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[a], Geraldine B Boylan[b], Ronit M Pressler[c], Boubou Hallberg[d], Mats Blennow[d], Mona C Toet[a], Floris Groenendaal[a], Linda S de Vries[a] *for the NEonatal seizure treatment with Medication Off -patent (NEMO)consortium*

a) Department of Neonatology, Wilhelmina Children's Hospital, University Medical Centre Utrecht, PO Box 85090, 3508 AB Utrecht, Netherlands.
b) Irish Centre for Fetal and Neonatal Translational Research, University College Cork, Wilton, co. Cork, Ireland.
c) Clinical Neurosciences, UCL-Institute of Child Health, 30 Guilford Street, London WC1N 1EH, UK.
d) Department of Neonatology, Karolinska University Hospital, SE-171 77 Stockholm, Sweden.

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Address correspondence to:

Linda S. de Vries,
Department of Neonatology
KE.04.123.1,
PO Box 85090,
3508 AB Utrecht,
The Netherlands,
Telephone: +31 (0)88 7554545,
Fax: +31 (0)88 7555320,
Email: l.s.devries@umcutrecht.nl

ABSTRACT

Objective

To investigate the role of EEG background activity, electrographic 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 72h, an MRI performed within the first two weeks and neurodevelopmental assessment at two years were evaluated. EEG background activity at age 24, 36 and 48h, 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 36h ($p=0.009$) and 48h after birth ($p=0.029$) and with severity of brain injury on MRI at 36h ($p=0.002$) and 48h ($p=0.018$). 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 36h and 48h and the negative predictive value (NPV) was 75% at 36h and 69% at 48h. 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 36h and 48h 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.

Key words: EEG, seizure burden, MRI, neurodevelopmental outcome, HIE, hypothermia

1 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 precooling 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]. Electrographic 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, electrographic seizure burden, and MRI in predicting neurodevelopmental outcome at two years of age in a single cohort of full-term infants with HIE in the era of therapeutic hypothermia.

2 METHODS

2.1 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 <48h; perinatal asphyxia (5 minutes Apgar $\leq$ 5, pH $\leq$ 7.10, BE $\geq$ 16 mmol/L, or resuscitation 10 min after birth); electrographic seizures not responding to 20 mg/kg phenobarbitone. Exclusion criteria: other diuretics or anticonvulsants; 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 phenobarbitone.

2.2 EEG

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 72h. 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 electrographic seizures in minutes), seizure onset (start of first electrographic seizure in hours after birth), seizure period (from start of first to end of last recorded electrographic 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 one hour epochs at the onset of EEG monitoring (baseline) and at 24, 36 and 48h 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 ≤10s, no clear sleep-wake cycling or clear asymmetry or asynchrony), severe abnormalities (discontinuous activity with interburst interval 10-60s, severe attenuation of background activity, no sleep-wake cycles), and isoelectric EEG (background activity <5uV or severe discontinuity with interburst interval >60s). Seizure burden was dichotomised into low (<18min) and high seizure burden (≥18min), the optimal cut-off for seizure burden for predicting an abnormal outcome was chosen as the point on the receiver operator curve closest to the (0,1) point.

2.3 MRI

An MRI (1.5 or 3T) 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 and 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 basal ganglia-thalamic (BGT) injury, predominant white matter/watershed (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.

2.4 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 two 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 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.

2.5 Statistical analysis

Statistical analysis was performed using SPSS 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 (<18min vs ≥18min) 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 efficiency (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.

3 RESULTS

A total of 30 infants were screened for the randomized study out of which 14 (47%) infants received bumetanide for seizures not responding to phenobarbitone; details of these infants have been published previously [27]. The remaining 16 infants were classed as screening failures with no electrographic seizure activity seen on the EEG after the first dose of phenobarbitone. 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 summarized in *Table 1*.

3.1 EEG

EEG was started at a median of 7.3h after birth (interquartile range [IQR] 3.3-14.9), the median duration of the EEG recordings was 71.4h (IQR 42.8-85.8). The EEG background activity at baseline and at 24h of age was mildly abnormal in 2 (7.7%) infants, moderately abnormal in 11 (42.3%), severely abnormal in seven (26.9%) and isoelectric in five (19.2%) *(Table 2).* At 36h 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 48h 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 36h and in six not at 48h. In one infant, the EEG deteriorated as the neonate had been given a bolus dose of midazolam at 47 hours. As a result, we did not include this time point in this neonate in our analysis and used a 46 hour time point instead. In 17 infants the EEG recordings (65.4%) showed electrographic seizure activity. Seizure data per infant is shown in *Table 2*.

3.2 MRI

MRI was performed at a mean age of 6.4 days (SD2.9) and a mean postmenstrual age of 41.1 weeks (SD1.3). 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.

3.4 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 months), 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.

