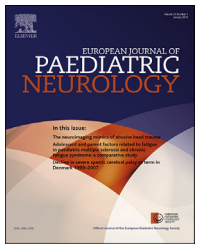
Official Journal of the European Paediatric Neurology Society



Original article

Electroencephalography and brain magnetic resonance imaging in asphyxia comparing cooled and non-cooled infants

Leen ATT. De Wispelaere [a,b,*], *Sabine Ouwehand* [a], *Marielle Olsthoorn* [c], *Paul Govaert* [a], *Liesbeth S. Smit* [a,d], *Rogier CJ. de Jonge* [a,e], *Maarten H. Lequin* [f], *Irwin K. Reiss* [a], *Jeroen Dudink* [a,g]

[a] *Department of Pediatrics, Division of Neonatology, Erasmus MC-Sophia Children's Hospital, Wytemaweg 80, 3015 CN, Rotterdam, the Netherlands*

[b] *Department of Neonatology, University Hospital Antwerp, Wilrijkstraat 10, 2650 Edegem, Belgium*

[c] *Department of Radiology, Erasmus MC-Sophia Children's Hospital, Wytemaweg 80, 3015 CN, Rotterdam, the Netherlands*

[d] *Department of Neurology, Erasmus MC-Sophia Children's Hospital, Wytemaweg 80, 3015 CN, Rotterdam, the Netherlands*

[e] *Department of Neonatology, VU University Medical Center, De Boelelaan 1105, 1081 HV, Amsterdam, the Netherlands*

[f] *Department of Radiology, Wilhelmina Children's Hospital, University Medical Center, Heidelberglaan 100, 3584 CX, Utrecht, the Netherlands*

[g] *Department of Pediatrics, Division of Neonatology, Wilhelmina Children's Hospital, University Medical Center, Heidelberglaan 100, 3584 CX, Utrecht, the Netherlands*

ARTICLE INFO

Article history:
Received 16 January 2018
Received in revised form
29 May 2018
Accepted 4 September 2018

Keywords:
Neonate
Therapeutic hypothermia
Hypoxic-ischemic encephalopathy
Neurodevelopmental outcome

ABSTRACT

Objective: The aim was to establish any differences in the predictive value of EEG and MRI for outcome in infants treated and not-treated with therapeutic hypothermia (HT) for perinatal asphyxia. We hypothesize that they are equally predictive and that combining both has the highest predictive value.

Study design: We retrospectively compared data of infants with hypoxic-ischemic encephalopathy (HIE) who received HT (n = 45) between September 2009 and December 2013 with those of infants with HIE born between January 2004 and August 2009, before HT was available (NT, n = 37). All received conventional and/or amplitude–integrated EEG during the first days and early MRI (day 4–5). Associations of EEG, MRI and severe neurodevelopmental outcome (death or Bayley's -2SD below mean), were tested with a multivariable logistic regression analysis, corrected for HT.

Results: Forty-eight hours' EEG background pattern had a PPV of 92% and a NPV of 81% in HT, versus 100% and 58% in NT. MRI had a PPV of 71% and a NPV of 93% in HT, versus 82% and 75% in NT. The adjusted OR for adverse outcome was 0.013 (95% CI 0.002–0.154,

* *Corresponding author*. Department of Neonatology, University Hospital Antwerp, Wilrijkstraat 10, 2650 Edegem, Belgium.
E-mail addresses: leendewispelaere@yahoo.com (L.ATT. De Wispelaere), 367751so@student.eur.nl (S. Ouwehand), mariel_olsthoorn@hotmail.com (M. Olsthoorn), govaert@icloud.com (P. Govaert), l.s.smit@erasmusmc.nl (L.S. Smit), r.dejonge@vumc.nl (R.CJ. de Jonge), m.h.lequin@umcutrecht.nl (M.H. Lequin), i.reiss@erasmusmc.nl (I.K. Reiss), j.dudink@umcutrecht.nl (J. Dudink).
https://doi.org/10.1016/j.ejpn.2018.09.001

p < 0.001) for EEG background normalization within 48 h and 32.19 (95% CI 4.84–214.25, p < 0.001) for abnormal MRI.

Conclusion: The predictive value of EEG and MRI is equal in cooled and non-cooled infants with HIE. Our data show a higher predictive value (death and severe outcome) for EEG compared to MRI. In HIE, persistent abnormal EEG background pattern until 48 h, combined with abnormal early MRI is strongly predictive for poor neurodevelopment.

Abbreviations

aEEG	amplitude-integrated EEG
BS	burst-suppression
BSID-II or BSID III-NL	Dutch Bayley Scales of Infant Development II or III
CI	confidence interval
CNV	continuous normal voltage
CP	cerebral palsy
cUS	cranial ultrasound
DNV	discontinuous normal voltage
EEG	electroencephalography
FT	flat trace
GMFCS	gross motor function classification system
HIE	hypoxic ischemic encephalopathy
HT	therapeutic hypothermia
LV	low voltage
MDI	mental developmental index
MRI	magnetic resonance imaging
NPV	negative predictive value
NT	normothermia
OR	Odds ratio
PDI	psychomotor developmental index
PPV	positive predictive value

1. Introduction

Hypoxic-ischemic encephalopathy (HIE) is a leading cause of newborn death and long-term disability.[1] Of the surviving children, one quarter is left with significant neurologic disability.[2] Therapeutic hypothermia (HT) reduces death rates and severe disability in term and late preterm newborns with HIE.[3] Early prediction of future neurodevelopmental outcome is important since the majority of HIE-related deaths are attributed to end-of-life decisions.[4] A combination of clinical scores and early biochemical markers has limited predictive value for outcome in HIE, the more so as HT induces changes in metabolism affecting these markers.

