Neonatal neuroimaging and neurophysiology predict infantile onset epilepsy after perinatal hypoxic ischemic encephalopathy

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Highlights

- 50% of survivors of severe neonatal HIE developed epilepsy by age 1 year
- Poor neonatal neurophysiology and MRI predict infantile onset epilepsy after HIE
- Frequent spikes in EEG at 4-8 weeks age predict infantile onset epilepsy after HIE

ABSTRACT

Purpose

To evaluate the accuracy of hypoxic ischemic encephalopathy (HIE) grade, and neonatal neurophysiological and neuroimaging measures for predicting development of infantile spasms syndrome (IS) or other postneonatal, infantile onset epilepsy after perinatal HIE.

Methods

We examined a population-based cohort of 92 consequent infants with moderate-to-severe HIE. The HIE grade and neonatal neuroimaging (MRI) and neurophysiology (EEG and somatosensory evoked potentials, SEPs) findings were compared to the development of IS or other epilepsy within the first year of life.

Results

Out of 74 surviving infants with follow-up information, five developed IS and one developed a focal onset epilepsy. They all had recovered from severe HIE. All survivors with inactive neonatal EEG (recorded within the first few postnatal days, n=4) or the most severe type of brain injury in MRI (n=3) developed epilepsy (positive predictive value, PPV 100%). Bilaterally absent SEPs had 100% sensitivity and 75% PPV for epilepsy. A combination of

absent SEPs and a poor MRI finding (combined deep and cortical gray matter injury) resulted in higher PPV (86%) without lowering sensitivity (100%). Follow-up EEGs showed recurrent epileptiform activity already between 1- and 2-months age in those that developed epilepsy, distinguishing them from those surviving without epilepsy.

Conclusions

Poor neonatal neuroimaging and neurophysiological findings provide accurate prediction for development of infantile onset epilepsy after HIE. Of the neonates with severe HIE, the ones with severe neonatal MRI and neurophysiological abnormalities need frequent follow-up, including repeated EEGs, for early detection of IS.

Keywords

Infantile spasms syndrome (IS), perinatal hypoxic ischemic encephalopathy, electroencephalography (EEG), somatosensory evoked potentials (SEPs), magnetic resonance imaging (MRI)

Abbreviations

aEEG, amplitude-integrated electroencephalography; EEG, electroencephalography; HIE, hypoxic-ischaemic encephalopathy; IBI, interburst interval; IS, infantile spasms syndrome; MRI, magnetic resonance imaging; NPV = negative predictive value, PPV = positive predictive value, SEP, somatosensory evoked potential; TH, therapeutic hypothermia

INTRODUCTION

Hypoxic-ischemic encephalopathy (HIE) due to perinatal asphyxia occurs in approximately 2,5 per 1000 live full-term births[1], and is one of the leading causes of neonatal deaths and severe developmental and neurological compromise[2]. HIE is also one of the most common causes of infantile spasms syndrome (IS)[3] accounting for 8-10% of all IS cases[4,5]. HIE is currently treated with therapeutic hypothermia, which despite its favorable effect on the overall outcome, does not affect the rate of postneonatal epilepsy[6–8] or IS[9]. Prediction of later epilepsy after perinatal HIE is, however, not straightforward, because the incidence of epilepsy may range from 0 to 100%, depending on the severity of HIE and/or the cohort being studied[9–12]. Yet, early diagnosis and control of IS are considered the key to a better prognosis[13–15].

In search for early diagnosis, several recent studies have attempted to identify predictive markers of later development of IS or other early onset epilepsies. Prior studies show that IS is associated with wide-spread structural abnormalities seen in magnetic resonance images (MRI)[12,16], or with a prolonged depression of cortical activity seen in the electroencephalogram (EEG)[17]. The only available study in consecutive cooled neonates showed a clear correlation between the severity of injury in MRI and the probability of developing IS, but could not unambiguously identify all infants that were to develop IS[12].

Recent works from our[18] and other centers[19] have supported the intuitively acceptable notion that a combination of early structural (MRI) and functional (neurophysiology) measures may provide better prediction of outcomes than either method alone. Here, we set out to study in a population-based cohort whether onset of IS or other epilepsy during the first year of life could be predicted at individual patient level with high precision already during the first few

days of life by combining information available from the routinely available clinical, neuroimaging and neurophysiological measures. Precise individual level prediction of which neonates will develop IS after HIE would not only enable earlier diagnosis and treatment, but also attempts to prevent IS, which could have beneficial effects on the neurodevelopment of these infants.