3.5 Association between EEG background activity, electrographic seizures, MRI and neurodevelopmental outcome

Neurodevelopmental outcome was significantly associated with EEG background activity at 36h ($p=0.009$) and 48h 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 24h. MRI was associated with EEG background activity at 36h ($p=0.002$) and 48h (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.

3.5.1 EEG background activity vs. MRI and neurodevelopmental outcome

EEG background activity at 36h after birth showed the strongest association with MRI and neurodevelopmental outcome (*Figure 1*). The background activity on EEG at 36h 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 48h after birth *(Table 3).* All infants with a severely abnormal or isoelectric EEG at 36h or 48h 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 36h 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 48h, 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 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 36h and 48h (1.8, $p=0.012$ and 2.1, $p=0.007$ respectively).

3.5.2 Seizure burden

The extent of electrographic 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 (<18min), 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 ($\geq$18min), two had mild MRI abnormalities (both normal outcome), six had moderate abnormalities (one hearing problems, three motor problems, two died) and one had near-total injury (died). A significant relation between seizure burden ($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).

3.5.3 MRI vs. neurodevelopmental outcome

MRI would have accurately predicted outcome in all but two out of 22 infants (*Table 2).* 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 cognition 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 [-2SD]) in this cohort *(Figure 3).* Two infants had a low MRI score, but scored below -2SD 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.

3.5.4 Predictive values

Predictive values of EEG background activity at 36h and 48h after birth, electrographic seizure burden and MRI for abnormal outcome are listed in *Table 3*. In 17 infants information on EEG background activity at 36h, 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 related with abnormal outcome in the presence of moderate-severe injury on MRI *(Figure 1).*

4 DISCUSSION

This is the first study using continuous video-EEG to investigate the role of EEG background activity, electrographic 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 36h and 48h after birth. The EEG was not predictive at earlier time points. Assessment of electrographic 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 one week of age instead of as early as 36h.

4.1 MRI

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 to MRI protocols of DWI-sequences 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.

4.2 EEG

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 six hours after birth[21], and when the EEG recovered within the first 24h 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 24h of life was not always associated with a poor outcome under hypothermia, while after 36h 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 midcooling time period was passed[40]. Hamelin *et al.* reported a reduction in PPV of a stage four EEG for death at 14-16h 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 three of life[39]. This delay in predictive property of EEG was also described for aEEG. Azzopardi *et al.* reported a slightly lower PPV (0.51) of aEEG in the first 6h of life compared to normothermic controls (0.59) 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 3-6h of age from 0.84 to 0.59, but the PPV in infants treated with hypothermia increased over time to 0.92 at 24-36h and was similar to normothermic patients at 48-60h[13]. Hallberg *et al.* also showed that severe aEEG abnormalities were only predictive of abnormal outcome after 36h[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 36h of age.

4.3 Seizure burden

Animal studies have suggested that seizures superimposed on moderate-severe hypoxic-ischemic 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, electrographic 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, and a decision to redirect care may be made before recovery of background activity often associated with occurrence of electrographic seizures, with no or limited superimposed electrographic seizures during the first days after birth. However, this observation needs further investigation in a larger cohort.

4.4

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 centers 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 36h 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 electrographic 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.

4.5 Conclusion

In conclusion, severely abnormal background activity on EEG at 36h and 48h 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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Table 1. Characteristics of all included infants.

	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)
HIE (Sarnat)[45] grade, 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

Table 2. EEG, MRI and Outcome Findings.