Electroencephalography (EEG) and neuroimaging techniques such as cranial ultrasound (cUS) and magnetic resonance imaging (MRI) can help predict outcome of neonates with HIE.[5] cUS correlates well with injury patterns seen on MRI and can be used for screening.[6,7] Since the original randomized controlled trials, no studies have compared both EEG and MRI during HT versus NT.[8,9] Our primary objective was to study differences in the predictive value of EEG and MRI for outcome in infants treated and not-treated with HT for perinatal asphyxia. We hypothesize that both are equally predictive and that combining EEG and MRI has the highest predictive value.

2. Materials and methods

2.1. Study population

A retrospective observational study was performed in which infants with HIE, admitted to the tertiary level neonatal intensive care unit of the Erasmus MC-Sophia Children's Hospital, Rotterdam, from January 2004 until December 2013, were included. Therapeutic hypothermia was implemented at our facility in September 2009. The inclusion criteria were the criteria used for the clinical indication for HT: gestational age of ≥36 weeks, perinatal asphyxia (5 min Apgar ≤5, (umbilical) blood gas <1 h after birth pH ≤ 7, BE ≥ 16, lactate ≥10 mmol/L, or resuscitation 10 min after birth), elevated encephalopathy score between 1 and 3 h postpartum (≥Thompson score 7 or Sarnat stage 2) or an abnormal EEG (discontinuous normal voltage (DNV), burst suppression (BS), low voltage (LV) or flat trace (FT)). Exclusion criteria were inborn errors of metabolism, major congenital malformations and no EEG within 48 h.

Data of infants fulfilling the criteria who did not receive cooling (NT) were compared to data of infants who received cooling (HT), independent of the era they were born in. Therapeutic HT started within 6 h after birth and consisted of 72 h of whole body cooling using the Criticool^{R} mattress (MTRE Advanced Technologies, Yavne, Israel) with target rectal temperature of 33.5 °C. The medical ethics review board of the Erasmus MC, Rotterdam, The Netherlands, approved retrospective analysis of anonymous patient data.

2.2. Patient data collection

Data were retrieved from the electronic patient data management system. Anticonvulsants were given according to the local seizure protocol, and the number of anticonvulsants needed was documented. Socio-economic status was ranked on the basis of the postal code area, as published by the Dutch National Institute for Public Health and the Environment.[10]

2.3. Electroencephalography, EEG

In children treated with HT, either conventional or two-channel EEG was started as soon as possible after birth and

maintained until the end of the rewarming phase. For the conventional EEG, nine electrode cups (Fp_{1-2}, C_z, C_{3-4}, T_{3-4}, O_{1-2}) were placed according to the restricted 10–20 system for electrode placement with a low-frequency filter set at 0.005–0.001 Hz, and a high frequency filter of 70 Hz (Nervus™ monitor, Taugagreining hf, Reykjavik, Iceland). Conventional EEG was maintained until normalization of background pattern in the NT infants. For the HT infants, conventional EEG was intermittently used together with continuous amplitude-integrated EEG (aEEG), which was introduced in the same time period as therapeutic hypothermia in our department. Amplitude-integrated EEG (BRAINZ BMR3 monitor, Natus Medical Incorporated, Pleasanton, USA) was performed by using four needle electrode or cups placed bilaterally on central and parietal locations (C_3-P_3 and C_4-P_4), and one frontal reference electrode. Impedance values were kept below 5 and 10 kOhm, respectively. Conventional EEG data were analyzed and scored by the attending clinical neurophysiologist specialized in neonatal EEG. Reports were retrospectively interpreted by researchers, and raw data were re-analyzed when necessary by a pediatric neurologist with extensive training in neurophysiology (LS). aEEG data were analyzed by two independent researchers (SO and LDW), blinded for clinical data. In eight cases of disagreement during the consensus meeting, a third investigator was consulted (LS).

The amplitude-integrated EEG background pattern was classified according to Hellström-Westas et al.[11] Conventional EEG was categorized according to the guidelines of the American Clinical Neurophysiology Society.[12] Continuous normal voltage (CNV) and discontinuous normal voltage (DNV) were considered "normal". Burst suppression (BS), low voltage (LV), flat trace (FT) or continuous seizures were classified as "abnormal" (Table 1). Seizures were defined as a sudden, repetitive and evolving EEG discharge pattern during at least 10 s. Sleep-wake cycling (SWC) was scored as present or absent.

2.4. *Magnetic resonance imaging, MRI*

Brain MRI (Signa HDx, 1.5 Tesla, General Electrics, US. Milwaukee) was performed within the first week after birth in all infants. MRI was analyzed based on T1, T2 and diffusion weighted images. Two independent investigators, a neonatologist (JD) and pediatric radiologist (ML), both with expertise in neonatal neuro-imaging, scored all MRI scans and were blinded for patient data and outcome. Afterwards a consensus was reached. The adapted Barkovich score was used, assessing basal ganglia/thalami including the myelination of the posterior limb of the internal capsule, and cortical regions including white matter and arterial watershed areas.[13] Absence of abnormalities was classified as "normal", and abnormalities were divided into "moderate" (injury of basal ganglia/thalamus or cortex), or "severe" (injury of both basal ganglia/thalamus and cortex).