PATIENTS AND METHODS

Patients (Table 1)

We first identified all neonates (n=100; 48 females) that were born at >36 gestational weeks and were treated for moderate or severe HIE at the Helsinki University Hospital between January 2011 and December 2016. We chose the 36-weeks gestational age limit based on previous publications on perinatal HIE[6]. An experienced neonatologist (MM) reviewed each newborn's medical records to determine the HIE grade[20] according to the the worst situation during the first four days of life. The Helsinki University Hospital is the only tertiary level referral center providing therapeutic hypothermia in the specific catchment area of Helsinki and Uusimaa, and hence the study cohort represents a birth cohort of N=122 647 during the six-year study period from 2011 to 2016.[21]

Initial exclusion criteria were diagnosed or suspected genetic abnormalities, inborn errors of metabolism, or major anatomical malformations. Further eight infants were excluded due to absent EEG and/or MRI information, resulting in the final study group of 92 neonates. Institutional Research Review Board at Helsinki Children's Hospital approved the study, including waiver of consent due to the retrospective and observational nature of the study. Some of the neonates were included in previous publications[18,22,23].

Of the 92 neonates, 76 received whole-body therapeutic hypothermia (target temperature 33-34 degrees Celsius) for 72 hours as part of their treatment strategy. Cooling was initiated according to the criteria of the TOBY trial[24]. Cooling was initiated without a preceding amplitude-integrated EEG (aEEG) recording if aEEG was not available at the moment. Sixteen neonates did not fulfill the criteria for cooling. The neonates received antiepileptic drugs according to hospital protocols for seizures detected in aEEG monitoring. Antiepileptic drugs used in the neonatal period were discontinued after seizure control or at discharge in all but one newborn.

Decisions on withdrawal of treatment

Decisions to withdraw intensive care were based on a combination of poor clinical condition including severe HIE, poor EEG, and severe MRI findings. Decisions were made after discussions with parents.

Neonatal neurophysiological recording

All neurophysiological and neuroimaging studies were performed according to hospital guidelines. The neonatal EEG and somatosensory evoked potentials (SEPs) were recorded according to clinical need at a median age of 82 hours (IQR 58 hours) following our in-house developed clinical routines[18,25,26]. We collected the EEG and SEP signals at 2000 Hz using the NicoletOne EEG system (Cardinal Healthcare/Natus, USA; acquisition bandwidth 0.053 to 500 Hz), Cz reference, and 21 channel EEG caps (sintered Ag/AgCl electrodes; Waveguard, ANT-Neuro, Germany). An additional electrode over the C7 vertebra detected the cervical SEP. The length of the recording (73 min ± 25 min) was determined by clinical need. We stimulated each median nerve at the wrist at 0.5 (n=27) or 1 Hz (n=64) rates using two disk electrodes and a battery powered portable electrical peripheral nerve stimulator

(Micromed Energy Light stimulator; Micromed, Italy) and pulse width of 0.2 ms. If standard stimulation was not possible due to intra-arterial lines, we stimulated the median nerve at the palm or elbow. The stimulation current was individually adjusted to just above the motor threshold. Two neonates only underwent unilateral median nerve stimulation because of Erb's paresis on the other side. Four neonates did not undergo SEPs.

Neonatal EEG background grading

Two EEG experts (PN and VM) blinded to the clinical information (except the gestational and postnatal ages) independently scored all neonatal EEGs for background pattern. In case of disagreement, a third expert (LL) scored the given data, and the final score was reached by consensus. The neonatal EEG scores were modified[18] from previously described criteria[27]: grade 4 = inactive trace (background activity <10 μV or severe discontinuity with interburst interval (IBI) > 60 s), grade 3 = severe abnormality (discontinuous activity with IBI 10 — 60 s, severe attenuation of background patterns, no sleep-wake cycle), grade 2 = moderate abnormality (sleep-wake cycling present, discontinuous activity in quiet sleep with IBI <10 s, or clear asymmetry or asynchrony), grade 1 = mild abnormality (continuous activity with slightly abnormal activity: e.g., mild asymmetry, or mild voltage depression), and grade 0 = normal.

Analysis of SEPs

As described in detail before[18,23,26], we averaged the neonatal EEG-SEPs offline in BESA® software (BESA GmbH, Germany) for epochs from –100 to 800 ms relative to stimulus onset without further filtering. An experienced clinical neurophysiologist (PN) blinded to the clinical information visually evaluated all SEPs using bipolar montages and electrical field maps. In case of disagreement with the original clinical report another clinical

neurophysiologist (LL) evaluated the given SEPs and consensus was reached through discussion.

Cervical SEP was identified in the neck electrode (C7, referenced to Fz) between 8 and 20 ms. Cortical SEP was observed as a salient response beginning within 100 ms from the stimulation in the contralateral centroparietal area coupled with a topographic pattern of electrical field that indicated a source at the contralateral primary somatosensory cortex i.e. a parietal negativity paired with a frontal positivity. SEPs were classified as bilaterally absent, unilaterally absent, or bilaterally present.

