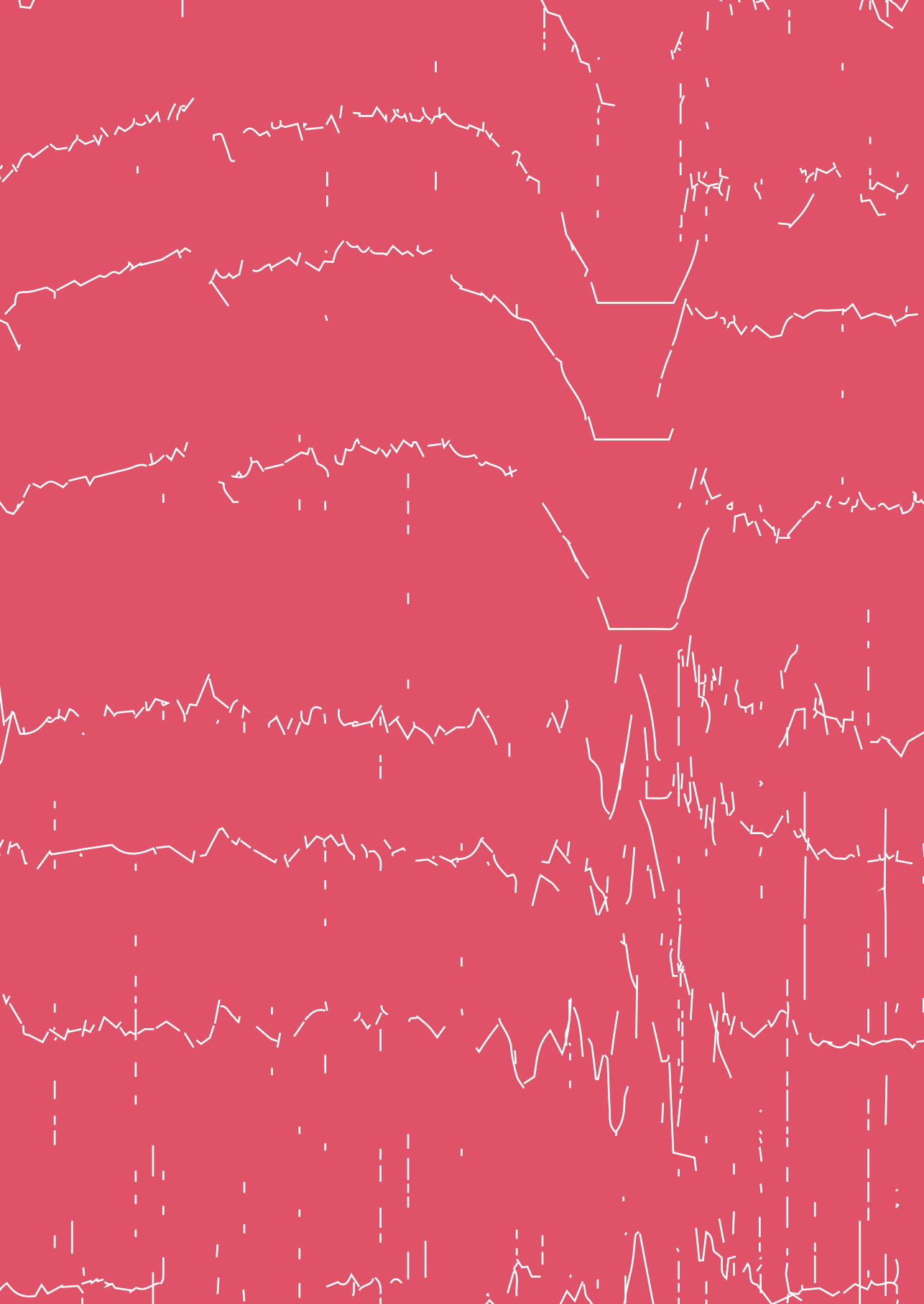
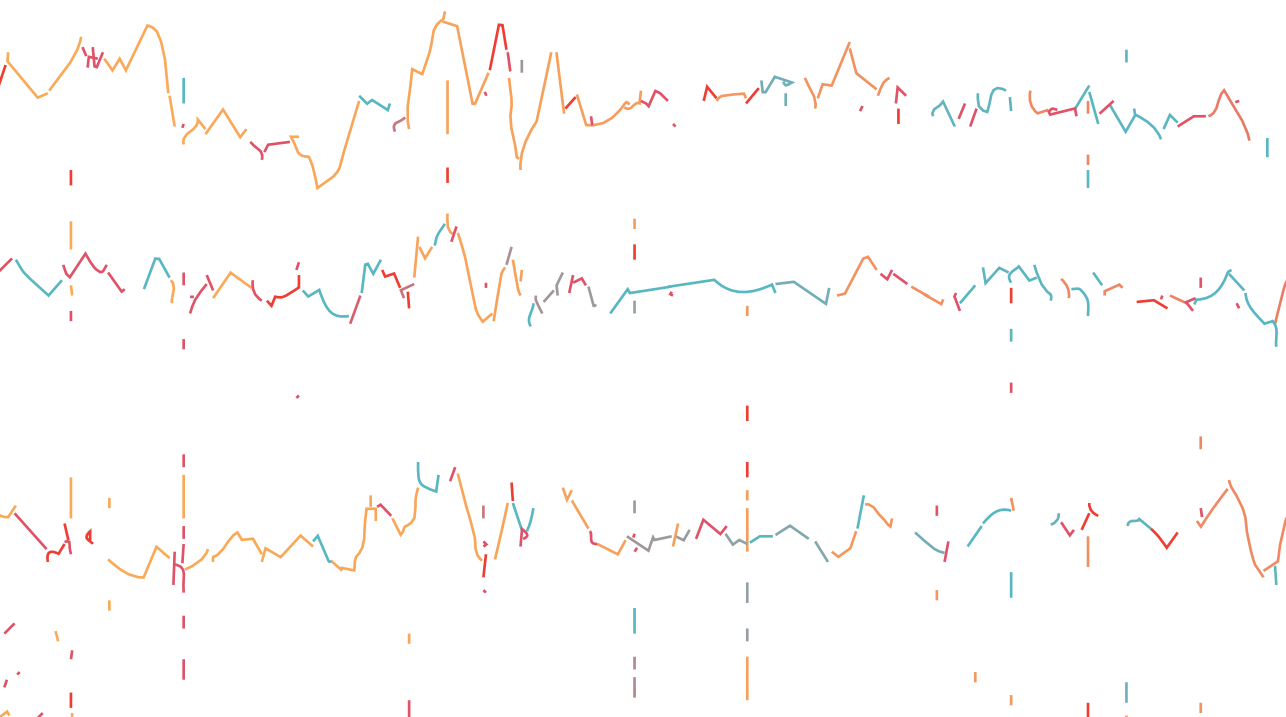


PART 3

Prognosis

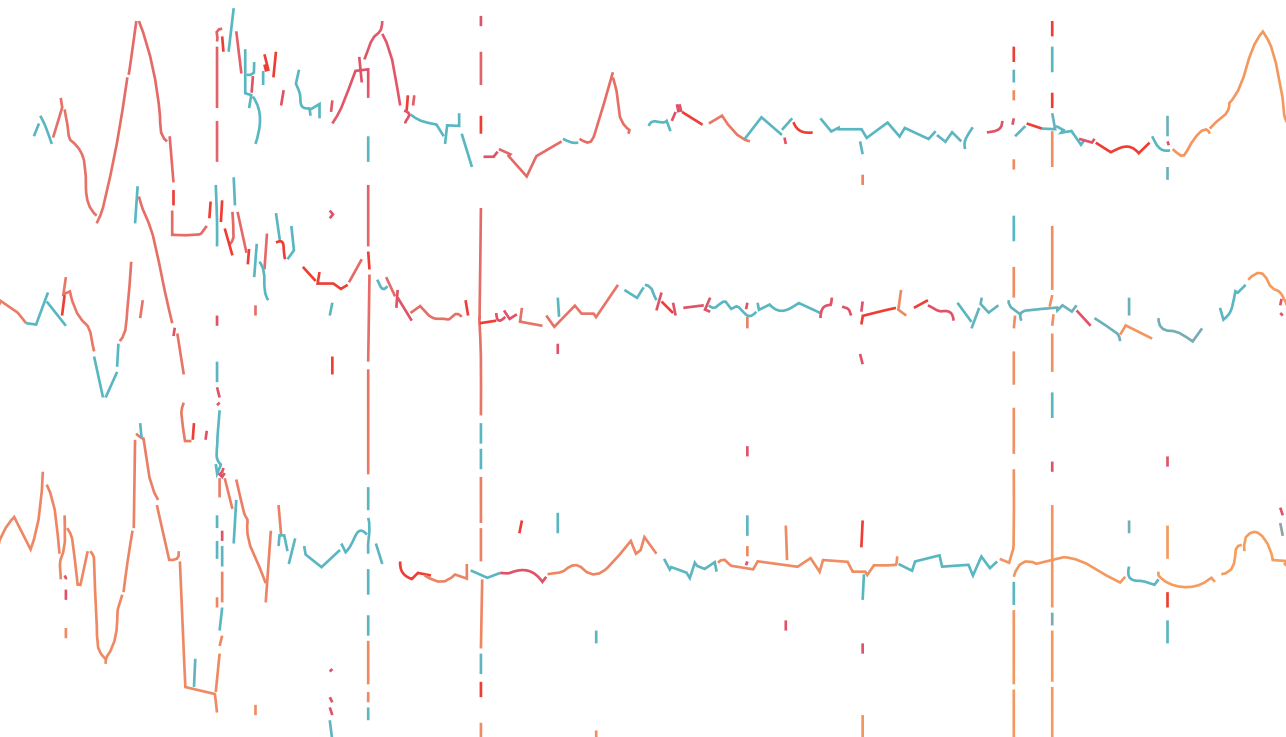
Chapter 9



Role of EEG background activity, seizure burden and MRI in predicting neurodevelopmental outcome in full-term infants with hypoxic-ischaemic encephalopathy in the era of therapeutic hypothermia

Lauren C. Weeke, Geraldine B. Boylan, Ronit M. Pressler, Boubou Hallberg, Mats Blennow, Mona C. Toet, Floris Groenendaal, Linda S. de Vries, for the NEonatal seizure treatment with Medication Off-patent (NEMO) consortium

Eur J Paediatr Neurol 2016;20:855-864



ABSTRACT

Introduction The aim was to investigate the role of EEG background activity, electroencephalographic seizure burden, and MRI in predicting neurodevelopmental outcome in infants with hypoxic-ischaemic encephalopathy (HIE) in the era of therapeutic hypothermia.

Methods Twenty-six full-term infants with HIE (September 2011 - September 2012), who had video-EEG monitoring during the first 72 h, an MRI performed within the first two weeks and neurodevelopmental assessment at 2 years were evaluated. EEG background activity at age 24, 36 and 48 h, seizure burden, and severity of brain injury on MRI, were compared and related to neurodevelopmental outcome.

Results EEG background activity was significantly associated with neurodevelopmental outcome at 36 h ($p = 0.009$) and 48 h after birth ($p = 0.029$) and with severity of brain injury on MRI at 36 h ($p = 0.002$) and 48 h ($p = 0.018$). All infants with a high seizure burden and moderate-severe injury on MRI had an abnormal outcome. The positive predictive value (PPV) of EEG for abnormal outcome was 100% at 36 h and 48 h and the negative predictive value (NPV) was 75% at 36 h and 69% at 48 h. The PPV of MRI was 100% and the NPV 85%. The PPV of seizure burden was 78% and the NPV 71%.

Conclusion Severely abnormal EEG background activity at 36 h and 48 h after birth was associated with severe injury on MRI and abnormal neurodevelopmental outcome. High seizure burden was only associated with abnormal outcome in combination with moderate-severe injury on MRI.

INTRODUCTION

Hypoxic-ischaemic encephalopathy (HIE) is still associated with abnormal neurological outcome, even after the introduction of therapeutic hypothermia. Twenty to almost 40% of infants die in the neonatal period following HIE, about 20% develop a severe disability such as cerebral palsy (CP) and 10% develop moderate impairment in motor or cognitive function later in life. [1-4] EEG and MRI are used more and more for predicting neurodevelopmental outcome in infants with HIE. The extent of EEG abnormalities, especially the background activity, [5-8] during the first days after birth has been associated with outcome in the pre-cooling era. The pattern of injury on MRI has been shown to be able to predict the severity and type of neurodevelopmental dysfunction later in life. [9-11] The combined use of EEG and MRI in predicting outcome has been studied in normothermia. [6] However, therapeutic hypothermia is now well established for neuroprotection in infants with HIE and has been shown to influence the predictive properties of EEG in particular. [12, 13] Seizures are a common phenomenon in HIE. [14, 15] Electroencephalographic seizures have been associated with more severe brain injury on MRI, [16-19] and clinical seizures have been associated with worse neurodevelopmental outcome compared to infants without seizures. [20] However, the role of seizures and seizure burden in predicting outcome of infants with HIE has not been studied. Our aim was to investigate the role of EEG background activity, electroencephalographic seizure burden, and MRI in predicting neurodevelopmental outcome at 2 years of age in a single cohort of full-term infants with HIE in the era of therapeutic hypothermia.

METHODS

Patients

Patients included in this study were participants of the "NEonatal seizure treatment with Medication Off-patent (NEMO) study", a European multicentre trial (September 2011 - September 2012) using bumetanide for seizure treatment in infants with HIE due to presumed perinatal asphyxia that had an MRI as part of routine clinical care. Inclusion criteria: gestational age (GA) of 37-43 weeks and postnatal age <48 h; perinatal asphyxia (5 min Apgar ≤5, pH ≤7.10, BE ≥16 mmol/l, or resuscitation 10 min after birth); electroencephalographic seizures not responding to 20 mg/kg phenobarbital. Exclusion criteria: other diuretics or antiepileptic drugs; major congenital anomalies; inborn errors of metabolism; genetic syndromes, and unacceptable abnormalities of electrolytes, total bilirubin or creatinine. If seizures were confirmed on EEG, written informed consent was

obtained and infants commenced the study protocol if seizures recurred after initial treatment with phenobarbital.

Electroencephalography

Patients were monitored with continuous video-EEG (Nicolet, Natus, Seattle, USA), with a minimum of eight channels using the 10-20 system of electrode placement modified for neonates where possible for at least 72 h. Two independent neurophysiologists, blinded to patient identity and MRI results reviewed the entire EEG for each neonate. The total seizure burden (accumulated duration of recorded electroencephalographic seizures in minutes), seizure onset (start of first electroencephalographic seizure in hours after birth), seizure period (from start of first to end of last recorded electroencephalographic seizure in hours), mean seizure duration in seconds, and the total number of seizures were calculated for each neonate. The background activity was graded in 1 h epochs at the onset of EEG monitoring (baseline) and at 24, 36 and 48 h after birth and categorised into four groups according to previously defined criteria [21, 22] with some adaptation according to the new ACNS guidelines [23]: normal/mild (continuous background activity with slightly abnormal activity e.g. mild asymmetries, mild voltage depression, poorly defined sleep-wake cycling), moderate (discontinuous activity with interburst interval ≤10 s, no clear sleep-wake cycling or clear asymmetry or asynchrony), severe abnormalities (discontinuous activity with interburst interval 10-60 s, severe attenuation of background activity, no sleep-wake cycles), and isoelectric EEG (background activity <5 μV or severe discontinuity with interburst interval >60 s). Seizure burden was dichotomised into low (<18 min) and high seizure burden (≥18 min), the optimal cut-off for seizure burden for predicting an abnormal outcome was chosen as the point on the receiver operating characteristic curve closest to the (0,1) point.

Magnetic resonance imaging

An MRI (1.5T or 3.0T) was performed as part of routine clinical care. Two independent reviewers blinded to patient identity and EEG results, assessed the MRIs using conventional T1- and T2-weighted images (T1WI and T2WI) and diffusion-weighted images (DWI) with apparent diffusion coefficient maps when available. In case of disagreement, consensus agreement was obtained with the help of a third reviewer. The infants were scored with the adapted Barkovich score, which assesses the basal ganglia and thalami (BGT), the white matter/watershed (WM/WS) areas and the myelination of the posterior limb of the internal capsule (PLIC). [19] Based on this score, infants were classified into five groups: no tissue injury, predominant BGT injury, predominant WM/WS injury, near-total brain injury. The severity of injury was estimated as mild, when the injury was small, focal and/

or unilateral or as moderate-severe, when the injury was more extensive and/or bilateral.

Neurodevelopmental outcome

A standardised protocol of neurological examination and the Bayley Scales of Infant and Toddler Development, third edition (BSITD-III) were used to assess outcome at 2 years of age. [24] Infants that were untestable with the BSITD-III, due to severe cerebral palsy (CP), were assigned a score of 54 for the BSITD-III composite score. CP was defined according to the criteria of Rosenbaum *et al*. [25] Motor function was classified using the Gross Motor Function Classification System. [26] Abnormal outcome was defined as a BSITD-III score <85 in all three subscales or <70 in any subscale, CP, epilepsy, moderate to severe impairment in motor, hearing, vision or communication function or death.

Statistical analysis

Statistical analysis was performed using SPSS version 20 (IBM Corp., Armonk, NY, USA). Fisher's exact test was used to investigate the association between neurodevelopmental outcome (normal vs. abnormal), EEG background activity (normal to moderate vs. severe and isoelectric), seizure burden (<18 min vs. ≥18 min) and MRI (normal and mild injury vs. moderate-severe and near-total). All variables were dichotomised. Quadratic regression was performed to test the relation between seizure burden, seizure number and MRI score. T-test was used to compare the mean MRI score between EEG background activity and outcome groups. For estimation of the predictive ability sensitivity, specificity, positive predictive value (PPV), negative predictive value (NPV) and accuracy (total number of correctly predicted individuals [true positive + true negative / all observations x 100]) were calculated for EEG background activity, seizure burden and MRI. A p-value of <0.05 was considered significant.

RESULTS

A total of 30 infants were screened for the randomised study out of which 14 (47%) infants received bumetanide for seizures not responding to phenobarbital; details of these infants have been published previously. [27] The remaining 16 infants were classed as screening failures with no electroencephalographic seizure activity seen on the EEG after the first dose of phenobarbital. An MRI was performed in 26 of the 30 screened infants. Twelve of these received bumetanide and 14 were screening failures. Clinical characteristics of these 26 infants are summarised in *table 1*.

Table 1. Patient characteristics

	Total (n = 26)
Male sex, n (%)*	15 (57.7)
Gestational age at birth (weeks), mean (SD)	40.4 (1.1)
Birth weight (grams), median (range)	3445 (2261-4750)
Apgar score at 5 min, median (range)†	4 (1-10)
First pH, mean (SD)	6.89 (0.20)
Sarnat grade,[45] n (%)^	
Grade 2	17 (65.4)
Grade 3	7 (26.9)
Therapeutic hypothermia, n (%)^	22 (84.6)

*Data available for 21 infants; †data available for 25 infants; ^data available for 24 infants.
SD: standard deviation; HIE: hypoxic-ischaemic encephalopathy.

Electroencephalography

EEG was started at a median of 7.3 h after birth (interquartile range [IQR] 3.3-14.9); the median duration of the EEG recordings was 71.4 h (IQR 42.8-85.8). The EEG background activity at baseline and at 24 h of age was mildly abnormal in two (7.7%) infants, moderately abnormal in 11 (42.3%), severely abnormal in seven (26.9%) and isoelectric in five (19.2%) *(table 2)*. At 36 h the EEG was mildly abnormal in six (23.1%), moderately abnormal in nine (34.6%), severely abnormal in three (11.5%) and isoelectric in three (11.5%). At 48 h the EEG was mildly abnormal in seven (26.9%), moderately abnormal in nine (34.6%), severely abnormal in three (11.5%) and isoelectric in one (3.8%). In one case, the EEG had a technical problem and was unreportable at all time points, in five cases there were no EEG recordings at 36 h and in six not at 48 h. In one infant, the EEG deteriorated as the neonate had been given a bolus dose of midazolam at 47 h. As a result, we did not include this time point in this neonate in our analysis and used a 46 h time point instead. In 17 infants the EEG recordings (65.4%) showed electroencephalographic seizure activity. Seizure data per infant is shown in *table 2*.

Magnetic resonance imaging

MRI was performed at a mean age of 6.4 days (SD 2.9) and a mean postmenstrual age of 41.1 weeks (SD 1.3). Eight infants had no tissue injury on MRI (30.8%), one had mild BGT injury (3.8%), five had mild WM/WS injury (19.2%), five had moderate-severe BGT injury (19.2%), three had moderate-severe WM/WS injury (11.5%) and three a near-total pattern of injury (11.5%). Due to movement artefacts it was impossible to assess the MRI in one infant.

Neurodevelopmental outcome

Three infants were lost to follow-up. Four (15.4%) infants died, due to redirection of care. The 19 survivors were assessed at a median age of 26 months (range 16-32), one infant was only assessed before 24 months (16 months). The BSITD-III was performed in 15 (78.9%), three infants could not be tested due to severe CP. In six infants the BSITD-III data was incomplete. In one infant with moderate hearing loss, the receptive language could not be tested. In five infants, with no impairments based on neurological examination, the motor function data was incomplete, in four the gross motor scaled score was not available, and in one the fine motor scaled score. Twelve infants (52.2%) had a good outcome with no impairments, 11 (47.8%) had a poor outcome. Four died, three developed CP with presumed cognitive impairment, two had moderate impairment in motor and cognitive function, one only had moderate motor impairment and one had moderate hearing impairment requiring a hearing aid.

Association between EEG background activity, electroencephalographic seizures, MRI and neurodevelopmental outcome

Neurodevelopmental outcome was significantly associated with EEG background activity at 36 h ($p = 0.009$) and 48 h after birth ($p = 0.029$), seizure burden ($p = 0.036$) and MRI ($p < 0.001$). No association was found between outcome and EEG at baseline or 24 h. MRI was associated with EEG background activity at 36 h ($p = 0.002$) and 48 h ($p = 0.018$), seizure burden ($p = 0.001$) and outcome ($p < 0.001$). No association was found between seizure burden and EEG background activity at any time point.