2.5. *Outcome*

Outcome measures were adapted from the TOBY trial.[14] Neurodevelopmental outcome at age two years was

Table 1 – Classification of amplitude-integrated EEG and conventional EEG background patterns in infants with hypoxic-ischemic encephalopathy.

	Amplitude-integrated EEG	Conventional EEG
Normal	**Continuous normal voltage, CNV** Continuous variable activity with minimum amplitude around 5–10 μV and maximum amplitude of 10–25 (to 50) μV	Continuous mixed frequency (theta and delta activity of 70–100 μV) Trace alternant during quiet sleep with quiet periods having a higher amplitude about 25 –50 μV (compared to < 25 μV in trace discontinue), and higher voltage bursts are about 50 –150 μV
	Discontinuous normal voltage, DNV Discontinuous background with minimum amplitude variable, but below 5 μV, and maximum amplitude at least 10 μV	Slow quiet sleep with a continuous high-amplitude delta activity over all regions Trace discontinue with interburst interval < 25 μV (often <10 μV)
Abnormal	**Burst Suppression, BS** Discontinuous background with minimum amplitude without variability at 0–2 μV and bursts with amplitude > 25 μV. EEG trace shows bursts of 1–3 s alternated with low activity	Burst suppression with prolonged interburst intervals with amplitudes < 5 μV, interspersed with invariant, non-reactive bursts. No normal grapho-elements are recognizable in bursts.
	Low voltage, LV Continuous background pattern of very low voltage (around or below 5 μV)	Low voltage EEG with amplitudes persistently <10 μV
	Flat trace, FT Primarily inactive (isoelectric tracing) background below 5 μV Primarily inactive (isoelectric tracing) background below 5 μV	Primarily inactive (isoelectric tracing) background below 5 μV

assessed with the Dutch Bayley Scales of Infant Development II or III (BSID-II or BSID-III-NL).[15] This resulted in a mental developmental index (MDI) and a psychomotor developmental index (PDI) with a mean of 100 and a standard deviation (SD) of 15. Neuromotor disability was based on the presence of cerebral palsy (CP), and functional disability was graded from one to five with the Gross Motor Function Classification System (GMFCS).[15] Since BSID-III is likely to generate a higher score than BSID-II, an adjustment was made by increasing the cut-off threshold for severe disability to a score less than 85.[16] Normal outcome was defined as MDI and PDI of at least 85 and/or a mild CP (GMFCS grade 1). Moderately abnormal outcome was defined as MDI or PDI between 70 and 85 and one or more of the following: CP with GMFCS grade 2, hearing impairment, visual impairment or (treated) epilepsy. Severely abnormal outcome was defined as MDI or PDI more than 2 SD below the mean, CP with GMFCS grade 3 to 5, or refractory epilepsy and death due to asphyxia. For statistical reasons a dichotomous classification was used: normal and adverse (moderate, severely abnormal and death).

2.6. Statistics

Data are presented as number (%), and continuous data as mean (SD) for normally distributed variables or as median (interquartile range, IQR) for not normally distributed variables. Independent sample *t*-test was used for normally distributed continuous variables; Mann–Whitney U test for not normally distributed continuous variables; and Chi-square or Fisher's exact test for categorical variables. Logistic regression modelling was used to calculate odds ratios (OR) and 95% confidence intervals (CI) for the association between EEG, MRI and outcome. Multivariable logistic regression analyses were performed after identifying confounders. Two sided P-values below 0.05 were considered as statistically significant. Statistical analysis was performed using SPSS statistics 21 (IBM Corp., Armonk, New York, 2012).

3. Results

3.1. Patient characteristics

Retrospectively, 89 infants were identified with HIE and had an indication for HT. Seven patients were excluded because of inborn errors of metabolism (n = 1), congenital cardiac abnormality (n = 1), low encephalopathy score (n = 3) or EEG started >48 h postpartum (n = 2). Of the 82 infants, 37 did not receive cooling (NT group) and 45 did receive cooling (HT group). Patients in the NT group were not cooled, either because they were born before HT became available (n = 34) or were admitted too late for HT (n = 3) (Fig. 1). Table 2 shows patients' characteristics.

3.2. EEG features, MRI patterns and outcome in HT versus NT group

Table 3 summarizes EEG and MRI findings in both groups. Patients are categorized according to the evolution of EEG background pattern, MRI pattern and outcome. More to the right and the bottom of the table means longer duration of abnormal EEG background pattern in combination with more