Follow-up EEGs

The follow-up EEGs were done according to clinical need at variable time points between 1-month and 1-year ages. We only included follow-up EEGs recorded at >44 weeks postmenstrual age. In the follow-up recordings, the EEG signals were collected at 250 Hz using Cz reference and 19 electrodes placed according to the international 10-20 system (NicoletOne EEG system, Cardinal Healthcare/Natus, USA). Two experienced clinical neurophysiologists (PN and LL) reviewed the follow-up EEGs (and videos of suspected seizures) and classified them according to the following criteria developed by the authors and based on their clinical experience and previous studies[28-30]: Score 3) IS: defined as hypsarythmia with or without a typical series of infantile spasms, or modified hypsarythmia or multiple independent spikes with a typical series of infantile spasms; Score 2) recurrent epileptiform activity defined as three or more 30-s episodes during sleep with i) ≥ 5 epileptiform discharges from at least two foci or ii) ≥ 10 epileptiform discharges from one focus, and not fulfilling the criteria for score 3; Score 1) not fulfilling criteria for score 2 or 3.

As response to treatment was beyond the scope of this study, we did not review further follow-up EEGs performed after an EEG that was diagnostic of IS (Score 3).

Magnetic resonance imaging (MRI)

All 92 neonates of the final study group underwent brain MRI with a 1.5Tesla scanner (89 neonates; Philips Intera Achieva, Philips Medical Systems, Best, The Netherlands) or a 3Tesla scanner (3 neonates; Siemens Magnetom Skyra, Siemens Healthcare GmbH, Erlangen, Germany) between 1 and 16 days age (median 5 days). The imaging protocol included T1-weighted axial, T2-weighted axial and coronal, and diffusion weighted axial images. An experienced pediatric neuroradiologist (author STS) blinded to the clinical information classified the MRIs into six categories according to previously published criteria[31]. Score 0 = normal. 1A = minimal cerebral lesions. 1B = more extensive cerebral lesions alone (no involvement of basal ganglia, thalamus or anterior or posterior limb of the internal capsule, and no area of watershed infarction). 2A = any involvement of the basal ganglia, thalamus, anterior or posterior limb of the internal capsule or watershed infarction (no other cerebral lesions). 2B = 2A + additional cerebral lesions. 3 = cerebral hemispheric devastation.

Outcome

The primary outcome measure was diagnosis of IS within the first year of life, and the secondary outcome measure was diagnosis of any epilepsy within the first year of life. The outcomes were determined by retrospective review of the medical records from follow-up visits to a neuropediatrician and re-review of follow-up EEGs when available (see paragraph 2.7.). As the follow-up visits were part of standard care, the neuropediatricians had access to all perinatal test results.

Statistics

We performed the statistical analysis with IBM SPSS Statistics software v. 23 (SPSS Inc, Chicago, IL, USA). We used the X^2 test or Fisher's exact test to compare the expected and observed frequencies. For between-groups comparisons we used one-way ANOVA, Mann-Whitney U test, or Kruskal-Wallis test depending on the number of groups and type (continuous vs. ordinal) of the data. Normality of continuous data was tested with Kolmogorov-Smirnov-test. For level of statistical significance, we chose $p < 0.05$. Finally, we calculated the accuracy, sensitivity, specificity, and positive (PPV) and negative predictive values (NPV), including 95% confidence intervals, for the prespecified clinical, neurophysiological and neuroimaging findings and their combinations to predict development of IS or other epilepsy within the first year of life.

RESULTS

Of the 92 included neonates, six were lost to follow-up and 12 died in the neonatal period (Figure 1, Table 1 and 2), leaving 74 survivors in the final analysis. Five of them (7%) developed IS (at a mean age of 3.8 months (SD 1.0 month), range 2- and 4.5 months), and one (1%) developed focal onset epilepsy (at 5-months age) within the one-year follow-up period. There were no significant group differences in the baseline characteristics between the neonates that developed epilepsy and those that survived without epilepsy (Table 1). However, the infant that developed focal onset epilepsy was the only one on antiepileptic drugs (levetiracetam) continuously from the neonatal period onwards.