Table 2. EEG, MRI and outcome findings

Case	Received bumetanide	HIE (Sarnat [45]) grade	MRI findings	EEG grade 24 h	Sedatives/ AED <24 h	EEG grade 36 h	Sedatives/AED 24-36 h	EEG grade 48 h
1	No	2	Poor quality MRI	3	Morphine, phenobarbital	2	Morphine	2
2	No	2	No injury	Poor quality EEG	Morphine, phenobarbital	Poor quality EEG	NR	Poor quality EEG
3	No	NR	No injury	2	Morphine, phenobarbital	NA	NA	NA
4	Yes	2	No injury	2	Morphine, phenobarbital	1	Morphine	1
5	No	2	No injury	1	Phenobarbital	1	No	1
6	No	2	No injury	2	Phenobarbital	1	No	1
7	No	2	No injury	2	Morphine, phenobarbital	1	NR	1
8	Yes	2	No injury	3	Morphine, phenobarbital	2	Morphine, phenytoin	2
9	No	2	No injury	3	Morphine, phenobarbital	NA	Morphine	NA
10	Yes	2	WM/WS mild	2	Morphine, phenobarbital	1	Morphine	1
11	Yes	2	WM/WS mild	3	Morphine, phenobarbital	2	Phenytoin	2
12	No	2	WM/WS mild	2	Morphine, phenobarbital	2	Morphine	2
13	Yes	2	WM/WS mild	2	Morphine, phenobarbital	2	Morphine	2*
14	No	2	WM/WS mild	2	Morphine, phenobarbital	2	Morphine	2
15	No	NR	BGT mild	3	Morphine, phenobarbital	2	NR	1
16	No	2	WM/WS mod-sev	1	Phenobarbital	1	NR	1
17	Yes	2	WM/WS mod-sev	2	Phenobarbital	Poor quality EEG	No	Poor quality EEG

Table 2. (continued)

Case	Sedatives/ AED 36-48 h	Duration EEG recording (h)	Seizure number	Seizure onset (h after birth)	Seizure period (h)	Seizure duration (s)	Seizure burden (min)	Outcome	Type of sequela
1	Morphine	77.45	0	NA	0	0	0	Normal	
2	NR	72.02	0	NA	0	0	0	Normal	
3	NA	8.00	0	NA	0	0	0	Normal	
4	Morphine	85.45	1	19.3	0	19.0	0.3	Normal	
5	No	79.32	3	11.5	0.4	88.0	4.4	Lost to follow-up	
6	No	26.75	1	28.1	0	51.0	0.9	Normal	
7	NR	86.52	0	NA	0	0	0	Normal	
8	Morphine	85.63	10	12.5	15.5	87.7	14.6	Normal	
9	Morphine	8.00	0	NA	0	0	0	Normal	
10	Morphine	86.48	4	7.0	4.4	784.3	52.3	Normal	
11	Phenobarbital	76.15	6	15.5	3.9	101.5	10.2	Moderate	Motor, communication
12	Morphine	93.48	0	NA	0	0	0	Moderate	Motor
13	Morphine, midazolam	104.68	5	13.7	31.5	12.8	1.1	Normal	
14	Morphine	120.07	4	4.4	2.9	658.8	43.9	Normal	
15	NR	91.52	0	NA	0	0	0	Normal	
16	NR	48.87	4	12.3	14.5	454.0	30.3	Lost to follow-up	
17	No	41.63	19	17.0	11.3	101.3	32.1	Moderate	Hearing

Table 2. (continued)

Case	Received bumetanide	HIE (Sarnat [45]) grade	MRI findings	EEG grade 24 h	Sedatives/ AED <24 h	EEG grade 36 h	Sedatives/AED 24-36 h	EEG grade 48 h
18	Yes	2	WM/WS mod-sev	3	Morphine, phenobarbital, midazolam, phenytoin, phenobarbital	3	Morphine	2
19	Yes	2	BGT mod-sev	2	Morphine, phenobarbital	2	Morphine, phenobarbital, phenytoin	2
20	Yes	3	BGT mod-sev	2	Morphine, phenobarbital	2	Morphine, phenobarbital	2
21	Yes	3	BGT mod-sev	4	Morphine, phenobarbital, midazolam	3	Morphine, phenobarbital, midazolam	3
22	Yes	3	BGT mod-sev	3	Morphine, phenobarbital	3	Morphine, phenobarbital	3
23	Yes	3	BGT mod-sev	4	Morphine, phenobarbital	4	Morphine, midazolam, lidocaine	3
24	No	3	Near-total	4	Morphine, phenobarbital	NA	NA	NA
25	No	3	Near-total	4	Morphine, phenobarbital	4	NR	NA
26	No	3	Near-total	4	Morphine, phenobarbital	4	NR	4

*EEG grade assessed at 46 h after birth since bolus of midazolam at 47 h impacted on EEG grade at 48 h.
BGT: basal ganglia and thalami; CP: cerebral palsy; mod-sev: moderate-severe; NA: not applicable; NR: not reported; WM/WS: white matter/watershed; EEG grade: 1=mildly abnormal, 2=moderately abnormal, 3=severely abnormal, 4=isoelectric.

Table 2. (continued)

Case	Sedatives/ AED 36-48 h	Duration EEG recording (h)	Seizure number	Seizure onset (h after birth)	Seizure period (h)	Seizure duration (s)	Seizure burden (min)	Outcome	Type of sequela
18	Morphine	61.77	64	6.0	41.8	278.3	296.8	Severe	CP
19	Morphine, midazolam	10.17	63	23.2	10.9	51.3	53.8	Lost to follow-up	
20	Morphine, phenobarbital	59.93	11	22.6	2.7	115.6	21.2	Died	
21	Morphine, midazolam, phenobarbital	82.87	44	17.6	24.2	128.3	94.1	Severe	CP
22	Morphine, phenytoin	57.53	34	26.2	15.3	76.9	43.6	Moderate	Motor, communi-cation
23	Morphine	70.77	30	13.3	34.1	54.9	27.5	Died (redirection of care)	
24	NA	59.43	5	21.6	3.7	754.6	62.9	Died (redirection of care)	
25	NR	23.80	0	NA	0	0	0	Died (redirection of care)	
26	NR	43.17	0	NA	0	0	0	Severe	CP

EEG background activity versus MRI and neurodevelopmental outcome

EEG background activity at 36 h after birth showed the strongest association with MRI and neurodevelopmental outcome *(figure 1)*. The background activity on EEG at 36 h after birth would have accurately predicted outcome in all but three out of 18 infants and in all but four out of 17 infants at 48 h after birth *(table 3)*. All infants with a severely abnormal or isoelectric EEG at 36 h or 48 h after birth had moderate-severe injury on MRI and an abnormal outcome. While all infants with no-mild EEG abnormalities had no-mild injury on MRI and a normal outcome. However, at 36 h three out of eight infants with a moderately abnormal EEG had an abnormal outcome. One of these infants had moderate-severe injury on MRI, the other two had mild WM/WS injury. At 48 h, four out of eight infants with a moderately abnormal EEG had an abnormal outcome. Two of these infants had moderate-severe injury on MRI, the other two had mild WM/WS injury. The adapted Barkovich score was significantly higher in infants with a severely abnormal or isoelectric EEG (6.8 and 6 respectively) compared to those with a mild to moderately abnormal EEG at 36 h and 48 h (1.8, $p = 0.012$ and 2.1, $p = 0.007$ respectively).

Seizure burden

The extent of electroencephalographic seizure burden (low vs. high) would have accurately predicted outcome in all but six out of 23 infants *(table 3)*. Relations between seizure burden, MRI and outcome could be analysed for 21 infants. Among the 26 infants included, one had an unassessable MRI, three were lost to follow-up and one EEG was unreportable. Twelve infants had a low seizure burden (<18 min), six had a normal MRI (all normal outcome); four had mild MRI abnormalities (two normal outcome, two motor problems); two had near-total injury (one died, one CP). Among the nine infants with a high seizure burden (≥18 min), two had mild MRI abnormalities (both normal outcome), six had moderate abnormalities (one hearing problems, three motor problems, two died) and one had near-total injury (died). A significant relation between seizure burden ($p = 0.04$) and seizure number ($p < 0.001$) and the adapted Barkovich score was found using quadratic regression analysis *(figure 2),* caused by two infants with a low seizure burden but a high MRI score (near-total injury).

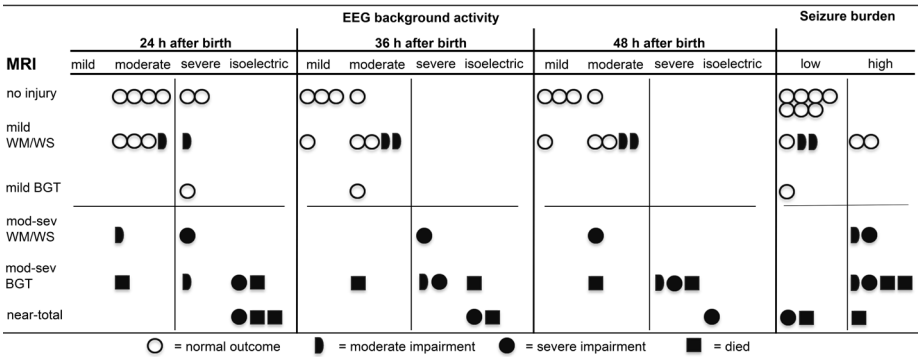


Figure 1. Association between EEG parameters (background activity and seizure burden), MRI and outcome. BGT: basal ganglia and thalami; WM/WS: white matter/watershed. NB. not in graph: one infant with severe EEG, no seizures, normal outcome, but no MRI pattern. One with normal MRI and normal outcome, but no information on EEG background activity and seizure burden. Three no outcome (one with normal MRI, moderate EEG and low seizure burden, one with moderate-severe WM/WS injury, moderate EEG and high seizure burden, one with moderate-severe BGT injury, moderate EEG and high seizure burden).

Table 3. Predictive values of EEG background activity at 36 and 48 h after birth, seizure burden, and MRI for abnormal outcome

	EEG 36 h (n = 18)*	EEG 48 h (n = 17)†	Seizure burden (n = 22)^	MRI (n = 22)$
Sensitivity	0.67	0.50	0.64	0.82
Specificity	1	1	0.82	1
PPV (%)	100	100	78	100
NPV (%)	75	69	69	85
Accuracy (%)	83	76	74	91

*Five infants did not have an EEG available at 36 h, three infants were lost to follow-up; †Six infants did not have an EEG available at 48 h, three infants were lost to follow-up; ^One EEG was unreportable, three infants were lost to follow-up; $One MRI was unassessable, three infants were lost to follow-up.

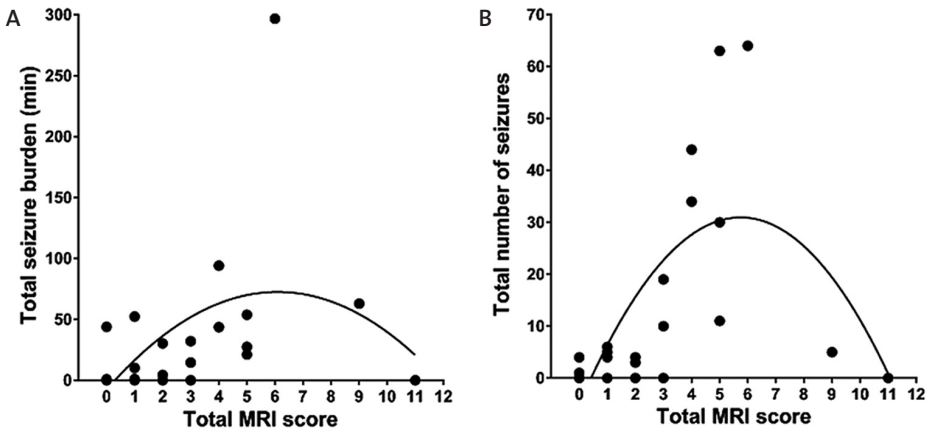


Figure 2. Quadratic regression MRI scoring system, seizure burden (A) and seizure number (B).

MRI versus neurodevelopmental outcome

MRI would have accurately predicted outcome in all but two out of 22 infants *(table 3)*. All infants with moderate-severe injury on MRI had a poor outcome, while all with no injury on MRI had a normal outcome. Of the infants with mild injury on MRI four out of six had a normal outcome. Infants with an abnormal outcome had significantly higher adapted Barkovich scores than infants with a normal outcome ($p = 0.001$). A significant negative correlation was found between the adapted Barkovich score, the BSITD-III motor ($r = -0.654$, $p = 0.021$) and cognitive composite score ($r = -0.780$, $p < 0.001$). A total MRI score above 3.5 for the adapted Barkovich score was associated with poor motor and cognitive function (BSITD-III <70 [-2 SD]) in this cohort *(figure 3)*. Two infants had a low MRI score, but scored below -2 SD on the BSITD-III motor composite score. Both infants had mild WM/WS injury, moderate EEG abnormalities and low seizure burden. In both infants, the MRI was performed on day 4-5, DWI was available, but the quality of both MRIs was moderate to poor.

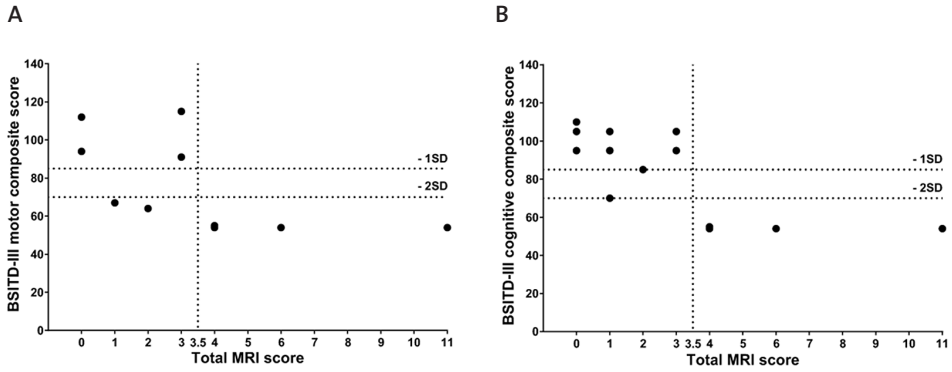


Figure 3. Correlation between the MRI scoring system and the BSITD-III motor (A) and cognitive composite score (B). A value below 70 (-2 SD) for both the BSITD-III motor and cognitive composite score was considered a poor outcome. The -1 SD line corresponds to a composite score of 85. The vertical lines define the total MRI score above which the MRI score is associated with a poor outcome (3.5).

Predictive values

Predictive values of EEG background activity at 36 h and 48 h after birth, electroencephalographic seizure burden and MRI for abnormal outcome are listed in *table 3*. In 17 infants information on EEG background activity at 36 h, seizure burden, MRI as well as neurodevelopmental outcome was available. Nine of these infants had an abnormal outcome. EEG alone was able to identify all but three infants with an abnormal outcome, MRI was able to identify an additional infant with a normal EEG but abnormal outcome. Seizure burden did not help in identifying infants at risk of having an abnormal outcome, since infants with a high seizure burden could have a normal outcome and vice versa. A high seizure burden was only associated with abnormal outcome in the presence of moderate-severe injury on MRI *(figure 1)*.

DISCUSSION

This is the first study using continuous video-EEG to investigate the role of EEG background activity, electroencephalographic seizure burden and MRI in predicting neurodevelopmental outcome in the same cohort of full-term infants with HIE in the era of therapeutic hypothermia. MRI was the best predictor of outcome, closely followed by the EEG background activity, but only at 36 h and 48 h after birth. The EEG was not predictive at earlier time points. Assessment of electroencephalographic seizure burden did not have additional predictive value since it was not as accurate in predicting outcome as MRI and EEG background activity and was only associated with abnormal outcome in the presence of moderate-severe injury on MRI. Our results confirm the findings of Li *et al*.[28] who also found MRI to be the best predictor of outcome followed by EEG background activity, but at 1 week of age instead of as early as 36 h.

Magnetic resonance imaging

The role of MRI in predicting outcome in infants with HIE has been described in several studies.[2, 9-11, 29-35] Clinically, the pattern of brain injury is most often used to predict the severity and type of neurodevelopmental dysfunction later in life.[9-11] BGT injury is associated with a worse outcome in general and with dyskinetic cerebral palsy or quadriplegia in particular, while WM/WS injury is most often associated with cognitive impairment, but this will only become apparent at school age.[9-11, 35-37] We found similar results in our study, infants with BGT and near-total injury had a worse outcome in general and motor deficits, but the follow-up period was too short and the study population too small to detect cognitive impairment and relate it to WM/WS injury. Hypothermia has led to a decrease in the extent and severity of injury, but not in the predictive properties of MRI.[2, 33, 38] This is confirmed by our study, since MRI was still the best predictor of outcome. Several MRI scoring systems have been used to quantify brain injury in full-term infants with HIE, the most commonly used scoring system is the one suggested by Barkovich *et al*.[29] However, the score was designed before the introduction of DWI sequences to MRI protocols and also the myelination of the PLIC is not part of the score. Rutherford *et al*.[32] and more recently Martinez-Biarge *et al*.[10] showed that abnormal signal intensity of the PLIC was a very strong predictor of outcome. Van Rooij *et al*.[19] used the Barkovich score but adjusted it and took the PLIC signal into account and also used the DWI sequence when performing the score, since DWI has been shown to be the most reliable sequence to assess injury in HIE in the first week of life.[30] The adapted Barkovich scoring system was associated with motor and cognitive outcome in our cohort and could, therefore, be used for outcome prediction. However, two infants had a low MRI score but impaired

motor outcome, which might be explained by the poor quality of the MRI resulting in an underestimation of the brain lesions, underlining the importance of a good quality MRI.

Electroencephalography

Many studies have reported on the predictive role of EEG in HIE. Under normothermia, a severely abnormal EEG was predictive of a poor outcome as early as 6 h after birth, [21] and when the EEG recovered within the first 24 h after birth this was associated with a good outcome. [22] Similar findings were described in studies on amplitude-integrated EEG (aEEG), which is used in many NICUs. [8,13] However, after the introduction of hypothermia for infants with HIE, the prognostic value of EEG background activity has changed. On conventional EEG, an inactive or burst suppression pattern in the first 24 h of life was not always associated with a poor outcome under hypothermia, while after 36 h these patterns were predictive of death or disability. [39,40] Nash *et al*. described that a burst suppression or extremely low voltage pattern was only associated with moderate-severe injury on MRI after the mid-cooling time period had passed. [40] Hamelin *et al*. reported a reduction in PPV of a stage four EEG for death at 14-16 h of age from 74% to 40% in infants treated with hypothermia, but the PPV of these infants increased over time and was similar to normothermic patients at day 3 of life. [39] This delay in predictive property of EEG was also described for aEEG. Azzopardi *et al*. reported a slightly lower PPV (51%) of aEEG in the first 6 h of life compared to normothermic controls (59%) and postulated that hypothermia lowered the PPV of very early aEEG due to its beneficial effect on neurological outcome. [12] Thoresen *et al*. reported a reduction in PPV at 3-6 h of age from 84% to 59%, but the PPV in infants treated with hypothermia increased over time to 92% at 24-36 h and was similar to normothermic patients at 48-60 h. [13] Hallberg *et al*. also showed that severe aEEG abnormalities were only predictive of abnormal outcome after 36 h. [41] These findings were confirmed by our results using continuous video-EEG, since we also did not find an association between EEG background activity and outcome prior to 36 h of age.

Seizure burden

Animal studies have suggested that seizures superimposed on moderate-severe hypoxic-ischaemic injury exacerbate brain injury in neonatal rats. [42] In humans, neonatal seizures due to hypoxia-ischaemia have been associated with long-term neurodevelopmental deficits, [17,20,43,44] such as cognitive and behavioural problems and epilepsy [16,17,43] and were even found to be an independent predictor of outcome after adjustment for brain injury on MRI in the pre-cooling era. [20] After the introduction of therapeutic hypothermia, electroencephalographic seizures have been related to severity of brain injury on MRI.

[16, 18] Moderate-severe injury was more common in infants with seizures, but 40% of infants with seizures did not have brain injury on MRI, suggesting that outcome after seizures is not uniformly poor in infants treated with hypothermia. [16] This is comparable to the findings in our study, moderate-severe injury was more common in infants with a high seizure burden and two out of 11 infants with a high seizure burden did not have brain injury on MRI and four out of these 11 infants had a normal outcome. In our study, using continuous 8-channel video-EEG and careful quantification of all seizures, seizure burden and seizure number showed a significant quadratic relation with the MRI score. This shows that infants with near-total brain injury and a high MRI score, may not always have a high seizure burden as most of these infants will have an isoelectric recording, with no or limited superimposed electroencephalographic seizures during the first days after birth, and a decision to redirect care may be made before recovery of background activity, which is often associated with occurrence of electroencephalographic seizures. However, this observation needs further investigation in a larger cohort. The strength of this study is that we evaluated the relationship between EEG, MRI and outcome in a single cohort of infants with HIE recruited from multiple centres across Europe using continuous video-EEG monitoring in the era of therapeutic hypothermia. The multicentre character was also a limitation of this study because of differences in MR scanners and MRI quality. Another limitation was the relatively small size of the study population, which did not allow multivariable analysis. Also, the incomplete BSITD-III data was a limitation. This study adds a comprehensive overview of the role of EEG, MRI and seizure burden in predicting outcome in infants with HIE in the era of therapeutic hypothermia to the literature. We provide additional evidence that under hypothermia the EEG background activity is a reliable predictor of outcome only after 36 h of age and show that the combined use of MRI and EEG is important in infants following HIE. Since MRI has been shown to be the best predictor of outcome, while continuous EEG monitoring helps to evaluate the severity of encephalopathy, detect and adequately treat electroencephalographic seizures, to minimize the seizure burden and help with decisions on redirection of care, for example when an infant is too unstable to be transported to the MRI. Lastly, we showed that a high seizure burden was not invariably associated with a poor outcome, but this was dependent on the extent of MRI abnormalities.

CONCLUSION

In conclusion, severely abnormal background activity on EEG at 36 h and 48 h after birth was associated with severe injury on MRI and abnormal neurodevelopmental outcome in our cohort of full-term infants with HIE. A high seizure burden was only associated with abnormal outcome in combination with moderate-severe injury on MRI.