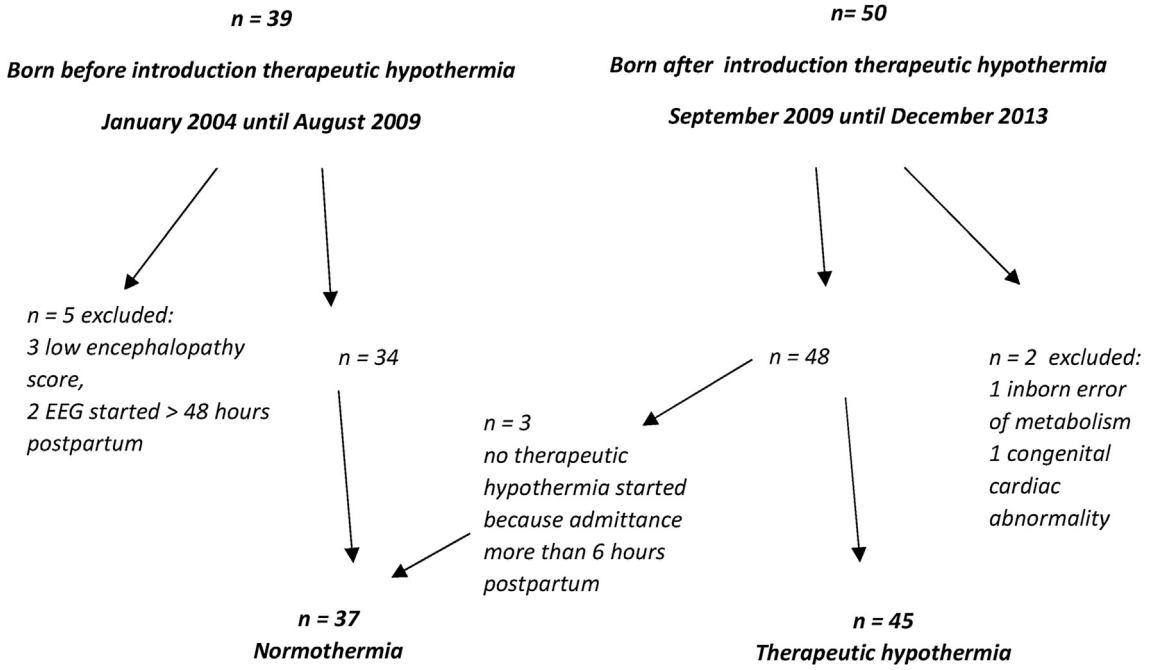


Fig. 1 – Flow chart patient enrollment.

Table 2 – Clinical characteristics of Normothermia and Hypothermia group.

	Normothermia (n = 37)	Hypothermia (n = 45)	p-value
Male gender, *n (%)*	21 (57)	22 (49)	0.63[a]
Gestational age (*weeks* $^{+\ days}$), *median (IQR)*	39^{+6} (38^{+6} – 41^{+3})	39^{+6} (38^{+1} – 40^{+4})	0.21[b]
Birth weight (*g*), *mean, SD*	3298 (477)	3294 (612)	0.97[b]
Delivery mode, *n (%)*			0.45[a]
Vaginal in hospital	4 (11)	6 (13)	
Vacuum extraction	7 (19)	10 (22)	
Emergency caesarean	19 (51)	26 (58)	
Home	7 (19)	3 (7)	
Inborn in Erasmus MC, *n (%)*	3 (8)	4 (9)	1[a]
Socio-economic status score, z-score, *SD*	−0.03 (1.17)	0.07 (1.16)	0.70[b]
Resuscitation in first 10 min, *n (%)*	24 (65)	27 (60)	0.82[a]
Apgar at 5 min, median *(IQR)*	4 (2–5) (*n* = 36)	3 (1–4) (*n* = 44)	0.33[a]
Lab results, *mean, SD*			
pH (lowest)	6.90 (0.19) (*n* = 36)	6.96 (0.24) (*n* = 43)	0.23[b]
Lactate (highest)	10.1 (6.1)	11.8 (6.2)	0.22[b]
Hypoxic-ischemic encephalopathy grade, *n (%)*[c]			
Mild	9 (24)	11 (24)	0.53[a]
Moderate	15 (41)	23 (51)	
Severe	13 (35)	11 (24)	
Type of EEG, *n (%)*			<0.001[a]
Conventional EEG	35 (95)	4	
aEEG	0	3	
Both	2	38 (83)	
Time until start EEG (hours), *median (IQR)*	11.0 (7.0–17.5)	5.5 (4.0–7.0)	<0.001[b]
Total duration EEG (hours), *median (IQR)*	61 (48–79)	87 (74–113)	<0.001[b]
Morphine use, *n (%)*	20 (54)	44 (98)	<0.001[a]
Inotropic agents use, *n (%)*	19 (51)	33 (73)	0.06[a]
No. of inotropes, median *(IQR)*	1 (0–1)	1 (0–2)	0.09[a]
Invasive ventilation, *n (%)*	31 (84)	43 (96)	0.13[a]
Time until MRI (days), *median (IQR)*	4 (4–6)	5 (4–5)	0.97[b]

Abbreviations: SD = standard deviations, IQR = interquartile range, EEG = electroencephalogram, aEEG = amplitude-integrated electroencephalogram, MRI = magnetic resonance imaging.

[a] Chi squared test or Fisher's Exact test for categorical data.

[b] Independent sample *t*-test for normally distributed continuous variables, Mann Whitney U test for not normally distributed continuous variables.

[c] According to Thompson and Sarnat.[39]

injury on MRI. Visually, more infants in HT group are positioned up and towards the left, reflecting less abnormal EEG and MRI patterns, compared to NT.

Table 4 compares EEG features, MRI injury patterns and outcome data between NT and HT.

Table 5 shows the predictive values of EEG features and MRI for adverse outcome. In both NT and HT, the PPV of EEG background pattern rises from 24 h to 48 h after the perinatal event. None of the infants of the HT group with an abnormal EEG background pattern at 48 h have a normal outcome. All infants with HIE (NT and HT) are included for the multivariable logistic regression analysis. The strongest predictors for outcome are EEG background pattern at 24 and 48 h and MRI, but not cooling. A normalizing EEG background pattern within 48 h decreases the odds for adverse outcome, regardless of cooling and adjusted for moderate/severe MRI.