Severe HIE, absence of SEPs, poor EEG and MRI scores were all significantly associated with developing postneonatal epilepsy ($p < 0.001$ for all measures, Table 2). Table 3 presents

the sensitivity, specificity, PPV, and NPV of different clinical, neurophysiological and neuroimaging parameters and their combinations for predicting the development of IS or other epilepsy within the first year of life. All the six neonates that developed epilepsy had severe HIE (more details in Table 4). Of the individual neurophysiological and neuroimaging markers, the overall accuracy was highest (97%) for inactive EEG (sensitivity 67%, PPV 100%) and bilaterally absent SEPs (sensitivity 100%, PPV 75%). When combined with severe HIE, the basal ganglia/thalamic and cortical injury in MRI (grades 2B and 3) also had 97% accuracy, 100% sensitivity and 75% PPV. Of all combinations, the accuracy was highest for bilaterally absent SEPs and simultaneous combined basal ganglia/thalamic and cortical injury in MRI (six out of seven developed epilepsy: accuracy 98%, sensitivity 100%, PPV 86%).

Follow-up EEGs between 2-weeks and 1-year age were available for a re-review in 9/12 infants with severe HIE (five of the six that developed epilepsy) and 25/62 with moderate HIE (median 2 recordings per infant, range 1 – 4; mean postnatal age at the earliest follow-up EEG 3.1 months, SD 1.5 months; Figure 2). The EEG did not normalize at any point in any of the infants that developed IS or focal onset epilepsy during infancy. In the infants that developed IS, the EEGs acquired after the neonatal period but within the first two months all already showed recurrent epileptiform activity (score 2). Likewise, all infants with such EEG finding between one- and two-months age developed epilepsy. By 2 to 5 months, the EEGs of these infants already showed findings diagnostic of IS (score 3). The infant with focal onset epilepsy did not undergo an EEG between 2- and 8-months age, but at 2- and 8-months age the EEG showed recurrent epileptiform activity (score 2, multifocal). On the contrary, in those infants that did not develop epilepsy, the follow-up EEGs were normal already from

early on; Only one of such infants had recurrent epileptiform activity (score 2) in his/her second follow-up EEG at 5-months age (Figure 2).

DISCUSSION

Our data show that HIE grade, neonatal MRI and neurophysiological findings and their combinations are all useful for evaluating which newborns affected by perinatal HIE will develop IS or other early postneonatal epilepsy. Recurrent epileptiform activity in follow-up EEGs during the first two postnatal months also distinguished those developing early epilepsy from those surviving the first year without epilepsy.

Incidence of IS and postneonatal epilepsy after moderate-to-severe HIE

In general concordance with recent literature on moderate-to-severe perinatal HIE, we found an overall incidence of 7% for IS (vs. 10%[9]) and 8% for any postneonatal epilepsy within the first year of life (vs. 6%[32]). Our study also clearly showed a distinction in the incidence of postneonatal epilepsy between neonates with moderate (0%) vs. severe (50%) HIE similar to prehypothermia era studies (moderate HIE 0-7% vs. severe HIE 60-100%)[10,11] and one study including both TH and normothermia treated neonates (moderate HIE 2% vs. severe HIE 20%).[33]

Neonatal MRI in prediction of IS after moderate-to-severe perinatal HIE

Previous studies showed that the combination of basal ganglia/thalamic and cortical injury, or total brain injury in MRI are associated with IS[12,16], whereas cortical injury without subcortical gray matter injury is associated with a low risk for short-term postneonatal epilepsy[12]. Our results from a population-based cohort are in full agreement with these previous findings in more selected cohorts as all neonates that developed IS or focal onset

epilepsy had either total brain injury including cortical and subcortical structures (MRI score 3) or basal ganglia/thalamic injury with associated cortical injury (MRI score 2B). Particularly, total brain injury seems to be highly predictive of epilepsy as all neonates with such finding developed early onset epilepsy in the present study as well as a previous study.[12]

Neonatal neurophysiology in prediction of IS after moderate-to-severe perinatal HIE

There is surprisingly little information on the predictive value of neonatal neurophysiological examinations to predict epilepsy after HIE. One small study showed in a selected cohort of 17 neonates treated at normothermia that prolonged EEG depression was predictive of IS[17]. Our study in a large, population-based cohort significantly extends these findings by showing that an inactive EEG background within the first few days was associated with postneonatal infantile onset epilepsy in all four survivors, and that bilaterally absent SEPs were able to depict all the six neonates that developed epilepsy with only two false positives. The high specificity of an inactive EEG for early epilepsy must be considered cautiously, however, as some of the EEGs in our study were recorded within the first two days of life. In general, a poor EEG/aEEG is only considered to be predictive of a poor outcome (usually defined as death, severe motor or cognitive disability, or severe epilepsy) if it fails to recover within the first postnatal day in normothermia treated[27] or within 36-48 hours in hypothermia treated newborns[19,34–37]. In our previous study, however, all newborns with an inactive EEG and bilaterally absent SEPs had a poor outcome even if the recordings were done within the first 48 hours after birth.[18] Hence, we suggest that the findings of the present study are interpreted so that an inactive neonatal EEG is highly predictive of infantile onset epilepsy at least when associated with absent SEPs.