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Chapter 10

Novel MRI score predicts neurodevelopmental outcome in infancy and at school age after perinatal asphyxia and therapeutic hypothermia

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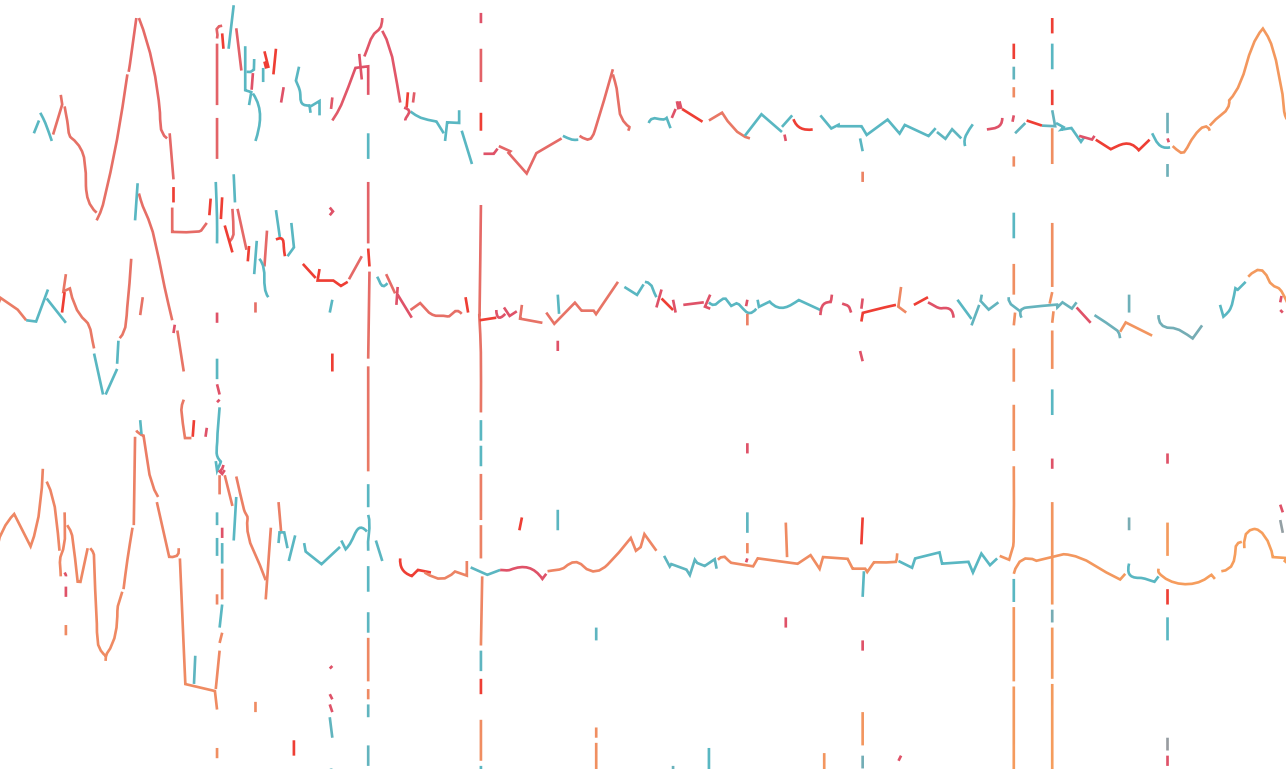
Submitted



ABSTRACT

Introduction MRI is an excellent predictor of outcome in neonatal hypoxic-ischaemic encephalopathy, but most MRI scores do not include diffusion-weighted images (DWI). An easily applicable score to quantify brain injury that includes DWI and correlates well with outcome in early and late childhood, does not yet exist. The objective was to develop an MRI brain injury score including DWI for full-term or near-term infants with therapeutic hypothermia, test it in a comparable cohort and relate this to outcome at 2 years and school age.

Methods Retrospective cohort study (cohort 1: the Netherlands 2008-2014, cohort 2: Sweden 2007-2012) including infants born ≥36 weeks gestational age (GA) treated with therapeutic hypothermia who had an MRI as part of routine clinical care. The MRI score consisted of three subscores: deep grey matter (GM), white matter/cortex (WM) and cerebellum (CB). Primary adverse outcome was defined as death, cerebral palsy, Bayley-III motor or cognitive composite scores at 2 years <85 or IQ at school age <85. Receiver operating characteristic analysis was performed for the MRI score. Multivariable logistic regression was used to calculate odds ratios (OR) and 95% confidence intervals (CI) for the association between the subscores and outcome.

Results In cohort 1 (n = 97, mean [SD] GA 39.9 [1.6]; median [IQR] Apgar score at 5 min 4 [2-5]) and cohort 2 (n = 76, mean [SD] GA 40.3 [1.4]; median [IQR] Apgar score at 5 min 3 [2-4]) the GM subscore was an independent predictor of adverse outcome at 2 years (cohort 1: OR 1.6, 95% CI 1.3-1.9, cohort 2: OR 1.4, 95% CI 1.2-1.6), and school age (cohort 1: OR 1.3, 95% CI 1.2-1.5, cohort 2: OR 1.3, 95% CI 1.1-1.6). The WM and CB subscore did not add to the predictive value. The AUC, negative and positive predictive value for the GM subscore was >0.83 in both cohorts.

Conclusion We developed a novel MRI brain injury score that uses DWI and assesses all relevant brain areas. In infants with therapeutic hypothermia after perinatal asphyxia the GM subscore can be used independently for prediction of outcome at 2 years and school age.

INTRODUCTION

Although therapeutic hypothermia after perinatal asphyxia has reduced the incidence of adverse outcome, 45% of infants still die or have neurodevelopmental impairment. [1-5] MRI and proton MR spectroscopy (^{1}H-MRS) have been shown to be excellent predictors of outcome [6] and are often used as bridging biomarkers for neurodevelopmental outcome in infants with hypoxic-ischaemic encephalopathy (HIE). [7, 8] Quantifying the extent of brain injury in these infants is important for objective and accurate prognostication and guiding decisions on redirection of care. Many existing MRI scores do not include diffusion-weighted images (DWI), while DWI has been shown to be the most reliable MRI sequence to assess injury during the first week after an hypoxic-ischaemic event. [4, 7-19] Early detection is important for selection for future additional neuroprotective strategies. Some abnormalities often encountered on MRIs of infants with HIE, such as intracranial haemorrhages, cerebellar lesions and ^{1}H-MRS abnormalities are also not included in existing scores, although separately known as being of additional value. We developed a novel score based on assessment of all MRI abnormalities of importance for prognostication in infants with HIE. The score was tested in two different international cohorts using T1WI, T2WI and DWI and was related to mortality and neurodevelopmental outcome at 2 years and school age.

METHODS

Patients

The ethics committees of both participating centres waived the requirement to obtain informed consent for this retrospective study with anonymised data. Infants born after a gestational age ≥36 weeks admitted to a level III neonatal intensive care unit (NICU) in the Netherlands (Cohort 1: January 2008 - March 2014, n = 97) and Sweden (Cohort 2: January 2007 - December 2009, n = 76) treated with therapeutic hypothermia for HIE due to presumed perinatal asphyxia (5 min Apgar ≤5, pH ≤7.10, BE ≥16 mmol/l, or resuscitation 10 min after birth) and examined by MRI as part of routine clinical care were included. Infants with major congenital anomalies, inborn errors of metabolism, and genetic syndromes were excluded.

MRI protocol

In both cohorts, MRI was performed on a 1.5T or 3.0T magnet (Philips Medical Systems, Best, the Netherlands, GE Healthcare, USA), within the first weeks after birth. Standard

MRI protocol included axial T1-weighted images (T1WI) or inversion recovery-weighted images, T2-weighted images (T2WI), DWI including apparent diffusion coefficient (ADC) mapping and ^{1}H-MRS in the basal ganglia and thalamus.

MRI score

A novel MRI score was designed based on existing scores and the patterns of brain injury reported in the literature *(supplemental table 1)*. The score assesses brain injury in three areas: (1) deep grey matter (GM, items scored: thalamus, basal ganglia, posterior limb of the internal capsule [PLIC], brainstem, perirolandic cortex, hippocampus. Maximum GM subscore: 23); (2) cerebral white matter/cortex (WM, items scored: cortex, cerebral white matter, optic radiation, corpus callosum, punctate white matter lesions [PWML], parenchymal haemorrhage. Maximum WM subscore: 21); and (3) cerebellum (CB, items scored: cerebellum, cerebellar haemorrhage. Maximum CB subscore: 8). Each item was scored for extent of injury: 0 (no injury), 1 (focal, <50%) or 2 (extensive, >50%) and for uni- (score of 1) or bilateral (score of 2) presence. A fourth group (additional [AD]) was included, assessing the presence of intraventricular or subdural haemorrhages and sinovenous thrombosis (0 if absent, 1 if present). Maximum AD subscore: 3. The total score was calculated by adding the four subscores (GM + WM + CB + AD. Maximum score: 55). In case ^{1}H-MRS was performed in the basal ganglia and thalamus, *N*-acetyl aspartate (NAA) and lactate were scored 0 (normal NAA peak, absent lactate peak) or 2 (reduced NAA peak, increased lactate peak), which was subsequently included in the GM subscore (maximum GM subscore: 25, maximum total score: 57). MRI examples for each item of the score are shown in *figure 1*.

Two reviewers blinded to outcome results (LV, LW) assessed all MRIs using the score described above. In case of disagreement consensus was obtained with the help of a third blinded reviewer (FG). To determine inter-rater reliability, two additional blinded paediatric radiologists (ML, LM) scored the injury on MRI on a sub-set of scans (n = 10).

Neurodevelopmental outcome

The Bayley Scales of Infant and Toddler Development, third edition (BSITD-III) was used to assess outcome at 2 years. [20] The Wechsler Preschool and Primary Scale of Intelligence, third edition, Dutch version (WPPSI-III-NL) [21] and Wechsler Intelligence Scale for Children, fourth edition, Swedish version (WISC-IV-SE) [22] was used to assess intelligence quotient (IQ) at school age. Severity of cerebral palsy (CP) was classified according to the gross motor function classification system (GMFCS). [23] For infants with CP that could not be tested with the BSITD-III a motor composite score was assigned, 70 (-2 standard

deviations [SD] on the BSITD-III) for GMFCS III, 45 (-3 SD) for GMFCS IV-V. For infants with severe CP (GMFCS V) that could not be tested with the BSITD-III, WPPSI-III-NL or WISC-IV-SE a cognitive composite score and IQ of 45 was assigned. Abnormal outcome was defined at 2 years as death, CP or a BSITD-III score <85 (-1 SD) for motor or cognitive composite score and at school age as death, CP or an IQ <85.

Statistical analysis

GraphPad Prism 6 (GraphPad Software Inc., La Jolla, CA, USA) was used to generate receiver operating characteristic (ROC) curves, calculate the area under the curve (AUC) and determine cut-off values for the MRI score (total- and subscores) based on the point on the ROC curve closest to the (0,1) point. Kappa values were calculated to determine the inter-rater reliability for MRI abnormality based on the cut-off values for abnormal outcome at 2 years for the total and subscores. Sensitivity, specificity, positive predictive value (PPV), negative predictive value (NPV) and accuracy (total number of correctly predicted individuals [true positive + true negative / all observations x 100]) were calculated. SPSS version 21 (IBM Corp., Armonk, NY, USA) was used to determine differences between the two cohorts in baseline characteristics using Mann-Whitney U test and chi-squared or Fisher's exact test and to perform univariate analyses and multivariable logistic regression to investigate the association between adverse outcome, the BSITD-III and IQ scores and the MRI subscores. A p-value of <0.05 was considered significant.

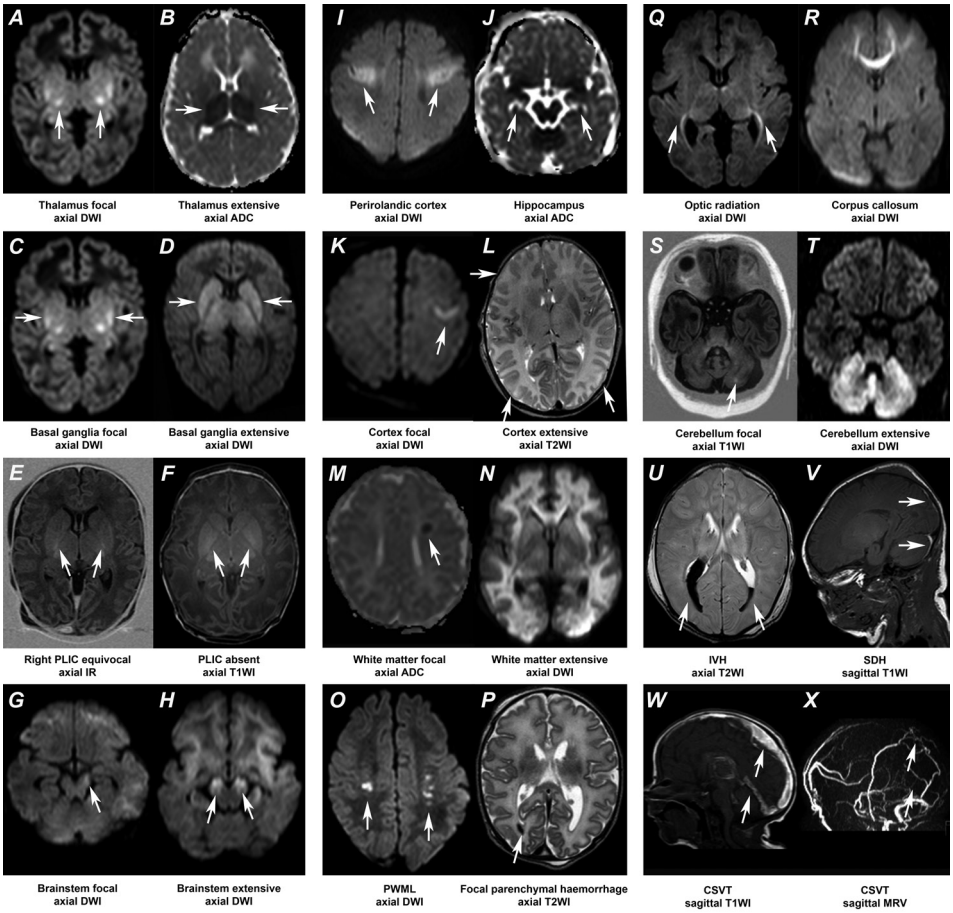

A
B
Thalamus focal
axial DWI
Thalamus extensive
axial ADC
I
J
Perirolandic cortex
axial DWI
Hippocampus
axial ADC
Q
R
Optic radiation
axial DWI
Corpus callosum
axial DWI
C
D
Basal ganglia focal
axial DWI
Basal ganglia extensive
axial DWI
K
L
Cortex focal
axial DWI
Cortex extensive
axial T2WI
S
T
Cerebellum focal
axial T1WI
Cerebellum extensive
axial DWI
E
F
Right PLIC equivocal
axial IR
PLIC absent
axial T1WI
M
N
White matter focal
axial ADC
White matter extensive
axial DWI
U
V
IVH
axial T2WI
SDH
sagittal T1WI
G
H
Brainstem focal
axial DWI
Brainstem extensive
axial DWI
O
P
PWML
axial DWI
Focal parenchymal haemorrhage
axial T2WI
W
X
CSVT
sagittal T1WI
CSVT
sagittal MRV

Figure 1. MRI examples of all items to be scored with the novel MRI score. The abnormalities of interest are marked by the white arrows. (A) Focal bilateral thalamic lesions (high signal intensity [SI]) on an axial diffusion-weighted image (DWI); (B) Extensive bilateral thalamic lesions (low SI) on an axial apparent diffusion coefficient (ADC) map; (C) Focal bilateral lesions (high SI) in the basal ganglia on an axial DWI; (D) Extensive bilateral lesions (high SI) in the basal ganglia on an axial DWI; (E) The posterior limb of the internal capsule (PLIC) is equivocal on both sides on an axial inversion recovery (IR) image; (F) Absent PLIC bilaterally seen as an inverted signal (low SI) on an axial T1-weighted image (T1WI); (G) Focal lesion (high SI) in the left cerebral peduncle on an axial DWI; (H) Extensive diffusion changes (high SI) in the cerebral peduncles bilaterally on an axial DWI; (I) Clear involvement (high SI) of the perirolandic cortex bilaterally on an axial DWI; (J) Bilateral involvement (low SI) of the hippocampus on an axial ADC map; (K) Focal involvement (high SI) of the left cortex on an axial DWI; (L) Extensive bilateral involvement of the cortex, seen as loss of the differentiation between the white matter and cortical grey matter in the occipital and frontal lobes bilaterally; (M) Focal unilateral abnormal signal (low SI) in the left periventricular white matter on an axial ADC map; (N) Extensive involvement of the white matter (high SI) on an axial DWI; (O) Bilateral punctate white matter lesions (PWML) seen as high SI on an axial DWI; (P) A small focal haemorrhage in the right occipital lobe (low SI) on an axial T2-weighted image (T2WI); (Q) Bilateral involvement of the optic radiation (high SI) on an axial DWI; (R) Involvement of the frontal part of the corpus callosum (high SI) on an axial DWI; (S) Focal lesion (high SI) in the left cerebellar hemisphere on an axial T1WI; (T) Extensive involvement of both cerebellar hemispheres (high SI) on an axial DWI; (U) Bilateral intraventricular haemorrhage (IVH) seen as low SI on an axial T2WI; (V) Subdural haemorrhage (SDH) supra- and infratentorial seen as high SI on a sagittal T1WI; (W) Cerebral sinovenous thrombosis (CSVT) seen as high SI at the location of the superior sagittal and straight sinus on a sagittal T1WI; (X) With corresponding lack of flow (lack of high SI) in those veins on an MR venography (MRV) in sagittal view.

RESULTS

MRI scans from cohort 1 were used to develop the score and scans from cohort 2 to test the score. The majority of scans were obtained in the first week after birth (81%). The quality of the scans was good (no movement artefacts, high resolution) in 85.6% for cohort 1 and 71.4% for cohort 2. The baseline characteristics are shown in *table 1*. Cohort 2 had a higher gestational age at birth, higher birth weights, lower Apgar scores at 5 min, fewer deaths, but a higher survival rate with impairment at 2 years compared to cohort 1. The inter-rater agreement for the total score and GM and WM subscore are shown in *table 2*. No cut-off values and therefore no inter-rater agreement could be determined for the CB and AD subscores.

MRI score and neurodevelopmental outcome

Adverse outcome at 2 years and school age

At 2 years, outcome data was available for all infants in both cohorts. The GM, WM and CB subscores were significantly associated with death or adverse outcome in both cohorts. The multivariable regression model included GM subscore as an independent predictor of death or impairment at 2 years of age in cohort 1 (OR 1.6, 95% CI 1.3-1.9 [$ß_0$ = -4.579, $ß_1$ = 0.456], including ^{1}H-MRS: OR 1.6, 95% CI 1.3-1.9 [$ß_0$ = -5.017, $ß_1$ = 0.443]) and cohort 2 (OR 1.4, 95% CI 1.2-1.6 [$ß_0$ = -2.310, $ß_1$ = 0.322]).

At school age (cohort 1: mean 5.9 years, cohort 2: mean 7.5 years), outcome data was available for 53 infants in cohort 1 (35 were too young, 1 not testable due to behavioural problems, 8 not tested for unknown reason) and 46 infants in cohort 2 (19 too young, 11 not tested for unknown reason). The GM and WM subscores were significantly associated with death or adverse outcome in both cohorts. The multivariable regression model included the GM subscore as an independent predictor of death or impairment at school age in both cohort 1 (OR 1.3, 95% CI 1.2-1.5 [$ß_0$ = -2.394, $ß_1$ = 0.292], including ^{1}H-MRS: OR 1.3, 95% CI 1.1-1.5 [$ß_0$ = -2.333, $ß_1$ = 0.262]) and cohort 2 (OR 1.3, 95% CI 1.1-1.6 [$ß_0$ = -2.747, $ß_1$ = 0.286]). The GM subscore was significantly correlated with the WM and CB subscore in both cohorts ($p < 0.001$). Entering WM and/or CB subscores in the models resulted in a reduction in the OR of the GM subscore, suggesting multicollinearity.

Table 1. Baseline characteristics of the two cohorts

	Cohort 1 (n = 97)	Cohort 2 (n = 76)	p-value
Gestational age (weeks), mean (SD)	39.9 (1.6)	40.3 (1.4)	0.060
Birth weight (grams), mean (SD)	3497 (610)	3708 (662)	0.039
Male, n (%)	53 (54.6)	36 (47.4)	0.342
Apgar at 5 min, median (IQR)	4 (2-5)	3 (2-4)	0.032
Sarnat grade, n (%)*			0.056
Grade 1	12 (12.4)	4 (5.3)	
Grade 2	67 (69.1)	62 (81.6)	
Grade 3	18 (18.6)	7 (9.2)	
MRI			
MRI age (day of life), median (IQR)	6 (5-7)	6 (5-8)	0.315
Total score, median (IQR)	6 (0-22)	3 (1-11.8)	0.122
GM subscore	0 (0-11.5)	0 (0-4.5)	
WM subscore	4 (0-8)	2 (0-7)	
CB subscore	0 (0-4)	0 (0-0)	
Outcome			
Died, n (%)	22 (22.7)	5 (6.6)	0.004
Age at BSITD-III assessment (months), mean (SD)	24.13 (0.42)	25.92 (1.68)	<0.001
BSITD-III motor composite score, mean (SD)	112 (12)	95 (23)†	<0.001
BSITD-III cognitive composite score, mean (SD)	107 (14)	95 (21)	<0.001
Age at IQ assessment (years), mean (SD)	5.9 (0.3)	7.5 (0.8)	<0.001
IQ, mean (SD)	102 (17)^	100 (19)§	0.555
Impairment at 2 years, n (%)	4 (4.1)	14 (18.4)	0.029
BSITD-III motor composite score <85	2 (2.1)	13 (17.1)	
BSITD-III cognitive composite score <85	2 (2.1)	10 (13.2)	
Impairment at 6 years, n (%)	4 (7.5)^	5 (10.9)§	0.773
CP	1 (1.9)	4 (8.7)	
IQ <85	3 (5.7)	4 (8.7)	

*Data available for 73 subjects in cohort 2; †data available for 57 subjects; ^data available for 53 subjects; §data available for 46 subjects. BSITD-III: Bayley Scales of Infant and Toddler Development, third edition; CB: cerebellum; CP: cerebral palsy; GM: grey matter; IQ: intelligence quotient; IQR: interquartile range; SD: standard deviation; WM: white matter.

Table 2. Agreement between raters 1 (LV, LW), 2 (ML) and 3 (LM) in determining MRI abnormality based on the optimal cut-off value for the total score and the GM and WM subscore

Score	Cut-off value	Rater 1 vs. 2	Rater 1 vs. 3	Rater 2 vs. 3
Total (incl. ^{1}H-MRS)	≥18.5	0.40 (0.58)	0.58 (0.80)	0.80 (0.80)
GM (incl. ^{1}H-MRS)	≥9.5	0.80 (0.80)	1.00 (1.00)	0.80 (0.80)
WM	≥4.5	0.78	1.00	0.78

GM: grey matter; ^{1}H-MRS: proton magnetic resonance spectroscopy; WM: white matter.

ROC curves for adverse outcome at 2 years and school age were plotted for the GM subscore as injury to the GM subscore was an independent predictor of outcome. AUC values with 95% confidence intervals and sensitivity and specificity for the cut-off values are shown in *table 3*. *Figure 2* shows the distribution of the individual scores in all infants with a normal versus an abnormal outcome at 2 years and school age in both cohorts. *Figure 3* shows the predicted probability for adverse outcome at 2 years and school age based on the GM subscore in cohort 1.