4. Discussion

Multivariable logistic regression analysis was used to study differences in the predictive value of EEG and MRI for outcome in infants treated and not-treated with HT for perinatal asphyxia. The predictive value of a normal background pattern at 48 h is higher in HT than NT (81% vs. 58%), as in previous studies.[17–21] An abnormal background pattern at 48 h had a PPV for adverse outcome of 92% and 100% in NT and HT, respectively. In literature, the PPV for EEG in HT ranges from 82% to 100%.[8,19,20]

The adjusted OR for adverse outcome of a normal EEG background pattern at 48 h and abnormal MRI is respectively 0.013 and 32 to 1. In literature, no data are available to compare OR, but in a recent study, the best predictor of outcome was MRI, with EEG background pattern at 36 h second best.[8] Another study also concluded that MRI was the best predictor, followed by background EEG at one week in HT.[22] Our data suggest that EEG background pattern at 48 h has a higher predictive value than MRI in the first week of life.

The type of EEG used differs significantly between the HT and NT groups. However, the classification of background patterns correlates well in both conventional EEG and aEEG.[5,23,24] All aEEG registrations displayed a double channel conventional EEG and therefore artefacts were easily recognized. More infants in the HT group had normalizing EEG background patterns within the first 48 h compared to NT, which could be the influence of hypothermia in reducing secondary injury to the brain.

Table 3 – Evolution of EEG background pattern, MRI and outcome in Normothermia[a] and Hypothermia group[b].

	EEG normal at start[c]	EEG normalizing within 24 hours	EEG normalizing between 24 and 48 hours	EEG persistently abnormal more than 48 hours
MRI normal				
Normothermia	○○●			○
Hypothermia	□□□ □□□ □□▣	□□□□	□	
MRI moderate				
Normothermia	○○○	○	○●●	○●✶✶✶✶✶✶✶✶
Hypothermia	□▣❖	□□□□□□	□□▣❖	▣▣■❖❖❖❖❖❖❖
MRI severe				
Normothermia	✶		✶	●● ✶✶✶✶✶✶✶✶✶✶
Hypothermia				❖❖❖❖❖❖❖❖

[a] Normothermia group: n = 37, ○ normal outcome , ● severely abnormal outcome, ✶died.
[b] Hypothermia group: n = 45, □ normal outcome, ▣ moderately abnormal outcome, ■ severely abnormal outcome, ❖ died. Every symbol equals a patient.
Abbreviations: EEG = electroencephalogram, MRI = magnetic resonance imaging.
[c] Start time of EEG monitoring varied in cooled infants between 4 and 6 hours, with one patient starting only 19 hours after birth.

Table 4 – EEG background pattern evolution, seizures, number of anticonvulsants, sleep wake cycling, MRI injury and outcome/withdrawal of intensive care comparing Normothermia versus Hypothermia group.

	Normothermia (n = 37)	Hypothermia (n = 45)	p-value
EEG background evolution, *n*			0.03[a]
Normal at start	7	12	
Normalizing < 24 h	1	10	
Normalizing between 24–48 h	4	5	
Abnormal > 48 h	25	18	
Seizures[d], *n (%)*	32 (86)	34 (76)	0.08[a]
Electrographical and clinical	23 (62)	14 (30)	
Clinical only	8 (22)	14 (30)	
Electrographical only	1 (3)	6 (13)	
Number of anticonvulsants[c], *median (IQR)*	2 (2–4)	1 (1–2)	0.003[a]
Sleep-wake cycling (SWC), *n %*	10 (27)	25 (58)	0.007[a]
Median time of SWC occurrence, *hours*	51.5	49.5	0.09[b]
MRI score, *n (%)*			0.02[a]
Normal	4 (11)	15 (33)	
Moderate	17 (46)	23 (50)	
Severe	16 (43)	8 (17)	
Basal ganglia/thalamus injury, *n %*	28 (76)	27 (60)	0.16[a]
White matter/watershed lesions, *n %*	21 (57)	12 (27)	0.007[a]
Outcome, *n (%)*[d]			
Normal	9 (24)	22 (49)	0.04[a]
Moderately abnormal	0	4 (9)	
Severely abnormal	6 (16)	2 (4)	
Death	22 (60)	17 (37)	0.08[a]
Withdrawal of intensive care, *n (%)*	25 (68)	20 (44)	0.04[a]

Abbreviations: EEG = electroencephalogram, MRI = magnetic resonance imaging. SWC = Sleep wake cycling, IQR = interquartile range.

[a] Chi squared test for categorical data.

[b] Independent sample t-test for normally distributed continuous variables, Mann Whitney U test for not normally distributed continuous variables.

[c] Add-on scheme for anti-seizure treatment: 1. Phenobarbital, 2. Midazolam, 3. Lidocain and 4. Levatiracetam.

[d] Of the surviving children, 4 infants of normothermia group and 3 infants of hypothermia group developed secondary epilepsy (not significant).

Table 5 – Predictive values, crude and adjusted OR of EEG features and MRI for outcome in Normothermia and Hypothermia group[a].