Case	Received bumetanide	HIE (Sarnat)[45] grade	MRI findings	EEG grade 24h	Sedatives/AED < 24h	EEG grade 36h	Sedatives/AED 24-36h	EEG grade 48h	Sedatives/AED 36-48h	Duration EEG recording (h)	Seizure number	Seizure onset (h after birth)	Seizure period (h)	Seizure duration (sec)	Seizure burden (min)	Outcome	Type of sequela
1	No	2	Poor quality MRI	3	Morphine. Phenobarbital	2	Morphine	2	Morphine	77.45	0	NA	0	0	0	Normal	
2	No	2	No injury	Poor quality EEG	Morphine. Phenobarbital	Poor quality EEG	NR	Poor quality EEG	NR	72.02	0	NA	0	0	0	Normal	
3	No	NR	No injury	2	Morphine. Phenobarbital	NA	NA	NA	NA	8.00	0	NA	0	0	0	Normal	
4	Yes	2	No injury	2	Morphine. Phenobarbital	1	Morphine	1	Morphine	85.45	1	19.3	0	19.0	0.3	Normal	
5	No	2	No injury	1	Phenobarbital	1	No	1	No	79.32	3	11.5	0.4	88.0	4.4	Lost to follow-up	
6	No	2	No injury	2	Phenobarbital	1	No	1	No	26.75	1	28.1	0	51.0	0.9	Normal	
7	No	2	No injury	2	Morphine. Phenobarbital	1	NR	1	NR	86.52	0	NA	0	0	0	Normal	
8	Yes	2	No injury	3	Morphine. Phenobarbital	2	Morphine. Phenytoin	2	Morphine	85.63	10	12.5	15.5	87.7	14.6	Normal	
9	No	2	No injury	3	Morphine. Phenobarbital	NA	Morphine	NA	Morphine	8.00	0	NA	0	0	0	Normal	
10	Yes	2	WM/WS mild	2	Morphine. Phenobarbital	1	Morphine	1	Morphine	86.48	4	7.0	4.4	784.3	52.3	Normal	
11	Yes	2	WM/WS mild	3	Morphine. Phenobarbital	2	Phenytoin	2	Phenobarbital	76.15	6	15.5	3.9	101.5	10.2	Moderate	Motor. communication
12	No	2	WM/WS mild	2	Morphine. Phenobarbital	2	Morphine	2	Morphine	93.48	0	NA	0	0	0	Moderate	Motor
13	Yes	2	WM/WS mild	2	Morphine. Phenobarbital	2	Morphine	2**	Morphine. Midazolam	104.68	5	13.7	31.5	12.8	1.1	Normal	
14	No	2	WM/WS mild	2	Morphine. Phenobarbital	2	Morphine	2	Morphine	120.07	4	4.4	2.9	658.8	43.9	Normal	
15	No	NR	BGT mild	3	Morphine. Phenobarbital	2	NR	1	NR	91.52	0	NA	0	0	0	Normal	
16	No	2	WM/WS mod-sev	1	Phenobarbital	1	NR	1	NR	48.87	4	12.3	14.5	454.0	30.3	Lost to follow-up	
17	Yes	2	WM/WS mod-sev	2	Phenobarbital	Poor quality EEG	No	Poor quality EEG	No	41.63	19	17.0	11.3	101.3	32.1	Moderate	Hearing
18	Yes	2	WM/WS mod-sev	3	Morphine. Phenobarital Midazolam Phenytoin Phenobarbital	3	Morphine	2	Morphine	61.77	64	6.0	41.8	278.3	296.8	Severe	CP
19	Yes	2	BGT mod-sev	2	Morphine. Phenobarbital	2	Morphine. Phenobarbital. Phenytoin	2	Morphine. Midazolam	10.17	63	23.2	10.9	51.3	53.8	Lost to follow-up	
20	Yes	3	BGT mod-sev	2	Morphine. Phenobarbital	2	Morphine. Phenobarbital	2	Morphine. Phenobarbital	59.93	11	22.6	2.7	115.6	21.2	Died	
21	Yes	3	BGT mod-sev	4	Morphine. Phenobarbital. Midazolam	3	Morphine. Phenobarbital. Midazolam	3	Morphine. Midazolam. Phenobarbital	82.87	44	17.6	24.2	128.3	94.1	Severe	CP
22	Yes	3	BGT mod-sev	3	Morphine Phenobarbital	3	Morphine Phenobarbital	3	Morphine Phenytoin	57.53	34	26.2	15.3	76.9	43.6	Moderate	Motor. communication
23	Yes	3	BGT mod-sev	4	Morphine. Phenobarbital	4	Morphine. Midazolam. Lidocaine	3	Morphine	70.77	30	13.3	34.1	54.9	27.5	Died (redirection of care)	
24	No	3	Near-total	4	Morphine. Phenobarbital	NA	NA	NA	NA	59.43	5	21.6	3.7	754.6	62.9	Died (redirection of care)	

25	No	3	Near-total	4	Morphine. Phenobarbital	4	NR	NA	NR	23.80	0	NA	0	0	0	Died (redirection of care)	
26	No	3	Near-total	4	Morphine. Phenobarbital	4	NR	4	NR	43.17	0	NA	0	0	0	Severe	CP

BGT=basal ganglia/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

**EEG grade assessed at 46h after birth. since bolus of midazolam at 47h impacted on EEG grade at 48h

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

	EEG 36h (n=18)[*]	EEG 48h (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
Efficiency (%)	83	76	74	91

[*]5 infants did not have an EEG available at 36h. 3 infants were lost to follow-up. [**]6 infants did not have an EEG available at 48h. 3 infants were lost to follow-up. [$]1 EEG was unreportable. 3 infants were lost to follow-up. [#]1 MRI was unassessable. 3 infants were lost to follow-up

Figure 1. Association between EEG Parameters (background activity and seizure burden). MRI and Outcome. BGT=basal ganglia/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 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).

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

Figure 3. Correlation between the MRI scoring system and the BSITD-III motor (A) and cognition composite score (B). A value below 70 (-2 SD) for both the BSITD-III motor and cognition 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).