Two-year BSITD-III composite scores and IQ at school age

The relation between the MRI scores and the BSITD-III motor and cognitive composite scores at 2 years or the IQ at school age was assessed on the pooled cohort (cohort 1 + 2), because the number of surviving infants with impairment was too small in the separate cohorts. At 2 years the BSITD-III motor composite score was not available for 14 infants and a motor composite score was assigned for five infants with CP in cohort 2. At school age, an IQ score was assigned for three infants with CP in cohort 2. The GM and WM subscores were significantly associated with a motor or cognitive composite score <85 at 2 years. None of the subscores was significantly associated with an IQ <85 at school age. The multivariable regression model included the GM subscore as an independent predictor of motor (OR 1.3, 95% CI 1.2-1.5 [$ß_0$ = -3.126, $ß_1$ = 0.294]) and cognitive impairment (OR 1.3, 95% CI 1.2-1.5 [$ß_0$ = -3.504, $ß_1$ = 0.290]) at 2 years. No analyses were performed on the scores including ^{1}H-MRS results as these were only available for cohort 1.

Table 3. Cross-tabulation of the MRI score results (based on the optimal cut-off values for the GM subscore) and outcome at 2 years and school age for cohort 1 (upper panel) and 2 (lower panel) and the estimates of diagnostic accuracy

Score	Cut-off value	Outcome		Diagnostic accuracy					
		Normal	Abnormal	AUC (95% CI)	Sensitivity	Specificity	PPV	NPV	Accuracy
Cohort 1									
Outcome at 2 years									
GM	<9.50	68	2	0.988 (0.973-1.000)	0.923	0.958	0.889	0.971	0.948
GM	≥9.50	3	24						
GM incl. ^{1}H-MRS	<11.50	61	2	0.989 (0.973-1.000)	0.923	0.953	0.889	0.968	0.944
GM incl. ^{1}H-MRS	≥11.50	3	24						
Outcome at school age									
GM	<11.50	25	4	0.945 (0.878-1.000)	0.846	0.926	0.917	0.862	0.887
GM	≥11.50	2	22						
GM incl. ^{1}H-MRS	<12.50	20	3	0.935 (0.855-1.000)	0.885	0.909	0.920	0.870	0.896
GM incl. ^{1}H-MRS	≥12.50	2	23						
Cohort 2									
Outcome at 2 years									
GM	<9.50	56	11	0.832 (0.708-0.955)	0.421	0.982	0.889	0.836	0.842
GM	≥9.50	1	8						
Outcome at school age									
GM	<11.50	36	5	0.861 (0.726-0.997)	0.500	1.000	1.000	0.878	0.891
GM	≥11.50	0	5						

AUC: area under the curve; CI: confidence interval; GM: grey matter; ^{1}H-MRS: proton magnetic resonance spectroscopy; NPV: negative predictive value; OR: odds ratio; PPV: positive predictive value.

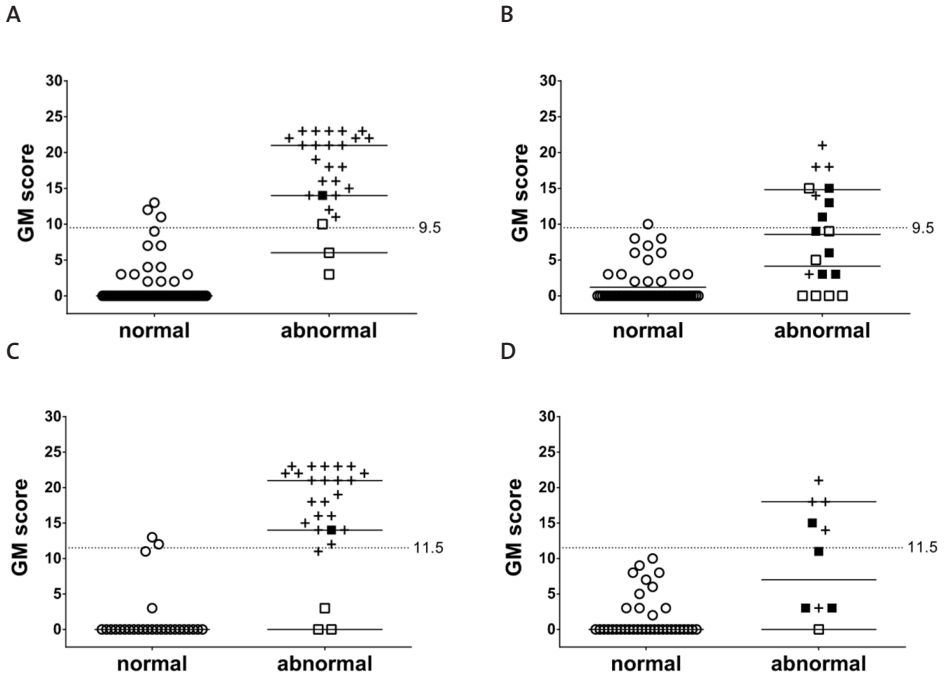


Figure 2. Individual score values on the GM subscore for infants with a normal (open circles) and infants with an abnormal outcome (death: black crosses; CP: black squares; other impairment: open squares) at 2 years of age (A, B) and school age (C, D) in cohort 1 (A, C) and cohort 2 (B, D). The black horizontal lines indicate the median. The dotted horizontal lines indicate the cut-off values for risk of adverse outcome.

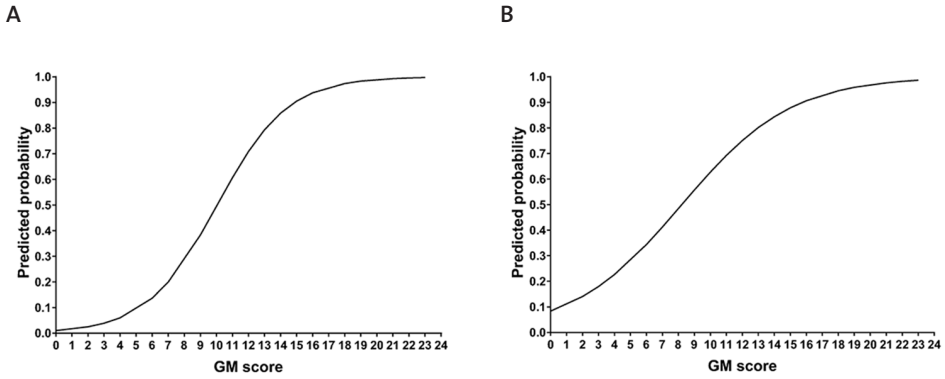


Figure 3. Predicted probability of death or impairment at 2 years (A) and school age (B) based on the GM subscore in cohort 1.

DISCUSSION

We developed an easily applicable, comprehensive MRI score that showed good predictive value in two independent, international cohorts, comprising a total of 173 infants treated with therapeutic hypothermia. In both cohorts, injury to the deep grey matter area was an independent predictor of adverse outcome at 2 years and school age. Therefore the GM subscore can be used for outcome prediction in hypothermia-treated infants with HIE. It had an AUC >0.99 in cohort 1. Reliable cut-off values with a sensitivity and specificity >0.85 and a predicted probability graph for risk of death or impairment at 2 years and school age are provided. These can be used to aid clinical decision making for future additional neuroprotective interventions and redirection of care or as an outcome measure in clinical trials. The inter-rater reliability was high and the predictive value remained good in cohort 2. The sensitivity of the GM subscore was not as good in cohort 2 (0.42 at 2 years and 0.50 at school age), however the specificity, PPV, NPV and accuracy remained good (>0.84). The reduction in sensitivity might be explained by the larger proportion of MRIs of moderate-poor quality in the cohort 2. A poor quality MRI could result in an underestimation of the brain lesions and a lower sensitivity, underlining the importance of a good quality MRI.

Two other studies related their MRI score to outcome at 2 years of age. [4, 18] Cheong *et al.* also found the basal ganglia/thalamus and PLIC score to be independent predictors of death or major disability at 2 years with an OR of 4.1. However, their scores had a low NPV (0.60). [4] Cavalleri *et al.* [18] used the summation score presented previously by Barkovich *et al.* [9] on DWI. This score showed a high sensitivity (1.00) but a low specificity (0.67) and is not applicable to scans obtained in the second week of life. At this stage, DWI abnormalities may no longer be visible, due to pseudonormalisation. [24] Two other previously published scores with a long-term follow-up did not provide predictive values. [12, 13] The remaining scores had a shorter follow-up period ranging from a few days after birth to 18 months of age. [9-11, 14-17] The predictive values of these scores ranged from 0.60 to 1.00. [10, 11, 14, 17] Only two had a good positive and negative predictive value of ≥0.87. [10, 11] However, both studies related MRI results to outcome at 12 months. This follow-up period may not be long enough to reveal the full extent of sequelae after HIE and these predictive values may, therefore, be an overestimation. Furthermore, the study by Jyoti *et al.* [11] only included 20 subjects and conclusions about the predictive value of their score should be considered with caution. Rutherford *et al.* [10] only used the signal of the PLIC to predict outcome, which may not be as reliable in infants with a gestational age of 36-37 weeks, in whom the PLIC is not yet myelinated or in infants who have their MRI before the end of the first week, when the signal intensity of the PLIC on T1WI or inversion recovery sequences may

still be normal. Only one other study related their score to outcome at school age in 124 cooled and non-cooled infants with HIE. [25] No injury on MRI had a sensitivity of 0.60 and a specificity of 0.92 for normal outcome and moderate-severe injury had a sensitivity of 0.81 and specificity of 0.78 for abnormal outcome. This study did not provide information on the predictive value of the score in infants with mild injury. Our score performs better than these previously described scores in predicting outcome. It also provides easy to use cut-off values and a predicted probability graph that can be used to determine the risk of adverse outcome in individual cases. We were, unfortunately, unable to assess and compare the quality of the score in the first versus the second week of life, because only a limited number of MRIs were performed in the second week of life. During the first week of life it is best to perform the score based on T1WI and T2WI combined with DWI, since DWI has been shown to be the most reliable sequence to assess injury in HIE in the first week of life. [19] In the second week of life DWI abnormalities may no longer be visible due to pseudonormalisation. [24] However, the score can also be performed using T1WI and T2WI only. ADC and ^{1}H-MRS measurements (lactate and NAA) in the basal ganglia can add significantly to the predictive properties of MRI. In contrast to ADC that shows pseudonormalisation after the first week, [24] ^{1}H-MRS measurements remain abnormal for a prolonged period of time. [26]

Clinically, the pattern of brain injury seen on MRI is most often used to predict the severity and type of neurodevelopmental dysfunction later in life. [27-30] Basal ganglia-thalamic injury has often been associated with a poor neurodevelopmental outcome and has primarily been associated with motor deficits. [27, 28, 30, 31] White matter or watershed injury has most often been associated with cognitive impairment, [27, 32, 33] which often does not become apparent until school age. [27, 33-35] Our results from a population of infants treated with therapeutic hypothermia confirm that injury to the deep grey matter area is associated with adverse outcome in general and impaired motor function. White matter injury was not included in the prediction model for outcome at 2 years, because many infants with a high WM subscore also had a high GM subscore. Only the GM subscore was included, suggesting that outcome was mainly determined by injury to the grey matter. These results support the findings of Harteman *et al*., who reported that extensive DWI changes in a watershed distribution tended not to be associated with adverse outcome at the age of 18 months in infants with HIE. Adverse outcome was only seen in the presence of associated lesions in the basal ganglia and thalami. [29] We found no association between the WM subscore and IQ at school age, which is different from other reports in the literature. [31, 33, 36, 37] However, these studies were performed in normothermic infants and included infants with isolated severe white matter injury. In our cohort, infants with isolated white matter injury had only mild to moderate lesions,

which did not have a significant impact on their cognition. For other populations, such as normothermic infants or infants with meningitis, the score can be used to perform a complete assessment of the brain and quantify injury. However, the predictive value of the score will be different and needs to be ascertained for each population separately.
The limitations of this study are the lack of ^{1}H-MRS measurements in cohort 2, therefore the score including ^{1}H-MRS measurements still needs validation in another cohort. Furthermore, not all eligible infants had follow-up at school age, which could have led to sampling bias. Also, a significant difference in age at WPPSI/WISC assessment was seen between cohort 1 and 2, however, regression analysis (data not shown) showed no relation between age at assessment and IQ. A difference in mortality and survival with impairment was observed between cohort 1 and 2 as well. However, the proportion of infants with adverse outcome, either death or impairment, was exactly the same. In cohort 1 more infants died due to redirection of care, but this was compensated by a higher number of infants that survived with impairment in cohort 2. The OR for prediction of adverse outcome was therefore not affected and remained stable in both cohorts and when infants who died were excluded. The inter-rater agreement was good for both the GM and WM subscore but not for the total score. This was caused by a discrepancy between the raters in three cases, determined by a difference in CB score in one case and in two cases the total score was high for all three raters, but 0.5 points below the cut-off level for abnormality for two raters. Finally, this novel score is dependent on MRI quality.

CONCLUSION

We developed a novel MRI score that uses DWI, assesses all relevant brain areas and was tested in two independent, international cohorts. In infants with therapeutic hypothermia after perinatal asphyxia, the GM subscore can be used for outcome prediction using the presented cut-off values or the predicted probability graphs. We also provide additional evidence that in this population outcome is mainly determined by injury to the deep grey matter area, independent of lesions to other areas of the brain such as the white matter.

Supplemental table 1. MRI scoring form

	Items	MRI sequence used to assess injury	Severity of injury		
	Grey Matter		**0**	**1**	**2**
1	Thalamus abnormal SI or diffusion restriction	T1WI/T2WI DWI	No	Focal (<50%)	Extensive (>50%)
	Specify location			Unilateral	Bilateral
2	Basal ganglia abnormal SI or diffusion restriction	T1WI/T2WI DWI	No	Focal (<50%)	Extensive (>50%)
	Specify location			Unilateral	Bilateral
3	PLIC myelination or diffusion restriction	T1WI/T2WI DWI	Normal or no diffusion restriction	Equivocal/partially myelinated or partial (<50%) diffusion restriction	Absent myelination or extensive (>50%) diffusion restriction
	Specify location			Unilateral	Bilateral
4	Brainstem (peduncles) abnormal SI or diffusion restriction	T1WI/T2WI DWI	No	Focal (<50%)	Extensive (>50%)
	Specify location			Unilateral	Bilateral
5	Perirolandic cortex diffusion restriction	DWI	No	Mild	Clear
	Specify location			Unilateral	Bilateral
6	Hippocampus diffusion restriction	DWI	No	Yes	
	Specify location			Unilateral	Bilateral
GM subscore					
	Basal ganglia NAA	^{1}H-MRS	Normal	Reduced	
	Basal ganglia Lactate	^{1}H-MRS	Absent	Increased	
GM subscore (incl. ^{1}H-MRS)					
	White matter/cortex		**0**	**1**	**2**
1	Cortex abnormal SI or diffusion restriction not being perirolandic cortex	T1WI/T2WI DWI	No	Focal (1 lobe)	Extensive (>1 lobe)
	Specify location			Unilateral	Bilateral
2	WM increased SI or diffusion restriction not being PWML	T1WI/T2WI DWI	No	Focal (1 lobe)	Extensive (>1 lobe)
	Specify location			Unilateral	Bilateral

Supplemental table 1. (continued)

	Items	MRI sequence used to assess injury	Severity of injury		
			0	1	2
3	PWML	T1WI/T2WI, DWI, SWI	No	< 6	≥ 6
	Specify location			Unilateral	Bilateral
4	Haemorrhage not being PWML	T1WI/T2WI, SWI	No	Single haemorrhage <1.5 cm	≥1.5 cm or multiple haemorrhages
	Specify location			Unilateral	Bilateral
5	Optic radiation diffusion restriction	DWI	No	Mild	Clear
	Specify location			Unilateral	Bilateral
6	Corpus callosum diffusion restriction	DWI	No	Yes	
WM subscore					
	Cerebellum		0	1	2
1	Cerebellum abnormal SI or diffusion restriction	T1WI/T2WI DWI	No	Focal (<0.5 cm)	Extensive (≥0.5 cm or multiple lesions)
	Specify location			Unilateral	Bilateral
2	Cerebellar haemorrhage	T1WI/T2WI, SWI	No	Single haemorrhage <0.5 cm	≥0.5 cm or multiple haemorrhages
	Specify location			Unilateral	Bilateral
CB subscore					
	Additional		0	1	2
1	IVH	T1WI/T2WI, SWI	No	Yes	
2	SDH	T1WI/T2WI	No	Yes	
3	CSVT	T1WI/T2WI, MRV	No	Yes	
AD subscore					
Total score (GM + WM + CB + AD score)					

AD: additional; CB: cerebellum; CSVT: cerebral sinovenous thrombosis; DWI: diffusion-weighted images; IVH: intraventricular haemorrhage; MRV: magnetic resonance venography; NAA: *N*-acetyl aspartate; PLIC: posterior limb of the internal capsule; PWML: punctate white matter lesions; SDH: subdural haemorrhage; SI: signal intensity; SWI: susceptibility-weighted images; T1WI: T1-weighted images; T2WI: T2-weighted images; WM: white matter.

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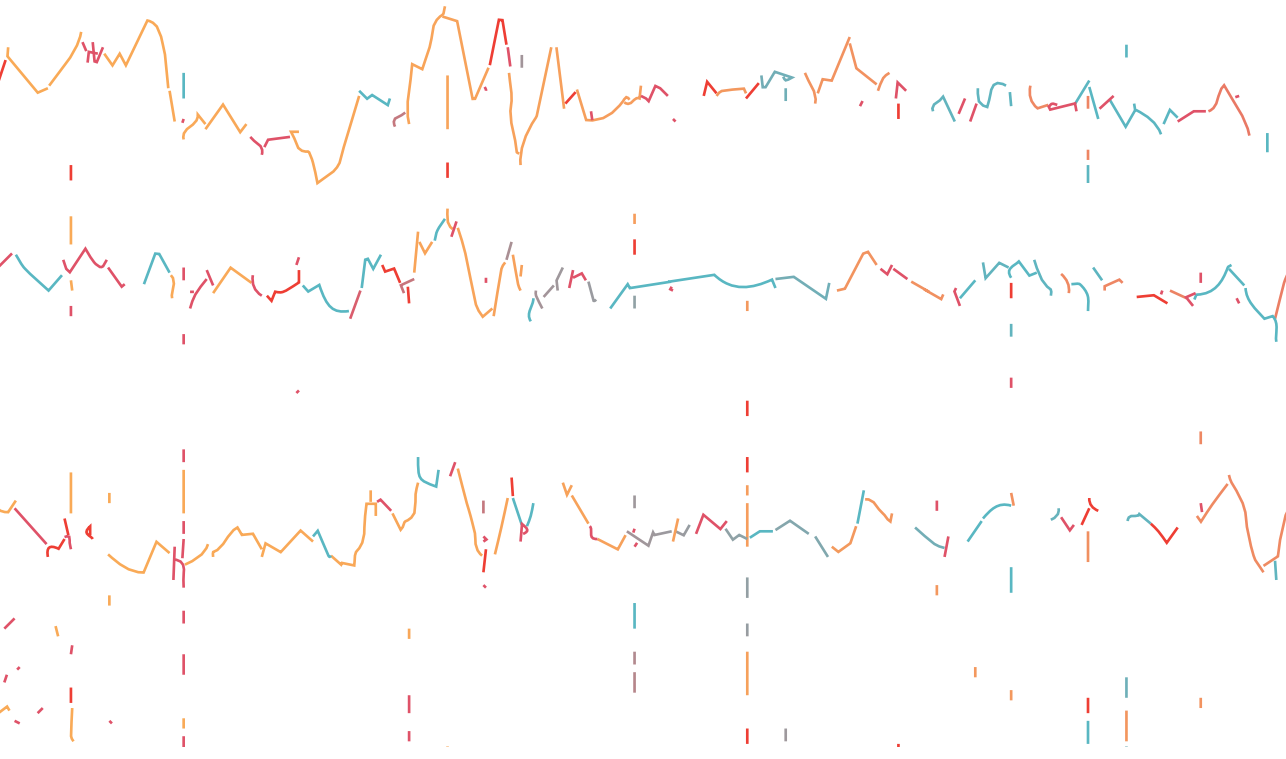
Chapter 11



Comparison of the Thompson encephalopathy score and amplitude-integrated EEG in infants with perinatal asphyxia and therapeutic hypothermia

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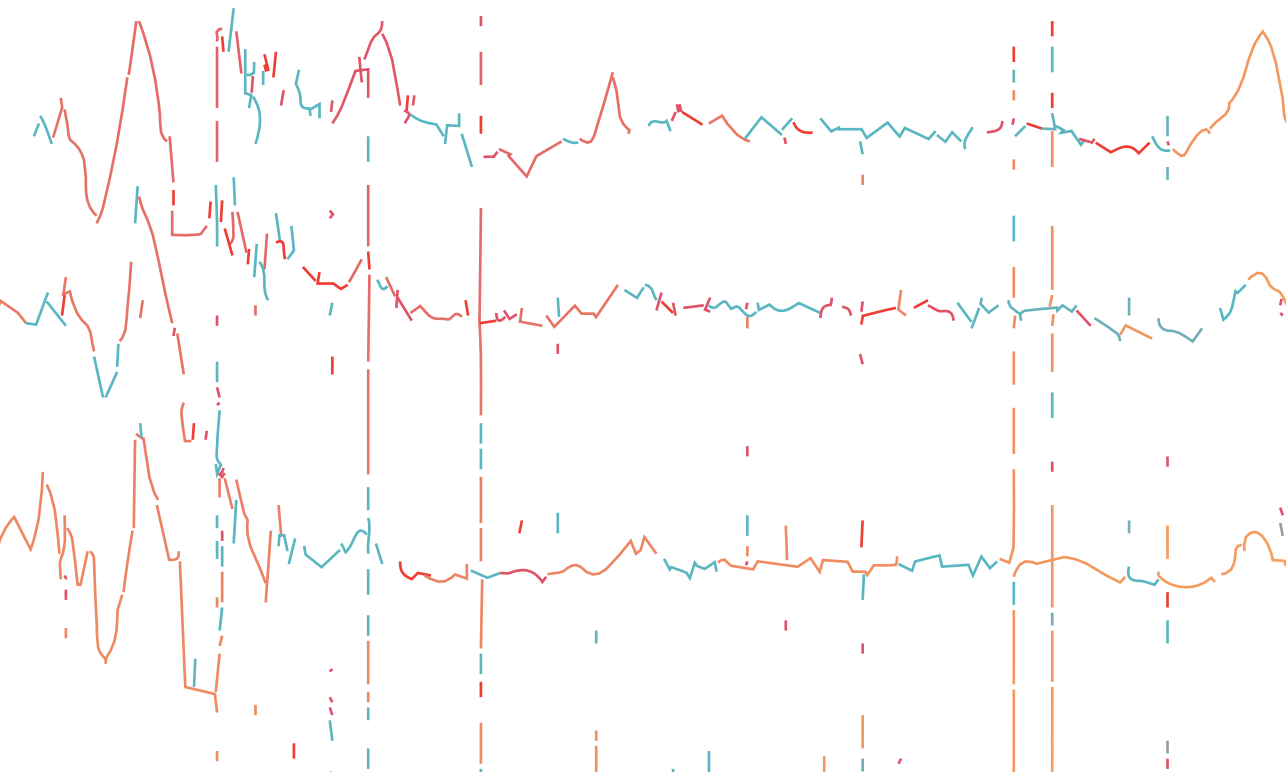

ABSTRACT

Introduction In previous studies clinical signs or amplitude-integrated electroencephalography (aEEG)-based signs of encephalopathy were used to select infants with perinatal asphyxia for treatment with hypothermia. The objective was to compare Thompson encephalopathy scores and aEEG, and relate both to outcome.