	Sensitivity (%)		Specificity (%)		PPV (%)		NPV (%)	
	NT	HT	NT	HT	NT	HT	NT	HT
EEG background pattern 24 h	26/28 (93)	20/23 (87)	6/9 (67)	19/22 (86)	26/29 (90)	20/23 (87)	6/8(75)	19/22(86)
EEG background pattern normalization within 24 h and adverse outcome								
Crude OR (95% CI) 0.026 (0.007–0.094), p < 0.001								
Adjusted[b] OR (95% CI) 0.006 (0.001–0.062), p < 0.001								
+Adjusted for moderate/severe MRI [c]: 0.011 (0.001–0.128), p < 0.001								
EEG background pattern 48 h	23/28 (82)	18/23 (78)	7/9 (77)	22/22 (100)	23/25 (92)	18/18 (100)	7/12 (58)	22/27 (81)
EEG background pattern normalization within 48 h and adverse outcome								
Crude OR (95% CI) 0.017 (0.003–0.083), p < 0.001								
Adjusted[b] OR (95% CI) 0.013 (0.002–0.094), p < 0.001								
+Adjusted for moderate/severe MRI [c]: 0.018 (0.002–0.154), p < 0.001								
Sleep wake cycling	24/28 (86)	18/23 (78)	6/9 (67)	21/22 (96)	24/27 (89)	18/19 (95)	6/10 (60)	21/26 (81)
Seizures	19/28 (68)	16/23 (70)	4/9 (44)	18/22 (82)	19/24 (79)	16/20 (80)	4/13 (31)	18/25 (72)
MRI	27/28 (96)	22/23 (96)	3/9 (33)	13/22 (59)	27/33 (82)	22/31 (71)	3/4 (75)	13/14 (93)
MRI moderate/severe and adverse outcome								
Crude OR (95% CI) 26.13 (5.39–126.83), p < 0.001								
Adjusted[b] OR (95% CI) 32.19 (4.84–214.25), p < 0.001								
+ Adjusted for EEG background pattern normalization ≤24h [c]: 6.143 (0.741–50.96), p = 0.09								
+ Adjusted for EEG background pattern normalization ≤48h [c]: 14.77 (1.93–113.10), p = 0.01								

Abbreviations: HT = therapeutic hypothermia, NT = normothermia, EEG = electroencephalogram, MRI = magnetic resonance imaging, OR = odds ratio.

[a] Sensitivity and positive predictive value of abnormal EEG background pattern at 24 or 48 h, no sleep-wake cycling, seizures and moderate/severe MRI, for adverse outcome, and specificity and negative predictive value vice versa. Numbers given for NT and HT infants separately.

[b] Adjusted for HT, gender, gestational age, birth weight Z-score, socio-economic status, 5-minutes Apgar, highest serum lactate, inotropes, inborn/outborn. All infants, NT and HT, are included.

[c] EEG background pattern normalization was additionally adjusted for moderate/severe MRI to identify collinearity and vice versa.

High seizure burden is independently associated with abnormal outcome. Unfortunately, total seizure burden analysis was impossible to perform because not all infants had full EEG and discharges can be missed on aEEG.[23,25,26]

EEG discontinuity is related to MRI in HT, but also shows an independent significant relationship with neurodevelopmental outcome.[9,27–29]

The MRI scans in our study were made at day 4–5. Since early scans may appear normal on conventional images, diffusion-weighted imaging (DWI) was performed.[30] The optimal timing for DWI in HIE is 3–7 days, after which pseudo-normalization can occur.[31] Magnetic resonance spectroscopy (MRS) sequences can have additive prognostic information, but were not systematically performed in our patients.[32]

In infants treated with HT, early (day 4) versus late MRI (≥1 week) both yielded 100% sensitivity for adverse outcome at age two years, but early MRI had a higher specificity.[33] MRI in the second week helps defining injury patterns, but it may be too late to redirect care. The strength of our study is that all MRIs were made at the same time-points. In the TOBY trial, HT was associated with significant reductions in abnormalities in the basal ganglia, thalami and white matter, but the reduction in cortical grey matter injury was not statistically significant.[34] However compared to our study, conventional MRI scans were made after the first week of life, so there were no early MRI scans made combined with diffusion weighed imaging techniques.

Our data revealed a preferential effect of HT on cortical regions, consistent with an earlier study.[35]

The 38% mortality rate among the cooled infants in the present study is slightly higher than those reported in the TOBY (26%) and CoolCap (33%) RCTs. Consistent with literature report, in our cohort deaths often resulted from withdrawal of intensive care.[4] This creates a possible bias, since a severely abnormal MRI is a surrogate biomarker for death, leading to a "self-fulfilling prophecy". Withdrawal of intensive care was significantly more frequent in the NT group (68% versus 44% in the HT group; $p = 0.04$). It is impossible to explore whether infants who died, would have had a good outcome when intensive care would not have been withdrawn. However, surviving infants with a bad prognosis according to both MRI and EEG eventually had a moderate or bad outcome.

Our data stress the importance of interpreting EEG and neuroimaging findings as complementary tools. In our NT group, one infant showed a persistent burst suppression pattern, although the MRI appeared to be normal and the outcome could be classified as normal. In all cases of persistent burst suppression patterns, one should be aware that serum levels of Morphine, or anti-seizure drugs such as Midazolam or Phenobarbital, may be toxic.[36,37] Unfortunately, serum levels were not determined in our cohort.