A previous study found neonatal status epilepticus to be an independent risk factor for postneonatal epilepsy[33], whereas in another study neonatal seizures did not retain significant association with IS after adjusting for HIE severity (mild vs. moderate-to-severe[9]). We decided not to evaluate the possible effect of neonatal seizures or status epilepticus, as the EEGs included in our study were of a relatively short duration (mean 73 min), which would not give a truthful estimation of the seizure burden.

Value of combining different neonatal predictors

To our knowledge, only one study has combined neonatal clinical, neurophysiological and neuroimaging findings to predict development of epilepsy in TH treated neonates with HIE.[38] They identified pH<6.8, burst-suppression EEG at 72 h, and deep gray matter injury in MRI as predictive factors for later epilepsy and showed that the more predictive factors a patient had the higher was the risk for developing epilepsy. They, however, extracted the tested features from medical charts instead of using well-defined and replicable classification systems of EEG and MRI, thus limiting the parameters that could be tested. They also did not consider IS as a separate entity. When using our classification systems, both neurophysiological and imaging findings were accurate predictors of early epilepsy in neonates with severe HIE, and combining them resulted in correct negative prediction in only one more patient. Hence, at least when combined with HIE grade either method (neurophysiology or neuroimaging) is probably sufficient to identify the neonates at greatest risk for early epilepsy that require more intensive follow-up.

EEG evolution towards IS

To evaluate whether follow-up EEGs could be useful for identifying the infants developing towards epilepsy we designed a follow-up EEG classification system based on our clinical

experience as well as the few previous studies[28-30] available. We chose multifocal/focal spiking as the criteria for Score 2 instead of modified hypsarythmia -level findings[29] to catch the highest-risk infants as early as possible, since the latency from the first EEG changes to spasms may be very short, especially in HIE infants.[28] In the infants that developed early postneonatal epilepsy, the initially highly abnormal EEGs never normalized completely after the neonatal period. Instead, all of these infants had recurrent epileptiform activity already in the earliest follow-up recordings between 1 and 2 months, followed by findings diagnostic of IS between 2 and 5 months. Importantly, recurrent epileptiform activity (score 2) was only seen in one of the infants surviving without epilepsy indicating that the proposed criteria could be useful for HIE follow-up as they were both sensitive and specific.

Comparing to earlier studies is not straightforward as there is no unified system for the EEG scoring. Still, our results seem to be highly concordant with previous studies[28,39] reporting that the EEGs of those HIE neonates that later developed IS, continued to have background abnormalities (e.g. discontinuity in quiet sleep) at age 1–2 months, and then multifocal discharges at 2–3 months, followed by hypsarrhythmia most frequently at 4–5 months. As the interval between appearance of spikes and hypsarrhythmia may be short[28,39], the neonates at greatest risk of IS based on neonatal neurophysiology/neuroimaging need frequent EEG follow-ups to enable early diagnosis and treatment of IS.

Limitations and strengths of the study

Though our data was collected from a relatively large tertiary level university hospital over six years, the study includes a fairly low number of neonates with HIE that eventually developed IS or other epilepsy. The incidences of IS and epilepsy are, however, comparable to those described for similar populations in the literature[9,32]. The low number of neonates

that developed epilepsy also resulted in somewhat large confidence intervals for the predictive values. Hence, future studies are necessary to confirm our findings in larger populations. Furthermore, as all examinations were done according to clinical need, there was variation in the timing of the neonatal tests and follow-up EEGs, and number of follow-up EEGs. Despite these limitations, however, we were able to detect very robust predictive markers for infantile onset epilepsy.

Strengths of our study are that it is population based and hence represents the whole spectrum of neonates with moderate-to-severe HIE, and as most of the newborns had their neurological and neurophysiological follow-up at our institution, we were able to determine their outcomes accurately in terms of epilepsy type unlike some previous studies relying on questionnaires.[33]

CONCLUSIONS

We demonstrate in a consequent, population-based cohort that amongst the neonates with severe HIE, those that will develop IS or other postneonatal epilepsy within the first year of life can be fairly accurately identified at individual level already in the neonatal period using neurophysiological and/or neuroimaging methods. Follow-up EEGs during the first few postnatal months further help to distinguish those developing towards early postneonatal epilepsy from those surviving the first year without epilepsy. Our results help in informing parents and in planning follow-ups for neonates with HIE. Importantly, IS started before the age of 5 months in all the five affected infants. Hence, frequent follow-ups including repeated EEGs are warranted particularly during the first months of life for those at highest risk. The present results also provide a foundation for studies investigating possibilities of preventing IS in neonates with HIE.