Methods Thompson scores, aEEG, and outcome were compared in 122 infants with perinatal asphyxia and therapeutic hypothermia. Of these 122 infants, 41 died and 7 had an adverse neurodevelopmental outcome. A receiver operating characteristics (ROC) analysis was also performed.

Results Thompson scores were higher in infants with more abnormal aEEG background patterns (ANOVA, $p < 0.001$). The ROC analysis demonstrated that a Thompson score of 11 or higher or an aEEG background pattern of continuous low voltage or worse was associated with an adverse outcome (AUC 0.84 for both).

Conclusion High Thompson scores and a suppressed aEEG background pattern are associated with an adverse outcome after perinatal asphyxia and therapeutic hypothermia. Further studies are needed to identify the best technique with which to select patients for therapeutic hypothermia.

INTRODUCTION

An intrapartum hypoxic-ischaemic event resulting in brain injury is a common cause of neonatal hypoxic-ischaemic encephalopathy (HIE). [1] An estimate of the incidence of HIE in developed countries is 1.5 per 1000 term live births, with a higher incidence in low-resource settings, with estimates ranging from 2.3 to 26.5 per 1000 live births. [2,3] Adverse outcomes are rare in infants with mild HIE while those with moderate to severe HIE will invariably develop major disability or die. [4,5] Besides cerebral palsy, major disability includes cognitive and developmental problems. [6,7] There is now sufficient data to conclude that hypothermia commencing by age 6 h is neuroprotective and is considered the standard of care for full-term infants who meet the trial entry criteria for therapeutic cooling. [8] This means that early identification of newborns at risk for HIE in referral centres is crucial.
The amplitude-integrated electroencephalography (aEEG) pattern obtained within 6 h of life in full-term asphyxiated infants has been shown to be useful for predicting neurodevelopmental outcome and was used in clinical trials to select infants for cooling. [9-11] Both positive (PPV) and negative predictive values (NPV) have been assessed at 3 and 6 h, [12,13] and were shown to be high in normothermic infants. Although the predictive value has changed in the era of hypothermia, the aEEG is still useful to select infants who will benefit from hypothermia. [14] Although it is easy to execute, bedside monitoring with aEEG is not available in the majority of the referral centres, even in developed countries, and may also be misleading due to artefacts. [15] Clinical assessment of HIE based on a Thompson score of more than 7 is also used as an entry criterion for cooling. [16] The Thompson score is a numeric scoring system that requires no equipment and no specific training with a high predictive value for outcome. [17] Recently, Thompson scores were analysed during or just before the start of hypothermia. [18-20] The primary aim of the present study was to compare Thompson scores and aEEG patterns in infants selected for therapeutic hypothermia, and relate these findings to outcome at 2 years of age.

METHODS

Patients

The ethics committee of the University Medical Center Utrecht waived the requirement to obtain informed consent for this retrospective study with anonymised data. From January 2008 to September 2014, 169 neonates with a gestational age of at least 36 weeks and clinical signs of perinatal asphyxia were treated with moderate hypothermia. All infants were admitted to the level III neonatal intensive care unit (NICU) of the

Wilhelmina Children´s Hospital in Utrecht, the Netherlands. Of these 169 infants, 134 without congenital malformations or syndromes, or need for extracorporeal membrane oxygenation, had aEEG and Thompson scores on admission and were available for retrospective analysis. Forty-one infants died while 81 survived and had a follow-up of at least 24 months, and these 122 were selected for the present study. Perinatal asphyxia was defined as described previously. [16] The decision to start therapeutic hypothermia was based on either a Thompson score >7 within 6 h after birth, or the presence of a discontinuous normal voltage (DNV) pattern on the aEEG with a lower amplitude limit below 5 μV, or a more suppressed aEEG background pattern. aEEG patterns were analysed as described previously, and scored as continuous normal voltage (CNV), DNV, burst suppression (BS), continuous low voltage (CLV), or flat trace (FT). [16] The data were retrieved from the medical files.

Neurodevelopmental outcome

Neurodevelopmental assessment was performed at regular intervals as described previously up to the age of 24 months. [21] Adverse outcome were considered as death, cerebral palsy, severe hearing or visual impairments, or an adverse neurodevelopment (Griffiths developmental quotient <88; Bayley Scales of Infant and Toddler Development, third edition [BSITD-III] cognitive composite score <85).

Statistical analysis

Thompson scores and aEEG patterns on admission were compared using analysis of variance (ANOVA) with a Bonferroni post hoc test. In addition, the association between aEEG patterns and Thompson scores on admission and outcome were analysed using receiver operating characteristics (ROC). The area under the curve (AUC) with 95% confidence intervals (CI) was calculated and the optimal cut-off values for predicting an adverse outcome were determined.

RESULTS

Clinical data

Clinical characteristics of the infants are given in *table 1*. Of the 122 infants, 81 (66.4%) survived and were seen at a follow-up age of at least 24 months. All deaths occurred after the redirection of care. The decision to redirect care was based on information obtained with clinical assessment, aEEG, and imaging (cranial ultrasound and/or MRI) as described previously. [22]

Thompson score versus aEEG

Twenty infants had a Thompson score of 7 or lower, of which 13 had a DNV (n = 12) or CNV (n = 1). The other seven had a BS pattern. Of the 102 infants with a Thompson score of more than 7, a BS pattern or worse was seen in 76. Box plots of the Thompson score versus aEEG patterns are presented in *figure 1*. A significant association between aEEG patterns and Thompson scores could be demonstrated (ANOVA, $p < 0.001$). The post hoc analysis using Bonferroni correction showed significant differences in Thompson scores between DNV and BS, and between BS and CLV/FT patterns. The differences in Thompson scores between CLV and FT patterns were not significant.

Table 1. Patient characteristics

	Total n = 122
Gestational age (weeks), median (IQR)	40.0 (2.2)
Birth weight (grams), median (IQR)	3500 (858)
Male/female	69/53
Apgar 1 min, median (IQR)	1 (2)
Apgar 5 min, median (IQR)	3 (4)
pH, median (IQR)	6.90 (0.25)
Lactate, median (IQR)	15.5 (8.0)
Sarnat grade on admission, n (%)	
Grade 1	16 (13.1)
Grade 2	70 (57.4)
Grade 3	36 (29.5)
Thompson >7, n (%)	102 (83.6)
aEEG BS or more severely depressed, n (%)	83 (68)
Died, n (%)	41 (33.6)
Follow-up 24 months or more	81 (66.4)

aEEG: amplitude-integrated EEG; BS: burst suppression; IQR: interquartile range.

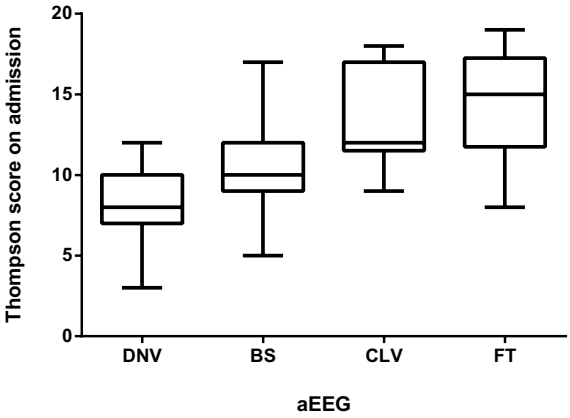

Figure 1. Thompson score on admission plotted against aEEG on admission. One infant with a CNV pattern was added to the DNV data.

Table 2. Thompson scores and aEEG versus outcome

A. Analysis of data obtained on admission based on previously described cut-off values				
	Sensitivity	**Specificity**	**PPV**	**NPV**
Thompson ≤7 vs. >7	0.96	0.24	0.45	0.90
aEEG DNV vs. BS, CLV, FT	0.94	0.49	0.54	0.92
B. Thompson scores and aEEG results vs. outcome				
Outcome	**Normal (n = 74)**	**CP, delayed development (n = 7)**	**Died (n = 41)**	
Thompson score, median (IQR)	9 (3)	10.5 (4)	15 (6)	
Thompson >7, n (%)	56 (75.7)	7 (100)	39 (95.1)	
Thompson ≤7, n (%)	18 (24.3)	0	2 (4.9)	
aEEG, n (%)				
DNV	36 (48.6)*	0	3 (7.3)	
BS	31 (41.9)	6 (85.7)	11 (26.8)	
CLV	2 (2.7)	0	7 (17.1)	
FT	5 (6.8)	1 (14.3)	20 (48.8)	

*Including one patient with a continuous normal voltage pattern. aEEG: amplitude-integrated EEG; BS: burst suppression; CLV: continuous low voltage; CP: cerebral palsy; DNV: discontinuous normal voltage; FT: flat trace; IQR: interquartile range; NPV: negative predictive value; PPV: positive predictive value.

Thompson score and aEEG versus outcome

The predictive values in *table 2A* are presented based on previously published cut-off values. [12, 17] The specificity and PPV of both cut-off values are low. Median Thompson scores were lowest in infants with a normal outcome and highest in infants who died *(table 2B)*. aEEG scores were most abnormal in infants who died *(table 2B)*. Of the two infants with a Thompson score of 7 or lower and an adverse outcome, one had a score of 7, and one a score of 4 within 2 h after birth. Both showed a rapid deterioration over the following hours. All three infants with a DNV pattern and an adverse outcome showed a rapid deterioration of the aEEG background pattern after admission.

ROC analysis of Thompson score and aEEG

Thompson scores and aEEG both showed an AUC of more than 0.80. The difference between them was not significant *(table 3)*.

Table 3. ROC analysis of Thompson score and aEEG versus outcome

	Cut-off value	AUC (95% CI)	Sensitivity	Specificity
Thompson score	11 or worse	0.84 (0.75-0.91)	0.76	0.83
aEEG	CLV or worse	0.84 (0.76-0.91)	0.60	0.93

aEEG: amplitude-integrated EEG; AUC: area under the curve; CI: confidence interval; CLV: continuous low voltage.

DISCUSSION

The present study compared Thompson scores and aEEG before initiating therapeutic hypothermia in infants with perinatal asphyxia who qualified for treatment with moderate hypothermia. Our data demonstrate a strong association between aEEG background pattern and Thompson score. Although the Thompson score contains many items on behaviour, (a)EEG is not part of the score. Like in the Apgar score, the predictive value of the items has not been tested separately. [23] Therefore, we cannot say which items of the Thompson score were most valuable in relation to outcome.

The Thompson score and aEEG background pattern have a similar predictive value for an adverse outcome. However, the aEEG may have several advantages over the Thompson score. First, the aEEG is a continuous measurement, and can identify (subclinical) seizures. Second, the aEEG can be sent out for a second opinion or expert revision. This is more difficult for the Thompson score, which needs to be captured on video for revision.

In our study, the Thompson score cut-off value to qualify for hypothermia was based on the original paper by Thompson *et al*. [17] Infants with a score of more than 7 on the first day of life had a higher risk of an adverse outcome. Horn *et al.* have analysed different cut-off levels of the Thompson score in predicting an abnormal 6-h aEEG. [19] At a level of ≥7 the sensitivity was 100% and specificity 66.7%, and at a level of ≥8 the sensitivity was 94.4% and specificity 75.0%. However, the Thompson score was measured when treatment with hypothermia had already commenced. Very high Thompson scores (≥16) at age 3-5 h during hypothermia were followed by a severely abnormal aEEG or death at 48 h. [18]

We confirmed that clinical assessment using the Thompson score before starting hypothermia is associated with outcome, since the highest scores were encountered in the infants who died. ROC analysis showed that a score of 11 or more was the cut-off level for an adverse outcome. A recent study investigating infants of the Dutch PharmaCool trial showed a comparable cut-off level. A Thompson score of 12 or more was associated with death before discharge. [20] aEEG background patterns were not analysed in that study.

In the past we and others have demonstrated that suppressed aEEG background patterns within the first 24 h were followed by an adverse outcome. [12, 13] When therapeutic hypothermia is used, the predictive value of the aEEG is delayed until 24-36 h. [14] Furthermore, while a BS pattern during normothermia was associated with an adverse outcome, in the period of hypothermia CLV or FT patterns are most likely to be followed by an adverse outcome. Interestingly, the Thompson score and aEEG have a comparable area under the ROC curve of approximately 0.83.

Both the Thompson score and aEEG obtained before starting hypothermia treatment had a lower specificity and PPV compared to previous studies in the period before hypothermia was used. Analysis with aEEG was slightly better than with the Thompson score. The lower specificity and PPV are attributed to the neuroprotective effects of hypothermia.
The present study does not answer the question of which of the two assessment systems, Thompson score or aEEG, is better in selecting neonates for treatment with hypothermia. It is unknown how many patients with either perinatal asphyxia and a low Thompson score or non-suppressed aEEG did not receive hypothermia, but nevertheless subsequently developed neonatal encephalopathy with seizures. In our cohort we observed that the Thompson score was ≤7 in 20 cases. If only the Thompson score had been used, these infants would not have been treated with hypothermia. Because of the aEEG findings, these cases were considered candidates for hypothermia. The Thompson score did not identify patients that were missed by aEEG in this study. This is in contrast with the findings of Sarkar *et al.*, but in agreement with our previous experience and that of others. [12, 13, 24]
A trial randomising infants with asphyxia to the Thompson score or the use of aEEG might answer the question of which technique is superior in selecting infants with perinatal asphyxia for treatment with hypothermia. Until then, both techniques may be used to select infants with perinatal asphyxia for therapeutic hypothermia. With the cut-off levels obtained through ROC analysis presented in this study, the Thompson score and aEEG may be used to select infants who might benefit from additional neuroprotective strategies in addition to hypothermia.

CONCLUSION

Both high Thompson scores and a suppressed aEEG background pattern are associated with an adverse outcome after perinatal asphyxia and therapeutic hypothermia. Further studies are needed to identify the best technique with which to select patients for therapeutic hypothermia. The novel cut-off levels presented in the present study may be used to select patients for additional neuroprotective strategies.

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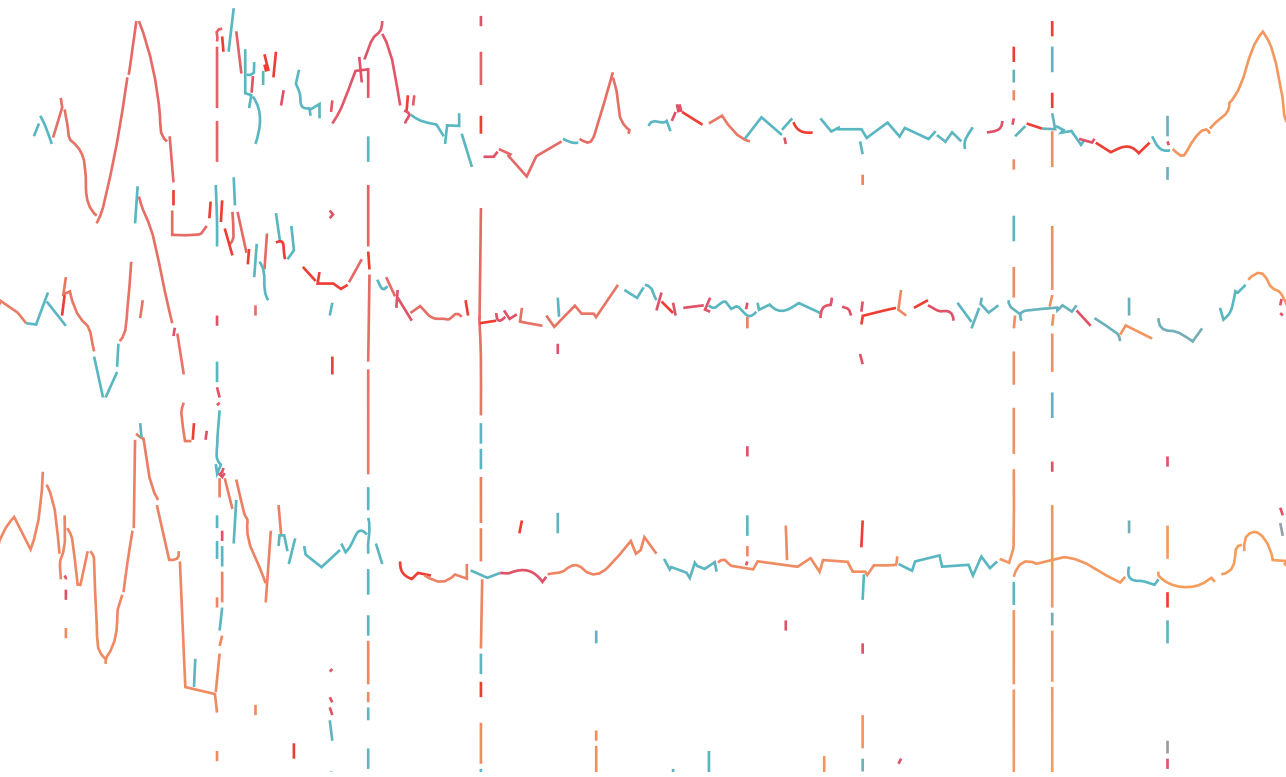
Chapter 12

Summarising discussion and future directions



The general aim of this thesis was to provide new insights to support evidence based guidelines for the diagnosis, treatment and prognosis of neonatal seizures. The second aim was to investigate and describe additional uses of EEG and amplitude-integrated EEG (aEEG) in the NICU. In Part 1 the use of EEG and neuro-imaging in the diagnostic process of neonatal seizures, as well as the additional uses of EEG were discussed. In Part 2 the safety and efficacy of seizure treatment with lidocaine were discussed. In Part 3 predictors of outcome in infants with hypoxic-ischaemic encephalopathy (HIE), the most common cause of seizures in full-term infants, were investigated.

PART 1 - DIAGNOSIS

Part 1.1 - Electroencephalography

EEG seizure morphology in extremely preterm infants - Chapter 2

What is known: EEG characteristics of ictal discharges in full-term and preterm infants have been described by a number of studies.[1-5] However, data on EEG seizure morphology in extremely preterm infants (born <28 weeks gestational age) is scarce. A variety of rhythmic patterns can be detected in the EEG of extremely preterm infants. However, not all patterns comply with the definition of subclinical seizure patterns.[6] They are rhythmic and last for >10 s, but they do not show evolution in amplitude and frequency. The incidence of seizures in preterm infants has been estimated at 4-48%.[7-10] This is higher than reported in full-term infants,[9] while the response rate to antiepileptic drugs (AEDs) seems to be significantly lower in preterm infants. As we have shown in *Chapter 8* of this thesis.

What this thesis adds: In *Chapter 2* we investigated 77 infants born <28 weeks gestational age, who were monitored prospectively with a two-channel EEG during the first 72 h after birth. Five different rhythmic EEG patterns could be recognised: ictal discharges, periodic epileptiform discharges (PEDs) and three other waveforms. Rhythmic EEG patterns were common (62.3%), but ictal discharges were only observed in 1.3%. The ictal discharges showed a clear increase in amplitude, decrease in frequency (0.7-2 Hz) and a change in morphology (sharp waves, rhythmic delta waves, spike-wave complexes). A clear change in the aEEG trace was seen as well. The presence of the three waveforms other than ictal discharges and PEDs was significantly associated with the infant's head position and are therefore more likely to be artefacts. PEDs were common (44%) and unrelated to head position, but they were also not associated with brain injury or poor cognitive outcome.

Implications and future research: The results from *Chapter 2* show that ictal discharges during the first 72 h of life are rare in extremely preterm infants, but not necessarily different in EEG morphology compared to full-term infants. Although the full spectrum of EEG seizure morphology in extremely preterm infants still needs to be determined, since EEG characteristics of ictal discharges in this age group have only been described in about 20 cases. [2, 4, 5] The incidence of EEG seizures was much lower in our study than previously reported. [7, 8, 10] A possible explanation for this difference could be that PEDs were considered seizures in those reports, since the incidence of PEDs in our cohort (44%) is strikingly similar to their incidence of seizures. However, PEDs were not related to brain injury in our cohort and outcome was normal independent of a high PED burden. This is contradictive to results from adult, paediatric and even full-term infant studies, where PEDs have been associated with brain lesions, epilepsy and poor outcome. [11-16] Our results suggest that PEDs are a benign phenomenon in extremely preterm infants and it could be postulated that PEDs are a form of spontaneous, hypersychronised activity that is related to normal brain development, but should not persist into infancy and childhood. To further explore the role of PEDs in preterm brain development, future research should focus on studying preterm infants at different gestational ages to assess the developmental changes in the presence of PEDs and compare it to other markers of brain development such as brain volumes, cortical development and functional and structural connectivity.

Depression of the aEEG background pattern: a sign of hypercapnia - Chapter 3

What is known: Acutely elevated carbon dioxide (CO_2) blood levels have a depressant effect on the central nervous system and can lead to coma in adults. [17-19] CO_2 narcosis has been described in adults with a $PaCO_2$ of 62-269 mm Hg, but the cerebral response to hypercapnia showed a broad inter-individual variability. [18] In preterm infants, the depressant effect of CO_2 on EEG has been described as well. Mild hypercapnia resulted in longer interburst intervals and decreased total EEG power. [20, 21] However, the phenomenon of CO_2 narcosis as seen in adults has not been described in infants.

What this thesis adds: In *Chapter 3* we show that profound depression of brain activity during acute severe hypercapnia as has been described in adults also occurs in infants. It can be detected by visual inspection of the aEEG trace, showing an acute depression of the background activity, and by quantitative analysis of the EEG, showing a decrease in cortical activity and an increase in cortical inactivity. No significant or clinically detectable changes in cerebral oxygenation and haemodynamic parameters such as heart rate, blood pressure and arterial oxygen saturation were seen during hypercapnia.