Another infant in the NT group had a normal EEG background pattern (DNV) at start and a normal MRI, but a severely abnormal outcome. Although MRI findings were classified as normal, corpus callosum diffusion restriction and subdural/subarachnoid blood was present. This addresses the limitations of both the MRI scoring system and classification of EEG background patterns used.[38] In this case, DNV was categorized as normal, but the pattern had not yet completely normalized to CNV. This is consistent with earlier research where some patients with DNV background pattern had abnormal outcome.[17]

One of the limitations of our study is the retrospective design, comparing two historical cohorts from two different era, where therapeutic strategies may have improved over time. A potential bias could be the admission of more mildly asphyxiated infants after HT was introduced. However, HIE grade and Apgar scores at 5 min did not significantly differ between the HT and NT groups. Furthermore, EEG background pattern at start did not differ significantly between both groups.

5. Conclusion

The predictive value of EEG and MRI remains the same in cooled and non-cooled infants with HIE. Our data show a higher predictive value for EEG compared to MRI. In HIE, persistent abnormal EEG background pattern until 48 h, combined with abnormal early MRI is strongly predictive for poor neurodevelopment.

What is already known on this topic

Therapeutic hypothermia (HT) reduces death and severe disability in neonatal hypoxic-ischemic encephalopathy (HIE). Electroencephalography (EEG) and magnetic resonance imaging (MRI) are reliable predictors of neurodevelopmental outcome in the pre-cooling era.

What this study adds

The predictive value of EEG and MRI remains the same in cooled and non-cooled infants with HIE. EEG background pattern is at least as predictive as MRI for adverse outcome. In HIE, persistent abnormal EEG background pattern until 48 h, combined with abnormal early MRI is strongly predictive for poor neurodevelopment.

Declaration of interest

There is no financial or personal interest or belief that could affect the objectivity of the authors. No potential conflicts do exist.

Appendix A. Supplementary data

Supplementary data to this article can be found online at https://doi.org/10.1016/j.ejpn.2018.09.001.

REFERENCES

1. Mortality GBD, Causes of Death C. Global, regional, and national age-sex specific all-cause and cause-specific mortality for 240 causes of death, 1990-2013: a systematic analysis for the Global Burden of Disease Study 2013. *Lancet* 2015;**385**(9963):117–71.