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CONFLICT OF INTEREST STATEMENT

None of the authors has any conflict of interest to disclose.

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FIGURE LEGENDS

Figure 1. Flow diagram of study inclusions. FU = follow-up.

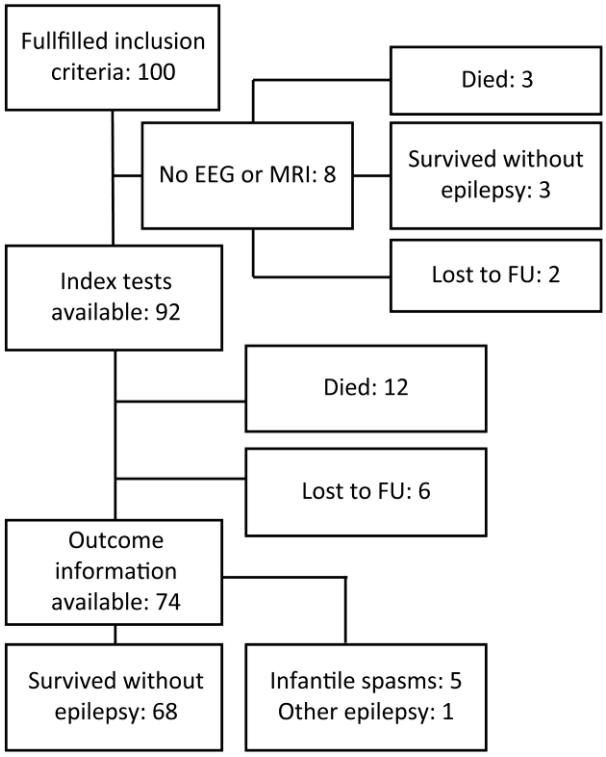

Figure 2. Follow-up EEG results. Each newborn is represented on the y-axis, and the x-axis indicates the timing of the follow-up EEGs. As the EEGs were done based on clinical need, their timing and number per infant is variable. Most infants had their first follow-up EEG within 6 months: ten infants had an EEG already before 2-months age (mean 1.6 months, SD 0.2 months), and 22 infants had their first EEG between 2- and 6-months age (mean 3,4 months, SD 0.8 months). Only two infants had their first EEG after 6 months (at 6.8- and 7.8-months age). The colors depict the EEG findings. Black = findings diagnostic of infantile spasms syndrome (IS i.e. score 3), gray = recurrent epileptiform activity (i.e. score 2), white = not fulfilling either of the prior criteria (i.e. score 1). For a thorough explanation of the scoring system see the methods section. As response to treatment was beyond the scope of

this study, we did not review the follow-up EEGs performed after an EEG that was considered diagnostic of IS (patients 1-4).