Implications and future research: The results from *Chapter 3* should alert clinicians to be aware that CO_2 mediated suppression of brain activity occurs in preterm and full-term infants and results in depression of the aEEG trace. Since EEG and aEEG are now widely used in the NICU and hypercapnia may not result in significant or clinically detectable changes in other continuously monitored parameters, such cerebral oxygenation, blood pressure and arterial oxygen saturation, a sudden aEEG depression should alert clinicians to perform a blood gas and check $PaCO_2$. Ideally, severe hypercapnia should be noted prior to aEEG depression by careful monitoring of CO_2 and ventilator parameters. Studies have suggested that preterm infants have the highest risk of EEG depression due to acute hypercapnia during the first two days after birth. [20, 21] In our cohort, however, we observed EEG depression due to acute hypercapnia at any gestational or chronological age, and even in full-term infants. As seen in adults, [18] a broad inter-individual variability in response to hypercapnia was seen in infants. Some infants showed severe visible aEEG depression and changes in quantitative EEG parameters, while others showed no visible depression to similar levels of hypercapnia and no or an inverse change in quantitative EEG parameters. Further studies using continuous CO_2 measurements are needed to investigate the precise relationship between CO_2 and brain activity and to determine at which $PaCO_2$ levels infants are at risk of severe depression of brain activity and whether this is related to poor outcome.

Part 1.2 - Neuro-imaging

Neuro-imaging in neonatal seizures - Chapters 4, 5 and 6

What is known: Neonatal seizures often reflect severe underlying brain injury. [22] Prognosis is primarily determined by the nature, site and extent of the underlying aetiology, [23-25] making accurate diagnosis and identification of associated brain lesions essential. Not surprisingly, neuro-imaging has been shown to be one of the most important factors for outcome prediction in infants with neonatal seizures. [26] Over the years, many studies have reported on brain imaging and neonatal seizures, but most studies focused on infants with a specific underlying problem and only a few studies reported on neuro-imaging findings in infants with neonatal seizures in general. [23, 25, 27, 28]

What this thesis adds: In *Chapter 4* we provide an overview of the spectrum of neuro-imaging findings in full-term infants with neonatal seizures in the light of the underlying aetiologies by review of the literature. In *Chapter 5* we investigated the underlying aetiology of seizures and the corresponding cranial ultrasound and MRI findings in a

cohort of 378 full-term or near-term infants with aEEG-confirmed neonatal seizures. In 93.7% an underlying aetiology was identified. In agreement with previous studies, HIE, intracranial haemorrhage and perinatal arterial ischaemic stroke were the most common aetiologies. [23, 24, 27, 29, 30] The diagnostic contribution of MRI compared to cranial ultrasound alone was assessed as well. In almost half of our study population (39.8%), MRI showed additional information compared to ultrasound, important for more focused diagnostic testing (e.g. metabolic disorders), more accurate prognosis (e.g. involvement of the corticospinal tracts in perinatal arterial ischaemic stroke, involvement of the basal ganglia in HIE) and genetic counselling (e.g. polymicrogyria and specific proton MR spectroscopy findings in Zellweger syndrome). In 11.9% of our population the diagnosis or important brain lesions would have been missed when only cranial ultrasound rather than a combination of ultrasound and MRI would have been used. In certain diagnoses, MRI is even more important: e.g. in CSVT, since early treatment is an option and MRI is required for confirmation of CSVT, [31] while ultrasound was able to make a correct diagnosis or reveal all brain lesions in 37.9%.

Implications and future research: The findings from *Chapter 5* should urge clinicians faced with neonatal seizures, to perform an MRI, preceded by cranial ultrasound as part of the diagnostic process. Cranial ultrasound is readily available, causes minimal disturbance of the infant and provides reliable information on, for example, the presence of intracranial haemorrhages, while MRI provides additional information on white matter abnormalities, myelination, the deep brain structures and migrational disorders. [22, 32, 33] Requirements are, however, that specific neonatal MRI sequences with thin slices are used, and that the MRI is reported by someone with experience. Diffusion-weighted images should always be included in the scanning protocol and an option to add MR angiography, venography, susceptibility-weighted images and proton MR spectroscopy to the protocol is preferred.

The number of infants with an unknown aetiology of seizures is becoming smaller with the increased use of MRI, but also because of advances in genetics. [34-37] With the expanding spectrum of newly found genetic causes of seizures, future research should focus on expanding the findings presented in *Chapter 5* and investigate and describe the neuro-imaging findings in those new diagnoses. *Chapter 6* of this thesis is a first step in doing so by describing the neuro-imaging findings in a distinct neonatal early-onset encephalopathy caused by autosomal recessive mutations in the *SLC13A5* gene. This specific epileptic encephalopathy is characterised by seizure onset in the first days after birth in otherwise healthy newborns after an uncomplicated pregnancy and delivery, refractory epilepsy with frequent status epilepticus, fever sensitivity, developmental

delay and teeth hypoplasia or hypodontia. [36, 37] However, little detailed information about the brain MRI features of these patients was available in previous studies. In *Chapter 6* we described the neuro-imaging findings in eight patients from five families with neonatal epilepsy and mutations in the *SLC13A5* gene. Seven out of eight patients included in our study had a characteristic MRI pattern, with punctate white matter lesions on their neonatal MRI scan, which were no longer visible at the age of 6 months, but led to gliotic scarring visible on MRI at the age of 18 months. Therefore, in otherwise healthy infants presenting with therapy resistant seizures in the first days after birth, without a clear history of HIE but with punctate white matter lesions on their neonatal MRI, a diagnosis of mutations in the *SCL13A5* gene should be considered.

PART 2 - TREATMENT

Safety of lidocaine for treatment of neonatal seizures - Chapter 7

What is known: Animal studies suggest that seizures may exacerbate brain injury in the developing brain. [38, 39] In humans, seizures have been related to more severe brain injury and poor outcome. [40, 41, 41-43] Rapid, protocol-driven therapy can reduce seizure burden. [44] However, data from randomised controlled trials to support the optimal treatment protocol are limited and no recommendations can be made. [45-47] Lidocaine promises to be an effective drug for seizures persisting despite first- and second-line therapy, [48-55] but is not widely used. [56] Presumably, due to the risk of cardiac events. Cardiac events have been reported in studies with neonates receiving lidocaine for seizures. [49-53, 55, 57, 58] The reported incidence varied from 0-4.8% and data were based on small, old studies, while new reduced dose regimens have been developed since.

What this thesis adds: In *Chapter 7* we show that lidocaine-associated cardiac events were rare in a cohort of 368 full-term and 153 preterm infants who received lidocaine for seizures. In only 1.3-1.9% observed cardiac events could be explained by the use of lidocaine, because (1) they occurred between 2 h after the start of lidocaine infusion and max. 12 h after cessation of infusion when the plasma level was expected to be >5 mg/l, (2) the symptoms matched expected symptoms based on pharmacodynamics [59-63] and (3) there were no contributing factors that could explain the cardiac events. After the introduction of new reduced dose regimens, the incidence decreased to 0.4%. The most common cardiac event associated with lidocaine was bradycardia. Important contributing risk factors for cardiac events were: unstable potassium, (congenital) cardiac dysfunction and concurrent phenytoin use.

Implications and future research: The findings from *Chapter 7* should reassure clinicians that lidocaine is safe to use as an antiepileptic drug in full-term and preterm infants with seizures, with regard to cardiac side effects. When customised dosing regimens are used the incidence is even lower than the reported background incidence of arrhythmias in the neonatal population (1-5%). [64, 65] Attention should be paid to electrolyte balance, administration to infants with congenital cardiac dysfunction should be limited and concurrent or subsequent phenytoin use avoided, since these are important contributing risk factors. Furthermore, when cardiac events occur during lidocaine administration the plausibility of a causal relation should be considered carefully. In previous studies all cardiac events during lidocaine administration were ascribed to lidocaine, without considering whether the lidocaine plasma concentration was sufficient to cause symptoms, whether symptoms could be explained by the pharmacodynamics of lidocaine or whether other possible causes or contributing factors were present. [49-53, 55, 57] Ideally, our results should be confirmed by prospective studies, with continuous blood pressure and ECG measurements.

Efficacy of lidocaine for treatment of neonatal seizures - Chapter 8

What is known: Lidocaine appears to be an effective drug for second- and third-line treatment of neonatal seizures, with seizure response rates ranging from 60-92%. [48-55] However, the numbers in these studies were small and the lidocaine treatment regimens differed.

What this thesis adds: In *Chapter 8* we investigated the response rate of seizures to lidocaine and compared it to midazolam in a cohort of 319 full-term and 94 preterm infants. We showed that lidocaine can reach a response rate of about 70%, but gestational age, the underlying aetiology and timing of administration influence the seizure response to treatment. Lidocaine had a significantly lower response rate in preterm infants, while infants with perinatal arterial ischaemic stroke or cerebral sinovenous thrombosis were more likely to have a good response to lidocaine than infants with another aetiology of seizures. The response rate to lidocaine was significantly higher compared to midazolam as second-line drug and slightly higher as third-line drug. Both lidocaine and midazolam had a higher response rate as third-line treatment, which could be due to a pharmacological synergistic mechanism between the two drugs.

Implications and future research: The findings in *Chapter 8* show that there is an urgent need to continue investigating treatments for seizures in neonates. The response rates to both lidocaine and midazolam should ideally be confirmed in a prospective multicentre

randomised controlled study. The possible synergistic or additive effect of antiepileptic drugs (AEDs) as well as the effect of timing of AED administration clearly needs further investigation. Also, the pathophysiological mechanisms of seizures in preterm infants need further investigation in order to develop better treatment strategies for these infants. Based on our results, a combination of lidocaine and midazolam may be a good treatment option for neonatal seizures refractory to phenobarbital. We would advise to start with lidocaine as a second-line AED, as lidocaine does not affect the EEG background activity as much as midazolam [66] and in the spontaneously breathing infant lidocaine is less likely to have an effect on respiratory drive. Furthermore, lidocaine-associated cardiac events, the main concern of lidocaine treatment, proved to be rare as we showed in *Chapter 7*. Midazolam should be given promptly if seizures persist.

PART 3 - PROGNOSIS

Predictors of outcome in infants with HIE - Chapter 9, 10 and 11

What is known: HIE is the most common cause of seizures in full-term infants and associated with abnormal neurological outcome. [67-71] Several predictors of outcome have been investigated in this population, such as neurological examination, EEG background activity, seizure burden and MRI. [41, 72-82] Attempts have been made to determine the best predictor of outcome, but this proved to be difficult since most predictors were investigated separately in studies with a limited number of infants.[83, 84] MRI and (a)EEG background activity were reported to be the most optimal predictors of outcome, but it was suggested that prediction of outcome could be improved substantially by evaluating multiple factors. [83, 84] Furthermore, most studies were performed in the pre-cooling era. However, therapeutic hypothermia is now well established for neuroprotection in infants with HIE and has been shown to influence the predictive properties of neurological examination and EEG in particular. [85-87]

What this thesis adds: In *Chapter 9* we used continuous video-EEG to investigate the role of EEG background activity, electroencephalographic seizure burden and MRI in predicting neurodevelopmental outcome in the same cohort of full-term infants with HIE in the era of therapeutic hypothermia. MRI was the best predictor of outcome, closely followed by the EEG background activity, but only at 36 h and 48 h after birth. The EEG was not predictive at earlier time points. Assessment of electroencephalographic seizure burden did not have additional predictive value since it was not as accurate in predicting outcome as MRI and EEG background activity and was only associated with abnormal

outcome in the presence of moderate-severe injury on MRI.

Since we confirmed that MRI is the best predictor of outcome in infants with HIE and it is often used as a bridging biomarker for outcome in clinical trials, [44, 88] quantifying the extent of injury for objective and accurate prognostication is important. In *Chapter 10* we present a novel, easily applicable, comprehensive MRI score that showed good predictive value in two independent, international cohorts, comprising a total of 173 infants treated with therapeutic hypothermia. In both cohorts, injury to the deep grey matter area was an independent predictor of adverse outcome at 2 years and school age. For the deep grey matter subscore, cut-off values and a predicted probability graph for the risk of adverse outcome, with an area under the curve, positive and negative predictive value >0.83, are provided.

In *Chapter 11* we compared the relation of the Thompson score, a standardised score for neurological examination of infants with HIE, and the aEEG background pattern with outcome at 2 years in 122 infants with HIE who were treated with therapeutic hypothermia. The Thompson score and aEEG background pattern were assessed before hypothermia was started. Higher Thompson scores were associated with worse aEEG background patterns, but for both the specificity and positive predictive value were lower than reported in the pre-cooling era. [73, 75] An effect which can be attributed to the neuroprotective effect of hypothermia. The predictive value of aEEG was slightly better compared with the Thompson score. A Thompson score of 11 or more and a continuous low voltage or flat trace aEEG pattern were associated with poor outcome despite therapeutic hypothermia.

Implications and future research: Chapter 9 provides a comprehensive overview of the role of EEG, MRI and seizure burden in predicting outcome in infants with HIE in the era of therapeutic hypothermia. We provide additional evidence that under hypothermia the EEG background activity is a reliable predictor of outcome only after 36 h of age and show that the combined use of MRI and EEG is important in infants following HIE. Since MRI has been shown to be the best predictor of outcome, while continuous EEG monitoring helps to evaluate the severity of encephalopathy, detect and adequately treat electroencephalographic seizures. To minimise the seizure burden and help with decisions on redirection of care, for example when an infant is too unstable to be transported to the MRI. We confirm that a high seizure burden is not invariably associated with a poor outcome, but this is dependent on the extent of MRI abnormalities. [40] The study presented in *Chapter 9* should be repeated in a large, multicentre cohort to confirm the

findings and to build a prediction model based on all important predictors of outcome in infants with HIE.

Chapter 10 provides a new comprehensive MRI scoring system to assess the extent of brain injury in infants following HIE. It can be used for reliable outcome prediction using the presented cut-off values or the predicted probability graph. We also provide additional evidence that outcome is mainly determined by injury to the deep grey matter, independent of lesions to other areas of the brain such as the white matter. We found no association between white matter injury and IQ at school age, which is different from other reports in the literature. [77, 81, 89-91] However, these studies were performed in normothermic infants and included infants with isolated severe white matter injury. In our cohort, infants with isolated white matter injury only had mild to moderate lesions, which did not have a significant impact on their cognition. The predictive value of the score is expected to be different in other populations such as normothermic infants or infants with meningitis. Therefore, future studies are needed to determine the predictive value of the score in each population separately. Also, the quality of the score in the second week of life still needs to be assessed, since the majority of scans in our cohort were performed in the first week after birth. During the first week after birth it is best to perform the score based on the T1- and T2-weighted images combined with diffusion-weighted images (DWI), since DWI has been shown to be the most reliable sequence to assess injury in HIE in the first week after birth. [92] However, in the second week after birth DWI abnormalities may no longer be visible due to pseudonormalisation. [93] The score should then be performed using T1- and T2-weighted images only, with the addition of proton MR spectroscopy measurements, since these remain abnormal for a prolonged period of time. [94]

The results from *Chapter 11* do not answer the question whether the Thompson score or the aEEG background pattern is better at selecting infants for treatment with hypothermia. A trial randomising infants with asphyxia to the use of the Thompson score or the aEEG is needed to answer this question. Until then, a combination of both techniques is recommended to select infants with perinatal asphyxia for therapeutic hypothermia. Although, it should be noted that the predictive value of aEEG was slightly better in our cohort and aEEG has the advantage of being a continuous measurement that can identify (subclinical) seizures in addition. The cut-off levels for the Thompson score and aEEG background pattern presented in *Chapter 11* could be used, however, to select patients who might benefit from additional neuroprotective strategies on top of hypothermia. Since these infants had a poor outcome despite therapeutic hypothermia.

FUTURE DIRECTIONS

Diagnosis - Electroencephalography

The increased use of EEG monitoring in NICUs creates new possibilities; it can aid in early recognition of brain injury or physiological changes such as hypercapnia as we showed in this thesis *(Chapter 3)*. However, it also poses new challenges when monitoring new populations such as extremely preterm infants. We have shown in this thesis that knowledge of EEG patterns in older infants and children cannot always be extrapolated to preterm infants *(Chapter 2)*. The full spectrum of EEG seizure morphology in extremely preterm infants still needs to be determined, since EEG characteristics of ictal discharges in this age group have only been described in a few cases. [2,4,5] The role of PEDs in preterm brain development needs to be explored further as well. Future research should focus on studying preterm infants at different gestational ages to assess the developmental changes in the presence of PEDs and compare it to other markers of brain development such as brain volumes, cortical development and functional and structural connectivity.

Regarding the effect of CO_2 on newborn brain activity, further studies using continuous CO_2 measurements are needed to investigate the precise relationship between CO_2 and brain activity and to determine at which $PaCO_2$ levels infants are at risk of severe depression of brain activity and whether this is related to poor outcome.

Diagnosis - Neuro-imaging

This thesis provides a comprehensive overview of neuro-imaging findings in neonatal seizures by review of the literature *(Chapter 4)* and investigation of a large cohort of infants with neonatal seizures *(Chapter 5)*. However, the spectrum of newly found causes of neonatal seizures is expanding, especially with advances in genetics. Therefore, future research should focus on expanding the findings presented in *Chapter 5* and investigate and describe the neuro-imaging findings in those new diagnoses.

Treatment

There is an urgent need to continue investigating treatments for seizures in neonates, since the efficacy and safety of available AEDs is not optimal. [45] We showed in a retrospective manner that lidocaine is safe to use in infants with respect to cardiac side effects *(Chapter 7)*. Ideally, our results should be confirmed by prospective studies, with continuous blood pressure and ECG measurements. We also showed that lidocaine could reach a response rate of about 70% *(Chapter 8)*, but this was dependent on gestational age, the underlying diagnosis and the timing of administration. Both lidocaine and

midazolam had a significantly higher response rate as third-line AED. This could be due to a pharmacological synergistic mechanism between lidocaine and midazolam or an additive effect of AEDs. The possible synergistic or additive effect of AEDs as well as the effect of timing of AED administration clearly needs further investigation. Also, the pathophysiological mechanisms of seizures in preterm infants need further investigation in order to develop better treatment strategies for these infants.

Prognosis

Predicting the future is an important part of neonatal care, but also the most challenging. The perfect tool for predicting outcome does not exist and studies in infants with HIE have shown that outcome prediction should be based on multiple factors. [83, 84] To develop a prediction model for infants with HIE, the study presented in *Chapter 9* should be repeated in a large, multicentre cohort and include neurological assessment, EEG and MRI as variables. The MRI score we presented in *Chapter 10* could be used as part of the prediction model. However, the score will first need to be optimised further by determining the predictive value in other populations and assessing the quality of the score when MRI is performed in the second instead of the first week of life. In addition, to answer the question of which method, the Thompson score or the aEEG, is best for selecting infants with HIE for treatment with hypothermia, a trial randomising infants with perinatal asphyxia to the use of the Thompson score or the aEEG is needed.

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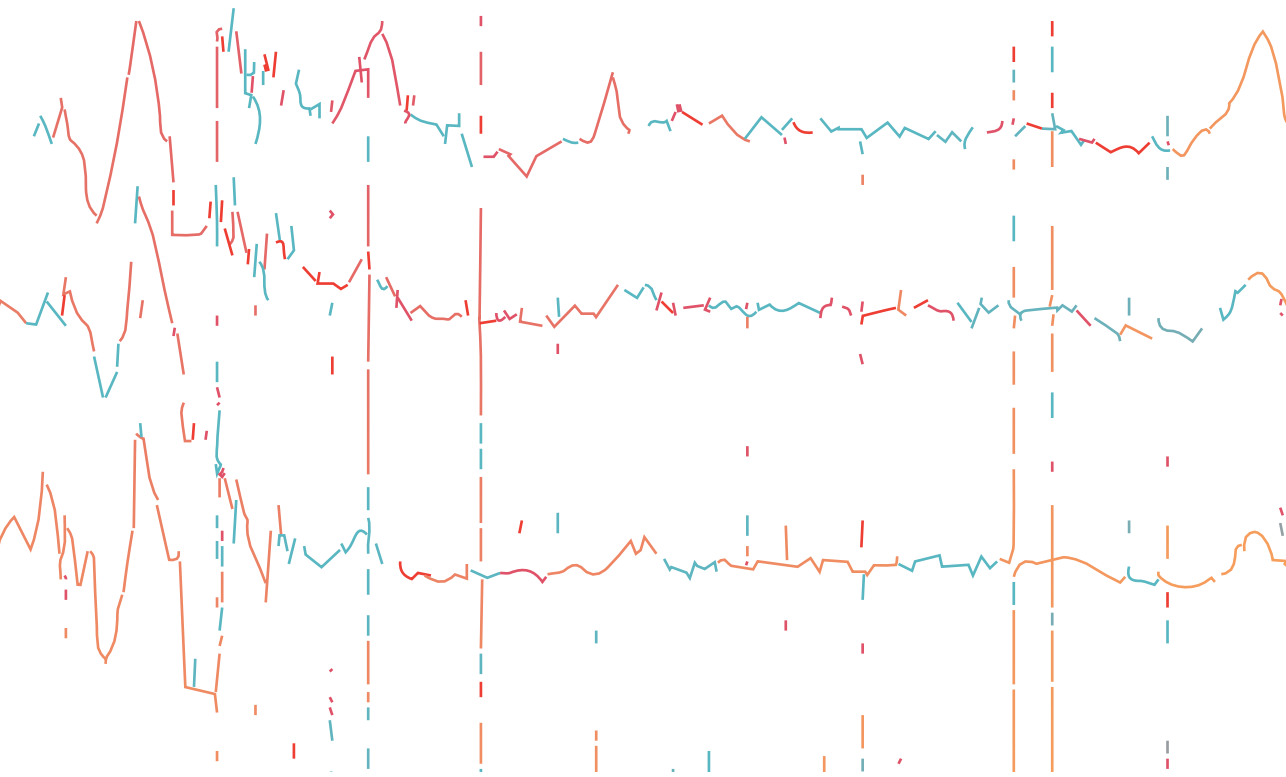
12

Chapter 13

Nederlandse samenvatting (summary in Dutch)



Het algemene doel van dit proefschrift was om nieuwe inzichten te creëren ter bevordering van evidence-based richtlijnen voor de diagnose, behandeling en prognose van neonatale epileptische aanvallen. Het tweede doel was om additionele toepassingen van elektro-encefalografie (EEG) en amplitude-geïntegreerd EEG op de neonatale intensive care unit (NICU) te onderzoeken. In *deel 1* bespraken we het gebruik van EEG en beeldvorming van de hersenen in het diagnostische proces van neonatale epileptische aanvallen, evenals de additionele toepassingen van EEG. In *deel 2* bespraken we de veiligheid en effectiviteit van lidocaïne ter bestrijding van neonatale epileptische aanvallen. In *deel 3* onderzochten we verschillende factoren die voorspellen hoe kinderen met hypoxisch-ischemische encefalopathie (HIE), de meest voorkomende oorzaak van epileptische aanvallen bij voldragen pasgeborenen, op latere leeftijd functioneren.