2. Azzopardi D, Strohm B, Marlow N, et al. Effects of hypothermia for perinatal asphyxia on childhood outcomes. *N Engl J Med* 2014;**371**(2):140–9.
3. Jacobs SE, Berg M, Hunt R, et al. Cooling for newborns with hypoxic ischaemic encephalopathy. *Cochrane Database Syst Rev* 2013;**1**, Cd003311.
4. Garcia-Alix A, Arnaez J, Cortes V, et al. Neonatal hypoxic-ischaemic encephalopathy: most deaths followed end-of-life decisions within three days of birth. *Acta Paediatr* 2013;**102**(12):1137–43.
5. van Laerhoven H, de Haan TR, Offringa M, Post B, van der Lee JH. Prognostic tests in term neonates with hypoxic-ischemic encephalopathy: a systematic review. *Pediatrics* 2013;**131**(1):88–98.
6. Barnette AR, Horbar JD, Soll RF, et al. Neuroimaging in the evaluation of neonatal encephalopathy. *Pediatrics* 2014;**133**(6):e1508–17.
7. Leijser LM, Vein AA, Liauw L, et al. Prediction of short-term neurological outcome in full-term neonates with hypoxic-ischaemic encephalopathy based on combined use of electroencephalogram and neuro-imaging. *Neuropediatrics* 2007;**38**(5):219–27.
8. Weeke LC, Boylan GB, Pressler RM, et al. 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. *Eur J Paediatr Neurol* 2016;**20**(6):855–64.
9. Dunne JM, Wertheim D, Clarke P, et al. Automated electroencephalographic discontinuity in cooled newborns predicts cerebral MRI and neurodevelopmental outcome. *Arch Dis Child Fetal Neonatal Ed* 2017;**102**(1):F58–64.
10. Frans K. *Statusontwikkeling van wijken in Nederland 1998–2010*. 2012. Available from: www.scp.nl/Onderzoek/Lopend_onderzoek/A_Z_alle_lopende_onderzoeken/Statusscores.
11. Hellstrom-Westas L, Rosen I. Continuous brain-function monitoring: state of the art in clinical practice. *Semin Fetal Neonatal Med* 2006;**11**(6):503–11.
12. Tsuchida TN, Wusthoff CJ, Shellhaas RA, et al. American clinical neurophysiology society standardized EEG terminology and categorization for the description of continuous EEG monitoring in neonates: report of the American Clinical Neurophysiology Society critical care monitoring committee. *J Clin Neurophysiol* 2013;**30**(2):161–73.
13. van Rooij LG, Toet MC, van Huffelen AC, et al. Effect of treatment of subclinical neonatal seizures detected with aEEG: randomized, controlled trial. *Pediatrics* 2010;**125**(2):e358–66.
14. Azzopardi DV, Strohm B, Edwards AD, et al. Moderate hypothermia to treat perinatal asphyxial encephalopathy. *N Engl J Med* 2009;**361**(14):1349–58.
15. Bayley N. *Bayley scales of infant and toddler development*. San Antonio: Harcourt Assessment, Psych. Corp.; 2006.
16. Jary S, Whitelaw A, Walloe L, Thoresen M. Comparison of Bayley-2 and Bayley-3 scores at 18 months in term infants following neonatal encephalopathy and therapeutic hypothermia. *Dev Med Child Neurol* 2013;**55**(11):1053–9.
17. Thoresen M, Hellstrom-Westas L, Liu X, de Vries LS. Effect of hypothermia on amplitude-integrated electroencephalogram in infants with asphyxia. *Pediatrics* 2010;**126**(1):e131–9.
18. Hallberg B, Grossmann K, Bartocci M, Blennow M. The prognostic value of early aEEG in asphyxiated infants undergoing systemic hypothermia treatment. *Acta Paediatr* 2010;**99**(4):531–6.
19. Massaro AN, Tsuchida T, Kadom N, et al. aEEG evolution during therapeutic hypothermia and prediction of NICU outcome in encephalopathic neonates. *Neonatology* 2012;**102**(3):197–202.
20. Cseko AJ, Bango M, Lakatos P, et al. Accuracy of amplitude-integrated electroencephalography in the prediction of neurodevelopmental outcome in asphyxiated infants receiving hypothermia treatment. *Acta Paediatr* 2013;**102**(7):707–11.
21. Pressler RM, Boylan GB, Morton M, Binnie CD, Rennie JM. Early serial EEG in hypoxic ischaemic encephalopathy. *Clin Neurophysiol* 2001;**112**(1):31–7.
22. Li J, Funato M, Tamai H, et al. Predictors of neurological outcome in cooled neonates. *Pediatr Int* 2013;**55**(2):169–76.
23. Toet MC, van der Meij W, de Vries LS, Uiterwaal CS, van Huffelen KC. Comparison between simultaneously recorded amplitude integrated electroencephalogram (cerebral function monitor) and standard electroencephalogram in neonates. *Pediatrics* 2002;**109**(5):772–9.
24. Awal MA, Lai MM, Azemi G, Boashash B, Colditz PB. EEG background features that predict outcome in term neonates with hypoxic ischaemic encephalopathy: a structured review. *Clin Neurophysiol* 2016;**127**(1):285–96.
25. Kharoshankaya L, Stevenson NJ, Livingstone V, et al. Seizure burden and neurodevelopmental outcome in neonates with hypoxic-ischemic encephalopathy. *Dev Med Child Neurol* 2016;**58**(12):1242–8.
26. Shah DK, Wusthoff CJ, Clarke P, et al. Electrographic seizures are associated with brain injury in newborns undergoing therapeutic hypothermia. *Arch Dis Child Fetal Neonatal Ed* 2014;**99**(3):F219–24.
27. Padden B, Scheer I, Brotschi B, et al. Does amplitude-integrated electroencephalogram background pattern correlate with cerebral injury in neonates with hypoxic-ischaemic encephalopathy? *J Paediatr Child Health* 2015;**51**(2):180–5.
28. Obeid R, Sogawa Y, Gedela S, et al. The correlation between a short-term conventional electroencephalography in the first day of life and brain magnetic resonance imaging in newborns undergoing hypothermia for hypoxic-ischemic encephalopathy. *Pediatr Neurol* 2017;**67**:91–7.
29. Elshorbagy HH, Azab AA, Kamal NM, et al. Value of electroencephalographic monitoring in newborns with hypoxic-ischemic encephalopathy treated with hypothermia. *J Pediatr Neurosci* 2016;**11**(4):309–15.
30. Sabir H, Cowan FM. Prediction of outcome methods assessing short- and long-term outcome after therapeutic hypothermia. *Semin Fetal Neonatal Med* 2015;**20**(2):115–21.
31. Lee YK, Penn A, Patel M, et al. Hypothermia-treated neonates with hypoxic-ischemic encephalopathy: optimal timing of quantitative ADC measurement to predict disease severity. *Neuroradiol J* 2017 Feb;**30**(1):28–35.
32. Thayyil S, Chandrasekaran M, Taylor A, et al. Cerebral magnetic resonance biomarkers in neonatal encephalopathy: a meta-analysis. *Pediatrics* 2010;**125**(2):e382–95.
33. Charon V, Proisy M, Bretaudeau G, et al. Early MRI in neonatal hypoxic-ischaemic encephalopathy treated with hypothermia: prognostic role at 2-year follow-up. *Eur J Radiol* 2016;**85**(8):1366–74.
34. Rutherford M, Ramenghi LA, Edwards AD, et al. Assessment of brain tissue injury after moderate hypothermia in neonates with hypoxic-ischaemic encephalopathy: a nested substudy of a randomised controlled trial. *Lancet Neurol* 2010;**9**(1):39–45.
35. Inder TE, Hunt RW, Morley CJ, et al. Randomized trial of systemic hypothermia selectively protects the cortex on MRI in term hypoxic-ischemic encephalopathy. *J Pediatr* 2004;**145**(6):835–7.
36. ter Horst HJ, Brouwer OF, Bos AF. Burst suppression on amplitude-integrated electroencephalogram may be induced by midazolam: a report on three cases. *Acta Paediatr* 2004;**93**(4):559–63.

37. van Leuven K, Groenendaal F, Toet MC, et al. Midazolam and amplitude-integrated EEG in asphyxiated full-term neonates. *Acta Paediatr* 2004;**93**(9):1221–7.
38. Rollins N, Booth T, Morriss MC, et al. Predictive value of neonatal MRI showing no or minor degrees of brain injury after hypothermia. *Pediatr Neurol* 2014;**50**(5):447–51.
39. Sarnat HB, Sarnat MS. Neonatal encephalopathy following fetal distress. A clinical and electroencephalographic study. *Arch Neurol* 1976;**33**(10):696–705.