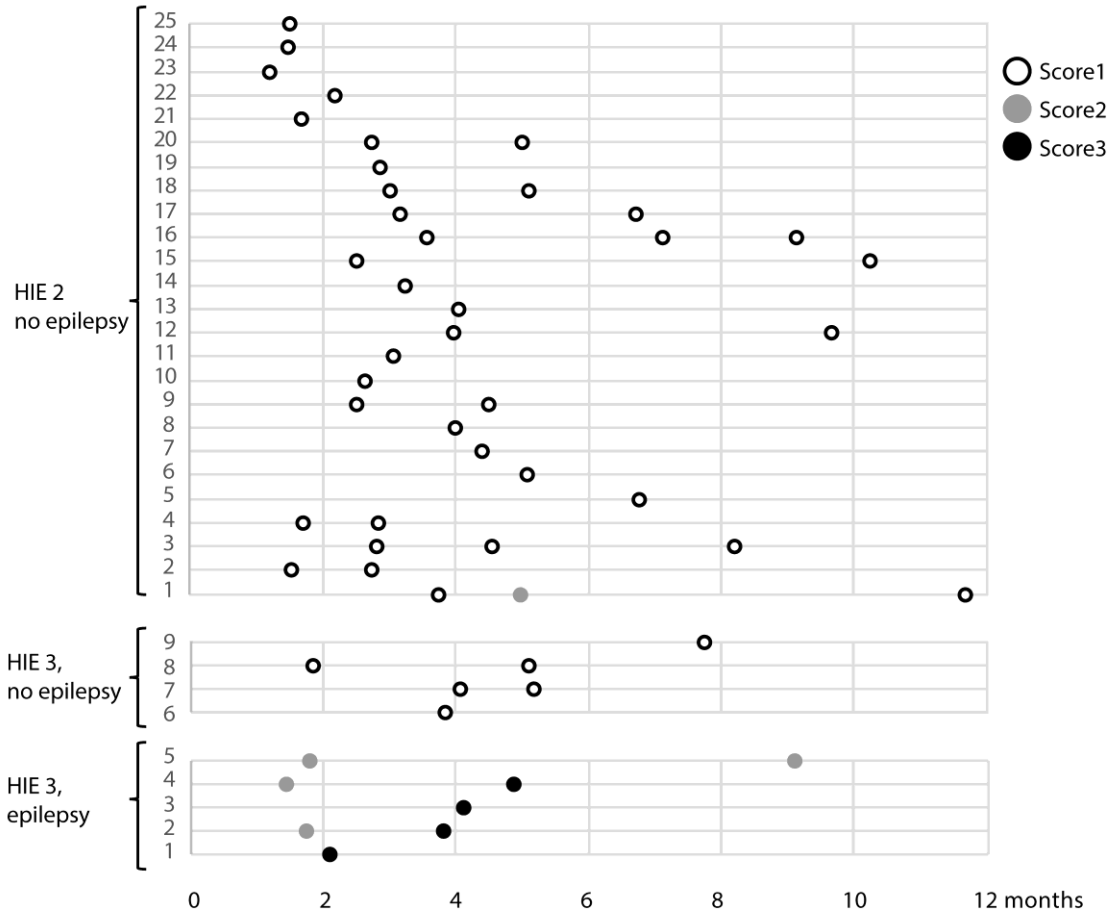

Table 1. Baseline characteristics of the study population.

Baseline characteristic	All n=92	Died n=12	Epilepsy n=6	No epilepsy n=68	Lost to FU n=6
Females	44 (48%)	5 (42%)	3 (50%)	34 (50%)	2 (33%)
Received TH	76 (83%)	10 (83%)	6 (100%)	54 (79%)	6 (100%)
GA (weeks)	39.6 [1.7]	39.8 [1.7]	40.3 [1.0]	39.6 [1.7]	39.5 [1.9]
Postmenstrual age at EEG-SEP (weeks)	40.1 [1.6]	40.0 [1.6]	40.6 [1.0]	40.1 [1.7]	40.0 [1.9]
Postnatal age at EEG-SEP (h)	81.5 {58}	40 {22}*	35.5 {78}	87 {52}	94 {33}
Postmenstrual age at MRI (weeks)	40.3 [1.7]	40.1 [1.7]	41.0 [1.0]	40.3 [1.7]	40.4 [2.2]
Postnatal age at MRI (h)	108 {61}	54.5 {21}**	99 {135}	112 {67}	138 {51}
Birth weight (g)	3400 [620]	3340 [550]	3340 [630]	3390 [620][a]	3670 [890][b]
Base excess	-13.0 [6.4]	-13.2 [11.4]	-13.5 [5.6][b]	-13.0 [5.5]	-11.70 [4.2][b]
pH	7.02 [0.17]	7.02 [0.25]	7.05 [0.13]	7.01 [0.16]	7.06 [0.14][b]
Apgar 1 min	1 {3}	0 {1}***	3 {3}	1{2}[a]	2{2}[b]
Apgar 5 min	3 {3}	0 {1}***	3 {5}	3{3}[a]	3{3}[b]
Apgar 10 min	5 {3}	1 {3}***	3.5 {5}	5{2}[a]	5{4}[b]

Data shown as n (%), mean [SD], or median {IQR}. There were no significant differences between the different outcome groups in sex, TH treatment (Chi square), GA, birth weight, BE or pH (ANOVA). *Postnatal ages at EEG-SEP ($p = 0.001$) and **MRI ($p < 0.001$) were significantly lower in the neonates that died (Kruskal-Wallis test) than in neonates surviving without epilepsy or those lost to follow-up. Post-hoc comparisons showed no difference to those developing with epilepsy. ***$p < 0.05$ Apgar scores were significantly lower in the neonates that died (Kruskal-Wallis test) than in neonates of the other groups. FU = follow-up, TH = therapeutic hypothermia, GA = gestational age, SEP = somatosensory evoked potential. a = not available for two neonates, b = not available for one neonate.

Table 2. Neurophysiological and MRI findings according to hypoxic-ischemic encephalopathy (HIE) severity and outcome.

	Table 2.	HIE 3 Total	Died	IS	Other Epi	No Epi	No FU	HIE 2 Total	No Epi	No FU
	n	26	12	5	1	6	2	66	62	4
EEG	Gr4	14	10	3	1	-	-	0	-	-
	Gr3	8	2	2	-	4	-	10	10	-
	Gr2-0	4	-	-	-	2	2	56	52	4
SEP	Bilaterally absent	18	10	5	1	2	-	0	-	-
(n=88*)	Unilaterally absent	1	1	-	-	-	-	1	1	-
	Present	6	1	-	-	4	1	62	58	4
MRI	3	8	5	2	1	-	-	-	-	-
	2B	11	6	3	-	2	-	6	6	-
	2A, 1A/B, 0	7	1	-	-	4	2	60	56	4

Data shown as n.