DEEL 1 - DIAGNOSE

Deel 1.1 - Elektro-encefalografie

De morfologie van epileptische aanvallen op EEG bij extreem vroeggeboren kinderen - Hoofdstuk 2

Wat is bekend: De EEG-kenmerken van epileptische ontladingen bij voldragen en premature neonaten zijn beschreven in een aantal studies. Gegevens over de morfologie van epileptische aanvallen op het EEG van extreem vroeggeboren kinderen (geboren na een zwangerschapsduur korter dan 28 weken) zijn echter schaars. Op het EEG van deze kinderen kunnen veel verschillende ritmische patronen worden gevonden, maar niet alle patronen voldoen aan de definitie van subklinische epilepsiepatronen. Deze patronen zijn ritmisch en duren weliswaar langer dan 10 seconden, maar ze vertonen geen opbouw in amplitude of frequentie. De incidentie van epileptische aanvallen bij prematuur geboren neonaten wordt geschat op 4-48%. Dit is hoger dan wordt gerapporteerd bij voldragen pasgeborenen, terwijl de effectiviteit van anti-epileptische medicatie (AED's) significant minder lijkt te zijn bij premature neonaten, zoals wij hebben laten zien in *Hoofdstuk 8* van dit proefschrift.

Wat dit proefschrift toevoegt: In *Hoofdstuk 2* hebben we 77 neonaten onderzocht die geboren zijn na een zwangerschapsduur korter dan 28 weken en die tijdens de eerste 72 uur van hun leven met een tweekanaals EEG zijn gemonitord. Op de EEG's van deze kinderen konden vijf verschillende ritmische patronen worden onderscheiden: epileptische ontladingen, periodische epileptiforme ontladingen (PED's) en drie andere

golfpatronen. Ritmische EEG-patronen kwamen veel voor (62,3%), maar epileptische ontladingen werden maar in 1,3% van de gevallen gezien. De epileptische ontladingen lieten een duidelijke toename in amplitude, afname in frequentie (0,7-2 Hz) en verandering in morfologie (scherpe golven, ritmische deltagolven, piek-golf-complexen) zien. Er werd ook een duidelijke verandering in het aEEG gezien. De drie golfpatronen, naast epileptische ontladingen en PED's, waren significant geassocieerd met de hoofdpositie van het kind en zijn daarom waarschijnlijk artefacten. PED's kwamen veel voor (44%) en waren niet gerelateerd aan de hoofdpositie, ze waren echter ook niet geassocieerd met hersenschade of een slechtere cognitie op latere leeftijd.

Implicaties en toekomstig onderzoek: De resultaten uit *Hoofdstuk 2* laten zien dat epileptische ontladingen zeldzaam zijn in de eerste 72 uur na geboorte bij extreem vroeggeboren kinderen. De morfologie op het EEG is echter te vergelijken met voldragen neonaten. De EEG-kenmerken van epileptische ontladingen bij deze extreme prematuren zijn echter tot op heden nog maar beschreven in ongeveer 20 casussen. Het volledige spectrum van de morfologie van epileptische aanvallen op EEG bij extreme prematuren moet daarom nog verder onderzocht worden. De incidentie van epileptische aanvallen was in onze studie veel lager dan voorheen is gerapporteerd. Dit verschil zou verklaard kunnen worden, doordat in eerdere studies PED's als epileptische aanvallen werden gezien. Het is opvallend dat de incidentie van PED's in ons cohort (44%) bijna gelijk is aan de incidentie van epileptische aanvallen in die studies. PED's waren echter niet gerelateerd aan hersenschade in ons cohort en het cognitief functioneren was normaal, ondanks een hoge PED *burden*. Dit is in tegenstelling tot resultaten uit studies met volwassenen, oudere kinderen en zelfs voldragen neonaten. In deze groepen waren PED's geassocieerd met hersenschade, epilepsie en matig neurologisch functioneren. Onze resultaten suggereren dat PED's een goedaardig fenomeen zijn bij extreme prematuren. PED's zouden een vorm van spontane, hypergesynchroniseerde activiteit kunnen zijn. Zulke activiteit lijkt nodig te zijn voor een normale ontwikkeling van het brein, maar moet niet blijven bestaan nadat een kind de voldragen leeftijd heeft bereikt. Om de rol van PED's in de ontwikkeling van het premature brein verder te onderzoeken zouden premature neonaten op verschillende leeftijden tot aan de voldragen leeftijd moeten worden onderzocht op het vóórkomen van PED's. Op die manier kan de verandering in het vóórkomen van PED's in kaart worden gebracht, die samenhangt met de ontwikkeling van het brein. Dit kan vervolgens vergeleken worden met andere markers van hersenontwikkeling zoals breinvolumes, ontwikkeling van de hersenschors en functionele en structurele connectiviteit.

Afvlakking van het aEEG-achtergrondpatroon: een teken van hypercapnie - Hoofdstuk 3
Wat is bekend: Een plotse stijging van het koolstofdioxidegehalte (CO_2-gehalte) in het bloed heeft een sederende werking op het centraal zenuwstelsel en kan leiden tot coma bij volwassenen. CO_2-coma is beschreven bij volwassenen bij een CO_2-gehalte in het bloed van 62-268 mm Hg. De reactie van het brein op een te hoog koolstofdioxidegehalte (hypercapnie) verschilt echter sterk per individu. Het sederend effect van CO_2 is ook bij te vroeg geboren kinderen beschreven. Milde hypercapnie leidde tot een langer interval tussen bursts van activiteit op het EEG (interburst-interval, IBI) en een lagere totale EEG-power (globaal minder hersenactiviteit). Het fenomeen CO_2-coma, zoals bekend bij volwassenen, is echter nooit eerder bij neonaten beschreven.

Wat dit proefschrift toevoegt: In *Hoofdstuk 3* laten we zien dat ernstige onderdrukking van de hersenactiviteit tijdens acute, ernstige hypercapnie, zoals beschreven bij volwassenen, ook bij neonaten optreedt. Het kan worden opgemerkt door het aEEG-kanaal te bekijken, dat een acute verslechtering van het achtergrondpatroon laat zien, of door kwantitatieve analyse van het EEG, dat een afname in activiteit en een toename in inactiviteit van de hersenschors laat zien. Tijdens de periodes met acute, ernstige verhoging van het CO_2-gehalte in het bloed werden geen significante veranderingen in de cerebrale oxygenatie en hemodynamische parameters zoals de hartactie, bloeddruk of de arteriële zuurstofsaturatie gezien.

Implicaties en toekomstig onderzoek: De resultaten uit *Hoofdstuk 3* zouden clinici moeten waarschuwen dat ernstige onderdrukking van de hersenactiviteit kan duiden op een te hoog CO_2 bij premature en voldragen neonaten (dit leidt tot een afvlakking van het aEEG). Een plotse verslechtering van het aEEG-achtergrondpatroon zou de medische staf moeten waarschuwen om een bloedgastest te doen om het CO_2-gehalte in het bloed te controleren. EEG en aEEG worden tegenwoordig veel gebruikt op de NICU en hypercapnie leidt niet altijd tot duidelijke veranderingen in andere parameters die continu gemeten worden, zoals de cerebrale oxygenatie, bloeddruk en arteriële zuurstofsaturatie. Idealiter zou ernstige hypercapnie moeten worden opgemerkt voordat het aEEG verslechtert, door het CO_2-gehalte en de beademingsparameters zorgvuldig in de gaten te houden. Een aantal studies hebben gesuggereerd dat premature neonaten het hoogste risico hebben op onderdrukking van de hersenactiviteit door hypercapnie tijdens de eerste twee dagen na de geboorte. Wij zagen echter in ons cohort zoals beschreven in *Hoofdstuk 3* dat onderdrukking van de hersenactiviteit door hypercapnie bij elke zwangerschapsduur en op elke leeftijd kan optreden, zelfs bij voldragen neonaten. Net als bij volwassenen zagen we dat de reactie op hypercapnie sterk verschilde per individu. Sommige kinderen

vertoonden ernstige afvlakking van het aEEG en duidelijke veranderingen in de kwantitatieve EEG-parameters. Bij andere kinderen was geen verandering waarneembaar in het aEEG bij vergelijkbare CO_2-waardes en geen verandering of een tegenovergestelde verandering in de kwantitatieve EEG-parameters. Verdere studies die gebruik maken van continue CO_2-metingen zijn nodig om de precieze relatie tussen CO_2 en hersenactiviteit te onderzoeken en te bepalen bij welke CO_2-waardes kinderen het risico lopen op ernstige onderdrukking van de hersenactiviteit. Eveneens moet onderzocht worden of deze onderdrukking later leidt tot bijvoorbeeld slechter functioneren op cognitief vlak.

Deel 1.2 - Beeldvorming van de hersenen

Beeldvorming van de hersenen bij neonatale epileptische aanvallen - Hoofdstuk 4, 5 en 6
Wat is bekend: Aan neonatale epileptische aanvallen ligt vaak ernstige hersenschade ten grondslag. De prognose wordt vooral bepaald door de aard, locatie en uitgebreidheid van de onderliggende hersenschade. Het stellen van de juiste diagnose en het opsporen van bijkomend hersenletsel is daarom essentieel. Het is dan ook niet verwonderlijk dat beeldvorming van de hersenen één van de belangrijkste factoren is voor het voorspellen van de uitkomst bij kinderen met neonatale epileptische aanvallen. De afgelopen jaren hebben vele studies geschreven over beeldvorming bij neonatale epileptische aanvallen, maar de meeste studies beschreven slechts een specifiek onderliggend probleem. Slechts een paar studies rapporteerden over de bevindingen bij beeldvorming van de hersenen van kinderen met neonatale epileptische aanvallen in het algemeen.

Wat dit proefschrift toevoegt: In *Hoofdstuk 4* geven we een overzicht van de mogelijke observaties bij beeldvorming van de hersenen van kinderen met neonatale epileptische aanvallen per onderliggende oorzaak. Dit deden we door de beschikbare literatuur te onderzoeken. In *Hoofdstuk 5* onderzochten we een groep van 378 kinderen die op (bijna) voldragen leeftijd geboren zijn en neonatale epileptische aanvallen hebben gehad, die bevestigd zijn op het aEEG. We bekeken welke oorzaken ten grondslag lagen aan de aanvallen en welke afwijkingen op de echografie en MRI van de hersenen daarbij hoorden. In 93,7% werd een onderliggende oorzaak gevonden. In overeenstemming met eerdere studies waren HIE, intracraniële bloeding en perinataal arterieel ischemisch infarct de meest voorkomende oorzaken. De bijdrage van MRI ten opzichte van echografie van de hersenen werd ook onderzocht. In 37,9% was hersenechografie voldoende om de diagnose te stellen of alle relevante hersenschade in beeld te brengen. In bijna de helft van onze studiepopulatie (39,8%) leverde MRI echter extra informatie op, die belangrijk

was voor meer gerichte diagnostische testen (bijv. metabole afwijkingen), preciezere bepaling van de prognose (bijv. betrokkenheid van de corticospinale banen bij perinataal arterieel ischemisch infarct en betrokkenheid van de basale kernen bij HIE) en genetische counseling (bijv. polymicrogyrie en specifieke proton MR-spectroscopie-bevindingen bij het syndroom van Zellweger). In 11,9% van onze populatie zou de diagnose of belangrijk hersenletsel gemist zijn als alleen hersenechografie zou zijn uitgevoerd in plaats van een combinatie van echografie en MRI. Bij bepaalde diagnoses, zoals een cerebrale sinustrombose, is MRI nog belangrijker, omdat vroege behandeling een optie is en MRI nodig is om de diagnose te bevestigen.

Implicaties en toekomstig onderzoek: De resultaten uit *Hoofdstuk 5* zouden clinici die geconfronteerd worden met neonatale epileptische aanvallen moeten aansporen om een MRI, voorafgegaan door echografie, van de hersenen te maken als onderdeel van het diagnostische proces. Hersenechografie is vaak gemakkelijk toe te passen, zorgt voor minimale verstoring van de neonaat en geeft betrouwbare informatie als het gaat om bijvoorbeeld hersenbloedingen. MRI geeft daarnaast extra informatie over eventuele witte stof-afwijkingen, myelinisatie, de diepere hersenstructuren en migratiestoornissen. Daarbij is het wel noodzakelijk dat MRI-sequenties specifiek voor neonaten en dunne plakken worden gebruikt en dat de MRI door een ervaren persoon wordt beoordeeld. Het MRI-protocol zou bij voorkeur binnen een week na het ontstaan van de aanvallen moeten plaats vinden en standaard diffusie-gewogen opnames moeten bevatten. MR-angiografie, venografie, susceptibility-gewogen opnames en proton MR-spectroscopie zouden op indicatie moeten kunnen worden toegevoegd.

Het aantal kinderen met neonatale epileptische aanvallen door onbekende oorzaak wordt steeds kleiner door het gebruik van MRI en ook door nieuwe ontwikkelingen in de genetica. Doordat er steeds meer nieuwe genetische oorzaken van epilepsie worden gevonden, zou toekomstige onderzoek zich moeten richten op het uitbreiden van de resultaten uit *Hoofdstuk 5*, door de bevindingen bij beeldvorming van de hersenen bij deze nieuwe diagnoses te onderzoeken en beschrijven. *Hoofdstuk 6* is een eerste stap in die richting, doordat de bevindingen bij beeldvorming van de hersenen bij kinderen met autosomaal, recessieve mutaties in het *SLC13A5*-gen worden beschreven. Deze mutaties leiden tot een specifieke neonatale *early-onset* encefalopathie, die wordt gekenmerkt door het ontstaan van epileptische aanvallen in de eerste dagen na geboorte bij verder gezonde neonaten, na een ongecompliceerde zwangerschap en bevalling, onbehandelbare epilepsie met frequente status epilepticus, koortsgevoeligheid, ontwikkelingsstoornissen en hypoplasie of hypodontie van de tanden. Gedetailleerde

informatie over de hersen-MRI-kenmerken was echter niet beschikbaar uit eerdere studies. In *Hoofdstuk 6* beschrijven we de bevindingen bij beeldvorming van de hersenen van acht kinderen uit vijf families met neonatale epilepsie en mutaties in het *SLC13A5*-gen. Zeven van de acht patiënten in onze studie hadden een karakteristiek MRI-patroon met puntlaesies in de witte stof van de hersenen op de neonatale MRI-scan, die niet meer zichtbaar waren op de leeftijd van 6 maanden, maar tot gliotische veranderingen (verlittekening van de hersenen) leidden op de leeftijd van 18 maanden. Bij kinderen die zich presenteren met onbehandelbare epileptische aanvallen in de eerste dagen na geboorte, zonder duidelijke kenmerken van HIE, maar met puntlaesies in de witte stof op hun neonatale MRI, zou daarom een diagnose van mutaties in het *SLC13A5*-gen moeten worden overwogen.

DEEL 2 - BEHANDELING

Veiligheid van lidocaïne voor het behandelen van neonatale epileptische aanvallen - Hoofdstuk 7

Wat is bekend: Dierexperimentele studies suggereren dat epileptische aanvallen hersenschade kunnen verergeren in het zich ontwikkelende brein. Bij mensen zijn epileptische aanvallen gerelateerd aan de ernst van de hersenschade en problemen in het functioneren op latere leeftijd. Snelle behandeling op basis van protocollen kan de totale duur van epileptische aanvallen *(seizure burden)* verminderen. Er zijn echter weinig gegevens uit gerandomiseerde en gecontroleerde studies beschikbaar om een optimaal behandelprotocol te ondersteunen en er kunnen daardoor geen aanbevelingen worden gedaan. Lidocaïne lijkt een effectief medicijn te zijn tegen epileptische aanvallen die blijven bestaan ondanks behandeling met anti-epileptische medicijnen. Het wordt echter in de westerse wereld niet veel gebruikt. Waarschijnlijk komt dit door het risico op cardiale complicaties, die beschreven zijn in een aantal studies bij neonaten die lidocaïne kregen in verband met epileptische aanvallen. De incidentie van deze cardiale complicaties varieert van 0% tot 4,8%, maar deze percentages zijn gebaseerd op verouderde, kleinschalige studies, terwijl er inmiddels nieuwe, lagere doseringsschema's zijn ontwikkeld.

Wat dit proefschrift toevoegt: In *Hoofdstuk 7* laten we zien dat lidocaïne-geassocieerd cardiale complicaties zeldzaam zijn in een groep van 368 voldragen en 153 premature neonaten die lidocaïne kregen in verband met epileptische aanvallen. Slechts in 1,3-1,9% konden de cardiale complicaties worden verklaard door het gebruik van lidocaïne, omdat

(1) ze optraden tussen 2 uur na de start van de lidocaïne-infusie en maximaal 12 uur na het stoppen van de infusie, wanneer de lidocaïneconcentratie in het bloed zeer waarschijnlijk >5 mg/l is, (2) de symptomen passen bij de verwachte symptomen op basis van farmacodynamiek en (3) er geen bijdragende factoren waren die de cardiale complicatie zouden kunnen verklaren. Na de introductie van nieuwe, lagere doseringsschema's daalde de incidentie naar 0,4%. De meest voorkomende cardiale complicatie die geassocieerd was met lidocaïnegebruik was bradycardie (te trage hartslag). Belangrijke bijdragende risicofactoren voor cardiale complicaties waren: instabiel kalium, (congenitale) cardiale dysfunctie en gelijktijdig gebruik van fenytoïne.

Implicaties en toekomstig onderzoek: De resultaten uit *Hoofdstuk 7* kunnen clinici geruststellen dat lidocaïne veilig is om te gebruiken als anti-epileptisch medicijn voor voldragen en premature neonaten met epileptisch aanvallen, met betrekking tot cardiale complicaties. Wanneer aangepaste doseringsschema's worden gebruikt is de incidentie lager dan de achtergrondincidentie van hartritmestoornissen in de neonatale populatie (1-5%). Voordat lidocaïne als anti-epileptisch medicijn gegeven wordt, moet aandacht worden besteed aan de elektrolytenbalans, (congenitale) cardiale dysfunctie en gelijktijdig gebruik van fenytoïne, omdat dit belangrijke bijdragende risicofactoren zijn. Daarnaast moet de waarschijnlijkheid van een causale relatie zorgvuldig worden onderzocht wanneer cardiale complicaties optreden tijdens het geven van lidocaïne. In eerdere studies werden alle cardiale complicaties die optraden tijdens de lidocaïne-infusie toegeschreven aan lidocaïne zonder te overwegen of de concentratie in het bloed wel hoog genoeg was om de symptomen te veroorzaken, of de symptomen verklaard konden worden op basis van de farmacodynamiek van lidocaïne en of er andere mogelijke oorzaken of bijdragende factoren waren. Idealiter zouden onze resultaten moeten worden bevestigd in prospectieve studies waarbij gebruik wordt gemaakt van continue bloeddruk- en ECG-registraties.

Effectiviteit van lidocaïne ter behandeling van neonatale epileptische aanvallen - Hoofdstuk 8

Wat is bekend: Lidocaïne lijkt een effectief middel te zijn voor de tweede- en derdelijnsbehandeling van neonatale epileptische aanvallen, met een effectiviteit variërend van 60-92%. Deze studies zijn echter gebaseerd op kleine groepen kinderen en de behandelschema's verschilden sterk in de studies.

Wat dit proefschrift toevoegt: In *Hoofdstuk 8* onderzochten we de effectiviteit van lidocaïne in een groep van 319 voldragen en 94 premature neonaten en we vergeleken

dit met de effectiviteit van midazolam, een ander veelgebruikt medicijn tegen neonatale epileptische aanvallen. Wij lieten zien dat lidocaïne in ongeveer 70% van de kinderen effectief was in het stoppen van de aanvallen, maar dat de effectiviteit afhankelijk was van (1) de zwangerschapsduur waarbij het kind geboren was, (2) de onderliggende oorzaak en (3) het moment waarop het middel gegeven werd. Lidocaïne had een significant mindere effectiviteit bij premature neonaten, terwijl kinderen met een perinataal arterieel ischemisch infarct of een cerebrale sinustrombose een grotere kans hadden op een goed effect van lidocaïne dan kinderen met een andere oorzaak van de epileptische aanvallen. Als tweedelijnsbehandeling (monotherapie, één medicijn gegeven) was de effectiviteit van lidocaïne significant beter dan de effectiviteit van midazolam. Als derdelijnsbehandeling (combinatie therapie, twee medicijnen tegelijk gegeven) was de effectiviteit van lidocaïne iets beter dan midazolam. Zowel lidocaïne als midazolam hadden een significant betere effectiviteit wanneer ze als derdelijnsbehandeling in combinatie met elkaar werden gegeven dan als tweedelijnsbehandeling als enige medicijn. Dit zou kunnen berusten op een farmacologisch synergistisch mechanisme tussen de twee medicijnen.

Implicaties en toekomstig onderzoek: De resultaten uit *Hoofdstuk 8* laten zien dat er dringend behoefte is aan meer onderzoek naar behandeling van neonatale epileptisch aanvallen. De effectiviteit van lidocaïne en midazolam zouden idealiter bevestigd moeten worden in een prospectieve, multicenter, gerandomiseerde, gecontroleerde studie. Het mogelijk synergistisch of additief effect van anti-epileptische medicatie (AED's) evenals het effect van de timing van het geven van AED's behoeft duidelijk nader onderzoek. Het pathofysiologisch mechanisme van epileptische aanvallen bij prematuren behoeft ook nader onderzoek om betere behandelstrategieën te ontwikkelen voor deze groep kinderen. Op basis van onze resultaten zou een combinatie van lidocaïne en midazolam een goede optie zijn voor de behandeling van neonatale epileptische aanvallen die niet voldoende reageren op behandeling met fenobarbital. Wij zouden adviseren om te beginnen met lidocaïne als tweedelijns-AED, omdat lidocaïne de achtergrondactiviteit van het EEG niet zoveel beïnvloedt als midazolam. Daarnaast heeft lidocaïne weinig effect op de ademdrive wanneer een kind nog spontaan ademt. Bovendien blijken lidocaïne-geassocieerde cardiale complicaties zeldzaam, waarover vaak de meeste bezorgdheid heerst bij behandeling met lidocaïne, zoals wij in *Hoofdstuk 7* lieten zien. Wanneer de aanvallen blijven bestaan zou midazolam spoedig moeten worden toegevoegd.

DEEL 3 - PROGNOSE

Uitkomstvoorspellers bij kinderen met HIE - Hoofdstuk 9, 10 en 11

Wat is bekend: HIE is de meest voorkomende oorzaak van epileptische aanvallen bij voldragen neonaten en wordt geassocieerd met afwijkingen in de neurologische ontwikkeling. Voor neonaten met HIE zijn verschillende uitkomstvoorspellers onderzocht, zoals neurologisch onderzoek, EEG-achtergrondactiviteit, de hoeveelheid epileptische aanvallen *(seizure burden)* en MRI. Men heeft geprobeerd vast te stellen wat de beste uitkomstvoorspeller is. Dit bleek echter moeilijk vast te stellen, doordat de meeste voorspellende factoren apart zijn onderzocht in verschillende studies met vaak maar een beperkt aantal kinderen. MRI en de achtergrondactiviteit op het (a)EEG werden genoemd als de meest optimale uitkomstvoorspellers, maar verschillende studies meenden dat het voorspellen van de uitkomst substantieel verbeterd kon worden, wanneer een combinatie van meerdere voorspellende factoren gebruikt zou worden. De meeste studies zijn echter uitgevoerd voordat therapeutische hypothermie beschikbaar was. Hypothermie is tegenwoordig de standaard voor neuro-protectie bij kinderen met HIE, maar het heeft wel invloed op de voorspellende waarde van met name het neurologisch onderzoek en EEG.

Wat dit proefschrift toevoegt: In *Hoofdstuk 9* gebruikten we continue video-EEG-registraties om de rol van de EEG-achtergrondactiviteit, de hoeveelheid epileptische aanvallen op EEG en MRI in het voorspellen van het neurologische ontwikkelen te bepalen in een groep voldragen neonaten met HIE in het tijdperk van therapeutische hypothermie. MRI was de beste uitkomstvoorspeller, op de voet gevolgd door de EEG-achtergrondactiviteit, maar alleen 36 en 48 uur na de geboorte. Het EEG was niet voorspellend op eerdere tijdsmomenten. Beoordeling van de hoeveelheid epileptische aanvallen op EEG had geen additionele voorspellende waarde. Het was niet zo precies in het voorspellen van de uitkomst als MRI en de EEG-achtergrondactiviteit en het was alleen geassocieerd met een afwijkende uitkomst wanneer er op de MRI matige tot ernstige schade gezien werd.

Omdat wij hebben bevestigd dat MRI de beste uitkomstvoorspeller is bij kinderen met HIE en MRI vaak als biomarker voor uitkomst wordt gebruikt in klinische trials, is het kwantificeren van de uitgebreidheid van hersenschade belangrijk voor het objectief en accuraat bepalen van de prognose. In *Hoofdstuk 10* presenteren wij een nieuwe MRI-score, die makkelijk toepasbaar is en het hele brein op alle belangrijke afwijkingen bij HIE beoordeelt. De MRI-score had een goede voorspellende waarde in twee onafhankelijke

internationale cohorten die samen 173 kinderen bevatten die behandeld zijn met hypothermie in verband met HIE. In beide cohorten was schade aan de diepe grijze stof een onafhankelijke voorspeller voor een afwijkende uitkomst op de leeftijd van 2 jaar en op de basisschoolleeftijd. Voor de diepe grijze stof subscore werden afkapwaardes voor het risico op een afwijkende uitkomst bepaald met een *area under the curve* en een positief en negatief voorspellende waarde >0.83.

In *Hoofdstuk 11* vergeleken we de relatie van de Thompson-score, een gestandaardiseerde score voor neurologisch onderzoek van kinderen met HIE, en het aEEG-achtergrondpatroon met de neurologische uitkomst op de leeftijd van 2 jaar in 122 kinderen met HIE die behandeld zijn met hypothermie. De Thompson-score en het aEEG-achtergrondpatroon werden beoordeeld voordat de hypothermie was gestart. Hogere Thompson-scores waren geassocieerd met slechtere aEEG-achtergrondpatronen, maar voor beide methodes was de specificiteit en de positief voorspellende waarde lager dan in het pre-hypothermie-tijdperk. Dit effect zou kunnen worden toegeschreven aan het neuroprotectieve effect van hypothermie. De voorspellende waarde van aEEG was iets beter dan die van de Thompson score. Een Thompson score van 11 of hoger en een *continuous low voltage* of *flat trace* aEEG-patroon waren geassocieerd met een matige uitkomst, ondanks hypothermie.

Implicaties en toekomstig onderzoek: Hoofdstuk 9 geeft een compleet overzicht van de rol van EEG, MRI en *seizure burden* in het voorspellen van de uitkomst bij kinderen met HIE in het hypothermie-tijdperk. Wij leveren extra bewijs dat de achtergrondactiviteit op EEG pas een betrouwbare uitkomstvoorspeller is vanaf 36 uur na de geboorte. Daarnaast laten wij zien dat het belangrijk is om een combinatie van MRI en EEG te gebruiken bij kinderen met HIE. MRI is bewezen als de beste uitkomstvoorspeller, terwijl continue EEG-monitoring kan helpen bij het beoordelen van de ernst van encefalopathie en bij het opsporen en adequaat behandelen van elektro-encefalografische epileptische aanvallen. Dit om de *seizure burden* te beperken en beslissingen over het wel of niet continueren van intensive care behandeling te ondersteunen, bijvoorbeeld wanneer een kind niet stabiel genoeg is om naar de MRI vervoerd te worden. Wij bevestigen dat een hoge *seizure burden* niet altijd geassocieerd is met een slechte uitkomst, maar dat dit afhankelijk is van de uitgebreidheid van de afwijkingen op MRI. De studie die in *Hoofdstuk 9* gepresenteerd werd, zou herhaald moeten worden in een grote, multicenter-studie om de bevindingen te bevestigen en om een predictiemodel te kunnen maken dat gebaseerd is op alle belangrijke uitkomstvoorspellers bij kinderen met HIE.

In *Hoofdstuk 10* presenteren we een nieuwe MRI-score om de uitgebreidheid van hersenschade bij kinderen met HIE te bepalen. De score kan gebruikt worden om een betrouwbare voorspelling voor de uitkomst te doen, door gebruik te maken van de gepresenteerde afkapwaardes. In dit hoofdstuk leveren we ook extra bewijs dat de uitkomst vooral bepaald wordt door schade aan de diepe grijze stof onafhankelijk van laesies in andere hersengebieden, zoals de witte stof. Anders dan resultaten uit eerdere studies, vonden wij geen relatie tussen schade aan de witte stof en het IQ op de schoolleeftijd. Deze studies zijn echter uitgevoerd bij kinderen die niet behandeld zijn met hypothermie en bij kinderen met ernstige, geïsoleerde witte stofschade. In ons cohort hadden kinderen met geïsoleerde witte stofschade slechts milde tot matige schade, wat geen significant effect had op hun cognitie. We verwachten dat de voorspellende waarde van de score anders is in andere populaties, zoals kinderen met HIE die niet behandeld zijn met hypothermie of kinderen met een meningitis. Om de voorspellende waarde van de score in deze populaties vast te stellen zijn vervolgstudies nodig. De kwaliteit van de score in de tweede levensweek moet ook nog worden beoordeeld, omdat de meerderheid van de scans in ons cohort in de eerste week na geboorte zijn gemaakt. Tijdens de eerste week na geboorte kan de score het best gedaan worden met T1-, T2- en diffusie-gewogen opnames, omdat gebleken is dat diffusie-gewogen opnames de meest betrouwbare beelden zijn in de eerste week na geboorte. In de tweede levensweek zijn diffusie-afwijkingen echter niet meer zichtbaar door het fenomeen pseudonormalisatie. De score zou dan op alleen T1- en T2-gewogen opnames gedaan moeten worden, in combinatie met proton-MR-spectroscopie metingen, omdat deze nog enige tijd afwijkend blijven.

De vraag of de Thompson-score of het aEEG-achtergrondpatroon beter is in het selecteren van kinderen voor behandeling met hypothermie, wordt niet beantwoord door de resultaten uit *Hoofdstuk 11*. Daarvoor zou een studie gedaan moeten worden die kinderen met perinatale asfyxie in de uren na de geboorte randomiseert tot het gebruik van ofwel de Thompson-score ofwel het aEEG. Tot die tijd zouden wij adviseren om een combinatie van beide technieken te gebruiken bij de selectie van kinderen met perinatale asfyxie voor behandeling met hypothermie. Daarbij moeten we wel benadrukken dat de voorspellende waarde van aEEG iets beter was dan die van de Thompson-score in ons cohort en dat het aEEG als voordeel heeft dat het een continue meting is en daarnaast subklinische epileptische aanvallen kan identificeren. De afkapwaardes voor de Thompson-score en het aEEG-achtergrondpatroon die in *Hoofdstuk 11* werden gepresenteerd, zouden wel kunnen worden gebruikt om patiënten te selecteren die mogelijk baat hebben bij additionele neuro-protectieve strategieën bovenop hypothermie, omdat deze kinderen een slechte uitkomst hadden, ondanks behandeling met hypothermie.

RICHTLIJNEN VOOR TOEKOMSTIG ONDERZOEK

Diagnose - Elektro-encefalografie

Het toegenomen gebruik van EEG-monitoring op de NICU biedt nieuwe mogelijkheden: het kan helpen bij het herkennen van hersenschade of fysiologische veranderingen, zoals een te hoog koolstofdioxidegehalte, zoals wij hebben laten zien in dit proefschrift *(Hoofdstuk 3)*. Het stelt ons echter ook voor nieuwe uitdagingen, wanneer we nieuwe populaties monitoren, zoals extreem vroeggeboren kinderen. In dit proefschrift hebben we laten zien dat kennis over EEG-patronen bij oudere neonaten en kinderen niet altijd één op één gebruikt kan worden bij premature neonaten *(Hoofdstuk 2)*. De EEG-kenmerken van epileptische ontladingen zijn in deze groep nog maar voor een paar casussen beschreven, waardoor het volledige spectrum van de morfologie van epileptische aanvallen op EEG bij extreme prematuren nog verder onderzocht moet worden. Ook de rol van PED's in de hersenontwikkeling van prematuren moet verder geëxploreerd worden. Toekomstige studies zouden daarom premature neonaten op verschillende leeftijden tot aan de voldragen leeftijd moeten onderzoeken op het vóórkomen van PED's. Op die manier kan de verandering in het vóórkomen van PED's in kaart gebracht worden die samenhangt met de ontwikkeling van het brein. Dit kan vervolgens vergeleken worden met andere markers van de hersenontwikkeling, zoals breinvolume, ontwikkeling van de hersenschors en functionele en structurele connectiviteit.

Verdere studies die gebruikmaken van continue CO_2-metingen zouden uitgevoerd moeten worden, om de precieze relatie tussen CO_2 en hersenactiviteit te onderzoeken en te bepalen bij welke CO_2-waardes kinderen het risico lopen op ernstige onderdrukking van de hersenactiviteit en of dit later leidt tot bijvoorbeeld slechter functioneren op cognitief vlak.

Diagnose - Beeldvorming van de hersenen

In dit proefschrift werd een compleet overzicht gegeven van de bevindingen die gedaan kunnen worden bij beeldvorming van de hersenen bij kinderen met neonatale epileptische aanvallen door een overzicht van de beschikbare literatuur te geven *(Hoofdstuk 4)* en door onderzoek van een grote groep kinderen met neonatale epileptische aanvallen *(Hoofdstuk 5)*. Het spectrum van nieuw ontdekte oorzaken van neonatale epileptische aanvallen breidt zich echter steeds uit, vooral door ontwikkelingen in de genetica. Daarom zou toekomstig onderzoek zich moeten richten op het uitbreiden van de resultaten uit *Hoofdstuk 5* door de bevindingen bij beeldvorming van de hersenen bij deze nieuwe diagnoses te onderzoeken en beschrijven.

Behandeling

Er is dringend behoefte aan meer onderzoek naar de behandeling van neonatale epileptische aanvallen, omdat de effectiviteit en veiligheid van de beschikbare AED's niet optimaal is. Wij lieten in een retrospectief onderzoek zien dat lidocaïne veilig is om te gebruiken bij neonaten met betrekking tot cardiale complicaties *(Hoofdstuk 7)*. Idealiter zouden onze resultaten moeten worden bevestigd in een prospectieve studie waarbij continue bloeddruk- en ECG-metingen beschikbaar zijn. Wij lieten ook zien dat lidocaïne een effectiviteit van 70% kon bereiken *(Hoofdstuk 8)*, maar dat dit afhankelijk was van de zwangerschapsduur, de onderliggende oorzaak en het moment waarop het middel gegeven werd. Zowel lidocaïne als midazolam hadden een betere effectiviteit als derdelijns- vergeleken met tweedelijns-behandeling, wat zou kunnen berusten op een farmacologisch synergistisch mechanisme tussen de twee medicijnen. Het mogelijk synergistisch of additief effect van AED's evenals het effect van de timing van het geven van AED's behoeft duidelijk nader onderzoek. Het pathofysiologisch mechanisme van epileptische aanvallen bij prematuren behoeft ook nader onderzoek om betere behandelstrategieën te ontwikkelen voor deze groep kinderen.

Prognose

Het voorspellen van de toekomst is een belangrijk onderdeel van neonatale zorg, maar ook het meest uitdagende. De perfecte uitkomstvoorspeller bestaat niet en studies bij kinderen met HIE hebben laten zien dat het voorspellen van de uitkomst op meerdere factoren gebaseerd zou moeten worden. Om een predictiemodel te kunnen ontwikkelen voor kinderen met HIE zou de studie in *Hoofdstuk 9* herhaald moeten worden in een grote, multicenter-studie, waarbij het neurologische onderzoek, EEG en MRI als variabelen worden meegenomen. De MRI-score die wij in *Hoofdstuk 10* hebben gepresenteerd zou gebruikt kunnen worden als onderdeel van het predictiemodel. De score moet daarvoor nog wel eerst geoptimaliseerd worden door de voorspellende waarde in andere populaties en de kwaliteit van de score in de tweede levensweek te bepalen.

Bovendien zou een studie die kinderen met perinatale asfyxie in de uren na de geboorte randomiseert tot het gebruik van ofwel de Thompson-score ofwel het aEEG gedaan moeten worden om te bepalen of de Thompson-score of het aEEG beter is in het selecteren van kinderen met HIE voor behandeling met hypothermie.

Chapter 14

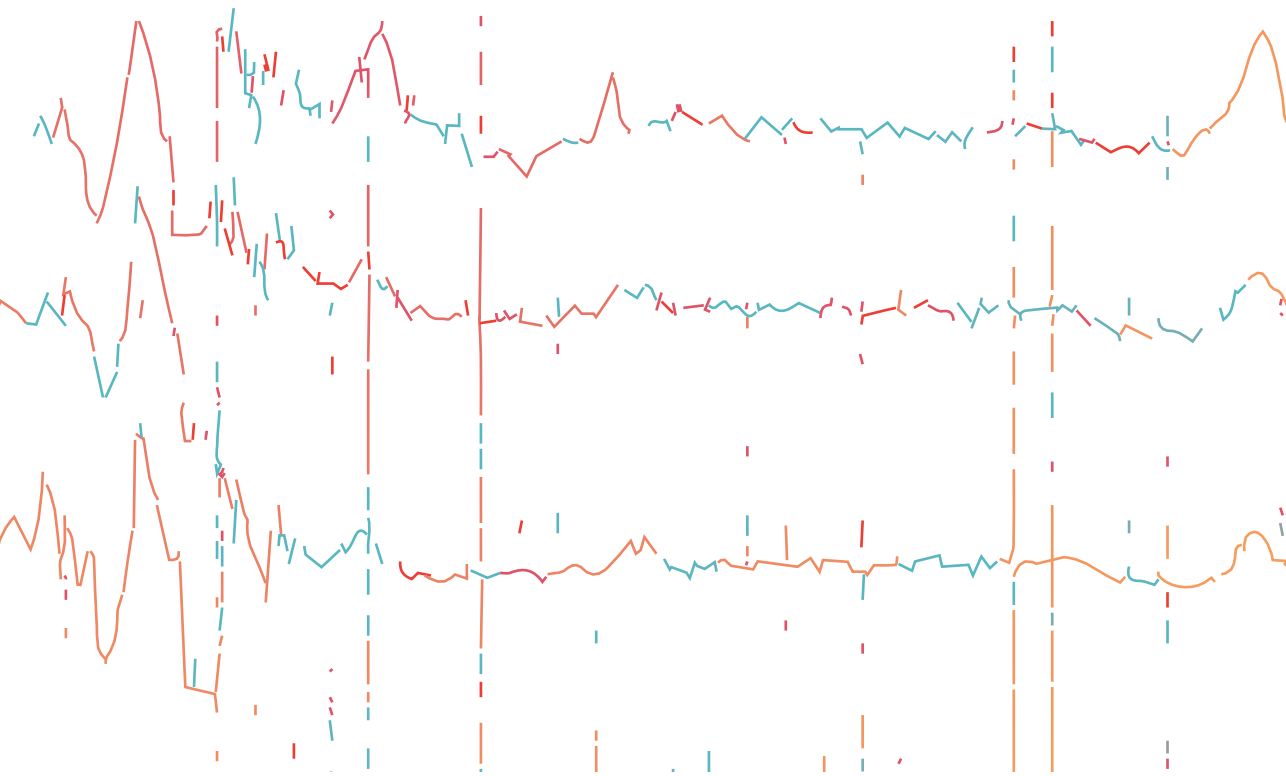
List of abbreviations

List of co-authors

List of publications

Curriculum vitae

Dankwoord (Acknowledgements)



LIST OF ABBREVIATIONS

^{1}H-MRS	Proton magnetic resonance spectroscopy
ACA	Anterior cerebral artery
AD	Additional
ADC	Apparent diffusion coefficient
AED	Antiepileptic drug
aEEG	Amplitude-integrated electroencephalography
AGS1-5	Aicardi-Goutières syndrome genes 1-5
ANOVA	Analysis of variance
ARX	Aristaless-related homeobox
AUC	Area under the curve
AV	Atrioventricular
AVM	Arteriovenous malformation
BFNC	Benign familial neonatal convulsions
BGT	Basal ganglia and thalami
BS	Burst suppression
BS-	Burst suppression sparse
BS+	Burst suppression dense
BSITD-III	Bayley Scales of Infant and Toddler Development, third edition
CB	Cerebellum
CDKL5	Cyclin-dependent kinase-like 5
Cho	Choline
CI	Confidence interval
CLV	Continuous low voltage
CNS	Central nervous system
CNV	Continuous normal voltage
CO_2	Carbon dioxide
CP	Cerebral palsy
cPVL	Cystic periventricular leukomalacia
CSF	Cerebrospinal fluid
CSVT	Cerebral sinovenous thrombosis
CT	Computed tomography
cUS	Cranial ultrasound
DNV	Discontinuous normal voltage
DQ	Developmental quotient

DTI	Diffusion tensor images
DWI	Diffusion-weighted images
ECG	Electrocardiogram
EE	Epileptic encephalopathy
EEG	Electroencephalography
EIEE	Early infantile epileptic encephalopathy
FiO_2	Fraction of inspired oxygen
FLAIR	Fluid attenuated inversion recovery
FT	Flat trace
FTOE	Fractional tissue oxygen extraction
GA	Gestational age
GLC	Germinolytic cyst
GM	Grey matter
GMFCS	Gross motor function classification system
HIE	Hypoxic-ischaemic encephalopathy
HSV	Herpes simplex virus
IBI	Interburst interval
ICH	Intracranial haemorrhage
IQ	Intelligence quotient
IQR	Interquartile range
ISI	InterSAT interval
ISP	InterSAT percentage
IVH	Intraventricular haemorrhage
LSV	Lenticulostriate vasculopathy
MABP	Mean arterial blood pressure
MCA	Middle cerebral artery
MRA	Magnetic resonance angiography
MRI	Magnetic resonance imaging
MRV	Magnetic resonance venography
NA	Not applicable
NAA	*N*-acetyl aspartate
NEMO	NEonatal seizure treatment with Medication Off -patent
NeoBrain	Neonatal Estimation of Brain Damage Risk and Identification of Neuroprotectants
NICU	Neonatal intensive care unit
NIRS	Near-infrared spectroscopy
NKH	Nonketotic hyperglycinaemia

NMDA	*N*-methyl D-aspartate
NPV	Negative predictive value
OR	Odds ratio
PAIS	Perinatal arterial ischaemic stroke
PCA	Posterior cerebral artery
PED	Periodic epileptiform discharge
PLIC	Posterior limb of the internal capsule
PMA	Postmenstrual age
Ppm	Parts per million
PPV	Positive predictive value
PVL	Periventricular leukomalacia
PWML	Punctate white matter lesions
ROC	Receiver operating characteristic
$rScO_2$	Regional cerebral oxygen saturation
SAH	Subarachnoidal haemorrhage
SaO_2	Arterial oxygen saturation
SAT	Spontaneous activity transient
SD	Standard deviation
SDH	Subdural haemorrhage
SI	Signal intensity
SSS	Superior sagittal sinus
STXBP1	Syntaxin-binding protein 1
SWI	Susceptibility-weighted images
T1WI	T1-weighted images
T2WI	T2-weighted images
TE	Echo time
WISC-IV-SE	Wechsler Intelligence Scale for Children, fourth edition, Swedish version
WM	White matter
WMI	White matter injury
WPPSI-III-NL	Wechsler Preschool and Primary Scale of Intelligence, third edition, Dutch version
WS	Watershed

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CURRICULUM VITAE

Lauren Weeke was born on August 30th 1986 in Blijham, the Netherlands, where she grew up with her younger brother. After graduating grammar school cum laude in 2004, she moved to Groningen to start medical school at the University of Groningen. During her medical training she discovered her love for traveling and performed two internships abroad: a clinical internship in tropical medicine at St. Carolus Hospital Mtinko, Tanzania and a research internship in paediatric pulmonology at the School of Paediatrics and Child Health, University of Western Australia in Perth, Australia under supervision of prof. Peter LeSouef. After graduating medical school cum laude in 2011, she decided to pursue a career in paediatrics and started working as a resident at the department of neonatology and paediatrics of the Isala Clinics in Zwolle under supervision of prof. dr. Paul Brand. Because she has a passion for teaching, she joined the medical school teaching staff of the University of Groningen in February 2013 for six months before she started her PhD on neonatal seizures at the department of neonatology of the Wilhelmina Children's Hospital in Utrecht in August 2013 under supervision of prof. dr. Linda de Vries, dr. Floris Groenendaal and dr. Mona Toet. During her PhD she worked as a research fellow for two European multicentre studies: the NEMO study investigating a new drug for neonatal seizures and the ANSeR study, investigating a new seizure detection algorithm for neonatal EEG. In October 2015 she spent three months in Leuven, Belgium at the department of neonatology of the UZ Leuven to increase her knowledge about preterm EEG and study new quantitative methods to assess neonatal EEG. In July 2017 she will start her training in paediatrics at the Erasmus MC-Sophia Children's Hospital in Rotterdam under supervision of prof. dr. Matthijs de Hoog.

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understand who we are,
what we are made of and where we want to go –

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Life can only be understood backwards,
but it must be lived forwards

Søren Kierkegaard