FU = follow-up, Epi = epilepsy, Gr = grade, IS = infantile spasms syndrome, SEP = somatosensory evoked potential.

* SEP not available in four

Table 3. The sensitivity, specificity, positive (PPV) and negative predictive values (NPV) with 95% confidence intervals of different clinical, neurophysiological and neuroimaging parameters and their combinations for predicting the development of epilepsy (infantile spasms syndrome (IS) or other epilepsy) within the first year of life.

	n 74	IS	Other epilepsy	Accuracy	Specificity	Sensitivity	PPV	NPV
HIE III	12	5	1	92 [83-97]	91 [82-97]	100 [54-100]	50 [32-68]	100 [-]
MRI 3	3	2	1	96 [89-99]	100 [95-100]	50 [12-88]	100 [-]	96 [91-98]
MRI 2B/3	14	5	1	89 [80-95]	88 [78-95]	100 [54-100]	43 [28-59]	100 [-]
EEG gr4	4	3	1	97 [91-100]	100 [95-100]	67 [22-96]	100 [-]	97 [92-99]
EEG gr3-4	16	5	1	86 [77-93]	85 [75-93]	100 [54-100]	38 [25-52]	100 [-]
SEP* bilaterally absent (all HIE III and EEG gr3-4)	8	5	1	97 [90-100]	97 [89-100]	100 [54-100]	75 [43-92]	100 [-]
HIE III & MRI 2B/3	8	5	1	97 [91-100]	97 [90-100]	100 [54-100]	75 [43-92]	100 [-]
HIE III & EEG gr3-4	10	5	1	95 [87-99]	94 [86 98]	100 [54-100]	60 [37-80]	100 [-]
MRI 2B/3 & SEP -/- (n=71)	7	5	1	99 [92-100]	98 [92-100]	100 [54-100]	86 [46-98]	100 [-]

Gr = grade, HIE = hypoxic-ischemic encephalopathy, SEP-/- = bilaterally absent somatosensory evoked potentials

* SEP not available in three of the 74 infants

Table 4. Details of the neurophysiological and neuroimaging findings of the patients with severe hypoxic ischemic encephalopathy (HIE).

Pat	EEG gr	SEP	Post-natal age at EEG-SEP [h]	CNS medication during EEG-SEP	MRI	Post-natal age at MRI [h]	Outcome epilepsy	Outcome other	Age at follow-up (mo)
1	4	-	81	PBT	3	83	IS	Dyskinetic CP (GMFCS V, CFCS V)	23
2	4	-	27	PBT, LEV	2B	35	IS	Spastic tetraplegic CP (GMFCS V, CFCS IV)	27
3	4	-	11	-	3	297	IS	Diplegic CP (GMFCS IV, CFCS IV)	25
4	3	-	44	(PBT), FEN	2B	115	IS	Severe CP (GMFCS V, CFCS IV-V)	21
5	4	-	23	(PBT), LEV, FEN	3	54	Epilepsy	Spastic tetraplegic CP (GMFCS V, CFCS IV-V)	28
6	3	-	149	(PBT)	2B	147	IS	Severe CP (GMFCS V, CFCS V)	30
7	2	+	56	PBT, LEV, MID, FEN	1B	91	No	Normal	12
8	3	+	104	PBT, FEN, DEX	2B	84	No	Spastic diplegic CP (GMFCS IV, CFCS IV)	27
9	3	-	85	PBT, LEV, MID, FEN	2B*	88	No	Spastic diplegic CP (GMFCS III, CFCS IV)	23
10	1	+	185	(PBT), FEN	1A**	383	No	Mild delay in motor skills	24
11	3	-	83	(PBT), MO	2A	130	No	Moderate dyskinetic CP (GMFCS II)	24
12	3	+	50	PBT, LEV, MID, FEN	2A	101	No	Spastic hemiplegic CP l.sin. (GMFCS I, CFCS III)	24

CNS = central nervous system, CP = cerebral palsy, SEP - / + = somatosensory evoked potentials absent / present, PBT = phenobarbital, (PBT) = loading dose of phenobarbital > 8 hours prior to EEG, FEN = phenytoin, MID = midazolam, LEV = levetiracetam, DEX = dexmedetomidine, MO = morphin, GMFCS = Gross Motor Function Classification System, CFCS = Communication Function Classification System. *In addition, right parietal epidural hemorrhage **Small hemorrhage in right caudothalamic sulcus and ventricle.